DEVELOPMENT AND CHARACTERIZATION OF THERMO-TRIGGERED IN-SITU GELLING VEHICLE FOR OPHTHALMIC DRUG DELIVERY

Submitted by

Saban Karmakar

B. Pharm

EXAM ROLL NO.: **M4PHP23009**

REGISTRATION NO.: **136622 OF 2016-2017**

Under the guidance of

Prof. (Dr.) Lakshmi Kanta Ghosh

Division of Pharmaceutics
DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY
Faculty of Engineering and Technology
JADAVPUR UNIVERSITY

Thesis submitted in partial fulfilment of the requirements for the

DEGREE OF MASTER OF PHARMACY

Department of Pharmaceutical Technology
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CERTIFICATE OF APPROVAL

This is to certify that **Saban Karmakar** (Exam Roll No. **M4PHP23009,** Reg. No. **136622 of 2016-17)** has carried out his **Master of Pharmacy** thesis on the topic **"DEVELOPMENT AND CHARACTERIZATION OF THERMO-TRIGGERED IN-SITU GELLING VEHICLE FOR OPHTHALMIC DRUG DELIVERY"** under my direct supervision in the Division of Pharmaceutics at the **Pharmaceutics Research Laboratory II** at the Department of Pharmaceutical Technology, Jadavpur University.

He has incorporated his findings into the thesis submitted by him in partial fulfilment of requirements for the award of the degree of Master of Pharmacy. I am satisfied that he has carried out this work with proper care and attention to my entire satisfaction.

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DECLARATION

I, Saban Karmakar, hereby declare that the thesis submitted in partial fulfilment of the requirements for the degree Master in Pharmacy at Jadavpur University is my original work. I further affirm that the research conducted and the content presented in this thesis complies with all academic ethics and integrity standards.

I assert that:

- The ideas, concepts, and findings presented in this thesis are the result of my independent research conducted under the guidance of my academic advisor, and have not been borrowed or copied from any other source.
- All sources, including books, journals, articles, and other materials referenced in this thesis, have been appropriately cited and documented in accordance with my university's academic integrity policy. Any direct quotations or paraphrased content from external sources are properly attributed.
- I have obtained all necessary permissions and approvals, where applicable, for the use of copyrighted materials, including images, figures, and tables, and have adhered to copyright laws and regulations when incorporating them into this thesis.
- The data presented in this thesis have been collected, analysed, and interpreted with transparency and integrity. I have avoided any data manipulation or misrepresentation, and I have disclosed any potential conflicts of interest.

By signing this declaration, I affirm my commitment to upholding the highest standards of academic integrity and ethics in the pursuit of knowledge and the dissemination of research findings.

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CHAPTER 01

INTRODUTION

INTRODUCTION

Eye as a Unique Organ

The eye is a unique organ that contains several different structures with specific physiological functions. The eye has protective mechanisms which include solution drainage, lacrimation, drug absorption via the vascularized conjunctiva, corneal barrier, melanin binding, aqueous humor flow and the blood-eye barrier [1, 2]. The corneal and the conjunctival surfaces are covered by continuous tear flow. The production and turnover of tears is essential for maintaining the health of the ocular surface in the face of environmental or body challenges. Tears cleanse, lubricate and nourish the surface of the eye and provide physical and immune protection against infection [1].

The wall of the eyeball (globe) consists of three primary layers:

I. the sclera, or outer layer (the fibrous protective layer with the transparent cornea anteriorly),
II. the uvea (uveal tract), or middle layer (having vascular and nutritive function, contains pigmented tissue consisting of the choroid, ciliary body and iris)
III. and the retina, or inner layer (which is the neural, sensory stratum of the eye)[3, 4].

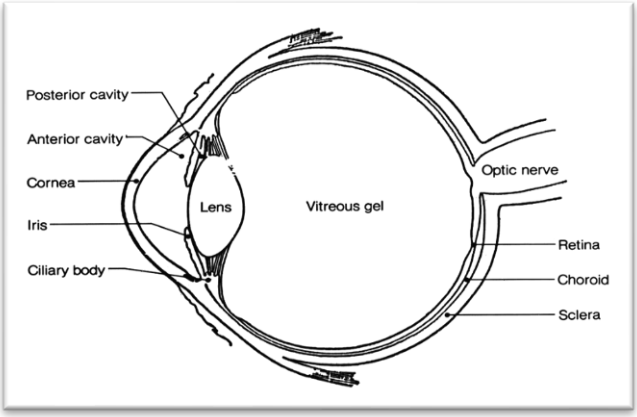

Figure 1.1: Cross section human eye

The eye surface is covered by a pre-corneal tear film composed of three layers: the adsorbed mucin layer, the middle aqueous layer and the superficial oily layer [5]. The tear film is by far the most dynamic structure [1]. The oily or lipid portion is secreted by the Meibomian glands and the mucins, a family of glycoproteins that are produced by the

conjunctival goblet cells [5]. The aqueous portion containing a salt solution is secreted by the main and accessory lacrimal glands [5, 6].

The drug in the pre-corneal area is absorbed by cornea, conjunctiva and sclera. Corneal permeability is the most important factor in determining the drug concentration in aqueous humor. The cornea is a transparent tissue, the clarity of which is due to its avascularity, non-keratinized epithelium, regular arrangement of epithelial and endothelial cells, and a relative corneal dehydration maintained by an endothelial pump mechanism [5]. It is indeed one of the most sensitive tissues of the body and this sensitivity serves a protective function [5, 7]. The cornea consists of five layers: epithelium, Bowman's membrane, stroma, Descemet's membrane and endothelium [6, 7]. However, only three of the layers (epithelium, stroma and endothelium) are significant with respect to barrier resistance.

Epithelium is a rate limiting barrier for transcorneal diffusion of most hydrophilic drugs [6]. 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 [8]. The corneal endothelium is responsible for maintaining normal corneal hydration. Stroma acts as diffusion barrier to highly lipophilic drugs owing to the hydrophilic nature of the former. Endothelium is lipoidal in nature which does not offer significant barrier to the transcorneal diffusion of most drugs [6].

The conjunctiva is a thin, vascularized mucus membrane that lines the inner surface of the eyelids and covers the anterior part of the sclera up to the cornea. Conjunctival uptake of a topically applied drug from tear fluid is typically an order of magnitude greater than corneal uptake [8]. The mucin coat, secreted by the goblet cells of the conjunctiva, covers the conjunctiva and corneal surfaces of the eye. This layer hydrates, cleanses, lubricates and serves as a defense against pathogens.

The sclera is the outer supporting layer of the globe and extends from the limbus at the margin of the cornea anteriorly to the optic nerve posteriorly, where it is contiguous with the dural sheath of the optic nerve [3, 7]. The composition of the sclera and cornea is identical; however, one layer is clear and the other opaque [4]. The sclera acts as a protective layer, maintains intraocular pressure and serves as the attachment site for the extraocular muscles [3].

Barriers during Ocular Drug Delivery

Usually, drugs administered systemically have poor access to the aqueous humor and the vitreous humor because of the blood-aqueous barrier and the blood-retinal barrier which prevent drugs from entering into the eye. Thereby, topical application of drugs to the eye is the most important and well-accepted route of administration for the treatment of various eye disorders [9].

This poor ocular bioavailability is attributed to several physiological factors, including limited permeability of the corneal membranes.

Furthermore, eye drops have a short precorneal residence time of 1–2 min because of their drainage through the nasolacrimal route and/or their systemic absorption via the highly vascular conjunctiva [10, 11].

Limitation of classical ODDS

The most common route of drug administration to the eye is topical instillation of eye drops because the price is low, and the usage is easy. However, the corneal uptake of drug from topically applied ocular formulations is low (i.e., <10%) [10, 11]. These preparations have some major drawbacks, such as short residence time, low bioavailability, and rapid precorneal drainage, drainage by gravity, normal tear turnover, enzymatic metabolism, conjunctival absorption, absence of controlled release and absence of bio-adhesive properties [12]. The low bioavailability of drugs is due to a large extent to nasolacrimal drainage precorneal drug loss. The rapid clearance into the eye of the topically applied drug often results in poor therapeutic response, hence the need for a frequent dosing regimen [13-17]. A high-frequency eye drop dosing regimen is associated with patient non-adherence [18]. Due to these drawbacks, long-acting ophthalmic drug delivery systems are required for better patient adherence, improved local bioavailability, and reduced dose concentration and dosing frequency of administration [19, 20].

Other dosage forms, which is ophthalmic ointments, provide prolonged contact with the eye surface recommended for bedtime use. But it may trigger some side effects such as foreign body sensation, blurred vision and inconvenience to the patient [21, 22].

Novel ODDS available in market

To overcome these drawbacks, the need for frequent application of the classical ODDS and pulse release various ophthalmic vehicles, such as inserts, ointments, suspensions, and aqueous gels, have been developed. It can lengthen the residence times of instilled dose and enhance ophthalmic bioavailability [23].

The residence time of an insert can almost be unlimited. An ocular insert may be designed to deliver the drug at a desired rate for a desired period of time, which determines the maximum contact time. Unfortunately the patient compliance of most inserts is rather low. [24, 25].

To overcome these problems, various ophthalmic vehicles, such as ointments, suspensions, micro and nanocarrier systems, inserts and liposomes have been investigated [26, 27].

Colloidal systems including liposomes and nanoparticles have the convenience of a drop, which is able to maintain drug activity at its site of action and is suitable for poorly water-soluble drugs.

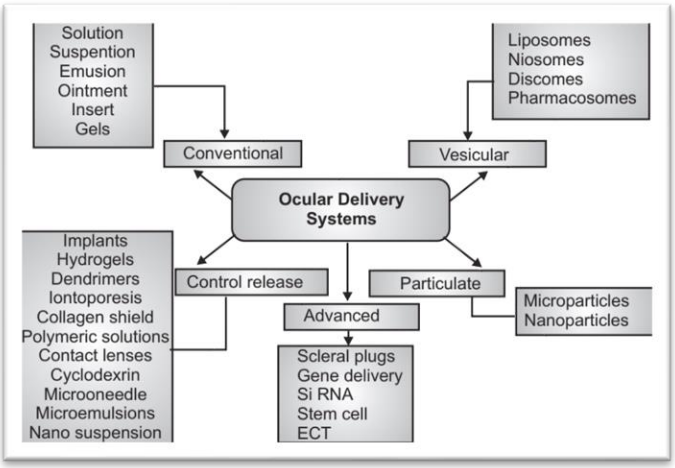

Figure 1.2: Ocular Drug Delivery Systems

In-situ Gelling System

Gel systems are better retained in the eye than conventional eye drops and are better tolerated by patients than inserts and ointments. Like ointments, gels are also difficult to

administer for some patients. In this respect in situ gels are interesting since these are conveniently dropped as a solution into the conjunctival sac, where they undergo a transition into a gel with its favourable residence time. The sol–gel transition occurs as a result of a chemical/physical change induced by the physiological environment.

The transition could be induced by a **shift in the pH** as for cellulose acetate phthalate [28] and for polycarbophil [29, 30]. Gelrite has another mechanism for sol–gel transitions: the gelation in the eye is due to the **presence of cations**, calcium ions especially have a marked effect on the gelling ability [31].

The efficacy of ophthalmic hydrogels is mostly based on an increase of ocular residence time by enhancing the viscosity and mucoadhesive properties. Since resulted swollen hydrogel is aqueous-based, it is very comfortable in the patient's eye. Because of this, in situ gels are preferred since they are conveniently dropped in the eye as a solution, where undergo transition into a gel [32].

In order to avoid the rapid dilution, formulations with an increased viscosity have been evaluated. Among them, the in-situ gel-forming formulations, which undergo phase transition from liquid to semisolid gel upon exposure to physiological environments, seem to be a promising tool.

Laddha and Mahajan also describe three ideal properties for in situ formulations: physical state, phase transition and strength of gel [14].

- First, the formulation should be a free-flowing liquid which allows ease of administration with reproducible dose delivery.
- Second, upon instillation, it should undergoes sol-to-gel formation by phase transition [33].
- And the third, formed gel should be strong enough to withstand the shear force in the cul-de-sac, which prolongs the residence time of the drug [34].

Stimuli that may trigger sol-gel phase transition of the polymer network on the ocular surface could be owing to physical (temperature, light) or chemical (ions, pH). One of the journals claims that among all in situ gel-forming systems, activation by change in ionic strength is most effective. The advantage is based on the fact that fluctuations in pH and temperature, which could cause changes in the gelation process, are not

associated with the ion activated system. These fluctuations in pH could cause ocular irritation, and storage conditions could lead to changes in temperature [15].

Amongst the natural polysaccharides that are considered as ion-activated polymers, the popular ones include gellan gum, kappa-carrageenan, alginates and xanthan gum. These polymers are able to interact with different cations that can be used to form ion-activated in situ gelling systems [35].

Thermo sensitive in-situ gel

These formulations should be a free-flowing liquid at room temperature to allow easily reproducible administration into the eye as a drop. They also should undergo in situ phase transition to form a strong gel that is capable of withstanding shear forces in the cul-de-sac and of sustaining drug release at physiological conditions [36].

In situ hydrogel

A modern effective strategy is the substitution of conventional eye drops with mucoadhesive hydrogel-based formulations can act as an to enhance drug retention and bioavailability. Hydrogels are hydrophilic molecules comprising a 3D network that can absorb and retain a large amount of water, making them important materials for drug delivery [37]. They can be used as vehicles to deliver drugs to the eye to prolong precorneal retention time and, in turn, improve ocular bioavailability.

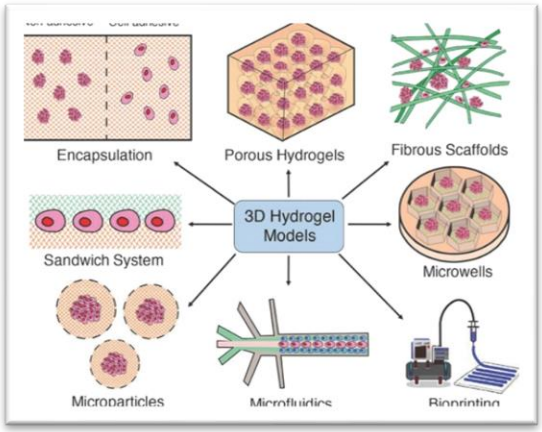

Figure 1.3: Hydrogel used in diverse field

Furthermore, in situ gelling systems can act as promising vehicles for intraocular and periocular injections, where they can create depots after injection into the vitreous humor

or in periocular tissues to provide sustained drug release to the posterior segment of the eye [38].

Advantages

An in situ gel drug delivery system provides several benefits, such as prolonged pharmacological duration of action, simpler production techniques, and low cost of manufacturing as compared to conventional drug delivery systems [10, 39].

Instillation of in situ gelling systems in the eye combines the merits of accurate dosing and easy administration of eye drops with prolonged retention in the eye and sustained drug delivery [40].

The in-situ gelling system consists of a stimuli-responsive polymer which displays sol-to-gel phase transitions in the eye due to a change in specific physicochemical parameters like pH, temperature, and electrolyte composition in the eye environment [41-45].

