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OPTHALMIC IN SITU GEL: A REVIEW

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ABSTRACT

The poor bioavailability of conventional ophthalmic formulations is due to rapid precorneal drug loss (through dilution and drainage from the eye). There are some static (different layers of the eye i. e. cornea, sclera, retina) and dynamic barriers (blood aqueous and blood retinal barrier) which also affect the bioavailability of drug. The problem can be overcome by using in situ forming ophthalmic drug delivery system prepared from polymer that exhibit reversible liquid–gel phase transition. In situ gels are the liquid preparations which upon instillation undergoes phase transition in cul-de-sac of the eye to form a viscous gel and this occurs due to the environmental changes in the eye (i.e. due to change in temperature, change in pH and ion induced change). This novel drug delivery system promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with mucosa. The primary requirement of a successful control release product focuses on increasing patient compliance, good stability and biocompatibility characteristics which make the in situ gel dosage forms very reliable. This review is to specify the basic anatomy and physiology of human eye, various approaches used for formulation of in-situ gels and polymers used in the formulation of in situ gels.

KEYWORDS: In situ gel, in situ gelling polymers, pH sensitive, temperature sensitive, ion sensitive.

INTRODUCTION.[1][3][14]

The eye is a unique organ, both anatomically and physiologically, containing several widely varied structures with different physiological functions that render the organ highly impervious to foreign substances. The conventional drug delivery such as suspension, ointment, solution show some drawbacks like increase pre-corneal drainage, blurred vision, low bioavailability low residence time. The absorption of drugs in the eye is severely limited by some protective mechanisms that ensure the proper functioning of the eye, and by other concomitant factors like, drainage of the instilled solutions, lachrymation and tear turnover, metabolism, tear evaporation, non-productive absorption/adsorption, limited corneal area and poor corneal permeability, binding by the lachrymal proteins.[1]

A major goal in ocular therapeutics is to circumvent structural obstacles and protective mechanisms of the eye to elicit desired pharmacological response.[3]

The specific aim of designing a therapeutic system is to achieve an optimal concentration of a drug at the active site for the appropriate duration. Ocular disposition and elimination of a therapeutic agent is dependent upon its physicochemical properties as well as the relevant ocular anatomy and physiology. A successful design of a drug delivery system, therefore, requires an integrated knowledge of the drug molecule and the constraints offered by the ocular route of administration.[3] Development of newer, more sensitive diagnostic techniques and novel therapeutic agents are needed to provide ocular delivery systems with high therapeutic efficacy as the conventional systems have some drawbacks which makes them less effective.

The various approaches that have been attempted to increase the bioavailability and the duration of the therapeutic action of ocular drugs can be divided into two categories. The first one is based on the use of sustained drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs. The second involves maximizing corneal drug absorption and minimizing precorneal drug loss.[3]

The development of in situ gel systems has received considerable attention over the past few years owing to the several advantages offered by this polymeric system, such as ease of administration and reduced frequency of administration, improved patient compliance and comfort. In situ gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange.[14]

ANATOMY AND PHYSIOLOGY OF HUMAN EYE.[1-5]

Owing to its design, human eye represents a gateway to the process called vision. Eyeball spherical in shape, houses many structures that work together to facilitate sight. The human eye is comprised of layers and internal structures, each of which performs distinct functions.

The eye is composed of two segments.[3]

The anterior segment consists of:

- The aqueous humor is a jelly-like substance located in the outer/front chamber of the eye. It is a watery fluid that fills the "anterior chamber of the eye" which is located immediately behind the cornea and in front of the lens. The aqueous humor is very slightly alkaline salt solution that has a high oxygen tension and about the same osmotic pressure as blood.
- Pupil generally appears to be the dark "centre" of the eye, but can be more accurately described as the circular aperture in the centre of the iris through which light passes into the eye.
- The iris is a thin circular contractile curtain located in front of the lens but behind the cornea. The iris is a diaphragm of variable size whose function is to adjust the size of the pupil to regulate the amount of light admitted into the eye.
- The ciliary muscle is a ring of striated smooth muscles in the eye's middle layer that controls accommodation for viewing objects at varying distances and regulates the flow of aqueous humour into schlemm's canal.

