DEVELOPMENT AND IN-VITRO EVALUATION OF THERMO-SENSITIVE IN-SITU OPHTHALMIC GEL

Submitted by

EKTA YADAV

B. Pharm

EXAM ROLL NO.: M4PHP22008

REGISTRATION NO.: 154286 OF 2020-2021

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
Faculty of Engineering and Technology
JADAVPUR UNIVERSITY
Kolkata-700032
Session: 2020-2022

CERTIFICATE OF APPROVAL

This is to certify that **EKTA YADAV** has carried out her M. Pharm thesis on the topic "**DEVELOPMENT AND IN-VITRO EVALUATION OF THERMO-SENSITIVE IN-SITU OPHTHALMIC GEL**" (Examination Roll No.: **M4PHP22008**) under my direct supervision in the division of **Pharmaceutics** at the **Department of Pharmaceutical Technology, Jadavpur University**. She has incorporated his finding 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 she has carried out this work with proper care attention to my entire satisfaction.

Prof. (Dr.) L. K. Ghosh
Project Supervisor
Division of Pharmaceutics
Department of Pharmaceutical Technology
Faculty of Engineering and Technology
Jadavpur University

Prof. Sanmoy Karmakar
Head of the Department
Department of Pharmaceutical Technology
Faculty of Engineering and Technology
Jadavpur University

Prof. Chandan Mazumdar
Dean
Faculty of Engineering and Technology
Jadavpur University

Evaluator

DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains literature survey and original research work by me, as part of my Master of Pharmacy studies.

All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name : EKTA YADAV

Roll No. : M4PHP22008

Thesis Title : **DEVELOPMENT AND IN-VITRO EVALUATION OF THERMO-SENSITIVE IN-SITU OPHTHALMIC GEL**

Date: 25th August, 2022

(EKTA YADAV)

CERTIFICATE OF APPROVAL

This is to certify that **EKTA YADAV** has carried out her M. Pharm thesis on the topic "**DEVELOPMENT AND IN-VITRO EVALUATION OF THERMO-SENSITIVE IN-SITU OPHTHALMIC GEL**" (Examination Roll No.: **M4PHP22008**) under my direct supervision in the division of **Pharmaceutics** at the **Department of Pharmaceutical Technology, Jadavpur University**. She has incorporated his finding 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 she has carried out this work with proper care attention to my entire satisfaction.

25/08/22

Prof. (Dr.) L. R. Ghosh
Project Supervisor
Division of Pharmaceutics
Department of Pharmaceutical Technology
Faculty of Engineering and Technology
Jadavpur University

25/8/22

Prof. Sanmoy Karmakar
Head of the Department
Department of Pharmaceutical Technology
Faculty of Engineering and Technology
Jadavpur University

25/8/22

Prof. Chandan Mazumdar
Dean
Faculty of Engineering and Technology
Jadavpur University

DEAN
Faculty of Engineering & Technology
JADAVPUR UNIVERSITY
KOLKATA-700 032

Evaluator

DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains literature survey and original research work by me, as part of my Master of Pharmacy studies.

All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name : EKTA YADAV

Roll No. : M4PHP22008

Thesis Title : **DEVELOPMENT AND IN-VITRO EVALUATION OF THERMO-SENSITIVE IN-SITU OPHTHALMIC GEL**

Date: 25th August, 2022

Ekta Yadav.

(EKTA YADAV)

ACKNOWLEDGEMENT

I take this opportunity to humble acknowledge the contributions of my supervisor, **Prof. (Dr.) L. K. Ghosh**, Division of Pharmaceutics, Department of Pharmaceutical Technology, Jadavpur University, Kolkata for his invaluable guidance and constant encouragement to materialize this piece of work and I express my heartfelt gratitude and thankfulness for his encouragement all through.

This acknowledgement will be incomplete if I do not express my sincere thanks to the team of our Pharmaceutics-II Laboratory, Department of Pharmaceutical Technology, Jadavpur University.

I extend my thanks to my co-guide **Dr. Manas Bhowmik** (Assistant Professor, Jadavpur University), **Prof. (Dr.) Dipankar Chattapadhyay** (Department of Polymer Science and Technology, University of Calcutta) and **Koushik Dutta** for providing polymers and chemicals and also my seniors of different laboratories and lab assistants for their help and guidance throughout my thesis work. I am also thankful to my batch mate Md. Zeeshanur Islam and my juniors Saban Karmakar, Rinchen Palzong Bhutia, Bikram Roy and Moumoyee Chakraborty.

I express my thanks to UGC, Ministry of Human Resource and Development, Government of India, for providing the financial assistance in the form of fellowship.

At last, but not the least, I express my sincere regards to my family for their inspiration and motivation throughout my studies and all they have done for me during my studies.

CONTENTS

ABBREVIATIONS vii
LIST OF FIGURES viii
LIST OF TABLES ix
INTRODUCTION 2
Ocular pharmacokinetics: barriers in drug delivery 4
Reasons for poor bioavailability of ocular product 5
In-situ forming gels 6
Advantages 6
Brief Insight 6
Mechanisms of drug release from hydrogels 7
Release mechanism from hydrogel matrices 7
Mechanisms of drug release from In-Situ hydrogels can be categorized as 7
Desired properties of polymers used in ophthalmic hydrogels as drug delivery systems 8
In-situ forming hydrogels 8
Methods of loading of hydrogels as drug carriers 8
Advantages of in-situ forming hydrogels 9
Poloxamers (Kolliphor®) 9
LITERATURE REVIEW 11
AIM OF WORK 16
DRUG AND POLYMER PROFILE 19
Atropine Sulphate 19
Description 20
Clinical pharmacology 21
Dosage and administration 23
Dosage forms and strengths 24
Contraindications 24
Warnings and precautions 24
Adverse reactions 24
Drug interactions 25
Uses in specific populations 25

Overdosage 25
Nonclinical toxicology 26
Poloxamer 407, Lutrol F 127 (Kolliphor® P 407) 27
Xanthan gum 29
Structure 29
Properties 30
Applications and Limitations 31
Guar Gum 33
Functions and Applications 34
METHODOLOGY 37
Preformulation studies 37
Materials used 37
Instruments used 37
Preparation of Artificial Tear Fluid (ATF) 37
UV Scanning for λ_{max} detection of Atropine sulphate 38
Standard Curve Preparation of Atropine sulphate: 38
Preparation of polymer solutions 39
Determination of gel temperature and Optimization of Kolliphor® P 407 concentration 40
Modification of Kolliphor® P 407 solutions 40
Effect of Drug on Gel Temperature 40
Conclusion of preformulation studies 41
Formulation Development and Evaluation 41
Preparation of sample solutions 41
Composition of *in-situ* gelling solutions 41
Determination of pH 41
In-vitro gelation studies 41
Thermo-gelling Property 42
Rheological Evaluation 42
Swelling study 42
In-vitro gel dissolution studies 43
In-vitro drug release studies 43
Release Kinetics 44
Statistical analysis 44
Clarity test 44

RESULTS AND DISCUSSIONS..... 46

Gel temperature 46

Thermogelling Properties.....47

Rheological properties 47

Swelling Study 48

***In-vitro* gel dissolution studies.....49**

***In-vitro* drug release studies 49**

Clarity test.....51

CONCLUSION 53

FUTURE WORK..... 55

REFERENCES 57

ABBREVIATIONS

SAS	Solution of atropine sulphate
PAS	Poloxamer atropine solution
ATF	Artificial tear fluid
PM	Poloxamer
XG	Xanthan gum
GG	Guar gum
GT	Gelation temperature
PEO	Polyethylene oxide
PPO	Poly propyleneoxide
PS	Poloxamer solution
TTM	Test tube tilting method
SI	Swelling index
TGP	Thermogelling property
CAT	Cellulose acetate phthalate

LIST OF FIGURES

Figure 1: Anatomy of eye 5
Figure 2: Structure of atropine sulphate 19
Figure 3: Structure of Poloxamer 27
Figure 4: Molecular structure of xanthan gum 29
Figure 5: Molecular structure of guar gum 33
Figure 6: UV Scanning for λ_{max} detection of Atropine sulphate 38
Figure 7: Standard Curve Preparation of Atropine sulphate 39
Figure 8: Preparation of polymer solutions 39
Figure 9: In-vitro drug release studies 43
Figure 10: Effect of temperature on viscosity of developed in-situ gel solution 47
Figure 11: In-vitro Gel swelling profile of developed in-situ gel formulation PAS in artificial tear fluid at 37°C (n=3) 48
Figure 12: In-vitro Gel dissolution profile of developed in-situ gel formulation PAS in artificial tear fluid at 37°C (n=3) 49
Figure 13: In-vitro drug release profile of developed in-situ gel formulation PAS, SAS and Tropin® in artificial tear fluid at 37°C (n=3) 50
Figure 14: Clarity Test shown by Test tube Tilting Method 51

LIST OF TABLES

Table 1: Composition of in-situ gelling solutions 41
Table 2: Characterisation of in-situ gel formulation (n=3) 46
Table 3: Comparison of in-vitro drug release profile of developed in-situ gel formulations with SAS and Topin® (n=3) 48
Table 4: Drug release kinetics of developed in-situ gel formulations (n=3)..... 50

Dedicated

to

My Beloved Family

CHAPTER 1

INTRODUCTION

INTRODUCTION

The most common route of drug delivery for the treatment of many eye diseases is topical application. The eyes are unique in their structural and functional characteristics, making them highly impenetrable to outside substances and materials. Blinking, baseline and reflex lachrymation, and drainage are all protective systems that remove foreign substances, including medicines, from the eye's surface. As a result, one of the most difficult challenges in medication delivery is directing pharmaceuticals to ocular tissues [1-3].

Traditional ocular dose forms, including as solutions and suspensions, have a number of disadvantages, including rapid precorneal drug clearance due to nasolacrimal drainage, the necessity for frequent treatment, and pulse release. Ophthalmic ointments, for example, are another dosing form [4][5].