Drug related parameter

Drug absorption is also dependent on the chemical nature of the drugs since corneal permeability depends on the molecular size and the hydrophobicity of the drug [46].

An insoluble drug in the form of a suspension has a longer contact time because of the slower removal of particles from the eye as compared with solution [47]

The most common ways to increase the residence time of a solution is to decrease the drainage rate by increasing the viscosity of the vehicle [46, 48]. This, however, only moderately affects the contact time of the drug.

Mechanism of drug release

It involves a combination of diffusion and erosion of the gel surface. Due to the hydrophilic nature of the gels, tears readily diffuse into the gel interior, where they rapidly leach out water soluble drugs.

Since the most common mechanism of drug release from hydrogels is passive diffusion, molecules of different sizes and characteristics would freely diffuse into and out of the hydrogel matrix during the loading and storage periods. The hydrophilic nature of a

hydrogel makes it highly different from non-hydrophilic polymer matrices with respect to the release behaviour of the incorporated agents.

Mechanisms of drug release from hydrogels can be categorized as:

I. Diffusion-controlled
II. Swelling-controlled
III. Chemically-controlled

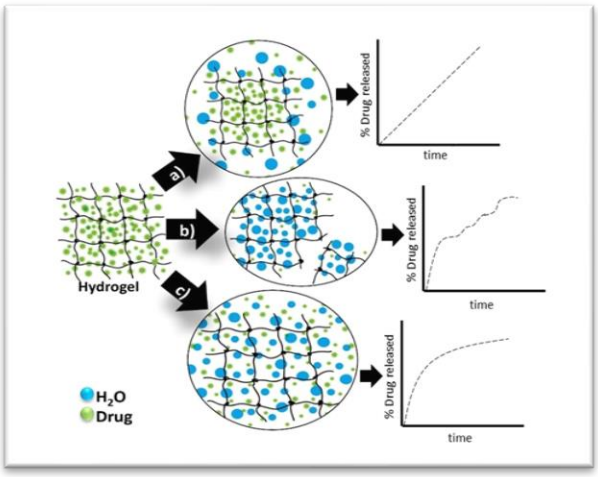

Figure 1.4: Drug release mechanism from hydrogel matrix

According to Fick's first law of diffusion (with constant or variable diffusion coefficients), diffusion-controlled behaviour is the most dominantly applicable mechanism to describe the drug release from hydrogels. The drug diffusion out of a hydrogel matrix is primarily dependent on the mesh size within the matrix of the gel, which, in turn, is affected by several parameters, including, mainly, the degree of crosslinking, the chemical structure of the composing monomers, and, when applicable, the type as well as the intensity of the external stimuli [49, 50].

Desired property of polymer

Thermoresponsive gelling systems are polymeric solutions that undergo sol–gel transitions in response to temperature changes. The lower critical solution temperature (LCST) is the minimum sol-gel transition temperature (Tsol–gel) of the polymer on its temperature concentration phase diagram, and depends on the interactions between water molecules and different hydrophilic/hydrophobic segments in the polymeric chain [51, 52].

Several natural and synthetic polymers exhibit thermos-responsive gelling behaviour at temperatures close to body temperature; thus, they can be used as injectable solutions or eye drops to achieve sustained drug delivery. For example,

- aqueous solutions of methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) exhibit an initial drop in viscosity upon heating, followed by solidification into hydrogels on continuous heating [53, 54].
- Chitosan-based thermoresponsive hydrogels were introduced by Chenite et al. as biocompatible sustained drug delivery systems [55] that resulted in the sustained delivery of latanoprost and ferulic acid to rabbit eyes [56, 57].
- Miyazaki et al. showed that an enzyme-degraded xyloglucan solution loaded with pilocarpine hydrochloride exhibited thermoresponsive gelling behaviour in rabbit eyes and increased the duration of the miosis [58].
- Poly(N-isopropylacrylamide) (pNIPAAm) is a thermoresponsive polymer with a LCST of~32 C, which can be tuned by grafting hydrophilic monomers [59]. For instance, Derwent et al. and Egbu et al. exploited pNIPAAm crosslinked with poly(ethylene glycol) diacrylate (PEGDA) with or without hyaluronic acid (HA) for the delivery of proteins to the posterior segment of eye, where the hydrogels displayed LCSTs ranging from 31 Cto36 C, depending on the PEGDA content [60, 61].
- Gao et al. synthesised a poly-(DL-lactic acid-co-glycolic acid)–PEG (PLGA-PEG) copolymer, which exhibited a Tsol–gel of 32 C and was used as an in situ gel for the ocular delivery of dexamethasone acetate [62].

Poloxamers are synthetic polymers that exhibit thermoresponsive behaviour, with a finely tunable Tsol–gel; thus, they are used for several pharmaceutical and biomedical applications [10, 63, 64]. They are commercially available as Pluronics1, Kolliphors1, and Lutrols1 [65]. Poloxamer 407 (P407) and poloxamer 188 (P188) are among the most commonly used poloxamers in ocular drug delivery as a result of their good solubility in water, clarity of their aqueous solutions, concentration- dependent viscosity, shear-thinning behaviour of their aqueous solutions, and their safety to the ocular tissues.

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CHAPTER 02

LITERATURE REVIEW

LITERATURE REVIEW

- Kurniawansyah IS, et al. created an in-situ ophthalmic gel forming technique using a combination of poloxamer 407 and hydroxypropyl methyl cellulose (HPMC) to administer chloramphenicole to the eyes over time. They used a 32 factorial design to optimise their formulation by putting different dependent variables (gelling capacity, pH, and viscosity) on different independent variables (poloxamer 407 and hydroxypropyl methyl cellulose (HPMC) concentration).
- Edsman K, Carlfors J, Petersson R. conducted rheological tests to analyse the gel and sol-gel transition of Poloxamer 407, an in situ gel. The gels' elasticity increased marginally as poloxamer concentration increased, and the temperature of the sol-gel transition decreased. The rheology of poloxamer solutions or gels combined with simulated tear fluid may be used to explain and connect the increase in contact time with increasing poloxamer concentration. The considerable concentration dependency of the sol-gel transition temperature coupled with the dilution that takes place in the eye led to the conclusion that the poloxamer system does not seem to be promising as an ocular in situ gel.
- Soliman KA, et al. represent prospective medication delivery systems for the eyes. To create a vehicle that gels at physiological temperatures, P407 and P188 may be combined in a variety of ratios. Extended in vitro release of a variety of medicines, with prolonged activity and enhanced bioavailability, has been shown with poloxamer-based in situ gels. Prior to further utilisation of poloxamers through the IVT pathway, additional research should be conducted into the minimal work that has been done so far in addressing posterior segment problems.
- Varshosaz J, Tabbakhian M, Salmani Z design a ciprofloxacin eye drop with mixtures of solutions of Pluronic with chitosan of different molecular weights (Mw) were prepared. Drug release study performed using membraneless dissolution and viscosity determined using Cup & Bob viscometer. Antimicrobial effect of the solutions was studied in nutrient agar in comparison to marketed solutions of ciprofloxacin using Pseudomonas aueroginosa and Staphylococcus aureus by the agar diffusion test using the cup-plate technique.
- Gratieri T, et al. developed an in situ forming gel comprised of the combination of a thermosetting polymer, poly (ethylene oxide)–poly (propylene oxide)–poly

(ethylene oxide) (PEO–PPO–PEO, poloxamer), with a mucoadhesive agent (chitosan). After a 10-min instillation of the poloxamer/chitosan 16:1 formulation in human eyes, 50–60% of the gel was still in contact with the cornea surface, which represents a fourfold increased retention in comparison with a conventional solution.

- Mansour M, et al. purpose of this study was to develop poloxamer-based in situ gelling formulations of ciprofloxacin hydrochloride (HCl) aiming at prolonging corneal contact time, controlling drug release, enhancing ocular bioavailability, and increasing patient compliance.
- Pardeshi SR, et al. studied the development of novel voriconazole (VCZ) loaded nanoparticles (NPs) for prolonged delivery for the management of ocular diseases. The in situ ophthalmic gel was prepared by incorporating NPs into carboxymethyl chitosan (CMCh) and poloxamer. After optimization of NPs the nanoparticle-loaded gel containing CMCh demonstrated enhanced antifungal activity against Candida albicans.
- Fathalla ZM, et al. aimed at preparing, characterising and evaluating in situ gel formulations based on a blend of two hydrophilic polymers i.e. poloxamer 407 (P407) and poloxamer 188 (P188) for a sustained ocular delivery of ketorolac tromethamine (KT). Drug-polymer interaction studies were performed using DSC and FT-IR. The gelation temperature (Tsol-gel), gelation time, rheological behaviour, mucoadhesive characteristics of these gels, transcorneal permeation and ocular irritation as well as toxicity was investigated.
- Chand et al., The polymers used in in situ gel should be nontoxic and non-absorbable from the gastrointestinal tract. It should adhere quickly to moist tissue and should possess some site-specificity, be a non-irritant to the mucous membranes, and possess a wide margin of safety both locally and systemically. Another important thing, the cost of the polymer should be not too high so that prepared dosage form remains competitive
- Saini et al., describe the ideal characteristics of polymers for the preparation of in situ ophthalmic gel. It should be biocompatible, capable of adhering to mucus membrane, have pseudoplastic behavior, has good tolerance and optical clarity, influences the tear behavior and the polymer should be capable of decreasing the viscosity with increasing shear rate.

- Aqueous solutions of Pluronic F127 and HPMC were prepared by dispersing them in distilled water with constant stirring. Ofloxacin was dissolved in glacial acetic acid and added to HPMC solution. HPMC drug solution was then poured into Pluronic solution with constant agitation and was allowed to stand at 4 °C for 24 h to make a clear solution.
- Satish KP, et al. also studied the in-situ gelling system included polyacrylic acid (Carbopol®980NF) as a phase transition polymer, hydroxypropyl methylcellulose (Methocel® K100LV) as a release retardant, and ion exchange resin as a complexing agent. To prevent drug and polyacrylic acid incompatibility, ciprofloxacin hydrochloride was complexed with ion exchange resin.
- Basavaraj K, et al also studied on the idea of in situ gelation caused by pH. An ocular delivery system for the non-steroidal anti-inflammatory medicine ketorolac tromethamine was developed and tested using polyacrylic acid (Carbopol® 934) as the gelling agent in conjunction with hydroxyl propyl methylcellulose (Methocel K4M), which served as a viscosity boosting agent.
- Yong Q. et.al. were also studied to extend the pre-corneal resident duration and the bioavailability of methazolamide (MTA), a thermosensitive in situ gelling vehicle was created. The gelling ingredients were analogues of poloxamer, and a cold technique was utilised to create the in-situ gel.
- Rathapon A, Suthira T, Asira F. were studied relating to DS in-situ gels, which are thermoresponsive diclofenac sodium ophthalmic in situ gels based on Pluronic F 127. They were made using the cold technique, and their pH, flowability, sol-gel transition temperature, gelling capacity, and rheological characteristics were all studied. Opthalmic absorptions in vivo were investigated in rabbits.
- Jagdish B, et al. were owing to the medication being pre-corneally eliminated by the usage of in-situ gel forming mechanism, standard opthalmic solutions display low bioavailability and therapeutic response. The aim of this study was to design an ion-activated in-situ gelation-based opthalmic administration system for the NSAIDS indomethacin. The gelling agent was Gelritegellan gum, a new opthalmic vehicle.

- Feng Cao, Xiaolin Z, and Qineng P. were studied about a thermosensitive and mucoadhesive in situ azithromycin (ATM) ophthalmic device. As gelling agents, poloxamer 407 (P407) and poloxamer 188 (Pl 88) were used. By using the salt effect, carbopol 974P (CP 974P) addition to the gelling systems could make ATM more soluble. Both in vitro and in vivo tests showed that this droppable gel outperformed ATM eye drops.
- Pluronic-g-poly (acrylic acid) copolymers were studied as a temperature-responsive in situ gelling vehicle for an ophthalmic drug delivery' system. The rheological properties and in vitro drug release of Pluronic-g-PAA copolymer gels, as well as the in vivo resident properties of such in situ gel ophthalmic formulations, were investigated.
- The potential of a chitosan solution as well as an in-situ gel- forming system comprised of poloxamer/chitosan as vehicles for enhanced corneal permeation and sustained release of fluconazole (FLU). Micro dialysis was employed in a rabbit model to evaluate the in vivo performance of the formulations. The in vitro release studies showed the sustained release of FLU from the poloxamer/chitosan formulation.
- A novel copolymer, poly(N-isopropylacrylamide)-chitosan (PNIPAAm- CS), was investigated for its thermosensitive in situ gel-forming properties and potential utilization for ocular drug deliven'. The thermal sensitivity and low critical solution temperature (LCST) were determined by the cloud point method. The in vivo ocular pharmacokinetics of timolol maleate in PNIPAAm-CS solution w'ere evaluated and compared to that in conventional eye drop solution by using rabbits according to the micro dialysis method.
- The rheological measurements and a small in vivo study of ocular residence times in humans were used to evaluate poloxamer as an ocular vehicle. An increasing concentration of pOloxamer resulted in a slightly increasing elasticity of the gels and a decreasing sol-gel transition temperature. The contact time increased with increasing concentration of poloxamer. In the present study rheological measurements were performed.
- Controlled release in situ gel consisting of carbopol and cellulose derivatives showed increased in viscosity, gelling capacity. Hydroxypropyl methyl cellulose combined with carbopol to reduce the concentration of the incorporated

carbopol. Study was designed control release opthalmic systems for ciprofloxacin based on polymeric carriers that undergo sol-to- gel transition upon change in pH and to prolong the effect of cirofloxicin.

CHAPTER 03

AIM OF STUDY

AIM OF STUDY

One of the most challenging issues for pharmaceutical researchers has remained to be the delivery of medications to the eyes. The eye's particular structure prevents medication molecules from reaching the essential area of action. Conventional treatments for treating vision-threatening ocular disorders, such as eye drops, suspensions, and ointments, cannot be considered optimum. Due to the eye's efficient manufacturing processes, ophthalmic solutions have relatively limited bioavailability when delivered. Over 90% of the formulations are provided as ocular solutions.

To retain a therapeutic medication level in the tear film or at the site of action, it is required to routinely administer eye drops. However, frequent use of extremely concentrated solutions could result in cellular damage and other undesirable side consequences.

The characteristic pulse entry type drug release seen with traditional preparations may be replaced with a more controlled, prolonged, and continuous drug administration employing a controlled release ocular drug delivery system. These systems may produce therapeutic efficacy with a lesser dosage and fewer systemic and ocular side effects. Such technologies include implants, ocuserts, collagen shields, nanoparticles, microspheres, and liposomes, however, the drawbacks of the foregoing systems are

- Difficulty of insertion into the ocular cavity causes poor patient compliance.
- Tissue discomfort and injury induced by penetration enhancers and collagen shields.
- Toxicity and irritation is caused by the insertion of foreign substances.

To solve such challenges, an alternate strategy of in situ gelling systems or phase transition systems which are instilled in a liquid state and shift to a gel or semisolid phase in cul-de-sac by the change in temperature is picked as a unique system.

Due to the gelling capabilities of the thermo-triggered in situ gelling system after instillation into the eye, the drainage of the formulation is limited compared to traditional ophthalmic eye drops.

