DEVELOPMENT AND EVALUATION OF THERMOSENSITIVE OPTHALMIC IN SITU GEL USING N-ISOPROPYLACRYLAMIDE GRAFTED LOCUST BEAN GUM

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CERTIFICATE

This is to certify that **SOURAV DEY** (Class Roll No.: 002111402039, Examination Roll No.: M4PHP23018 and Reg. No.: 139988 of 2017-2018), has carried out the research work on the subject entitled "***DEVELOPMENT AND EVALUATION OF THERMOSENSITIVE OPTHALMIC IN SITU GEL USING N-ISOPROPYLACRYLAMIDE GRAFTED LOCUST BEAN GUM***" under my supervision in the Pharmaceutics Research Laboratory in the Department of Pharmaceutical Technology of this university. He has incorporated his findings into this thesis of the same title, being submitted by him, in partial fulfillment of the requirements for the degree of **Master of Pharmacy** of Jadavpur University. He has carried out this research work independently and with proper care and attention to my entire satisfaction.

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I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as part of his Master of Pharmaceutical Technology 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 materials and results that are not original to this work.

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CHAPTER-I

INTRODUCTION

1. Introduction:

One of the most visually dangerous eye infectious disorders is bacterial keratitis[1]. If vigorous and proper medication is not promptly started, many patients experience a poor clinical outcome because the avascular corneal stroma is particularly prone to bacterial infection. There have been reports of corneal perforations, which can happen in less than 24 hours, when invasive microorganisms like *Pseudomonas aeruginosa* and *Staphylococcus aureus* are present. Ofloxacin, a second-generation fluoroquinolone derivative, is used to treat bacterial keratitis, keratoconjunctivitis, acute and subacute conjunctivitis, and other external eye infections.[2] Ofloxacin should be applied topically to the affected eye every four hours, or hourly in cases of severe infection. The recommended dosage for this is 1-2 drops of a 0.3% w/v solution. The ocular distribution of the medicine has proven to be one of the most difficult challenges for a pharmaceutical scientist due to the particular structure of the eye, which prevents the entry of drug molecules into the desired spot. More than 90% of ophthalmic medicines available on the market are eye drops. [3]. However, due to the high tear fluid turnover and quick precorneal clearance of the medicine, eye drops frequently have low bioavailability and therapeutic response [2]. The administration of eye drops frequently is correlated with patient noncompliance. If the medicine solution drained from the eye is consistently absorbed from the nasolacrimal duct, adding extra medication to the formulation to address bioavailability issues could be harmful [4]. Short precorneal residence time, poor corneal penetration, and low ocular bioavailability are the main drawbacks of this type of dosage form. [5]. Many ophthalmic vehicles, such viscous solutions, ointments, gels, or polymeric inserts, have been developed to lengthen the ocular residence time of topical eye treatments in an effort to solve these issues. These vehicles have improved the corneal contact time to varied degrees. But these formulations have not been well received because of obscured eyesight (for example, ointments) or low patient compliance (for example, inserts). Therefore, achieving adequate ocular bioavailability of a medicine after topical administration is still difficult and requires attention. The creation of an in situ gelling delivery system, which is supplied as a liquid and transforms into a semisolid gel upon exposure to the physiological environment, can solve these issues. [6]. The Latin word in-situ, which means "in its original place or in position," is the source of the phrase. The polymeric in situ gel has received more attention due to its benefits over traditional ophthalmic formulations, including simplicity of administration, decreased frequency of administration, and greater patient compliance. In situ gels can be divided into ion-activated, thermosensitive and pH-sensitive types [7]. The

thermosensitive in situ gel, which is one of three varieties of in situ gels, has a higher level of safety than the other two gels. Ion-sensitive and pH-sensitive gels have been said to irritate the eyes more, which could harm conjunctival cells[8]. Therefore, thermosensitive in situ gels, which undergo a sol-gel transition upon temperature change due to changes in the intermolecular interaction, are more promising for sustained ocular drug delivery [6]. There are various thermosensitive materials available with different gelling mechanisms and properties.

PNIPAAm is a commonly used negative thermosensitive polymer that has recently aroused a lot of scientific curiosity due to its increasing solubility with decreasing temperature and ability to cause volume phase shift by generating hydrogen bonds[9]. PNIPAAm forms a stretched spiral elastic shape in the aqueous media; polymer molecules create hydrogen bonds with one another as a result of hydrogen bonding with water molecules. Its structure combines hydrophilic amide (-CONH-) groups and hydrophobic isopropyl (-CH(CH3)2) side groups. PNIPAAm can be modified to create thermosensitive, in situ gels by copolymerizing with a variety of other hydrophobic or hydrophilic polymers. PNIPAAm has an LCST of 32 °C, which is somewhat lower than the average body temperature of 37 °C. The interactions between these groups and solvent molecules are the main causes of the temperature-responsive behaviour. As a result, as soon as the LCST hits body temperature, PNIPAAm transforms from a solution to a gel state. A larger LCST results from copolymerization with more hydrophilic monomers, which makes the polymer more hydrophilic and causes the polymer to interact with water in more important ways. In contrast, copolymerization with more hydrophobic monomers reduces LCST. PNIPAAm is suited for biomedical applications because of this characteristic, including controlled wound dressings, tissue engineering scaffolds, and drug delivery systems. Because the produced polymer matrices are brittle and unable to cling to the bottom of the vial, the poly(N-isopropylacrylamide) is not employed directly. [10]. Due to the viscosity-increasing properties of natural polysaccharides, the grafted copolymer displays remarkable adhesion qualities. So, to create graft copolymer, locust bean gum (LBG), a high molecular weight branch polysaccharide that is obtained from the seeds of the carob tree *Ceratonia silique*, is utilized[11]. They are referred to as galactomannan because they are a non-starch polysaccharide made up of galactose and mannose in a 1:4 ratio.

1.1 Phase transition for PNIPAAm:

Poly(N-isopropyl-acrylamide) and other N-substituted acrylamide polymers (PNIPAAM) have balanced hydrophilic and hydrophobic regions below LCST.[9] The gel-polymer/water system's total energy is lowered due to hydrophobic polymers enveloped by water molecules below LCST. The salvation and transition capacity of PNIPAAm in cold water increases when the temperature is raised off its LCST (LCST-32–34 ˚C), leading to the "coil to globule" of the polymeric chain's (CG) transition. The CG transition is in charge of phase inversion into rich layers. Polymeric/water phases further exhibit volume phase transition (VPT). A loss in entropy of water molecules enveloping the hydrophilic polymeric chain is counter balanced by an increase in enthalpy owing to hydrogen bonding between the hydroxyl groups surrounding the polymeric chain's hydrophobic sections. Hydrophobic hydration is a process that allows a hydrophobic polymer to stay hydrated in an aqueous environment. If the temperature is increased above LCST, water molecules leave the polymer chain and form a globule structure. As a result, the PNIPAAm–polymer is hydrated, and a definite volume phase transition is observed. The phase separation initially occurs due to PNIPAM molecule incorporation into larger aggregates via several mechanisms and factors, e.g., dewetting caused by solvent fluctuations, cooperative hydration, the aqueous medium's energy state, endothermic heat, precipitation polymerization, etc. The hydrogen bond between water molecules and PNIPAAm is weaker due to the temperature rising above LCST, leading to the formation of an unstable solution.

1.2 The Role of the Hydrogen Bonding Interactions:

At a cloud point, PNIPAAm exhibits a unique volume phase transition from a hydrated state called a hydrophilic state with an expanded structure to a shrunken dehydrated state called a collapsed structure[12]. The presence of hydrophilic and hydrophobic groups inside the neutral polymer is responsible for this reversible sol–gel behavior of PNIPAAm homopolymer in water solutions. The reversibility of the hydrophilic/hydrophobic states occurs by varying the temperature below or above the LCST value (32 °C) (Fig1.1). The LCST is the temperature above which the gel becomes insoluble in an aqueous environment. LCST depends on the critical gel concentration (CGC). Then, at its CGC, solvated PNIPAAm molecules will exhibit aqueous insolubility upon heating above the LCST. The LCST is mainly dependent on the hydrogen bonding between water molecules and the structure of functional monomer units of PNIPAAm polymer; i.e., N–H and C=O linkages. Thus, the incorporation of hydrophilic units

typically increases the volume-phase transition temperature (VPTT), whereas the addition of hydrophobic units has the opposite effect.

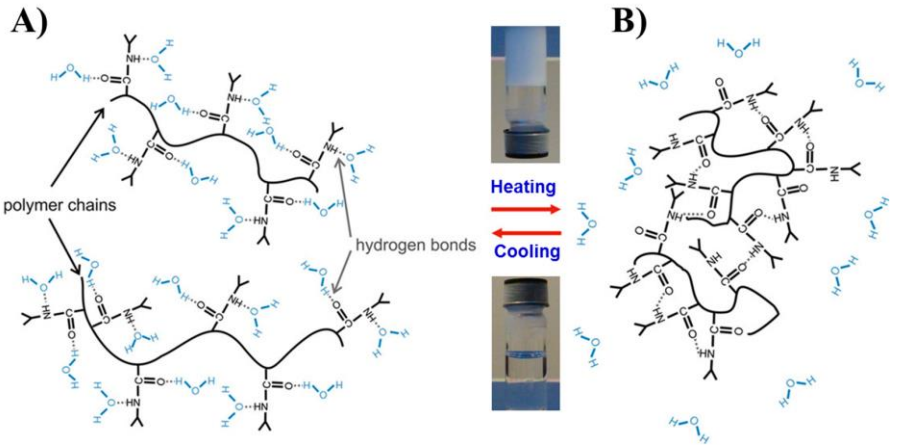


Fig 1.1: Difference in hydrogen bonding before and after gel formation

1.3 Biocompatibility and Biodegradability of PNIPAAm Based in situ Gel:

Polymers' physical and chemical characteristics (such as melting point, glass transition temperature, crystallinity, storage modulus, etc.) have an impact on how biodegradable they are. The use of PNIPAAm hydrogel in clinical medicine has been constrained by its poor biodegradability. By adding various biodegradable monomers and/or crosslinkers or natural polymers like poly(amino acids), polysaccharides, and proteins, as well as synthetic polymers like poly(esters), poly(caprolactone), and poly(ethylene glycol) (PEG), various methods for the preparation of biodegradable PNIPAAm-based hydrogels are being investigated. Both biodegradability and biocompatibility are necessary features depending on the application target (for example, in medication delivery and cell encapsulation). Animal cells are extremely biocompatible with PNIPAAm. In situ PNIPAAm-gelatin gel production was documented by Matsuda and colleagues in rat subcutaneous tissue. They histologically studied the rat fibroblast development for up to 12 weeks following organogel injection. The inflammatory response manifested at the time of injection but went away after two weeks, and the fibroblasts within the gel expanded and multiplied. This study establishes the viability of implanting PNIPAAm-gelatin gel as a cell scaffold in vivo.

1.4 Bacterial Keratitis: Bacterial keratitis is a serious ocular infectious disease that can lead to severe visual disability [13](Fig 1.2). The underlying health of the cornea and the pathogenicity of the infecting bacteria are two factors that frequently affect the severity of a corneal infection. If aggressive and adequate therapy is not promptly started, many patients experience poor clinical outcomes. Particularly vulnerable to bacterial infection is the avascular corneal stroma[14]. There have been reports of corneal perforations, which can happen in less than 24 hours, when invasive microorganisms like Pseudomonas aeruginosa and Staphylococcus aureus are present. Since most ocular pathogens cannot penetrate an intact epithelial corneal layer that is shielded by the eyelids and the tear film, corneal infections are mostly caused by a breakdown of one of the defensive mechanisms that maintain the integrity of the ocular surface. The host's defense mechanisms, both passive and active, shield ocular tissues from bacterial invasion. Although lysozymes, lactoferrin, betalysin, orosomucoid, and ceruloplasmin have been isolated within the lacrimal fluid, the precise role of each of these enzymes has not yet been established. Complement activation and enzyme secretion within the lacrimal fluid are parts of the natural host defense mechanisms. Bacteria can enter the cornea at any time after the passive systems of defense have failed or the corneal epithelium has been damaged. Interleukin 8 (IL-8) is the main factor that causes polymorphonuclear cell attraction, which takes place 8–10 hours after corneal injury. This IL-8 secretion may also be in charge of the subsequent growth of neovascularization. By using a slit lamp to examine the cornea, inflammatory cell infiltration causes corneal cloudiness that encircles the infected tissues. The limbus, which is surrounded by many lymphoid T and B cells that are a component of the mucosal associated lymphoid tissues (MALT), is where the particular immunity originates. Immunoglobulins that are secreted support bacterial phagocytosis as well.

