FORMULATION DESIGN AND OPTIMIZATION OF TOPICAL MICROEMULSION IN SITU GELLING SYSTEM: DRUG DELIVERY TO POSTERIOR SEGMENT OF THE EYE

A THESIS SUBMITTED TO

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MASTER OF PHARMACY
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BY

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CERTIFICATE

This is to certify that ***Ms. Moorti S. Khodakiya*** *has prepared her thesis entitled "Formulation design and optimization of topical microemulsion in situ gelling system: Drug delivery to posterior segment of the eye", in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under our guidance. She has carried out the work at the Department of Pharmaceutics & Pharmaceutical Technology, Institute of Pharmacy, Nirma University.*

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DECLARATION

I declare that the thesis entitled "Formulation design and optimization of topical microemulsion in situ gelling system: Drug delivery to posterior segment of the eye", has been prepared by me under the guidance of Prof. (Dr.) Tejal A. Mehta, Professor & Head and Mr. Jigar Shah, Assistant Professor, Department of Pharmaceutics & Pharmaceutical technology, Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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ABSTRACT

The purpose of this research was to develop the microemulsion based in situ gelling system of gatifloxacin for prophylaxis and treatment of the posterior segment diseases like endophthalmitis. Gatifloxacin is well reported for the treatment of endophthalmitis, it has better penetration into ocular tissues and high potency compared to many of the same class drugs. Unlike halogenated fluoroquinolones it is safe to use in higher dose and is devoid of phototoxicity. So, Gatifloxacin was used as a model drug. Formulation approach provides better absorption, penetration, retention and improves bioavailability of the drug. The average conc. reached into vitreous humor from topical microemulsion in situ gelling formulation was ~0.4 µg/ml, which is far more than concentration required for therapeutic effect (i.e. >0.1 µg/ml or > >MIC_{90} for *S. Epidermidis*, a pathogen commonly responsible to cause endophthalmitis). Thus, novel microemulsion based in situ gelling formulation could be potential drug delivery system for treatment of posterior segment diseases like endophthalmitis.

1. AIM OF PRESENT INVESTIGATION

Posterior segment ocular diseases are the most prevalent causes of visual impairment. These diseases include Age-related macular degeneration, Proliferative vitreo-retinopathy, Diabetic retinopathy and Endophthalmitis. However, the ocular drug market is dominated by anterior segment drug therapies (e.g. antibiotics, anti-inflammatory agents, diagnostics, and intraocular pressure decreasing anti-glaucoma drugs), typically in eye drop formulations (Del Amo and Urtti, 2008).

Endophthalmitis is an infection of intraocular fluids like vitreous humor and ocular tissues (Kresloff, Castellarin and Zarbin, 1998). To combat the disease, the formulation which provides sufficient concentration in posterior segment is required.

Routes of administration in treatment of posterior segment diseases are intra-vitreal injection, systemic administration, periocular administration and topical administration (Vidyashankar et al., n.d.). Intra-vitreal injection remains most favourable route to treat Endophthalmitis. But, it has drawbacks like frequent puncturing; retinal tissue damage due to drug accumulation; patient non compliance; very expensive and required hospitalization. Systemic and periocular medication requires high dose to maintain MIC_{90} in posterior tissues and leads to more toxicity (Ogura, 2001) (Del Amo and Urtti, 2008). So, topical ocular route remains most favourable to treat ocular diseases.

The anatomic and physiologic barriers of the eye render drug delivery to the posterior segment tissues a major challenge. Topical ocular delivery of drugs can achieve therapeutic concentrations in the anterior segment (cornea, anterior chamber, iris, crystalline lens, and ciliary body) and less effectively to the posterior segment (vitreous humor, retinal pigmented epithelium, retina and choroid) (Hughes et al., 2005).

Conventional ophthalmic formulation like eye drops exhibits many drawbacks like rapid pre-corneal drainage and poor bioavailability. They can not provide sufficient concentration of drug in posterior tissues (Urtti, 2006). Novel formulations like liposomes, niosomes and other microparticulate systems are commonly employed for invasive delivery and they are very expensive (Kaur et al., 2004). This indicates

strong need to formulate a dosage form, which is economic and efficient to overcome the drawbacks of existing ophthalmic formulations.

The in situ drug delivery system and colloidal formulation like microemulsion has potential to use in ocular delivery. Microemulsion provides better permeation of drug through the membrane and provides improved bioavailability (Bagwe et al., 2001) (Anna and Joanna, 2005). The in situ drug delivery system decreases pre-corneal drainage, increase the contact time of formulation with eye and prolong the release in ocular tissues. Again, in situ gelling system has advantage of delivering accurate and reproducible quantities, in contrast to already gelled formulations. To exploit the benefits of these two dosage forms, microemulsion based in situ gelling system was developed as a new vehicle for ophthalmic drug delivery (Gan et al., 2009). The essential idea is to encapsulate the drug in droplets that form a microemulsion, and then disperse the drug-loaded droplets in a polymer solution that gels upon triggering by the electrolyte present in the tear fluid.

This novel dosage form have advantages of improved bioavailability due to efficient penetration of nano sized globules into deep tissues of eye and gelling reduces the drainage of instilled volume, prolongs the action of drug and minimize the toxicity. Thus, the formulation might be use as an alternative to intra-vitreal injection for delivery of drug to posterior segment.

Gatifloxacin is well reported for the treatment of endophthalmitis, it has better penetration into ocular tissues and high potency compared to many of the same class drugs (Conventry healthcare company, 2008). Unlike halogenated flouroquinolones, it is safe to use in higher dose and is devoid of phototoxicity (Gardner, 2010). It is also reported that Gatifloxacin has more ocular tolerability compared to Moxifloxacin (Donnenfeld et al., 2004). So, Gatifloxacin was used as a model drug to formulate microemulsion based in situ gelling system.

Chapter 2
Introduction

2. INTRODUCTION

2.1 ANATOMY OF THE EYE

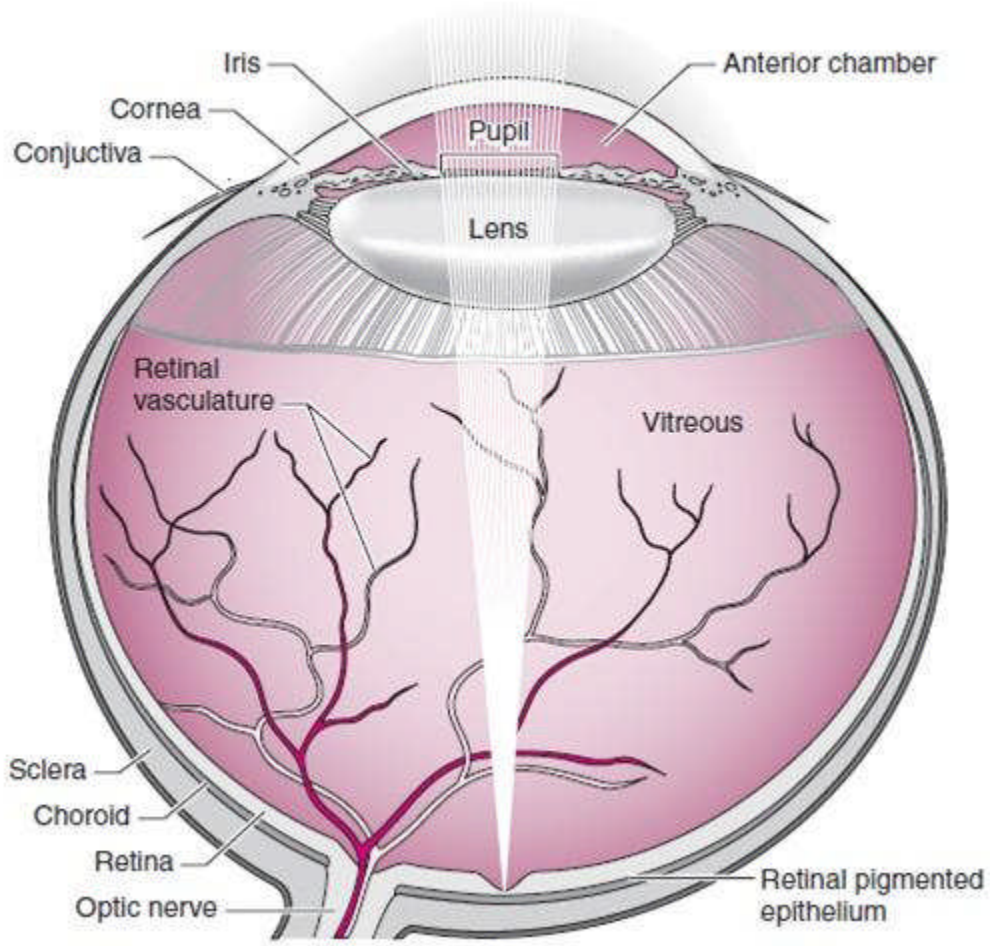
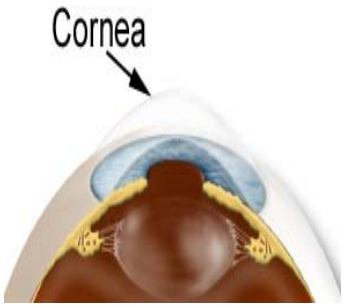
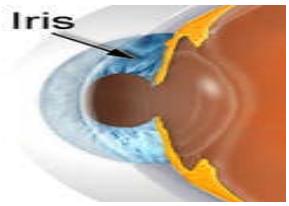
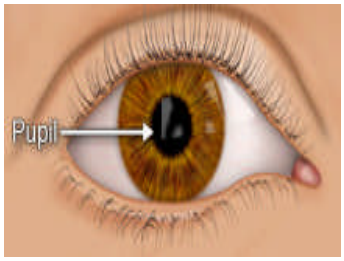
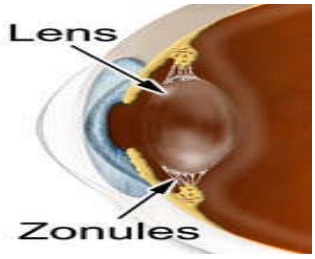
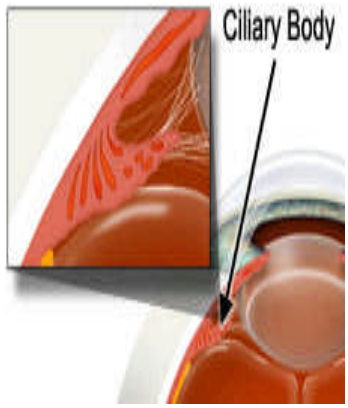
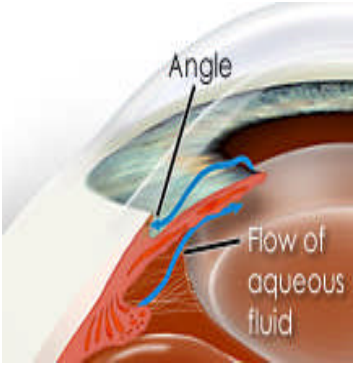
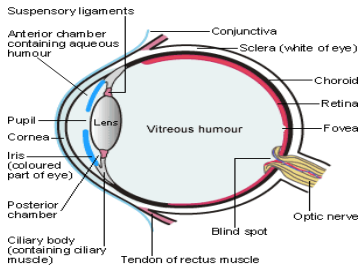
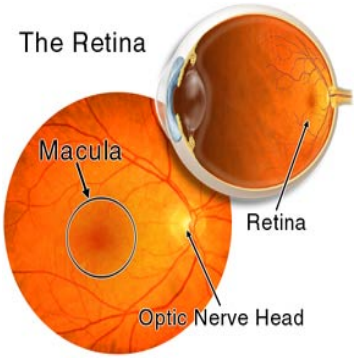


Figure 1: Anatomy of the Eye

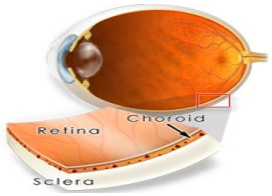
(Courtesy: Pharmacotherapy – A Pathophysiological Approach by Joseph T. et al)

In order to develop an effective ophthalmic delivery system, a good understanding of the anatomy and physiology of the eye is necessary. Figure 1 shows a cross section through the human eye. The eye composes of two segments. The anterior segment consists of iris, pupil, ciliary body and aqueous humor. The posterior segment consists of the lens, sclera, choroid, vitreous humor, retina and optic nerve.

CONJUCTIVA 	The conjunctiva is the thin, transparent tissue that covers the outer surface of the eye. It begins at the outer edge of the cornea, covering the visible part of the sclera, and lining the inside of the eyelids. It is nourished by tiny blood vessels that are nearly invisible to the naked eye. The conjunctiva also secretes oils and mucous that moistens and lubricates the eye.
CORNEA 	The cornea is the transparent, dome-shaped window covering the front of the eye. It is a powerful refracting surface, providing 2/3 of the eye's focusing power. Because there are no blood vessels in the cornea, it is normally clear and has a shiny surface. The cornea is extremely sensitive - there are more nerve endings in the cornea than anywhere else in the body.
IRIS 	The colored part of the eye is called the iris. It controls light levels inside the eye similar to the aperture on a camera. The round opening in the center of the iris is called the pupil. The iris is embedded with tiny muscles that dilate and constrict the pupil size.
PUPIL 	The pupil is the opening in the center of the iris. The size of the pupil determines the amount of light that enters the eye. The pupil size is controlled by the dilator and sphincter muscles of the iris. Doctors often evaluate the reaction of pupils to light to determine a person's neurological function.
LENS 	The crystalline lens is located just behind the iris. Its purpose is to focus light onto the retina. The nucleus, the innermost part of the lens, is surrounded by softer material called the cortex. The lens is encased in a capsular-like bag and suspended within the eye by tiny "guy wires" called zonules.

CILIARY BODY 	The ciliary body lies just behind the iris. Attached to the ciliary body are tiny fibers "guy wires" called zonules. The crystalline lens is suspended inside the eye by the zonular fibers. Nourishment for the ciliary body comes from blood vessels which also supply the iris. One function of the ciliary body is the production of Aqueous humor, the clear fluid that fills the front of the eye. It also controls accommodation by changing the shape of the crystalline lens.
AQUEOUS HUMOR 	The aqueous humor is the thin, watery fluid that fills the space between the cornea and the iris (anterior chamber). It is continually produced by the ciliary body, the part of the eye that lies just behind the iris. This fluid nourishes the cornea and the lens and gives the front of the eye its form and shape. The production and drainage of aqueous fluid determines the eye's intraocular pressure (IOP).
VITREOUS HUMOR 	The vitreous humour is a transparent jelly-like mass located behind the lens. It acts as a "suspension" for the lens so that the delicate lens is not damaged. It helps to maintain the shape of the posterior chamber of the eyeball and keep the retina in place.
RETINA 	The retina is a multi-layered sensory tissue that lines the back of the eye. It contains millions of photoreceptors that capture light rays and convert them into electrical impulses. These impulses travel along the optic nerve to the brain where they are turned into images. There are two types of photoreceptors in the retina: rods and cones. The cones are contained in the macula, the portion of the retina responsible for central vision.

CHOROID	The choroid lies between the retina and sclera. It is composed of layers of blood vessels that nourish the back of the eye. The choroid connects with the ciliary body toward the front of the eye and is attached to edges of the optic nerve at the back of the eye.



2.2 ANATOMICAL AND PHYSIOLOGICAL CONSIDERATIONS IN TOPICAL DELIVERY

2.2.1 PRECORNEAL AREA

2.2.1.1 Precorneal tear film

Corneal transparency and good visual function require a uniform eye surface. This is achieved by the tear film, which covers and lubricates the cornea and the external globe. It is about 7 – 8 μm thick and is the first structure encountered by topically applied drugs. The trilaminar structure of the tear film is shown in Figure 2. Attached to the glycocalix of the corneal/conjunctival surface is a mucous layer, which consists mainly of glycoproteins. It plays an important role in the stability of the tear film as well as in the wetting of the corneal and conjunctival epithelium. The middle aqueous layer constitutes about 98% of the tear film. It is composed of water, electrolytes, and various proteins such as lipocalin, lysozyme, and lactoferrin. The outermost lipid layer prevents the evaporation of the tear fluid. It consists of sterol esters, triacylglycerols, and phospholipids and is spread over the aqueous layer during blinking (Rupenthal and Alany, 2008).

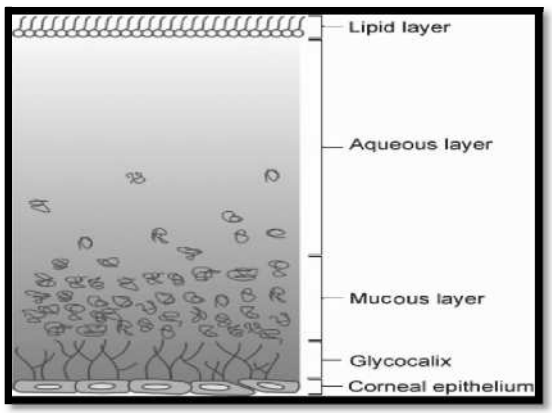


Figure 2: Structure of the precorneal tear film (Courtesy: Pharmaceutical Manufacturing Handbook - Production and Processes Ed by Shayne Cox Gad)

2.2.1.2 Nasolacrimal drainage system

The lacrimal gland, which is situated in the superior temporal angle of the orbit, is responsible for most of the tear fluid secretion. Secreted fluid is spread over the surface of the cornea during blinking and ends up in the puncta when the upper eye lid approaches the lower lid. The blinking process creates a suction mechanism which results in tears flowing through the lacrimal canaliculi into the lacrimal sac. Fluid from the lacrimal sac then drains into the 12 mm long nasolacrimal duct, which empties into the inferior nasal passage. This passage is a highly vascular area and is responsible for most of the systemic drug absorption and subsequent systemic side effects of topically administered drug (Jarvinen, Jarvinen and Urtti, 1995)

The cul-de-sac normally holds 7–9µL of tear fluid, with the normal tear flow rate being 1.2–1.5µL/min (Chastain, 2003). Loss from the precorneal area by drainage, tear fluid turnover, and noncorneal absorption plays an important role in determining the ocular bioavailability of a drug (Worakul and Robinson, 1997). As the drainage rate is much faster than the ocular absorption rate, most of the topically applied drug is eliminated from the precorneal area within the first minute. Tear production can be divided into basal, reflex, and emotional tearing. Reflex tearing can be induced by many pharmaceutical/formulation factors, including the drug itself as well as pH and tonicity of ocular dosage form (Rupenthal and Alany, 2008).

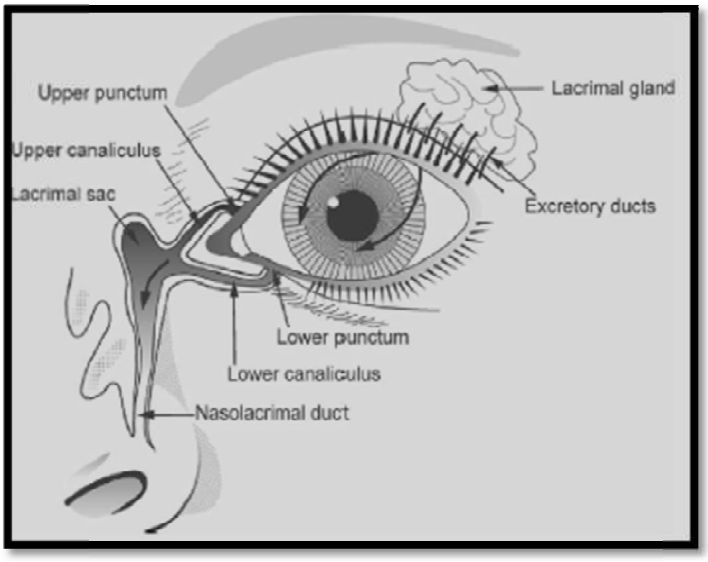


Figure 3: Schematic of the nasolacrimal drainage system

(Courtesy: Pharmaceutical Manufacturing Handbook - Production and Processes Ed by Shayne Cox Gad)

2.2.2 TRANSPORT BARRIERS IN THE EYE

Topical administration is the most common route for ocular drug delivery. Consequently, the cornea, conjunctiva, and sclera form the most essential barriers for drug penetration into the intraocular tissues.

Cornea:

The cornea is composed of five layers: epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium. It is an important mechanical barrier protecting the intraocular tissues. It is considered to be the main pathway for ocular penetration of topically applied drugs (Vellonen, Mannermaa and Urtti, 2006). However, due to its unique structure, with the hydrophilic stroma sandwiched between the highly lipophilic epithelium and the less lipophilic endothelium, the penetration of compounds through the cornea depends on their n-octanol – water partition coefficient. Only drugs with a partition coefficient between 10 and 100 that show both lipid and water-soluble properties can readily pass through the cornea (Vellonen, Mannermaa and Urtti, 2006) (Macha, Hughes and Mitra, 2003).

