Design, Development and Evaluation of Ophthalmic Solid Inserts and In Situ Gels of Gatifloxacin

THESIS

Submitted in partial fulfilment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY

By
VENUGOPAL KOLACHINA

Under the supervision of
PROF. RANENDRA N. SAHA

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE
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CERTIFICATE

This is to certify that the thesis entitled "**Design, Development and Evaluation of Ophthalmic Solid Inserts and In situ Gels of Gatifloxacin**" and submitted by **VENUGOPAL KOLACHINA**, ID No. 2001PHXF406 for award of Ph.D. Degree of the Institute, embodies original work done by him under my supervision.

Date:

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Dedicated
To
My Family

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Summary

Conventional formulations for ophthalmic therapy have several drawbacks, like drug loss due to spillage, tear over flow, corneal impermeability and nasolachrymal drainage. Thus design of novel ophthalmic delivery systems to overcome these draw backs is necessary. Ophthalmic inserts and in situ gel formulations will reduce the drug wastage due to spillage and releases the drug in controlled manner for longer periods, there by enhances the ophthalmic bioavailability. Objectives of the present research work were to prepare, characterize and evaluate the designed ophthalmic inserts and in situ gels of gatifloxacin. This work is presented in following chapters.

As per the need of present work, various methods viz, UV spectrophotometric, spectrofluorimetric and HPLC methods were developed and validated. The proposed methods were accurate, precise and sensitive. Solubility and partition coefficients of gatifloxacin were found to be pH dependent. At all the levels of concentrations studied the degradation rate was less and at neutral pH the degradation was found to be minimal. At neutral pH, gatifloxacin has shown good thermal stability and extensive photodegradation. The DSC and FT-IR studies confirmed the compatibility of drug with all the excipients studied. Gatifloxacin, alone and in combination with different excipients, was stable for more than 5 months in accelerated conditions and for 24 months at controlled room temperature conditions.

Different controlled release inserts were prepared using various polymers alone and in combination. The polymers used were different viscosity grades of hydroxypropylmethyl cellulose (HPMC), different molecular weight grades of poly(ethylene oxide) (PEO), hydroxyethyl cellulose (HEC) and different grades of Eudragit (Eu S100, Eu L100 and Eu L100-55). The designed ophthalmic inserts of gatifloxacin showed good physical properties indicating that the method of preparation of formulation is suitable for preparing good quality solid inserts. Drug release was found to depend on both polymeric network and microenvironment pH of the polymeric matrix. The drug release was extended upto 18 hr for formulations prepared with HPMC K15M and Eu S100 with satisfactory bioadhesion strength. Maximum bioadhesion strength was obtained for formulations prepared with PEO 900 and PEO 2000 in combination with Eu S100. Sterilization method has not affected the drug content and drug release rate. The drug release rate and drug content had not changed significantly even after storage at ambient conditions for 24 months.

In situ gel formulations were prepared by cold method using Pluronic® F-127 (PL F127) alone and in combination with HEC and different molecular weight grades of PEO. The prepared in situ gel formulations have showed reversible thermogelation. The physical properties like gelation temperature, spreadability, viscosity and content uniformity were

reproducible, indicating the reproducibility of the method of preparation of in situ gels. The gels with PL F127 alone and in combination with PEO and HEC were showing pseudoplastic flow. The addition PEO has decreased the PL F127 concentration required to show same effect. Best formulation was showing extension of release upto nearly 4 hr. The main mechanism of drug release was gel erosion. Formulations with 2.5 % w/w of PEO 900 and 1 % w/w PEO 7000 in combination 15 % w/w PL F127 showed maximum bioadhesion strength. Sterilization by autoclaving and storage at refrigerated temperatures had no effect on rheological and drug release characters of gels. The formulations were stable at refrigerator temperature for entire period of study.

The apparent permeability coefficient was found to be higher in pH 7.2, i.e. pH of tear fluid. Benzalkonium chloride enhanced the corneal permeability by 2.4 folds. The studied formulation additives have not affected the corneal permeability. The corneal penetration was enhanced by 3.8 times with PL F127.

Ocular tolerance and toxicity study in rabbits conclude that except insert formulation prepared with Eu S100 alone, all other formulations were found to be weakly irritant products. The insert formulations with PEO 900 and PEO 2000 in combination with Eu S100, the C_{max} was increased by 5 and 3.9 folds respectively, when compared to marketed eye drop preparation. AUC was enhanced by 8.3 and 9.4 folds respectively. The in situ gels with PEO 900 (2.5 %) and PEO 7000 (1 %) in combination with PL F127 (15 %), the C_{max} was increased by 5.5 and 5.8 folds respectively. For both these formulations AUC_{rel} was found to be 12.

From reported MIC_{90} values of ocular pathogens, parameters like C_{max}:MIC_{90} and AUC_{0-t}:MIC_{90} for different formulations was calculated. Insert formulations containing PEO 900 and PEO 2000 combined with Eu S100, have shown C_{max}:MIC_{90} > 10, AUC_{0-t}:MIC_{90} > 75 and several folds increase in AUC_{eff} for most of the organisms. Similarly, in situ gel formulations containing PEO 900 or PEO 7000 combined with PL F127, have shown Cmax:MIC90 > 10, AUIC > 75 and several folds increase in AUC_{eff} against most of the organisms. These results indicate that the designed formulations were effective against most of the infection causing organisms.

In vivo studies showed several folds increase in pharmacokinetic parameters for insert and in situ gel formulations as compared with marketed product. The enhancement of bioavailability of the designed formulations is due to better absorption, controlled drug release in precorneal area and reduction in drug loss. From the above studies it may be concluded that the designed formulations are promising with improved therapeutic effectiveness against ophthalmic infections as compared to that of marketed eye drops.

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List of Abbreviations/Symbols

% CDR	Percentage cumulative drug released
% GD	Percentage gel dissolved
% RSD	Percentage relative standard deviation
% RTD	Percentage remaining to be degraded
% w/v	Percentage weight per volume
% w/w	Percentage weight per weight
ΔQ	Change in amount of drug in receiving chamber in permeability studies
Δt	Change in time
$\mu g.g^{-1}$	Microgram per gram
$\mu g.ml^{-1}$	Microgram per milliliter
A	Area
ACN	Acetonitrile
AHQC	Aqueous humor high quality control sample
AIC	Akaike's information criteria
ALQC	Aqueous humor low quality control sample
AMQC	Aqueous humor medium quality control sample
ANOVA	Analysis of variance
APC	Apparent permeability coefficient
AT	Accelerated temperature conditions
AUC	Area under the curve
$AUC_{0-\infty}$	Area under the curve from '0' time to '∞' time
AUC_{0-t}	Area under the curve from '0' time to 't' time
$AUC_{aqueous\ humor}$	Area under the curve from aqueous humor vs. time profile
AUC_{eff}	Effective area under the curve
AUC_{rel}	Relative area under the curve
AUIC	AUC of concentration-time curve to minimum inhibitory concentration
AUMC	Area under the moment curve
BKC	Benzalkonium chloride
BODI	Bioadhesive ophthalmic drug insert
BSS	Balanced salt solution
BUTL	O-butyryl ester prodrug of tilisolol
$CaCl_2.2H_2O$	Calcium chloride dihyrate
CAP	Cellulose acetate phthalate
CDER	Center for drug evaluation and research
CDS	Chemical delivery systems
CH-HCl	Chitosan hydrochloride
Cl/F	Total systemic drug clearance
Cl_R	Total renal clearance
C_{max}	Maximum drug concentration in pharmacokinetic study
C_{min}	Minimum plasma drug concentration
C_o	Initial permeant concentration in permeability studies

CP	Carbopol
CPC	Cetylpyridium chloride
cPs	Centi poises
CRT	Control room temperature conditions
DC	Sodium deoxycholate
DCM	Dichloromethane
DL	Detection limit
DNA	Deoxy nucleic acid
DSC	Differential scanning calorimeter
$Dynes.cm^{-2}$	Dynes per sqaure centimeter
EC	Ethylcellulose
EDTA	Ethylenediaminetetraacetic acid
Eu L100	Eudragit L100
Eu L100-55	Eudragit L100-55
Eu S100	Eudragit S100
F	Shear stress
$F_{calculated}$	Calculated *F*-value
$F_{critical}$	Critical or Tabulated *F*-value
f_e	Fraction of each dose excreted unchanged in the urine
F_{max}	Maximum detachment for in bioadhesion test
FT	Refrigerator temperature conditions
FT-IR	Fourier transformed infrared
G	Shear rate
HA	Hyaluronic acid
HA-Na	Sodium hyaluronic acid
HCl	Hydrochloric acid
HEC	Hydroxyethyl cellulose
HEMA	2-Hydroxyethylmethacrylate
HL %	Hydration level in percentage
HP-β-CD	Hydroxypropyl-beta-cyclodextrin
HPC	Hydroxypropyl cellulose
HP-guar	Hydroxypropyl-guar
HPLC	High performance liquid chromatography
HPM	Poly (2-hydroxy-propyl methacrylate)
HPMC	Hydroxypropyl methylcellulose
HPMC K100M	Metalose® 90SH 100000SR - viscosity 100000 cPs
HPMC K15M	Metalose® 90SH 15000SR - viscosity 15000 cPs
HPMC K4M	Hydroxypropylcellulose - viscosity 4000 cPs
HPTLC	High performance thin layer chromatography
HQC	High quality control sample
hr	Hours
IC_{50}	Concentration required for 50% inhibition
IOL	Intraocular hydrophilic acrylic lens
IOP	Intraocular pressure

IP	Indian Pharmacopeia
$J.g^{-1}$	Joules per gram
K	Release rate constant
K_2HPO_4	Di-potassium hydrogen phosphate
KCl	Potassium chloride
kDa	Kilo dalton
K_{deg}	First order degradation rate constant
KH_2PO_4	Potassium dihydrogen phosphate
kHz	Kilo hertz
LQC	Lower quality control sample
M	Molarity
MATP	Mono amine-terminated poloxamer
MC	Methylcellulose
MDR efflux	Multi drug resistance efflux mechanism
$mg.ml^{-1}$	Milligram per milliliter
$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
MIC	Minimum inhibitory concentration
MIC_{90}	Minimum inhibitory concentration required to kill 90% population
min	Minutes
ml	Milliliter
mM	Millimolar
$mOsm.l^{-1}$	Milli osmols per liter
mPa.sec	Milli pascal second
MQC	Medium quality control sample
MRT	Mean residence time
MSSR	Mean sum of the squared residuals
N	Newton
$N.cm^{-2}$	Newton per square centimetre
$N.sec.cm^{-2}$	Newton second per square centimetre
$Na_2HPO_4.2H_2O$	Di sodium hydrogen phosphate dihydrate
NaH_2PO_4	Sodium dihydrogen phosphate
$NaH_2PO_4.2H_2O$	Sodiumm dihydrogen phosphate dihyrate
$NaHCO_3$	Sodium hydrogen carbonate
NaOH	Sodium hydroxide
$ng.ml^{-1}$	Nanogram per milliliter
NIR	Near infrared
OImax	Maximum ocular irritating index
OLCS	Ophthalmic lyophilisate carrier system
PAA	Poly (acrylic acid)
PalTL	O-palmitoyl ester prodrug of tilisolol
P_{app}	Apparent partition coefficient
PBS	Phosphate buffered saline
PEO	Poly(ethylene oxide)
PEO 200	Polyox® WSR N-80

PEO 300	Polyox® WSR N-750
PEO 900	Polyox® WSR-1150
PEO 2000	Polyox® WSR N-60K
PEO 5000	Polyox® WSR Coagulant
PEO 7000	Polyox® WSR-303
P-gp	P-glycoprotein
p*I*	Isoelectric point or pH
pK_a	Dissociation constant
PL F127	Poloxamers 407 or Pluronic® F-127
PMA	Phenyl mercuric acetate
POE	Postoperative endophthalmitis
PRK	Photorefractive keratectomy
PTFE	Polytetrafluoroethylene
PVA	Poly(vinyl alcohol)
PVP	Polyvinylpyrrolidine (Povidone)
q.i.d	Four times daily
QL	Quantitation limit
R^2	Regression coefficient
R_f	Retention factor
rhEGF	Human epithelial growth factor
RPE	Retinal pigment epithelium
RP-HPLC	Reverse phase high performance liquid chromatography
rpm	Rotations per minute
SA	Sodium alginate
SD	Standard deviation
SDS	Sodium dodecyl sulphate
sec	Second
SHQC	Serum high quality control sample
SLQC	Serum lower quality control sample
SMQC	Serum medium quality control sample
SSR	Sum of the squared residuals
STF	Simulated tear fluid
t	Time
$t_{1/2}$	Biological half-life
$t_{90\%}$	Time taken for 90% of the drug to dissolved or time taken for 90% of the drug to remain
TBAHS	Tetrabutyl ammonium hydrogen sulphate
$t_{calculated}$	Calculated t-value
$t_{critical}$	Critical or Tabulated t-value
TDW	Triple distilled water
TEA	Triethyl amine
TEER	Trans epithelial electrical resistance
THM	Thiomersal
TLC	Thin layer chromatography

T_{max}	Time at which maximum drug concentration achieved in pharmacokinetic study
TMC	*N*-Trimethylchitosan
TPA	Texture Profile Analysis
t_R	Retention time
$T_{sol-gel}$	Gelation temperature or sol-gel transition temperature
TSP	Tamarind seed polysaccharide
Tw20	Tween 20
US FDA	United States Food and Drug Administration
USP	United States Pharmacopeia
UV	Ultraviolet
V_d/F	Volume of distribution at steady state
Vis	Visible
$W.cm^{-2}$	Watt per square centimeter
XG	Xyloglucan
°C	Degree centigrade
Δ	Difference
η′	Apparent viscosity
λ	Wavelength
λ_{em}	Emission wavelength
λ_{exc}	Excitation wavelength
λ_{max}	Maximum absorption wavelength
λ_{max}	Wavelength maximum
Ω	Ohm

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Chapter 1

Introduction, Literature Survey and Objectives

1.1. Ophthalmic drug delivery

The **"EYE"** is considered as the 'window to the brain' and thereby holds special status among sensory organs. Millions of people worldwide are affected by a wide variety of ocular diseases and conditions, many of which cause serious vision impairment or irreversible blindness (Clark and Yorio, 2003). The research on ocular therapy, in recent years, mainly focuses on improvement of existing ophthalmic dosage forms, development of novel drug delivery systems and on discovery of newer and safer drug molecules.

The delicate anatomical nature and the protective physiological mechanisms of the eye against the administered products are the major factors for the challenges encountered. Due to these constraints, effectively 1 % or even less of the instilled dose of the drug is locally absorbed. Many attempts have been made to improve ocular drug bioavailability by several techniques, but still there is ample scope for development.

1.2. Diseases Affecting the Eye

Ocular disorders range from infections to conditions that produce blindness. These conditions affect the anterior surface of the eye to those that affect the posterior pole of the eye. Some of the diseases with respect to the anatomy of eye given in Table 1.1. A complete, elaborate and extensive discussion of anatomy and physiology has been given in literature (Chandran, 2003).

Table 1.1: Various parts of the eye with the main diseases affecting them (Clark and Yorio, 2003).

Ocular Tissue	Disease
Cornea	Keratitis (an inflammation of the cornea, caused by bacterial, viral or fungal pathogens), stromal edema and ulcer, epithelial bulbar neovascularization
Cornea and lens	Ametropia, myopia, hyperopia, astigmatism
Eye lids and Meibomian cysts of the eyelid	Blepharitis, infection, hordeolum, sty and chalazia
Precorneal and conjunctiva	Dry eye, allergic, bacterial and viral conjunctivitis (an inflammation of the conjunctiva that may be caused by bacterial and viral infection, pollen and other allergens, smoke and pollutants), subconjunctival hemorrhage, herpes simplex and dacryocystitis
Conjunctiva, sclera, and episclera	Scleritis
Internal diseases	Glaucoma, cataract
Anterior uveitis	Iritis
Vitreo-retinal diseases	Endophthalmitis, viral retinitis, proliferative vitreoretinopathy, macular degeneration

1.2.1. Fluoroquinolone therapy in ocular infections

Ocular infections are common and can occur almost anywhere on or within the eye. These infections can be self-limiting or can become vision-threatening. Microbes normally present on the eye can become pathogenic when the eye is damaged or when the natural defenses of the eye are compromised. In addition, infections can occur when eye tissues are exposed to new pathogens (Clark and Yorio, 2003). Keratitis and endophthalmitis are among the leading causes of visual loss and ocular morbidity worldwide (Seal, 1998). Fluoroquinolones have found widespread use in therapy of ocular bacterial infections since they possess a broad spectrum of antibacterial activity and can be administered topically or by other ocular routes and these agents result in fewer side effects than when fortified aminoglycosides or cephalosporins are used (O'Brien et al., 1995). They have been shown in most situations to be equivalent to combination therapy and because of their effectiveness against poly-resistant organisms (Ligtvoet and Wickerhoff-Minoggio, 1985; Stevens et al., 1991). Commonly used fluoroquinolones in ophthalmology include ciprofloxacin, ofloxacin, norfloxacin, lomefloxacin, levofloxacin, sparfloxacin and pefloxacin. Other fluoroquinolones such as gatifloxacin, moxifloxacin and tosufloxacin are gaining importance because of low resistance and broader spectrum of activity (Smith et al., 2001).

(a) Conjunctivitis and blepharitis

Bacterial conjunctivitis and blepharitis are common external ocular infections that can be caused by many types of micro-organisms. Everett et al. (1995) found 92 % of bacterial strains isolated from 173 patients with blepharitis were Gram-positive compared with 8 % that were Gram-negative. They also found that 75 % of micro-organisms (289 of 385 bacterial isolates) isolated from patients with conjunctivitis were Gram-positive. Several studies in literature showed that the fluoroquinolones are effectively used for treatment of conjunctivitis and blepharitis (Everett et al., 1995; Hofman et al., 1995; Agius-Fernandez et al., 1998; Friedlander, 1998).

(b) Bacterial keratitis

Fluoroquinolone use has become particularly predominant in the treatment of presumed bacterial keratitis, an important cause of ocular morbidity and visual loss. In patients where no specific organism has been identified, the choice of antibacterials with good broad spectrum activity, such as the fluoroquinolones where there is an 85 to 95 % bacterial susceptibility (Jones et al., 2000), has increased their use in the treatment of ocular infections (Smith et al., 2001). Bower et al. (1996) found the cefazolin-fluoroquinolone

combination had greater predicted susceptibility than any of the single agent therapies. The cefazolin/fluoroquinolone combination was comparable to a cefazolin/gentamicin combination. In contrast, fluoroquinolone monotherapy has shown equivalence to fortified tobramycin/cefazol in the treatment of bacterial keratitis (O'Brien et al., 1995; Baker et al., 1996). However, fluoroquinolones may also be associated with more complications in patients with large corneal ulcers (Gangopadhyay et al., 2000).

(c) Endophthalmitis

Postoperative endophthalmitis (POE) is rare but serious complication that may rise from any surgical procedure disrupting the integrity of the globe. The organisms causing post-surgical endophthalmitis are *S. aureus*, *S. epidermidis* and *S. pneumoniae* (Sande, 1981; Speaker et al., 1991; Kowalski et al., 1998). The prophylactic use of antibiotics before, during and/or after the operation is common (Kleinmann et al., 2006a; Kleinmann et al., 2006b; Kleinmann et al., 2006c; Kleinmann et al., 2007). A variety of antibacterial agents are administered via oral, intravitreal and topical route. Oral fluoroquinolones after intravitreal route may help maintain intravitreal concentrations to reduce the need for further intravitreal injections.

1.3. The barriers for ophthalmic drug delivery

1.3.1. Precorneal barriers

Poor ocular bioavailability is attributed to (i) rapid nasolachrymal drainage, (ii) non-productive absorption through conjunctiva, (iii) permeability and metabolic barriers imposed by corneal epithelia, (iv) inherent physicochemical insufficiencies of the drug molecules, (v) increased tear turnover upon instillation of the formulation and (vi) binding of drug to tear fluid proteins. These factors are schematically represented in the Figure 1.

After topical administration of an ophthalmic drug solution, the drug is firstly mixed with the lachrymal fluid. The contact time of drug with ocular tissues is relatively short (1-2 min) because of the permanent production of lachrymal fluid (0.5-2.2/~l/min). The excess volume of the instilled fluid is flown to the nasolachrymal duct rapidly. Drainage of lachrymal fluid during blinking (every 12 sec) towards the nasolachrymal duct induces a rapid elimination of conventional dosage forms (Ahmed and Patton, 1985, 1986, 1987; Ahmed et al., 1987; Urtti and Salminen, 1993).

Another source of non-productive drug removal is its systemic absorption instead of ocular absorption. Systemic absorption may take place either directly from the conjunctival sac via local blood capillaries or via absorption across the mucosa in the nasal cavity (Urtti et

al., 1985; Chang and Lee, 1987; Urtti et al., 1994). Anyway, most of the small molecular weight drug dose is absorbed into systemic circulation rapidly in few minutes. This contrasts the low ocular bioavailability of less than 5 % (Urtti and Salminen, 1993).

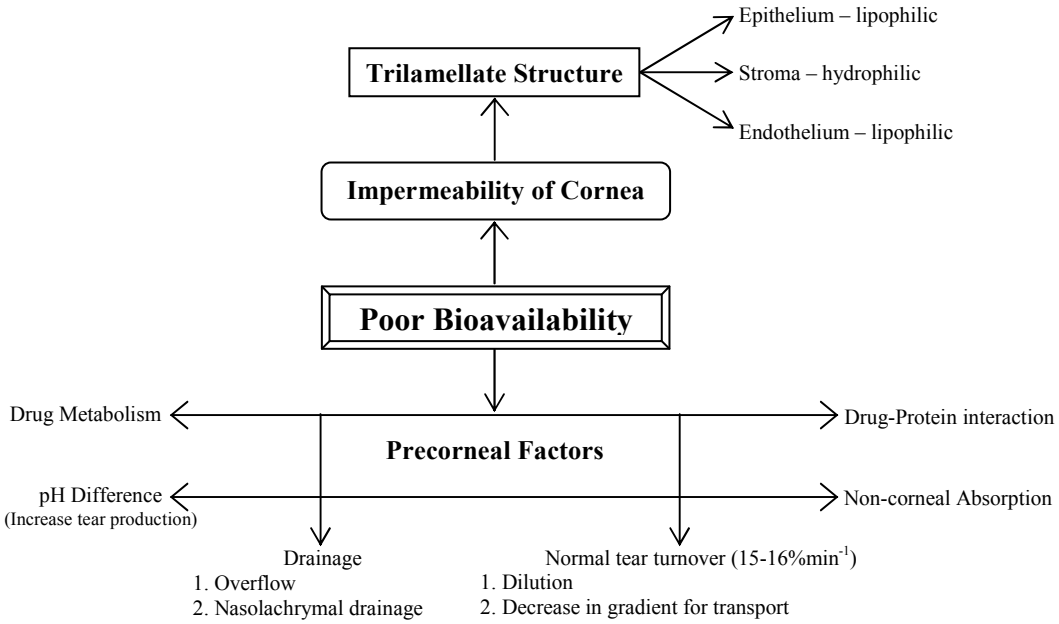

Figure 1.1: Physiological barriers of ophthalmic drug delivery (Kaur and Smitha, 2002).

Corneal epithelium limits drug absorption from the lachrymal fluid into the eye (Maurice and Mishima, 1984). The most apical corneal epithelial cells from tight junctions that limit the paracellular drug permeation (Hornof et al., 2005). The tight junctions of the corneal epithelium serve as a selective barrier for small molecules and they prevent the diffusion of macromolecules via the paracellular route. Lipophilic drugs prefer the transcellular route, while hydrophilic drugs penetrate primarily through the paracellular pathway, which involves passive or altered diffusion through intercellular spaces. Lipophilic drugs have typically at least an order of magnitude higher permeability in the cornea than the hydrophilic drugs (Huang et al., 1983). The transcorneal penetration is also hindered by the binding of the drug to the corneal tissues. The cornea may act as a drug reservoir, slowly releasing the drug into the aqueous humour, where levels decrease very slowly (Romanelli et al., 1994). The stroma beneath the epithelium is a highly hydrophilic layer. Clearly then, the more lipophilic the drugs are, the more resistance they will find crossing the stroma. The more hydrophilic a drug, the more resistant the epithelium, whereas the stroma and endothelium are limited in their resistance. Despite the tightness of the corneal epithelial layer, transcorneal permeation is the main route of drug entrance from the lachrymal fluid to the aqueous humor (Figure 1.1).

Owing to the relative leakiness of the membrane, rich blood flow and large surface area, conjunctival uptake of a topically applied drug from tear fluid is typically an order of magnitude greater than corneal uptake (Ahmed and Patton, 1985; Hamalainen et al., 1997; Prausnitz and Noonan, 1998). Drug absorption across the bulbar conjunctiva has gained increasing attention recently, since conjunctiva is also fairly permeable to the hydrophilic and large molecules (Geroski and Edelhauser, 2001). In both membranes, cornea and conjunctiva, principles of passive diffusion have been extensively investigated, but the role of active transporters is only sparsely studied. The drug is absorbed into the retina-choroid via an extracorneal or sclero-conjunctival route; the iris and ciliary body are presumably supplied via both the transcorneal and the extracorneal pathways. Both transconjunctival absorption and transnasal absorption after drainage via the nasolachrymal duct are generally undesirable, not only because of the loss of active ingredient into the systemic circulation, but also because of possible side-effects, for instance the effects on the heart when beta-blockers are administered for the treatment of glaucoma (Meseguer et al., 1994).

Physicochemical drug properties, such as lipophilicity (Schoenwald and Huang, 1983), solubility, molecular size and shape (Grass and Robinson, 1988a, 1988b; Grass et al., 1988; Huang et al., 1989; Liaw and Robinson, 1992), charge (Liaw et al., 1992; Rojanasakul et al., 1992; Liaw and Robinson, 2003) and degree of ionization (Sieg and Robinson, 1977; Maren and Jankowska, 1985; Brechue and Maren, 1993) affect the route and rate of permeation in cornea. Some authors consider the optimum solubility for corneal absorption is found in drugs with an octanol/buffer distribution coefficient in the range of 100-1000, i.e. more lipophilic than hydrophilic (Schoenwald and Ward, 1978; Schoenwald and Huang, 1983). Moreover, unionized species usually penetrate the lipid membranes more easily than the ionized form.

The medication, upon instillation, stimulates the protective physiological mechanisms, i.e., tear production, which exert a formidable defense against ophthalmic drug delivery. The drug moiety is subjected to the negative influences of tears, such as (i) an increased tear turnover leading to an increased spillage and dilution of the drug; (ii) reflex blinking causing an accelerated clearance via tears; (iii) binding of the drug molecule to the tear proteins thus reducing the effective concentration of drug in contact with the cornea; and (iv) buffering action of the carbonic acid and weak organic acids present in tears affecting the extent of ionized/unionized forms of the drug and hence its bioavailability. A drop of an aqueous solution, irrespective of the instilled volume, is eliminated completely from the eye and the eye returns to the normal lachrymal lake volume within 5-6 min of its application. This results in a very short period during which the drug has access to the ocular tissues (Kaur and

Kanwar, 2002).

1.3.2. Blood ocular barriers

The eye is protected from the xenobiotics in the blood stream by blood-ocular barriers. These barriers have two parts, blood-aqueous barrier and blood-retina barrier. The anterior blood-eye barrier is composed of the endothelial cells in the uvea. This barrier prevents the access of plasma albumin into the aqueous humor, and limits also the access of hydrophilic drugs from plasma into the aqueous humor. Inflammation may disrupt the integrity of this barrier causing the unlimited drug distribution to the anterior chamber (Urtti, 2006).

The posterior barrier between blood stream and eye is comprised of retinal pigment epithelium (RPE) and the tight walls of retinal capillaries (Maurice and Mishima, 1984; Hornof et al., 2005). Unlike retinal capillaries the vasculature of the choroid has extensive blood flow and leaky walls. Drugs easily gain access to the choroidal extravascular space, but thereafter distribution into the retina is limited by the RPE and retinal endothelia. Despite its high blood flow the choroidal blood flow constitutes only a minor fraction of the entire blood flow in the body. Therefore, without specific targeting systems only a minute fraction of the intravenous or oral drug dose gains access to the retina and choroid.

1.4. Formulation approaches to improve ocular therapy

Currently, the challenge faced by scientific community in ophthalmic pharmaceutical research is to improve ocular bioavailability from less than 1-5 % to at least 15-20 % (Saettone et al., 1996). Numerous strategies were developed to increase the bioavailability of ophthalmic drugs. The emphasis is given to maximize precorneal drug absorption by minimizing precorneal drug loss, increasing the drug residence time by developing novel drug delivery systems and enhance the corneal permeability by virtue of permeation enhancers. The benefits to the patient should be easy to administer, prolong the contact time with ocular tissues, reduce frequency of administration, reduce the spillage, be acceptable to the patients, non-toxic, reduce side-effects, non-irritant and comfortable. The additives used should not affect the corneal permeability of the drug and vision of the patient (Van-Ooteghem, 1987).

The use of a water-soluble polymer to enhance the contact time and possibly also the penetration of the drug was first proposed by Swan (Swan, 1945). Although eye-drops represent 90 % of all ophthalmic dosage forms, there is a significant effort directed towards development of new drug delivery systems to overcome the disadvantages of eye-drops. All

novel ophthalmic drug delivery systems are intended to increase the bioavailability of the drug, to sustain/control the drug delivery to the pre and intraocular tissues and to reduce systemic side effects. The primary determinant of efficacy for a controlled release system applied to the eye is the time during which the drug remains in contact with the cornea.

1.4.1. Liquid dosage forms

(a) Viscous drug solutions

Conventional aqueous solutions topically applied to the eye have the disadvantage that most of the instilled drug is lost within the first 15-30 sec after instillation. A simple and most popular approach to prolong the precorneal residence time thereby increasing the ocular bioavailability is to increase vehicle viscosity by incorporating soluble polymers into an aqueous solution. The increased vehicle viscosity reduces the drainage of the formulation, thus the drug, into the nasolachrymal duct and slower elimination from the preocular area and hence a greater transcorneal penetration of the drug into the anterior chamber (Saettone et al., 1982). Viscous vehicles increase the contact time of the preparations to varying degrees, but so far no marked sustaining effect has been attained (Saettone et al., 1989). Acetazolamide formulated in carboxy methyl cellulose (CMC), compared with the saline solution of the drug in patients with unilateral open-angle glaucoma, was found to have a longer duration of action. However, the results were not spectacular and were significant only when using high drug concentrations (Kaur and Kanwar, 2002). Corneal permeability of acetazolamide was enhanced with polyvinyl alcohol (PVA) viscous preparations (Kaur et al., 2004). Viscous solutions of ofloxacin prepared using chitosan hydrochloride (CH-HCl) and N-carboxymethyl chitosan have maintained drug levels for 2.4 folds more time than reference vehicle (Di Colo et al., 2004).

Nevertheless, viscosity alone cannot significantly prolong the residence time. This can be considered, in part, as the premise of using bioadhesive polymers to enhance drug absorption. The capacity of some polymers to adhere to the mucin coat covering the conjunctiva and the corneal surfaces of the eye forms the basis for ocular mucoadhesion. Due to interactions with the mucus layer or the eye tissues, an increase in the precorneal residence time of the preparation, therefore prolongs the residence time of a drug in the conjunctival sac. In these systems clearance is controlled by the much slower rate of mucus turnover than the tear turnover rate.

Bioadhesive polymers are usually macromolecular hydrocolloids with numerous hydrophilic functional groups and possess the correct charge density (Robinson and Mlynek, 1995). These bioadhesive polymers can be natural, synthetic, or semi-synthetic in nature.

Ocular bioavailability of rufloxacin has been enhanced with tamarind seed polysaccharide (TSP) (Burgalassi et al., 2006). Viscosity inducing and bioadhesive polymer like sodium hyaluronate (Aragona et al., 2002; Vico et al., 2005), trehalose (Matsuo et al., 2002), hydroxypropyl-guar (HP-guar) (Ubels et al., 2004; Hartstein et al., 2005) were used to prepare artificial tears for the treatment of conditions like preservative induced damage and dry eye syndrome.

(b) Aqueous suspensions and oily preparations

Insoluble drugs can be delivered effectively by using these strategies. Suspensions are dispersions of finely divided relatively insoluble drug substances in an aqueous vehicle containing suitable suspending and dispersing agents. Because of a tendency for the particles to be retained in the cul-de-sac, the contact time and duration of action of a suspension exceed those of a solution (Le Bourlais et al., 1998). While the retention increases with an increase in the particle size, as does the irritation of the eye, the rate of dissolution of the suspended drugs increases with decreasing particle size. Thus an optimum particle size has to be selected for each type of drug, and it is recommended that the particles in an ophthalmic suspension should be lower than 10 mm in size (Sieg and Robinson, 1975). The spillover and drainage of a suspension leading to the loss of both solution and suspended solid can also affect the drug availability and absorption. Moreover, a change in crystal structure, i.e., polymorphism, may occur during storage, resulting in an alteration in the suspension characteristics causing solubility changes reflected in an increased or decreased bioavailability. Ocular bioavailability of ketorolac was enhanced by two folds when sesame oil and soybean oil based formulations were instilled into rabbit eyes (Malhotra and Majumdar, 2005).

(c) Vesicular or colloidal systems

These systems were exploited for both intraocular and topical administration. The rationale for the development of various particulate systems for the delivery of ophthalmic drugs was based on possible entrapment of the particles in the ocular mucus layer and the interaction of bioadhesive polymer chains with mucins inducing a prolonged residence, and slow release. Furthermore, controlled drug release and enhanced absorption or even endocytosis in the case of nanoparticles have been found to improve bioavailability (Kreuter, 1993; Le Bourlais et al., 1995; Zimmer and Kreuter, 1995; Le Bourlais et al., 1998; Saettone et al., 1999; Alonso and Sanchez, 2003). The carriers used should be biocompatible, non-irritant and biodegradable. Various vesicular or colloidal systems used for ocular therapy are

liposomes, microemulsions, niosomes, pharmacosomes, microparticles, nanoparticles and dendrimers.

When appropriately formulated for ophthalmic delivery, the particles are retained in the ocular cul-de-sac and the drug released at a rate that is neither too fast nor too slow to allow adequate drug penetration into ocular tissues. They can be used to target the drug molecule to a specific tissue. The particle size of ophthalmic controlled-release formulations has proved to be very important in balancing between the drug release rate, bioavailability improvement, patient comfort and ease of use (Shekunov et al., 2007). Nanoparticles (typically about 300 nm) without bioadhesion can be eliminated from the precorneal site almost as quickly as aqueous solutions. Microparticles (mean diameter 1-3 μm) may be better suited for controlled release, but the presence of coarse particle fraction above 25 μm makes them less tolerable and can cause irritation to the eye. One of the main challenges in developing such particulate systems is the manufacturing complexity and particle size control during large-scale manufacturing (Ding, 1998; Burrow et al., 2002).

1.4.2. Semi-solid dosage forms

(a) Ointments

Ointments are useful as drug carriers for improving bioavailability, sustaining drug release and improving drug stability. There is more flexibility in the choice of drug to be incorporated into an ointment base, as even drugs with low water solubility can be suitably delivered to the eye. The ointments are reported to sustain on the surface of the eye for up to 2-4 or even 8 hr after application (Gebhardt and Kaufman, 1995). Ophthalmic ointments containing different sorption promoters have also been formulated and reported to show significantly higher release rates, relative to ointments without these promoters. However, dosage variability with ointments is greater than with solutions and the ointments interfere with vision unless their use is limited to bedtime instillation. Drug molecules may be entrapped within the ointment base or may not be released at the site of action due to a favorable partitioning towards the base (Kaur and Kanwar, 2002). Flavin adenine dinucleotide sodium ointment applied topically in humans extended the drug levels upto 1 hr (Takaoka et al., 2004).

(b) Aqueous gels/hydrogels

The use of highly viscous aqueous solutions leads to an improvement in the precorneal retention of drugs and a better miscibility with the lachrymal fluid. It also leads to a reduction in the dosage frequency due to enhanced bioavailability. As a consequence, there

is a decrease in the drug concentration required, with a concomitant reduction in the potential for side effects. A number of water-soluble or insoluble natural, synthetic, and semi-synthetic viscous vehicles have been developed during the last 50 years (Kaur and Kanwar, 2002).

Bioadhesive polymers enhance the possibility of interactions with mucus thereby increase residence time of drugs. An optimal concentration of mucoadhesive polymer will produce maximum adhesion (Lee et al., 2000). The drug should be slowly released during its stay in the cul-de-sac. Water soluble drugs will release fast, so alternative approaches were developed by incorporating slightly soluble drug complexes, micelles, and liposomes into the gel. Another possibility is the non-covalent or covalent binding of the drug molecule or a (PEG)–drug conjugate to the polymer chains (Ludwig, 2005). Tethering of long PEG chains on acrylate based hydrogels improves mucoadhesion properties due to enhanced anchoring of the chains with the mucus layer (Bures et al., 2001). Pilocarpine HCl or chloramphenicol loaded co-polymeric hydrogels constituting of vinylpyrrolidone and methacrylic or acrylic acid repeat units have extended the drug release (Barbu et al., 2005). Important disadvantages are irritation, blurred vision, sticky eyelids, poor control of drug release, prone to bacterial contamination and difficulty in administration (Kaur and Kanwar, 2002; Ludwig, 2005).

(c) In situ gelling systems

In situ gelling systems are viscous hydrogel systems, which undergo a sol-gel phase transition after exposure to the physiological conditions in the cul-de-sac, forming a viscoelastic gel. These systems have advantages like easy, accurate and reproducible administration of a dose compared to the application of preformed gels and they increase the patient compliance due to ease of administration and low frequency of administration (Ludwig, 2005). Different polymers exhibiting reversible phase transitions were used in these systems.

The phase transition is triggered by the pH of the tears (e.g. carbomer, cellulose acetate phthalate (CAP) latex) (Gurny et al., 1985; Ke et al., 2001; Srividya et al., 2001; Xu et al., 2002; Sultana et al., 2006; Wu et al., 2007), the temperature at the eye surface (e.g. methyl cellulose (MC), xyloglucan (XG), poloxamer 407) (Miyazaki et al., 2001; Wei et al., 2006) or the monovalent and divalent cations present in the tear film (e.g. alginic acid, sodium alginate, gellan gum) (Demailly et al., 2001; Balasubramaniam et al., 2003; Trinquand et al., 2003; El-Kamel et al., 2006; Liu et al., 2006b; Takiyama et al., 2006). These polymers were used alone and in combination with other in situ gel forming polymers, viscosity enhancing agents and bioadhesive polymers (Kaur and Kanwar, 2002). Once gelled, the formulation resists the natural drainage process from the precorneal area. Residence at the

site of drug absorption is prolonged and subsequently, the bioavailability of the drug is increased (Rozier et al., 1989, 1997). The rate of in situ gel formation is important because between instillation in the eye, and before a strong gel is formed, the solution or weak gel is prone to elimination by the fluid mechanics of the eye (Carlfors et al., 1998).

CAP latex coagulates when its native pH of 4.5 is raised by the tear fluid to pH 7.4. Gelrite® is a low-acetyl gellan gum, which forms a clear gel in the presence of mono- or divalent cations. The electrolytes of the tear fluid and especially Na^{+}, Ca^{2+} and Mg^{2+} cations are particularly suited to initiate gelation of the polymer when instilled as a liquid solution into the cul-de-sac (Ludwig, 2005). Recent advances in these formulations were compiled in Table 1.2.

Poloxamers or Pluronics®, block copolymers, undergo thermal gelation or sol-gel transition in the 25-35°C temperature range. Below transition temperature, poloxamer solutions allow a comfortable and precise delivery by the patient in the cul-de-sac, where thermogelation occurs (Koller and Buri, 1987). Due to inherent surface active properties, poloxamers were employed as solubilizer, and proposed as artificial tears (Koller and Buri, 1987; Saettone et al., 1988; Chetoni et al., 2000; Liaw et al., 2001).

In order to reduce the total polymer content of a formulation and to improve the rheological behaviour and gelling characters of the delivery system, combination of polymers were employed. Several researchers explored the advantage of using various in situ gelling polymers with different phase transition mechanisms in ophthalmic drug delivery. Desai and Blanchard (1998) showed that the addition of MC and hydroxypropylmethylcellulose (HPMC) to Pluronic® F-127 (PL F127) gels slowed down the gel dissolution rate and pilocarpine release. Miotic response was extended in rabbits for the gels compared to the aqueous solution.

Poloxamers were tested as a vehicle for various drugs or drug complexes such as liposomes or cyclodextrins (Le Bourlais et al., 1995; Bochot et al., 1998; Kim et al., 2002). Also various polymers such as water-soluble cellulose derivatives (Desai and Blanchard, 1998), polysaccharides (Miyazaki et al., 2001), poly(acrylic acid) (Lin and Sung, 2000) and hyaluronan (Wei et al., 2002) were added to poloxamer gels.

Copolymerization of the bioadhesive polymer poly(acrylic acid) (PAA) with Pluronic®, a thermally-induced, phase-separating graft polymer, has been reported to yield a bioadhesive vehicle with a prolonged residence time plus a prolonged drug release period in contact with mucosal surfaces such as the eye (Robinson and Mlynek, 1995).

Table 1.2: In situ gelling systems developed and studied in recent past.

Drug/Marker	Polymer(s)	Characters	In vitro / In vivo Model	Inference	Reference
Pilocarpine	Carbopol (CP) 934P and PL F127	pH-triggered gelling and thermally reversible gelation	Rabbit	Increase in $AUC_{0\text{-}6\ hr}$ (Δ pupil diameter vs. time) by 1.85 fold compared with an aqueous solution.	(Lin and Sung, 2000)
Pilocarpine	XG and PL F127	Thermally reversible gelation	Rabbit	Increase in AUC (response vs. time) was 1.36 and 1.45 folds with XG and PL F127 respectively.	(Miyazaki et al., 2001)
Ofloxacin	CP 940 and HPMC E50LV	pH-triggered gelling and viscosity enhancement	Rabbit	Formulation was non-irritating and sustained the drug release for 8 hr.	(Srividya et al., 2001)
Carteolol	Alginic acid	Ion activated gelation	Human	Alginate formulation given once daily was as effective as standard solution given twice daily with out blurring effect.	(Demailly et al., 2001)
Ciprofloxacin	CP, HPMC and dodecylmaltoside	pH-triggered gelling, viscosity enhancement and penetration enhancer	Rabbit	Corneal permeation was 10 folds more in formulations with penetration enhancer. The aqueous humor drug levels were sustained for longer periods.	(Ke et al., 2001)
Human epithelial growth factor (rhEGF)	rhEGF/ hydroxypropyl-beta-cyclodextrin (HP-β-CD) inclusion complex in PL F127, Pluronic® F-68 (PL F68)	Stable, bioadhesive and thermally gelling properties	Rabbit	AUC (tear concentration vs. time) of poloxamer gel containing the inclusion complex was 3.8 folds greater than that of rhEGF solution.	(Kim et al., 2002)
Timolol maleate	PL F127 in combination with HPMC, MC and sodium CMC	Reversible thermal gelation and viscosity enhancement	Rabbit	More than 2 fold increase in $AUC_{aqueous\ humor}$ for MC and PL F127 formulations.	(El-Kamel, 2002)
^{99m}Technetium	PL F127, PL F68 and Sodium hyaluronic acid (HA-Na)	Bioadhesive and thermally gelling properties	Rabbit	AUC (remaining activity vs. time) for 0.2 % HA-Na solution and mixed Pluronic gel were improved by 2.8 and 3.6 fold, respectively, in comparison to reference solution.	(Wei et al., 2002)
Pilocarpine	Carbomer, PVA	pH-triggered gelling	Rabbit	Different formations with same strength have shown same lowering of intraocular pressure (IOP) effect.	(Xu et al., 2002)

(Contd..)

Table 1.2

Drug/Marker	Polymer(s)	Characters	In vitro / In vivo Model	Inference	Reference
Carteolol	Alginic acid	Ion activated gelation	Human	Alginate formulation given once daily was as effective as standard solution given twice daily well tolerated by glaucoma patients who require chronic treatment.	(Trinquand et al., 2003)
Indomethacin	Gelrite®	Ion activated gelation	Rabbit	Drug release was sustained for 8 hr. Improvement in the clinical parameters up to 24 hr.	(Balasubramaniam et al., 2003)
Ciprofloxacin	Mono amine-terminated poloxamer (MATP) and hyaluronic acid (HA) coupled graft copolymers	Bioadhesive, thermally gelling and tissue regeneration properties	Rabbit	Formulation (graft copolymer with 13.99 wt. % of HA) has shown drug release up to 18 hr.	(Cho et al., 2003)
Carteolol hydrochloride	Gelrite®	Ion activated gelation	Rabbit	More than 2 fold increase in AUC of aqueous humor concentration vs. time.	(El-Kamel et al., 2006)
Timolol maleate	Gellan gum	Ion activated gelation	Dog	Gels reduced the IOP at the same time systemic side effects were also not observed.	(Takiyama et al., 2006)
Gatifloxacin and ^{99m}Technetium	Sodium alginate (SA) and HPMC	Ion activated gelation and viscosity enhancement	Rabbit	More than 3 fold increase in $AUC_{0\text{-}10\ min}$ of activity remaining vs. time.	(Liu et al., 2006b)
Pefloxacin mesylate	CP 934P and MC A4M	pH-triggered gelling and viscosity enhancement	Rabbit	Formulations maintained aqueous humor concentrations more than MIC for 12 hr.	(Sultana et al., 2006)
Timolol	Sodium deoxycholate (DC) penetration enhancer	Thermo gelation and penetration enhancer	Rabbit	Gels improved ocular bioavailability by several folds. In corporation of DC increased bioavailability by 2 folds.	(Wei et al., 2006)
Puerarin	CP 980, HPMC E4M and HP-β-CD	pH-triggered gelling and solubility enhancement	Rabbit	$AUC_{aqeous\ humor}$ of gels has enhanced by 2 fold compared to aqueous solution.	(Wu et al., 2007)
Puerarin	21 % w/v poloxamer 407/5 % w/v poloxamer 188) and CP (0.1 % w/v or 0.2 % w/v) CP 1342P NF	pH-triggered gelling and thermally reversible gelation	Rabbit	Diffusion controlled release of puerarin upto 8 hr. Combined solutions retained drug in tear and reduced IOP for longer periods than alone formulations.	(Qi et al., 2007)

1.4.3. Solid ophthalmic dosage forms

Ophthalmic inserts are solid ophthalmic dosage forms that are more effective, require less frequent administration, avoid pulsed release and diminish the number of additives needed. Ophthalmic inserts are defined as sterile preparations, with a solid or semisolid consistency and, whose size and shape are especially designed for ophthalmic application. Films, erodible and non-erodible inserts, rods and shields are the most logical delivery systems aimed at remaining for a long period of time in front of the eye. From a therapeutic point of view, inserts have been a success in the improvement of accurate dosing, and drug bioavailability and by the reduction of systemic absorption, and consequently side effects. They are composed of polymeric support containing the drug, which can be incorporated as dispersion or solution in the polymeric support. The inserts ensure a sustained release suited to topical or systemic treatment, by prolonging the contact time between the preparation and the conjunctival tissue. Addition of bio/mucoadhesive polymers in the inserts will further improve the residence time of dosage form. Inserts suffers drawbacks like high cost, not well tolerated or accepted by patients, due to difficulties encountered in the application, psychological factors, interference with vision, and the intense foreign body sensation (Ludwig, 2005). Ophthalmic inserts can be classified into three groups insoluble, soluble and bioerodible ophthalmic inserts (Gurtler and Gurny, 1995).

In insoluble ophthalmic inserts, the polymeric support used is not soluble and the device has to be removed after the specified period of use. This is the major disadvantage of these systems. The insoluble ophthalmic inserts can be sub classified into diffusional systems, osmotic systems and the hydrophilic contact lenses (Gurtler and Gurny, 1995).

Soluble and biodegradable ophthalmic inserts offer a great advantage of being entirely soluble so that they do not need to be removed from their site of application, thus limiting the interventions to insertion only. The polymers used in these devices can be natural, semisynthetic or synthetic. Polymers include collagen, collagen derivatives, cross-linked collagen, chitin, chitosan, chitosan derivatives, pectin, sodium alginate, polyacrylic acids, HPMC, hydroxypropylcellulose (HPC), CMC, PVA, polyvinylpyrrolidine (PVP), ethyl-vinyl acetate copolymers, hydroxy alkyl acrylate derivatives, methyl hydroxyethyl cellulose, hydroxy propyl sucrose, Eudragit, poly(ethylene oxides), polyester derivatives, poly(orthoesters), polycarbonate derivatives, polyorthocarbonates, poly(carboxylic acid)derivatives, cross linked gelatin derivatives (Gelfoam) etc. (Gurtler and Gurny, 1995). Recent advances in these formulations are compiled in Table 1.3.

Table 1.3: Insert systems developed and studied in recent past.

Drug/Marker	Polymer(s)	Characters	In vitro / In vivo Model	Inference	Reference
Gentamicin sulphate	HPC, ethylcellulose (EC), CP 974P and CAP	Bioadhesive ophthalmic drug inserts (BODI) - extruded formulations (length 5 mm, diameter 2 mm, weight 20.5 mg, drug content 5 mg) with controlled drug release	Rabbit and Dog	These formulations were well tolerated by rabbits. Gentamicin concentrations were maintained for 72 hr above effective levels where as eye drops achieved concentration for 15 min.	(Gurtler et al., 1995a)
Gentamicin sulphate	HPC, EC, CP 974P and CAP	BODI - extruded formulations (length 5 mm, diameter 2 mm, weight 20.5 mg, drug content 5 mg) with controlled drug release	Rabbit and Dog	The AUC_{eff} (the efficacy area under the curve) and the t_{eff} (the efficacy time) were always much higher in the case of BODIs than for the eye drop solution both in dogs and rabbits.	(Gurtler et al., 1995b)
Oxytetracycline HCl	Silicone elastomer (polyacrylic acid or polymethacrylic acid were grafted on polydimethylsiloxane)	Cylindrical devices (diameter 0.9 mm, length 6-12 mm, weight 3-8 mg, drug content 0.8 mg) mucoadhesive controlled release system	Rabbit	Ocular retention of grafted devices was significantly higher compared to un-grafted inserts. Drug tear levels of 20-30 $\mu g.ml^{-1}$ were measured for several days for grafted devices. This concentration is 10-30 folds above the MIC_{90} values for common ocular pathogens.	(Chetoni et al., 1998)
Gentamicin sulphate	HPC, HPMC, EC, CP 974P and CAP	BODI - extruded formulations (length 5 mm, diameter 2 mm, weight 20.5 mg, drug content 5 mg) with controlled drug release	Rabbit and Dog	Drug co-precipitated and solid dispersed with EC and CAP reduced the solubility and prolonged t_{eff}.	(Baeyens et al., 1998a)
Dexamethasone and Gentamicin sulphate	HPC, HPMC, EC, CP 974P and CAP	BODI - extruded formulations (length 5 mm, diameter 2 mm, weight 20.5 mg, drug content 5 mg) with controlled drug release	Rabbit and Dog	Both the drug levels were above effective for 10 hr later only gentamicin levels were there for 50 hr due to co-precipitation with CAP.	(Baeyens et al., 1998b)
Indomethacin	Water-soluble cellulose derivatives and polyvinyl alcohol	Inserts by film casting and compression moulding. Diffusion controlled drug release	In vitro	Compression moulding showed higher release rates.	(Karatas and Baykara, 2000)

(Contd..)

Table 1.3

Drug/Marker	Polymer(s)	Characters	In vitro / In vivo Model	Inference	Reference
Ofloxacin	High molecular weight (400 kDa) linear poly(ethylene oxide) (PEO) mixed with Eudragit L100 (Eu L100) neutralized at 17 % or 71 % with NaOH	Gel-forming erodible inserts (Diameter 6 mm, Weight 20 mg, Drug 0.3 mg). Eudragit is to modulate the swelling and erosion rate of the insert	Rabbit	Instantaneously adhere to the mucosa, and gradually formed well-tolerated mucoadhesive gels which spread. As compared to PEO alone combination formulation has enhanced the AUC_{eff} by 132 folds and C_{max} by 62 folds.	(Di Colo et al., 2001a)
Ofloxacin	Different grades of PEO	Swellable and bioadhesive polymer with controlled drug release	Rabbit	Formulations have enhanced the AUC_{eff} by 11 folds and C_{max} by 4 folds with respect to the eye drops.	(Di Colo et al., 2001b)
Ofloxacin	Different grades of PEO	Bioadhesive controlled release system	Rabbit	Increase in $AUC_{aqueous\ humor}$ was 12.5 folds.	(Di Colo et al., 2001b)
Tilisolol and Prodrugs	Poly (2-hydroxy-propyl methacrylate) (HPM) and O-butyryl ester prodrug of tilisolol (BUTL) and the O-palmitoyl ester prodrug of tilisolol (PalTL)	Hydrogels insert films of lipophilic prodrugs	Rabbit	$AUC_{aqueous\ humor}$ / AUC_{plasma} of BUTL inserts has enhanced by 3 fold compared to TL inserts.	(Kawakami et al., 2001)
Gentamicin sulphate	HPC, EC, CP 974P and CAP	BODI - extruded formulations (length 5 mm, diameter 2 mm, weight 20.5 mg, drug content 5 mg) with controlled drug release	Dog	With single instillation of insert conjunctivitis and keratoconjunctivitis sicca could be cured.	(Baeyens et al., 2002)
Prednisolone (PDS), oxytetracycline hydrochloride (OTH) and gentamicin sulphate (GTS)	Different grades of PEO	Swellable, bioadhesive and controlled release gel-forming erodible inserts	In vitro	PDS and OTH were released by erosion mechanism and GTS by diffusion mechanism.	(Di Colo and Zambito, 2002)

(Contd..)

Table 1.3

Drug/Marker	Polymer(s)	Characters	In vitro / In vivo Model	Inference	Reference
Ofloxacin (OFX) and sodium fluorescein	CH-HCl for microspheres were used to prepared inserts with different grades of PEO	Swellable, bioadhesive and controlled release gel-forming erodible inserts	Rabbit	AUC_{eff} (AUC in the aqueous humour for concentrations >MIC_{90}) was 12.5 folds more than marketed preparation.	(Di Colo et al., 2002)
Fluorescein	Lyophilised HPMC on polytetrafluoroethylene (PTFE)	No preservatives, higher and long term stability, no pH adjustment, and easy handling	Human	Bioavailability of formulation increased to that of solutions.	(Dinslage et al., 2002)
Sodium fluorescein	Drum dried waxy maize starch and CP 974PR	Bioerodible bioadhesive minitablet (diameter 2 mm, weight 6 mg) controlled release systems	Human	High fluorescein levels in tears were observed for several hours. With increase in compression force the C_{max} has not effected but T_{max} increased.	(Weyenberg et al., 2003)
Ciprofloxacin HCl	Sodium alginate films with Eudragit RL 100, RS 100 and/or polyvinyl acetate as rate controlling membrane	Controlled drug release films	Rabbit	Aqueous humor drug concentration was maintained above MIC for 4 to 4.5 days.	(Charoo et al., 2003)
Tilisolol	Polypropylene tape on the one side of the polymer disc of poly(2-hydroxypropyl methacrylate)	Swellable polymer, with controlled drug release	Rabbit	Uncoated side facing bulbar conjunctiva/sclera increased AUC in sclera 6.5 fold and decreased AUC in conjunctival 3.4 fold compared to uncoated side facing palpebral conjunctiva.	(Sasaki et al., 2003)
Fluorescein and two diclofenac salts	Poly(acrylic acid) (PAA) and thiolated PAA (PAA–cysteine conjugated)	Swellable and bioadhesive polymer with controlled drug release	Human	AUC values for both inserts were comparable, but the C_{8h} and t_{max} value were increased for the thiolated PAA formulations. Tear fluorescein was observed till 8 hr.	(Hornof et al., 2003)
Fluorescein	Lyophilised HPMC on PTFE	No preservatives, higher and long term stability, no pH adjustment, and easy handling	Human	Three doses were well tolerated. Ocular bioavailability was improved in the cornea and anterior chamber for up to 7 hr.	(Lux et al., 2003)

(Contd..)

Table 1.3

Drug/Marker	Polymer(s)	Characters	In vitro / In vivo Model	Inference	Reference
Fluorescein	Lyophilised HPMC on PTFE	No preservatives, higher and long term stability, no pH adjustment, and easy handling	Human	Mean anterior chamber concentrations were up to 16 times higher with the lyophilisate.	(Steinfeld et al., 2004)
Ciprofloxacin	Drum dried waxy maize starch and CP 974PR	Bioerodible bioadhesive minitablet (diameter 2 mm, weight 6 mg) controlled release systems	Human	Drug levels in tear film remained above its MIC for at least 8 hr. Gamma-irradiated sterilization suited minitablets.	(Weyenberg et al., 2004a)
Ciprofloxacin	Drum-dried waxy maize starch (containing 99.9 % amylopectin) with CP 974P and sodium stearyl fumarate	Ocular gelling minitablets with prolonged release properties	Human	Non irritating to mucosa. Mean tear concentration was 135.2 $\mu g.g^{-1}$ at 300 min after application of the minitablet and the drug level was extended upto 8 hr.	(Weyenberg et al., 2004b)
Atropine and fluorescent dye	Metallic coil coated with polymer and drug depot	Controlled drug release	Human	Convenience of dosage form was checked.	(Pijls et al., 2004)
-	Calcium alginates with a different hydroxyethylcellulose (HEC)	Bioadhesive and control release	Human	Devices were well tolerated except for initial hyperemia and feeling of foreign body in eye.	(Fuchs-Koelwel et al., 2004)
Fluorescein, Pilocarpine and Tropicamide	Ophthalmic lyophilisate carrier system (OLCS) lyophilised HPMC/sodium hyaluronate/mannitol on PTFE	No preservatives, higher and long term stability, no pH adjustment, and easy handling	Human	Reduction in IOP was significantly increased in case of OLCS formulations with both the drugs.	(Suverkrup et al., 2004)
Bupivacaine	HA and HA-Na	Bioadhesive control release	Human	Local anaesthesia was achieved for 20 min.	(Mahe et al., 2005)
Sodium fluorescein	Lyophilised HPMC on PTFE	No preservatives, higher and long term stability, no pH adjustment, and easy handling	Human	Lyophilisate formulations showed increase in corneal content as compared to eye drops.	(Lux et al., 2005)
Corticosteroid	Microinfusion pump	Controlled drug pumping	Rabbit	Eyes treated with corticosteroid delivered by pump recovered faster than those treated with topical corticosteroid.	(Kwon et al., 2005)
Pefloxacin mesylate	Polyvinyl acetate K-30 coated with Eudragit RL 100 or RS 100 as rate controlling membrane (dibutyl phthalate as a plasticizer)	Soluble and controlled release inserts	Rabbit	The in vivo (precorneal area) drug release was extended up to 5 days.	(Sultana et al., 2005)

(Contd..)

Table 1.3

Drug/Marker	Polymer(s)	Characters	In vitro / In vivo Model	Inference	Reference
Sodium fluorescein	Drum dried waxy maize® starch, Amioca® starch and CP 974P	Bioerodible bioadhesive minitablet (diameter 2 mm, weight 6 mg) controlled release systems	Human	Fluorescein was released for 12 hr in lower fornix without any mucosal irritation.	(Weyenberg et al., 2006)
Chloramphenicol, atropine, norfloxacin, or pilocarpine	Acrylic acid-functionalized chitosan reacted with either N-isopropylacrylamide or 2-hydroxyethyl methacrylate monomers,	Hybrid polymeric hydrogels with bioadhesive properties	Rabbit	Chitosan-based polymer containing 2-hydroxyethyl methacrylate was best.	(Verestiuc et al., 2006)
Dexamethasone and Tobramycin	*N*-trimethylchitosan (TMC) for microspheres were used to prepared inserts with different grades of PEO	Erodible inserts (weight, 20 mg; diameter, 6 mm; drug dose, 0.3 mg)	Rabbit	$AUC_{0\text{-}240\ min}$ was 36 folds more than marketed preparation.	(Zambito et al., 2006)
Ciprofloxacin HCl	Gelatin films (glycerine as a plasticizer) coated rate controlling ethyl cellulose membrane (dibutyl phthalate as a plasticizer)	Soluble and controlled release inserts	Rabbit	In vivo (precorneal) drug release was extended upto 12 hr.	(Mundada and Shrikhande, 2006)
Ketotifen fumarate	Soft contact lens was prepared by co-polymerization of monomers (acrylic acid, acrylamide, 2-hydroxyethylmethacrylate (HEMA), N-vinyl 2-pyrrolidinone, Polyethylene glycol (200) dimethacrylate)	Drug reservoir within contact lenses for controlled drug release	In vitro	High drug loading was achieved using formulation containing poly(AA-co-AM-co-NVP-co-HEMA-co-PEG200DMA) copolymer.	(Venkatesh et al., 2007)

1.4.4. Ophthalmic sprays

Ophthalmic sprays were also reported to improve the bioavailability of drugs. Delivery of pilocarpine (4 % solution) via a spray (single application) to closed eyelids was found to be an effective delivery method for intraocular miosis (Doe and Campagna, 1998). In four patients out of total 14 patients group, vitamin B_{12} of 100 nM concentration was detected in aqueous humor, when nebulizer was used. Vitamin B_{12} was not detected in any of the patients in control group (Kahn, 2005).

1.5. Chemical approaches to improve ocular therapy

1.5.1. Permeation enhancers

Corneal permeability can be facilitated by increasing the permeability of the corneal epithelial membrane (paracellular pathway), which is limited by high transepithelial electrical resistance (TEER) i.e., 12-16 $k\Omega.cm^2$ (Marshall and Klyce, 1983). Penetrability of the cornea can be increased by the following methods, (a) adjustment of the actual acidity in such a way that pH of the preparation makes it possible to produce the optimal portion of non-ionized particles; (b) incorporation of absorption enhancers into the composition of the preparation, and (c) production of prodrugs or ionic pairs (Masteikova et al., 2004). Permeation enhancers or absorption promoters like amphiphilic substances and chelating agents will modify the integrity of the corneal epithelium transiently, thereby increases the drug corneal permeability. At low concentrations, the amphiphilic substances will partition into cell membrane and causes polar defects in lipid bilayer. This disruption and fluidization of the ordered lipid layer results in an increased penetration of drugs. At high concentrations in cell membrane they will extract phospholipids and proteins, thereby increases the membrane permeability (transcellular permeability) to drugs and ions. Calcium ions are important for the regulation of tight junctions, Ca^{+2} ions complexes with chelating agents and dilate the intercellular spaces. Chelating agents will loosen the tight junctions of the corneal epithelium and enhances the paracellular penetrations of drugs (Sasaki et al., 1999).

Surfactants (e.g. benzalkonium chloride (BKC), chlorhexidine, bile salts - sodium dodecyl sulfate, dioctyl sodium sulfosuccinate, Tween 20, Tween 40, Tween 81, Triton X-100, Spans 20, Spans 40, Spans 81, Aptet 100, G 1045, Brij-35, Brij-58, Brij-78, Brij-98, Myrj-52, Myrj-53), bile acids (e.g. sodium cholate, sodium taurocholate, sodium glycodeoxycholate, sodium taurodeoxycholate, taurocholic acid, taurourso-deoxycholic acid, deoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid), fatty acid (e.g. capric acid, sorbic acid), ionophore (e.g. lasalocid), preservatives (e.g. BKC, chlorhexidine digluconate, benzyl alcohol, chlorobutanol, 2-phenylethanol, parabens, propyl parabens), chelating agent

(e.g. ethylenediaminetetraacetic acid - EDTA) and others (e.g. azone, hexamethylene lauramide, hexamethylene octanamide, decylmethyl sulfoxide, saponin, cyclodextrin, cyclodextrin derivatives) were used as a corneal permeation enhancers (Sasaki et al., 1999; Kaur and Kanwar, 2002; Kaur and Smitha, 2002).

BKC and EDTA enhanced the permeation of ketorolac tromethamine (Malhotra and Majumdar, 2002), gatifloxacin (Rathore and Majumdar, 2006) and moxifloxacin (Pawar and Majumdar, 2006) in excised rabbit, goat and buffalo cornea. In the presence of Transcutol P (0.005-0.03 %), the maximum increase in the permeability coefficient (in rabbit cornea) was 1.5, 1.5, 3.0 and 3.3 fold for ribavirin, gatifloxacin, levofloxacin hydrochloride and enoxacin, respectively (Liu et al., 2006a).

1.5.2. Prodrugs and ion pairing agents

Prodrugs are defined as pharmacologically inactive derivatives of drug molecules that are chemically or enzymatically converted to the active parent drugs. These are prepared to enhance the partitioning and corneal bioavailability of topically applied drugs. Drugs were chemically modified to make them more selective, site-specific and safe. The lipophilicity and hence the corneal penetration of a drug can also be increased through ion-pair formation. Ion-pair association is between large organic ions of opposite charge. The ions are transferred better when associated rather than individually. These are called chemical delivery systems (CDS) (Sasaki et al., 1996; Sasaki et al., 1999; Kaur and Kanwar, 2002).

Cyclosporin attached with dipeptide sarcosine-serine (or lysine)-(acyloxy)alkyl-oxy-carbonyl, carrying a phosphate group or an ammonium group were synthesized. Both prodrugs enhanced the solubility and readily converted into drug in both rabbit and human tears (Lallemand et al., 2005a; Lallemand et al., 2005b).

In recent past, Mitra and group, has synthesized large number of amino acid, amino acid derivative and lipophilic (acyl ester) prodrugs of acyclovir and ganciclovir. They extensively studied the in vitro stability of prodrugs, solubility enhancement by prodrugs, prodrug conversion to drug, corneal permeability of prodrugs, the metabolism of prodrugs at precorneal site and in corneal membrane, the mechanism of drug/prodrug permeation (carrier mediated transport systems), drug efflux mechanisms (P-gp mediated efflux, MDR efflux pumps, transmembrane phosphoglycoprotein efflux pumps) and identified different inhibits for enzymes responsible for precorneal prodrug conversion. These studies were conducted on reconstructed human and rabbit cell lines, isolated rabbit and human cornea and in vivo in rabbits (Anand and Mitra, 2002; Tirucherai et al., 2002; Majumdar et al., 2003; Tirucherai and Mitra, 2003; Majumdar et al., 2005; Anand et al., 2006; Karla et al., 2006; Katragadda et

al., 2006a, 2006b).

Sorbic acid was used as ion pairing agent for timolol maleate and corneal permeability was studied in rabbits. More than 2 fold increase in $AUC_{aqueous\ humor}$ was observed for ion-paired timolol (Higashiyama et al., 2004).

1.5.3. Cyclodextrins

Cyclodextrins act as true carriers by keeping the hydrophobic drug molecules in solution and delivering them to the surface of the ocular membrane, where the relatively lipophilic membrane has a much lower affinity for the hydrophilic cyclodextrin molecules and therefore they remain in the aqueous vehicle system or aqueous tear fluid (Kaur and Kanwar, 2002). Hydrophilic cyclodextrins are non-toxic upon topical application, as shown by various animal species and humans (Fromming and Szejtli, 1994). Solubility of tropicamide was enhanced by HP-β-CD, there by mydriatic activity studies showed an improved bioavailability in rabbits (Cappello et al., 2001). Using HP-β-CD in formulation, stability, biological activity and tolerance of ciprofloxacin hydrochloride was improved in rabbits (Nijhawan and Agarwal, 2003). Solubility and corneal permeability of zinc diethyldithiocarbamate (Wang et al., 2004b) and disulfiram (Wang et al., 2004a) have been enhanced using HP-β-CD and thereby enhanced the ocular bioavailability in rabbit aqueous humor was enhanced and prevents the development of cataracts in rats. Latanoprost solutions with and without cyclodextrin have shown same efficacy in humans, but, the stability of drug has enhanced enormously for cyclodextrin formulation (Gonzalez et al., 2007).

1.6. Physical approaches to improve ocular therapy

This approach can be accomplished by exposing the eye to iontophoresis and phonophoresis. One major advantage of this approach is that the flux of penetrant can be precisely controlled by varying the applied physical power, thus the drug therapy can be more accurately tailored for the patient.

Iontophoresis, a noninvasive technique for introducing ionic drugs into tissues by means of an electric current. In the field of ophthalmology, there are two types of iontophoresis, transcorneal and transscleral. Transcorneal iontophoresis can deliver and sustain high concentrations of drugs into the anterior segment of the eye, particularly into the cornea and the aqueous humor. Several drugs like gentamicin, tobramycin, vancomycin, vidarabine monophosphate, acetylsalicylic acid, ketoconazole etc. were delivered using transcorneal iontophoresis in rabbits (Sasaki et al., 1999; Kralinger et al., 2003; Mullenax et al., 2003).

Phonophoresis, a technique for enhancing drug permeability by means of ultrasound. The in vitro permeability of rabbit cornea increased by 2.6 times for atenolol, 2.8 for carteolol, 1.9 for timolol and 4.4 times for betaxolol, after 60 min of ultrasound (20-kHz) exposure. Ultrasound application appeared to produce epithelial disorganization and structural changes in the corneal stroma (Zderic et al., 2002). In vitro corneal permeability studies of few beta-blockers and fluorescein were studied after exposure to ultrasound frequency of 880 kHz and intensities of 0.19-0.56 $W.cm^{-2}$ with exposure duration of 5 min. Corneal permeability increased with increase in intensity. The surface cells of corneal epithelium exposed to ultrasound appeared swollen and showed indications of membrane rupture (Zderic et al., 2004).

1.7. Ideal ophthalmic drug delivery

Conventional eye drops suffer from several drawbacks due to physiological conditions in the eye and other barriers. Blinking, baseline and reflex lachrymation, and nasolachrymal drainage remove foreign substances rapidly, including drugs, from the surface of the eye. So, eye drops suffer quick elimination and low bioavailability (1-5 %) and required to be administered frequently to maintain the drug levels for prolonged periods, often as high as 8 times a day. But the frequent use of highly concentrated solutions may induce toxic systemic side effects and cellular damage at the ocular surface. Novel ophthalmic formulation would enhance bioavailability by reducing the drug precorneal wastage, prolonging the residence time and sustaining the drug release, with less patient inconvenience. Consequently, delivery systems that prolong the residence time of the applied dose in the conjunctival sac would be expected to reduce systemic drug absorption. This project has targeted to design novel ocular delivery systems for gatifloxacin, a broad spectrum antibacterial agent.

1.8. Objective of the work

Gatifloxacin is a broad spectrum antibiotic used for treatment of number of ophthalmic complications. Several topical gatifloxacin eye drop formulations, 0.3 % w/v, are in the market. As discussed in previous sections, they have few disadvantages.

Therefore present research aims at design and development of novel ophthalmic drug delivery systems, to improve ocular therapy of gatifloxacin by increasing residence time of the formulation and by controlling/sustaining/modifying the drug release from the dosage forms.

Objectives of this work were to

- Perform preformulation studies of gatifloxacin
- Prepare and optimize gatifloxacin loaded soluble and bioadhesive ophthalmic solid insert systems using biocompatible polymers alone and in combination and study the physical parameters and in vitro drug release of the formulation
- Prepare and optimize gatifloxacin loaded bioadhesive and in situ gel forming systems using biocompatible polymers alone and in combination and study the physical parameters and in vitro drug release of the formulation
- Study the stability of selected formulations
- Study microbiological activity of selected formulations
- Perform in vitro corneal permeability studies in excised goat cornea
- Perform in vivo safety and toxicity studies in rabbit model
- Carryout pharmacokinetic studies on rabbits upon topical administering selected gatifloxacin formulations and determining drug levels in aqueous humor and blood

The proposed work required analysis and estimation of drug at different levels of sensitivity at various stages. As formulation design and optimization essentially needs a suitable and sensitive analytical method (s), it was also planned to develop and validate analytical methods for various studies. Thus for preformulation, formulation characterization and in vivo samples following analytical methods were planned to be developed.

- UV Spectrophotometric method
- Spectrofluorimetric method
- High performance liquid chromatographic (HPLC) method
- HPLC bioanalytical method

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Chapter 2

Drug Profile - Gatifloxacin

2.1 Gatifloxacin

Gatifloxacin is a fourth generation synthetic broad-spectrum 8-methoxy fluoroquinolone antibacterial agent. For the first time, Bristol-Myers Squibb introduced Gatifloxacin tablets in 1999 under the proprietary name Tequin® (200 and 400 mg) for the treatment of respiratory tract infections, having licensed the drug from Kyorin Pharmaceutical Company of Japan. In 2003, Zymar® eye-drop formulation (Gatifloxacin - 0.3 % w/v) was introduced by Allergan in US market and this product was approved by FDA for the treatment of bacterial conjunctivitis (Keam et al., 2005).

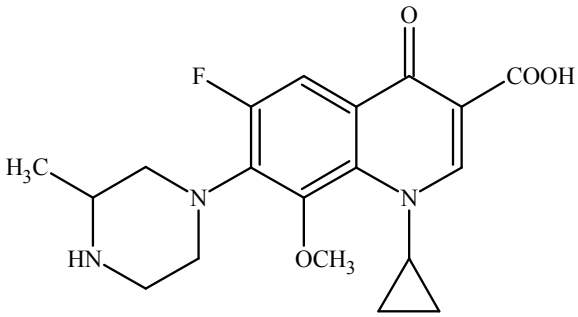

Figure 2.1: Structure of Gatifloxacin.

2.2. Physicochemical properties

Chemically, gatifloxacin is (±)-1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid. Structure is given in Figure 2.1. Its empirical formula is $C_{19}H_{22}FN_3O_4$ and its molecular weight is 375.39. Gatifloxacin is a crystalline powder and is white to pale yellow in color. It exists as a racemate, with no net optical rotation. The solubility of the compound is pH dependent. The maximum aqueous solubility (40-60 $mg.ml^{-1}$) occurs at a pH range of 2 to 5. The reported dissociation constants (pK_{a1} and pK_{a2}) were 5.94 and 9.21, respectively (Bristol Myers Squibb Company, 1999; Allergan Inc, 2003; Physician desk reference, 2003; Rx List, 2004).

2.3. Pharmacology

2.3.1. Therapeutic uses

Gatifloxacin was found to be active against ophthalmic infections such as bacterial conjunctivitis, bacterial blepharitis, bacterial keratitis, corneal keratitis and ulcers, vitreo-retinal diseases (endophthalmitis) and postoperative endophthalmitis (Allergan Inc, 2005; Jensen et al., 2005). Gatifloxacin was also found to be effective against the community-acquired pneumonia, acute exacerbations of chronic bronchitis, acute sinusitis, genitourinary

tract infections, urinary tract infections, sexually transmitted diseases and uncomplicated skin and skin structure infections (Physician desk reference, 2003).

2.3.2. Dosage and administration

Gatifloxacin is orally administered in the form of tablets with dose of 200 to 400 mg per day. The drug is also available in the form of small volume parentrals for intravenous infusion and it is commercially packed in single-use vials with a concentration of 10 $mg.ml^{-1}$ (Bristol Myers Squibb Company, 1999; Physician desk reference, 2003; Rx List, 2004).

Gatifloxacin ophthalmic solution 0.3 % w/v administered topically single 2 drop dose, then 2 drops 4 times daily for 7 days and finally 2 drops 8 times daily for 3 days. Gatifloxacin 0.3 % w/v ophthalmic solution administered at a dosing regimen of 1 drop 4 times daily has been used safely for the treatment of patients with bacterial conjunctivitis, for which it is US FDA approved currently (Jensen et al., 2005). The recommended dosage regimen for the treatment of bacterial conjunctivitis is days 1 and 2 – one drop every two hours in the affected eye(s) while awake, up to 8 times daily; days 3 through 7 – instill one drop up to four times daily while awake (Allergan Inc, 2003, 2005).

2.3.3. Mechanism of action and resistance

Fluoroquinolone class of antimicrobial compounds binds to DNA gyrase or topoisomerase IV inside bacterial cells (Bauernfeind, 1997). Like other new fluoroquinolones, gatifloxacin has a dual mechanism of action, inhibiting both bacterial DNA gyrase [a type II topoisomerase, that is involved in the replication, transcription and repair of bacterial DNA] and topoisomerase IV [a heterotetramer, known to play key role in the partitioning of the chromosomal DNA during cell division] (Zhanel et al., 2002). The primary target depends on type of bacteria. DNA gyrase is considered to be the primary site of fluoroquinolone action in many bacteria; however, resistance studies suggest that topoisomerase IV is the primary target in gram-positive bacteria such as *Streptococcus pneumoniae* and *Staphylococcus aureus* (Takei et al., 1998; Zhanel et al., 2002).

In vitro studies showed that gatifloxacin was more potent among the other quinolones tested in inhibiting DNA gyrase from *Escherichia coli* and topoisomerase IV from *S. aureus* [concentration required for 50 % inhibition (IC_{50}) values of 0.1 and 13.8 $mg.l^{-1}$, respectively] (Takei et al., 1998). Gatifloxacin selectivity ([IC_{50} for mammalian topoisomerase II]/[IC_{50} for bacterial topoisomerase II]) was 19 for *S. aureus* topoisomerase IV and >2400 for *E. coli* DNA gyrase, higher than that of all other quinolones tested.