Because the human eye is an isolated organ with problematic medication administration, the ocular drug delivery system is considered vital and complex. Furthermore, due to quick and widespread drug removal from pre-corneal lachrymal fluid via solution drainage, lachrymation, and non-productive absorption by conjunctiva, traditional ophthalmic formulations have a short pre-corneal residence period and poor bioavailability. Various attempts have been made to manufacture stable, sustained-release in situ gels in order to overcome the difficulties associated with standard ophthalmic formulations. Newer research in ophthalmic drug delivery systems is focusing on incorporating a variety of drug delivery technologies, such as developing systems that not only extend the time the vehicle is in contact with the ocular surface but also extend the time the vehicle is in contact with the ocular surface. Changes in a certain physico-chemical parameter (pH, temperature, ion-sensitive) by which the medication is released in a sustained and regulated manner determine the development of gels. In situ gel, nanosuspension, nanoparticulate system, liposomes, niosomes, dendrimers, ocular iontophoresis, collagen shield, minidisc, ocular film, implants, ocuserts, and other innovative dosage forms are available. Because of the limitations of the ocular route, such as non-productive absorption, impermeability of medications to the cornea, drainage, induced lachrymation, and tear turns over, developing ocular drug delivery devices has always

been difficult. Topical medicine application to the eye is a well-established method of treating a variety of ocular illnesses such as dryness, conjunctivitis, keratitis, and eye fever. New ways of drug delivery have been investigated [6-8].

Low drug absorption and short therapeutic effect duration are caused by physiological limits imposed by the eye's protective mechanism. Because the permeability of the cornea is determined by the molecular size and hydrophobicity of medications, the chemical nature of the drugs has an impact on drug absorption. To improve drug effectiveness, choose a dosage form that extends the time the drug is in contact with the eye. This can contribute to improved patient compliance by increasing bioavailability, reducing systemic absorption, and reducing the need for frequent drug delivery. Increasing the viscosity of the medication is one way to increase the pre-corneal residence duration. Gelling systems or, in this case, in situ gel dosing can be used to produce this situation [9][10].

The achievement of desirable drug levels at the target site, particularly within the anterior chamber, is a common difficulty encountered during the development of ocular delivery systems. for a sufficient period of time in the eye's hollow This is primarily owing to the intricate structure and increased risk of infection. Corneal barriers prevent any foreign chemicals from entering the ocular tissues. Eye drops, ointments, gels, and polymeric ophthalmic vehicles are among the several forms of ophthalmic vehicles. In order to improve the pre-corneal residence time, ocular implants were created. In situ gel drug delivery methods are among the several ocular dosage forms that have been explored thus far. During the previous few decades, there has been a lot of research in this field. In situ gels are appealing because they can be used in a variety of situations [11].

Designing formulations and delivery systems for topically applied ophthalmic drugs is challenging. The search for novel ways of delivering therapeutically active agents has been the focus of attention in the past 2 to 3 decades. For any non-invasive route of administration, the need to prolong the duration of residence of the dosage form is evident. This is especially true in the area of ophthalmic drug delivery. An effective ocular drug delivery device must be easy to use, comfortable for the patient, and must improve the therapeutic performance of the drug over conventional dosage forms.

Some ocular diseases may be treated by systemic dosage forms, either by oral ingestion or parenteral injections. However, the systemic routes present the great disadvantage of exposing all organs of the body to the action of the drug, thus leading to unwanted side effects [12].

Topical application of drugs to the eye is the most popular and well-accepted route of administration for the treatment of various eye disorders. Except for the skin, the eye is the most easily accessible site for topical administration of a medication. Various systems have been designed to maximize ocular absorption of ophthalmic drugs. The two main strategies for improving ocular absorption are increasing the corneal permeability and prolonging the contact time on the ocular surface [12].

Drugs are commonly applied to the eye for a localized action on the surface or in the interior of the eye. Poor bioavailability of drugs from ocular dosage forms is mainly due to the precorneal loss factors, which include tear dynamics, non-productive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the corneal epithelial membrane. Due to these physiological and anatomical constraints, only a small fraction of the drug is ocularly absorbed. The effective dose of medication administered ophthalmically may be altered by varying the strength, volume, or frequency of administration of the medication or the retention time of medication in contact with the surface of the eye. So far, attempts have been made to improve ocular drug bioavailability by extending drug residence time in the conjunctival sac and improving drug penetration across the cornea, the major pathway of drug entry into the internal eye [13-15].

Ocular pharmacokinetics: barriers in drug delivery

The ocular tissues can be reached either by local or systemic drug administration. The tissue barriers limit the access of drugs to their targets (Figure 1). The blood–aqueous barrier, composed of the uveal capillary endothelia and ciliary epithelia, limits the access of compounds from the systemic circulation to the anterior chamber, whereas the blood–retina barrier limits drug diffusion from the systemic blood to the retina and vice versa. The barrier has two components: outer and inner blood–retina barriers that

are formed by the retinal pigment epithelium (RPE) and the tight retinal capillary walls, respectively [16].

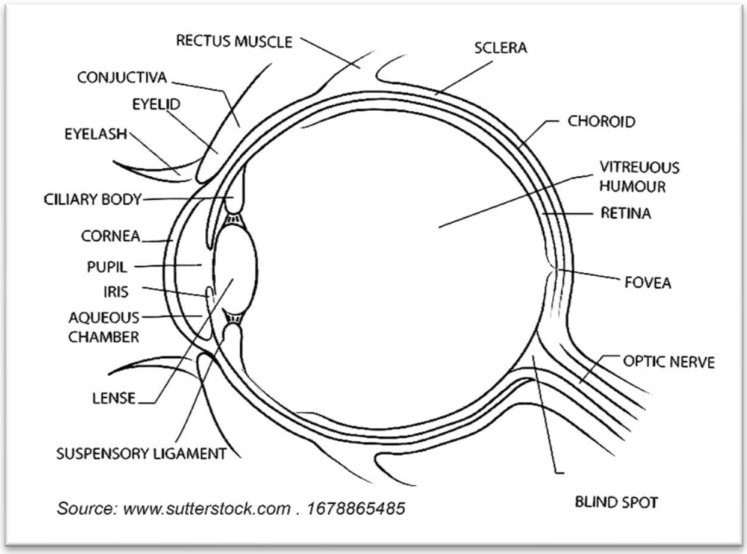

Figure 1: Anatomy of eye

Reasons for poor bioavailability of ocular product

- Poor ocular drug bioavailability is the result of ocular anatomical and physiological constraints, which include the relative im-permeability of the corneal epithelial membrane, tear dynamics and nasolacrimal drainage.
- Most of the topically applied drugs are washed off from the eye by various mechanisms include lacrimation, tear dilution and tear turn over resulting in low ocular bioavailability of drugs.
- The residence time of most conventional ocular solutions ranges between 5 and 25 minutes. Only 1-10% of topically applied drug is absorbed.
- The corneal epithelium is the main barrier of drug absorption into the eye.
- Only few percent of the applied dose are delivered into ocular tissues, the major part of the dose is eliminated due to tear turn over or may be absorbed

systemically through the nasolacrimal duct.This maycause systemic side effects varying from mild to life threatening events.

Systemic absorption can be minimized by

A) Reducing the instilled volume,

B) Controlling drug release,

C) Prodrug derivatization,

D) Adding vasoconstrictive agents

In-situ forming gels

In situ forming hydrogels are liquids upon instillation and undergo a phase transition to form a viscoelastic gel in response to environmental changes like temperature, pH, and electrolyte composition.[17]

Advantages

a) In situ forming hydrogels are attractive as conventional ocular solutions because of facile dosing as a liquid.

b) Ensures complete and rapid ocular coverage.

c) They also allow for accurate and reproducible quantities to be administered to the eye in contrast to pre-gelled formulations.

d) Prolonged contact time and increased bioavailability.

e) Improved level of patient acceptance.

f) Once or twice day dosing is the great advantage.

Brief Insight

A major problem in ocular therapeutics is the attainment of an optimal drug concentration at the site of action. Various systems have been designed during the past two decades to maximize ocular absorption of ophthalmic drugs.[18]

The various approaches attempted in the early stages can be divided into two main categories: bioavailability improvement and controlled release drug delivery.[18]

A more desirable dosage form is one that can be delivered in the form of a drop and causes little to no refractive index problem for vision. Current research efforts are focused on the design and evaluation of ocular delivery systems that are easy to administer, require decreased administration frequency, and provide controlled and possibly sustained release, to increase therapeutic efficacy and patient compliance.[19]

Major progress has been made in ophthalmic gel technology for the development of droppable in-situ forming gels. In the present era, sol-gel technology has the most promising applications in drug delivery systems as well as in industries When used in an ocular drug delivery system, hydrogels are expected to provide prolonged corneal contact time and also provide reduced precorneal drug loss and convenience in administration as compared to eye drops, suspensions, or ointments.[20]

Mechanisms of drug release from hydrogels

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.[21]

Release mechanism from hydrogel matrices

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.[22]

Mechanisms of drug release from In-Situ hydrogels can be categorized as

i) Diffusion-controlled

ii) Swelling-controlled

iii) Chemically-controlled

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.[23]

Desired properties of polymers used in ophthalmic hydrogels as drug delivery systems

- Chemical compatibility with the other excipients and the drug
- Stability after prolonged storage and, if possible, heat stability permits sterilization by autoclaving.
- Absence of ocular irritation or toxicity
- There is little or no vision difficulty.
- Promotion of precorneal retention due to viscosity or bio adhesive properties.
- Physicochemical properties (pH, osmolality) compatible with ocular use.

In-situ forming hydrogels

In-situ forming hydrogels are liquid upon instillation and undergo phase transition in the cul-de-sac to form viscoelastic gel, and this provides a response to environmental changes.[17]

Methods of loading of hydrogels as drug carriers

In this method, a polymer is allowed to swell in a suitable drug solution or a drug is added to a preformed hydrogel and diffuses through the polymeric network.[17]

Advantages of in-situ forming hydrogels

- Prolonging the corneal contact time of the drug and decreasing its drainage rate, thus increasing ocular bioavailability.
- It lowers the frictional resistance between the cornea and eyelids during blinking, there by exerting a lubricating effect.

Three methods have been employed to cause phase transition.

(i) Gelling triggered by change in pH

(ii) Gelling triggered by change in temperature

(iii) Gelling triggered by change in ionic strength

Poloxamers (Kolliphor®)

The Poloxamers consist of more than 30 different non-ionic surface-active agents. These polymers are ABA-type triblock copolymers composed of polyethylene oxide (PEO) (A) and polypropylene oxide (PPO) units (B). The Poloxamer series covers a range of liquids, pastes, and solids, with molecular weights and ethylene oxide–propylene oxide weight ratios varying from 1100 to 14,000 and 1:9 to 8:2, respectively. Poloxamers, also known as Kolliphor®, are the most widely used thermal setting polymers in ophthalmology. They are formed by a central hydrophobic part (polyoxy-propylene) surrounded by a hydrophilic part (ethylene oxide). Depending on the ratio and the distribution along the chain of the hydrophobic and hydrophilic subunits, several molecular weights are available, leading to different gelation properties. Kolliphor® P407, which gives colourless and transparent gels, is the most commonly used polymer in pharmaceutical technology.