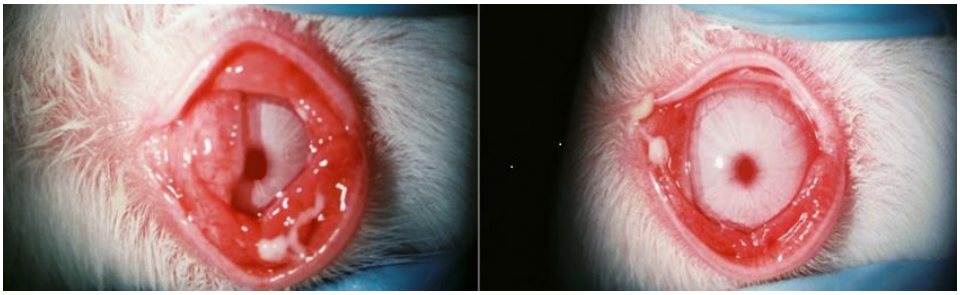


Fig 1.2: Bacterial keratitis induced in rabbit model

1.5 In situ gel- The word *in-situ* is derived from a Latin term that means “in its original place or in position” [6]. When exposed to physiological conditions including pH, temperature, and ionic strength in the environment, in situ gel undergoes a phase shift from liquid to semisolid

gel. When injected into the eye, in-situ forming ophthalmic gels are liquids that undergo rapid gelation in the eye's cul-de-sac in reaction to environmental changes to generate viscoelastic gels (Fig 1.3)[15]. lastly release the drug slowly under physiological conditions. Additionally, the in-situ gel's residence period will be prolonged, and the drug will be given gradually. These factors increase bioavailability, reduce systemic absorption, and need fewer frequent doses, which improves patient compliance. In-situ gelling devices have also demonstrated several additional potential benefits such an easy manufacturing process, convenience of administration, and delivery of a precise dose.

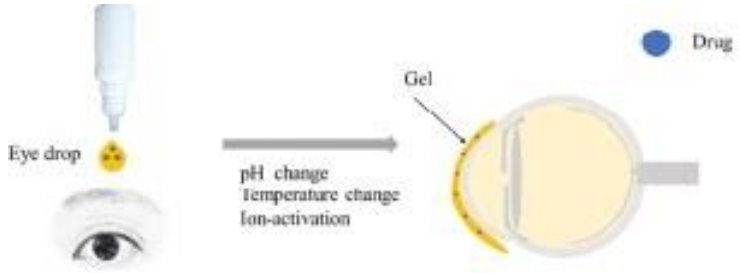


Fig 1.3 :In situ ophthalmic gel

1.6 Temperature responsive in situ gel- Based on the stimuli, in situ gels can be divided into ion-activated, thermosensitive and pH-sensitive types [7]. The thermosensitive in situ gel has superior safety than the other two in situ gels. Ion-sensitive and pH-sensitive gels have reportedly been found to irritate the eyes more, which can harm conjunctival cells. For prolonged ocular drug administration, thermosensitive in situ gels are therefore more promising since they go through a sol-gel transition when heated or cooled due to changes in the intermolecular interaction(Fig 1.4). The oldest, most well-studied, and most popular kind of stimuli-responsive gel is the temperature sensitive ophthalmic in-situ gel [15]. It may be applied to the eye in liquid form with ease and precision without irritating the eye or impairing vision. The gel is created at precorneal temperature (35 °C) to withstand the dilution of injected medication by lachrymal fluid without causing rapid precorneal elimination. A good thermo-responsive ocular in-situ gel has been advised to have the gelation temperature above room temperature and go through the gel-sol transition at a pre-corneal temperature to avoid having to store it in the refrigerator before use, which could occasionally cause eye irritation due to its cold nature.

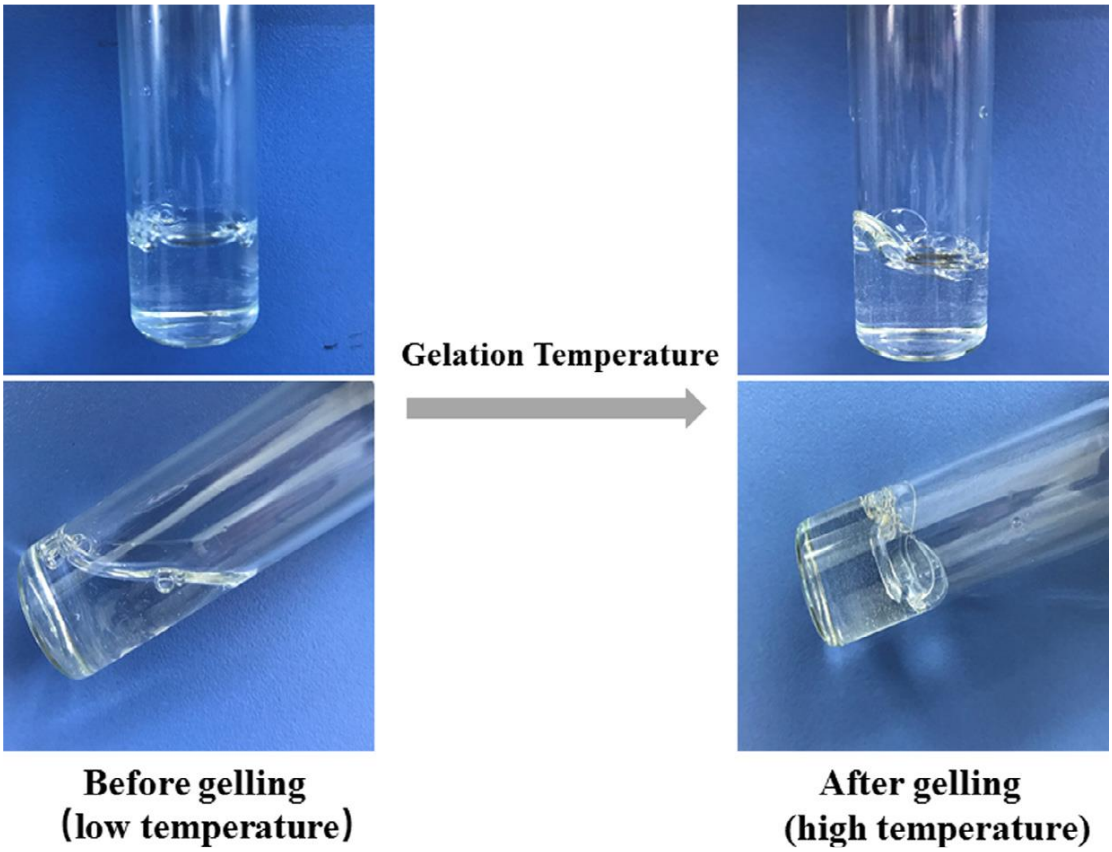


Fig 1.4: Temperature responsive in situ gel

1.7 Temperature responsive polymer and their mechanism of gelation:

1.7.1 Poloxamers (Pluronic): Poloxamers are a triblock copolymer poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide) (PEO-PPO- PEO) exhibiting amphiphilic nature because of hydrophilic ethylene oxide domains and hydrophobic propylene oxide domains [15](Fig 1.5). The triple block of copolymers PEO-PPO- PEO (Pluronics or Poloxamers) undergo gelation at body temperature in concentrations above 15% (w/w) [16] . The main theories put out to explain the sol-gel phase shift at a higher temperature include progressive polymer desolvation, accelerated micellar aggregation, and increased polymeric network entanglement. [17].

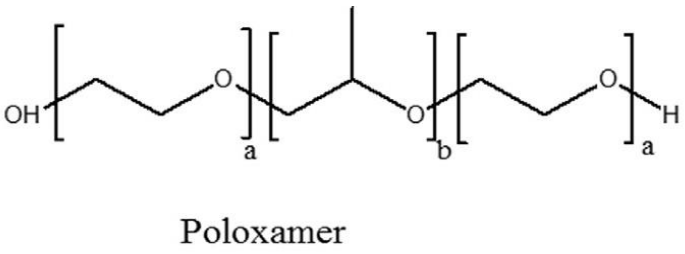


Fig 1.5: Structure of poloxamer

1.7.2 Poly(N-isopropylacrylamide)(PNIPAAm): PNIPAAm is a commonly used negative thermosensitive polymer that has recently aroused a lot of scientific curiosity due to its increasing solubility with decreasing temperature and ability to cause volume phase shift by generating hydrogen bonds[9]. PNIPAAm forms a stretched spiral elastic shape in the aqueous media; polymer molecules create hydrogen bonds with one another as a result of hydrogen bonding with water molecules. Its structure combines hydrophilic amide (-CONH-) groups and hydrophobic isopropyl (-CH(CH3)2) side groups[Fig 1.6]. PNIPAAm can be modified to create thermosensitive, in situ gels by copolymerizing with a variety of other hydrophobic or hydrophilic polymers. PNIPAAm has an LCST of 32 °C, which is somewhat lower than the average body temperature of 37 °C. The interactions between these groups and solvent molecules are the main causes of the temperature-responsive behaviour and it can be modified by copolymerizing with some distinct hydrophobic or hydrophilic polymers to develop thermo-sensitive, in situ gels. Poly(N-isopropyl-acrylamide) and other N-substituted acrylamide polymers (PNIPAAM) have balanced hydrophilic and hydrophobic regions below LCST. The gel-polymer/water system's total energy is lowered due to hydrophobic polymers enveloped by water molecules below LCST. The salvation and transition capacity of PNIPAAm in cold water increases when the temperature is raised off its LCST (LCST-32–34 ˚C), leading to the "coil to globule" of the polymeric chain's (CG) transition.

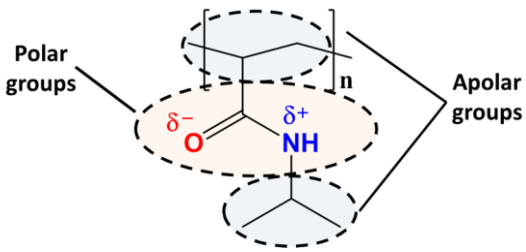
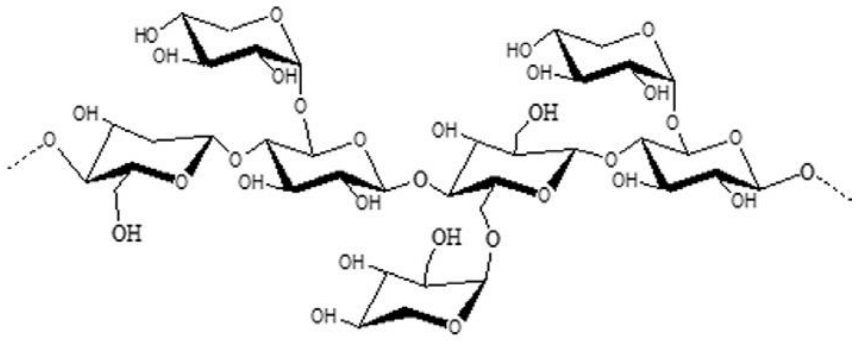


Fig 1.6: Structure of N-isopropylacrylamide

1.7.3 Xyloglucan: Xyloglucan is a polysaccharide obtained from tamarind seeds, therefore it is often named tamarind seed polysaccharide (TSP), which when partially degraded by β-

galactosidase displays thermally reversible gel formation in diluted aqueous solution[15](Fig 1.7). The sol-gel transition temperature is varying with the degree of galactose degradation.



Xyloglucan

Fig1.7: Structure of Xyloglucan

1.8 Objective of the work:

The objective of the present work was to develop a temperature sensitive in situ gelling ophthalmic delivery system of ofloxacin, a second generation fluoroquinolone derivative used in external infections of the eye such as acute and subacute conjunctivitis, bacterial keratitis and keratoconjunctivitis [2]. The topical ophthalmic dose of ofloxacin is 1-2 drops of a 0.3% solution in the affected eye every 4 h or hourly in the case of severe infection. A solution of poly(NIPAAm-g-LBG) was investigated as a vehicle for the formulation of in situ gel of ofloxacin (0.3% w/v) which would gel when instilled into the eye and provide sustained release of drug during treatment in ocular infections.

REFERENCES:

1. Schaefer F, Bruttin O, Zografos L, Crosier YG. Bacterial keratitis: a prospective clinical and microbiological study. Br J Ophthalmol 2001;85:842–847.

2. Srividya B, Cardoza RM, Amin PD. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. J Control Release. 2001; 73: 205–211.

3. Okur NU, Yozgatli V, Okur ME , Yoltaş A , Siafaka PI. Improving therapeutic efficacy of voriconazole against fungal keratitis: Thermo-sensitive in situ gels as ophthalmic drug carriers. J Drug Deliv Sci Technol. 2019; 49: 323–333.

4. Zhu M, Wang J, Li N. A novel thermo-sensitive hydrogel-based on poly(*N*-isopropylacrylamide)/hyaluronic acid of ketoconazole for ophthalmic delivery. Artif Cells Nanomed Biotechnol. 2018; 46: 1282–1287.

5. Lai JY, Hsieh AC. A gelatin-g-poly(N-isopropylacrylamide) biodegradable in situ gelling delivery system for the intracameral administration of pilocarpine, Biomaterials 2012; 33: 2372-2387.

6. Gadad AP, Wadklar PD, Dandghi P, Patil A. Thermosensitive In situ Gel for Ocular Delivery of Lomefloxacin. Indian J. Pharm. Educ. Res. 2016; 50: 96-105.

7. Wei Y, Li C, Zhu Q, Zhang X, Guan J, Mao S. Comparison of thermosensitive in situ gels and drug-resin complex for ocular drug delivery: In vitro drug release and in vivo tissue distribution. Int. J. Pharm. 2020; 578 : 119184.

8. Kawase K, Lin W, Aoyama Y , Yamamoto T , Shimazawa M , Hara H. Effects of Timolol-Related Ophthalmic Solutions on Cultured Human Conjunctival Cells. Jpn J Ophthalmol 2010; 54: 615–621.