Resistance to fluoroquinolones is being seen more frequently and corresponds with their increasing use (Goldstein et al., 1999). The 4 main mechanisms of quinolone resistance are point mutations in gyrA and gyrB (codes for A & B subunits of DNA gyrase), point mutation in the grlA gene (code for A subunit of topoisomerase IV), membrane-associated efflux proteins (coded by gene norA), and a locus which confers quinolone resistance in *Staphylococcus aureus* (cfxB-ofxC or flqA) (Kaatz et al., 1993; Goldstein et al., 1999; Hooper, 1999; Wang et al., 2003). Resistance to gatifloxacin in vitro develops via multiple step mutations and occurs at a general frequency of between 1 x 10^{-7} to 10^{-10}. Fourth-generation fluoroquinolones require two mutations to establish resistance, one in the topoisomerase IV and a second one in the DNA gyrase gene; it was also predicted that these fluoroquinolones would provide better coverage against gram-positive bacteria that were already resistant to current fluoroquinolones which required only a single mutation to establish resistance (Courvalin and Depardieu, 2000; Drlica, 2001; Ince and Hooper, 2001; Hooper, 2002). Mino de Kaspar et al. (2005) as studied the resistance developed by different antibacterials for normal and multiresistant ocular surface flora. This study demonstrated that the order of the frequency of resistance of normal ocular surface flora as follows: gatifloxacin = moxifloxacin < gentamicin = tobramycin = levofloxacin = neomycin < ciprofloxacin = ofloxacin < erythromycin. The resistance rate among the multiresistant bacteria as follows: gatifloxacin = moxifloxacin < neomycin = gentamicin = tobramycin = levofloxacin < ciprofloxacin = ofloxacin = erythromycin. The mechanism of action of fluoroquinolones including gatifloxacin is different from that of aminoglycoside, macrolide, and tetracycline antibiotics. Therefore, gatifloxacin may be active against pathogens that are resistant to these antibiotics. There is no cross-resistance between gatifloxacin and the aforementioned classes of antibiotics. Cross resistance has been observed between systemic gatifloxacin and some other fluoroquinolones (Bristol Myers Squibb Company, 1999; Allergan Inc, 2003; Physician desk reference, 2003; Rx List, 2004).

2.3.4. Spectrum of anti-bacterial activity

Gatifloxacin exhibits in vitro MIC_{90} of 2 $\mu g.ml^{-1}$ or less (systemic susceptible breakpoint) against most (≥ 90 %) strains of the ocular pathogens, like aerobic gram-positive bacteria (*Listeria monocytogenes*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus viridans* Group, *Streptococcus* Groups C, F, G), aerobic gram-negative bacteria (*Acinetobacter lwoffii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherichia coli*, *Citrobacter freundii*, *Citrobacter koseri*, *Haemophilus parainfluenzae*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*,

Morganella morganii, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Proteus mirabilis*, *Proteus vulgaris*, *Serratia marcescens*, *Vibrio cholerae*, *Yersinia enterocolitica*), anaerobic microorganisms (*Bacteroides fragilis*, *Clostridium perfringens*) and other microorganisms (*Chlamydia pneumoniae*, *Legionella pneumophila*, *Mycobacterium marinum*, *Mycobacterium fortuitum*, *Mycoplasma pneumoniae*) (Bristol Myers Squibb Company, 1999; Allergan Inc, 2003; Physician desk reference, 2003; Rx List, 2004).

The fourth-generation fluoroquinolones (gatifloxacin and moxifloxacin) were more potent (lower minimum inhibitory concentrations) than the second- and third-generation fluoroquinolones for gram-positive bacteria but comparable for gram-negative bacteria. Among moxifloxacin and gatifloxacin, moxifloxacin has shown less MIC_{90} in gram-positive bacteria, but in some of gram negative bacteria gatifloxacin has shown lesser MIC_{90} (Mather et al., 2002; Kowalski et al., 2003). From a microbiological point of view, a low minimum inhibitory concentration to an antibiotic would offer two theoretical advantages, (1) more effective killing of a bacterium as lethal tissue levels exceed the minimum inhibitory concentration and (2) reduced selection for resistance because sublethal dosing is less likely.

Kaliamurthy et al. (2005) have reported 369 isolates of gram-positive and 129 strains of gram-negative bacteria in ocular specimens. Ninety-nine percent of gram-positive and 92 % of gram-negative bacterial strains was found to be susceptible in vitro to gatifloxacin, whereas lower percentages of gram-positive and gram-negative bacteria were found to be susceptible to other antibiotics (ofloxacin, ciprofloxacin, norfloxacin, gentamicin, tobramycin, neomycin, chloramphenicol, erythromycin, tetracycline and amikacin). MICs were ranging from 0.03 to 2 $\mu g.ml^{-1}$ for moxifloxacin and gatifloxacin (Schlech and Alfonso, 2005). MICs were ranging from 0.9 to 2.7 $\mu g.ml^{-1}$ with percentage susceptibility in the rage of 88 to 100 %. Gatifloxacin was proved to be active against *Candida* species, which causes fungal keratitis (Ozdek et al., 2006).

Based on the clinical data, Allergan claimed that the Zymar® solution is indicated for the treatment of bacterial conjunctivitis caused by susceptible strains of aerobes, gram-positive organism (*Corynebacterium propinquum*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus mitis*, *Streptococcus pneumoniae*) and aerobes, gram-negative organisms (*Haemophilus influenzae*) (Bristol Myers Squibb Company, 1999; Allergan Inc, 2003; Physician desk reference, 2003; Rx List, 2004).

Gatifloxacin was found to be most effective in the treatment of keratitis caused by *Mycobacterium chelonae* (Sandoval et al., 2004; Sarayba et al., 2005), *Pseudomons aeruginosa* (Jensen et al., 2005), multi drug-resistant *Staphylococcus aureus* (Costello et al., 2006) and *Streptococcus pneumoniae* (Donnenfeld et al., 2003; Donnenfeld et al., 2006) in

rabbit model. An aggressive treatment regimen with Zymar® (1 drop in both eyes every 15 minutes for 5 hr - 21 doses) was successfully used for the treatment of infections caused by moderately and highly gatifloxacin resistant *Staphylococcus aureus* in the rabbit keratitis model (Romanowski et al., 2005). Topical gatifloxacin solution has reduced the inflammation, infection and culture positive endophthalmitis induced by *Staphylococcus aureus* in rabbits (Fernandez et al., 2006). Gatifloxacin showed significantly better action against gram-positive *cocci* both in vitro and in vivo when compared with ciprofloxacin. Clinical studies on patients with bacterial keratitis, revealed that the significantly higher proportion of ulcers in the gatifloxacin group exhibited complete healing compared with those in the ciprofloxacin group (39 eyes [95.1 %] versus 38 [80.9 %]) and the ulcers caused by these pathogens were healed significantly in the gatifloxacin group. Mean time taken for healing of ulcer and the efficacy against gram-negative bacteria did not significantly differ between the two groups (Parmar et al., 2006).

2.3.5. Pharmacokinetic properties

The pharmacokinetic profiles of gatifloxacin, given by oral and intravenous route, are summarized in Table 2.1. Gatifloxacin is well absorbed after oral administration. Its absolute bioavailability is approximately 96 % and is apparently not dependant on the dose.

Table 2.1: Gatifloxacin pharmacokinetics (mean ± SD) in healthy volunteers after multiple-dose oral and intravenous administration (Nakashima et al., 1995; Fish and North, 2001).

Pharmacokinetic Parameters	**Oral Gatifloxacin 400 mg** †	**Intravenous Gatifloxacin** ‡ **400 mg**
No. of subjects	18	5
C_{max} ($mg.l^{-1}$)	4.2 ± 1.3	5.5 ± 1.0
C_{min} ($mg.l^{-1}$)	0.4	-
T_{max} (hr)	1.0 (0.5 to 4.0)	1.0 (0.5 to 1.0)
Half-life (hr)	7.1 ± 0.6	7.4 ± 1.6
V_d/F ($l.kg^{-1}$)	1.7 ± 0.2	1.5 ± 0.2
$AUC_{0-\infty}$ ($mg.hr.l^{-1}$)	34.4 ± 5.7	35.1 ± 6.7
Cl/F ($l.hr^{-1}$)	11.9 ± 1.9	9.5 ± 2.0
Cl_R ($l.hr^{-1}$)	9.1 ± 2.8	9.7 ± 2.6
f_e (%)	80 ± 12	84 ± 14

C_{max} = maximum plasma drug concentration
C_{min} = minimum plasma drug concentration
T_{max} = time to reach C_{max}
V_d/F = volume of distribution at steady state
$AUC_{0-\infty}$ = area under the plasma concentration-time curve from time zero to infinity
Cl/F = total systemic drug clearance
Cl_R = total renal clearance
f_e = fraction of each dose excreted unchanged in the urine
† Steady state pharmacokinetic parameters
‡ 10 $mg.ml^{-1}$ of drug administered as a infusion for 60 min

On ocular application, gatifloxacin achieved a C_{max} of 4.5 $\mu g.ml^{-1}$ (T_{max} = 0.5 hr) in rabbit cornea upon single dose and 7.8 $\mu g.ml^{-1}$ (T_{max} = 0.5 hr; AUC_{0-24} - 28.7 $\mu g.hr.ml^{-1}$) upon multiple doses (q.i.d. for 3 days). C_{max} of gatifloxacin achieved in aqueous humor was 0.27

$\mu g.ml^{-1}$ (T_{max} = 1 hr) upon single dose and 0.54 $\mu g.ml^{-1}$ (T_{max} = 0.5 hr; AUC_{0-24} - 1.05 $\mu g.hr.ml^{-1}$) upon multiple doses (q.i.d. for 3 days). Gatifloxacin concentrations at cornea and anterior chamber were found to be at levels higher than MIC_{90} reported for the major ocular pathogens (Batoosingh et al., 2003). Levine et al. (2004) have found that when gatifloxacin applied topically in rabbits following two protocols, i.e., keratitis protocol (every 15 min for 4 hr, aqueous sample taken 10 min after last sample) and cataract prophylaxis protocol (q.i.d. for 10 days, aqueous sample taken 1 hr after last sample), the aqueous humor concentration was found to be 7.57 (4.75 to 10.86) $\mu g.ml^{-1}$ and 1.207 (0.44 to 2.44) $\mu g.ml^{-1}$, respectively.

In a recent study (Kleinmann et al., 2006a) when lens of rabbit eye were surgically replaced by intraocular hydrophilic acrylic lens (IOL) soaked for 24 hr in gatifloxacin solution (3 $mg.ml^{-1}$), the aqueous humor drug concentration was found to be 16.6, 13.9 and 8.1 $\mu g.ml^{-1}$ at 2, 4 and 6 hr respectively. Similarly when collagen shields were soaked in gatifloxacin solution (5 $mg.ml^{-1}$) for 10 min and placed on surface of rabbit cornea, the aqueous humor drug concentration was found to be 6.32 ± 2.67 and 1.39 ± 1.13 $\mu g.ml^{-1}$ at 3.5 and 6 hr respectively (Kleinmann et al., 2006b). Both the above studies achieved extended drug release from the dosage forms.

In study on humans, application of multiple doses (q.i.d. for 3 days) of gatifloxacin solution, the aqueous humor concentration was found to be 0.63 ± 0.30 $\mu g.ml^{-1}$ (Solomon et al., 2004; Solomon et al., 2005b). However, application of multiple doses (q.i.d. for 1 day) of same gatifloxacin solution produced the aqueous humor concentration of 1.02 $ng.ml^{-1}$ (McCulley et al., 2004). In a study on human, application of multiple doses (q.i.d. for 1 day) of gatifloxacin solution before phacoemulsification and IOL insertion, the aqueous humor concentration achieved was 0.95 ± 0.15 $ng.ml^{-1}$ (Aronowicz et al., 2005). On administration of one drop of gatifloxacin eye drop solution every 10 min for 4 times, 1 hr prior to cataract surgery, the aqueous humor concentration was found to be 0.48 ± 0.34 $\mu g.ml^{-1}$ (Kim et al., 2005a; Kim et al., 2005b). When gatifloxacin topical eye drops were applied over 1 hr and q.i.d. for 3 days preoperatively, it lead to a drug vitreous concentration of 0.001 ± 0.0003 and 0.008 ± 0.006 $\mu g.ml^{-1}$, respectively (Costello et al., 2006).

Oral administration of gatifloxacin tablets (2 tablets of 400 mg), when administered at 12 hr intervals to patients preoperatively, the serum, vitreous and aqueous humor concentrations were found to be 5.14 ± 1.36, 1.34 ± 0.34 and 1.08 ± 0.54 $\mu g.ml^{-1}$, respectively. Vitreous and aqueous humor concentrations were found to be 26.17 and 21.02 %, respectively when compared with serum gatifloxacin concentration. Aqueous humor concentration of gatifloxacin was found to be 0.26–0.63 $\mu g.ml^{-1}$, following topical q.i.d.

dosing a day before surgery in patients undergoing a routine cataract surgery (Hariprasad et al., 2002, 2003).

2.3.6. Toxicity and tolerability

In ophthalmic clinical studies, 5-10 % population have been reported conjunctival irritation and increased lacrimation; 1-4 % of patients have been reported chemosis, conjunctival hemorrhage, dry eye, eye discharge, eye irritation, eye pain, eyelid edema, headache, red eye and reduced visual acuity. Before giving this drug, patients should be checked with hypersensitivity reactions, prolongation of QT interval in electrocardiogram, uncorrected hyperkalemia and patients taking antiarrhythmic agents. Fluoroquinolones are not used in children and pregnant or lactating women. Unlike other fluoroquinolones, gatifloxacin have little to no phototoxic potential (Allergan Inc, 2003; Mah, 2004; Allergan Inc, 2005).

Use of ophthalmic solutions of gatifloxacin 0.3 % w/v did not result in clinically significant epithelial toxicity in healthy human corneas after dosing regimens of 4 times a day for 7 days or hourly for 10 hr dosing regimens. Hourly use of gatifloxacin 0.3 % for 10 hr resulted in a mild increase in conjunctival hyperemia (Price et al., 2005). In humans, Gatifloxacin 0.3 % with BKC demonstrated greater ocular tolerability in comparison to the group receiving moxifloxacin 0.5 % without BKC (Donnenfeld et al., 2004a; Donnenfeld et al., 2004b). Gatifloxacin has not delayed corneal epithelial wound healing in rabbits after anterior keratectomy (Fernandez de Castro et al., 2005). Whereas, moxifloxacin delays corneal epithelial wound healing in comparision to gatifloxacin in patients undergoing photorefractive keratectomy (PRK) (Solomon et al., 2005a). Gatifloxacin 0.3 % showed statistically significantly greater ocular biocompatibility than moxifloxacin 0.5 %, even at frequent dosing interval (Kaufman et al., 2006).

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Chapter 3

Analytical Method Development

3.1. Introduction

Accurate and precise estimation of drug is an integral part of any formulation development process. This can be achieved only by use of appropriate and sensitive analytical method for the estimation of drug in formulations and in different samples of study. If the analytical method is not sensitive and selective, there is a possibility of erroneous results, which will lead to false conclusions. Therefore, proper analytical method development is first step for formulation development process.

Since, gatifloxacin is not official in any of the pharmacopoeias, no official method was available. A survey of literature has revealed few UV-spectrophotometric (Reddy et al., 2004; Patel et al., 2005; Salgado and Oliveira, 2005; Ilango et al., 2006), colorimetric (Ilango et al., 2006; Mali et al., 2006), microbiological (Salgado et al., 2006), HPLC (Lakshmi and Muthukumaran, 2005; Salgado and Lopes, 2006; Santoro et al., 2006), high performance thin layer chromatographic (HPTLC) (Shah et al., 2004; Motwani et al., 2006; Suhagia et al., 2006) and capillary electrophoresis (Sane et al., 2005) methods for estimation of gatifloxacin in bulk drug and formulations. Microbiological assay, spectrofluorimetric and HPLC methods (with electron-spray tandem mass spectrometry, ultraviolet and fluorescence detectors) were reported for the estimation of gatifloxacin in biological fluids such as plasma, serum, urine and semen (Borner et al., 2000; Vishwanathan et al., 2001; Liang et al., 2002; Trampuz et al., 2002; Overholser et al., 2003; Nguyen et al., 2004; Khan et al., 2005; Ocana et al., 2005; Al-Dgither et al., 2006; Cavazos et al., 2006).

A sensitive and selective HPLC method is required for error free estimation of drug content in ophthalmic formulations and dissolution samples of very less concentration of drug. Even though chromatographic methods are very sensitive and useful, they are time consuming for routine drug analysis. Thus, simple, inexpensive, sensitive and rapid analytical methods are required for routine estimation of drug in large number of samples. UV-spectrophotometric and spectrofluorimetric methods are suitable for routine and quick estimation of gatifloxacin in preformulation study samples, formulations, dissolution and in vitro corneal permeability samples. Analysis can be faster with above mentioned techniques without compromising much on sensitivity.

Thus for the present work, UV-spectrophotometric, spectrofluorimetric and an ion-pair RP-HPLC methods were developed for estimation of gatifloxacin for various purposes. The developed methods were validated according to the available guidelines (International Conference on Harmonisation, 1996; US Pharmacopoeia XXIII, 2005). Suitable statistical tests were (Bolton, 1997) performed on validation data.

3.2. Materials and equipment/instruments

3.2.1. Materials

Pure gatifloxacin was obtained as gift sample from Dr. Reddy's Laboratories Ltd., Hyderabad, India, and Venkar Chemicals Pvt. Ltd., Hyderabad, India. Triethyl amine (TEA), Acetonitrile (ACN) and Methanol of HPLC grade were purchased from Spectrochem, Mumbai. Chemicals (analytical reagent and HPLC grade) and excipients like, BKC, calcium chloride dihyrate ($CaCl_2.2H_2O$), citric acid, dextrose, D-glucose, dichloromethane (DCM), di-potassium hydrogen phosphate (K_2HPO_4), di-sodium hydrogen phosphate dihyrate ($Na_2HPO_4.2H_2O$), glacial acetic acid, hydrochloric acid (HCl), iron oxide yellow, lactose, magnesium stearate, magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), methyl cellulose, microcrystalline-cellulose, ortho phosphoric acid, potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), sodium citrate trihydrate, sodium dihydrogen phosphate dihyrate ($NaH_2PO_4.2H_2O$), sodium dodecyl sulphate (SDS), sodium hydrogen carbonate ($NaHCO_3$), sodium hydroxide (NaOH), starch, sulphuric acid, talc, tetrabutyl ammonium hydrogen sulphate (TBAHS), titanium oxide, tri-sodium acetate, were purchased either from BDH, India or Himedia, India or Qualigens, India or SD-fine chemicals, India. For buffer preparations, in-house prepared triple distilled water (TDW) was used. For HPLC, aqueous phases (buffers and TDW) were filtered through 0.22 µ filters (Millipore, USA). The specialized excipients like Eudragit® (L100, S100 and L100-55), hydroxypropylcellulose (Klucel® LF Pharm - viscosity 75-150 cPs), hydroxypropylmethylcellulose (Metalose® 90SH 15000SR - viscosity 15000 cPs; Metalose® 90SH 100000SR - viscosity 100000 cPs) and polyethylene oxide Water-Soluble Resins (PEO 200: Polyox® WSR N-80; PEO 300: Polyox® WSR N-750; PEO 900: Polyox® WSR-1150; PEO 2000: Polyox® WSR N-60K; PEO 5000 - Polyox® WSR Coagulant; PEO 7000: Polyox® WSR-303), were gifted by Aurobindo Laboratories Ltd., India or by Signet Chemicals Ltd., India or by IPCA Laboratories Ltd., India and by Ranbaxy Research Laboratories Ltd., India. HPMC K4M (4000 cPs) and HEC was purchased from Sigma chemicals, USA.

Formulations containing gatifloxacin: Gaity-200 tablets, labeled claim of 200 mg of gatifloxacin per tablet (Dr. Reddy's Laboratories Ltd., India), Gatilox concentrated injection, labeled claim of gatifloxacin 10 $mg.ml^{-1}$ (Solares, Sun Pharma, India) and Gatilox sterile eye drops, labeled claim of gatifloxacin 0.3 % w/v in aqueous buffered vehicle (Sun Pharma. Ind. Ltd., India) were collected from local Indian market. In-house, ophthalmic solution of 0.3 % w/v strength was prepared in the laboratory using phosphate buffer saline (pH 7.4) as vehicle, under aseptic conditions. Gatifloxacin ophthalmic inserts of 150 µg strength was prepared in the laboratory using hydroxypropyl methylcellulose as polymeric carrier. Apart from

common excipients Gaity-200 tablets contain excipients like iron oxide yellow and titanium oxide. Gatilox concentrated injection contains excipients like dextrose and water for injection. In-house prepared gatifloxacin eye drops and Gatilox sterile eye drops contain 0.01 % w/v of BKC. All other chemicals and reagents used were of analytical grade. Composition of buffers and reagents is given in appendix.

3.2.2. Equipment/instruments

A double-beam Jasco (Japan) UV-Vis-NIR spectrophotometer, model V570 connected to computer loaded with spectra manager software and a double-beam Perkin Elmer UV-Vis spectrophotometer, model LAMBDA EZ210 connected to computer loaded with PESSW software (Version 1.2 and Revision E) were used for method development and for intermediate precision respectively. Both the instruments have an automatic wavelength accuracy of 0.1 nm and matched quartz cells of 10 mm path length.

A spectrofluorimeter (Jasco FP777, Japan) loaded with in-built software and equipped with a 150 W Xenon lamp, using 10 mm quartz cells was used for spectrofluorimetric method. Measurement parameters used were: excitation band width - 3 nm, emission band width - 5 nm, photo-multiplier tube response - high, slit width - 0.5 nm, λ_{exc} = 292 ± 1 nm and λ_{em} = 482 ± 1 nm.

HPLC equipment (Jasco, Japan) consisted of model PU-1580 intelligent HPLC pumps, UV-1575 model intelligent UV/Visible detector, FP-1520 model intelligent Fluorescence detector and AS-1559 model intelligent auto sampler. Separation was performed on a Hibar® LiChrospher® 100 RP-18e (250×4 mm, 5 µm particle size) column under isocratic conditions. Chromatograms were analysed using Borwin software provided with the system. The pH of the solutions was adjusted with Elico pH meter (Elico, India).

3.3. UV Spectrophotometric method

3.3.1. Experimental

(a) Optimization of media

Different media were investigated to develop a suitable UV-spectrophotometric method for the analysis of gatifloxacin in formulations. For selection of media the criteria employed were sensitivity of the method, ease of sample preparation, solubility of the drug, cost of solvents and applicability of method to various purposes. Absorbance of gatifloxacin in the selected medium at respective wavelength was determined and apparent molar absorptivity and sandell's sensitivity were calculated according to the standard formulae.

(b) Calibration standards

Primary stock solution of 100 μg.ml^{-1} of gatifloxacin was prepared in phosphate buffer (pH 7.4) by dissolving 5 mg of gatifloxacin in 50 ml media. For preparation of different concentrations, aliquots of stock solutions were transferred into a series of 10 ml standard flasks and volumes were made with respective media. Five different concentrations were prepared in the range of 1-18 μg.ml^{-1} of gatifloxacin in the phosphate buffer for standard graph. Gatifloxacin was estimated at 286 nm. Spectra of different spectra are shown in Figure 3.1 and the calibration data are presented in Table 3.1. To establish linearity of the proposed method, nine separate series of solutions of the drug (1-18 μg.ml^{-1}) were prepared from the stock solutions and analysed. Average absorbance of each concentration was substituted in regression equation to calculate corresponding predicted concentration. The data was subjected to least square regression analysis. ANOVA test (one-way) at 95 % level of significance was performed based on the absorbance values observed for each pure drug concentration during the replicate measurement of the standard solutions.

(c) Analytical validation

To study the specificity and selectivity of method, gatifloxacin solutions (5 μg.ml^{-1}) were prepared in selected media along with and without excipients separately. All the solutions were scanned from 450 to 200 nm at a speed of 400 nm.min^{-1} and checked for change in the absorbance at respective wavelengths. To check the effect of stock solution, different stock solutions (drug concentration 100 μg.ml^{-1}) were prepared in balanced salt solution (BSS), simulated tear fluid (STF) and pH 7.4 phosphate buffered saline (PBS). Aliquots of these stock solutions were used to prepare a concentration of 10 μg.ml^{-1} in selected media and analysed. In a separate study, drug concentration of 5 μg.ml^{-1} was prepared independently from pure drug stock and commercial sample stock in selected media and analysed (n = 5). Paired *t*-test at 95 % level of significance was performed to compare the means of absorbance.

As a part of determining accuracy of the proposed method, different levels of drug concentrations (lower quality control sample [LQC] - 2 μg.ml^{-1}, medium quality control sample [MQC] - 7 μg.ml^{-1} and high quality control sample [HQC] - 15 μg.ml^{-1}) were prepared from independent stock solution and analysed (n = 9). Solutions were analysed by proposed method and predicted concentrations were calculated using regression equation. Accuracy was assessed as the mean percentage recovery and percentage bias (% Bias = 100 × [(predicted concentration – nominal concentration)/nominal concentration]) (Table 3.2). To give additional support to accuracy of the developed assay method, standard addition method

was done. In this study, different concentrations of pure drug (2, 5 and 10 $\mu g.ml^{-1}$) were added to a known pre-analysed formulation sample (5.25 $\mu g.ml^{-1}$) and the total concentration was determined using the proposed method (n = 3). The percent recovery of the added pure drug was calculated as, % Recovery = [(Cv-Cu)/Ca] × 100, where Cv is the total drug concentration measured after standard addition; Cu, drug concentration in the formulation; Ca, drug concentration added to formulation.

Precision was determined by studying repeatability and intermediate precision. Repeatability was determined by using different levels of drug concentrations (same concentration levels as taken in accuracy study), prepared from independent stock solution and analysed (n = 9) (Table 3.2). Inter day and intra day variation and instrument variation were taken to determine intermediate precision of the proposed method. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. Same protocol was followed for three different days to study inter-day variation (n = 27). One set of different levels of the concentrations were re-analysed using Perkin Elmer instrument by proposed method (n = 3). The percentage relative standard deviation (% RSD) of the predicted concentrations from the regression equation was taken as precision (Table 3.3).

The detection limit (DL) and quantitation limit (QL) of gatifloxacin by the proposed method was determined using calibration standards. DL and QL were calculated as $3.3\sigma/S$ and $10\sigma/S$ respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation. Robustness of the proposed method was determined by (a) changing pH of the media by ± 0.1 units and (b) stability of the gatifloxacin in the selected medium at room temperature for 36 hr. Three different concentrations (LQC, MQC and HQC) were prepared in selected media with different pH. Mean percentage recovery was determined.

d) Estimation of drug content of formulations

Twenty tablets were weighed and pulverized. Amount of the powder equivalent to 10 mg of gatifloxacin was taken and extracted with media separately for 30 min. These solutions were diluted suitably to prepare a 100 $\mu g.ml^{-1}$ concentration in selected media. Finally solutions were filtered through Whatman filter paper number 40 and the filtrate was suitably diluted to prepare a 5 $\mu g.ml^{-1}$ concentration in selected media and the samples were analysed using proposed method.

Three vials of gatifloxacin injection were emptied into a sterile container and aliquot of injection equivalent to 1 mg of drug was taken and diluted with media to get a 10 $\mu g.ml^{-1}$ concentration and the samples were analysed.

Three containers of gatifloxacin eye drop solution were emptied into a sterile container and aliquot of eye drop solution equivalent to 120 µg of gatifloxacin was taken and diluted with media to get a 12 $\mu g.ml^{-1}$ concentration and the samples were analysed. Five replicates were prepared in all the cases (Table 3.4).

3.3.2. Results and discussion

(a) Optimization of media

For media optimization various aqueous media like 0.1 M hydrochloric acid, acetate buffers (pH 3.6 to 5.8), phosphate buffers (pH 5.8 to 8.0) and 0.1 M sodium hydroxide were investigated. Gatifloxacin showed pH dependent UV absorption spectra. Addition of acetonitrile/methanol in various proportions with various aqueous media did not improve the sensitivity of the method. The final decision of using phosphate buffer (pH 7.4) as a media was based on criteria like: sensitivity of the method, cost, ease of preparation and applicability of the method to in vitro drug release studies. The spectra of different concentrations of gatifloxacin in the phosphate buffer are shown in Figure 3.1. The λ_{max} of gatifloxacin in phosphate buffer (pH 7.4) was found to be 286 nm. No change in absorption spectra was observed at 1 and 36 hr. Apparent molar absorptivity and Sandell's sensitivity of drug were found to be 2.62×10^4 $l.mol^{-1}.cm^{-1}$ and 0.0143 $\mu g.cm^{-2}/0.001A$ respectively.

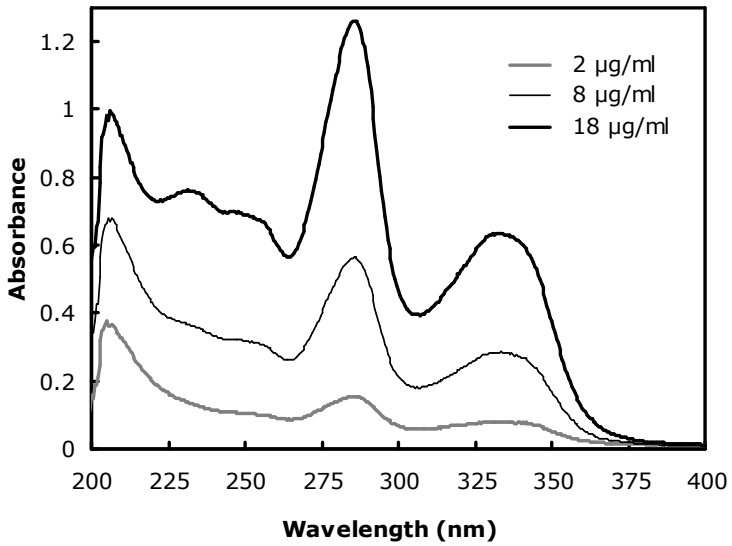

Figure 3.1: Absorption spectra of different concentrations of gatifloxacin in phosphate buffer (pH 7.4).

(b) Calibration curve

The linear regression equation obtained was: Absorbance = 0.0684 × Concentration ($\mu g.ml^{-1}$) + 0.0050. Different standard concentrations and their absorbencies were shown in the Table 3.1. At all the concentration levels the standard deviation was low and the percentage relative standard deviation (% RSD) did not exceed 3.50. The predicted concentrations were nearly matching with the nominal concentration. In selected medium the linearity range was found to be 1-18 $\mu g.ml^{-1}$. The standard error values of slope and intercept were found to be 1.20×10^{-4} and 7.12×10^{-4} respectively. The individual values of slope and intercept were found to be within the 95 % confidence limits. Goodness of fit of regression equation was supported by high regression coefficient value (0.9998), low standard error of estimate (2.95×10^{-3}) and low $F_{calculated}$ value (3.01×10^{-4}) than $F_{critical}$ (2.21 at 8 and 36 d.f.). Lower values of parameters like standard error of slope, intercept and estimate indicated high precision of the proposed method.

Table 3.1: Calibration data of gatifloxacin by UV-spectrophotometric method.

Drug Con. ($\mu g.ml^{-1}$)	Abs. at 286 nm (Mean ± SD) †	% RSD	Predicted Con. ($\mu g.ml^{-1}$)
1	0.0742 ± 0.0026	3.50	1.01
5	0.3453 ± 0.0025	0.72	4.98
8	0.5506 ± 0.0040	0.73	7.98
12	0.8301 ± 0.0066	0.80	12.06
18	1.2348 ± 0.0074	0.60	17.98

† Each value is average of nine separate determinations

(c) Analytical validation

The UV-spectrum of gatifloxacin was not changed in the presence of various excipients. There was no difference in absorbencies or spectra of drug solutions (10 $\mu g.ml^{-1}$) prepared from variety of stock solutions. Absorption spectrum of pure drug sample was matching with the marketed formulation sample. The $t_{calculated}$ (1.32) were found to be less than that of the $t_{critical}$ value (2.31 at 8 d.f.), indicating that statistically there was no significant difference between mean absorbance of solutions prepared from pure drug sample and marketed formulation samples. Therefore proposed method is specific and selective for the drug.

Percentage bias ranged from -0.38 to 0.61 (Table 3.2). The high mean % recovery values (nearly 100) and their low standard deviation values (SD < 1.4) indicated high accuracy. The validity and reliability of the proposed method was evaluated by recovery studies from standard addition method. The mean percentage recoveries (% RSD value is

given in the parenthesis) for 2, 5 and 10 µg.ml^{-1} concentrations in standard addition studies were found to be 101.16 (2.47), 99.59 (0.52) and 100.11 (0.69) respectively. These results revealed accuracy of the proposed method.

Repeatability (% RSD) ranged from 0.40 to 1.39 % at all three levels of concentrations (Table 3.2). In intermediate precision study, RSD values were not more than 1.78 % in all the cases (Table 3.3). RSD values were low, indicating the excellent precision of the method.

Table 3.2: Accuracy and precision data for the UV-spectrophotometric method.

Level	Predicted con. (µg.ml^{-1})			% Recovery (Mean ± SD)	% Bias
	Range	Mean ± SD †	% RSD		
LQC	1.98 - 2.07	2.01 ± 0.03	1.39	100.51 ± 1.40	-0.38
MQC	6.98 - 7.07	7.01 ± 0.03	0.40	100.14 ± 0.40	-0.10
HQC	14.81 - 15.01	14.91 ± 0.07	0.48	99.40 ± 0.48	0.61

† Each value is average of nine separate determinations

Table 3.3: Result of intermediate precision study of UV-spectrophotometric method.

Level	Intra-day repeatability % RSD (n = 3)			Inter-day repeatability % RSD (n = 27)	Inter-instrument repeatability % RSD (n = 6)
	Day 1	Day 2	Day 3		
LQC	0.55	0.95	0.54	1.13	1.00
	0.74	0.73	0.94		
	1.14	0.78	1.01		
MQC	1.20	0.85	0.19	0.89	0.91
	0.37	0.36	0.40		
	1.40	0.83	0.63		
HQC	1.78	1.14	1.06	1.08	1.17
	0.31	0.21	1.13		
	1.08	1.05	1.30		

DL and QL were found to be 0.103 µg.ml^{-1} and 0.312 µg.ml^{-1}, respectively. Variation of pH of the selected media by ± 0.1 did not have any significant effect on absorbance. The mean % recovery (± SD) was found to be 100.08 (± 1.56). The gatifloxacin solution in selected media exhibited no spectrophotometric changes for 36 hr when kept at room temperature.

d) Estimation of drug content of formulations

The assay values of gatifloxacin in different formulations ranged from 100.18 to 102.29 % with standard deviation not more than 0.87. Assay values of formulations were found to be same as mentioned in the label claim, suggesting that the interference of excipient

matrix is insignificant in estimation of gatifloxacin by proposed method. The estimated drug content with low values of standard deviation established the precision of the proposed method (Table 3.4).

Table 3.4: Result of estimation of drug in commercial products by UV-spectrophotometric method.

Commercial products	Amount found † (Mean ± SD)	% Assay † (Mean ± SD)
Gaity-200 tablets (200 mg)	204.58 ± 1.68 mg	102.29 ± 0.84
Gatilox concentrated injection (10 $mg.ml^{-1}$)	10.07 ± 0.09 $mg.ml^{-1}$	100.68 ± 0.87
Ophthalmic solution (0.3 % w/v = 3 $mg.ml^{-1}$) ‡	3.01 ± 0.02 $mg.ml^{-1}$	100.18 ± 0.51

† Each value is average of five separate determinations; ‡ In-house prepared formulation

3.4. Spectrofluorimetric method

3.4.1. Experimental

(a) Optimization of medium

Different pH media alone and in combination with different organic solvents, in various proportions, were tried. For selection of medium the criteria employed was based same parameters mentioned in section 3.3.2.a.

(b) Calibration curve

Primary stock solution of 200 $\mu g.ml^{-1}$ of gatifloxacin was prepared in 0.01 M hydrochloric acid (pH 2.0). Using aliquot of primary stock solution, secondary stock solution of 1 $\mu g.ml^{-1}$ was prepared in same 0.01 M hydrochloric acid. For preparation of different concentrations, aliquots of secondary stock solution were transferred into series of 10 ml standard flasks and volume was made with 0.01 M hydrochloric acid. Five different concentrations (20-160 $ng.ml^{-1}$) of gatifloxacin were prepared for calibration curve. Relative fluorescence intensity was measured at λ_{exc} of 292 nm and λ_{em} of 482 nm against blank (Figure 3.2 and Table 3.5). To establish linearity of the proposed method, six separate series of solutions of the drug in selected medium were prepared from the stock solution and analysed. Average intensity of each concentration was substituted in regression equation to calculate corresponding predicted concentration. Least square regression analysis was done for the obtained data. ANOVA test (one-way) at 95 % level of significance was performed based on the relative fluorescence intensity values observed for each pure drug concentration during the replicate measurement of the standard solutions.

(c) Analytical validation

Gatifloxacin solutions (100 $ng.ml^{-1}$) were prepared in the selected medium with and without excipients separately. All the solutions were scanned (400 nm/min) for emission spectrum by fixing the λ_{exc} at 292 nm and checked for the change in emission spectrum. To check the effect of stock solution, different secondary stock solutions (drug concentration 1 $\mu g.ml^{-1}$) were prepared in BSS, STF and pH 7.4 PBS. Aliquots of these stock solutions were used to prepare a concentration of 150 $ng.ml^{-1}$ in selected media and analysed. In a separate study drug concentration of 150 $ng.ml^{-1}$ was prepared independently from pure drug stock and formulation sample stock in selected medium and analysed (n = 5). Paired *t*-test at 95 % level of significance was performed to compare the means of relative fluorescence intensity.

For determining accuracy of the proposed method, different levels of drug concentrations (LQC - 25 $ng.ml^{-1}$, MQC - 75 $ng.ml^{-1}$ and HQC - 150 $ng.ml^{-1}$) were prepared independently from stock solution and analysed (n = 6). For standard addition study, different concentrations of pure drug (20, 40, 60, 80 and 100 $ng.ml^{-1}$) were added to a known pre-analysed formulation sample (drug concentration of 50.3 $ng.ml^{-1}$) and the total concentration was determined using the proposed method (n = 3). Calculations were same as mentioned in the experimental section 3.3.1.c.

Repeatability was determined by using different levels of drug concentrations (as mentioned in accuracy), prepared from independent stock solution and analysed (n = 6) (Table 3.6). Inter day and intra day variation and analyst variation were studied to determine intermediate precision of the proposed method. Different levels of drug concentrations in triplicates were prepared at two different times in a day and studied for intra-day variation. Same protocol was followed for three different days to study inter-day variation (n = 18). Different analysts prepared different solutions on different days. % RSD of the predicted concentrations from the regression equation was taken as precision (Table 3.7).

DL and QL were calculated in the same manner mentioned in the experimental section of UV-spectrophotometric method. Robustness of the proposed method was determined by (a) changing pH of the media by ± 0.1 units and (b) stability of drug in the selected medium at room temperature for 8 hr. Three different concentrations (LQC, MQC and HQC) were prepared in different pH media and mean percentage recovery was determined.

d) Estimation of drug content of formulations

Twenty tablets were weighed and pulverized. Amount of the powder equivalent to 5 mg of gatifloxacin was taken and extracted with selected medium for 30 min. The solution

was diluted suitably to prepare a 200 $\mu g.ml^{-1}$ concentration. This primary stock solution was filtered through Whatman filter paper number 40 and the filtrate was further diluted suitably to prepare a secondary stock solution of 1 $\mu g.ml^{-1}$ concentration. Aliquot of the secondary stock solution was diluted to a concentration of 50 $ng.ml^{-1}$ and the samples were analysed using proposed method.

Three vials of gatifloxacin injection were emptied into a sterile container and aliquot of injection equivalent to 5 mg of drug was taken and diluted in the selected medium to get a 200 $\mu g.ml^{-1}$ concentration primary stock. Rest of the sample preparation was same as used for tablets and samples were analysed.

Three containers of gatifloxacin eye drop solution were emptied into a sterile container and aliquot of eye drop solution equivalent to 300 µg of drug was taken and diluted in the selected medium to get a 3 $\mu g.ml^{-1}$ concentration primary stock. Aliquot of the primary stock solution was diluted to a concentration of 150 $ng.ml^{-1}$ and the samples were analysed. Five replicates were prepared in all the cases. The results are presented in Table 3.8.

3.4.2. Results and discussion

(a) Optimization of media

For media optimization various aqueous media like 0.01 M hydrochloric acid, acetate buffers (pH 3.6 to 5.8), phosphate buffers (pH 5.8 to 8.0) and 100 mM sodium hydroxide were investigated. Addition of acetonitrile/methanol in various proportions with various aqueous media did not improve the sensitivity of the method. Sensitivity was found to be maximum in 0.01 M hydrochloric acid medium. The final decision of using 0.01 M hydrochloric acid (pH 2.0) as a medium was based on criteria mentioned in section 3.3.2.a. The excitation and emission spectra of gatifloxacin in 0.01 M hydrochloric acid are shown in Figure 3.2.

(b) Calibration curve

Different concentrations and their relative fluorescence intensities were shown in the Table 3.5. At all the concentration levels the SD was low and the % RSD did not exceed 2.4. The predicted concentrations were nearly matching with the nominal concentration. In selected medium the linearity range was found to be 20 - 160 $ng.ml^{-1}$. The linear regression equation obtained was: Fluorescence intensity = 36.05 × Concentration ($ng.ml^{-1}$) + 12.60. According to linear regression analysis, the standard error values of slope and intercept were found to be 8.38×10^{-2} and 24.46 respectively. The individual values of slope and intercept

were found to be within the 95 % confidence limits. Goodness of fit of regression equation was supported by high regression coefficient value (0.9998), low standard error of estimate (0.591) and low $F_{calculated}$ value (2.59×10^{-3}) than $F_{critical}$ value (2.62 at 5 and 24 d.f.). Lower values of parameters like standard error of slope, intercept and estimate indicated high precision of the proposed method.

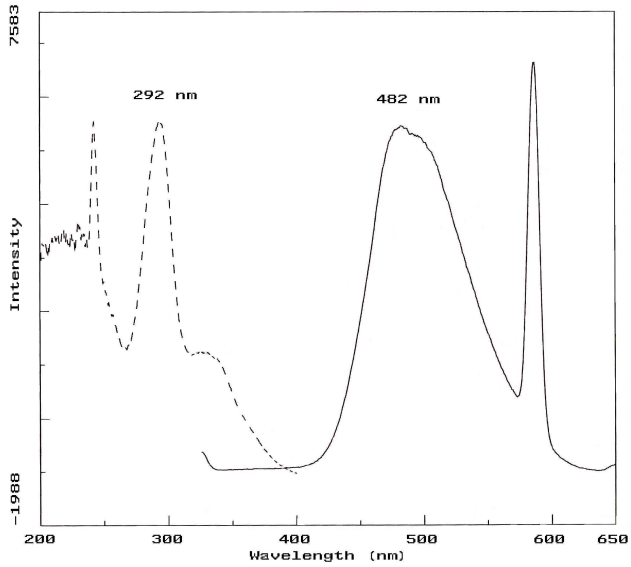

Figure 3.2: Excitation and emission spectra of gatifloxacin. Excitation spectra indicated by (- - -) and emission spectra indicated by (—). Concentration of gatifloxacin was 160 ng.ml^{-1} in 0.01 M hydrochloric acid medium.

Table 3.5: Calibration data of gatifloxacin by spectrofluorimetric method.

Con. (ng.ml^{-1})	Mean relative fluorescence intensity (Mean ± SD) †	% RSD	Predicted Con. (ng.ml^{-1})
20	731 ± 17	2.33	19.93
60	2180 ± 17	0.78	60.12
100	3624 ± 71	1.96	100.19
140	5051 ± 85	1.68	139.76
160	5781 ± 54	0.93	160.03

† Each value is average of six separate determinations

(c) Analytical validation

The emission spectrum of gatifloxacin was not changed in the presence of common excipients in selected medium. There was no difference in relative intensities or emission spectra of drug solutions (150 ng.ml^{-1}) prepared from variety of stock solutions. When the relative fluorescence intensity of same concentration of pure drug sample and formulation sample were compared by paired *t*-test, the $t_{calculated}$ (0.24) was found to be less than that of the $t_{critical}$ (2.31 at 8 d.f), indicating that statistically there was no significant difference between mean relative fluorescence intensities. The interference of excipients was

insignificant in the estimation of drug. Therefore proposed method was specific and selective for the drug.

At all the three concentration levels, % bias ranged from -0.22 to 0.06 % (Table 3.6). The high mean % recovery values (99.78 to 100.06) and their low standard deviation values (SD < 1.2) represented accuracy of the method. In standard addition method, the mean percentage analytical recoveries (± SD) for 20, 40, 60, 80 and 100 $ng.ml^{-1}$ concentrations were found to be 100.54 (± 1.65), 98.70 (± 0.70), 99.22 (± 1.96), 101.47 (± 0.95) and 100.38 (± 0.57), respectively. This result revealed the validity and reliability of the proposed method.

In repeatability study, the RSD was ranged from 0.15 to 1.14 % (Table 3.6). At all three concentration levels, precision showed satisfactory levels. Intermediate precision expresses within-laboratory variations in different days and by different analysts. Results of intermediate precision study, RSD values for each set (all three levels) were given in Table 3.7. In all the cases the RSD values were not more than 2.15 %. RSD values within the acceptable range indicating that this method have excellent repeatability and intermediate precision.

Table 3.6: Accuracy and precision data for the spectrofluorimetric method.

Level	Predicted con. ($ng.ml^{-1}$)			% Recovery (Mean ± SD)	Bias (%)
	Range	Mean ± SD †	% RSD		
LQC	24.44 - 25.50	25.02 ± 0.29	1.14	100.06 ± 1.14	0.06
MQC	72.24 - 75.10	74.83 ± 0.65	0.87	99.78 ± 0.87	-0.22
HQC	149.59 - 150.28	149.99 ± 0.23	0.15	99.99 ± 0.15	-0.01

† Each value is average of six separate determinations

Table 3.7: Result of intermediate precision study of spectrofluorimetric method.

Level	Intra-day repeatability - % RSD (n = 3)			Inter-day repeatability % RSD (n = 18)
	Day 1	Day 2	Day 3	
LQC	0.91	1.35	1.32	0.52
	0.66	1.43	0.38	
MQC	0.04	0.03	0.01	0.37
	2.15	0.09	0.02	
HQC	0.04	0.03	0.01	0.18
	0.01	0.06	0.02	

DL and QL were found to be 5.48 and 16.61 $ng.ml^{-1}$ respectively. Robustness was found to be very high as variation of pH of the selected media by ± 0.1 did not have any significant effect on relative fluorescence intensity. The mean % recovery (± SD) was found to be 99.94 (± 0.83). The gatifloxacin solution in selected medium exhibited no change in emission spectra for 8 hr when kept at room temperature.

d) Commercial formulations

The proposed method was evaluated by estimation of gatifloxacin in pharmaceutical formulations. The assay values of gatifloxacin for different formulations ranged from 99.83 to 101.59 % with standard deviation not more than 1.17. Assay values of formulations were very close to the label claim. This indicated no interference of excipient matrix in estimation of gatifloxacin by the proposed method (Table 3.8).

Table 3.8: Result of estimation of drug in commercial products by spectrofluorimetric method.

Commercial products	Amount found (Mean ± SD) †	% Assay (Mean ± SD) †
Gaity-200 tablets (200 mg)	203.17 ± 0.81 mg	101.59 ± 0.41
Gatilox concentrated injection (10 $mg.ml^{-1}$)	9.98 ± 0.07 $mg.ml^{-1}$	99.83 ± 0.67
Ophthalmic solution (0.3 % w/v = 3 $mg.ml^{-1}$) ‡	3.00 ± 0.02 $mg.ml^{-1}$	99.89 ± 0.67

† Each value is average of five separate determinations. ‡ - Formulation was prepared in laboratory.

3.5. Ion-pair RP-HPLC method

3.5.1. Experimental

(a) Chromatographic condition

The mobile phase consisted of aqueous phase (25 mM citrate buffer, 10 mM TBAHS and 10 mM SDS in triple distilled water – pH adjusted to 3.5) and ACN (52:48 % v/v). Before use, the mobile phase was filtered through 0.22 µm filter and degassed by sonication. Injection volume was 20 µl. Flow rate was adjusted to 1 $ml.min^{-1}$, the sensitivity was 0.0005 absorbance unit full screen (AUFS) and the wavelength of UV detector was set at 292 nm. All the experiments were conducted at room temperature at approximately 25 °C. The system was stabilized for at least 1 hr before analysis.

(b) Optimization of mobile phase

Different pH media (pH 3 to 5 orthophosphoric acid-triethyl amine buffer, pH 3 to 5 ammonium acetate buffer, pH 3 to 5 phosphate buffer and pH 3 to 5 citrate buffer) in combination with different organic solvents (ACN and methanol) and ion pairing agents in various proportions (TEA, SDS and TBAHS) were tried. For selection of media the criteria employed was gatifloxacin peak properties (Retention time (t_R), asymmetric factor) sensitivity (height and area) ease of sample preparation, and applicability of the method for various purposes.

(c) Calibration curve

Primary stock solution of 50 μg.ml^{-1} of gatifloxacin was prepared in mobile phase. From an aliquot of primary stock, secondary stock solution of 1 μg.ml^{-1} was prepared in mobile phase. For preparation of different concentrations, aliquots of primary or secondary stock solutions were transferred into a series of 5 ml standard flasks and volume was made with mobile phase. Six different concentrations (50-1000 ng.ml^{-1}) of gatifloxacin were prepared for calibration curve. Twenty micro liter of each concentration was injected and area of the peak was determined (Table 3.9). To establish linearity of the proposed method, six separate series of solutions of the drug in selected medium were prepared from the stock solution and analysed. Average area of each concentration was substituted in regression equation to calculate corresponding predicted concentration. Least square regression analysis was done for the obtained data. ANOVA test (one-way) at 95 % level of significance was performed based on the peak area observed for each pure drug concentration during the replicate measurement of the standard solutions.

d) Analytical validation

To study selectivity of the method, gatifloxacin solutions (50 μg.ml^{-1}) were prepared in mobile phase with and without excipients separately. All the solutions were diluted suitably with mobile phase to get a drug concentration of 1000 ng.ml^{-1} and analysed. Blank containing only excipient was also injected after similar dilutions were made and checked for interference near the drug peak. To check the effect of stock solution, different stock solutions (drug concentration 50 μg.ml^{-1}) were prepared in BSS, STF and pH 7.4 PBS. These stocks were further diluted appropriately to get a concentration of 500 ng.ml^{-1} in mobile phase and analysed. In a separate study drug concentration of 500 ng.ml^{-1} was prepared independently from pure drug stock and formulation sample stock in selected medium and analysed (n = 5). Paired *t*-test at 95 % level of significance was performed to compare the means of area.

As a part of determining accuracy of the proposed method, different levels of drug concentrations (LQC - 75 ng.ml^{-1}, MQC - 400 ng.ml^{-1} and HQC - 900 ng.ml^{-1}) were prepared independently from stock solution and analysed (n = 6). Further, different concentrations of pure drug (50, 100, 250 and 500 ng.ml^{-1}) were added to a known pre-analysed formulation sample (drug concentration of 507.9 ng.ml^{-1}) and analysed using proposed method (n = 5) to check accuracy (Table 3.10). Calculations were same as mentioned in the experimental section 3.3.1.c.

Repeatability was determined by using different levels of drug concentrations (as mentioned in accuracy), prepared from independent stock solution and analysed (n = 6) (Table 3.10). Intermediate precision was done by similar procedure mentioned in the experimental section 3.4.1c (n = 18). % RSD of the predicted concentrations from the regression equation was taken as precision (Table 3.11).

DL and QL were determined using signal to noise ratio. The noise levels were determined at 1 min before and after drug's t_R. A signal to noise ratio of 3 and 10 were taken as DL and QL respectively. The QL samples were prepared in replicates (n =5) using same procedure followed for calibration standards and analysed. Robustness of the proposed method was determined by (a) varying pH of the media by ± 1 units and (b) bench-top stability and stock solution stability of gatifloxacin at room temperature for 36 hr.

e) Estimation of drug content of formulations

Twenty tablets of each brand were weighed, pulverized and amount of the powder equivalent to 5 mg of gatifloxacin was taken accurately and sonicated with mobile phase for 5 min. The solution was diluted suitably to prepare a 50 $\mu g.ml^{-1}$ concentration. This primary stock solution was filtered through Whatman filter paper number 40. Aliquot of the primary stock solution was diluted to a concentration of 500 $ng.ml^{-1}$ with mobile phase and the samples were analysed using proposed method.

Three vials of gatifloxacin injection were emptied into a sterile container and aliquot of injection equivalent to 5 mg of drug was taken and diluted in mobile phase to get a 50 $\mu g.ml^{-1}$ concentration primary stock. Rest of sample preparation was the same as used for tablets and samples were then analysed.

Three containers of gatifloxacin eye drop solution were emptied into a sterile container and aliquot of gatifloxacin ophthalmic solution equivalent to 1.5 mg of drug was taken and diluted in mobile phase to get a 15 $\mu g.ml^{-1}$ concentration of primary stock. Aliquot of the primary stock solution was diluted to a concentration of 300 $ng.ml^{-1}$ with mobile phase and the samples were analysed.

Twenty inserts were weighed, pulverized, and amount of the powder equivalent to 150 µg of gatifloxacin was taken and sonicated with the mobile phase for 5 min. The solution was diluted suitably to prepare a 15 $\mu g.ml^{-1}$ concentration. Rest of sample preparation was the same as used for ophthalmic solution and the samples were analysed. Five replicates were prepared in all the cases (Table 3.12).

3.5.2. Results and discussion

(a) Mobile phase composition optimization

In the mobile phase containing citric acid buffer (pH 3.5) and ACN (50:50 % v/v), t_R and asymmetric factor of drug were found to be 3.60 min and 1.83 respectively. Addition of ion-pairing reagents (SDS and TBAHS), to this mobile phase increased t_R of the drug and reduced the asymmetric factor. When TBAHS concentration was changed from 0 to 20 mM, keeping SDS concentration 10 mM, t_R decreased from 7.35 to 4.04 min and asymmetric factor decreased from 1.43 to 1.22. However, opposite effect was observed when SDS concentration was varied (0 to 20 mM) by keeping TBAHS concentration at 10 mM. T_R increased from 2.24 to 6.22 min and asymmetric factor decreased from 1.38 to 1.18. Based on these experiments the optimum peak parameters were obtained with 10 mM SDS and 10 mM TBAHS. T_R of gatifloxacin was decreased from 11 to 4.6 min with increase in concentration of ACN from 40 to 50 % in mobile phase (with 10 mM SDS and 10 mM TBAHS), but there was no effect on area and asymmetric factor of the drug. t_R, area, height and asymmetric factor of the drug were not affected with change in pH of mobile phase (with 10 mM SDS and 10 mM TBAHS) from 3 to 4. Incorporation of TEA, methanol and phosphate buffer lowered response and increased asymmetric factor. Thus, the final optimized mobile phase consisted 52 % aqueous phase (25 mM citrate buffer, 10 mM SDS and 10 mM TBAHS at pH 3.5) with 48 % ACN.

(b) Calibration curve

Different concentrations and their corresponding area were shown in the Table 3.9. At all the concentration levels the standard deviation of area was very low and the relative standard deviation (% RSD) did not exceed 1.96 %. Chromatograms of three different concentrations were shown in the Figure 3.3. The predicted concentrations were nearly matching with the nominal concentration. In selected mobile phase the linearity range was found to be 50-1000 $ng.ml^{-1}$.

The linear regression equation obtained was: Area (μV.sec) = 105.5 × Concentration ($ng.ml^{-1}$) – 695.8. According to linear regression analysis, the standard error values of slope and intercept were found to be 0.2 and 108.3 respectively. The individual values of slope and intercept were found to be within the 95 % confidence limits. Goodness of fit of regression equation was supported by high regression coefficient value (0.9996), low standard error of estimate (6.73), low mean sum of the squared residuals value (44.87) and low $F_{calculated}$ value (1.05×10^{-4}) than $F_{critical}$ value (2.53 at 5 and 30 d.f.). Lower values of parameters like

standard error of slope, intercept and estimate indicated high precision of the proposed method.

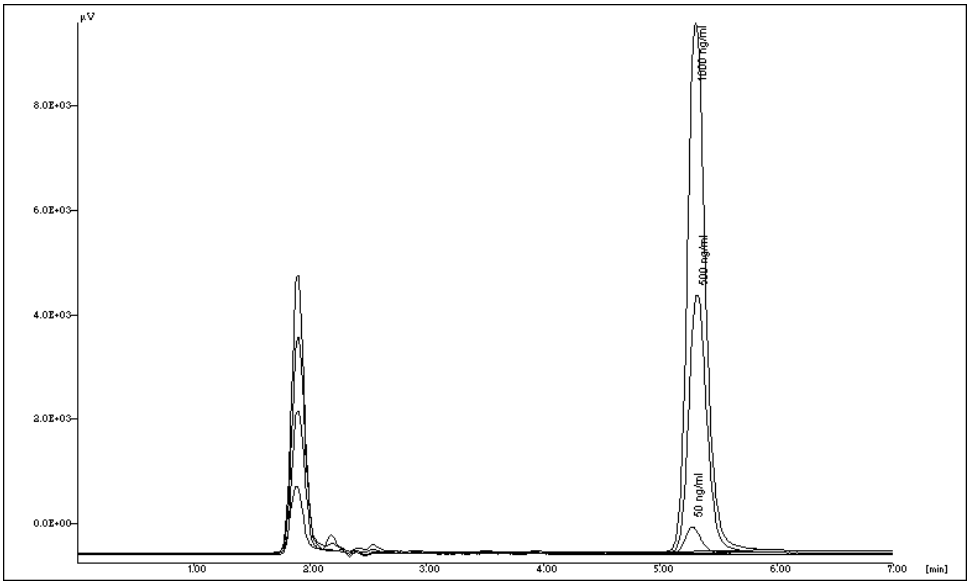

Figure 3.3: Chromatogram of different concentration of gatifloxacin. Mobile phase: aqueous phase (25 mM citrate buffer, 10 mM TBAHS and 10 mM SDS – pH adjusted to 3.5) and ACN – 52:48 % v/v.

Table 3.9: Calibration data of gatifloxacin by HPLC method.

Con. ($ng.ml^{-1}$)	Mean Area (µV.sec) (Mean ± SD) †	% RSD	Predicted Con. ($ng.ml^{-1}$)
50	5024.9 ± 92.6	1.84	52.2
100	10090.9 ± 115.9	1.15	102.2
250	25262.6 ± 276.9	1.10	246.0
500	51533.0 ± 1011.9	1.96	495.0
750	78224.5 ± 624.4	0.80	747.9
1000	105306.9 ± 658.7	0.63	1004.6

† Each value is average of six separate determinations

(c) Analytical validation

The blank samples of excipients did not show any interference near the drug peak. Figure 3.4 shows the chromatograms of gatifloxacin with hydroxypropyl methylcellulose in 1:50 proportion and its blank and this excipient did not interfere with the drug peak. Similar results were observed with all the other excipients (chromatograms not shown). In presence of the excipients, peak characters of the drug (t_R, area and asymmetric factor) were not affected. From all the excipient-drug combinations studied the mean % recovery (± SD) value was found to be from 98.19 % (± 0.98) to 101.16 % (± 1.72). This indicated that there is no significant interference of excipients in the estimation of drug by proposed method. There was no difference in areas or chromatogram of drug solutions (500 $ng.ml^{-1}$) prepared from variety of stock solutions. When the areas of same concentration of pure drug sample and

formulation sample were compared by paired *t*-test, the $t_{calculated}$ (1.27) was found to be less than that of the $t_{critical}$ (2.31 at 8 d.f.), indicating that statistically there was no significant difference between areas. Therefore the proposed method was specific and selective for the drug.

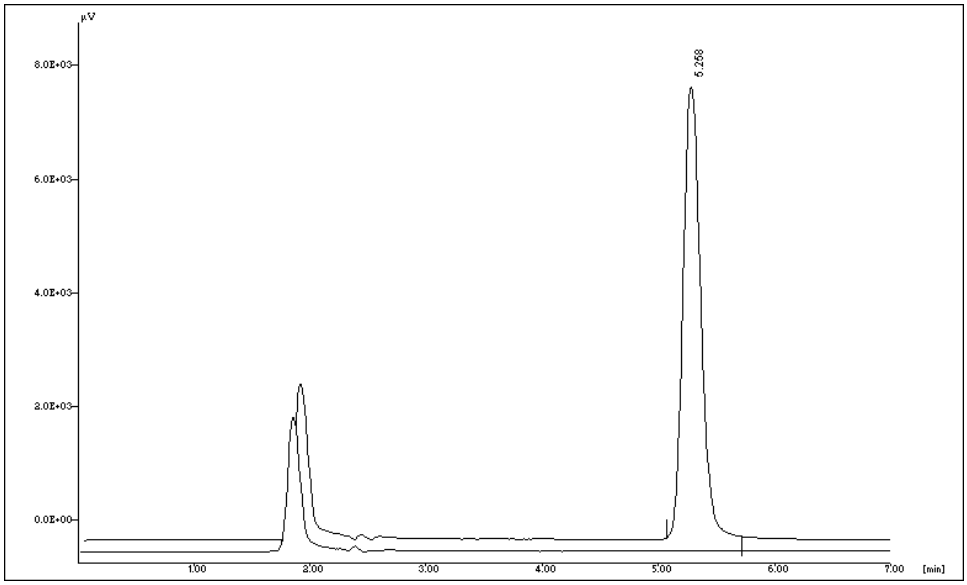

Figure 3.4: Chromatograms of gatifloxacin with hydroxypropyl methylcellulose in 1:50 proportion and blank. Mobile phase: aqueous phase (25 mM citrate buffer, 10 mM TBAHS and 10 mM SDS – pH adjusted to 3.5) and ACN – 52:48 % v/v.

At all the three concentration levels, % bias ranged from -0.44 to 0.33 (Table 3.10). The nearly 100 % mean recovery values and their low standard deviation values (SD < 1.40) represented accuracy of the method. In standard addition method, the mean percentage analytical recoveries (± SD) for 50, 100, 250 and 500 ng.ml^{-1} concentrations were found to be 101.88 (± 1.81), 99.39 (± 0.87) 101.48 (± 0.96) and 100.41 (± 0.66) respectively. This result revealed the validity and reliability of the proposed method.

Table 3.10: Accuracy and precision data for the HPLC method.

Level	Predicted con. (µg.ml^{-1})			% Recovery (Mean ± SD)	Bias (%)
	Range	Mean ± SD †	% RSD		
LQC	73.67 - 76.77	75.24 ± 0.95	1.26	100.33 ± 1.27	0.33
MQC	381.94 - 405.18	398.24 ± 5.54	1.39	99.56 ± 1.40	-0.44
HQC	887.38 - 912.94	902.55 ± 5.87	0.65	100.28 ± 0.65	0.28

† Each value is result of six separate determinations

In repeatability study, the RSD values ranged from 0.65 to 1.39 % (Table 3.10). RSD values were significantly low for intermediate precision; intra-day variation was not more

than 1.52 %, while inter-day variation was less than 1.11 % (Table 3.11). Lower RSD values indicated the repeatability and intermediate precision of the method.

Table 3.11: Result of intermediate precision study of HPLC method.

Level	Intra-day repeatability - % RSD (n = 3)			Inter-day repeatability % RSD (n = 18)
	Day 1	Day 2	Day 3	
LQC	0.21	0.60	1.26	0.75
	0.15	1.18	1.50	
MQC	0.85	0.41	0.14	1.11
	0.35	1.52	0.29	
HQC	0.37	0.42	0.29	0.31
	0.32	1.47	0.34	

DL and QL were found to be 6.50 $ng.ml^{-1}$ and 17.38 $ng.ml^{-1}$ respectively. Figure 3.5 shows the chromatogram of gatifloxacin having concentration at QL – 17.4 $ng.ml^{-1}$. On repeated injections, the characteristics of the drug peak (t_R, area and asymmetric factor) were not affected. The mean % recovery (± SD) of the drug at QL was found to be 102.33 (± 2.65), representing the accuracy and precision of the method. Robustness was found to be very high as variation of pH of the selected media by ± 0.5 did not have any significant effect on t_R (5.20 ± 0.04), asymmetric factor (1.22 ± 0.02) and % drug recovery (98.55 ± 2.30).

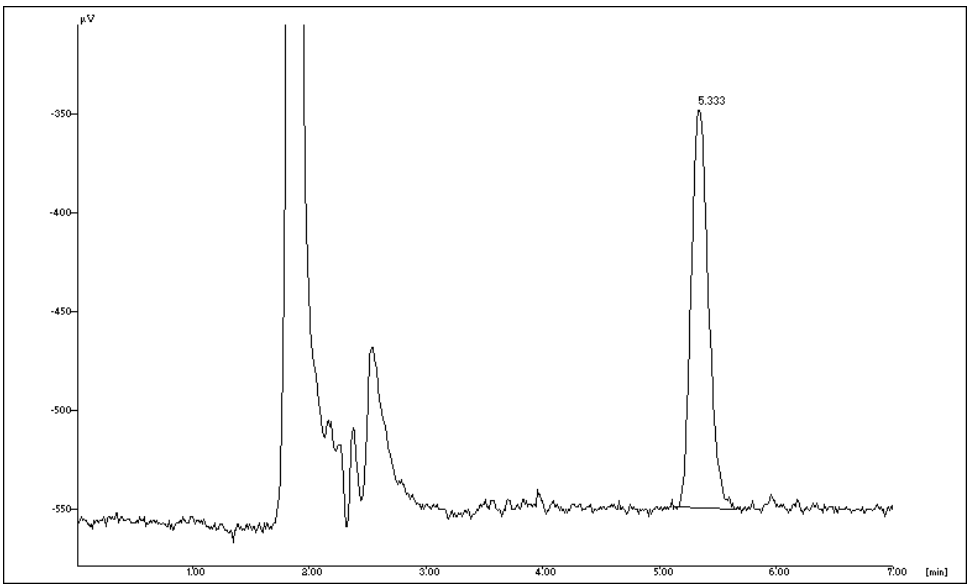

Figure 3.5: Chromatogram of gatifloxacin (QL - 17.4 $ng.ml^{-1}$). Mobile phase: aqueous phase (25 mM citrate buffer, 10 mM TBAHS and 10 mM SDS – pH adjusted to 3.5) and ACN – 52:48 % v/v.

Different concentrations of bench-top gatifloxacin solutions and stock solutions of gatifloxacin showed RSD values not more than 2.56 %, indicating robustness of the proposed method. These solutions exhibited no change in chromatographic characters (t_R, asymmetric

factor and area) at least till 36 hr at room temperature. During this period no extra peaks were observed in the chromatograms at all concentrations.

d) Estimation of drug content of formulations

The proposed method was evaluated by estimation of gatifloxacin in pharmaceutical formulations. The assay values of gatifloxacin for different formulations ranged from 99.12 to 103.69 % with standard deviation not more than 2.00. Assay values of formulations were very close to the label claim. This indicated that the interference of excipient matrix is insignificant in estimation of gatifloxacin by the proposed method (Table 3.12). This result supports the applicability of this method to a variety of formulations.

Table 3.12: Result of estimation of drug in commercial products by HPLC method.

Commercial products	**Amount found (Mean ± SD)** †	**% Assay (Mean ± SD)** †
Gaity-200 tablets (200 mg)	204.94 ± 0.82 mg	102.47 ± 0.41
Gatilox concentrated injection (10 $mg.ml^{-1}$)	9.91 ± 0.02 $mg.ml^{-1}$	99.12 ± 0.21
Gatilox eye drops (0.3 % w/v = 3 $mg.ml^{-1}$)	3.11 ± 0.03 $mg.ml^{-1}$	103.69 ± 0.99
Ophthalmic inserts (150 µg) ‡	153.85 ± 2.12 µg	102.57 ± 1.41

† Each value is the average of five separate determinations; ‡ Formulation was prepared in laboratory

3.6. Ion-pair RP-HPLC bio-analytical method

3.6.1. Experimental

(a) Chromatographic condition

The mobile phase consisted of aqueous phase (25 mM citrate buffer, 10 mM TBAHS and 10 mM SDS in triple distilled water – pH adjusted to 3.5) and ACN (55:45 % v/v). Before use, the mobile phase was filtered through 0.22 µm filter and degassed by sonication. Injection volume was 20 µl. Flow rate was adjusted to 1 $ml.min^{-1}$ and the response of fluorescence detector was optimized at 10X gain and 1X attenuation. The excitation wave length and emission wavelength of detector was set at 293 nm and 450 nm respectively. All the experiments were conducted at room temperature at approximately 25°C. The system was stabilized for at least 1 hr before analysis. Mobile phase composition was decided based on the t_R of the drug.

(b) Serum and aqueous humor sample collection

New Zealand rabbits weighing from 1.5 to 2 kg, free of any signs of ocular inflammation or gross abnormality, were used. Animal procedures conformed to the protocol approved by Institutional Animal Ethics Committee (Protocol No. IAEC/REC/5/2). Rabbits

were anaesthetised by intramuscular administration of ketamine HCl (35-50 $mg.kg^{-1}$) and xylazine (5-10 $mg.kg^{-1}$). Xylocaine solution was used as a local anesthetic. Aneket® (Ketamine - 50 $mg.ml^{-1}$; Neon laboratories Ltd., Mumbai, India), Xylaxin (Xylazine - 20 $mg.ml^{-1}$; Indian Immunologicals Ltd., Guntur, India) and Xylocaine® topical solution (Lidocaine hydrochloride - 42.7 $mg.ml^{-1}$; Astrazeneca Pharma, Bangalore, India) were used for anaesthetia. Aqueous humor (upto 100 µl) was withdrawn through the limbus by a syringe with a 30G needle attached to a 1 ml syringe. For collection of blood samples, unanaesthetised rabbits were kept in restraining boxes and a single puncture was made into marginal ear vein using a 22G needle. Blood was kept at room temperature in order to enable clotting of cell constituents and the serum was separated from the blood by centrifugation at 4,000 rpm for 10 min using Remi desktop centrifuge, Remi Instruments, India. The samples were stored at-20°C until analysis and all the samples were analysed within 15 days.

(c) Calibration curve

Primary stock solution of 50 $\mu g.ml^{-1}$ of gatifloxacin was prepared in TDW. From an aliquot of primary stock, secondary stock solution of 10 $\mu g.ml^{-1}$ was prepared in TDW. Analytical standards in the ranging from 50 to 2000 $ng.ml^{-1}$ and 20 to 120 $ng.ml^{-1}$ were prepared in similar manner given in previous section for rabbit aqueous humor and serum samples, respectively. Rabbit aqueous humor standards were ranging from 50 to 2000 $ng.ml^{-1}$ and rabbit serum standards were ranging from 20 to 120 $ng.ml^{-1}$, were prepared along with respective blank samples.

Frozen aqueous humor samples were thawed at room temperature. An aliquot (10 µl) of different drug solutions in TDW was added to 40 µl of blank aqueous humor sample and vortex mixed for 2 min to prepare different aqueous humor standards. Drug was extracted efficiently by one-step precipitation method. To 50 µl of drug spiked aqueous humor standard 150 µl of ACN was added and vortex mixed for 2 min and centrifuged at 10,000 rpm for 10 min at 4°C using Remi cooling compufuge (model CPR24 with servo controlled voltage stabilizer) Remi Instruments, India. The clear supernatant was aspirated and evaporated to dryness using Heto vacuum centrifuge, Max Dry Lyo, Heto-Holten, Denmark. The evaporated samples were reconstituted with ACN and 20 µl was injected into HPLC. Blank samples were processed in similar manner.

Frozen serum samples were thawed at room temperature. An aliquot (50 µl) of drug solution in TDW was added to 200 µl of blank serum sample and vortex mixed for 2 min. Using different concentrations of drug solutions, different serum standards were prepared. Drug was extracted efficiently by one-step precipitation method. To 250 µl of drug spiked

serum standard 750 µl of ACN was added and vortex mixed for 5 min and centrifuged at 10,000 rpm for 10 min at 4°C. The clear supernatant was aspirated and evaporated to dryness. The evaporated samples were reconstituted with ACN and 20 µl was injected into HPLC. Blank samples were processed in similar manner.

Recovery of gatifloxacin was assessed from rabbit aqueous humor and rabbit serum at different concentration levels. Recovery was calculated by using the following formula: % Relative Recovery = [(determined concentration of biomatrix sample)/(determined concentration of analytical standard)] × 100.

d) Analytical validation

Specificity was determined as non-interference at the t_R of gatifloxacin by proteins and others. Blank rabbit serum and aqueous humor samples from five different individual animals were processed and analysed.

As a part of determining accuracy of the proposed method, different levels of drug concentrations were prepared independently and analysed (n = 6). Quality control samples at three levels viz. 250 $ng.ml^{-1}$ (aqueous humor lower quality control sample - ALQC), 1000 $ng.ml^{-1}$ (aqueous humor medium quality control sample - AMQC) and 1750 $ng.ml^{-1}$ (aqueous humor higher quality control sample - AHQC) were prepared in aqueous humor by above mentioned procedure. Quality control samples at three levels viz. 20 $ng.ml^{-1}$ (serum lower quality control sample - SLQC), 50 $ng.ml^{-1}$ (serum medium quality control sample - SMQC) and 90 $ng.ml^{-1}$ (serum higher quality control sample - SHQC) were prepared in serum by above mentioned procedure. The concentrations were measured using the standard curve. The percentage recovery and percentage bias were taken as a measure for accuracy. Recovery was calculated by using the following formula: % Recovery = [(observed concentration)/(nominal concentration)] × 100. Percentage bias was calculated by using the following formula: % Bias = [(observed concentration – nominal concentration)/(nominal concentration)] × 100.

Repeatability was determined by using different levels of drug concentrations (as mentioned in accuracy), prepared independently and analysed (n = 6). Intermediate precision was done by similar procedure mentioned in the experimental section 3.3.1.c (n = 18). % RSD of the predicted concentrations from the regression equation was taken as precision.

DL and QL were determined using signal to noise ratio. A signal to noise ratio of 3 and 10 were taken as DL and QL respectively. The QL samples were prepared in replicates (n =5) using same procedure followed for calibration standards and analysed. Robustness of the proposed method was determined by checking the freeze thaw stability, post-extraction stability and long-term stability (Kole, 2006).

Freeze thaw stability of gatifloxacin in rabbit serum and aqueous humor samples was determined by preparing sample of strengths, mentioned in accuracy, in triplicates per cycle. Total four sets were prepared, one set was used for zero time reading and remaining sets were stored at -20°C. One cycle freezing step was done for at least 24 hr and thawing was achieved by keeping sample at 25 ± 3°C for 60 min. The % deviation from the initial concentration was calculated by the following formula: % Deviation = [(concentration after freeze thaw cycle – initial concentration)/(initial concentration)] × 100.

Post-extraction stability of gatifloxacin in rabbit serum and aqueous humor samples was determined by preparing sample of strengths, mentioned in accuracy, and analysed at different time points. Processed samples were kept at room temperature (25 ± 3°C) in the sample rack of the auto-injector and analysed at 3, 6, 12 and 24 hr. The % deviation from the initial concentration was calculated by the following formula: % Deviation = [(observed concentration – initial concentration)/(initial concentration)] × 100.

Long-term stability of gatifloxacin in rabbit serum and aqueous humor samples was determined by preparing sample of strengths, mentioned in accuracy, stored at -20°C and analysed at different time points. Samples were analysed at 1^{st}, 3^{rd}, 7^{th} and 15^{th} day of storage. Samples were thawed by at 25 ± 3°C for 60 min. The % deviation from the initial concentration was calculated by the following formula: % Deviation = [(observed concentration – initial concentration)/(initial concentration)] × 100.

3.6.2. Results and discussion

The chromatograms of the blank, test and spiked standard of rabbit aqueous humor and rabbit serum samples were shown in the Figure 3.6. and 3.7.

The final optimized mobile phase consisted of 55 % aqueous phase (25 mM citrate buffer, 10 mM SDS and 10 mM TBAHS at pH 3.5) with 45 % ACN. In this mobile phase, t_R and asymmetric factor of drug were found to be 6.50 min and 1.01 respectively. One step precipitation technique gave higher recoveries (Table 3.13). Other than drug no extra peaks were observed near drug peak and no interference from the protein impurities of the biomatrices, metabolites and degraded impurities near drug peak. This was evident from the chromatograms shown in the Figure 3.6. and 3.7.

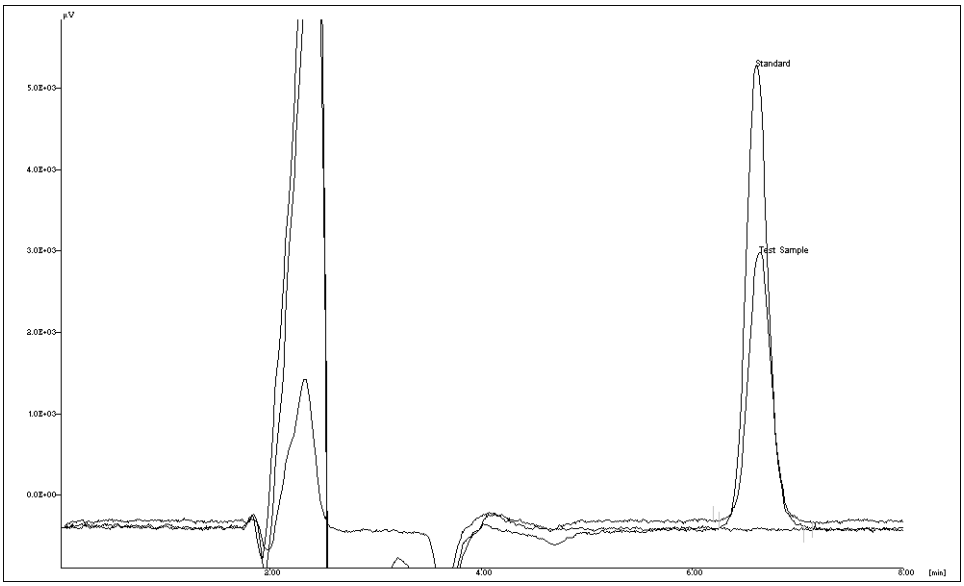

Figure 3.6: Overlay of chromatograms of blank, test sample and standard of gatifloxacin in rabbit aqueous humor.