The in-situ gelling technique allows extended contact time and sustained distribution of the medication, consequently, reduction in frequency of administration and enhanced patient compliance are the key benefits.

The ocular conjunctivitis produced by different microorganisms produces lasting conjunctival damage, corneal ulcers, and systemic infections.

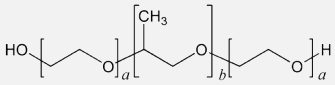
The aim of our current study is the formulation and evaluation of an in-situ ophthalmic gel of Atropine Sulphate (as a model drug) reserved principally for severe bacterial conjunctivitis. The objective of the present work is to develop a temperature-triggered in-situ gelling ophthalmic delivery system with a higher degree of retention and lower elimination rate based on Kolliphor® P 407. Xanthan gum and guar gum have been investigated as additives that optimize the gelling temperature for the formulation of eye drops that would gel when instilled into the eye and provide controlled release of the drug at body temperature even below 35°C when instilled into the eye.

CHAPTER 04

DRUG AND POLYMER PROFILE

POLYMER PROFILE

Poloxamer

Synonym	**Lutrol F 127, Pluronic F 68**
Structure	
Chemical name	Polyethylene-Polypropylene Glycol
Empirical formula	HO(C2H4O)a(C3H6O)b(C2H4O)aH
Molecular weight	8400.00
Functional category	Emulsifying agent Sensitize drug resistant cancers to chemotherapy
Description	White to off white granule
Properties	
Physical state	white powder
Solubility in water	soluble in water
Solvent solubility	soluble in methanol and chloroform mixture
HLB value	29.0

Introduction

Poloxamer block copolymers were first presented in the late 1950s and have subsequently been suggested for a variety of medicinal uses [1].They are included in both the American and European Pharmacopoeias [2]. This copolymer group is made up of ethylene oxide (EO) and propylene oxide (PO) blocks organised in a triblock structure EOxPOyCH2O]y[CH2 CH2O]xOH, where y is greater than 14. These copolymer registered trademarks (for example, Pluronic, Synperonic, or Tetronic) span a wide spectrum of liquids, pastes, and solids. They are made by sequentially polymerizing PO and EO monomers in the presence of sodium or potassium hydroxide [3]. The block copolymers may be purified by chromatographic fractionation. The amphiphilic qualities of these copolymers are described by their HLB values (hydrophilic lipophilic balance), which are significantly dependent on x and y values. Size, lipophilicity, and hydrophilicity may all be readily adjusted by modifying the values of these characteristics.

Poloxamer 407, a thermogelling in situ gel, was investigated in this study. Poloxamer 407 is a block co-polymer composed of poly(oxy ethylene) and poly(oxy pro-pylene). The sol-gel transition is induced by an increase in temperature, but it is dependent on polymer concentration and the presence of additives such as salts and polymers (Miller and Drabik, 1984; Vadnere et al., 1984; Gilbert et al., 1987).

Chemical structure

Chemical structure of poloxamers Poloxamers are non-ionic surfactants with a triblock copolymer structure comprising two hydrophilic poly(ethylene oxide) (PEO) blocks with a hydrophobic poly(propylene oxide) (PPO) block [4, 5]. Their different PEO:PPO proportions contribute to their variable physicochemical properties.

Nomenclature

Poloxamers have a unique nomenclature system comprising three digits, where the first two digits represent the approximate molecular weight (Mwt) of the PPO block divided by 100, whereas the third digit represents the approximate weight percentage of the PEO divided by 10. By contrast, Pluronics1 are nominated by a letter representing their physical state followed by a three-digit number that depends on the PEO:PPO weight fraction. Pluronics1 are given a letter (L) for liquid, (P) for paste, and (F) for flakes. The first two digits represent the approximate Mwt of the PPO block divided by 300, whereas the third digit represents the approximate weight percentage of the PEO divided by 10[6].

Thermo responsive behaviour

The most accepted mechanism to explain the thermos-gelification of poloxamers is that it results from interactions between different segments of the copolymer [7, 8]. The poloxamer copolymer molecules aggregate into micelles. These micelles are spherical with a dehydrated polyoxypropylene (PPO) core with an outer shell of hydrated swollen polyoxyethylene (PEO) chains [9]. An increase in the temperature leads to dehydration and conformational changes at the hydrophobic chains regions, increasing chain friction and entanglement of the polymeric network [10, 11]. More unbound water is available at the hydrophilic regions of the gel [12]; therefore, the external PEO chains interpenetrate extensively in the gel. At this point, gelation has occurred, and the micelles remain apparently intact and orderly packed, which has been described as ‘‘hard-sphere crystallization” [13].

Given their surfactant properties, poloxamer molecules self-associate, forming micelles at a certain concentration known as the critical micelle concentration (CMC). During micelle formation, the PPO groups interact together via van der Waals forces to form the hydrophobic micelles core, whereas the PEO groups occupy the micelle shell, interacting with water molecules via hydrogen bonds [14]. Temperature increases favour interactions between PPO groups as well as polymer desolvation, thus enhancing micelle formation

at lower polymer concentrations [14]. Upon further heating of the micellar aqueous solution, poloxamer micelles aggregate at a certain temperature and the system fluidity decreases abruptly, leading to gel formation. This process is reversible because cooling converts the gel back to its original sol state [15]. Over the past three decades, the thermoresponsive behaviour of poloxamers has been thoroughly investigated with respect to the development of sensitive and precise techniques for the determination of Tsol–gel, as well as the investigation of different molecular and formulation variables affecting their thermoresponsive gelling behaviour.

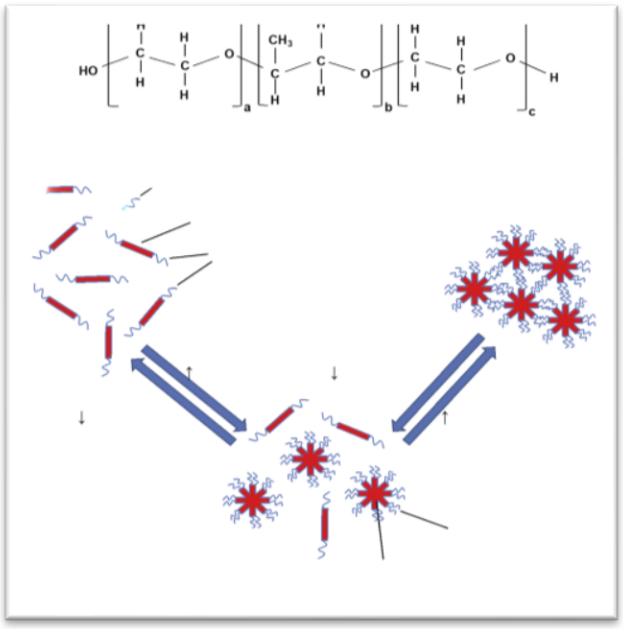


Figure 1 *Schematic of molecular phase changes exhibited when the temperature is changed.[16]*

Pharmaceutical importance

Sterilization: Sterilisation by autoclaving (120-C, 15 min, 1 bar) appears compatible and does not significantly alter viscosity characteristics of Poloxamer 407 solution, which is interesting to prepare sterile formulations (i.e., ophthalmic or injectable) [17]. Nevertheless, no studies have been conducted to specify possible degradation of this copolymer during autoclaving.

Solubility increase: Poloxamer 407 facilitates solubilisation of poorly watersoluble molecules like indomethacin or insulin [18]. Solubility of piroxicam in water was increased by 11-fold by adding 22.5% w/w Poloxamer 407 . It appears more effective than polyol or polysorbate. Incorporation of Poloxamer 407 in solid dispersion of poorly

water-soluble molecules, like nifedipine or piroxicam, led to marked improvement and thus promoted faster and more complete dissolution [19].

Drug delivery Vehicle: Poloxamer has been evaluated as vehicles for drugs; lipophilic drugs, especially, have favourably slow release rates from poloxamer vehicles (Gilbert et al., 1986). Poloxamer has also been evaluated as an ophthalmic vehicle in animal models (Miller and Donovan, 1982)[20]

Stabilization Property: Poloxamer 407 promotes stabilisation of included drugs in particular proteins. Poloxamer 407 also reduces the propensity for peptide unfolding in relation with low CMC and lack of electrostatic binding. The three-dimensional structure of proteins is better preserved in the presence of Poloxamer 407 [21]. Micro-or nanoparticles were optimised by addition of Poloxamer 407.

Adhesive property: Defining the (bio)adhesive characteristics is of great importance when prolonged residence-time is required, in particular with topical formulations (e.g., rectal, cutaneous or ophthalmic preparations). (Bio)adhesive force generally increases with gel strength and its value is modified by the same parameters (i.e., temperature and Poloxamer 407 concentration). The presence of various solvents or ionic agents may alter the adhesion characteristics of poloxamer formulations as it has been previously noted. Because of its adhesion-promoting action, NaCl has been included in some Poloxamer 407 gels to prolong residence-time in the site of administration . Experimental models determine the detachment force using different inert or physiological media and are completed with in vivo preliminary behaviour studies conducted with animals.

Drawback

Poloxamer 407 is a well-known stimuli-responsive polymer type with thermoresponsive behaviour. It is commonly used as an eye drug delivery system as it could prolong drug release for eye tissue [22]. However, the major drawback of poloxamer 407 alone is low mucoadhesive activity [23]. In addition, adding excessive concentrations of poloxamer 407 can induce hypertriglyceridemia in the eye [24]. The addition of other polymer like xanthan gum and guar gum can reduce the concentration of poloxamer 407 needed to form in situ gel gelation [16, 25]. Thereby, improving mucoadhesive activity and reducing the risk of hypertriglyceridemia induction in the eye. [26]

Moreover, previous reports have revealed that higher concentrations of P407 are required in a formulation when used on its own; such concentrations were found to be irritant to the eye. In order to overcome this challenge, researchers adopted the approach of blending P407 with other polymers like xanthan gum, guar gum methyl cellulose, chitosan, and P188 in order to decrease the total concentration of P407 used, improve its gelling characteristics as well as mechanical properties of P407 and reduce its ocular irritation potential [4]

Furthermore, it was previously reported that P407 at a concentration of 18% (w/v) or higher, has the ability to transform from a low viscosity solution into a gel under the ambient temperature [27, 28]. However, at this concentration the solution will lose its gelation ability after being diluted by lacrimal fluid upon instillation into the eye. Hence, 25% (w/w) P407 can be used in order to ensure the completion of the phase transition process of the polymer under ocular physiological condition. But, under these circumstances, the gelation temperature will be lower than room temperature and P407 solution have to be stored in the fridge, which makes it inconvenient for use [29]

Xanthan Gum

Property	Value
Physical state	Dry, cream-colored powder
Moisture (%)	8-15
Ash (%)	7-12
Nitrogen (%)	0.3- 1.0
Acetate content (%)	1.9-6.0
Pyruvate content (%)	1.0-5.7
Monovalent salt (g L^{-1})	3.6-14.3
Divalent salt (g L^{-1})	0.085-0.17
Viscosity (cp)	13-35

Introduction

The xanthan gum is a high molecular weight hetero polysaccharide gum made by a pure culture fermentation of a carbohydrate with the bacterium Xanthamonas campestris.

Xanthan gum is a hetero polymer composed mostly of repeating unit of pentasaccharide produced by two glucose units, two mannose units, and one glucuronic acid unit, with the molar ratio 2.8:2.0:2.0.[30]

Source

Xanthomonas campestris, a bacteria often found on cabbage plants, produces xanthan gum, a high molecular weight polymer that is naturally occurring. The free-flowing, white to cream-colored xanthan gum powder is soluble in both hot and cold water but insoluble in the majority of organic solvents. Even at low concentrations, xanthan gum solutions exhibit much higher viscosity than other polysaccharide solutions. It is more effective as a thickening and stabiliser because of this characteristic. Although not thixotropic, xanthan gum solutions are rather pseudoplastic.[31]

The pseudoplasticity of xanthan gum facilitates processing, assures high pourability, and improves sensory attributes in finished goods. The pH-variations resistance of xanthan gum solutions means that they maintain their stability in both acidic and alkaline environments. Furthermore, xanthan gum outperforms the majority of other water-

soluble polysaccharides in terms of heat stability. Since xanthan gum has no flavour, it has no impact on the flavour of other culinary components.[32]

Structure

A high molecular weight polysaccharide called xanthan gum is created when Xanthomonas campestris bacteria are used in pure culture aerobic fermentation of carbohydrates. It is a polysaccharide with a long chain and several trisaccharide side chains. D-glucose units that are -(1, 4)-linked make up the main chain. Two mannose units and one glucuronic acid unit are found in the side chains, which are joined by the main chain's alternating glucose residues. The placement of this group in the chemical structure depends on the bacterial strain and the fermentation circumstances, and the terminal D-mannose residues may have a pyruvate role. An acetyl function may be found in the side chain's non-terminal D-mannose unit. The side chain of this polymer contains pyruvic acid and glucuronic acid groups, which give the polymer its anionic properties.

Solubility

Xanthan gum is a cream-colored powder that has a high viscosity even at low concentrations and is soluble in hot or cold water. In addition to being utilised as a gelling agent, stabilising agent, suspending agent, and viscosity enhancing agent, xanthan gum has been intensively researched as a potential polymeric material in several floating drug delivery technologies.[33]

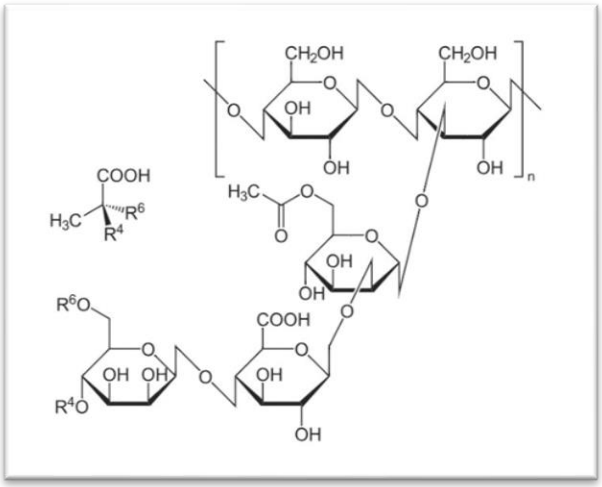


Figure 2: Structure of Xanthan Gum

Bio pharmaceutical application

Natural polymers are used in a wide range of pharmaceutical formulations, including the creation of solid monolithic matrix systems, films, implants, beads, nanoparticles, microparticles, inhalable, and injectable systems. Polymeric materials play several functions in these dosage forms, including binders, matrix formers, drug release modifiers, coatings, viscosity enhancers, stabilisers, emulsifiers, suspending agents, disintegrators, solubilizers, gelling agents, and bioadhesives. Because of the unique properties of xanthan gum, including as its gelling ability and interactions with other polysaccharides, there is growing interest in its biopharmaceutical use. The emulsifying, thickening, stabilising, film forming, and gelling characteristics of xanthan gum allow it to be used in medicinal applications.[34]

Property

- Xanthan gum is an excellent thickening agent. It exhibits pseudoplastic rheological characteristics, which means as shear is increased, viscosity is reduced. Once the shear is removed, the starting viscosity is recovered. The reason this occurs with xanthan gum is the ability of the xanthan molecules to form aggregates through hydrogen bonds and polymer entanglement (Sworn 2000). At low shear rates, xanthan solutions are highly ordered, entangled, stiff molecules. As shear is increased, the aggregates are interrupted and individual polymer molecules align in the direction of the shear force, which results in the pseudoplastic conditions. (Vanderbilt 2000; Deis 2001).
- As little as 0.1% xanthan gum will significantly increase viscosity. When 1.0% xanthan gum is used, an almost gel-like consistency will be observed at rest (Sworn 2000; Vanderbilt 2000). However, when shear is applied, it exhibits the same rheological properties seen at a lower concentration. Generally, xanthan gum is stable over the pH range 2 to 12 (Dziesak 1991; Sworn 2000; Vanderbilt 2000). At pH below 2 and above 12, viscosity tends to 25 decreases slightly. However, change in viscosity is dependent on the concentration of xanthan gum. The lower the concentration, the more profound the decrease in viscosity (Sworn 2000; Vanderbilt 2000).
- Xanthan gum, unlike many other food gums, is stable at a range of temperatures. The viscosity will not change significantly between ambient temperature and a definitely “melting temperature”, which is usually around 60ºC. (Sworn 2000;

Vanderbilt 2000). At the melting temperature, a sharp decrease in viscosity is seen due to a reversible molecular conformation change (Sworn 2000).