The solution behaves as a mobile viscous liquid at room temperature (b25 °C), but transforms into a semisolid transparent gel at body temperature (37 °C). [25]

CHAPTER 2

LITERATURE REVIEW

LITERATURE REVIEW

1. Rathapon A, Suthira T, Asira F. were studied about Pluronic F127-based thermoresponsive diclofenac sodium ophthalmic in situ gels (DS in-situ gel). They were prepared by cold method and investigated their physicochemical properties i.e., pH, flowability, sol–gel transition temperature, gelling capacity and rheological properties. In-vivo opthalmic absorptions was studied in rabbits.

2. Yong Q. et.al. were also studied about thermo sensitive in situ gelling vehicle was prepared to increase the pre-corneal resident time and the bioavailability of methazolamide (MTA). Poloxamer analogue were used as the gelling agents, and the in-situ gel was obtained by using a cold method. The gelation temperature, rheological properties, in vitro release as well as in vivo evaluation of the optimized formulations were investigated.

3. Basavaraj K, et al also studied on the concept of pH-triggered in situ gelation. Polyacrylic acid (Carbopol® 934) was used as the gelling agent in combination with hydroxyl propyl methylcellulose (Methocel K4M) which acted as a viscosity enhancing agent was formulated and evaluated an ophthalmic delivery system for a non-steroidal anti-inflammatory drug, ketorolac tromethamine.

4. Feng Cao, Xiaolin Z, and Qineng P. were studied about a thermo sensitive and mucoadhesive in situ gelling ophthalmic system of azithromycin (ATM). Poloxamer 407 (P407) and poloxamer 188 (P188) were used as gelling agents. Addition of Carbopol 974P (CP 974P) to the gelling systems could increase the solubility of ATM by salt effect In vitro and in vivo indicated that this droppable gel performed better than ATM eye drop did.

5. Jagdish B, et al. were studied the poor bioavailability and therapeutic response exhibited by the conventional opthalmic solutions due to pre-corneal elimination of the drug by the use of in-situ gel forming system. The purpose of this work was to develop an opthalmic delivery system of the NSAIDS indomethacin, based on the concept of ion-activated in-situ gelation. Gelritegellan gum, a novel opthalmic vehicle, was used as the gelling agent.

6. Satish KP, et al. also studied Polyacrylic acid (Carbopol®980NF)as phase transition polymer, hydroxypropyl methylcellulose (Methocel® K100LV) as a release retardant was used for the in-situ gelling system, and ion exchange resin as a complexing agent. Ciprofloxacin hydrochloride was complexed with ion exchange resin to avoid incompatibility between drug and polyacrylicacid.

7. 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.

8. 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). Microdialysis 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.

9. The Pluronic F127 (PF127) based formulations of timolol maleate (TM) were developed with the aim. Which is enhancing ocular bioavailability The effect of isotonicity agents and PF127 concentrations on the rheological properties of the prepared formulations was examined. In an

attempt to reduce the concentration of PF127 without compromising the in situ gelling capabilities, various viscosity enhancing agents were added to PF127 solution containing 0.5% TM. The viscosity and the ability of PF127 gels to deliver TM. In vivo study showed that the ocular bioavailability of TM, measured in albino rabbits.

10. 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.

11. The concentration of S(−)-satropane in dialysates was measured by using liquid chromatography/tandem mass spectrometry (LC–MS/MS). Unlike the common solution prepared in normal saline, in which the level of S(−)-satropane in aqueous humour increased rapidly after instillation and reached the maximal level within 1 h, S(−)-satropane exhibited 3.2-fold greater in the in situ forming gel.

12. 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 delivery. 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 were evaluated and compared to that in conventional eye drop solution by using rabbits according to the micro dialysis method.

13. 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.

CHAPTER 3

AIM OF WORK

AIM OF WORK

One of the most difficult tasks for pharmaceutical researchers has continued to be the delivery of drugs into the eyes. The eye's distinctive structure prevents drug molecules from reaching the necessary region of action. Conventional methods for treating vision-threatening ocular illnesses, such as eye drops, suspensions, and ointments, cannot be regarded as ideal. Due to the eye's effective production mechanisms, ophthalmic solutions have very low bioavailability when administered. Over 90% of the formulations are offered as ocular solutions.

To keep a therapeutic drug level in the tear film or at the site of action, it is necessary to frequently insert eye drops. However, repeated use of highly concentrated solutions might result in cellular harm and other harmful adverse effects.

The typical pulse entry type drug release observed with conventional preparations can be replaced by a more controlled, sustained, and continuous drug delivery using a controlled release ocular drug delivery system. These systems can achieve therapeutic action with a smaller dose and fewer systemic and ocular side effects. Such systems include implants, ocuserts, collagen shields, nanoparticles, microspheres, and liposomes, but the limitations of the above systems are

- Poor patient compliance and difficulty of insertion into the ocular cavity.
- Tissue irritation and damage caused by penetration enhancers and collagen shields.
- Toxicity is caused by insertion of foreign substances like albumin and polybutyl cyanoacrylates, as in the case of nanoparticles and microspheres.
- Cell toxicity and ocular irritation.

To overcome such problems, an alternative approach of in situ gelling systems or phase transition systems which are instilled in a liquid form and shift to a gel or semisolid phase in cul-de-sac by change in temperature is selected as a novel system.

Due to the gelling capability of the thermo-triggered in situ gelling system after instillation in to the eye, the drainage of formulation is minimal compared to conventional ophthalmic eye drops.

The in-situ gelling system provides longer contact time and sustained delivery of the drug, hence decrease in frequency of administration and improved patient compliance are the major advantages.

The ocular conjunctivitis caused by various microorganism causes permanent conjunctival damages, corneal ulceration, systemic infections.

The aim of the current work is formulation and evaluation of in-situ ophthalmic gel of Atropine Sulphate (as 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 based on Kolliphor® P 407. Xantahan gum and guar gum has been investigated as additives 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 37°C when instilled into the eye.

CHAPTER 4

DRUG AND POLYMER PROFILE

DRUG AND POLYMER PROFILE

Atropine Sulphate

Atropine is a tropane alkaloid and anticholinergic medication used to treat certain types of nerve agent and pesticide poisonings as well as some types of slow heart rate, and to decrease saliva production during surgery. It is typically given intravenously or by injection into a muscle. Eye drops are also available which are used to treat uveitis and early amblyopia. The intravenous solution usually begins working within a minute and lasts half an hour to an hour.Large doses may be required to treat some poisonings [29].

Common side effects include a dry mouth, large pupils, urinary retention, constipation, and a fast heart rate. It should generally not be used in people with angle closure glaucoma. While there is no evidence that its use during pregnancy causes birth defects, that has not been well studied. It is likely safe during breastfeeding. It is an antimuscarinic (a type of anticholinergic) that works by inhibiting the parasympathetic nervous system [30].

IUPAC name:

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

Structure:

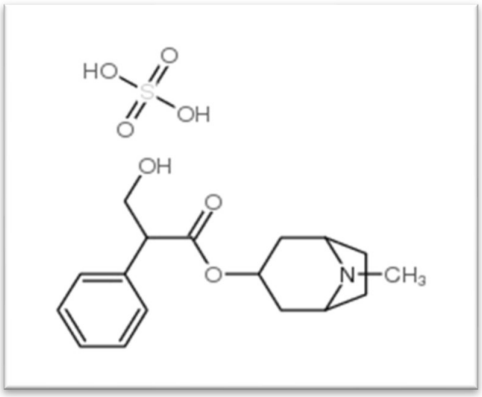

Figure 2: Structure of atropine sulphate

Formula	$C_{17}H_{23}NO_3$
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

Description

Atropine Sulfate Injection, USP is a sterile, nonpyrogenic isotonic solution of atropine sulphate monohydrate in water for injection with sodium chloride sufficient to render the solution isotonic. It is administered parenterally by subcutaneous, intramuscular, or intravenous injection. Each milliliter (mL) contains 0.1 mg (adult strength) or 0.05 mg (pediatric strength) of atropine sulphate monohydrate equivalent to 0.083 mg (adult strength) or 0.042 mg (pediatric strength) of atropine, and sodium chloride. 9 mg may contain sodium hydroxide and/or sulfuric acid for pH adjustment 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 ingredient is present in amounts insufficient to affect the serum electrolyte balance of sodium (Na+) and chloride (Cl-) ions. The solution contains no bacteriostat, antimicrobial agent, or added buffer (except for pH adjustment) and is intended for use only as a single-dose injection. When smaller doses are required, the unused portion should be discarded. The chemical formula for Atropine Sulfate, USP is 1 H, 5 H-Tropan-3--ol ()-tropate (ester), sulphate (2:1) (salt) monohydrate, $(C17H23NO3)_2 \cdot H_2SO_4 \cdot H2O$, colourless crystals or white crystalline powder, very soluble in water. It has the following structural formula: Atropine, a naturally occurring belladonna alkaloid, is a racemic mixture of equal parts of d-and 1-hyocyamine, whose activity is due almost entirely to the levo isomer of the drug. Sodium Chloride, USP, also known as NaCl, is a white crystalline powder that is freely soluble in water. The syringe is moulded from a specially formulated

polypropylene. Water permeates from inside the container at an extremely slow rate, which will have an insignificant effect on solution concentration over the expected shelf life. Solutions in contact with the plastic container may leach out certain chemical components from the plastic in very small amounts. However, biological testing was supportive of the safety of the syringe material [31].

Clinical pharmacology

Mechanism of Action: Atropine is an antimuscarinic agent since it antagonises the muscarine-like actions of acetylcholine and other choline esters. Atropine inhibits the muscarinic actions of acetylcholine on structures innervated by postganglionic cholinergic nerves and on smooth muscles which respond 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 structures that are stimulated or inhibited by muscarine (i.e., exocrine glands and smooth and cardiac muscle). Responses to postganglionic cholinergic nerve stimulation may also be inhibited by atropine, but this occurs less readily than with responses to injected (exogenous) choline esters [32].

