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A Survey on Ocular in Situ Gel: An Original Medication Conveyance Framework

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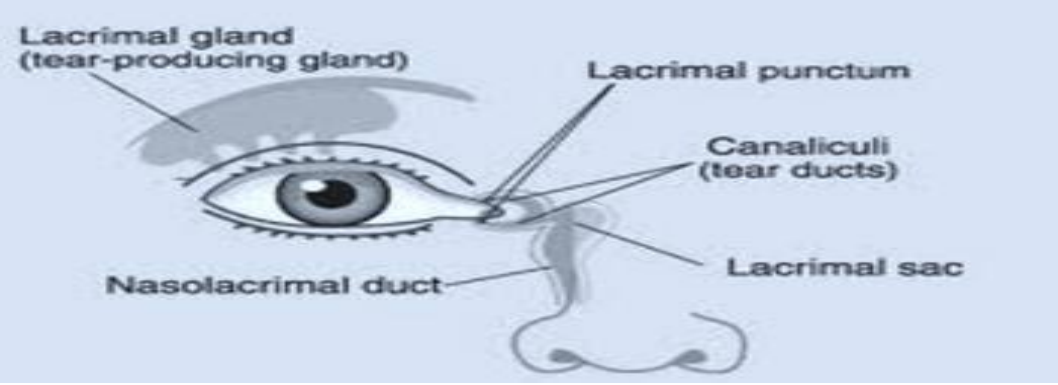
ABSTRACT

In medication delivery system for the eyes the difficult part is to administer drug to the complex anatomical structure of eye. The residence time and bioavailability are the two major problems in conventional ophthalmic drug delivery system while designing a dosage form (eye drops, eye lotions, eye ointments) in order to address eye conditions. To combat these problems a novel drug a technique for delivering drugs termed In Situ Gel eye medication has been developed. Delivery system which is safe easy and effective. Furthermore bioavailability, precorneal residence duration can be increased and frequency of administration, side effect, drug loss owing to tearing can be decreased. In-situ gel production system are methods of delivering drugs that, before being administered to the body, are in solution form but require in situ gelation to change into a gel when activated by environmental stimuli including temperature, pH, and other factors.

INTRODUCTION

A form of visual treatments conveyance framework is thought of however significant and testing as natural eye seems to be a disengaged organ where the conveyance of medication is very troublesome. Also, the traditional ocular preparation show due to quick and widespread drug removal from pre-corneal lachrymal fluids by arrangement seepage, lachrymation, and ineffective conjunctival absorption, there is a short pre-corneal residence period and poor bioavailability.[1] A significant amount of precorneal drug loss by nasal lachrymal drainage is the cause of the inadequate bioavailability of drugs from the conventional delivery system. A frequent dosage schedule is required because of the frequent clearance of the topically administered drug into the eye, which frequently leads to a short duration of pharmacological activity. Additionally, 50% to 100% of an implanted dose may be drained through the nasal lachrymal duct and undergo systemic absorption. This might result in a higher chance of unintended systemic harmful consequences.[2]

Reaching and maintaining the ideal medication concentration at the intended site of action in the eye is one of the primary challenges with ocular drug administration. Many ophthalmic dose forms have been studied, including ointments, gels, eye drop solutions, and ocular inserts, to extend the period that medications remain in the ocular cavity following topical administration to the eye. The corneal contact period has been somewhat extended with these formulations. However, they have not been widely embraced because of impaired vision and low patient compliance brought on by ointments and inserts, respectively.[3]



Utilizing polymers that will boost the viscosity of the solution to enhance medication retention on the surface of the cornea.[4]

