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Insitu Biodegradable Polymer Gel for Drug Delivery in the Eye

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Abstract: Effective delivery of the drug to particular regions of the anterior(front) or posterior(back) region of the eye has proven to be a significant problem because of multiple protective barrier and eradication processes correlated to the distinct anatomical and physiological characteristics of the eye. Conventional delivery systems for administering medications to the eyes have resulted in poor ocular bioavailability of less than 5%. To overcome the drawbacks of conventional eye drops, in situ gel systems are administered as an eye drop and go through a solution-gel transition in cul-de-sac. Transitioning to a gel state on the corneal surface prolongs the contact time. It has also advantages of solutions such as accuracy, reproducibility of dosing, or ease of administration with better patient compliance. Biodegradable polymers have several benefits for developing drug delivery systems, as they are biodegradable, biocompatible, and readily available from renewable sources. This study's goal was to show the potential of an optimized in-situ gelling system to enhance therapeutic effectiveness in ocular therapy by prolonging residence time while minimizing side effects.

Keywords: Biodegradable Polymer, In-Situ Gel, Ocular Drug Delivery, Thermosensitive In-Situ Gel, PH Triggered In-Situ Gel, Ion- Activated In-Situ Gel.

1. Introduction

The core problem often experienced during the development of an ocular drug delivery system is maintaining the desired drug concentration at the target site for a long enough period, particularly in the eye's anterior chamber. This is because the extremely selective corneal barrier and complicated structure of the eye prevent any exogenous substance from entering the ocular tissue[1][2]. To enhance the precorneal residence period, various ophthalmic carriers including eyedrops, ocular polymeric inserts, gel, and ointment have been created[3]. In-situ gel systems have been the focus of major research over the past few decades among the various ocular dosage form examined thus far. In-situ gels are appealing because they can be delivered effectively as a drop of solution into the eye's conjunctiva sac. Where they undergo a phase transition into gel form upon exposure to either the tear fluid's PH, temperature of the eye's surface or ion present in the tear fluid [4], [5]. Changes to gel form in the corneal surface prolong the residency period in the eye resulting in improved bioavailability of the eye by reducing quick precorneal eliminations, primarily because of nasolacrimal leakage and blinking of eye[6]. Through precorneal clearance, it can also help to decrease the poor compliance brought on by repeated dosing as well as the possibility of unfavorable side effects connected to systemic drug absorption[7].

Polymers, particularly biodegradable polymers, offer many advantages specifically to improve therapeutic ophthalmic formulation. Its mucoadhesive characteristic, particularly around the cornea and conjunctiva, is the main advantage. This makes it possible for a formulation to spend an extended period of time on the corneal epithelial surface, which enhances drugs penetration. Renewable natural

resources like fungi, plants and animal can be used to obtain naturally occurring polymer[8]. They are non-immunogenic or poisonous, and in-vivo enzymes can break them down. Their metabolites are less poisonous to living things, and they can also release the drugs in controlled manner because they can swell[8]. Natural polymers utilized for in-situ gel systems include - gellan gum, chitosan, alginic acid, pectin, guar gum, Carbopol, xyloglucan, xanthum gum, HPMC, and poloxamer[9]. mucoadhesive and viscous gel is generated on the ocular surface after introducing the solutions containing stimuli-responsive polymers like PH-responsive polymer, thermos-sensitive polymer, and ion-activated polymer[10]. The review primarily focuses on the introduction of in-situ gel, in-gelling mechanism, stimuli-responsive in-situ gel systems, various polymers utilized, their properties, and their evaluation.

The ocular system's anatomy

The eye has 2 main segments: (a) anterior/front part - includes conjunctiva, ciliary muscle, iris, pupil, anterior chamber, cornea, aqueous humor, trabecular meshwork, and lens. (b) The posterior/back part contains vitreous humor, sclera, choroid, retina, optic nerve, and macula. [figure.1] [11]

The cornea, the clearest and most transparent portion of the eye, forms the eye's front layer. Lacrimation and tear production also remove any foreign substance from the eye and administered formulation [12]. Tears keep the cornea moist, which is made up of several layers of tissue (including corneal epithelium, bowman's layer, the stroma, descemet's membrane, and endothelium). The outer film is corneal epithelium[11], [13]. The permeability of the cornea is the most essential factor in determining the concentration of drugs in the aqueous humor. Drug diffusion is limited by the epithelium for hydrophilic medicines. Due to stroma's hydrophilic characteristic, it serves as an obstacle to the medication that is extremely lipophilic[14]. Stroma inhibits the dispersion of hydrophobic molecules because it is mainly composed of hydrophilic collagen that is charged and well-organized [15].

The sclera is the eye's white portion and is covered by the conjunctiva, a clear and thin membrane that lines the inside of the eyelid It is made up of goblet cells and stratified epithelium (non-keratinized). Its main functions are to protect the eye by secreting mucus that guards against the introduction of pathogens and lubricates the eye [11]. The conjunctiva of a human has a surface area that is 17 times greater than that of the cornea. This makes it possible for more drugs to be absorbed through the conjunctiva. As a result, drugs generally have more permeability through the conjunctiva compared to the cornea. Blood capillaries and lymphatics in the conjunctiva, can lead to significant drug loss in the blood circulation and reduce the total ocular bioavailability of the drug, decreasing the capacity of drugs to be absorbed through the conjunctiva [16].