Pharmacodynamics: Atropine-induced parasympathetic inhibition may be preceded by a transient phase of stimulation, especially in the heart where small doses first slow the rate before characteristic tachycardia develops due to paralysis of vagal control. Atropine has a stronger and longer-lasting effect on the heart, intestine, and bronchial muscle than scopolamine, but it has a weaker effect on the iris, ciliary body, and certain secretory glands. Unlike the latter, atropine in clinical doses does not depress the central nervous system but may stimulate the medulla and higher cerebral centers. Although mild vagal excitation occurs, the increased respiratory rate and (sometimes) increased depth of respiration produced by atropine are more probably the result of bronchial dilatation. Accordingly, atropine is an unreliable respiratory stimulant, and large or repeated doses may depress respiration. Adequate doses of atropine abolish various types of reflex vagal cardiac slowing or asystole. The drug

also prevents or abolishes bradycardia or asystole produced by injection of choline esters, anticholinesterase agents or other parasympathomimetic drugs, and cardiac arrest produced by stimulation of the vagus. Atropine may also lessen the degree of partial heart block when vagal activity is an etiologic factor. In some patients with complete heart block, the idioventricular rate may be accelerated by atropine; in others, the rate is stabilized. Occasionally, a large dose may cause atrioventricular (A-V) block and nodal rhythm. Atropine Sulfate Injection, USP in clinical doses, counteracts the peripheral dilatation and abrupt decrease in blood pressure produced by choline esters. However, when given by itself, atropine does not exert a striking or uniform effect on blood vessels or blood pressure. Systemic dosing slightly raises systolic and lower diastolic pressures and can produce significant postural hypotension. Such doses also slightly increase cardiac output and decrease central venous pressure. Occasionally, therapeutic doses dilate cutaneous blood vessels, particularly in the "blush" area (atropine flush), and may cause atropine "fever" due to suppression of sweat gland activity in infants and small 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 [33].

Pharmacokinetics: Atropine disappears rapidly from the blood following injection and is distributed throughout the body. Exercise, both prior to and immediately following intramuscular administration of atropine, significantly increases the absorption of atropine due to increased perfusion in the muscle and significantly decreases the clearance of atropine. The pharmacokinetics of atropine are nonlinear after intravenous administration of 0.5 to 4 mg. Atropine's plasma protein binding is about 44% and saturable in the 2-20 μg/mL concentration range. Atropine readily crosses the placental barrier and enters the foetal circulation but is not found in amniotic fluid. Much of the drug is destroyed by enzymatic hydrolysis,

particularly in the liver; from 13 to 50% is excreted. unchanged in the urine. Traces are found in various secretions, including milk. The major metabolites of atropine are noratropine, atropin-n-oxide, tropine, and tropic acid. The metabolism of atropine is inhibited by organophosphate pesticides. Specific Populations The elimination half-life 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 effect on the pharmacokinetics and pharmacodynamics (heart rate changes) of atropine [34].

Adult Dosage: Recommended Dosage Use Dose (adults) Repeat Antisialagogue or other antivagal 0.5 to 1 mg 1-2 hours Organophosphorus or muscarinic mushroom poisoning 2 to 3 mg 20-30 minutes Bradyasystolic cardiac arrest 1 mg 3-5 minutes; 3 mg maximum total dose [34].

Indications: Atropine or atropine sulfate carries FDA indications for anti-sialagogue/anti-vagal effect, organophosphate/muscarinic poisoning, and bradycardia. It was originally synthesized from the plant Atropa belladonna which is where the drug derives its name.

Atropine Sulfate Injection, USP, is indicated for temporary blockade of severe or life-threatening muscarinic effects, e.g., as an antisialagogue, an antivagal agent, an antidote for organophosphorus or muscarinic mushroom poisoning, and to treat bradyasystolic cardiac arrest [34].

Dosage and administration

General Administration Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not administer unless solution is clear and seal is intact. Discard unused portion. Intravenous administration is usually preferred, but subcutaneous, intramuscular, and endotracheal administration are possible. For administration via an endotracheal tube, dilute 1-2 mg in no more than 10 mL of sterile water or normal saline. Titrate based on heart rate, PR interval, blood pressure and symptoms. 2.3 Pediatric Dosage Dosing in pediatric populations has not been well studied. Usual initial dose is 0.01 to 0.03 mg/kg [34].

Dosage forms and strengths

Injection: 0.05 mg/mL and 0.1 mg/mL in Ansyr™ Plastic Syringes

Contraindications

None.

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 [34].

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 [34].

Viscid Plugs

Atropine may cause inspissation of bronchial secretions and formation of viscid plugs in patients with chronic lung disease [34].

Adverse reactions

The following adverse reactions have been identified during post-approval use of atropine sulfate. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. Most of the side effects of atropine are directly related to its antimuscarinic action. Dryness of the mouth, blurred vision,

photophobia and tachycardia commonly occur. Anhidrosis can produce heat intolerance. Constipation and difficulty in micturition may occur in elderly patients. Occasional hypersensitivity reactions have been observed, especially skin rashes which in some instances progressed to exfoliation [35].

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 [35].

Uses in specific populations

Pregnancy

Animal reproduction studies have not been conducted with atropine. It also is not known whether atropine can cause fetal harm when given to a pregnant woman or can affect reproduction capacity.

Nursing Mothers

Trace amounts of atropine was found in breast milk. The clinical impact of this is not known. Geriatric Use An evaluation of current literature revealed no clinical experience identifying differences in response between elderly and younger patients. In general, dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy [35].

Overdosage

Excessive dosing may cause palpitation, dilated pupils, difficulty in swallowing, hot dry skin, thirst, dizziness, restlessness, tremor, fatigue and ataxia. Toxic doses lead to restlessness and excitement, hallucinations, delirium and coma. Depression and circulatory collapse occur only with severe intoxication. In such cases, blood pressure declines and death due to respiratory failure may ensue following paralysis and coma. The fatal adult dose of atropine is not known. In pediatric populations, 10 mg or less may be fatal. In the event of toxic overdosage, a short acting barbiturate or diazepam

may be given as needed to control marked excitement and convulsions. Large doses for sedation should be avoided because central depressant action may coincide with the depression occurring late in atropine poisoning. Central stimulants are not recommended. Physostigmine, given as an atropine antidote by slow intravenous injection of 1 to 4 mg (0.5 to 1 mg in pediatric populations), rapidly abolishes delirium and coma caused by large doses of atropine. Since physostigmine is rapidly destroyed, the patient may again lapse into coma after one to two hours, and repeated doses may be required. Artificial respiration with oxygen may be necessary. Ice bags and alcohol sponges help to reduce fever, especially in pediatric populations. Atropine is not removed by dialysis [35].

Nonclinical toxicology

Carcinogenesis, Mutagenesis, Impairment of Fertility Studies have not been performed to evaluate the carcinogenic or mutagenic potential of atropine or its potential to affect fertility adversely [36].

Poloxamer 407, Lutrol F 127 (Kolliphor® P 407)

Synonym

Lutrol F 127, Pluronic F 68

Structure

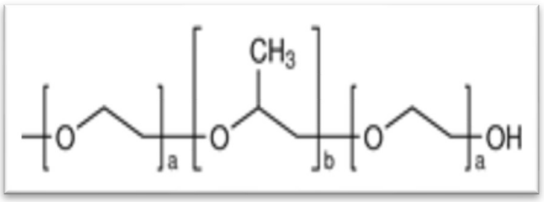

Figure 3: Structure of Poloxamer

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

Biological effects of poloxamer

Originally thought to be inert carrier molecules work led by Kabanov has recently shown that some of these polymers have a very real effect on biological systems independently of the drug they are transporting. The poloxamers have been shown to incorporate into cellular membranes affecting the micro viscosity of the membranes [38].

Stability and storage conditions

It is stable under ordinary conditions, and should be stored in a well-closed container and protected from light [38-42].

Safety

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipient [43].

Handling precautions

Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe dust [44-47].

Xanthan gum

Xanthan gum is a hydrocolloid that is produced by the microorganism, Xanthomonas campestris. Xanthomonas campestris is a bacterium which is naturally found on the cabbage plant, which produces slimy and gummy colonies.23 The microorganism produces these slimy and gummy colonies which are called extracellular polysaccharides. These polysaccharides are released from the bacterium cell because no covalent bonds are formed to the cell wall. The extracellular polysaccharide released from Xanthomonas campestris is called xanthan gum. Xanthan gum is produced by the process of submerged aerobic fermentation using glucose as the primary carbohydrate source. The xanthan gums are recovered, purified, dried and milled into a white powder [48-50].

Structure

Xanthan gum is an anionic linear hydrocolloid with a (1□ 4) linked β-D-glucose backbone, as seen in cellulose. However, unlike cellulose it has a large side unit on every other glucose unit at location C-3. The side unit, a trisaccharide, contains a glucuronic acid residue linked (1□4) to a terminal mannose unit and (1□2) to a second mannose which connects to the glucose backbone (Sworn 2000). The mannose unit connected to the backbone usually contains an acetyl group. Approximately 50% of the terminal mannose molecules carry a pyruvic acid residue. (Kovac P 1977; Zirnsak MA 1999; Sworn 2000). The primary structure is shown in Figure 4 [51].

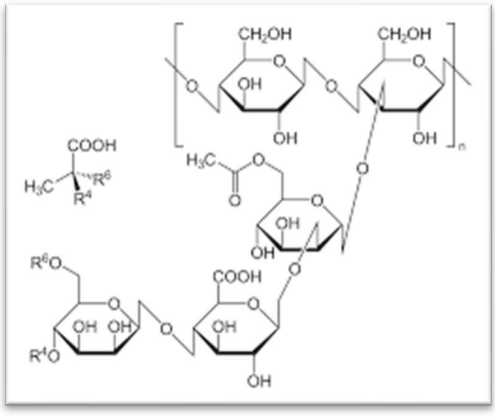

Figure 4: Molecular structure of xanthan gum

Properties

- 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) [48-52].
- 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) [52].
- 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) [52].

- 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 [53].
- Although xanthan gum is not a gelling agent, it can form elastic, thermos-reversible 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) [55].

Applications and Limitations

- Xanthan gum is approved for food use as a stabilizer, emulsifier, thickener, suspending agent, and foam enhancer (Sanderson 1996)
- It also adds volume and moisture, which leads to higher crumb strength with less crumbling. The use of xanthan gum in a mix application facilitates better moisture retention, better stabilization and structure formation Anon 1989).
- The xanthan gum can provide a very rapid viscosity development as well as enhancing body and evenly suspending particles. Xanthan gum provides stability, syneresis control and consistent viscosity when exposed to freeze-thaw cycles (Sanderson 1996).
- It can be added to frozen products like whipped toppings, batters, soufflés, gravies, and entrees. Another use of xanthan gum is in retorted products, due to the stability over a wide range of temperatures. Although the viscosity would be low at high retort temperatures, upon cooling the original viscosity would return.
- In addition to this reason, the xanthan can improve the filling process and reduce splashing (Sworn 2000). Syrups and toppings also have a use for xanthan gum. It allows these products to have excellent pouring and cling properties as well as good stability and uniform suspension of ingredients. In general terms, xanthan thins under shear in the mouth, facilitating flavor release (Chinachoti 1995) [54].