9. Ansari MJ, Rajendran RR , Mohanto S, Agarwal U, Panda K, Dhotre K, Manne R , A. Deepak , Zafar A , Yasir M and Pramanik S. Poly(N-isopropylacrylamide)-Based Hydrogels for Biomedical

Applications: A Review of the State-of-the-Art. Gels 2022; 8: 1-45.

10. Lai JY, Hsieh AC. A gelatin-g-poly(N-isopropylacrylamide) biodegradable in situ gelling delivery system for the intracameral administration of pilocarpine, Biomaterials 2012; 33: 2372-2387.

11. Kaity S, Isaac J, Kumar P.M, Bose A, Wong T.W ,Ghosh A. Microwave assisted synthesis of acrylamide grafted locust bean gum and its application in drug delivery; Carbohydr. Polym. 2013; 98: 1083– 1094.

12. Lanzalaco S, Armelin E. Poly(N-isopropylacrylamide) and Copolymers: A Review on Recent Progresses in Biomedical Applications. Gels 2017; 3: 1-31.

13. Bourcier T, Thomas F, Borderie V, Chaumeil C, Laroche L. Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. Br J Ophthalmol 2003;87:834–838.

14. Schaefer F, Bruttin O, Zografos L, Crosier YG. Bacterial keratitis: a prospective clinical and microbiological study. Br J Ophthalmol 2001;85:842–847.

15. Wu Y, Liu Y, Li X, Kebebe D, Zhang B, Ren J, Lu J, Li J, Du S, Liu Z. Research progress of in-situ gelling ophthalmic drug delivery system. Asian J. Pharm. Sci. 2019; 14: 1–15

16. Almeida H ,Amaral MH ,Lobao P . Temperature and pH stimuli-responsive polymers and their applications in controlled and self regulated drug delivery. J App Pharm Sci 2012;2:1–10.
17. Laddha UD ,Mahajan HS . An insight to ocular in situ gelling systems. Int J Adv Pharmaceut 2017;6:31–40.

CHAPTER-II

LITERATURE REVIEW

2. Literature Review

Kaity et al. developed an acrylamide grafted copolymer of locust bean gum by microwave irradiation using ceric ammonium nitrate(CAN) as a redox initiator [1]. The grafting process was optimized in terms of irradiation time, amount of initiator and acrylamide keeping fixed the amount of locust bean gum. This optimized grafted gum was used to prepare a controlled release matrix tablet of buflomedil hydrochloride(BH) and it was compared with the HPMC based matrix tablet of BH. Both the tablets followed a non-Fickian diffusion controlled drug release process. Hence it can be concluded that acrylamide grafted locust bean gum can be used as rate controlling hydrophilic polymer for controlled release matrix tablets.

Giri et al. developed polyacrylamide grafted locust bean gum via the conventional method using ceric ammonium nitrate (CAN) as a free radical initiator to improve flocculation efficiency [2]. The flocculation efficacy of the grafted copolymer was studied in kaolin suspension. The grafting process was optimized in terms of initiator concentration, monomer concentration, polymer concentration, reaction time and reaction temperature keeping fixed the concentration of other parameters. The grafted copolymer had an improved intrinsic viscosity. So, it can be used as a superior viscosifier. The flocculation efficiency of the grafted copolymer was noticeable in kaolin suspension.

Coronado et al. developed an interpenetrating polymer network(IPN) by using poly(*N*-isopropyl acrylamide) and hyaluronic acid(HA) [3]. The LCST of the obtained hydrogel was found between 34.4 to 35.5 ˚C. the functional groups of the hydrogel were identified by FT-IR and 1H-NMR. It should be noted that the LCST of the hydrogel was maintained at the same temperature after the inclusion of HA biopolymer. The rheological study confirmed the viscoelastic characteristics of the hydrogel. Furthermore, it can be concluded that this hydrogel can be used as a nontoxic injectable hydrogel.

Andrei et al. developed a novel injectable thermosensitive hydrogel with improved both water retention and drug release profile by adding dextran to an aqueous solution of poly(N-isopropyl acrylamide)[4]. The addition of hydrophilic polysaccharide improved water retention of the hydrogel from 12% in the absence of dextran to about 40-55%. Also, the burst effect of the drug release can be prevented by the dextran containing hydrogel using 5-fluorouracil as a model drug.

Santos et al. synthesized new pH and temperature sensitive semi-interpenetrated polymer network(semi-IPN) by combining cross-linked poly(N-isopropyl acrylamide) (PNIPAAm) with hyaluronic acid(HA) [5]. The LCST of the hydrogel was found between 33-34 ˚C and did

not change upon the introduction of HA. This semi-IPN hydrogels had significantly greater and faster swelling at 25 ˚C and a more complete deswelling at 37 ˚C than PNIPAAm hydrogel. The presence of HA allowed a more complete release of the drug, Gentamycin, at physiological conditions.

Xiao et al. prepared hydrogel based on linear HPMC and cross-linked PNIPAAm by the semi-IPN method [6]. It was found that the content of HPMC had an impact on the swelling ratio of the hydrogel. Furthermore, the modified PNIPAAm hydrogel exhibited a faster shrinking rate upon a temperature rise from 25 ˚C to 45 ˚C. All the results suggested that this hydrogel would be a potential thermosensitive gel for drug delivery.

Zhang et al. designed and synthesized a novel thermosensitive hydrogel by graft copolymerization of N-isopropyl acrylamide (NIPAAm) and biodegradable carboxymethyl chitosan (CMCS) [7]. In comparison with the conventional PNIPAAm hydrogels, the newly developed hydrogels have improved thermoresponsive properties, including enlarged water content at room temperature and faster deswelling/swelling rate upon heating. This thermosensitive and biodegradable hydrogel may have potential applications in controlled drug delivery systems.

Srividya et al. formulated sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system using Carbopol 940 as a gelling agent and Methocel E50LV as a viscosity-enhancing agent [8]. The formulation was liquid at pH 6.0 and underwent rapid gelation upon raising the temperature to pH 7.4. The gel formed in situ offered sustained drug release over a period of 8 h. The formulation was therapeutically efficacious. The developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through prolonged precorneal residence time and its ability to sustain drug release. Furthermore, ease of administration and reduction in dosing frequency result in better patient compliance.

Gadad et al. developed a thermosensitive in situ gel for ocular delivery of lomefloxacin using Pluronic F127, Pluronic F68 and sodium alginate [9]. The FTIR result revealed that the drug is compatible with the excipients of the formulations. The rheological study confirmed sol to gel transition at physiological eye temperature and it was also observed that all formulations exhibited pesudoplastic rheology as evidenced by a decrease in viscosity with an increase in angular velocity. Prepared in situ gels formed instantaneously when contacted with STF and formed gel would enhance the contact time of the drug which in turn prolongs the residence time of the drug. In vitro release data indicated the drug release in a controlled manner over a

period of 8 hr. All the formulations were best expressed by Higuchi's model and the release mechanism was Fiction release.

Zhu et al. developed a thermosensitive in situ gelling formulation of ketoconazole based on poly(N-isopropyl acrylamide/hyaluronic acid [10]. The gelation temperature was found to be 33 ˚C. In vitro release indicated that the release of ketoconazole from in situ gels was moderate without a burst effect. From in vivo antimicrobial study, it can be concluded to have a better cure percent in clinical profile and negative growth of Candia albicans. Therefore, this novel dosage form is able to prolong residence time and release the drug in a controlled manner.

Lai et al. synthesized aminated gelatin grafted with carboxylic end-capped poly(N-isopropyl acrylamide) via a carbodiimide-mediated coupling reaction for intracameral administration of pilocarpine [11]. This in situ gel forming drug delivery system had given an increase in performance over conventional eye drops. The grafted copolymer possessed better thermal gelation ability and adherence and might allow a sustained release of pilocarpine and enhance the ocular drug bioavailability. This novel dosage form helped to improve the efficacy of glaucoma treatment by prolonging the pharmacological responses. These findings suggested that the thermoresponsive and biodegradable gelatin-g-PNIPAAm may have potential application as an injectable formulation for intraocular drug delivery.

Pentlavalli et al. developed thermoresponsive copolymers of alginate-g-NIPAAm that are injectable, biodegradable and biocompatible and are synthesized via free radical graft copolymerization technique [12]. The LCST was found to be at 32°C. the copolymers had the ability to control the release of model drugs. The hydrogel system showed very good biocompatibility and ability to encapsulate pBMSCs without affecting their differentiation capability. It was observed that 70% of the grafted copolymers were degraded after 8 weeks. Therefore, the synthesized grafted copolymer has the potential to be used as an injectable hydrogel for drug delivery and bone tissue engineering applications.

This study reported the development of chitosan-g-poly(N-isopropylacrylamide) via carbodiimide-mediated formation of amide linkages between chitosan and carboxyl-terminated poly(N-isopropyl acrylamide) as an in situ gelling delivery system for the intracameral administration of pilocarpine [13]. The amount of thermo-responsive polymer chains grafted onto the chitosan backbone was greatly affected by varying the feeding quantity of carboxyl-terminated PNIPAAm in the synthesis, thereby determining the LCST and enzymatic degradability of grafted copolymers. It was observed that the increase in grafting ratio facilitated temperature responsive gelation behavior and drug encapsulation at physiological

conditions. Slow degradation of the grafted copolymer was also responsible for the delayed release of pilocarpine, which in turn allowed the concentration of the released drug could reach a minimum therapeutic level in the treatment of glaucoma during the total period of the study. These chitosan-based thermogels can be potentially used as ophthalmic carriers for prolonged drug release and improved delivery performance.

Abreu et al. developed NIPAAm grafted cashew gum via radical polymerization technique to generate a thermoresponsive copolymer for a drug delivery system [14]. The grafted copolymers formed nanoparticles at room temperature via self-assembly as the particles were dispersed in distilled water and they showed thermal responsiveness. At temperatures below LCST, the nanoparticle sizes were small, but above the LCST the particles aggregated, increasing the particle size. The cell viability in the presence of the nanoparticles was good and the graft copolymer showed good potential for application as a epirubicin delivery matrix. Nesseem developed an in situ gelling drug delivery system by combining pluronic F127 and pluronic F68, with sparfloxacin to examine the influence of sodium hyaluronate, a mucoadhesive polysaccharide, on the healing property due to bacterial keratitis [15]. It was observed that the developed formulations were therapeutically efficacious, and provided sustained release of the medication over a period of 24 hours. The release behavior of all formulations was non-Fickian anomalous release. The different formulations were used to overcome the pathological alterations, produced by bacterial infections. A better improvement in artificially induced bacterial conjunctivitis in rats' cornea was observed with the developed formulae; thus it can be considered as a viable alternative to conventional eye drops.

REFERENCES

1. Kaity S, Isaac J, Kumar P.M, Bose A, Wong T.W ,Ghosh A. Microwave assisted synthesis of acrylamide grafted locust bean gum and its application in drug delivery; Carbohydr. Polym. 2013; 98: 1083– 1094.

2. Giri TK, Vishwas S, Tripathi,DK. Synthesis of Grafted Locust Bean Gum Using Vinyl Monomer and Studies of Physicochemical Properties and Acute Toxicity; J. Nat. Prod. 2016; 6: 179-187.

3. Coronado R, Pekerar S, Lorenzo AT , Sabino MA. Characterization of thermo-sensitive hydrogels based on poly(N-isopropylacrylamide)/hyaluronic acid. Polym. Bull. 2011; 67: 101–124.

4. Andrei M, Turturica G, Stanescu P.O,Teodorescu M. Thermosensitive injectable hydrogels from poly(Nisopropylacrylamide)–dextran aqueous solutions:Thermogelation and drug release properties. Soft Mater. 2016; 14: 162–169.

5. Santos JR, Alves NM Mano JF. New Thermo-Responsive Hydrogels Based On Poly (N-Isopropylacrylamide)/ Hyaluronic Acid Semi-Interpenetrated Polymer Networks: Swelling Properties And Drug Release Studies. J Bioact Compat Polym 2010; 25: 169-184.

6. Xiao Y, Xia C,Duan G, Zhao X. Preparation and Characterization of Thermo-Sensitive Hydroxypropyl-Methyl Cellulose / Poly (N-isopropylacrylamide) Hydrogel. Adv Mat Res 2011; 194-196: 773-776.

7. Zhang H, Zhong H, Zhang L, Chen S, Zhao Y, Zhu Y. Synthesis and characterization of thermosensitive graft copolymer of N-isopropylacrylamide with biodegradable carboxymethylchitosan. Carbohydr. Polym. 2009; 77: 785–790.

8. Srividya B, Cardoza RM, Amin PD. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. J Control Release. 2001; 73: 205–211.

9. Gadad AP, Wadklar PD, Dandghi P, Patil A. Thermosensitive In situ Gel for Ocular Delivery of Lomefloxacin. Indian J. Pharm. Educ. Res. 2016; 50: 96-105.

10. Zhu M, Wang J, Li N. A novel thermo-sensitive hydrogel-based on poly(*N*-isopropylacrylamide)/hyaluronic acid of ketoconazole for ophthalmic deliveryArtif Cells Nanomed Biotechnol. 2018; 46: 1282–1287.

11. Lai JY, Hsieh AC. A gelatin-g-poly(N-isopropylacrylamide) biodegradable in situ gelling delivery system for the intracameral administration of pilocarpine, Biomaterials 2012; 33: 2372-2387.