The eye's posterior and frontal chambers are filled with a transparent liquid called aqueous humor. It's a non-vascular form that needs to be translucent in order for light to pass through and nourish the cornea [11]. The aqueous humor has a higher concentration of ascorbate (vit. C) compared to the plasma, with levels approximately 15 times higher, and it has PH 7.2 [13]. Its primary functions include supplying nutrition to the eye, cleaning non-vascular tissue of waste, regulate internal eye pressure, which is necessary to maintain the cornea's convex shape [17].

The eye's white part, known as the sclera, is composed of collagen fibers and is opaque and elastic in character[11]. Hydrophilic substances often have higher permeability through the sclera than through the conjunctiva and cornea; this is because diffusion through the sclera primarily occurs through a proteoglycan (aqueous media) or leaky space in the collagen network, instead of the cellular membrane [16]. Sclera provides a protected external film, controlling eye pressure/IOP as a result it is acting as the extraocular muscle's connection point [14].

The retina is a multilayered, complicated structure made up of nerve fibers, vascular, glial, and neural cells [13]. Due to its composition, the retina poses a huge barrier to the ocular administration of drugs with higher molecular weight [16].

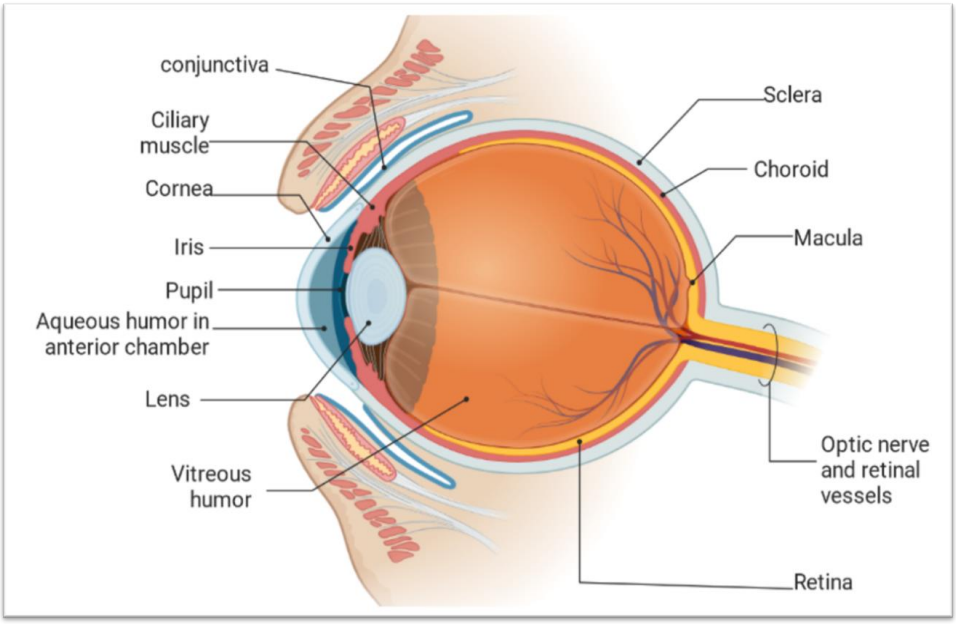

Figure .1- the anatomy of the human eye

In-situ gelling system's importance[18 ,19]

- Its unique 'sol-gel transition' helps the drug's sustained and controlled release.
- Because of gel formation, extends the drug's duration in the body.
- Bioavailability of the drug will be more.
- It minimizes the frequency of the dosage of medication administered to the body.
- Only a small dosage of the medication is needed, and there won't be any drug buildup or adverse effects.
- It reduces the wastage of the drug.
- The optimal dose form is a liquid that can maintain drug release and stay on the cornea for an extended period of time.

In situ gel system's advantage [20]– [22]

- Control and sustain drug release.
- Increase patient compliance and comfort.
- Convenience in medicine administration.
- A patient who is unconscious may receive this treatment.
- Use of natural polymers provides biocompatibility and biodegradability.
- Minimize the frequency of doses and toxicity.
- Improve bioavailability.
- Natural polymers have the natural characteristics of a biocompatible, biodegradable, and biologically recognizable moiety, supporting cellular activities.
- In situ gel is designed to show bio-adhesiveness for promoting drug targets, particularly across mucus membranes, and for administering drugs without pain.

Polymer's ideal characteristics for making of in situ gel[23, 24]

- The polymers must be highly compatible and free of any hazardous effects.
- It should have the potential to adhere to mucosal membranes.
- The behavior needs to be pseudo-plastic.
- Must be able to reduce the viscosity as increases in rate of shear.
- It is preferable to have better tolerance and optical clarity.