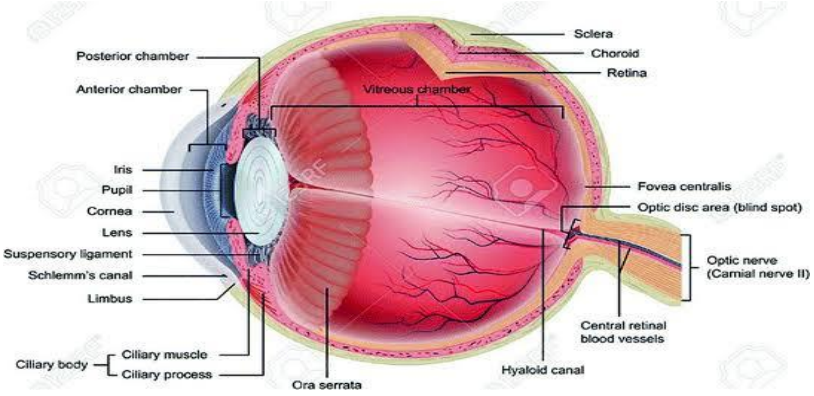
The individual polymer chains of hydro gels cross link chemically or physically to produce a polymeric network that absorbs a significant amount of water yet is insoluble in aqueous solutions. More so than any other form of synthetic biomaterial, their high water content makes them more like to natural living tissue and enhances biocompatibility.[5] The use of dry swelling polymeric networks as drug delivery systems for oral, nasal, and other routes of administration is made possible by the hydrogels' capacity for drug loading and release. The polymer's capacity to absorb water and swell makes it prone to the liquid-gel transition.[6] There are two types of hydro gels

1. Preformed hydro gels

2. In situ forming gels preformed hydro gels are straightforward viscous solutions that don't change after being administered. Hydrogels that are prefabricated are used to replace tears. Following administration, the preformed hydro gels cause lachrymation, crusting of the eyelid, and impaired vision.[7] Therefore, it is possible to "Inject as eye drops and undergo an immediate gelation when in contact with the eye" with In situ gels. When in situ-forming gels are injected, they are liquid. In the ocular cul-de-sac, they undergo a phase transition to become viscoelastic gel, which allows them to react to changes in the surrounding environment. To increase medication retention on the ocular surface, three distinct types of polymers develop. The release of drugs from in situ gels is contingent upon multiple stimuli, such as temperature, pH, ion-induced, and so on. In situ gel formation depends on a variety of mechanisms, including physical and chemical ones.[8]

Anatomy of eye

The adult eyeball, often referred to as a spherical globe, is only approximately spherical in shape, with its largest diameter being 24mm antero-posteriorly.[9][10] Among the human body's most intricate organs is the eye. Three distinct layers exist in the human eye. The cornea and sclera make up the outer region. In addition to refracting and transmitting light to the lens and retina, the cornea shields the deeper portions of the eye from infection and structural deterioration. The sclera creates a layer of connective tissue that keeps the eye structurally stable and shields it from external and internal stresses. The limbus is where the cornea and sclera are joined. The conjunctiva, a translucent mucous membrane, covers the visible portion of the sclera. The iris, ciliary body, and choroid make up the central layer of the eye. The ciliary body regulates the power and shape of the lens and is the source of aqueous production; the choroid is a vascular layer that supplies oxygen and nutrients to the outer retinal layers; and the iris controls the size of the pupil, which affects the quantity of light that reaches the retina. The retina, a sophisticated, multi-layered system of neurons that absorbs and processes light, is the innermost layer of the eye. The aqueous, vitreous, and lens are the three transparent structures that are encircled by the ocular layers.[11]



PASSAGE OF FORMULATION ADMINISTERED THROUGH EYE

The overall course of medication retention into the eye from the precorneal region (portion site) following skin visual organization is very intricate. The old-style succession of occasions includes drug instillation, weakening in tear liquid, dispersion through mucin layer, corneal entrance (stroma, endothelium, epithelium), and move from cornea to watery hum or. Following assimilation, drug disseminates to the site of activity (For instance, the iris-ciliary body). Equal retention through the conjunctiva/sclera gives an extra pathway to eye tissues in any case, for most medications, is minor contrasted and corneal ingestion. Likewise, ineffective, contending, and equal pathways (e.g., nasolacrimal waste or fundamental ingestion through the conjunctiva) work to divert drug from the eye and breaking point the time considered the retention cycle. Additionally, in certain species, like the hare, non-useful retention into the nictitating layer can happen. Figure 1 presents a synopsis of these precorneal occasions, alongside a somewhat improved visible of the energy in the cornea, watery humour, and front section.[12]

CLASSIFICATION OF OPHTHALMIC DRUG DELIVERY SYSTEMS

I. Conventional delivery systems
- Eye drops
- Ointments and Gels
- Ocuserts and Lacrisert

II. Drug delivery to anterior segment
- Contact lens
- Cal du sac inserts
- Subconjunctival/ Episcleral implants

III. Drug delivery to posterior segment
- Intravitreal implants (e.g., Duraser Technology system, Novadu Technology, I- vatio TA, NT-501)
- Injectable Particulate Systems (RETAAC, Cortiject, Visudyne)