12. Pentlavalli S, Chambers P, Sathy BN, O'Doherty M, Chalanqui M, Kelly DJ, Donahue TH, McCarthy HO, Dunne NJ. Simple Radical Polymerization of Poly(Alginate-Graft- N-Isopropylacrylamide) Injectable Thermoresponsive Hydrogel with the Potential for Localized and Sustained Delivery of Stem Cells and Bioactive Molecules, Macromol. Biosci. 2017; 1700118: 1-12.

13. Lai JY, Luo LJ. Chitosan-g-poly(N-isopropylacrylamide) copolymers as delivery carriers for intracameral pilocarpine administration, Eur J Pharm Biopharm. 2017; 113: 140–148.

14.Abreua CMWS, Paulab HCB, Seabrac V, Feitosaa JPA, Sarmentoc B, Paulaa RCMD. Synthesis and characterization of non-toxic and thermo-sensitivepoly(N-

isopropylacrylamide)-grafted cashew gum nanoparticles as apotential epirubicin delivery matrix ; Carbohydr. Polym. 2016; 154: 77–85.

15. Nesseem DI. Ophthalmic delivery of sparfloxacin from in situ gel formulation for treatment of experimentally induced bacterial keratitis; Drug Test Anal, 2011; 3: 106–115.

CHAPTER-III

MATERIALS AND METHODS

3 MATERIALS

Ofloxacin was kindly gifted by Crest Life Science Pvt. Ltd, Himachal Pradesh, India. N-isopropylacrylamide (NIPAAm) was obtained from Sisco Research Laboratories Pvt. Ltd, Maharashtra, India. Locust Bean Gum (LBG) was procured from HiMedia Laboratories Pvt. Ltd, Nashik, India. Ceric Ammonium Nitrate (CAN) was obtained from Loba Chemie Pvt. Ltd, Maharashtra, India. All other solvents and reagents were of analytical grade and used without further modification.

METHODS

3.1 Preparation of Simulated Tear Fluid (STF)

In a 100 ml volumetric flask 30 ml distilled water was taken. All the reagents were taken in the required quantity. The regents were dissolved by continuous shaking. Finally, the volume was made up to 100 ml.

Table 3.1: Composition of simulated tear fluid: For 100 ml [1]

Component	Quantitity
Sodium chloride	0.68 g
Sodium bicarbonate	0.22 g
Calcium chloride dehydrate	0.008 g
Potassium chloride	0.14 g
Distilled deionized water	Upto 100 ml

3.2 Preparation of Standard Curve of ofloxacin in STF

The standard curve of ofloxacin was plotted in STF by making serial dilutions from the stock solution of 0.1 mg/ml [2]. Diluted solutions were analyzed at 287 nm. Absorbance was determined using a UV–vis spectrophotometer at 287 nm (UV 2450, Shimadzu, Japan). The quantity of ofloxacin released at various time intervals was calculated from standard curves drawn in STF.

3.3 Synthesis of N-isopropyl acrylamide grafted LBG

0.2 g of LBG was taken in a beaker containing 15 ml of water and it was left overnight to become hydrated. 0.4 g of NIPAAm was dissolved in 4 ml of water and both solutions were transferred into the reaction vessel. They were mixed thoroughly for 1 h with the help of a magnetic stirrer. 0.0005-0.002 M of CAN was taken into 0.2-0.8 M of nitric acid and it was transferred into the vessel under continuous stirring. Then the reaction was continued for 4 h at room temperature under continuous nitrogen purging. After the reaction was completed, the

semisolid mass was immersed in excess of acetone to get the precipitated product of the grafted copolymer. To remove the homopolymer associated with the grafted copolymer the residue mass was washed several times with methanol and water at a ratio of 70:30. The product was collected and dried completely until constant weight was obtained. The grafting parameters were calculated using the following equations [3].

$$\%\ Grafting\ (\%G) = \frac{(W_g - W_c)}{W_c} \times 100 \text{.....(1)}$$

$$\%\ Grafting\ Efficiency\ (\%GE) = \frac{(W_g - W_c)}{W_m} \times 100 \text{.....(2)}$$

Where W_g, W_c, and W_m denote the weight of pure grafted copolymer, pure polysaccharide and monomer respectively.

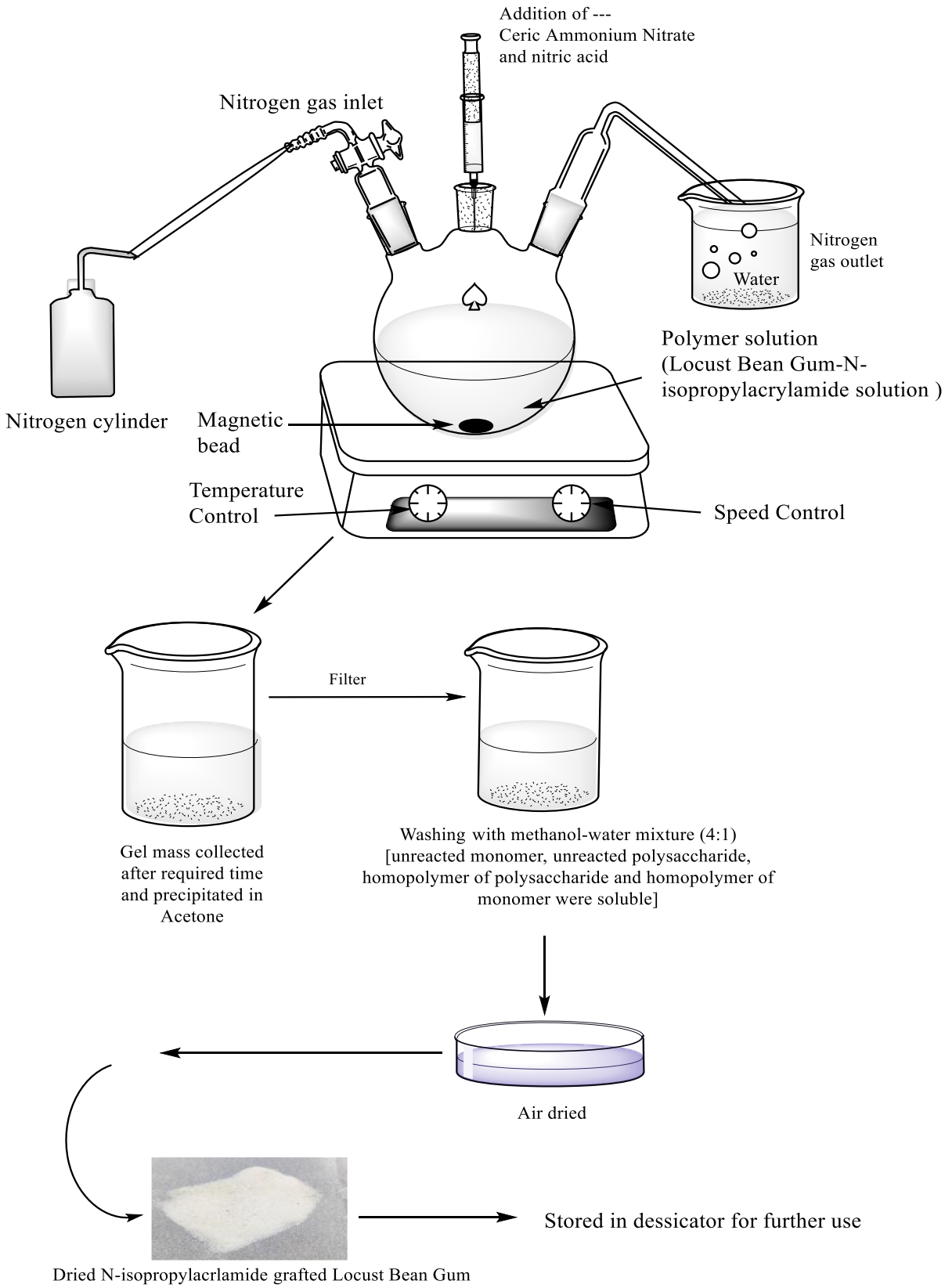


Fig 3.1: Synthesis mechanism of N-isopropylacrylamide grafted Locust bean gum

3.4 CHARACTERIZATION

3.4.1 Fourier-transformed infrared spectroscopy (FT-IR)

Analysis of FTIR spectra of LBG, NIPAAm, NIPAAm-g-LBG, were performed in a FTIR spectrophotometer (Bruker) at 4000–400 cm^{-1} [4]. Preparation of samples involved mixing samples with potassium bromide and converting them into pellets in a hydraulic press.

3.4.2 Nuclear Magnetic Resonance (NMR)

The 13C cross-polarization magic angle spinning (CP-MAS) solid-state NMR of LBG and NIPAAm-g-LBG was studied to confirm the grafting of LBG [4]. NMR spectra of LBG and NIPAAm-g-LBG samples (50 mg) were conducted on a JEOL ECX 500 MHz NMR spectrometer at a frequency of 10 kHz. The sample holder was a zirconium made 3.2 mm rotor. The referencing for the 13C spectrum is done by using Tetramethyl silane (TMS). TMS was separately filled in the rotor and the referencing was done externally.

3.5 Preparation of in situ gel Forming Solutions

The weighed quantities of grafted copolymers were taken in a test tube and left overnight for swelling in distilled water [2]. Then it was dissolved by stirring with the help of a glass rod. Ofloxacin (0.3%w/v) was added to the polymeric solution with continuous stirring. Benzalkonium chloride was added as a preservative to the final solution.

3.6 Evaluation

3.6.1 Clarity Test

The prepared formulations were visually checked for clarity against a black and white background under good light, with the contents set in motion with a swirling action.

3.6.2 pH determination

pH of each formulation was determined by using a digital pH meter (Toshcon Toshniwal) immediately after preparation. The digital pH meter was calibrated by using pH 4 and pH 7 standard buffers.

3.7 Gelling Capacity

The gelling capacity was determined the according to the reported method with modification [6]. 50 ml of STF was taken into a small beaker and the temperature was maintained at physiological body temperature (37 °C). A small drop of the formulation was placed into the beaker and the time was noted to form the gel and also the formed gel to get solubilized by observing visually.

3.8 Gelling Temperature

The gelling temperature of the developed in situ ophthalmic gel was determined by test tube inversion technique [5]. 2 ml of the formulation was placed in a test tube which was immersed in a beaker containing 50 ml of water. Then the temperature was increased gradually from 25 to 50 ˚C. The temperature at which the solution inside the test tube stopped moving upon inversion of the test tube was noted as gelling temperature.

3.9 Viscosity Study

The Brookfield viscometer was used to obtain the viscosity of the developed formulation at different temperatures keeping the rpm fixed and different rpm keeping the temperature fixed [6]. In a tube, 5 ml of the cold formulation was taken and the spindle associated with the viscometer was dipped up to the mark inside the tube. The setup was arranged so cautiously that the temperature was maintained at a fixed value as well as increased gradually from 25 to 40 °C. Viscosity was noted down at different rpm and temperatures.

3.10 In vitro dissolution study

The in vitro dissolution study was carried out on both sides opened test tube with a diameter of 1.5 cm. The dialysis membrane, previously soaked overnight, was tied to one end of the test tube [6]. The test tube was placed into a beaker containing 50 ml of STF as dissolution media. The test tube is to be placed in such a way that the dialysis membrane just touches the surface of the dissolution media. 1 ml of the optimized formulation containing the drug ofloxacin (0.3 % w/v) was taken into the opened side of the test tube. The whole assembly was placed onto a magnetic stirrer and the solution was rotated by using a magnetic bead at 50 rpm. The temperature was maintained at 37 °C. 1 ml of aliquot was withdrawn at specified time intervals and replaced with freshly prepared STF. Samples were diluted properly and absorbances were taken at a particular wavelength by using a UV-Visible spectrophotometer (UV 2450, Shimadzu, Japan). The study was conducted for marketed eye drops, and ophthalmic solution and developed a formulation at the same concentration of the drug. This study was conducted in triplicate. The obtained data were fitted into zero order, first order, and Higuchi models to analyze the mechanism of drug release and release rate kinetics. The best fit model was selected by comparing the obtained R^2 values.

3.11 Ex vivo permeation study

The ex vivo permeation study was conducted in the same way as mentioned above with the same optimized formulation. But the dialysis membrane was replaced with the goat cornea [6]. Goat corneas were used to study permeation across the corneal membrane. Goat eyes were collected previously from the slaughter house and transported to laboratory in cold condition. They were maintained at 4 ˚C in normal saline water. Cornea was separated from the scleral tissue and washed cautiously with saline water. This cornea was used as a lipophilic-hydrophilic barrier for permeation study. Samples were collected, and diluted properly and absorbances were taken at a particular wavelength by using a UV visible spectrophotometer. This study was also conducted for marketed eye drops, ophthalmic solutions, and the developed formulation at the same concentration of the drug. This study was also conducted in triplicate. The flux (Jss, μg/cm^2.h) and Permeability coefficient (PC, cm/h) were calculated by using equations 3 and 4 respectively.

$$Flux(Jss) = \frac{Amount\ of\ drug\ permeated(\mu g)}{Time(h) * Area\ of\ corneal\ membrane} \quad \text{.....(3)}$$

$$Permeability\ Coefficient(PC) = \frac{Flux}{Initial\ amount\ of\ drug} \quad \text{.....(4)}$$

References

1. Okur NU, Yozgatli V, Okur ME , Yoltaş A , Siafaka PI. Improving therapeutic efficacy of voriconazole against fungal keratitis: Thermo-sensitive in situ gels as ophthalmic drug carriers. J Drug Deliv Sci Technol. 2019; 49: 323–333.