Mechanism of in-situ gel

Various methods, such as temperature-sensitive, PH-sensitive, and ion-activated systems can be used to create in-situ gels. Utilis temperature-sensitive polymers in temperature-triggered in-situ gel systems go through gelling. If the temperature exceeds low critical solution temperature (LCST), which is where the polymer resides as a liquid below LCST[25]. In-situ gel that is PH-induced, which contains polymer with an acidic or alkaline functional group that undergoes a sol-gel(S-G) phase transition when the environment goes from a lower PH to higher PH [26]. The ion-activate systems, sometimes referred to as an osmotically induced in-situ gel system, go through an S-G transition as a result of alteration in ionic concentrations, typically Mg2+, Na+, Ca2+, which are mono/ bivalent cations within the tear-film, trigger this transition[27]. The S-G transition has also been demonstrated to be triggered by photon polymerization and enzymatic cross-linking[27,28]

Stimuli-responsive in-situ gel system

1. Temperature-activated in-situ gelling systems

The oldest, most widely studied, and most popular form of stimuli-responsive gel is temperature-sensitive in-situ gel. It may be applied to the eye accurately and readily in a liquid state without irritating the eye or blurry eyesight. To withstand the dilution of lachrymal fluid without being rapidly eliminated from the eye after administration of a drug, the gel is created at a precorneal temperature of 35°C [29]. For effective use, an excellent thermo-reactive in-situ gel for the eye must gel above room temperature and proceed through the S-G transition at 35°C to prevent refrigeration before installation, which can cause irritation of the eye due to its cold characteristic (Table1) [30].

Table 1 – Few examples of thermos-sensitive in-situ gel system

Drug	polymer	Main discovery	Ref.
Brinzolamide	Poloxamer F127, Carbopol 934P	The controlled delivery of medication over an 8h period using S-G at 33.2 ± 1.1 °C	[31]
Ofloxacin	Pluronic F-127 /Pluronic F-68	In vivo, evaluation on rabbits revealed 20%(W/W) Pluronic F127 had better retention performance than Pluronic F68. 20 wt% Pluronic F127 forms a transparent gel under physiological conditions with less irritation.	[32]
Ketorolac tromethamine	Poloxamer 407(Pluronic F-127) and HPMC K4M	Increased ocular bioavailability, extends its residency period without causing irritation to the eye.	[33]
Sparfloxacin	Pluronic (PF127, PF68)	Both in vitro and in vivo showed promising antimicrobial activity.	[34]
Fluconazole	Poloxamer407, tween80, Carbopol943	ophthalmic dosage had better in-vivo absorption than regular eye drops.	[35]
Lomefloxacin	Pluronic F127, Pluronic F68 and sodium alginate	Shows slow discharge of medication for 8h.	[36]
Methazolamide	Poloxamer 40/ and poloxamer P188	Show enhanced retention of the medication compared to eyedrop.	[37]
Diclofenac sodium	Pluronic F127 and PF 68, Carbopol 940	In aqueous humor, diclofenac sodium's bioavailability was greatly improved.	[38]
Dorzolamide hydrochloride	Poloxamer 407, poloxamer 188	In situ, gel enhances the ocular bioavailability of the medication is and superior to conventional ocular eye drops.	[39]

2. PH-triggered in-situ gelling system

This in-situ gel system made up of PH-responsive polymer, is a polyelectrolyte containing either a basic (ammonium salt) or acidic (carboxylic/sulfonic) group that may release or receive proton in-reaction to changes in the environment's PH. At PH7.4, that is tear fluid's PH, the formulation forms a gel instead of remaining in its usual solution as it does at PH4.4. The most often utilized PH-responsive polymers-polyacrylic acid (PAA, Carbopol940), polycarbophil, and cellulose acetate phthalate (CAP). (Table2) [40]

Table 2 – Example of PH- triggered in-situ gel systems

Drug	Polymer	Main discovery	Ref.
Baicalin	Carbopol 974P, HPMC E4M	Greater capacity to keep the drug stable, higher ocular bioavailability, and sustained release of the medication(baicalin) compare to eyedrops.	[41]
Ciprofloxacin hydrochloride	Sodium alginate, HPMC K4M and ES0LV	Add the benefit of sustained released medication and improved patient compliance	[42]
Norfloxacin	Carbopol 934P	Adequate mucoadhesive, anti-bacterial effectiveness without ocular irritation.	[43]
Timolol maleate	Carbopol and chitosan	Displayed a controlled release of drug beyond the 24-hour period.	[44]
Brimonidine	Carbopol 974P and HPMC E4M	Increase brimonidine's effectiveness and decrease its systemic absorption.	[45]
Gatifloxacin	Carbopol 940 with HPMC and HPMC K15M	Sustained release of medication for 8hrs.	[46]
moxifloxacin	Carbopol/HPMC	Enhanced ocular bioavailability and precorneal residence duration.	[47]

3. Ion-activated in-situ gelling system

Ion-activated in-situ gel system creates a crosslinking using cations that are naturally present in tear fluid like Na+, Mg2+ and Ca2+, resulting in the gel's formation on the ocular surface, which prolongs the contact time with the cornea(table-3). [40,48]

The most commonly utilized polymers in the ion-activated in-situ gelling systems-gellan gum (Gelrite®), hyaluronic acid, and sodium alginates[49]

Table 3 - Example of an ion-activated in-situ gel system.