IV. Physical devices
- Iontophoresis
- Micro- electromechanical intra ocular drug delivery devices

V. Vesicular system
- Liposome
- Niosomes
- Discomes
- Pharmacosomes

VI. Controlled delivery systems
- In situ gel systems/ Phase transition systems
- Iontophoresis
- Dendrimer
- Contact lens
- Collagen shield
- Micro emulsion
- Nanosuspensions
- Micro needle

VII. Particulates
- Nanoparticles
- Micro particles

VIII. Advanced delivery systems
- Cell encapsulation
- Gene therapy
- Stem cell therapy
- Protein and Peptide therapy
- Scleral plug therapy
- siRNA therapy
- Oligonucleotide therapy
- Aptamer[13][14]

Mechanism of In Situ Gelling System

1) Temperature induced in situ gel system

A rise in temperature can cause certain polymers and hydrogels to go from a sol to a gel state. There is no need for an external temperature to initiate the sol to gel conversion because the body temperature is sufficient. The system should be able to adapt to slight temperature variations. There are three types of temperature-sensitive hydrogels: thermally reversible gels, positively thermosensitive gels, and negatively thermosensitive gels. Certain hydrogels have a lower critical solution temperature and are negative temperature sensitive, meaning that heating them above this point will cause them to contract. [15]. Temperature changes improve the gelling of these solutions, prolonging the drug release. Temperature changes result in hydration state changes that lead to a volume phase transition when the polymer molecule's intra- and intermolecular hydrogen bonding is preferred over water solubilization. Drug polymer, which at room temperature is in solution form and turns into gel at body temperature, can be used to achieve this state.[16] The upper critical solution temperature is reached at which certain polymers become soluble. The compound becomes insoluble due to inter- and intramolecular hydrogen bonding caused by the volume phase transition brought on by the change in hydration state.

Example- Poloxamer- Since poloxamer is a thermosetting polymer, increasing its concentration increases the drug's elasticity and contact time while decreasing the conversion of sol to gel.[17][18]

2) PH induced in situ gel systems

In this instance, a pH shift caused the sol to gel transition. The polymers with basic or acidic groups that either receive or release protons when the pH changes are those that exhibit pH-dependent transition. Weakly basic (cationic) groups exhibit shrinkage at lower pH values, whereas weakly acidic (anionic) groups exhibit swelling in response to pH increases. Anionic polymers that are sensitive to pH include carbopol, carbomer, and its variants.[19] When the formulation is injected into the eye, its pH changes from 4.4 to 7.4 because of the shift in pH. The formulation is in solution form at pH 4.4.

Example: derivatives of carbomer, cross-linked polyacrylic, etc. [20]

3) Ion induced In situ gel systems

Sometimes polymers may convert from sol to gel in presence of various ions. Some polysaccharides come under ion sensitive polymers. It is believed that the osmotic gradient across the gel surface determines the pace of gelation. The sol-to-gel transition in the eye may be influenced by the solution's osmolality. Generally

speaking, tear fluid contains mono- or divalent cations that combine to produce a transparent aqueous polymer solution gel.[21]An anionic polymer known as gellan gum gels when exposed to mono- and divalent cations. The sol to gel transition is often started by the Na, Ca, and Mg ions found in the tear fluid. Alginic acid gels when a divalent cation (Ca) is present.
Example- gellan gum.[22]