- Pseudoplasticity is also important for the mouthfeel and visual aesthetics of the product and for its utility as a processing aid. Xanthan gum's pseudoplastic properties allow for easy mixing and pumping during the production of food products in addition to providing food systems with long-term stability.
- Xanthan gum is stable over a wide pH range, temperature, and exposure to enzymes. Therefore, it can be used in many different products. However, xanthan gum is a thickening agent not a gelling agent. Therefore, it cannot form a gel network [56].

Guar Gum

Guar gum is found in the endosperm of the seeds of the guar plant, Cyamopsis tetragonoloba, which is milled in order to obtain guar gum (Meer1977; Wielinga 2000). Guar gum is a neutral hydrocolloid with linear chains of Dmannopyranosyl units with D-galactopyranose substituents protruding by (1→6) linkages. For every galactose residue there are approximately two mannose residues [57].

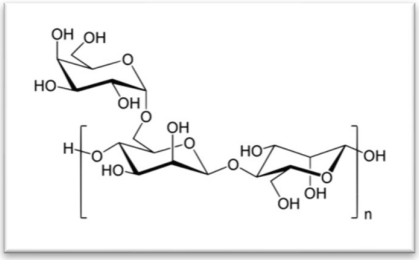

Figure 5: Molecular structure of guar gum

Guar gum is highly substituted which allows for good hydration and hydrogen-bonding activity. Water can easily "slip" between the molecules in order to hydrate or dissolve the gum. The molecular weight of guar gum is between 220,000 and 300,000 (Hoyt 1966). Guar gum has a higher degree of galactose substitution (40%) than locust bean gum (20-23%) (Maier 1992). The galactose content of galactomannans has been studied to show that it strongly influences the behavior of each hydrocolloid. Low galactose content leads to stronger synergistic interactions with other hydrocolloids as well as a stronger gelling capacity independently based upon interactions of smooth areas of the 31mannan backbone (Dea 1977; McCleary 1985). The higher galactose content leads to prevention of strong cohesion of the main backbone, so no extensive junction zones or crystalline regions can be formed. Another factor that influences physical behavior of the galactomannan is the distribution of galactose units along the mannan backbone. Guar gum is evenly substituted, which means there are no smooth and hairy regions of the mannan backbone. On average, for every two molecules of mannose, a galactose side unit is attached (Meer 1977). A two to one mannose-galactose ratio leaves small galactose uninhibited mannose areas, which has been shown to have lesser functionality

(McCleary 1979; Launay1986). Whereas locust bean gum, on average, has a four to one ratio, which should exhibit greater functionality. Richardson et al (1998) reported that two galactomannans, with the same average galactose content but with different mannose-galactose ratios, would exhibit different degrees of functionality (Richardson 1998). The galactomannan with the broader distribution of galactose units would be more functional because they contain a greater proportion of chains with lower galactose content (McCleary 1979; Launay 1986) [58][59].

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 [60-63].

CHAPTER 5

METHODOLOGY

METHODOLOGY

Preformulation studies

Materials used

Drug: Atropine sulphate (S. D. Fine-Chem Ltd., Mumbai), Topin (Batch no. JTN-009, Ordain Health Care Global Pvt Ltd. Tamilnadu, India)

Gelling agent: Kolliphor® P 407 (SIGMA Life Science), Xanthan Gum and Guar Gum (S. D. Fine-Chem Ltd., Mumbai)

Salts of artificial tear fluid: NaCl, $NaHCO_3$, $CaCl_2.2H_2O$ (Merck Life Science Pvt Ltd., Mumbai)

Membrane: Dialysis Membrane-60 (LA390, Av. Flat width-25.27mm, Av. Diameter-15.9mm, Capacity approx.-1.99ml/cm, HiMedia, Mumbai, india)

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 (Toki Sangyo 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

Preparation of Artificial Tear Fluid (ATF)

0.67g NaCl, 0.20g $NaHCO_3$ and 0.008g $CaCl_2,2H_2O$ were dissolved in a beaker containing water and make the amount with addition of water quantity required to100gm. The prepared solution was Artificial Tear Fluid [64].

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 ATF was prepared and scanned from a wavelength of 190-400 nm in a UV-Visible Spectrophotometer using appropriate blank. The λ_{max} was found to be 206 nm, which was used for spectrophotometric determination of Atropine sulphate in test samples [64].

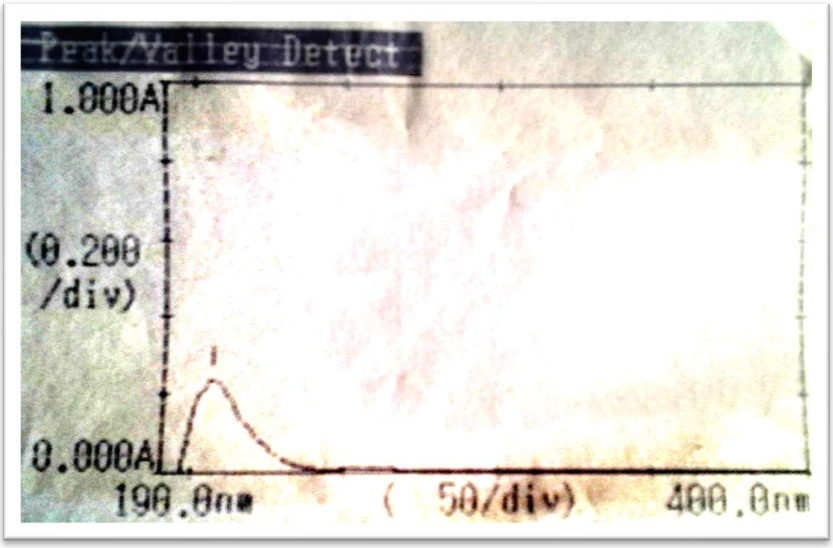

Figure 6: UV Scanning for λ_{max} detection of Atropine sulphate

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 ATF. The different concentration of drug taken and corresponding absorbances are plotted. The corresponding standard curve generated by linear regression analysis along with the mathematical equation representing the curve is given in Figure 7 [65].

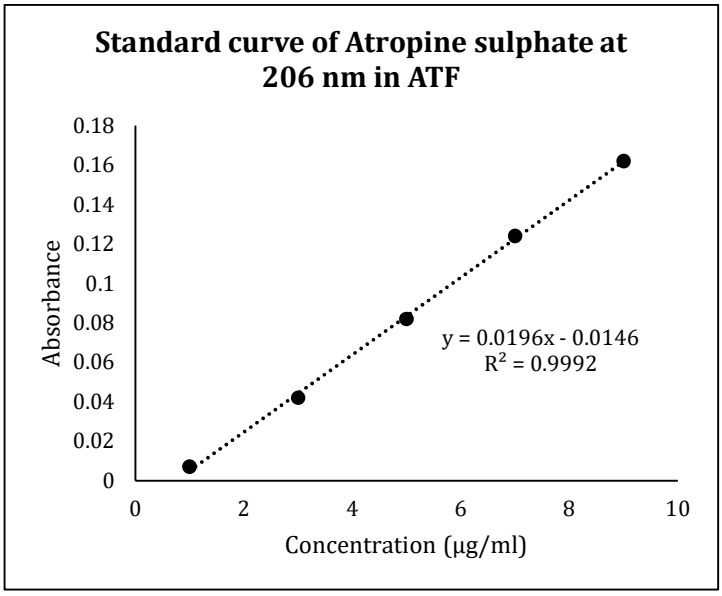

Figure 7: Standard Curve Preparation of Atropine sulphate

Preparation of polymer solutions

The Kolliphor® P 407 solutions (1-20% w/v) were prepared by dispersing the required amount of Kolliphor® P 407 in the desired volume of cold water to form a homogenous dispersion by continuous stirring. The dispersion was kept in a refrigerator for at least 24 hours to get a homogenous dispersion [66].

Figure 8: Preparation of polymer solutions

Determination of gel temperature and Optimization of Kolliphor® P 407 concentration

Kolliphor® P 407 solutions of different concentrations (1-20%) 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% Kolliphor® P 407 solutions were under gone gelation above 25ºC and below body temperature i.e., 37ºC [67].

Modification of Kolliphor® P 407 solutions

Kolliphor® P 407 solution was showing no gelation below 18%. Modifications of the Kolliphor® P 407 solutions below 18% were done by addition of xanthan and guar in different possible ratios and concentrations. The xanthan and guar mixture in the ratio of 7: 3 was capable to convert Kolliphor® P 407 solution below 18% to clear gel. Modification of Kolliphor® P 407 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. It was found that only xanthan: guar ::7:3 was capable to converted the Kolliphor® P 407 solution below 18% into gel below body temperature i.e., 37ºC. All other xanthan: guar ratios produced hazy gels. In addition of xanthan-guar mixture 14-17% of Kolliphor® P 407 solutions were getting gelation. But only 17% Kolliphor® P 407with xanthan-guar mixture solution was droppable liquid and it was taken for formulation development [68].

Effect of Drug on Gel Temperature

The polymer solutions were subjected to gelation study with addition of 1%w/v of atropine sulphate. No effect on gelation was observed [68].

Conclusion of preformulation studies

On the basis of preformulation studies, it was concluded that 18-20% of Kolliphor® P 407 solution was converted to *in-situ* gel. The xanthan and guar mixture were capable to convert Kolliphor® P 407 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 Kolliphor® P 407 solution modified with xanthan and guar at a ratio of 7:3 undergone thermo-triggered sol-gel transition. The Kolliphor® P 407 solution below 14-16% was found to be non-droppable highly viscous solution. But only 17% Kolliphor® P 407with xanthan-guar mixture solution was droppable liquid at room temperature. So, Kolliphor® P 407 solutions 17% was considered for formulation development [64-68].

Formulation Development and Evaluation

Preparation of sample solutions

A specific amount of xanthan gum: guar gum in the ratio of 7:3 was dispersed into the 17% Kolliphor® P 407 solutions. 1% Atropine sulphate was dissolved into the modified Kolliphor® P 407 solution to obtain sample *in-situ* gelling solutions [69].

Composition of *in-situ* gelling solutions

Table 1: Composition of in-situ gelling solutions

Formulation	Xanthan (%w/v)	Guar (%w/v)	Poloxamer (% w/v)	Deionized water (ml)
PAS	0.191	0.082	17	100

Determination of pH

Formulation was taken in a beaker and pH of gel was determined by digital pH meter.

In-vitro gelation studies

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 [70].

Thermo-gelling Property

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 (presoaked 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 upto the point of immobilization of the PAS [70].

Rheological Evaluation

The viscosity of the gel solutions was measured with the Brookfield Viscometer (Viscometer TV-10, Spindle-M3-Cord-22) equipped with temperature controller for viscosity measurement and also to measure the gelation temperature. The sample was placed in the sample container for 5 min so that it reaches the constant temperature. The viscosity of the samples was measured at different temperature [70].