Drug	Polymer	Main discovery	Refe.
Gatifloxacin	Alginate and HPMC	Compared to the traditional ophthalmic solution, it has better bioavailability in the eye & a prolonged residency duration in aqueous humor.	[50], [51]
Fluconazole	HPBCD complexed gellan gum and κ-carrageenan	Show efficient controlled release of fluconazole and strong bioadhesive qualities.	[52]
Acetazolamide	Gellan gum with xanthan gum, HPMC or Carbopol	Increase the effectiveness of therapy and the duration of IOP reduction is longer in comparison to commercial eye drops and oral tablets.	[53]
Terbinafine hydrochloride	Gellan gum	Significant increases in Cmax, extended mean residence duration, postponed tmax with enhanced bioavailability.	[54]

Antisense oligodeoxynucleotide	Gellan gum and carrageenan	The highest shrinkage of the wound, minimal stromal edema and hypercellularity	[55]

*IOP-Intraocular pressure

4. multi-stimuli responsive in-situ gel

Table 4- Few examples of multi-stimuli responsive in-situ gelling systems

Drug	Polymer	stimuli	Main discovery	Ref.
Sparfloxacin	Sodium alginate, methylcellulose	PH, Ion - sensitive	Quickly forms a gel, when the PH was increased to 7.4, in vitro sustained release of medication for a duration of 24hr, greatly improved corneal permeability.	[56]
Nepafenac	Carboxymethyl chitosan (CMC), poloxamer	Thermosensitive and PH-induced	A 32-33°C is the gelation temperature and slows down the rate of drug diffusion.	[57]
Timolol	Chitosan and gellan gum	The polymer that is PH and ion-activated	Improved trans-corneal medication permeation and extended retention time at the cornea, also nonirritant.	[58]
Levofloxacin	Sodium alginate, chitosan	Ion and PH-activated	Observed greater retentiveness, nonirritant, and better therapeutic activity compared to eye drops.	[59]
ciprofloxacin	Carbopol/HPMC, poloxamer	PH-induced and thermosensitive	Enhanced therapeutic effectiveness, show sustained release of the medication for a duration of 8h.	[60]

Polymers utilized in the in situ gelling system

Pectin

Pectin is a polysaccharide and its major component is α-(1,4)-D galacturonic acid residue [Figure.2]. Lower methoxy pectin (degree of esterification <50%) quickly forms a gel in aqueous solutions when free calcium ion is present, crosslinking the galacturonic acid chain according to the egg-box concept[61]. Pectin forms gel in the presence of an H+ ion, a form of bivalent ion, and typically calcium ions are needed to make the gel that is suitable for use as drug delivering vehicle. Pectin is primarily used in the formulation because it's aqueous soluble, hence the formulation does not need organic solvent[62]. When it is orally administered, the bivalent cation of the stomach causes the change of pectin to gel form. pectin has also been utilized in the in-situ gel system to release medications from the formulation for a longer time [63].

In this context in 2011, Vijaya et al., synthesized an in-situ gel for ocular administration of azithromycin. In this formulation they have taken different concentration of pectin (1-5% w/v) and various ratios of HPMC and SCMC. Rheological behaviour and gelling ability served as the primary criteria for formulation optimization. They have assessed the pH, drug release and antimicrobial effectiveness. They proposed a satisfactory result for the formulation on pH, gelation in simulated tear fluid, clarity and rheological qualities. They found that this formulation inhibits staphylococcus aureus growth successively and proven to have no harmful effects on rabbit eyes. According to the results of this investigation, the formulations CP2 with pectin at a concentration of 3% w/w, and SCMC concentration of 0.5% w/v have shown most promising for extending the release and effectiveness of azithromycin[64] . Similarly, Golnar Rahimzadeh et al., in 2021 created bacteriophage containing in-

situ gel eye drop for treatment of pseudomonas aeruginosa keratoconjunctivitis. In this study they prepared In -situ gel forming eye drop contain phage, pectin, NaCMC etc. When the chosen formulation was administered in to the eye, its viscosity increased. In groups where no in-situ gel forming formulation was utilised, the histological results revealed edoema, abscesses, and loss of the corneal stroma structure. Re-epithelialization and normal corneal structure were observed in the group treated with the in-situ gel forming formulation. After applying the formulation to the rabbit eye, they concluded that phage- containing in situ eye drop formulations that form gel in the eye can be used to treat *P. aeruginosa* keratoconjunctivitis.[65]

Guar gum

Guar gum, which occurs naturally and is made from the endosperm of the seeds, is also known as guaran. Guar gum is miscible in water but immiscible in hydrocarbon, fat, ester, alcohol, and ketone. These demonstrate it disperses in both hot and cold water and that it can dissolve in hot and cold water at low concentrations to generate colloidal solutions. It has byproducts employed in target delivery systems to create coated matrix systems, hydrogels, and nano-microparticle. Guar gum also has derivatives that are good in targeting the colon, such as graft polymers like polyacrylamide. Additionally, it is employed as a polymer in matrix tablets with control delivery[66].