- The specific "melting temperature" is dependent upon the ionic strength of the solution. If viscosity is lost due to an increase in temperature, it is reversible and as the solution cools, the initial viscosity will return.
- Depending the concentration of xanthan gum, salts may either decrease or increase viscosity. At 0.25% xanthan gum concentration or below, monovalent salts may cause a slight decrease in viscosity. When a higher concentration is used, the salt actually increases the viscosity. Many divalent salts, such as calcium or magnesium, affect viscosity similarly (Sworn 2000).
- Unlike most hydrocolloids, xanthan gum is not degraded by enzymes. Frequently enzymes, such as proteases, pectinases, cellulases and amylases, are found in many food systems. It is believed the arrangement of the trisaccharide side unit is responsible for this enzyme resistance (Sworn 2000). The side unit prevents enzymes from attacking the β-(1-4) linkages located on the backbone. Therefore, xanthan gum can be used in food products containing active enzymes.
- Although xanthan gum is not a gelling agent, it can form elastic, thermosreversible gels when combined with locust bean gum. High viscosities are achieved when combined with galactomannans such a locust bean gum and guar gum (Dziesak 1991).

Guar Gum

Property	Description
Chemical Structure	Mannose and galactose units linked by glycosidic bonds
Solubility	Soluble in cold and hot water
Viscosity	High viscosity, effective thickener
pH Sensitivity	Maximum thickening at pH 5-7
Hydrocolloid	Absorbs and retains water, thickening agent
Rheological Behavior	Non-Newtonian, pseudoplastic
Ionic Properties	Interacts with ions, influenced by salts
Synergistic Effects	Enhances properties when combined with other gums or thickeners
Stability	Stable over a wide range of temperatures and pH levels
Film-Forming	Can form films when dried

Introduction

Guar gum is produced by milling the seeds of the Cyamopsis tetragonoloba guar plant, which contains guar gum (Meer 1977; Wielinga 2000). Guar gum is a neutral hydrocolloid having linear chains of D-mannopyranosyl units and substituents that protrude by (1–6) links from D-galactopyranose. There are around two mannose residues for every galactose residue.

Due to the high level of substitution in guar gum, it has strong hydration and hydrogen bonding activity. It is simple for water to "slip" between the molecules and hydrate or dissolve the gum. Guar gum has a molecular weight of 220,000 to 300,000 (Hoyt, 1966). Galactose substitution in guar gum is greater (40%) than in locust bean gum (20-23%) (Maier 1992). Galactose content in galactomannans has been investigated and shown to have a significant impact on how each hydrocolloid behaves. Low galactose concentration promotes both higher independent interactions across smooth 31mannan backbone regions and stronger synergistic interactions with other hydrocolloids (Dea 1977; McCleary 1985).

As a result of the main backbone's weaker cohesiveness due to the increased galactose concentration, no substantial junction zones or crystalline areas may develop. The placement of galactose units along the mannan backbone is another aspect that affects the physical behaviour of the galactomannan. On average, for every two molecules of mannose, a galactose side unit is connected to guar gum, therefore there are no smooth and hairy portions of the mannan backbone (Meer 1977). Mannose-to-galactose ratios of two to one result in tiny patches of uncontrolled galactose, which has been linked to lower functioning (McCleary 1979; Launay 1986). Comparatively, locust bean gum often has a four to one ratio, which should be more useful. Two galactomannans with the same average galactose concentration but varied mannose-galactose ratios would display varying degrees of functionality, according to Richardson et al. (1998) (Richardson 1998). Because they include a higher percentage of chains with lower galactose concentration, galactomannans with a wider distribution of galactose units would be more functional (McCleary 1979; Launay 1986).

Functions and Applications

- Guar gum is used as a thickener and stabilizer in the food industry as a result of its hydration and water-binding properties.
- It is used as a stabilizer at a concentration of 3.0% in ice cream, ice pops, and sherbet. It improves the body, texture, chewiness, and heat-shock resistance by binding free water (Wielinga 2000).
- It is used in conjunction with agar to prevent fat migration during storage as well as controlling syneresis. As a thickener it is sometimes added to salad dressings, pickle and relish sauces.
- Guar gum can also be used in dietetic beverages or low carbohydrate products due to its suspending ability and improving body of thin and watery products. (Meer1977). An advantage of guar gum is its cold-water solubility which allows viscous pseudoplastic solutions to form when hydrated in cold water (Deis 2001).
- Its viscosity is dependent upon factors such as time, temperature, concentration, pH, ionic strength, and type of agitation. Maximum viscosity is reached during the temperature range of 25-40ºC, with higher temperatures increasing the rate at which maximum viscosity is achieved. However, too high a temperature will degrade the gum and normal function will not be carried out.

- Guar gum is stable over a wide range of pHs, with its optimal rate of hydration between pH 7.5-9. The maximum viscosity will remain stable between the pH range of 1 to 10.5. Another advantage of guar gum is its ability to be compatible with salts over a wide range of electrolyte concentrations. For instance, guar gum with borate ions, the borate ions act as cross-linking agents with guar gum to form structural gels.
- It is also a good emulsifier due to the amount of galactose substituents. Guar gum exhibits stability during freeze-thaw cycles as it is able to retard ice crystal growth by slowing mass transfer across solid and liquid interfaces (Chaplin 2003).
- Guar gum is easily hydrated and is an economical stabilizer and thickener, it has some limitations as well. Unlike locust bean gum, it does not form gels. Guar gum is stable over a wide pH range, however if both temperature and pH are at extreme points, it could lead to degradation. For instance, at a pH 3 and temperature of 50ºC, guar gum starts to degrade.

DRUG PROFILE

Atropine Sulphate

Formula	**C17H23NO3**
Molar mass	676.8 g/mol
Melting point	189-192 °C (A)(lit.)
Boiling point	135 °C
Density	1.1172 approx
Refractive index	1.6900
Storage temp.	0-6°C
Color	Crystals

Introduction

Atropine is a tropane alkaloid and anticholinergic medicine used to treat some forms of nerve agent and pesticide poisonings as well as certain types of sluggish heart rate, and to suppress saliva production during surgery. It is often delivered intravenously or by injection into a muscle. Eye drops are also available which are used to treat uveitis and early amblyopia. The intravenous solution generally starts functioning within a minute and lasts half an hour to an hour. Large dosages may be necessary to treat certain poisonings.

Common adverse effects include a dry mouth, wide pupils, urine retention, constipation, and a rapid heart rate. It should normally not be utilised in persons with angle closure glaucoma. While there is no indication that its usage during pregnancy causes birth abnormalities, that has not been widely examined. It is likely safe while nursing. It is an antimuscarinic (a sort of anticholinergic) that operates by suppressing the parasympathetic nervous system.

IUPAC name:

[(1S,5R)-8-methyl-8-azabicyclo [3.2.1] octan-3-yl] 3-hydroxy-2- phenylpropanoate; sulfuric acid.

Structure:

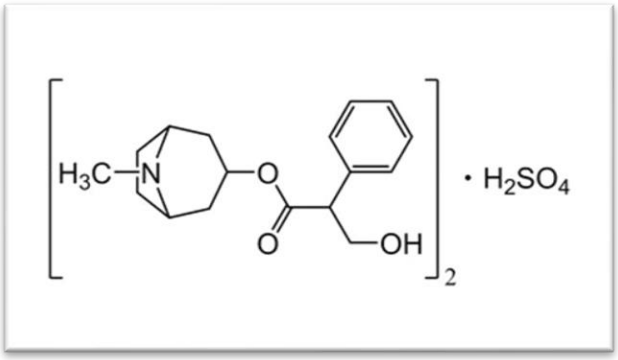


Figure 3: Structure of atropine sulphate

Description

Atropine Sulfate Injection, USP is a sterile, nonpyrogenic isotonic solution of atropine sulphate monohydrate in water for injection with sodium chloride sufficient to make the solution isotonic. It is delivered parenterally via subcutaneous, intramuscular, or intravenous injection. Each milliliter (mL) includes 0.1 mg (adult strength) or 0.05 mg (pediatric strength) of atropine sulphate monohydrate corresponding to 0.083 mg (adult strength) or 0.042 mg (pediatric strength) of atropine, and sodium chloride. 9 mg may include sodium hydroxide and/or sulfuric acid for pH correction of 0.308 mOs.mol/mL (calc.). pH 4.2 (3.0 to 6.5). Sodium chloride added to the solution isotonic for injection of the active component is present in proportions inadequate to impact the serum electrolyte balance of sodium (Na+) and chloride (Cl-) ions. The solution includes no bacteriostat, antimicrobial agent, or additional buffer (save for pH correction) and is designed for use exclusively as a single-dose injection. When lesser dosages are necessary, the leftover part should be thrown. [35, 36]

The chemical formula for Atropine Sulfate, USP is 1 H, 5 H-Tropan-3-ol ()-tropate (ester), sulphate (2:1) (salt) monohydrate, (C17H23NO3)2 ·H2SO4 ·H2O, colourless crystals or white crystalline powder, extremely soluble in water. It has the following structural formula:

Atropine, a naturally occurring belladonna alkaloid, is a racemic combination of equal amounts of d-and 1-hyocyamine, whose action is attributable almost exclusively to the levo isomer of the drug. Sodium Chloride, USP, often known as NaCl, is a white crystalline powder that is easily soluble in water. The syringe is manufactured from a specifically designed polypropylene. Water seeps from within the container at a very slow pace, which will have a minor influence on solution concentration throughout the predicted shelf life. Solutions in contact with the plastic container may leak out some chemical components from the plastic in very minute volumes. However, biological testing proved supportive of the safety of the syringe material.[37]

Mechanism of Action:

Atropine is an antimuscarinic drug as it antagonises the muscarine-like activities of acetylcholine and other choline esters. Atropine suppresses the muscarinic activities of acetylcholine on tissues innervated by postganglionic cholinergic neurons and on smooth muscles which react to endogenous acetylcholine but are not so innervated. As with other antimuscarinic agents, the major action of atropine is a competitive or surmountable antagonism, which can be overcome by increasing the concentration of acetylcholine at receptor sites of the effector organ (e.g., by using anticholinesterase agents which inhibit the enzymatic destruction of acetylcholine). The receptors antagonised by atropine are the peripheral tissues that are activated or inhibited by muscarine (i.e., exocrine glands and smooth and cardiac muscle). reactions to postganglionic cholinergic nerve stimulation may also be blocked by atropine, although this happens less quickly than with reactions to injected (exogenous) choline esters.[36, 37]

Pharmacodynamics:

Atropine-induced parasympathetic inhibition may be followed by a transitory period of stimulation, notably in the heart where tiny dosages initially decrease the pulse before typical tachycardia occurs owing to paralysis of vagal regulation. Atropine has a greater and longer-lasting impact on the heart, gut, and bronchial muscle than scopolamine, although it has a lesser effect on the iris, ciliary body, and some secretory glands. Unlike the latter, atropine at therapeutic quantities does not depress the central nervous system but may stimulate the medulla and higher cerebral regions. Although moderate vagal excitement occurs, the increased respiratory rate and (occasionally) greater depth of breathing generated by atropine are more probably the effect of bronchial dilatation. Accordingly, atropine is an inconsistent respiratory stimulant, and high or repeated

dosages may reduce breathing. Adequate dosages of atropine eradicate different forms of reflex vagal cardiac slowing or asystole. The medicine also prevents or abolishes bradycardia or asystole generated by injection of choline esters, anticholinesterase compounds or other parasympathomimetic drugs, and cardiac arrest produced by stimulation of the vagus. [35]

Atropine may also lower the degree of partial heart block when vagal activity is an etiologic component. In some individuals with total heart block, the idioventricular rate may be raised by atropine; in others, the rate is stable. Occasionally, a big dosage may produce atrioventricular (AV) block and nodal rhythm. Atropine Sulfate Injection, USP at clinical quantities, counteracts the peripheral dilatation and sudden fall in blood pressure induced by choline esters. However, when administered by itself, atropine does not produce a noticeable or uniform impact on blood vessels or blood pressure. Systemic dosage marginally elevates systolic and reduces diastolic pressures and may create substantial postural hypotension. Such dosages also marginally boost cardiac output and lower central venous pressure. Occasionally, therapeutic dosages widen cutaneous blood vessels, notably in the "blush" region (atropine flush), and may produce atropine "fever" owing to inhibition of sweat gland activity in newborns and young children. The effects of intravenous atropine on heart rate (maximum heart rate) and saliva flow (minimum flow) after I.V. administration (rapid, constant infusion over 3 min.) are delayed by 7 to 8 minutes after drug administration, and both effects are non-linearly related to the amount of drug in the peripheral compartment. Changes in plasma atropine levels following intramuscular administration (0.5 to 4 mg doses) and heart rate are closely overlapping, but the time course of the changes in atropine levels and behavioural impairment indicates that pharmacokinetics is not the primary rate-limiting mechanism for the central nervous system effect of atropine.[38]

Pharmacokinetics:

Atropine dissipates swiftly from the circulation upon injection and is dispersed throughout the body. Exercise, both before to and immediately after intramuscular injection of atropine, considerably enhances the absorption of atropine due to enhanced perfusion in the muscle and significantly lowers the clearance of atropine. The pharmacokinetics of atropine are nonlinear following intravenous injection of 0.5 to 4 mg. Atropine’s plasma protein binding is roughly 44% and saturable in the 2-20 µg/mL

concentration range. Atropine quickly passes the placental barrier and reaches the foetal circulation but is not detectable in amniotic fluid. Much of the medication is eliminated by enzymatic hydrolysis, notably in the liver; from 13 to 50% is excreted. unaltered in the urine. Traces are discovered in many secretions, including milk. The primary metabolites of atropine include noratropine, atropin-n-oxide, tropine, and tropic acid. The metabolism of atropine is hindered by organophosphate insecticides. Specific Populations The elimination halflife of atropine is more than doubled in children under two years and the elderly (>65 years old) compared to other age groups. There is no gender influence on the pharmacokinetics and pharmacodynamics (heart rate alterations) of atropine.[37, 38]

Dosage and administration

Recommended adults dose Repeated antisialagogue or other antivagal 0.5 to 1 mg 1-2 hours Organophosphorus or muscarinic mushroom poisoning 2 to 3 mg 20-30 minutes Brady systolic cardiac arrest 1 mg 3-5 minutes; 3 mg maximum total dosage. Dosing in paediatric populations has not been adequately investigated. Usual first dosage is 0.01 to 0.03 mg/kg.[35, 38]

General Administration Parenteral medication preparations should be evaluated visually for particle matter and discolouration before to administration, whenever solution and container permit. Do not administer until solution is clear and seal is intact. Discard unused part. Intravenous administration is normally preferable, however subcutaneous, intramuscular, and endotracheal delivery are conceivable. For delivery through an endotracheal tube, dilute 1-2 mg in no more than 10 mL of sterile water or normal saline. Titrate depending on heart rate, PR interval, blood pressure and symptoms.[39]

Warnings and precautions

- **Tachycardia:** When the recurrent use of atropine is essential in patients with coronary artery disease, the total dose should be restricted to 2 to 3 mg (maximum 0.03 to 0.04 mg/kg) to avoid the detrimental effects of atropine-induced tachycardia on myocardial oxygen demand.
- **Acute Glaucoma:** Atropine may precipitate acute glaucoma.
- **Pyloric Obstruction:** Atropine may convert partial organic pyloric stenosis into complete obstruction.