2. Pujitha C , Jyothi JS , Sucharitha J , Lakshmi G , Rao AL. Formulation and characterization of ofloxacin opthalmic GEL. Indian J Pharm Pharmacol. 2017;4(2):105-109.

3. Zhang HF, Zhong H, Zhang LL, Chen S, Zhao YJ, ZhuYL. Synthesis and characterization of thermosensitive graft copolymer of N-isopropylacrylamide with biodegradable carboxymethylchitosan. Carbohydr. Polym. 2009; 77: 785–790.

4. Mukherjee K, Dutta P, Giri TK. Al3+/Ca2+ cross-linked hydrogel matrix tablet of etherified tara gum for sustained delivery of tramadol hydrochloride in gastrointestinal milieu. Int. J. Biol. Macromol. 2023; 232: 123448.

5. Zhu M, Wang J, Li N. A novel thermo-sensitive hydrogel-based on poly(*N*-isopropylacrylamide)/hyaluronic acid of ketoconazole for ophthalmic deliveryArtif Cells Nanomed Biotechnol. 2018; 46: 1282–1287.

6. Gadad AP, Wadklar PD, Dandghi P, Patil A. Thermosensitive In situ Gel for Ocular Delivery of Lomefloxacin. Indian J. Pharm. Educ. Res. 2016; 50: 96-105.

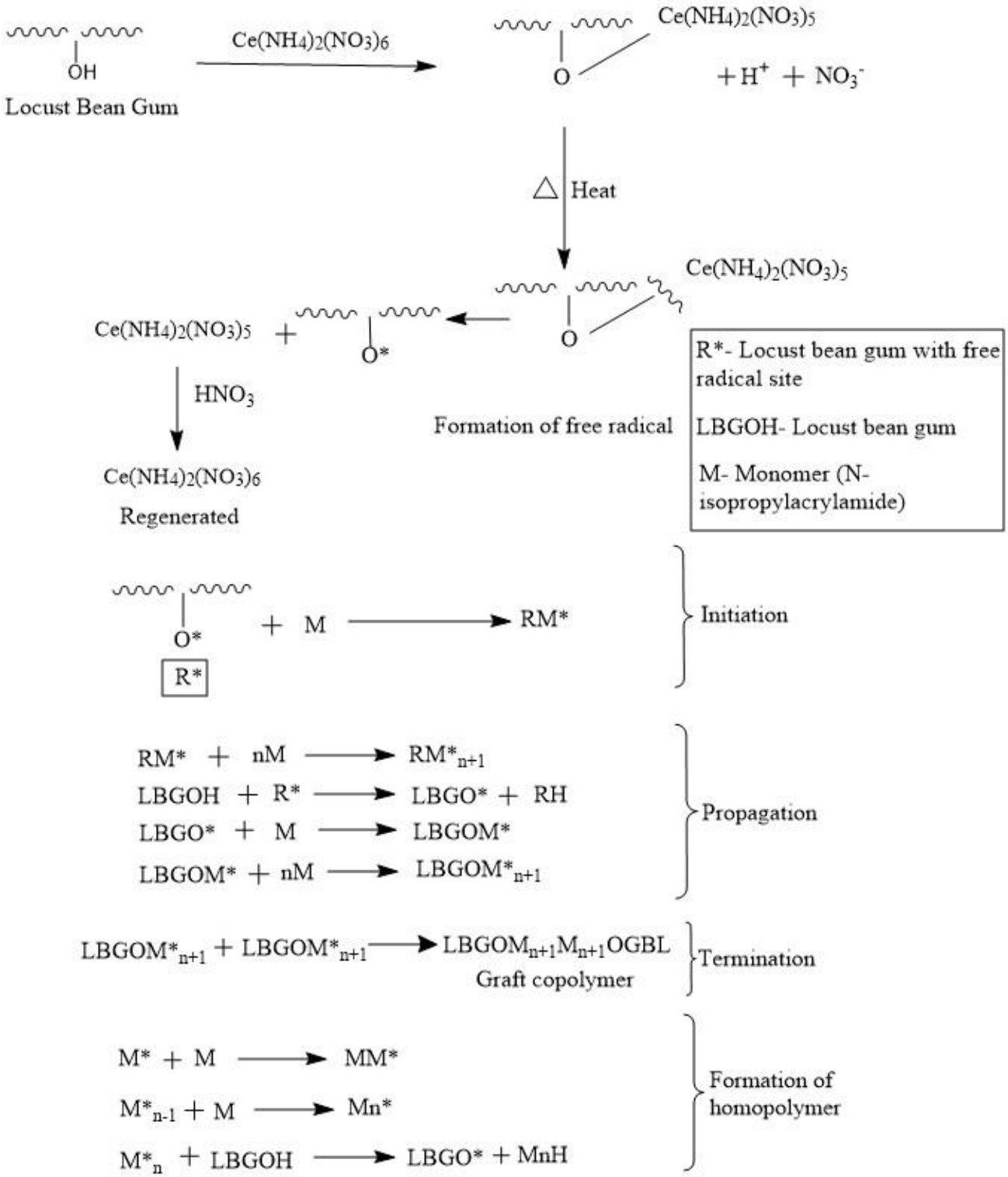
CHAPTER-IV

RESULTS, DISCUSSION & CONCLUSION

4. Results and Discussion:

4.1 Synthesis of *N*-isopropylacrylamide grafted LBG

The temperature sensitive NIPAAm grafted LBG was synthesized by free radical graft copolymerization technique. As an initiator ceric ammonium nitrate (CAN) is used to induce free radical graft copolymerization. In the presence of nitric acid, ceric (IV) ion attacks the polysaccharide chain and generates an LBG-ceric complex [1]. The ceric (IV) ions in the complex are reduced to ceric (III) ions by oxidizing hydrogen atoms onto the LBG backbone and generating a free radical site onto the polysaccharide chain. An optimum amount of initiator is required to induce free radical graft copolymerization. The grafting of NIPAAm onto LBG is affected by how many free radicals react with the monomer. The LBG free radical is chemically coupled to the NIPAAm by a covalent bond. A similar mechanism was obtained by other researchers [2,3]. The reaction mechanisms has been shown in scheme 1.



Scheme 1: Synthesis mechanism of N-isopropylacrylamide grafted locust bean gum

4.2 Characterization

4.2.1 Solid State NMR

NMR spectra of LBG and grafted LBG were depicted in Fig 4.1. NMR spectrum of LBG exhibited three distinct peaks for the mannan unit at δ = 61.9 ppm (C-6); broadened signals between δ ~ 69–81 ppm accumulating the signals of δ = 69.7, 71.9, 75.5, and 80.2 ppm (C-2, C-3, C-4 and C-5). Some notches on the peak reveal evidence of signal merging at δ = 101.1 ppm (for C-1 of mannan unit) [4]. The peaks for the respection C atoms of galactose moiety overlapped with the peaks of the mannan unit. The existing literature revealed that C atoms of galactose unit in LBG exhibited peak at δ = 59.6 (C-6), 68.5 (C-3, C-4), 70.3 (C-5), 99.4(C-1) ppm [5]. In the NMR spectra of NIPAAm-g-LBG, some intense peaks were observed at δ=23.95 ppm corresponding to the carbon of CH_3, at 42.87 ppm to the C-N carbon, at 128.14 and 133 ppm to the C=C (sp2 hybridized carbon) carbon and at 166.14 ppm to the carbon of carbonyl group respectively. Except for the peaks at 128.14 and 133 ppm for the carbon of C=C (sp2 hybridized carbon), all the peaks of LBG and NIPAAm were present in the NMR spectrum of poly(NIPAAm-g-LBG) copolymer. After the grafting of NIPAAm into the LBG backbone, the sp2 hybridized carbon atom was converted to a sp3 hybridized carbon atom. As a result, the peaks at 128.14 ppm and 133 ppm were shifted to 42.3 ppm and 42.5 ppm respectively and overlapped with the peak at 42.9 ppm. Similar signals for grafted gum have been reported earlier [6].

A **B**

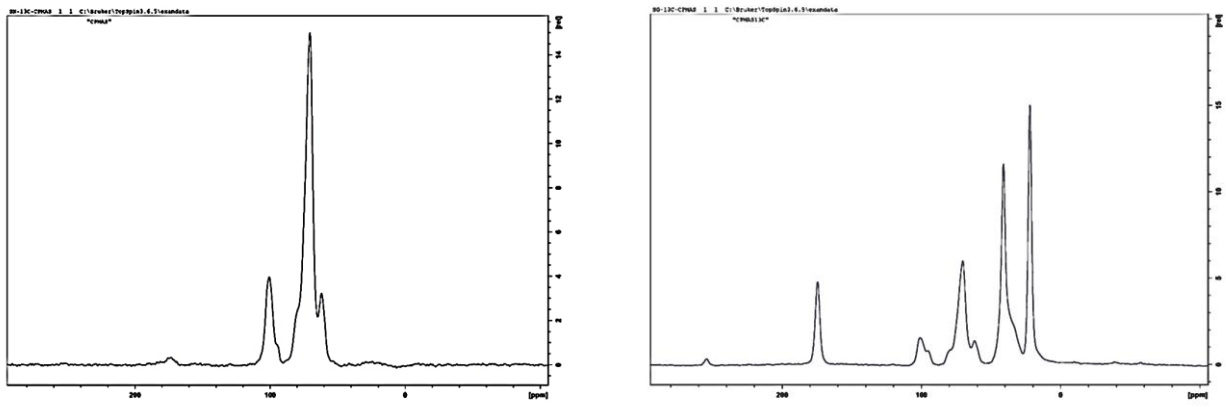


Fig 4.1: NMR spectra of LBG(A) and Grafted LBG(B)

4.2.2 FTIR characterization of poly(NIPAAm-graft-LBG)

The FTIR spectra of LBG and grafted LBG were represented in Fig 4.2 The FTIR spectrum of LBG demonstrated a broad absorption band at 3300 cm^{-1}due to stretching of -OH groups, a peak at 2931.80 $cm^{-1}$for CH_2 stretching, peaks within a range of 1000–1100 cm^{-1}(1026, 1096 and 1145 cm^{-1}) due to C- O- H stretching, and a peak at 1400.03 cm^{-1} for CH_2 bending. Similar IR spectrums of LBG are available in the literature [7]. NIPAAm showed some characteristics peaks at 2974 cm^{-1}(symmetric vibration of mehyl groups), 2878 cm^{-1} (asymmetric vibration of methyl groups), 1659 cm^{-1} (stretching vibration of C=O groups), 1548 cm^{-1}(bending vibration of N-H groups), and a doublet at 1455 and 1410 cm^{-1}(symmetric vibration of isopropyl groups). Similar absorption bands of NIPAAm have been reported by other authors [6]. Therefore, the presence of characteristics absorption bands at 2986, 2880, 1648, and 1540 cm^{-1} as well as a doublet at 1394 and 1364 cm^{-1} in the FTIR spectrum of NIPAAm-g-LBG is clear evidence of grafting of NIPAAm into LBG backbone. Broadband between 3400-3200 cm-1 was also observed due to the overlapping of the O-H stretching band of LBG and the N-H stretching band of NIPAAm. It suggests that the NIPAAm monomer was successfully grafted into LBG backbone.