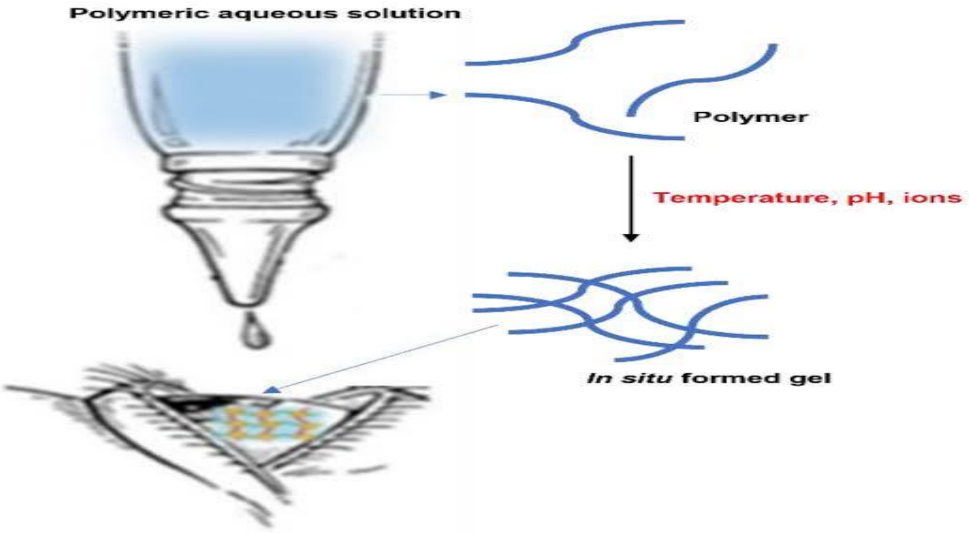


Figure No.1 - Shows mechanism and example of various stimuli sensitive polymer.

Table.1 - Stimuli Sensitive Polymer

Stimuli	Mechanism	Examples
Temperature	At room temperature (20–250C), the formulation is liquid; when it comes into contact with bodily fluids (35–370C), it gels.	Poloxamer/ phloronics Xyloglucan cellulose derivative.
pH Change	pH rises from 4.2 to 7.4 during the sol to gel transition. A hydrogel network is formed when a polymer and mucin form a hydrogen bond at a higher pH.	Pseudolatexes carbomer (acrylic acid) cellulose Acetate, phthalate latex.
Ion induced	A rise in the ionic strength gel causes the formulation to change from liquid to gel. Complexation with polyvalent cations (such as Ca+) in lacrimal fluid causes formation.	Chitosan, Gellan Gum/ Gel rite Alginate.

Classification of in situ gel polymers

Based on their origin, polymers are classified or the mechanism of gelation. According to a source in situ, gelling systems classified into two types: [23][24][25][26]

1. Natural polymers (E. g., chitosan, Alginic acid, gellan gum, Carrageenan, xyloglucan, pectin, sodium hyaluronate, Guar gum, xanthan gum, etc.)

2. Synthetic or semi-synthetic polymers (E. g., hydroxyl propyl methylcellulose, Cellulose acetate phthalate, Polyacrylic acid, methylcellulose, poly (lactic-co-glycolic acid, poloxamers).

1. **Natural polymers**

Alginic acid, also known as sodium alginate, is a linear block copolymer polysaccharide made of β-D-mannuronic acid and α-L-glucuronic acid residues connected by 1,4-glycosidic bonds. It is hydrophilic, non-toxic, and biodegradable. It is employed as a delivery system for eye formulations. When exposed to divalent cations (Ca+2, Mg+2), alginate cross-links the carboxylate groups to form a solid gel that is resistant to erosion by tear fluid.[27][28]

- **Carrageenan.**

As gelatine, it's utilized as a natural cold and cough cure. Three categories are distinguished based on the sulphate group number and position: [29][30][31]

a. Iota carrageenan: When calcium or potassium ions are present, it forms an elastic gel, and it dissolves entirely in hot water.

b. Kappa carrageen an: It resembles locust bean gum in that it is soluble in hot water and forms a "gel" when potassium ions are present.

c. Lambda carrageen an: It is totally soluble in cold water and generates very viscous solutions, although it does not cause gel formation.

- **Chitosan**

It is a cationic, pH-dependent, biodegradable, biocompatible, thermosensitive, and amino polysaccharide that is produced when chitin is alkaline-deacetylated. Chitosan gels as a result of pH and

temperature variations. Because of the electrostatic interaction between the negatively charged mucosal surfaces and the positively charged chitosan, it possesses good mucoadhesive characteristics. Gels with electrostatic forces produced at low critical solution temperatures because of severe hydrophobic contacts. Chitosan gels with the use of displaying polymers at the highest critical solution temperature. Availability, non-toxicity, affordability, and other factors make this polysaccharide the second most often used polymer after cellulose.[32][33][34][35][36]

Guar gum, often known as guar While it is insoluble in alcohols, ketones, lipids, hydrocarbons, and ester, it is soluble in water. In tiny doses, it creates highly viscous colloidal solutions with both hot and cold water, demonstrating improved dispersibility. There is a reversible shift in gel formation due to temperature variations.[37]

- **Gellan gum**

Commercially marketed as Gel rite or Kelcogel, this gellan gum is a linear, water-soluble, extracellular, hetero, anionic polysaccharide that forms gel in the presence of metal cations (mono or divalent), just like alginate. Cross-linking gelation is induced by divalent cations (Ca+2 or Mg+2) and monovalent cations (Na+ or K+).