- **Complete Urinary Retention:** Atropine may lead to complete urinary retention in patients with prostatic hypertrophy.
- **Viscid Plugs:** Atropine may cause inspissation of bronchial secretions and formation of viscid plugs in patients with chronic lung disease.

Drug interactions

Mexiletine: Atropine Sulfate Injection decreased the rate of mexiletine absorption without altering the relative oral bioavailability; this delay in mexiletine absorption was reversed by the combination of atropine and intravenous metoclopramide during pretreatment for anesthesia.[39]

Overdosage

Excessive dose may produce palpitation, dilated pupils, trouble in swallowing, heated dry skin, thirst, dizziness, restlessness, tremor, weariness and ataxia. Toxic dosages lead to restlessness and excitation, hallucinations, delirium and coma. Depression and circulatory collapse occur only with acute intoxication. In such circumstances, blood pressure drops and death due to respiratory failure may result after paralysis and unconsciousness. The lethal adult dosage of atropine is not known. In pediatric groups, 10 mg or less may be deadly. In the case of toxic overdosage, a short acting barbiturate or diazepam may be administered as required to manage pronounced excitation and convulsions. Large dosages for sedation should be avoided because central depressive activity may coincide with the depression occurring late in atropine overdose. Central stimulants are not advised. Physostigmine, administered as an atropine antidote by gradual intravenous injection of 1 to 4 mg (0.5 to 1 mg in pediatric populations), quickly abolishes delirium and coma produced by massive doses of atropine. Since physostigmine is quickly degraded, the patient may again go into coma within one to two hours, and additional dosages may be necessary. Artificial respiration using oxygen may be essential. Ice bags and alcohol sponges aid to lower fever, particularly in pediatric populations. Atropine is not eliminated by dialysis.[39]

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CHAPTER 05

METHODOLOGY

METHODOLOGY

Materials used:

I. Drug: Atropine sulphate (S. D. Fine-Chem Ltd., Mumbai), Topin® (Batch no. JTN-009, Ordain Health Care Global Pvt Ltd. Tamilnadu, India)
II. Gelling agent: Kolliphor® P 407 (poloxamer) (SIGMA Life Science), Xanthan Gum and Guar Gum (S. D. Fine-Chem Ltd., Mumbai)
III. Salts of artificial tear fluid: NaCl, $NaHCO_3$, CaCl2.2H2O (Merck Life Science Pvt Ltd., Mumbai)
IV. Membrane: Dialysis Membrane-60 (LA390, Av. Flat width-25.27mm, Av. Diameter-15.9mm, Capacity approx.-1.99ml/cm, HiMedia, Mumbai, india)
V. Solvent: Doubled distilled water

Instruments used:

- UV-Vis Spectrophotometer (Model No. 2450, Shimazdu, Japan)
- Magnetic stirrer (Model No. 1MLH, Remi and Techno makes, Mumbai)
- Micropipette (0.5-10µl) (Accupipet V19347)
- Balance (Metler Toledo)
- Brookfield Viscometer (Tokisangyo Company Ltd)
- Magnetic Stirrer (REMI)
- Franz Diffusion Cell (Local purchase)
- Temperature Controlled water bath (Multi Spam TC 44)
- Thermometer (JRM, Kolkata)
- Test tubes, Pipettes, Beakers, Volumetric Flasks, Conical Flasks
- Digital pH meter (Chem Line)

Preparation of Simulated Tear Fluid (STF):

Simulated tear fluid was made using: NaCl, $NaHCO_3$, $CaCl_2 2H_2O$ in water up to 100 g [1], with an ionic strength of 0.188. Required amount of materials are mentioned in *table 5.1*.

Table 5.1: Composition of STF

Sodium Chloride (NaCl)	0.67 g
Sodium Bicarbonate (NaHCO3)	0.20 g
Calcium Chloride Dihydrate (CaCl2,2H2O)	0.008 g
Water (q.s.)	100 g

UV scanning for λ_{max} detection of atropine sulphate:

To determine the wavelength of maximum absorbance (λ_{max}) of atropine sulphate for developing the analytical procedure for its spectrophotometric determinations, a solution of suitable concentration of the drug in STF was prepared and scanned between wavelength of 190-400 nm in a UV-Visible Spectrophotometer.

The **λ_{max} was found to be 206 nm**, which was used for spectrophotometric determination of atropine sulphate in test samples.

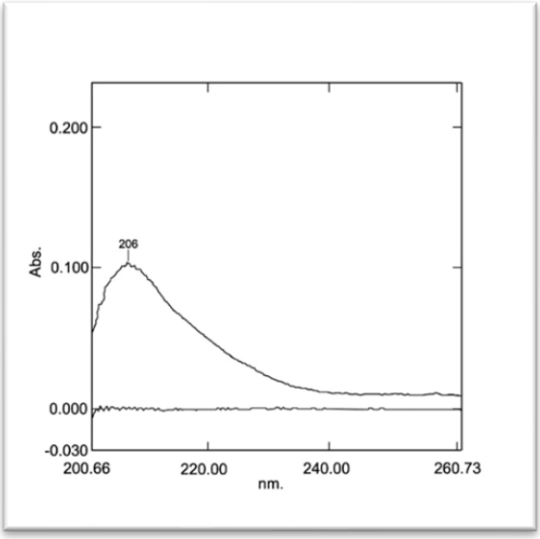

Figure 5.1: UV Scanning for λ_{max} detection of atropine sulphate

Standard curve preparation of atropine sulphate:

For quick and accurate analysis of Atropine sulphate by spectrophotometric method at the determined λ_{max}, an operating calibration curve or standard curve was prepared. A series of standard samples were prepared by using STF. The different concentration of drug (2-16µg/ml) taken and corresponding absorbances are plotted. The corresponding standard curve generated (*figure 5.2)* by linear regression analysis along with the mathematical equation has been calculated and mentioned in *table 5.2.*

Table 5.2: Summarization of standard curve

Slope (m)	**0.02170**
Intercept (c)	0.02992
Regression Coefficient (R^2)	0.9965

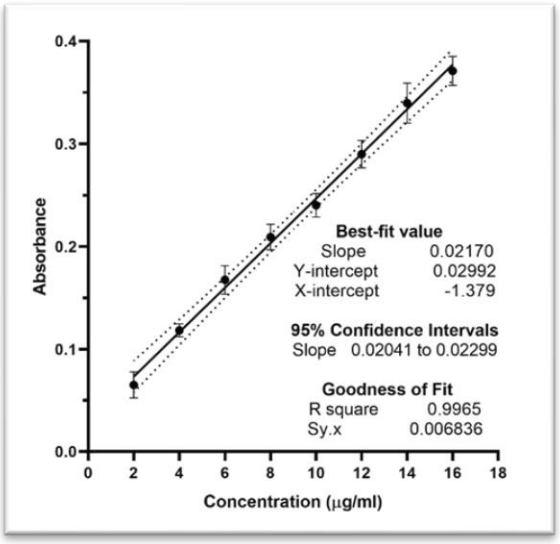

Figure 5.2 Standard Curve of Atropine sulphate at λ_{max} = 207nm

Preparation of polymer solutions

The poloxamer solutions were prepared on a weight basis by using the modified cold method [2]. Ice cold double distill water at 4 °C was taken in a stoppered Erlenmeyer flask and placed on magnetic stirrer in an ice bath with a bead inside and required amount of poloxamer (10-20% w/v) was added at a slow and constant rate until a clear solution of homogenous dispersion is observed. The bead was removed and the solution was kept in fridged for at least 24 h to ensure the complete relaxation and hydration of the poloxamer molecules.

Figure 5.3: Preparation of polymer solutions

Preformulation Studies

Determination of gel temperature and Optimization of poloxamer concentration:

Poloxamer solutions of different concentrations (10-20%) were subjected to in-vitro gelation study by the test tube tilting method. The test tube containing test solution was kept in a water bath, with a thermal cycle of 20°C to 40°C. The gelation temperatures were noted and verified repeatedly by observation of non-flowing state of the solution.

Only 18-20% poloxamer solutions were under gone gelation above 25°C and below body temperature (i.e., 37°C).

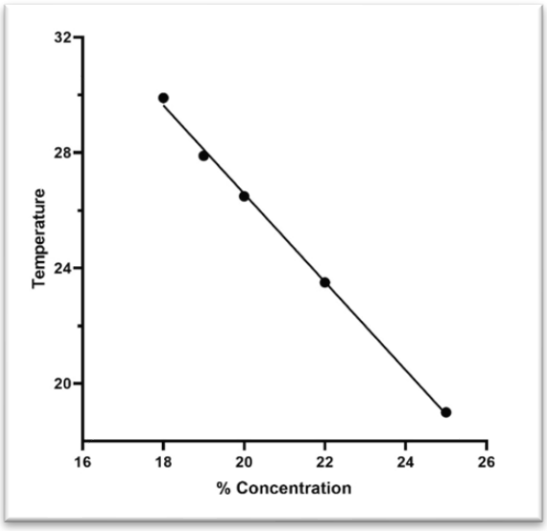

Figure 5.4: Relation between gelation temperature and concentration of poloxamer

Modification of poloxamer solutions below 18% was done by addition of xanthan and guar in different possible ratios and concentrations. Then solutions were subjected to in-vitro gelation study by the test tube tilting method. The test tube containing test solution was kept in a water bath, with a thermal cycle of 20°C to 40°C. The gelation temperatures were noted and verified repeatedly by observation of non-flowing state of the solution.

The xanthan and guar mixture in the ratio of 7:3 was capable to convert poloxamer solution below 18% to clear gel.

It was found that only XG-GG in a ratio of 7:3 was capable to converted the poloxamer solution below 18% into gel below body temperature (i.e., 37°C). All other xanthan-guar ratios either producing hazy gels, or their gel temperatures cannot be optimized correctly which means they cannot be liquid state in room temperature (=25°C) and in gel state in eye temperature (~35°C) simultaneously.

In addition of XG-GG mixture 12-17% of poloxamer solutions were getting gelation. But only 17% poloxamer with XG-GG mixture solution was droppable clear liquid and it was taken for formulation development.

The gelation temperature was confirmed by rotating magnetic siring method, viscosity measurement and rheological properties.

Effect of STF

The ionic strength of tear fluids and their dilution effect on poloxamer solutions has also been taken into consideration before optimizing the formulation. Based on our dosing amount and tear fluid present in our eyes. We have diluted our formulation with STF in a ratio 50:7 [3, 4] and gelling temperature was observed.

Effect of drug addition

Before making optimum poloxamer preparation, the effect of other formulation components on $T_{sol–gel}$ has been taken into consideration as reported in literature [5-8] drug addition may influence the $T_{sol–gel}$ of the poloxamer formulation.

In our study the polymer solutions with addition of 1% (w/v) of atropine sulphate shows no effect on gelation was observed.

Conclusion of Preformulation Studies:

On the basis of preformulation studies, it was concluded that only 18-20% (w/v) of poloxamer solution itself can be converted into gel. The xanthan and guar mixture were capable to convert poloxamer solution below 18% to gel.

Optimization of the effect of xanthan and guar ratio was also done in the development of in-situ sol-gel formulation. The preformulation studies showed that the poloxamer solution modified with xanthan and guar at a ratio of 7:3 undergone thermo-triggered sol-gel transition.

The poloxamer solution below 14-16% was found to be non-droppable highly viscous solution. But only 17% poloxamer xanthan-guar mixture solution was droppable liquid at room temperature.

So, poloxamer solutions 17% was considered for formulation development.

Development of the formulation

A specific amount of xanthan gum: guar gum in the ratio of 7:3 was dispersed into the 17% poloxamer solutions. 1% Atropine sulphate was dissolved into the modified poloxamer solution to obtain sample in-situ gelling solutions.

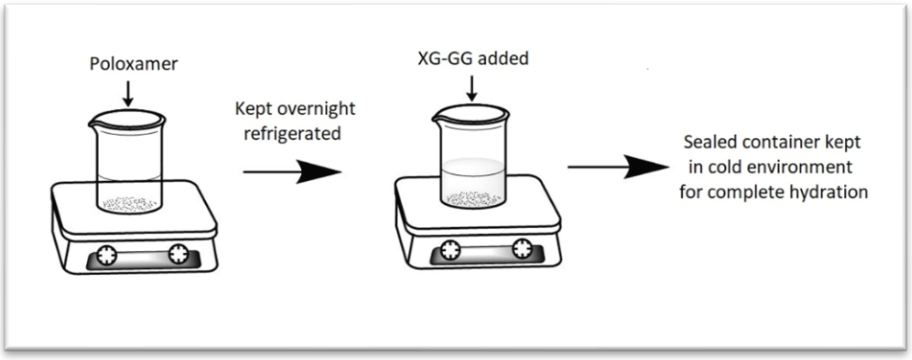

Figure 5.5: Schematic diagram formulation development

Table 5.3: Composition of formulation

Xanthan (%w/v)	Guar (% w/v)	Poloxamer (% w/v)	Deionized water (ml)
0.28	0.12	17	100

Evaluation of the formulation

Organoleptic/clarity test

Organoleptic evaluation was done by looking at the changes in the color, odor, and clarity of the formulation visually on the day of preparation and the 3rd, 5th, 7th, 14th, 21th, and 28th days of storage [9].

The appearance of the prepared in situ gel systems was determined visually. Clarity of the *in-situ* gel was observed against a white and black background for presence of any particulate matter [10-12].

Figure 5.6: Clarity Test

pH

pH test was measured using a calibrated digital pH meter (Chem Line) at pH 4 and 7 [10, 12-14]. The measurements of pH were carried out at room temperature. The pH ranges expected to maintain stability of the in situ gel preparations are 5–7.8 [9].