Swelling study

The cell containing artificial tear fluid was used as swelling medium equilibrated at 37°C. A specific volume of formulated solutions was packed in one end open F N drop tube dialysis membrane 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, [71-72]

$$\%St = [(W_t - W_0) \times 100]/W_0$$

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 [71-72].

In-vitro gel dissolution studies

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 ATF 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 [71-74].

In-vitro drug release studies

A Franz diffusion cell was used for *in-vitro* release study of Atropine sulphate from PAS and marketed product Topin®. The dissolution medium used was artificial tear fluid. The dialysis membrane, previously soaked overnight in the dissolution medium, was tied to one end of the specifically designed glass cylinder, i.e. donor of the Franz diffusion cell. The donor was fitted into the cell in such a fashion that the membrane just touches the upper surface of the dissolution media filled in 40 ml receiver chamber of the cell maintained at 37ºC. The stirring rate was maintained at 20 rpm. 1 ml of formulation was accurately pipetted and placed over the dialysis membrane. Aliquots, each of 1 ml starting from 5 minute, were withdrawn at hourly interval for 10 hours and replaced by an equal amount of artificial tear fluid. The Atropine sulphate content of the aliquots was analyzed by UV spectrophotometer at 206 nm. 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 [75].

Figure 9: In-vitro drug release studies

Release Kinetics

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. M t / M ∞ = K t n, where M_t/M_∞ is the fractional solute release, *t* is the release time, *K* is a kinetic constant characteristic of the drug/polymer system, and *n* is an exponent that characterizes the mechanism of release of tracers.[1] For film matrix, if the exponent *n* = 0.45, then the drug release mechanism is Fickian diffusion, and if 0.45 <*n*< 0.89, then it is non-Fickian or anomalous diffusion. An exponent value of 0.89 is indicative of Case-II Transport or typical zero-order release [73] [75-80].

Statistical analysis

The percentage of drug release at 4 hours of the developed formulations were analyzed 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 4.03, Graph Pad Software Inc, sandiego, CA). The analysis was based on the comparison of the drug release from marketed product TROPIN® with developed formulation PAS in *in-vitro* studies [76].

Clarity test

To inspect the visual clarity of the formulation clarity test was performed. In order to check visual disturbances after instillation of the formulation into the eye, test was performed against white and dark background [64-66].

CHAPTER 6

RESULTS AND DISCUSSIONS

RESULTS AND DISCUSSIONS

Table 2: Characterisation of in-situ gel formulation (n=3)

Formulation	Appearance	pH	Thermo-gelling time (Sec.)	% Swelling at 6h	Gel temperature (Test tube tilting) (°C)	Gel temperature (Viscosity) (°C)	Viscosity at gel temperature (cP)
PAS	Clear	6.97±0.2	9.33±1.5	51.18±7.1	33±1	32.7	121

Gel temperature

Table 1 and Table 2 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 [71]. 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. 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 0.273% 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 25ºC. 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 0.273% w/v [64-69].

From the table 2, it was observed that little variation in gel temperature between test tube tilting method and viscosity was observed [73].

Thermogelling Properties

The thermogelling properties of developed formulation was evaluated and result is shown in table 2. It was observed that the formulation was converted to gel within 8-11 Seconds. The solutions exhibited gel-sol transition on cooling [74-76].

Rheological properties

From the Table 2 and Figure 10 it was clear that the developed formulations converted to gel at the temperature at which viscosity was sharply increased. It was found to be 32.7°C [77].

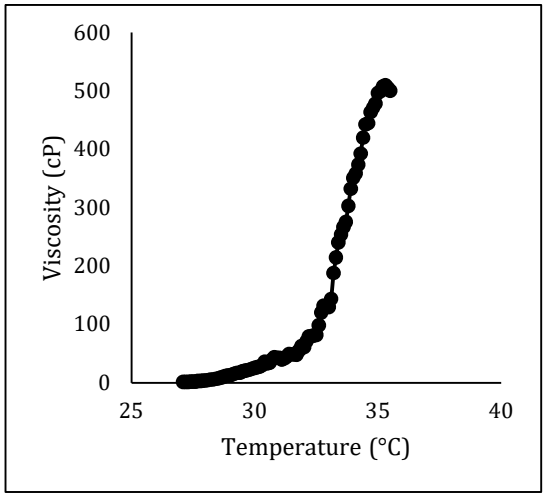

Figure 10: Effect of temperature on viscosity of developed in-situ gel solution

Table 3: Comparison of in-vitro drug release profile of developed in-situ gel formulations with SAS and Topin® (n=3)

Time (min)	Cumulative % drug release		
	SAS	Topin®	PAS
240	99.94±0.27	100.4±0.39	67.85±2.59**
600	-	-	100.68±2.6

Values were expressed as mean±S.E for three replications. Values differs significantly from SAS and Tropine® (**p<0.01)

Swelling Study

The *in-situ* gelling formulations were intended for ophthalmic delivery; hence their swelling behaviour was evaluated at 37°C and in artificial tear fluid. Since Kolliphor® P 407 is a temperature sensitive polymer in the view of gel formation, the degree of swelling is influenced by the eye environment. The developed formulation was observed to be stable gels during the period of swelling up to 6 hours. On comparing the rate of swelling of the developed *in-situ* formulation, it was observed that the swelling was following constant rate with time. The percentage of swelling after 6 hours was about 51% (Table 2 and Figure 11) [78].

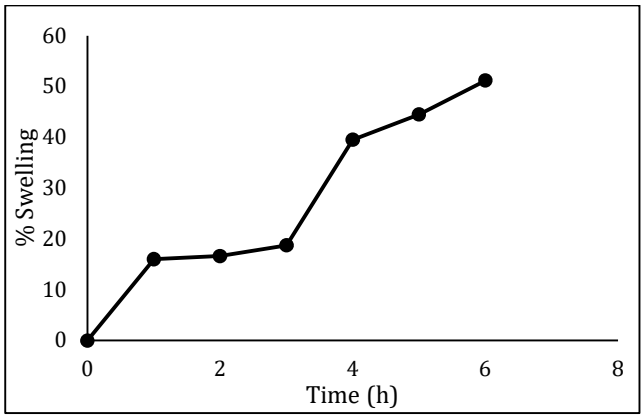

Figure 11: In-vitro Gel swelling profile of developed in-situ gel formulation PAS in artificial tear fluid at 37°C (n=3)

In-vitro gel dissolution studies

The gel dissolution study was conducted using developed in-situ gel formulation at 37°C for 6 hours. The percentage weight loss of the gel was considered as percentage gel dissolution. From the Figure 12 it was found to about 92% dissolution of the gel after 6 hours of studies [64-66].

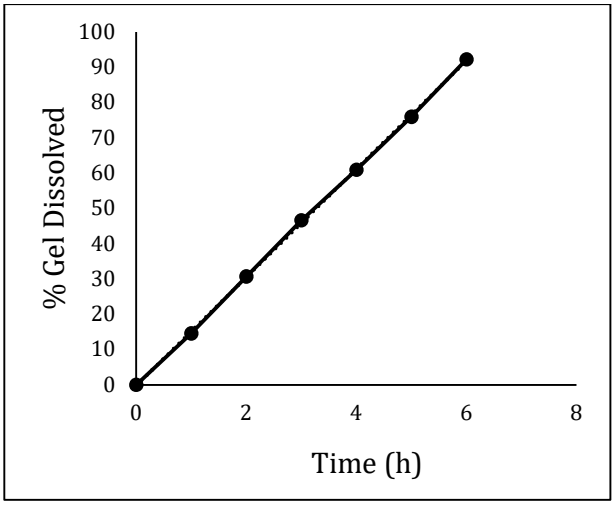

Figure 12: In-vitro Gel dissolution profile of developed in-situ gel formulation PAS in artificial tear fluid at 37°C (n=3)

In-vitro drug release studies

The cumulative percentage release of Atropine sulphate as a function of time profiles from SAS, Topin® and PAS have been shown in Figure 13. It had been observed that PAS sustains the drug release process from the gel matrices. When the formulations 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 68% 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 100% drug at 10 hours. The release data of PAS was significantly ($^{**}p<0.001$) differ from SAS and Topin® at 5% level of significance after 4 hours of release study (Table 3). The different kinetic equations (Zero order, First order and Higuchi's equation) were applied to interpret the release pattern from *in-situ* gel formulation PAS. From the Table 4, it was found that the *in-vitro* drug release was best explained by Higuchi's kinetics, as the plots showed the highest linearity $r^2 > 0.98$ followed by first order and zero order. All the kinetic data were fitted to the Korsmeyer-Peppas Equation. More acceptable linearity $r^2 > 0.971$ was observed for PAS. The value of release exponent 'n' was found to be 0.36, which appeared to indicate Fickian drug diffusion mechanism shown in Table 4 [64-71].

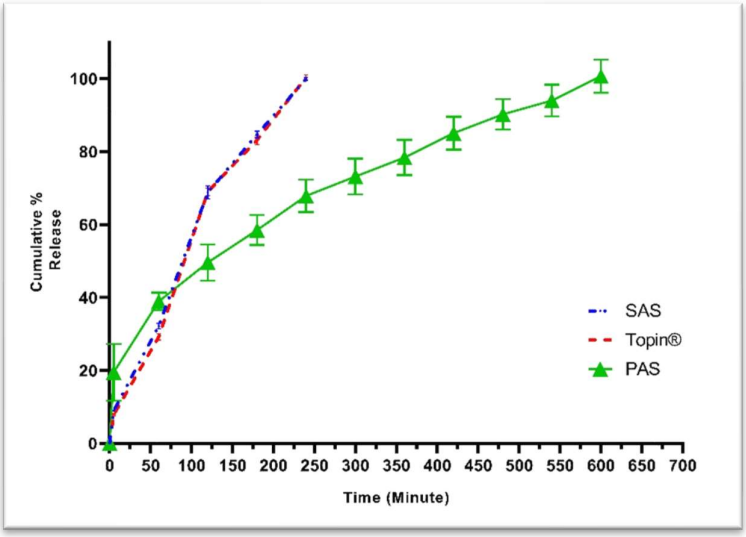

Figure 13: In-vitro drug release profile of developed in-situ gel formulation PAS, SAS and Tropin® in artificial tear fluid at 37°C (n=3)

Table 4: Drug release kinetics of developed in-situ gel formulations (n=3)

Formulation	Zero- order	First- order	Higuchi	Korsmeyer-peppas	
	r^2	r^2	r^2	r^2	N
APS	0.897±0.01	0.940±0.001	0.982±0.01	0.971±0.04	0.36±0.05

Clarity test

The clarity of the in-situ gels was determined against dark and black background and the in-situ gelling formulation PAS was found to be visually clear (Figure 14) [78-80].