In 2013, Manas Bhowmik et al., synthesized (PM)poloxamer-407 based in situ gelling ophthalmic drug delivery system. Here they have added (XG)xanthan gum and (GG)guar gum in to PM and developed different formulations. They discovered that at PM concentration below 18%, XG and GG with a weight ratio of 3:7 could turn PM solution into gel below body temperature. The in vitro drug release investigation demonstrates that the modified poloxamer solutions released the medication more gradually than those that did not contain XG–GG. According to the findings, the created in siu gelling formulations with PM and XG-GG might be a preferable option to traditional eye drops [67]. Similarly, Dasankoppa F.S. et al., in 2012 created in situ gel based on cationic guar and hydroxypropyl guar for delivery of ocular drugs. For the preparation of this formulation, they have taken linezolid, cationic guar, HPG and benzalkonium chloride. The optimal formulation was determined to be CG-HPG2, which demonstrates good physical stability following sterilization and storage which also maintained the flow behaviour index and consistency index. The persistent release of the medication for up to 12 hrs was shown by the in-situ gel. Data on the stability of the formulation over a six-month period at high temperatures showed that it was stable. They concluded that the in situ, which is based on a derivative of guar gum, demonstrated extended corneal residence duration and sustained release of medication and might be utilized as alternative to the traditional formulation of eye drops with assured enhancement in bioavailability.[68]

Carbopol

polyacrylic acid polymer is known as carbopol, converted into gel-form when PH was increased from 4.0 - 7.4. Carbopol exists in solution when PH is acidic whereas at alkaline PH, it changes to a thin gel. HPMC combined with Carbopol increases the solution's viscosity and decreases the solution's acidity. Contrasting several forms of Carbopol (Carbopol 940, 934, 941, and 910) 47 found that Carbopol 940 had better clarity and appearance [Figure.2] [69].

Indrajeet G, et al., in 2010, formulated and assessed different in-situ gels of gatifloxacin using mucoadhesive polymers. They used gatifloxacin, sodium alginate, mucoadhesive polymers such as poloxamer 407, Carbopol 974P, and hydroxyethyl cellulose to prepare the formulation. Employing 3^2 factorial design, they examined the combined impact of two independent formulation variables in the creation of in-situ gel. To visually depict the impact of independent variables on the evaluation parameters, a surface plot was also made. The created formulations demonstrated a six hour of sustained drug release[70]. Lekhraj V, et al., in 2010, the current study aims to address these issues by creating an in-situ gel forming system of flurbiprofen that was based on the pH-induced and ion activated in-situ gelation. Sodium alginate, Carbopol and HPMC were used to prepare flurbiprofen In-situ gels using simple dispersion technique. The gel was then assessed for gelling capacity, rheology, isotonicity, PH, in-vitro and in-vivo investigations. The created formulations exhibited a five hour of sustained drug release [71].

Xyloglucan

Xyloglucan (polysaccharide) extracted from the seed's endosperm, it also known as tamarind gum. Xyloglucan contains three oligomers with varying numbers of galactose side chains, such as heptasaccharide, octasaccharide, and nonsaccharide. In diluted aqueous solution, it is moderately decomposed by β-galactosidase and show reversible gelation of temperature. Depending on how much galactose is degraded, the temperature of sol-gel transition varies. Because of its non-toxicity, biocompatible and biodegradable characteristics, it delivers the drugs via the oral, rectal, and ocular routes [Figure.2] [72].

S. Miyazaki et.al., 2001 formulated in situ gel to deliver pilocarpine hydrochloride to the eyes. Here they have taken Xyloglucan, Pilocarpine hydrochloride etc. has shown that pilocarpine can be released invitro from gels made by heating xyloglucan sols (1.0, 1.5 and 2.0% w/w) to 34 °C and injected into rabbit eyes, that left in the eyes for at least six hours. Sustained pilocarpine release was found from all gels, and the length of miotic response increased as the concentration of xyloglucan increased. A 1.5% w/w xyloglucan formulation produced a response that was comparable to a 25% w/w Pluronic F127 formulation[73]. Hitendra S. Mahajan et.al., (2015) formulated gel forming ocular delivery of ciprofloxacin. They prepared the formulation using xyloglucan (2%) with the solvent casting method. And evaluated the formulation for percentage of drug content, thickness, surface pH, swelling, in vitro drug release and mechanical strength. Found the % drug content is 95.45 ± 0.25%and having uniform thickness (0.20 ± 0.07 μm). After a full day, the formulation's cumulative percentage of drug releases was 98.85. They suggested that xyloglucan could serve as a visible film forming polymer for ciprofloxacin delivery in to the eyes[74].

Gellan gum

An anionic heteropolysaccharide, gellan gum can be utilized to create an ion-sensitive hydrogel. It secretes through the bacterium Sphingomonas elodea. It is composed of glucuronic acid, glucose and rhamnose are all joined to make a tetra-saccharide molecule. Deacetylated gellan gum or gelrite is produced by treating gellan gum with alkali to eliminate the acetyl group[75]. Gelrite produces gel when there are calcium ions present. The electrolyte of tear film specifically Na^{+}, Mg^{2+}, and Ca^{2+} cations are especially known to make the polymers form a gel when a liquid solution of the polymer is injected into the **cul-de-sac**[76]. The process of gel formation includes the creation of double helical junction zones, which are then aggregated to form three-dimensional networks through complexes with cations and water-based hydrogen bonds [Figure.2] [28].