Gelling capacity

In vitro gelling capacity of the formulation was determined by placing 2 mL of freshly prepared simulated tear fluid (STF) (35°C 1°C) in a vial. We accurately measured 20 µL of in situ gel formulation that was added to STF with mild agitation, which prevented the gel formation from breaking up. Gelling was observed visually. The time taken for its gelling and melting was noted [9, 10, 15].

Figure 5.7: Gelling capacity

In-vitro Gelation Studies

Different techniques have been used for the determination of $T_{sol–gel}$.

The determination of gel temperature of PAS was conducted with test tube tilting method by observing the non-flowing state of the solution. The test tube containing test solution was kept in a water bath, with a thermal cycle of 20°C to 40°C. The gelation temperatures were recorded by visual inspection repeatedly. The PAS solution undergoes gelation below the body temperature [16].

10ml of the sample solution and a magnetic bar were put into a transparent vial that was placed on a heating-magnetic stirrer. A thermometer with accuracy of 0.1 °C was immersed in the sample solution. The solution was heated at the rate of 2°C/min with the continuous stirring of 500 rpm. The temperature was determined as gelation temperature, at which the magnetic bar stopped moving due to gelation [17-19].

We can determine a more accurate gelation temperature with the help of a Brookfield viscometer. The sample was taken in a flat bottom glass tube with an internal diameter 11mm connected with a thermal cycler. Temperature was increased slowly and uniformly at a slow rate. The viscosity is recorded with the viscometer. A sharp increase in viscosity confirms gelation temperature [20].

It can also be determined by rheometer[21-23].

Each sample was measured at least in triplicate. Both of these visual observation methods offer rapid determination of approximate Tsol– gel with minimal equipment, yet, their results are not reliable enough in terms of accuracy and precision [20].

Thermo-gelling Property/Gelation Time (Test-tube Tilting Method)

An experiment was designed to determine the sol-gel transition time of different formulations in the following fashion. 1ml of in-situ gelling solutions of different formulations maintained at 25°C were poured over a dialysis membrane (pre-soaked with ATF) which was tightly bound at one end of a thin-walled glass cylinder of 11mm internal diameter. Tube containing the solution was then immersed into a water bath set at 37°C. Sol-gel transition time was then measured by frequently tilting the glass cylinder up to the point of immobilization of the PAS[24].

Thermo-gelling Property/Gelation Time (Slanting Slide Method)

To mimic the actual ocular physiological environment for determining a more accurate gelling time a set-up has been made shown in *figure 5.9*. A biological membrane has been tightly placed on a glass slide with an inclination 45°. Both of them are maintain physiological temperature of 36°C with a constant flow of water connected to a thermoregulated water bath. The membrane was kept moist with a constant very slow flow rate of STF to keep the slide wet which also ensure the amount of STF present our eye when our formulation has been administered. 60µl of the formulation is dropped perpendicular to the glass slide and the time taken to stop the formulation from flowing has been recorded.

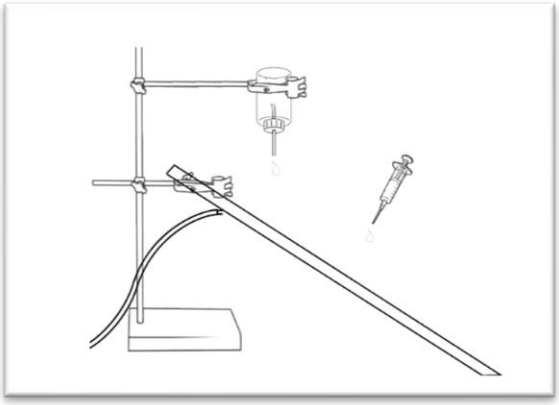

Figure 5.8: Measurement of gel temparature SSL

Viscosity

It can be done using Brookfield Viscometer (Viscometer TV-10, Spindle-M3-Cord-22) equipped with a temperature-controlled mechanism, where the sample is heated slowly and uniformly. The viscosity of the samples was measured at different temperature (and also to measure the gelation temperature). At a constant shear rate (at 20rpm represented eye lid blinking) and Tsol–gel is determined as the temperature at which the sample exhibits an abrupt increase in viscosity [20].

However, the rate of temperature increase has a crucial effect on the accuracy of this method, where rapid temperature increases can result in a higher false $T_{sol–gel}$ being recorded, because of the time elapsed during gel-network formation and the subsequent increase in sample viscosity.

Viscosity was also studied with a rheometer.

Figure 5.9: Viscosity measurement: Brookfield viscometer attached with thermal cycler

Rheological measurements

The solid–gel transition temperature (Tsol/gel) of each formulation under examination was measured using an Anton Paar MCR102 rheometer with a stainless-steel measuring cone CP40-1D (40 mm diameter and 1 angle and a gap of 0.084mm between the cone and plate) and temperature ramp step oscillation procedure. Samples were carefully applied to the lower plate of the rheometer, ensuring that formulation shearing was minimized, and allowed to equilibrate for at least 5 min prior to analysis. Silicone oil was added to the surface of the sample to prevent evaporation of solvent.

In all oscillation experiments, the strain amplitude value was obtained from the linear viscoelastic region of the samples analysed at 25° and 35° C, in which they had lower and higher strength, respectively. The linear viscoelastic region was identified as the region where stress was directly proportional to strain, while the storage modulus (G') remained constant. Following application of a constant stress, frequency of 0.14 Hz, a temperature sweep analysis was performed over the temperature range of 30-45°C, with the temperature being increased at 2°C/min.

[The evaluation was done at constant shear rate d(gamma)/dt = 50 1/s, Speed [1/min] = 8.38, Csr [min/s]: 5.9635, Css [Pa/mNm]: 59.5756]

$T_{sol–gel}$ can be determined more accurately by operating the rotational viscometer in oscillation mode, where the sample temperature is increased slowly with concurrent measurement of both the elastic (storage, G') and the viscosity (loss, G'') moduli. The

elastic modulus is proportional to the energy stored and returned on oscillation, whereas the viscosity modulus is proportional to the energy dissipated in friction. Therefore, G' >G'' in predominantly elastic solids, whereas G'' > G' in predominantly viscous liquids. Tsol–gel can be determined as the point at which G' and G'' intersect [21-23]. The storage modulus (G') and loss modulus (G'') were then determined using RHEOPLUS v3.62 software provided by Anton Paar.

The analyses were performed on at least three replicates of each formulation. The Tsol/gel was considered to be the temperature at which the two moduli were equal (G' and G'' crossover)[25, 26].

Figure 5.10: Rheological measurement: Anton Paar MCR102 rheometer

Rheological profile stress vs strain

The rheological behaviour of the gels was studied in two conditions in the physiologic (37°C) and non-physiologic (25°C) conditions. An ideal gel should show a Newtonian flow in non-physiological condition while, pseudoplastic properties at physiological conditions [28].

Effect of dilution on Gelling temperature

The measurements were made at 30-45°C, the temperature in the conjunctival sac of the eye. To mimic the properties in the eye, if all applied polymer solution (40µl) was immediately mixed with the available tear fluid (7µl), which would be the worst case scenario, the polymer solution was mixed with simulated tear fluid in a ratio of 40:7 [18].

Bio-adhesive Study

Bio-adhesion is an important property of ocular gels to enhance their retention within the eye, prolong drug release, and minimise formulation clearance. The bio-adhesive forces of all the prepared formulae were determined using the bio-adhesive force measuring device. Earlier invitro methods of testing mucoadhesive force was developed by Choi et al. using rectal membrane them [27] and Shastri et al. using sheep cornea [28].

Our device is a modified balance that was developed in our laboratory according to previously reported methods [29-31]. The mucoadhesive force of the formulae under examination was determined by measuring the force required to detach the formulation from a mucin disc using the measuring device. But in our study, we used biological membrane in place of mucin disc to measure the bio-adhesive force.

At the left arm of the balance, membranes were horizontally glued to the both stages of the modified balance by adhesive. The membranes were hydrated with STF for 30s prior to bio-adhesion testing. A drop of our formulation was placed on the lower vertically movable stage of the balance. The in situ forming gel sample was exposed to an electric lamp as a source of heat to allow its gelation. The lower stage was then elevated till the surface of the sample came in contact with the biological membrane. Both the in-situ gel and the hydrated membranes were left in contact for 1 min using a preload to establish the contact between them and allow the formation of an adhesive bond. Excess surface liquid was removed by gentle blotting.

After completion of the preload time, weight was put on and gradually increased by filling water at a constant flowrate of 8.45ml/s on the right pan when the membranes were detached from the tested sample, time is noted and the weight required to detach the tested sample from the biological membrane was calculated. The results were the mean of three runs.

The detachment force (dyne/cm2) was determined using the following equation [32]:

$$\text{Detachment force (dyne/cm}^2\text{)} = \frac{m.g}{A}$$

where m is the weight in grams;

g is acceleration due to gravity taken as 980 cm/sec^2; and

A is the area of the membrane (area of contact).

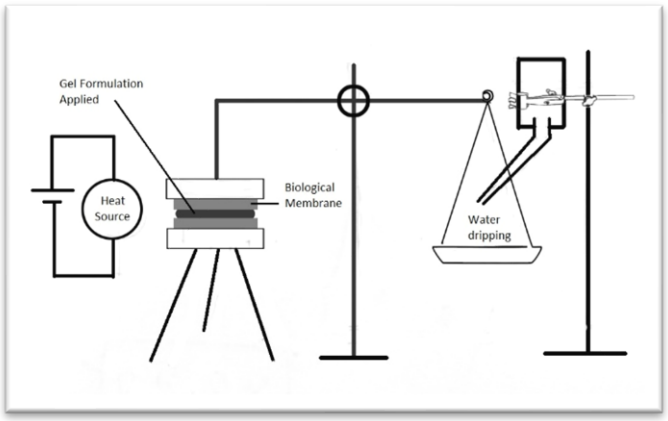

Figure 5.11: Experimental setup Bio-adhesive study

Swelling study

The cell containing artificial tear fluid was used as swelling medium equilibrated at 37°C. A two end open glass tube is taken and one end is tied with biological membrane. A specific volume of formulated solutions was placed inside the tube on the biological membrane and put into the swelling medium. At specific time intervals the tubes were removed from the medium and weight was recorded. Studies were conducted with a cell, equipped with thermo-jacket to maintain constant temperature.

The swelling of the polymer gel as a function of time was determined by using the following relationship,

$$\%S_t = \frac{(W_t - W_0)}{W_t} \times 100$$

Where, S_t is swelling at time 't',

W_0 is the initial weight of the gelling solution and

W_t is the final weight of the gel.

Gel Dissolution Study

Pre-weighed glass vial containing 1ml of developed in-situ gel formulation was kept at 37°C. The weight of the gel was calculated. 1ml of STF pre-warm at 37°C was placed above the formed gel and kept in such a way over the water bath that the temperature of the whole assembly was constant at 37°C. At hourly interval the water was discarded and

weight of the gel was recorded up to 6 hours. The percentage weight loss of the gel was considered as percentage gel dissolution.

In vitro drug dissolution study

The in vitro release set-ups use Franz-cell apparatus. The in-situ gelling formulation an aliquot (1 mL) of the formulation equivalent to 10 mg/mL atropine was transferred to a donor chamber in Franz-diffusion cell; it was then occluded with parafilm. The receptor chamber (42 mL volume) was filled with STF pH 7.4 in such a fashion that the membrane just touches the upper surface of the dissolution media and stirred constantly using small magnetic bar at 20rpm.

Donor and receptor chambers were separated by means of a biological membrane pre-soaked in the receptor medium overnight prior to the experiment. The temperature was maintained at 35 C ± 0.5 C.

Samples (1 mL each) were withdrawn at predetermined time points for up to 8h, and replaced with an equal volume of the receptor medium. The experiments were carried out in triplicate and the samples containing atropine were analyzed by UV spectrophotometer at 206 nm. [33].

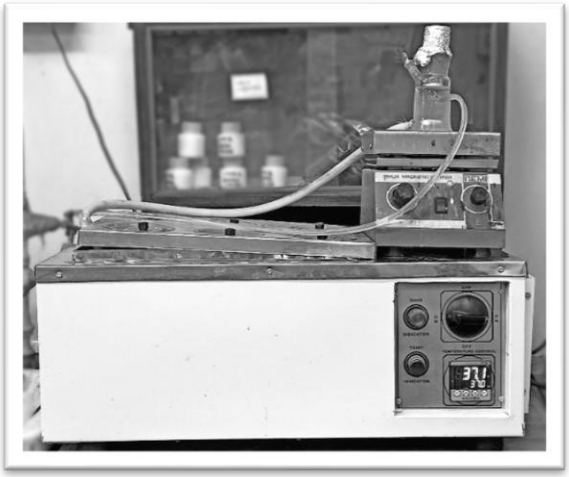

Figure 5.12: In-vitro drug release study carried out in franz diffusion cell

Release Kinetics

Various models like zero order, first order, Higuchi models, and Korsemeyer and Pappas were tested for explaining the kinetics of drug release based on the release data.

To evaluate the mechanism of drug release from in-situ gel, data of drug release were plotted in Korsemeyer et al equation as log cumulative percentage of drug released vs. log time, and the exponent n was calculated through the slope of the straight line.

Mt/M∞ is the fractional solute release, t is the release time, K is a kinetic constant characteristic of the drug/polymer system.

- Zero-order release:

$$\frac{M_t}{M_\infty} = kt$$

- First-order release:

$$ln(1 - \frac{M_t}{M_\infty}) = -kt$$

- The Higuchi square root of time model has been derived from Fick's first law of diffusion and is suited for the modelling of drug release from a homogeneous planar matrix, assuming that the matrix does not dissolve:

$$(\frac{M_t}{M_\infty})^2 = kt$$

- The Korsmeyer-Peppas equation:

$$\frac{M_t}{M} = kt^n$$

n is the diffusion exponent that characterizes the mechanism of release.

According to this equation For film matrix, if

- n=0.45 the drug release mechanism is Fickian diffusion,
- 0.45<n<0.89 the Non-Fickian or anomalous diffusion and if
- 0.8<n<1 a zero-order mechanism is governing the drug release mechanism from the gels [34].

An exponent value of 0.89 is indicative of Case-II Transport or typical zero-order release.

In- Vivo residential Study

The studies were conducted using New Zealand albino rabbit of both sex with weights of 2.9–3.6 kg. The rabbits were kept in restraining boxes throughout the course of each experiment. All tests were performed in the same room under standard lighting

conditions. After 1 h of acclimatization, the basal pupil diameters of the eyes were measured three times using a metric ruler to establish a baseline for both eyes.

The differences in pupil diameter were calculated for each pair of readings. The mean value of the differences was used to convert post instillation data to the baseline corrected values. The gel solutions were kept at room temperature prior to filling the pipette to facilitate this procedure. Developed in situ gel solution were tested on 3 groups each containing three rabbits.

- Group A: Formulation (containing drug as test),
- Group B: Control vehicle (without drug as control) and
- Group C: Topin® (reference standard)

At first, 30µl of control vehicle was administered in the left eye of each animal to eliminate the effect of the polymer if any, followed by instillation of test solution or Topin® to the right eye. The solutions were placed in the lower conjunctivital sac, approximately midway between the inner and outer canthus.