Conjunctiva:

The conjunctiva is a thin and vascular mucous membrane consisting of two to three layers of epithelial cells overlying a loose, highly vascular connective tissue. The tight junctions present on the apical surface of the epithelium act as the main barrier for drug penetration (molecules > 20,000 Da) across the tissue, although not as tight as the corneal epithelium, which is impermeable to molecules larger than 5000 Da (Huang, Tseng and Kenyon, 1989) (Greaves and Wilson, 1993) . The conjunctiva covers the anterior surface of the globe (bulbar conjunctiva), with the exception of the cornea, and is folded at the fornix (fornix conjunctiva) to form the palpebral conjunctiva, which lines the inner surface of the eyelids. The bulbar conjunctiva represents the first barrier for permeation of topically applied drugs via the noncorneal route (Ahmed and Patton, 1985).

Sclera:

The sclera is the outermost firm coat of the eye that serves as a protective barrier for the sensitive inner parts. It is composed of the same type of collagen fibers as the

corneal stroma. However, the fibers are arranged in an irregular network rather than a lattice pattern, which makes the tissue appear opaque compared to the transparent cornea. The white sclera constitutes the posterior 5/6th of the globe, whereas the transparent cornea comprises the anterior 1/6th of the globe (Macha, Hughes and Mitra, 2003).

Protective blood – ocular barriers:

The blood-ocular barriers can be divided into blood-aqueous barrier and blood-retinal barrier (Urtti, 2006). The blood-aqueous barrier is located in anterior part of the eye and is formed by endothelial cells of the blood vessels in iris and nonpigmented cell layer of the ciliary epithelium (Hornof, Toropainen and Urtti, 2005). It regulates the solute exchange between the blood and the intraocular fluid, preventing unspecific passage of solutes that could influence the transparency of the ocular tissues. The outward movement into the systemic blood circulation is less restricted, allowing especially small and lipophilic drug molecules to enter the uveal blood circulation (Jumbe and Miller, 2003). These molecules are consequently removed more rapidly from the anterior chamber than larger, hydrophilic molecules, which are eliminated by the aqueous humor turnover only (Urtti and Salminen, 1993). The blood – retinal barrier can be found in the posterior part of the eye. It prevents toxic molecules, plasma components, and water from entering the retina. It also forms a barrier for passage of systemically administered drugs into the vitreous, typically resulting in only 1 – 2% of the drug's plasma concentration in the intraocular tissues (Duvvuri, Majumdar and Mitra, 2003) (Vellonen, Mannermaa and Urtti, 2006).

2.3 COMMON DISORDERS OF THE EYE (Garg, 2008)

1. **Cataract**
2. **Conjuctival and scleral disorders**
    - Allergic Conjuctivitis
    - Episcleritis
    - Infectious Conjuctivitis
    - Noncancerous Growths
    - Scleritis
    - Trachoma

3. **Corneal disorders**
    - Bullous Keratopathy
    - Corneal Ulcer
    - Herpes Simplex Keratitis
    - Herpes Zoster Ophthalicus
    - Keratoconjuctivitis Sicca
    - Keratoconus
    - Keratomalacia
    - Peripheral Ulcerative Keratitis
    - Superficial Punctate Keratitis
4. **Eyelid and tearing disorders**
    - Blepharitis
    - Canaliculitis
    - Chalazion
    - Dacryocystitis
    - Dacryostenosis
    - Entropion and Ectropion
    - Eyelid Tumors
    - Stye (Hordeolum)
    - Trichiasis
5. **Eye socket disorders**
    - Infections of the Orbit
    - Inflammation of Orbit
    - Proptosis
    - Tumors of the Orbit
6. **Glaucoma**
7. **Injuries to eye**
    - Blunt Injuries to the Eye
    - Chemical Burns to the Eye
    - Corneal Abrasions and Foreign Bodies
    - Eyelid Lacerations
    - Fractures of the Orbit
    - Lacerated Eyeball
    - Traumatic Iritis and Chemical Iritis

8. **Optic nerve disorders**
    - Ischemic Optic Neuropathy
    - Optic Neuritis
    - Papilledema
    - Toxic Amblyopia (Nutritional Amblyopia)
9. **Refractive disorders**
10. **Retinal disorders**
    - Age-related Macular Degeneration
    - Blockage of Central Retinal Arteries and Veins
    - Cancers affecting the Retina
    - Diabetic Retinopathy
    - Endophthalmitis
    - Epiretinal Membrane disorder
    - Hypertensive Retinopathy
    - Retinitis Pigmentosa
11. **Uveitis**

2.4 ENDOPHTHALMITIS

2.4.1 ENDOPHTHALMITIS: INTRODUCTION

Endophthalmitis is infection inside the eye. It is uncommon disease. It is caused by organisms that have entered the eye through a surgical incision (Pijl et al., 2010) or an injury to the eyeball or, less often, has travelled through bloodstream into the eye. Infection in the bloodstream has many possible causes, such as intravenous drug use, an abscess (a collection of pus), skin ulcers, infections such as pneumonia or sepsis, or surgery anywhere in the body. Infection is usually due to bacteria, but fungi or protozoa may also be responsible. Viruses can also cause extensive eye infections, but these are not usually classified as endophthalmitis (Garg, 2008).

2.4.2 COMMON TYPES OF ENDOPHTHALMITIS

Two broad categories of endophthalmitis are Infectious Endophthalmitis and Non-infectious Endophthalmitis (Kresloff, Castellarin and Zarbin, 1998). The infectious endophthalmitis are further distinguished into endogenous and exogenous infections. Exogenous infection results from introduction of organisms into the eye through a surgical or traumatic penetrating wound. Endogenous (or metastatic) infection is

caused by organisms that enter the eye via the bloodstream. Both categories of infection are extremely serious, threatening blindness and even loss of the globe (Greenwald, 2008).

Table 1: Classification of Endophthalmitis (Kresloff, Castellarin and Zarbin, 1998)

Infectious Endophthalmitis	Endogenous
	Postoperative • Acute postoperative endophthalmitis • Delayed-onset endophthalmitis (onset >6 weeks postoperatively • Conjunctival filtering bleb–associated
	Posttraumatic
Noninfectious Endophthalmitis	Sterile uveitis
	Phacoanaphylactic Endophthalmitis
	Sympathetic ophthalmia

2.4.3 CAUSATIVE ORGANISMS OF ENDOPHTHALMITIS

The term endophthalmitis is applied to bacterial, fungal or protozoal infection involving intraocular tissues (retina, uveal tract, or lens) or fluids (vitreous or aqueous) (Greenwald, 2008). 94.2% of isolates from post–operative Endophthalmitis are gram positive bacteria (70% due to *Staphylococcus epidermidis*). Other common pathogens are *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Propionibacterium acnes*, *Haemophilus influenzae*, *E coli*, *Bacillus cereus*, *Neisseria gonorrhoea*, and *Proteus mirabilis* (Hariprasad et al., 2004).

2.4.4 SIGNS AND SYMPTOMS

Symptoms of endophthalmitis may be severe and include pain, redness in the white of the eye, extreme sensitivity to bright light, and partial or complete loss of vision. Other most common signs of endophthalmitis are decreased vision, mild to moderate anterior chamber reaction, hypopyon (pus in the eye), vitritis, variable pain, conjunctival hyperaemia, chemosis, lid oedema, corneal oedema, poor fundal glow (Vidyashankar et al., n.d.). Figure 4 shows 78-year-old man's eye with the symptoms of endophthalmitis.

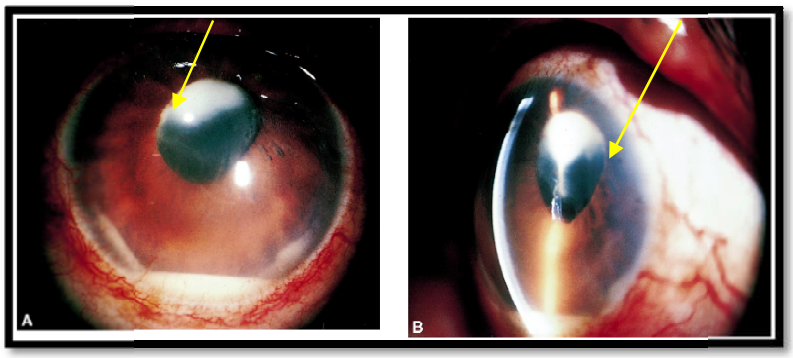


Figure 4: *Staphylococcus epidermidis* chronic endophthalmitis in a 78-year-old man. Note the white plaque posterior to the posterior chamber and hypopyon. *A:* Frontal view. *B:* Tangential view.
(Courtesy: Survey of Ophthalmology, Michael S. Kresloff et al.)

2.4.5 DETECTION AND DIAGNOSIS

The diagnosis is based on the symptoms, an examination of the eye, cultures, and sometimes antibody or DNA testing. Cultures may be taken from the aqueous humor and the vitreous humor and plated on sheep blood agar, chocolate agar, thioglycolate broth, and Sabrouraud's dextrose media and smears are treated with Giemsa and Gram staining to determine which organism is responsible and which drugs are most active against them (Raichand and Peyman, 1982).

2.4.6 DRUGS USED IN ENDOPHTHALMITIS

There are four main characteristics that one should evaluate in an ocular antibiotic prior to making a selection. First, the drug must be able to effectively penetrate into the intraocular tissues. Second, the drug must be of adequate potency, which, in the case of antibiotics, is measured by the mean inhibitory concentration (MIC). Third, there must be minimal bacterial resistance to the drug. Finally, the drug should be able to rapidly eradicate bacteria, before infection is able to set in (Busquets, 2007). Serious vision-threatening infections like Endophthalmitis require the empirical use of broad spectrum antibiotics. Two forth-generation fluoroquinolones, Gatifloxacin (Zymar) and Moxifloxacin (Vigamox), appear to provide better coverage for gram-positive and resistant organisms than the third-generation fluoroquinolone, levofloxacin (Quixin, Iquix), and the second-generation fluoroquinolones, Ciprofloxacin (Ciloxan) and Ofloxacin (Ocuflox) (Conventry healthcare company, 2008). Moxifloxacin and Gatifloxacin offer improved spectrum of activity along with

increased penetration into ocular tissues, and delayed propensity to the development of bacterial antibiotic resistance (Mah, 2004). In term of photosensitivity of drug, unlike the halogenated fluoroquinolones (i.e. Clinafloxacin, Sitafloxacin, and Lomefloxacin), Moxifloxacin and Gatifloxacin do not have a chlorine atom at the C8 position; instead, they have a methoxy group that is not associated with photosensitivity (Gardner, 2010). Therefore Moxifloxacin or Gatifloxacin can be best option for the treatment of endophthalmitis. Previous research also suggests that Gatifloxacin 0.3% is significantly better tolerated than Moxifloxacin 0.5% as measured by degree of conjunctival hyperaemia and vascularity, ocular irritation and pain. Moxifloxacin also induce pupillary miosis, may be due to prostaglandin release in the anterior chamber (Donnenfeld et al., 2004). So, Gatifloxacin would be better option for the treatment.

2.4.7 TREATMENT OPTIONS (Vidyashankar et al., n.d.)

1. **Anti microbial therapy**
   - Intra-vitreal
   - Topical
   - Peribulbar or Intra cameral
   - Systemic
2. **Anti inflammatory therapy (NSAIDS & corticosteroids)**
   - Intra-vitreal
   - Topical
   - Systemic
3. **Supportive therapy**
   - Anti glaucoma medication
   - Vitamins
4. **Vitrectomy**

2.4.8 THERAPEUTIC CHALLENGES

Anatomic barriers and the delicate nature of the interior of the eye are factors to consider during treatment. Key anatomic barriers that prevent adequate treatment of endophthalmitis are the inner and outer blood–retinal barrier and the blood–aqueous humor barrier, collectively called the blood–ocular barrier. The blood–ocular barrier, similar to the blood–brain barrier, consists of tight junctions between the endothelial

cells and basement membrane of retinal capillaries and retinal pericytes. It protects the interior of the eye from assault by cells, macromolecules, and drugs & prevents the entrance and subsequent activity of most systemic antimicrobial and anti-inflammatory drugs (Ogura, 2001). Intraocular barriers can be bypassed by direct injection of drugs into the vitreous. Photoreceptors and other cells of the retina are exquisitely sensitive to insult, and high doses of antimicrobial agents necessary to sterilize the eye may have toxic effects on the retina, potentially disrupting the biochemical pathways necessary for vision (Jacquin et al., 2001). Systemic administration of drugs has been used to treat some vitreoretinal diseases. But previous studies reported a very small amount of drugs could reach the eye after systemic administration. A large systemic amount is required to obtain a therapeutic level of drug concentration in the eye (Ogura, 2001).

2.5 OCULAR DRUG DELIVERY

2.5.1 HISTORY

Eye drops have already been used at the times of Cleopatra for the treatment of ocular conditions. For example, Belladonna (i.e. atropine) was used as a mydriatic in ancient Egypt. The eye drops must be administered frequently, their ocular bioavailability is low (less than 5% of the dose is absorbed), and the posterior segment cannot be treated with them. For these reasons, prolonged action dosage forms with improved ocular absorption have been developed. The first polymeric inserts that release the drug over prolonged period were used already in the late 1800s in the U.K. These gelatine inserts released cocaine for the purpose of local ocular anaesthesia. Soluble ophthalmic drug inserts (SODI) were introduced in the 1960s in the Soviet Union. They were manufactured as several versions that contained drugs such as pilocarpine and mydriatics. The matrix of an acrylate-based co-polymer dissolved during a couple of hours after its application to the conjunctival sac. Pilocarpine releasing Ocusert (Alza, USA) was introduced in the early 1970s in the Western world. This system released the drug for a week at constant rate through ethylene vinyl acetate (EVA) membranes (Armaly and Rao, 1973). Later, another insert, Lacrisert (Merck and Co. Inc., USA), was introduced for the treatment of dry eye syndrome (Cordonnier, 1984). These inserts did not become popular in the out-patient use because the elderly patients had difficulties to use them, and occasionally the insert was expelled from the

conjunctival sac during sleep. This motivated the development of liquid state delivery system that forms a timolol releasing gel after its instillation as an eye drop. Another product releases betaxolol to the tear fluid by ion-exchange from the surface of microspheres. These approaches can modestly delay drug release and prolong drug action. Importantly, the posterior ocular tissues cannot be treated with these topical ocular delivery methods. The need to treat the posterior segment of the eye revived the interest in ocular controlled release systems. Vitrasert (Bausch & Lomb, USA), a polymeric ganciclovir implant, was introduced already in the 1990s for the treatment of opportunistic viral retinitis in the AIDS patients (Del Amo and Urtti, 2008).

2.5.2 CONSTRAINTS TO TOPICAL OCCULAR DRUG DELIVERY

Due to the accessibility of the eye surface, topical administration of ophthalmic medications is the most common method for treatment of eye diseases. However, the unique anatomy and physiology of the eye renders it difficult to achieve an effective drug concentration at the target site. Therefore, efficient delivery of a drug to past the protective ocular barriers accompanied with minimization of its systemic side effects remains a major challenge (Rupenthal and Alany, 2008).

Drugs are mainly eliminated from the precorneal lacrimal fluid by solution drainage, lacrimation and nonproductive absorption to the conjunctiva of the eye (Figure 5). These factors and the corneal barrier limit the penetration of the topically administered drug into the eye.

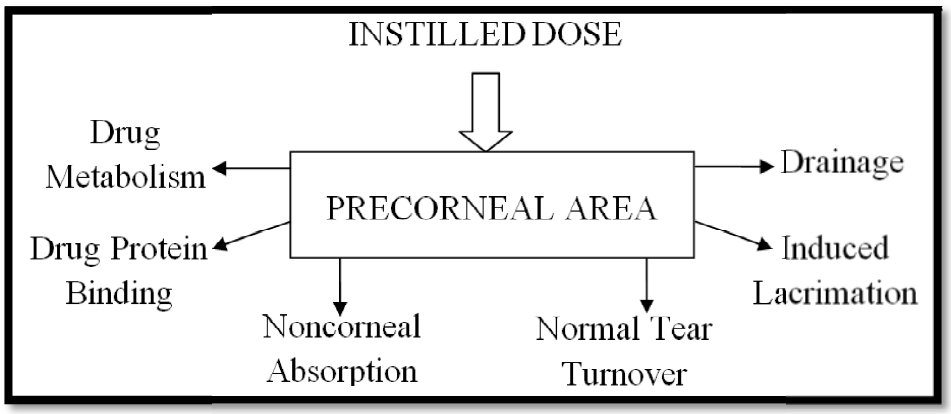


Figure 5: Precorneal factors that affect bioavailability of topically administered ophthalmic formulations

(Courtesy: Advanced drug delivery reviews, Thorsteinn Loftssona et al.)

Only a few percent of the applied dose is delivered into intraocular tissues, while the major part (50-100%) of the dose is absorbed systemically. The major loss of drug is through solution drainage into the nose, which leads to systemic absorption as shown in Figure 6 (Loftssonaa and Ja¨rvinenb, 1999). In addition, the fraction of drug available for absorption is severely limited by physiological constraints such as reflex tearing and reflex blinking that exist in the precorneal area (Lee and Robinson, 1986).

Productive absorption from topical delivery has been described as occurring by two routes: the corneal and non-corneal (conjunctival/scleral) pathways (Figure 7). An aqueous instilled dose leaves the precorneal area within 5 min of instillation in humans and less than 3% of the instilled dose reaches the aqueous humor. The low fraction of applied drug reaching the anterior chamber further undergoes rapid elimination from the intraocular tissues and fluids by distribution into non-target tissues and as a consequence of aqueous humor flow. Further posterior movement from corneal absorption is thought to be prevented by the iridolenticular diaphragm and the aqueous humor flow (Hughes et al., 2005).

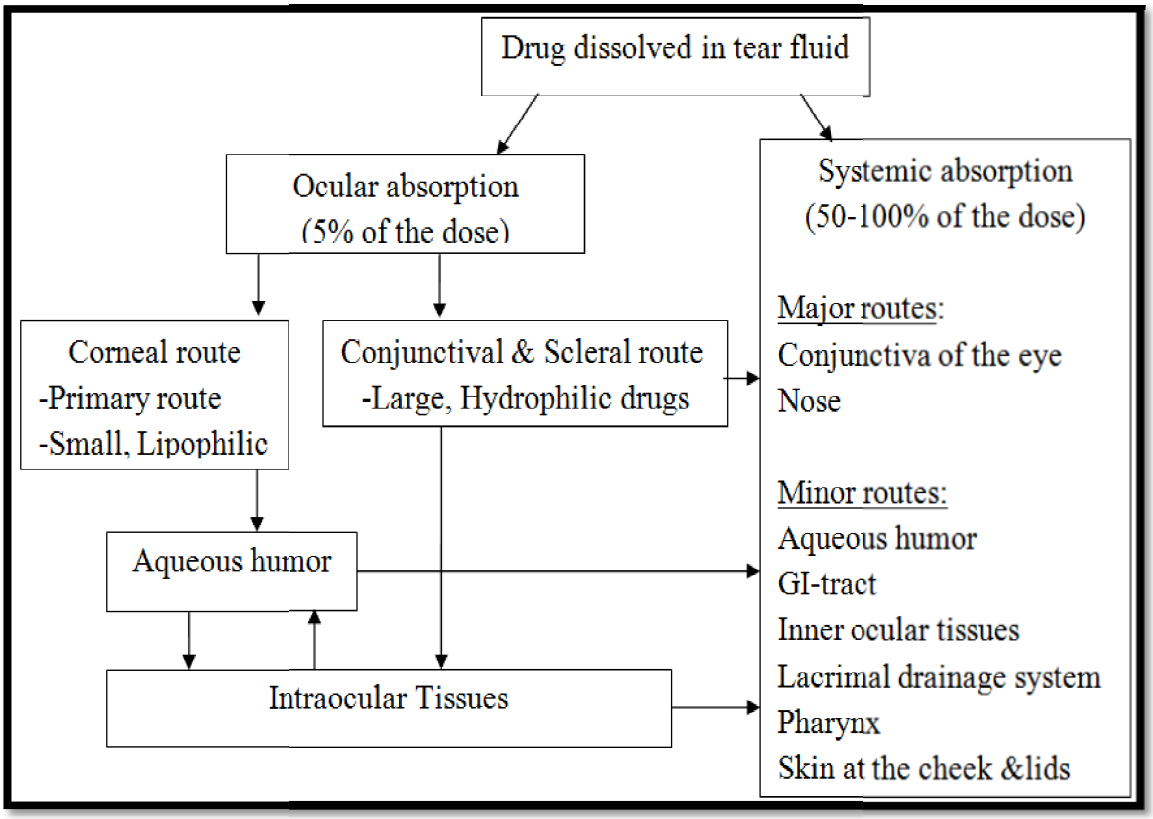


Figure 6: Absorption routes of a topically applied ophthalmic drug

(Courtesy: Advanced drug delivery reviews, Thorsteinn Loftssona et al.)