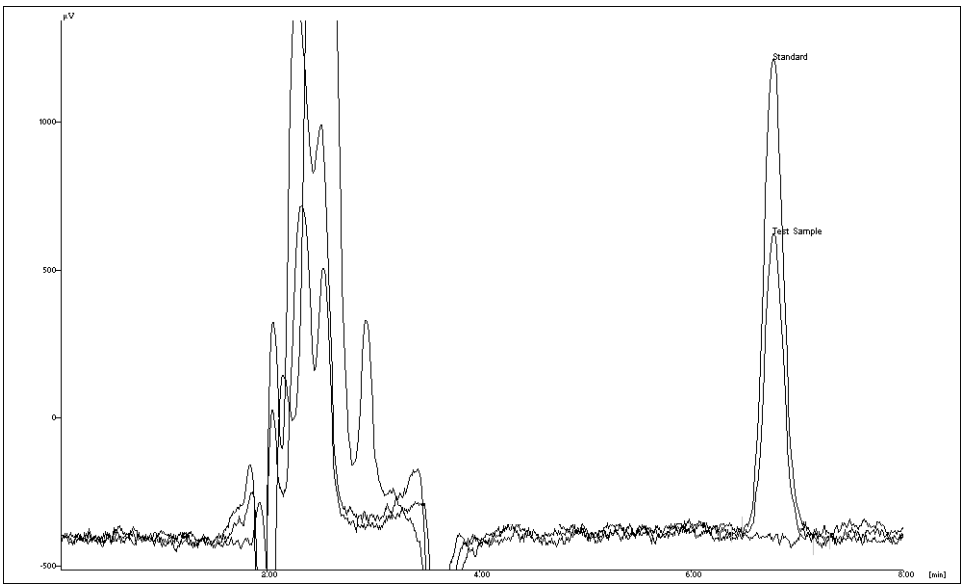

Figure 3.7: Overlay of chromatograms of blank, test sample and standard of gatifloxacin in rabbit serum.

(a) Calibration curve

Different concentrations and their corresponding areas were shown in the Table 3.13. At all the concentration levels the standard deviation of area was low and the relative standard deviation (% RSD) found to be below 6.5 %. Linearity range was found to be 50-2000 $ng.ml^{-1}$ and 20-120 $ng.ml^{-1}$ in aqueous humor and serum respectively.

In aqueous humor, the linear regression equation obtained was: Area (µV.sec) = 745.49 × Concentration ($ng.ml^{-1}$) + 210.12. The standard error values of slope and intercept were found to be 2.22 and 148 respectively. Goodness of fit of regression equation was supported by high regression coefficient value (0.999), low standard error of estimate (19.72) and low mean sum of the squared residuals value (385.06).

In serum, the linear regression equation obtained was: Area (μV.sec) = 754.66 × Concentration ($ng.ml^{-1}$) + 285.90. The standard error values of slope and intercept were found to be 6.14 and 26.57 respectively. Goodness of fit of regression equation was supported by high regression coefficient value (0.993), low standard error of estimate (2.89) and low mean sum of the squared residuals value (8.62). Lower values of parameters like standard error of slope, intercept and estimate indicated high precision of the proposed method.

Table 3.13: Calibration data of gatifloxacin in rabbit biomatrices by HPLC method.

Con. ($ng.ml^{-1}$)	**Area (μV.sec) † (Mean ± SD)**	**% RSD**	**% Relative recovery (Mean ± SD)**
Rabbit aqueous humor			
50	37484.6 ± 1975.4	5.27	99.67 ± 5.05
100	75180.1 ± 3492.8	4.65	98.82 ± 4.60
500	373605.6 ± 19427.5	5.20	96.09 ± 5.00
750	559697.2 ± 25421.5	4.54	99.10 ± 4.50
1250	930651.2 ± 10888.3	1.17	98.65 ± 1.15
1500	1116127.6 ± 6330.8	0.57	98.99 ± 0.56
2000	1493510.0 ± 1752.2	0.79	99.61 ± 0.78
Rabbit serum			
20	15314.7 ± 793.6	5.18	92.04 ± 4.86
40	30078.3 ± 1739.7	5.78	90.47 ± 5.28
60	45438.5 ± 1602.9	3.53	95.70 ± 3.40
80	62424.2 ± 2798.5	4.48	97.88 ± 4.41
100	74502.8 ± 2577.8	3.46	93.41 ± 3.24
120	90915.8 ± 1940.3	2.13	97.02 ± 2.08

† Each value is average of six separate determinations

(c) Analytical validation

Selectivity of the developed method can be confirmed since no significant interference peaks were observed at the t_R of gatifloxacin in the blank rabbit aqueous humor and serum samples (Figure 3.8 and 3.9).

In aqueous humor, at all the three concentration levels, % bias ranged from 0.63 to 3.12 % (Table 3.14). Good recovery was observed at all concentration level studied with SD less than 5, establishing accuracy of the developed method. In serum, at all the three concentration levels, % bias ranged from -3.05 to 1.55 % (Table 3.14). Good recovery was observed at all concentration levels studied with SD less than 9, indicating high accuracy.

In repeatability study of aqueous humor samples, the RSD was ranged from 2.24 to 4.47 % (Table 3.14). In the intermediate precision study, RSD values were not more than 7.47 % for intra-day variation, while inter-day variation was not more than 2.06 % (Table 3.15).

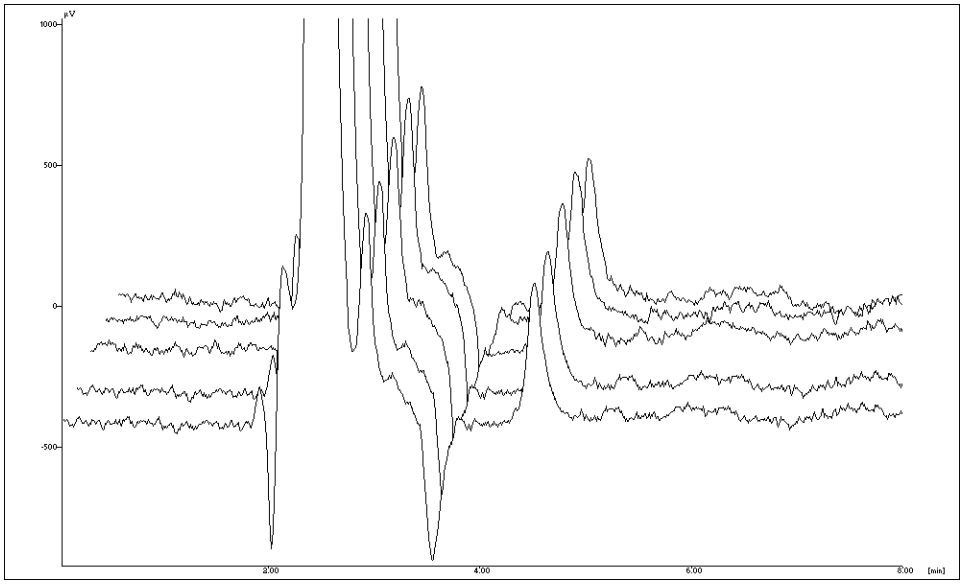

Figure 3.8: Overlay of chromatograms of blank samples of rabbit aqueous humor.

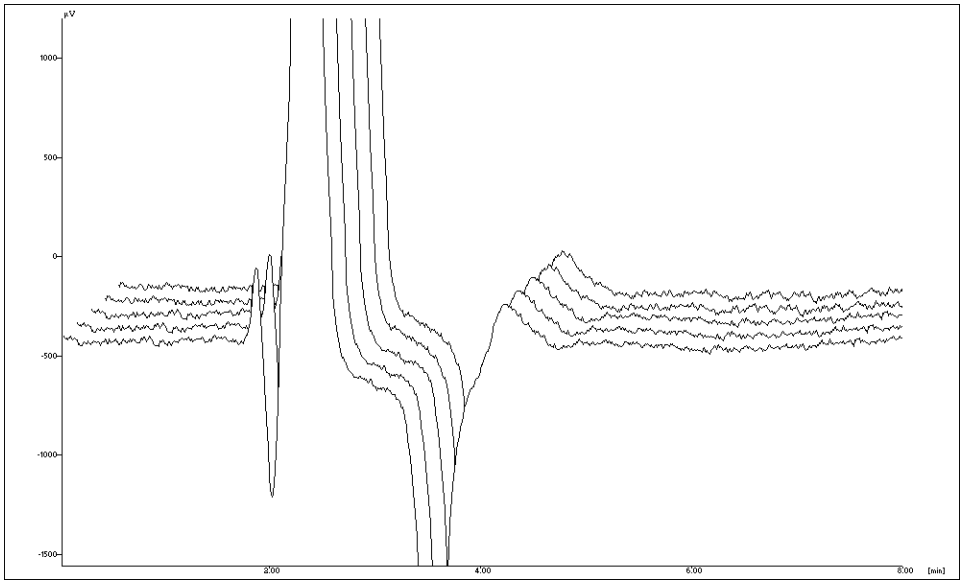

Figure 3.9: Overlay of chromatograms of blank samples of rabbit serum.

Table 3.14: Accuracy and precision data for the HPLC method.

Level	Predicted con. ($ng.ml^{-1}$)			% Recovery (Mean ± SD)	Bias (%)
	Range	Mean ± SD †	% RSD		
ALQC	239.38 - 285.77	257.81 ± 11.52	4.47	103.12 ± 4.61	3.12
AMQC	950.98 - 1099.55	1014.83 ± 50.21	4.95	101.48 ± 5.02	1.48
AHQC	1677.90 - 1832.59	1761.06 ± 39.53	2.24	100.63 ± 2.26	0.63
SLQC	15.98 - 21.75	19.39 ± 1.61	8.29	96.95 ± 8.03	-3.05
SMQC	43.01 - 53.85	49.12 ± 3.08	6.26	98.24 ± 6.15	-1.76
SHQC	84.70 - 100.21	91.40 ± 3.69	4.04	101.55 ± 4.10	1.55

† Each value is result of six separate determinations

In repeatability study of serum samples, the RSD was ranged from 4.04 to 8.29 % (Table 3.14). In the intermediate precision study, RSD values were not more than 11.36 % for intra-day variation, while inter-day variation was not more than 4.03 % (Table 3.15). Lower RSD values indicated the repeatability and intermediate precision of the method.

Table 3.15: Result of intermediate precision study of HPLC method.

Level	Intra-day repeatability - % RSD (n = 3)			Inter-day repeatability % RSD (n = 18)
	Day 1	Day 2	Day 3	
ALQC	3.91	1.13	5.38	1.79
	5.12	5.14	4.67	
AMQC	1.10	4.16	5.05	2.06
	2.65	2.69	7.47	
AHQC	0.46	1.71	1.97	1.25
	2.22	0.58	3.01	
SLQC	6.97	5.76	4.11	4.03
	11.36	8.95	9.75	
SMQC	5.26	6.67	8.45	2.46
	8.41	3.01	5.12	
SHQC	5.39	3.14	4.32	0.76
	2.48	3.39	5.37	

In aqueous humor, DL and QL were found to be 3.28 $ng.ml^{-1}$ and 9.95 $ng.ml^{-1}$ respectively. On repeated injections at QL, the characteristics of the drug peak (t_R, area and asymmetric factor) were not affected. The mean % recovery (± SD) of the drug at QL was found to be 101.51 % (± 4.38). In serum, DL and QL were found to be 5.39 $ng.ml^{-1}$ and 16.33 $ng.ml^{-1}$ respectively. On repeated injections at QL, the characteristics of the drug peak (t_R, area and asymmetric factor) were not affected. The mean % recovery (± SD) of the drug at QL was found to be 96.04 % (± 7.20). This represents the accuracy and precision of the method.

In freeze-thaw stability studies of gatifloxacin in aqueous humor and serum, the % deviation at all levels of concentrations was not exceeding 7.38 and 8.70 respectively (Figure 3.10). In the reconstituted samples in aqueous humor and serum, gatifloxacin was found to stable for 24 hr, with not more than 9.34 and 6.91 % deviation respectively (Figure 3.11). The frozen samples of gatifloxacin were found to be stable for 15 days, with not more than 7.90 and 9.21 % deviation respectively (Figure 3.12).

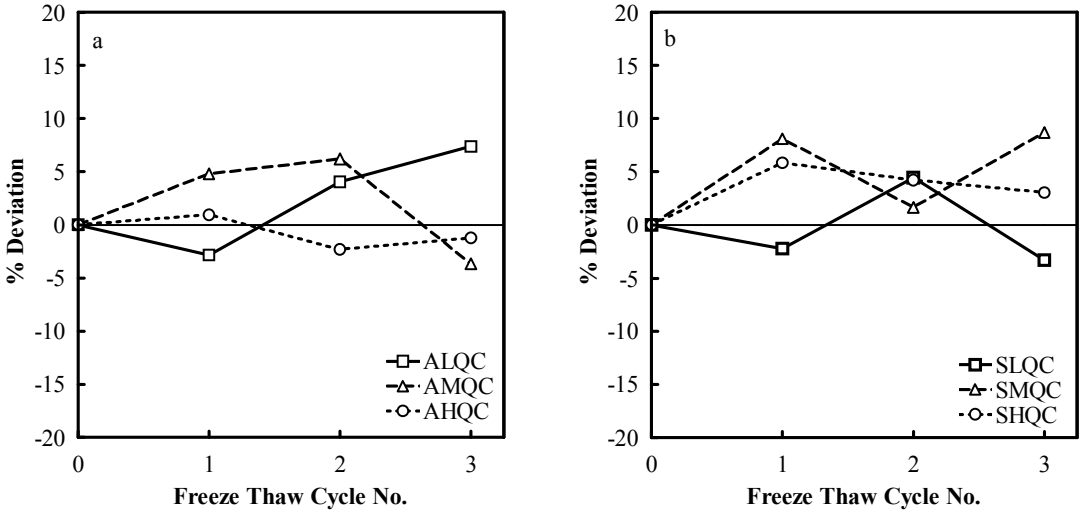

Figure 3.10: Freeze-thaw stability of gatifloxacin in (a) rabbit aqueous humor and (b) rabbit serum.

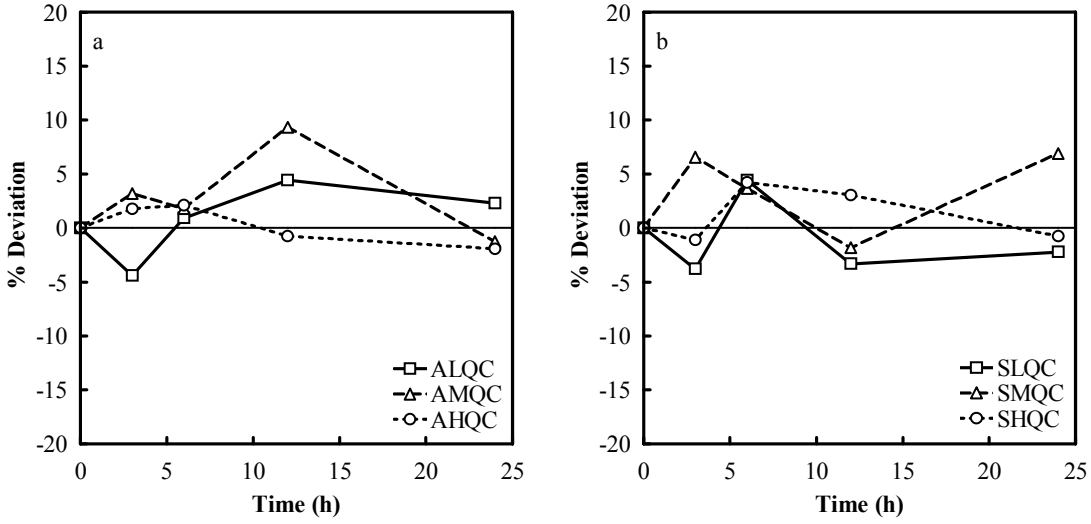

Figure 3.11: Post-extraction stability of gatifloxacin in (a) rabbit aqueous humor and (b) rabbit serum.

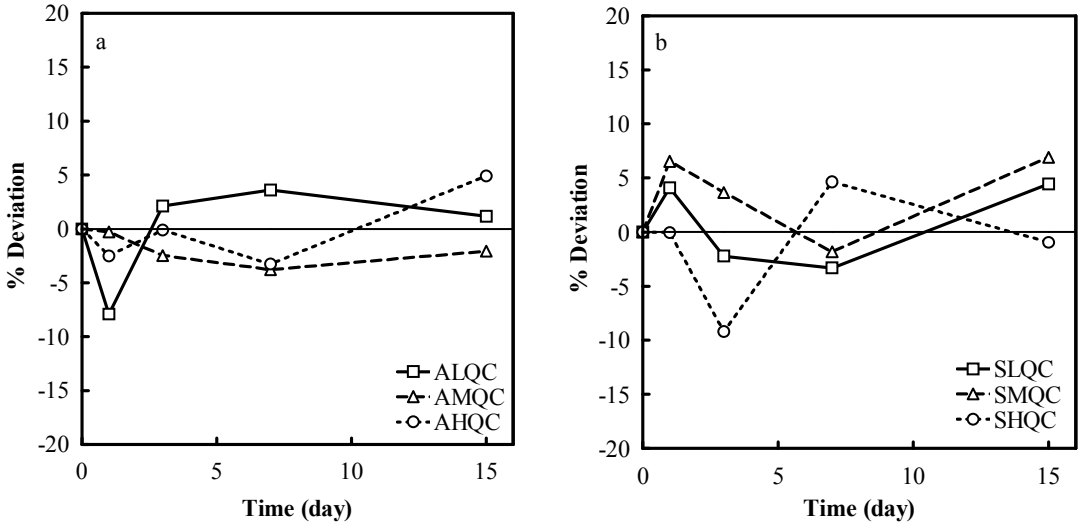

Figure 3.12: Long-term stability of gatifloxacin in (a) rabbit aqueous humor and (b) rabbit serum.

The comparision of values obtained at zero time and those up to study period of each stress condition showed maximum deviation of ± 10 % (well below the acceptable limits of < ± 20 % at LQC and < ± 15 % at all other QC levels). During these stress studies, no extra peaks were observed in the chromatograms at all concentrations, indicating no degradation and metabolism of the drug.

3.6. Conclusion

The developed, spectrophotometric, spectrofluorimetric and ion-pair RP-HPLC methods were sensitive, accurate and precise and can be used for analysis of gatifloxacin in bulk, pharmaceutical formulations, for in vitro drug release studies of ophthalmic formulations and corneal permeation studies. There is no interference of excipients in all these methods. The developed ion-pair RP-HPLC method for in vivo sample analysis was selective, sensitive, accurate, and precise and can be used for analysis of gatifloxacin in vivo samples. The developed methods were cheaper and more sensitive than reported methods.

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Chapter 4

Preformulation Studies

4.1. Introduction

Preformulation study is very critical for any formulation development process. Prior to proceeding to design any dosage form for a drug, it is important to determine certain physicochemical and other related properties of the bulk drug. A thorough understanding of these properties may ultimately provide a rationale for formulation development process and useful for trouble shooting in formulation development. These studies generally include generation of information on pH-solubility, partition coefficient, dissociation constant of the drug, solution state stability (including pH-stability) and solid state stability (including excipient compatibility). Scientific approach of preformulation study can make formulations easy, cheaper and effective.

4.2. Materials, equipment/instruments

4.2.1. Materials

Materials are same as mentioned in section 3.2.1 of chapter 3. Composition of buffers and reagents is given in appendix.

4.2.2. Equipment/instruments

A constant temperature shaker water bath (MAC instruments, India) was used for solubility studies and partition coefficient studies. For dissociation constant measurements, UV-Vis-spectrophotometer (Jasco, Japan) was used (for details refer to section 3.2.1). Samples were stored in Frost-free- 200 L Godrej refrigerator for the studies at refrigerated conditions. Humidity chamber (MAC Instruments, India) was used to maintain 40°C ± 2°C/75 % ± 5 % RH conditions. Higher temperature studies (60°C) were done in hot air oven (MAC Instruments, India). All pH measurements were performed using Elico pH meter (Elico, India) equipped with combination glass electrode filled with potassium chloride gel and with auto temperature adjustments. pH-Meter was calibrated with three standards of pH 4.00, 7.00 and 9.20. Thermal analysis was performed using a Shimadzu (Japan) differential scanning calorimeter (DSC) (Model: DSC-60; integrator: TA-60WS thermal analyzer; integrating software: TA-60WS collection monitor version 1.51; analysis software: TA60; principle: heat flux type; temperature range: -150 to 600°C; heat flow range: ± 40 mW; temperature program rate: 0-99°C.min^{-1}; atmosphere: inert nitrogen at 30 ml.min^{-1}). Characterization and compatibility studies were done using Shimadzu Fourier Transform Infrared (FT-IR) Spectrophotometer (model - IRPrestige-21), Shimadzu Corporation, Japan. The IRSolutions, version 1.10, software was used for IR data processing and plotting. Eluted TLC plates were checked in UV-Fluorescence chamber (Superfit, India).

4.3. Methods

4.3.1. Solubility study

Solubility of gatifloxacin was determined using different aqueous and non aqueous media. Different media used were TDW, ethanol, methanol, acetonitrile and acetone. Sufficient quantity of drug was added to 2 ml of each media in micro centrifuge tubes and kept for shaking at 37°C. All the samples were kept in triplicates. At frequent intervals of time these containers were checked for presence of insoluble drug. At different points of time (12, 24 and 48 hr) the samples were taken and centrifuged at 4000 rpm at 37°C for 10 min. The samples were kept for equilibration for 10 min, and then the supernatant was aspirated, diluted suitably and analysed by UV-spectrophotometric method as described in chapter 3. Study was continued till the last solubility values were matched.

Solubility of gatifloxacin was determined using different unbuffered pH media. Different unbuffered pH media used were 0.1 M HCl (pH 1.2), 0.01 M HCl (pH 2.0), TDW pH adjusted with HCl (pH 5.53), TDW (pH 6.5), TDW pH adjusted with NaOH (pH 6.84), TDW pH adjusted with NaOH (pH 7.41), TDW pH adjusted with NaOH (pH 8.25), 0.01 M NaOH (pH 11.9) and 0.1 M NaOH (pH 12.5). All the samples were kept in triplicates. At different point of time (3, 6 and 12 hr) the samples were taken, processed and analysed.

Solubility of gatifloxacin was determined using different pH buffer media. Different pH media used were pH 5.8, 6.0, 6.5, 6.8, 7.0, 7.2, 7.4 and 8.0 buffers (Indian Pharmacopoeia, 1996; US Pharmacopoeia XXIII, 2005). Sufficient quantity of NaCl was added to maintain uniform ionic strength (0.3 M) in all the media. All the samples were kept in triplicates. At different points of time (12, 24 and 48 hr) the samples were taken and analysed.

4.3.2. Determination of apparent partition coefficient

Apparent partition coefficient of gatifloxacin was determined using octanol/pH 7.4 buffer and chloroform/pH 7.4 buffer in the ratio of 1:1. All the studies were conducted only after presaturation of both aqueous and organic phases for 24 hr. Gatifloxacin solution (500 µg/ml) was prepared in aqueous medium. Initial drug concentration in aqueous phase was determined using UV-spectrophotometric and HPLC methods as described in chapter 3, after suitable dilution. Drug solution (1 ml) was added to organic phase (1 ml) in separate containers and kept for shaking at 37°C in triplicates. Samples were taken at different time points like 1, 2, 12, 24 and 48 hr. These samples were centrifuged at 4000 rpm at 37°C for 10 min. The mixture samples were kept for equilibration for 10 min, then the aqueous phase was

separated, suitably diluted and analysed by UV-spectrophotometric and HPLC methods as described in chapter 3. The apparent partition coefficient was calculated by the following equation: Apparent partition coefficient (P_{app}) = C_o/C_a; Where, C_o = Concentration of drug in oily and/or organic phase; C_a = Concentration of drug in aqueous phase.

Further, to study effect of pH on P_{app}, study was carried using buffers of different of pH (5.8, 6.0, 6.5, 6.8, 7.0, 7.2, 8.0 and 8.85) and octanol systems (1:1). Study was also carried using buffers of different of pH (5.70, 6.80, 7.60 and 8.3) and chloroform systems (1:1). P_{app} was determined using octanol/water and chloroform/water systems (1:1). Gatifloxacin solutions (concentration - 500 µg/ml) were prepared in corresponding aqueous media. Rest of the procedure was same as above.

4.3.3. Determination of dissociation constants (pK_a)

UV-spectrophotometric method for determination of pK_a depends on the neutral and ionic species having different spectra. When this criterion is met, excellent precision can be obtained in determination of pK_a (Barbosa et al., 2001). Primary stock solution of gatifloxacin (1000 µg/ml) was prepared by dissolving sufficient amount drug in 25 ml of TDW. Different pH media were prepared by adjusting pH of TDW, with 1M HCl for acidic side and 1M KOH for alkaline side. Total ionic strength was adjusted to 0.3 M using NaCl. Immediately after pH of the media was adjusted, aliquot volume of stock solution were transferred to 10 ml standard volumetric flask and diluted with different pH media to give drug concentration of 10 µg/ml. The resulting solutions were scanned in the wavelength range of 600-200 nm at speed of 200 nm/min using UV-Vis-spectrophotometer. The UV-spectra were examined to determine the wavelength at which there was a maximum change in absorbance as the pH was varied. The absorbance vs. wavelength profiles of the resulting solutions were immediately obtained. Absorbance values at the wavelength were plotted against pH. First derivative of absorbance respective to pH ($\Delta Abs/\Delta pH$) was plotted against mean pH and the pK_a was taken as the peak and/or valley (Pinsuwan et al., 1999). Each determination was performed in triplicate at 25°C ± 0.5°C.

4.3.4. Stability study

Both solution state stability and solid state stability of gatifloxacin were determined. Solution state stability study was done with different buffer systems to establish pH-stability profile and thermal study was done at different temperatures. Solid state stability was done in presence of different polymers and excipients at three different temperatures. All the stability data was fitted into different mathematical models and best fit was selected based on the

parameters like regression coefficient, mean sum of the squared residuals, and Akaike's information criteria (AIC) (Snehalatha, 2006).

(a) Solution state stability study

To establish pH-stability profile (or pH-rate profile - degradation rate constant vs. pH profiles), different pH media (pH 2, 3, 4, 5, 6, 6.5, 7, 7.4, 8 and 12.5) were prepared according to IP and USP (Indian Pharmacopoeia, 1996; US Pharmacopoeia XXIII, 2005). Sufficient quantity of NaCl was added to maintain uniform ionic strength (0.3 M) in all the media. Drug concentrations of 80, 800 and 8000 µM were prepared in different pH media. These solutions were filled into ampoules, sealed and stored at 25°C ± 2°C. The study was done in triplicate. Samples were taken at different time points, diluted suitably and analyzed by UV-spectrophotometric and selected samples were analysed by HPLC methods as described in chapter 3. To establish thermal stability and photostability, drug concentrations of 80, 800 and 8000 µM were prepared in pH 7.4 buffer. The study was done in triplicate. These solutions were filled into ampoules, sealed and stored at 40 ± 2°C and 60 ± 2°C. For photostability, the concentrations mentioned above were kept in natural sun-light. Samples were taken at different time points, diluted suitably and analyzed by UV-spectrophotometric and selected samples were analysed by HPLC methods as described in chapter 3. At different time points the solutions were spotted on a TLC plate (GF254) and eluted using mobile phase (dichloromethane : 2-propanol : tetrahydrofuran : 25 % aqueous ammonia = 4:6:3:3). Gatifloxacin dissolved in acetone was taken as control in all TLC studies.

(b) Solid state stability study

Differential scanning calorimetry (DSC) study was carried out for unprocessed gatifloxacin, gatifloxacin grinded for 5 min, 10 min, recrystallized using isopropyl alcohol (IPA) and acetone. After processing all the samples were passed through sieve number 80. Around 2 mg of the material was taken and sealed in standard non-hermetic aluminium pans with lid. The temperature range of measurement was 30°C to 300°C with a heating rate of 5°C.min^{-1}. After measurement, temperature was decreased back to starting temperature by cooling. Exothermic peaks recorded in the thermograms are directed upwards and endothermic peaks downwards. In another study, the heating cycle experiment was done to determine the temporary and/or permanent changes occurred to gatifloxacin during measurement of thermogram. In this experiment, same sample was heated from 35 to 175°C at 5°C.min^{-1}, then cooled to 145°C at 5°C.min^{-1} and again heated to 225°C at 5°C.min^{-1}.

DSC study was carried out for gatifloxacin, individual excipients and combination of gatifloxacin with excipients (mixture in the ratio of 1:1). The samples were assayed using UV-spectrophotometric method as described in chapter 3. Around 2 to 6 mg of the material was taken and sealed in standard aluminium pans with lid. The temperature range of measurement was 30°C to 300°C with a heating rate of 5°C.min^{-1}. These samples were stored at room temperature (CRT) for 12 months and the study was repeated.

The drug alone, polymer alone and in combination with different excipients (mixed in the ratio of 1:1) was taken and subjected to FT-IR studies. These samples were properly diluted with dried KBr and IR spectra were acquired in range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The data was processed using Kubelka Munk method.

Gatifloxacin (# 150 passed) and different excipients short listed for the preparation of ophthalmic formulations were physically mixed in a ratio of 1:100. Excipients used for this study were HPMC (different viscosity grades), HEC, different molecular weight grades PEO, Eudragit (Eu L100 and Eu S100), PL F127 and sodium alginate (SA). The drug and its corresponding physical mixtures with different excipients were prepared carefully by geometric mixing, filled in vials and kept at different temperature conditions, refrigerated temperature (FT: 5 ± 2°C), room temperature (CRT: 25 ± 2°C/60 ± 5% RH) and at accelerated condition (AT: 40 ± 2°C/75 ± 5 % RH). The samples were taken at predetermined time points and analyzed for drug content after suitable dilution with UV-spectrophotometric method as described in chapter 3.

4.4. Results and discussion

4.4.1. Solubility

The solubility data of gatifloxacin in different solvents is presented in Table 4.1. Among the non polar solvents, acetone and acetonitrile were showing highest solubility (20.02 ± 0.97 mg.ml^{-1} and 18.91 ± 0.17 mg.ml^{-1} respectively).

Table 4.1: Solubility of gatifloxacin in TDW and different non-aqueous media at 37°C ± 0.5°C.

Media	Solubility (mg.ml^{-1}) (Mean ± SD)
TDW	2.98 ± 0.04
Methanol	2.29 ± 0.22
Ethanol	2.09 ± 0.04
Acetonitrile	18.91 ± 0.17
Acetone	20.02 ± 0.98

Each value is average of 3 independent determinations

The pH-solubility profile of gatifloxacin in buffered and unbuffered pH media are shown in Figure 4.1 & 4.2 and Table 4.2 & 4.3, respectively.

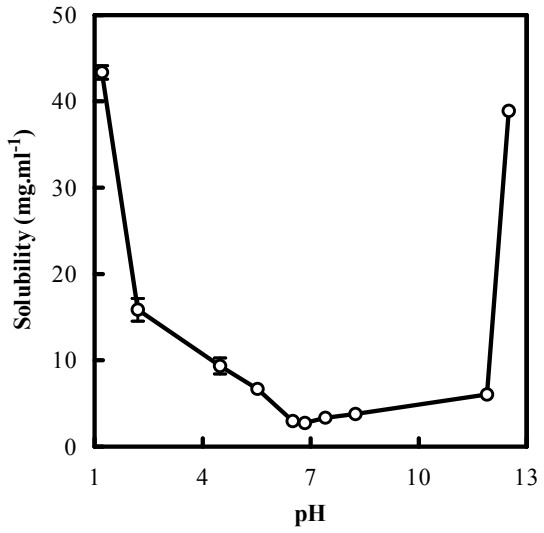

Figure 4.1: Solubility of gatifloxacin different pH media (unbuffered) at 37°C ± 0.5°C.

Table 4.2: pH-Solubility profile of gatifloxacin in different pH media (unbuffered).

pH	Solubility (mg.ml^{-1}) (Mean ± SD)
1.2	43.38 ± 0.77
2.2	15.87 ± 1.32
4.48	9.37 ± 0.93
5.53	6.67 ± 0.60
6.5	2.98 ± 0.04
6.84	2.75 ± 0.43
7.41	3.36 ± 0.21
8.25	3.78 ± 0.34
11.9	6.06 ± 0.36
12.5	38.89 ± 0.13

Each value is average of 3 independent determinations

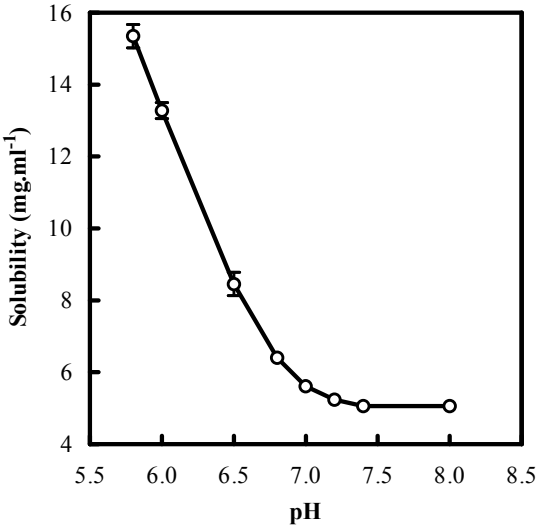

Figure 4.2: pH-Solubility profile of gatifloxacin (buffered pH media) at 37°C ± 0.5°C.

Table 4.3: pH-Solubility profile of gatifloxacin.

Buffer system	Solubility (mg.ml^{-1}) (Mean ± SD)
pH 5.8 buffer	15.35 ± 0.33
pH 6.0 buffer	13.27 ± 0.22
pH 6.5 buffer	8.46 ± 0.33
pH 6.8 buffer	6.40 ± 0.10
pH 7.0 buffer	5.61 ± 0.04
pH 7.2 buffer	5.24 ± 0.03
pH 7.4 buffer	5.07 ± 0.14
pH 8.0 buffer	5.06 ± 0.12

Each value is average of 3 independent determinations

Solubility of gatifloxacin was found to be pH dependent. In extreme pH it shows higher solubility (43.38 ± 0.77 and 38.89 ± 0.13 mg.ml^{-1} in pH 1.2 and 12.3 respectively). This is due to an increase in the concentration of the ionized species of the drug in extreme pH and a decrease of the unionized form. Low solubility was observed in neutral pH. Solubility was found to be higher in the case of buffered pH media as compared to un-buffered media. Drug in buffered media was showing relatively higher solubility as compared

to the unbuffered media. The obtained saturated solutions of gatifloxacin in some of the solvents and pH media (extreme pH) were yellow in colour at end of the study.

4.4.2. Apparent partition coefficient

The equilibrium partition of gatifloxacin between chloroform/pH 7.4 buffer and octanol/pH 7.4 buffer was achieved in 2 hr. The partition coefficients for chloroform/water and octanol/water systems were 0.781 ± 0.006 and 0.208 ± 0.021 respectively. P_{app} of gatifloxacin in octanol/buffer system is pH dependent. The results were given in Table 4.4 and Figure 4.3.

Table 4.4.Apparent partition coefficient data of gatifloxacin in different pH media system.

Chloroform/Aqueous media systems			**Octanol/Aqueous media systems**		
Aqueous Media	**P_{app} (Mean ± SD)**	**Log P_{app} (Mean ± SD)**	**Aqueous Media**	**P_{app} (Mean ± SD)**	**Log P_{app} (Mean ± SD)**
pH 5.7 buffer	0.597 ± 0.007	-0.22 ± 0.01	pH 5.8 buffer	0.050 ± 0.006	-1.30 ± 0.05
pH 6.8 buffer	0.711 ± 0.050	-0.15 ± 0.03	pH 6.0 buffer	0.084 ± 0.002	-1.07 ± 0.01
pH 7.4 buffer	0.751 ± 0.003	-0.12 ± 0.00	pH 6.5 buffer	0.140 ± 0.009	-0.85 ± 0.03
pH 8.0 buffer	0.741 ± 0.020	-0.13 ± 0.01	pH 6.8 buffer	0.123 ± 0.003	-0.91 ± 0.01
Water	0.781 ± 0.006	-0.11 ± 0.00	pH 7.0 buffer	0.154 ± 0.010	-0.81 ± 0.03
			pH 7.2 buffer	0.154 ± 0.010	-0.81 ± 0.03
			pH 7.4 buffer	0.143 ± 0.007	-0.84 ± 0.02
			pH 8.0 buffer	0.174 ± 0.002	-0.76 ± 0.00
			pH 8.85 buffer	0.149 ± 0.011	-0.83 ± 0.03
			Water	0.208 ± 0.021	-0.68 ± 0.04

Each value is average of 3 independent determinations

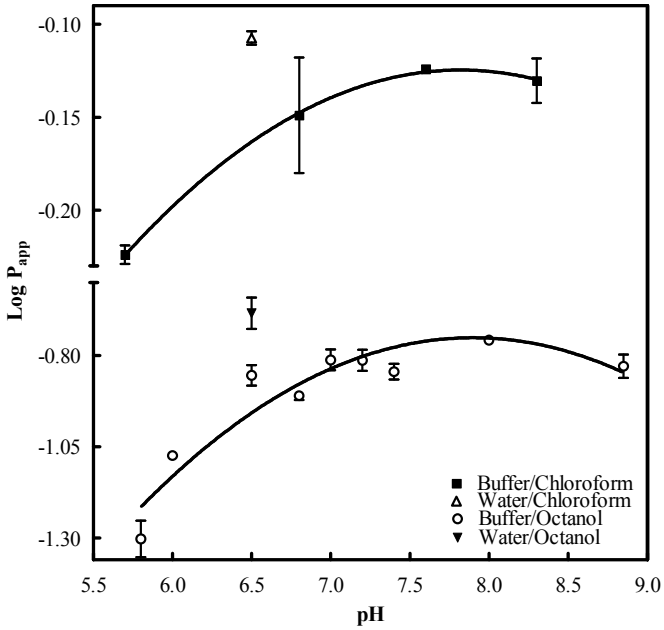

Figure 4.3: pH-Partition coefficient profile of gatifloxacin.

The pH-partition curve has a parabolic shape, typical profile of zwitterionic compounds (Takacs-Novak et al., 1995). From the Figure 4.3 it is evident that in lower pH (5.8 and 6.0), drug is showing relatively less partitioning and in higher pH (6.8 to 8.85), drug is showing relatively more partitioning. In acidic environment the drug might be in ionized state due to the presence of basic functional groups and in neutral pH drug might exist in a zwitterionic form. Partition coefficient of gatifloxacin was higher in the case of chloroform/buffer system rather than octanol/buffer system. Because chloroform is an acidic solvent, drug partitions more strongly into chloroform as compared to n-octanol, an amphoteric solvent (Rivera et al., 2000).

4.4.3. Dissociation constant (pK_a)

The reported pK_{a1} and pK_{a2} were 5.94 and 9.21, respectively and were determined by potentiometric titration method (Bristol Myers Squibb Company, 1999; Allergan Inc, 2003). Gatifloxacin showed pH dependent spectra (Figure 4.4).

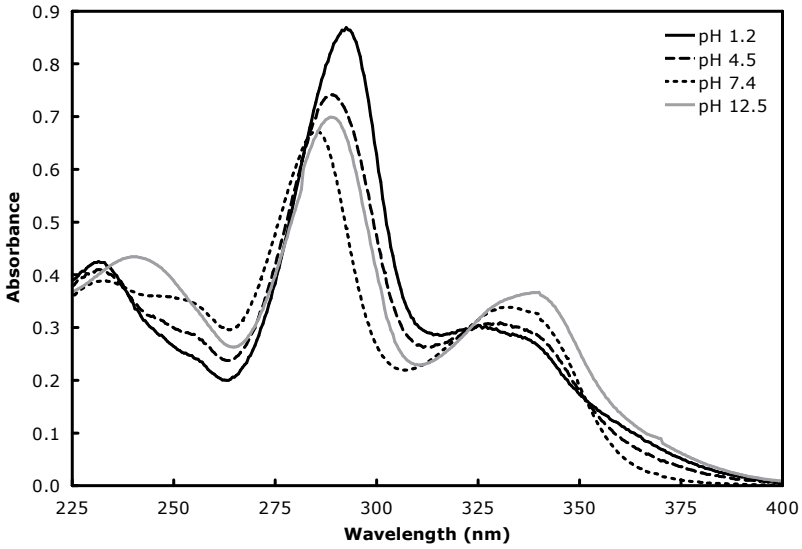

Figure 4.4: Spectra of gatifloxacin in different pH media.

Different wavelengths were selected for the determination of pK_a, based on the region where maximal changes in the spectra with respect to pH were observed. The selected wavelengths were 370, 365, 360, 330, 305, 300, 292, 286, 265, 263 and 260 nm (Table 4.5). At a given wavelength absorbance of same concentration of gatifloxacin was changing with pH (representative plot given in Figure 4.5a). Peaks and/or valley in the first derivative method were taken as pK_a (representative plots given in Figure 4.5 b and c). The resulted pK_a vales at different wavelengths and their mean data were presented in the Table 4.5.

At all the wavelengths studied the obtained pK_a values were same. The pK_{a1} (5.47 ± 0.21) of gatifloxacin is due to protonation of nitrogen of piperazinyl group and pK_{a2} (9.12 ± 0.07) is due to deprotonation of carboxylic acid group and the isoelectric pH (pI) was found be 7.30 ± 0.08. The low SD values represents that the UV-spectrophotometric method was reliable and reproducible. The values obtained by UV-spectrophotometric method were similar to that of reported method.

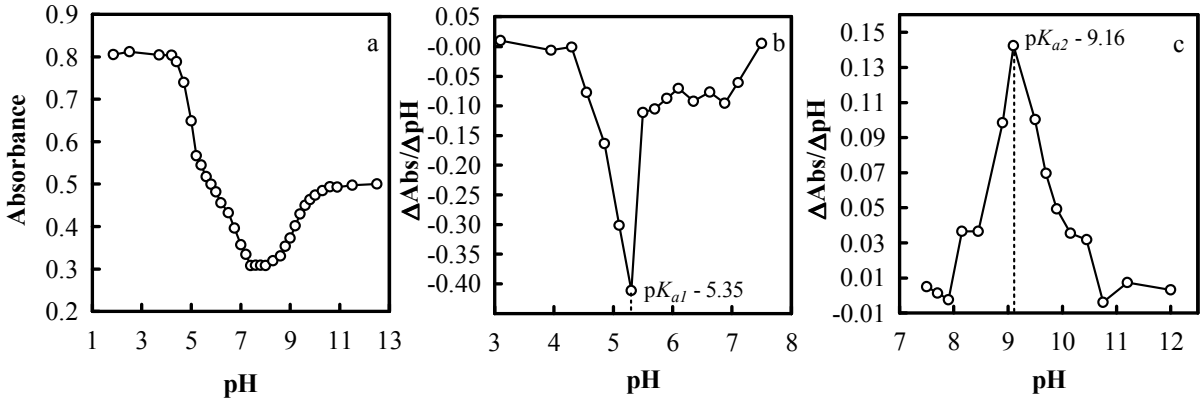

Figure 4.5: Plots of (a) pH-absorbance profile of gatifloxacin at 300 nm, (b) first derivative curve for pK_{a1} at 300 nm and (c) first derivative curve for pK_{a2} at 300 nm.

Table 4.5: Result of pK_a determination of gatifloxacin at different wavelengths.

Wavelength (nm) →	**370**	**365**	**360**	**305**	**300**	**292**	**286**	**265**	**263**	**260**	**Mean ± SD** †
pK_{a1}	5.50	5.52	5.54	5.34	5.35	5.45	6.01	5.35	5.33	5.31	5.47 ± 0.21
pK_{a2}	9.14	9.14	9.12	9.18	9.16	9.12	8.95	9.09	9.11	9.17	9.12 ± 0.07
pI	7.32	7.33	7.33	7.26	7.26	7.29	7.48	7.22	7.22	7.24	7.30 ± 0.08

† - The mean value was obtained from 10 different wavelengths

4.4.4. Stability

(a) Solution state stability

The Log percent remaining to be degraded (Log % RTD) versus time profiles were linear for all plots at all pH's indicating first order degradation. Best fit was supported by lower mean sum of squared residuals (MSSR) and AIC. Degradation rate constants (K_{deg}) obtained from the slopes of the curves were used to determine $t_{90\%}$ at various pH. The data was given in Table 4.6.

At all the levels of concentrations the degradation rate was pH dependent. At neutral pH the degradation was found to be minimal and at acidic and alkaline conditions the degradation rate was slightly high. For gatifloxacin concentration of 80 µM, the K_{deg} values were ranging from 19.08 × 10^{-3} (pH 6.5) to 39.56 × 10^{-3} (pH 12.5) $month^{-1}$ and $t_{90\%}$ values

were ranging from 2.66 to 5.52 month. For gatifloxacin concentration of 800 µM, the K_{deg} values were ranging from 25.36 × 10^{-3} (pH 7.4) to 58.08 × 10^{-3} (pH 2.0) $month^{-1}$ and $t_{90\%}$ values were ranging from 2.19 to 4.15 month. For gatifloxacin concentration of 8000 µM, the K_{deg} values were ranging from 23.69 × 10^{-3} (pH 7.0) to 58.48 × 10^{-3} (pH 12.5) $month^{-1}$ and $t_{90\%}$ values were ranging from 1.8 to 4.45 month. From the pH-rate profiles (Figure 4.6) of different concentrations it is evident that the drug was showing minimal degradation at around neutral pH (6 to 8). With increase in drug concentration from 80 to 8000 µM the pH-rate profiles showed similar pattern.

Table 4.6: First order reaction kinetics of pH-stability of gatifloxacin.

Concentration (µM)	pH	$K_{deg} \times 10^3$ ($month^{-1}$) (Mean ± SD) †	$t_{90\%}$ (month)	R^2	MSSR	AIC
80	2	34.97 ± 1.06	3.01	0.962	0.18	4.98
	3	33.35 ± 1.07	3.16	0.958	0.13	2.59
	4	29.72 ± 1.05	3.55	0.854	0.27	8.08
	5	27.57 ± 1.06	3.82	0.849	0.10	0.47
	6	21.69 ± 1.15	4.86	0.957	0.04	-7.73
	6.5	19.08 ± 1.15	5.52	0.976	0.01	-15.14
	7	23.31 ± 1.07	4.52	0.946	0.03	-9.29
	7.4	21.95 ± 1.03	4.80	0.953	0.01	-15.31
	8	26.36 ± 1.05	4.00	0.882	0.11	0.78
	12.5	39.56 ± 1.05	2.66	0.917	0.41	11.48
800	2	58.08 ± 1.03	2.19	0.947	0.26	7.79
	3	48.84 ± 1.07	2.16	0.980	0.05	-5.92
	4	41.34 ± 1.05	2.55	0.923	0.18	4.95
	5	41.27 ± 1.15	2.55	0.709	0.19	5.33
	6	26.76 ± 1.15	3.94	0.954	0.03	-8.56
	6.5	29.41 ± 1.08	3.58	0.986	0.01	-19.59
	7	28.22 ± 1.07	3.73	0.948	0.04	-7.00
	7.4	25.36 ± 1.03	4.15	0.935	0.03	-9.68
	8	25.41 ± 1.05	4.15	0.748	0.17	4.39
	12.5	43.22 ± 1.00	2.44	0.872	0.56	14.05
8000	2	56.26 ± 1.15	1.87	0.951	0.34	9.95
	3	57.11 ± 1.07	1.85	0.955	0.16	4.18
	4	47.78 ± 1.05	2.21	0.634	0.41	11.58
	5	45.12 ± 1.06	2.34	0.907	0.11	0.96
	6	37.22 ± 1.15	2.83	0.933	0.07	-2.73
	6.5	32.32 ± 1.08	3.26	0.899	0.08	-1.36
	7	23.69 ± 1.15	4.45	0.881	0.09	-1.08
	7.4	25.32 ± 1.04	4.16	0.963	0.02	-14.69
	8	32.40 ± 1.07	3.25	0.954	0.05	-5.89
	12.5	58.48 ± 1.06	1.80	0.946	0.30	8.88

† Each value is average of 3 independent determinations

The retention factor (R_f) for gatifloxacin was found to be 0.38 in the given mobile phase. There was no difference in the R_f values of gatifloxacin alone and gatifloxacin in different buffers. In acidic pH, at all concentration levels, TLC has shown one extra spot other than gatifloxacin under UV wavelength of 254 nm after 5 months of study with R_f value of 0.12. In basic pH, TLC has shown one extra spot other than gatifloxacin after 5 months of study with R_f value of 0.09. In neutral pH no extra spot was observed till the end of the study. This shows the stability of gatifloxacin at neutral pH conditions. These results correlate with the pH stability study of gatifloxacin.

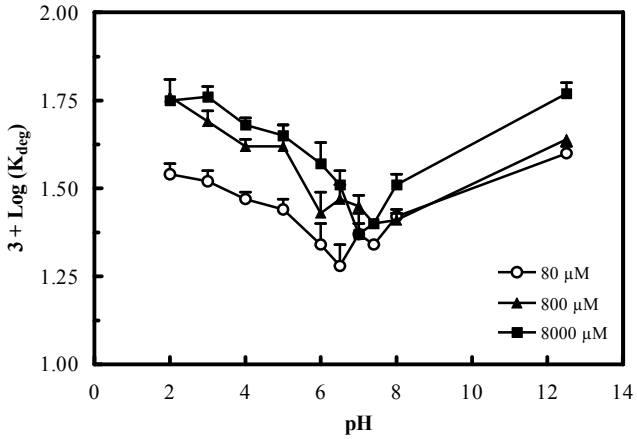

Figure 4.6: pH-Rate profile of gatifloxacin.

At different temperatures and sunlight, the Log % RTD versus time profiles were linear for all plots indicating first order degradation. Best fit was supported by lower MSSR and AIC (Table 4.7).

Table 4.7: First order reaction kinetics of thermal degradation and photostability studies of gatifloxacin in pH 7.4 phosphate buffer.

Concentration (µM)	Condition	$K_{deg} \times 10^3$ (month^{-1}) (Mean ± SD) †	$t_{90\%}$ (month)	R^2	MSSR	AIC
80	25°C	21.95 ± 1.03	4.80	0.953	0.01	-15.31
	40°C	39.83 ± 1.57	2.65	0.859	0.45	7.83
	60°C	60.65 ± 1.02	1.74	0.969	0.19	1.81
	Natural light	329.32 ± 10.50	0.32	0.959	0.29	-7.98
800	25°C	25.36 ± 1.03	4.15	0.935	0.03	-9.68
	40°C	44.21 ± 0.54	2.38	0.955	0.08	3.96
	60°C	70.98 ± 0.73	1.48	0.972	0.11	8.30
	Natural light	355.10 ± 21.21	0.30	0.956	0.09	-17.23
8000	25°C	25.32 ± 1.04	4.16	0.963	0.02	-14.69
	40°C	46.14 ± 1.97	2.28	0.866	1.18	4.31
	60°C	73.55 ± 1.93	1.43	0.915	0.69	1.73
	Natural light	354.49 ± 9.58	0.30	0.968	0.96	1.65

† Each value is average of 3 independent determinations

Degradation rate constants obtained from the slopes of the curves were used to determine $t_{90\%}$ at various conditions. At all the levels of concentrations the degradation rate was temperature dependent. At 40°C, the K_{deg} values were ranging from 39.83×10^{-3} to 46.14×10^{-3} $month^{-1}$ and $t_{90\%}$ values were ranging from 2.28 to 2.65 month. At 60°C, the K_{deg} values were ranging from 60.65×10^{-3} to 73.55×10^{-3} $month^{-1}$ and $t_{90\%}$ values were ranging from 1.48 to 2.74 month. The drug was found to degrade rapidly under sunlight. The K_{deg} values for different concentrations were ranging from 329.32×10^{-3} to 355.10×10^{-3} $month^{-1}$ and $t_{90\%}$ values were ranging from 0.30 to 0.32 month. The photodegradation of gatifloxacin was not concentration dependent at neutral pH. After 2 months of study at 40°C, TLC has shown one extra spot other than gatifloxacin with R_f value of 0.09. After 1 month, samples stored at 60°C showed one extra spot in TLC, with R_f value of 0.09. Samples stored under sunlight conditions, at all concentration levels, TLC has shown one extra spot other than gatifloxacin after 7 days of study with R_f value of 0.07. In neutral pH, at low temperature no extra spot was observed till the end of the study indicating high stability.

(b) Solid state stability

Thermograms of unprocessed gatifloxacin, processed gatifloxacin, polymers alone and polymer drug combinations were shown in Figure 4.7 to 4.10 and thermal parameters were given in Table 4.8. Gatifloxacin showed a melting point at 186 to 189°C by visual melting point apparatus. A part of material was melting at 168 to 172°C. When unprocessed gatifloxacin was subjected to DSC the melting endotherm was observed at 189.4°C. Another endotherm and exotherm were observed between 165 to 175°C (Figure 4.7 a), which represents another form of gatifloxacin.

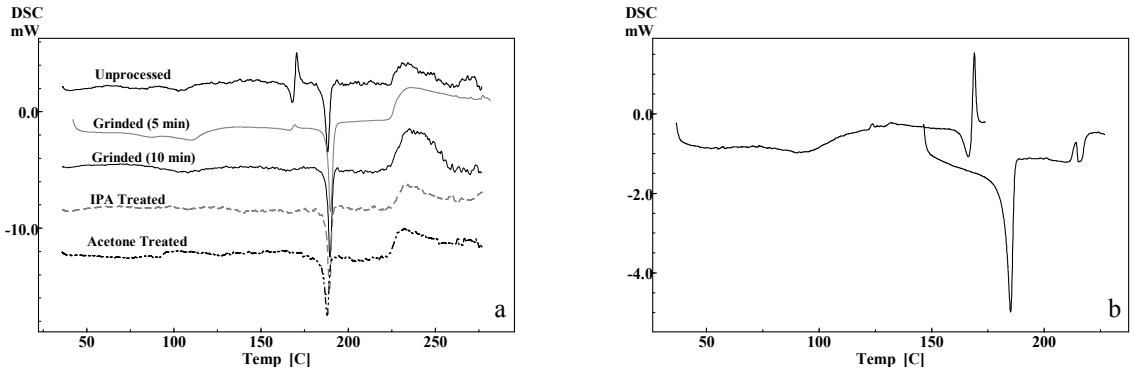

Figure 4.7: Thermograms of gatifloxacin (a) unprocessed, grinded and solvent treated gatifloxacin (b) three heating cycles.

This form was minimized on grinding for 5 minutes and disappeared when grinded for 10 minutes, without affecting the drug melting. Similar effect was seen in the case gatifloxacin treated with IPA and acetone. DSC plot of gatifloxacin showed that used gatifloxacin have two polymorphic forms and these have distinctly different melting points of ~170 and ~189°C. First form was shown to be unstable and it converts to second form by grinding or by recrystallizing in different solvents. The endotherm corresponding to first form had disappeared when the sample was heated again, which represents that this form converted to second from irreversibly (Figure 4.7 b). The sum of enthalpies of first and second endothermic was 82.75 $J.g^{-1}$ and where as for processed drug endotherm enthalpy was 82.69 $J.g^{-1}$, indicating that the first form had converted to the second. The energy associated with the endotherm between 188 and 191°C for grinded and treated gatifloxacin was same.

Figures 4.8 to 4.10 represent thermograms of gatifloxacin, excipient and physical mixtures of gatifloxacin with different polymers selected for study. Melting endotherms of gatifloxacin were well preserved in most of the cases. However, there were slight changes in the peak shape with little broadening or shifting towards the lower temperature, which could be attributed to the mixing process that lowers purity of each component in the mixture as also reported by Verma and Garg (2004). Thermal properties of different thermograms were tabulated in Table 4.8.

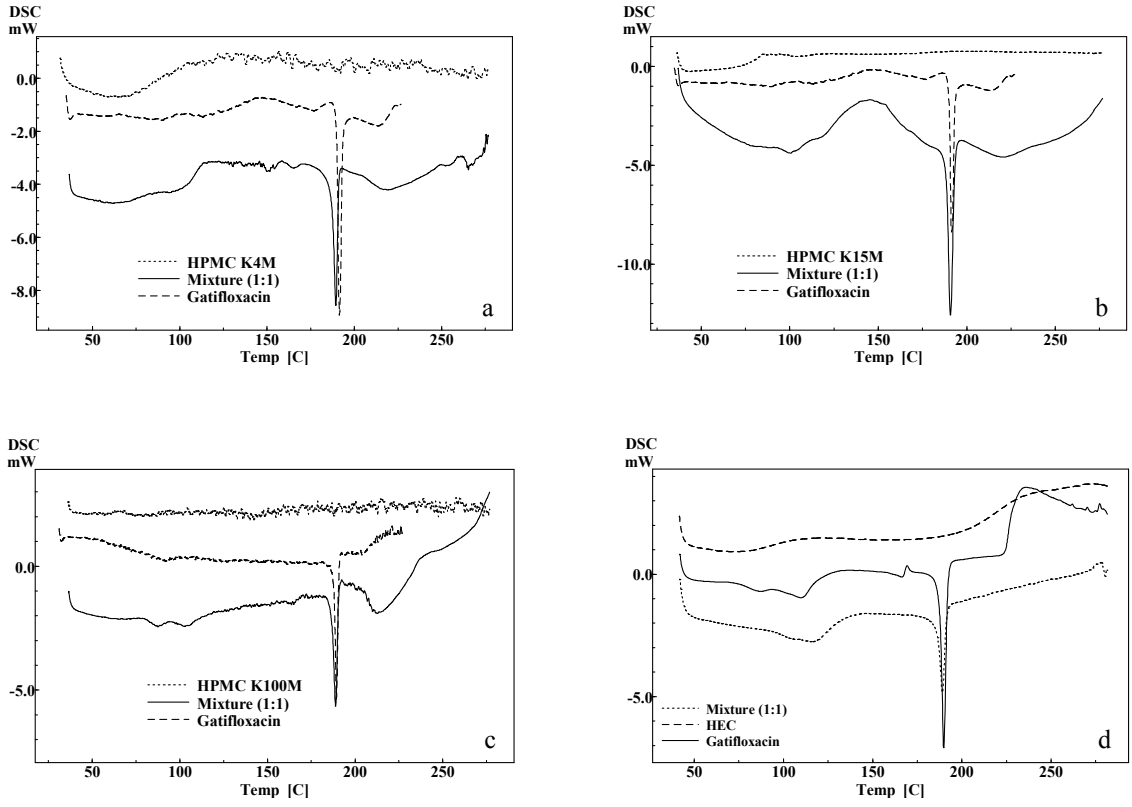

Figure 4.8: Thermograms of gatifloxacin alone, polymer alone and combination (a) gatifloxacin with HPMC K4M; (b) gatifloxacin with HPMC K15M; (c) gatifloxacin with HPMC K100M; (d) gatifloxacin with HEC.

Figure 4.8 a-c shows the thermograms of pure gatifloxacin, HPMC (different viscosity grades) and physical mixtures (1:1). Polymers were not showing any peaks and the endothermic peak of gatifloxacin was retained in the physical mixture with no change in enthalpy value (Table 4.8). Similar results were observed with HEC mixtures (Figure 4.8d). Based on the results, it was concluded that gatifloxacin is compatible with HPMC and HEC.

The DSC thermogram of different grades of PEO showed an endothermic peak ranging from 68 to 72°C (Figure 4.9 a-f). Endothermic peak of gatifloxacin was shifted slightly towards lower temperature; peak was broadened and small change in shape. However, enthalpy values of drug had not changed significantly (Table 4.8), no considerable incompatibility between drug and PEO.

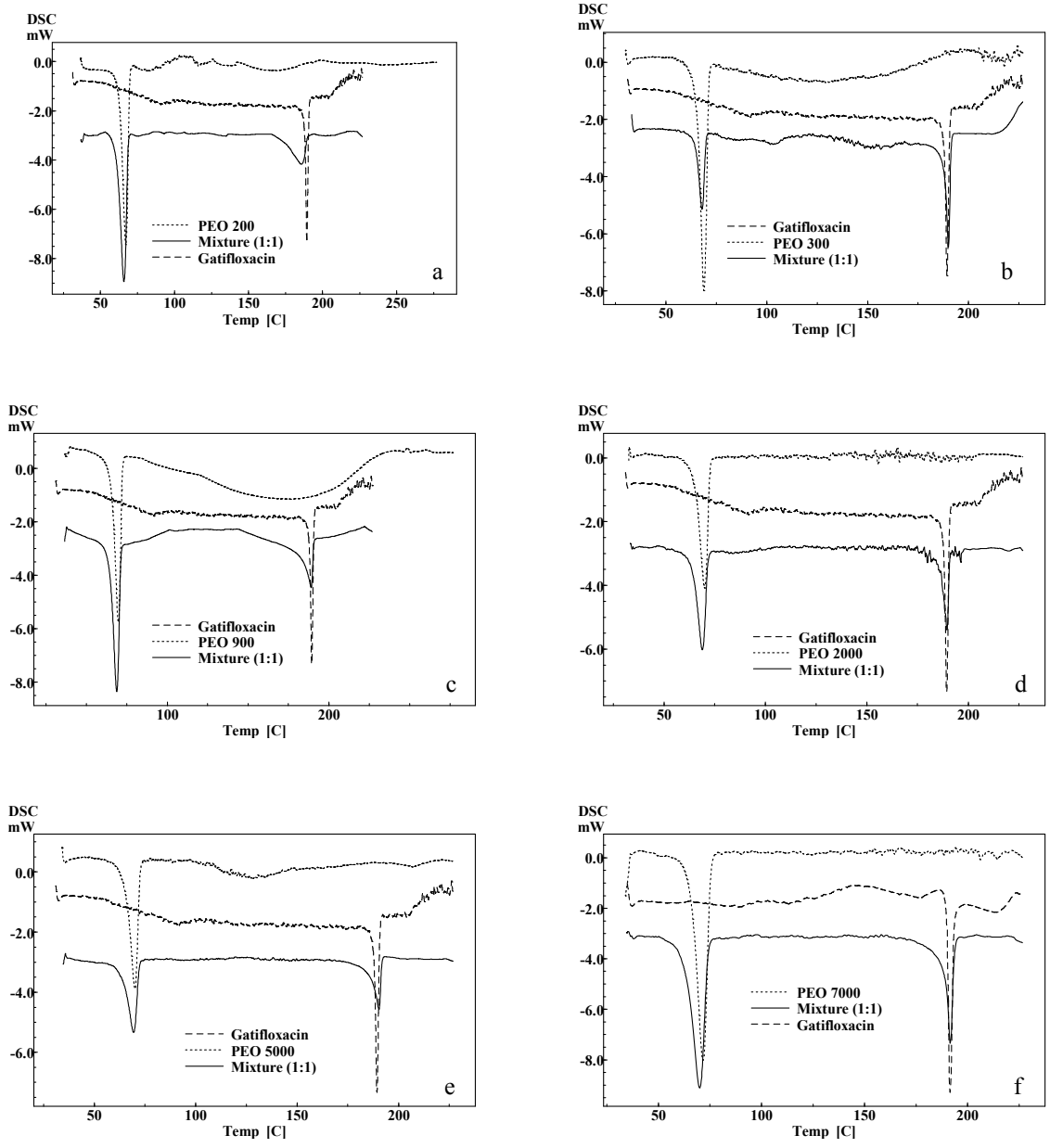

Figure 4.9: Thermograms of gatifloxacin alone, polymer alone and combination (a) gatifloxacin with PEO 200; (b) gatifloxacin with PEO 300; (c) gatifloxacin with PEO 900; (d) gatifloxacin with PEO 2000; (e) gatifloxacin with PEO 5000; (f) gatifloxacin with PEO 7000.

Table 4.8: Thermal properties of drug alone, polymer alone and in combination (mixture in the ratio of 1:1).

Sample	Wt. (mg) †	Assay (%) ‡	Amt. (mg) •	Peak	Onset (°C)	Peak (°C)	End set (°C)	Heat (mJ)	Heat ($J.g^{-1}$)
Gatifloxacin (GT)	1.78	99.89	1.78	Endo	188.19	189.4	190.69	145.25	81.69
HPMC K4M	2.49	-	2.49	No Peak	-	-	-	-	-
GT + HPMC K4M	5.53	96.65	2.67 ¨	Endo	186.91	189.4	190.94	211.74	79.23
HPMC K15M	2.88	-	2.88	No Peak	-	-	-	-	-
GT+HPMC K15M	5.37	99.74	2.68 ¨	Endo	186.57	188.72	190.5	216.58	80.87
HPMC K100M	2.64	-	2.64	No Peak	-	-	-	-	-
GT + HPMC K100M	5.38	102.87	2.77 ¨	Endo	186.26	188.84	190.45	220.55	79.70
HEC	2.80	-	2.80	No Peak	-	-	-	-	-
GT + HEC	5.67	96.15	2.73 ¨	Endo	185.21	187.71	189.38	211.07	77.43
PEO 200	2.66	-	2.66	Endo	62.87	67.1	69.46	437.78	164.58
GT + PEO 200	4.61	-	2.39 ¨	Endo	60.95	65.81	68.4	357.59	161.35
		96.15	2.22 ¨	Endo	173.26	185.63	189.58	161.2	72.74
PEO 300	2.76	-	2.76 ¨	Endo	65.28	68.86	71.18	418.04	151.46
GT + PEO 300	3.47	-	1.66	Endo	65.77	67.90	69.72	261.7	144.83
		104.15	1.81 ¨	Endo	188.12	190.04	191.34	128.14	70.91
PEO 900	2.67	-	2.67	Endo	65.71	69.74	74.29	380.73	142.60
GT + PEO 900	4.84	-	2.44 ¨	Endo	64.56	68.78	71.17	330.68	137.79
		99.17	2.40 ¨	Endo	184.51	189.01	190.59	169.32	70.55
PEO 2000	1.99	-	1.99	Endo	65.32	70.06	72.52	275.89	138.64
GT + PEO 2000	3.84	-	1.87 ¨	Endo	65.47	68.89	71.56	245.88	124.60
		102.78	1.97 ¨	Endo	185.69	189.48	190.86	139.94	70.91
PEO 5000	2.11	-	2.11	Endo	65.68	70.09	72.44	291.07	137.95
GT + PEO 5000	4.08	-	1.98	Endo	64.83	69.42	72.13	258.06	122.68
		103.11	2.10 ¨	Endo	186.76	190.38	191.75	149.46	71.05
PEO 7000	2.16	-	2.16	Endo	65.62	70.67	73.16	314.42	145.56
GT + PEO 7000	4.30	-	2.12 ¨	Endo	64.95	69.38	72.24	299.83	137.33
		101.55	2.18 ¨	Endo	187.99	189.88	191.22	155.52	71.23
Eu L100	2.41	-	2.41	No Peak	-	-	-	-	-
GT + Eu L100	6.07	99.32	3.01 ¨	Endo	180.98	187.28	190.39	219.79	72.91
Eu S100	2.06	-	2.06	No Peak	-	-	-	-	-
GT + Eu S100	4.26	97.77	2.08	Endo	185.19	187.19	189.15	150.39	72.22
PL F127	1.98	-	1.98	Endo	55.06	57.46	58.80	174.94	88.35
GT + PL F127	4.10	-	2.10 ¨	Endo	51.28	55.98	58.35	158.91	79.59
		97.40	2.00 ¨	Endo	181.80	187.41	191.4	110.11	55.15
SA	2.66	-	2.66	No Peak	-	-	-	-	-
GT + SA	6.72	99.74	3.35 ¨	Endo	188.53	190.83	193.29	273.22	81.53

† weight taken in pan; ‡ Mean of 3 determinations and RSD values of all samples were not more than 3.5%; • amount of sample taken in pan based on the total amount taken and/or assay value of gatifloxacin; ¨ Amount calculated from gatifloxacin assay value; 'Endo' is endotherm.

Figure 4.10 a-c shows the thermograms of pure gatifloxacin, Eu L100, Eu S100, SA and physical mixtures (1:1). Polymers were not showing any peaks and the endothermic peak

of gatifloxacin was retained in the physical mixture with no change in enthalpy value (Table 4.8). This indicates there is no possible incompatibility between drug and studied excipients.

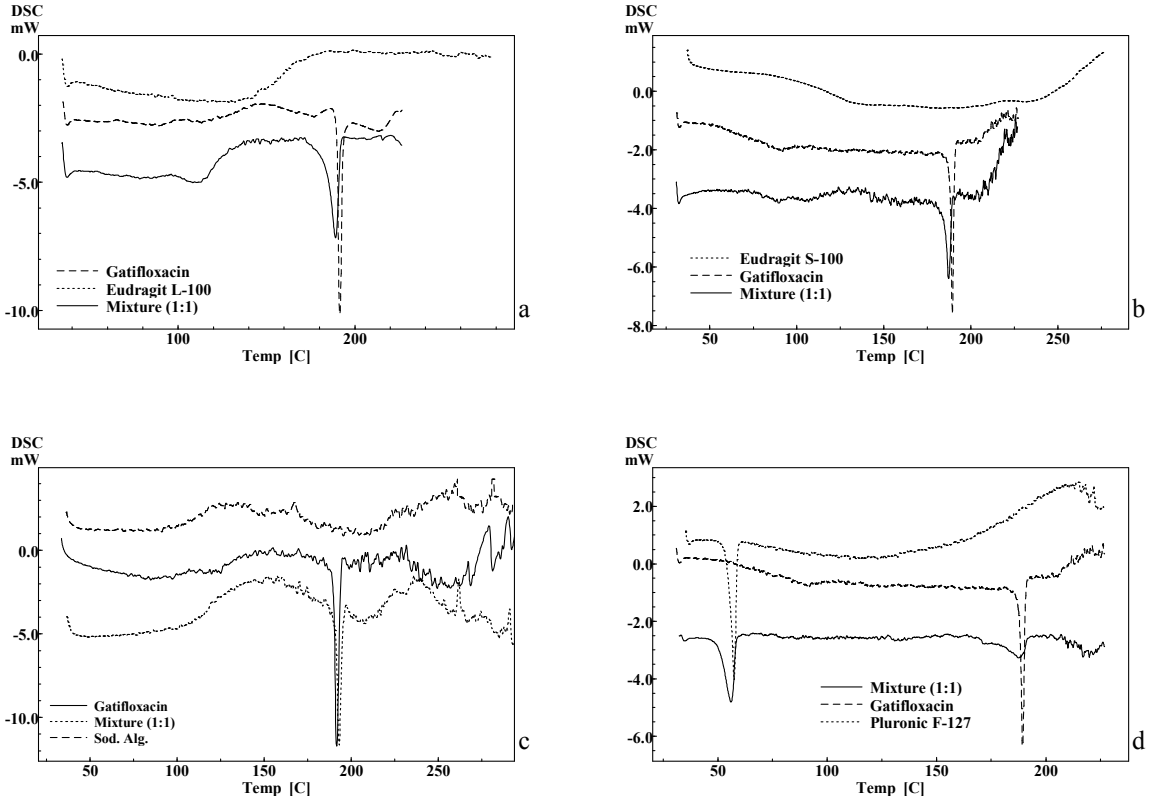

Figure 4.10: Thermograms of gatifloxacin alone, polymer alone and combination (a) gatifloxacin with Eudragit L-100 (Eu L100); (b) gatifloxacin with Eudragit S-100 (Eu S100); (c) gatifloxacin with sodium alginate (SA); (d) gatifloxacin with Pluronic F-127 (PL F127).

The thermogram of PL F127 showed an endothermic peak at 55.98°C (Figure 4.10 d). Endothermic peak of gatifloxacin was shifted slightly towards lower temperature; peak was broadened with small change in shape. However, enthalpy value of drug was lowered (Table 4.8) and that can be due lower melting polymer. The enthalpy was reduced but the position of endotherm of drug was not changed significantly, emphasis that there was no considerable incompatibility between drug and PL F127.

Few important IR bands of gatifloxacin were identified. An IR band was observed at 1729 cm^{-1} which can be assigned to the C=O stretch. Broad IR band was observed 3300 to 2500 cm^{-1}, assigned to carboxylic O−H stretch. The C−H stretches of the CH_2 and CH_3 represented by strong bands at 2854 and 2938 cm^{-1}. The bands at 1620 and 1533 cm^{-1} were assigned to aromatic C=C and/or C=N stretch. A broad weak band representing aliphatic N−H stretch was observed at 3428 cm^{-1}. A weak IR band representing C−O stretch of aromatic alkyl ether was observed at 1232 cm^{-1}. A strong IR band representing C−O stretch of alkyl ether was observed at 1152 cm^{-1}. The C−H bending of CH_2 and CH_3 represented by

strong bands at 1366 and 1450 cm^{-1}. These IR bands were in agreement with the reported FT-IR spectra of gatifloxacin (Wang et al., 2006). In all the drug-excipient combinations studied, these important bands were identified, which suggested very less to no chemical interaction between the excipients and gatifloxacin. FT-IR study gives more evidence that there is no chemical incompatibility between drug and excipients studied, even though the enthalpy of gatifloxacin was lowered with some excipients (lower melting range excipients) in DSC study.

Physical mixtures of gatifloxacin with different formulation excipients were prepared in the ratio of 1:100. The degradation rate has followed first order reaction kinetics. Table 4.9 gives the degradation rate kinetics of drug alone and drug in different combinations. In refrigerated temperature, gatifloxacin and all combinations were stable for entire period of study. The degradation of drug stored at CRT and AT followed first order kinetics, which is evident by high regression value and low AIC. The K_{deg} values of pure gatifloxacin were found to be 0.63×10^{-3} and 3.56×10^{-3} $month^{-1}$ at CRT and AT conditions, respectively. The $t_{90\%}$ was found to be 166.9 and 29.6 months respectively.

The K_{deg} values for all the combinations ranged from 0.5×10^{-3} to 0.72×10^{-3} $month^{-1}$ when stored in CRT conditions. The highest rate was observed for gatifloxacin in combination with PEO 200 and lowest rate was observed for gatifloxacin in combination with HPMC K4M. At these storage conditions the $t_{90\%}$ was ranging from 145.8 to 210.9 months. Gatifloxacin, alone and in combination with different excipients, was stable for more than 24 months at this condition.

Table 4.9: First order reaction kinetics of incompatibility studies of gatifloxacin with different excipients.

Gatifloxacin (GT) + Excipient (1:100)	**CRT (25 ± 2°C and 60 ± 5 % RH)**				**AT (40 ± 2°C and 75 ± 5 % RH)**			
	$K_{deg} \times 10^3$ ($month^{-1}$) †	**$t_{90\%}$ (month)**	**R^2**	**AIC**	**$K_{deg} \times 10^3$ ($month^{-1}$) †**	**$t_{90\%}$ (month)**	**R^2**	**AIC**
GT	0.63 ± 0.03	166.9	0.978	-7.8	3.56 ± 0.12	29.6	0.981	-8.3
GT + Eu L100	0.65 ± 0.02	161.6	0.938	-8.4	3.39 ± 0.05	31.1	0.967	-7.6
GT + Eu S100	0.58 ± 0.01	182.0	0.958	-8.0	3.41 ± 0.02	30.9	0.984	-9.0
GT + HEC	0.52 ± 0.01	202.0	0.958	-8.4	3.50 ± 0.05	30.1	0.995	-7.6
GT + HPMC K100M	0.54 ± 0.01	196.0	0.979	-8.3	3.38 ± 0.07	31.2	0.975	-7.6
GT + HPMC K15M	0.53 ± 0.01	197.2	0.969	-8.3	3.66 ± 0.07	28.8	0.965	-7.6
GT + HPMC K4M	0.50 ± 0.01	210.9	0.953	-8.3	3.48 ± 0.08	30.3	0.989	-7.5
GT + PEO 200	0.72 ± 0.01	145.8	0.937	-8.3	5.08 ± 0.03	20.8	0.990	-9.6
GT + PEO 900	0.70 ± 0.00	151.3	0.982	-9.2	4.07 ± 0.04	25.9	0.954	-8.1
GT + PEO 7000	0.67 ± 0.01	158.2	0.955	-7.9	3.70 ± 0.04	28.4	0.963	-8.2
GT + PL F127	0.70 ± 0.01	149.7	0.974	-7.6	4.10 ± 0.02	25.7	0.993	-9.8
GT + SA	0.62 ± 0.01	170.9	0.939	-8.3	3.21 ± 0.06	32.9	0.975	-7.6

† Each value is average of 3 independent determinations

The K_{deg} values for all the combinations were ranging from 3.21×10^{-3} to 5.08×10^{-3} $month^{-1}$ when stored in AT conditions. The highest rate was observed for gatifloxacin in combination with PEO 200 and lowest rate was observed for gatifloxacin in combination with SA. At these storage conditions the $t_{90\%}$ varied from 20.8 to 32.9 months. Gatifloxacin, alone and in combination with different excipients, was stable for more than 5 months at this condition.

The increase in temperature had not affected the drug stability of combinations as compared to the pure drug. At both CRT and AT storage conditions, the degradation rate of different combinations did not show drastic difference with gatifloxacin pure drug. However, the combinations having lower melting point excipients showed slightly high degradations rates, when compared to that of pure drug at both storage conditions. They can be stored at RT and at FT conditions for longer period of time. This study gives valuable information regarding the stability of gatifloxacin in presence of selected excipients at various storage conditions and for deciding formulation excipients.

4.5. Conclusions

Solubility of gatifloxacin is pH dependent. In extreme pH it shows higher solubility. This is due to an increase in the fraction of the ionized species of the drug in extreme pH. Among the non polar solvents studied, it was showing highest solubility in acetone and acetonitrile. The P_{app} in octanol/water was 0.208 ± 0.021. P_{app} in octanol/buffer system was pH dependent, pH-partition curve was found to be bell shaped. Highest partitioning was observed in neutral pH. Drug has shown two pK_a values, which is in agreement with reported potentiometric method. The pK_{a1} (5.47 ± 0.21) of gatifloxacin is due to protonation of nitrogen of piperazinyl group and pK_{a2} (9.12 ± 0.07) is due to deprotonation of carboxylic acid group.

The pH-stability profiles of gatifloxacin at all concentration levels studied were following first order degradation kinetics. At all the levels of concentrations the degradation rate was pH dependent. At neutral pH the degradation was found to be minimal and at acidic and alkaline conditions the degradation rate was slightly high. Same was confirmed from TLC. At neutral pH, gatifloxacin has shown good thermal stability and extensive photodegradation. The DSC and FT-IR studies confirmed the compatibility of drug with all the excipients studied. Gatifloxacin, alone and in combination with different excipients, was stable for more than 5 months in AT conditions and for 24 months at CRT conditions.

The rapid photodegradation can be handled by selecting appropriated containers for storage. Apart from physiological barriers of eye, the low partition coefficient of the drug can

be another factor for low ophthalmic bioavailability. Therefore formulation approaches to increase the drug residence time will enhance the permeation of drug into ocular tissue and will improve the ophthalmic bioavailability.