The posterior segment consists of:

- The sclera (white portion of the eye) is the tough white sheath that forms the outer-layer of the ball and can withstand the intra-ocular tension constantly maintained in the eye.
- The conjunctiva is a thin transparent mucous epithelial barrier, lines the inside of the eyelids. The conjunctiva is composed of two layers: an outer epithelium and its underlying stroma (substantia propria). The conjunctiva contributes to the formation of the tear film by way of secreting substantial electrolytes, fluid, and mucins.
- The cornea is a strong clear bulge located at the front of the eye. It has an important optical function as it refracts light entering the eye which then passes through the pupil and onto the lens (which then focuses the light onto the retina). Non vascular in nature, oxygen and nutrients are transported by aqueous humour and is richly supplied with free nerve endings. Withstand the intra-ocular tension constantly maintained in the eye.
- The lens is a transparent structure enclosed in a thin transparent capsule. It is located behind the pupil of the eye and encircled by the ciliary muscles. It helps to refract light travelling through the eye (which first refracted by the cornea). The lens focuses light into an image on the retina. Oxygen and nutrients are transported by aqueous humour as is non vascular.
- The vitreous humour (also known as the vitreous body) is located in the large area that occupies approximately 80% of each eye in the human body. The vitreous humour is a perfectly transparent thin-jelly-like substance that fills the chamber behind the lens of the eye. Non vascular structure to which oxygen and nutrients are transported by aqueous humour.
- The retina is located at the back of the human eye. The retinal "screen" is therefore a light-sensitive structure lining the interior of the eye. It contains photosensitive cells (called rods and cones) and their associated nerve fibers that convert the light they detect into nerve impulses that are then sent onto the brain along the optic nerve.
- The choroid layer is located behind the retina and absorbs unused radiation and nourishes the outer portions of the retina. It is a thin, highly vascular (i.e. it contains blood vessels) membrane that is dark brown in colour and contains a pigment that absorbs excess light and so prevents blurred vision.
- The optic nerve (a bundle of over 1 million nerve fibers) is responsible for transmitting nerve signals from the eye to the brain.

Lachrymal apparatus.[13]

Consists of four structures: Lachrymal glands, lachrymal canals, lachrymal sac, naso- lachrymal duct. The lachrymal fluid (7µl, pH 7.4) secreted by the lachrymal glands is emptied on the surface of the conjunctiva of the upper eyelid at a turnover rate of 16% per min. It washes over the eyeball and is swept up by the blinking action of the eyelids. Thus the eyeball is continually irrigated by a gentle stream of lachrymal fluid that prevents it from becoming dry and inflamed.

A schematic diagram of the human eye is depicted in Figure1:

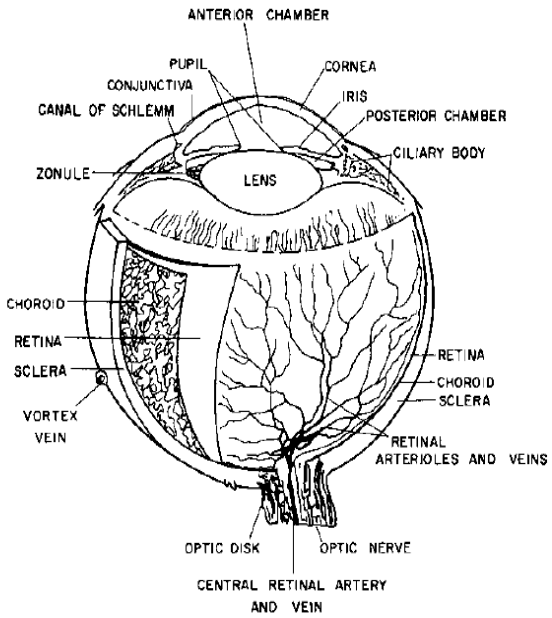

Figure 1: Anatomical structure of the human eye[3]

BARRIERS FOR OCULAR DELIVERY[3-5]

1. Drug loss from the ocular surface

After instillation, the flow of lacrimal fluid removes instilled compounds from the surface of the eye. Even though the lacrimal turnover rate is only about 1 µl/min the excess volume of the instilled fluid is flown to the nasolacrimal duct rapidly in a couple of minutes. Another source of non-productive drug removal is its systemic absorption instead of ocular absorption. Systemic absorption may take place either directly from the conjunctival sac via local blood capillaries or after the solution flow to the nasal cavity. Drug absorption into the systemic circulation decreases the drug concentration in lacrimal fluid extensively.

2. Lacrimal fluid-eye barriers.

Corneal epithelium limits drug absorption from the lacrimal fluid into the eye. The corneal epithelial cells form tight junctions that limit the paracellular drug permeation. Therefore, lipophilic drugs have typically at least an order of magnitude higher permeability in the cornea than the hydrophilic drugs. In general, the conjunctiva is leakier epithelium than the cornea and its surface area is also nearly 20 times greater than that of the cornea.