Pupil diameters were taken at pre-determined times and increased pupil diameters were determined using baseline corrected values. The extent of total pharmacological response of the in-situ gels, area under the percentage increase in pupil diameter (AUC) was calculated using trapezoidal rule. Data were presented as the arithmetic means of three experiments with standard error mean (SE).

The efficiency of the gel formulations was estimated by the peak mydriatic response (PR), peak response time (PRT) and the AUC after administration of the respective formulation. The duration of mydriatic response (DR) was defined as the time interval between administration of the treatment and the time at which the pupil diameter returned to its normal pre-treatment value.

The animal studies were permitted by the Institutional Animal Ethics Committee (IAEC no. JU/IAEC-22/41 and CPCSEA Registration no.1805/GO/Re/S/15/CPCSEA dated 11.03.2015)[24, 35].

Figure 5.13: In-vivo study measuring pupil diameter

Statistical analysis

The percentage of drug release at 4 hours and pharmacodynamic parameters of the developed formulations were analysed by one way ANOVA followed by Dunnette's post hoc test of significance where $p<0.05$ and $p<0.01$ were considered to be significant and highly significant respectively (Graph Pad prism Software, Version 8.03, Graph Pad Software Inc, sandiego, CA). The analysis was based on the comparison of the drug release and pharmacodynamic parameters from marketed product Topin® with developed formulation PAS in in-vitro studies.

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CHAPTER 06

REASULT AND DISCUSSION

REASULT & DISCUSSION

Charecterization

Table 6.1: Charecterization of developed formulation

Formulation	Formulation
Appearance	Clear
pH	7.6 ±0.3
Thermo-gelling time (Sec.) TTL	8.33 ±0.33
Thermo-gelling time (Sec.) SSL	3.4±0.5
% Swelling at 6h	30.59 ±12.23
Gel temperature (Test tube tilting) (°C)	35.0±0.6
Gel temperature (Viscosity) (°C) Brookfield	35.8
Viscosity at gel temperature (Pa.s) Brookfield	0.747±0.220
Gel temperature (Viscosity) (°C) Rheometer	34.53
Viscosity at gel temperature (Pa.s) Rheometer	1.279

Gel temperature

Table 6.1 and *table 6.6* shows the effect of Xanthan gum-guar gum on gelling temperature of 17% Kolliphor® P 407 solution. It had been found from the study that increment of Xanthan gum-guar gum concentration significantly lowered the gelling temperature of the polymeric system below the normal body temperature. It seemed that this lowering of gel temperature may be due to conformational changes in polymer chain. A polymer molecule in aqueous dispersion exhibits stronger interaction(s) with water than those with hydrogen bonds between water molecules. When temperature is increased, some of the original hydrogen-bonding network (including cage like structures) formed by water molecules is destroyed. This is due to the decrease of solubility in water leads to more hydrophobic-hydrophobic interaction between polymer molecules.

Thus, upon increase in polymer concentration it is be easier for a polymer solution to meet the requirement for the critical number of hydrophobic aggregates to form a gel, so

that the sol-gel transition occurs at a lower temperature. It was also found that less than 18% Kolliphor® P 407 solution alone cannot undergo sol-gel transition. The addition of Xanthan gum-guar gum in 17% Kolliphor® P 407 increased the total polymer content results in fewer free water molecules available around polymer chains and a stronger hydrophobic environment achieved by the 17% Kolliphor® P 407 for conversion into gel. It had been observed from the study that addition of 0.4% w/v Xanthan gum-guar gum in 17% Kolliphor® P 407 was capable to ensure the gelling temperature appearing below body temperature and the solutions were free flowing liquid to allow reproducible instillation into the eye as drops at 25°C. Also, it had been observed that the in-situ gelling solutions become non-flowable when Xanthan gum-guar gum in 17% Kolliphor® P 407 was more than of 0.4% w/v.

From the *table 6.6*, it was observed that little variation in gel temperature between test tube tilting method, magnetic stirring method and viscosity measurement was observed. Confirmatory gel temperature study was done by rheometer. Rheological analysis confirms the gel temperature of the developed formulation was found to be 34.53°C.

Fourier Transform Infrared (FTIR)

From the *Figure 6.1a*, it was observed that all the major peaks of the individual polymers (S1, S2, S3) are distinctly present in their mixture (S4). For the drug, we have matched it with the FTIR profiles of IP. Observed similarity between them indicates the purity of our API.

From the *Figure 6.1b* we can also observe presence all the major peaks of S4 and S5 in their mixture S6. From this FTIR studies we can clearly conclude drug and polymers were distinctly appeared in their mixture. So, no such interaction was there between composite materials and with the drug in our formulation.

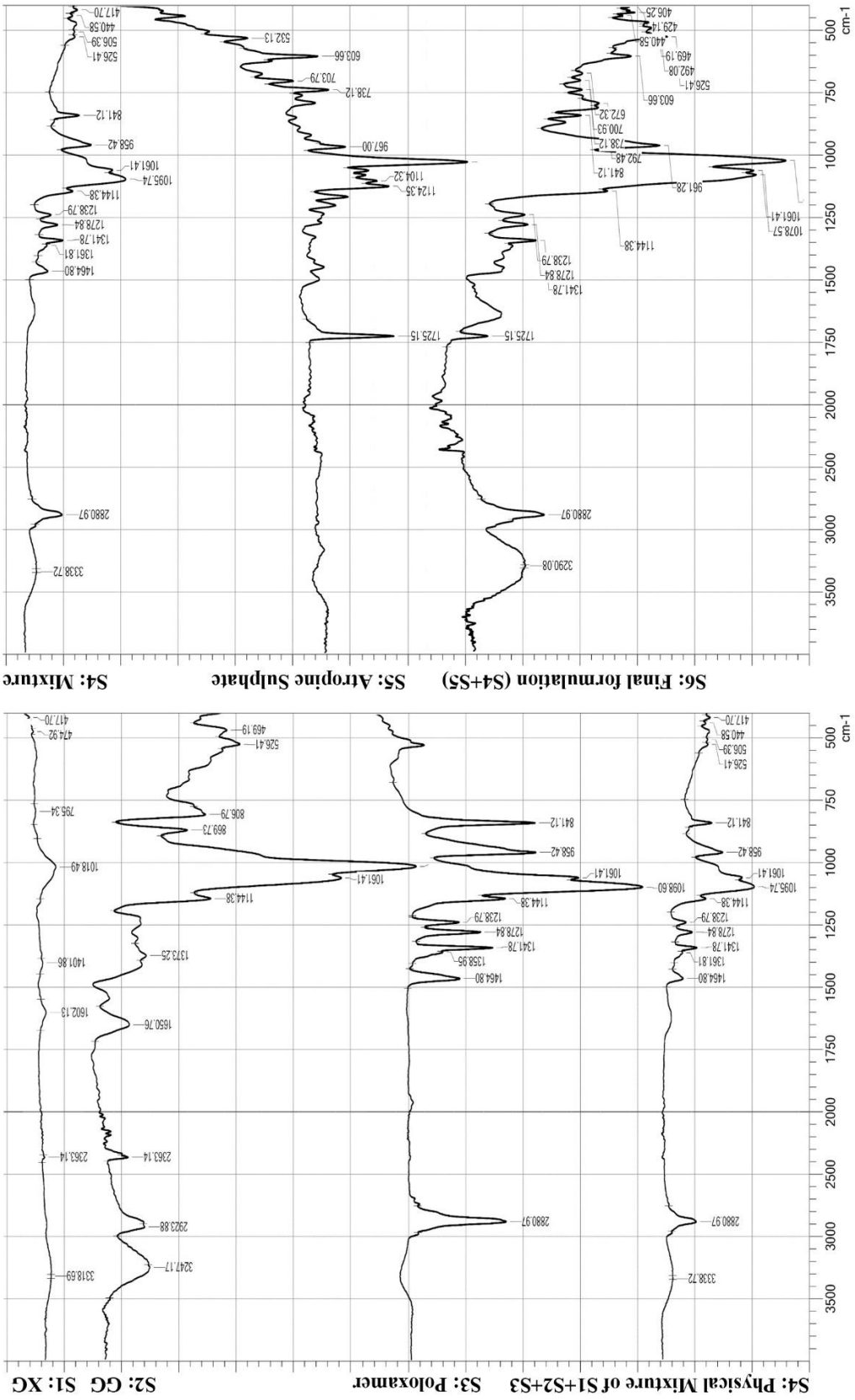

Figure 6.1: Comparison of IR data of the components in the formulation

Evaluation of the formulation

Organoleptic Test

The appearance of the prepared *in-situ* gel systems was determined visually against a white and black background. It was observed that on the day of preparation of the formulation, it was an odourless, slightly greenish, opaque liquid. During storage of the formulation as the hydration of the polymers takes places it appeared as colourless, odourless and transparent after 21days. Detailed observations are tabulated in *table 6.3*.

Table 6.2: Observation table for Organoleptic Test

Day	Color	Odour	Clarity
0,3,5,7	Slightly Greenish/ Cream colour	Odourless	Opaque
14	Colorless	Odourless	Translucent
21,28	Colorless	Odourless	Transparent

pH

pH of the formulation takes an important role in ophthalmic drug delivery. The pH of tear fluid is around 7.4. The pH of the developed formulation was near to the pH of tear fluid (*table 6.4*) that does not cause irritation to eye.

Table 6.3: Observation Table for pH of the formulation

Formulation without drug	7.8±0.3
Formulation with drug	7.6±0.3

Gelling capacity

From the *table 6.1* and *table 6.5* it was confirmed that the developed in-situ gelling solution undergo gelation within 4-8 second. The melting of the formed gel was observed to be within 12 minutes under experimental condition. The solutions exhibited gel-sol transition on cooling.

Table 6.4: Observation table for Gel strength

Time taken for gelling	4s
Time taken for gel melting	753.5±31.4

In-vitro gelation studies

Different techniques were used to study in-vitro gelation study. Variation in gelation temperature was observed in different techniques may be due to the difference in thickness of the in-situ gel layer exposed in different techniques.

Table 6.5: Summary table gelation temperature

Test Tube Tilting Method	35.0±0.6°C
Magnetic Bar Stirring Method	35.4±2.1°C
Brookfield Viscometer	35.8±0.1°C
Rheometer	34.53°C

Thermo-gelling property

The thermos-gelling properties of developed formulation was evaluated and result is shown in *Table No. 6.1*. It was observed that the formulation was converted to gel within 7-8 Seconds. The solutions exhibited gel-sol transition on cooling.

Viscosity

Viscosity study of the developed formulation has been conducted using Brookfield viscometer and verified by Rheometer. At the gel temperature the viscosity of the developed formulation was found to be 0.747 Pa.s in Brookfield viscometer (*figure 6.2*) whereas it was 1.279 Pa.s in Rheometer (*figure 6.3*). So, this difference is due to this difference may be due to the quantity of composite employed and the thickness of gel layer exposed.

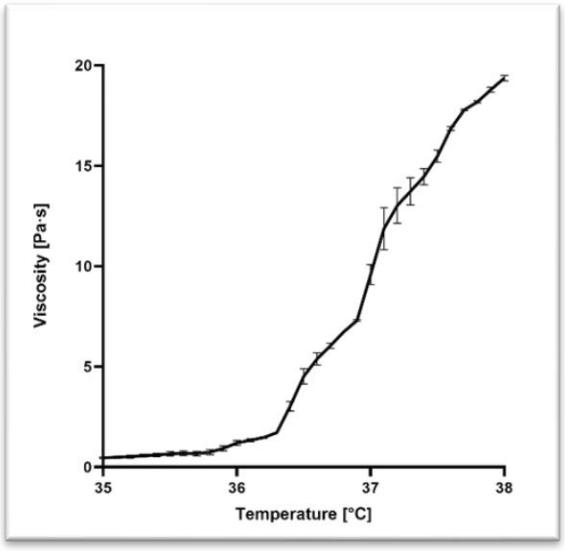

Figure 6.2: Measurement of viscosity with time (Brookfield Viscometer)

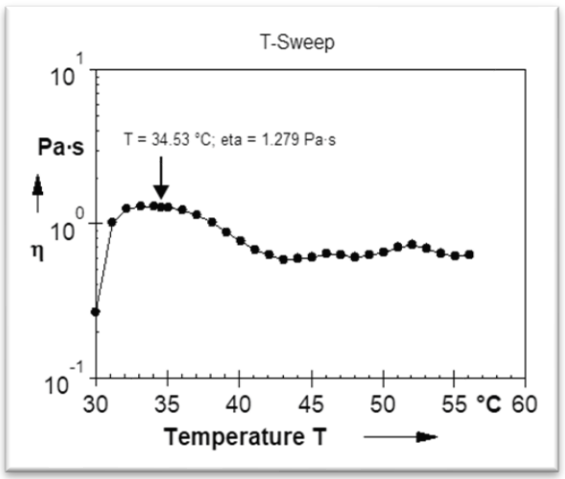

Figure 6.3: Measurement of viscosity with time (Rheometer)

Rheological measurements

The viscosity increased significantly with increase in temperature of our formulation. At non physiological condition (at 35°C) it nearly shows the property of newtonian fluid. But in physiological condition after formation gel of the prepared formulations it exhibited a characteristic pseudoplastic (shear thinning) flow behaviour (*figure 6.4*).

This is a desirable characteristic for ophthalmic in-situ gel. During blinking of our eye lid when shear stress will be applied the viscosity of the gel will decrease. So, this will not cause any hindrance to eye blinking. Again, when the lid is open the gel structure will regain which ensure the sustained release of drug from the gel.

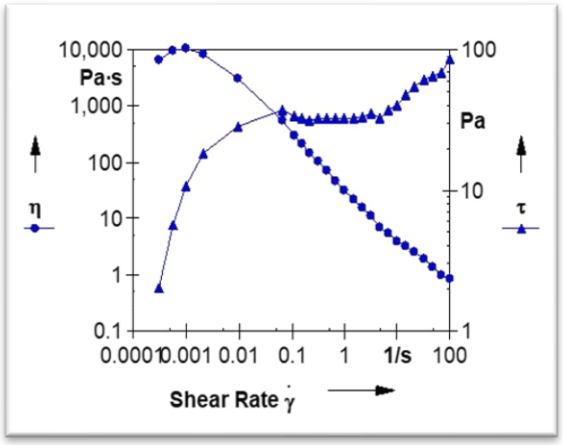

Figure 6.4: Rheological profile of the formulation after gel formation

Effect of dilution on Gelling temperature

On dilution of the formulation with specified amount of freshly prepared STF a slight increase in gelling temperature and slight decrease in viscosity of the gel has been observed. It does not show any major effect on the sustained release characteristics of formulated in-situ gel.