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Chapter 5

Ophthalmic Solid Insert Formulations: Development and In Vitro Characterization

5.1. Introduction

Ophthalmic inserts offer many advantages over conventional dosage forms with increased ocular residence, controlled/extended release, accurate dosing, exclusion of preservatives and increased shelf life (Attia et al., 1988; Hume et al., 1994; Bharath and Hiremath, 1999). It also ensures better patient compliance due to lower frequency of administration and lower incidence of systemic side effects (Maichuk, 1975a, 1975b; Maichuk, 1979; Ozawa et al., 1983; Grass et al., 1984). In recent past, to enhance ocular bioavailability, several novel strategies (films, minitablets, coated minitablets, one-side-coated minitablets, extruded inserts and drug imprinted soft contact lens) were developed and studied using existing and new polymers.

Sasaki et al. (2003) prepared and studied film type inserts containing tilisolol, o-butyryl ester prodrug of tilisolol and o-palmitoyl ester prodrug of tilisolol using hydrogel forming polymer, HPM. Prodrug containing inserts produced enhanced bioavailability in rabbits and reduced the systemic drug levels (Kawakami et al., 2001). Further, they had prepared a unique one-side-coated insert that released the drug from uncoated side only. Tilisolol containing HPM inserts were prepared and a hydrophobic, impermeable polypropylene tape was attached on one side. Ocular application of these formulations produced constant concentrations of tilisolol in the tear fluid over 180 min.

Similarly, sodium alginate films with Eudragit RL 100, RS 100 and/or polyvinyl acetate as rate controlling membrane were prepared with ciprofloxacin HCl. Drug concentration was continuously maintained above MIC for 4 to 4.5 days in rabbit aqueous humor (Charoo et al., 2003). Polyvinyl acetate K-30 films with pefloxacin mesylate coated with Eudragit RL 100 or RS 100 as rate controlling membrane has also been reported (Sultana et al., 2005). The in vivo (precorneal area) drug release was extended up to 5 days. In another study, Ciprofloxacin HCl, loaded into gelatin based films, which were coated with rate controlling membrane of ethyl cellulose have also been reported (Mundada and Shrikhande, 2006). Here, the in vivo (precorneal area) drug release was extended up to 12 hr.

In yet another study, ofloxacin ophthalmic inserts (diameter 6 mm, weight 20 mg, drug 0.3 mg) containing different grades of PEO alone and in combination with methacrylates were prepared and tested in rabbit model (Di Colo et al., 2001a, 2001b). These formulations instantaneously adhered to the mucosa, gradually formed the gel in the eye and controlled the drug release for extended periods. Drug release from these dosage forms was controlled and the mechanism of release was based on the nature of the drug. For hydrophobic drugs, like prednisolone and oxytetracycline hydrochloride, the drug release was governed mainly by erosion mechanism. For hydrophilic drug like, gentamicin sulphate, the

drug release was governed mainly by diffusion (Di Colo and Zambito, 2002). Further, ofloxacin loaded chitosan hydrochloride microspheres were embedded in inserts prepared with different grades of PEO (Di Colo et al., 2002). Dexamethasone loaded *N*-trimethylchitosan microspheres were embedded in inserts prepared with PEO (Zambito et al., 2006). These strategies lead to an enhancement of ocular bioavailability of drugs studied by several folds.

Fluorescein, pilocarpine and tropicamide loaded inserts were prepared by lyophilization on to hydrophobic PTFE support using excipients like HPMC, sodium hyaluronate and mannitol. These inserts require no preservative, no pH adjustment and are easy to handle. They increased the stability of drug for long terms, well tolerated in humans, increased the marker/drug availability for extended periods and significantly increased reduction in intra ocular pressure (IOP) to that of solutions (Dinslage et al., 2002; Lux et al., 2003; Steinfeld et al., 2004; Suverkrup et al., 2004; Lux et al., 2005).

Further, drum dried waxy maize® starch and CP 974P based inserts (diameter 2 mm, weight 6 mg) were prepared and loaded with sodium fluorescein and ciprofloxacin. High fluorescein levels were observed in human tears for several hours. Drug levels in human tears remained above its MIC for at least 8 hr (Weyenberg et al., 2003; Weyenberg et al., 2004a). Application of drum dried waxy maize® starch, CP 974P and sodium stearyl fumarate based inserts of diameter 2 mm and weight 6 mg, into humans, by topical route, showed ciprofloxacin level of tears above MIC upto 8 hr (Weyenberg et al., 2004b). Application of drum dried waxy maize® starch, Amioca® starch and CP 974P sodium stearyl fumarate based inserts (diameter 2 mm, weight 6 mg) into humans, by topical route, showed sodium fluorescein levels in tears upto 12 hr without any mucosal irritation (Weyenberg et al., 2006).

Dexamethasone and gentamicin sulphate containing BODI formulations (length 5 mm, diameter 2 mm, weight 20.5 mg and drug content 5 mg) were prepared by extrusion method using HPC, EC, CP 974P and CAP. These formulations were well tolerated by rabbits, the drug concentrations were maintained above required levels for more than 50 hr. The bioavailability was enhanced several fold and was capable of healing external ocular diseases such as conjunctivitis, keratoconjunctivitis sicca and superficial corneal ulcers in dogs (Gurtler et al., 1995a; Gurtler et al., 1995b; Baeyens et al., 1998a; Baeyens et al., 1998b; Baeyens et al., 2002).

Chetoni (1998) reported oxytetracycline hydrochloride ocular inserts (diameter 0.9 mm, length 6-12 mm, weight 3-8 mg, drug content 0.8 mg) that were fabricated using silicone elastomer (polyacrylic acid or polymethacrylic acid grafted on polydimethylsiloxane) that maintained the drug levels for several days and enhanced the bioavailability.

Several existing and novel polymers were used for better ophthalmic drug delivery by solid inserts. Properties of important polymers are given below.

PEO as a non-ionic homopolymer of ethylene oxide, represented by the formula $(CH_2CH_2O)_n$, where n represents the average number of oxyethylene groups (number of repeating units). Chemical structure of PEO was shown in Figure 5.1. Different grades of PEO, number of monomers, molecular weights and their viscosities were given in Table 5.1. These polymers are included in the FDA Inactive Ingredients Guide (sustained-release tablets) (Inactive Ingredients Guide, 1996; Owen, 2005).

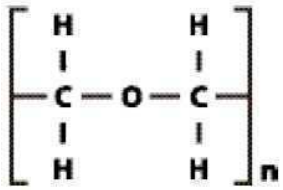

Figure 5.1: Chemical structure of PEO, where n represents the average number of oxyethylene groups which can vary from 2275 to 159000.

Table 5.1: Different grades of PEO, their number of monomers, molecular weights and their viscosities (Owen, 2005).

PEO	Polyox® grade	n	Approximate Mol. Wt. (kDa)	Poly(ethylene oxide) viscosity at 25°C (mPa.sec)		
				5 % solution	2 % solution	1 % solution
100	WSR N-10	2275	100	30-50	-	-
200	WSR N-80	4500	200	55-90	-	-
300	WSR N-750	6800	300	600-1200	-	-
400	WSR N-3000	9100	400	2250-4500	-	-
600	WSR 205	14000	600	4500-8800	-	-
900	WSR 1105	20000	900	8800-17600	-	-
1000	WSR N-12K	23000	1000	-	400-800	-
2000	WSR N-60K	45000	2000	-	2000-4000	-
4000	WSR 301	90000	4000	-	-	1650-5500
5000	WSR Coagulant	114000	5000	-	-	5500-7500
7000	WSR 303	159000	7000	-	-	7500-10000

PEO exhibits good compressibility and thus is easy for the manufacture of matrix tablets. In contact with an aqueous medium, PEO hydrates and gels superficially, the polyether chains of PEO forming strong hydrogen bonds with water. Drug release from PEO matrices is controlled by polymer swelling and erosion, or drug diffusion through the gel, or by both the processes. Various release patterns can be achieved depending on the PEO molecular weight and physicochemical properties of the drug (Ludwig, 2005).

HPMC can be described as a partly O-methylated and O-(2-hydroxypropylated) cellulose, and its chemical name is cellulose 2-hydroxypropyl methyl ether. Chemical

structure of HPMC was shown in Figure 5.2. Molecular weight is approximately 10-1500 kDa. Methocel K4M, Metalose® 90SH 15000SR and Metalose® 90SH 100000SR were having viscosity of 4000, 15000 and 100000 cPs, respectively. For convenience the different grades of HPMC were coded as HPMC K4M (Methocel K4M), HPMC K15M (Metalose® 90SH 15000SR) and HPMC K100M (Metalose® 90SH 100000SR).

HPMC polymers are included in the FDA Inactive Ingredients Guide (ophthalmic preparations; oral capsules, suspensions, syrups, and tablets; topical and vaginal preparations) (Inactive Ingredients Guide, 1996; Harwood, 2005b).

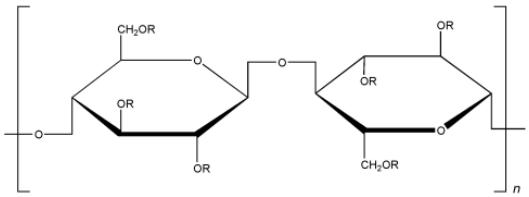

Figure 5.2: Chemical structure of HPMC, where R is H, CH_3, or $CH_3CH(OH)CH_2$.

Hydroxyethyl cellulose (HEC) can be described as a partially substituted poly(hydroxyethyl) ether of cellulose, and its chemical name is cellulose 2-hydroxyethyl methyl ether. Chemical structure of HEC was shown in Figure 5.3. It is available in several grades that vary in viscosity and degree of substitution; some grades are modified to improve their dispersion in water.

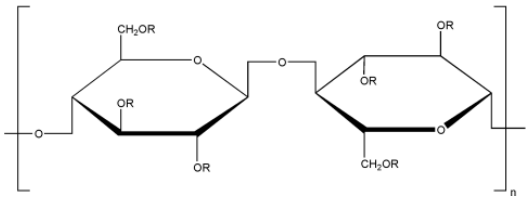

Figure 5.3: Chemical structure of HEC, where R is H or $[—CH_2CH_2O—]_nH$.

This polymer was included in the FDA Inactive Ingredients Guide(ophthalmic preparations; oral syrups and tablets; otic and topical preparations) (Inactive Ingredients Guide, 1996; Harwood, 2005a).

Polymethacrylates (Eudragit) are synthetic cationic and anionic polymers of dimethylaminoethyl methacrylates, methacrylic acid, and methacrylic acid esters in varying ratios. Different types of Eudragits are commercially available in the form of dry powder, aqueous dispersion or organic solution.

Chemically (Figure 5.4), Eudragit L100 (Eu L100) is a methacrylic acid and methyl methacrylate copolymer having a mean relative molecular mass of about 135000. The ratio of carboxylic groups to ester groups is about 1:1 and it is soluble at and above pH 6. Chemically Eudragit S100 (Eu S100) is a methacrylic acid and methyl methacrylate copolymer. The ratio

of carboxylic groups to ester groups is about 1:2 and it is soluble at and above pH 7. Chemically Eudragit L100-55 (Eu L100-55) is a methacrylic acid and ethyl acrylate copolymer having a mean relative molecular mass of about 250000. The ratio of carboxylic groups to ester groups is about 1:1 and it is soluble at and above pH 5.5. Eudragits are included in the FDA Inactive Ingredients Guide (oral capsules and tablets) (Inactive Ingredients Guide, 1996; Chang et al., 2005).

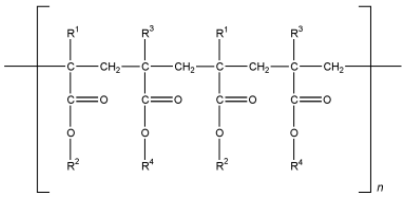

Figure 5.4: Chemical structure of Eudragit. Eudragit L and Eudragit S - R^1, $R^3 = CH_3$, $R^2 = H$ and $R^4 = CH_3$.

Small, mucoadhesive solid dosage forms, in particular gel-forming minitablets and erodible inserts, show interesting in vivo performances and allow for therapeutic levels to be obtained over an extended period of time in the tear film and anterior chamber. Sustained release can be modulated by the composition and manufacturing procedure. Mucoadhesive minitablets or inserts are promising ocular drug delivery systems to treat external and intraocular eye infections, and diseases that require frequent eye drops instillation in order to maintain therapeutic drug levels. The objective of the present study was to develop a solid ophthalmic insert formulation containing gatifloxacin. HPMC, HEC, Eudragit and PEO were investigated as matrix systems for the formulations, which swell when instilled into the cul-de-sac of the eye and provide sustained release of the drug. There is no report in literature on the usage of HPMC, HEC and PEO in combination with Eudragit for ophthalmic purpose, especially for gatifloxacin.

5.2. Experimental

5.2.1. Materials

For buffer preparations, in-house prepared triple distilled water (TDW) was used. All other materials used were same as given in chapter 3. Composition of buffers and reagents is given in appendix.

5.2.2. Insert preparation

The drug and excipients were passed through #150 and #60 sieves respectively, dried under vacuum, weighed and used for preparation of inserts. The schematic diagram of method of insert preparation was given in Figure 5.5. Materials were weighed for 100 inserts

according to the formula given in the Table 5.2 to 5.5. These formulae were designed in such way that each insert contains 0.15 mg of drug.

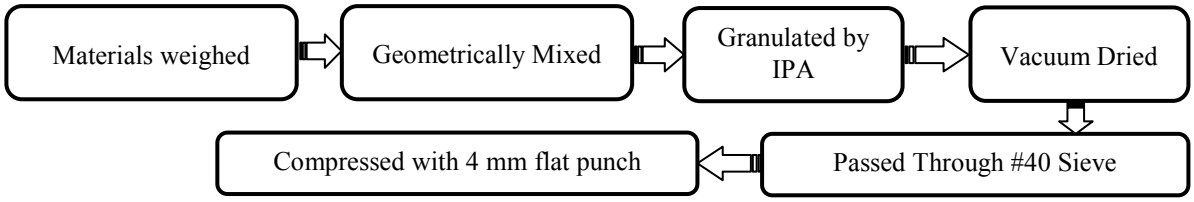

Figure 5.5: Schematic representation of method of fabrication of ophthalmic solid inserts.

Drug was geometrically mixed, first with hydrophilic polymers and then with methacrylate polymers (where ever they are present in the formula). These blends were granulated using isopropyl alcohol and thoroughly mixed to let the solvent evaporate. The residual solvent was removed completely by drying under vacuum at room temperature till constant weight. The dried granules were passed through #40 sieve and compressed using 4 mm diameter flat-faced punch using tablet compression machine (Cadmach, Ahmedabad). The inserts obtained were 4 mm in diameter and the thickness of the inserts were dependent on drug to excipient ratio. The prepared inserts were stored in a self-sealable air tight polyethylene bags and sterilized by ethylene oxide in 3-4 cycles. In each cycle inserts were exposed to ethylene oxide gas for 30 min at 40°C followed by 12 hr of aeration time. They were stored at controlled room temperature till further usage.

5.2.3. Effect of various parameters

Inserts were prepared with combinations of hydrophilic, hydrophobic and anionic polymer(s) and the proportions were given in the Table 5.2 to 5.5.

(a) Effect of granulating agent

Effect of various granulating agents (acetone and isopropyl alcohol - IPA) on physical properties and drug release characters of inserts were studied. Acetone and IPA were tried in batches G_1H1_{80} - A and G_1H1_{80} - IPA, respectively.

(b) Hydrophilic polymer content

Inserts were prepared with varying proportion of hydrophilic polymers (HPMC K4M, HPMC K15M, HPMC K100M, HEC, PEO 200, PEO 300, PEO 900, PEO 2000, PEO 5000 and PEO 7000) alone, to check the effect of hydrophilic polymer on formulation character.

(c) Anionic polymer content

Inserts were prepared with varying proportion of anionic polymer (Eu S100) alone, to study the effect of anionic polymer on formulation characteristics.

(d) Combination of anionic and hydrophilic polymers

Inserts were prepared with varying proportion of anionic polymer (Eu S100), different grades of methacrylate polymers (Eu L100 Eu L100-55 and Eu S100) and in combination with varying proportions of hydrophilic polymers, to study the effect on formulation characters.

(e) Combination of hydrophobic polymer and hydrophilic polymers

In the preparation of batch with ethyl cellulose ($G_1H1_{40}EC_{40}$), initially drug and ethyl cellulose (20 cPs) were dissolved in acetone, mixed thoroughly and solvent was removed by vacuum drying. The obtained powder was processed similar to the procedure mentioned above for the preparation of inserts were prepared (schematic diagram given in Figure 5.6.).

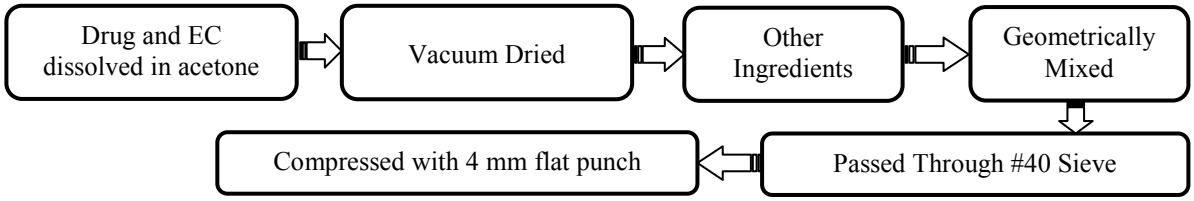

Figure 5.6: Schematic representation of method of fabrication of ophthalmic solid inserts (with EC).

(f) Amount of gatifloxacin

To check the effect of drug loading, separate set of batches were prepared with increased drug loading. Materials were weighed for 100 inserts according to the formula given in the Table 5.5. These formulations were prepared in such a way that each insert contained 0.3 mg of drug. The inserts were prepared by similar procedure as given above. Inserts were prepared with varying proportion of methacrylate polymers alone and in combination with hydrophilic polymers.

5.2.3. Evaluation of Inserts

(a) Weight variation and dimensions

Twenty inserts of each batch were weighed individually and weights were noted using weighing balance (Mettler Toledo, Model - AG135, GMBH, Switzerland). Diameter and thickness were determined using vernier caliper.

(b) Crushing strength/hardness

Crushing strength/hardness of the prepared inserts was determined by Texture profile analysis using a Texture analyser (TA-XT2, Stable Micro Systems, UK) fitted with a 30 kg load cell (force resolution of 0.1 g, force measurement accuracy of 0.001 % and a distance resolution of 0.001 mm) using a 4 mm diameter analytical probe (Stainless steel cylindrical flat bottom probe - SMS P/4). The probe was set to penetrate a distance of 1 mm with speed of 0.1 $mm.sec^{-1}$ and withdrawn at a speed of 10 $mm.sec^{-1}$. The crushing strength is the maximum force of the force-time plot. Study was carried out in triplicates.

(c) Friability

The friability of the inserts was determined by subjecting pre-weighed 20 inserts to falling shocks for 4 min in an friabilator (Campbell Electronics), set at a speed of 25 rpm. The inserts were reweighed and the percentage friability was calculated.

(d) Bioadhesion study

Freshly excised conjunctival mucosa was obtained from male/female goats from the local slaughterhouse. The animals were sacrificed by decapitation. The tissue was then removed, placed in BSS and stored in refrigerated condition till further usage. The tissue was thawed at the time of use by immersion in a bath of BSS at ambient temperature.

Conventional method

The experimental setup for determination of bioadhesion strength of inserts was adaptation of the reported methods (Ch'ng et al., 1985; Yong et al., 2001) with some modifications. The setup consisted of a analytical balance shown schematically in Figure 5.7. The left pan was removed and a teflon block (6 cm height × 6.2 cm diameter) with a downward perpendicular extension (2.5 cm height × 1.5 cm diameter) was suspended using non-elastic wire. The conjunctival mucosal tissue was tied to a lower block (8 cm height × 6.2 cm diameter) with a corresponding upward perpendicular extension. The lower block was kept in a beaker filled with STF maintained at 37°C, so that the fluid is in contact with the outer surface of the mucosal tissue in order to keep it moistened and to simulate ocular environment. The inserts were attached to upper block using glue, which was rested on to the membrane on the lower block and 40 g of weight was applied for 15 min. The weight was then removed and water was added drop wise to the tared beaker in right pan using micropipette at 5 $ml.min^{-1}$. The water was added until the two supports separated due to

breaking of the adhesive bond. The time taken for the addition of water was varied from 3 to 5 min. The force of detachment was calculated using the weight required to detach the insert from the mucosal tissue. The experiment was done thrice for each formulation. The variables such as contact force and contact time were studied and optimized using blank formulations.

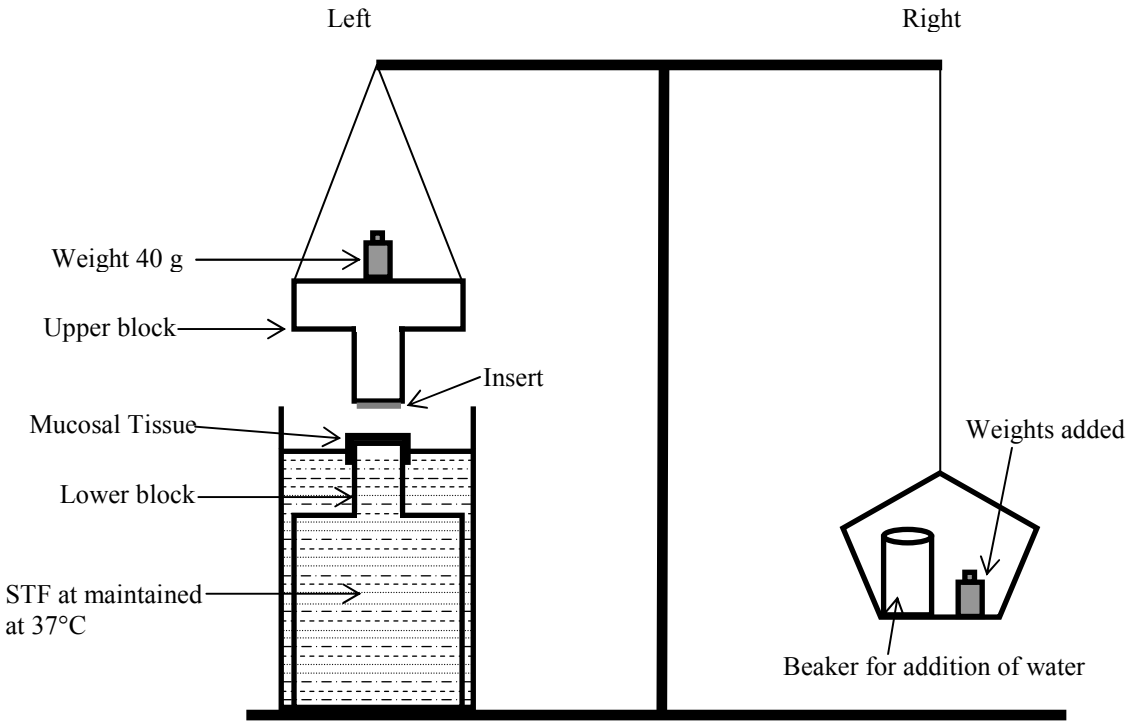

Figure 5.7: Schematic diagram for setup of conventional bioadhesion testing.

Texture analyser method

Tensile test using texture analyser (TA-XT2) is a useful technique that has been extensively employed as a valid mean for mechanical characterization of pharmaceutical mucoadhesive solid and semisolid dosage forms (Eouani et al., 2001). The experimental setup for determination of bioadhesion strength of gels and inserts was in-house modification of the reported methods (Eouani et al., 2001; Yong et al., 2001). The setup is given schematically in Figure 5.8. The instrument variables such as contact force, contact time and speed of withdrawal of the probe were studied using blank formulations.

The mucosal tissue was held using clips on a holder immersed in STF maintained at 37°C, so that the fluid is in just contact with the tissue surface of the mucosal tissue. The pre-test speed, the test speed, and the withdrawal speed of the probe were set up at 0.1, 0.1, and 0.5 mm.sec^{-1} respectively, with an acquisition rate of 200 points per sec.

The inserts were attached to probe using glue. The study was carried out at a temperature of 37°C. The probe used was a stainless steel cylindrical probe (SMS P/4) having a diameter of 4 mm. The probe was rested onto the tissue and 40 g of weight was applied for

30 sec. After lapse of time the probe was withdrawn at specific rate to a height of 10 mm. The mucoadhesive performance of the samples were determined by measuring the resistance to the withdrawal of the probe (maximum detachment force, F_{max}, in Newton 'N') reflecting the mucoadhesion characterization of the formulations with mucus. The areas under the force-time curves (AUC in 'N.sec.cm^{-2}') were also determined to represent the work required for detachment of the two systems (mucosa and formulations), i.e. work of adhesion. Measurements were carried out in triplicates using fresh inserts.

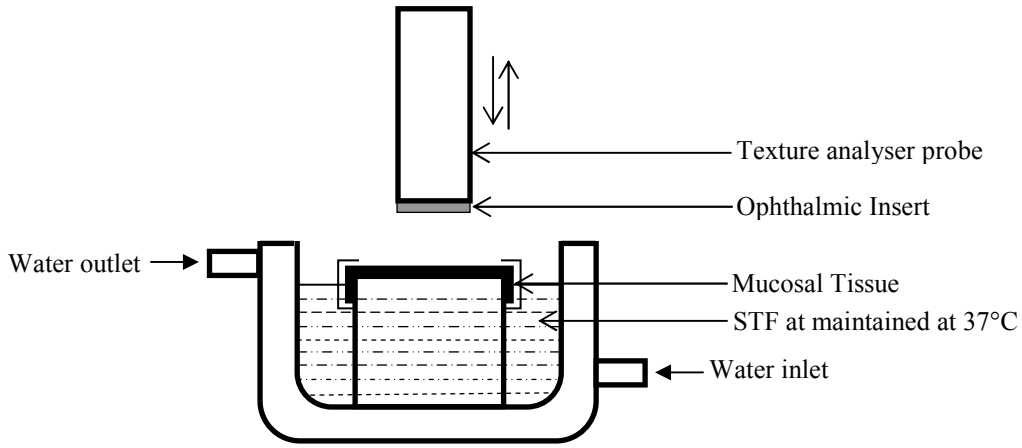

Figure 5.8: Schematic representation of bioadhesion testing by texture analyser.

(e) Assay of inserts

Twenty inserts were weighed individually and pulverized. Amount of the powder equivalent to 1.5 mg of gatifloxacin was taken in a 100 ml volumetric flask and sonicated with the pH 7.4 PBS. After sonication for 2 hr. the volume was made up with PBS to prepare a 15 µg.ml^{-1} concentration. These solutions/dispersions were filtered through #40 Whatman filter paper and analysed using UV-spectrophotometric method as described in chapter 3.

(f) Test for sterility

All the sterilized formulations were tested for sterility using USP method (US Pharmacopoeia XXIII, 2005). The representative samples of the formulations were powdered under aseptic conditions. The resulting powders were tested for the absence of microbial load.

(g) In vitro drug release studies

As there was no official apparatus for evaluating ocular dosage forms, vials kept in oscillating water bath were employed to study the release of drug from the inserts. This dissolution method was reported (Weyenberg et al., 2003) to be the most appropriate to obtain a suitable in vivo simulation. An insert was weighed, and transferred to a glass vial

containing 2 ml isotonic phosphate buffer solution (pH 7.4). To avoid water evaporation, the vials were covered with caps and placed in an oscillating (40 cycles per minute) water bath at 37 ± 0.5°C. Aliquots of 1 ml were withdrawn throughout the experiment at different time points (30, 45, 60, 90, 120, 180, 240, 300, 360, 480, 720, 1080 and 1440 min), and replaced by an equal volume of fresh buffer solution. The samples were centrifuged at 4000 rpm for 10 min. After suitable dilution, samples were analysed by UV-spectrophotometric method as described in chapter 3. Percentage cumulative drug release (% CDR) was plotted against time.

In vitro drug release data was analysed using GraphPad Prism version 4.00 (Trial) for windows, GraphPad software, San Diego California USA. Different kinetic models (zero order, first order, Higuchi and Peppas) were fitted for the release data and best fit was decided based on R^2, MSSR and AIC. AIC can be used to determine the 'best' model from a number of alternatives. The formula used for the calculation is AIC = [N * ln(SSR)] + [2 * M]; where N is the number of data points, SSR is the sum of the squared residuals and M is the number of adjustable parameters. It is a measure of the goodness of fit between calculated and observed data. The lower the value of the AIC, the better the model describes the data (Yamaoka et al., 1978). The data upto 60 % of drug release was used for Peppas model fitting. Time taken for 50 % drug release ($t_{50\%}$) was also determined based on best fit model equation.

(h) Textural analysis of swelling behavior

The swelling behavior of the formulations was investigated through textural analysis of swollen inserts. Textural analysis was performed using a texture analyser (TA-XT2). Inserts were placed in glass vials under conditions identical to those described above for in vitro drug release. The hydrated inserts were removed at predetermined intervals, touched lightly with tissue paper, and subjected to textural profiling to determine gel layer thickness into the entire matrix. All measurements were carried out in triplicate for each time point and inserts were discarded. The force-displacement-time profiles associated with the penetration of a 3 mm round-tipped stainless steel probe (cylindrical probe SMS P/3) into the swollen matrices were monitored at a data acquisition rate of 200 points per second as previously described (Jamzad et al., 2005). Probe approached the sample at pretest speed of 0.1 mm. sec^{-1}. Once a trigger force of 5 g was detected (at contact of the probe with insert) the probe was advanced into the sample at a test speed of 0.1 $mm.sec^{-1}$ until the maximum force of 30 g was reached. After the set load was reached, the probe was withdrawn at a 0.5 $mm.sec^{-1}$. Swollen thickness was determined by measuring the total probe displacement value recorded

and by the observation of textural profiles. Percent axial swelling was calculated according to the following equation: Axial swelling (%) = [Swollen thickness − Original thickness] × 100/[Original thickness]. The original thickness of the inserts was determined using vernier calipers before commencement of study.

(i) In vitro Microbiological Studies

Microbiological studies determine the in vitro activity of the optimized formulation and marketed eye drops against microorganism. Clinical isolate of *Pseudomonas aeruginosa* was used as a test microorganism (MIC - 50 $\mu g.ml^{-1}$). *Pseudomonas aeruginosa* was provided by Dr. G. Nath, Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University (BHU), Varanasi, India. Test organism was seeded by swab method on the layer of solidified nutrient media (Muller Hinton agar II media, Himedia, Mumbai) in a petri plate. Cups were made on the solidified media with the help of sterile borer of 5 mm diameter. The sterile inserts were placed in the cups and 50 µl of sterile water for injection was added. Volume of the marketed eye drop formulation containing equivalent amount of gatifloxacin as that of insert formulation was transferred into a separate cup. After keeping the petri plates at room temperature for 30 min, the plates were incubated at 37°C for 18 hr. The zone of inhibition was measured. Both marketed and optimized formulations were tested in similar manner. All the formulations were tested in triplicate.

5.2.4. Batch reproducibility

Two more batches of each formulation were prepared and evaluated for physical and in vitro release characters as described previously. Batch reproducibility was accessed by comparing the results of all the three batches prepared.

5.2.5. Stability studies

The effect of sterilization method on the stability of the drug was also determined by comparing physical parameters, drug content and in vitro drug release before and after sterilization. Few best formulations from each polymer were selected for this study. The mean assay values of inserts, before and after sterilization, were compared using *t*-test at $P \leq 0.05$.

The selected formulations were sealed in airtight cellophane packets and stored at ambient as well as accelerated storage conditions as per ICH guidelines. The samples were taken at initial time point and analysed for drug content after suitable dilution with UV-spectrophotometric or HPLC methods as described in chapter 3. Formulations were stored at

different conditions like room temperature (CRT: 25 ± 2°C/60 ± 5 % RH), accelerated condition (AT: 40 ± 2°C/75 ± 5 % RH) and refrigerated temperature (FT: 5 ± 2°C). Samples (in triplicate) were withdrawn from each batch at predetermined time intervals (0.5, 1, 3 and 6 months for AT condition; 1, 3, 6, 12, 18 and 24 months for CRT and FT condition) and analyzed for physical characters and in vitro release behavior. The physical properties such as appearance, crushing strength and friability were evaluated as per the specifications enlisted in previous sections and compared with the initial values. The drug content in triplicate was determined for each formulation by the UV-spectrophotometric method described in chapter 3 after suitable dilutions. The % RTD was plotted against time and the K_{deg} and $t_{90\%}$ (time for reaching 90 % of the drug content) were calculated based on the degradation rate kinetics at different storage conditions. The in vitro release profiles were studied as per the specifications enlisted in previous sections and compared with their respective initial release profiles.

5.2.6. Statistical analysis of data

The data were statistically evaluated by *t*-test and one way ANOVA at $P \leq 0.05$, where ever required. If null hypothesis was rejected in one way ANOVA, all pairs of columns were evaluated by Bonferoni test.

5.3. Results and discussion

5.3.1. Physical characterization of the inserts

The designed drug loaded polymeric inserts were found to have good physical properties and the results are presented in the Table 5.2 to 5.5. The inserts prepared with HPMC alone, PEO alone and their combinations with Eu S100 were smooth and off-white in color. The inserts prepared with HEC alone and HEC with Eu S100 in combination were smooth and yellowish in color.

In case of inserts prepared with HEC, different viscosity grades of HPMC and PEO, direct compression led to formation of inserts with good physical properties, but the formula containing Eudragit lead to friable inserts. Thus, all the formulations were prepared by a non-aqueous wet granulation technique. When acetone was used as a granulating agent, the material was found to dry rapidly during granulation. However, when acetone was replaced with IPA, it was found to produce cohesive granules with better physical properties.

Formulations have been manufactured with uniform weight, which was indicated by low SD values. The prepared inserts were showing weight variation ranging from ± 0.02 to ±

0.89 mg. The thickness of inserts was found to be ranging from 0.4 to 1.2 mm depending on the drug and polymer proportion. The inserts prepared with 1:20 (insert weight - 3 mg) drug to polymer proportion were showing thickness of 0.4 mm and with 1:120 (insert weight - 18 mg) drug to polymer proportion were showing thickness of 1.2 mm. The relative standard deviation for thickness was not more than 7.21 %. The diameter of inserts was uniform (RSD < 1 %). The content uniformity of the inserts was achieved, as assay values were in between 95 to 105 % with SD value not more than 5 %.

The prepared inserts with weight less than 9 mg (G_1H1_{20}, G_1H1_{40}, G_1E3_{20}, G_1E3_{40}, $G_2H1_{20}E3_{20}$, $G_2H2_{20}E3_{20}$, $G_2H3_{20}E3_{20}$, $G_2P1_{20}E3_{20}$, $G_2P3_{20}E3_{20}$ and $G_2P6_{20}E3_{20}$) showed very less crushing strength (0.71 to 5.12 N) and high friability (≥ 1.45 %). In the case of inserts prepared with Eu S100 alone in the proportion of 1:60 and 1:80, the friability was found be nearly 1 %. The formulations prepared with combination of HPMC and Eu S100, showed crushing strength of more than 20 N and were less friable. The formulations prepared with HEC and Eu S100 combination, showed crushing strength less than 13 N, but they have shown very less friability. The moisture content of the formulations immediately after fabrication was not more than 2 %. None of the formulations showed any microbial load and all the batches studied passed the sterility testing.

Table 5.2: Formulation code, composition and various physical parameters of ophthalmic inserts (0.15 mg per insert) prepared using HPMC (different viscosity grades) and Eudragit (different grades), alone and in combination.

Code	Drug : Polymer	Ratio	Drug Content (mg)	Weight (mg) (Mean ± SD)[†]	% Assay (Mean ± SD)[‡]	Crushing Strength (N) (Mean ± SD) [Π]	Friability (%) [Ψ]
GC	GT : Lactose	1 : 80	0.15	12.36 ± 0.50	99.23 ± 1.77	10.45 ± 1.65	0.78
G_1H1_{80} - A	GT : HPMC K4M	1 : 80	0.15	12.83 ± 0.63	99.61 ± 2.65	18.67 ± 2.63	0.75
G_1H1_{80} - IPA	GT : HPMC K4M	1 : 80	0.15	12.59 ± 0.35	103.87 ± 2.36	22.56 ± 0.99	0.35
→							
G_1H1_{20}	GT : HPMC K4M	1 : 20	0.15	3.21 ± 0.11	102.32 ± 0.74	2.95 ± 0.03	2.11
G_1H1_{40}	GT : HPMC K4M	1 : 40	0.15	5.97 ± 0.26	98.65 ± 1.25	2.86 ± 0.01	3.65
G_1H1_{60}	GT : HPMC K4M	1 : 60	0.15	9.12 ± 0.33	99.11 ± 0.32	15.87 ± 0.64	0.45
G_1H1_{80}	GT : HPMC K4M	1 : 80	0.15	12.59 ± 0.35	103.87 ± 2.36	22.56 ± 0.99	0.35
G_1H2_{80}	GT : HPMC K15M	1 : 80	0.15	12.77 ± 0.18	102.14 ± 1.94	30.98 ± 0.68	0.36
G_1H3_{80}	GT : HPMC K100M	1 : 80	0.15	12.19 ± 0.44	98.77 ± 1.47	32.11 ± 1.23	0.45
→							
G_1E3_{20}	GT : Eu S100	1 : 20	0.15	2.98 ± 0.11	98.65 ± 3.65	0.77 ± 0.01	3.45
G_1E3_{40}	GT : Eu S100	1 : 40	0.15	6.12 ± 0.25	101.32 ± 0.36	5.12 ± 0.23	2.45
G_1E3_{60}	GT : Eu S100	1 : 60	0.15	9.02 ± 0.37	100.65 ± 1.13	18.35 ± 0.55	0.96
G_1E3_{80}	GT : Eu S100	1 : 80	0.15	12.05 ± 0.39	100.54 ± 3.98	22.74 ± 1.77	0.87
→							
$G_1H1_{40}E1_{40}$	GT : HPMC K4M : Eu L100	1 : 40 : 40	0.15	12.75 ± 0.43	101.49 ± 0.44	25.67 ± 1.10	0.48
$G_1H1_{40}E2_{40}$	GT : HPMC K4M : Eu L100-55	1 : 40 : 40	0.15	13.25 ± 0.49	102.65 ± 1.56	24.65 ± 0.95	0.49
$G_1H1_{40}E3_{40}$	GT : HPMC K4M : Eu S100	1 : 40 : 40	0.15	12.54 ± 0.37	100.26 ± 0.23	25.11 ± 2.36	0.36
$G_1H1_{40}EC_{40}$	GT : HPMC K4M : EC	1 : 40 : 40	0.15	12.17 ± 0.52	101.77 ± 2.01	22.15 ± 1.24	0.23

GT - Gatifloxacin; Eu L100 - Eudragit L-100; Eu L100-55 - Eudragit L-100-55; Eu S100 - Eudragit S-100; [†] - Mean of 3 batches (20 inserts from each batch); [‡] - Mean of 3 batches (10 inserts from each batch); [Π] - Mean of 3 batches (3 inserts from each batch); [Ψ] - 20 inserts were used.

Table 5.3: Formulation code, composition and various physical parameters of ophthalmic inserts (0.15 mg per insert) prepared using HEC or PEO 2000 alone and Eudragit (different grades) in combination with HPMC (different grades), HEC and PEO 2000.

Code	Drug : Polymer	Ratio	Drug Content (mg)	Weight (mg) (Mean ± SD)[†]	% Assay (Mean ± SD)[‡]	Crushing Strength (N) (Mean ± SD) [Π]	Friability (%) [Ψ]
$G_1H1_{20}E3_{60}$	GT : HPMC K4M : Eu S100	1 : 20 : 60	0.15	12.60 ± 0.43	98.02 ± 3.65	23.12 ± 1.55	0.41
$G_1H1_{60}E3_{20}$	GT : HPMC K4M : Eu S100	1 : 60 : 20	0.15	13.26 ± 0.65	102.40 ± 2.24	20.67 ± 0.12	0.36
$G_1H1_{40}E3_{20}$ →	GT : HPMC K4M : Eu S100	1 : 40 : 20	0.15	8.42 ± 0.15	99.21 ± 3.77	18.67 ± 1.74	0.78
$G_1H2_{20}E3_{60}$	GT : HPMC K15M : Eu S100	1 : 20 : 60	0.15	11.21 ± 0.26	101.26 ± 1.70	28.12 ± 2.12	0.36
$G_1H2_{40}E3_{40}$	GT : HPMC K15M : Eu S100	1 : 40 : 40	0.15	13.59 ± 0.34	99.14± 2.18	31.56 ± 0.23	0.12
$G_1H2_{60}E3_{20}$ →	GT : HPMC K15M : Eu S100	1 : 60 : 20	0.15	13.97 ± 0.39	99.56 ± 2.56	30.15 ± 0.91	0.45
$G_1H3_{20}E3_{60}$	GT : HPMC K100M : Eu S100	1 : 20 : 60	0.15	12.17 ± 0.52	101.77 ± 2.01	32.44 ± 0.12	0.31
$G_1H3_{40}E3_{40}$	GT : HPMC K100M : Eu S100	1 : 40 : 40	0.15	13.16 ± 0.15	99.78 ± 1.50	30.99 ± 2.11	0.38
$G_1H3_{60}E3_{20}$ →	GT : HPMC K100M : Eu S100	1 : 60 : 20	0.15	13.35 ± 0.12	98.60 ± 5.65	30.02 ± 0.12	0.34
G_1HE_{80}	GT : HEC	1 : 80	0.15	13.12 ± 0.54	99.41 ± 0.54	12.12 ± 0.56	0.45
$G_1HE_{40}E1_{40}$	GT : HEC : Eu L100	1 : 40 : 40	0.15	12.35 ± 0.51	102.60 ± 1.38	7.99 ± 0.87	0.34
$G_1HE_{40}E2_{40}$	GT : HEC : Eu L100-55	1 : 40 : 40	0.15	12.63 ± 0.02	99.15 ± 0.56	8.45 ± 1.23	0.56
$G_1HE_{40}E3_{40}$ →	GT : HEC : Eu S100	1 : 40 : 40	0.15	12.32 ± 0.09	100.65 ± 0.89	8.78 ± 0.67	0.34
G_1P4_{80}	GT : PEO 2000	1 : 80	0.15	12.21 ± 0.23	102.19 ± 2.38	27.44 ± 3.27	0.46
$G_1P4_{40}E1_{40}$	GT : PEO 2000 : Eu L100	1 : 40 : 40	0.15	11.59 ± 0.46	99.31 ± 2.01	28.32 ± 1.41	0.48
$G_1P4_{40}E2_{40}$	GT : PEO 2000 : Eu L100-55	1 : 40 : 40	0.15	12.97 ± 0.48	101.01 ± 1.91	27.12 ± 0.11	0.37
$G_1P4_{40}E3_{40}$	GT : PEO 2000 : Eu S100	1 : 40 : 40	0.15	13.67 ± 0.19	98.97 ± 1.98	26.57 ± 0.77	0.23

GT - Gatifloxacin; Eu L100 - Eudragit L-100; Eu L100-55 - Eudragit L-100-55; Eu S100 - Eudragit S-100; [†] - Mean of 3 batches (20 inserts from each batch); [‡] - Mean of 3 batches (10 inserts from each batch); [Π] - Mean of 3 batches (3 inserts from each batch); [Ψ] - 20 inserts were used.

Table 5.4: Formulation code, composition and various physical parameters of ophthalmic inserts (0.15 mg per insert) prepared using combination of Eudragit and different grades of PEO.

Code	Drug : Polymer	Ratio	Drug Content (mg)	Weight (mg) (Mean ± SD)[†]	% Assay (Mean ± SD)[‡]	Crushing Strength (N) (Mean ± SD) [Π]	Friability (%) [Ψ]
G_1P1_{40}	GT : PEO 200	1 : 40	0.15	6.36 ± 0.18	95.87 ± 0.77	27.87 ± 0.95	0.56
$G_1P1_{40}E3_{40}$	GT : PEO 200 : Eu S100	1 : 40 : 40	0.15	13.45 ± 0.27	102.39 ± 2.07	26.87 ± 1.65	0.37
$G_1P1_{40}E3_{80}$	GT : PEO 200 : Eu S100	1 : 40 : 80	0.15	17.77 ± 0.49	96.15 ± 1.91	33.68 ± 1.35	0.28
→							
G_1P2_{40}	GT : PEO 300	1 : 40	0.15	6.19 ± 0.02	98.13 ± 1.01	28.75 ± 1.65	0.51
$G_1P2_{40}E3_{40}$	GT : PEO 300 : Eu S100	1 : 40 : 40	0.15	12.15 ± 0.21	97.94 ± 1.18	25.87 ± 2.65	0.45
$G_1P2_{40}E3_{80}$	GT : PEO 300 : Eu S100	1 : 40 : 80	0.15	18.25 ± 0.89	102.59 ± 1.73	34.78 ± 1.54	0.65
→							
G_1P3_{40}	GT : PEO 900	1 : 40	0.15	6.41 ± 0.27	101.56 ± 0.83	28.11 ± 3.15	0.41
$G_1P3_{40}E3_{40}$	GT : PEO 900 : Eu S100	1 : 40 : 40	0.15	12.54 ± 0.23	99.72 ± 2.65	26.19 ± 0.54	0.38
$G_1P3_{40}E3_{80}$	GT : PEO 900 : Eu S100	1 : 40 : 80	0.15	18.26 ± 0.65	103.20 ± 1.94	34.78 ± 2.15	0.45
→							
G_1P4_{40}	GT : PEO 2000	1 : 40	0.15	6.21 ± 0.26	101.06 ± 2.98	29.01 ± 4.65	0.40
$G_1P4_{40}E3_{80}$	GT : PEO 2000 : Eu S100	1 : 40 : 80	0.15	18.97 ± 0.49	103.96 ± 1.56	34.76 ± 1.87	0.15
→							
G_1P5_{40}	GT : PEO 5000	1 : 40	0.15	6.17 ± 0.22	101.47 ± 1.93	28.65 ± 0.99	0.42
$G_1P5_{40}E3_{40}$	GT : PEO 5000 : Eu S100	1 : 40 : 40	0.15	14.56 ± 0.64	95.12 ± 1.25	26.78 ± 1.11	0.12
$G_1P5_{40}E3_{80}$	GT : PEO 5000 : Eu S100	1 : 40 : 80	0.15	19.35 ± 0.12	97.60 ± 3.49	33.48 ± 2.38	0.45
→							
G_1P6_{40}	GT : PEO 7000	1 : 40	0.15	6.54 ± 0.17	103.82 ± 1.21	29.32 ± 2.18	0.56
$G_1P6_{40}E3_{40}$	GT : PEO 7000 : Eu S100	1 : 40 : 40	0.15	12.12 ± 0.45	99.15 ± 1.61	26.11 ± 1.32	0.23
$G_1P6_{40}E3_{80}$	GT : PEO 7000 : Eu S100	1 : 40 : 80	0.15	17.26 ± 0.65	101.90 ± 2.08	34.87 ± 1.68	0.12

GT - Gatifloxacin; Eu L100 - Eudragit L-100; Eu L100-55 - Eudragit L-100-55; Eu S100 - Eudragit S-100; [†] - Mean of 3 batches (20 inserts from each batch); [‡] - Mean of 3 batches (10 inserts from each batch); [Π] - Mean of 3 batches (3 inserts from each batch); [Ψ] - 20 inserts were used.

Table 5.5: Formulation code, composition and various physical parameters of ophthalmic inserts (0.3 mg per insert) prepared using Eudragit alone, combination of Eudragit and HPMC, HEC or PEO (different grades).

Code	Drug : Polymer	Ratio	Drug Content (mg)	Weight (mg) (Mean ± SD)[†]	% Assay (Mean ± SD)[‡]	Crushing Strength (N) (Mean ± SD) [Π]	Friability (%) [Ψ]
G_2E3_{40}	GT : Eu S100	1:20	0.30	6.31 ± 0.04	97.15 ± 2.08	4.35 ± 1.44	2.12
G_2E3_{80}	GT : Eu S100	1:40	0.30	12.77 ± 0.06	98.31 ± 1.52	18.61 ± 0.95	0.65
→							
$G_2H1_{40}E3_{40}$	GT : HPMC K4M : Eu S100	1:20:20	0.30	12.36 ± 0.19	102.06 ± 2.28	21.45 ± 1.65	0.46
$G_2H2_{40}E3_{40}$	GT : HPMC K15M : Eu S100	1:20:20	0.30	12.54 ± 0.11	98.97 ± 1.57	31.90 ± 1.27	0.42
$G_2H3_{40}E3_{40}$	GT : HPMC K100M : Eu S100	1:20:20	0.30	12.16 ± 0.1	99.18 ± 1.54	29.84 ± 1.20	0.39
$G_2HE_{40}E3_{40}$	GT : HEC : Eu S100	1:20:20	0.30	12.45 ± 0.12	99.52 ± 1.15	7.86 ± 1.41	0.85
→							
$G_2H1_{20}E3_{20}$	GT : HPMC K4M : Eu S100	1:10:10	0.30	6.04 ± 0.04	99.75 ± 1.34	0.71 ± 0.08	2.65
$G_2H2_{20}E3_{20}$	GT : HPMC K15M : Eu S100	1:10:10	0.30	6.1 ± 0.09	97.83 ± 2.21	0.72 ± 0.04	3.11
$G_2H3_{20}E3_{20}$	GT : HPMC K100M : Eu S100	1:10:10	0.30	6.09 ± 0.1	100.58 ± 2.39	0.84 ± 0.02	3.48
→							
$G_2P1_{40}E3_{40}$	GT : PEO 200 : Eu S100	1:20:20	0.30	12.6 ± 0.29	100.88 ± 3.09	26.39 ± 0.29	0.38
$G_2P2_{40}E3_{40}$	GT : PEO 300 : Eu S100	1:20:20	0.30	12.74 ± 0.24	99.69 ± 2.61	29.58 ± 2.64	0.29
$G_2P3_{40}E3_{40}$	GT : PEO 900 : Eu S100	1:20:20	0.30	12.12 ± 0.13	100.08 ± 1.87	21.55 ± 1.28	0.45
$G_2P4_{40}E3_{40}$	GT : PEO 2000 : Eu S100	1:20:20	0.30	12.09 ± 0.17	99.83 ± 2.14	27.67 ± 3.96	0.24
$G_2P5_{40}E3_{40}$	GT : PEO 5000 : Eu S100	1:20:20	0.30	12.34 ± 0.18	101.90 ± 2.27	25.02 ± 6.65	0.62
$G_2P6_{40}E3_{40}$	GT : PEO 7000 : Eu S100	1:20:20	0.30	12.44 ± 0.17	102.73 ± 2.16	25.71 ± 0.44	0.36
→							
$G_2P1_{20}E3_{20}$	GT : PEO 200 : Eu S100	1:10:10	0.30	6.15 ± 0.12	101.57 ± 2.66	3.01 ± 0.54	1.45
$G_2P3_{20}E3_{20}$	GT : PEO 900 : Eu S100	1:10:10	0.30	6.11 ± 0.03	100.91 ± 1.18	2.86 ± 0.17	1.56
$G_2P6_{20}E3_{20}$	GT : PEO 7000 : Eu S100	1:10:10	0.30	6.01 ± 0.04	101.43 ± 1.41	3.25 ± 0.92	1.87

GT - Gatifloxacin; Eu L100 - Eudragit L-100; Eu L100-55 - Eudragit L-100-55; Eu S100 - Eudragit S-100; [†] - Mean of 3 batches (20 inserts from each batch); [‡] - Mean of 3 batches (10 inserts from each batch); [Π] - Mean of 3 batches (3 inserts from each batch); [Ψ] - 20 inserts were used.

5.3.2. In vitro drug release

The drug release from the inserts was shown in the Figures 5.8 to 5.32. Among all the models tried, data were best fitted into Peppas model, which is evident from low AIC and MSSR values. Kinetic parameters, such as release rate constant (K), exponential component (n), $t_{50\%}$, R^2, MSSR and AIC for Peppas model are presented in Table 5.6 to 5.9.

(a) Effect of granulating agent

Formulations prepared with HPMC K4M alone by granulation with IPA and acetone has resulted in similar drug release profiles (Figure 5.9).

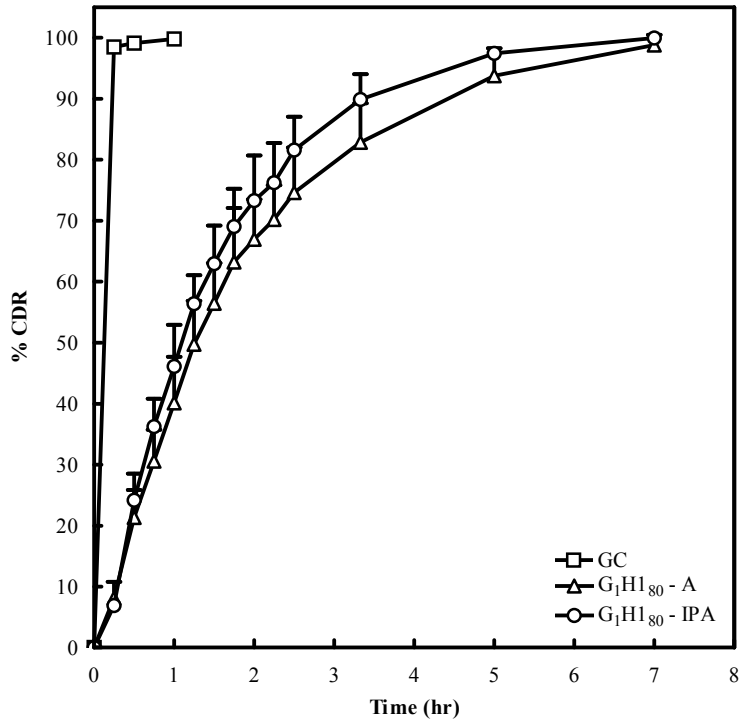

Figure 5.9: In vitro drug release from insert formulations (150 µg) prepared with different granulating agents. Each data point is average of three inserts from different batches.

As compared to control, the drug release was extended upto 3.5 hr. Peppas release rate constant was found to be 0.39 $hr^{-0.79}$ and 0.88 $hr^{-0.84}$ for G_1H1_{80} - A and G_1H1_{80} - IPA, respectively (Table 5.6). The $t_{50\%}$ was found to be 1.36 and 1.15 hr for G_1H1_{80} - A and G_1H1_{80} - IPA, respectively. From the 'n' values the drug release mechanism was found to be anomalous non-Fickian diffusion.

(b) Effect of HPMC and Eudragit alone

Hydrophilic matrix, when exposed to aqueous liquid, the surface polymer hydrates to form a viscous gel-layer (Melia et al., 1994). The gel layer forms a diffusional barrier that retards further water uptake and the release of the dissolved drug. Water-soluble drugs are

released primarily by diffusion of dissolved drug molecules across the gel layer, whilst poorly water-soluble drugs are released predominantly by the erosion mechanism (Alderman, 1984).

In our study, with increase in proportion of HPMC K4M the drug release extended (Figure 5.10).

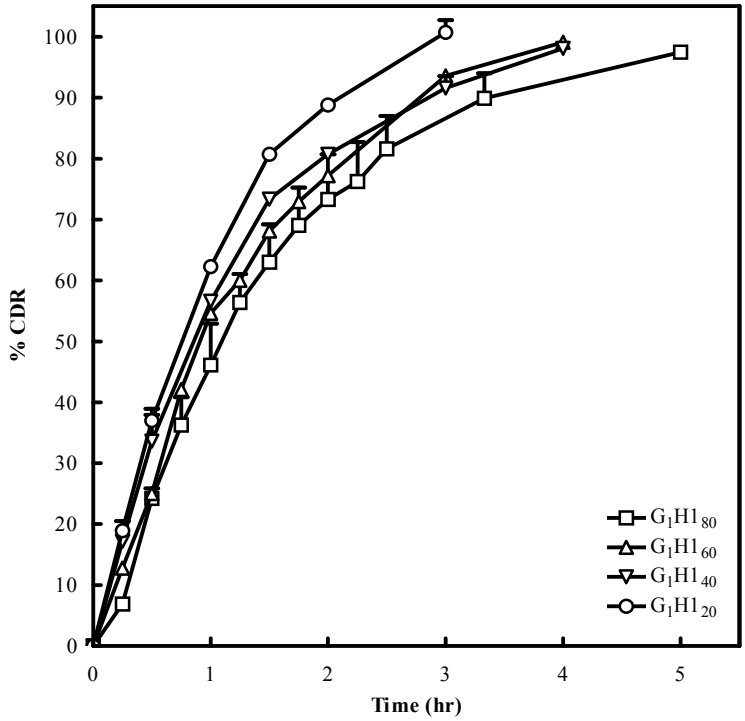

Figure 5.10: In vitro drug release from insert formulations (150 µg) prepared with different proportions of HPMC K4M. Each data point is average of three inserts from different batches.

The release rate constants were found to be 0.63 $hr^{-0.82}$, 0.57 $hr^{-0.82}$, 0.50 $hr^{-0.85}$ and 0.44 $hr^{-0.88}$ for formulations prepared with 1:20, 1:40, 1:60 and 1:80 drug to polymer proportions respectively (Table 5.6). The $t_{50\%}$ values for these formulations were found to be 0.76, 0.85, 1.00 and 1.15 hr. Modest decrease in drug release was observed with increase in HPMC K4M proportion due to the formation of a thicker gel layer with increase in polymer content resulting in retardation in drug release. From the 'n' values the drug release mechanism was found to be anomalous non-Fickian diffusion indicative of simultaneous swelling and erosion of the matrix.

High viscosity grade and hydrophilic polymers, swells and form a strong gel network, which imparts strength to dosage form and at the same time control the release of drug by diffusion mechanism (Siepmann and Peppas, 2001; Katzhendler et al., 2004). With increase in viscosity of HPMC grades the drug release was not affected much in our case (Figure 5.11). The release rate constants were found to be 0.44 $hr^{-0.88}$, 0.38 $hr^{-0.92}$ and 0.36 $hr^{-0.89}$ for formulations prepared with HPMC K4M, HPMC K15M and HPMC K100M in 1:80

proportions, respectively (Table 5.6). The $t_{50\%}$ values for these formulations were found to be 1.15, 1.34 and 1.47 hr. From the 'n' values the drug release mechanism was found to be again of anomalous non-Fickian type. The drug release was not much extended from the insert formulations containing different grades of HPMC. It was extended only up to 7 hr in case of HPMC K4M. Further, there was no marked difference in drug release from formulations containing different grades of HPMC. On the other hand, the release rate constant and $t_{50\%}$ value were found to be 0.41 $hr^{-0.84}$ and 1.26 hr for inserts prepared with HEC 1:80 proportion respectively indicating faster release from these inserts on account of the relatively more hydrophilic nature of HEC resulting in rapid swelling of the matrix (Figure 5.14). From the 'n' values the drug release mechanism was again found to be anomalous non-Fickian type.

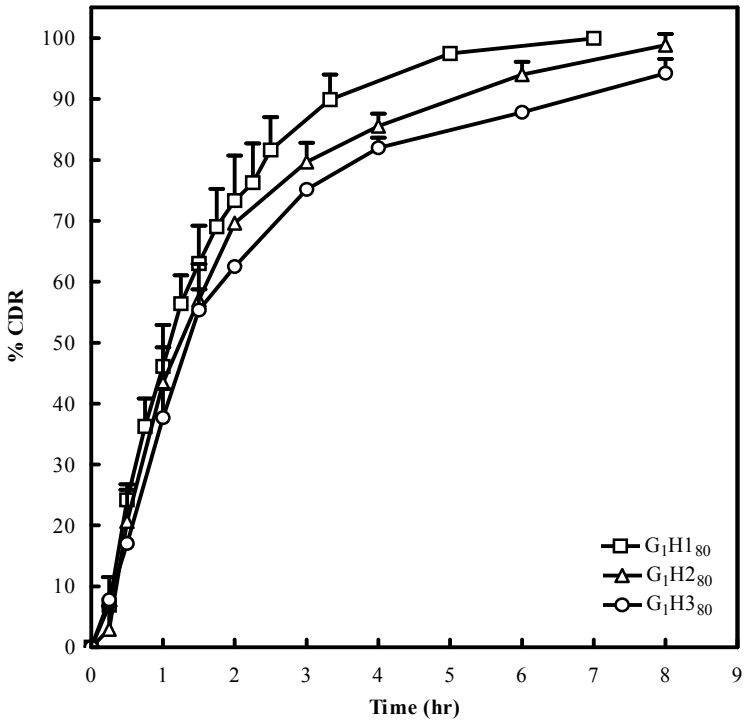

Figure 5.11: In vitro drug release from insert formulations (150 µg) prepared with different viscosity grades of proportions of HPMC. Each data point is average of three inserts from different batches.

With increase in proportion of Eu S100 the drug release extended beyond 24 hr (Figure 5.12). More than 75 % of drug was released from inserts prepared with 1:20 proportion within 0.5 hr. This can be attributed to low hardness of the insert. The release rate constants were found to be 0.37 $hr^{-0.52}$, 0.22 $hr^{-0.57}$ and 0.13 $hr^{-0.63}$ for formulations prepared with 1:40, 1:60 and 1:80 drug to polymer proportions and the $t_{50\%}$ values were 1.80, 4.08 and 9.14 hr respectively (Table 5.6). Decrease in drug release was observed with increase in Eu S100 proportion due its hydrophobic nature. From the 'n' values the drug release mechanism was found to be anomalous non-Fickian diffusion. But, during in vitro drug release study of

these inserts, those containing higher proportion of Eu S100 were found to exhibit excessive particle shredding, indicating unacceptability for ophthalmic purpose.

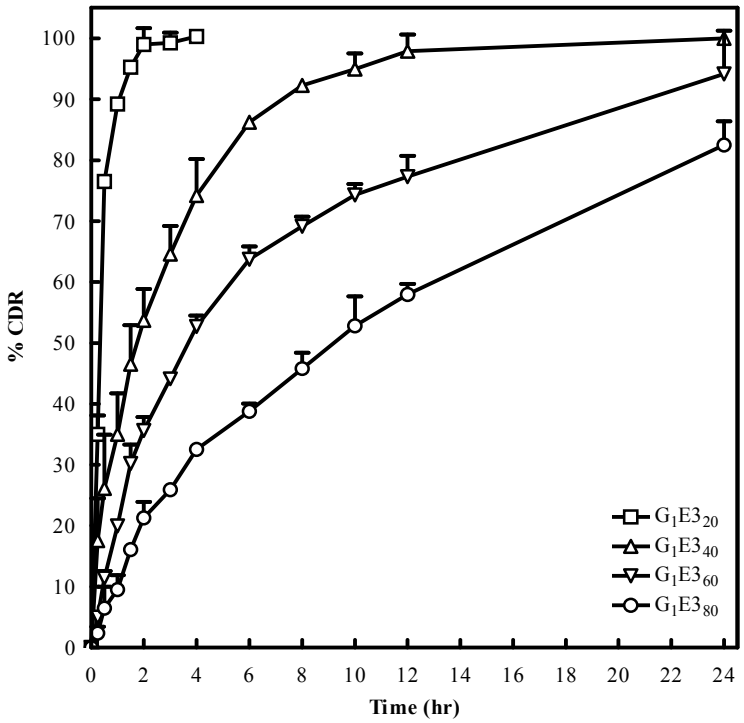

Figure 5.12: In vitro drug release from insert formulations (150 µg) prepared with different proportions of Eu S100. Each data point is average of three inserts from different batches.

Table 5.6: Results of drug release data from inserts (prepared using different granulating agents, different proportions of HPMC K4M, different grades of HPMC and different proportions of Eu S100) fitted into Peppas model.

Code	K (h^{-n}) (Mean ± SD) †	n	$t_{50\%}$ (h)	R^2	MSSR	AIC
G_1H1_{80} - A	0.39 ± 0.01	0.79	1.36	0.983	7.31	19.90
G_1H1_{80} - IPA	0.44 ± 0.02	0.88	1.15	0.979	8.97	17.36
G_1H1_{20}	0.63 ± 0.02	0.82	0.76	0.996	1.26	2.69
G_1H1_{40}	0.57 ± 0.02	0.82	0.85	0.996	1.03	2.10
G_1H1_{60}	0.50 ± 0.02	0.85	1.00	0.978	8.34	14.73
G_1H1_{80}	0.44 ± 0.02	0.88	1.15	0.979	8.97	17.36
G_1H2_{80}	0.38 ± 0.03	0.92	1.34	0.967	19.26	16.79
G_1H3_{80}	0.36 ± 0.02	0.89	1.47	0.979	9.28	13.14
G_1HE_{80}	0.41 ± 0.01	0.84	1.26	0.993	3.55	8.34
G_1E3_{40}	0.37 ± 0.01	0.52	1.80	0.996	1.00	2.00
G_1E3_{60}	0.22 ± 0.02	0.57	4.08	0.978	10.11	22.82
G_1E3_{80}	0.13 ± 0.01	0.63	9.14	0.991	3.14	14.58

† Each data is average of three determinations.

(c) Effect of HPMC and HEC in combination with anionic or hydrophobic polymers

The in vitro drug release from the inserts prepared with combination of HEC and Eudragits (1:40:40) were shown in Figure 5.13.

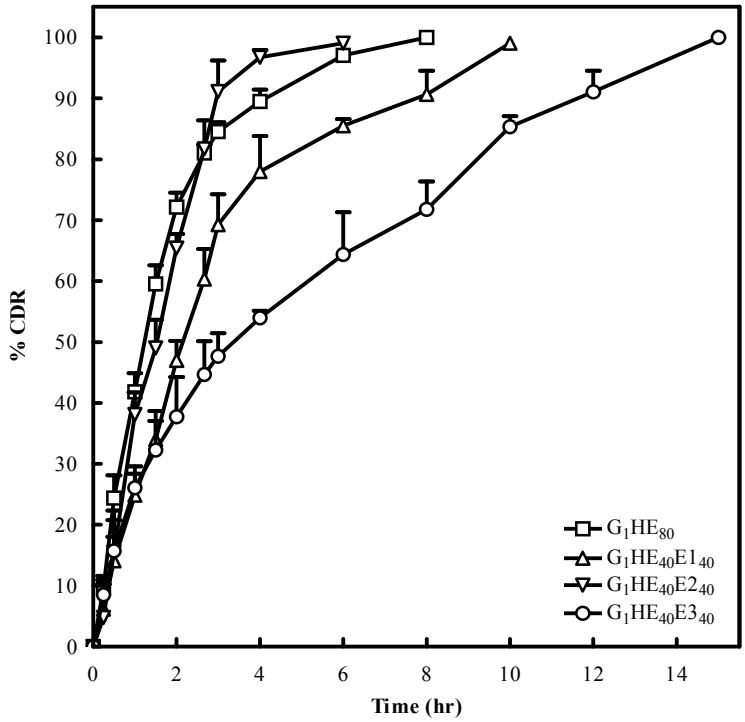

Figure 5.13: In vitro drug release from insert formulations (150 µg) prepared with combination of HEC and Eu L100, Eu L100-55 and Eu S100. Each data point is average of three inserts from different batches.

The release rate constants were found to be 0.24 $hr^{-0.94}$, 0.33 $hr^{-0.99}$ and 0.25 $hr^{-0.54}$ for formulations prepared with HEC in combination with Eu L100, Eu L100-55 and Eu S100 and the $t_{50\%}$ values were found to be 2.15, 1.50 and 3.51 hr respectively (Table 5.7). The drug release mechanism was found to be anomalous non-Fickian diffusion type. The acid to ester ratio in Eu L100, L100-55 and S100 is 1:1, 1:1 and 1:2 respectively. It is evident from the literature that the Eudragits reduces the micro-environmental pH depending on the acid to ester ratio of Eudragit (Tatavarti et al., 2004; Chang et al., 2005). Gatifloxacin showed pH dependent solubility with low solubility in neutral pH and higher solubility in acidic pH. Due to high acidic group proportion of Eu L100 and Eu L100-55 micro-environmental pH of the matrices will be acidic leading to higher drug solubility thereby resulting in faster release. The relatively lesser acidic group substitution in Eu S100 does not reduce micro-environmental pH of the matrices to the same extent, hence good retardation in drug release was observed for formulations containing HEC with Eu S100 ($G_1HE_{40}E3_{40}$).

The in vitro drug release from the inserts prepared with combination of HPMC K4M and Eudragits (1:40:40) and HPMC K4M and ethylcellulose (1:40:40) were shown in Figure 5.14.

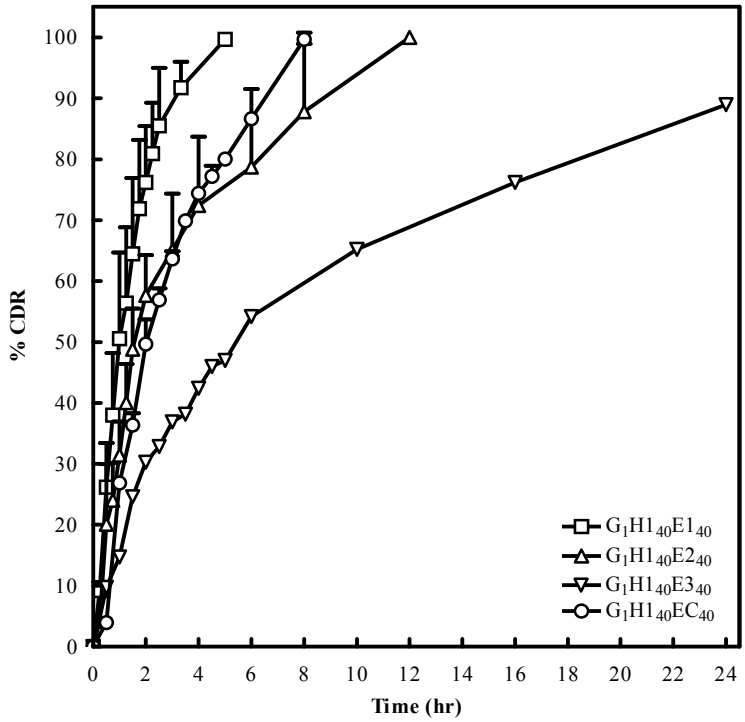

Figure 5.14: In vitro drug release from insert formulations (150 µg) prepared with combination of HPMC K4M and Eu L100, Eu L100-55, Eu S100 and ethylcellulose. Each data point is average of three inserts from different batches.

The release rate constants were found to be 0.47 $hr^{-0.87}$, 0.32 $hr^{-0.71}$ and 0.19 $hr^{-0.55}$ for formulations prepared with HPMC K4M in combination with Eu L100, Eu L100-55 and Eu S100 respectively (Table 5.7). The $t_{50\%}$ values for these formulations were 1.08, 1.84 and 5.63 hr and the drug release mechanism was found to be anomalous non-Fickian type. Drug release from inserts containing HPMC in combination with Eu S100 ($G_1H1_{40}E3_{40}$) has shown extended drug release beyond 24 hr. This finding was similar to the one observed with HEC in combination with Eu S100 implying similar matrix behaviour as noted above.

With decrease in proportion of Eu S100, there is increase in drug release due to decrease in the relative hydrophobicity of the matrix (Figure 5.15 and 5.16). The release rate constants were found to be 0.18 $hr^{-0.49}$, 0.19 $hr^{-0.55}$ and 0.27 $hr^{-0.79}$ and $t_{50\%}$ values were found to be 7.68, 5.63 and 2.21 hr for inserts prepared with HPMC K4M in combination with Eu S100 in 1:20:60, 1:40:40 and 1:60:20 respectively. In Eu S100, the micro-environmental pH will be towards neutral side and it is relatively insoluble (Chang et al., 2005) causing slower drug release. An increase in matrix porosity due to the dissolution of the polymer is the predominating factor due to which the drug release is enhanced (Tatavarti et al., 2004). By

replacing a portion of the hydrophilic polymer with the less soluble polymer, the drug release was reduced due to lowering in matrix porosity.

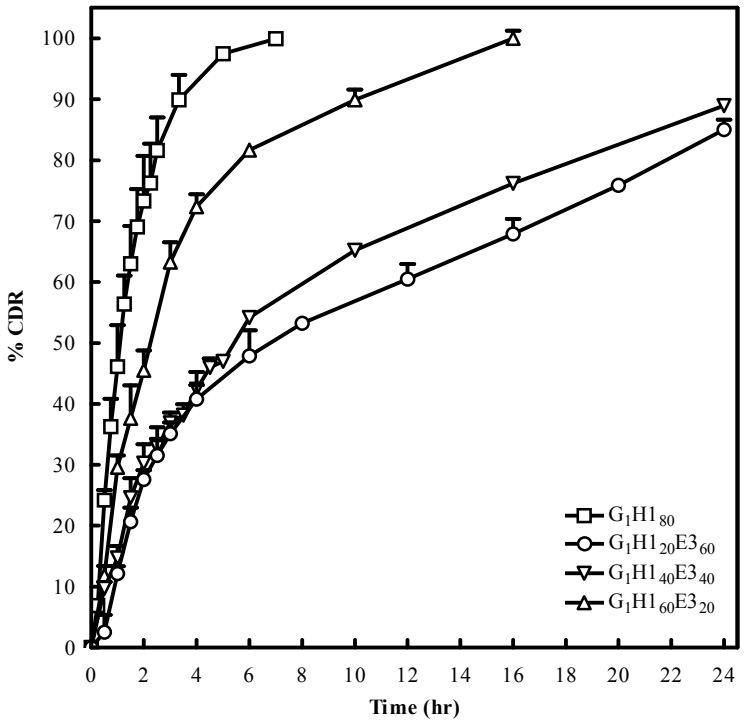

Figure 5.15: In vitro drug release from insert formulations (150 µg) prepared with combination of HPMC K4M and Eu S100 in different proportions. Each data point is average of three inserts from different batches.

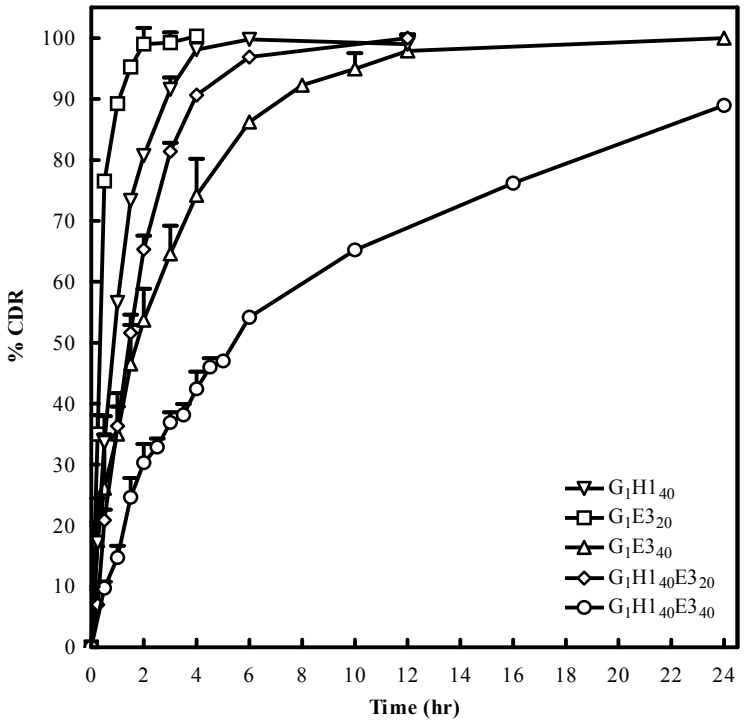

Figure 5.16: In vitro drug release from insert formulations (150 µg) prepared with HPMC K4M alone, Eu S100 alone and combination of HPMC K4M and Eu S100 in different proportions. Each data point is average of three inserts from different batches.

The release rate constants were found to be 0.57 $hr^{-0.82}$, 0.36 $hr^{-0.89}$ and 0.19 $hr^{-0.55}$ for formulations prepared with HPMC K4M in combination with Eu S100 in 1:40:0, 1:40:20 and 1:40:40 respectively (Table 5.6 and 5.7). The $t_{50\%}$ values for these formulations were found to be 0.76, 1.46 and 5.63 hr. With increase in proportion of Eu S100 the drug release was extended from 6 hr to beyond 24 hr and the mechanism of drug release was again observed to be diffusion controlled.

Similar effects were observed in case of combination of different viscosity grades of HPMC and Eu S100 (Figure 5.17 to 5.19). The release rate constants were found to be 0.38 $hr^{-0.92}$, 0.17 $hr^{-0.51}$, 0.13 $hr^{-0.64}$ and 0.26 $hr^{-0.86}$ for formulations prepared with HPMC K15M in combination with Eu S100 in 1:80:0, 1:20:60, 1:40:40 and 1:60:20 respectively (Table 5.6 and 5.7). The $t_{50\%}$ values for the same formulations were found to be 1.34, 8.78, 7.80 and 2.19 hr. The release rate constants were found to be 0.36 $hr^{-0.89}$, 0.15 $hr^{-0.51}$, 0.13 $hr^{-0.58}$ and 0.29 $hr^{-0.65}$ for formulations prepared with higher viscosity grade of HPMC (K100M) in combination with Eu S100 in similar proportions as above (Table 5.6 and 5.7). The $t_{50\%}$ values were found to be 1.47, 10.05, 9.66 and 2.30 hr respectively.

The results in the Figure 5.13 to 5.19 imply that the optimum proportion of Eu S100 and hydrophilic polymer (HPMC K4M, HPMC K15M, HPMC K100M and HEC) is essential for delaying the drug release. The best formulations were found to be $G_1H1_{40}E3_{40}$, $G_1H2_{40}E3_{40}$, $G_1H3_{40}E3_{40}$ and $G_1HE_{40}E3_{40}$.