3. Blood-ocular barriers.

The eye is protected from the xenobiotics in the blood stream by blood-ocular barriers. These barriers have two parts: blood-aqueous barrier and blood-retina barrier. The anterior blood-eye barrier is composed of the endothelial cells in the uvea (The middle layer of the eye beneath the the sclera. It consists of the iris, ciliary body, and choroid). This barrier prevents the access of plasma albumin into the aqueous humor, and also limits the access of hydrophilic drugs from plasma into the aqueous humor. The posterior barrier between blood stream and eye is comprised of retinal pigment epithelium (RPE) and the tight walls of retinal capillaries. Unlike retinal capillaries the vasculature of the choroid has extensive blood flow and leaky walls. Drugs easily gain access to the choroidal extravascular space, but thereafter distribution into the retina is limited by the RPE and retinal endothelia. Unlike blood brain barrier, the blood-eye barriers have not been characterised in terms of drug transporter and metabolic enzyme expression.

Overview of Opthalmic Drug Delivery Systems[15,17]

Table 1: Different routes and dosage forms for ocular drug delivery

Sr no.	Route	Dosage Forms	Benefits	Constraints
1	Topical	Solutions,	Ease of administration	Poor bioavailability, suitable only for anterior segment, blurring vision.
		Suspensions	Patient compliance. Best for drug with slow dissolution.	Drug properties decide Performance. Loss of both solution & Suspended solid.
		Ointments	Flexibility in drug choice. Improved drug stability. Resistance to nasolacrimal drainage Inhibition of dilution by tears.	Sticking of eyelids. Blurred vision. Poor patient compliance. Drug choice limited by partition coefficient.
		Emulsions	Prolonged release of drug from vehicle	Blurred vision. Patient's non-compliance. Possible oil entrapment.
		Gels	Comfortable. Less blurred vision	Matted eyelids after use. No rate control on diffusion
2	Subconjunctival	Injectables	Delivery of large molecular size drugs, sustained release of drug	Patient non-compliance, suitable for only water soluble drugs
3	Retrobulbar	Injectables (used For anesthetization)	-	Perforation of globe, patient non- compliance
4	Peribulbular	Injectables (used For anesthetization)	Avoidance of perforation of globe	Non-compliance in pediatrics patients and patient with mental disorders
5	Intracameral	Injectables	Sustained delivery to aqueous humor	Patient non-compliance.
6	Intraviteral	Injectables	Sustained delivery of drug to posterior segment of the eye	Patient non-compliance.

Fate of Formulation Administered Through Eye.[3]
The general process of absorption into the eye from the precorneal area (dose site) following topical ocular administration is quite complex. The classical sequence of events involves drug instillation, dilution in tear fluid, diffusion through mucin layer, corneal penetration (epithelium, stroma, endothelium), and transfer from cornea to aqueous humor. Following absorption, drug distributes to the site of action (e.g., iris-ciliary body).

Parallel absorption via the conjunctiva/sclera provides an additional pathway to eye tissues but, for most drugs, is minor compared with corneal absorption. Also, nonproductive, competing, and parallel pathways (e.g., nasolacrimal drainage or systemic absorption via the conjunctiva) work to carry drug away from the eye and limit the time allowed for the absorption process. Moreover, in some species, such as the rabbit, non-productive absorption into the nictitating membrane can occur. Figure 1 presents a summary of these precorneal events, along with a relatively simplified view of the kinetics in the cornea, aqueous humor, and anterior segment.

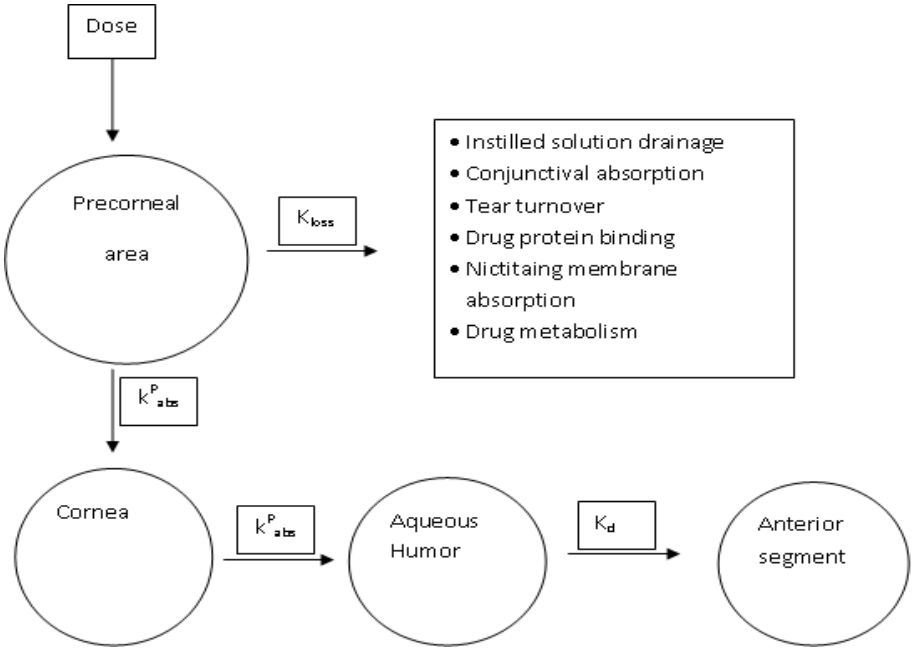

Figure 2. Model showing precorneal and intraocular events following topical ocular administration of the drug.[3]

Factors Responsible for Poor Ocular Bioavaibility of Drugs.[13]
Factors mainly responsible for poor ocular bioavailability following topical instillation are precorneal drainage and the lipoidal nature of the corneal epithelium.