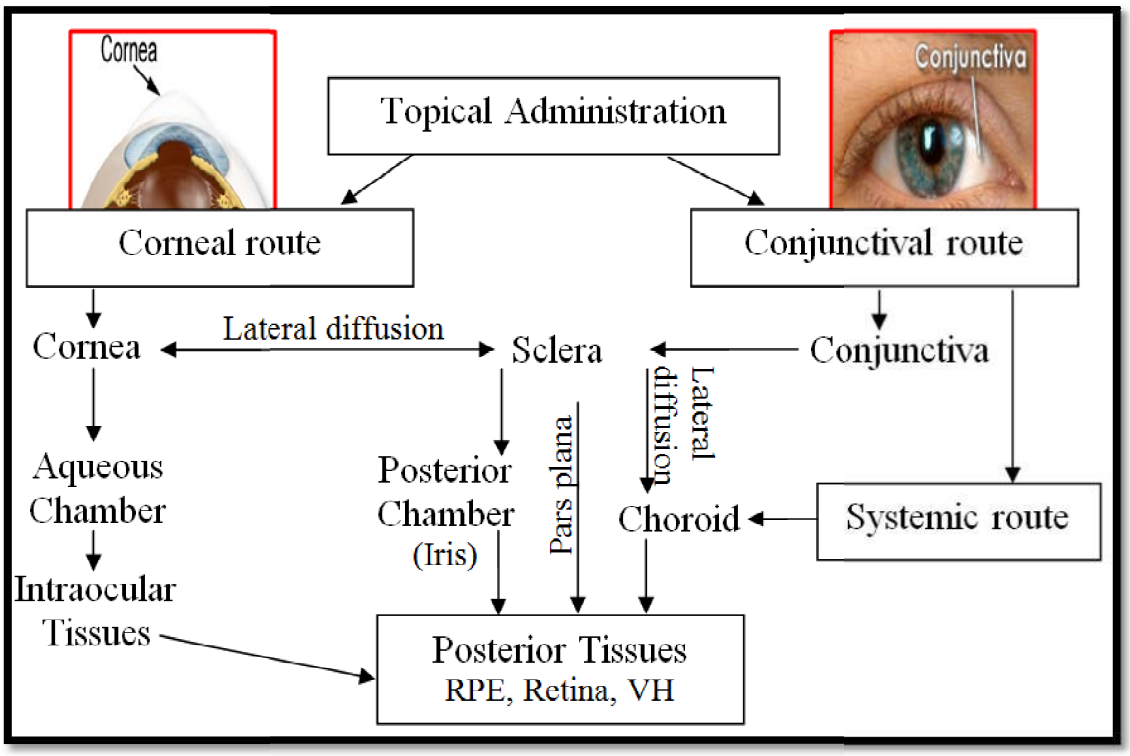


Figure 7: Drug distribution pathways through the corneal and conjunctival/sclera routes following topical administration

(Courtesy: Advanced drug delivery reviews, Patrick M. Hughes et al.)

2.5.3 DRAINAGE OF INSTILLED SOLUTIONS

Drainage, in particular, is very rapid and generally limits ocular contact at the site of absorption to about 3–10 minutes (Worakul and Robinson, 1997). However, the lag time (the time for drug to traverse the cornea and appear in aqueous humor) is sufficiently long to extend time to maximal concentration in the aqueous to between 20 and 60 minutes for most drugs (Worakul and Robinson, 1997). Due to rapid loss of drug from the precorneal region, less than 10%, and more typically less than 1–2%, of a topical dose is absorbed into the eye. At the same time, systemic absorption can be as high as 100%, indicating that most of the drug dose is unavailable for efficacy (Chastain, 2003).

Following the instillation of an applied eye-drop (25– 50 µl) onto the pre-corneal area, the greater part of the drug solution is rapidly drained from the eye surface and the solution volume returns to the normal resident tear volume of 7.5 µl (Chrai et al., 1973). Thereafter, the preocular solution volume remains constant, but drug concentration decreases due to dilution by tear turnover and corneal and non-corneal

absorption (Patton and Robinson, 1976). The value of the first-order rate constant for the drainage of eye drops from pre-corneal area is typically between 0.5 and 0.7 min-1 in rabbits and about 1.5 min-1 in human. Drainage rates decrease with increased viscosity of eye-drop solutions and increase with increased eye drop volumes. Normal tear turnover is about 0.07 min-1 (0.5 ml/min) in rabbits and 0.16 min-1 (1.2 ml/min) in human. Thus, under normal conditions, i.e. when the tear volume is normal, the rate of tear turnover has a minor role in the removal of instilled solutions from the pre-corneal area. However, ocular administration of an irritating drug or vehicle increases the drug loss from the pre-corneal area due to an increased tear flow rate (induced lacrimation) (Loftssonaa and Ja¨rvinenb, 1999).

2.5.4 PROTEIN BINDING

Protein binding of drugs in the tear fluid is another factor affecting drug bioavailability (Conrad et al., 1978). The total protein content of human tears ranges from 0.6 to 2.0 % w/v, with the major components being albumin, globulin and lysozyme. Drug binding to tear proteins results a reduction in the free drug concentrations available for absorption. Protein-bound drug can also be lost due to drainage.

2.5.5 NON-PRODUCTIVE AND SYSTEMIC ABSORPTION

Topically applied ophthalmic drugs remain simultaneously in contact with three absorptive membranes: the cornea, conjunctiva and nasal mucosa, resulting in ocular as well as systemic absorption (Mikkelson, Chrai and Robinson, 1973). Routes that lead to the removal of drug from the precorneal area and do not result in direct ocular uptake are referred to as nonproductive absorption pathways. These noncorneal pathways, which are in parallel with corneal absorption, include conjunctival uptake and drainage via the nasolacrimal duct. Both lead to systemic absorption by way of conjunctival blood vessels in the former case or via the nasal mucosa and gastrointestinal tract in the latter case (Chastain, 2003).

2.5.6 CLASSIFICATION OF THE ROUTES FOR OCULAR DRUG ADMINISTRATION (Del Amo and Urtti, 2008)

- **Invasive drug administration to intraocular cavities**
  - Intravitreal surgery (at the pars plana)

- Repeateda intravitreal injections
- Intracameral surgery (capsular bag)
- Subretinal injection
- Repeateda suprachoroidal injections
- Repeateda intracameral injections

- **Invasive periocular and scleral modes of drug administration**
  - Intrascleral surgery
  - Episcleral surgery
  - Repeateda periocular injections
  - Repeateda subconjuctival injections
  - Transscleral diffusion from controlled release systems
- **Non-invasive methods**
  - Topical administration on the eye
  - Oral administration
- **Systemic administration**
  - Intravenous infusion and injection
  - Per oral

[a] The repetition is needed to accomplish long-term ocular treatment.

2.6 OCULAR DRUG DELIVERY APPROACHES

2.6.1 CONVENTIONAL DOSAGE FORMS

Conventional dosage forms such as solutions, suspensions (micronized drug < 10 μ m), and ointments account for almost 90% of the currently accessible ophthalmic formulations on the market (Lang, 1995) (Le Bourlais et al., 1998). They offer some advantages such as their ease of administration by the patient, ease of preparation, and the low production costs. However, there are also significant disadvantages associated with the use of conventional solutions in particular, including the very short contact time with the ocular surface and the fast nasolacrimal drainage, both leading to a poor bioavailability of the drug.

2.6.2 POLYMERIC DELIVERY SYSTEMS

Polymeric systems used for ocular drug delivery can be divided into three groups: viscosity enhancing polymers, which simply increase the formulation viscosity,

resulting in decreased lacrimal drainage and enhanced bioavailability; mucoadhesive polymers, which interact with the ocular mucin, therefore increasing the contact time with the ocular tissues; and in situ gelling polymers, which undergo sol-to-gel phase transition upon exposure to the physiological conditions present in the eye.

Viscosity -enhancing polymers:

In order to reduce the lacrimal clearance (drainage) of ophthalmic solutions, various polymers have been added to increase the viscosity of conventional eye drops, prolong precorneal contact time, and subsequently improve ocular bioavailability of the drug (Trueblood et al., 1975) (Lee and Robinson, 1986). Among the range of hydrophilic polymers investigated in the area of ocular drug delivery are polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP), cellulose derivates such as methylcellulose (MC), polysaccharides, poly methylvinyl ether maleic anhydride and polyacrylic acids (carbopols) (Chrai and Robinson, 1974) (Saettone et al., 1982) (Trueblood et al., 1975). Typically, these polymers are high molecular weight molecules (5,000 - 100,000 Da), that cannot cross biological membranes.

Mucoadhesive polymers:

Bioadhesion refers to the attachment of a drug molecule or a delivery system to a specific biological tissue by means of interfacial forces. If the surface of the tissue is covered by a mucin film, as is the case for the external globe, it is more commonly referred to as mucoadhesion. Cationic polymers (like chitosan), which are able to interact with the negative sialic acid residues of the mucin, would probably show better mucoadhesive properties than anionic or neutral polymers (Lehr et al., 1992). The most commonly used bioadhesives are macromolecular hydrocolloids with numerous hydrophilic functional groups capable of forming hydrogen bonds (such as carboxyl, hydroxyl, amide, and sulfate groups) (Robinson and Mlynek, 1995). Hyaluronic acid is also most promising mucoadhesive polymer in ocular drug delivery (Saettone et al., 1989).

In situ gelling systems:

The use of preformed gels still has drawbacks that can limit their interest for ophthalmic drug delivery. They do not allow accurate and reproducible administration

of drugs and after administration; they often produce blurred vision, crusting of eyelids, and lacrimation. In situ gelling approach amalgamates the advantages of both solutions and gels, such as accuracy and facility of administration of the former and prolonged residence time of the latter. Thus in situ gels can be instilled as eye drops and undergo an immediate gelation when in contact with the eye. In situ forming gels are liquid upon instillation and undergo phase transition in the ocular cul-de-sac to form viscoelastic gel and this provides a response to environmental changes.

Three methods have been employed to cause phase transition on the surface: change in temperature, change in pH, and change in electrolyte composition. Polymers such as Poloxamers (Pluronic®) (Pan et al., 2008) (Zheng et al., 2002), polysaccharides, N-isopropylacrylamide copolymers, poly(ethylene oxide)/(D,L-lactic acid-co-glycolic acid) copolymers (Jean-Christophe and Eve, 2004), Methylcellulose and hydroxypropyl methylcellulose (HPMC), Xyloglucan (Attwood et al., 2001), Carbomer (Carbopol®) (Wu et al., 2007), Pseudolatexes, Gelrite (Gellan Gum) (Gan et al., 2009) and Alginates undergo phase transitions due to changes in their microenvironment and have been investigated for applications in ocular drug delivery.

2.6.3 COLLOIDAL DRUG DELIVERY SYSTEM

Colloidal carriers are small particulate systems ranging in size from 100 to 400 nm. As they are usually suspended in an aqueous solution, they can easily be administered as eye drops, thus avoiding the potential discomfort resulting from bigger particles present in ocular suspensions or from viscous/sticky preparations (Mainardes et al., 2005). Most efforts in ophthalmic drug delivery have been made with the aim of increasing the corneal penetration of the drug. Colloidal particles are preferably taken up by the corneal epithelium via endocytosis (Calvo et al., 1996). Cornea acts as a drug reservoir, slowly releasing the active compound present in the colloidal delivery system to the surrounding ocular tissues (Lallemand et al., 2003).

Nanoparticles:

Nanoparticles are defined as submicrometer sized polymeric colloidal particles ranging from 10 to 1000 nm in which the drug can be dissolved, entrapped, encapsulated, or adsorbed (Kreuter, 1990). Depending on the preparation process, nanospheres or nanocapsules can be obtained. Nanospheres have a matrix like

structure where the drug can either be adsorbed at the surface of the particle or be dispersed/dissolved in the matrix. Nanocapsules, on the other hand, consist of a polymer shell and a core, where the drug can either be dissolved in the inner core or be adsorbed onto the surface (Mainardes and Silva, 2004).

The most commonly used biodegradable polymers in the preparation of nanoparticulate systems for ocular drug delivery are poly-alkylcyanoacrylates, poly-ε-caprolactone, and polylactic-co-glycolic acid copolymers (Marchal-Heussler et al., 1992). The major limiting issues for the development of nanoparticles include the control of particle size and drug release rate as well as the formulation stability. There is only one microparticulate ocular delivery system in the market, Betoptic S 0.25%. It is obtained by binding of betaxolol to ion exchange resin particles and is found to be bioequivalent to the Betoptic 0.5% solution in lowering the intraocular pressure (Ding, 1998).

Liposomes:

Liposomes are potentially valuable as ocular drug delivery systems due to their simplicity of preparation and versatility in physical characteristics. However, their use is limited by instability (due to hydrolysis of the phospholipids), limited drug loading capacity, technical difficulties in obtaining sterile preparations and blurred vision due to their size and opacity (Lee et al., 1985). In addition, liposomes are subject to the same rapid precorneal clearance as conventional ocular solutions, especially the ones with a negative or no surface charge (Nagarsenker, Londhe and Nadkarni, 1999). Positively charged liposomes, on the other hand, exhibit a prolonged precorneal retention due to electrostatic interactions with the negative sialic acid residues of the mucin layer (Nagarsenker, Londhe and Nadkarni, 1999) (Meisner and Mezei, 1995). There have been several attempts to use liposomes in combination with other newer formulation approaches, such as incorporating them into mucoadhesive gels or coating them with mucoadhesive polymers (Meisner and Mezei, 1995).

Niosomes:

Niosomes are nonionic surfactant vesicles which exhibit the same bilayered structures as liposomes. In order to circumvent some of the limitations encountered with liposomes, such as their chemical instability, the cost and purity of the natural

phospholipids, and oxidative degradation of the phospholipids, niosomes have been developed. Niosomes are biocompatible, biodegradable, and nonimmunogenic (Carafa, Santucci and Lucania, 2002). They were also shown to increase the ocular bioavailability of hydrophilic drugs significantly more than liposomes. This is due to the fact that the surfactants in the niosomes act as penetrations enhancers. A modified niosomes also called discomes, which vary from the conventional niosomes in size and shape. The larger size of the vesicles (12-60 μm) prevents their drainage into the nasolacrimal drainage system. Furthermore, their disclike shape provides them with a better fit in the cul-de-sac of the eye (Kaur et al., 2004).

Microemulsions:

Microemulsions (MEs) are colloidal dispersions composed of an oil phase, an aqueous phase, and one or more surfactants. They are optically isotropic and thermodynamically stable and appear as transparent liquids as the droplet size of the dispersed phase is less than 150 nm. One of their main advantages is their ability to increase the solubilization of lipophilic and hydrophilic drugs accompanied by a decrease in systemic absorption (Schmalfuß, Neubert and Wohlrab, 1997). They can also prolong the release of drug over definite period (Gan et al., 2009). Moreover, MEs are transparent systems thus enable monitoring of phase separation and/or precipitation. In addition, MEs possess low surface tension and therefore exhibit good wetting and spreading properties. The presence of surfactants is advantageous due to an increase in cellular membrane permeability, which facilitates drug absorption and bioavailability (Bagwe et al., 2001). Surfactants most frequently utilized for the preparation of MEs are poloxamers, polysorbates, and polyethylene glycol derivatives (Attwood, 1994). Caution needs to be taken in relation to the amount of surfactant incorporated, as high concentrations can lead to ocular toxicity.

2.6.4 OTHER DRUG DELIVERY SYSTEMS

Many other ocular delivery approaches have been investigated, including the use of prodrugs, penetration enhancers, cyclodextrins, cell encapsulation as well as different types of ocular inserts. In addition, iontophoresis, which is an active drug delivery, utilizing electrical current of only 1-2 mA to transport ionized drugs across the cornea, offers an effective, noninvasive method for ocular delivery.

Another more recent approach is the use of dendrimers in ocular therapy. Dendrimers are synthetic spherical molecules named after their characteristic treelike branching around a central core with a size ranging from 2 to 10 nm in diameter (Esfand and Tomalia, 2001). PAMAM (polyamidoamine) has been the most commonly studied dendrimer system for ocular use (Cloninger, 2002).

2.6.5 SOLID POLYMERIC DEVICES

Solid ocular dosage forms such as films, erodible and nonerodible inserts, rods, and collagen shields have been developed to overcome the typical pulse-entry-type drug release associated with conventional ocular dosage forms. This pulse entry is characterized by a transient overdose, a relatively short period of appropriate dosing, followed by a prolonged period of underdosing. Ocular inserts were developed in order to overcome these disadvantages by providing a more controlled, sustained, and continuous drug delivery by maintaining an effective drug concentration in the target tissues and minimizing the number of applications (Sultana et al., 2006).

The difficulty of insertion by the patient, foreign body sensation, and inadvertent loss of inserts from the eye make these systems less popular, especially among the elderly. Furthermore, the high cost involved in manufacture prevented the insert market from taking off (Calonge, 2001). Two products, Alza Ocusert and Merck Lacrisert, have been marketed, although Ocusert is no longer available.

Ocusert is a membrane-controlled reservoir system for the treatment of glaucoma. It contains pilocarpine and alginic acid in the core reservoir, sandwiched between two transparent, lipophilic ethylenevinyl acetate (EVA) rate-controlling membranes, which allow the drug to diffuse from the reservoir at a precisely determined rate for a period of seven days. This system is nonbiodegradable and must therefore be removed after use. Lacrisert, on the other hand, is a soluble minirod of hydroxypropylmethyl cellulose without any active ingredient. The system is placed in the conjunctival sac, where it softens within an hour and completely dissolves within 14 – 18 h. Lacrisert stabilizes and thickens the precorneal tear film and prolongs the tear film break-up time, which is usually accelerated in patients with dry-eye syndrome (keratoconjunctivitis sicca) (Ranade and Hollinger, 2003).

Numerous studies have also been performed on soluble collagen shields. Collagen shields are fabricated from porcine scleral tissue, which has a similar collagen composition to that of human cornea. Drug loading is typically achieved by soaking the collagen shield in the drug solution prior to application. They are designed to slowly dissolve within 12, 24, or 72 h. Collagen shields have attracted much interest as potential sustained ocular drug delivery systems over the last years (Lee, 1990).

2.6.6 MARKETED OPHTHALMIC DELIVERY SYSTEMS BASED ON RECENT FORMULATION APPROACHES

A critical look at the literature (Table 2) shows promising role of In Situ gelling system as an ocular drug delivery. ME based products will also find their way to successful market following the successful introduction of oral ME Sandimmune Neoral, Norvir and Fortovase.