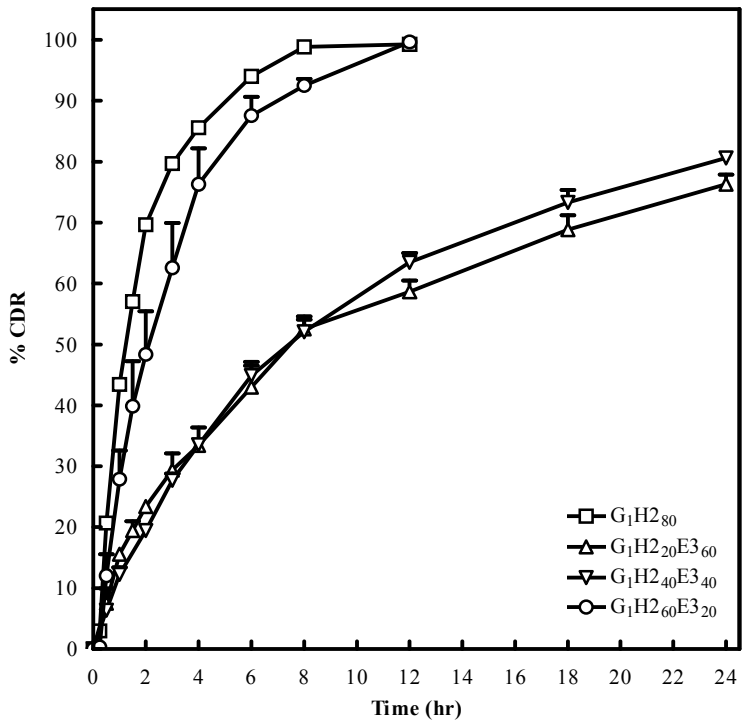

Figure 5.17: In vitro drug release from insert formulations (150 µg) prepared with HPMC K15M alone and combination of HPMC K15M and Eu S100 in different proportions. Each data point is average of three inserts from different batches.

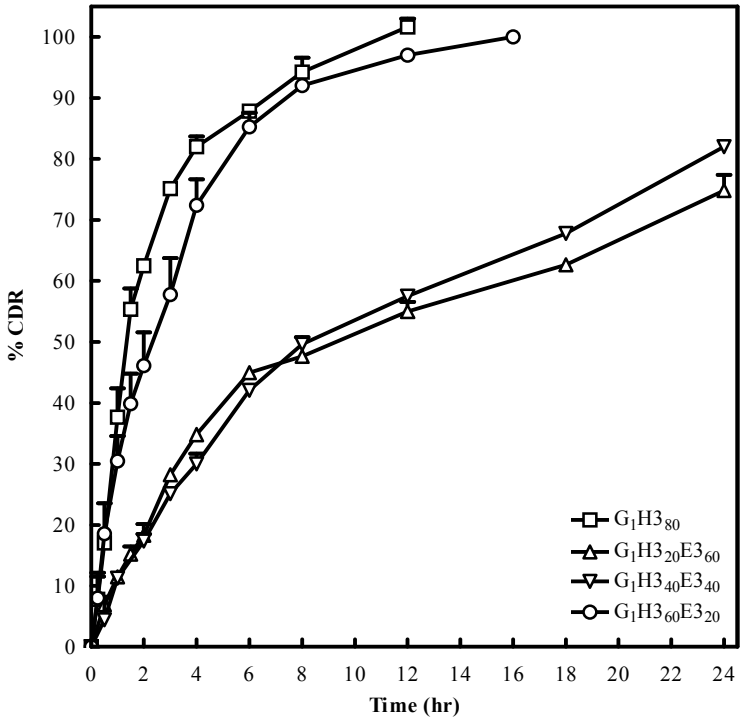

Figure 5.18: In vitro drug release from insert formulations (150 µg) prepared with HPMC K100M alone and combination of HPMC K100M and Eu S100 in different proportions. Each data point is average of three inserts from different batches.

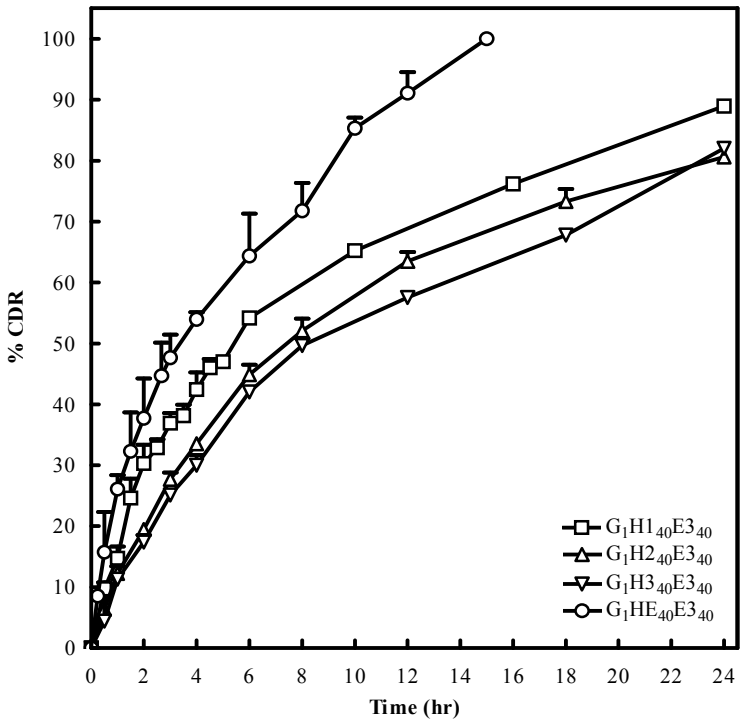

Figure 5.19: In vitro drug release from insert formulations (150 µg) prepared with combination of HPMC K4M, HPMC K15M, HPMC K100M, HEC and Eu S100 in 1:40:40 proportion. Each data point is average of three inserts from different batches.

Table 5.7: Results of drug release data from inserts (prepared using different proportions of HPMC in combination with Eudragit and different proportions of HEC in combination with Eudragit) fitted into Peppas model.

Code	K (h^{-n}) (Mean ± SD) †	n	$t_{50\%}$ (h)	R^2	MSSR	AIC
$G_1H1_{40}E1_{40}$	0.47 ± 0.01	0.87	1.08	0.979	7.37	13.98
$G_1H1_{40}E2_{40}$	0.32 ± 0.02	0.71	1.84	0.946	19.85	25.91
$G_1H1_{40}E3_{40}$	0.19 ± 0.01	0.55	5.63	0.978	5.16	21.69
$G_1H1_{40}EC_{40}$	0.25 ± 0.03	0.87	2.25	0.958	19.34	22.74
$G_1HE_{40}E1_{40}$	0.24 ± 0.01	0.94	2.15	0.998	0.98	1.85
$G_1HE_{40}E2_{40}$	0.33 ± 0.02	0.99	1.50	0.984	7.84	12.29
$G_1HE_{40}E3_{40}$	0.25 ± 0.01	0.54	3.51	0.989	3.13	12.27
$G_1H1_{40}E3_{20}$	0.36 ± 0.01	0.89	1.46	0.993	3.04	7.56
$G_1H1_{20}E3_{60}$	0.18 ± 0.02	0.49	7.68	0.943	21.12	35.55
$G_1H1_{60}E3_{20}$	0.27 ± 0.02	0.79	2.21	0.984	4.53	9.56
$G_1H2_{20}E3_{60}$	0.17 ± 0.01	0.51	8.78	0.985	5.56	19.15
$G_1H2_{40}E3_{40}$	0.13 ± 0.01	0.64	7.80	0.992	2.97	10.71
$G_1H2_{60}E3_{20}$	0.26 ± 0.03	0.86	2.19	0.966	15.42	18.41
$G_1H3_{20}E3_{60}$	0.15 ± 0.02	0.51	10.05	0.957	14.64	28.84
$G_1H3_{40}E3_{40}$	0.13 ± 0.01	0.58	9.66	0.976	9.92	22.65
$G_1H3_{60}E3_{20}$	0.29 ± 0.01	0.65	2.30	0.986	3.87	10.12

† Each data is average of three determinations.

(d) Effect of PEO alone and in combination with anionic polymers

In the case of inserts prepared with different grades of PEO in 1:40 proportion, the entire amount of drug was released within 2 hr (Figure 5.20). In formulations prepared with PEO 200 to 2000, 50% of drug was released within 0.25 hr. Where as the drug release was extended upto 1 hr in case of formulations prepared with PEO 5000 and PEO 7000.

Combination of PEO 2000 with Eu L100 and Eu L100-55 marginally extended the drug release (Figure 5.21 and Figure 5.22). Similar to the earlier results, in the inserts prepared with PEO 2000 in combination with Eu S100 in 1:40:40 proportion the drug release was extended upto 12 hr. The release rate constants were found to be 0.42 $hr^{-0.90}$, 0.39 $hr^{-0.67}$, 0.49 $hr^{-0.78}$, 0.32 $hr^{-0.57}$ and 0.21 $hr^{-0.47}$ for formulations prepared with PEO 2000 alone (1:80), PEO 2000 with Eu L100 (1:40:40), PEO 2000 with Eu L100-55 (1:40:40) and PEO 2000 with Eu S100 (1:40:80) respectively and the $t_{50\%}$ values were 1.23, 1.43, 1.02, 2.20 and 6.39 hr (Table 5.8). With increase in proportion of Eu S100 the drug release was extended from 0.5 to 20 hr and the mechanism of drug release was shifted more towards diffusion controlled. This may be due to the decreased swelling of the matrix in presence of Eu S100 resulting in Fickian type diffusion.

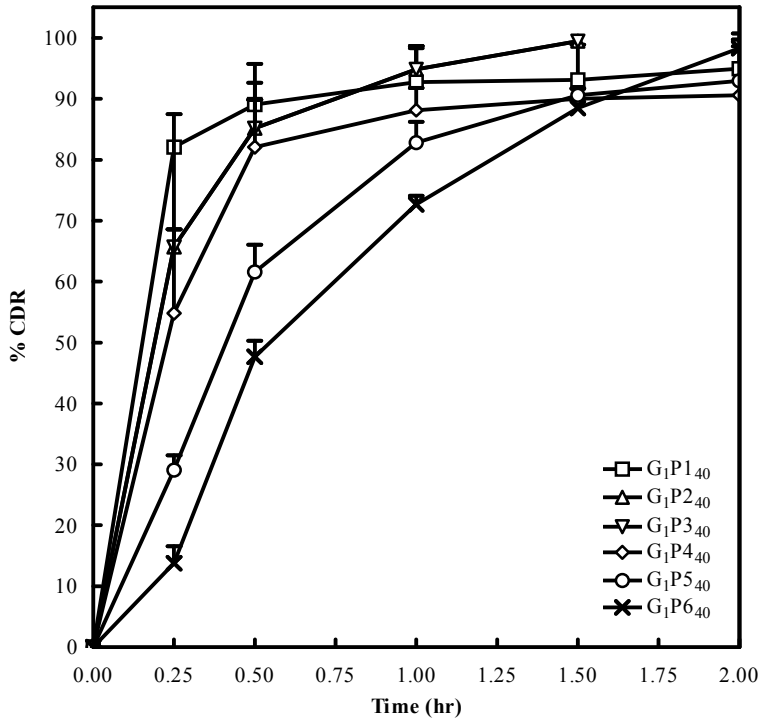

Figure 5.20: In vitro drug release from insert formulations (150 µg) prepared with different grades of PEO in 1:40 proportion. Each data point is average of three inserts from different batches.

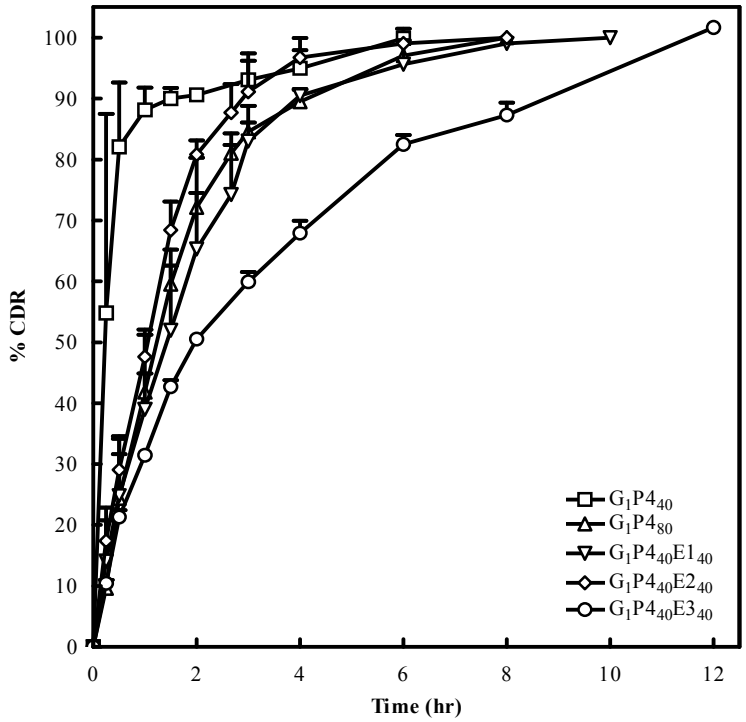

Figure 5.21: In vitro drug release from insert formulations (150 µg) prepared with PEO 2000 alone and combination of PEO 2000 and Eu L100, Eu L100-55 and Eu S100 in different proportions. Each data point is average of three inserts from different batches.

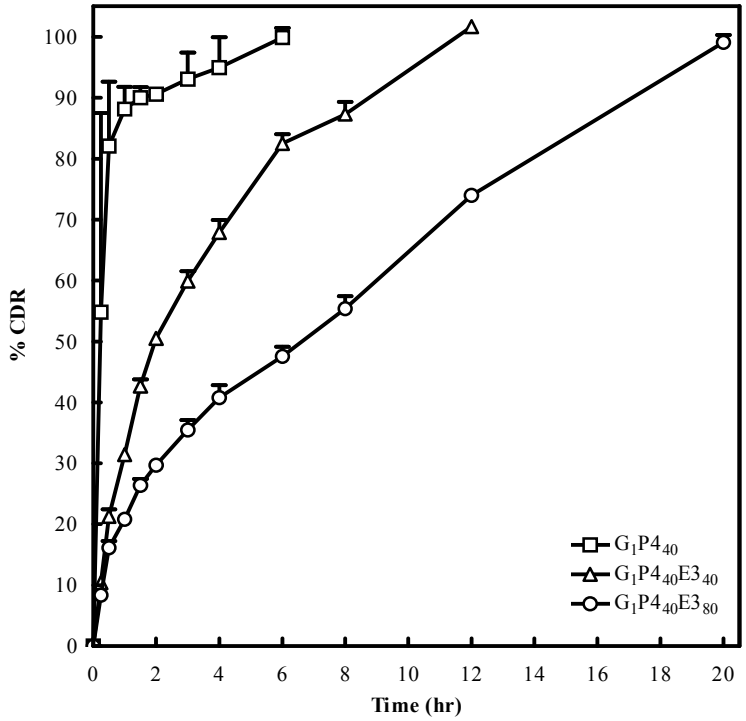

Figure 5.22: In vitro drug release from insert formulations (150 µg) prepared with PEO 2000 alone and combination of PEO 2000 and Eu S100 in different proportions. Each data point is average of three inserts from different batches.

Similarly, with addition of Eu S100 to different PEO grades the drug release was extended (Figure 5.22 to 5.28). The release rate constants were found to be 0.57 $hr^{-0.43}$, 0.48 $hr^{-0.81}$, 0.31 $hr^{-0.69}$, 0.29 $hr^{-0.53}$ and 0.28 $hr^{-0.56}$ for formulations prepared with PEO 200, 300, 900, 5000 and 7000 in combination with Eu S100 in 1:40:40, respectively and the $t_{50\%}$ values were 0.74, 1.05, 2.00, 2.76 and 2.80 hr (Table 5.8).

The release rate constants were found to be 0.23 $hr^{-0.71}$, 0.20 $hr^{-0.78}$, 0.18 $hr^{-0.76}$, 0.16 $hr^{-0.68}$ and 0.12 $hr^{-0.75}$ for formulations prepared with PEO 200, 300, 900, 5000 and 7000 in combination with Eu S100 in 1:40:80, respectively (Table 5.8). For the same formulations $t_{50\%}$ values were found to be 2.90, 3.28, 3.74, 5.54 and 6.47 hr. The increase in Eu S100 has extended the drug release. With increase in proportion of Eu S100 the drug release was extended from 0.5 to 16 hr and the mechanism of drug release was shifted more towards diffusion controlled. By replacing a portion of the hydrophilic polymer with the less soluble polymer like Eu S100, a lowering in matrix porosity is seen there by reducing the drug release.

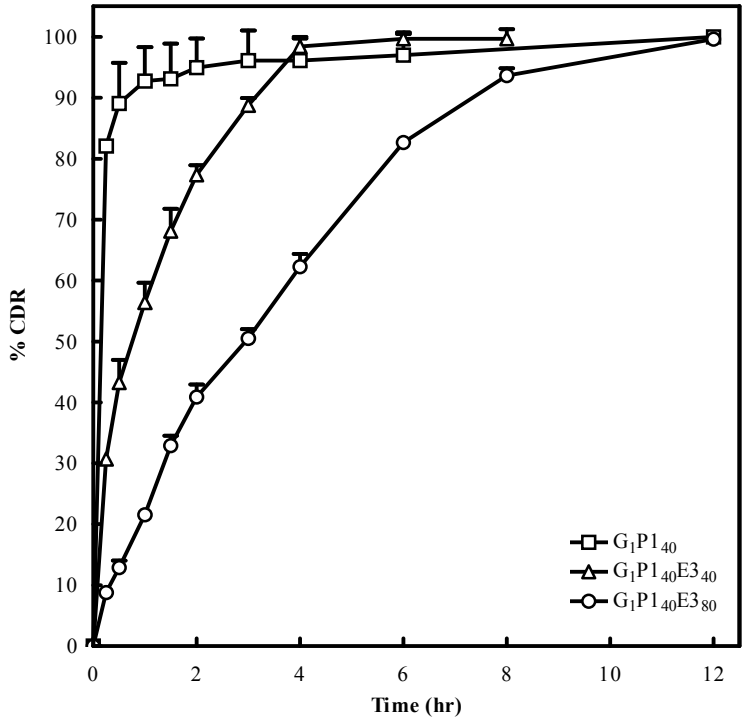

Figure 5.23: In vitro drug release from insert formulations (150 µg) prepared with PEO 200 alone and combination of PEO 200 and Eu S100 in different proportions. Each data point is average of three inserts from different batches.

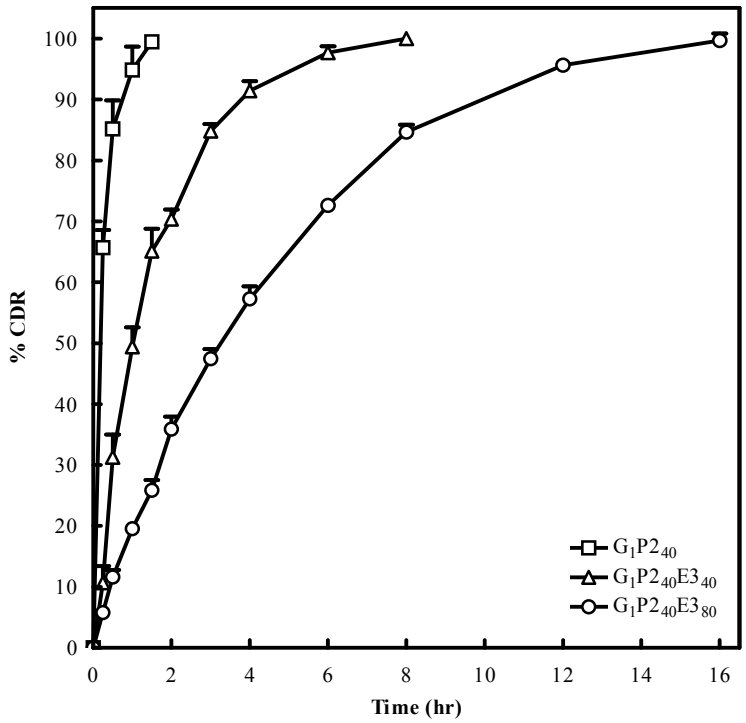

Figure 5.24: In vitro drug release from insert formulations (150 µg) prepared with PEO 300 alone and combination of PEO 300 and Eu S100 in different proportions. Each data point is average of three inserts from different batches.

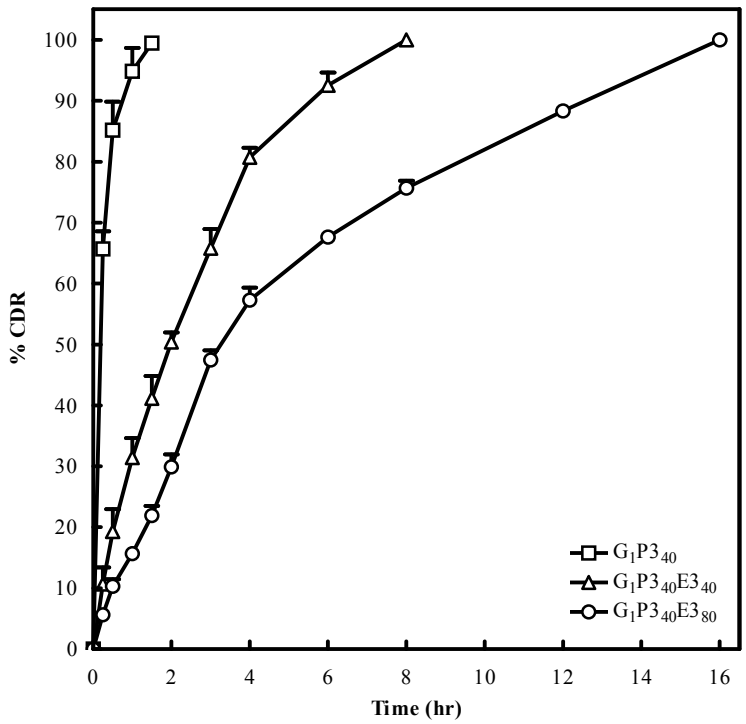

Figure 5.25: In vitro drug release from insert formulations (150 µg) prepared with PEO 900 alone and combination of PEO 900 and Eu S100 in different proportions. Each data point is average of three inserts from different batches.

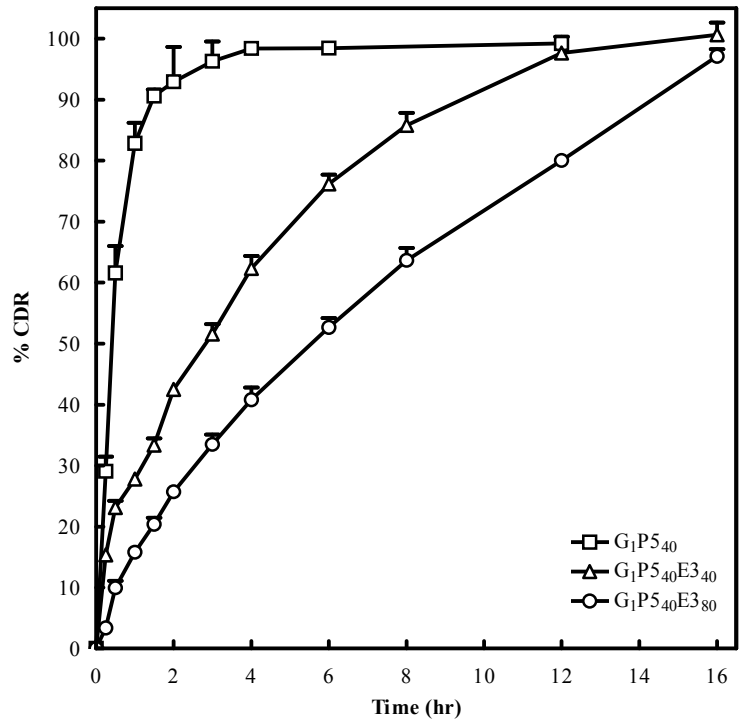

Figure 5.26: In vitro drug release from insert formulations (150 µg) prepared with PEO 5000 alone and combination of PEO 5000 and Eu S100 in different proportions. Each data point is average of three inserts from different batches.

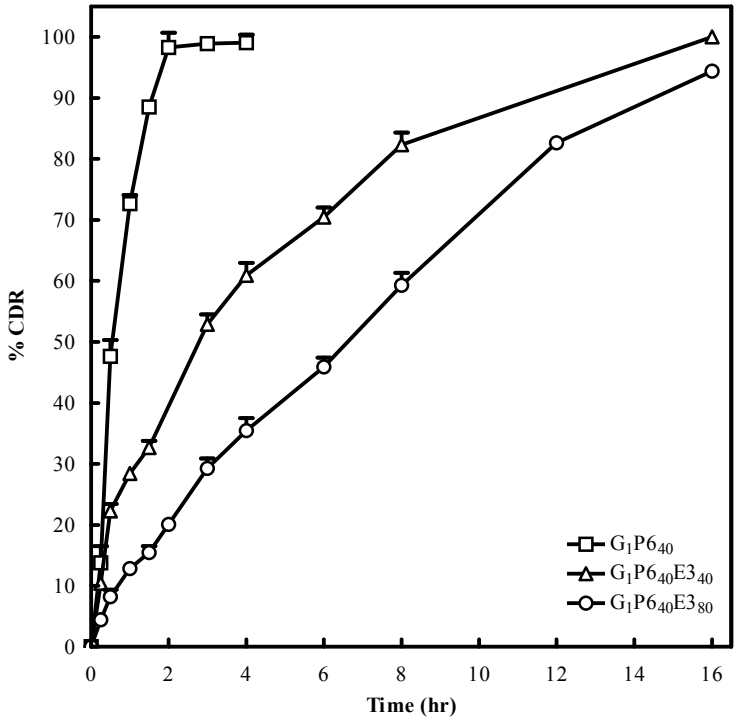

Figure 5.27: In vitro drug release from insert formulations (150 µg) prepared with PEO 7000 alone and combination of PEO 7000 and Eu S100 in different proportions. Each data point is average of three inserts from different batches.

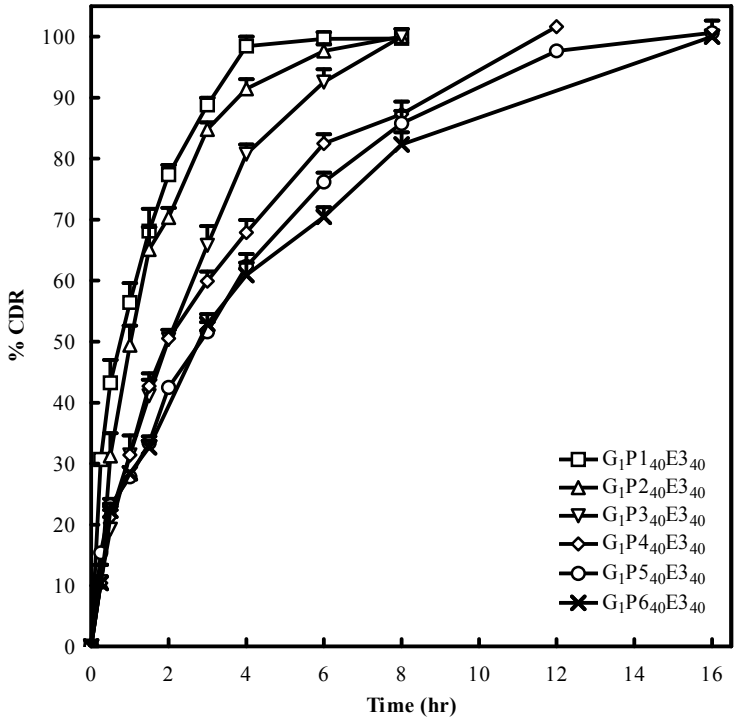

Figure 5.28: In vitro drug release from insert formulations (150 µg) prepared with different grades of PEO in combination with Eu S100 in 1:40:40 proportions. Each data point is average of three inserts from different batches.

Table 5.8: Results of drug release data from inserts (prepared using different proportions of PEO 2000 in combination with Eudragit and different grades of PEO in combination with Eudragit) fitted into Peppas model.

Code	K (h^{-n}) (Mean ± SD) †	n	$t_{50\%}$ (h)	R^2	MSSR	AIC
G_1P4_{80}	0.42 ± 0.01	0.90	1.23	0.993	2.49	5.65
$G_1P4_{40}E1_{40}$	0.39 ± 0.01	0.67	1.43	0.995	2.12	6.50
$G_1P4_{40}E2_{40}$	0.49 ± 0.01	0.78	1.02	0.997	1.01	2.06
$G_1P4_{40}E3_{40}$	0.32 ± 0.01	0.57	2.20	0.985	5.55	13.99
$G_1P4_{40}E3_{80}$	0.21 ± 0.01	0.47	6.39	0.994	1.18	3.48
$G_1P1_{40}E3_{40}$	0.57 ± 0.01	0.43	0.74	0.998	0.46	-1.11
$G_1P1_{40}E3_{80}$	0.23 ± 0.01	0.71	2.90	0.993	2.22	7.57
$G_1P2_{40}E3_{40}$	0.48 ± 0.03	0.81	1.05	0.974	10.95	11.57
$G_1P2_{40}E3_{80}$	0.20 ± 0.01	0.78	3.28	0.996	1.15	2.98
$G_1P3_{40}E3_{40}$	0.31 ± 0.00	0.69	2.00	0.999	0.33	-4.74
$G_1P3_{40}E3_{80}$	0.18 ± 0.02	0.76	3.74	0.977	10.41	20.75
$G_1P5_{40}E3_{40}$	0.29 ± 0.01	0.53	2.76	0.986	3.24	10.22
$G_1P5_{40}E3_{80}$	0.16 ± 0.00	0.68	5.54	0.997	1.01	2.08
$G_1P6_{40}E3_{40}$	0.28 ± 0.01	0.56	2.80	0.987	4.04	10.38
$G_1P6_{40}E3_{80}$	0.12 ± 0.00	0.75	6.47	0.997	0.80	-0.04

† Each data is average of three determinations.

(e) Effect of dose on different combinations

At higher dose (300 µg per insert), HPMC (different viscosity grades) and HEC in combination with Eu S100 retarded the drug release (Figure 5.29).

The release rate constants were found to be 0.17 $hr^{-0.51}$, 0.24 $hr^{-0.57}$, 0.20 $hr^{-0.50}$, 0.18 $hr^{-0.53}$ and 0.25 $hr^{-0.82}$ and the $t_{50\%}$ values were 8.72, 3.56, 5.90, 6.90 and 2.40 hr for formulations prepared with Eu S100 alone (1:80), Eu S100 in combination of with HPMC K4M, HPMC K15M, HPMC K100M and HEC in 1:20:20 proportion, respectively (Table 5.9). The n values indicate that these entire formulations released the drug by diffusion mechanism except for formulations prepared with Eu S100 with HEC. The drug release was extended from 12 hr to beyond 24 hr.

Inserts prepared with Eu S100 alone, combination of Eu S100 and HPMC K15M and combination of Eu S100 and HPMC K100M, extended the drug release beyond 24 hr. Formulations prepared with combination of Eu S100 and HPMC K4M, extended the drug release upto 24 hr. Inserts prepared with combination of Eu S100 and HEC, extended the drug release upto 12 hr.

The release rate constants were found to be 0.31 $hr^{-0.62}$, 0.50 $hr^{-0.65}$, 0.33 $hr^{-0.68}$ and 0.37 $hr^{-0.78}$ for formulations prepared with Eu S100 alone (1:20), Eu S100 in combination of with HPMC K4M, HPMC K15M and HPMC K100M in 1:10:10 proportion, respectively

(Table 5.9). For the same formulations the $t_{50\%}$ values were found to be 2.13, 1.00, 1.83 and 1.46 hr. The n values indicate that these entire formulations release drug by non-Fickian diffusion mechanism. The drug release was extended from 8 to 12 hr (Figure 5.30). Hence it can be concluded that increase in drug load from 150 to 300 µg per insert increased the drug release from ophthalmic dosage forms containing HPMC and HEC in combination with Eu S100 in similar proportions. This can be attributed to the reduction in drug to polymer proportion. In formulations prepared with 1:10:10 proportion of drug to hydrophilic polymer to Eu S100, the drug release was faster than 1:20:20 proportion.

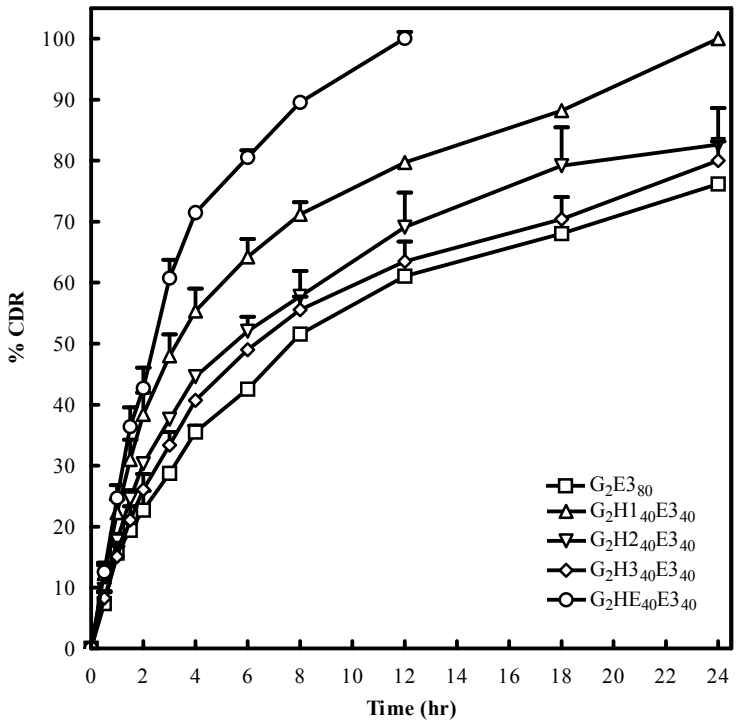

Figure 5.29: In vitro drug release from insert formulations (300 µg) prepared with of Eu S100 alone and Eu S100 in combination with hydrophilic polymers (HPMC K4M, HPMC K15M, HPMC K100M and HEC) in 1:20:20 proportion. Each data point is average of three inserts from different batches.

In case of the formulations prepared with Eu S100 in combination with PEO 200, 300, 900, 2000, 5000 and 7000 in 1:20:20 proportion (Table 5.9), with increase in molecular weight of PEO, the drug release was extended from 3.5 to 9 hr (Figure 5.31). The release rate constants for were found to be 0.40 $hr^{-0.48}$, 0.39 $hr^{-0.53}$, 0.35 $hr^{-0.50}$, 0.33 $hr^{-0.51}$, 0.28 $hr^{-0.55}$ and 0.26 $hr^{-0.53}$ and the $t_{50\%}$ values were 1.55, 1.64, 2.07, 2.30, 2.82 and 3.39 hr respectively. The drug release was found to be Fickian diffusion. The extension of drug release was maximum with highest molecular weight of PEO.

With increase in molecular weight of PEO the drug release was extended from 1 to 2.5 hr (Figure 5.32). The release rate constants were found to be 0.70 $hr^{-0.54}$, 0.62 $hr^{-0.66}$ and 0.53 $hr^{-0.68}$ for formulations prepared with Eu S100 in combination with PEO 200, 900 and 7000 in 1:10:10 proportion, respectively (Table 5.9). The $t_{50\%}$ values for the same

formulations were found to be 0.53, 0.73 and 0.92 hr. The drug release was found to be non-Fickian diffusion.

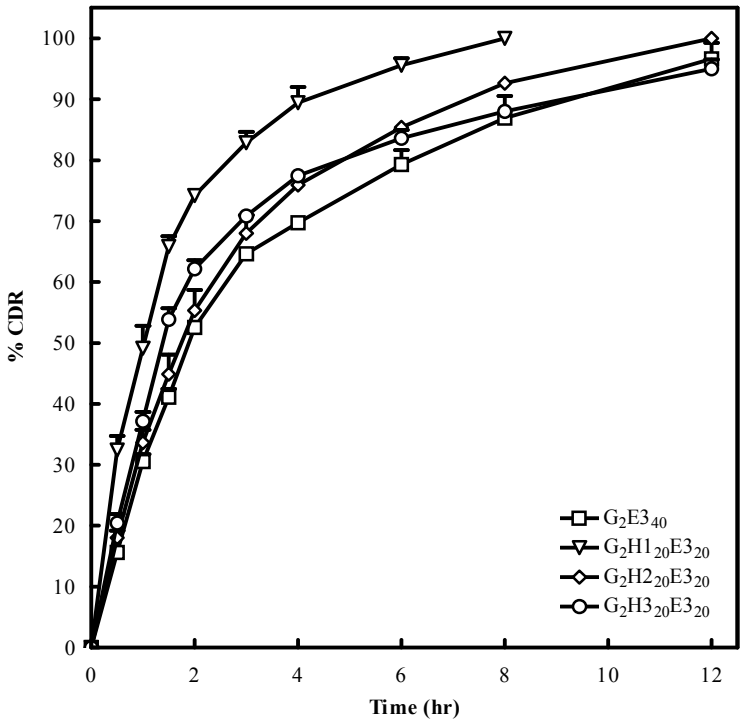

Figure 5.30: In vitro drug release from insert formulations (300 µg) prepared with of Eu S100 alone and Eu S100 in combination with hydrophilic polymers (HPMC K4M, HPMC K15M and HPMC K100M) in 1:10:10 proportion. Each data point is average of three inserts from different batches.

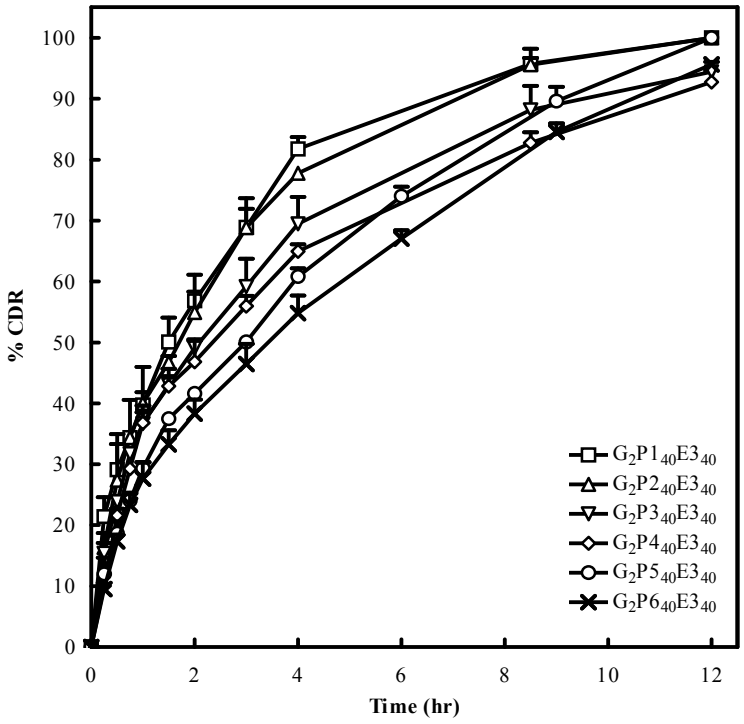

Figure 5.31: In vitro drug release from insert formulations (300 µg) prepared with of different grades of PEO in combination with Eu S100 in 1:20:20 proportion. Each data point is average of three inserts from different batches.

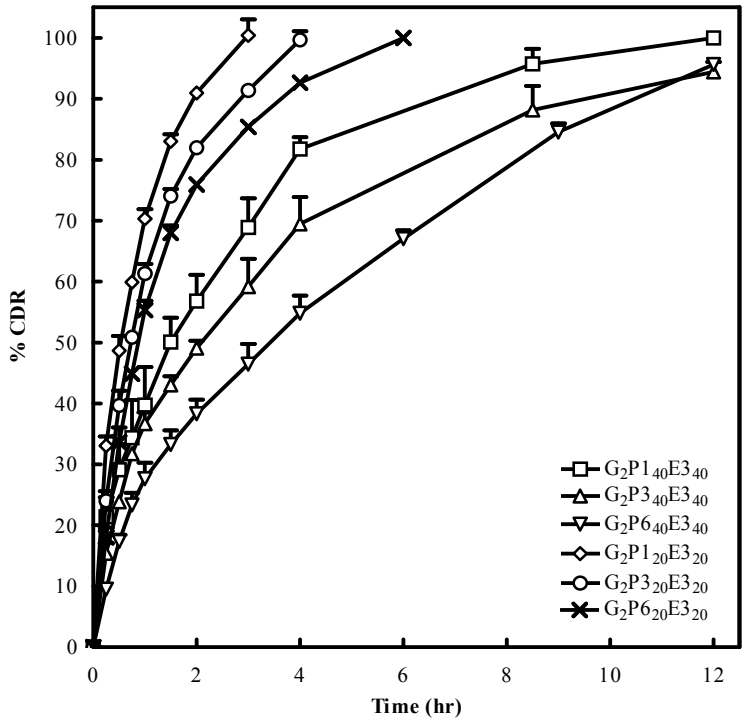

Figure 5.32: In vitro drug release from insert formulations (300 µg) prepared with of different grades of PEO in combination with Eu S100 in different proportions. Each data point is average of three inserts from different batches.

Table 5.9: Results of drug release data from inserts (prepared using Eudragit alone, PEO (different grades), HEC and HPMC (different grades) in combination with Eudragit) fitted into Peppas model.

Code	K (h^{-n}) (Mean ± SD) †	n	$t_{50\%}$ (h)	R^2	MSSR	AIC
G_2E3_{40}	0.31 ± 0.02	0.62	2.13	0.968	11.51	16.66
G_2E3_{80}	0.17 ± 0.01	0.51	8.72	0.983	6.29	20.38
$G_2H1_{40}E3_{40}$	0.24 ± 0.02	0.57	3.56	0.977	6.63	15.25
$G_2H2_{40}E3_{40}$	0.20 ± 0.01	0.50	5.90	0.981	6.33	18.60
$G_2H3_{40}E3_{40}$	0.18 ± 0.01	0.53	6.90	0.979	6.64	19.04
$G_2HE_{40}E3_{40}$	0.25 ± 0.01	0.82	2.40	0.995	1.32	3.40
$G_2H1_{20}E3_{20}$	0.50 ± 0.01	0.65	1.00	0.997	0.51	-0.02
$G_2H2_{20}E3_{20}$	0.33 ± 0.01	0.68	1.83	0.988	3.50	8.26
$G_2H3_{20}E3_{20}$	0.37 ± 0.02	0.78	1.46	0.988	3.10	6.52
$G_2P1_{40}E3_{40}$	0.40 ± 0.00	0.48	1.55	0.998	0.38	-4.86
$G_2P2_{40}E3_{40}$	0.39 ± 0.01	0.53	1.64	0.994	1.47	4.72
$G_2P3_{40}E3_{40}$	0.35 ± 0.01	0.50	2.07	0.995	1.49	5.20
$G_2P4_{40}E3_{40}$	0.33 ± 0.01	0.51	2.30	0.971	8.40	19.02
$G_2P5_{40}E3_{40}$	0.28 ± 0.01	0.55	2.82	0.995	1.27	3.89
$G_2P6_{40}E3_{40}$	0.26 ± 0.01	0.53	3.39	0.995	1.60	6.23
$G_2P1_{20}E3_{20}$	0.70 ± 0.01	0.54	0.53	0.999	0.07	-5.93
$G_2P3_{20}E3_{20}$	0.62 ± 0.01	0.66	0.73	0.999	0.23	-3.89
$G_2P6_{20}E3_{20}$	0.53 ± 0.01	0.68	0.92	0.988	3.50	8.26

† Each data is average of three determinations.

The prepared inserts showed dose depended drug release profiles. Optimum drug release was observed for formulations prepared with hydrophilic polymer in combination with Eu S100 in 1:40:40 proportion for 150 µg strength and 1:20:20 proportion for 300 µg strength.

5.3.3. Swelling studies

Swelling studies of prepared formulations by texture analyser revealed that they have good swelling capacity. The results of these studies were presented in Figure 5.33.

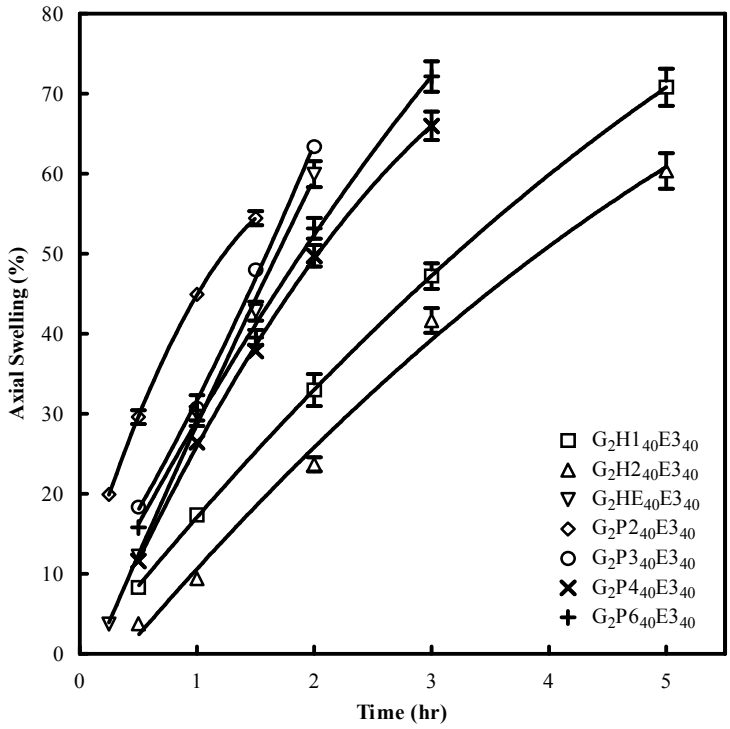

Figure 5.33: Swelling studies results of insert formulations (300 µg) prepared with of different grades of PEO and HPMC in combination with Eu S100 in 1:20:20 proportion. Each data point is average of three inserts.

Axial swelling studies showed that the formulations containing PEO take up water readily and swell at faster rate than the formulations prepared with HPMC and HEC. Faster drug release from the formulations containing PEO can be attributed to its swelling. At any given time point, formulations prepared with low molecular weight PEO ($G_2P2_{40}E3_{40}$) have shown highest swelling when compared with the other formulations. This is because PEO 200 hydrates faster than other polymers and thereby has highest water absorbing capacity. As the molecular weight of PEO increased further, the % of swelling decreased. This might be the reason for the slow release rate of gatifloxacin from $G_2P4_{40}E3_{40}$, and $G_2P6_{40}E3_{40}$ formulations. The Peppas release rate constants for these preparations were 0.33 $hr^{-0.51}$ and

0.26 $hr^{-0.53}$ respectively. Slow release of drug from these formulations can also substantiated by $t_{50\%}$, calculated from the Peppas model, which are 2.30 and 3.39 hr respectively. The $t_{50\%}$ for the formulations prepared with low molecular weight of PEO are 1.64 and 2.07 hr indicating faster release from these preparations. Presence of HPMC (4000 cps) in the formulation decreased the swelling when compared with PEO grades there by retarding the drug release (Peppas rate constant for $G_2H1_{40}E3_{40}$ is 0.24 $hr^{-0.57}$ and $t_{50\%}$ is 3.56 hr). Replacing the HPMC 4000 with HPMC 15000 further decreased the % axial swelling of the formulation.

In a nut shell, swelling studies of various formulations have helped to clarify the mechanism of drug release from the prepared inserts.

5.3.4. Bioadhesion testing

(a) Conventional bioadhesion testing

The hydration time and the weight applied were optimized by a series of pre-experiments. Bioadhesion testing was conducted on different formulations using goat conjunctival tissue. The force of detachment was determined and compared for the formulations and the data was given in Table 5.10 and depicted in Figures 5.34 to 5.36.

Table 5.10: Result of bioadhesion test conducted on ophthalmic inserts by conventional method. Each data point is average of three inserts.

Code	Force of Detachment ($N.cm^{-2}$) (Mean ± SD)	Code	Force of Detachment ($N.cm^{-2}$) (Mean ± SD)
G_1H1_{20}	0.266 ± 0.020	G_1P1_{40}	0.369 ± 0.004
G_1H1_{40}	0.287 ± 0.013	G_1P2_{40}	0.487 ± 0.013
G_1H1_{60}	0.297 ± 0.011	G_1P3_{40}	0.569 ± 0.043
G_1H1_{80}	0.302 ± 0.027	G_1P4_{40}	0.529 ± 0.022
G_1H2_{80}	0.327 ± 0.002	G_1P4_{80}	0.569 ± 0.034
G_1H3_{80}	0.338 ± 0.033	G_1P5_{40}	0.465 ± 0.025
G_1E3_{20}	0.010 ± 0.000	G_1P6_{40}	0.427 ± 0.014
G_1E3_{80}	0.020 ± 0.002	$G_1P1_{40}E3_{40}$	0.379 ± 0.010
$G_1H1_{40}E1_{40}$	0.277 ± 0.027	$G_1P2_{40}E3_{40}$	0.470 ± 0.036
$G_1H2_{40}E3_{40}$	0.289 ± 0.020	$G_1P3_{40}E3_{40}$	0.578 ± 0.014
$G_1H3_{40}E3_{40}$	0.308 ± 0.014	$G_1P4_{40}E3_{40}$	0.517 ± 0.013
G_1HE_{80}	0.327 ± 0.009	$G_1P5_{40}E3_{40}$	0.477 ± 0.032
$G_1HE_{40}E3_{40}$	0.289 ± 0.027	$G_1P6_{40}E3_{40}$	0.447 ± 0.008

The insert prepared with Eudragit alone did not show any bioadhesion (Figure 5.34). With increase in the proportion of HPMC K4M from 1:20 to 1:80, detachment force increased significantly from 0.266 to 0.302 $N.cm^{-2}$, due to increased polymer proportion.

With increase in viscosity of HPMC from 4000 to 100000 cPs the detachment force increased, but not to significant level (Figure 5.34).

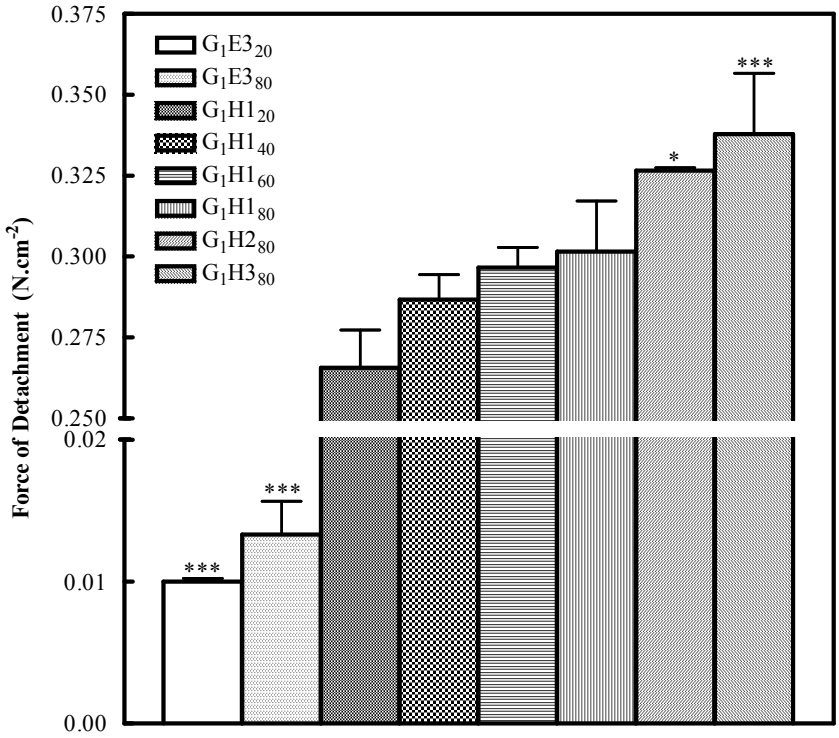

Figure 5.34: Results of the mucoadhesion test; detachment force as function of different polymers alone (Eudragit and HPMC) in different proportions. Each data point is average of three inserts. (* – *P < 0.05*; *** – *P < 0.001*; data compared with G_1H1_{20})

Detachment force of different HPMC grades in combination with Eu S100 was shown in Figure 5.35. Both HPMC and HEC were showing same detachment force. Addition of Eu S100 to the formulations led to lesser detachment force as compared to formulations prepared with HPMC and HEC alone. However, this reduction was not significant.

Detachment force for insert containing different PEO grades alone and combination with EU S100 was shown in the Figure 5.36 a and b. Increase in ratio of PEO from 1:40 to 1:80 increased the detachment force from 0.529 to 0.569 $N.cm^{-2}$, due to increased bioadhesive polymer proportion. The detachment force increased from 0.369 to 0.569 $N.cm^{-2}$ with increase in molecular with of PEO from 700 to 900 kDa. However, detachment force decreased from 0.569 to 0.427 $N.cm^{-2}$with further increase in molecular weight of PEO from 900 to 7000. Similar effect was observed with inserts containing combination of PEO and Eu S100. As the number of chains increases with increase in the molecular weight, the possibility of polymer-polymer interactions increases, there by reduces the number of penetrating/entangling polymer chains per unit mucus volume. Least polymer-polymer interaction and more penetrating/entangling polymer chains per unit mucus volume are evident for the polymers having lower molecular weights. Therefore bioadhesion will be low

in the case of higher molecular polymers than lower molecular weight polymers (Bremecker, 1983; Di Colo et al., 2001b).

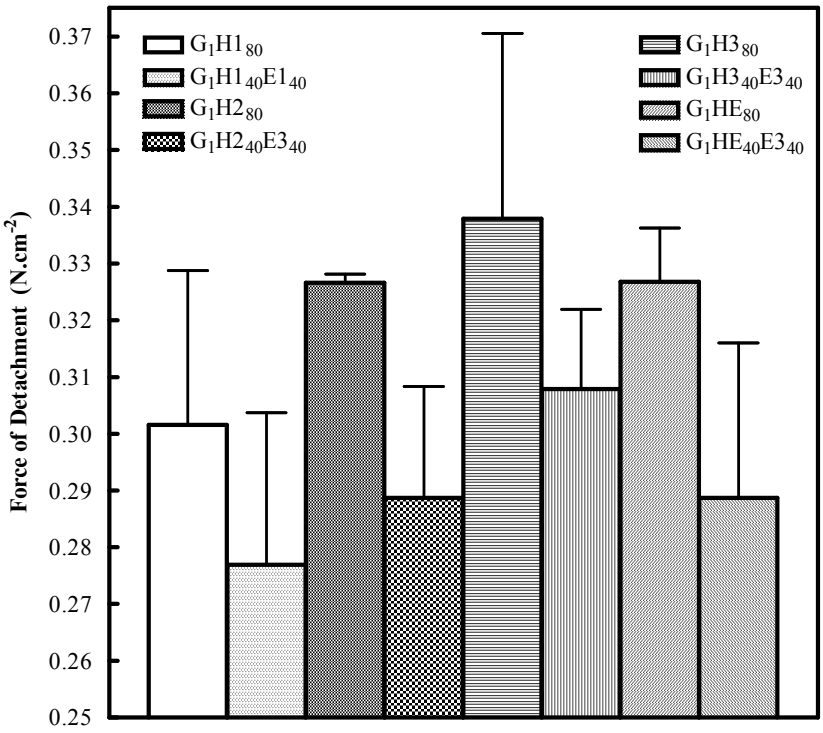

Figure 5.35: Results of the mucoadhesion test; detachment force for inserts prepared with different hydrophilic polymers alone and in combination with Eu S100. Each data point is average of three inserts.

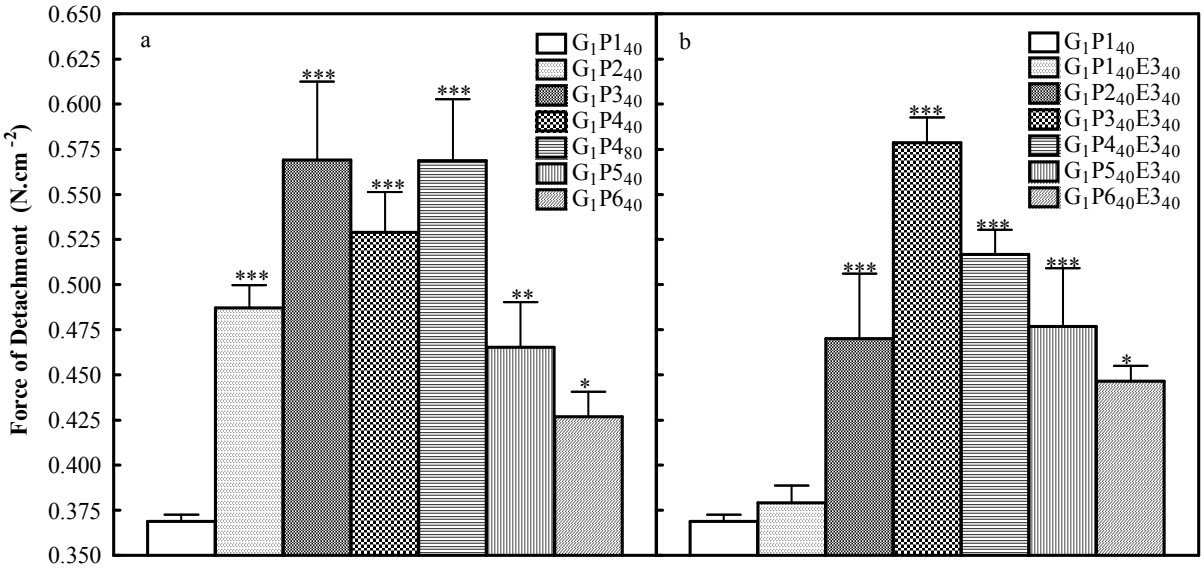

Figure 5.36: Results of the mucoadhesion test; detachment force for inserts prepared with (a) different grades of PEO alone and (b) different grades of PEO in and in combination with Eu S100. Each data point is average of three inserts. (* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$; data compared with G_1P1_{40})

Addition of Eu S100 to the formulations has not changed the detachment force significantly as compared PEO alone formulations. These results show that the Eu S100 was not interfering with the bioadhesion character of the hydrophilic polymers.

(b) Bioadhesion testing by texture analyser

According to Lejoeux et al. (1988), when the bioadhesive power of different solid or viscous systems is put to comparison, the work required to detach the bioadhesive system from the substrate, equivalent to the area under the force-distance curve, is a more sensitive parameter than the detachment force. Therefore the work of adhesion and F_{max} was determined using texture analyser. The hydration time and the weight applied were optimized by a series of pre-experiments.

F_{max} and work of adhesion data are given in Table 5.11 and depicted in Figure 5.37. The inserts prepared with combination of hydrophilic and methacrylate polymers were studied for mucoadhesiveness using goat conjunctival tissue.

Using HPMC with higher viscosity grade from 4000 to 100000 cPs, the F_{max} increased moderately from 2.49 to 3.16 $N.cm^{-2}$ and work of adhesion increased from 1.4 to 2.01 $N.sec.cm^{-2}$. Inserts having formulations with increase in molecular weight of PEO from 200 to 900 kDa, the F_{max} increased from 2.71 to 4.69 $N.cm^{-2}$ and work of adhesion from 2.29 to 3.92 $N.sec.cm^{-2}$. There after the increase in molecular weight of PEO from 900 to 7000 kDa, decreased the F_{max} and work of adhesion.

Table 5.11: Result of bioadhesion testing conducted on texture analyser on different insert formulations. Each data point is average of three inserts.

Code	F_{max} ($N.cm^{-2}$)	Work of Adhesion ($N.sec.cm^{-2}$)
$G_2H1_{40}E3_{40}$	2.49 ± 0.39	1.40 ± 0.15
$G_2H2_{40}E3_{40}$	2.95 ± 0.13	1.89 ± 0.18
$G_2H3_{40}E3_{40}$	3.16 ± 0.11	2.01 ± 0.05
$G_2HE_{40}E3_{40}$	1.42 ± 0.07	1.08 ± 0.06
$G_2P1_{40}E3_{40}$	2.71 ± 0.07	2.29 ± 0.07
$G_2P2_{40}E3_{40}$	3.71 ± 0.33	3.52 ± 0.12
$G_2P3_{40}E3_{40}$	4.69 ± 0.01	3.92 ± 0.01
$G_2P4_{40}E3_{40}$	4.12 ± 0.06	3.65 ± 0.16
$G_2P5_{40}E3_{40}$	3.24 ± 0.23	2.83 ± 0.13
$G_2P6_{40}E3_{40}$	2.35 ± 0.20	2.14 ± 0.13
$G_2P3_{20}E3_{20}$	3.39 ± 0.15	2.92 ± 0.06

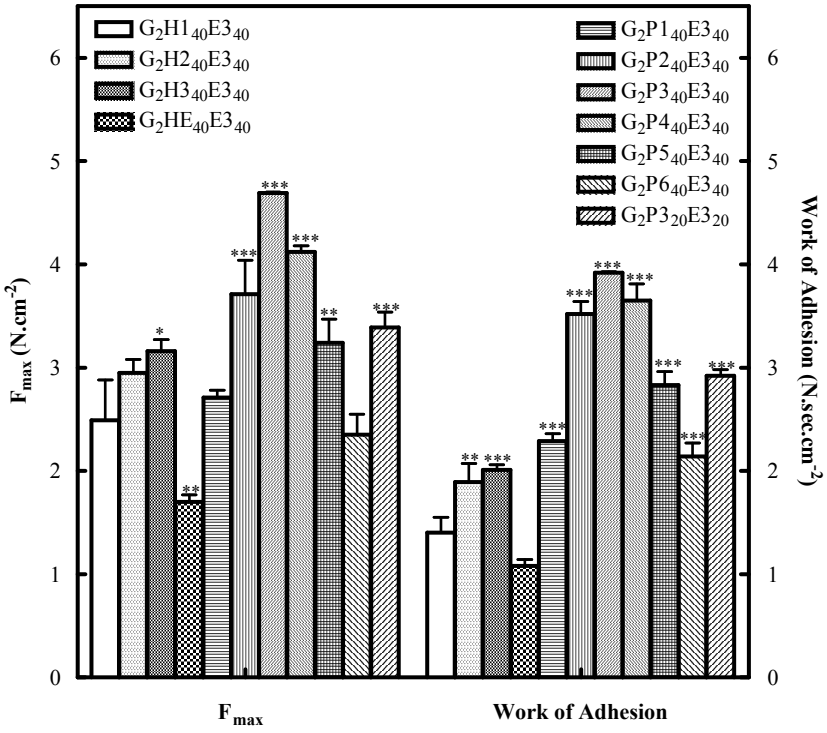

Figure 5.37: Results of the mucoadhesion test by texture analyser; F_{max} and work of adhesion for inserts prepared with different hydrophilic polymers alone and in combination with Eu S100. Each data point is average of three inserts. (* – *P < 0.05*; ** – *P < 0.01*; *** – *P < 0.001*; data compared with $G_2H1_{40}E3_{40}$)

With increase in insert weight and polymer proportion (from 1:20 to 1:40), the F_{max} and work of adhesion increased by 1.38 and 1.34 folds respectively. The results are in agreement with conventional bioadhesion testing.

5.3.5. In vitro microbiological testing

The selected insert formulations showed antimicrobial activity when tested microbiologically by cup plate technique. Zone of inhibitions were clearly identified and areas of zone of inhibition were determined. Areas of zone of inhibition for prepared insert formulations were compared with Gatilox (marketed formulation). Mean areas (± SD) of zone inhibition were found be 272.09 (± 19.16), 233.23 (± 22.37) and 278.48 (± 22.68) mm^2 at 18 hr for marketed, $G_1H2_{40}E3_{40}$ and $G_1P3_{40}E3_{40}$ inserts formulations respectively. At 24 hr. for the same formulations the mean areas (± SD) of zone inhibition were 275.13 (± 22.75), 257.75 (± 20.95) and 299.48 (± 12.15) mm^2. Statistically insignificant difference was observed between the developed formulation and marketed formulation at both 18 hr and 24 hr. The study provided the evidence that the drug released from the formulation has not changed its anti-microbial activity.

5.3.6. Batch reproducibility

The physical properties of the inserts from all three batches were evaluated in the same manner as for the original batch formulations. The inserts showed low standard deviation values for the drug content, weight variation and crushing strength for three different batches prepared separately (Tables 5.2 to 5.5). These low standard deviation values for all physical properties showed that there was excellent batch-to-batch reproducibility. No significant difference was observed in the release profiles of the formulations between different batches as indicated by the low standard deviation values of the percent cumulative release data at different time points obtained from the replicate release studies of the samples.

5.3.7. Stability studies

The drug content of the formulations before and after sterilization was given in Table 5.12. The comparison of mean assay values of inserts, before and after sterilization, by *t*-test showed that there was no significant difference. The drug release from the insert formulations before and after sterilization was not showing any difference.

The degradation kinetics of gatifloxacin in insert formulations at different storage conditions were presented in Table 5.13. The stability data of formulations was best fitted into first-order rate kinetics, which was evident from high regression coefficient, low MSSR and low AIC values. The $t_{90\%}$ was calculated based on the K_{deg} of gatifloxacin in CRT conditions.

Table 5.12: Effect of sterilization on assay of the drug in designed insert formulations containing gatifloxacin. Each data point is average of three inserts.

Formulation Code	Amount in µg before sterilization (Mean ± SD)	Amount in µg after sterilization (Mean ± SD)
G_1E3_{80}	151.50 ± 0.15	150.81 ± 5.97
$G_1H1_{40}E3_{40}$	151.53 ± 1.35	150.39 ± 0.35
$G_1H2_{40}E3_{40}$	148.95 ± 1.28	148.71 ± 3.27
$G_1H3_{40}E3_{40}$	150.51 ± 3.06	149.67 ± 2.25
$G_1HE_{40}E3_{40}$	152.12 ± 1.35	150.98 ± 1.34
$G_1P3_{40}E3_{40}$	150.27 ± 2.99	149.58 ± 3.98
$G_1P4_{40}E3_{40}$	148.99 ± 2.98	148.46 ± 2.97

Formulation Code	Amount in µg before sterilization (Mean ± SD)	Amount in µg after sterilization (Mean ± SD)
G_2E3_{80}	310.53 ± 2.53	308.19 ± 6.48
$G_2H1_{40}E3_{40}$	306.67 ± 3.85	306.18 ± 6.84
$G_2H2_{40}E3_{40}$	298.28 ± 1.73	296.91 ± 4.71
$G_2H3_{40}E3_{40}$	298.61 ± 0.64	297.54 ± 4.62
$G_2HE_{40}E3_{40}$	300.83 ± 1.48	298.56 ± 3.45
$G_2P3_{20}E3_{40}$	301.92 ± 5.64	300.24 ± 5.61
$G_2P4_{40}E3_{40}$	301.77 ± 1.47	299.49 ± 6.42

At accelerated conditions (AT) the degradation rate constant for the drug was found to be 3.33×10^{-3}, 3.63×10^{-3}, 3.74×10^{-3}, 3.16×10^{-3}, 4.02×10^{-3} and 3.96×10^{-3} $month^{-1}$ for formulations (containing 150 µg) prepared with HPMC K4M, HPMC K15M, HPMC K100M, HEC, PEO 900 and PEO 2000 in combination with Eu S100 respectively. At these

conditions the degradation rate constant for the drug was found to be 4.54×10^{-3}, 3.82×10^{-3}, 3.87×0^{-3}, 3.94×10^{-3}, 4.28×10^{-3} and 3.70×10^{-3} $month^{-1}$ for formulations (containing 300 µg) prepared with HPMC K4M, HPMC K15M, HPMC K100M, HEC, PEO 900 and PEO 2000 in combination with Eu S100 respectively. The degradation rate was found to be 3.24×10^{-3} for formulations (containing 300 µg) prepared with Eu S100 alone in the proportion of 1:40. The drug release from the insert formulations at zero time and last time point was not showing significant difference. There was increase in moisture content of the formulations after storage at accelerated conditions for 6 months. But, moisture content even in worst case was not more than 7.23 %.

Table 5.13: First order degradation kinetics parameters of gatifloxacin in insert formulations.

Code	Storage Condition	$K_{deg} \times 10^3$ ($month^{-1}$) † (Mean ± SD)	R^2	MSSR	AIC	$t_{90\%}$ (month)
G_2E3_{80}	CRT	0.53 ± 0.01	0.996	0.19	-30.44	199.89
	AT	3.24 ± 0.02	0.996	0.47	-7.19	
$G_1H1_{40}E3_{40}$	CRT	0.51 ± 0.01	0.995	0.37	-16.91	204.65
	AT	3.33 ± 0.07	0.996	0.42	-8.87	
$G_2H1_{40}E3_{40}$	CRT	0.45 ± 0.01	0.992	0.06	-56.84	232.73
	AT	4.54 ± 0.24	0.993	3.11	21.00	
$G_1H2_{40}E3_{40}$	CRT	0.52 ± 0.01	0.996	0.23	-26.79	202.54
	AT	3.68 ± 0.09	0.996	0.54	-5.19	
$G_2H2_{40}E3_{40}$	CRT	0.45 ± 0.01	0.999	0.03	-69.82	236.75
	AT	3.82 ± 0.21	0.998	2.76	19.25	
$G_1H3_{40}E3_{40}$	CRT	0.51 ± 0.01	0.996	0.22	-28.06	208.24
	AT	3.74 ± 0.09	0.996	0.53	-5.62	
$G_2H3_{40}E3_{40}$	CRT	0.53 ± 0.01	0.997	0.04	-63.57	197.10
	AT	3.87 ± 0.21	0.995	2.95	20.22	
$G_1HE_{40}E3_{40}$	CRT	0.51 ± 0.02	0.995	0.28	-22.47	207.21
	AT	3.16 ± 0.08	0.995	0.81	0.83	
$G_2HE_{40}E3_{40}$	CRT	0.50 ± 0.01	0.998	0.04	-65.20	211.35
	AT	3.94 ± 0.22	0.992	3.79	23.99	
$G_1P3_{40}E3_{40}$	CRT	0.67 ± 0.02	0.995	0.54	-9.09	157.24
	AT	4.02 ± 0.15	0.995	1.30	7.93	
$G_2P3_{40}E3_{40}$	CRT	0.65 ± 0.01	0.997	0.06	-55.71	161.77
	AT	4.28 ± 0.24	0.992	3.83	24.13	
$G_1P4_{40}E3_{40}$	CRT	0.48 ± 0.02	0.995	0.14	-37.39	219.54
	AT	3.96 ± 0.15	0.995	1.35	8.55	
$G_2P4_{40}E3_{40}$	CRT	0.52 ± 0.03	0.992	0.80	-0.71	201.32
	AT	3.70 ± 0.09	0.996	0.63	-3.01	

† Each data is average of three determinations.

The drug has shown degradation rate constant similar to that of the bulk drug (reported in chapter 4) at accelerated conditions. All the products were stable till 6 months of the study. The $t_{90\%}$ calculated based on the K_{deg} of AT was above 10 months, which indicates that the drug is stable under the accelerated conditions.

In the formulations stored at room temperature, the degradation rate was found to be 0.51×10^{-3}, 0.52×10^{-3}, 0.51×10^{-3}, 0.57×10^{-3}, 0.67×10^{-3} and 0.48×10^{-3} $month^{-1}$ for formulations (containing 150 μg) prepared with combination of HPMC K4M, HPMC K15M, HPMC K100M, HEC, PEO 900 and PEO 2000 combination with Eu S100 respectively. At the same storage conditions the degradation rate constant for formulations (300 μg) prepared with HPMC K4M, HPMC K15M, HPMC K100M, HEC, PEO 900 and PEO 2000 combination with Eu S100 was found to be 0.45×10^{-3}, 0.45×10^{-3}, 0.53×10^{-3}, 0.50×10^{-3}, 0.65×10^{-3} and 0.52×10^{-3} $month^{-1}$ respectively. The degradation rate was found to be 0.53×10^{-3} for formulations (containing 300 μg) prepared with Eu S100 alone in the proportion of 1:40. The drug release from the insert formulations at zero time and last time point was not showing significant difference. There was increase in moisture content of the formulations after storage at CRT conditions. But, moisture content even in worst case was not more than 3.11 %.

The $t_{90\%}$ calculated based on K_{deg} in CRT condition for all combinations was found to be above 150 months. None of the products have shown considerable degradation in the entire period of real time stability study (at CRT conditions).

5.4. Conclusions

The designed ophthalmic inserts of gatifloxacin showed good physical properties indicating that the method of preparation of formulation is suitable and acceptable for preparing good quality solid inserts. Wet granulation method produced ophthalmic inserts with optimum thickness and reproducible content uniformity. Physical appearance, insert hardness, friability, weight variation, and drug content uniformity of all formulations were found to be satisfactory. The insert manufacturing method was relatively simple and can be easily adopted in conventional formulation manufacturing units in industries on a commercial scale.

Drug release from gatifloxacin loaded ophthalmic inserts was affected by nature of polymers (hydrophilic or anionic polymer), proportion, combination (hydrophilic and anionic polymer) and drug loading. In the present study a series of formulations was developed with different release rates and duration. The duration of gatifloxacin release was extended from 1 hr to beyond 24 hr by varying the polymer type, polymer ratio and polymer combination.

Drug release was found to depend on both polymeric network and micro-environment pH of the polymeric matrix. The formulations with HPMC and HEC in combination with Eu S100 have shown non-Fickian diffusion mechanism for drug release and formulations with PEO in combination with Eu S100 have shown Fickian diffusion mechanism for drug release. The drug release was affected by rate of swelling. Formulations containing non-ionic hydrophilic polymer in combination with Eu S100 has shown maximum extension of drug release.

The low standard deviation values for all physical properties showed that there was excellent batch-to-batch reproducibility. Sterilization method has not affected the drug content and drug release rate. When stored at CRT for 24 months, the drug release rate and drug content had not changed significantly.

The drug release was extended upto 24 hr for formulations prepared with Eu S100 alone 1:40 proportion (G_2E3_{80}). These formulations have shown very weak bioadhesive strength. The drug release was extended upto 18 hr for formulations prepared with HPMC K15M and Eu S100 in 1:20:20 proportion ($G_2H2_{40}E3_{40}$). Maximum detachment force and work of adhesion were found to be satisfactory for this formulation. Maximum bioadhesion strength was obtained for formulations prepared with PEO 900 and Eu S100 in 1:20:20 proportion ($G_2P3_{40}E3_{40}$). Based on bioadhesion strength, next best formulation was PEO 2000 and Eu S100 in 1:20:20 proportion ($G_2P4_{40}E3_{40}$). The drug release was extended upto 9 hr from these formulations.

Formulations showing extension of drug release between 9 to 24 hr and/or good bioadhesion strength were considered for further studies. The selected formulations were G_2E3_{80}, $G_2H2_{40}E3_{40}$, $G_2P3_{40}E3_{40}$ and $G_2P4_{40}E3_{40}$.

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Chapter 6

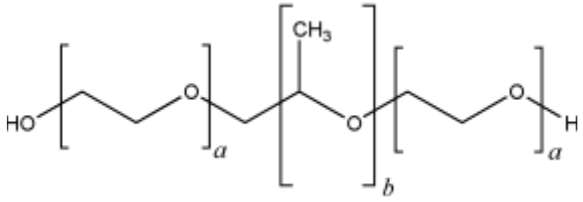
Ophthalmic In situ Gel Formulations: Development and In Vitro Characterization

6.1. Introduction

Although eye drops represent 90 % of all ophthalmic dosage forms, there is a significant effort directed towards development of new drug delivery systems to overcome the disadvantages of eye drops. In situ gel systems are viscous hydrogel systems which undergo a sol-gel phase transition after exposure to the physiological conditions in the cul-de-sac, forming a viscoelastic gel. The polymers used exhibit reversible phase transitions. In situ gel systems increase patient compliance and ease of administration as compared to other novel dosage forms.

The phase transition of the different polymers used in these systems is triggered by the pH of the tears (e.g. carbomer, cellulose acetate phthalate latex) (Gurny et al., 1985; Ke et al., 2001; Srividya et al., 2001; Xu et al., 2002; Sultana et al., 2006a; Wu et al., 2007), the temperature at the eye surface (e.g. methyl cellulose, xyloglucan, poloxamer 407) (Miyazaki et al., 2001; Wei et al., 2006) or the monovalent and divalent cations present in the tear film (e.g. alginic acid, sodium alginate, gellan gum - Gelrite®) (Demailly et al., 2001; Balasubramaniam et al., 2003; Trinquand et al., 2003; El-Kamel et al., 2006; Liu et al., 2006; Takiyama et al., 2006). These polymers were used alone and in combination with other in situ gel forming polymers, viscosity enhancing agents and bioadhesive polymers (Kaur and Kanwar, 2002). With these systems, the drug residence time can be increased to 24 hr. Once gelled, the formulation resists the natural drainage process from the precorneal area. Residence at the site of drug absorption is prolonged and subsequently, the bioavailability of the drug is increased (Rozier et al., 1989, 1997). Faster conversion of sol-gel in the cul-de-sac will reduce the drug loss due to spillage or tear turn over.

Pluronic® or poloxamer is a triblock copolymers, consists of hydrophilic ethylene oxide (EO) and hydrophobic propylene oxide (PO) blocks arranged in a basic PEO-PPO-PEO structure. The poloxamer polyols are a series of closely related with general formula $HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH$ (Figure 6.1). The letter 'F', stands for the physical form of the poloxamer, i.e. flakes (Collett, 2005).



Name		a	b	Average molecular weight
Poloxamer 188	Pluronic® F-68	80	27	7680 to 9510
Poloxamer 407	Pluronic® F-127	95-105	54-65	9840 to 14600

Figure 6.1: General formulae for poloxamers, chemical composition and molecular weight.

The unique characteristic of these copolymers is its reverse thermal gelation behaviour. Due to inherent surface active properties, mucomimetic properties and optical clarity, poloxamers were employed as solubilizer and proposed as artificial tears (Koller and Buri, 1987; Saettone et al., 1988; Chetoni et al., 2000; Liaw et al., 2001). The poloxamers are reported to be well tolerated and non-toxic even though large amounts (20-30 %) of polymer are required to obtain a suitable gel (Gilbert et al., 1986; Koller and Buri, 1987). Five grades were included in the European Pharmacopoeia (PhEur, 2005), US Pharmacopoeia (US Pharmacopoeia XXIII, 2005) and included in the FDA Inactive Ingredients Guide (IV injections; inhalations, ophthalmic preparations; oral powders, solutions, suspensions, and syrups; topical preparations) (Inactive Ingredients Guide, 1996). These polymers are suitable for incorporation of both hydrophilic and hydrophobic drugs for prolonged drug release.