- Binding by the lachrymal proteins.
- Drainage of the instilled solutions.
- Lachrimation and tear turnover.
- Limited corneal area and poor corneal penetration.
- Metabolism.
- Tear evaporation and permeability.
- Non-productive absorption/adsorption.

This can be minimized by developing delivery systems which provide controlled and targeted drug delivery for prolonged. Conventional ophthalmic formulations such as solutions and suspensions exhibit poor bioavailability. Over the last decade, numerous drug delivery systems have been developed to overcome the limitations of conventional dosage forms.

Characteristics Required To Optimize Drug Delivery Systems.[13,17]
• Good corneal penetration.
• Prolonged contact time with corneal tissue.
• Simplicity of installation for the patient.
• Non- irritative and comfortable form (the viscous solution should not provoke lachrimation and reflex blinking).

IN SITU GELLING SYSTEM.[13,18]
The word in situ is derived from Latin which means ‘in its original place or in position’.
This novel drug delivery system promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with mucosa, that problems generally encountered in semisolid dosage forms. In situ hydrogels can be instilled as eye drops and undergo an immediate gelation when in contact with the eye. In situ forming hydrogels are liquid upon instillation and undergo phase transition in the ocular cul-de-sac to form viscoelastic gel and this provides a response to environmental changes.

Advantages of In Situ forming gel.[12,13,17,18]

1. Less blurred vision as compared to ointment.
2. Decreased nasolacrimal drainage of the drug which may causes undesirable side effects due to systemic absorption (i.e. reduced systemic side effects).
3. The possibility of administering accurate and reproducible quantities, in contrast to already gelled formulations and moreover promoting precorneal retention.
4. Sustained, Prolonged drug release and maintaining relatively constant plasma profile.
5. Reduced dosing frequency compared to preformed gel. Reduced number/frequency of applications hence improved patient compliance and comfort.
6. Generally more comfortable than insoluble or soluble insertion.
7. Increased bioavailability due to increased precorneal residence time and absorption.
8. Avoidance of hepatic first pass.

Approaches for In Situ Gelling System.[13,14,18]

Ideally, an in situ gelling system should be a low viscous, free flowing liquid to allow for reproducible administration to the eye as drops, and the gel formed following phase transition should be strong enough to with stand the shear forces in the cul de sac and demonstrated long residence times in the eye. In order to increase the effectiveness of the drug a dosage form should be chosen which increases the contact time of the drug in the eye. This may then prolonged residence time of the gel formed in situ along with its ability to release drugs in sustained manner will assist in enhancing the bioavailability, reduce systemic absorption and reduce the need for frequent administration leading to improved patient compliance.

There are four broadly defined mechanisms used for triggering the in situ gel formation of biomaterials:

- Physiological stimuli (e.g., temperature and pH),
- Physical changes in biomaterials (e.g., solvent exchange and swelling),
- Chemical reactions (e.g., enzymatic, chemical and photo-initiated polymerization).

1. In situ formation based on physiological stimuli

Thermally triggred system.[14,18]

Temperature-sensitive hydrogels are probably the most commonly studied class of environment-sensitive polymer systems in drug delivery research. The use of a biomaterial whose transitions from sol-gel is triggered by increase in temperature is an attractive way to approach in situ formation. The ideal critical temperature range for such system is ambient and physiologic temperature, such that clinical manipulation is facilitated and no external source of heat other than that of body is required for trigger gelation. A useful system should be tolerable to account for small differences in local temperature, such as might be encountered in appendages at the surface of skin or in the oral cavity. Three main strategies are exists in engineering of thermoresponsive sol-gel polymeric system. For convenience, temperature-sensitive hydrogels are classified into negatively thermosensitive, positively thermosensitive, and thermally reversible gels.

Negative temperature-sensitive hydrogels have a lower critical solution temperature (LCST) and contract upon heating above the LCST. Polymers with low critical temperature (LCST) transition between ambient and physiologic temperature is used for this purpose. One of the most extensively investigated polymers that exhibit useful LCST transition is poly(N-isopropyl acrylamide) (PNIPAAm). PNIPAAm is a water soluble polymer at its low LCST, but hydrophobic above LCST, which result on precipitation of PNIPAAm from the solution at the LCST. Pluronics are poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO) triblock copolymer that are fluid at low temperature, but forms thermo responsible gel when heated as a consequences of a disorder-order transition in micelle packing which makes these polymers suitable for in situ gelation.