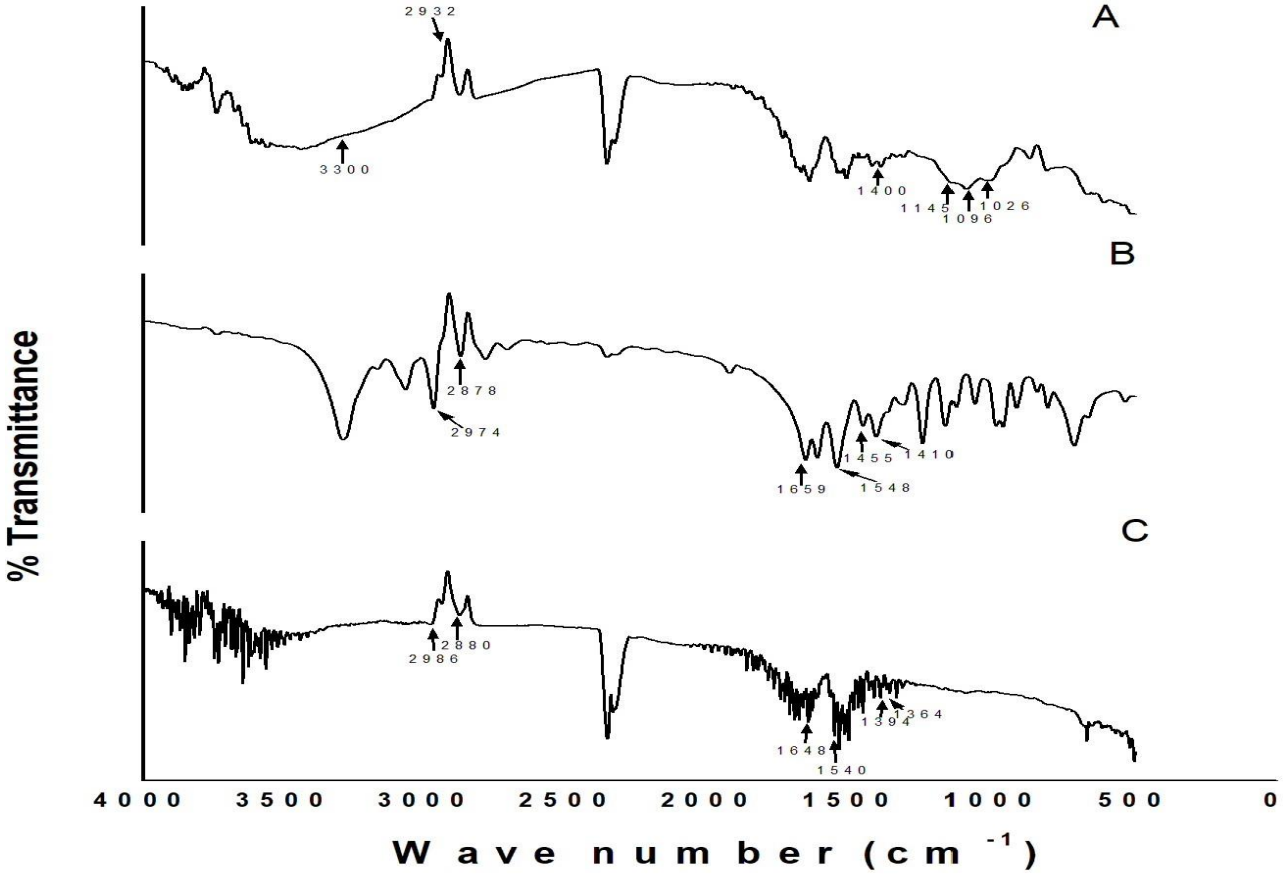


Fig 4.2: FTIR spectra of A) LBG B) NIPAAm C) Grafted copolymer

4.3 Viscosity

Phase transition temperature is an important parameter for in situ gel-forming polymer, which determines the potential utilization of the polymer in ocular drug delivery [8]. The phase transition of a thermosensitive polymer is a result of the change in its hydrophilic–hydrophobic balance. Below the LCST the hydrogen-bonding interactions of the polymer with water molecules determine its solubility. When raising the temperature these interactions are weakened and above the LCST the hydrophobic interactions become predominant. Changes in the structure of the polymer chain that increase its overall hydrophobicity result in decreasing in the LCST of the polymer solution. An acceptable ophthalmic thermogelling solution must have a gelation temperature in the range of 32-37℃ so as to be in liquid form at room temperature and to form a gel phase instantly in the ocular cavity. The relationship between the sol–gel transition intrinsic viscosity and temperature are shown in Fig 4.3.

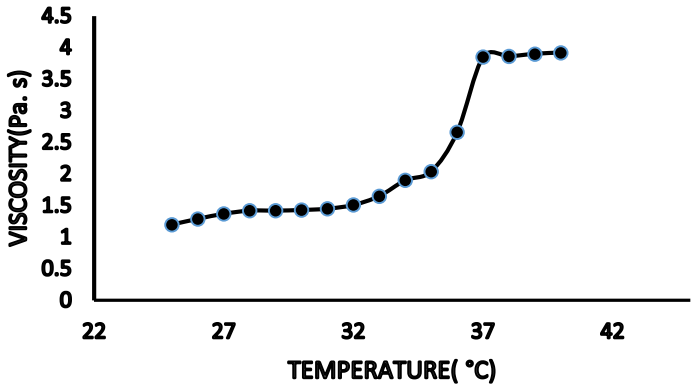


Fig 4.3: Temperature versus Viscosity curve

At a fixed RPM when the temperature was increased gradually from 25-40 ˚C, a drastic change in the viscosity was observed at the point of LCST (Table 4.1). The formulation was in solution state at room temperature but when the temperature was raised up to 37℃, the solution was converted into gel at the point of LCST. Gadad et al. developed a thermosensitive in situ gel for ocular delivery of lomefloxacin using Pluronic F127, Pluronic F68 and sodium alginate [9]. The rheological study confirmed sol to gel transition at physiological eye temperature (37 °C) and it was also observed that all formulations exhibited pesudoplastic rheology as evidenced by a decrease in viscosity with an increase in angular velocity. Prepared in situ gels formed instantaneously when contacted with STF and formed gel would enhance the contact time of

the drug which in turn prolongs the residence time of the drug. Zhu et al. developed a thermosensitive in situ gelling formulation of ketoconazole based on poly(N-isopropyl acrylamide/hyaluronic acid [10]. The gelation temperature was found to be 33 ˚C.

The viscosity of in situ gel formulation was studied at different angular velocities (Fig 4.4). At 25℃, viscosity was not changed significantly with an increase in rpm because it was in a liquid state throughout the study (Table 4.2).

Table 4.1: At RPM 30 TEMP vs VISCOSITY

TEMP	25	26	27	28	29	30	31	32	33
VISCOSITY	1.2	1.29	1.37	1.42	1.42	1.43	1.45	1.51	1.65

TEMP	34	35	36	37	38	39	40
VISCOSITY	1.9	2.04	2.66	3.85	3.86	3.9	3.92

Table 4.2: At 25 °C RPM vs VISCOSITY

RPM	12	20	30	50	60	100
VISCOSITY	1.42	1.35	1.2	1.1	1.03	0.945

At 37℃ in situ gel formulation showed non Newtonian flow behavior at different shear stress (Table 4.3). At the higher shear rate, the non-Newtonian flow shows lesser resistance. At rest, the polymer has a long entangled chain and exhibits high viscosity. High internal resistance with an increase in shear rate, the molecular chain disentangles and exhibits shear thinning behavior (low internal resistance). Moreover, increasing the shear rate decreases the interaction between the molecules and consequently reduced viscosity.

Table 4.3: At 37 °C RPM vs VISCOSITY

RPM	12	20	30	50	60	100
VISCOSITY	6.38	4.8	3.85	2.9	2.55	0.19

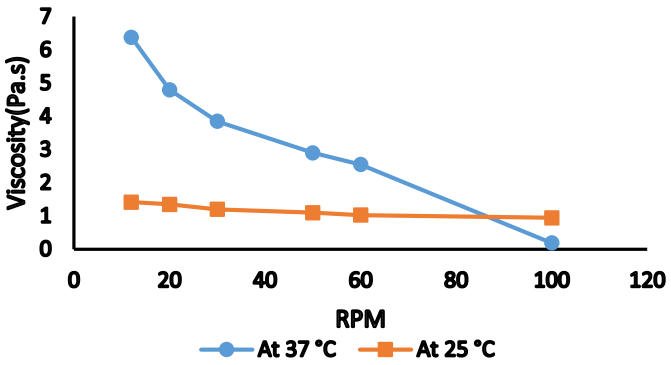


Fig 4.4: Viscosity of thermosensitive in situ gel formation at different shear rates

The ocular shear rate is very large ranging from 0.03 S^{-1} during inter-blinking periods to 4250-28 550 S^{-1} during blinking [11]. So, viscoelastic fluids with a viscosity that is high under the conditions of low shear rate and low under the conditions of high shear rate are preferred. Therefore, in situ gel formulations with pseudoplastic behavior are preferred for ophthalmic drug delivery, and polymer solutions with pseudoplastic behavior show a decreasing viscosity trend with increasing shear rates.

4.4 Construction of Calibration Curve of Ofloxacin

The standard curve data of ofloxacin has shown good linearity over a concentration range of 2-10 µg/ml with R^2 value of 0.9995 The equation was y=0.0842x This was further utilized for in vitro drug release and ex vivo corneal permeation study.

Table 4.4: Standard curve of Ofloxacin

Conc(µg/ml)	Abs 1	Abs 2	Abs 3	Avg Abs	SD
2	0.178	0.175	0.169	0.174	0.004583
4	0.345	0.341	0.339	0.341667	0.003055
6	0.508	0.505	0.503	0.505333	0.002517
8	0.657	0.67	0.667	0.664667	0.006807
10	0.842	0.856	0.838	0.845333	0.009452

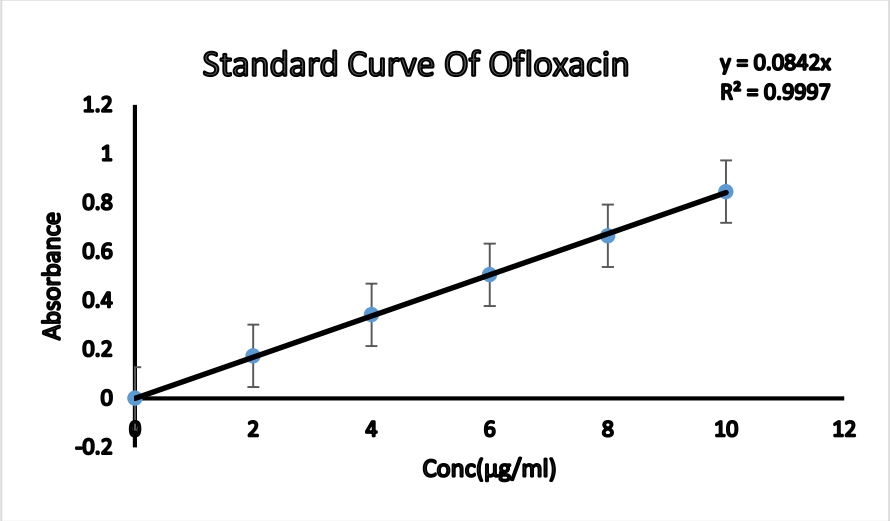


Fig 4.5: Standard curve of Ofloxacin

4.5 Optimization of the grafting conditions

The different grafting parameters such as monomer, initiator, and nitric acid concentrations were obtained to optimize grafting reaction conditions.

4.5.1 Effect of Initiator Concentration

CAN concentration varied from 0.0005M to 0.002 M. Increase in CAN concentration initially led to an increase in %GE and %G beyond which both %GE and %G declined (Table 4.5, Fig 4.6) . The rise in %GE and %G might be due to the initiator attacking the polysaccharide backbone and generating free radical sites, which will initiate grafting. At high concentrations, the reduction in %GE and %G might be due to an increase in the number of LBG radicals terminated prior to NIPAAm addition [1]. Furthermore, at higher initiator concentrations the formation of homopolymer competes with the grafting reaction [12]. A similar result was observed for the ceric ion induced graft copolymerization of hydroxyl propyl guar gum [13]. So the optimum initiator concentration is 0.001M.

Table 4.5: %GE and %G at different CAN concentration.

Code	CAN concentration (M)	Amount (mg)	Yield (g)	(%GE)	%G
F1	0.0005	60	0.210	2.50%	5%
F2	0.001	120	0.240	10%	20%
F3	0.002	240	0.170	0%	0%

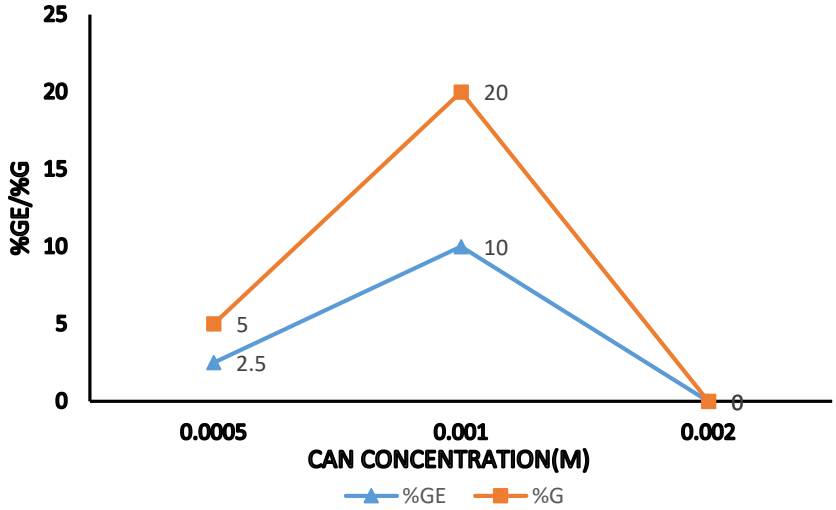


Fig 4.6: %GE and %G at different CAN concentration

4.5.2 Effect of Nitric acid concentration

The concentration of nitric acid was varied from 0.2M to 0.8M keeping fixed concentrations of all other reagents, time, and temperature. The %G and %GE increase with an increase of acid concentration up to 0.4M and decreases beyond this concentration(Table 4.6, Fig 4.7). The optimum nitric acid concentration was 0.4M. The role of nitric acid in grafting of NIPAAm onto LBG is explained by the fact that ceric ion in water is believed to react in the following manner:

$Ce^{4+} + H_2O \rightarrow [Ce(OH)_3]^{3+} + H^+$

$2[Ce(OH)_3]^{3+} \rightarrow [Ce\text{-}O\text{-}Ce]^{6+} + H_2O$

In the beginning the %G and %GE increases with increasing the concentration of H^+. This result is attributed to the increase in concentration of Ce^{4+} and $[Ce(OH)_3]^{3+}$ at the expense of

$[Ce\text{-}O\text{-}Ce]^{6+}$. Ceric ions, being smaller in size , form complex with LBG more efficiently than $[Ce\text{-}O\text{-}Ce]^{6+}$. At higher concentration of H^+, the equilibria shifts towards formation of more $[Ce\text{-}O\text{-}Ce]^{6+}$. The similar result was observed for the ceric ion induced graft copolymerization of acrylamide onto Cassia tora Gum.[12]