Double-helical junction zones are formed during the gelation process, and then the double-helical segment aggregates to create three-dimensional networks through complexation with cations and hydrogen bonding with water. It is one of the most often utilized polymers in the creation of in situ gels.[38]

- **Pectin**

A family of linear polysaccharides with cationic properties includes residues of α-(1, 4)-Dgalacturonic acid. Pectin will gel in the presence of H+ ions, which are a source of mono, divalent, and trivalent ions. It does not apply to organic solvents; it is only applicable to formulations that dissolve in water. Pectin and pectin acid monovalent cations (alkali metal salts) dissolve in water. Conversely, trivalent and divalent cationic salts dissolve poorly or not at all in water. Water has the tendency to hydrate dry powdered pectin, and when it is mixed with a water-soluble carrier, the clusters become soluble and form clumps, or semi-dry packages. The percentage of carbonyl groups that esterify with methanol is known as the degree of methylation, or DM. There are two types of pectin based on the extent of esterification:[39][40][41][42][43]

a. Low methoxy pectins; less than 50% of the carboxyl groups methyl ate the pectin's.

b. High methoxy pectin's; more than 50% of the carboxyl groups methyl ate the pectin's.

- **Sodium hyaluronate**

It is a type of hyaluronic acid sodium salt that is soluble in water. It is an endogenous, natural polysaccharide that helps the body produce collagen and keeps its flexibility. Additionally, it lessens the likelihood of oxidation and improves formulation stability.[44][45][46]

- **Xyloglucan or tamarind gum**

Because it is non-toxic, biocompatible, and biodegradable, xyloglucan is a plentiful hemicellulose polysaccharide that may be used in a variety of delivery strategies. Bgalactosidase partially breaks it down, and the thermo responsive process causes it to gel. When taken orally, it has a gelation period of up to minutes and permits cold gelation in the stomach. Similar to polyloxamer, it gels when heated, refrigerated, or cooled from a higher temperature. Over a broad pH range, xyloglucan can gel in the presence of sugars (40–65%) or alcohols. However, the combination of 20% alcohols significantly reduces the sugars to produce a gel.[47][48][49]

- **Thiolated chitosan or thiomers**

Compared to other polymers, thiol groups now have substantially stronger sticky (mucoadhesive) characteristics. Thiomers interact with mucus glycol proteins or cysteine-rich sub-domains by forming gels that enter the physiological environment through the straightforward oxidation process that cross links intra- and inter-disulfide linkages. These are the most promising hydrophilic, cationic, multifunctional macromolecules; unlike chitosans, they also improve permeability. Its positive charges interact with cell membranes to reorganize the structural makeup of proteins linked to tight junctions. In addition, it demonstrates a strong, cohesive character.[50][51][52][53]

- **Xanthan gum**

Xanthan gum is soluble in hot and cold water and has good stability in both acidic and alkaline environments. Both pyruvic and glucuronic acid groups are present, which causes it to have an anionic nature.[54][55]

2. Synthetic or semi-synthetic polymers

- **Cellulose acetate phthalate (CAP)**

CAP is another name for pseudo latex. It is synthetic latex that is made by dispersing an already-existing polymer in an aqueous solution. Since latex is a free-running solution at pH 4.4 and coagulation tears fluid raises the pH to pH 7.4, it is a pH-sensitive, cross-linked polyacrylic polymer with potentially beneficial features for prolonged medication delivery to the eye. In γ-scintigraphy, CAP is employed to track the duration of ocular residency of an ophthalmic solution, and organic solvents are not used during manufacture.[56]

- **Hydroxypropyl methylcellulose (HPMC)**

This polymer is mucoadhesive, thermoreversible, and biocompatible. Because of its high swelling capacity and thermal gelation qualities, it is a kind of cellulose ether that is used in oral drug delivery systems and hydrophilic matrices. When HPMC and carbopol are combined, the solution's viscosity is increased and its acidity is decreased. Higher temperatures cause HPMC to gel because of the interaction between the hydrophobic and hydrophilic polymer components. It was actively participating in the creation of an aqueous solution for topical eye therapy. Formulating a vaginal mucoadhesive film with CR of S-nitroso glutathione and its effects on the gelling behavior turned out to be crucial.[57][58][59]