The structure of PL F127 is EO_{100}-PO_{65}-EO_{100} (70 % hydrophilic units and 30 % hydrophobic units) with an average molecular weight of 12600 and HLB value of 22 (Moghimi and Hunter, 2000; Kabanov et al., 2002). They undergo thermal gelation or sol-gel transition in 25-35°C temperature range (Koller and Buri, 1987).

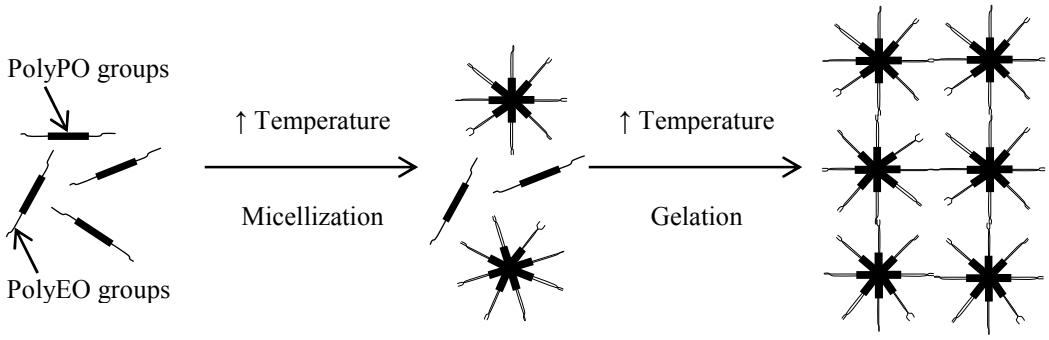


Figure 6.2: Schematic representation of the association mechanism of Poloxamer 407 in water (Dumortier et al., 2006b).

The phenomenon of thermogelling is perfectly reversible and is characterised by a sol-gel transition or gelation temperature ($T_{sol\text{-}gel}$). Below this temperature, the sample remains fluid when the temperature increases the solution becomes semi-solid. The thermogelation results from interactions between different segments of the copolymer (Dumortier et al., 1991; Dumortier et al., 2006a). As temperature increases, Poloxamer 407 copolymer molecules aggregate into micelles. This micellization is due to the dehydration of hydrophobic PO blocks, which represents the very first step in the gelling process (Figure 6.2). These micelles are spherical with a dehydrated polyPO core with an outer shell of hydrated swollen polyEO chains (Juhasz et al., 1989). This gelation was attributed to the ordered packing of micelles. This micellization is followed by gelation for sufficiently concentrated samples. At higher concentrations face centred cubic and body centred cubic

packing of micelles were reported by Liu and Chu (2000). These micellar cubic structures and possible micellar entanglements produce high viscosity, partial rigidity and slow dissolution of the gels. Below transition temperatures, poloxamer solutions allow a comfortable and precise delivery by the patient in the cul-de-sac and immediate gelling at body temperatures will increase the residence time and ocular absorption of the drug (Miller and Donovan, 1982; Koller and Buri, 1987; Katakam et al., 1997; Desai and Blanchard, 1998; Edsman et al., 1998; Lin and Sung, 2000; Moghimi and Hunter, 2000; Kabanov et al., 2002; Ludwig, 2005).

Miyazaki et al. (2001) found that the PL F127 gel has enhanced the therapeutic action of pilocarpine by 1.45 folds. Edsman et al. (1998), reported that the poloxamer 407 system is not as promising as an in situ gel due to the strong concentration dependence of the sol-gel transition temperature, combined with the dilution that occurs in the eye.

In order to improve the efficiency of in situ forming gels, polymers were used in combination with poloxamer or poloxamer grafted polymers are used for preparation of gels. In fact these strategies reduce the total poloxamer content in formulation, improve the rheological behavior and gelling properties of the delivery system, there by extending the drug release. The addition of bioadhesive polymers will improve the retention of the drug in the precorneal area.

rhEGF and HP-β-CD inclusion complex loaded in PL F127 and PL F68 gel system improved the bioavailability by 4 times when compared to rhEGF solution (Kim et al., 2002).

Desai and Blanchard, (1998) showed that addition of methylcellulose and hydroxypropylcellulose to PL F127 systems extended the drug release, which in fact enhanced the therapeutic response of the pilocarpine. Various polymers such as polysaccharides (Miyazaki et al., 2001), water-soluble cellulose derivatives (Desai and Blanchard, 1998; El-Kamel, 2002), poly(acrylic acid) (Lin and Sung, 2000) and hyaluronan (Wei et al., 2002) were added to poloxamer gels.

Copolymerization of the bioadhesive polymer poly(acrylic acid) with Pluronic®, has been reported to yield a bioadhesive vehicle with a prolonged residence time plus a prolonged drug release period in contact with mucosal surfaces such as the eye (Robinson and Mlynek, 1995). Drug release from poloxamer-graft-hyaluronic acid was extended up to 20 hr (Cho et al., 2003). MATP and HA coupled graft copolymers were showing both thermal gelling and bioadhesive properties extended ciprofloxacin release up to 18 hr (Cho et al., 2003). Poloxamers were tested as a vehicle for various drugs or drug complexes such as liposomes or cyclodextrins for ocular drug delivery (Le Bourlais et al., 1995; Bochot et al., 1998; Kim et al., 2002).

PEO are polymers of high molecular weight poly(ethylene oxides) which are non-ionic and hydrophilic. PEO has been used to improve the viscosity of formulations intended for the eye and also has got good mucoadhesive properties.

In situ gelling and mucoadhesive formulations show interesting in vivo performances and allow for therapeutic levels to be obtained over an extended period of time in the tear film and anterior chamber. Ease of administration, tolerance and patient acceptability will be more for these of dosage forms. In situ gelling character, bioadhesion and sustained release can be modulated by the composition of various components in the formulation. In situ gels are promising ocular drug delivery systems to treat external and intraocular eye infections, and diseases that require frequent eye drops instillation in order to maintain therapeutic drug levels.

Apart from insert type of solid dosage forms, the study has also been done on in situ gel systems for ocular delivery of gatifloxacin using PL F127 in combination with HEC or PEO. There is no report in literature on the usage of HEC and PEO in combination with PL F127 for improvement in the gel properties for ophthalmic purpose, especially for gatifloxacin.

6.2. Experimental

6.2.1. Materials

For buffer preparations, in-house prepared triple distilled water (TDW) was used. All other materials used were same as given in chapter 3. Composition of buffers and reagents is given in appendix.

6.2.2. Gel preparation

Gels were prepared on weight basis using the cold method (Schmolka, 1972) with minor modification. Vehicle used for preparation of in situ gels was 0.34 M phosphate buffered (pH 7.4). Isotonicity of gels was adjusted with help of sodium chloride. The schematic representation of method of gel preparation was given in Figure 6.3. PL F127 was added slowly to part of media, maintained at 5°C, with continuous stirring. This part was refrigerated for 18 hr for complete hydration and dissolution of PL F127. In another part of media, drug was dissolved with continuous stirring. To this part of media, PEO or HEC were added, as selected, slowly with constant stirring and dissolved completely. Then the PL F127 part was added to drug part slowly with rigorously stirring. BKC (0.02 % w/w), a preservative, was added with stirring and the temperature was maintained at 5°C. Formulations were made up with phosphate buffer on weight basis to give a drug

concentration of 0.3 % w/w. The formulations were sterilized by autoclaving at 121°C, 15 psig for 20 min. The preparations were protected from light and stored at 2-8°C.

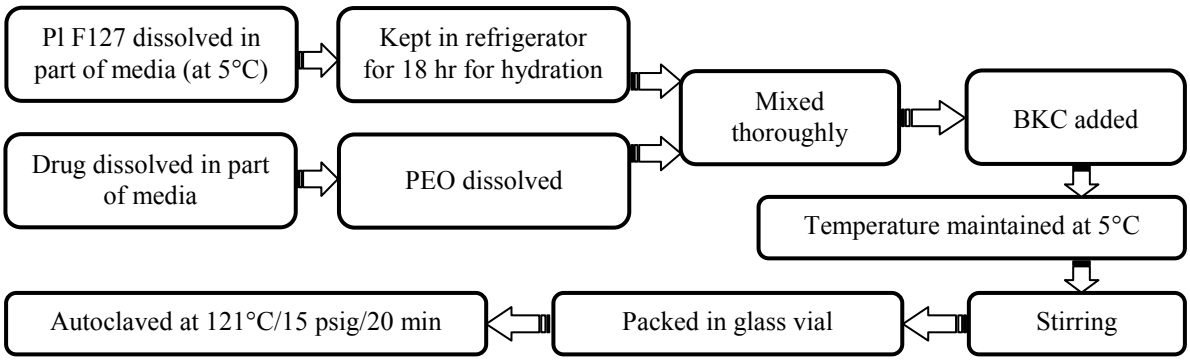


Figure 6.3: Schematic representation of method of preparation of in situ gel formulations.

6.2.3. Effect of various parameters

Formulae of different gel formulations prepared were given in Table 6.1. These gels were checked for the effect on various physical and in vitro properties. HPMC (15 cPs) gel, concentration of 1 % w/w, was used as control formulation. Effects of various concentrations of PL F127 alone and in combination with different concentrations of PEO or HEC on characters of gels were studied.

(a) Effect of PL F127 concentration

Gel systems with different concentrations of PL F127 (14, 15, 16, 17, 18, 20, 22 and 25 % w/w) were prepared to study the effect of concentration.

(b) Effect of PEO 900 concentration

Formulations containing different concentrations of PEO 900 (0.5, 1, 1.5, 2, 2.5 and 3 % w/w) in combination with of PL F127 (15 % w/w) were prepared.

(c) Effect of PL F127 concentration in combination with 1 % w/w PEO 900

Gels with different concentrations of PL F127 (12, 13, 14, 15, 16, 18, 20, 22 and 25 % w/w) in combination with PEO 900 (1 % w/w) were prepared and studied.

(d) Effect of vehicle

The gels with 15 % w/w concentration of PL F127 alone and with 15 % w/w concentration of PL F127 in combination with 1 % w/w PEO 900 were prepared separately in different media, triple distilled water (TDW) and phosphate buffer.

Table 6.1: Composition of prepared in situ gel formulations containing gatifloxacin.

Formulation Code	Drug (% w/w)	Composition of base						
		HPMC (% w/w)	PL F127 (% w/w)	PEO Grade	PEO (% w/w)	HEC (% w/w)	BKC (% w/w)	Vehicle
HP_1	0.3	1		-	-	-	0.02	PBS
F_{14}	0.3	-	14	-	-	-	0.02	PBS
F_{15}	0.3	-	15	-	-	-	0.02	PBS
F_{16}	0.3	-	16	-	-	-	0.02	PBS
F_{17}	0.3	-	17	-	-	-	0.02	PBS
F_{18}	0.3	-	18	-	-	-	0.02	PBS
F_{20}	0.3	-	20	-	-	-	0.02	PBS
F_{22}	0.3	-	22	-	-	-	0.02	PBS
F_{25}	0.3	-	25	-	-	-	0.02	PBS
$F_{12}P3_1$	0.3	-	12	900	1	-	0.02	PBS
$F_{13}P3_1$	0.3	-	13	900	1	-	0.02	PBS
$F_{13}P3_2$	0.3	-	13	900	2	-	0.02	PBS
$F_{14}P3_1$	0.3	-	14	900	1	-	0.02	PBS
$F_{14}P3_2$	0.3	-	14	900	2	-	0.02	PBS
$F_{15}P1_1$	0.3	-	15	200	1	-	0.02	PBS
$F_{15}P3_{0.5}$	0.3	-	15	900	0.5	-	0.02	PBS
$F_{15}P3_1$	0.3	-	15	900	1	-	0.02	PBS
$F_{15}P3_2$	0.3	-	15	900	2	-	0.02	PBS
$F_{15}P3_{2.5}$	0.3	-	15	900	2.5	-	0.02	PBS
$F_{15}P3_3$	0.3	-	15	900	3	-	0.02	PBS
$F_{15}P5_1$	0.3	-	15	5000	1	-	0.02	PBS
$F_{15}P6_1$	0.3	-	15	7000	1	-	0.02	PBS
$F_{16}P3_1$	0.3	-	16	900	1	-	0.02	PBS
$F_{17}P3_1$	0.3	-	17	900	1	-	0.02	PBS
$F_{18}P3_1$	0.3	-	18	900	1	-	0.02	PBS
$F_{20}P3_1$	0.3	-	20	900	1	-	0.02	PBS
$F_{22}P3_1$	0.3	-	22	900	1	-	0.02	PBS
$F_{25}P3_1$	0.3	-	25	900	1	-	0.02	PBS
$F_{15}HE_1$	0.3	-	15	-	-	1	0.02	PBS
$F_{15}HE_2$	0.3	-	15	-	-	2	0.02	PBS
$F_{15}HE_3$	0.3	-	15	-	-	3	0.02	PBS
TWD-F_{15}	0.3	-	15	-	-	-	0.02	TDW
TWD-$F_{15}P3_1$	0.3	-	15	900	1	-	0.02	TDW

HPMC : Hydroxy propyl methyl cellulose 15 cPs grade; PL F127 : Pluronic® F-127; PEO 200 : Polyox® WSR N-80; PEO 900 : Polyox® WSR-1105; PEO 5000 : Polyox® WSR Coagulant; PEO 7000 : Polyox® WSR-303; HEC : Hydroxy ethyl cellulose mol wt 250,000; BKC : Benzalkonium chloride; PBS : pH 7.4 Phosphate buffer isotonicity adjusted with sodium chloride.

(e) Effect of different grades of PEO

Formulations containing 1 % w/w of different viscosity grades of PEO (200, 900, 5000 and 7000) in combination with of PL F127 (15 % w/w) were prepared and studied.

(f) Effect of different concentrations of HEC

Formulations containing different concentrations of HEC (1, 2 and 3 % w/w) in combination with of PL F127 (15 % w/w) were prepared for study.

6.2.4. Evaluation of formulations

(a) Assay of developed gel systems

The weight of different gel equivalent to 3 mg of drug was taken in a 100 ml volumetric flask and PBS (pH 7.4) was added to it and thoroughly shaken. Solutions were filtered through Whatman filter paper #40. Aliquot volume was withdrawn from the filtrate, suitably diluted and analysed by using UV-spectrophotometric method (chapter 3).

(b) Rheological studies

Rheological studies were done using Brookfield RVDV-II+ Pro viscometer (Brookfield Engineering Laboratory, Inc., Middleboro) connected to a computer with software Brookfield Wingather V2.4. Water jacketed small sample adapter, with a coaxial-cylindrical spindle and chamber combination (SC4-21/13R), was optimized for rheological studies of gels. Temperature was maintained with external water bath; with a temperature of precision ± 0.2°C. Gels were diluted (1:1) with STF for viscosity measurements. The STF was prepared according to the procedure given by Rozier et al. (1989). By changing the angular rpm, the gel system was exposed to different shear rates ranging from 0.01 to 186 sec^{-1} at constant temperature. The same was repeated at different temperatures. Each sample was analysed in triplicates.

The shear rates (G) versus shear stress (F) data were plotted to determine the flow type. The exponential formula for determining the Pseudoplastic flow (non-newtonian) was as follows: $F^N = \eta' \times G$; where G is shear rates ($Dynes.cm^{-2}$), F is shear stress (sec^{-1}), η' is apparent viscosity (mPa.sec) and N is fluidity index. The exponent N rises as flow becomes increasingly non-Newtonian. When N = 1, the equation reduces to Newtonian flow equation (Martin et al., 2001; Vandamme and Brobeck, 2005).

(c) Determination of gelation temperature ($T_{sol-gel}$)

Methods for determination of $T_{sol-gel}$ were based on the literature reports (Ryu et al., 1999; Kim et al., 2002; Fawaz et al., 2004; Dumortier et al., 2006a; Dumortier et al., 2006b) with minor modifications according to the lab conditions.

Magnetic bar method

Poloxamer solutions were heated progressively (1°C.min^{-1}), from 5°C to 50°C, with constant stirring (100 rpm). The temperature was set up using a thermostated bath and was controlled inside the sample with a precise thermometer (± 0.1°C). When the magnetic bar (length 25 mm, diameter 6 mm) stopped moving due to gelation, the temperature displayed was considered to be $T_{sol\text{-}gel}$.

Rheological method

The $T_{sol\text{-}gel}$ of some selected formulations was determined by using Brookfield RVDV-II+ Pro viscometer also. To maintain the constant shear rate of 0.09 sec^{-1} rotational speed was controlled. Based on the preliminary studies, this value was chosen to get a precise determination of $T_{sol\text{-}gel}$ value. The temperature was incremented at a rate of 1°C.min^{-1}, from 10°C to 50°C to locate the sol-gel transition point. $T_{sol\text{-}gel}$ was determined with and without STF dilution. The gelling temperature was determined graphically as the inflection point on the curve of the apparent viscosity as a function of the temperature. Each preparation was tested thrice to check the repeatability of the measurement.

(d) Pourability and spreadability

Pourability was determined by measuring the ease with which a formulation flows from the container after shaking. For determination of spreadability, 2 g of preheated (at 37°C) formulation was added between two glass plates of 12 cm × 12 cm dimensions. The area spread was determined when 1.5 g of weight was applied for 2 min. The spreadability was given as cm^2.min^{-1} (Chandran, 2003). Each formulation was checked trice.

(e) Texture profile analysis (TPA)

The mechanical properties of selected formulations were examined using texture profile analysis described by Jones and co workers (Jones et al., 1996a; Jones et al., 1996b; Jones et al., 1997; Jones et al., 1999; Jones et al., 2000; Jones et al., 2002) and Tamburic and Craig (1997). All the selected formulations were transferred into aluminium containers (67 mm height and diameter of 37.5 mm) up to fixed height and care was taken to avoid the introduction of air into the samples. Texture profile analysis was performed using a Texture Analyser TA-XT2 (Stable Micro Systems, UK) fitted with a 30 kg load cell and using a 10 mm diameter analytical probe (Delrin cylindrical flat bottom probe - SMS P/10). The machine has a force resolution of 0.1 g, force measurement accuracy of 0.001 % and a distance resolution of 0.001 mm. In texture profile analysis mode the probe was twice compressed into each sample at a defined rate to a depth of 15 mm. Initially, the probe has

moved from a 44 mm distance at a pre-test speed of 1 mm.sec^{-1} towards the gel, immediately after sensing 5 g of trigger force it has attained the test-speed of 2 mm.sec^{-1}. After travelling a distance of 15 mm into gel, the probe was withdrawn from the gel at post-test speed (2 mm.sec^{-1}). A delay period (15 sec) was allowed between the end of the first and the beginning of the second compression. All analysis were performed at least in triplicates. The study was carried out at 37 ± 1°C. From the resultant force-time plots, several mechanical parameters are derived. These are (i) hardness or stiffness (the force required to attain a given deformation) was given as the peak force required to compress the gel, (ii) compressibility (the work required to deform the sample during the first compression of the probe) was given as a positive AUC of force-time plot due to first compression, (iii) adhesiveness (the work required to overcome the attractive forces between the surface of the sample and the surface of the probe) was given as a negative AUC of force-time plot due to withdrawal of probe in first cycle and (iv) cohesiveness or elasticity was defined as the ratio of the area under the force-time curve produced on the second compression cycle to that on the first compression cycle, where successive compressions are separated by defined recovery period.

(f) Bioadhesion study

Conventional method

Separate mucosal tissues were tied on to both upper and lower block, 2 g of gels were placed on lower block (Figure 6.4), and then the rest of the procedure was same as mentioned in earlier chapter. The experiment was done thrice for each formulation.

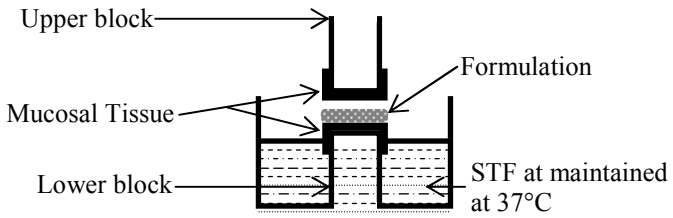


Figure 6.4: Schematic representation of conventional bioadhesion testing setup for gels.

Texture Analyser method

The probe used was a delrin cylindrical probe (SMS P/10) having a diameter of 10 mm and rest of machine setting were same that mentioned in earlier chapter. Separate mucosal tissue were tied on to probe and held using clips on a holder (Figure 6.5). Two g of gels were placed on lower mucosal tissue, and then the rest of the procedure was same as mentioned in earlier chapter. The experiment was done thrice for each formulation. Blank was performed without formulation on fresh tissue.

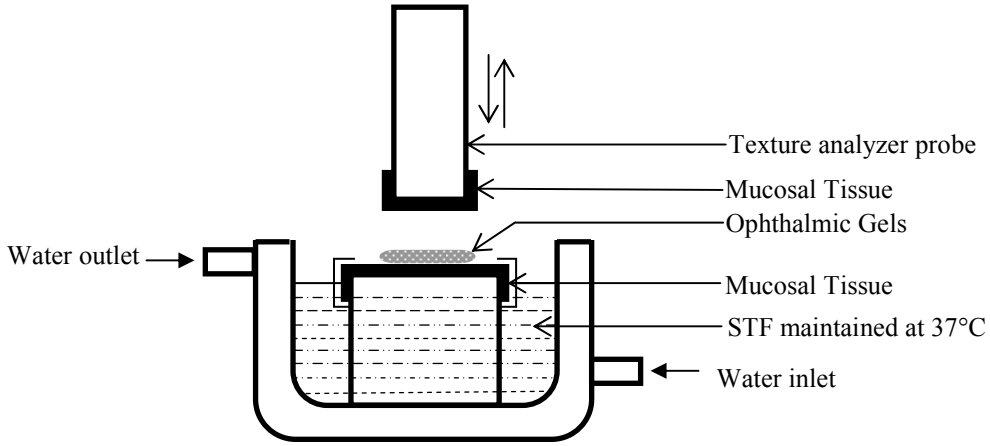


Figure 6.5: Schematic representation of setup of bioadhesion test of in situ gels using texture analyser.

(f) Test for sterility

All the sterilized formulations were tested for sterility using USP method (US Pharmacopoeia XXIII, 2005). The representative samples of the formulations were taken and tested for the absence of microbial load.

(g) In vitro drug release and erosion studies

Method 1 - Membrane less dissolution mode

In vitro drug release studies of in situ gel systems were performed at 37°C ± 0.5°C using membrane less dissolution model to simulate the conditions experienced by formulations during their precorneal residence time. This drug release model was reported to have an advantage of discriminating formulations based on their character (Bhardwaj and Blanchard, 1996; El-Kamel, 2002; Wenzel et al., 2002; Zhang et al., 2002). The dissolution media was freshly prepared STF (pH 7.4) and was pre-equilibrated at 37°C. After the formulations were converted to gel at 37°C in vial, 1 ml of dissolution media was added carefully to it. The whole set up was stirred at 20 cycles per min. At different time intervals the entire dissolution media was sampled out carefully without disturbing the gel layer and replaced with pre-equilibrated fresh dissolution media. Each experiment was performed in triplicate. The samples were analysed by UV-spectrophotometric and spectrofluorimetric methods as described in chapter 3. % CDR was plotted against time. In vitro drug release data was processed in similar manner mentioned in chapter 5.

Method 2 - In vitro gel erosion and/or gel dissolution studies

Procedure was adopted from the literature (Wenzel et al., 2002; Zhang et al., 2002) with minor modifications. Experiment was set-up as per above-mentioned conditions. In

addition to the described procedure, the vials with gels were weighed prior to start of study. At different time intervals the entire dissolution media was removed carefully without disturbing gel layer and the vials were weighed after each removal of the release medium. Each experiment was performed in triplicate. The differences in weight of the vials between time points yielded the amount of the gel dissolved and/or eroded. The percentage gel dissolved can be calculated using following formula: % Gel Dissolved (% GD) = [1 – (G_{t+1}/G_t)] × 100; where G_t is weight of gel at time 't', G_{t+1} is weight of gel at time 't+1' (next time point). Percentage gel dissoluted was plotted against time. Erosion data was analysed using GraphPad Prism version 4.00 (Trial) for windows, GraphPad software, San Diego California USA.

Method 3 - Modified USP XXIII dissolution apparatus

The in vitro release of drug from the formulations through a suitably treated dialysis membrane (cut off between 12-14 kDa and pore size of 2.4 nm, Himedia Lab. Pvt. Ltd., Mumbai, India) was determined using previously reported method (Bottari et al., 1974; Sultana et al., 2006b) with minimal changes. Freshly prepared STF (pH 7.4) was used as a dissolution media. The holder contains a body with 19 mm internal diameter and 10 mm depth with a diffusion surface area of 2.84 cm^2. A 2 g weight of the gel formulation was accurately pipetted into holder and then sealed with dialysis membrane. Before sealing air bubbles were avoided in gel formulations. The gel loaded container was attached to the teflon support and immersed in 150 ml dissolution medium maintaining at 37°C ± 0.5°C with a paddle rotating at a speed of 50 rpm. Samples, each 10 ml in volume, were withdrawn at regular intervals and replaced by an equal volume of receptor medium. The samples were analysed by UV-spectrophotometric and spectrofluorimetric methods as described in chapter 3. % CDR was plotted against time. In vitro drug release data was processed in similar manner mentioned in chapter 5.

(h) In vitro microbiological studies

Both marketed and optimized formulations were tested in same manner mentioned in earlier chapter. Volume of the formulations with equivalent amount of gatifloxacin was transferred into the cup. All the formulations were tested in triplicate.

6.2.5. Batch reproducibility

Two more batches of each selected formulation were prepared and their quality and respective release characters were evaluated under the same conditions as prescribed

previously. The physical properties of the gels for all three batches were evaluated in the same manner as that of original batch formulations.

6.2.6. Stability studies

The effect of sterilization method on the stability of the drug was also determined by comparing the physical parameters, drug content and in vitro drug release before and after sterilization. Few selected formulations from each polymer combination were used for this study.

The selected formulations were transferred in airtight and light protected glass vials and stored at ambient as well as accelerated storage conditions as per ICH guidelines. The samples were taken at initial time point and analysed for drug content after suitable dilution with UV-spectrophotometric and HPLC methods as described in chapter 3. Formulations were stored at different conditions like room temperature (CRT: 25 ± 2°C/60 ± 5 % RH), accelerated condition (AT: 40 ± 2°C/75 ± 5 % RH) and refrigerated temperature (FT: 5 ± 2°C). The samples in triplicate were withdrawn from each batch at predetermined time intervals (0.5, 1, 3 and 6 months for AT condition; 1, 3, 6, 12 and 18 months for CRT and FT condition) and analyzed for physical characters and in vitro release behavior. The physical properties such as appearance, $T_{sol\text{-}gel}$, rheological properties and spreadability were evaluated as per the specifications enlisted in previous sections and compared with the initial values. The drug content in triplicate was determined after suitable dilutions. The % RTD was plotted against time and the K_{deg} and $t_{90\%}$ were calculated based on the degradation rate kinetics at different storage conditions. The in vitro release profiles were studied as per the specifications enlisted in previous sections and compared with their respective initial release profiles.

6.2.7. Statistical analysis of data

The data were statistically evaluated by *t*-test and one way ANOVA at $P \leq 0.05$ where ever required. If null hypothesis was rejected in one way ANOVA, all pairs of columns were evaluated by Bonferoni test.

6.3. Results and discussion

6.3.1. Physical characterization of the in situ gels

The designed drug loaded in situ gels were found to have good physical properties and the results are presented in the Table 6.2. The in situ gels prepared with PL F127 alone,

at all concentration levels, were colourless and transparent at temperatures above and below $T_{sol\text{-}gel}$. These gels were free flowing and showed excellent pourability at temperatures below $T_{sol\text{-}gel}$. With the temperature reaching $T_{sol\text{-}gel}$ the pourability reduced and above $T_{sol\text{-}gel}$ the gels were non-flowing. The gels with lower concentrations of PL F127 (F_{14}) have shown good spreadability, same as that of control (HP_1). In gels with PL F127 alone, as the concentration increased from 14 to 16 % w/w the spreadability reduced and further higher concentrations the spreadability remained constant (7 to 8 $cm^2.min^{-1}$).

The in situ gels prepared with PL F127 in combination with all grades of PEO, at all concentration levels, were colourless and translucent at temperatures below and above $T_{sol\text{-}gel}$. Pourability of these gels was excellent at temperatures below $T_{sol\text{-}gel}$ and reduced with increase in temperature. The gels with 12 % w/w of PL F127 in combination with 1 % w/w PEO 900 ($F_{12}P3_1$) have shown spreadability lower than that of control. As the concentration of PL F127 increased from 12 to 14 % w/w in combination with 1 % w/w PEO 900 the spreadability reduced and at higher concentrations (above 15 % w/w) the spreadability reached constant (7 to 9 $cm^2.min^{-1}$). The gels with 13 % w/w of PL F127 in combination with 2 % w/w PEO 900 ($F_{13}P3_2$) have shown spreadability same as that of 16 % w/w PL F127 alone (F_{16}). As the concentration of PL F127 increased from 13 to 15 % w/w in combination with 2 % w/w PEO 900 ($F_{13\text{-}15}P3_2$), the spreadability reduced (from 10 to 7 $cm^2.min^{-1}$). As the concentration of PEO 900 increased from 0 to 3 % w/w in combination with 15 % w/w PL F127 ($F_{15}P3_{0\text{-}3}$), the spreadability reduced from 18 to 6 $cm^2.min^{-1}$.

The in situ gels prepared with PL F127 in combination with HEC, at all concentration levels, were pale yellow colour and translucent at temperatures below and above $T_{sol\text{-}gel}$. These gels were flowing freely and showed excellent pourability at temperatures below $T_{sol\text{-}gel}$ and were non-flowing with the temperature reaching $T_{sol\text{-}gel}$. The gels with 15 % w/w of PL F127 in combination with 1 % w/w HEC ($F_{15}HE_1$) have shown spreadability lower than that of control. As the concentration of HEC increased from 0 to 3 % w/w in combination with 15 % w/w PL F127, the spreadability reduced from 18 to 10 $cm^2.min^{-1}$.

The gels prepared with 15 % w/w PL F127 alone and 15 % w/w PL F127 in combination with 1 % w/w of PEO 900 in TDW showed good physical properties and the spreadability values were similar that of formulations prepared with phosphate buffer. None of the formulations showed any microbial load and all the batches passed the sterility testing. The content uniformity of the in situ gels was very high. Assay values of the in situ gels were in between 97 to 103 % with SD value not more than 3 %. Low SD values of all these physical parameters indicate that the method of production of the in situ gels was reproducible.

Table 6.2: Result of assay, gelling temperature, rheological properties and bioadhesion by conventional method.

Code	Appearance	$T_{sol-gel}$ * (°C)	Rheological Properties$^{\Pi}$			Spreadability$^{\Pi}$ ($cm^2.min^{-1}$) (Mean ± SD)	Detachment Force$^{\Psi}$ ($N.cm^{-2}$) (Mean ± SD)	% Assay * (Mean ± SD)
			Apparent Viscosity (mPa.sec) (Mean ± SD)	N	Flow Character			
HP_1	Colourless and Transparent	NGP	14.4 ± 0.8	0.98	Newt	24.2 ± 0.5	Control	97.81 ± 1.77
F_{14}	Colourless and Transparent	NGP	118.6 ± 6.7	0.97	Newt	22.3 ± 0.9	0.016 ± 0.002	100.35 ± 0.48
F_{15}	Colourless and Transparent	34.1 ± 0.8	4662.1 ± 92.4	1.65	non-Newt	18.7 ± 0.2	0.024 ± 0.003	100.65 ± 0.48
F_{16}	Colourless and Transparent	31.6 ± 0.5	17132.8 ± 235.2	1.95	non-Newt	8.7 ± 0.1	0.026 ± 0.002	100.28 ± 2.45
F_{17}	Colourless and Transparent	28.1 ± 0.2	18021.8 ± 471.6	1.99	non-Newt	7.7 ± 0.4	0.027 ± 0.002	97.65 ± 2.03
F_{18}	Colourless and Transparent	26.6 ± 0.8	19572.0 ± 840.6	2.00	non-Newt	7.2 ± 0.1	0.028 ± 0.002	99.97 ± 0.78
F_{20}	Colourless and Transparent	25.9 ± 0.6	20137.9 ± 158.3	1.98	non-Newt	8.1 ± 0.2	0.038 ± 0.001	101.39 ± 0.88
F_{22}	Colourless and Transparent	24.3 ± 1.1	20693.0 ± 72.2	1.97	non-Newt	7.9 ± 0.3	0.049 ± 0.005	98.64 ± 1.23
F_{25}	Colourless and Transparent	22.4 ± 0.9	22566.5 ± 1154.5	2.01	non-Newt	7.6 ± 0.2	0.059 ± 0.001	100.35 ± 0.76
$F_{12}P3_1$	Colourless and Translucent	NGP	165.6 ± 8.6	1.06	Newt	18.7 ± 0.8	0.154 ± 0.004	97.23 ± 1.36
$F_{13}P3_1$	Colourless and Translucent	33.3 ± 0.2	1268.9 ± 66.5	1.20	non-Newt	10.4 ± 0.3	0.160 ± 0.014	98.49 ± 0.77
$F_{13}P3_2$	Colourless and Translucent	29.1 ± 0.5	1587.9 ± 84.18	1.25	non-Newt	8.7 ± 0.3	0.185 ± 0.013	101.32 ± 0.85
$F_{14}P3_1$	Colourless and Translucent	29.7 ± 1.0	2635.9 ± 41.4	1.45	non-Newt	8.4 ± 0.2	0.168 ± 0.003	99.32 ± 1.21
$F_{14}P3_2$	Colourless and Translucent	26.8 ± 0.8	3055.4 ± 65.8	1.48	non-Newt	7.9 ± 0.2	0.179 ± 0.010	102.65 ± 0.29
TWD-F_{15}	Colourless and Transparent	40.0 ± 0.9	124.6 ± 6.5	0.97	Newt	17.3 ± 0.5	0.022 ± 0.002	99.65 ± 1.65
TWD-$F_{15}P3_1$	Colourless and Translucent	29.2 ± 0.8	10417.9 ± 138.5	1.85	non-Newt	9.2 ± 0.3	0.127 ± 0.010	101.21 ± 0.43

(Contd..)

NGP : No gelation point; $T_{sol-gel}$: Gelation temperature; $^{\Pi}$ - Measured at 37°C - Mean of 3 batches (3 determinations from each batch); Viscosity calculation: $F^N = \eta' \times G$; Where, F = Shear Stress ($Dynes.cm^{-2}$), G = Shear Rate (sec^{-1}), η' = Apparent viscosity and N = viscosity exponent or fluidity index; Newt : Newtonian system; non-Newt : non-Newtonian - Pseudoplastic system; $^{\Psi}$ - mean of 3 batches (3 determinations from each batch) by conventional method; * - Determined by magnetic bar method and some gels were verified by rheological method and the value mean of 3 batches (3 determinations from each batch).

Table 6.2

Code	Appearance	$T_{sol-gel}$ * (°C)	Rheological Properties$^{\Pi}$			Spreadability$^{\Pi}$ ($cm^2.min^{-1}$) (Mean ± SD)	Detachment Force$^{\Psi}$ ($N.cm^{-2}$) (Mean ± SD)	% Assay * (Mean ± SD)
			Apparent Viscosity (mPa.sec) (Mean ± SD)	N	Flow Character			
$F_{15}P3_{0.5}$	Colourless and Translucent	30.4 ± 0.3	14437.8 ± 803.1	1.81	non-Newt	10.1 ± 0.3	0.159 ± 0.012	99.23 ± 1.34
$F_{15}P3_{1}$	Colourless and Translucent	27.3 ± 0.6	16893.1 ± 450.7	1.83	non-Newt	8.7 ± 0.4	0.189 ± 0.005	100.65 ± 0.98
$F_{15}P3_{2}$	Colourless and Translucent	23.1 ± 0.2	20839.0 ± 887.1	1.84	non-Newt	7.7 ± 0.2	0.208 ± 0.020	99.36 ± 0.22
$F_{15}P3_{2.5}$	Colourless and Translucent	17.7 ± 1.2	22666.2 ± 705.9	1.78	non-Newt	7.5 ± 0.4	0.257 ± 0.017	102.01 ± 0.31
$F_{15}P3_{3}$	Colourless and Translucent	15.4 ± 0.6	23577.5 ± 875.8	1.87	non-Newt	6.1 ± 0.2	0.277 ± 0.021	101.61 ± 0.39
$F_{15}P1_{1}$	Colourless and Translucent	32.8 ± 0.8	12822.5 ± 714.7	1.88	non-Newt	7.7 ± 0.2	0.129 ± 0.006	98.56 ± 0.56
$F_{15}P5_{1}$	Colourless and Translucent	25.8 ± 0.6	24575.8 ± 750.4	1.89	non-Newt	7.9 ± 0.4	0.235 ± 0.002	101.77 ± 1.57
$F_{15}P6_{1}$	Colourless and Translucent	24.1 ± 0.2	25868.1 ± 267. 1	1.82	non-Newt	7.1 ± 0.0	0.302 ± 0.008	99.12 ± 0.85
$F_{16}P3_{1}$	Colourless and Translucent	26.5 ± 0.5	18613.0 ± 397.9	1.96	non-Newt	8.9 ± 0.3	0.190 ± 0.015	99.67 ± 0.68
$F_{17}P3_{1}$	Colourless and Translucent	24.5 ± 0.8	19016.4 ± 406.5	2.00	non-Newt	7.6 ± 0.4	0.193 ± 0.008	98.91 ± 1.63
$F_{18}P3_{1}$	Colourless and Translucent	21.5 ± 0.1	19838.9 ± 238.4	2.01	non-Newt	7.4 ± 0.3	0.192 ± 0.012	100.05 ± 0.84
$F_{20}P3_{1}$	Colourless and Translucent	19.5 ± 0.3	21554.5 ± 421.2	1.99	non-Newt	7.7 ± 0.3	0.195 ± 0.010	102.41 ± 0.91
$F_{22}P3_{1}$	Colourless and Translucent	16.2 ± 0.6	21471.6 ± 1202.2	1.98	non-Newt	7.4 ± 0.3	0.196 ± 0.006	98.95 ± 0.98
$F_{25}P3_{1}$	Colourless and Translucent	15.1 ± 0.7	24773.0 ± 368.9	2.02	non-Newt	7.2 ± 0.4	0.198 ± 0.009	100.41 ± 0.49
$F_{15}HE_{1}$	Pale yellow and Translucent	29.8 ± 0.9	13478.6 ± 234.6	1.87	non-Newt	13.4 ± 0.3	0.105 ± 0.005	98.88 ± 0.97
$F_{15}HE_{2}$	Pale yellow and Translucent	29.3 ± 0.5	14681.6 ± 189.6	1.85	non-Newt	10.7 ± 0.3	0.169 ± 0.004	101.69 ± 0.72
$F_{15}HE_{3}$	Pale yellow and Translucent	27.6 ± 0.3	14755.0 ± 42.7	1.85	non-Newt	10.4 ± 0.3	0.168 ± 0.004	99.41 ± 1.03

$T_{sol-gel}$: Gelation temperature; $^{\Pi}$ - Measured at 37°C - Mean of 3 batches (3 determinations from each batch); Viscosity calculation: $F^N = \eta' \times G$; Where, F = Shear Stress ($Dynes.cm^{-2}$), G = Shear Rate (sec^{-1}), η' = Apparent viscosity and N = viscosity exponent or fluidity index; Newt : Newtonian system; non-Newt : non-Newtonian - Pseudoplastic system; $^{\Psi}$ - mean of 3 batches (3 determinations from each batch) by conventional method; * - Determined by magnetic bar method and some gels were verified by rheological method and the value mean of 3 batches (3 determinations from each batch).

6.3.2. Gelation temperature

Poloxamer solutions with optimum concentration were converted to gels at or above gelation temperature. $T_{sol\text{-}gel}$ of prepared gels varied from 15°C to 40°C depending on the concentration of PL F127 and other viscolysing agents (Table 6.2). The results of the magnetic bar method were completely matched with the rheological method and both the methods were reproducible. Results were given in Table 6.2 and Figure 6.6 to 6.10.

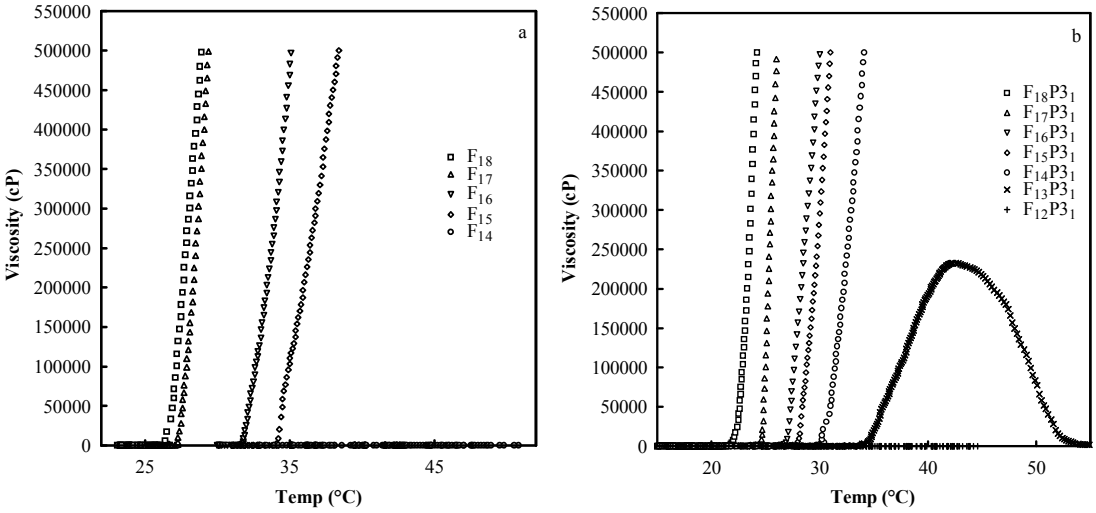


Figure 6.6: Change in viscosity with respect to temperature in gels with (a) varying PL F127 concentration alone, (b) varying PL F127 concentration in combination with 1 % w/w PEO 900.

In gels with PL F127 alone, increase in concentration of PL F127 from 15 to 25 % w/w decreased the $T_{sol\text{-}gel}$ from 34°C to 22°C (Figure 6.6a and 6.7). Solutions containing less than 15 % w/w PL F127 did not form gels over the tested temperature range. This observation was in concordance with data available in the literature (Wei et al., 2002; Dumortier et al., 2006b). Following equation was drawn from the concentration of PL F127 (C_{F127}) and $T_{sol\text{-}gel}$: $T_{sol\text{-}gel} = 95.07 - 5.95 \times C_{F127} + 0.12 \times (C_{F127})^2$ with regression coefficient of 0.951, MSSR of 0.70 and AIC of -0.50.

Different concentrations of PEO 900 in combination with different PL F127 concentrations have been tested. In gels with combination of 1 % w/w PEO 900 and increase in concentration of PL F127 from 13 to 25 % w/w decreased the $T_{sol\text{-}gel}$ from 33°C to 15°C (Figure 6.6b and 6.7). Solutions containing less than 12 % w/w PL F127 in combination with 1 % w/w PEO 900 did not form gels over the tested temperature range. Following equation was drawn from the concentration of PL F127 (C_{F127}) and $T_{sol\text{-}gel}$: $T_{sol\text{-}gel} = 83.27 - 5.12 \times C_{F127} + 0.10 \times (C_{F127})^2$ with regression coefficient of 0.991, MSSR of 0.32 and AIC of -8.26. As compared to gels containing PL F127 alone, gels containing PL F127 in combination with

PEO 900 showed less $T_{sol\text{-}gel}$. It was evident from the literature that the molecules interfering with the intra molecular hydrogen bonding of polyEO groups of PL F127 affects the $T_{sol\text{-}gel}$ (Dumortier et al., 1991; Wei et al., 2002; Dumortier et al., 2006a; Dumortier et al., 2006b). It was assumed that the added PEO, a hydrophilic polymer with good number of ethylene oxide groups, would reduce the intra-molecular bonding between polyEO groups of PL F127 thereby increasing the hydrophobic interaction between polyPO blocks resulting in micellization at lower temperature. Therefore, $T_{sol\text{-}gel}$ will decrease in presence of PEO.

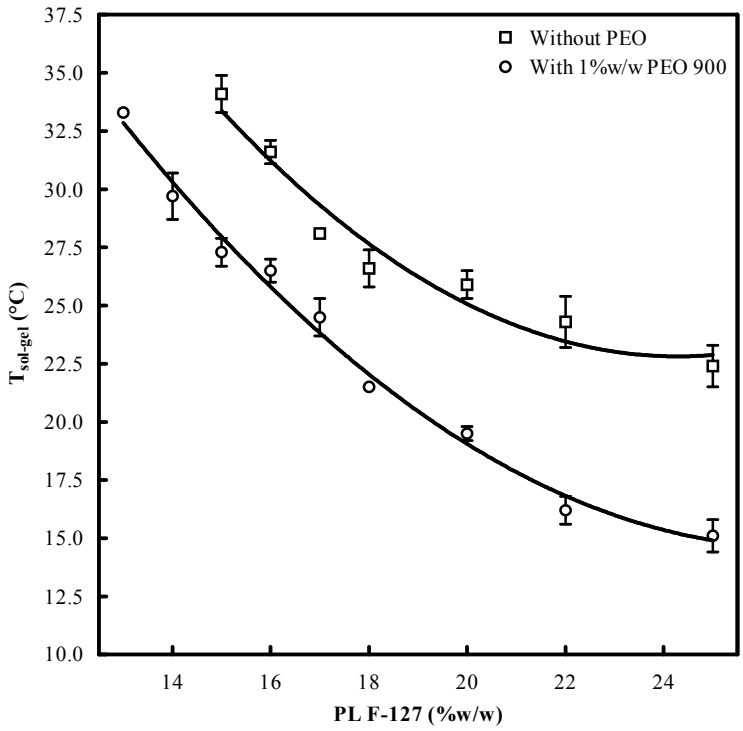


Figure 6.7: Gelation temperature ($T_{sol\text{-}gel}$) as function of PL F127 concentration in gels with PL F127 alone and PL F127 in combination with 1 % w/w PEO 900. Each data point is average of three separate determinations.

In gels with 13 % w/w and 14 % w/w of PL F127 in combination with 2 % w/w PEO showed $T_{sol\text{-}gel}$ of 29.1°C and 26.8°C respectively (Table 6.2). Transition temperatures were lower than gels prepared with 13 % w/w and 14 % w/w of PL F127 alone and in combination with 1 % w/w PEO. In gels with 15 % w/w PL F127, $T_{sol\text{-}gel}$ reduced with increase in PEO 900 concentration from 0 to 3 % w/w from 34°C to 15°C (Figure 6.8a and 6.9). Following equation was drawn from the concentration of PEO 900 (C_{PEO}) and $T_{sol\text{-}gel}$ in gels prepared with 15 % w/w PL F127 in combination with different concentrations of PEO 900: $T_{sol\text{-}gel} = 33.85 - 6.12 \times C_{PEO}$ with regression coefficient of 0.987, MSSR of 0.56 and AIC of -1.50.

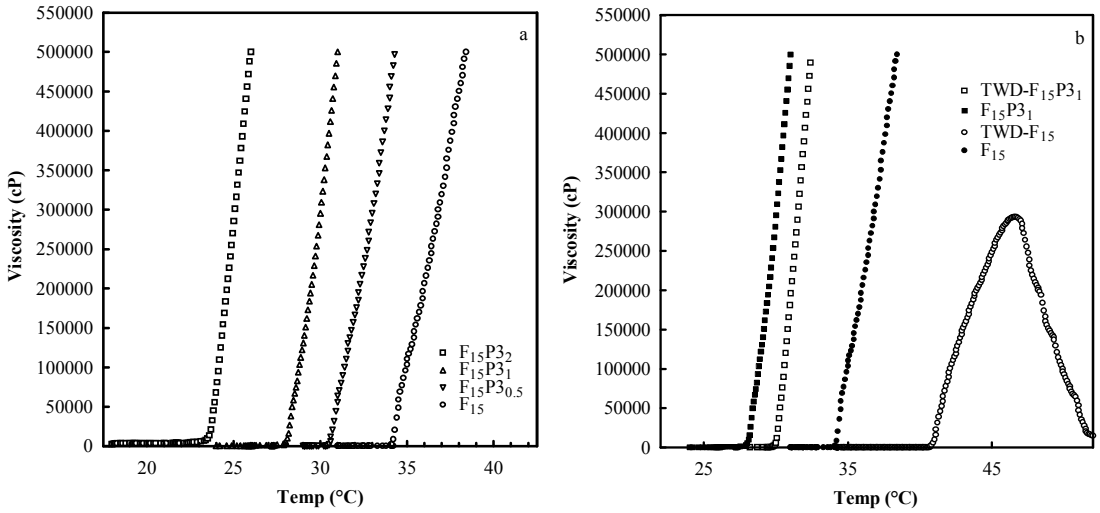


Figure 6.8: Change in viscosity with respect to temperature in gels with (a) varying PEO 900 concentration in combination with 15 % w/w PL F127 concentration, (b) 15 % w/w PL F127 alone and in combination with 1 % w/w PEO 900 prepared in phosphate buffer and triple distilled water (TDW).

In gels with 15 % w/w PL F127, $T_{sol\text{-}gel}$ decreased with increase in HEC concentration from 0 to 3 % w/w from 34°C to 27°C (Figure 6.8a and 6.9). Following equation was drawn from the concentration of HEC (C_{HEC}) and $T_{sol\text{-}gel}$ in gels prepared with 15 % w/w PL F127 in combination with different concentrations of HEC: $T_{sol\text{-}gel} = 33.20 - 2.00 \times C_{HEC}$ with regression coefficient of 0.872, MSSR of 0.74 and AIC of 0.70.

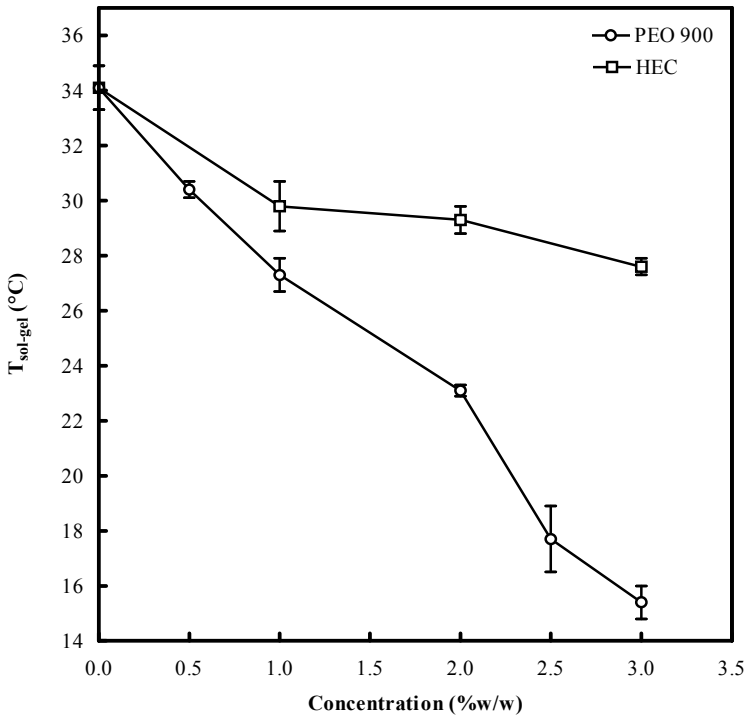


Figure 6.9: Gelation temperature ($T_{sol\text{-}gel}$) of in situ gels as function of PEO 900 and HEC concentration in combination with 15 % w/w PL F127. Each data point is average of three separate determinations.

$T_{sol\text{-}gel}$ for gels prepared with 15 % w/w PL F127 in buffer, 15 % w/w PL F127 in TDW, 15 % w/w PL F127 and 1 % w/w PEO 900 in buffer, 15 % w/w PL F127 and 1 % w/w PEO 900 in TDW was found to be 34.1°C, 40°C, 27.3°C and 29.2°C, respectively (Figure 6.8b and 6.10a). The gels prepared in TDW showed higher $T_{sol\text{-}gel}$ as compared to gels prepared in phosphate buffer saline. This result was in agreement with literature reports that the presence of ionic substances (benzalkonium chloride, sodium chloride and sodium buffer salts) reduces the $T_{sol\text{-}gel}$ of PL F127 gels (Edsman et al., 1998; Choi et al., 1999; Pisal et al., 2004; Dumortier et al., 2006b). The gels prepared with 15 % w/w PL F127 in combination with 1 % w/w different grades of PEO showed decrease in $T_{sol\text{-}gel}$ with increase in molecular weight of PEO (Figure 6.10b).

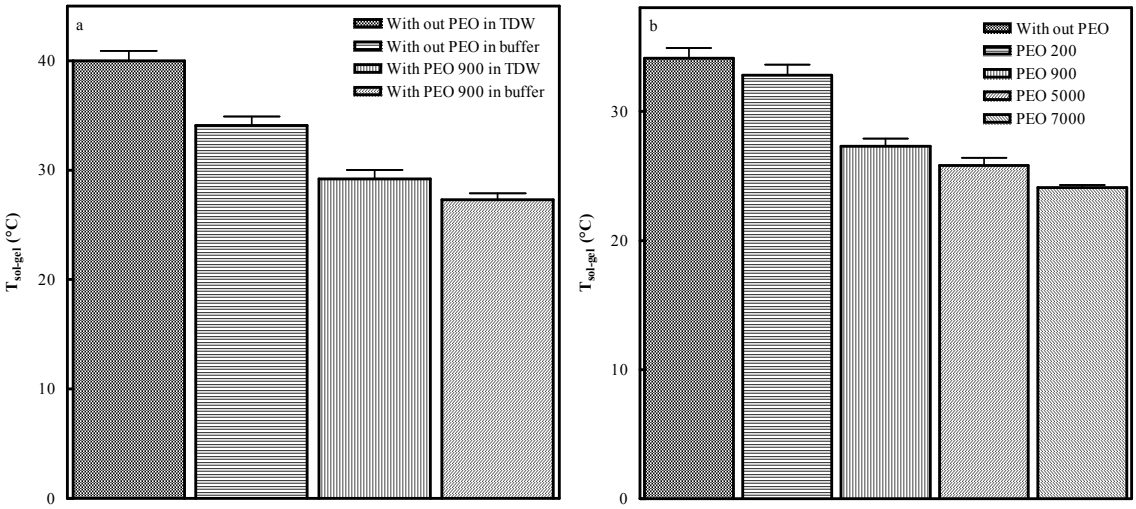


Figure 6.10: Effect of excipients and vehicle on gelation temperatures of gels (a) with 15 % w/w PL F127 alone and in combination with 1 % w/w PEO 900 prepared in phosphate buffer and triple distilled water (TDW), (b) with 15 % w/w PL F127 alone and in combination with 1 % w/w of different grades of PEO in phosphate buffer. Each data point is average of three separate determinations.

6.3.3. Rheological studies

The flow properties of any given system can have, in theory, a significant effect on the behaviour of that system when instilled into the eye. Most rheological studies made on in situ gels use the viscosity of the gels as the rheological parameter (Charrueau et al., 2001). Table 6.2 shows the viscosity of the formulations at 37°C and its corresponding rheological system. Formulations prepared with HPMC (HP_1) showed very less viscosity when compared to other formulations. Formulation was following Newtonian flow as the N value is nearing to 1.

At lower temperatures (below $T_{sol\text{-}gel}$) gels exhibited Newtonian flow (Linear relationship between shear rate and shear stress) and above $T_{sol\text{-}gel}$, they exhibited Pseudoplastic flow, which is represented by the rheogram given in the Figure 6.11a and 6.11b

for gatifloxacin gel system containing 15 % w/w of PL F127 and 2 % w/w PEO 900. These results are in agreement with literature reports (Edsman et al., 1998). With increase in temperature, the flow property of the gel changed from Newtonian to Pseudoplastic. As shown in the Figure 6.11b an increase in shear rate, the shear stress increased exponentially (upward curve) and on removal of shear rate, the shear stress decreased in same path (downward curve). There was no hysteresis loop observed, indicating the elasticity of the gel systems.

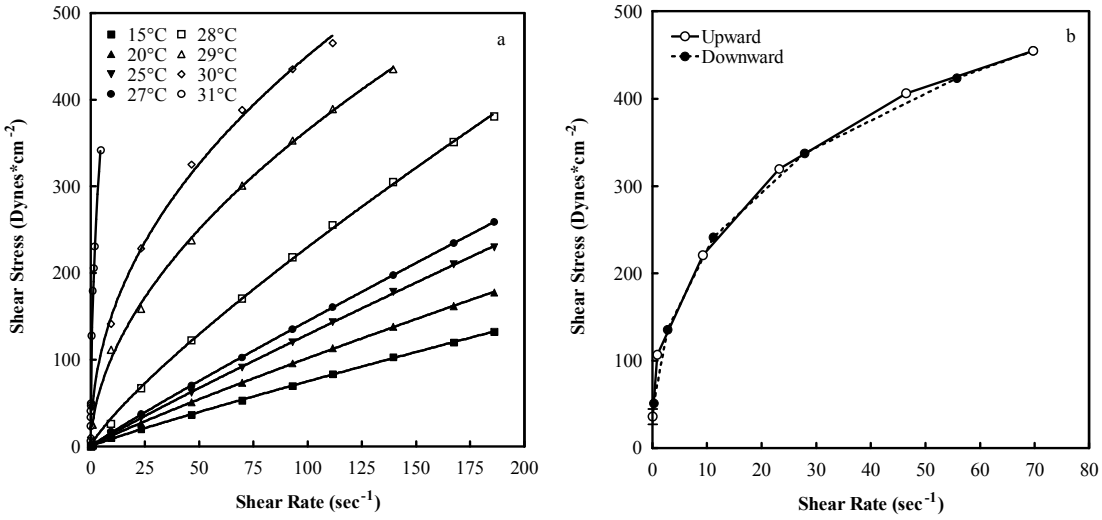


Figure 6.11: Rheograms of (a) gatifloxacin gel ($F_{15}P3_2$) at different temperatures and (b) gatifloxacin gel ($F_{15}P3_2$) with upward and downward curves at 37°C.

As observed, with the increase in concentration of PL F127 in the system, viscosity measured at 37°C increased significantly. There was increase in viscosity for formulations prepared with 14 % w/w PL F127 to 25 % w/w PL F127 from 118.6 to 22566.5 mPa.sec.

Depending on the polymer concentration, the rheological behaviour of PL F127 preparations changed. Preparation F_{14} remained fluid at 37°C and showed tremendous increase in viscosity and gelling capacity with non-Newtonian flow (Pseudoplastic - shear thinning system) properties with increase in polymer concentration. Their apparent viscosities increased with the polymer concentration. They were 4662.1, 17132.8, 18021.8, 19572.0, 20137.9, 20693.0 and 22566.5 mPa.sec for 15, 16, 17, 18, 20, 22 and 25 % w/w, respectively. There was significant difference in viscosity observed between the formulations prepared using pH 7.4 phosphate buffer and triple distilled water. Preparation F_{15}, which is prepared, using pH 7.4 PBS, showed high viscosity (4662.1 mPa.sec) when compared with TDW-F_{15} prepared using triple distilled water (124.6 mPa.sec). The same result was observed with $F_{15}P3_1$ formulation also.

Although PL F127 provides thermosensitive nature to the system, gel strength was found to be very low when used alone. To increase the residence time of the formulation in cul-de-sac and provide continuous release for longer time higher gel strength is required which keeps the gel intact in the precorneal area. To provide gel strength to the formulations, along with PL F127 another hydrophilic polymer PEO was incorporated. Ratio of PEO and the type of PEO used in the preparation were changed and their effect on the rheological properties was studied. When the preparation ($F_{12}P3_1$) containing 12 % w/w of PL F127 in combination with 1 % w/w PEO 900 was studied, viscosity of the system was low (165.6 mPa.sec) with Newtonian flow. With increase in the concentration of PL F127, by keeping PEO 900 (1 % w/w) constant, there was significant increase in viscosity and showed non-Newtonian (Pseudoplastic) flow (Table 6.2).

Based on these experiments, 15 % w/w concentration of PL F127 was selected as optimum concentration as this is the minimum concentration of PL F127 showing high viscosity and Pseudoplastic flow. While in literature concentrations as high as 25 % w/w of PL F127 are reported (Dumortier et al., 2006b). Once the concentration of PL F127 was optimised, PEO 900 concentration was changed from 0.5 to 3 % w/w and it was found that viscosity of the formulation increased tremendously from 14437.8 to 23577.5 mPa.sec. Fluidity index, N values were more than 1 indicating non-Newtonian flow (Table 6.2).

To know the effect of PEO type on the rheological properties, formulation ($F_{15}P1_1$) was prepared using PEO 200 (1 % w/w) along with 15 % w/w PL F127. Viscosity and strength of this system is less than that of the formulations with PEO 900 and also formulations with PEO 900 exhibited good gel strength. PEO 5000 and 7000 (1 % w/w) concentration were also tried in preparations along with 15 % w/w PL F127. There was increase in viscosity of the formulation with change in PEO molecular weight from 200 to 7000 kDa.

Gels were also prepared using HEC in different concentrations (1 to 3 % w/w) along with PL F127 (15 % w/w) ($F_{15}HE_1$, $F_{15}HE_2$, $F_{15}HE_3$). There was increase in viscosity of the formulation when combined with HEC as compared to PL F127 alone. However, increase in HEC concentration has not improved the viscosity significantly. All the preparations containing HEC were showing Pseudoplastic flow.

The administration of ophthalmic formulations should have as little effect as possible on the Pseudoplastic character of the precorneal film (Bothner et al., 1990). Since the ocular shear rate is very high, ranging from 0.03 sec^{-1}, during inter-blinking periods, to 4250-28500 sec^{-1}, during blinking (Kumar and Himmelstein, 1995), viscoelastic fluids with a viscosity that is high under low shear rate conditions and low under the high shear rate conditions are

often preferred. Several prepared formulations showed Pseudoplastic flow and thus have advantages.

6.3.4. Texture profile analysis

The mechanical properties of each preparation determined using texture analyser and data was presented in Figure 6.12 and Table 6.3. Gel preparation with PL F127 alone (F_{15}) did not show good gel strength and also did not produce any value for parameters like hardness, adhesiveness, compressibility and cohesiveness. This was shown by flat line in Figure 6.12, which is parallel to x-axis. But for those formulations containing PEO, the strength was high as indicated in Figure 6.12.

As observed, incorporating PEO in the formulations along with PL F127 increased the hardness, adhesiveness, compressibility and cohesiveness drastically. Cohesiveness values for the formulations containing PEO indicated that these gels have good elastic properties, which is in agreement with the rheological studies.

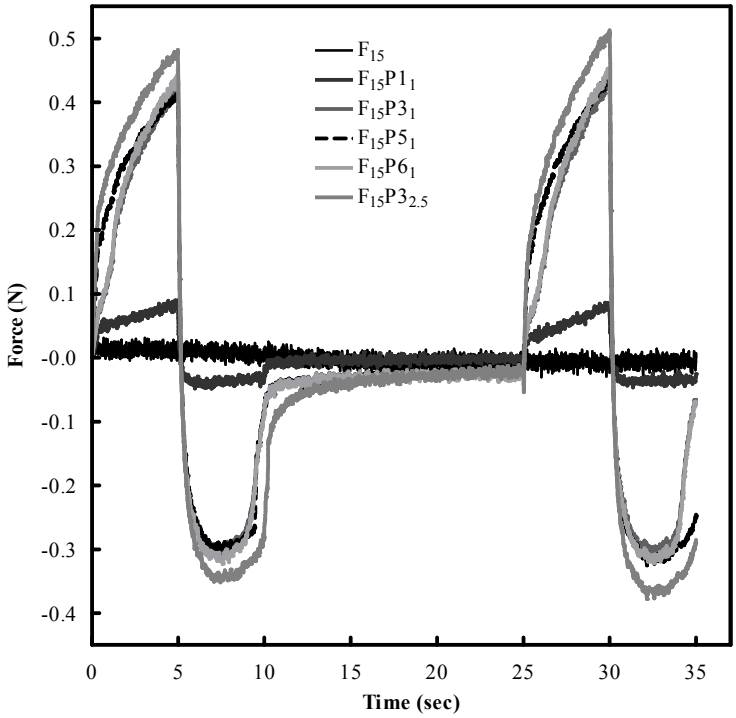


Figure 6.12: Result of texture analysis profile of different gels.

Increasing the molecular weight of PEO has increased the hardness and elasticity of the gels. When PEO 200 was replaced with PEO 900, in the formulation, there was significant increase in hardness, adhesiveness, compressibility and cohesiveness. There was no significant improvement or increase in these parameters with increase in PEO molecular weight more than 900 kDa. With increase in concentration of PEO 900 from 1 to 2.5 % w/w

there was increase in hardness, adhesiveness and compressibility for the in situ gels. But the elasticity of the gels was unaffected. Maximum hardness, adhesiveness, compressibility and cohesiveness values were established by $F_{15}P3_{2.5}$ containing 2.5 % w/w of PEO 900 with 15 % w/w of PL F127.

Table 6.3: Texture profile analysis and bioadhesion data (texture analyser method) of different gel formulations. Each data point is average of three separate determinations.

Code	Hardness (N)	Adhesiveness (N.mm)	Compressibility (N.mm)	Cohesiveness	F_{max} (N.cm^{-2})	Work of Adhesion (N.sec.cm^{-2})
F_{15}	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00	0.271 ± 0.052	0.123 ± 0.027
$F_{15}P1_1$	0.090 ± 0.02	0.374 ± 0.02	0.622 ± 0.08	0.864 ± 0.01	0.336 ± 0.051	0.127 ± 0.015
$F_{15}P3_1$	0.434 ± 0.03	3.312 ± 0.42	2.760 ± 0.15	0.991 ± 0.01	0.628 ± 0.183	0.330 ± 0.043
$F_{15}P5_1$	0.415 ± 0.01	3.332 ± 0.15	3.140 ± 0.76	1.018 ± 0.01	0.836 ± 0.141	0.403 ± 0.030
$F_{15}P6_1$	0.452 ± 0.02	3.450 ± .033	2.874 ± 0.20	0.991 ± 0.01	1.190 ± 0.090	0.522 ± 0.190
$F_{15}P3_{2.5}$	0.482 ± 0.02	4.214 ± 0.29	3.704 ± 0.40	1.003 ± 0.01	1.045 ± 0.019	0.437 ± 0.096

These results confirm that the presence of PEO in the formulation in combination with PL F127 provides elasticity of the preparation and improves hardness, adhesion and compressibility of the gels. All these results confirm that the presence of PEO in gels increased the gel strength of the formulation with increase in viscosity.

6.3.5. Bioadhesion testing

(a) Conventional bioadhesion testing

Bioadhesion study done using conventional method was able to differentiate gels based on their adhesion strength. Force of detachment calculated for different gels was given in Table 6.2 and depicted in Figures 6.13 to 6.16 as bar graphs.

Formulations prepared with PL F127 alone have low adhesion strength among all formulations studied. As the concentration of PL F127 in the formulation increases, the force required for detachment of gel from the tissue also increased. Among these formulations, F_{25} has shown highest bioadhesion (Figure 6.13). In formulations with increasing concentration of PL F127 (12 to 25 % w/w) along with 1 % w/w PEO 900 there was 3 to 15 fold increase in bioadhesion strength. No significant increase in detachment force was observed on increasing PL F127 above 15 % w/w (Figure 6.13).

Figure 6.14 shows the force of detachment required to separate tissue from the formulations prepared with PL F127 in combination with different concentration of HEC and different concentration of PEO using both TDW and PBS as a vehicle.

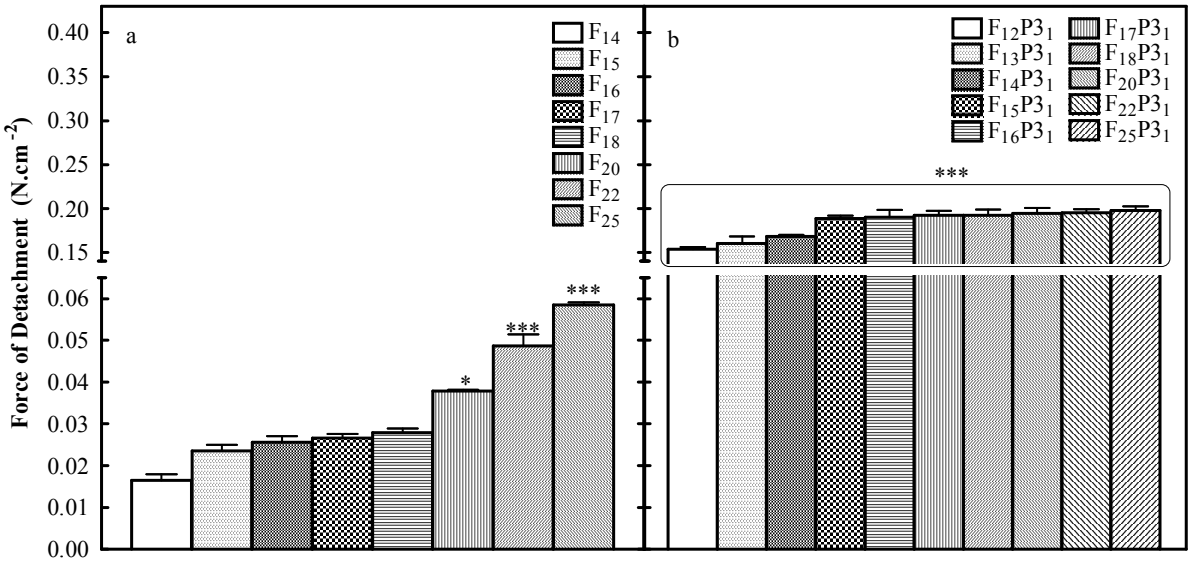


Figure 6.13: Force of detachment obtained from conventional bioadhesion testing for different in situ gel formulations prepared with (a) different concentrations of PL F127 alone (b) different concentrations of PL F127 in combination with 1 % w/w PEO. Each data point is average of three separate determinations. (* – *P* < *0.05*; *** – *P* < *0.001*; block represents *P* < *0.001*; data compared with F_{14})

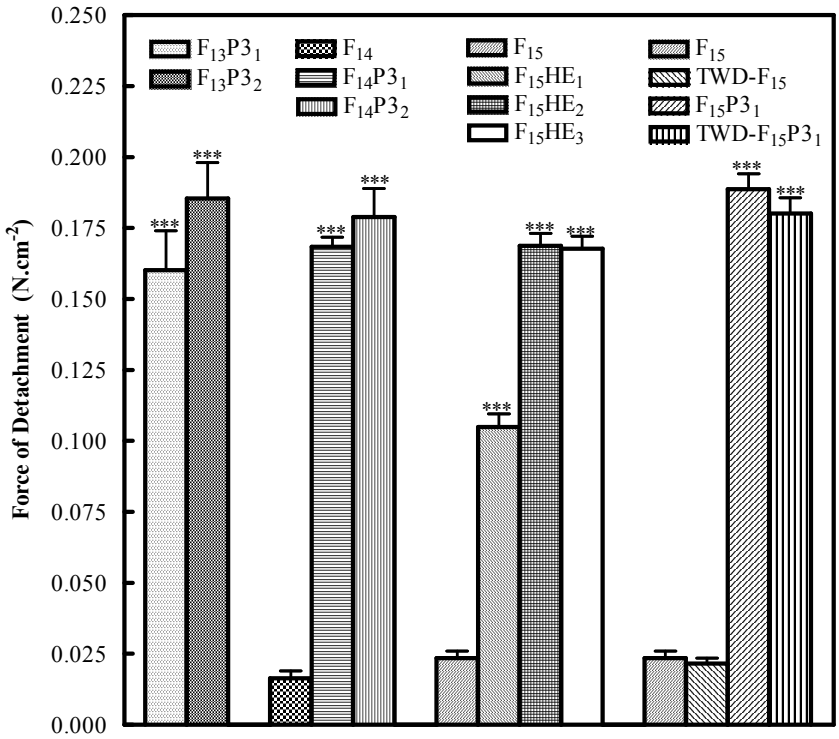


Figure 6.14: Force of detachment obtained from conventional bioadhesion testing for different in situ gel formulations prepared with PL F127 alone, in combination with different concentration of PEO and HEC and in PBS and TDW. Each data point is average of three separate determinations. (*** – *P* < *0.001*; data compared with F_{14})

The detachment force for formulations prepared with 13 % w/w PL F127 and 1 to 2 % w/w PEO 900 ($F_{13}P3_{1\text{-}2}$) was 3-fold more than that of the formulations prepared with that of 25 % w/w of PL F127 alone (F_{25}). With increase in concentration of PEO 900 from 1 to 2 %

w/w in combination with 14 % w/w of PL F127 ($F_{14}P3_{1-2}$) the detachment force increased by 8 fold as compared to 14 % w/w PL F127 alone (F_{14}).

The force of detachment required was more for the formulations prepared with PL F127 and HEC combination. With the increase in concentration of HEC in the formulation up to 2 % w/w, force required for detachment also increased. Above this percentage there was no further increase in the detachment force. There was no significant difference in force detachment between the formulations prepared with triple distilled water and buffer.

The effect of PEO concentration on the bioadhesion strength was studied and shown in Figure 6.15. PL F127 concentration was kept constant but PEO concentration was changed from 0.5 to 3 % w/w. Highest bioadhesion was shown by the formulation containing PEO 3 % w/w when compared with other formulations shown here. There was around 6 folds increase in bioadhesion strength observed with the incorporation of PEO 900 at 0.5 % w/w ($F_{15}P3_{0.5}$) concentration when compared with F_{15}. Further increase in PEO concentration, increased the bioadhesion as indicated by force of detachment.

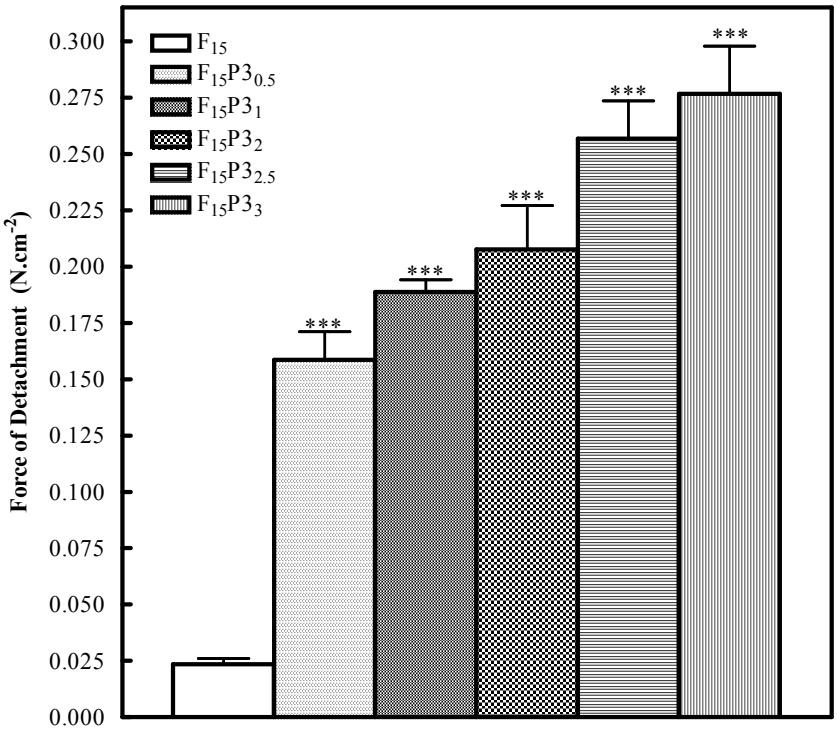


Figure 6.15: Force of detachment obtained from conventional bioadhesion testing for different in situ gel formulations prepared with 15 % w/w of PL F127 and increasing concentrations of PEO 900 (0.5 to 3 % w/w). Each data point is average of three separate determinations. (*** – *P < 0.001*; data compared with F_{15})

Different molecular weight of PEO has given variable detachment force in the present study. Increase in the molecular weight of the PEO in the formulation lead to increased force

required for detachment of the formulation from the tissue. Gels with highest molecular weight grade PEO has shown highest force of detachment of around 0.3 $N.cm^{-2}$ (Figure 6.16).

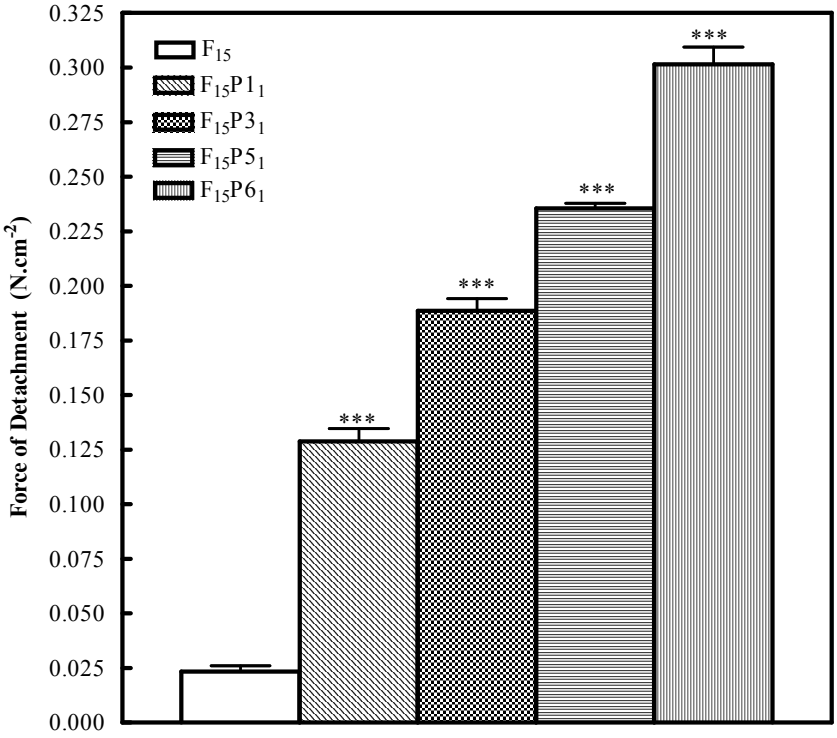


Figure 6.16: Force of detachment obtained from conventional bioadhesion testing for different in situ gel formulations prepared with 15 % w/w of PL F127 and increasing molecular weight of PEO (200, 900, 5000 and 7000). Each data point is average of three separate determinations. (*** – $P < 0.001$; data compared with F_{15})

(b) Bioadhesion testing by texture analyser

Figure 6.17 indicates the F_{max} and work of adhesion force shown by selected formulations and the data was given in Table 6.3. In this study also, formulations prepared with only PL F127 required minimum force for detachment and with the incorporation of PEO detachment the force required was increased. With the increase in the molecular weight of PEO force required for detachment also increased. The increase in concentration of PEO 900 increased F_{max} and work of adhesion. These results are in compliance with the previously discussed results obtained with conventional method.