Table 4.6: %GE and %G at different nitric acid concentration:

Code	Concentration (M)	Quantity(µl)	Yield(g)	%GE	%G
F4	0.2	50	0	0%	0%
F5	0.8	200	0.218	4.50%	9%
F6	0.6	150	0.23	7.50%	15 %
F7	0.4	100	0.24	10%	20 %

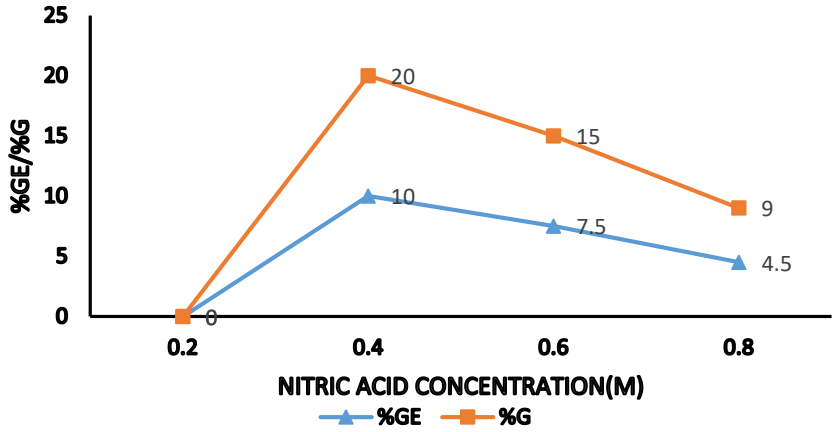


Fig 4.7: %GE and %G and different nitric acid concentration

4.5.3 Effect of monomer concentration on Grafting Parameters:

Monomer content was varied from 0.4 g to 2 g. (Table 4.7, Fig 4.8). The monomer content could not be extended further because increase in monomer concentration leads to decrease in grafting parameters. The %GE and %G were found to increase as the monomer content increased. This is due to greater availability of free radical sites on LBG backbone to monomer molecule. However, at higher monomer concentration decrease in grafting parameters might be due to the higher affinity of monomer for its homopolymer over LBG free radicals. Thus

most of the monomers were taken up for the formation of homopolymer on increasing the NIPAAm concentration. The similar result was observed earlier.[12, 14]

Table 4.7: %GE and %G at different monomer concentration

code	Monomer amount(g)	Yield(g)	%GE	%G
F8	0.4	0.24	10%	20%
F9	1	0.272	18%	36%
F10	2	0.32	30%	60%

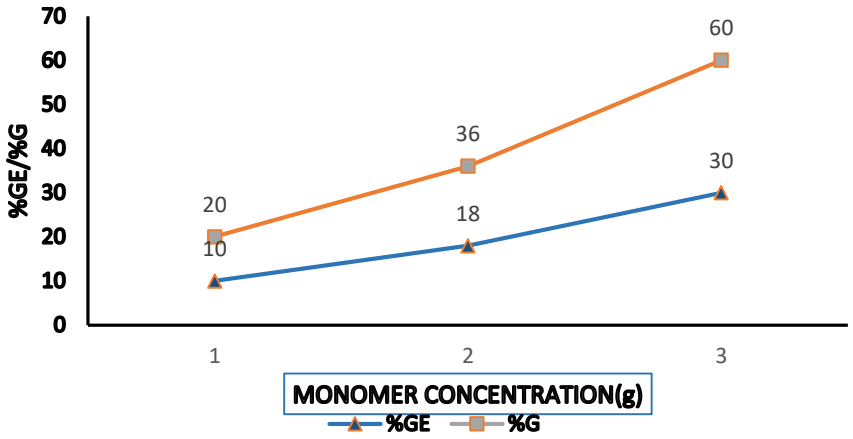


Fig 4.8: %GE and %G and different monomer concentration

4.6 Development of in situ Gel:

The use of the NIPAAm in situ gelling system is substantiated by the property of this polymer's aqueous solution to transform into a stiff gel when the temperature is raised above LCST. The two main criteria of in situ gelling systems are viscosity and gelling capacity. LBG is used to enhance the viscosity of the formulation and also to incorporate the biodegradability nature of the grafted copolymer. It should be noted that the formulation should have optimum viscosity. It will allow easy instillation into the eye as a liquid drops which would undergo rapid gelation when temperature is raised above LCST. Furthermore, the gel formed in situ should preserve its integrity without eroding or dissolving for a prolonged period of time. A concentration of 1.5% w/v of the optimized grafted polymer was selected to form in situ ophthalmic gel as it had satisfactory attributes of viscosity and gelling capacity.

4.7 Evaluation

4.7.1 Clarity

After careful visual inspection against black and white background all the formulations were found to be free from any suspended particulate matter.

4.7.2 pH

The pH of all prepared in situ gel was found to be in the range of 7.0-7.4 which was within the range of physiological pH of the eye and hence would not create any irritation upon instillation into the eye. Tears pH was about 7.4 and varied in the range of 5.2 to 9.3 in disease conditions [15]. However, the eye can tolerate the formulations without buffering in the range of 3.5-8.5, and after administration tears adjust the physiologic pH.

4.8 Gelling Capacity

All temperature sensitive formulations showed immediate gelation and retained for a prolonged period of time. The formulations should have an optimum gelling capacity, so that after instillation into the eye as a liquid it would undergo rapid sol to gel transition and would retain its integrity without dissolving or eroding for an extended period of time [9]. A similar result was found in the thermosensitive in situ gel for the ocular delivery of lomefloxacin [9].

4.9 Gelation Temperature

The gelation temperature of all the formulations was in the range of 35-37 ˚C(Table 4.8). The concentration of CAN and HNO_3 was optimized earlier. It was observed that upon increment in the amount of monomer (NIPAAm), the gelling temperature was decreased. This may be due to the presence of more no of monomer units in the case of 1:5 and 1:10(ratios of polymer and monomer) as compared to 1:2. The temperature sensitive nature is mainly shown by NIPAAm, so the presence of more no of monomer unit results in a decrease in gelling temperature. Ideally in situ thermosensitive ophthalmic gel which is free flowing liquid at room temperature and turns into gel at physiological eye surface temperature is considered optimized as gelation at lower or higher physiological temperature will either cause difficulty in instillation or rapid precorneal elimination [9]. Thus by variation in the concentration of monomer units, the gelling temperature can be attained in the physiological eye surface temperature. A similar result was found in the thermosensitive in situ gel for the ocular delivery of lomefloxacin [9]. So the formulation which contains 1:2 (LBG: NIPAAm) showing gelation at temperature 37 ˚C, is considered optimized. The final solution of the optimized one was selected for further study on the basis of gelation time, temperature, viscosity and %G. 1.5% w/v of the grafted copolymer was used to prepare in situ gel forming solution because it had

gelation temperature near physiological body temperature and optimum consistency to administer easily into the eye.

Table 4.8: Gelling temperature of various formulations

Code	Polymer(g)	Monomer(g)	CAN(M)	HNO_3(M)	Gelling Temperature(°C)
F1	0.2	0.4	0.0005	0.4	37
F2	0.2	0.4	0.001	0.4	37
F3	0.2	0.4	0.002	0.4	37
F4	0.2	0.4	0.001	0.2	37
F5	0.2	0.4	0.001	0.8	37
F6	0.2	0.4	0.001	0.6	37
F7	0.2	0.4	0.001	0.4	37
F8	0.2	0.4	0.001	0.4	37
F9	0.2	1	0.001	0.4	36
F10	0.2	2	0.001	0.4	35

4.10 In vitro drug release

The cumulative percentage release profiles of optimized formulation obtained was compared with that of a marketed formulation and drug solution. In case of marketed eye drops almost all the drug was released within 7 hours whereas ophthalmic drug solution gave the therapeutic effect upto 8 hours. Initially, optimized formulation possessed rapid release, gradually approaching constant values for the rest of the time [16]. Thus, conforming to the controlled release behavior of the formulation. The initial quick release (burst effect) would be beneficial as it would help achieve the therapeutic concentration of the drug in a minimum time, and the constant release later on would then provide a sustained and controlled release of the drug. The Burst effect might be due to the initial migration of the drug toward the surface of the matrix. Drug release from in situ gel formulation was much slower than that from an aqueous eye drop solution with no polymer excipients. It was reported that drug release depends on two parameters: the polymer type and its concentration.[17] Due to the increasingly tangled character of the polymeric network, the release of Ofloxacin through the formulation decreased as the polymer content increased. Additionally, the amount of water entering the formulation with a high polymer content was decreased, which lowered the rates of both erosion and dissolution. Additionally, at increasing polymeric concentrations, the chain structure of the gels

became denser, which reduced the region where the active material could travel. Finally, as the solids content grew, the degree of swelling also increased, which inhibited drug diffusion from the swelled matrix. The influence of the viscosity due to the addition of polymeric substance on the diffusion of the drug can be described by the Stokes Einstein equation. This demonstrates that an increased viscosity of the formulation results in slower diffusion of the drug across the gel matrix and into the receptor medium. A similar observation was observed by other authors, where the viscosity of the gel represented the main factor controlling drug diffusion [18]. The in vitro drug release conditions may be very different from those likely to be encountered in the eye.[19] However, the outcomes clearly demonstrate that ofloxacin can be retained by the gels and will not be released prematurely. Due to the shearing effect of the eyelid and eyeball movements in the cul-de-sac, the gels will likely dissolve more quickly there. The in vitro release data were kinetically analyzed according to Zero order, first order, diffusion controlled mechanism (Higuchi model), and Krosmeyer-Peppas model. The linear regression analysis is summarized in Table 4.9

Table 4.9: In vitro drug release kinetics of Ofloxacin from in situ gel system

Model	**Equation**	**R^2**
Zero order	$Q = Q_0 + kt$	0.402
First order	$logQ = \log Q_0 + \frac{kt}{2.303}$	-16.73
Higuchi	$Q = K_H + \sqrt{t}$	0.9816
Korsmeyer–Peppas	$\frac{M_t}{M_\infty} = K_P \times t^n$	0.9954

The Coefficient of determination(R^2) values for the in situ gel indicated that the Korsmeyer–Peppas model was suitable for its release mechanism. The cumulative percent of drug released as a function of time from in situ gel formulation and marketed eye drops are shown in Fig 4.9.

Table 4.10: Cumulative percentage of drug released as a function of time (For marketed eye drop):

Time	CPR1	CPR2	CPR3	Mean	STD
1	27.5138559	25.32565	28.65499	27.16483	1.691887
2	43.3056215	42.35465	45.23698	43.63242	1.468696
3	66.3301663	67.56262	66.54896	66.81391	0.657558
4	76.9319082	78.65543	75.69846	77.09526	1.485238

5	85.3444181	88.56419	86.25985	86.72282	1.659062
6	90.3325416	92.45129	90.25411	91.01265	1.246515
7	100.3365	101.8547	102.3652	101.5188	1.055253

Table 4.11: Cumulative percentage of drug released as function of time (For drug solution):

Time	CPR1	CPR2	CPR3	MEAN	STD
1	29.6912114	28.65231	30.54615	29.62989	0.948404
2	45.922407	47.25645	45.25987	46.14624	1.016935
3	56.5281077	55.65456	58.95461	57.04576	1.709841
4	66.3380839	67.59875	68.54126	67.4927	1.105412
5	78.3016627	77.58944	78.54951	78.14687	0.4984
6	86.1243072	84.52331	85.24157	85.2964	0.801903
7	93.0839272	91.56665	92.54986	92.40014	0.769641
8	99.5566112	100.1895	101.246	100.3307	0.853497

Table 4.12: Cumulative percentage of drug released as function of time (For in situ gel):

Time(hr)	CPR(1)	CPR(2)	CPR(3)	Avg CPR	STDEV
1	14.58333333	12.70833333	13.54166667	13.61111111	0.939427
2	22.16666667	24.00416667	23.1875	23.11944444	0.920638
3	31.97916667	29.27083333	30.3125	30.52083333	1.366133
4	38.64583333	35.46666667	36.94583333	37.01944444	1.590861
5	43.76666667	41.15	41.825	42.24722222	1.35847
6	48.35	45.475	46.7875	46.87083333	1.43931
7	54.675	51.32916667	52.66666667	52.89027778	1.684088
8	60.275	57.49166667	58.64583333	58.80416667	1.398406
9	64.50833333	63.75833333	64.1	64.12222222	0.375494
10	67.97083333	68.25416667	67.975	68.06666667	0.162393
24	92.10416667	97.19166667	94.82083333	94.70555556	2.545708