A positive temperature sensitive hydrogel has an upper critical solution temperature (UCST), such hydrogel contracts upon cooling below the UCST. Polymer networks of poly(acrylic acid) (PAA) and polyacrylamide (PAAm) or poly(acrylamide-co-butyl methacrylate) have positive temperature dependence of swelling.

The most commonly used thermoreversible gels are these prepared from poly(ethylene oxide)-b-poly(propylene oxide)-bpoly(ethylene oxide) (Pluronics®, Tetronics®, poloxamer). Polymer solution is a free flowing liquid at ambient temperature and gels at body temperature. Novel "protein polymers" called as ProLastins, which undergo an irreversible sol gel transition, when injected as a solution into the body, the material forms a firm, stable gel within minutes. It remains at the site of injection providing absorption times from less than one week to many months. Such a system would be easy to administer into desired body cavity.

pH triggered systems[14,18]

Another formation of in situ gel based on physiologic stimuli is formation of gel is induced by pH changes. All the pH-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH. The polymers with a large number of ionizable groups are known as polyelectrolytes. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups. The most of anionic pHsensitive polymers are based on PAA (Carbopol®, carbomer) or its derivatives. Likewise poly vinyl acetaldiethyl amino acetate (AEA) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition. Drug formulated in liquid solutions have several

limitations including limited bioavailability and propensity to be easily removed by tear fluid. To minimize this factors and maximize this drug delivery by making a poly(acrylic acid) (PAA) solution that would be gel at pH 7.4, by that we found that at concentrations high enough to cause gelation, however, the low pH of PAA solution would cause damage to surface of eye before being neutralized by the lacrimal fluid. This problem was solved by partially by combining PAA with HPMC, a viscous enhancing polymer, which resulted in pH responsive polymer mixtures that was sol at pH 4 and gel at pH 7.4. Mixtures of poly(methacrylic acid) (PMA) and poly(ethylene glycol) (PEG) also has been used as a pH sensitive system to achieve gelation.

2. In situ formation based on physical mechanism[14,18]

Swelling

In situ formation may also occur when material absorbs water from surrounding environment and expand to occur desired space. One such substance is myverol 18-99 (glycerol mono-oleate), which is polar 1400 lipid that swells in water to form lyotropic liquid crystalline phase structures. It has some Bioadhesive properties and can be degraded in vivo by enzymatic action.

Diffusion

This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. Nmethyl-pyrrolidone (NMP) has been shown to be useful solvent for such system.

3. In situ formation based on chemical reactions[14]

Chemical reactions that results in situ gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes, and photo-initiated processes.

Ionic cross linking

Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones .While k-carrageenan forms rigid, brittle gels in reply of small amount of K+, icarrageenan forms elastic gels mainly in the presence of Ca2+. Gellan gum commercially available as Gelrite® is an anionic polysaccharide that undergoes in situ gelling in the presence of mono- and divalent cations, including Ca2+, Mg2+, K+ and Na+. Gelation of the low-methoxy pectins can be caused by divalent cations, especially Ca2+. Likewise, alginic acid undergoes gelation in presence of divalent/polyvalent cations e. g. Ca2+ due to the interaction with glucuronic acid block in alginate chains. That is, gelation is triggered by the presence of cations (Na+, Mg++, Ca++) in the tear fluid. These can be achieved by polymers like sodium alginate, gellan gum. Gelation is occurred by ionic interaction of polymer and divalent ions of tear fluid. When anionic polymers come in contact with cationic ions, it converts to form gel.

Enzymatic cross-linking

In situ formation catalysed by natural enzymes has not been investigated widely but seems to have some advantages over chemical and photochemical approaches. For example, an enzymatic process operates efficiently under physiologic conditions without need for potentially harmful chemicals such as monomers and initiators. Intelligent stimuli-responsive delivery systems using hydrogels that can release insulin have been investigated. Cationic pH-sensitive polymers containing immobilized insulin and glucose oxidase can swell in response to blood glucose level releasing the entrapped insulin in a pulsatile fashion. Adjusting the amount of enzyme also provides a convenient mechanism for controlling the rate of gel formation, which allows the mixtures to be injected before gel formation.