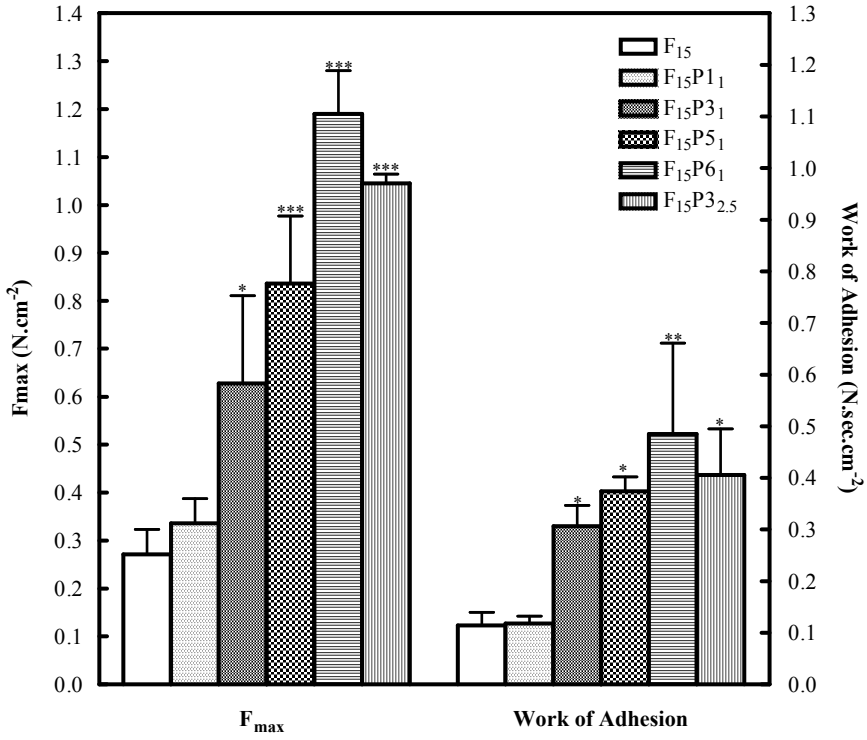


Figure 6.17: Result of bioadhesion testing using texture analyser. Each data point is average of three separate determinations. (* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$; data compared with F_{15})

6.3.6. In vitro drug release

(a) Membrane free method

The drug release from the in situ gels were shown in the Figures 6.18 to 6.24. Among all the models tried, data were best fitted into first order release kinetics, which is evident from low AIC and MSSR values. All the kinetic parameters, release rate constant (K), $t_{50\,\%}$, R^2, MSSR and AIC for first order release model were presented in Table 6.4.

Control formulation (HP_1) released the drug immediately (Figure 6.18). Where as, formulations prepared with PL F127 alone and in combination with PEO retarded the drug release. Figure 6.18 shows the release profiles of formulations prepared with different concentrations of PL F127. Increase in concentration of PL F127 from 15 to 25 % w/w retarded the release of gatifloxacin from 45 to 180 min. This retardation was due to the increase in viscosity of the formulation with increase in concentration of PL F127 (Table 6.2). Increase in viscosity reduces the penetration of dissolution medium into the system thereby decreasing the drug release. The PL F127 gels are viewed as consisting of large populations of micelles and aqueous channels in which the incorporated solute may be released by its passage through the aqueous channels and due to gel dissolution (Zhang et al., 2002). The decrease in the rate of release with an increase in the PL F127 concentration could be attributed to the increase in the number and size of the micelles formed at the higher

polymer concentration. This could cause a greater tortuosity in the aqueous phase of the gel structure (Gilbert et al., 1986) and a slower rate of dissolution.

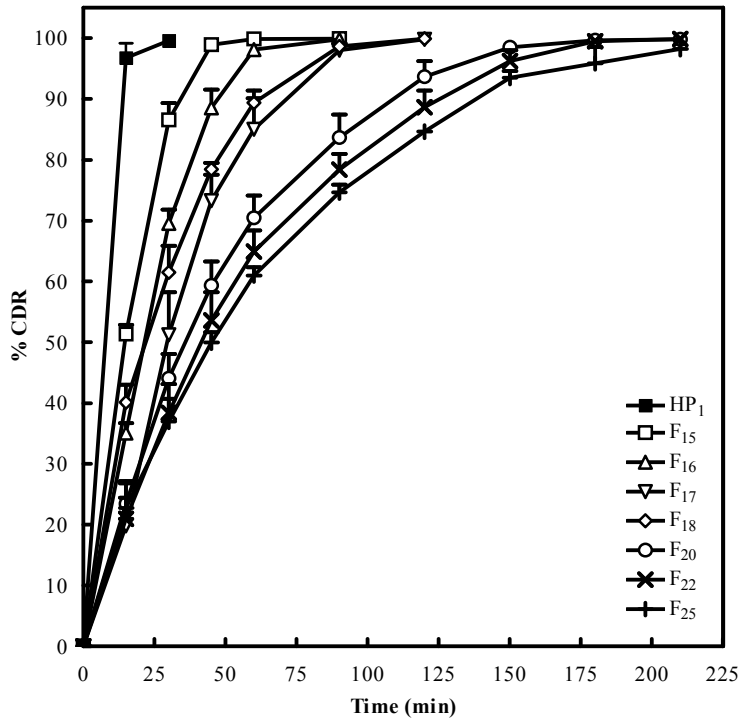


Figure 6.18: Result of in vitro drug release from gatifloxacin gels containing different concentrations of PEO 900 in combination with 14 % w/w of PL F127. Each data point is average of three separate determinations.

In addition, higher poloxamer concentrations results in a shorter inter-micellar distance, leading to greater number of cross-links between neighbouring micelles and a greater number of micelles per unit volume (Bhardwaj and Blanchard, 1996). For gels prepared with different concentrations of PL F127 (15 to 25 % w/w), the release rate constant was found to decreased from 52.41×10^{-3} to 14.86×10^{-3} min^{-1} and $t_{50\ \%}$ was found to increased from 13.23 to 46.64 min.

Effect of PEO 900 concentration on the release of drug from the gels, was also studied and profiles were shown in Figure 6.19. It shows that with increase in concentration of PEO in the preparation there is decrease in the drug release from gel matrix. Incorporating 0.5 % w/w of PEO in formulation containing 15 % w/w of PL F127, extended the release of gatifloxacin from the gel upto 90 min. Increase in the PEO concentration from 0.5 to 1 % w/w extended the release for nearly 120 min. Further increase in the concentration to 2.5 % w/w significantly decreased the release as indicated in the Figure 6.19. There was no further control on the release with the increase in the PEO concentration beyond 2.5 % w/w. As no viscosity increase observed beyond 2.5 % w/w of PEO 900, there was no further retardation of release.

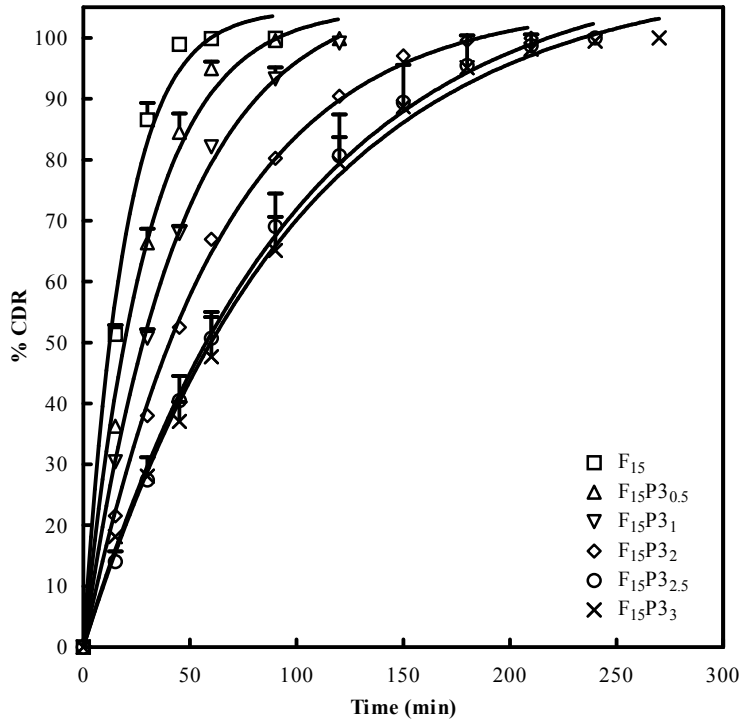


Figure 6.19: Result of in vitro drug release from gatifloxacin gels containing different concentrations of PEO 900 in combination with 15 % w/w of PL F127. Each data point is average of three separate determinations.

It has been reported in literature that drug diffusion from a poloxamer gel is through extramicellar aqueous channels. The microviscosity of the water channels controls drug diffusion and release from the poloxamer gels. Another inert, hydrophilic polymer would decrease the amount of free water in the water channels, and affect molecular orientation of the gel matrix. The length and size of the diffusional channels also controls drug release. Therefore, additives that would change the microviscosity and molecular orientation of aqueous channels will modify drug release kinetics (Chen-Chow and Frank, 1981; Zhang et al., 2002). PEO swells in aqueous media forming disordered three-dimensional physical networks and will cause molecule entanglement forming tightly orientated gel structure. This will increase the microviscosity of the aqueous channels and gel strength of the formulations, which indeed will extended the drug release. Possibility of extensive hydrogen bonding between polyEO groups of PEO and PL F127 could be another factor that can extend the drug release from the gel formulations. These mechanisms were supported by the fact that with increase in concentrations of PEO 900 (from 0.5 to 3 % w/w) in combination with 15 % w/w PL F127 the release rate constants were found to be reduced from 33.62×10^{-3} to 10.07×10^{-3} min^{-1} and $t_{50\,\%}$ was found to be increased from 20.61 to 68.81 min. Similar results were obtained with gels prepared with 13 and 14 % w/w Pl F127 in combination with 1 % and 2 % w/w of PEO 900 (Figure 6.20). Maximum control of drug release for more than 240 min was

shown by the preparation containing 2.5 % w/w concentration of PEO 900 in combination with 15 % w/w of PL F127 ($F_{15}P3_{2.5}$).

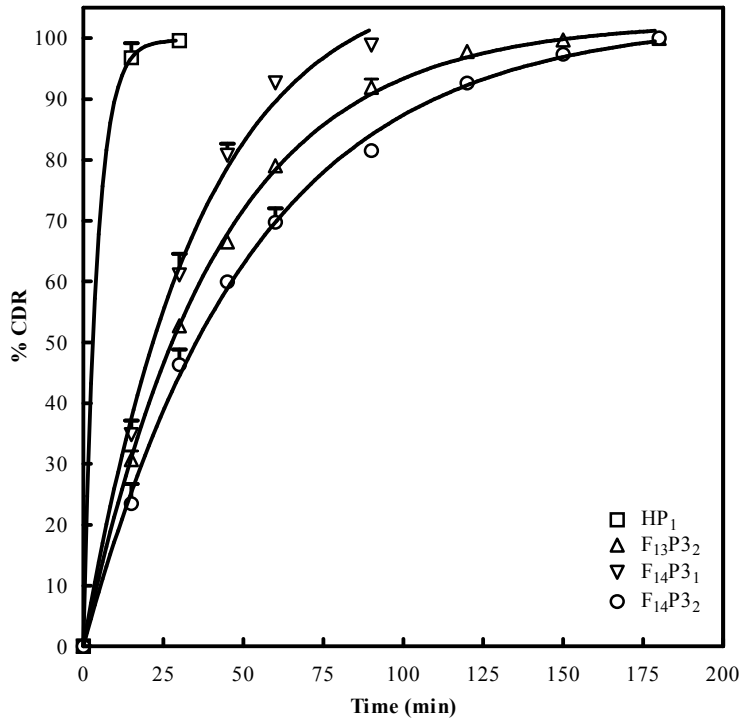


Figure 6.20: Result of in vitro drug release from gatifloxacin gels containing different concentrations of PEO 900 in combination with 13 and 14 % w/w of PL F127. Each data point is average of three separate determinations.

With the increase in the concentration of PL F127 from 14 to 25 % w/w in combination with 1 % w/w PEO 900 the drug release was retarded (Figure 6.21). This can be attributed to increase in viscosity. The release rate constants were found to be reduced from 27.58×10^{-3} to 14.77×10^{-3} min^{-1} and $t_{50\,\%}$ was found to be increased from 25.13 to 46.94 min. Maximum control of drug release for more than 200 min was shown by the preparation containing 25 % w/w concentration of PL F127 in combination with 1 % w/w of PEO ($F_{25}P3_1$). The drug release from the gels with 25 % w/w concentration of PL F127 with 1 % w/w PEO ($F_{25}P3_1$) was less retarded than the gels prepared with 15 % w/w concentration of PL F127 with 2.5 % w/w of PEO ($F_{15}P3_{2.5}$).

Preparations with increasing concentration of PL F127 from 13 to 15 % w/w in combination with 2 % w/w PEO 900 controlled the release of drug from the gels. In vitro release profiles for these preparations were shown in Figure 6.22. Preparation with 13 % w/w of PL F127 ($F_{13}P3_2$) retarded upto 1 hr whereas formulation with 14 % w/w PL F127 ($F_{14}P3_2$) retarded up to 1.5 hr. With increase in concentration of PL F127 from 13 to 15 % w/w, the release rate constants were found to be decreased from 24.04×10^{-3} to 15.81×10^{-3} min^{-1} and $t_{50\,\%}$ was found to be increased from 28.84 to 43.85 min (Table 6.4). When combined with 2

% w/w of PEO 900, even the lower concentration of PL F127 was showing reversible thermogelation property; therefore control of drug release was observed.

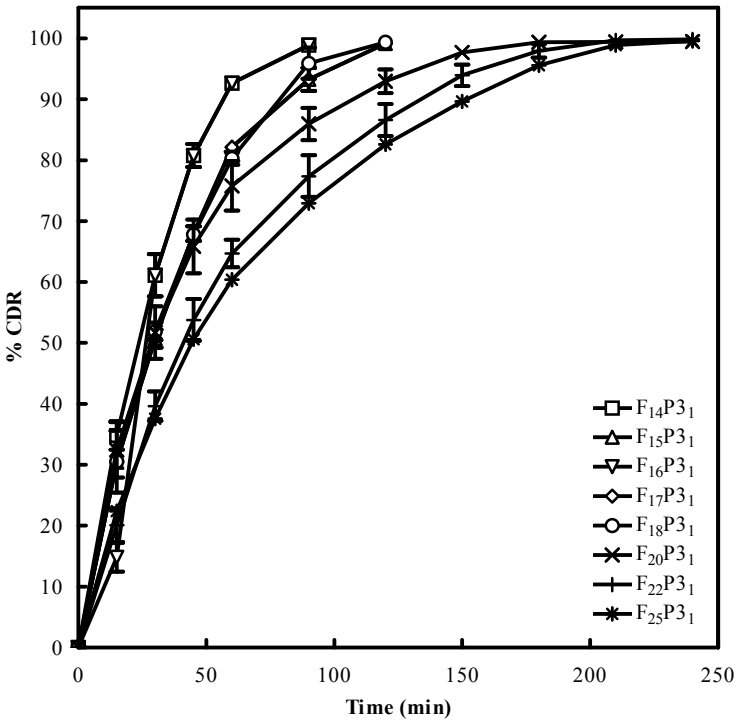


Figure 6.21: Result of in vitro drug release from gatifloxacin gels containing 1 % w/w PEO 900 in combination with different concentrations of PL F127. Each data point is average of three separate determinations.

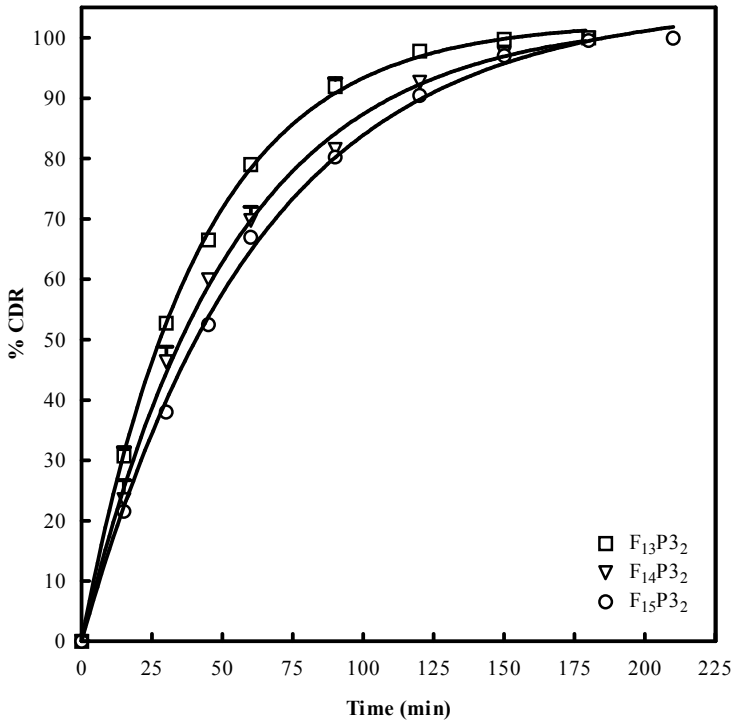


Figure 6.22: Result of in vitro drug release from gatifloxacin gels containing 2 % w/w PEO 900 in combination with different concentrations of PL F127. Each data point is average of three separate determinations.

In vitro release profiles of formulations prepared with 15 % w/w PL F127 alone and in combination with different concentration of HEC were compared and presented in the Figure 6.23. Incorporation of HEC in to the preparation retarded the release and increase concentration of HEC decreased the release of gatifloxacin.

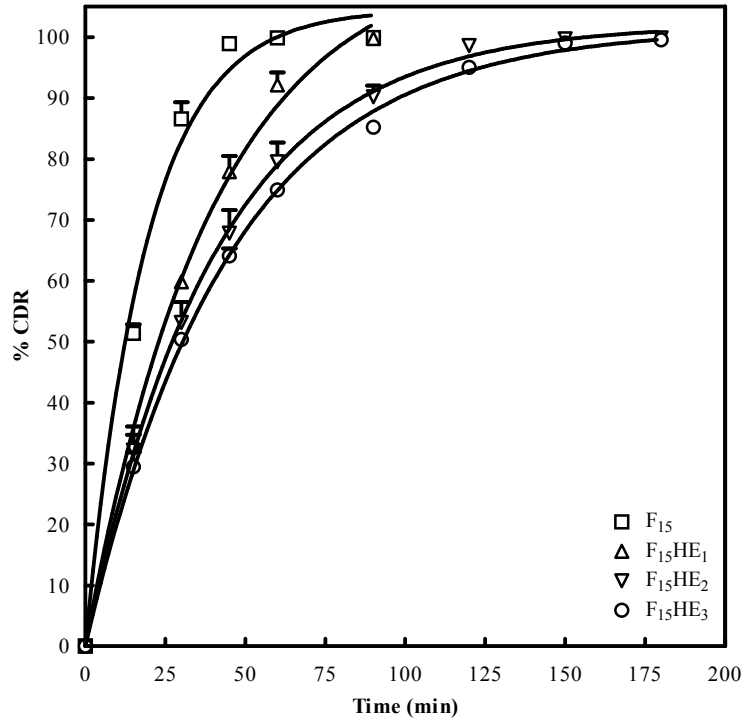


Figure 6.23: Result of in vitro drug release from gatifloxacin gels containing different concentrations of PEO 900 in combination with 14 % w/w of PL F127. Each data point is average of three separate determinations.

Effect of molecular weight of PEO on the release of drug from the gels was also studied and profiles were shown in Figure 6.24. There was no significant difference in the drug release from the formulations prepared with only 15 % w/w of PL F127 (F_{15}) and preparation with combination of PL F127 and 1 % w/w of PEO 200 ($F_{15}P1_1$). By replacing PEO 200 with same concentration of PEO 900 in the formulation keeping PL F127 constant ($F_{15}P3_1$), the release of drug from the system was extended to 120 min. Changing PEO type to higher molecular weight PEO 5000 ($F_{15}P5_1$) further extended the release. There was no difference in the release of drug from the formulations prepared with PEO 5000 ($F_{15}P5_1$) and 7000 ($F_{15}P6_1$).

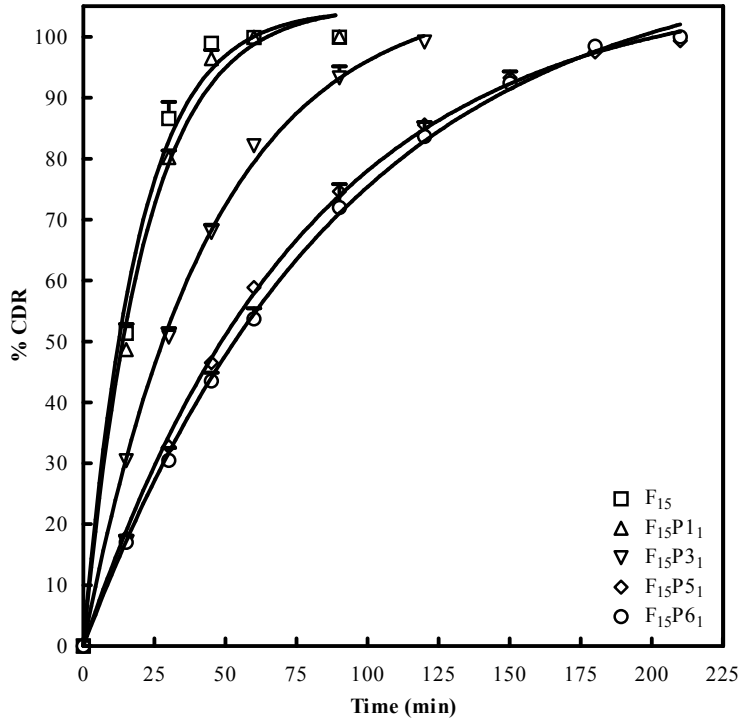


Figure 6.24: Result of in vitro drug release from gatifloxacin gels containing different concentrations of PEO 900 in combination with 14 % w/w of PL F127. Each data point is average of three separate determinations.

Table 6.4: Result of in vitro drug release data fitting into first order release kinetics.

Code	K (min^{-1}) × 10^3 (Mean ± SD) †	$t_{50\,\%}$ (min)	R^2	MSSR	AIC
F_{15}	52.41 ± 6.41	13.23	0.991	0.08	17.27
F_{16}	32.15 ± 5.15	21.56	0.987	0.06	19.16
F_{17}	20.68 ± 3.67	33.52	0.981	0.04	24.43
F_{18}	31.57 ± 1.15	21.95	0.999	0.72	4.30
F_{20}	18.75 ± 0.45	36.97	0.999	0.97	2.32
F_{22}	15.57 ± 0.35	44.51	0.999	1.28	-0.49
F_{25}	14.86 ± 0.40	46.64	0.999	0.98	2.23
$F_{15}P3_{0.5}$	33.62 ± 2.95	20.61	0.993	0.12	17.14
$F_{15}P3_1$	22.35 ± 1.04	31.02	0.999	0.59	5.72
$F_{15}P3_2$	15.81 ± 0.51	43.85	0.999	0.61	6.89
$F_{15}P3_{2.5}$	10.27 ± 0.43	67.47	0.998	0.46	10.55
$F_{15}P3_3$	10.07 ± 0.54	68.81	0.996	0.22	20.17
$F_{14}P3_1$	27.58 ± 2.57	25.13	0.996	0.21	11.49
$F_{16}P3_1$	20.08 ± 8.48	34.53	0.950	0.01	27.66
$F_{17}P3_1$	22.35 ± 1.04	31.02	0.999	0.59	5.72
$F_{18}P3_1$	22.01 ± 0.87	31.49	0.999	0.81	3.46
$F_{20}P3_1$	24.08 ± 0.56	28.79	0.999	0.81	4.33
$F_{22}P3_1$	16.05 ± 0.35	43.18	0.999	1.06	1.39
$F_{25}P3_1$	14.77 ± 0.48	46.94	0.998	0.55	8.63

(Contd..)

† Each data is average of three determinations.

Table 6.4

Code	K (min^{-1}) × 10^3 (Mean ± SD) †	$t_{50\,\%}$ (min)	R^2	MSSR	AIC
$F_{13}P3_2$	24.04 ± 0.48	28.84	0.999	1.51	-1.69
$F_{14}P3_2$	18.82 ± 0.67	36.84	0.998	0.58	6.90
$F_{15}HE_1$	25.14 ± 2.25	27.58	0.997	0.24	10.47
$F_{15}HE_2$	24.71 ± 0.50	28.05	0.999	1.43	-1.22
$F_{15}HE_3$	22.29 ± 0.58	31.10	0.999	0.93	2.68
$F_{15}P1_1$	46.49 ± 4.70	14.91	0.994	0.12	14.83
$F_{15}P5_1$	12.74 ± 0.36	54.42	0.999	1.03	1.73
$F_{15}P6_1$	10.91 ± 0.34	63.52	0.999	0.98	2.16

† Each data is average of three determinations.

In gels with increase in concentration from 15 to 25 % w/w of PL F127 alone, the $t_{50\,\%}$ was reduced. There exists a linear relationship between $t_{50\,\%}$ and $T_{sol\text{-}gel}$ in this range. The slope was found to be 2.28 and the regression coefficient was found to be 0.813 (Figure 6.25a). In gels with increase in concentration of PEO 900 from 0 to 3 % w/w in combination with 15 % w/w PL F127, the $t_{50\,\%}$ was reduced. A linear relationship with high regression coefficient was observed with slope of 3.21 (Figure 6.25b). In gels with increase in concentration from 14 to 25 % w/w of PL F127 in combination with 1 % w/w PEO 900, the $t_{50\,\%}$ was reduced but the relationship between $t_{50\,\%}$ and $T_{sol\text{-}gel}$ was showing poor linear regression coefficient (Figure 6.25c). In gels with increase in molecular weights of PEO (1 % w/w) in combination with 15 % w/w PL F127, the $t_{50\,\%}$ was reduced. A linear relationship with regression coefficient of 0.889 and slope of 5.53 was observed (Figure 6.25d). These relationships indicate that the drug release from the in situ gels was depended on the gelation temperature of the formulation, which again dependent on concentration of PL F127, concentration of PEO and molecular weight of PEO.

Maximum control of drug from gels was observed from $F_{15}P3_{2.5}$, $F_{15}P3_3$ and $F_{15}P6_1$. These formulations showed high $t_{50\,\%}$ value (>60 min) and lesser release rate (≈ 10 × 10^{-3} hr^{-1}). There was no significant difference in these parameters between $F_{15}P3_{2.5}$ and $F_{15}P3_3$ formulations.

Incorporating PEO in the formulation along with PL F127 increased the viscosity and gel strength thereby controlling the drug release. With the increase in the molecular weight of PEO used in the formulation, there was increase in mucoadhesion of the formulation and this might also contribute to extended drug release in vivo.

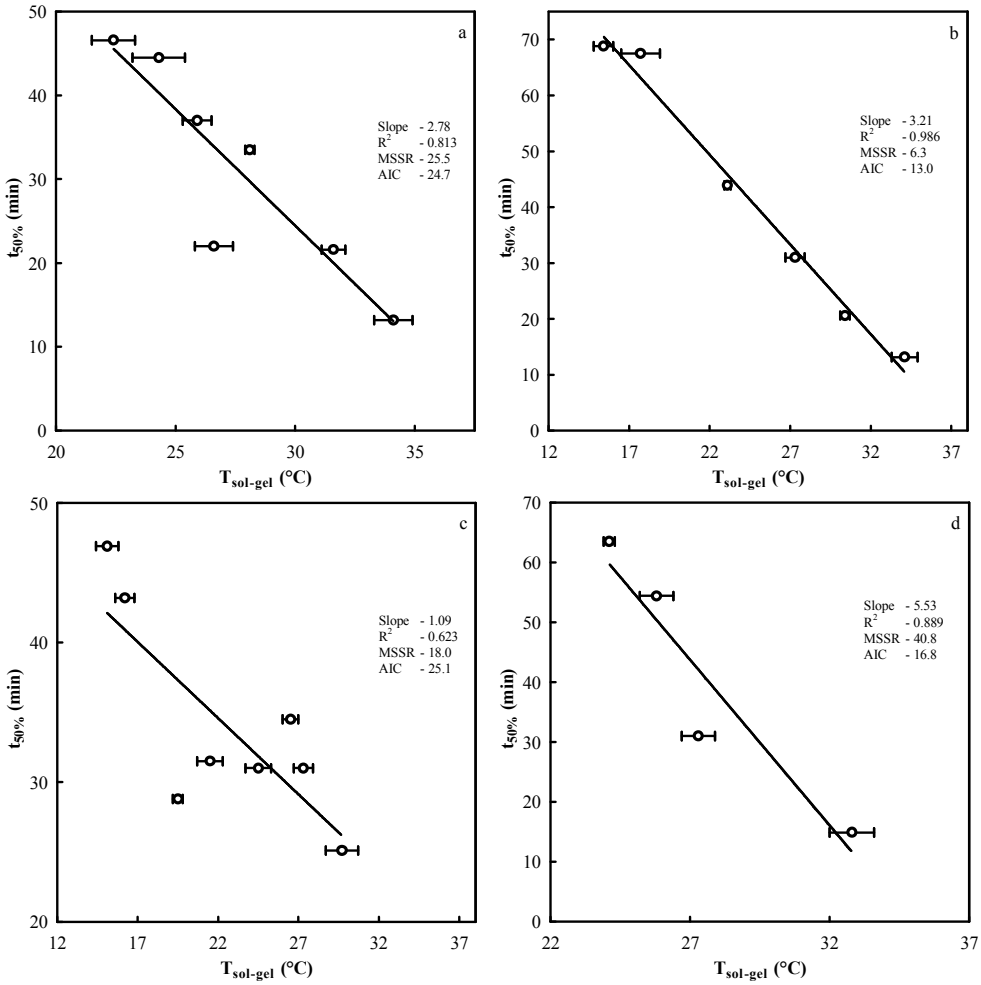


Figure 6.25: Correlation between $T_{sol-gel}$ and $t_{50\ \%}$ for gatifloxacin ophthalmic in situ gels (a) with 15 to 25 % w/w of PL F127 alone, (b) with 15 % w/w concentration of PL F127 in combination with increase in concentration of PEO 900 from 0 to 3 % w/w, (c) with 14 to 25 % w/w concentrations of PL F127 in combination with 1 % w/w of PEO 900 and (d) with concentration of 15 % w/w PL F127 in combination with 1 % w/w of different molecular weights of PEO.

(b) Gel erosion or dissolution studies

The %GD-time plots were shown in Figure 6.26 to 6.28. The gels were dissolved at different rates depending on the concentrations of PL F127 alone and in combination of different concentrations of PEO. Gels with 15 to 25 % w/w concentrations of PL F127 alone have shown different gel dissolution rates. The gels were dissolved within 90 min of the study.

Gels with 0.5 to 3 % w/w concentrations of PEO in combination with 15 % w/w of PL F127 have shown concentration dependent gel dissolution rates (Figure 6.26). Fifty percent of gels were eroded within 50 min for formulations prepared with 0.5 and 1 % w/w concentrations of PEO 900 and for formulations prepared with 2 % w/w and above concentrations of PEO 900 were dissolved within 150 min.

Gels with 15 to 25 % w/w concentrations of PL F127 in combination with 1 % w/w of PEO 900 have shown different gel dissolution rates (Figure 6.27). Fifty percentages of gel were dissolved within 25 min for gels having 14 to 18 % w/w of PEO 900. Gels with still higher concentrations of PL F127 gels have dissolved within 50 min.

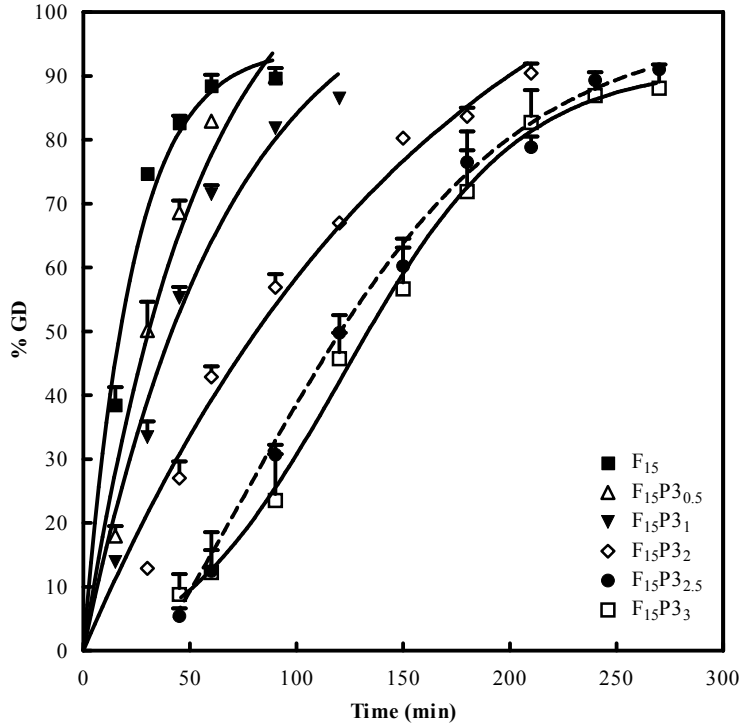


Figure 6.26: Gel dissolution of gatifloxacin gels containing different concentration of PEO 900 with 15 % w/w PL F127. Each data point is average of three separate determinations.

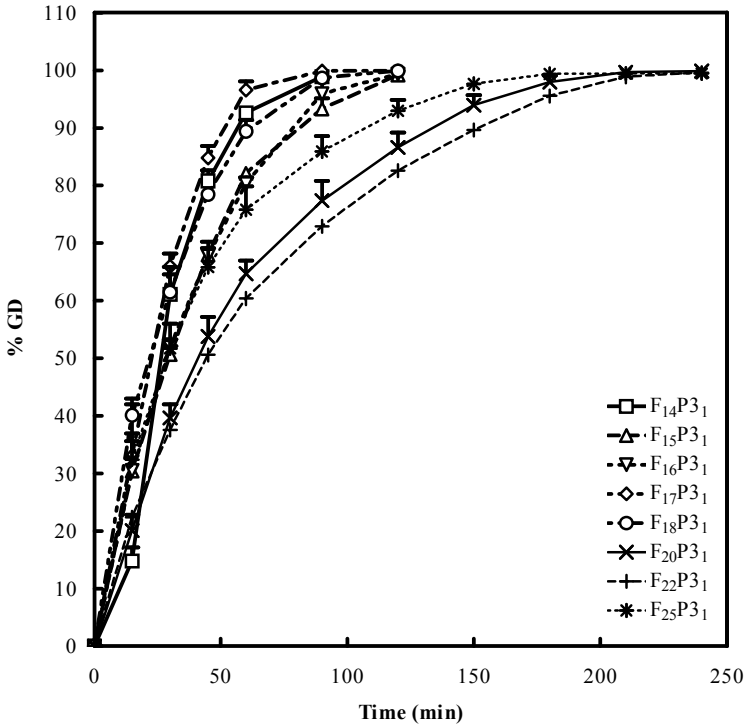


Figure 6.27: Gel dissolution of gatifloxacin gels containing different concentration of PL F127 with 1 % w/w PEO 900. Each data point is average of three separate determinations.

Gels with 15 % w/w concentrations of PL F127 in combination with 1 % w/w of different grades of PEO have shown molecular weight dependent gel dissolution rates (Figure 6.28). Fifty percent of gel was dissolved within 20 min for gels containing lower molecular weights of PEO (200 kDa). Fifty percentages of gels were dissolved in formulations containing molecular weight of 900, 5000 and 7000 kDa of PEO were dissolved in 30, 60 and 60 min respectively.

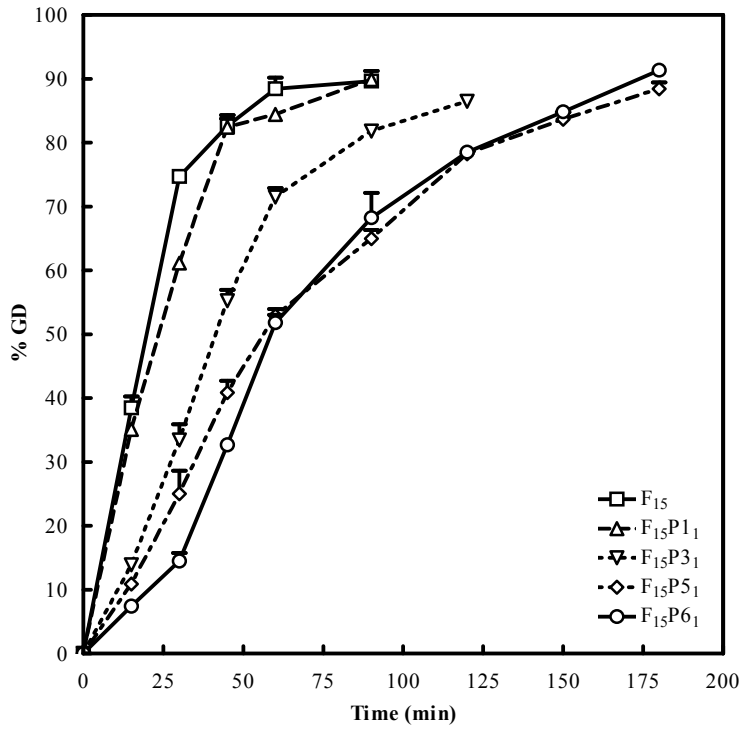


Figure 6.28: Gel dissolution of gatifloxacin gels containing different molecular weights of PEO with 15 % w/w PL F127. Each data point is average of three separate determinations.

As the gel dissoluted during the release study, there was increase in the drug release from the gels. The % CDR was plotted as a function of the % GD in Figure 6.29. Relation between the % CDR and % GD for all gel preparations was linear with the difference in slope, with high regression value ($R^2 > 0.9$), low MSSR and AIC values (Figure 6.29 and Table 6.5).

As the concentration of Pl F127 (alone) increased from 15 to 20 % the slope values remained near to 1 (Figure 6.29a and Table 6.5). This indicates that the overall rate of drug release is controlled by dissolution of the gel. Beyond 20 % PL F127 (22 and 25 %) the slope value reduced to 0.8, indicating change in mechanism of drug release with increase in concentration of PL F127. Linear regression analysis showed best fit with slope value of 0.86. With addition of 1% PEO 900 to gels containing different concentrations of Pl F127 (16

to 25 %), the obtained slope values were ranging from 0.43 to 0.84 (Figure 6.29c and Table 6.5). This indicates that the drug release from the gels is controlled not only by erosion but also by other mechanisms like diffusion through aqueous channels. Linear regression analysis showed poor fit with slope value of 0.75. Addition of high molecular weight hydrophilic polymers effects the microviscosity of diffusional channels of PL F127 gels and orientation of gel structure (Zhang et al., 2002).

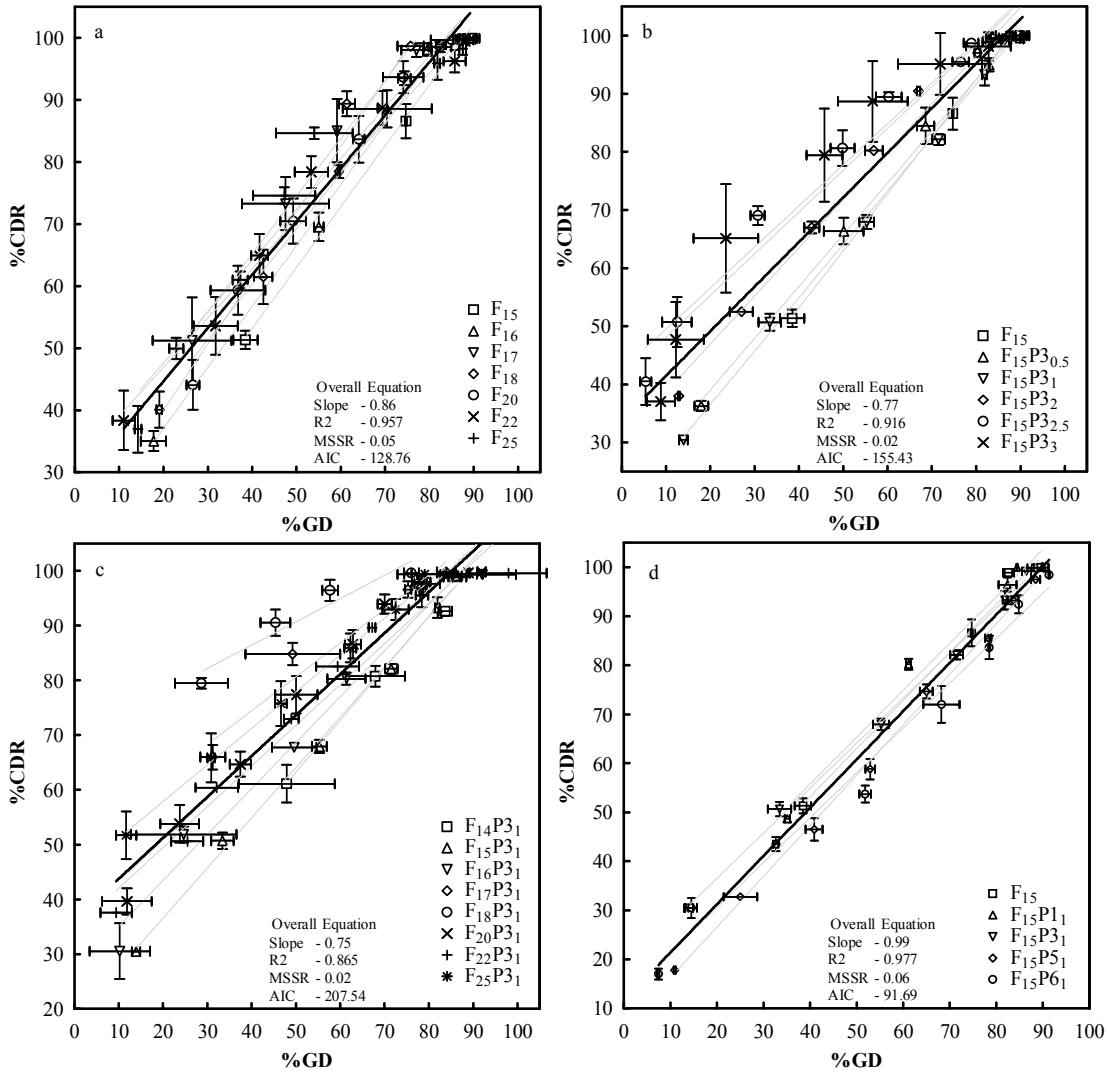


Figure 6.29: Correlation between % GD and % CDR in gatifloxacin gels (a) with 15 to 25 % w/w of PL F127 alone, (b) with 15 % w/w concentration of PL F127 in combination with increase in concentration of PEO 900 from 0 to 3 % w/w, (c) with 14 to 25 % w/w concentrations of PL F127 in combination with 1 % w/w of PEO 900 and (d) with concentration of 15 % w/w PL F127 in combination with 1 % w/w of different molecular weights of PEO.

With change in molecular weight of PEO the slope has not been affected (Figure 6.29d and Table 6.5); therefore drug release was highly dependent on gel dissolution. Linear regression analysis of overall data showed best fit with slope value of 0.99.

With increase in concentration of HEC (1 to 3%) in combination with 15 % Pl F127, the slope reduced from 0.92 to 0.68 (Table 6.5). With increase in concentration of PEO 900 (0.5 to 2%) in combination with Pl F127 gels (15 %), the slope did not changed and it was near to 0.9 (Figure 6.29b and Table 6.5). Linear regression analysis showed best fit with slope value of 0.75 and high R^2. Beyond 2% of PEO 900 (2.5 and 3 %) the slope reduced to 0.7. Similar results were observed with 2 % w/w PEO in combination with 13 and 14 % w/w PL F127 gels (Table 6.5). These results indicated that addition of hydrophilic polymers to PL F127 gels causes increase in microviscosity of the aqueous channels and subsequently diffusion of drug through these aqueous channels will also contribute to the controlled drug release.

Therefore the drug release from these gels was controlled not only by gel dissolution but also by the drug diffusion through extramicellar aqueous channels. These results are in concomitance with earlier reported mechanisms (Johnston et al., 1992; Wenzel et al., 2002).

Table 6.5: Correlation between % CDR and % GD in gatifloxacin gels.

Code	Slope † (Mean ± SD)	R^2	MSSR	AIC	Code	Slope † (Mean ± SD)	R^2	MSSR	AIC
F_{15}	0.98 ± 0.06	0.988	0.24	9.09	$F_{14}P3_1$	0.95 ± 0.08	0.986	0.34	6.27
F_{16}	0.98 ± 0.05	0.992	0.22	9.51	$F_{16}P3_1$	0.84 ± 0.06	0.977	0.07	17.63
F_{17}	0.93 ± 0.06	0.992	0.41	5.57	$F_{17}P3_1$	0.62 ± 0.10	0.952	0.12	10.56
F_{18}	1.06 ± 0.09	0.977	0.10	13.48	$F_{18}P3_1$	0.43 ± 0.09	0.920	0.21	8.21
F_{20}	0.90 ± 0.05	0.983	0.15	17.00	$F_{20}P3_1$	0.76 ± 0.05	0.967	0.07	25.87
F_{22}	0.79 ± 0.04	0.986	0.15	16.98	$F_{22}P3_1$	0.75 ± 0.05	0.965	0.06	26.78
F_{25}	0.82 ± 0.07	0.954	0.05	26.49	$F_{25}P3_1$	0.67 ± 0.01	0.997	1.41	-1.10
$F_{15}P3_{0.5}$	0.89 ± 0.03	0.997	0.58	4.74	$F_{15}HE_1$	0.92 ± 0.03	0.997	0.50	5.49
$F_{15}P3_1$	0.92 ± 0.02	0.997	0.59	5.18	$F_{15}HE_2$	0.71 ± 0.03	0.991	0.39	8.64
$F_{15}P3_2$	0.83 ± 0.05	0.982	0.12	19.08	$F_{15}HE_3$	0.68 ± 0.05	0.973	0.12	16.75
$F_{15}P3_{2.5}$	0.71 ± 3 0.05	0.967	0.07	23.59					
$F_{15}P3_3$	0.72 ± 0.07	0.936	0.03	33.19	$F_{15}P1_1$	0.96 ± 0.08	0.980	0.13	12.27
					$F_{15}P5_1$	1.03 ± 0.02	0.998	0.67	5.19
$F_{13}P3_2$	0.73 ± 0.04	0.985	0.23	12.22	$F_{15}P6_1$	0.92 ± 0.04	0.986	0.09	21.06
$F_{14}P3_2$	0.56 ± 0.06	0.951	0.09	16.20					

† Each data is average of three determinations.

(c) Modified USP XXIII dissolution apparatus

The % CDR from the gels versus time profiles are given in the Figure 6.30. The drug was released at slower rate than the membrane free in vitro drug release method. The solution of gatifloxacin has released the drug completely by 16 hr.

The release data of drug from the in situ gel forming formulations was best fitted into Peppas model and the parameters are given in Table 6.6. The best fit of the data was supported by parameters like high regression coefficient (≥ 0.993), low MSSR (ranging from 0.33 to 5.01) and low AIC (ranging from 11.83 to 28.50). For all the formulations the Peppas release rate constant was ranging from 0.37 $hr^{-0.74}$ to 0.083 $hr^{-0.72}$. The $t_{50\,\%}$ was ranging from 12.02 to 33.53 hr. The 'n' value was in the range of 0.64 to 0.74, indicating the drug release from all these formulations was by anomalous non-Fickian diffusion mechanism. With increase in the molecular weight and/or viscosity of PEO (from 200 to 7000 kDa) the release rate was reduced and the $t_{50\,\%}$ was increased from 12.45 to 28.84 hr, which can be attributed to the increase in viscosity and gel strength of the formulations. However, between the formulations with 1 % w/w of PEO 5000 and of PEO 7000, the $t_{50\,\%}$ was not significantly different. The increase in concentration of PEO 900 from 1 to 2.5 % w/w, the release rate was decreased and the $t_{50\,\%}$ was increased form 16 to 33.53 hr.

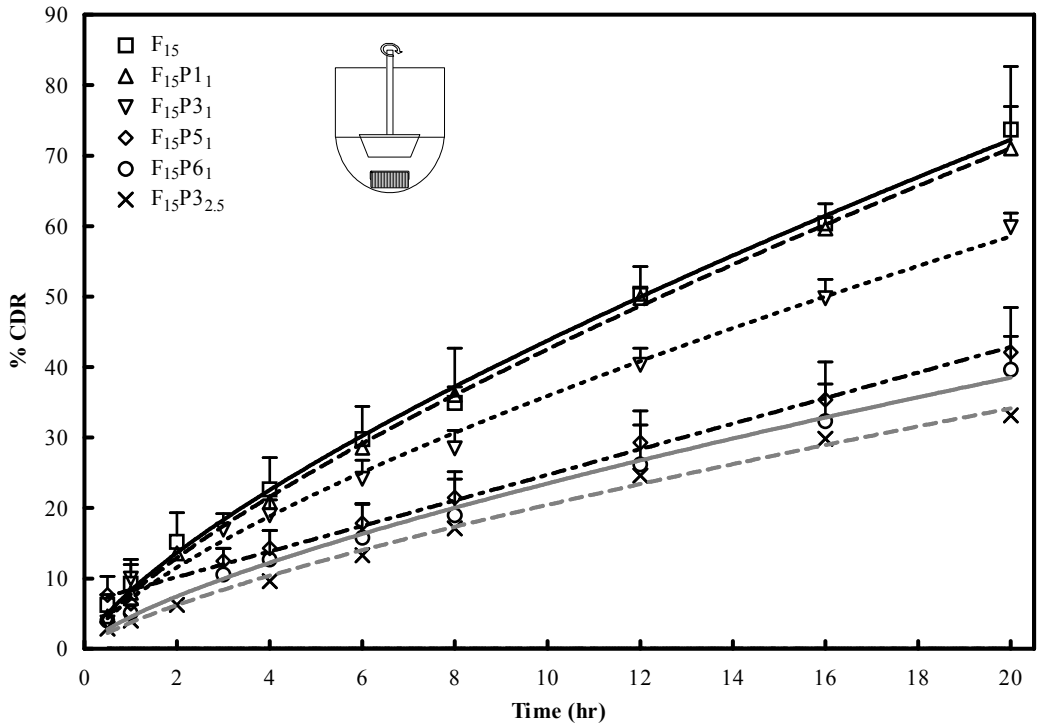


Figure 6.30: In vitro drug release form gatifloxacin containing in situ gel forming systems using modified dissolution testing. Each data point is average of three separate determinations.

Initially the drug release from the gels with high gel strength was slow, whereas for gel with low gel strength the drug release was high (Figure 6.28). For formulations F_{15} and $F_{15}P1_1$ (low gel strength) the drug release was more or less equal. Upto 3 hr the drug release from $F_{15}P3_1$ formulation was same as that of $F_{15}P1_1$ and this effect can be attributed to drug release from superficial layers of the gel. Past 3 hr the drug release was retarded form $F_{15}P3_1$

due to controlled drug diffusion through stronger gel network. The gels with higher molecular weight of PEO and high concentration of PEO 900 gel strength ($F_{15}P5_1$, $F_{15}P6_1$ and $F_{15}P3_{2.5}$) the drug release was much slower than rest of the formulations studied. The drug release was much slower in this type of dissolution as compared to membrane less dissolution model, but the comparative effect between the formations was same.

Table 6.6: In vitro drug release kinetics of in situ gel systems and drug solution in PBS in modified dissolution apparatus.

Formulation Code	Rate constant (h^{-n})	n	$t_{50\,\%}$ (h)	R^2	MSSR	AIC
F_{15}	0.083	0.72	12.02	0.997	1.56	25.76
$F_{15}P1_1$	0.077	0.74	12.45	0.999	0.33	11.83
$F_{15}P3_1$	0.071	0.71	16.00	0.993	2.11	28.50
$F_{15}P5_1$	0.061	0.64	27.40	0.985	5.01	17.23
$F_{15}P6_1$	0.045	0.71	28.84	0.995	0.63	17.65
$F_{15}P3_{2.5}$	0.037	0.74	33.53	0.995	0.54	16.20

6.3.7. Microbiological testing

The selected in situ gel formulation showed antimicrobial activity when tested microbiologically by cup plate technique. Zone of inhibitions were clearly identified and areas of zone of inhibition were determined. Area of zone of inhibition for prepared gel formulation was compared with Gatilox (marketed formulation). Mean areas (± SD) of zone inhibition were found be 272.09 (± 19.16) and 270.75 (± 10.99) mm^2 at 18 hr for marketed and in situ gel ($F_{15}P3_2$) formulations respectively. For the same formulations the mean areas (± SD) of zone inhibition were 275.13 (± 22.75) and 284.27 (± 29.42) mm^2 at 24 hr. Statistically insignificant difference was observed between the developed formulation and marketed formulation at both 18 and 24 hr. The study provided the evidence that the drug released from the formulation has not changed its anti-microbial activity.

6.3.8. Batch reproducibility

The physical properties of the in situ gels from all three batches were evaluated in the same manner. The in situ gels showed low standard deviation values for the transition temperature, drug content, viscosities and spreadability from three different batches prepared separately (Tables 6.2). These low SD values for all physical properties showed that there was excellent batch-to-batch reproducibility. No significant difference was observed in the release profiles of the formulations between different batches as indicated by the low

standard deviation values of the percent cumulative release data at different time points obtained from the replicate release studies of the samples.

6.3.9. Stability studies

Assay values of formulations before and after sterilization were given in Table 6.7. The comparison of these values of gels before and after sterilization, by *t*-test showed insignificant difference and there is no effect of sterilization on the drug content of the formulation. The difference in the drug release from the gels before and after sterilization was not significant indicating the formulations are robust and could withstand autoclave sterilization conditions. There was no change in $T_{sol\text{-}gel}$ and rheological properties with sterilization. Therefore sterilization by autoclave method has not affected the drug content, physical characters and drug release of the prepared formulations.

Table 6.7: Effect of sterilization on assay of the drug in designed in situ gel formulations containing gatifloxacin. Each data point is average of three separate determinations.

Code	Amount in $mg.ml^{-1}$ before sterilization (Mean ± SD)	Amount in $mg.ml^{-1}$ after sterilization (Mean ± SD)	Code	Amount in $mg.ml^{-1}$ before sterilization (Mean ± SD)	Amount in $mg.ml^{-1}$ after sterilization (Mean ± SD)
F_{15}	3.06 ± 0.01	3.04 ± 0.02	$F_{15}P3_3$	3.06 ± 0.00	3.05 ± 0.01
F_{20}	3.05 ± 0.01	3.04 ± 0.03	$F_{15}P5_1$	3.08 ± 0.02	3.05 ± 0.05
F_{25}	3.03 ± 0.03	3.01 ± 0.02	$F_{15}P6_1$	3.00 ± 0.01	2.97 ± 0.03
$F_{14}P3_1$	3.01 ± 0.01	2.99 ± 0.04	$F_{20}P3_1$	3.08 ± 0.01	3.07 ± 0.03
$F_{15}P1_1$	2.98 ± 0.02	2.96 ± 0.02	$F_{25}P3_1$	3.04 ± 0.01	3.01 ± 0.01
$F_{15}P3_{0.5}$	3.00 ± 0.02	2.98 ± 0.04	$F_{15}HE_1$	2.97 ± 0.01	2.97 ± 0.03
$F_{15}P3_2$	2.98 ± 0.02	2.97 ± 0.01	$F_{15}HE_3$	3.02 ± 0.01	2.98 ± 0.03

The stability of gatifloxacin present in gel formulations at different storage conditions is showed in Table 6.8. The stability data of formulations was best fitted into first-order rate kinetics, which was evident from R^2, low MSSR and low AIC values. The $t_{90\%}$ was calculated based on the K_{deg} of gatifloxacin in FT conditions.

When stored at accelerated conditions, the degradation rate constants were found to be 51.25×10^{-3} and 41.58×10^{-3} $month^{-1}$ for formulations prepared with 15 and 25 % w/w PL F127 alone respectively. In same concentrations of PL F127 in combination with 1 % w/w PEO 900, the degradation rate constants were found to be 41.77×10^{-3} and 43.35×10^{-3} $month^{-1}$ respectively. The degradation rate constants were found to be 41.68×10^{-3}, 41.77×10^{-3}, 39.46×10^{-3} and 40.66×10^{-3} $month^{-1}$ for formulations prepared with 15 % w/w PL F127 in combination with 0.5, 1, 2 and 2.5 % w/w of PEO 900 respectively. The degradation rate constants were found to be 39.91×10^{-3} and 38.60×10^{-3} $month^{-1}$ for formulations

prepared with 15 % w/w PL F127 in combination with 1 and 3 % w/w of HEC respectively when stored at accelerated conditions. The degradation rate constants were found to be 42.89 $\times 10^{-3}$, 41.77 $\times 10^{-3}$, 37.74 $\times 10^{-3}$ and 39.29 $\times 10^{-3}$ $month^{-1}$ for formulations prepared with 15 % w/w PL F127 in combination with 1 % w/w of PEO 200, 900, 5000 and 7000 respectively.

Statistically insignificant difference in degradation rates, at accelerated conditions, was observed with increase in concentration of PL F127, increase in concentration of PEO in combination with PL F127, increase in concentration of HEC in combination with PL F127 and increase in molecular weight of PEO in combination with PL F127. All the formulations were showing similar degradation rate constant, which is almost equal to the degradation rate constant of bulk drug. Formulations stored at accelerated conditions were found to be stable for 2 months.

In the formulations stored at room temperature (CRT), the degradation rate constants were found to be 23.13 $\times 10^{-3}$ and 23.66 $\times 10^{-3}$ $month^{-1}$ for formulations prepared with 15 and 25 % w/w PL F127 respectively. In similar concentrations, gels of PL F127 in combination with 1 % w/w PEO 900, the degradation rate constants were found to be 24.61 $\times 10^{-3}$ and 25.48 $\times 10^{-3}$ $month^{-1}$ respectively. The degradation rate constants were found to be 25.23 $\times 10^{-3}$, 24.61 $\times 10^{-3}$, 23.64 $\times 10^{-3}$ and 23.87 $\times 10^{-3}$ $month^{-1}$ for formulations prepared with 15 % w/w PL F127 in combination with 0.5, 1, 2 and 2.5 % w/w of PEO 900 respectively. The degradation rate constants were found to be 23.62 $\times 10^{-3}$ and 22.95 $\times 10^{-3}$ $month^{-1}$ for formulations prepared with 15 % w/w PL F127 in combination with 1 and 3 % w/w of HEC respectively. The degradation rate constants were found to be 26.15 $\times 10^{-3}$, 24.61 $\times 10^{-3}$, 23.88 $\times 10^{-3}$ and 27.76 $\times 10^{-3}$ $month^{-1}$ for formulations prepared with 15 % w/w PL F127 in combination with 1 % w/w of PEO 200, 900, 5000 and 7000 respectively. Statistically insignificant difference in degradation rates, at CRT conditions, was observed for all the formulations. All the formulations were showing similar degradation rate constant, which is almost equal to the degradation rate constant of bulk drug. Formulations stored at accelerated conditions were found to be stable for 4 months.

The $t_{90\%}$ calculated for formulations stored at accelerated conditions and room temperature were around 2 and 4 months respectively, indicating that the formulations could not withstand the accelerated and room storage conditions. The physical properties of the gels like $T_{sol\text{-}gel}$ and rheological properties were affected at accelerated conditions and room temperature storage conditions.

Table 6.8: First order degradation kinetics parameters of gatifloxacin in in situ gel formulations.

Formulation Code	Storage Condition	$K_{deg} \times 10^3$ (month^{-1}) † Mean (± SD)	R^2	MSSR	AIC	$t_{90\%}$ (month)
F_{15}	FT	1.23 ± 0.08	0.988	0.00	-141.8	86.0
	CRT	23.13 ± 4.22	0.997	0.13	-44.1	
	AT	51.25 ± 3.94	0.999	0.02	-69.2	
F_{25}	FT	1.21 ± 0.64	0.917	0.07	-60.1	87.3
	CRT	23.66 ± 5.05	0.995	0.13	-44.9	
	AT	41.58 ± 12.41	0.998	0.20	-25.1	
$F_{15}P1_1$	FT	1.26 ± 0.31	0.882	0.11	-48.7	83.9
	CRT	26.15 ± 6.03	0.993	0.18	-37.5	
	AT	42.89 ± 13.99	0.998	0.26	-20.4	
$F_{15}P3_{0.5}$	FT	1.18 ± 0.83	0.931	0.02	-96.5	88.9
	CRT	25.23 ± 3.37	0.998	0.05	-65.8	
	AT	41.68 ± 13.36	0.998	0.24	-21.7	
$F_{15}P3_1$	FT	1.27 ± 0.28	0.933	0.07	-59.6	82.7
	CRT	24.61 ± 5.64	0.994	0.24	-30.4	
	AT	41.77± 0.13	0.998	0.26	-25.4	
$F_{15}P3_2$	FT	1.31 ± 0.39	0.870	0.04	-73.2	80.5
	CRT	23.64 ± 3.62	0.998	0.07	-61.5	
	AT	39.46 ± 10.85	0.999	0.16	-29.3	
$F_{15}P3_{2.5}$	FT	1.29 ± 0.34	0.816	0.08	-55.9	81.5
	CRT	23.87 ± 6.00	0.994	0.21	-33.3	
	AT	40.66 ± 15.60	0.997	0.27	-19.7	
$F_{15}P5_1$	FT	1.19 ± 0.67	0.815	0.06	-64.8	88.9
	CRT	23.88 ± 3.58	0.998	0.08	-55.8	
	AT	37.74 ± 14.40	0.998	0.29	-18.6	
$F_{15}P6_1$	FT	1.23 ± 0.40	0.889	0.07	-59.5	86.0
	CRT	24.76 ± 4.37	0.997	0.15	-42.3	
	AT	39.29 ± 12.75	0.998	0.20	-25.0	
$F_{25}P3_1$	FT	1.19 ± 0.26	0.884	0.07	-58.9	88.9
	CRT	25.48 ± 5.53	0.995	0.15	-42.2	
	AT	43.35 ± 12.63	0.998	0.21	-24.5	
$F_{15}HE_1$	FT	1.25 ± 0.50	0.921	0.20	-35.2	84.2
	CRT	23.62 ± 7.25	0.991	0.35	-21.2	
	AT	39.99 ± 10.28	0.995	0.42	-11.5	
$F_{15}HE_3$	FT	1.22 ± 0.50	0.963	0.08	-57.2	86.4
	CRT	22.95 ± 4.46	0.996	0.14	-42.4	
	AT	38.60 ± 12.95	0.998	0.21	-24.1	

† Each data is average of three determinations.

When stored at refrigerated temperature, the degradation rate constants were found to be in the range of 1.19 to 1.26 × 10^{-3} month^{-1} for all the formulations prepared with different concentration of PL F127 alone and in combinations with PEO and HEC. The drug was found be stable for the entire period of study. High $t_{90\%}$ values were obtained for formulations

stored at FT. The calculated $t_{90\%}$ values for the formulations were found to be in the range of 80 to 90 months. At FT, all formulations in the current study shown low degradation rate constant, indicating the stability of the formulations at this temperature conditions. The values are given in the Table 6.8. All the products were stable till 18 months of the study stored at FT. The $t_{90\%}$ calculated based on the K_{deg} of FT was above 80 months, which indicates that the drug is stable under the refrigerated conditions. From the formulations stored at FT, the drug release from the gel formulations at zero time and last time point did not show significant difference and the physical properties of the gels were not changed.

6.4. Conclusion

The prepared in situ gel formulations containing PL F127 in combination with hydrophilic polymers were translucent. The physical properties like $T_{sol\text{-}gel}$, spreadability, viscosity and content uniformity were consistent, indicating the reproducibility of the method of preparation. The gels have showed reversible thermogelation at their respective $T_{sol\text{-}gel}$ and were affected by concentration of PL F127, PEO 900 and vehicle. The concentration of PL F127 plays an important role in maintaining the $T_{sol\text{-}gel}$. Minimum of 15 % w/w of PL F127 was required to show thermogelation when it was used alone. Whereas in combination with PEO 900 (2 % w/w), the concentration of Pl F127 required for gelation reduced.

With increase in PEO 900 concentration and increase in molecular weight of PEO the gels in combination with 15 % w/w PL F127, reduced $T_{sol\text{-}gel}$, increased viscosity, bioadhesive strength and gel strength. Above $T_{sol\text{-}gel}$, the gels showed Pseudoplastic flow.

In membrane-free in vitro drug release studies, the drug release data fitted into first order release kinetics. Increase in the concentration of PL F127 alone from 15 to 25 % w/w increased $t_{50\,\%}$ drastically (13.23 to 46.64 min) and decreased the release rate of the drug from gels. Formulations containing different types of PEO along with PL F127 also retarded the release. Increase in the PEO 900 concentration from 0.5 to 3 % w/w in the preparation by keeping PL F127 constant (15 % w/w) shown retardation of drug release and $t_{50\,\%}$ increased from 20 to 60 min. In gels with PL F127 concentration 12 to 18 % w/w and presence of PEO 900, increased the $t_{50\,\%}$. Incorporating HEC did not extended the drug release when compared to PEO in combination with PF127. Best formulation was showing extension of release upto nearly 240 min. The addition PEO has decreased the PL F127 concentration required to show same extension of drug release. The drug release was affected by $T_{sol\text{-}gel}$ and good correlation was established between $T_{sol\text{-}gel}$ and % CDR. Linear relationships between % CDR and % GD indicated gel erosion as the main mechanism of drug release. Sterilization by autoclaving and storage at refrigerated temperatures had no effect on rheological and drug release characters

of gels. The formulations were stable at refrigerated temperature for entire period of study. Slower drug release was observed with USP modified in vitro drug release technique.

As the antimicrobial efficacy is not affected, these in situ gel systems could serve as better alternatives to conventional ophthalmic preparations to improve the bioavailability. The selected formulations were $F_{15}P3_{2.5}$ and $F_{15}P6_{1}$. These formulations have gelation temperatures at 18°C and 24°C (near to room temperature) respectively. They showed extension of drug release upto 3.5 to 4 hr and/or good bioadhesion and gel strength were considered for further studies.

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Chapter 7

In vitro Corneal Permeability and In vivo Bioavailability Studies

7.1. Introduction

Drug delivery systems are required to be studied in vivo for their pharmacokinetic behaviour and therapeutic efficacy. The evaluation of drug delivery systems requires a study of the bioavailability in animal models and/or human volunteers. Ocular bioavailability studies provide valuable information about formulations functions at precorneal barriers and absorption of drug into ocular tissues of the healthy animal. This is particularly important as it is expected that ophthalmic inserts and in situ gelling systems would enhance the precorneal drug residence time, reduce the drug loss and change pharmacokinetic profile of drugs with better availability. Moreover this is also important for antibacterial drug delivery systems in order to monitor aqueous humor concentration profile which should be maintained above MIC. Ocular bioavailability studies can be done directly by measuring the drug concentration levels in various ocular tissues including aqueous humor.

One of the main disadvantages in assessing new ophthalmic delivery systems is that it is virtually impossible to carry out *in vivo* studies on an intact human, partly because it is difficult to sample fluids and tissues without causing severe distress and damaging the tissue itself. The most commonly used animal model for ocular studies has been the albino rabbits, because of many similar anatomical and physiological factors of the rabbit and human eye (Table 7.1). Rabbits have been used as an animal model in most ocular experiments.

Table 7.1: Comparison of pharmacokinetic factors between rabbit and human eye (Worakul and Robinson, 1997).

Pharmacokinetic factors	Rabbit	Human
Tear volume (µl)	5-10	7-30
Tear turn over rate (µl.min^{-1})	0.5-0.8	0.5-2.2
Spontaneous blinking rate	4-5 times/hr	6-15 times/min
Lachrymal punctum/puncta	1	2
Bowman's membrane partially	absent	Present
Nictitating membrane	Present	Absent
pH of lachrymal fluids	7.3-7.7	7.3-7.7
Turnover rate of lachrymal fluids (% min^{-1})	7	16
Lachrymal volume (µl)	7.5	7.0
Buffering capacity of lachrymal fluids	Poor	Poor
Milliosmolarity of tear (mOsm.l^{-1})	305	305
Initial drainage rate constant (min^{-1})	0.55	1.6
Corneal thickness (mm)	0.35-0.45	0.52-0.54
Corneal diameter (mm)	15	11-12
Corneal surface area (cm^2)	1.5-2.0	1.04
pH of aqueous humor	8.2	7.1-7.3
Aqueous humor volume (ml)	0.25-0.3	0.1-0.25
Aqueous humor turnover rate (µl.min^{-1})	3-4.7	2-3
Protein content of tear (%)	0.5	0.7
Protein content of aqueous humor (mg.ml^{-1})	0.55	30
Ratio of conjunctival surface and corneal surface	9	17

In the present study, in vitro corneal permeability of gatifloxacin was studied in the presence and absence of various penetration enhancers and formulation excipients, to check their performance. The developed formulations were screened for ophthalmic tolerance and toxicity in rabbit eye. The ocular bioavailability and pharmacokinetics of gatifloxacin from ophthalmic inserts and in situ gelling systems were studied. A comparison was made between developed novel formulations and marketed formulations. These formulations were administered into healthy rabbit eye by topical route and drug levels were determined at different time points in the aqueous humor and serum. Different pharmacokinetic and pharmacodynamic parameters were used to study the possible performance of the developed formulations and their therapeutic effectiveness.

7.2. Experimental

7.2.1. Materials

For buffer preparations, in-house prepared triple distilled water (TDW) was used. All the chemicals and solvents were of analytical grade and purchased from CDH (Mumbai, India), Qualigens (Mumbai, India) and SD-Fine Chem (Mumbai, India). Composition of buffers and reagents is given in appendix.

7.2.2. Animal/tissue

New Zealand white rabbits weighing from 1.5 to 2 kg were provided by the Animal Housing Facility of Birla Institute of Technology and Sciences, India. Animals housed in standard cages in light-controlled room at 25 ± 1°C and 50 ± 5 % RH, were given a standard pellet diet and water ad libitum. All studies were conducted in accordance with protocols, reviewed and approved by the Institutional Animal Ethics Committee (IAEC). The serial numbers of the approved protocols (date of approval 12-03-2005) were IAEC/REC/5/2 and IAEC/REC/5/3 for ocular in vivo and ocular safety studies, respectively. Animals free of any signs of ocular inflammation or gross abnormality, were used.

Corneas were obtained from goats, sacrificed in local slaughter house. Eyes were carefully enucleated and washed with ice-cold pH 7.4 PBS and warm BSS to remove any traces of blood and debris. The excised eyes were stored at refrigerated conditions in BSS till further usage. The mean time between enucleation and the experimental procedure was 2 ± 1 days (range 1 to 3 days).

7.2.3. In vitro corneal permeation studies

Cornea was carefully excised, leaving some portion of the sclera attached to it so that

mounting on the diffusion apparatus could be facilitated. Excised goat cornea was fixed between donor and receptor compartments of modified Franz diffusion cell in such a way that its epithelial surface faced the donor compartment and then clamped tightly (Chandran, 2003). The corneal area available for diffusion was 0.49 ± 0.05 cm^2. The receptor compartment (endothelial side) was filled with 8 ml of freshly prepared phosphate buffered saline, and all air bubbles were expelled from the compartment. An aliquot (1 ml) of test solution was placed on the cornea, and the opening of the donor cell was sealed with parafilm, while receptor fluid was kept at 37 ± 0.5°C using external circulating water. Constant hydrodynamic conditions were maintained inside the receptor chamber by stirring using teflon-coated magnetic bead. One ml of sample was withdrawn from the receptor at different time points like 15, 30, 60, 90, 120, 150 and 180 min. The media was replaced with equal volume of fresh preheated media to ensure sink conditions. Each experiment was performed in triplicates. The samples were suitably diluted and analyzed for gatifloxacin content by using UV-spectrophotometric or spectrofluorimetric methods given in chapter 3.

The apparent permeability coefficient (APC) in units $cm.sec^{-1}$, defined by the expression: $APC = \Delta Q/[\Delta t \times C_o \times A \times 60]$; where A, the exposed corneal surface area and C_o, the initial permeant concentration, were calculated from the steady-state slopes of linear plots of the amount of drug in receiving chamber (Q) vs time (t). The steady state flux in units $\mu g.cm^{-2}.min^{-1}$, defined by the expression: steady state flux $= \Delta Q/[\Delta t \times A]$ (Schoenwald and Huang, 1983; Saettone et al., 1996).

The percent cumulative permeation was calculated as follows: % Permeation = [Cumulative amount of drug permeated in receptor × 100]/[Initial amount of drug in donor] (Rathore and Majumdar, 2006).

The corneal hydration level was determined both on untreated cornea (removed no later then 30 min after the animal death) and the cornea removed after experiment. The corneas were carefully removed from the apparatus and surface water was removed by gently blotting with filter paper. Each cornea (free from adhering sclera) was weighed, soaked in 1 ml of methanol, dried overnight at 90°C and reweighed till constant weight. From the difference of weights corneal hydration was calculated. The percent corneal hydration level (HL %) was obtained as follows: $HL\ \% = [1-W_d/W_w]\times 100$, where W_d and W_w are the dry and wet corneal weights, respectively. The corneal hydration of untreated cornea was used as control.

Gatifloxacin (0.3 %w/v) solutions were prepared in different pH buffers to study the effect of pH on corneal permeability. The buffers used (pH 5.8, 6.0, 6.5, 6.8, 7.0, 7.2, 7.4 and 8.0) were prepared according to the standard procedures. Corneal permeability of different

concentrations of gatifloxacin (0.1, 0.2 and 0.3 %w/v) prepared in pH 7.4 PBS was studied. Drug solutions (0.3 %w/v), with different penetration enhancers were prepared to study their influence on corneal penetration. The penetration enhancers and their concentrations were chosen as per the literature reports (Furrer et al., 2000; Furrer et al., 2002a; Furrer et al., 2002b). Penetration enhancers used in the present study were 0.001 %w/v of cetylpyridium chloride (CPC), 0.5 % w/v of Tween 20 (Tw20), 0.01 %w/v phenyl mercuric acetate (PMA), 0.01 %w/v of thiomersal (THM), disodium EDTA (0.01 and 0.5 %w/v) and BKC (0.01, 0.02 and 0.03 %w/v). Drug solutions (0.3 %w/v), with different formulation additives were also prepared and studied for their effect on corneal penetration. Drug solutions (0.3 %w/v), with different formulation additives were prepared to study their influence on corneal penetration. Formulation additives used were 0.1 %w/v of HPMC K4M, 0.1 % w/v of HEC, 0.1 %w/v of Eu S100, 0.1 %w/v of PEO 200, 0.1 %w/v of PEO 900 and 0.5 %w/v of PL F127. These corneal permeability data were compared with the data obtained from 0.3 % w/v gatifloxacin solution in pH 7.4 PBS.

7.2.4. Ocular toxicity and tolerability studies

Ocular irritation studies were performed according to the Draize technique (Draize et al., 1944; Vandamme and Brobeck, 2005) using 24 rabbits divided into 8 groups. The objective of this study was to quantitatively and qualitatively assess ocular tolerance and toxicity following a single application of formulations (eye drops and in situ gel systems of 100 µl, single insert having drug of 300 µg) and observed at various time points for 7 days. The selected formulations were Gatilox marketed eye drops (F1), four insert formulations (G_2E3_{80}, $G_2H2_{40}E3_{40}$, $G_2P3_{40}E3_{40}$ and $G_2P4_{40}E3_{40}$) and two in situ gelling formulations ($F_{15}P3_{2.5}$ and $F_{15}P6_1$). During the experiments animals were placed in restraining boxes, allowed to move their heads freely and eye movements were not restricted. After application of formulation the eye lids were gently closed for about 10 sec. The observations and ocular reactions (redness, conjunctival chemosis, discharge, iris and corneal lesions) were scored at 1, 24, 48, 72, 96 hr and 7 days after application. The scoring system for measuring ocular toxicity is given Table 7.2.

At each time point, the mean scores (A, B, C, D, E and F) were determined using three rabbits scores. The lesion scores for different ocular tissues (N1, N2 and N3) at different time points were determined using mean score values (A, B, C, D, E and F). Out of different lesion score values (N1, N2 and N3) at different time points, the highest value was used to calculate the maximum ocular irritating index (OImax). All the formulae for these calculations are given in Table 7.2.

Table 7.2: The scoring system followed for measuring ocular tolerability and toxicity (Vandamme and Brobeck, 2005).