Table 2: List of Marketed ophthalmic delivery systems based on recent formulation approaches (Rupenthal and Alany, 2008)

FORMULATION APPROACH	POLYMER/BASE	PRODUCT	COMPANY
Suspension/ microparticulates	Carbomer ion exchange resin	Betoptic S	Alcon
Ointments	Wool fat, paraffin	Polyvise	Alcon
	Liquid paraffin, White soft paraffin	LacriLube	Allergan
Viscosity enhancers/ mucoadhesives	Polyethylene glycol (PEG), Propylene glycol (PG), HP-guar	Systane	Alcon
	Dextran, HPMC	Bion Tears	Alcon
	Carboxymethylcellulose sodium (CMC-Na)	Refresh Celluvise	Allergan
	Carboxymethylcellulose sodium (CMC-Na)	Refresh Liquigel	Allergan

Viscosity enhancers/ mucoadhesives	Polyvinyl alcohol (PVA)	Liquifilm Tears	Allergan
	HPMC	Lacrigel	Sunways
	Carbomer	Viscotears	Novartis
	Carbomer, PVA	Nyogel	Novartis
	Hyaluronic acid (HA)	Hy-Drop	Bausch & Lomb Fidia Oftal
	Sodium hyaluronate	Vismed	TRB Chemedica
	Carbomer	Pilopine HS	Alcon
	Polyacrylic acid (PAA)	Fucithalmic	Leo Pharma
In Situ gelling systems	Gellan gum	Timoptic XE	Merck
	Polycarbophil	DuraSite	InSite Vision
	Polyacrylic acid, Poloxamer	Smart Hydrogel	Advanced Medical Solutions
Prodrugs	Dipivefrin hydrochloride (epinephrin prodrug)	Propine	Allergan
Ocular inserts	Alginic acid	Ocusert	Alza
	Hydroxylpropyl cellulose	Lacrisert	Merck
	Silicone elastomer	Ocufit SR	Escalon Medical
	Collagen shield	MediLens	Chiron
		ProShield	Alcon

2.6.7 ADVANTAGES AND DISADVANTAGES OF THE CURRENT AND POTENTIAL DRUG DELIVERY SYSTEMS

Table 3: Advantages and Disadvantages of the current and potential drug delivery systems (Del Amo and Urtti, 2008)

DRUG DELIVERY SYSTEM	ADVANTAGES	DISADVANTAGES
Drops	- Easy to apply - The least invasive of the methods - Good patient acceptance	- Poor ocular bioavailability - Sometimes short duration of action - Ineffective to treat diseases of the posterior segment of the eye - The high concentrations or frequent instillations may lead to ocular and systemic toxicity - Sometimes low patient compliance
Systemic administration	- More effective to treat diseases of the posterior segment of the eye than drops	- Most of the administered drugs do not bypass blood ocular barriers - Side effects: systemic toxicity
Intravitreal, periocular and subconjuctival injections	- Improve drug absorption over systemically and topically delivered agents - More safety drug delivery to the posterior segment of the eye than systemic administration (no systemic toxicity) - Drug delivery to the target sites of the eye	Injections display first-order kinetics (this rapid rise may cause difficulties with toxicity, and drug efficacy can diminish as the drug concentration falls below the targeted range) - Injections have short half-life (few hours) and should be administered repeatedly

		- Side effects: repeated injections can cause pain, discomfort, IOP increases, intraocular bleeding, increased chances for infection, and the possibility of retinal detachment; the major complication for intravitreal injection is endophtalmitis - Poor acceptance by patient
Implants	- An alternative to repeated injections because they increase half-life of the drug and may help to minimize peak plasma level; - they might improve patient acceptance and compliance - Stabilization of the drug - The non-biodegradable implants are more controllable delivery profile and longer periods of drug release than biodegradable ones - The biodegradable implants do not need to be removed	- the insertion of devices is invasive and associated with ocular complications (retinal detachment and intravitreal hemorrhage for intravitreal implant) - The non-biodegradable require surgery to harvest the device once is depleted of the drug (risk of ocular complications) - The biodegradable implants have a final uncontrollable 'burst' in their drug release profile
Microparticles, nanoparticles and liposomes	- Stabilization of the drug - Increase half-life of drugs (the frequency of injections diminishes)	- Side effects: risk associated with injections and vitreous clouding

	- Decrease peak concentration resulting in decreasing the toxicity Localized delivery of drug (RPE cells) - Improved patient compliance and convenience	
Cell encapsulation	- Long-lasting and continuous expression of the given protein (avoiding repeated injections) without genetic alteration of the host tissues - Delivery directly to the target site (limiting toxicity) - Easy retrieval of the implant when desired (making the treatment reversible) - Improve patient compliance	- Side effects: invasive method with the complications related to the surgical insertion and removal - Patient acceptance to be seen
Iontophoresis	- Non-invasive method and easy to use - May combine with other drug delivery systems - Ability of modulate dosage - Good drug penetration to anterior and posterior segment of the eye - Good acceptance by patients	- No sustained half-life: requires repeated administrations - Side effects: mild pain in some cases, but no risk of infections or ulcerations - Risk of low patient compliance because the frequent administrations that may be needed

2.7 EMULSION

2.7.1 EMULSION - INTRODUCTION

Emulsion consists of two immiscible liquids (e.g. oil and water) that are brought together into one pseudo phase by using surfactants. They are prepared using shearing force or shaking (Bibette et al., 2002). The O/W emulsion consists of oil droplets dispersed in water phase. Similarly, W/O emulsion consists of water droplets dispersed in the oil phase.

The word "Emulsion" can be found in "Macroemulsion" as well as in "Microemulsion" (Nielloud and Marti-Mestres, 2000). The differences between macroemulsion and microemulsion (Figure 8) are listed in Table 4 (Talegaonkar et al., 2008).

Table 4: Difference between Microemulsion and Macroemulsion

S.NO	PROPERTY	MICROEMULSION	MACROEMULSION
1	Appearance	Transparent	Cloudy
2	Optical Isotropy	Isotropic	Anisotropic
3	Interfacial tension	Ultra low	High
4	Microstructure	Dynamic (interface is continuously and spontaneously fluctuating)	Static
5	Droplet size	20-200 nm	> 500 nm
6	Stability	Thermodynamically stable, long shelf-life	Thermodynamically unstable (kinetically stable), will eventually phase separate
7	Phases	Monophasic	Biphasic
8	Preparation	Facile preparation, relatively lower cost for commercial production	Require a large input of energy, higher cost
9	Viscosity	Low viscosity	Higher viscosity

Figure 8: Difference between Microemulsion and Macroemulsion

2.7.2 TYPES OF EMULSION

Emulsions can be commonly classified as water-in-oil (W/O) emulsion or oil-in-water (O/W) emulsion. Generally, hydrophilic surfactant forms O/W emulsion easily and hydrophobic surfactant is likely to form W/O emulsion.

Double emulsions are "emulsions of emulsions". The droplets of double emulsions contain a number of inner droplets and are much greater in size as compared with the droplets of single emulsions. Two main types of double emulsions are: O/W/O emulsions and W/O/W emulsions.

Microemulsions can be classified into three types (Figure 9): W/O microemulsion, O/W microemulsion and bicontinuous microemulsion (Alany and Wen, 2008). Bicontinuous microemulsions consist of a net structure which is twined by oil, water, and surfactants.

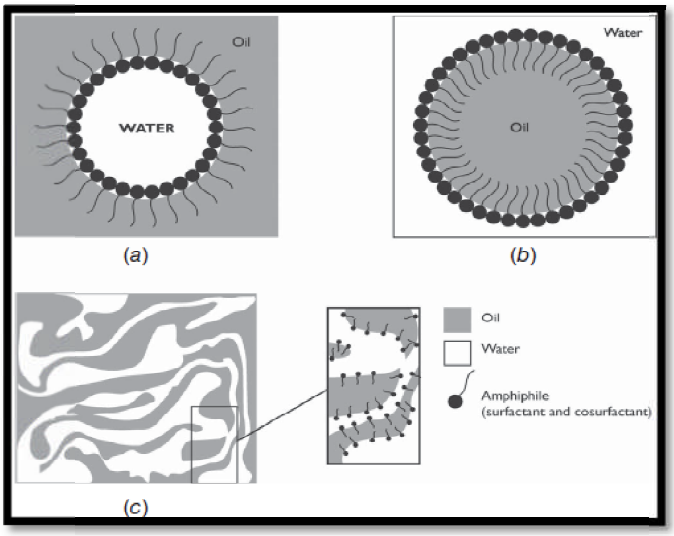


Figure 9: Diagrammatic representation of different types of ME systems: (a) w/o ME, (b) o/w ME, (c) water-and-oil bicontinuous ME

(Courtesy: Pharmaceutical Manufacturing Handbook - Production and Processes Ed. by Shayne Cox Gad)

2.8 MICROEMULSION

2.8.1 MICROEMULSION AS DRUG DELIVERY VEHICLE

Microemulsions have unique physical properties. They are composed of water, oil and a mixture of surfactants making a homogeneous, optically isotropic and thermodynamically stable solution. Microemulsions can be sterilized by filtration and their production is relatively simple and inexpensive. Because of these properties, they have attracted a great interest as drug delivery vehicles. Microemulsions can be applied as liquid membrane carriers to transport lipophilic substances through an aqueous medium or to carry hydrophilic substances across lipoidal medium. They are proposed for oral, topical, dermal, transdermal, parentenal and pulmonary administration of drugs. Although microemulsions have been known for a long period, their potential as vehicles for topical ocular drug delivery has been investigated only within the last decade (Anna and Joanna, 2005). Preparing a pharmaceutical acceptable dosage form demands a clear understanding of the microemulsion structure, phase behaviour, factors leading to its thermodynamic stability, factors influencing drug release from the formulation, requirements of ideal microemulsion excipients, and the potential uses and limitations of the microemulsion system (Tenjarla, 1999).

2.8.2 MICROEMULSIONS ARE NOT NANOEMULSIONS

The main difference between microemulsions and nanoemulsions is that microemulsions are self-assembling nano-scale emulsions whereas nanoemulsions are nano-scale emulsions formed by energy input, generally from mechanical devices or from the chemical potential of the components (Maria et al., 2005).

Microemulsions are isotropic solutions of oil and water and are prepared using a high surfactant concentration of around 40 percent under gentle stirring or shaking. Microemulsions form spontaneously without mechanical shear (Mason et al., 2006). An extremely high concentration of surfactants ensures self-assembling with particle size at the nano-scale level.

Nano-emulsion generating processes are divided into two groups. The first gathers together the ‘high-energy’ processes which use high mechanical shear to reach very small droplet sizes, whereas the second ‘low-energy’ process benefits from the

intrinsic physico-chemical properties of surfactants for generating nano-emulsions (Anton, 2010).

Unlike microemulsions, nanoemulsions are thermodynamically unstable systems as the interfacial tension between oil and water phase is high. It has high kinetic stability against creaming or sedimentation and a large interfacial area (Maria et al., 2005).

2.8.3 MECHANISM OF FORMING MICROEMULSIONS

The formation of a ME system can be explained using a simplified thermodynamic approach and with reference to the equation

$$\Delta G = \gamma\, \Delta A - T\Delta S$$

Where, ΔG = Free energy of ME formation

γ = Interfacial tension at oil-water interface

ΔA = Change in interfacial area (associated with reducing droplet size)

S = System entropy

T = Absolute temperature

The process of ME formation is associated with a reduction in droplet size, which results in an increase in the value of ΔA due to an overall increase in surface area that is associated with droplet size reduction. This is compensated by a very low interfacial tension that is normally achieved by using relatively high amphiphile concentrations. Furthermore, the process of ME formation is accompanied by a favorable entropy contribution (increased value of ΔS) that is due to the mixing of the two immiscible phases, surfactant molecules partitioning in favour of the interface rather than the bulk and monomer-micelle surfactant exchange. The net outcome is a negative value for ΔG which translates into a spontaneous ME formation (Lawrence and Rees, 2000).

Whether an o/w or w/o ME forms is dependent to a great extent on the volume fraction of oil and water as well as the nature of the interfacial film as reflected by the geometry of the amphiphile molecules forming the film. It follows that the presence of o/w ME droplets is more likely to happen in systems where the oil volume fraction is low, whereas w/o ME droplets form when the water volume fraction is low and oil is present in abundance. Interestingly, in systems containing comparable amounts of

water and oil, a bicontinuous ME may exist (Figure 9 *c*). In such systems both oil and water exist as microdomains that are separated by an amphiphile - stabilized interface with a zero net curvature.

Theory of self-assembly of surfactant molecules

It is based solely on geometric considerations. Accordingly, if the volume of the surfactant is *v,* its head group surface area *a,* and its length *l,* it follows that when the critical packing parameter (CPP = *v/al)* has values between 0 and 1, o/w MEs are likely to be formed. On other hand, when CPP is greater than 1, w/o MEs are favoured. When using surfactants with critical packing parameters close to unity (CPP ≈ 1) and at approximately equal volumes of water and oil, the mean curvature of the interfacial film approaches zero and droplets may merge into a bi-continuous structure (Mitchell and Ninham, 1981).

The ratio of hydrophilic and hydrophobic groups of the surfactant molecules, that is, their hydrophile-lipophile balance (HLB), is also important in determining interfacial film curvature and consequently the structure of the ME. The HLB system has been used for the selection of surfactants to formulate MEs and accordingly the HLB of the candidate surfactant blend should match the required HLB of the oily component for a particular system. In brief, a match in the lipophilic part of the surfactant used with the oily component should be favourable (Prince, 1977) (Alany and Wen, 2008).

2.8.4 THE APPLICATIONS OF MICROEMULSIONS

During the last two decades, microemulsions have been extensively researched because of their tremendous potential in many applications. The role of microemulsions in drug delivery is major concern for pharmaceutical development.

Oral delivery

Microemulsions have the potential to enhance the solubilization of the poorly soluble drugs and overcome the dissolution related bioavailability problems. This is particularly important for the BCS class II or class IV drugs. The successful formulation of such drugs is highly dependent on the performance of the formulated product. Microemulsions act as super solvent of these drugs and can be optimized to ensure consistent bioavailability. In addition, they can be used for the delivery of

hydrophilic drugs including macromolecules such as proteins and peptides. This is due to the existence of polar, nonpolar and interfacial domains which allow encapsulation of drugs with varying solubility. Moreover, these systems have been reported to protect the incorporated drugs against oxidation, enzymatic degradation (Sariciaux, Alan and Sado, 1995) and enhance the membrane permeability (Swenson and Curatolo, 1992). Presently, Sandimmune Neoral® (Cyclosporine A), Fortovase® (Saquinavir), Norvir® (Ritonavir), etc. are the commercially available oral SMEDDS formulations.

Parenteral delivery

The formulation of lipophilic and hydrophobic drugs into parenteral dosage forms has proven to be difficult. O/W microemulsions are beneficial in the parenteral delivery of sparingly soluble drugs where the administration of suspension is not desirable. They provide a means of obtaining relatively high concentration of these drugs which usually requires frequent administration. Other advantages are that they exhibit a higher physical stability in plasma than liposomes or other vesicles (Shaw, 1991) and the internal oil phase is more resistant against drug leaching. Several sparingly soluble drugs have been formulated into o/w microemulsion for parenteral delivery (Shaw, 1991) (Kyung-Mi and Chong-Kook, 1999) (Lee et al., 2002) (Rhee et al., 2007) (Ryoo et al., 2005) (Hwang et al., 2004) (Zhao et al., 2005) (Jumma and Muller, 1998). Microemulsions can also be used as intravenous delivery systems for the fat soluble vitamins and lipids in parenteral nutrition (Jumma and Muller, 1998).

Topical delivery

Microemulsions have been reported to enhance the transdermal permeation of drugs significantly compared to conventional formulations such as solutions, gels or creams (Kriwet and Muller-Goymann, 1995) (Trotta, 1999). They are able to incorporate both hydrophilic (5-fluorouracil, apomorphine hydrochloride, diphenhydramine hydrochloride, tetracaine hydrochloride, methotrexate etc.) and lipophilic drugs (estradiol, finasteride, ketoprofen, meloxicam, felodipine, triptolide etc.) and enhance their permeation (Schmalfuß, Neubert and Wohlrab, 1997) (Alvarez-Figueroa and Blanco-Mendez, 2001) (Trotta, Morel and Gasco, 1997) (Chen, Chang and Weng, 2004) (Gupta, Jain and Varshney, 2005) (Elena, Paola and Maria, 2001) (Rhee et al., 2001) (Yuan et al., 2006).

The advantages of microemulsion for the transdermal delivery of a drug are: A large amount of drug can be incorporated in the formulation due to the high solubilizing capacity that might increase thermodynamic activity towards the skin (Hua et al., 2004), the permeation rate of the drug from microemulsion may be increased, since the affinity of a drug to the internal phase in microemulsion can be easily modified to favour partitioning into stratum corneum, using different internal phase, changing its portion in microemulsion (Kreilgaard, 2002), the surfactant and co surfactant in the microemulsions may reduce the diffusional barrier of the stratum corneum by acting as penetration enhancers (Rhee et al., 2001), the percutaneous absorption of drug will also increase due to hydration effect of the stratum corneum if the water content in microemulsion is high enough.

Opthalmic delivery

Microemulsions offer a promising alternative in ocular drug delivery (Alany et al., 2006). In point of view of production and sterilization, microemulsions are simple and inexpensive. Moreover, they are comprised of aqueous and oily components and therefore can accommodate both hydrophilic as well as lipophilic drugs. Water-in-oil microemulsions may be of value as vehicles for ocular drug delivery of irritant hydrophilic compounds as they appear to have a protective effect (Alany et al., 2006).

Microemulsions could become especially favourable for water-continuous ophthalmological carrier systems because of their aqueous consistence, their transparency and thermodynamical stability. Further advantages result from a possible improvement of solubility and stability of drugs with a potential increase in bioavailability, especially for poorly soluble drugs. In addition, no impairment of visibility can be expected in comparison with eye oils. Because of these circumstances the compliance to the patient could be improved (Keipert and HaBe, 1997). The most used surfactants in the preparation of ophthalmic microemulsions are the poloxamers, polysorbates, tyloxapol, polyethylene glycol and their derivatives (Vandamme, 2002).

Periodontal delivery

The periodontium, which anchors the teeth to the jaws, consists of the gingiva, periodontal ligament, cementum and alveolar bone (Newman, Takei and Carranza,

2002). It is normally in a balanced state with the periodontal microbiota in the dental plaque. Human periodontal diseases (i.e. gingivitis and periodontitis) result from heterogenous etiologies, including changes to the complex biofilm in the subgingivial microenvironment, social and behavioral modulations, and genetic or epigenetic traits of the host's immune and inflammatory responses. Periodontitis is a chronic inflammatory disease that is characterized by destructive inflammatory processes affecting the supporting structures of the teeth, causing resorption of alveolar bone and formation of periodontal pockets (Houshmand et al., 2009). It is a major cause of tooth loss. The microemulsion formulation comprising local anaesthetic in oil form, surfactant, water and optionally a taste masking agent could be used as a local anaesthetic for pain relief within the oral cavity in conjunction with periodontal scaling and root planning (Talegaonkar et al., 2008). The formulation can overcome the problem with the existing topical products (jelly, ointment or spray) such as lack of efficacy due to inadequate depth of penetration, too short duration and difficulties in administration due to spread, taste etc. Microemulsion alone or in conjunction with in situ gelling system is promising tool for drug delivery in periodontitis.

Nasal delivery

Microemulsions are now being studied as a delivery system to enhance penetration/uptake across nasal mucosa. Addition of a mucoadhesive polymer helps in prolonging the residence time on the mucosa. Nasal route for administration of diazepam microemulsion might be a useful approach for the rapid onset of action during the emergency treatment of status epilepticus due to better penetration and improved bioavailability (Lianly, Nandi and Kim, 2002).

2.8.5 SCALE-UP AND MANUFACTURE

Scale-up and manufacture of microemulsion is easier. This is because of the intrinsic properties microemulsion. Two characteristics, spontaneous formation and thermodynamic stability are helpful in the scale-up and manufacture processes (Tenjarla, 1999) (De Villiers, Aramwit and Kwon, 2008). Because of the advantages of microemulsion, the manufacturing process only needs very basic mixing equipment to provide mild agitation to form micelles. And the preparation does not require careful in-process control needed in the manufacture of other formulations.

2.9 IN SITU GELLING SYSTEMS

2.9.1 IN SITU FORMING GELS: INTRODUCTION

In situ gelling systems are viscous polymer-based liquids that exhibit sol-to-gel phase transition on the ocular surface due to change in a specific physicochemical parameter (Ionic strength, Temperature, pH). They are highly advantageous over preformed gels as they can easily be applied in liquid form but are capable of prolonging residence time of the formulation on the surface due to gelling (Krauland, Leitner and Bernkop Schnurch, 2003). The principal advantage of in situ gelling systems is easy, accurate, and reproducible administration of a dose compared to application of preformed gels (Zignani, Tabatabay and Gurny, 1995) (Gan et al., 2009).

Methods to cause phase transition on the surface and polymers employed are:

1. Temperature induced gelation:

Polymers that may undergo sol-to-gel transition triggered by a change in temperature are Poloxamers (Pluronic®), Methylcellulose and Hydroxypropyl methylcellulose (HPMC), polysaccharides, N-isopropylacrylamide copolymers, poly(ethylene oxide)/(D,L-lactic acid-co-glycolic acid) copolymers, Xyloglucan etc. They rapidly undergo thermal gelation when the temperature is raised to that of the body temperature (~37 ° C), while they remain liquid at low temperature (temp <250C) (Wei et al., 2002).

2. pH Induced gelation:

Polymers that may undergo sol-to-gel transition triggered by a change in pH are cellulose acetate phthalate (CAP) and cross-linked polyacrylic acid derivates such as carbopols, methacrylates and polycarbophils. CAP latex is a free running solution at pH 4.4 which undergoes sol-to-gel transition when the pH is raised to pH ~7.2. This is due to neutralization of the acid groups contained in the polymer chains, which leads to a massive swelling of the particles (Gurny, Ibrahim and Buri, 1993).

3. Osmotically induced / electrolyte triggered gelation:

Polymers that may undergo sol-to-gel transition triggered by electrolytes in the body fluid are Gelrite (Gellan Gum) and Alginates. They are an anionic polysaccharide. On

contact with cations in body fluid the formulation will form a clear gel. This is caused by cross linking of the negatively charged polysaccharide by monovalent and divalent cations (Na^+, K^+, Ca^{++}). The gel strength of these polymers increases proportionally with the amount of mono or divalent cations present in the body fluid (Greaves et al., 1990).

2.9.2 IN SITU GEL AS DRUG DELIVERY CARRIER

Oral delivery

Oral administration of aqueous solutions containing either gellan gum or sodium alginate and calcium in complexed form, results in the formation of gels in rabbit and rat stomachs as a consequence of the release of the calcium ions in the acidic environment, which function as depots for the release of drug over a period of 6 h (Attwood, Kubo and Miyazaki, 2003). Xyloglucan gels also have potential as vehicles for oral delivery. Gel may be formed in situ (at body temp.) by oral administration of a chilled solution that can sustain the release of drug (Attwood et al., 1999).