Photo-polymerisation

Photo-polymerisation is commonly used for in situ formation of biomaterials. A solution of monomers or reactive macromer and initiator can be injected into a tissues site and the application of electromagnetic radiation used to form gel. Acrylate or similar polymerizable functional groups are typically used as the polymerizable groups on the individual monomers and macromers because they rapidly undergo photopolymerisation in the presence of suitable photoinitiator. Typically long wavelength ultraviolet and visible wavelengths are used. Short wavelength ultraviolet is not used often because it has limited penetration of tissue and biologically harmful. A ketone, such as 2,2-dimethoxy-2-phenyl acetophenone, is often used as the initiator for ultraviolet photo- polymerization, where as camphorquinone and ethyl eosin initiators are often used in visible light systems. These systems can be designed readily to be degraded by chemical or enzymatic processes or can be designed for long term persistence in vivo. Photo polymerizable systems when introduced to the desired site via injection get photocured in situ gel with the help of fiber optic cables and then release the drug for prolonged period of time. The photo-reactions provide rapid polymerization rates at physiological temperature. Furthermore, the systems are easily placed in complex shaped volumes leading to an implant formation.

IN SITU GELLING POLYMERS[13][19-23]

A polymer used in in situ gels should have following characteristics:

1. It should be biocompatible.
2. It should be capable of adherence to mucus and non irritating.
3. It should have pseudo plastic behaviour.
4. It should influence the tear behavior.
5. The polymer should be capable of decrease the viscosity with increasing shear rate there by offering lowered viscosity during blinking & stability of the tear film during fixation.

Some of the most important polymers used as in-situ gelling agents are described in table.[19-21]

Table 2: Different in situ gelling polymers.

POLYMER	MECHANISM	PROPERTIES
In Temperature Sensitive In Situ Gelling System:		
1. POLOXAMER/ PLURONICS	At room temperature (25^0C), it behaves as viscous liquid and is transformed to transparent gel when temperature increases (37^0C). At low temperature, it forms small micellar subunit in solution and increase in temperature results increase in viscosity leads to swelling to form large micellar cross linked network.	Poloxamers or pluronic are the series of commercially available difunctional triblock copolymers of non-ionic nature. They comprise of a central block of relatively hydrophobic polypropylene oxide surrounded on both sides by the blocks of relatively hydrophilic poly ethylene oxide. The pluronic triblock copolymers are available in various grades differing in molecular weights and physical forms.
2. CELLULOSE DERIVATIVES (Methyl Cellulose, Hydroxy Propyl Methyl Cellulose, Ethyl Hydroxy Ethyl Cellulose)	Gelation of cellulose solution is caused by hydrophobic interactions between molecules containing methoxy substitution. At low temperature, molecules are hydrated and little polymer-polymer interaction occurs, whereas at high temperature, polymers lose their water of hydration	Cellulose is a linear homopolymer polysaccharide consisting of D-anhydroglucopyranose units joined together by β-1,4-glycosidic bonds. Extensive intramolecular and intermolecular hydrogen bonding present in cellulose renders it insoluble in water. Various cellulose ethers (CEs) have been prepared by etherification of the three hydroxyl groups on anhydroglucose units of cellulose producing water-soluble derivatives.
In pH Sensitive In Situ Gelling System:		
1. CARBOPOL	At specific pH there is Electrostatic, hydrophobic interaction and Hydrogen bonding takes place, hence leads to interdiffusion. The observed phase transition for carbopol solution was mediated by the variation of pH from 4.0 to 7.4 and can be attributed to ionization of Carbopol polymer.	Carbopol is the lightly crosslinked commercial form of Poly(acrylic acid), which stays in solution form at acidic pH but forms a low viscosity gel at alkaline pH. As the concentration of carbopol increases, due to its acidic nature it causes irritation to the eye. Addition of viscosity enhancer like HPMC, MC will reduce the concentration without affecting its gelling property.
2. POLYCARBOPHILS	Polycarbophil is insoluble in water, but its high swelling capacity in a neutral medium permits the entanglement of the polymer chains with the mucus layer. The nonionized carboxylic acid groups of polycarbophil bind to the mucin by means of hydrogen bonds	Polycarbophil is also the lightly crosslinked commercial form of Poly(acrylic acid) exhibits stronger mucoadhesion same as Carbopol. As concentration increases, acidic nature may cause lacrimation, hence combination of polymers are used.
In Ion Sensitive In Situ Gelling System		
1. GELLAN GUM/GELRITE	Gellan gum produce a cation induced in situ gelation (Ca2+, Mg 2+, K+, Na+) due to the cross linking between negatively charged helices and mono or divalent cations (Na+, Ca+, Mg+) present in tear fluid.	Gellan gum is anionic heteropolysaccharide that is, tetrasaccharide repeat unit of 2 β-D-glucoses, 1 β-D-glucuronate, and 1 α-L-rhamnose. GelriteR is a low-acetyl Gellan gum, which forms a clear gel in the presence of mono- or divalent cations. It has the tendency of gelation which is temperature dependent or cations induced.
2. ALGINATES	The monomers of alginate (β-D-mannuronic acid (M) and α-	It consist of (1→ 4) linked β- D-mannuronic acid and α-L-guluronic acid.