Balasubramaniam J et.al., (2003) formulated an indomethacin ophthalmic delivery system based on gellan gum. They've used Indomethacin, Gelrite® gellan gum etc. The medication was tested for drug content, pH and clarity and got a satisfactory result. When the gellan concentration was raised above 0.5% and cooled to 40 °C, gelation resulted. They concluded that the medication was therapeutically effective and delivered prolonged drug release in rabbit eye for 8hrs in-vitro [77]. Lina Zhu et.al., (2023) formulated in situ gel for ocular delivery of ketotifen. They have prepared the formulation by mixing Ketotifen fumarate, deacetylase gellan gum etc and tested on male and female white rabbits. The investigations were conducted on its stability, rheological properties etc. The solution's ideal viscosity allowed for a smooth liquid drop and the ionic interaction caused a quick sol-gel transition. Over a course of 180 days of storage, there were very little changes to the formulation's initial viscosity values. The release of ketotifen from in situ gels showed a sustained characteristic, according to the in vitro release studies. Scintigraphy investigations revealed that the formulation's residence time could be extended by deacetylase gellan gum. When compared to a standard drop, in situ gels showed a sustained characteristic and prolonged drug effects behaviour at the same dose [78].

Alginic acid

Alginate is a natural polysaccharide. Its 1,4-glycosidic linkage connects the residues of β-D-mannuronic acid and α-L-glucuronic acid to form an unbranched block copolymer polysaccharide. When sequential glucuronic residuals in the α-L-glucuronic acid blocks of the alginate chain work together in a cooperative mechanism, diluted aqueous solution of alginate forms solid gels with additions of bi- and trivalent metal ions[79]. The higher guluronic acid content in alginate results in superior gelling

characteristics and reduces the amount of polymer needed to make a stiff gel[28]. Alginic acid is used in ophthalmic formulation, due to its desirable biological characteristics including being biodegradable and non-toxic[80].

Yuejiang Liu, et.al., (2010), formulated In-situ gelling gelrite/alginate formulations as vehicles for delivering medications to the eyes. They have prepared the formulation by using gelrite, sodium alginate etc, they have characterized the preparation by in vitro release and in vivo in rabbit eye. They have discovered that the ideal concentration of gelrite solution for in-situ gel was 0.3%(w/w) while 1.4%(w/w) and the ideal concentration for alginate solution. Under physiological condition, the combination of 0.2% Gelrite and 0.6% alginate solutions demonstrated a notable increase in gel strength. They found that the gelerite/alginate solution was non-irritating had a higher capacity to retain drug than either Gelrite or alginate alone, according to both in vitro and in vivo investigations[81].

Aparna B, et.al.,(2011), developed and evaluated an ophthalmic delivery system based on the idea of ion-activated in-situ gelation for the antiglaucoma drug dorzolamide hydrochloride (2%w/v). Sodium alginate (1 and 2 %w/v). Hydroxy propyl cellulose (0.1,0.2 and 0.3 %w/v), which served as a viscosity-enhancing agent, was combined with sodium alginate (1 and 2 %w/v) as a gelling agent. According to In-vitro release investigation, the drug was retained more effectively by the alginate/HPC solution than by either alginate or HPC solutions alone. Viscosity, in vitro diffusion studies and intraocular pressure investigations was conducted on the formulation. The medication was released steadily over a ten-hour period by the formulations, which was also stable and therapeutically effective [82].

Xanthum gum

Xanthum gum is an extremely hydrophilic natural heteropolysaccharide that is created by fermenting a gram-negative bacterium *Xanthomonas campestris*[83]. This extracellular polysaccharide with a high molecular weight has a primary structure consisting of a cellulosic backbone of (β- D-glucose residue) and an oligosaccharides side chain (β-D-mannose β-D-glucuronic acid-α-D-mannose) connected to the main chain's alternate glucose residuals. It dissolves in both hot and cold water as well as in both alkaline and acidic environments. In alkaline conditions, it shows better stability[84].

In this study in. 2008 Shivanand Swamy P.et al., formulated a novel in situ gum based ophthalmic drug delivery system of linezolid. Here they have taken hydroxypropyl guar, xanthan gum, Carbopol 934P, sodium alginate, Linezolid etc for the formulation of the in-situ gel. and have used suitable concentration of buffering agent to adjust the PH to 7.4. They evaluated the formulation for PH measurement, drug content estimation, clarity, rheological study, in vitro diffusion study, gelling capacity, antibacterial activity, eye irritation testing, isotonicity testing, on albino rabbit. They found that, the created formulations showed sustained release of the medication over a six-hour period, extending its residence time. Additionally, the drug is non-irritating and does not cause ocular damage or unusual clinic sign.[85]

Cellulose derivatives

Cellulose is made up of a glucan chain and contains a repetitive unit of β-(1, 4)-D-glucopyranose. Hydroxyethyl cellulose, sodium carboxymethyl cellulose (NaCMC), methylcellulose, and HPMC are some examples of natural polymers utilized in topical ophthalmic preparation that display a temperature-sensitive sol-gel phase transition[86]. At lower temperature, it behaves as a liquid but transforms into a gel when heated. Cellulose derivatives have a high phase transition temperature and can be reduced by physical or chemical alteration[87]. The transition temperature of MC ranges from 40°C-50°C, while for HPMC it ranges from 75-90°C. The gelation temperature of MC can be decreased to 32-34°C by adding sodium chloride while lowering the hydroxypropyl molar substitution in HPMC can lower the transition temperature to 40°C. At low temperatures (30^0C) solutions are liquid, and as temperatures increased (between 40^0c to 50^0C) gelation takes place[88].