- **Methylcellulose (MC)**

It is also a derivative of cellulose and is employed as an in situ gelling polymer. At low temperatures, a number of cellulose derivatives remain liquid, but when heated, they gel. The aqueous solutions of MC and HPMC, for instance, have a phase transition into gels between 40–50 °C and 75–90 °C, respectively. The phase transition temperature of MC and HPMC, on the other hand, is lower than the physiological temperature due to chemical and physical modifications made to the polymers. Gelation of HPMC and MC solutions is caused by hydrophobic interactions between molecules containing methoxy groups. Macromolecular hydration at lower

temperatures leads to polymer-polymer interaction. As the heat resulting in lower viscosity increases, the hydration is gradually lost. The polymers begin to associate and thicken at the point where sufficient dehydration occurs, indicating the creation of a network structure. A solution is liquid at low temperatures (30 °C), and gelation happens at higher temperatures (40–50 °C).[60][61][62]

- **Polyacrylic acid (PAA)**

Commercially, PAA is referred to as carbopol. In ophthalmology, it is frequently utilized to improve pre-corneal retention. Compared to other cellulose derivatives, it can have superior mucoadhesive qualities. After comparing many grades, including carbopol 910, 934, 940, 941, etc., it was determined that 940 demonstrated superior one.[63][64][65]

- **Poly (lactic-co-glycolic acid) or PLGA**

This polymer is both biodegradable and biocompatible. It is a polyglycolic acid (PGA) and polylactic acid (PLA) synthetic copolymer. These systems are available on the market as implants, microparticles, and in situ implants and are used for controlled drug delivery. Because of its extensive clinical experience, PLGA is one of the most capable polymers employed in tissue engineering and drug delivery applications.[66][67][68]

- **Poloxamers**

Poloxamers are employed in thermosensitive in situ gels and are marketed under the trade name pluronic. It lengthens the duration of medication residence and has great thermal setting properties. It is a tri-block copolymer made of two polyethylene oxides (PEO) and one polypropylene oxide (PPO) that dissolves in water. Because Pluronic F127 forms translucent gels and is colorless, it is the most widely used poloxamer polymer in the pharmaceutical industry. PEO makes up 70% of it, whereas PPO makes up 30%. To improve the bioavailability and residence time of the ocular medications, an in situ gelling vehicle consisting of a copolymer pluronic F127-g-poly (acrylic acid) was employed.[69][70][71]

- **Poloxamines**

Tronics is the usual term for poloxyamines. These ethylene and propylene oxide tetra functional block copolymers are biocompatible. PEO-PPO's four arms, which are connected by an ethylenediamine group to produce X-shaped poloxamines, appear to be essential for tetronics' potential to be osteoinductive. Up till now, it has made dual use of temperature- and pH-responsive micelles and gels. No other polymer has been found to be osteoinductive on its own. When their molecular weight rises, hydrophilic compounds become more compatible and are more cytocompatible than hydrophobic ones.[72][73][74][75]

- **Poly (N-isopropyl acryl amide) or PNIPAAm**

This thermosensitive polymer exhibits a reversible phase change between 32 and 35 °C, which is more in line with the temperature that humans can tolerate. therapeutic objectives.[76][77][78][79]

ADVANTAGES OF IN SITU DRUG DELIVERY SYSTEM

1. More comfortable than formulations that are insoluble and insoluble.
2. Less visual blurriness than with ointments.
3. Precorneal residence duration increases and nasolacrimal drainage decreases as a result of increased viscosity.
4. Frequent instillation is not necessary because of the longer residence period.
5. Ease of administration.[80][81]
6. Better precision in dosage. to get around the negative effects of conventional systems' pulsed dosing.
7. To distribute drugs in a controlled and continuous manner.
8. To lengthen the corneal contact time in order to enhance the drug's ocular absorption. Effective adhesion to the corneal surface can accomplish this.
9. To get beyond barriers that protect, such as lacrimation, drainage, and absorption through the conjunctiva.
10. To make the patient more comfortable, increase their compliance, and enhance the medication's therapeutic effect.
11. To give the delivery system greater housing.[82]

EVALUATION OF OCULAR IN SITU GEL

Ocular in situ gel can be tested for various parameters in order to ensure that prepared formulation satisfy safety guidelines for ocular drug delivery system (ODDS).