	L- glucuronic acid (G) are arranged as M-M block or G-G block with alternating sequence (M-G) block. Upon interaction of G block of polymer with calcium moieties in tear fluid, resulting in the formation of homogenous gel.	A prolonged precorneal residence of formulations containing alginic acid looked for, not only based on its ability to gel in the eye but also because of its mucoadhesive properties.
3. XANTHAN GUM	The anionic character of this polymer is due to the presence of both glucuronicacid and pyruvic acid groups in the side chain which results in gel formation when comes in contact with (ions present in) tear fluid.	The primary structure of this naturally produced cellulose derivative contains a cellulosic backbone (β-D glucoseresidues) and a trisaccharide side chain of β-D-mannose-β-D-glucuronicacid-α-D-mannose attached with alternate glucose residues of the main chain. The anionic character of this polymer is due to the presence of both glucuronic acid and pyruvic acid groups in the side chain.

EVALUATION PARAMETERS[6,8,10,11,14,28,33,35]

Following evaluation parameters followed for In-situ gel for ocular delivery:

1. Clarity

The clarity of the formulations before and after gelling will be determined by visual inspection of the formulations under fluorescent light, alternatively against white and black backgrounds.

2. pH

The pH of the prepared in situ gelling system after addition of all the ingredients will be measured using pH meter.

3. Gelling capacity

The gelling ability of the prepared formulations will be determined either visually or by SEM.

By visual inspection- The gelling capacity is determined by pouring a drop of the solution in a vial containing 2 ml of artificial tear fluid which should be freshly prepared and equilibrated at 37°C, and both the time of gelation and the time taken for the gel formed to dissolve will be noted. The composition of the artificial tear fluid.[6]

Table 3: Composition of Artificial tear fluid.

Sr no.	Ingredients	Qty taken
1.	NaCl	0.670g
2.	$NaHCO_3$	0.200g
3.	$CaCl_2$	0.008 g
4.	Purified Water	q. s. 100 g
Physiological pH (7.4±0.2) adjusted by adding the required amount of 0.1 N HCl.		

By SEM- SEM studies the surface morphology of the formulations at solution state and at gel state. By SEM image we can study compact and loose surface morphology of In-situ gel which helps in finding the gelation time of in situ gel.

4. Viscosity and Rheological studies

Viscosity of the instilled formulation is an important factor in determining the residence time of drug in the eye. Rheology of formulation need to be determined before and after gelation by using either the Brookfield's viscometer (RVT model) or Cone and plate geometry viscometer (Brookfield RVCP DV-III). The formulation before gelling should have viscosity from 5 to 1000 mpas. After ion gel activation in the eyes it will have viscosity of about 50-50,000 mpas. The samples are analysed both at room temperature at 25 °c and thermo stated at 37 °c ± 0.5 °c by a circulating bath connected to viscometer adaptor prior to each measurement. Also rheological study needs to be performed for formulations with and without drug to analyze the effect of addition of drug on rheological behaviour of polymer blend. Angular velocity run from 10-100 rpm. The hierarchy of shear rates was reversed and the average of two readings was used to calculate viscosity.

5. Drug content

It is determined by taking 1ml of the formulation and diluting it to 100ml with distilled water. 1 ml was withdrawn and further diluted to 10 ml with distilled water. Concentration was determined at 200-400nm by using UV visible spectroscopy.

6. Isotonicity Evaluation

Isotonicity is important characteristic of the ophthalmic dosage forms. Isotonicity has to be maintained to prevent tissue damage or irritation of eye. All ophthalmic dosage forms are subjected to isotonicity testing, since they exhibited good release characteristics, optimum gelling capacity and the requisite viscosity. Formulations are mixed with few drops of blood and observed under microscope at 45X magnification and compared with standard marketed ophthalmic formulation.

7. In Vitro Drug Release Profile

can be studied by either of the following method:

By using dialysis tube

This study is performed in the Dialysis tube containing 1 ml of the formulation, which is then suspended in beaker at 37 ± 0.50C containing 100 ml artificial simulated tear fluid (pH 7.4) under continuous stirring at 20 RPM to stimulate the blinking effect. Dialysis membrane (0.22 µm pore size), previously soaked overnight in simulated tear fluid is mounted by tied and sandwiched between the donor and receiver compartment.

Aliquots of 1 ml withdrawn at different time intervals and equal volumes of fresh media added to replace the withdrawn samples. Withdrawn samples analyze by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using an equation generated from standard calibration curve. The percentage cumulative drug release (% CDR) calculated. The obtained data is further subjected to curve fitting for drug release data.