Parenteral delivery

Biodegradable injectable in situ forming drug delivery systems represent an attractive alternative to microspheres and implants as parenteral depot systems. These devices may offer attractive opportunities for protein delivery and could possibly extend the patent life of protein drugs. The controlled release of bioactive macromolecules via in situ forming systems has a number of advantages, such as ease of administration, less complicated fabrication, and less stressful manufacturing conditions for sensitive drug molecules. For these reasons, a number of polymeric drug delivery systems (Thermoplastic paste, Thermogelling system, Polymer precipitation) with the ability to form a drug reservoir at the injection site are under investigation (Kissel et al., 2004).

Nasal delivery

One of the strategies used in nasal drug delivery is decrease the mucocilliary clearance (MCC) by the use of gel/mucoadhesive formulations to prolong the residence time at the nasal absorption site and thereby facilitate the uptake of the drug. Ordinary gels are difficult to administer and an accurate drug dose cannot be

measured while mucoadhesive powders are not highly favored products. They can cause irritation on the nasal mucosa and give a gritty feel to the tissue, besides the difficulty and the cost of manufacturing powder with specified particle size/morphology. A nasal mucoadhesive *in situ* gel appears very attractive since it is fluid-like prior to nasal administration and can thus easily be instilled as a drop allowing accurate drug dosing, and gel formation prevents MCC and allows time for absorption of drug resulting improved bioavailability (Zaki et al., 2007).

Ocular delivery

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of drug may be overcome by the use of in situ gel-forming systems that are instilled as drops into the eye and undergo a sol-gel transition in the cul-de-sac. Various drugs like Gatifloxacin (Doijad et al., 2006), Timolol maleate, Pilocarpine, ofloxacin, Pefloxacin mesylate, Indomethacin, Ciprofloxacin (Budai et al., 2007), Puerarin (Wu et al., 2007), have been developed as in situ drug delivery system.

Periodontal delivery

Systemic administration has been useful in treating periodontal pockets diseases, but repeated and long term use of systemic drugs is fraught with potential danger including resistant strains and superimposed infections. These may be overcome by the in situ gelling drug delivery system with the aim of improving patient compliance, therapeutic efficacy, reduced dosage regimen and targeted drug action with mucoadhesive and biodegradable polymeric systems (Rawat, Warade and Lahoti, 2010).

Vaginal delivery

Vagina has been used for a long time as a route for delivery of several drug classes such as antimicrobials, labor inducers, spermicides, and sexual hormones with the purpose of obtaining a local pharmacological effect. Ideal vaginal drug delivery systems should be easy to handle, minimal leakage and long local residence after administration, cost-effective, nonirritant and safe even after repeated use. Most vaginal dosage forms, like liquid preparations, creams/gels, tablets, films and tampons, etc., are not satisfactory for either difficult self-administration or rapid

clearance from the application site. In recent decades, environmentally sensitive gels, in particular thermosensitive gels have represented an important improvement for drug delivery to special cavities including vagina.

Poloxamers, especially poloxamer 407, have been the most commonly encountered thermosensitive material for their advantages such as easy availability, simple method for gel preparation and drug loading and good compatibility with various drug and pharmaceutical excipients (Lu et al., 2009).

2.10 MICROEMULSION BASED GEL AS A DRUG DELIVERY CARRIER

Properties of microemulsions, e.g. enhanced drug solubility, protection against enzymatic hydrolysis, ease of manufacturing and permeation enhancement ability are exploited in pharmaceutics, and they play an important role in drug delivery systems. However, most of the microemulsions possess a very low viscosity and therefore their application, especially in pharmaceutical industry may be restricted due to inconvenient use. To overcome this disadvantage, some gelling agents are added into the microemulsion to form microemulsion-based gels (MBGs) (Yang et al., 2009).

Transdermal and dermal delivery

Microemulsion based hydrogel (MBH) could expected as a promising drug delivery system. It was reported that compared with the commercial cream, MBH could significantly enhance the permeation of penciclovir in vitro and in vivo. Furthermore, the MBH presented the excellent ability of slow-release and weaker irritation (Zhai et al., 2009).

Ibuprofen was formulated into many topical preparations to reduce the adverse side effects and avoid the hepatic first-pass metabolism. But it is difficult to maintain effective concentrations by topical delivery of ibuprofen due to its poor skin permeation ability (Yang et al., 2006). In order to enhance the permeation of drug and retention at site of application MBG have been explored (Yang et al., 2006) (Walde et al., 1997).

Vaginal delivery

Fluconazole (FLZ) is the primary treatment option for virtually all forms of susceptible *Candida* infections in vagina. Currently, FLZ is available as oral tablets

(Diflucan®, Pfizer Inc., NY) for the treatment of vulvovaginal candidiasis but there are no FLZ formulations available for the vaginal delivery. Recently, it has been demonstrated that Gynazole- 1® (a vaginal cream containing imidazole antifungal agent) is more effective than oral fluconazole therapy with respect to fast relief from symptoms. The hydrophobic nature of FLZ poses problems in a suitable topical dosage form for vaginal delivery. The solubilisation of FLZ in microemulsions may improve its vaginal availability. However, adherence of dosage form to the vaginal mucosa and increases the residence time of FLZ in vagina can be imparted by gelling of FLZ microemulsion using bioadhesive agent (Patravale and Bachhav, 2009). Thus, microemulsion based bioadhesive gel of drugs can be explored for vaginal delivery.

Ocular delivery

Contact lenses made from microemulsion and surfactant-laden hydrogels can be used for extended delivery of Cyclosporin A at therapeutic dosages. Also, surfactant-laden hydrogels can go through all the processing steps that a typical contact lens goes through including monomer extraction, autoclaving and packaging, and provide extended drug release at therapeutic dosages (Chauhan and Kapoor, 2008).

2.11 MICROEMULSION BASED IN SITU GELLING SYSTEM

2.11.1 MICROEMULSION BASED IN SITU GELLING SYSTEM: POTENTIAL IN OCULAR DELIVERY

Microemulsions are used to formulate poorly water-soluble drugs since their structure allow solubilization of lipophilic drugs in the oil phase. Also, they allow better penetration and provide improved bioavailability. In situ gel-forming systems are viscous liquids that shift to a gel phase upon exposure to physiological conditions. The principal advantage of this formulation is the possibility of delivering accurate and reproducible quantities, in contrast to already gelled formulations, and promoting precorneal retention. Exploiting benefits of these two dosage forms, microemulsion based in situ gelling system can serve as a new vehicle for ophthalmic drug delivery. The essential idea is to encapsulate the drug in droplets that form a microemulsion and then disperse the drug-loaded droplets in a polymer solution that gels upon triggering by the electrolyte, pH or Temperature (Gan et al., 2009).

2.11.2 RATIONALE OF THE SYSTEM IN TOPICAL TREATMENT OF ENDOPHTHALMITIS

Endophthalmitis is the infection of posterior chamber of Eye. Topical application of contemporary available marketed formulations has various drawbacks in treatment of endophthalmitis due to rapid and extensive precorneal loss. Typically, less than 5% of the drug applied penetrates the cornea/sclera and reaches the intraocular tissue, with the major fraction of the dose applied often absorbed systemically through the conjunctiva and nasolacrimal duct. This can result in undesirable systemic side effects. Other routes like periocular (oral and systemic) medications require high dose to achieve and maintain MIC90 in vitreous humor hence causing higher toxicity, and Intra-vitreal injection is remain major treatment option for treatment of endophthalmitis. It also has drawbacks like patient non-compliance, very expensive, requires hospitalization, invasive technique, frequent puncturing etc. In addition, due to accumulation of drug at posterior tissues, it causes retinal tissue damage.

Thus, amalgamating the advantages of microemulsion (improve penetration and bioavailability) and in situ gelling system (higher residence time and prolongation of drug release), better formulation could be developed that can deliver the drug to posterior segment of the eye and has potential to treat retinal disorders like endophthalmitis.

2.12 DRUG PROFILE (Sweetman, 2009) (Drug bank, 2010)

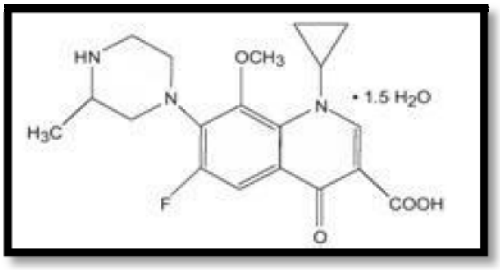
Name: Gatifloxacin Sesquihydrate

Chemical Name: (±)-1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid sesquihydrate.

Molecular Formula: C19H22FN3O4 . 1.5 H2O

Molecular Weight: 402.42

Structural Formula:



Physical and Chemical Properties: It is dull white solid having melting point 182-1850C, its predicted solubility is 2.32 mg/ml (solubility is pH dependent) and Experimental log P is 2.6.

Mechanism of Action: The bactericidal action of Gatifloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, and recombination.

Adverse effects and Precautions: Symptomatic hyperglycaemia and/or hypoglycaemia have been reported in patients (usually diabetics) taking Gatifloxacin (orally). Although in most cases the blood-glucose disturbance was reversible, fatalities have been reported. Gatifloxacin should not be given to diabetic patients. Other risk factors for developing blood-glucose disturbances include older age (patients 65 years of age or over), renal impairment, or use of other drugs that alter blood-glucose concentrations, particularly hypoglycaemics. Patients with risk factors should have their blood-glucose concentrations closely monitored and if signs or symptoms of glucose disturbances develop, gatifloxacin should be stopped.

Interactions: Use of gatifloxacin with drugs that alter blood-glucose concentrations increases the risk of blood-glucose disturbances.

Pharmacokinetics: Gatifloxacin is readily absorbed from the gastrointestinal tract with an absolute bioavailability of 96%. Peak plasma concentrations occur within 1 to 2 hours of an oral dose. Gatifloxacin is widely distributed into body tissues and is about 20% bound to plasma proteins. It undergoes limited metabolism and has an elimination half-life of 7 to 14 hours. Gatifloxacin is excreted primarily unchanged in the urine with less than 1% as metabolites. About 5% is also excreted unchanged in the faeces. Distribution into milk occurs in animals.

Topical Ocular Pharmacokinetics: In corneal tissues, Gatifloxacin (0.3%) achieved a maximum concentration (Cmax) of 4.5µg/mL (tmax = 0.5 hr) after single dose, and 7.8µg/mL (tmax = 0.5 hr) after multiple doses, corneal tissue concentrations integrated over 24 hr after multiple doses was AUC = 28.7µg·hr/mL. Cmax of Gatifloxacin (0.3%) in aqueous humor was 0.27µg/mL (tmax = 1 hr) after a single dose, and 0.54µg/mL (tmax = 0.5 hr) after multiple doses (Batoosingh et al., 2003).

Uses and Administration: Gatifloxacin is a fluoroquinolone antibacterial with actions and uses similar to those of ciprofloxacin. It is given orally, or by intravenous infusion as a 2mg/mL solution over 60 minutes, for the treatment of susceptible infections, including respiratory- and urinary-tract infections and skin infections. The usual adult dose is 400 mg once daily. A single dose of 400 mg or a dose of 200 mg daily for 3 days may be adequate for uncomplicated urinary-tract infections. For details of reduced doses to be used in renal impairment, a single dose of 400 mg may also be given for the treatment of uncomplicated gonorrhoea. Gatifloxacin is also used as 0.3% eye drops for the treatment of bacterial conjunctivitis.

Preparations:

Proprietary Preparations

Arg.: Gatif; Tequin†; Zymaran; ***Austral.:*** Tequin; ***Braz.:*** Tequin; Zymar; ***Canad.:*** Tequin; Zymar; ***Chile:*** Starox†; Zymar; ***Ger.:*** Bonoq†; ***India:*** Biogat†; Gaticin; Gatiquin; Gatt; Zyquin; ***Indon.:*** Gaticin; Gatimax; ***Jpn:*** Gatiflo; ***Malaysia:*** Tequin†; ***Mex.:*** Tequin; Zymar; ***NZ:*** Tequin; ***Philipp.:*** Tequin; Zymar; ***S.Afr.:*** Tequin; ***Singapore:*** Tequin†; Zymar; ***Thai.:*** Tequin†; Zymar; ***USA:*** Tequin†; Zymar. **Multi-ingredient:** ***India:*** Gatiquin Oz Kit.

2.13 POLYMER PROFILE

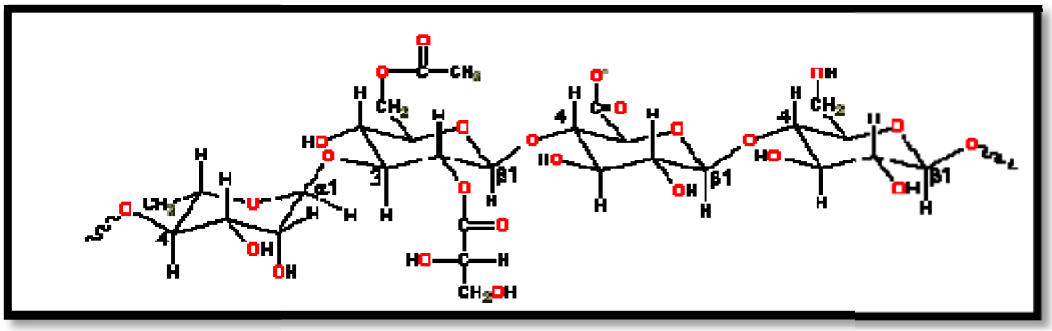
Polymeric preparations for ophthalmic use needs to be biocompatible, inert, non-irritant to ocular tissues, mechanically strong, comfortable to the patient, capable of achieving high drug loading, safe from accidental release, simple to administer and remove and easy to fabricate and sterilize.

2.13.1 KELCOGEL F

Synonyms: Gellan gum, gelrite

Chemical nature: The repeating unit of the polymer is a tetrasaccharide which consists of two residues of D-glucose and one of each residue of L-rhamnose and D-glucuronic acid. The tetrasacharide repeat has the following structure:

Structural Formula:



[D-Glc (β1→4) D-GlcA(β1→4)D-Glc(β1→4)L-Rha(α1→3)]n

Molecular weight: Approximately 500,000

Description: Gellan gum is a high molecular weight polysaccharide gum produced by a pure culture fermentation of a carbohydrate by *Pseudomonas elodea,* purified by recovery with isopropyl alcohol, dried, and milled.

Physico-chemical Characteristics:

- Gellan gum is a water soluble, off-white powder.
- It forms gels when positively charged ions (i.e., cations) are added. Thus, the thickness and texture of gellan gum in various products can be controlled by

manipulating the addition of potassium, magnesium, calcium, and/or sodium salts.

- Melting temperature can be modified to either be below or above 100° C depending on the types and concentrations of ions present.
- Heat and pH stable (pH 3.5 – 10).
- High gel strength, high clarity, excellent film former, low use level, thermally reversible gel.

Functional category: Thickening agent, gelling agent, stabilizer

Solubility: Soluble in water, forming a viscous solution; insoluble in ethanol

Stability: KELCOGEL F gellan gum has demonstrated good stability over a wide range of pH from less than pH 3 to at least pH 13. At very low pH, acid hydrolysis can occur leading to depolymerization, especially at elevated temperatures. Because the gel strength of gellan gum is governed by the type and concentration of ions, its gel strength may vary with changing pH.

Incompatibility: KELCOGEL® F gellan gum is compatible with up to 20-35% nonionic surfactants. Anionic and amphoteric surfactants tend to salt out gellan gum above a surfactant concentration of 15%. KELCOGEL F gellan gum is an anionic polymer and, therefore, tends to be incompatible with cationic surfactants.

Applications: Gellan gum can be used to produce easy-to-swallow solid dosage forms, such as gels and coated tablets, and to modify the rate of release of active ingredients from tablets and capsules. Gellan gum is also conveniently used for controlled or sustained release of various drugs and also for microencapsulation preparation. It is used in liquid dosage forms, semi-solid dosage forms, Intact Gel Dosage Forms - Self-standing, self-lubricated, easy to swallow, oral dosage form

Regulatory status: KELCOGEL® F gellan gum products are manufactured to food GMPs and are tested to ensure compliance with the purity criteria defined in the monograph for gellan gum in the current edition of the US Pharmacopoeia / National Formulary.

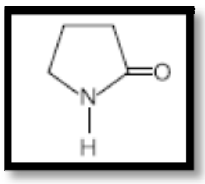
2.14 EXCIPIENT PROFILE

2.14.1 SOLUPHORE P

Chemical nature: Pyrrolidone-2 dist.

Molecular Formula: C4H7NO

Molecular weight: 85.1

Structural Formula: 

Properties: Soluphor P is a colourless or slightly coloured liquid which solidifies at room temperature and has a characteristic odour. It is soluble in water and a number of organic solvents, e.g. ethanol, isopropyl alcohol and aromatic hydrocarbons. Solutions of Soluphor P in water of up to 50% have viscosity of no more than 4 mPas.

Functional category: Surfactant and solvent

Stability: The product can be kept in the unopened original container for at least 12 months at room temperature.

Storage and packaging: In order to avoid discoloration storage below 25°C is recommended. The sensitivity of the substance to traces of iron is countered by the use of a 200-kg metal drum with a removable PE inner container.

Safety: No particular hazards known. Not irritating to the skin. Not irritating to the eyes. Ingestion may cause gastrointestinal disturbances.

Incompatibility: Incompatible with strong acids, oxidizing agents. Exothermic reaction is a hazardous reaction. React with acids.

Applications: Used as a surfactant in topical formulations. Soluphor P is used in veterinary injection preparations as a solvent together with water and/or in combination with low-molecular polyvinylpyrrolidone (Kollidon ® 12 PF or Kollidon 17 PF). Likewise it suggests itself for use in solutions for oral application.

Regulatory status: Soluphor P is produced in accordance with the GMP guidelines and meets the requirements of the current Ph. Eur. monograph "Pyrrolidone".

2.14.2 LABRASOL

Chemical nature: Glyceryl and polyethylene glycol esters; Caprylocaproyl Macrogolglycerides (Polyoxylglycerides)

Properties:

Form - Oily liquid

Colour - Roughly white

Odour - Light

Boiling point/Boiling range - > 150°C

Flash point - > 150°C

Self igniting - Product is not selfigniting

Relative density - 1,060 - 1,070 (20°C)

Solubility in / Miscibility with water - Soluble

Organic solvents - Soluble in many organic solvents.

Functional category: Surfactant and Co-surfactant

HLB value: 14

Stability and reactivity: No decomposition if used according to specifications. Avoid mixture of the products with strong acids, oxidants and alkalis.

Storage and packaging: Store in its original package hermetically closed. Recommended packing or flasks materials are polyethylene.

Safety: No particular hazards known. Not irritating to the skin. Not irritating to the eyes. Ingestion may cause gastrointestinal disturbances.

Incompatibility: Incompatible with strong acids, oxidizing agents. Incomplete combustion releases monoxide carbon and dioxide carbon.

Applications: ***Labrasol®*** is used in oral and topical formulations. It is a solubilizer/bioavailability enhancer for oral formulations, and can be used in

Self Emulsifying Lipidic Formulations (SELF) as a surfactant. It is used in topical ointments, microemulsions, emulsions and gels. It is also used in transdermal patches.

Regulatory status: EP, USP-NF, FDA IIG**.** All compounds of the substance are recorded in the US inventory: TSCA (Toxic Substance Control Act). All compounds of the substance are recorded into european inventory EINECS (European Inventory of Existing Chemical Substances)-Directives 79/831/EEC, sixth modification of directive 67/548/EEC.

2.14.3 PECEOL

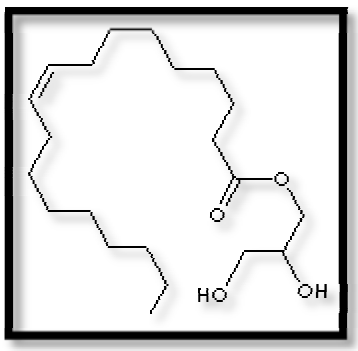
Chemical nature: Glycerol mono-oleate

Synonyms: Glycerol Oleate; Glyceryl Monooleate; Glyceryl oleate; (Z)-1-Oleoyl-Sn-glycerol; 1, 2, 3-propanetriol, 9-Octadecenoic acid; Glycerol Monoleate; Monoolein;

Molecular Formula: C21H40O4

Molecular weight: 356.54

Structural Formula:



Properties: Amber, oily liquids which may be partially solidified at room temperature, practically insoluble in water, freely soluble in methylene chloride.

Functional category: Oily vehicle

HLB value: 6

Stability: Stable under ordinary conditions.

Storage and packaging: It should be stored in an airtight container, protected from light under dry conditions and at room temperature. It is packaged as 180 kg net in drums.

Safety: No particular hazards known. Not irritating to the skin and the eyes.

Incompatibility: It reacts violently with acetic anhydrides in the presence of a catalyst.