In this study 2016, Nadia morsi et al, formulate Thermosensitive in situ gel containing Ketorolac tromethamine loaded nanodispersion for extended ophthalmic delivery, in this formulation they had used ketorolac tromethamine, Eudragit RL 100(EG) and PVA, HPMC etc. The combination of 20% Pluronic F-27 and 14% Pluronic F-127/1.5 % HPMC K4m produced the best gelling capacity. Here lowering the Pluronic F-127 concentration led to an increase in the in-situ gel's gelling temperature and

gelation time. By adding HPMC, the mucoadhesive strength was greatly enhanced. They stated that ketorolac tromethamine in situ gel exhibits improved residence times, better ocular availability and sustained release without irritating the eyes[89]. Wu. h., et al (2011), design and evaluation of baicalin containing in situ Ph-triggered gelling system for sustained ophthalmic drug delivery, in this preparation they had used Carbopol 974P, hydroxypropyl methylcellulose E4M where Carbopol was used as gelling agent and hydroxypropyl methylcellulose E4M was utilized as viscosity enhancer. The rheological behaviour showed an enhancement in gel strength, and the formulation presented sustained release of the drug over 8-h period. They concluded that an in-situ Ph-triggered gelling system have good ability to keep baicalin stable and drug release is retained than marketed baicalin eye drops to increase the bioavailability of ocular[90].

Chitosan

Gelling of chitosan occurs by temperature and PH-sensitive changes. It's a naturally occurring material found in shells of shrimps and crabs, and it is a polycationic polymer that is biodegradable, thermosensitive, and generated via alkaline deacetylation of chitin. Chitosan is a cationic polymer that is PH-dependent and biocompatible, remain to dissolve in aqueous solution up to PH 6.2. Because of precipitation, the hydrated gel will form when aqueous solutions of chitosan is neutralized to a PH greater than 6.2 [91]. Chitosan-based thermosensitive gels containing various polyols, including glycerol, sorbitol, and ethylene glycol have become very popular [92].

The main primary amino group of chitosan is derivatized by the thiol group to produce Thiolated Chitosan (TCS). The TCS-based medication delivery systems are attracting interest due to their potent mucoadhesive characteristics and extended drug release capabilities. Due to the production of inter and intra-molecular disulfide linkages as a result of the thiol groups oxidation at physiological PH-value, TCS exhibits in-situ gelling characteristics [Figure.2] [93].

Yanxia Cao et al., (2007) formulated poly(N-isopropylacrylamide)-chitosan (PNIPAAM-CS) in situ gel forming technology for administration of ophthalmic drug. They used Chitosan, *N*-isopropylacrylamide ,2,2′-Azobis(isobutylonitrile), 3-mercaptopropionic acid and other ingredients to create the formulation**.** They then used rabbits to compare it to a standard eye drop solution and determine how well it worked**.** They discovered that the PNIPAAM-CS solution's Cmax of timolol maleate in aqueous solution was 11.2 μg/ml, was two-times higher than that of the typical eye drop, in addition with increased area under the curve. Over a 12-hour period, it was more effective at lowering intraocular pressure (IOP) than a typical eye drops of the same dose. Additionally, the MTT experiment demonstrated that PNIPAAM–CS has negligible cytotoxicity at concentrations between 0.5–400 μg/ml. They proposed that PNIPAAM–CS could be a viable temperature-sensitive in situ gel-forming substance for ocular distribution, and it might increase the effectiveness, bio-availability and adherence of several eye medications[94]. ANUJA T. KADAM et al., (2017) developed and evaluated modified chitosan drug administration using in-situ gel for the eyes. They created the formulation by combining Moxifloxacin HCl, Modified chitosan, Polaxomer 407 etc using cold technique, then tested for their release kinetics, gelation temperature, rheological study, in vitro drug release tests, and drug content. All of the in -situ formulation batches were found to have appropriate pH ranges between 6.2 and 0.2 and drug contents between 98.8 and 0.2, demonstrating uniform drug distribution. Phase change temperature and in vitro drug release both decrease as the concentration of each polymeric component rises. The proposed formulation has a lasting effect and preserved its qualities against bacterial infection, according to the antibacterial effectiveness of the chosen formulation against staphylococcus aureus. They concluded that the formulation contained the right combination of modified chitosan and poloxomer 407 to sustained the drug release from in situ gel containing moxifloxacin HCl[95].

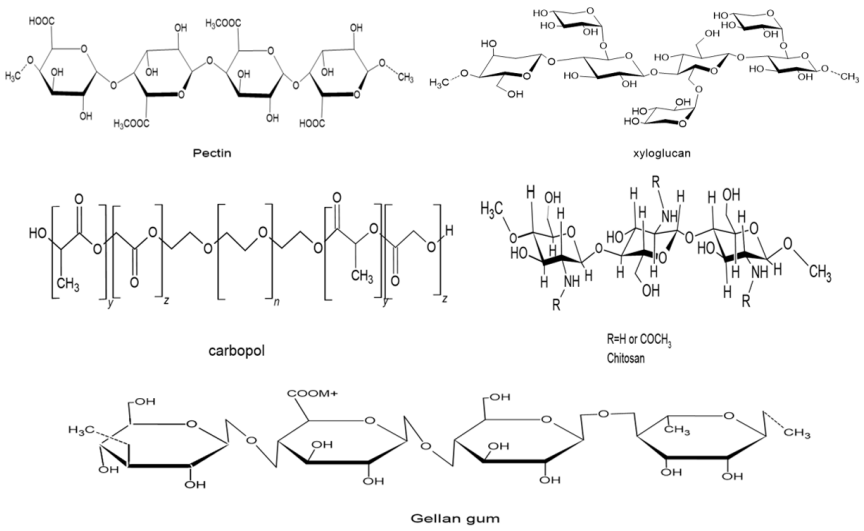

Figure.2- Structure of in-situ gel polymer

Evaluation of in situ gels

For ocular treatments, the in-situ gel should be evaluated for the following parameters.