By using franz diffusion cell

In vitro release studies can also be carried out by using bi-chambered donor receiver compartment model (Franz diffusion cell). In this method 1ml of solution spread uniformly on a dialysis membrane, which is then contacted with receptor medium which is stirred continuously at 20 rpm to simulate blinking action of eyelids membrane (0.22 µm pore size), previously soaked overnight in simulated tear fluid is mounted by tied and sandwiched between the donor and receiver compartment. Aliquots of 1 ml withdrawn at different time intervals and equal volumes of fresh media added to replace the withdrawn samples. Withdrawn samples analyze by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using an equation generated from standard calibration curve. The percentage cumulative drug release (% CDR) calculated. The obtained data is further subjected to curve fitting for drug release data.

8. Ex vivo drug release studies

Goat corneas are used to examine the permeation across the corneal membrane. The cornea is carefully removed along with a 5-6 mm of surrounding scleral tissue and washed with cold saline. The washed corneas are kept in cold freshly prepared solution of tear buffer of pH 7.4. The study is carried out by using Franz-diffusion cell in such a way that the cornea side is continuously remained in an intimate contact with formulation in the donor compartment. The receptor compartment is filled with STF pH 7.4 at 34˚C ± 0.5˚C. The receptor medium is stirred on a magnetic stirrer. The samples are withdrawn at different time intervals and analyzed for drug content. Receptor phase is replenished with an equal volume of STF (pH 7.4) at each time interval.

9. Ocular irritation studies

Ocular irritancy studies are performed on male albino rabbits, weighing 1-2 kg. The studies were carried out with the guidelines of Council for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

The Draize-irritancy test is generally performed for the ocular irritation potential of the ophthalmic product prior to marketing. Mostly, left eye of each rabbit was used for test while the right eye was served as control. According to the Draize test, the amount of solution applied to the eye is normally 100µl is placed into the lower cul-de-sac. After dosing, the lids were held together for few seconds in order to avoid loss of the dosage form by lacrimation. The observation of the redness, swelling and irritation was done at time interval of 1hr, 24hrs, 48 hrs, 72hrs, and 1week after administration. The sterile formulation is administered twice a day for a period of 7 days ,and a cross-over study is carried out (a 3 day washing period with saline was carried out before the cross over study).

10. Sterility

All ophthalmic preparations should be sterile therefore the test for sterility is very important evaluation parameter. The sterility test is performed according to Indian Pharmacopoeia. Direct inoculation method is used , 2 ml of liquid from test container is removed with a sterile pipette or with a sterile syringe or a needle. The test liquid is then aseptically transferred to fluid thioglycolate medium (20 ml) and soyabean-casein digest medium (20 ml) separately. The liquid is mixed with the media. The inoculated media is incubated for not less than 14 days at 30°C to 35°C in the case of fluid thioglycolate medium and 20°C to 25°C in the case of soyabean-casein digest media.

11. Accelerated stability studies

Formulations are placed in ambient coloured vials and sealed with aluminium foil for a short terms accelerated stability study at 25°C to 28°C ambient temperature (temperature in the working area), 4±1°C (refrigerated temperature) and 37±2°C (temperature in the incubator) as per International Conference on Harmonization (ICH) states guidelines. Samples are analysed every month for clarity, pH, gelling capacity, drug content, rheological evaluation, and in vitro dissolution.

12. Drug Polymer interaction study:

Interaction studies can be performed in three ways one is by using UV, second is by taking IR spectra and third is by using DSC instrument.

In first method by UV the solutions of Polymer and drug prepared separately and in combinations and are autoclaved. The ultraviolet spectra taken before and after autoclaving using double beam ultraviolet visible spectrophotometer. Compare both the spectra for any possible change in solution content due to interactions between different ingredients.

In the second method the IR spectra was taken by using FTIR spectrophotometer. The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi on KBr-press and the spectra was scanned in the wave number range of 6000-400 cm-1. The FTIR graph of pure drug and combination of drug with excipient are recorded, then compared.

In the third method DSC scan is runned for individual component and the mixture for the interaction study. The interaction studies were carried out to check any possible physiochemical interaction among the formulation ingredients.

If UV spectra, IR spectra and DSC graph of the ingredients before and after mixing found to be identical and no additional peak emerged or existent peak shifted that confirms the formulation ingredients were compatible to each other and no physicochemical reactions taking place.

CONCLUSION

The development of in situ stimuli activated gel-forming systems for ophthalmic drug delivery provides simplest and best gel-forming systems and have been proved advantageous over other conventional dosage forms. These advantages include sustained and prolonged release of drug (like hydrogel), good stability, biocompatibility, ease of instillation (like solution), etc.. It is an ideal system that maintains effective level of drug for the longer duration following a single application and offers the primary requirement of a successful controlled release product. Use of biodegradable and water soluble polymers for the in situ gel formulations can make them more acceptable and excellent drug delivery systems with minimum chances of irritation, and hence improved patient compliance.

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