Applications:

- Oily vehicle for use in self-emulsifying lipid formulations to obtain a coarse dispersion i.e. Emulsion (SEDDS) or a fine dispersion i.e. microemulsion (SMEDDS).
- Bioavailability enhancer: increased oral bioavailability is potentially associated with the long chain fatty acids present in its composition and selective absorption of highly lipophilic active pharmaceutical ingredients by the lymphatic transport system reducing hepatic first-pass metabolism.
- Good solvent for lipophilic active pharmaceutical ingredients.
- Glycerol Fatty Acid Esters are used as emulsifiers or oiling agents for foods, spin finishes and textiles; antifoaming and antistatic agents for plastics; and lubricants, water treatment, metal working fluids, and dispersing agents. End applications include cosmetics, foods, personal care products, medicine, pesticides, paper making, plastics and paints.

Regulatory status: Peceol is produced in accordance with the GMP guidelines and meets the requirements of the current Ph. Eur. monograph “glycerol mono-oleate".

3. REVIEW OF LITERATURE

3.1 MICROEMULSION

REFERENCE	POLYMER/ EXCIPIENTS USED	WORK DONE
(Lv, Zheng and Tung, 2005)	Span20/80, Tween20/80, *n*-butanol and Isopropyl palmitate (IPP)/Isopropyl myristate (IPM).	They developed o/w microemulsion for ophthalmic use. They showed that the stability of the chloramphenicol in the o/w microemulsion was increased remarkably compared to eye drops.
(Alany et al., 2007)	Polyoxyethylene sorbitan mono-oleate, Sorbitan mono laurate and Ethyl oleate.	They determined that incorporation of pilocarpine hydrochloride did not affect the phase behaviour. Also, the miotic response and duration of action were greatest in case of ME and LC formulations indicating high ocular bioavailability.
(HaBe and Keipert, 1997)	Isopropyl Myristate, Lecithin and Polyethylene glycol 200.	They developed ocular o/w microemulsion and evaluate for pH value, refractive index, viscosity, and physiological compatibility. They also observed prolonged pilocarpine release and improved bioavailability.
(Anna and Joanna, 2005)	Isopropyl myristate, Soybean lecithin (Epikuron 200), Polysorbate 80, Cremophor EL, n-butanol & triacetine	They had shown that microemulsion systems containing Epikuron 200 and Cremophor EL, partly or fully met the requirements of eye drops but formulations with n-butanol was not acceptable for ocular use.

(Alany et al., 2006)	Ethyl oleate (Crodamol EO), Sorbitan mono-laurate (Crill 1), Polyoxyethylene 20 sorbitan mono-oleate (Crillet 4), alkanol & alkandiols	They revealed from Precorneal clearance studies that the retention of colloidal and coarse dispersed systems was significantly greater than an aqueous solution. Crill 1, Crillet 4 super and Crodamol EO were found to be practically non-irritant, while alkanol or alkanediol were irritant to Chorionic Allantoin Membrane.
(Dalmora, Dalmora and Oliveira, 2001)	*N*- hexadecyl-*N*,*N*,*N*-trimethylammonium bromide (HTAB), Ethyl alcohol and Isopropyl myristate	They had shown that piroxicam microemulsion exhibits prolonged drug release effects, providing inhibition of the inflammation for 9 days after a single dose. They also shown improved bioavailability compared to the buffered piroxicam (42.2%).
(Chen et al., 2004)	Oleic acid, Tween 80 and Propylene glycol	They determined that triptolide-loaded microemulsions showed an enhanced in vitro permeation through mouse skins compared to an aqueous solution of 20% propylene glycol containing 0.025% triptolide. They had also shown that aqueous solution of 20% propylene glycol containing 0.025% triptolide exhibits significant skin irritation compared to triptolide-loaded microemulsions.

3.2 IN SITU GELLING SYSTEM

REFERENCE	POLYMER/ EXCIPIENTS USED	WORK DONE
(Gratieri et al., 2010)	Poloxamer 407 and Chitosan	They showed that chitosan improves the mechanical strength, mucoadhesive properties and texture properties of poloxamer formulations and also conferred fourfold increase in ocular retention of in situ gel formulation in comparison with a conventional solution.
(Qi et al., 2007)	Poloxamer 407, Poloxamer 188 and Carbopol 1342P NF	They had shown that ocular bioavailability can be increased more readily by using the in situ gelling and mucoadhesive vehicle. They also controlled release of puerarin from the formulation over a period of 8 h.
(Miyazaki et al., 2001)	Xyloglucan and Pluronic F127	They had prepared thermoreversible in situ gel of xyloglucan for the ocular delivery of pilocarpine hydrochloride. Sustained release of pilocarpine and improved miotic response than aqueous buffer solution containing the same drug concentration was observed.
(Di et al., 2008)	Pluronic F127-*g* poly (acrylic acid) copolymers (Pluronic-*g*-PAA Copolymer)	They showed by in vivo experiments on rabbits that the drug resident time and the total resident amount in conjunctiveal sac increased by 5 folds for in situ gel than eye drops. They also shown that in situ gel prolong the drug resident time and thus improve bioavailability.

(Cao et al., 2007)	Poly(N-isopropylacrylamide) –chitosan (PNIPAAm–CS) copolymer	They determined in vivo ocular pharmacokinetics of timolol maleate in PNIPAAm–CS solution and compared to that in conventional eye drop solution by using rabbits. The Cmax and intra-ocular pressure (IOP) reducing capacity of timolol maleate in PNIPAAm–CS in situ gel was two-fold higher than that of the conventional eye drops.
(Ganguly and Dash, 2004)	Chitosan and Glyceryl monooleate (GMO)	They had shown that drug release from the gel followed a matrix diffusion controlled mechanism. They also determined that inclusion of GMO enhanced the mucoadhesive property of chitosan by three to sevenfold.
(Mayol et al., 2008)	Hyaluronic acid and Poloxamers 407	They had studied the influence of hyaluronic acid (HA) on the gelation and mucoadhesive properties of poloxamers. They had shown that formulated system prolong and control acyclovir release for more than 6 h.
(Itoh et al., 2008)	Xyloglucan and Pectin	They evaluate plasma levels of paracetamol in rats after oral administration of a in situ gelling system containing 1.5% (w/w) xyloglucan and 0.75% (w/w) pectin and conferred that in situ gelling system provides more sustained release and higher drug bioavailability compared to that of a 1.5% (w/w) xyloglucan solution.

3.3 MICROEMULSION BASED IN SITU GELLING SYTEM

REFERENCE	POLYMER/ EXCIPIENTS USED	WORK DONE
(Ma et al., 2008)	Poloxamer 407	They had shown that desorption kinetics of vitamin A palmitate cationic microemulsion in situ gel (VAP/CM-ISG) exhibited longer corneal retention time and smaller contact angle compared with Oculotect gel. Irritation test showed a good ocular compatibility of VAP/CM-ISG.
(Zhao et al., 2007)	Pluronic F127, Isopropyl myristate (IPM), Span20/Tween20	They observed that the viscosities of the gels increased and the gelation temperatures decreased with increasing Pluronic F127 concentration. Chloramphenicol loading had little effect on the viscosities and gelation temperatures of the microemulsion based in situ gel. The microstructures of the microemulsion droplets were maintained in the microemulsion gel after the addition of Poloxamer F127.
(Chaudhari et al., 2010)	Capryol 90, Labrasol, Ethanol, Pluronic F127 and Carbopol 934P	They did ex-vivo permeation study of an optimized thermoreversible mucoadhesive microemulsion based in situ gel (TMMIG) formulation across nasal sheep mucosa. They revealed that 90.1%, 67.4%, 50.1% Raloxifene Hydrochloride (RLX) was released from microemulsion, TMMIG and RLX suspension respectively at 180

		minutes. They also showed increase in bioavailability of RLX.
(Gan et al., 2009)	Castor oil, Solutol HS 15 (surfactant), glycerol, water and Kelcogel.	They design a novel microemulsion in situ gelling system for ophthalmic delivery of a lipophilic drug, cyclosporine A (CsA). They observed by in vivo study in rabbits that the $AUC_{0\rightarrow 32h}$ of CsA in cornea for the microemulsion Kelcogel® system was approximately 3 fold greater than for a simple CsA emulsion. Moreover, at 32 h after administration, CsA concentrations delivered by the microemulsion Kelcogel® system remained at therapeutic levels in the cornea.

3.4 GATIFLOXACIN

REFERENCE	POLYMER/ EXCIPIENTS USED	WORK DONE
(Liu et al., 2006)	Alginate (Kelton®), HPMC (Methocel E50Lv).	They had shown that poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid pre-corneal elimination of the drug may be overcome by the use of **in situ gel**-forming systems. They also showed that alginate/HPMC solution retained the drug better than the alginate or HPMC E50Lv solutions alone.
(Mohammed et al., 2010)	Chitosan, Acetic acid, Glutaraldehyde.	They had developed chitosan **strips** containing Gatifloxacin (10%, 20% and 30% to the weight of polymer) by solution casting method using 1% v/v acetic acid in water. Further strips containing 30% gatifloxacin were cross-linked by exposing vapours of 2% v/v glutaraldehyde to extend the release. The formulation release drug up to 19 days.
(Shah, Patel and Patel, 2010)	Gelucire 39/01, Gelucire 43/01 and Ethyl cellulose (EC).	They had developed and optimize a **controlled-release multiunit floating system** of gatifloxacin, a potential drug for eradication of *H. Pylori* infection responsible for gastric and duodenal ulcers, using Gelucire 39/01, and Gelucire 43/01 as lipid carriers.

(Patel et al., 2009)	Hydroxypropropyl methylcellulose (HPMC), Methylcellulose (MC), Ethyl cellulose (EC).	They had formulated **ocular inserts** of gatifloxacin sesquehydrate which showed increased residence time and prolong drug release.
(Kesavan, Nath and Pandit, 2010)	Sodium alginate, Sodium carboxymethyl-cellulose (NaCMC).	They had formulated sodium alginate based **ophthalmic mucoadhesive system** of gatifloxacin and shown that the formulated systems provided sustained release of the drug over 12-hr in vitro. Significant reduction in total bacterial count was also observed between control and treatment groups.
(Motwani et al., 2008)	Chitosan, Sodium alginate.	They had developed **mucoadhesive chitosan-sodium alginate nanoparticles** for topical ocular delivery. Fast release was observed during the first hour followed by a more gradual drug release during a 24-h period following a non-Fickian diffusion process

Chapter 4

Materials and Methods

4. MATERIALS AND METHODS

4.1. MATERIALS AND REAGENTS

Table 5: List of Materials

MATERIALS	VENDOR'S NAME
Gatifloxacin Sesquihydrate(GFS)	Kindly Gifted by Cadila Pharmaceuticals, India
Labrasol (Polyethylene glycol-8-glycol caprylate)	Kindly gifted by Gattefosse, France
Transcutol (Diethylene glycol monoethyl ether)	
Labrafil M 2125 (PEG -6 corn oil)	
Capryol 90 (Propylene glycol monocaprylate)	
Peceol (Glycerol monooleate)	
Cremophor EL (Polyethoxylated castor oil)	Kindly gifted by BASF, Germany
Soluphor p (2-Pyrrolidone)	
Kelcogel F (Gellan gum), Food grade	Kindly gifted by CP Kelco UK Ltd, UK
Isopropyl Myristate	Central drug house Pvt. Ltd, India
Castor oil	Central drug house Pvt. Ltd, India
Oleic acid	Central drug house Pvt. Ltd, India
Tween 20	S.D.Fine-chem Ltd, India
Tween 80	S.D.Fine-chem Ltd, India
Span 80	S.D.Fine-chem Ltd, India
Propylene glycol	S.D.Fine-chem Ltd, India
PEG 400	Central drug house Pvt. Ltd, India

Benzylkonium chloride	Finar chemicals Pvt. Ltd, India
Mannitol	Central drug house Pvt. Ltd, India
Carbopol 934	Central drug house Pvt. Ltd, India
Sodium alginate	Central drug house Pvt. Ltd, India
Sodium bicarbonate	Central drug house Pvt. Ltd, India
Sodium chloride	Central drug house Pvt. Ltd, India
Sodium hydroxide	Central drug house Pvt. Ltd, India
Methanol AR	S.D.Fine-chem Ltd, India
Calcium chloride dihydrate	Central drug house Pvt. Ltd, India
HPLC-grade acetonitrile (99.9% pure)	S.D.Fine-chem Ltd, India
Orthophosphoric acid	S.D.Fine-chem Ltd, India
Potassium dihydrogen ortho phosphate	Central drug house Pvt. Ltd, India
HPLC grade Methanol	S.D.Fine-chem Ltd, India
Membrane filter 0.45 µm	Merck India Ltd, India
Membrane filter 0.22 µm	Merck India Ltd, India

4.2 EQUIPMENTS USED

Table 6: List of Equipments

INSTRUMENTS	VENDOR'S NAME
Digital balance	Citiweigh- Tejas exports, India
Electronic Weighing Balance	Shimadzu corporation Ltd. Japan
Mechanical Stirrer	Remi motors Ltd. India
Vortex shaker	Remi motors Ltd. India
Hot air oven	EIE Instruments Pvt. Ltd., India
Ultraviolet spectrophotometer	Shimdzu UV 1800 corporation, Japan
Refrigerated micro centrifuge	Rajendra Electrical Industries Ltd, India
Humidity control oven	Nova Instruments Pvt. Ltd, India
Ultrasonicator	Trans-o-Sonic D-Compact, India
pH meter	Analab scientific instruments, India.
Malvern-Zetasizer	Nano ZS90, Malvern Instruments Ltd, UK
Brookfield viscometer	Brookfield Engineering Laboratories, USA
Q T S Texture Analyser	Brookfield Engineering Laboratories, USA
Fourier Transformed Infra Red spectrophotometer	Spectrum- GX, Perkin elmer, USA
Transmission Electron Microscope	Tecnai 20, Philips, Holland

4.3 IDENTIFICATION OF GATIFLOXACIN

4.3.1 MELTING POINT ANALYSIS

The melting point range of the Gatifloxacin was determined by thiel's tube method and it was compared with reported melting point range of standard Gatifloxacin (Drug bank, 2010).

4.3.2 UV SPECTROPHOTOMETRIC ANALYSIS

UV Spectra was scanned for 10µg/ml drug solution from 200-400 nm in 100 mM Phosphate buffer (pH 7.4) using UV-Visible spectrophotometer. The wavelength maxima were found and it was compared with reported wavelength maxima of standard Gatifloxacin (Venugopal and Saha, 2005).

4.4 DEVELOPMENT OF ANALYTICAL METHOD FOR ESTIMATION OF GATIFLOXACIN

To have quantitative data on various studies such as purity, evaluation of the drug, compatibility studies, in-vitro diffusion studies etc. It is essential to develop analytical methods which are precise, specific and accurate. Therefore the following analytical methods were developed and validated for Gatifloxacin and other additives.

4.4.1 SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF GATIFLOXACIN

Spectroscopic method was developed to analyze Gatifloxacin content in the product.

4.4.1.1 Calibration curve for analysis of gatifloxacin from in-vitro release medium

- **Preparation of stock solution**

50 mg of Gatifloxacin was weighed into 50 ml volumetric flask and was dissolved in sufficient simulated tear fluid (pH 7.4), sonicated and volume was made up to 50 ml.

- **Preparation of dilutions**

1st dilution: 1ml of the stock solutions were pipetted out into a 100 ml volumetric flask and the volume was maintained up to the mark with simulated tear fluid (pH7.4).

2 nddilution: Suitable aliquots of the first dilution solutions were pipetted out into a 10 ml volumetric flask and the volume was maintained up to the mark with simulated tear fluid (pH 7.4).

The absorption maxima (λ_{max}) was determined by scanning 10µg/ml solution against the reagent blank on UV-visible spectrophotometer and the absorbance maxima was found out at 286 nm. The absorption of all the prepared solutions was then measured at the absorption maxima, 286 nm, against the reagent blank. The readings were recorded in triplicate and the experiment was repeated on 3 consecutive days using freshly prepared stock solutions each time. Mean values (n=3) along with the standard deviation was recorded and the regressed calibration curve is developed.

Table 7: Preparation of simulated tear fluid (pH 7.4) (Rozier et al., 1989)

INGREDIENTS	QUANTITY
Sodium chloride (NaCl)	6.7 g
Sodium bicarbonate($NaHCO_3$)	2.0 g
Dihydrated Calcium chloride($CaCl_2.2H_2O$)	0.08 g
Purified bidistilled water	q.s. to 1000 ml

4.4.1.2 Calibration curve of gatifloxacin for determination of drug content in microemulsion

A standard stock solution of GFS was prepared in methanol by dissolving 50 mg GFS in 50 ml methanol, suitable dilutions were made from the standard solution to get concentrations in the range of 1-10µg/ml. The absorption maxima (λ_{max}) was determined by scanning 10 µg/ml solution against the reagent blank on UV-visible spectrophotometer and the absorbance maxima was found out at 294 nm. The absorption of all the prepared solutions was then measured at the absorption maxima, 294 nm, against the reagent blank. The readings were recorded in triplicate and the experiment was repeated on 3 consecutive days using freshly prepared stock solutions each time. Mean values (n=3) along with the standard deviation are recorded and the regressed calibration curve was developed.

4.4.2 HPLC METHOD FOR THE ESTIMATION OF GATIFLOXACIN IN VITREOUS HUMOR (Davis et al., 2010)

4.4.2.1 Chromatographic conditions (Patel and Krishnaveni, 2010)

- **Instrument used:** Shimadzu HPLC with UV-detector
- **Analytical Column:** Phenomenex C-18, 150×4.6 mm, 5µ
- **Flow rate:** 1.0 ml/minute
- **Oven temperature:** Ambient (25^{o} C)
- **Wavelength:** 293 nm
- **Injection volume:** 20 µl
- **Run time:** 25 minute
- **Retention time:** Approximately 17.0 min. for Gatifloxacin
- **Mobile phase:** Acetonitrile: Potassium dihydrogen ortho-phosphate, 25 mM (pH 2.5 adjusted by ortho-phosphoric acid) mixture in ratio 15:85
- **Diluting solvent:** Methanol and water

4.4.2.2 Linearity and Range

To obtain the linearity of the method, aliquots of Gatifloxacin standard solutions were added into blank rabbit vitreous humor to get final concentrations of 0.5, 1, 2, 3, 4 and 5µg/ml respectively (Davis et al., 2010). The linearity was calculated by plotting the peak areas of standard against the concentrations of the added drug standards.

- **Preparation of standard solutions**

Accurately weighed Gatifloxacin (10 mg) was placed in 10 ml volumetric flask, 7 ml of diluting solvent (methanol) was added and sonicate for 10 minutes and then volume was made 10 ml with diluting solvent (methanol). From the stock solution, 0.1 ml of solution was pipetted out and diluted to 10 ml with diluting solvent (water). The resultant solution obtained was 10µg/ml.

- **Sample preparation**

Aliquots of 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 ml of 10 µg/ml solution of Gatifloxacin were pipetted out into 1.5 ml eppendorp tubes and 0.05 ml of vitreous humor was added to each of them. 2 ml of acetonitrile was then added to each of them for vitreous precipitation (Pang et al., 2006). The mixture was centrifuged at 10,000 rpm for 15 min at 4 ^{0}C. The supernatant was transferred to another eppendorp tubes and volume was made up to 1 ml with diluting solvent (water) to give concentration of 0.5, 1, 2, 3, 4 and 5µg/ml respectively. The resultant solutions were subjected to analyze by the proposed HPLC method.

4.5 PRELIMINARY STUDIES TO FORMULATE ME

A selection of components for microemulsions suitable for pharmaceutical use involves a consideration of their toxicity and, if the systems are to be used topically, their irritation and sensitivity properties. The ionic surfactants are generally too toxic to be used for preparation of microemulsions (Attwood, 1994); therefore, non ionic surfactants, such as the poloxamers, polysorbates, polyethylene glycol are preferred.

4.5.1 SCREENING OF SURFACTANTS AND OILS

The solubility of Gatifloxacin in various oils, surfactants, and co surfactants was determined respectively. An excess amount of drug was incorporated to different excipient and visual inspection was carried out to screen the excipient where drug shows maximum solubility (Vandamme, 2002). Furthermore quantification was carried out in those selected excipients.

4.5.2 SOLUBILITY STUDIES AND SELECTION OF SURFACTANT AND OIL COMPONENT

The solubility of Gatifloxacin in various oils, surfactants, and co-surfactants was determined visually and then they were quantified. Each of selected vehicles (1.5 ml) was added to each eppendorp tube containing an excess of GFS. After sealing, the mixture was heated at 40 ^{0}C in water-bath to facilitate the solubilization and mixed using a vortex mixer. Mixtures were shaken on shaker bath at 25 ^{0}C for 48 h. After reaching equilibrium, each tube was centrifuged at 12,000 rpm for 10 min (Zhai et al., 2009), then 0.5 ml supernatant was taken with micropipette, and the content of

Gatifloxacin was quantified by UV-Visible spectrophotometer 294 nm after dilution with methanol.