1. Visual appearance and clarity

Under fluorescent light against a white and black background, the created in situ formulation's visual appearance and clarity are examined for the presence of any particle matter.

2. pH

PH has an impact on the drug's stability and solubility in ocular preparations. It should be designed so that the patient won't experience any irritation after ingestion and that the formulation will be stable at that pH. A digital pH meter 32 is used to measure it.

3. Gelling capacity

A drop of the formulation is added to a vial holding 2.0 ml of freshly made simulated tear fluid to measure the formulation's gelling capability. The gelling time is then recorded.[83]

4. Isotonicity

Tonicity is a crucial component of ophthalmic formulations that must be preserved to avoid damaging tissue or irritating the eyes. It speaks of the osmotic pressure that dissolves salts in water exert. The osmotic pressure of an ophthalmic formulation should be between 290 and 310 mOsmol/kg. The osmometer is used to measure tonicity.[84][85]

5. In vitro drug release study

To analyze drug release in vitro, Franz diffusion cell is used. Freshly manufactured artificial tear fluid (ATF) is inserted into the receptor compartment. The dialysis membrane sits between the donor and receptor compartments. The entire assembly is maintained at 37 oC ± 0.5 oC in a medium that is thermostatically controlled to replicate in vivo circumstances. Continuous stirrer rate of 20 rpm is the medium. One milliliter of the formulation is put in the donor chamber. At regular intervals, 0.5 ml of the sample is removed and replaced with ATF before being analyzed using an HPLC or UV spectrophotometer.[86][87]

6. Rheological studies

The primary purpose of the Brookfield viscometer is to measure the viscosity of in situ ophthalmic gels. Before

and after gelation, viscosity is measured by progressively increasing angular velocity from 0.5 to 100 rpm.[88]

7. Texture analysis

Using a texture profile analyzer, the cohesiveness, stiffness, and consistency of in situ gel are evaluated. This mostly shows the gel's strength and ease of use. Texture analysis yields data on hardness, compressibility, and adhesiveness that can be related to a number of characteristics, such as the ease of removal from the container, the product's ability to spread evenly over the corneal surface, and its capacity to cling to the mucous layer to extend the duration of residency.[89]

8. Tran corneal permeability study

Goat eye cornea is used to test the drug's permeability through the cornea. Fresh goat eyeballs in their entirety are purchased from a nearby butcher shop and brought into the lab in a standard saline solution at 4oC. After gently removing the cornea and 2-4 mm of the surrounding sclera tissue, a saline solution wash is applied. The excised cornea is positioned so that its epithelial surface faces the donor compartment, between the donor and receptor compartments of the Franz diffusion cell. Artificial tear fluid (ATF) is freshly manufactured and placed into the receptor compartment. The entire assembly is set up on a thermostatically controlled magnetic stirrer, which maintains both the temperature (37 ± 0.5 °C) and stirring rate (20 rpm). The donor compartment is filled with 1 ml of the prepared mixture. At predefined intervals of one to five hours, samples (0.5 ml) are removed and replaced with the same volume of ATF. After that, samples are diluted up to 10 ml and examined using an HPLC or UV spectrophotometer.[90][91]

9. Ocular irritation study

As there is ban on Raise study in many countries ocular irritation study of in situ formulation can be performed by one of the following method.

10. Histological study

In order to assess the impact of in situ formulation on corneal structure and investigate the possibility of irritation, corneas are extracted from recently slaughtered goat eyes and incubated at 37°C for a duration of five hours. The positive control is a 0.1% (w/w) solution of sodium dodecyl sulphate (SDS) in phosphate buffer saline (PBS). Following incubation, corneas are promptly fixed in formalin (8%, w/w) and cleaned with PBS. After being dehydrated in a gradient of alcohol, tissues are melted paraffin-filled and hardened into blocks. Haematoxylin and eosin (H&E) is used to cut sections. Cross sections are examined under a microscope to look for any changes.[92]

11. Hen's Egg Test

The test for chorioallantoic membrane (HETCAM) involves incubation the eggs for ten days at 37°C and a relative humidity of roughly 70%, with an automated rotating process that occurs once per hour. Following the incubation time, a part of each egg shell is removed, and to prevent capillary damage during removal, a drop of water is placed onto the membrane of the air sack. After being cautiously exposed to 0.1 ml or 0.1 gram of test compounds for 30 seconds, the CAM is cleaned off with regular saline solution. 1% SDS solution (positive control) and saline solution (negative control) are applied to CAM simultaneously. After five minutes, each CAM is examined under a microscope to check for coagulation, lysis, and hemorrhage. For every CAM, an irritation score (IS) is determined using the formula below: = 301 - *h*300×5 + (301 -)300×7 + 301 - 300×9. The following scheme is used to assign an irritation score: 1 denotes a little reaction, 2 a moderate reaction, and 3 a severe reaction.[93][94]