Conjunctiva	
Chemosis: eyelids and/or nictitating membrane (A)	
Absence of swelling	0
Slight swelling more than usual (including the nictitating membrane)	1
Swelling with eversion of the eyelids	2
Swelling with eyelids half closed	3
Swelling with eyelids more than half-closed or completely closed	4
Watering: (B)	
Absence of watering	0
Slight watering	1
Watering with moistening of the eyelids and the hairs immediately around the eyelids	2
Watering with moistening of the eyelids and the hairs on wide area around the eye	3
Redness: determined on the palpebral and ocular conjunctiva (C)	
Normal vessels	0
Vessels clearly more bloodshot than normal	1
Vessels difficult to distinguish individually: bright red colouring, diffuse	2
Vessels difficult to distinguish individually: dark red colouring, diffuse	3
N1 = "conjunctiva" result = (A+B+C) × 2 with a maximum = 20	
Iris (D)	
Normal of swelling	0
Obviously more contracted than usual, congestion, swelling, the iris continuing to react to light (one or more of these characteristics)	1
No reaction to light, bleeding, marked destruction (one or more of these characteristics)	2
N2 = "iris" result = D × 5 with a maximum = 10	
Cornea	
Opaqueness: only the areas with most marked injuries were taken into account	
Degree of corneal opacity (E)	
Absence of visible damage on immediate examination (loss of the bright or polished appearance)	0
Presence of one translucent area (diffuse or scattered), details of iris clearly visible	1
Presence of one translucent area easily identifiable, details of iris slightly masked	2
Presence of one opalescent area, no detail of visible iris, contour of pupil scarcely discernible	3
Presence of total corneal opacity leading to an invisible iris and pupil	4
Opacity area (F)	
A quarter (or less but not equal to zero)	1
Between a quarter and a half	2
Between a half and tree-quarters	3
Between a tree-quarters and the whole area	4
N3 = "cornea" result = E × F × 5 with a maximum = 80	
OImax = Maximum N1 + Maximum N2 + Maximum N3; with a maximum = 110	
OImax ≤ 15	Weakly irritant product
15 < OImax ≤ 30	Moderately irritant product
30 < OImax ≤ 50	Irritant product
OImax > 50	Very irritant product

7.2.5. Pharmacokinetic studies

Based on in vitro toxicity and tolerability study, the formulations selected for in vivo pharmacokinetic studies were three insert formulations ($G_2H2_{40}E3_{40}$, $G_2P3_{40}E3_{40}$ and

$G_2P4_{40}E3_{40}$) and two in situ gelling formulations ($F_{15}P3_{2.5}$ and $F_{15}P6_1$) along with Gatilox marketed eye drops (F1) for comparision. New Zealand rabbits weighing from 1.5 to 2.5 kg, free of any signs of ocular inflammation or gross abnormality, were used. Gatifloxacin levels in aqueous humor were monitored at different time points after a single application (eye drops and in situ gel systems of 100 µl, single insert having drug of 300 µg) of the various formulations into the conjunctival sac. After application of formulations in the cul-de-sac the eye lids were held in closed position for 10 sec. Depending on the formulation characters samples were withdrawn at different time points 0.5, 1, 2, 3, 4, 6, 8, 12, 18 and 24 hr, post-dose. Before paracentesis, rabbits were anaesthetised by intramuscular administration of Aneket® and Xylaxin. Xylocaine® topical solution was used as a local anaesthetic. Aqueous humor (upto 80-100 µl) was withdrawn through the limbus by a syringe with a 30G needle attached to a 1 ml syringe. The samples were stored at -20°C until further usage. Frozen aqueous humor samples were thawed at room temperature. Drug was extracted efficiently by one-step precipitation method. To 50 µl of aqueous humor sample, 150 µl of ACN was added and vortexed for 2 min and centrifuged at 10,000 rpm for 10 min at 4°C using Remi cooling compufuge (Model CPR24, Remi Instruments, India). The clear supernatant was aspirated and 20 µl was analysed by bioanalytical HPLC method as described in chapter 3. Aqueous humor blank samples were processed in similar manner. At the same time points blood samples were collected by making a single puncture into marginal ear vein using a 22G needle. Blood was kept at room temperature in order to enable clotting of cell constituents and the serum was separated from the blood by centrifugation at 4,000 rpm for 10 min at room temperature using Remi desktop centrifuge, Remi Instruments, India. The samples were stored at -20°C until analysis. Frozen serum samples were thawed at room temperature before analysis. Drug was extracted efficiently by one-step precipitation method. To 250 µl of serum sample, 750 µl of ACN was added and vortexed for 5 min and centrifuged at 10,000 rpm for 10 min at 4°C. The clear supernatant was aspirated and 20 µl was analysed by bioanalytical HPLC method as described in chapter 3. Serum blank samples were processed in similar manner.

7.2.6. Data analysis

Results of the in vivo studies were statistically evaluated by *t*-test with $P \leq 0.05$ as the level of significance. Pharmacokinetic parameters were assessed using WinNonlin (version 2.1) software. The aqueous humor concentrations and time data obtained from the study were fitted in non-compartmental model. Pharmacokinetic parameters like T_{max}, C_{max}, Area under the curve (AUC), Area under the moment curve (AUMC), Mean residence time (MRT), were

calculated using this software. Relative area under the curve (AUC_{rel}) was calculated as the ratio between AUC of test formulation to that of standard formulation.

7.3. Pharmacokinetic and pharmacodynamic relationship

Ophthalmic products with higher concentrations of antibiotics offer better assurance that the antibiotic can be present at or above the MIC for a longer time. The most popular models that indicate the correlation between pharmacokinetics and pharmacodynamics for fluoroquinolone antibacterial formulations for ophthalmic purpose are the ratio of maximum concentration produced to minimum inhibitory concentration (C_{max}:MIC_{90}), the effective area under curve (AUC above the MIC – AUC_{eff}) and the ratio of AUC of concentration-time curve to minimum inhibitory concentration (AUC_{0-t}:MIC_{90} or AUIC) (Schlech and Alfonso, 2005). MIC_{90} values of gatifloxacin for ocular pathogens isolated from clinical isolates were collected from literature reports (Mather et al., 2002; Kowalski et al., 2005) and used for calculation of above mentioned parameters.

7.4. Results and discussion

7.4.1. In vitro corneal permeability studies

(a) Effect of pH of the medium

The plots between cumulative amount of drug permeated versus time were found to be linear with R^2 in the range 0.979 - 0.991 (Figure 7.1). The permeability of drug has shown lag time of 20.8, 29.8, 30.47, 25.7, 17.3, 30.7, 33.7 and 27.3 min in pH 5.8, 6.0, 6.5, 6.8, 7.0, 7.2, 7.4 and 8.0, respectively.

The mean cumulative percentage drug permeated (± SD) through cornea after 180 min was found to be 0.62 (± 0.02), 0.98 (± 0.03), 1.26 (± 0.04), 1.26 (± 0.04), 1.41 (± 0.24), 1.54 (± 0.22), 1.37 (± 0.04) and 1.46 (± 0.08) in pH 5.8, 6.0, 6.5, 6.8, 7.0, 7.2, 7.4 and 8.0, respectively. The mean steady state flux (± SD) of the drug at 180 min in pH 5.8, 6.0, 6.5, 6.8, 7.0, 7.2, 7.4 and 8.0, was 0.23 (± 0.02), 0.29 (± 0.02), 0.38 (± 0.02), 0.38 (± 0.02), 0.42 (± 0.04), 0.46 (± 0.07), 0.48 (± 0.02) and 0.50 (± 0.04) $\mu g.cm^{-2}.min^{-1}$ respectively.

Corneal permeability of gatifloxacin was found to be pH dependent (Figure 7.2). The APC was ranging from 14.51×10^{-7} to 34.15×10^{-7} $cm.sec^{-1}$ (Table 7.3). APC values for buffers pH 5.8 and 6.0 were found to be 14.51×10^{-7} and 19.02×10^{-7} $cm.sec^{-1}$ respectively. The permeability coefficient at pH 6.5 was enhanced to 23.95×10^{-7} $cm.sec^{-1}$. The APC values for buffers 6.5, 6.8 and 7.0, were more or less same. Further, increase of pH to 7.2 the value was increased to 30.68×10^{-7} $cm.sec^{-1}$. Still increase in pH upto 8.0 did not produce any appreciable change in APC.

At lower pH, the APC obtained was low due to the higher ionization of the drug and lower partition coefficient. An increase in the pH of the solution from pH 5.8 to pH 7.2 increased the cumulative amount of drug permeated at 180 min, the steady state flux at 180 min and apparent permeability coefficient. Gatifloxacin has a pK_{a1} of 5.44 for the carboxyl group and a pK_{a2} of 9.12 for the piperazinyl group. The drug has an isoelectric point of 7.29. The octanol/water partition coefficient of gatifloxacin at pH 5.8 is 0.050, whereas at pH 7.2 the value increases to 0.154. Thus, as the pH of the solution is shifted toward neutrality (i.e., the pH of tears), a larger fraction of the drug exists in an un-ionized and/or zwitterionic state that is lipid soluble. Having higher lipid solubility at neutral pH would promote drug permeation through the cornea. These results were in agreement with the literature reports (Rathore and Majumdar, 2006).

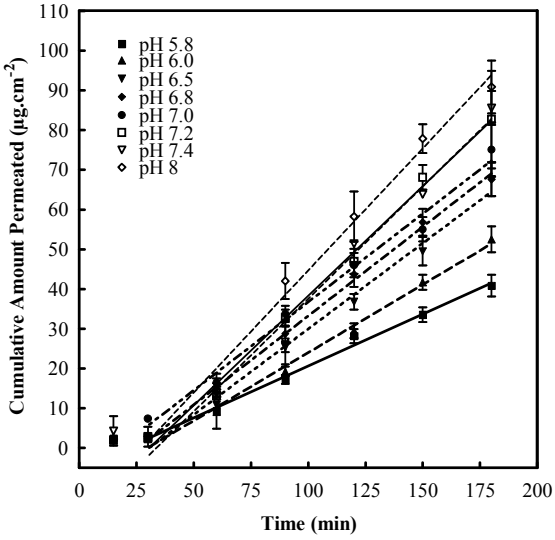

Figure 7.1: In vitro corneal permeability profiles of gatifloxacin (0.3 %w/v) in different pH media.

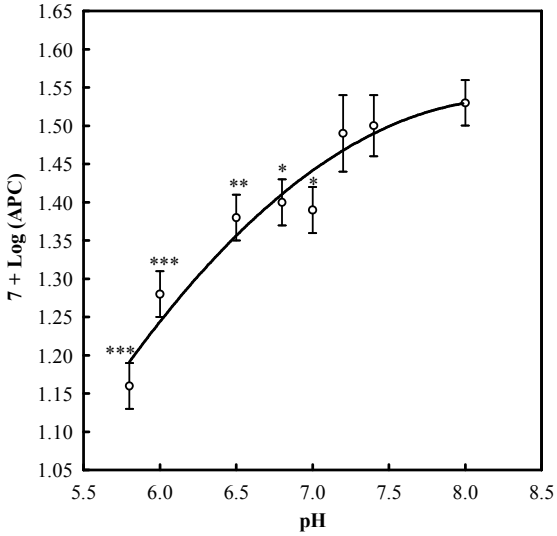

Figure 7.2: Change in APC with respect to pH of the medium. (* - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$)

Table 7.3: Apparent permeability coefficients of gatifloxacin through goat cornea in different pH media and in different concentrations in pH 7.4 buffer.

pH	APC × 10^7 (cm.sec^{-1}) (Mean ± SD) †
5.8	14.51 ± 0.89
6.0	19.02 ± 1.08
6.5	23.95 ± 1.57
6.8	24.87 ± 1.48
7.0	24.69 ± 1.56
7.2	30.68 ± 3.34
7.4	31.44 ± 3.32
8.0	34.15 ± 2.12

Concentration of drug (%w/v) in pH 7.4 Phosphate buffer	APC × 10^7 (cm.sec^{-1}) (Mean ± SD) †
0.1	13.85 ± 0.52
0.2	23.21 ± 0.83
0.3	31.44 ± 3.32

† Each value is average of three independent determinations.

The mean corneal hydration (± SD) was found to be 80.7 (± 0.4), 82.8 (± 0.6), 81.0 (± 0.6), 80.7 (± 0.3), 80.8 (± 0.3), 80.7 (± 0.7), 80.6 (± 0.7), 80.7 (± 0.6) and 81.3 (± 0.4) % for control, pH 5.8, 6.0, 6.5, 6.8, 7.0, 7.2, 7.4 and 8.0 samples, respectively (Figure 7.3). As per literature reports the normal goat corneal hydration levels were around 80% (Malhotra and Majumdar, 2001, 2002, 2005; Ahuja et al., 2006; Pawar and Majumdar, 2006; Rathore and Majumdar, 2006). The corneal hydration was significantly high in acidic pH and could lead to corneal irritation. Corneal hydration remained in the normal range when the pH of the solution was between 6.0 and 8.0, indicating minimal corneal irritation and toxicity.

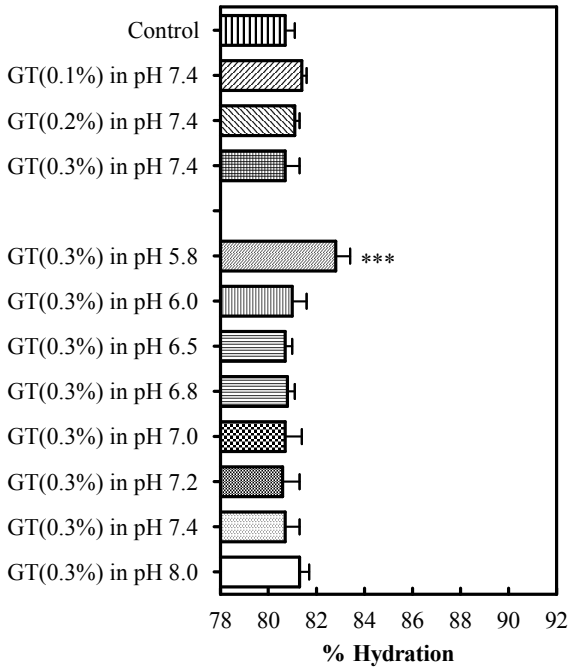

Figure 7.3: Corneal hydration level for control and gatifloxacin (GT) in various pH. (*** - $P < 0.001$)

(b) Effect of drug concentration

Plots between cumulative amount of drug permeated versus time (Figure 7.4) were linear with R^2 in the range of 0.985 - 0.997. The permeability of drug has shown lag time of 27.7, 31.2 and 33.7 min with 0.1, 0.2 and 0.3 %w/v of gatifloxacin, respectively.

The mean cumulative percentage drug permeated (± SD) calculated at 180 min was 0.610 (± 0.02), 1.00 (± 0.03) and 1.37 (± 0.04) for 0.1, 0.2 and 0.3 %w/v of drug respectively. The mean steady state flux (± SD) of the drug obtained at 180 min was 0.21 (± 0.01), 0.34 (± 0.01) and 0.48 (± 0.02) $\mu g.cm^{-2}.min^{-1}$ for 0.1, 0.2 and 0.3 %w/v of drug respectively.

Corneal permeability was found to be concentration dependent and there exists a linear relationship between Log (APC) and drug concentration with high regression

coefficient value of 0.975 (Figure 7.5). The mean APC (± SD) of the drug was calculated and found to be 13.85 (± 0.52) × 10^{-7}, 23.21 (± 0.83) × 10^{-7} and 31.44 (± 3.32) × 10^{-7} cm.sec^{-1} for 0.1, 0.2 and 0.3 %w/v of drug respectively (Table 7.3).

The results showed that an increase in drug concentration resulted in increase in permeability till the study time (180 min). An increase in drug concentration from 0.1 % to 0.3 % w/v has been found to increase the cumulative amount permeated through excised goat cornea by more than 2 times at 180 minutes. There was 2 folds increase in steady state flux and APC with increase in drug concentration from 0.2 to 0.3 % w/v. The APC of 0.3 % w/v was significantly higher to that of the APC obtained for 0.1 and 0.2 % w/v. This can be attributed to increase in gradient with increase in concentration.

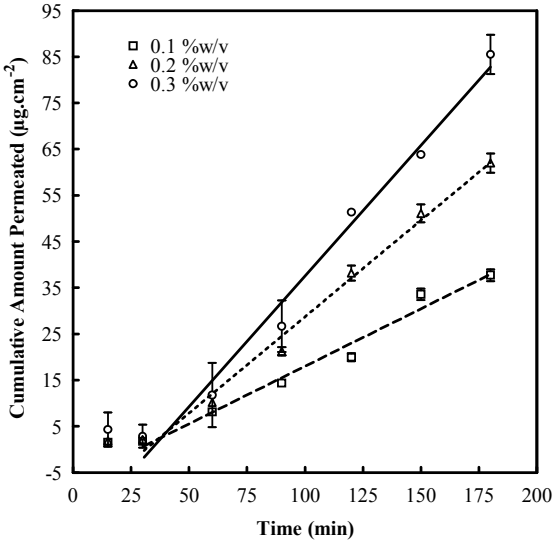

Figure 7.4: In vitro corneal profiles of gatifloxacin (in pH 7.4 phosphate buffer) in concentrations.

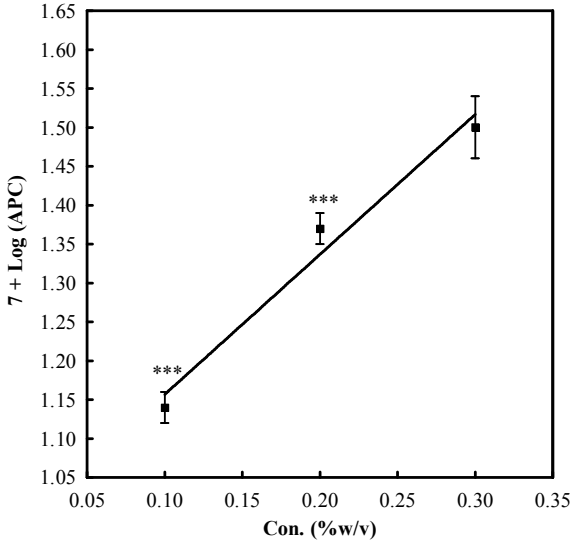

Figure 7.5: Change in APC with respect to drug concentration. (*** - $P < 0.001$)

The mean corneal hydration (± SD) was found to be 80.7 (± 0.4), 81.4 (± 0.2), 81.1 (± 0.2), and 80.7 (± 0.6) % in control, 0.1, 0.2 and 0.3 %w/v, respectively (Figure 7.3). However, an increase in drug concentration did not affect the corneal hydration and was found to lie within the normal range.

(c) Effect of penetration enhancers

Different penetration enhancers were studied for their effect on corneal permeability and corresponding cumulative amount permeated vs time plots were given in Figures 7.6 and 7.7. The profiles in combination with different penetration enhancers were found to be linear with R^2 in the range 0.982-0.994. The permeability of drug has shown lag time of 22.4, 20.2, 23.2, 24.3, 24.6, 31.0 and 32.4 min in the case of gatifloxacin (0.3 %w/v) in combination

with permeation enhancers, CPC (0.001 %w/v), PMA (0.01 %w/v), THM (0.01 %w/v), EDTA (0.01 %w/v), EDTA (0.5 %w/v), BKC (0.01 %w/v), BKC (0.02 %w/v) and BKC (0.03 %w/v), respectively.

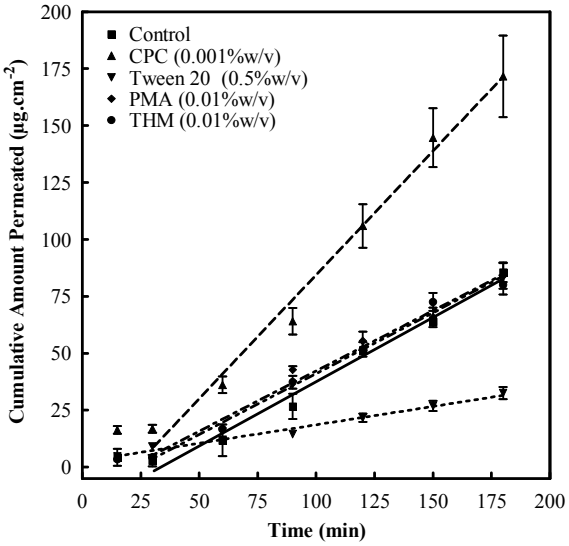

Figure 7.6: In vitro corneal profiles of gatifloxacin (in pH 7.4 phosphate buffer) with and without penetration enhancers (CPC, Tw 20, PMA and THM).

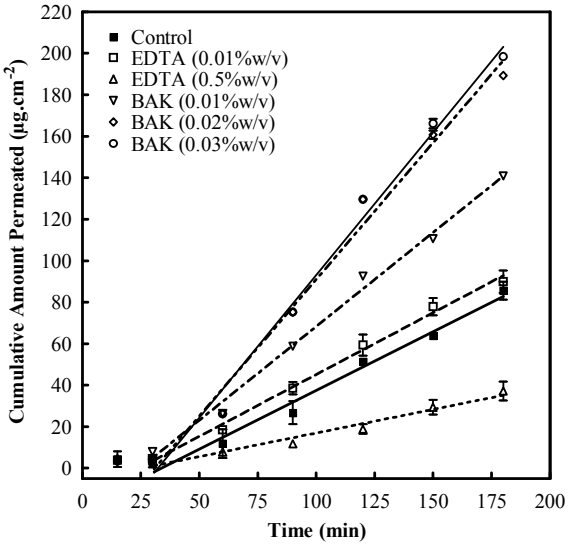

Figure 7.7: In vitro corneal profiles of gatifloxacin (in pH 7.4 phosphate buffer) with and without penetration enhancers (EDTA and BKC).

The mean cumulative percentage drug permeated (± SD) at 180 min was 2.30 (± 0.07), 0.59 (± 0.07), 1.35 (± 0.02), 1.28 (± 0.03), 1.44 (± 0.03), 0.67 (±0.01), 2.36 (± 0.03), 3.17 (± 0.01) and 3.32 (± 0.01) in the case of gatifloxacin (0.3 %w/v) in combination with permeation enhancers, CPC (0.001 %w/v), Tween 20 (0.5 %w/v), PMA (0.01 %w/v), THM (0.01 %w/v), EDTA (0.01 %w/v), EDTA (0.5 %w/v), BKC (0.01 %w/v), BKC (0.02 %w/v) and BKC (0.03 %w/v), respectively.

The mean steady state flux (± SD) of the drug at 180 min was found to be 0.95 (± 0.10), 0.18 (± 0.01), 0.47 (± 0.03), 0.44 (± 0.02), 0.50 (± 0.03), 0.21 (± 0.03), 0.78 (± 0.01), 1.05 (± 0.00) and 1.10 (± 0.00) $\mu g.cm^{-2}.min^{-1}$ for drug in combination with CPC (0.001 %w/v), Tween 20 (0.5 %w/v), PMA (0.01 %w/v), THM (0.01 %w/v), EDTA (0.01 %w/v), EDTA (0.5 %w/v), BKC (0.01 %w/v), BKC (0.02 %w/v) and BKC (0.03 %w/v), respectively.

The APC of the drug in combination with several penetration enhancers was ranging from 8.93×10^{-7} to 76.47×10^{-7} $cm.sec^{-1}$ (Figure 7.6 and 7.7, Table 7.4). Permeation enhancers like PMA, THM and EDTA (0.01 %w/v) has not produced any significant enhancement of the corneal permeability of gatifloxacin. Non-ionic surfactant, Tween 20, and EDTA, in high concentration (0.5 %w/v), has reduced the in vitro corneal penetration of gatifloxacin by 3.5 and 2.5 folds, where as a cationic surfactants, CPC (0.001 %w/v) and

BKC (0.03 %w/v), enhanced the permeability by ~2 folds. With increase in concentration of BKC from 0.01 to 0.03 %w/v, the corneal permeability was enhanced by 1.6 to 2.4 times. The results observed with EDTA and BKC were in agreement with the literature reports (Rathore and Majumdar, 2006).

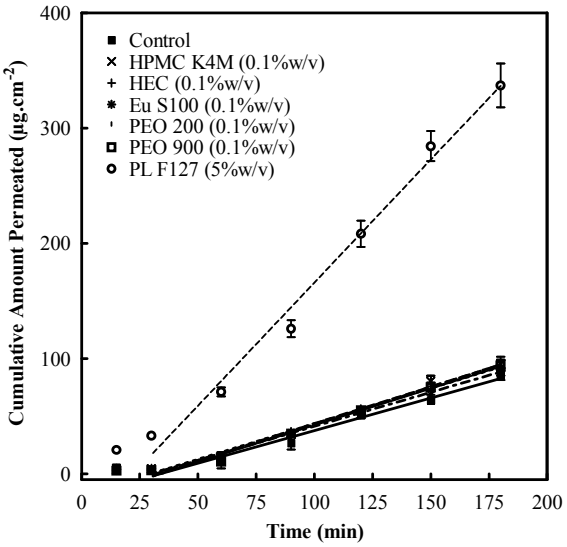

Figure 7.8: In vitro corneal profiles of gatifloxacin (in pH 7.4 phosphate buffer) with and without formulation additives.

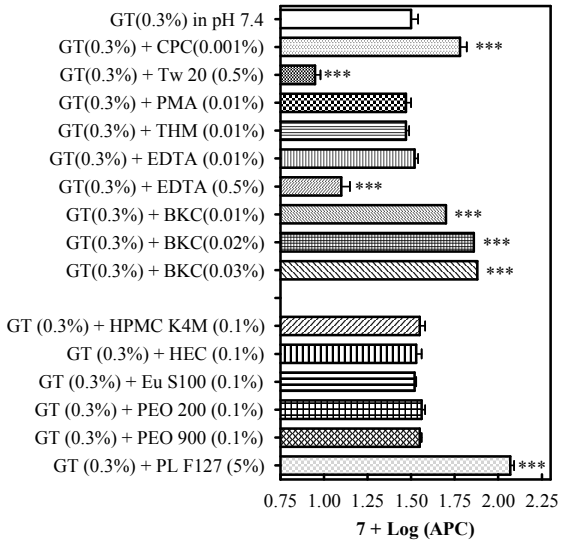

Figure 7.9: Change in APC of gatifloxacin (0.3 %w/v) with respect to different excipient combinations in pH 7.4 media. (*** - $P < 0.001$)

Table 7.4: Apparent permeability coefficients of gatifloxacin through goat cornea in combination with different excipients and in pH 7.4 phosphate buffer. Each value is average of three independent determinations.

Drug (0.3 %w/v) + Penetration enhancer (concentration)	**APC × 10^7 ($cm.sec^{-1}$) (Mean ± SD) †**
CPC (0.001 %w/v)	60.42 ± 5.77
Tw 20 (0.5 %w/v)	8.93 ± 0.72
PMA (0.01 %w/v)	29.42 ± 2.00
THM (0.01 %w/v)	29.72 ± 1.51
EDTA (0.01 %w/v)	33.11 ± 1.88
EDTA (0.5 %w/v)	12.64 ± 1.54
BKC 1 (0.01 %w/v)	50.32 ± 0.55
BKC 2 (0.02 %w/v)	73.22 ± 0.29
BKC 3 (0.03 %w/v)	76.47 ± 0.26

Drug (0.3 %w/v) + Formulation additive (concentration)	**APC × 10^7 ($cm.sec^{-1}$) (Mean ± SD) †**
HPMC K4M (0.1 %w/v)	35.47 ± 2.17
HEC (0.1 %w/v)	34.25 ± 2.47
Eu S100 (0.1 %w/v)	32.77 ± 0.43
PEO 200 (0.1 %w/v)	36.04 ± 1.46
PEO 900 (0.1 %w/v)	35.39 ± 1.05
PL F127 (5 %w/v)	118.62 ± 5.53

† Each value is average of three independent determinations.

As the penetration enhancers modify corneal membrane character, the corneal hydration levels in the case of all penetration enhancers was significantly higher than control and gatifloxacin (0.3 %w/v) solution in pH 7.4 buffer (Figure 7.10). Corneal permeability was increased by 2 fold with very low concentration of CPC. However, the corneal hydration level at 180 min was significantly higher than control, indicating possibility of corneal

toxicity of CPC. A concentration of 0.02 %w/v of BKC has enhanced the corneal permeability of gatifloxacin at the same time caused less corneal hydration.

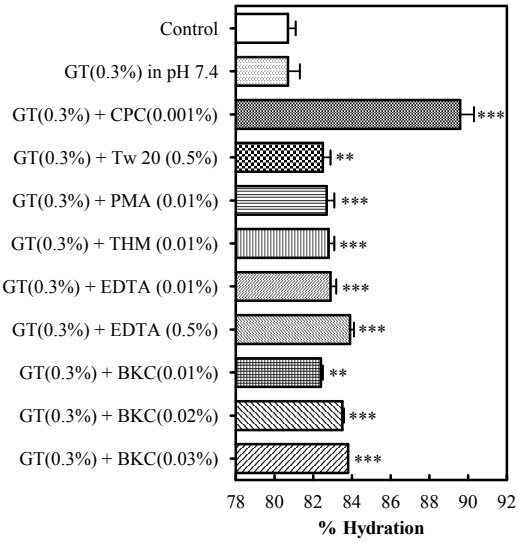

Figure 7.10: Corneal hydration level for control, gatifloxacin alone and in combination with permeation enhancers in pH 7.4 phosphate buffer. (** - $P < 0.01$; *** - $P < 0.001$)

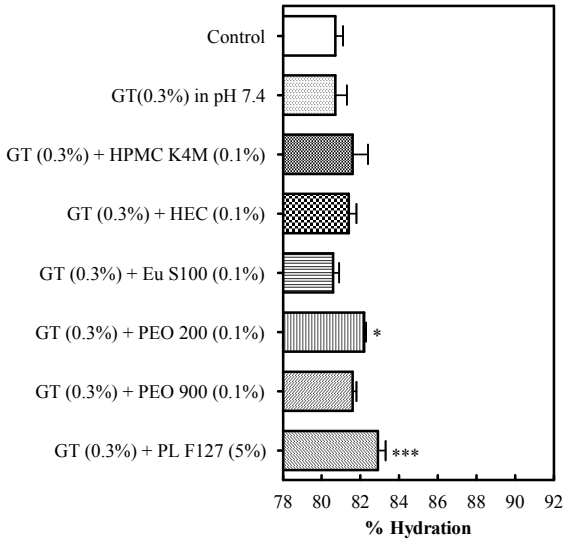

Figure 7.11: Corneal hydration level for control, gatifloxacin alone and in combination with formulation additives in pH 7.4 phosphate buffer. (* - $P < 0.05$;*** - $P < 0.001$)

(d) Effect of formulation additives

Effect of different formulation additives used in the formulation of inserts and gels on the corneal permeability was studied and the profiles were given in Figures 7.8 & 7.9 and data was presented in Table 7.4. The plots between cumulative amount of drug permeated versus time, in combination with different excipients, were found to be linear with R^2 in the range 0.985-0.991. The permeability of drug has shown lag time of 31.7, 29.5, 30.0, 33.8, 33.8 and 22.3 min in the case of gatifloxacin (0.3 %w/v) in combination with formulation additives, HPMC K4M (0.1 %w/v), HEC (0.1 %w/v), Eu S100 (0.1 %w/v), PEO 200 (0.1 %w/v), PEO 900 (0.1 %w/v) and PL F127 (5 %w/v) %w/v, respectively.

The mean cumulative percentage drug permeated (± SD) at 180 min was found to be 1.46 (± 0.02), 1.41 (± 0.01), 1.32 (± 0.01), 1.40 (± 0.05), 1.39 (± 0.04) and 5.74 (± 0.17) in the case of gatifloxacin (0.3 %w/v) in combination with formulation additives, HPMC K4M (0.1 %w/v), HEC (0.1 %w/v), Eu S100 (0.1 %w/v), PEO 200 (0.1 %w/v), PEO 900 (0.1 %w/v) and PL F127 (5 %w/v) %w/v, respectively.

The mean steady state flux (± SD) of the drug at 180 min was 0.53 (± 0.04), 0.51 (± 0.04), 0.50 (± 0.01), 0.53 (± 0.02), 0.52 (± 0.02) and 1.87 (± 0.11) $\mu g.cm^{-2}.min^{-1}$ in the case of gatifloxacin (0.3 %w/v) in combination with formulation additives, HPMC K4M (0.1 %w/v), HEC (0.1 %w/v), Eu S100 (0.1 %w/v), PEO 200 (0.1 %w/v), PEO 900 (0.1 %w/v) and PL F127 (5 %w/v) %w/v, respectively.

The mean APC (± SD) of the drug was ranging from 32.77 × 10^{-7} to 118.62 × 10^{-7} cm.sec^{-1} in the case of gatifloxacin (0.3 %w/v) in combination with formulation additives. There is no significant change in the APC values of control, HPMC K4M (0.1 %w/v), HEC (0.1 %w/v), Eu S100 (0.1 %w/v), PEO 200 (0.1 %w/v) and PEO 900 (0.1 %w/v). APC value was found to be increased by 3.7 folds when drug was added in combination with PL F127 (5 %w/v) and this can be attributed to the surface active characters of the PL F127. There was no significant difference in the corneal hydration levels in presence of formulation additives except for PEO 200 and PL F127 (Figure 7.11).

7.4.2. Ocular toxicity and tolerability studies

Study suggested that except formulation G_2E3_{80} (Inserts with Eu S100 alone), all the formulations can be considered as weakly irritant (Figure 7.12).

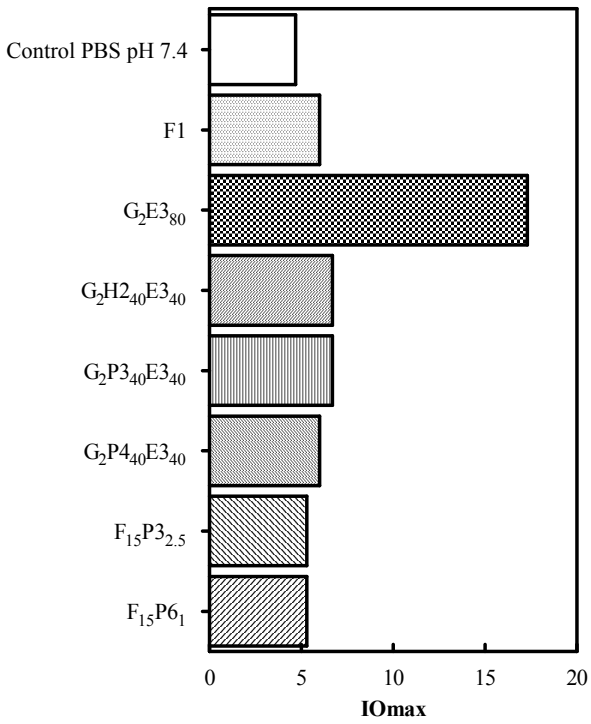

Figure 7.12: Ocular toxicity and tolerance values for different formulations.

The inserts containing only Eu S100 formulation was categorised as moderately irritating product, which can be attributed to the shredding of insoluble particles from the dosage form during the residence time in cul-de-sac. Therefore this product was not taken to next steps of study. There was no watering reflux observed for insert formulations ($G_2P3_{40}E3_{40}$, $G_2P4_{40}E3_{40}$ and $G_2H2_{40}E3_{40}$) once the formulations were hydrated when administered to the eye. However, these formulations have not caused any major change in

blinking frequency. This result was in agreement with the report of Di Colo et al. (2001a; 2001b; 2002; Di Colo and Zambito, 2002). The marketed formulations and in situ gelling formulations (F1, $F_{15}P3_{2.5}$ and $F_{15}P6_1$) did not induce watering reflex and high frequency blinking. This result was in agreement with the report of Furrer et al. (2000). Therefore, the developed formulations ($G_2P3_{40}E3_{40}$, $G_2H2_{40}E3_{40}$, $G_2P4_{40}E3_{40}$, $F_{15}P3_{2.5}$ and $F_{15}P6_1$) were well tolerated by rabbit eye and were not observed to cause any short-term toxicity.

7.4.3. Pharmacokinetics studies

The level of gatifloxacin concentrations in aqueous humor upon instillation of 100 µl of 0.3 %w/v marketed eye drops (F1) and inserts containing 300 µg of gatifloxacin ($G_2P3_{40}E3_{40}$, $G_2P4_{40}E3_{40}$ and $G_2H2_{40}E3_{40}$) are presented in Figure 7.13 and Table 7.5.

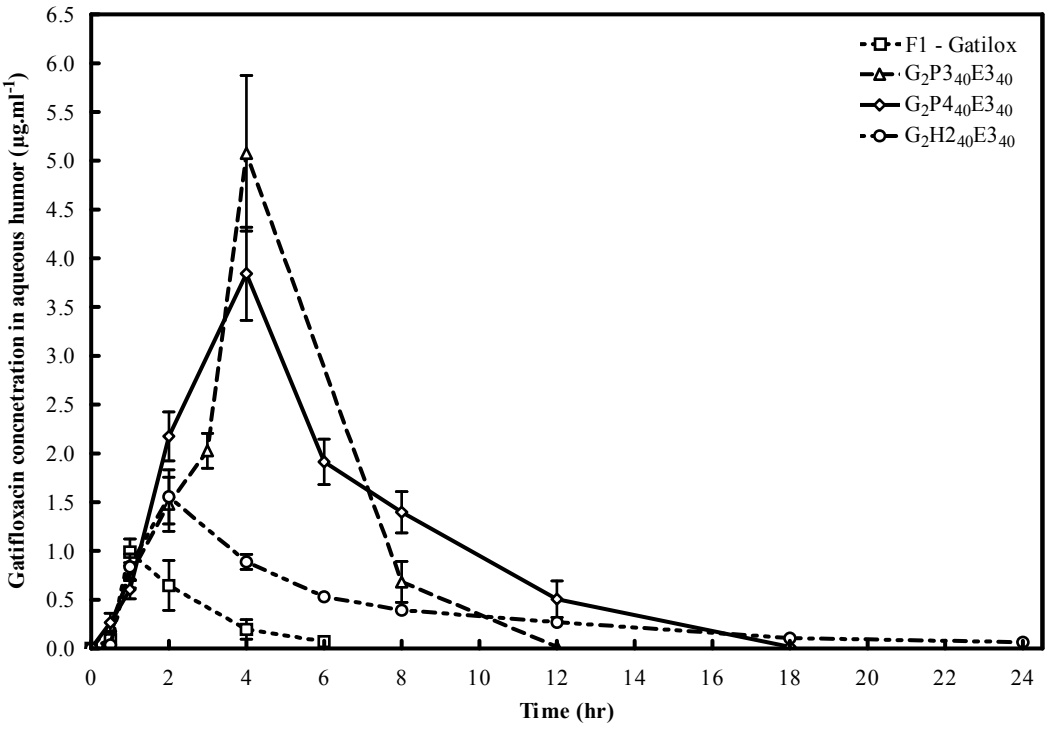

Figure 7.13: In vivo profiles of different gatifloxacin insert formulations.

Maximum concentration (C_{max}) of 0.99 µg.ml^{-1} was achieved in 1 hr (T_{max}) for F1 marketed preparation. Elimination rate constant (calculated from terminal portion) obtained for F1 was 0.53 hr^{-1} indicating faster disappearance of drug from the aqueous humor. MRT of F1 formulation in aqueous humor was 2.45 hr and $AUC_{0-\infty}$ was 2.36 µg.hr.ml^{-1} (Table 7.5).

The pharmacokinetic parameters of the studied formulations were compared with that of marketed preparation (F1). The insert formulation with PEO 900 in combination with Eu S100 ($G_2P3_{40}E3_{40}$), the C_{max} was increased by 5 folds and the T_{max} to 4 hr. The MRT of the

drug was enhanced by 1.7 times, which can be attributed to the sustained drug release from the dosage form. AUC_{rel} was found to be 8.3, indicating enhancement of ocular bioavailability. The insert formulation with PEO 2000 in combination with Eu S100 ($G_2P4_{40}E3_{40}$), the C_{max} was increased by 3.9 folds and the T_{max} to 4 hr. The MRT of the drug was enhanced by 2.3 times, which can be attributed to the slower and sustained drug release and better availability from the dosage form. AUC_{rel} was found to be 9.4, indicating enhancement of bioavailability. The physical observation of the formulations containing PEO after topical administration showed that the dosage forms were swollen completely and translucent gel layer was formed adhering to the conjunctival sac of the eye.

Table 7.5: Pharmacokinetic parameters obtained from aqueous humor concentration vs. time profiles upon single topical administration of different formulations of gatifloxacin.

Pharmacokinetic Parameters	**F1 – Gatilox**	**$G_2P3_{40}E3_{40}$**	**$G_2P4_{40}E3_{40}$**	**$G_2H2_{40}E3_{40}$**
T_{max} (hr) †	1.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	2.00 ± 0.00
C_{max} ($\mu g.ml^{-1}$) †	0.99 ± 0.14	5.08 ± 0.80	3.84 ± 0.48	1.56 ± 0.28
Elimination Rate Constant (hr^{-1}) † (calculated from terminal portion)	0.53 ± 0.08	0.71 ± 0.08	0.38 ± 0.02	0.14 ± 0.01
$AUC_{0-\infty}$ ($\mu g.hr.ml^{-1}$) †	2.36 ± 0.42	19.67 ± 1.73	22.19 ± 1.24	9.65 ± 0.53
$AUMC_{0-\infty}$ ($\mu g.hr^2.ml^{-1}$) †	5.77 ± 0.48	83.01 ± 0.91	126.59 ± 7.47	75.23 ± 12.31
MRT (hr) †	2.45 ± 0.32	4.22 ± 0.04	5.70 ± 0.79	7.80 ± 0.75
AUC_{rel}	1.0	8.3	9.4	4.1

† Each value is average of three independent determinations.

The insert formulation with HPMC in combination with Eu S100 ($G_2H2_{40}E3_{40}$), the C_{max} was increased by 1.6 folds and the T_{max} to 2 hr. The C_{max} was for this formulation was low when compared to PEO 900 and 2000. This can be attributed to the slower drug release from the formulation. However, bioavailability, indicated by $AUC_{0-\infty}$, of these formulations was enhanced by 1.7 folds and the mean residence time (MRT) of drug by 3.2 times, which will produce longer duration of action. The drug levels in aqueous humor were detectable till 24 hr post topical administration. AUC_{rel} was found to be 4.1, indicating enhancement of ocular bioavailability.

These results show that the insert formulations improved the bioavailability of gatifloxacin by reducing the drug loss and controlling the drug release in to the precorneal area.

Similarly, Figure 7.14 and Table 7.6 show the levels of gatifloxacin concentration in aqueous humor after instillation of 100 µl of 0.3 %w/v marketed eye drops (F1) and 100 µl of 0.3 %w/v in situ gelling systems ($F_{15}P3_{2.5}$ and $F_{15}P6_1$).

The in situ gelling formulation with PEO 900 in combination with PL F127 ($F_{15}P3_{2.5}$),

the C_{max} was increased by 5.5 folds and the T_{max} to 4 hr. The MRT of the drug was enhanced by 1.4 times. AUC_{rel} was found to be 11.8. The in situ gelling formulation with PEO 7000 in combination with PL F127 ($F_{15}P6_1$), the C_{max} was increased by 5.8 folds and the T_{max} to 2 hr. The MRT of the drug was enhanced by 1.6 times. AUC_{rel} was found to be 11.8. Low elimination rate constant indicates the slow drug disposition from aqueous humor.

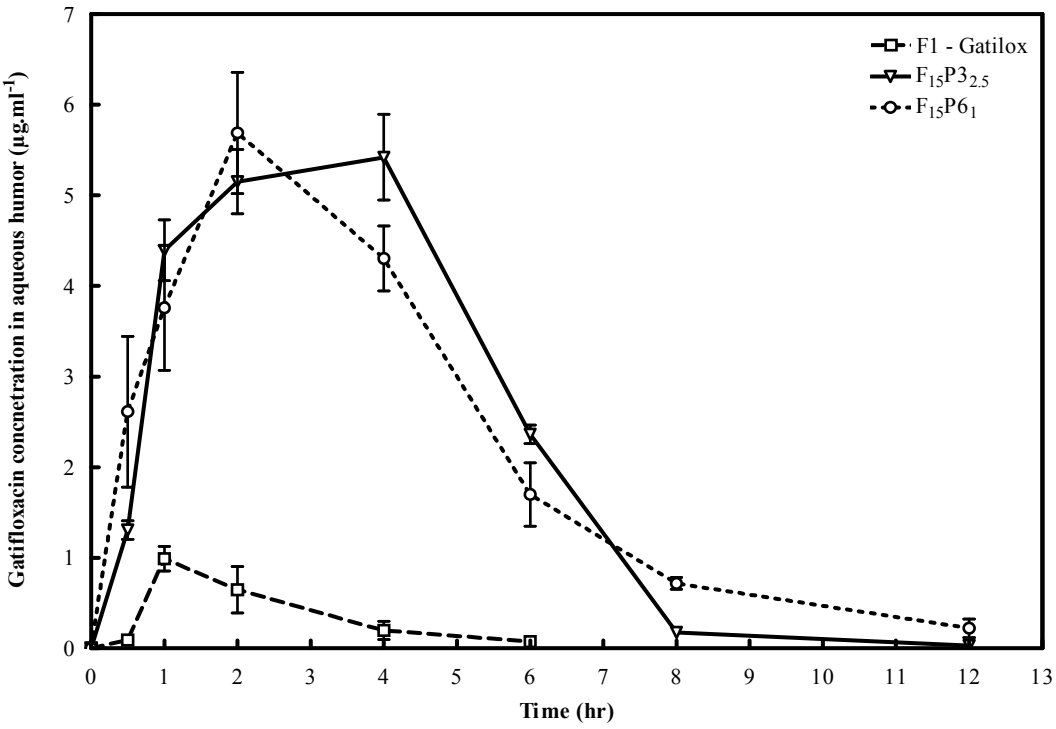

Figure 7.14: In vivo profiles of different gatifloxacin in situ gel formulations.

Table 7.6: Pharmacokinetic parameters obtained from aqueous humor concentration vs. time profiles upon single topical administration of different formulations of gatifloxacin. Each value is average of three independent determinations.

Pharmacokinetic Parameters	F1 – Gatilox	$F_{15}P3_{2.5}$	$F_{15}P6_1$
T_{max} (hr) †	1.00 ± 0.00	4.00 ± 0.00	2.00 ± 0.00
C_{max} (µg.ml^{-1}) †	0.99 ± 0.14	5.42 ± 0.47	5.69 ± 0.67
Elimination Rate Constant (hr^{-1}) † (calculated from terminal portion)	0.53 ± 0.08	0.67 ± 0.04	0.34 ± 0.02
$AUC_{0-\infty}$ (µg.hr.ml^{-1}) †	2.36 ± 0.42	27.87 ± 3.83	27.89 ± 2.50
$AUMC_{0-\infty}$ (µg.hr^2.ml^{-1}) †	5.77 ± 0.48	96.36 ± 8.40	107.32 ± 6.43
MRT (hr) †	2.45 ± 0.32	3.46 ± 0.30	3.85 ± 0.09
AUC_{rel}	1.0	11.8	11.8

† Each value is average of three independent determinations.

These results indicate that the in situ gelling formulations improved the bioavailability of gatifloxacin by increasing the corneal permeability, reducing the drug loss in precorneal area and controlling the drug release in to the precorneal area.

Designed insert and in situ gel formulations ($G_2P3_{40}E3_{40}$, $G_2H2_{40}E3_{40}$, $G_2P4_{40}E3_{40}$, $F_{15}P3_{2.5}$ and $F_{15}P6_1$) showed higher aqueous humor concentration levels of gatifloxacin than those reported (Batoosingh et al., 2003; Levine et al., 2004) in literature. Designed in situ gel formulations ($F_{15}P3_{2.5}$ and $F_{15}P6_1$) showed aqueous humor concentration levels comparable to those reported by Kleinmann et al. (2006).

Among all the formulations studied, only marketed preparation (F1), and in situ gelling systems, $F_{15}P3_{2.5}$ and $F_{15}P6_1$, have shown the serum drug concentrations at 0.5 and 1 hr. All other insert formulations ($G_2P3_{40}E3_{40}$, $G_2H2_{40}E3_{40}$, $G_2P4_{40}E3_{40}$) have not shown any gatifloxacin concentrations in detectable range. The serum drug levels observed at 0.5 hr, for both in situ gelling formulations ($F_{15}P3_{2.5}$ and $F_{15}P6_1$), were significantly lower than that of marketed preparation. The serum drug concentration for the formulation $F_{15}P3_{2.5}$, at 1 hr, was same as that of marketed preparation. However, for the formulation $F_{15}P6_1$, the serum drug concentration at 1 hr, was well below the limit of detection. The gatifloxacin applied topically has not reached the systemic circulation.

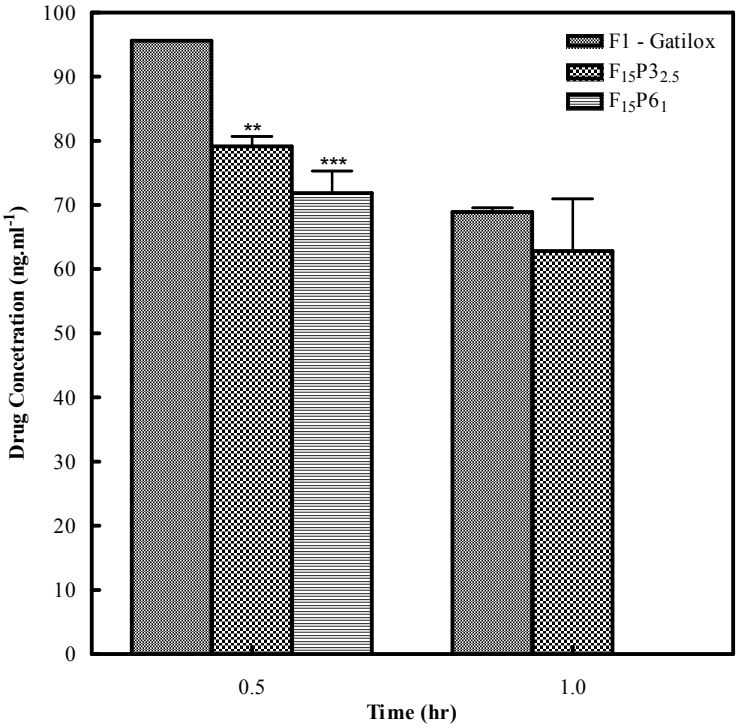

Figure 7.15: Serum gatifloxacin concentrations of gatifloxacin marketed preparation and in situ gelling systems after topical application. (** - $P < 0.01$; *** - $P < 0.001$)

7.5. Pharmacokinetic and pharmacodynamic relationship

The pharmacokinetic and pharmacodynamic properties of each antibiotic determine the in vivo relationship between the drug and the pathogen necessary to achieve optimal

antibiotic efficacy. For concentration-dependent agents, such as fluoroquinolones, higher drug concentrations (i.e., above the MIC) may result in more rapid and extensive bacterial eradication, considering that the pathogen is sensitive to a given antibiotic. The ratio of C_{max}:MIC_{90} ≥ 10 will ensure rapid bacterial killing and prevent regrowth of resistant gram-negative bacterial populations. An AUIC value of 75 or above for fluoroquinolones, indicates that these agents would be effective in eradicating specific organisms. The AUC_{eff} will represent the effect of both the concentration dependent and time dependent antibiotics (Schlech and Alfonso, 2005). For an antibiotic to achieve optimal efficacy, it must reach the pathogen infected tissue and remain there for sufficient time at a concentration or above required for bactericidal action. These parameters determine how well the antibiotic penetrates ocular tissues like cornea, conjunctiva or aqueous humor.

The calculated pharmacokinetic and pharmacodynamic parameters for the ophthalmic inserts and ophthalmic in situ gelling systems were given in the Table 7.7 and 7.8. C_{max}:MIC_{90} value for marketed preparation was less than 10 for most of the organisms listed, except few. AUIC values were also less than 57 except for Gram negative organisms. This indicates that the marketed preparation is not effective against the organisms causing infection.

Insert formulations, PEO 900 combined with Eu S100 ($G_2P3_{40}E3_{40}$) and PEO 2000 combined with Eu S100 ($G_2P4_{40}E3_{40}$), have shown C_{max}:MIC_{90} > 10, AUIC > 75 and several folds increase in AUC_{eff} for most of the organisms. The results indicate that these formulations are effective against most of the infection causing organisms except few resistant strains like *Staphylococcus aureus*, CoagNeg *Staphylococcus* and *Enterococci* species.

C_{max}:MIC_{90} and AUIC values for the insert formulations, HPMC combined with Eu S100 ($G_2H2_{40}E3_{40}$), were less than 10 and 75 respectively. There was increase in AUC_{eff} but the increment was less when compared with other insert formulations. The results indicate that this formulation is effective against infection causing organisms like susceptible *Staphylococcus aureus*, susceptible CoagNeg *Staphylococcus*, *Bacillus* species and few Gram negatives only. Even though the $G_2H2_{40}E3_{40}$ formulation has shown much controlled release, it failed to meet the required values for parameters like C_{max}:MIC_{90} and AUIC, which indicates the in-effectiveness against ophthalmic infections.

Table 7.7: Pharmacokinetic and pharmacodynamic parameters of marketed and ophthalmic solid insert formulations containing gatifloxacin against clinical isolates.

Ophthalmic Clinical Isolates	MIC_{90} ($\mu g.ml^{-1}$)	F1 - Gatilox			$G_2P3_{40}E3_{40}$			$G_2P4_{40}E3_{40}$			$G_2H2_{40}E3_{40}$		
		C_{max}: MIC_{90}	AUIC	AUC_{eff}	C_{max}: MIC_{90}	AUIC	AUC_{eff}	C_{max}: MIC_{90}	AUIC	AUC_{eff}	C_{max}: MIC_{90}	AUIC	AUC_{eff}
Bacterial Endophthalmitis Isolates †													
Resistant *Staphylococcus aureus* ‡	3.5	0.3	1.3	0.0	1.5	5.6	1.5	1.1	6.3	0.1	0.4	2.6	0.0
Susceptible *Staphylococcus aureus* ¨	0.11	9.0	42.8	1.6	46.1	178.6	18.4	34.9	201.3	20.2	14.1	83.5	6.7
CoagNeg *Staphylococcus* FQR ‡	2	0.5	2.4	0.0	2.5	9.8	5.9	1.9	11.1	3.8	0.8	4.6	0.0
CoagNeg *Staphylococcus* FQS ¨	0.09	11.0	52.3	1.7	56.4	218.3	18.6	42.7	246.1	20.6	17.3	102.0	7.1
Streptococcus pneumoniae	0.22	4.5	21.4	1.2	23.1	89.3	17.2	17.5	100.7	18.5	7.1	41.7	5.1
Streptococcus viridans	0.25	4.0	18.8	1.1	20.3	78.6	16.9	15.4	88.6	18.0	6.2	36.7	4.7
Beta-hem Streptococcus	0.25	4.0	18.8	1.1	20.3	78.6	16.9	15.4	88.6	18.0	6.2	36.7	4.7
Enterococci species	0.38	2.6	12.4	0.7	13.4	51.7	15.6	10.1	58.3	16.2	4.1	24.2	3.4
Bacillus species	0.09	11.0	52.3	1.7	56.4	218.3	18.6	42.7	246.1	20.6	17.3	102.0	7.1
Gram negatives	0.06	16.5	78.5	1.9	84.6	327.4	18.9	64.1	369.1	21.1	25.9	153.0	7.8
Bacterial Conjunctivitis Isolates Π													
Resistant *Staphylococcus aureus* ‡	64	0.0	0.1	0.0	0.1	0.3	0.0	0.1	0.3	0.0	0.0	0.1	0.0
Susceptible *Staphylococcus aureus* ¨	0.125	7.9	37.7	1.5	40.6	157.2	18.2	30.7	177.2	20.0	12.4	73.4	6.5
Streptococcus pneumoniae	0.19	5.2	24.8	1.3	26.7	103.4	17.5	20.2	116.6	18.9	8.2	48.3	5.5
Haemophilus species	0.25	4.0	18.8	1.1	20.3	78.6	16.9	15.4	88.6	18.0	6.2	36.7	4.7

AUIC = AUC_{0-t} / MIC_{90}; † - (Mather et al., 2002); ‡ - Resistance to ciprofloxacin and ofloxacin as determined by disk diffusion; ¨ - Susceptible to ciprofloxacin and ofloxacin as determined by disk diffusion; Π - (Kowalski et al., 2005)

Table 7.8: Pharmacokinetic and pharmacodynamic parameters of marketed and ophthalmic in situ gel forming formulations containing gatifloxacin against clinical isolates.

Ophthalmic Clinical Isolates	MIC_{90} ($\mu g.ml^{-1}$)	F1 - Gatilox			$F_{15}P3_{2.5}$			$F_{15}P6_1$		
		C_{max}: MIC_{90}	AUIC	AUC_{eff}	C_{max}: MIC_{90}	AUIC	AUC_{eff}	C_{max}: MIC_{90}	AUIC	AUC_{eff}
Bacterial Endophthalmitis Isolates †										
Resistant *Staphylococcus aureus* ‡	3.5	0.3	1.3	0.0	1.5	8.0	6.1	1.6	7.8	4.5
Susceptible *Staphylococcus aureus* ¨	0.11	9.0	42.8	1.6	49.3	253.0	26.6	51.7	247.7	25.9
CoagNeg *Staphylococcus* FQR ‡	2	0.5	2.4	0.0	2.7	13.9	13.7	2.8	13.6	11.4
CoagNeg *Staphylococcus* FQS ¨	0.09	11.0	52.3	1.7	60.2	309.2	26.8	63.2	302.7	26.2
Streptococcus pneumoniae	0.22	4.5	21.4	1.2	24.6	126.5	25.7	25.9	123.8	24.6
Streptococcus viridans	0.25	4.0	18.8	1.1	21.7	111.3	25.4	22.8	109.0	24.3
Beta-hem Streptococcus	0.25	4.0	18.8	1.1	21.7	111.3	25.4	22.8	109.0	24.3
Enterococci species	0.38	2.6	12.4	0.7	14.3	73.2	24.4	15.0	71.7	22.8
Bacillus species	0.09	11.0	52.3	1.7	60.2	309.2	26.8	63.2	302.7	26.2
Gram negatives	0.06	16.5	78.5	1.9	90.4	463.8	27.1	94.8	454.1	26.5
Bacterial Conjunctivitis Isolates Π										
Resistant *Staphylococcus aureus* ‡	64	0.0	0.1	0.0	0.1	0.4	0.0	0.1	0.4	0.0
Susceptible *Staphylococcus aureus* ¨	0.125	7.9	37.7	1.5	43.4	222.6	26.5	45.5	218.0	25.8
Streptococcus pneumoniae	0.19	5.2	24.8	1.3	28.5	146.5	25.9	29.9	143.4	25.0
Haemophilus species	0.25	4.0	18.8	1.1	21.7	111.3	25.4	22.8	109.0	24.3

AUIC = AUC_{0-t} / MIC_{90}; † - (Mather et al., 2002); ‡ - Resistance to ciprofloxacin and ofloxacin as determined by disk diffusion; ¨ - Susceptible to ciprofloxacin and ofloxacin as determined by disk diffusion; Π - (Kowalski et al., 2005)

In situ gelling formulations, PEO 900 combined with PL F127 ($F_{15}P3_{2.5}$) and PEO 7000 combined with PL F127 ($F_{15}P6_1$), have shown C_{max}:MIC_{90} > 10, AUIC > 75 and several folds increase in AUC_{eff} against most of the organisms. This shows that these formulations are effective against most of the infection causing organisms except resistant *Staphylococcus aureus* and resistant CoagNeg *Staphylococcus*.

7.6. Conclusion

Corneal permeability of gatifloxacin was found to be pH dependent and was found to be maximum at pH 7.2, the pH of the tear fluid. Gatifloxacin showed concentration dependent permeability, permeability increased with increase in drug concentration. Penetration enhancers, BKC and CPC enhanced the corneal permeability by 2.4 and 1.9 folds respectively. But, CPC showed indications for possible corneal toxicity. The studied formulation additives have not affected the corneal permeability. The corneal penetration was enhanced by 3.8 times with PL F127.

According to the ocular tolerance and toxicity study, except insert formulation having only Eu S100 (G_2E3_{80}), all the other formulations were found to be weakly irritant products. The developed formulations ($G_2H2_{40}E3_{40}$, $G_2P3_{40}E3_{40}$, $G_2P4_{40}E3_{40}$, $F_{15}P3_{2.5}$ and $F_{15}P6_1$) were well tolerated in rabbit eye and they have not showed any toxicity.

In vivo studies showed that the MRT and AUC_{rel} for insert and in situ gelling formulations were higher when compared with marketed product. This indicates the enhanced bioavailability of the prepared formulations by reducing drug loss, better absorption and controlled drug release in pre-corneal area. The enhancement of bioavailability was higher in the case of in situ gel formulations as compared to insert formulations.

Correlation of the pharmacokinetic and pharmacodynamic data showed that the insert and in situ gelling formulations are effective against most of the microorganisms. All the above studies suggested that the prepared formulations are promising with improved therapeutic effectiveness against ophthalmic infections when compared to that of marketed preparation.

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Chapter 8

Conclusions

8.0. Conclusions

In the recent years there is a significant effort directed towards development of better drug delivery systems to overcome the disadvantages of eye drops, to control drug delivery and to improve ophthalmic therapy. Thus, it was planned to design better ocular delivery systems for gatifloxacin, a fourth generation board spectrum fluoroquinolone antibacterial agent.

The in-house developed spectrophotometric, spectrofluorimetric and ion-pair RP-HPLC methods were sensitive and facilitated the accurate and precise estimation of gatifloxacin in bulk and formulations including drug release and corneal permeation studies. The developed HPLC method for in vivo sample analysis was selective, sensitive, accurate, precise and can be used to analyse gatifloxacin in in vivo samples. There was no interference of excipients/biomatrix in all these methods. The developed methods found to have advantages over reported methods.

Solubility, apparent partition coefficient and stability of gatifloxacin were pH dependent. Highest partition coefficient was observed in neutral pH. Drug has shown two pK_a values, 5.44 and 9.12. The DSC and FT-IR studies confirmed the compatibility of drug with all the excipients studied. Gatifloxacin, alone and in combination with different excipients, was stable for more than 5 months at accelerated conditions and for 24 months at controlled room temperature conditions.

The designed ophthalmic inserts of gatifloxacin showed good physical properties indicating suitability and reproducibility of non-aqueous granulation technique. The duration of gatifloxacin release was extended from 1 to 24 hr by varying the polymer type and combination. Drug release was found to depend on both polymeric network and microenvironment pH of the polymeric matrix. The drug release was extended upto 18 hr for formulations prepared with HPMC K15M and Eu S100 in 1:20:20 proportion ($G_2H2_{40}E3_{40}$). Highest bioadhesion strength was obtained for formulations prepared with PEO 900 and Eu S100 in 1:20:20 proportion ($G_2P3_{40}E3_{40}$). Formulations showing extension of drug release upto 9 to 24 hr and good bioadhesion strength were considered for further studies.

The method of in situ gel preparation was reproducible. The gels showed reversible thermogelation. Viscosity, bioadhesive strength and gel strength increased with increase in concentration of PEO 900 and molecular weight of PEO in combination with 15 % w/w PL F127. Bioadhesion was found to be highest in gels containing 15 % w/w PL F127 and 1 % w/w PEO 7000. The drug release was extended upto 4 hr in formulation with 15 % w/w PL F127 and 1 % w/w PEO 900. Sterilization by autoclaving and storage at refrigerated condition had no effect on rheological and drug release character of gels. Formulations

showing extension of drug release upto 4 hr and good bioadhesion strength were considered for further in vivo studies. The anti microbial activity of the drug was not affected in the prepared formulations.

Corneal permeability of gatifloxacin was found to be pH dependent and was found to be maximum at pH 7.2, the pH of the tear fluid. PL F127 enhanced corneal permeability by 3.8 times. The developed formulations ($G_2H2_{40}E3_{40}$, $G_2P3_{40}E3_{40}$, $G_2P4_{40}E3_{40}$, $F_{15}P3_{2.5}$ and $F_{15}P6_1$) were well tolerated in rabbit eye with no apparent toxicity.

The insert formulations with PEO 900 and PEO 2000 in combination with Eu S100, the C_{max} was increased by 5 and 3.9 folds respectively, when compared to marketed eye drop preparation. AUC_{rel} data were found to be 8.3 and 9.4 respectively, indicating enhancement of ocular bioavailability. The insert formulation with HPMC in combination with Eu S100, the C_{max} was increased by 1.6 folds. The C_{max} was for this formulation was low when compared to PEO 900 and 2000 due to slower drug release from the formulation. For this formulation MRT was enhanced by 3.2 times and AUC_{rel} was found to be 4.1.

The in situ gelling formulation with PEO 900 (2.5 %) and PEO 7000 (1 %) in combination with PL F127 (15 %), the C_{max} was increased by 5.5 and 5.8 folds respectively. For both these formulations AUC_{rel} was found to be 12, indicating enhancement of ocular bioavailability.

The enhancement of bioavailability with insert and in situ gel formulations is due to reduced drug loss and controlled drug release in precorneal area. On comparision of pharmacokinetic data with reported MIC values it can be concluded that the developed formulations would be effective against most of the micro-organisms.

All the above studies indicate that the prepared formulations are promising with improved therapeutic effectiveness against ophthalmic infections when compared to that of marketed preparation. The designed insert formulations were found to be stable for 24 months at ambient conditions and in situ gel formulations were found to be stable for 24 months at refrigerated temperature. Drug content, physical properties and in vitro drug release properties of designed formulations were not affected at the respective conditions.

However, further drug to polymer ratio in the inserts may be reduced by means of decreasing dimensions, with out affecting the extension of drug release. In case of in situ gels, further work can be done to extend the drug release still longer by varying the polymer combination or proportion. In vivo therapeutic efficacy needs to be studied in the infection induced animals. Also, clinical trial may be done in human volunteers to study pharmacokinetics parameters, effectiveness and specificity.

Appendix

Buffers and Reagents

- *Acetate Buffer (pH 4.1):* 0.75 g of sodium acetate trihydrate was dissolved in 400 ml to TDW and 9.8 ml of 2 M glacial acetic acid solution was added and volume was made up to 500 ml with TDW.
- *Acetate Buffer (pH 4.5):* 1.50 g of sodium acetate trihydrate was dissolved in 400 ml to TDW and 7.00 ml of 2 M glacial acetic acid solution was added and volume was made up to 500 ml with TDW.
- *Acetate Buffer (pH 5.1):* 2.55 g of sodium acetate trihydrate was dissolved in 400 ml to TDW and 3.2 ml of 2 M glacial acetic acid solution was added and volume was made up to 500 ml with TDW.
- *Acetate Buffer (pH 5.5):* 3.00 g of sodium acetate trihydrate was dissolved in 400 ml to TDW and 1.5 ml of 2 M glacial acetic acid solution was added and volume was made up to 500 ml with TDW.
- *Aqueous phase for HPLC with ion-pair reagents - pH 3.5 (Citrate buffer-25 mM; TBAHS-10 mM; SDS-10 mM):* 4.80 g of citric acid, 3.40 g of tri-sodium citrate, 3.40 g of TBAHS and 2.88 g of SDS was dissolved in 750 ml of TDW one after another, then volume was made upto 1000 ml using TDW. Amount of TBAHS and SDS was varied depending on mobile phase requirement.
- *Balanced salt solution (BSS):* 86.1 mg of $CaCl_2.2H_2O$, 200 mg of KCl, 204.9 mg of $MgSO_4.7H_2O$, 123.5 mg of $NaH_2PO_4.2H_2O$, 3.94 g of NaCl and 500 mg D-glucose was dissolved in 500 ml of TDW one after another. The pH of the solution was made to 7.2 using 0.1 M sodium hydroxide.
- *Buffers for solubility and partition coefficient studies:* Solution A – 17.80 g of $Na_2HPO_4.2H_2O$dissolved in 1000 ml of TDW (0.1 M). Solution B – 13.60 g of KH_2PO_4 dissolved in 1000 ml of TDW (0.1 M). Both the solutions were mixed proportionately to get different pH media and NaCl was added and dissolved to adjust the ionic strength to 0.3. Solution A + Solution B + NaCl (16.4 ml + 183.6 ml + 2.15 g - pH 5.80; 30.2 ml + 209.8 ml + 2.45 g - pH 6.00; 66.2 ml + 139.8 ml + 1.60 g - pH 6.50; 97 ml + 103 ml + 1.21 g - pH 6.80;121 ml + 79 ml + 0.92 g - pH 7.00; 146 ml + 60 ml + 0.70 g - pH 7.20; 160 ml + 42 ml + 0.49 g - pH 7.40; 187 ml + 12.8 ml + 0.15 g - pH 8.00).
- *Citrate buffer (25 mM):* 4.80 g of citric acid and 3.40 g of tri-sodium citrate was dissolved in 750 ml of TDW one after another, then volume was made upto 1000 ml using TDW.
- *Glacial acetic acid solution (0.1 M):* 50 ml of 2 M glacial acetic acid solution was transferred to a 1000 ml volumetric flask and volume was made up to the mark with TDW.

- *Glacial acetic acid solution (2 M):* Concentrated glacial acetic acid, 57.30 ml was transferred to a 500 ml volumetric flask and volume was made up to the mark with TDW.
- *Hydrochloric acid solution (0.01 M):* Hydrochloric acid solution (0.1 M), 100 ml was transferred to a 1000 ml volumetric flask and volume was made up to the mark with TDW.
- *Hydrochloric acid solution (0.1 M):* Concentrated hydrochloric acid, 8.58 ml was transferred to a 1000 ml volumetric flask and volume was made up to the mark with TDW.
- *Hydrochloric acid solution (1 M):* Concentrated hydrochloric acid, 8.58 ml was transferred to a 100 ml volumetric flask and volume was made up to the mark with TDW.
- *IP Buffer - Phosphate buffered saline (pH 6.8):* 1.00 g of Na_2HPO_4, 2.00 g of KH_2PO_4 and 8.50 g of NaCl was dissolved in 900 ml of triple distilled water, adjust pH with orthophosphoric acid or sodium hydroxide and then volume was made upto 1000 ml using TDW.
- *IP Buffer - Phosphate buffered saline (pH 7.4) - PBS:* 2.33 g of Na_2HPO_4, 0.19 g of KH_2PO_4 and 8.00 g of NaCl was dissolved in 900 ml of triple distilled water, adjust pH with orthophosphoric acid or sodium hydroxide and then volume was made upto 1000 ml using TDW.
- *Ortho phosphoric acid (0.1 M):* Ortho phosphoric acid, 6.78 ml was transferred to a 1000 ml volumetric flask and volume was made up to the mark with TDW.
- *Simulated tear fluid (STF) of pH 7.4*: The STF contained 0.67 g of sodium chloride, 0.20 g of sodium bicarbonate, 0.008 g of calcium chloride and water to a volume of 100 ml. The pH of the solution was adjusted to 7.4 with 1 N hydrochloric acid and was stable at ambient conditions
- *Simulated Tear Fluid (STF):* 6.7 g of NaCl, 2.0 g of $NaHCO_3$ and 0.08 g $CaCl_2.2H_2O$ were dissolved in 1000 ml of TDW one after another. The pH of the solution was adjusted to 7.4 using 1 M hydrochloric acid solution. These salt quantities are equal to 138 mM Na^+, 23.8 mM HCO_3^- and 0.72 mM Ca^{+2}.
- *Sodium hydroxide (0.1 M):* 4.00 g of NaOH was dissolved in 750 ml of triple distilled water and then volume was made upto 1000 ml using TDW.
- *Sodium phosphate buffers (0.1 M):* Solution A – 15.6 g of $NaH_2PO_4.2H_2O$ dissolved in 1000 ml of TDW (0.1 M). Solution B – 14.20 g of Na_2HPO_4 dissolved in 1000 ml of TDW (0.1 M). Both the solutions were mixed proportionately to get different pH media. Solution A + Solution B (185 ml + 15 ml - pH 5.8; 106.3 ml + 93.7 ml - pH 6.8; 49 ml + 151 ml - pH 7.4).
- *Sulphuric acid solution (0.01 M):* 100 ml of 0.1 M sulphuric acid solution was transferred to a 1000 ml volumetric flask and volume was made up to the mark with TDW.

- *Sulphuric acid solution (0.1 M):* Concentrated sulphuric acid, 2.72 ml was transferred to a 1000 ml volumetric flask and volume was made up to the mark with TDW.
- *USP Buffer - Phosphate buffer (pH 7.4):* 6.80 g of KH_2PO_4 was dissolved in 500 ml of triple distilled water and 195.5 ml of NaOH (0.2 M) was added, then volume was made upto 1000 ml using TDW.

List of Publications and Presentations

Publications

1. K. Venugopal, M. Snehalatha, B. Girish and R.N. Saha, "Development and Validation of Ion-Pairing RP-HPLC Method for the Estimation of Gatifloxacin in Bulk and Formulations", April 2007, Journal of Chromatographic Sciences, Volume 45, Issue 4, 166-172.
2. K. Venugopal, M. Snehalatha and R.N. Saha, "New, rapid and Sensitive Spectrofluorimetric Method for the Estimation of Gatifloxacin in Bulk and Formulations", November-December 2006, Indian Journal of Pharmaceutical Sciences, Volume 68, Issue 6, 726-730.
3. K. Venugopal, P. Haritha, M. Snehalatha and R.N. Saha, "In situ Ophthalmic Gels of Gatifloxacin: Development and In-vitro Evaluation test", AAPS 2006, The AAPS Journal, Vol. 8, No. S2, Abstract R6188.
4. K. Venugopal and R.N. Saha, "New, simple and validated UV-spectrophotometric methods for the estimation of gatifloxacin in bulk and formulations", November-December 2005, Il Farmaco, Volume 60, Issues 11-12, 906-912.
5. K. Venugopal, K. Udaya Kanth, M. Snehalatha and R.N. Saha, “Studies on effect of some permeation enhancers on transcorneal permeability of gatifloxacin”, AAPS 2005, The AAPS Journal, Vol. 7, No. S2, Abstract W4098.
6. K. Venugopal, K. Udaya Kanth, M. Snehalatha and R.N. Saha, “Design of gatifloxacin extended release ophthalmic inserts using HPMC and Eudragit”, AAPS 2005, The AAPS Journal, Vol. 7, No. S2, Abstract M1231.

Paper Presentations

1. K. Venugopal, P. Haritha, J. Varun, C. Chetan, M. Snehalatha and R.N. Saha, "Pluronic® F-127 and Poly(ethylene oxide) based ophthalmic in situ gelling systems of gatifloxacin: In vitro and in vivo evaluation", 2007 AAPS Annual Meeting and Exposition to be held in San Diego, CA, USA between 11th to 15th November, 2007, Accepted, No. AM-07-03185.
2. K. Venugopal, P. Haritha, M. Snehalatha and R.N. Saha, "Hydroxypropylmethylcellulose and Poly(ethylene oxide) based ophthalmic polymeric disc formulations of gatifloxacin: In vitro and in vivo evaluation", 2007 AAPS Annual Meeting and Exposition to be held in San Diego, CA, USA between 11th to 15th November, 2007, Accepted, No. AM-07-03003.

3. K. Venugopal, P. Haritha, M. Snehalatha and R.N. Saha, "In situ Ophthalmic Gels of Gatifloxacin: Development and In-vitro Evaluation test", 2006 AAPS Annual Meeting and Exposition held in San Antonio, Texas, USA between 29th October to 2nd November, 2006.
4. K. Venugopal, K. Udaya Kanth, M. Snehalatha and R.N. Saha, "Studies on effect of some permeation enhancers on transcorneal permeability of gatifloxacin", AAPS Annual Meeting and Exposition held in Nashville, Tennessee, USA between 6th to 10th November, 2005.
5. K. Venugopal, K. Udaya Kanth, M. Snehalatha and R.N. Saha, "Design of gatifloxacin extended release ophthalmic inserts using HPMC and Eudragit", AAPS Annual Meeting and Exposition held in Nashville, Tennessee, USA between 6th to 10th November, 2005.
6. K. Venugopal, M. Snehalatha and R.N. Saha, "Design and studies of novel ophthalmic inserts for extended release of gatifloxacin", 32nd Annual Meeting and Exposition of Controlled Release Society held in Miami Beach, USA between 18th to 22nd June, 2005.
7. K. Venugopal, K. Udaya Kanth and R.N. Saha, "Enhancement of transcorneal permeability of gatifloxacin using permeation modifiers", International Symposium on Recent Advances in Drug Design and Delivery Systems, 2005, Pilani, India.
8. K. Venugopal and R.N. Saha, "Studies of novel ophthalmic inserts for extended release of gatifloxacin", Sixth International Symposium on Advances in Technology and Business Potential of New Drug Delivery Systems, 2005, Mumbai, India.
9. K. Venugopal, M. Snehalatha and R.N. Saha, "New analytical method for the estimation of gatifloxacin in formulations by UV-Vis spectrophotometry", 55th Indian Pharmaceutical Congress, 2003, Chennai.
10. K. Venugopal and R.N. Saha, "Novel ocular drug delivery systems", International Symposium on Emerging Trends in Genomics and Proteonomics Education and Research, 2003, BITS, Pilani.
11. K. Venugopal, M. Snehalatha and R.N. Saha, "Simultaneous estimation of nimesulide and paracetamol by using derivative UV-spectrophotometry", 54th Indian Pharmaceutical Congress, 2002, Pune.

Biography of Venugopal Kolachina

Mr. Venugopal Kolachina has completed his bachelor degree in Pharmacy (B. Pharm) from K.V.S.R. Siddhartha College of Pharmaceutical Sciences, Vijayawada, A.P. in the year 1999 and Post graduation (M. Pharm, Pharmaceutical Technology) from Department of Pharmaceutical Technology, National Institute of Pharmaceutical Education and Research, Mohali, Punjab in 2001. He has been working as a research scholar at BITS, Pilani from 2002 to 2007. He has published/presented research articles in well renowned journals and presented papers in national and international conferences.

Biography of Prof. Ranendra N. Saha

Dr. Ranendra N. Saha is Professor of pharmacy and Dean, Faculty Division III and Educational Development Division, BITS, Pilani. He obtained his B. Pharm and M. Pharm (Pharmaceutics) degrees from Jadavpur University, Kolkata and Ph.D. from BITS, Pilani. He has more than 26 years of teaching and research experience and guided several doctoral, M. Pharm and B. Pharm students. He has many publications in reputed international and national journals and presented papers in international and national conferences in India and abroad. He has successfully completed several government and industry sponsored projects and continuing so. Dr. Saha has developed commercial products for industries and transferred technologies of production to industries and filed patents. He is expert member to various committees of UGC and other agencies and selection committee members of CSIR laboratories and several universities and colleges. He is also member of Board of studies of several universities and colleges and visiting Professor to few universities.