4.5.3 VISUAL INSPECTION OF MISCIBILITY OF SELECTED SURFACTANT WITH OTHER CO SURFACTANTS

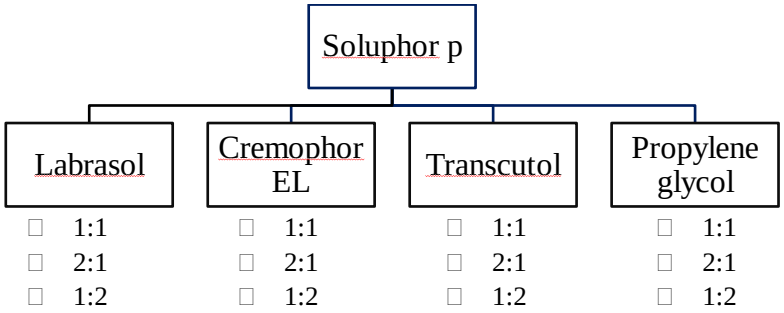
Co-surfactants were screened by miscibility tests with tested surfactant. Visual inspection was carried out and the formation of interphase between two liquids was noted. Weaker the interphase formed, higher the miscibility between them. Co-surfactant with higher miscibility with surfactant was required so as to form large ME domain.

4.6 SELECTION OF CO SURFACTANT

Some studies have shown that mixtures of two surfactants can enlarge microemulsion region significantly in pseudo-ternary phase diagram (Ping et al., 2005). Therefore, mixture of surfactants was used in this research to explore enlargement of ME region.

From preliminary studies, Peceol was selected as oil component and Soluphor p was selected as a surfactant. Various combinations of Peceol and soluphor P with different co-surfactants were screened for the formation of microemulsion.

Soluphor p was blended with each co-surfactant in fixed weight ratios (1:2, 1:1, 2:1). Aliquots of each Soluphor p and co-surfactant mixture (S_{mix}) were then mixed with Peceol at room temperature (25^0 C). The ratio of oil to S_{mix} was varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 7:3, 2:8 and 1:9. Then, water was added to above mixture in 5% increment and checked for formation of microemulsion or liquid crystal or gel. The resulting MEs were tightly sealed and stored at ambient temperature. Their physical stability was measured by observing periodically the occurrence of phase separation.



For each Surfactant to Cosurfactant ratio, different Smix-Oil proportions (9:1, 8:2 …1:9) were screened for the formation of microemulsion.

4.7 DETERMINATION OF EFFECT OF DIFFERENT PROPORTION OF FORMULATION COMPONENTS ON THE FORMATION OF MICROEMULSION

Among Labrasol, Cremophor EL, Transcutol and Propylene glycol, Labrasol was selected as a co surfactant. So, resulting formulation components were: Oil= Peceol, Surfactant= Soluphor p (S), Co-surfactant= Labrasol (L) and Aqueous phase.

Effect of different proportions of components i.e. S: Co S, Smix: Oil etc. on the formation of microemulsion (i.e. ease of formation of ME, particle size, stability of formed ME etc.) were thoroughly observed and evaluated.

PREPARATION OF MICROEMULSION:

Various batches were prepared and screened as shown in Table 8. Soluphor p was blended with Labrasol in fixed weight ratios (1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:4 and 1:5). Aliquots of each S_{mix} were then mixed with oil at room temperature (25^0 C). The ratio of oil to the S_{mix} was varied as 9:1, 8.5:1, 8.5:1.5, 8:2, 7.5:2, 7.5:2.5, 7:2.5, 7:3, 6.5:3, 6.5:3.5, 6:3.5, 6:4, ….., 1:9. Water was added to above mixture in 5% increment till the total of components mixture become 100% and checked for the formation of microemulsion. Stability of formed ME was also monitored.

4.8 DETERMINATION OF EFFECT OF DRUG LOADING ON THE FORMATION OF ME

Previous research work shown that drug loading can influence the particle properties and microemulsion formation (Ping et al., 2005). The drug was loaded in oily phase and influence of drug loading on the formation of microemulsion was determined.

Calculation of Dose:

- Drug: Gatifloxacin Sesquihydrate
- Dose: 0.3% w/v or 3 mg/ml

	GATIFLOXACIN	GATIFLOXACIN SESQUIHYDRATE
Mol wt.	375.40 g/mol	402.42 g/mol
Dose	3 mg/ml	3.22 mg/ml

Formulation of drug loaded Microemulsion

Appropriate amount of oil, surfactant and co surfactant was mixed together in accordance to ME domain, and equilibrated with gentle vortex shaking to get the initial concentrate. Then appropriate GFS (Gatifloxacin Sesquihydrate) was dissolved in the initial concentrate under ultra-sonication. Then water was added in the resulting mixture with 5% increment at room temperature (Zhai et al., 2009).

To obtain blank ME, no GFS was added in the above process before adding water. The particle size of drug loaded ME and ME without drug were analysed and compared.

4.9 DEVELOPMENT OF PSEUDO-TERNARY PHASE DIAGRAM

The pseudo-ternary phase diagram of oil, surfactant, and co surfactant were constructed to obtain the components and their concentration ranges that can result in large existence area of ME. A titration technique was employed for the preparation of the pseudo ternary phase diagrams (Barrow, 1996). The titration begins by fixing two components and varying the third component.

4.10 EVALUATION OF MICROEMULSIONS

4.10.1 VISCOSITY

The rheological property of the microemulsion formulations was evaluated using small sample adaptor and spindle no. 18 of the Brookfield Viscometer (LVDV-I Prime model). The viscosity was measured at 0.5 rpm speed. Evaluations were conducted in triplicate.

4.10.2 PARTICLE SIZE DISTRIBUTION

The particle size distribution of the oil droplets in the microemulsion was analyzed using a Dynamic Light Scattering (DLS) technique by Malvern-Zetasizer (Nano ZS90) without dilution at 25 ^{0}C.

DLS technique, also known as Photon Correlation Spectroscopy, is one of the most widely used methods to measure the size of nanoparticles. This technique assumes

that all the particles are in Brownian motion in the solution and that all the particles are very small and spherical. Scattering of light (normally a laser) takes place when particles are hit by light. The particle size can be determined based on the physical properties of the scattered light: the angular distribution, frequency shift, the polarization and the intensity of the light (Berne, 1975).

4.10.3 PERCENTAGE TRANSMITTANCE

The percent transmittance of the system was measured using colorimeter. % transmission was set to zero using filter and % transmission was set to 100% using transparent cuvette filled with water. Then, different microemulsion samples were putted in the transparent cuvette and % transmission is noted.

4.10.4 SOLUBILITY OF GATIFLOXACIN IN O/W MICROEMULSION

An excess amount of Gatifloxacin was introduced to 1 ml of microemulsion in eppendorp tube. After sealing, the mixture was heated at 40^0 C in water-bath to facilitate the solubilization and mixed using a vortex mixer. Mixtures were shaken with shaker bath at 25^0 C for 48 h. After reaching equilibrium, each tube was centrifuged at12,000 rpm for 10 min (Zhai et al., 2009), then 0.5 ml supernatant was taken and the content of Gatifloxacin was quantified by UV-Visible spectrophotometer at 294 nm after dilution with methanol.

4.10.5 DRUG CONTENT

Drug content in formulation was determined by dissolving 100µl quantity of formulation in 10 ml of methanol. The solution was then filtered through 0.45µm membrane filter and analyzed for Gatifloxacin content by UV-visible spectrophotometer at 294 nm.

4.10.6 DILUTION POTENTIAL

The prepared formulation was diluted 10 times with continuous media and the effect of dilution on transmittance was checked. Occurrence of phase separation was also noted.

4.10.7 CONDUCTIVITY AND ZETA POTENTIAL

Conductivity and zeta potential of the microemulsion formulations was determined at 25 ^{0}C using Malvern Zetasizer. Different types of cuvette were utilized for the measurement of particle size and zeta potential.

4.10.8 pH MEASUREMENTS

The pH was measured for each formulation using a pH meter, which was calibrated before use with buffered solutions of pH 4 and pH 7.

4.10.9 STABILITY ASSESSMENT

Stability of microemulsion, both as a function of time and storage temperature was routinely evaluated by visual inspection of the samples initially on a daily and later on a weekly basis. Stable systems were identified as those free of any physical change, such as phase separation, flocculation or precipitation. Particle size of the microemulsion upon storage was also determined to assess microemulsion stability in terms of drastic changes in the mean droplet diameter due to droplet coalescence or aggregation. Stability was monitored at ambient temperature (Constantinides and Scalart, 1997).

Stability of microemulsion was also checked by centrifugation at 12000 rpm for 30 mins and then the clarity, phase separation and concentration of drug were investigated (Zhai et al., 2009). Occurrence of phase separation of the microemulsion on centrifugation suggests that the system is not stable.

4.10.10 IN VITRO PERMEATION STUDY

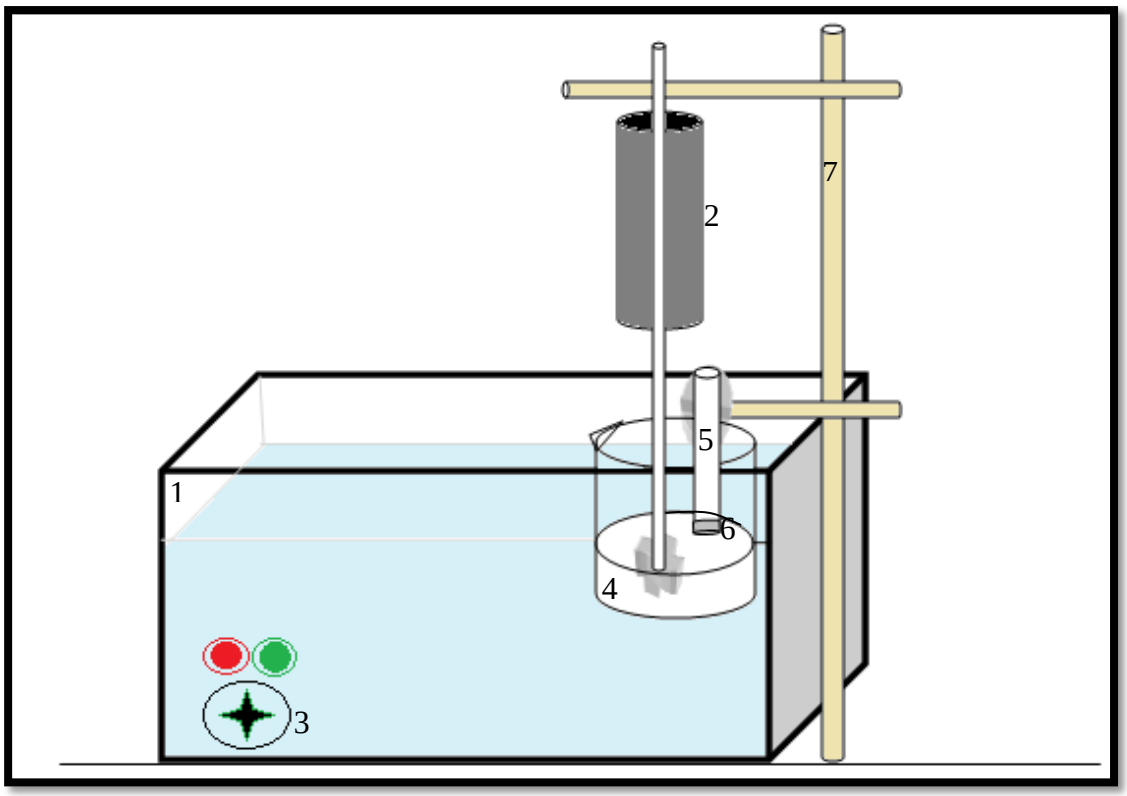


Figure 11: Diffusion Assembly Set up

(1=Water bath with thermostat; 2=Mechanical stirrer connected with rpm display; 3=Temperature control knob; 4=Receiver compartment filled with STF; 5=Donor compartment; 6=Diffusion membrane; 7=Holder/Stand)

To determine the in vitro permeation of GFS, assembly was set as shown in Figure 11. 100 ml of artificial tear fluid was placed in a beaker and mounted vertically in a water bath at 34 ± 0.1 ^{0}C. 1ml of formulation was added to donor compartment tied with the cellophane membrane at lower end. The temperature and stirring rate were remained at 34 ^{0}C and 75 rpm, respectively. Aliquots of 5 ml were withdrawn from the release medium and replaced by an equal volume with STF at each sampling time. The amount of GFS was determined by UV-visible spectrophotometer.

Cumulative amount of drug (Qn, µg/cm^2) in the receiver compartment was plotted as a function of time (t, min), and the cumulative amount of Gatifloxacin permeated through membrane from Gatilox Eye drop solution and formulated microemulsion was determined based on the following equation (Zhu et al., 2008):

$$Qn = (Cn * V_0 + \sum_{i=1}^{n-1} Ci * Vi) / S$$

Where, *Cn* stands for the drug concentration of the receiver medium at each sampling time, *Ci* for the drug concentration of the *i* th sample, and V_0 and V_i stand for the volumes of the receiver solution and the sample, respectively, *S* for the effective diffusion area.

4.11 FORMULATION OPTIMIZATION BY MIXTURE DESIGN

4.11.1 THREE COMPONENT MIXTURE DESIGN

The objective of modelling the phase diagrams is to quantify the effect of composition (Different amounts of components) on the particle size. A successful "mixture design" shows the statistical approach to obtain the relationship between the particle size distribution and the amounts of various components. In this method, the pseudo-ternary phase diagrams were plotted and several points was selected within the ME region for particle size measurement. The method can be explained briefly with the help of Figures 12 and 13.

As shown in Figure 12, a triangular region (shaded area) was selected arbitrarily within the ME region. The constraint that the proportions of different components must sum to 100% should be satisfied. Note that in the selected triangular region, the oil phase is less than 20% w/w, the water phase is between 44% w/w and 60% w/w, and the surfactant phase is between 36% w/w and 52% w/w. Following all these constraints, the points (composition) can be selected according to Figure 13. Three vertexes (Run 1, Run2, Run 3), three halfway points between vertices (Run 4, Run5, Run 6), and the centre point (Run 7). Each vertex represents a formulation containing the maximum amount of one component, with the other two components at a minimum level. The halfway point between the two vertices represents a formulation containing the average of the minimum and maximum amounts of the two ingredients represented by two vertices. The centre point represents a formulation containing one-third of each ingredient (Zhai et al., 2009). The particle size distribution can be obtained for each of the composition points. Then the regressions models was constructed using Microsoft office excel 2007. The minimum particle size can be inferred from the regression model and an optimal composition can be calculated in a statistical way.

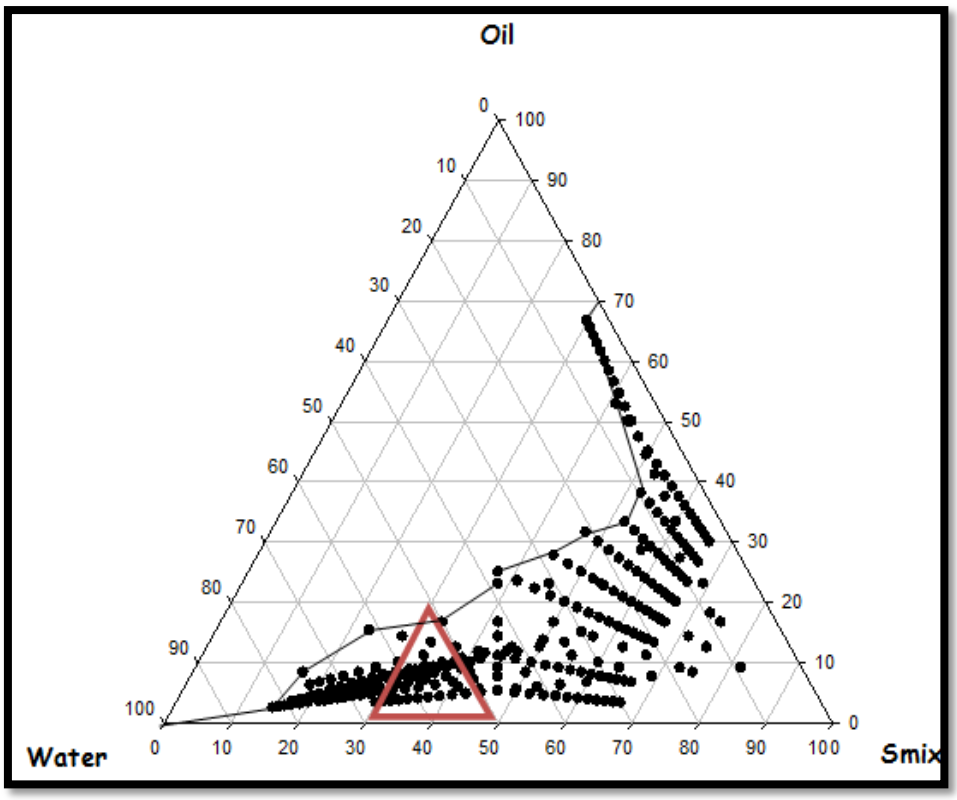


Figure 12: Sketch map for mixture design

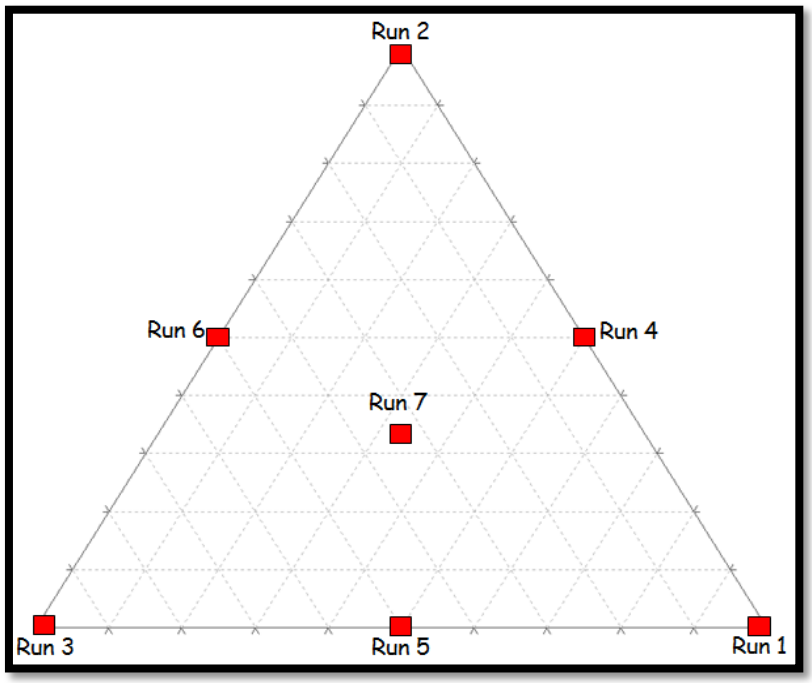


Figure 13: Distribution for each of the run in a mixture design

Three component simplex design was run with one checkpoint, as shown in Table 37.

The particle size values represent the average diameter of globules particle in the microemulsion. The simplex coefficients were obtained from regressions models using Microsoft office excel 2007, the values of simplex coefficients was putted into simplex equation resulting in the response equation that may be used to predict the response of combinations of mixture components in the system. With the aid of Design expert software, response was calculated over the simplex space, and a contour diagram was developed.

4.11.2 EQUATION OF SIMPLEX DESIGN

$$Y= b_1A + b_2B + b_3C+ b_{12}AB + b_{13}AC + b_{23}BC + b_{123}ABC$$

Where, Y is response (here, Particle size); A, B and C is transformed proportion of Smix, Oil and water respectively; b*i* are simplex coefficients.

4.11.3 VALIDATION OF APPLIED DESIGN

The design was validated by an extra design check point.

One point was selected within the design i.e. Smix = 37%, Oil = 18%, Water = 45%. The actual formulation components were converted into the transformed proportion. The values of the transformed proportions were placed into the response equation and theoretical response was found out. Then response of the check point formulation was practically obtained and compared with theoretical response. Closeness of the result justifies the validation of the design (Bolton and Bon, 2004).

4.12 FORMULATION OF IN SITU GELLING SYSTEM

4.12.1 SELECTION OF POLYMERS FOR IN SITU OPHTHALMIC GEL FORMULATION

Preliminary studies were carried out to select a suitable polymer system, which is capable of producing in situ ophthalmic gels of desirable physical property. Different formulations were prepared with the use of polymers like Kelcogel F (Food grade), Sodium alginate and Carbopol 934p. Kelcogel F and Sodium alginate were meant for electrolyte triggered in situ gelling system and Carbopol 934p was meant for pH sensitive in situ gelling system as well as it was tried in combination for mucoadhesion purpose. These preliminary studies were carried out to find out the

suitable proportion of polymer blends to be used for the manufacturing of in situ ophthalmic gel and to derive the range of polymer blends for desired property.