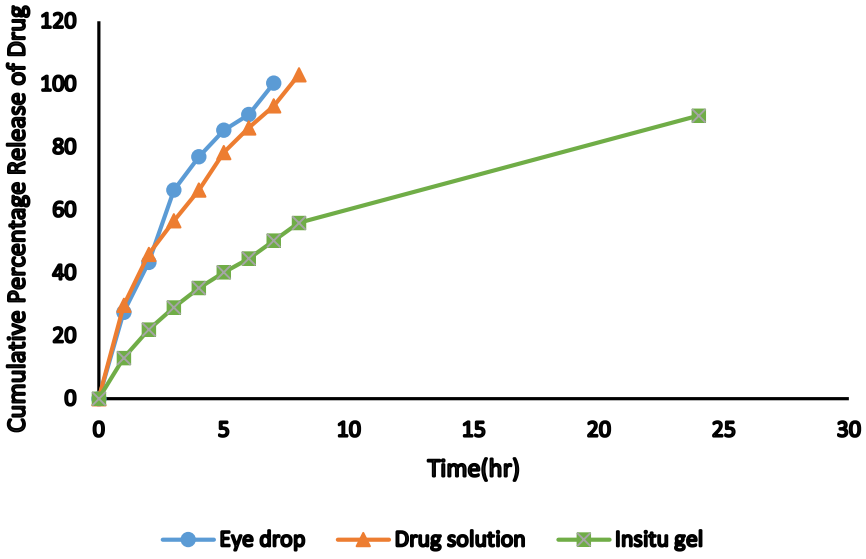


Fig 4.9: The cumulative percent of drug released as a function of time

4.11 Ex vivo corneal permeation study

This ex vivo permeation study of the in situ gel aimed to investigate the information about the extent of transport of drug through the isolated corneal membrane.[20] Ex vivo permeation was conducted for 6 h using fresh goat cornea. Corneal integrity and barrier properties when mounted on the Franz diffusion cell are assumed for 6 hours. The permeation profile of Ofloxacin from in situ gel and from commercial eye drop through goat corneal membrane is depicted in Figure 4. The steady increase in the drug permeation with time was observed in ex-vivo.[21] The permeation profile was fitted to various kinetic models. It was found to fit with zero order kinetics(Table 4.13) i.e. the rate of permeation was independent of the amount of drug permeated at various time points. The permeation of the optimized formulation through goat cornea after 6 h was about 55%. It was observed that the drug diffused through the corneal membrane was less as compared to the drug diffused through the dialysis membrane. This may be due to the cornea being made up of epithelium (lipophilic), stroma (hydrophilic), and endothelium (less lipophilic than epithelium) which acts as a lipophilic-hydrophilic barrier for corneal penetration while dialysis membrane acts as a mechanical barrier. A similar observation was achieved by Gadad et al. in thermosensitive in situ gel for ocular delivery of lomefloxacin [9]. It was observed that the flux(Jss) of the marketed formulation, drug solution and in situ gel were 58.70, 57.35, and 58.36 µg/cm^2.h respectively.

The permeability coefficients(PC) of the marketed formulation, drug solution, and in situ gel were 0.0196, 0.0191, and 0.0195 cm/h respectively. So, there was no such difference in the permeation of the drug of the three formulations through the corneal membrane. The cumulative percent of drug permeated as a function of time from in situ gel formulation and marketed eye drops are shown in Fig 4.10.

Table 4.13: Ex vivo drug permeation kinetics of Ofloxacin from in situ gel system

Model	Equation	R^2
Zero order	$Q = Q_0 + kt$	0.9982
First order	$logQ = \log Q_0 + \frac{kt}{2.303}$	0.1824
Higuchi	$Q = K_H + \sqrt{t}$	0.8128

Table 4.14: Permeation of Drug (For marketed eye drop):

Time	CPP1	CPP2	CPP3	MEAN	STD
1	2.375296912	3.456522153	2.512635622	2.781485	0.588619
2	4.798099762	4.93512653	4.54122132	4.758149	0.199968
3	5.091053048	5.251654198	6.44561556	5.596108	0.740065
4	13.89944576	12.21365235	12.55461423	12.88924	0.891322
5	16.34996041	17.54623143	18.24694123	17.38104	0.959218
6	19.23990499	20.14563214	21.44123695	20.27559	1.106405

Table 4.15: Permeation of Drug (For drug solution):

Time	CPP1	CPP2	CPP3	MEAN	STD
1	1.97941409	2.036451265	2.489689569	2.168518309	0.279600686
2	4.5922407	4.78458588	4.958646324	4.778490967	0.183278836
3	6.46476643	6.984451223	7.854855213	7.101357622	0.702379553
4	9.16468725	9.874555126	10.32633521	9.788525864	0.585582848
5	15.083135 4	13.56489655	14.25461586	14.3008826	0.760176135

6	21.116389 5	20.14423662	21.00316452	20.7545969	0.531610503

Table 4.16: Permeation of Drug (For in situ gel):

Time	CPP 1	CPP 2	CPP 3	Mean	STD
1	2.850356	4.552652	3.549854	3.650954	0.855639
2	7.657957	6.227237	6.542437	6.80921	0.751742
3	11.13539	10.11085	11.01237	10.75287	0.559399
4	14.85986	13.47585	12.45879	13.59817	1.205196
5	19.98575	15.51861	17.45655	17.65363	2.240083
6	23.24466	17.20111	21.4588	20.63485	3.10488

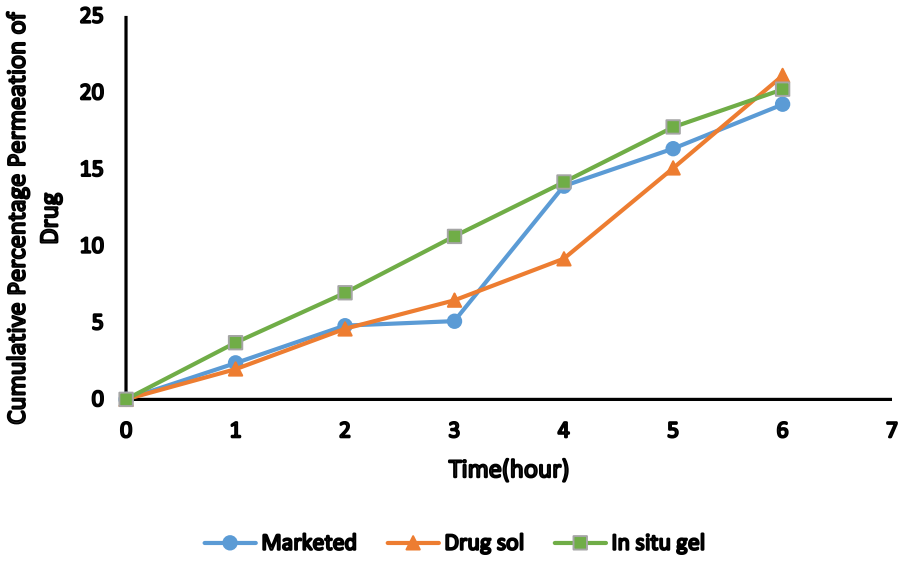


Fig4.10: Permeation of drug at different types of formulation

Conclusion:

In this study, a novel type of in situ gel was synthesized using NIPAAm and LBG by free radical graft copolymerization technique. Ofloxacin, a broad spectrum antibacterial agent used in the treatment of ocular infections was successfully formulated as temperature sensitive in situ ophthalmic gel (0.3% w/v) using NIPAAm as gel forming agent and LBG (natural polysaccharide) as viscosity enhancing agent. The formulation was liquid below the physiological body temperature and underwent rapid gelation upon raising the temperature to

the physiological body temperature (37 ˚C). The rheological study confirmed the gelation temperature at physiological eye temperature. The pH of all the formulations was between 7 to 7.4. So, upon instillation into the eye, it would not cause any irritation. The drug content of all the formulations was between 95-105 % which ensures dose uniformity in every drop. The formed gel would enhance the ocular contact time of Ofloxacin which in turn ensures the prolonged residence time of the drug. From the rheological study, it was observed that all the formulations exhibited pseudoplastic flow as evidenced by a decrease in viscosity with increasing in angular velocity. The formed in situ gel afforded sustained drug release over a period of 24 hours. Formulation F8 was considered the optimized one as it showed gelation temperature at physiological body temperature and 94 % drug release at the end of 24 hours. All the formulations in this study were best expressed by Higuchi's model as the plots showed good linearity. The linearity of the plot also indicated that the release profile of the drug was diffusion controlled. In peppas model n value for the formulation below 0.5 indicated that the release profile was the Fickian release. The developed in situ gel is a viable alternative to conventional eye drops because of enhanced bioavailability through longer precorneal residence time and the ability to sustain drug release. It also afforded ease of administration and decrease frequency of administration resulting in better patient compliance.

References:

1. Giri TK, Vishwas S, Tripathi,DK. Synthesis of Grafted Locust Bean Gum Using Vinyl Monomer and Studies of Physicochemical Properties and Acute Toxicity. J. Nat. Prod. 2016; 6: 179-187.

2. Kim SY, Cho SM, Lee YM, Kim SJ. Thermo- And Ph-Responsive Behaviors Of Graft Copolymer And Blend Based On Chitosan And N-Isopropylacrylamide. J. Appl. Polym. Sci. 2000; 78: 1381–1391.

3. Abreua CMWS, Paula HCB, Seabra V, Feitosaa JPA, Sarmentoc B, Paula RCM. Synthesis and characterization of non-toxic and thermo-sensitivepoly(N-isopropylacrylamide)-grafted cashew gum nanoparticles as a potential epirubicin delivery matrix. Carbohydr. Polym. 2016; 154: 77–85.

4. Kaity S, Ghosh A. Carboxymethylation of locust bean gum: Application in interpenetrating polymer network microspheres for controlled drug delivery. Ind. Eng. Chem. Res. . 2013; 52: 10033–10045.

5. Vieira MC, Gil AM. A solid state NMR study of locust bean gum galactomannan and Konjac glucomannan gels. Carbohydr. Polym. 2005; 60: 439–448.

6. Dutta K, Das B, Orasugh JT, Mondal D, Adhikari A, Rana D, Banerjee R, Mishra R, Kar S, Chattopadhyay D, Bio-derived cellulose nanofibril reinforced poly(N-isopropylacrylamide)-g-guar gum nanocomposite: An avant-garde biomaterial as a transdermal membrane. Polymer. 2018; 135: 85-102.

7. Chakravorty A, Barman G, Mukherjee S, Sa B. Effect of carboxymethylation on rheological and drug release characteristics of locust bean gum matrix tablets. Carbohydr. Polym. 2016; 144: 50–58.

8. Cao Y, Zhang C, Shen W, Cheng Z, Yu L, Ping Q. Poly(N-isopropylacrylamide)–chitosan as thermosensitive in situ gel-forming system for ocular drug delivery. J Control Release. 2007; 120: 186–194.

9. Gadad AP, Wadklar PD, Dandghi P, Patil A. Thermosensitive In situ Gel for Ocular Delivery of Lomefloxacin. Indian J. Pharm. Educ. Res. 2016; 50: 96-105.

10. Zhu M, Wang J, Li N. A novel thermo-sensitive hydrogel-based on poly(*N*-isopropylacrylamide)/hyaluronic acid of ketoconazole for ophthalmic delivery. Artif Cells Nanomed Biotechnol. 2018; 46: 1282–1287.

11. Shastri DH, Patel LD, Parikh RK. Studies on In situ Hydrogel: A Smart Way for Safe and Sustained Ocular Drug Delivery. J Young Pharm. 2010; 2(2): 116-120.

12. Sharma BR, Kumar V, Soni PL. Ceric Ammonium Nitrate-Initiated Graft Copolymerization of Acrylamide onto Cassia tora Gum. J. Appl. Polym. Sci. 2002; 86: 3250–3255.

13. Nayak BR, Singh RP. Synthesis and Characterization of grafted hydroxyl propyl guar gum by ceric ion. Eur. Polym. J., 2001; 37: 1655-1660.

14. Giri TK, Pure S, Tripathi DK, Synthesis of graft copolymers of acrylamide for locust bean gum using microwave energy: swelling behavior, flocculation characteristics and acute toxicity study. Polimeros. 2015; 25: 168-174.

15. Chowhan A, Ghosh B, Giri T K. Composite Ion Responsive In Situ Gel Based on Gellan/ Carboxymethyl Cellulose Blend for Improved Gelation and Sustained Acyclovir Ocular Release. Indian J. Pharm. Sci.. 2023; 85: 581-590.

16. Sultana Y, Aqil M, Ali A. Ion-Activated, Gelrite Based in Situ Ophthalmic Gels of Pefloxacin Mesylate: Comparison with Conventional Eye Drops. Drug Deliv. 2006; 13: 215–219.

17. Ranch K, Patel H, Chavda L , Koli A , Maulvi F, Parikh RK, Development of in situ Ophthalmic gel of Dexamethasone Sodium Phosphate and Chloramphenicol: A Viable Alternative to Conventional Eye Drops. J. Appl. Pharm. Sci. 2017; 7: 101-108.

18. Pandit NK , Wang D. Salt effects on the diffusion and release rate of propranolol from poloxamer 407 gels. Int. J. Pharm. 1998; 167: 183–189.

19. Srividya B, Cardoza RM, Amin PD. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. J Control Release. 2001; 73: 205–211.

20. Pramanik A, Sahoo RN, Nanda A, Mohapatra R, Singh R, Mallick S. Ocular Permeation and Sustained Anti-inflammatory Activity of Dexamethasone from Kaolin Nanodispersion Hydrogel System. Curr. Eye Res. 2018; 43: 828-838.

21. Pathak MK, Chhabra G, Pathak K. Design and development of a novel pH triggered nanoemulsified in-situ ophthalmic gel of fluconazole: Ex-vivo transcorneal permeation, corneal toxicity and irritation testing. Drug Dev. Ind. Pharm. 2013; 39: 780–790.