12. In vivo Scintigraphy Studies

An established method for assessing ocular retention duration in vivo is gamma scintigraphy. Human volunteers are preferred for this study despite the rabbit being the frequently recommended animal model for evaluating ophthalmic formulations. This is because humans and rabbits have different physiological characteristics, particularly with regard to blinking rate.[95]

13. Accelerated stability study A

To ascertain the physical stability of the formulation under accelerated storage circumstances, an in situ stability study is conducted in accordance with ICH principles. The formulation process is exposed to high temperatures and humidity levels of 25±1°C/60%RH, 30±1°C/65%RH, and 40±2°C/75±5%RH. After0,30,60, and 90 days, samples are removed, and their active drug content is assessed.[96]

14. Sterility testing

One crucial evaluating factor for ophthalmic preparations is their sterility. The Indian Pharmacopoeia is followed for performing the sterility test. The liquid from the test container is extracted in 2 milliliters using a sterile pipette, sterile syringe, or sterile needle as part of the direct inoculation procedure. The test liquid is aseptically transferred to separate 20 ml containers of the soybean-casein digest medium and the fluid thioglycolate media. The medium is combined with the liquid. For a minimum of 14 days, the infected media is incubated at 30°C to 35°C for fluid thioglycolate medium and 20°C to 25°C for soybean-casein digest media.[97]

15. Gel strength

The gel strength is assessed using a rheometer. As stated in the formulation, the gel is made in a beaker using sol form. The beaker containing the gel is lifted at a specific rate to force the probes into the gel gradually. The probe's depth of immersion below the gel surface can be used to measure the variations in load from gel to empty space.[98]

SUMMARY

Despite of the difficulties in visual medication conveyance, over the past few years, many inventive methodologies are being created to beat the issues related with ordinary of ophthalmic arrangements. The in-situ gelling framework is one the promising and broadly concentrated on techniques that could draw out precorneal residence time and deal the supported delivery drug conveyance, in this manner work on visual bioavailability and restorative adequacy and lessen foundational as simulation. Moreover, because of its medication discharge supporting capacity and decline the recurrence of organization, in-situ gel could work on persistent consistence. In-situ gel detailing with various improvements responsive polymers that have high awareness to change in pH, temperature, and particle focus are utilized. Nonetheless, the mix of at least two improvements responsive polymers in a similar detailing is known to display more noteworthy consistence and

worked on restorative viability. In addition, investigating the blend of various medication conveyance draws near (for example nanoparticles stacked in-situ gelling) to create in-situ gel has been the alluring procedures to further develop ocular delivery system.

As the eye is the most fundamental part of the body, the security issues of ophthalmic plans is fundamentally significant. The dominant parts of the cytotoxicity and peevishness concentrates on remembered for this audit showed that no huge adjustments or sign of toxicity because of the use of in-situ gel. Nonetheless, further investigations are needed to assess the conceivable harmfulness because of rehashed and long-haul applications and materials for the readiness of nanoparticles in nano-gel frame works. Also, the expanded consistency of in-situ gel might causea few restrictions like blurred vision and uneasiness to patient bringing about a quicker disposal because of reflex tears and flickers. So, basic control of the consistency ought to be taken into thought during planning and advancement of in-situ gel definition to the tolerable level.

Regardless of the promising capability of in-situ gel in visual medication conveyance, just a set number of medications as in-situ gel are at present in clinical use. Thus, further work sought to be done to investigate this medication conveyance framework for the clinical utilization of other ophthalmic medications. As of now, the majority of the ophthalmic in-situ gels were planned uniquely for the definitions containing of single dynamic fixing. Later on, a few more appropriate systems ought to be produced for the recipe comprising of different fixings like Traditional Chinese Medicine specifically, which includes a multi-target way to deal with produce their activity. Ultimately, later on, we expect the advancement of new and more dependable in-situ framing polymers which might be receptive to a few biochemical markers related with the illness states of the eye.

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