4.12.2 OPTIMIZATION OF IN SITU GEL FORMULATION

The selected polymers Sodium alginate, Kelcogel F and Carbopol 934P were used in different concentrations and proportions to formulate different batches of in situ ophthalmic gels. The formulation batches (F1 to F13) were prepared (Table 38) and evaluated for different parameters like gelling capacity, viscosity, % change in viscosity from normal conditions to physiologic conditions, transparency, dropping capacity, adhesiveness etc. Among the evaluated batches, those batches which have given ideal results as an in situ ophthalmic gel formulation were further evaluated for drug release by the diffusion studies. The best polymer giving desired physical characteristics, contact time and drug release was identified from the optimization studies and was further optimized to formulate microemulsion based in situ gelling system.

Table 38: Composition of in situ gel formulation batches (F1-F13)

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
Gatifloxacin (%w/v)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sod. Alginate (%w/v)	0.5	0.7	0.9	–	–	–	–	–	–	–	–	0.5	0.1
Kelcogel F (% w/v)	–	–	–	0.3	0.5	0.7	–	–	–	0.3	0.5	–	0.3
Carbopol (%w/v)	–	–	–	–	–	–	0.1	0.2	0.3	0.1	0.1	0.1	–
BAK	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Mannitol (%w/v=5%)	4.86 g/100ml	4.86 g/100ml	4.86 g/100ml	4.86 g/100ml	4.86 g/100ml	4.86 g/100ml	4.86 g/100ml	4.86 g/100ml	4.86 g/100ml	4.86 g/100ml	4.86 g/100ml	4.86 g/100ml	4.86 g/100ml
Bidistilled Water	q.s to 50 ml	q.s to 50 ml	q.s to 50 ml	q.s to 50 ml	q.s to 50 ml	q.s to 50 ml	q.s to 50 ml	q.s to 50 ml	q.s to 50 ml	q.s to 50 ml	q.s to 50 ml	q.s to 50 ml	q.s to 50 ml
1 N NaOH	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

4.13 CHARACTERIZATION OF IN SITU GELLING SYSTEM

4.13.1 pH MEASUREMENTS

The pH was measured for each formulation using a pH meter, which was calibrated before use with buffered solutions of pH 4 and 7.

4.13.2 DRUG CONTENT

Drug content in formulation was determined by dissolving 100 µl quantity of formulation in 10 ml of methanol. The solution was then filtered through 0.45 µm membrane filter and analyzed for Gatifloxacin content by UV-visible spectrophotometer at 294 nm.

4.13.3 TONICITY TEST

Tonicity of the formulation was determined using Rat blood. The principle that blood cells shrinks in hypertonic solution, they raptures in hypotonic solution and retain its shape in isotonic solution (normal saline) was utilize. Effect of formulation with isotonic agent (5% mannitol), and formulation without isotonic agent on blood cells was measured and compared with blood cells in normal saline.

4.13.4 RHEOLOGICAL STUDIES

The viscosity of the prepared formulations was determined at different angular velocities at $34 \pm 1\ ^{0}C$ using small sample adaptor of the Brookfield Viscometer (LV model). A typical run involved changing the angular velocity from 0.5 to 100 rpm at a controlled ramp speed. After 6 s at 0.5 rpm, the velocity was successively increased to 100 rpm, with a similar period at each speed. The angular velocity was then decreased (100-0.5 rpm) for a similar period of 6 s (Gan et al., 2009). The average of two readings was used to calculate the viscosity. To evaluate the viscosity change after administration, rheological measurements were taken after diluting the GFS formulations with artificial tear fluid (0.67% NaCl, 0.2% $NaHCO_3$, 0.008% $CaCl_2$ $2H_2O$ (Rozier et al., 1989)) in 25:7 ratio(application volume, 25 µl; normal volume of tear fluid in the eye, 7 µl).

4.13.5 GELLING CAPACITY

The gelling capacity was determined by placing a drop of the formulation in a petridish containing 2 ml of simulated tear fluid (STF) freshly prepared and equilibrated at 37°C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve.

Table 39: Criteria for measurement of Gelling capacity

-	No Gelation
+	Gels after a few min., dissolved rapidly
++	Gelation immediate, remains for nearly 5 hours
+++	Gelation immediate, remains for nearly 8 hours
++++	Gelation immediate, remains for more than 8 hours

4.13.6 PARTICLE SIZE DETERMINATION

Gatifloxacin is poorly water soluble drug; it has insoluble particles within the in situ gelling system. So, aseptic recrystallization and particle size reduction or appropriate method to solubilise or control the particle size is required to comply with standards for ophthalmic suspensions.

4.13.7 GEL STRENGH DETERMINATION

Gel strength was determined using a Brookfield Texture Analyzer (USA) in compression mode. Formulations with STF (50+14 ml) were transferred into cylindrical holder shown in Figure 14(a), taking care to avoid the introduction of air into the samples. A cylindrical analytical probe (38 mm diameter) was forced down into each sample at a defined rate (30 mm/min) and to a defined depth (10 mm). At least three replicate analyses of each sample were performed with the simulated tear fluid. From the resulting load–time plots, the gel strength (the maximum force required to attain a given deformation i.e. peak load) and adhesive force (the work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe) were derived (Lopez et al., 2010).

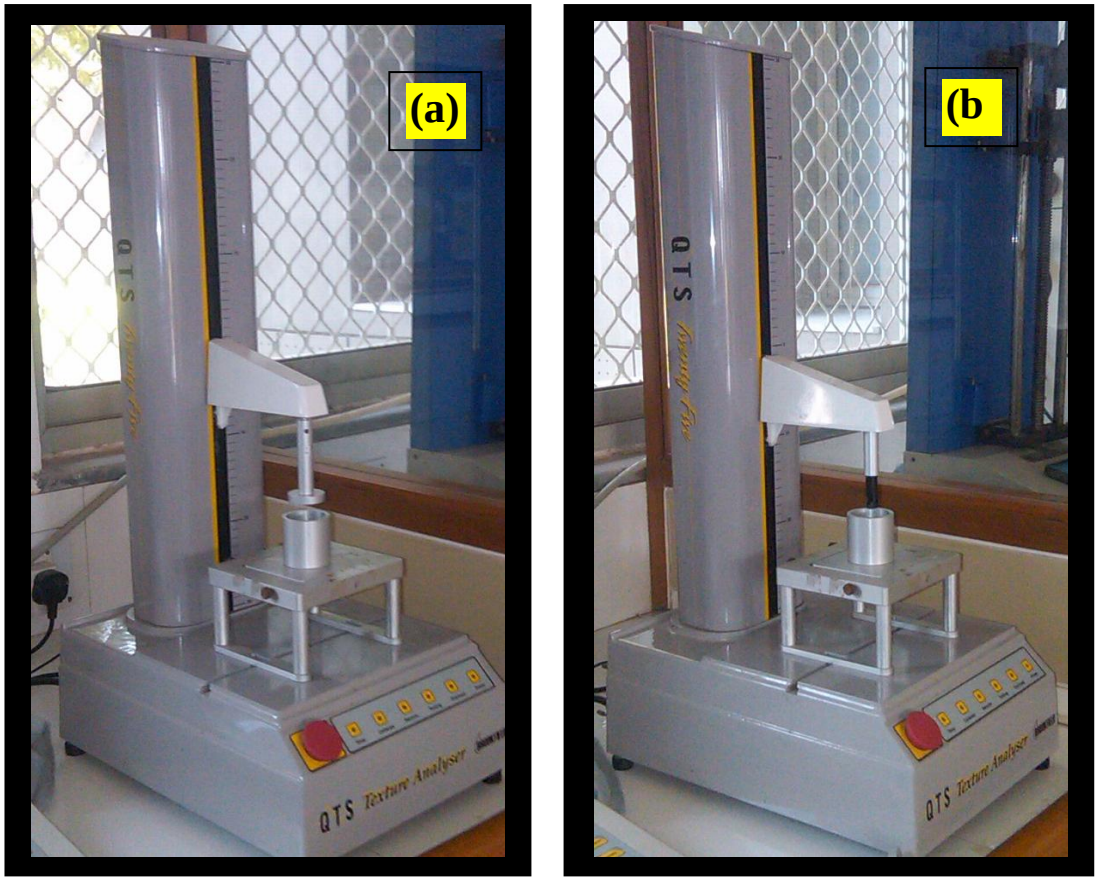


Figure 14: Brookfield Texture Analyzer

4.13.8 IN VITRO RELEASE STUDIES

To determine the in vitro release of GFS, assembly was set as shown in Figure 11. 100 ml of artificial tear fluid was placed in a beaker and mounted vertically in a water bath at 34±0.1 ^{0}C. 1ml of formulation was added to donor compartment tied with the cellophane membrane at lower end. The temperature and stir rate were remained at 34 ^{0}C and 75 rpm, respectively. Aliquots of 5 ml were withdrawn from the release medium and replaced by an equal volume with STF at each sampling time. The amount of GFS release was determined by UV-visible spectrophotometer and cumulative amount release, % was plotted as a function of time (*t*, min).

4.14 FORMULATION OF MICROEMULSION IN SITU GELLING SYSTEM

In previous sections, suitable compositions of microemulsion and in situ gelling system were individually screened for the desired properties and they were used for further optimization of ME based in situ gelling system.

Step 1- Appropriate amount of oil (12%), surfactant (12%) and co surfactant (24%) was mingled together in accordance to ME domain, and equilibrated with gentle vortex shaking to get the initial concentrate. Then appropriate GFS was dissolved in the initial concentrate under ultra-sonication (Zhai et al., 2009).

Step 2- Kelcogel F was dispersed in sufficient deionized water until it completely dissolved using magnetic stirrer (with the application of heat to allow complete hydration of polymer) to obtain final Kelcogel F concentrations of 0.3% and 0.5%.

Step 3- Deionized water with Kelcogel F was then added (in place of water in ME composition) in small increments (≤5% v/v) to the mixture of Smix/oil (initial concentrate) at room temperature. After each water addition, the mixture was stirred in vortex shaker for 1-2 min.

4.15 EVALUATION AND OPTIMIZATION OF MICROEMULSION IN SITU GELLING SYSTEM

4.15.1 DRUG CONTENT

Drug content in formulation was determined by dissolving 100µl quantity of formulation in 10 ml of methanol. The solution was then filtered through 0.45µm membrane filter and analyzed for Gatifloxacin content by UV-visible spectrophotometer at 294 nm.

4.15.2 pH MEASUREMENTS

The pH was measured for each formulation using a pH meter, which was calibrated before use with buffered solutions of pH 4 and 7.

4.15.3 PARTICLE SIZE DISTRIBUTION

The particle size distribution of the oil droplets in the microemulsion was analyzed using a Dynamic Light Scattering (DLS) technique by Malvern-Zetasizer (Nano ZS90) without dilution at 25 ^{0}C (Gan et al., 2009).

4.15.4 TRANSMISSION ELECTRON MICROGRAPHIC STUDIES

Images were recorded with transmission electron microscope (Tecnai 20, Philips, Holland). Analysis was performed at 25±2 ^{0}C. Microemulsions before/after mixing

with 0.3% Kelcogel F solution were dyed with phosphotungstic acid for visualization (Gan et al., 2009).

4.15.5 RHEOLOGICAL STUDIES

The viscosity of the optimized formulation was determined at different angular velocities at $34 \pm 1\ ^{0}C$ using small sample adaptor of the Brookfield Viscometer (LV model). A typical run involved changing the angular velocity from 0.5 to 100rpm at a controlled ramp speed. After 6 s at 0.5 rpm, the velocity was successively increased to 100 rpm, with a similar period at each speed. The angular velocity was then decreased (100-0.5 rpm) for a similar period of 6 s. The average of two readings was used to calculate the viscosity. Evaluations were conducted in triplicate.

To evaluate the viscosity change after administration, rheological measurements were taken after diluting the GFS formulation with artificial tear fluid (0.67% NaCl, 0.2% NaHCO3, 0.008% CaCl2· 2H2O (Rozier et al., 1989)) in 25:7 ratio (application volume, 25 µl; normal volume of tear fluid in the eye, 7 µl).

4.15.6 BIOADHESION STUDY

Texture analysis was performed using a Brookfield Texture Analyzer (USA) in compression mode. Formulations with STF (50+14 ml) were transferred into cylindrical holder as shown in Figure 14(b), taking care to avoid the introduction of air into the samples. A cylindrical analytical probe (12 mm diameter) attached at lower end with Goat's corneal membrane was forced down into the sample at a defined rate (30 mm/min) and to a defined depth (10 mm). From the resulting report, adhesive force (the work necessary to overcome the attractive forces between the surface of the sample and the surface of corneal membrane) were derived.

4.15.7 IN VITRO RELEASE STUDIES

Study of diffusion kinetics through an excised goat cornea

Whole eye ball of goat was transported from the local butcher shop to the laboratory in cold ($4\ ^{0}C$) normal saline within one hour of slaughtering of the animal. The cornea was carefully excised along with 2 to 4 mm of surrounding scleral tissue and was

washed with cold normal saline till the washing was free from protein (Ahuja, Singh and Majumdar, 2008).

Isolated cornea was tied to the lower end of donor compartment with the help of thread such that surrounding scleral tissue clamped with donor compartment to fix the membrane at lower end. The cornea was tied in such a way that its epithelial surface faced the donor compartment (Ahuja, Singh and Majumdar, 2008). The corneal area available for diffusion was 0.785 cm^2.

The receptor compartment was filled with 100 ml of freshly prepared STF (pH 7.4). One ml of test formulation was placed on the cornea. The temperature and stir rate were remained at 34 ^{0}C and 75 rpm, respectively. Aliquots of 5 ml were withdrawn from the release medium and replaced by an equal volume with STF at each sampling time. The amount of GFS was determined by UV-visible spectrophotometer.

The experiments were conducted using in situ gel forming microemulsion of Gatifloxacin with Kelcogel F concentration of 0.3% and 0.5%.

4.15.8 STERILITY TESTING

The final optimized formulation means for the animal study must be sterile. Sterility test was carried out according to USP method with the help of Fluid-Thioglycolate Medium (FTM) and Soya bean-Casein Digest Medium (SCDM). The media was sterilized by moist heat sterilization in an autoclave at 121°C for 20 min at 15 lb pressure. The sterilized formulation (1ml) was then added to each sterilized media (9 ml each) under laminar flow in the aseptic area. After that, the media were incubated in incubator (FTM at 34±1°C in bacteriological incubator and SCDM at 23±1°C in fungal incubator) for 1 day. The test tubes were checked after 24 hours.

On the next day Soya bean-Casein Digest Medium (SCDM) in 2%w/v of agar were made and autoclaved and plated. 4 plates were inoculated with solutions from test tubes of FTM by streaking using a wire loop and another 4 plates with solutions from test tubes of SCDM. Another 4 plates served as blank. All the plates were then incubated at 34±1°C. The plates were checked every 24 hours up to 3 days. Absence of turbidity and plaque shows the formulation is sterile.

4.16 IN VIVO EVALUATION

4.16.1 EYE IRRITATION TEST IN RABBIT

New Zealand white rabbits weighing 1.5–2.5 kg were provided by the animal experimental department of torrent research center. The animals were housed in standard cages in a light controlled room at 19±1 ^{0}C and 50±5% RH and were fed a standard pellet diet and water. All studies were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), Department of Pharmacy, Nirma University (Project registration no. 883/ac/05/CPCSEA). Rabbits were treated twice a day (one drop in the right eye) with optimized formulation for 3 days. The left eyes served as controls and were treated with saline. The ocular condition was recorded every day and at 1 h after the last administration. According to the Draize test, ocular irritation scores for every rabbit were calculated by adding together the irritation scores for the cornea, iris and conjunctiva.

The eye irritation score was obtained by dividing the total score for all rabbits by the number of rabbits. Irritation was classified according to four grades: practically non-irritating, score 0–3; slightly irritating, score 4–8; moderately irritating, score 9–12; and severely irritating (or corrosive), score 13–16 (Gan et al., 2009).

4.16.2 DETERMINATION OF VITREOUS PENETRATION OF DRUG

All animal procedures were performed at the Nirma University following an animal use protocol approved by the Institutional Animal Ethics Committee (IAEC), Department of Pharmacy, Nirma University (Project registration no. 883/ac/05/CPCSEA). The experiments were performed with the help and guidance of pharmacology personnel at the Institute.

Rabbits were marked as 1 to 4. Rabbits were treated three times a day (instillation of one drop in the right eye) with optimized formulation for 3 days. The left eyes served as controls and were treated with marketed eye drops (GATILOX).

Vitreous humor collection procedure

It is same as application of intra-vitreal injection. But the difference is, instead of application, blank injection is injected and vitreous humor is collected up to the mark in the syringe (previously calibrated 50 µl and 100 µl with the help of micropipettes).

Each rabbit was anaesthetized by i.m injection of mixture of Xylazine HCl (10 mg/kg) and Ketamine HCl (50 mg/kg) (Yagci et al., 2007). When rabbit was anaesthetized, 2% lidocaine was applied in eye at injection site to provide local anaesthesia. Then eye lash and eye liners/lids were wiped with 5% povidone solution maintaining standard of care to give intra-vitreal injection. 5% povidone was also added at injection site to prevent any infection to induce. Then 4mm distance from the limbus was measured with the help of callipers and intravitreal injection was given with 31 gauze insulin syringe and vitreous humor was collected. The depth of the insertion is 5 mm (Meyer et al., 2009).

Samples of vitreous humor were taken at 30 min, 2 h, 4 h and 6 h after the last instillation of the formulation and samples were analysed by HPLC for estimation of drug concentration in vitreous humor.

Chapter 5
Results and Discussion

6. CONCLUSION

In this study we investigated the potential of a microemulsion in situ electrolyte-triggered gelling system for specific delivery of Gatifloxacin sesquihydrate to posterior ocular tissue and fluids. Compared to two other formulations, in situ gelling system and eye drop solutions, the Gatifloxacin microemulsion based in situ gelling system exhibited better penetration into ocular tissues, higher Gatifloxacin levels in vitreous humor and prolonged residence in the cornea.

After 45 minutes of last dosing in three times a day for 3-days regimen, a sufficient Gatifloxacin concentration (>>MIC_{90} for most of the pathogens responsible for Endophthalmitis) was detected in the vitreous humor, which suggests potential of Gatifloxacin microemulsion in situ gelling system for delivery of drug to the posterior chamber of eye. Ocular irritation test revealed good compatibility of the system. Therefore, it is concluded that the Gatifloxacin microemulsion in situ gelling system might represent an alternative for treatment of Endophthalmitis.

7. SUMMARY

- Solubility of drug in different oils and surfactants was determined. Gatifloxacin showed higher solubility in Peceol and Soluphor p. So, they were selected as oil and surfactant component respectively for formulation of microemulsion.

- The influence of different co surfactants (Labrasol, Transcutol, Propylene glycol and Cremopher EL) on the formation of microemulsion was studied. Combination of Labrasol with soluphor p enhanced the region of ME domain in the phase diagram.

- The effect of drug (Gatifloxacin) loading on the selected system, Peceol + Soluphor p + Labrasol + Water was also studied. The loading of drug did not significantly affect the particle size of globules and existence region of ME.

- The pseudo-ternary phase diagrams for different surfactant to co surfactant ratio were successfully developed for the following system: Peceol (Oil) + Soluphor p (Surfactant) + Labrasol (Co surfactant) + Water. The system showed the largest ME region in pseudo-ternary phase diagram when surfactant to co surfactant ratio was 1:2.

- A successful "mixture design" or simplex lattice design was developed to obtain the relationship between the particle size distribution and components of the mixture in the formulation. A simplex equation was obtained and contour plot of response was plotted over simplex space.

- The in situ gelling system was developed using different polymers and optimized by evaluating transparency, dropping capacity, drug content, pH, viscosity, % change in viscosity at physiological condition and drug release studies. Formulations with Kelcogel F in 0.3% and 0.5% concentration were selected as optimized batches.

- The microemulsion in situ gelling system was developed and evaluated for physico-chemical parameters, bioadhesive force of with goat's cornea, drug release kinetics through goat's cornea, ocular irritation studies in rabbits and vitreous penetration of drug in rabbit's eye after topical instillation. Peceol (12%), Soluphor p (12%), Labrasol (24%), Water (52%) with 0.3% Kelcogel F was found to be better system.

8. FUTURE PROSPECTIVE

This research was executed *in vitro* as a preliminary step to develop an optimal formulation system. More intensive *In vivo* research in animals and human are required to select an optimal formulation for delivery of drug to the posterior tissues and fluids.

The prospects are:

- Determination of In vivo-In vitro correlation of drug release studies.
- Determination of pharmacokinetics of drug in vitreous humor after topical administration.
- Clinical trials to determine concentration of drug in vitreous humor of human eye.
- Comparison of vitreous penetration of drug in rabbit and human eye.
- Stability of microemulsion based in situ gelling formulations.

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