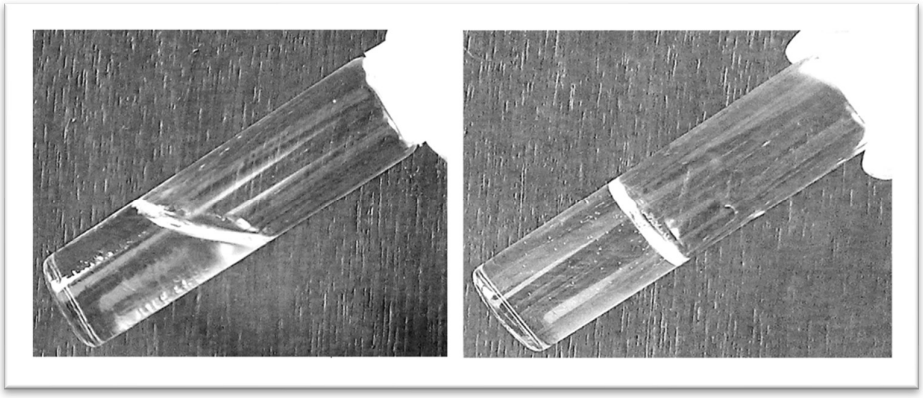

Figure 14: Clarity Test shown by Test tube Tilting Method

CHAPTER 7

CONCLUSION

CONCLUSION

Atropine sulphate was successfully formulated as thermos-sensitive *in-situ* gel-forming eye drops using 17% Kolliphor® P 407 as gelling agent. The formulation was liquid at 25°C and rapidly converted to gel at 37°C when xanthan:guar::7:3 was used to modified the 17% Kolliphor® P 407 solution since 17% Kolliphor® P 407 alone does not formed gel on its own. The modified solution was liquid at 25°C and underwent gelation below body temperature keeping the solution free flowing at room temperature. The gel temperature, swelling and rheological properties of solution were not affected by the incorporation of the drug. The *in-vitro* gel dissolution and drug release showed that the modified Kolliphor® P 407 solution PAS released drug at more sustained rate. Swelling, drug release profile, viscosity etc showed that the formulations are having excellent sustained drug release profile. The developed formulation was a viable alternative to conventional eye drops by virtue of their ability to sustained drug release profile and may decreased frequency of administration resulting in better patient acceptance. On the basis of the observations, it was concluded that the developed *in-situ* gel-forming eye drops may be better alternative than conventional eye drops.[64][78]

CHAPTER 8

FUTURE WORK

FUTURE WORK

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

In future the following investigations are planned to be continued:

1. Extensive *in-vivo* studies
2. Stability Studies

CHAPTER 9

REFERENCES

REFERENCES

1. Al-Bazzaz, Al-Kotaji. Ophthalmic in-situ sustained gel of ciprofloxacin, preparation and evaluation study. Int J Appl Pharm 2018;10:153-61.
2. Wu Y, Liu Y, Li X, Kebebe D, Zhang B, Ren J, *et al.* Research progress of in-situ gelling ophthalmic drug delivery system. Asian J Pharm Sci 2019;14:1-15.
3. Fathalla MA, Vangala ZA, Longman M, Khaled KA, Hussein AK, ElGarhy OH, Alany RG. Poloxamer-based thermoresponsive ketorolac tromethamine in situ gel preparations: design, characterisation, toxicity and transcorneal permeation studies. Eur J Pharm Biopharm 2017;114:119-34.
4. Preethi GB, Narendra E. Formulation and evaluation of in situ mucoadhesive ophthalmic hydrogel for sustained delivery of pefloxacin mesylate. Int J Pharm Pharm Sci 2015;7:345-50.
5. Anuja TK, Rahul LJ, Pradnya BS, Satwashila SK. Design and evaluation of modified chitosan based in situ gel for ocular drug delivery. Int J Pharm Pharm Sci 2017;9:87-91.
6. EL-Kamel AH. In vitro and in vivo evaluation of Pluronic F127- based ocular delivery system for timolol maleate. International journal of pharmaceutics 2002; 24 (1):47–55.
7. Varshosaz J, Tabbakhian M, Salmani Z. Designing of a Thermo sensitive Chitosan/Poloxamer In Situ Gel for Ocular Delivery of Ciprofloxacin. The Open Drug Delivery Journal 2008; 2:61-70.
8. Nalla A and Chinnala KM. *In - situ* ophthalmic drug delivery systems – An Overview , Indo Am. J. Pharm. Sci. 2016; 3(3): 202-208.
9. Peppas NA, Langer R. New challenges in biomaterials. Science 1994; 263(5154):1715-1720.
10. Swapnil S. A review on polymers used in novel in situ gel formulation for ocular drug delivery and their evaluation. Journal of biological and scientific opinion 2003; 1(2):132-137.
11. Varela-Ferna´ndez R, Dı´az-Tome´ V, Luaces-Rodrı´guez A, Conde-Penedo A, Garcı´a-Otero X, Luzardo-A´lvarez A, et al. Drug Delivery to the Posterior Segment of the Eye:Biopharmaceutic and Pharmacokinetic Considerations. Pharmaceutics. 2020; 12(3):269. https://doi.org/10.3390/pharmaceutics12030269 PMID: 32188045.

12. Jacob S, Nair AB, Shah J. Emerging role of nanosuspensions in drug delivery systems. Biomaterials research. 2020; 24:3-. https://doi.org/10.1186/s40824-020-0184-8 PMID: 31969986.
13. Souto EB, Dias-Ferreira J, Lo´pez-Machado A, Ettcheto M, Cano A, Camins Espuny A, et al. Advanced Formulation Approaches for Ocular Drug Delivery: State-Of-The-Art and Recent Patents. Pharmaceutics.2019; 11(9). Epub 2019/09/11. https://doi.org/10.3390/pharmaceutics11090460 PMID: 31500106;PubMed Central PMCID: PMC6781321.
14. Makwana SB, Patel VA, Parmar SJ. Development and characterization of in-situ gel for ophthalmic formulationcontaining ciprofloxacin hydrochloride. Results in Pharma Sciences. 2016; 6:1–6. https://doi.org/10.1016/j.rinphs.2015.06.001 PMID: 26949596.
15. Wu Y, Liu Y, Li X, Kebebe D, Zhang B, Ren J, et al. Research progress of in-situ gelling ophthalmic drug delivery system. Asian Journal of Pharmaceutical Sciences. 2019; 14(1):1–15. https://doi.org/10.1016/j.ajps.2018.04.008 PMID: 32104434.

16. 16.Asasutjarit R, Thanasanchokpibull S, Fuongfuchat A, Veeranondha S. Optimization and evaluation of thermoresponsive diclofenac sodium ophthalmic in situ gels. International journal of pharmaceutics. 2011 Jun 15;411(1-2):128-35.
17. 17.Qian Y, Wang F, Li R, Zhang Q, Xu Q. Preparation and evaluation of in situ gelling ophthalmic drug delivery system for methazolamide. Drug development and industrial pharmacy. 2010 Nov 1;36(11):1340-7.
18. 18.Nanjawade BK, Manjappa AS, Murthy RS, Pol YD. A novel pH-triggered in situ gel for sustained ophthalmic delivery of ketorolac tromethamine. Asian J Pharm Sci. 2009;4(3):189-99.
19. Cao F, Zhang X, Ping Q. New method for ophthalmic delivery of azithromycin by poloxamer/carbopol-based in situ gelling system. Drug delivery. 2010 Oct 1;17(7):500-7.
20. Balasubramaniam J, Kant S, Pandit JK. In vitro and in vivo evaluation of Gelrite® gellan gum-based ocular delivery system for indomethacin. ACTA PHARMACEUTICA-ZAGREB-. 2003 Dec 1;53(4):251-62.

21. Jain SP, Shah SP, Rajadhyaksha NS, Singh PS PP, Amin PD. In situ ophthalmic gel of ciprofloxacin hydrochloride for once a day sustained delivery. Drug development and industrial pharmacy. 2008 Jan 1;34(4):445-52.
22. Ma WD, Xu H, Nie SF, Pan WS. Temperature-responsive, Pluronic-g-poly (acrylic acid) copolymers in situ gels for ophthalmic drug delivery: rheology, in vitro drug release, and in vivo resident property. Drug development and industrial pharmacy. 2008 Jan 1;34(3):258-66.
23. Gratieri T, Gelfuso GM, de Freitas O, Rocha EM, Lopez RF. Enhancing and sustaining the topical ocular delivery of fluconazole using chitosan solution and poloxamer/chitosan in situ forming gel. European journal of pharmaceutics and biopharmaceutics. 2011 Oct 1;79(2):320-7.
24. El-Kamel AH. In vitro and in vivo evaluation of Pluronic F127-based ocular delivery system for timolol maleate. International journal of pharmaceutics. 2002 Jul 8;241(1):47-55.
25. Al-Kassas RS, El-Khatib MM. Ophthalmic controlled release in situ gelling systems for ciprofloxacin based on polymeric carriers. Drug delivery. 2009 Apr 1;16(3):145-52.
26. Fu J, Feng X, Yuan H, Yan L, Kuang X, Xia Z, Gao X, Yu C, Lu Y, Chen HZ. Study of ocular pharmacokinetics of in situ gel system for S (−)-satropane evaluated by microdialysis. Journal of pharmaceutical and biomedical analysis. 2008 Nov 4;48(3):840-43.
27. Cao Y, Zhang C, Shen W, Cheng Z, Yu LL, Ping Q. Poly (N-isopropylacrylamide)–chitosan as thermosensitive in situ gel-forming system for ocular drug delivery. Journal of controlled release. 2007 Jul 31;120(3):186-94.
28. Edsman K, Carlfors J, Petersson R. Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. European journal of pharmaceutical sciences. 1998 Apr 1;6(2):105-1.
29. Avetisov SE, Fisenko VP, Zhuravlev AS, Avetisov KS. [Atropine use for the prevention of myopia progression]. Vestn Oftalmol. 2018;134(4):84-90. [PubMed]
30. Smulyan H. The Beat Goes On: The Story of Five Ageless Cardiac Drugs. Am J Med Sci. 2018 Nov;356(5):441-450. [PubMed]