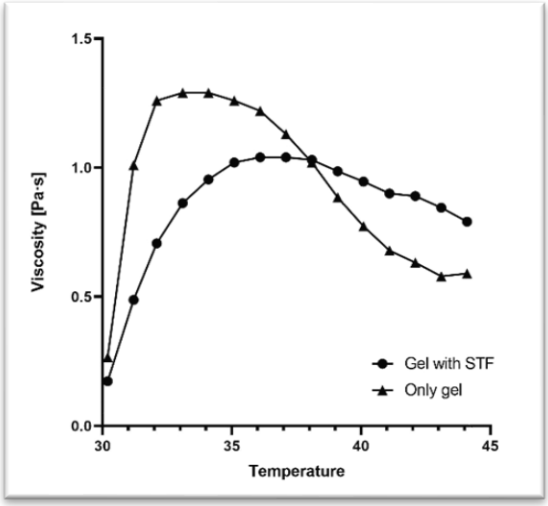

Figure 6.5: Effect of dilution on gelation temperature

Bio-adhesiveness

The experiment was performed in triplicate and result is calculated using their mean. The force required to detach two membranes was 183.25±10.25 g.wt.

The area of each membrane exposed to the formulation is 6.25±0.01 cm^2.

$$\text{Detachment force (dyne/cm}^2) = \frac{183.25*980}{6.25} = \mathbf{28,733.6 dyne/cm^2}$$

Swelling study

The swelling performance revealed that after 6hours 35.72% increase in gel weight was observed (*figure 6.6*). The rate of swelling per hour was found to be 12.33% which is compatible with eye environment since lachrymal turnover and secretion rate are 6.5-10.7 µl/min and 0.5-2.22 µl/min respectively. So, it does not facilitate eye dryness.

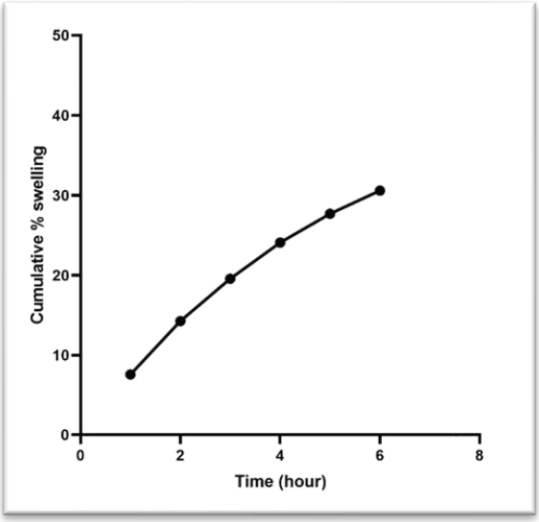

Figure 6.6: Swelling Study (35.72% swelling in 6h at a rate 12.33%/hour)

Gel Dissolution Study

From the *figure 6.7* it was observed that about 81% of developed in-situ gel dissolved after 6h in the experimental condition.

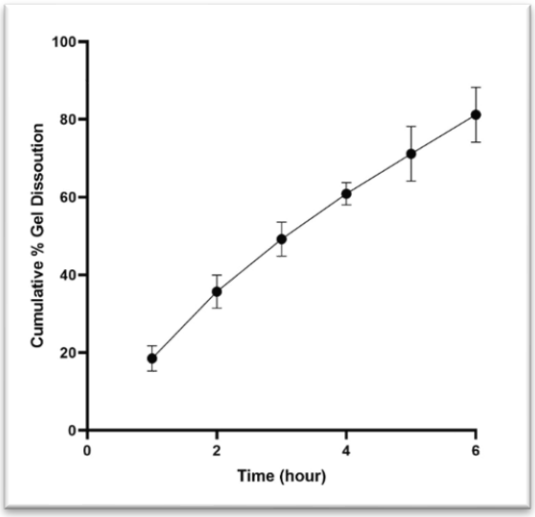

Figure 6.6: Gel Dissolution Study (81% dissolution in 6h)

In-vitro Drug Release

The in-vitro drug release profiles of the developed formulation (Poloxamer Atropine Sulfate-PAS), marketed product Topin® and drug solution (Solution of Atropine Sulfate-SAS) as a function of time have been shown in *figure 6.8*.

It was observed that drug release was more sustain from PAS gel matrix. When the formulation come in contact with the artificial tear fluid at 37°C and gelation occurs, a pre hydrated gel matrix was formed in which water penetration and hydration becomes the rate limiting step of drug release process. If water penetration was faster, hydration and drug release would be faster, i.e., sustained drug release would not be achieved. The reason behind the extension of drug release time with corresponding developed PAS seems to be the increase in viscosity as a result of slower penetration of solvent into the core region, hence slower drug diffusion occurred.

In the view point of sustained drug release of all developed formulation PAS (about 41% drug release) showed better drug release profile than SAS and Topin® (about 100% drug released) after 4 h. It was observed that the developed formulation PAS released 56% drug at 8 hours.

Table 6.6: In-vitro drug release study

Time (min)	SAS	Topin®	PAS
240	99.94±0.27	100.4±0.39	41.06±4.97****
480	-	-	55.97±6.25

% drug releases of SAS, Topin and PAS at 4h was compared by one way ANOVA followed by Dunnette's post hoc test of significance where $p < 0.05$ and $p < 0.01$ are considered to be significant and highly significant respectively (Graph Pad prism Software, Version 8.0.1, Graph Pad Software Inc., San Diego, CA). The release data of PAS at 4 hours was significantly (****p<0.0001) differ from SAS and Topin® at 5% level of significance (*table 6.7*).

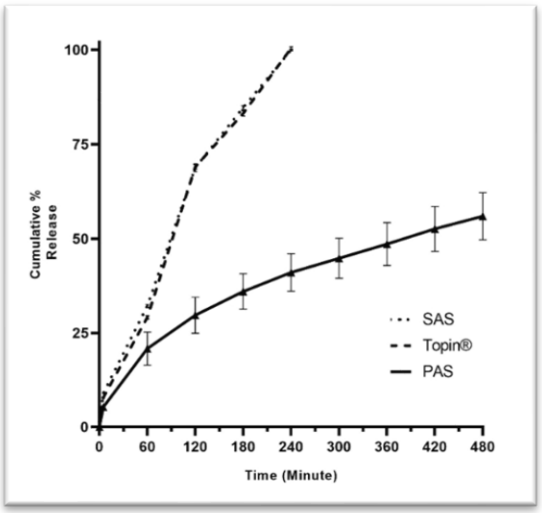

Figure 6.7: In-vitro drug release study

Release Kinetics

All the in-vitro release data were analysed by different kinetic equations (Zero order, first order and Higuchi's equation) to interpret the release pattern from in-situ gel formulation PAS. From the *table 6.8*, it was observed that the in-vitro drug release was best described by Higuchi's model, since the highest linearity $r^2 > 0.997$ was found followed by first order and zero order. All the release data were fitted to the Korsmeyer-Peppas equation and acceptable linearity ($r^2 > 0.971$) was observed for developed formulation PAS. The release exponent 'n' was found to be 0.48, indicating non-Fickian drug diffusion that

indicates diffusion with slightly erosion mechanism associated (Anomalous diffusion) from the formed gel at eye environment.

Table 6.7: Release kinetics

Zero-order	First-order	Higuchi	Korsmeyer-peppas	
r^2	r^2	r^2	r^2	n
0.91±0.016	0.965±0.003	0.997±0.003	0.997±0.002	0.48±0.04

In-vivo Studies

Figure 6.9 shows the pharmacological response as a percentage increase in pupil diameter vs. time profiles for developed formulation and Topin®. All the pharmacodynamic parameters like Area under the pharmacological response curve, peak response time, peak response and duration of response of the developed formulation was compared with Topin® by one way ANOVA followed by Dunnette's post hoc test of significance where $p < 0.05$and $p < 0.01$ are considered to be significant and highly significant respectively (Graph Pad prism Software, Version 8.0.1, Graph Pad Software Inc., San Diego, CA).

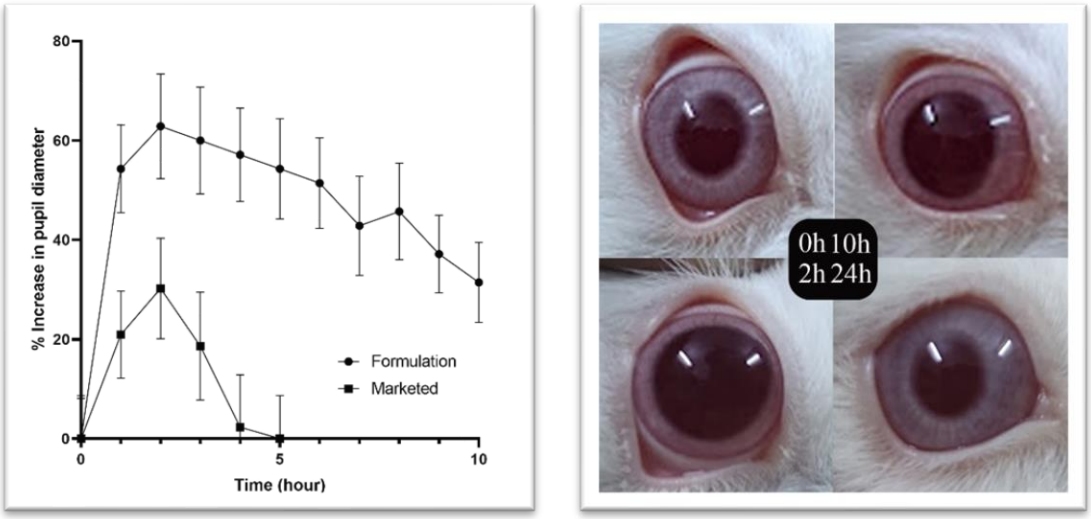

Figure 6.8: Mydriatic response of developed in situ gel formulation

From the *table 6.9*, it is found that all the parameters, area under the pharmacological response curve (***p<0.001), peak response (**p<0.01) and duration of response (****p < 0.0001) are significantly different from Topin® except peak response time.

Table 6.8: In-vivo performance of developed formulation as compared to Topin®

Formulation	AUC	PRT	PR	DR
Topin®	117.25±10.60	2	42.5±3.86	4.70±0.58
Formulation	487.30±89.20***	2±1	63.40±9.4**	>10.00****

The results indicated that 4 times increase in pharmacological response, 1.5 times in peak response and 2 times in duration of response are observed for developed formulation in a 10 hours of study protocol relative to Topin®. From the results, it is concluded that the developed in-situ gelling formulation comprising of poloxamer solution along with XG–GG may significantly increase the residential time into the corneal surface and thus improve ocular bioavailability.

CHAPTER 07

CONCLUSION

CONCLUSION

Thermo-triggered in-situ gelling vehicle based on poloxamer with xanthan gum guar gum has been successfully developed using Atropine Sulphate as a model drug. Poloxamer was used as a gelling vehicle. The aquas poloxamer solution at 18% and above concentration was droppable liquid at room temperature and underwent fast gelation in exposure to the eye environment. Below the 18% poloxamer concentration, the solution was unable to gelation alone.

To reduce the poloxamer concentration in the development of an in-situ gelling vehicle or to make a non-gellable poloxamer solution to an in-situ gellable one, additive polymers, Xanthan gum and Guar Gum were studied at different ratios and concentrations. The non-gellable poloxamer (17-10%) solution was studied with XG-GG mixture. The mixture of XG-GG at 7:3 ratio was able to convert non-gellable poloxamer solution to in situ gel. Other ratios of XG-GG were found to produce hazy gels. It was observed that only 17% poloxamer with 0.4% XG-GG at 7:3 ratio form in situ clear gel droppable at room temperature. All other compositions with poloxamer below 17% and appropriate concentrations (in situ gellable) of XG-GG at 7:3 ratio were non-droppable.

The developed solution was droppable liquid at room temperature and underwent gelation at 34.53°C within 4-8 seconds and form a clear film matrix. It facilitates in accurate dosing, patient compliance and no interference in vision.

The pH of the formulation was determined to be 7.6 which is near to our physiological ophthalmic pH 7.4. It does not create any irritation due to the high buffer capacity of tear fluid which neutralizes the high pH immediately since the instilled dose will be 1-2 drops.

The rate of swelling was found to be 12.33% per hour which is compatible with eye environment due to the tear turnover and secretion rate are 6.5-10.7 µl/min and 0.5-2.22 µl/min respectively. So, it does not facilitate eye dryness.

From the rheological study, it was found that developed in-situ gel following pseudoplastic flow with 1.279 Pa.S viscosity at gel temperature. Therefore, no hindrance in the eyelid blinking will be occurred.

The gel temperature, swelling and rheological properties of solution were not affected by the incorporation of the drug.

In-vitro drug release studies revealed that the developed in-situ gel formulation deliver drug as a sustained manner in 8 hours (56% release) experimental period. After 4h of dissolution, only 41% of drug was released from the developed formulation whereas 100% drug released was observed from pure drug solution (SAS) and marketed product Topin®. The % drug release at 4h from developed formulation was analysed by one way ANOVA followed by Dunnette's post hoc test at 5% level of significance as compared with SAS and Topin® where $p < 0.05$ and $p < 0.01$ are considered to be significant and highly significant respectively. The release data of PAS at 4 hours was significantly (****$p<0.0001$) differ from SAS and Topin®.

The in-vitro release data were analysed by Zero order, First order and Higuchi's equation and it was observed that the release was best described by Higuchi's model, since the highest linearity r2 > 0.997 was found followed by first order and zero order. From the Korsmeyer-Peppas equation, the acceptable linearity ($r^2 > 0.971$) was observed for developed formulation PAS and the release exponent 'n' was found to be 0.48 indicating non-Fickian drug diffusion or Anomalous diffusion mechanism associated from the formed gel at eye environment.

The pharmacological response as a percentage increase in pupil diameter vs. time profiles for developed formulation and Topin® was studied. All the pharmacodynamic parameters like Area under the pharmacological response curve, peak response time, peak response and duration of response of the developed formulation was compared with Topin® by one way ANOVA followed by Dunnette's post hoc test of significance where $p < 0.05$and $p < 0.01$ are considered to be significant and highly significant respectively. It was found that all the parameters, area under the pharmacological response curve (***$p<0.001$), peak response (**$p<0.01$) and duration of response (****$p < 0.0001$) are significantly different from Topin® except peak response time. The results indicated that 4 times increase in pharmacological response, 1.5 times in peak response and 2 times in duration of response are observed for developed formulation in 10 hours of study relative to Topin®. Finally, in-vivo studies in rabbit confirmed a prolonged retention time of this formulation.

Finally, it was concluded that the developed in-situ gelling formulation comprising of poloxamer solution along with XG–GG may significantly increase the residential time into the corneal surface and thus improve ocular bioavailability. So, the developed in situ

gelling vehicle may be a viable alternative to conventional eye drops by virtue of their ability to sustain drug release profile and may decrease frequency of administration resulting in better patient acceptance.

CHAPTER 08

FUTURE WORK

FUTURE WORK

In this research work temperature sensitive ophthalmic in-situ gelling vehicle had been developed and characterized successfully.

Due to short duration of this project work evaluation of prepared in-situ ophthalmic gel has been limited to gelation study, viscosity, rheology, swelling, gel dissolution, in-vitro drug release and in-vivo residential study.

For further improvement and making this a commercial product many more testing is needed to be done.

In the future the following investigations are planned to continued:

- Mucoadhesive study
- Stability Study
- Texture Profile
- Study with other drugs
- Anti-microbial study
- Study on human