Gelling Strength

From the sol form, a predetermined quantity of gel is made in the beaker. The beaker with the gel is then lifted at a consistent rate while a rheometer probe is slowly inserted into the gel. Variations in the load on the probe can be recorded based on the depth of the probe's immersion below the surface of the gel [61], [96], [97].

Sol to gel transitions temperature and gelation time

S-G transition temperature should be calculated for an in-situ gel developing system. The gelation time refers to the duration it requires to notice the initial formation of gel in the in-situ gel. For thermosensitive in-situ gels, their gelation behavior should be checked at body temperature[61]

Clarity & PH

The clarity of the formulation can be measured by visual observation on white and black backgrounds. A calibrated PH meter was used to measure different gel's PH at 25 ±0.5°C according to standard protocol [61].

Viscosity and Rheology

Viscosity and rheology characteristics of polymeric formulation whether in gel or solution form using artificial tissue fluid (depending on the route of administration) measured using several viscometers. These formulations should have sufficient viscosity to ensure patient compliance [61], [98].

Texture Analysis

The texture profile analyzer was employed to evaluate the cohesion, firmness, and consistency of the formulation, which mostly reflects the syringeability of the solution, ensuring easy administration in vivo. In order to maintain close contact with the surface-like tissue, a greater degree of adhesiveness in the gel is required[61], [99].

Fourier Transform Infra-Red Spectroscopy (FTIR) and Thermal Analysis

FTIR is utilized to examine the compatibility of constituents in the formulation. Different scanning calorimetry (DSC) is employed to observe any variation in the thermograms in comparison to the pure substances utilized, which indicate interactions[61], [99], [100].

In-Vitro Drug Release Study

The drug release experiments are conducted with a plastic dialysis cell, which consists of 2 half cells-receptor compartment, and a donor compartment. The cellulose membrane helps in the separation of the two half-cells. The donor compartment contains the formulation's solution form. Then the formed cell is agitated in an incubator horizontally. At specified intervals, the receptor solution is sampled and then replaced with freshly made media. An analytical approach is used to examine the drug release from this receptor solution[61], [100].

Drug Content

To achieve good bioavailability, the drug must be distributed uniformly. The drug content is measured using a simultaneous method with a UV-Visible spectrophotometer. In this procedure, dilute of 1 ml of drug formulation with 100 ml of a PH 7.4 artificial tear fluid (ATF) solution. 1 ml of aliquot was taken out and again diluted to make 10 ml of ATF solution. Finally, a UV-Visible spectrophotometer is used to calculate the concentration[61], [101].

Accelerated Stability Study

For a short-term accelerated stability study, the preparations are stored in vials of ambient color and are sealed with aluminum foil, following the guidelines stated by the international conference on Harmonization (ICH). The study is conducted using a relative humidity of 75 ±5% and temperature of 40 ±2°C. Sample are examined each month to assess clarity, in-vitro dissolution, PH, gelling capacity, drug content, and rheological properties [61].

In vivo ocular Irritancy Test

The Draize test is a method used to assess the potential ocular irritation caused by ophthalmic products before they are marketed. In this test, a volume of 100µl of the substance is applied to the lower cul-de-sac of the eye, and different criteria including redness, swelling, and watering of the eye are observed at a predetermined interval of 1 hour, 24 hours, 48 hours, 72 hours, and 1-week following administration. The study is conducted on three male rabbits weight between 1.5 to 2 kg. After a 3-day saline washing interval, the sterile formulation is administered into the eye twice a day for 7 days and a crossover study is performed. The rabbits are periodically observed for any sign of eye irritation [61], [98].

Antibacterial Activity

The antibacterial spectrum of the In -situ gels developed for treating infectious eye diseases is studied. The concentration of antibiotics utilized to determine the bacteria's microbial growth should be compared to that produced by the known amount of a typical antibiotic formulation. The serial dilution method is used to conduct the microbiological assay[102].

2. Conclusion

The utilization of polymeric gel for controlled drug release offers several benefits compared to conventional dosage form and is very reliable because of its sustained and prolonged release of medication, which exhibits high stability and biocompatibility characteristics. the contact period with ocular tissue is lengthened with the aid of in-situ gel. This leads to increased patient compliance because it not only increases bioavailability but also reduces dose frequency.

The introduction of biodegradable polymers significantly enhances the efficiency of in-situ gel. Formulators can experiment with the release characteristics of in-situ gels due to their flexibility. The formulator can make the drug release to be controlled, sustained, or prolonged as needed. This controlled drug release characteristic of the dosage form increases patient acceptance and increase patient compliance, as it minimizes the dosing frequency with fewer side effects.

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