31. Drugs and Lactation Database (LactMed) [Internet]. National Library of Medicine (US); Bethesda (MD): 2006. Atropine. [PubMed]
32. Drugs and Lactation Database (LactMed) [Internet]. National Library of Medicine (US); Bethesda (MD): 2006. Belladonna. [PubMed]
33. Wu PC, Chuang MN, Choi J, Chen H, Wu G, Ohno-Matsui K, Jonas JB, Cheung CMG. Update in myopia and treatment strategy of atropine use in myopia control. Eye (Lond). 2019 Jan;33(1):3-13. [PMC free article] [PubMed]
34. Menezes RG, Usman MS, Hussain SA, Madadin M, Siddiqi TJ, Fatima H, Ram P, Pasha SB, Senthilkumaran S, Fatima TQ, Luis SA. Cerbera odollam toxicity: A review. J Forensic Leg Med. 2018 Aug;58:113-116. [PubMed]
35. Krzyzak M, Regina A, Jesin RC, Deeb L, Steinberg E, Majlesi N. Anticholinergic Toxicity Secondary to Overuse of Topricin Cream, a Homeopathic Medication. Cureus. 2018 Mar 05;10(3):e2273. [PMC free article] [PubMed]
36. Hansen M, Eriksson C, Skarica B, Meckler G, Guise JM. Safety events in pediatric out-of-hospital cardiac arrest. Am J Emerg Med. 2018 Mar;36(3):380-383. [PMC free article] [PubMed]
37. Squires N, Wills A, Rowson J. The management of drooling in adults with neurological conditions. Curr Opin Otolaryngol Head Neck Surg. 2012 Jun;20(3):171-6. [PubMed]
38. Wichterle O., Lim D. Hydrophilic Gels for Biological Use. Nature. 1960;185:117–118. doi: 10.1038/185117a0. [CrossRef] [Google Scholar]
39. Buwalda S.J., Boere K.W., Dijkstra P.J., Feijen J., Vermonden T., Hennink W.E. Hydrogels in a historical perspective: From simple networks to smart materials. J. Control. Release. 2014;190:254–273. doi: 10.1016/j.jconrel.2014.03.052. [PubMed] [CrossRef] [Google Scholar]
40. Gioffredi E., Boffito M., Calzone S., Giannitelli S.M., Rainer A., Trombetta M., Mozetic P., Chiono V. Pluronic F127 hydrogel characterization and biofabrication in cellularized constructs for tissue engineering applications. Procedia Cirp. 2016;49:125–132. doi: 10.1016/j.procir.2015.11.001. [CrossRef] [Google Scholar]
41. Almeida M., Magalhães M., Veiga F., Figueiras A. Poloxamers, poloxamines and polymeric micelles: Definition, structure and therapeutic applications in cancer. J. Polym. Res. 2018;25:31. doi: 10.1007/s10965-017-1426-x. [CrossRef] [Google Scholar]

42. Aguilar M.R., Elvira C., Gallardo A., Vázquez B., Román J.S. Smart Polymers and Their Applications as Biomaterials. In: Ashammakhi N., Reis R.L., Chiellini E., editors. Topics in Tissue Engineering. Volume 3 Biomaterials and Tissue Engineering Group; Oulu, Finland: 2007. [Google Scholar]
43. Laryngoscope. 2000 Oct;110(10 Pt 1):1694-7 - PubMed
44. Int J Pharm. 2001 Oct 23;229(1-2):75-86 - PubMed
45. Biopharm Drug Dispos. 1997 Oct;18(7):623-33 - PubMed
46. Med Sci Sports Exerc. 1997 Nov;29(11):1416-21 - PubMed
47. Drug Dev Ind Pharm. 1999 Aug;25(8):897-904 - PubMed
48. HPMC K4M. Journal of Applied Pharmaceutical Science, 2012. 2(05): p. 100-105.
49. Jian, H., et al., Galactomannan (from Gleditsia sinensis Lam.) and xanthan gum matrix tablets for controlled delivery of theophylline: in vitro drug release and swelling behavior. Carbohydrate Polymers, 2012. 87(3): p. 2176-2182.
50. Talukdar, M. and R. Kinget, Swelling and drug release behaviour of xanthan gum matrix tablets. International Journal of Pharmaceutics, 1995. 120(1): p. 63-72.
51. Talukdar, M., et al., In vivo evaluation of xanthan gum as a potential excipient for oral controlled-release matrix tablet formulation. International journal of pharmaceutics, 1998. 169(1): p. 105-113.
52. Shiledar, R.R., A.A. Tagalpallewar, and C.R. Kokare, Formulation and in vitro evaluation of xanthan gum-based bilayered mucoadhesive buccal patches of zolmitriptan. Carbohydrate polymers, 2014. 101: p. 1234-1242.
53. Abu-Huwaij, R., et al., Formulation and in vitro evaluation of xanthan gum or carbopol 934-based mucoadhesive patches, loaded with nicotine. AAPS PharmSciTech, 2011. 12(1): p. 21-27.
54. Park, C.R. and D.L. Munday, Evaluation of selected polysaccharide excipients in buccoadhesive tablets for sustained release of nicotine. Drug development and industrial pharmacy, 2004. 30(6): p. 609-617.
55. Jaipal, A., et al., Interaction of calcium sulfate with xanthan gum: effect on in vitro bioadhesion and drug release behavior from xanthan gum based buccal discs of buspirone. Colloids and Surfaces B: Biointerfaces, 2013. 111: p. 644-650.
56. Azhar, S.A., et al., Studies on directly compressed ondansetron hydrochloride mucoadhesive buccal tablets using gelatin, chitosan and xanthan gum along with Fukuda, M., N.A. Peppas, and J.W. McGinity, Properties of sustained release hot-melt

extruded tablets containing chitosan and xanthan gum. International journal of pharmaceutics, 2006. 310(1): p. 90-100.

57. McCleary BV. Enzymic hydrolysis, fine structure and gelling interaction of legume seed D-galacto-D-mannans. Carbohydr Res. 1979;71:205–230. doi: 10.1016/S0008-6215(00)86071-1. [CrossRef] [Google Scholar]
58. McCleary BV. Galactomannan quantitation in guar varieties and seed fractions. Lebensm. Wiss. Technol. 1981;14:188–191. [Google Scholar]
59. McCleary BV, Clark AH, Dea ICM, Rees DA. The fine structures of guar and carob galactomannans. Carbohydr Res. 1985;139:237–260. doi: 10.1016/0008-6215(85)90024-2. [CrossRef] [Google Scholar]
60. McKiernan BJ (1957) The role of gums in stabilizers. Paper presented at the Michigan Dairy Manufacturer's Annual Conference, Michigan State University, East Lansing, Michigan
61. Burchard W. Light scattering. In: Murphy R, editor. Physical techniques for the study of food biopolymers S B. London: Blackie Academic and Professional; 1994. pp. 151–213. [Google Scholar]
62. Burrell JR. Pickles and sauces. Food Manuf. 1958;33:10–13. [Google Scholar]
63. Butt MS, Shahzadi N, Sharif MK, Nasir M. Guar gum: a miracle therapy for hypercholesterolemia, hyperglycemia and obesity. Crit Rev Food Sci Nutr. 2007;47:389–396. doi: 10.1080/10408390600846267.
64. M. Bhowmik, S. Das, D. Chattopadhyay, L.K. Ghosh, Scientia Pharmaceutica 79 (2011) 351–358.
65. O. Séchoy, G. Tissié, C. Sébastian, F.Maurin,J.Y. Driot, C. Trinquand,International Journal of Pharmaceutics 207 (2000) 109–116.
66. M.K. Bain, M. Bhowmik, S.N. Ghosh, D. Chattopadhyay, Journal of Applied Polymer Science 113 (2009) 1241–1246.
67. G.H. Hsiue, R.W. Chang, C.H. Wang, S.H. Lee, Biomaterials 24 (2003) 2423–2430.
68. Y.B. Schuetz, R. Gurny, O. Jordan, European Journal of Pharmaceutics and Biopharmaceutics 68 (2008) 19–25.
69. Z. Jiang, Y. You, X. Deng, J. Hao, Polymer 48 (2007) 4786–4792.
70. J. Lee, Y.H. Bae, Y.S. Sohn, B. Jeong, Biomacromolecules 7 (2006) 1729–1734.

71. G.M. Zentner, R. Rathi, C. Shih, J.C. McRea, M.-H. Seo, H. Oh, B.G. Rhee, J.Mestecky, Z.Moldoveanu,M.Morgan, S.Weitman,Journal ofControlledRelease 72 (2001) 203–215.
72. P. Alexandridis, J.F. Holzwarth, T.A. Hatton, Macromolecules 27 (1993) 414–2425.
73. J. Juhasz, V. Lenaerts, P. Raymond, H. Ong, Biomaterials 10 (1989) 265–268.
74. S.C. Miller, B.R. Darbik, International Journal of Pharmaceutics 18 (1984) 269–276.
75. J.C. Gilbert, C. Washington, M.C. Davies, J. Hadgraft, International Journal of Pharmaceutics 40 (1987) 93–99.
76. T.T. Gratieri, G.M. Gelfuso, M.E. Rocha, V.H. Sarmento, O.D. Freitas, R.F.V. Lopez, European Journal of Pharmaceutics and Biopharmaceutics 75 (2010) 186–193.
77. T. Ur-Rehman, S. Tavelin, G. Grübner, International Journal of Pharmaceutics 409 (2011) 19–29.
78. T. Ur-Rehman, S. Tavelin, G. Grübner, International Journal of Pharmaceutics 394 (2010) 92–98.
79. M. Bhowmik, S. Das, J. Sinha, S. Bag, D. Chattopadhyay, L.K. Ghosh, Asian Journal in Chemistry 22 (2010) 2147–2154.
80. J. Varshosaz, N. Tavakoli, F. Kheirolahi, American Association of Pharmaceutical Scientists 7 (2006) E1–E7.
81. V.F. Patel, N.M. Patel, Drug Development and Industrial Pharmacy 33 (2007) 327–334.
82. V.R. Sinha, R. Kumria, Drug Development and Industrial Pharmacy 30 (2004) 143–150.
83. R.C. Rowe, P.J. Sheskey, P.J. Weller, Handbook of Pharmaceutical Excipients, 5th ed., Royal Pharmaceutical Society of Great Britain, London, 2003.
84. M.K. Bain, D. Maity, N.K. Bera, S.N. Ghosh, M. Bhowmik, D. Chattopadhyay, International Journal of Biological Macromolecules 50 (2012) 565–572.
85. M. Bhowmik, M.K. Bain, L.K. Ghosh, D. Chattopadhyay, Pharmaceutical Development and Technology 16 (2011) 385–391.
86. F. Artzner, S. Geiger, A. Olivier, C. Allais, S. Finet, F. Agnely, Langmuir 23 (2007) 5085–5092.

NOTES