ABSTRACT
Eye is the most important organ of body. The conventional ophthalmic dosage forms consist of 90% of currently available ophthalmic formulations. The major drawback associated with conventional ophthalmic formulations is quick precorneal drug loss resulting in poor bioavailability and therapeutic response, because of high tear fluid turnover and dynamics. Various attempts were made to improve ophthalmic drug bioavailability. In-situ-forming gels are solutions, instilled as drops into the eye and undergo a sol to gel transition in the cul-de-sac thus improved ocular bioavailability by increasing the contact time with corneal tissue, thereby reducing the frequency of administration required in case of conventional ophthalmic solutions, thus optimizing ocular therapy. Lot of novel temperature, pH, and ion induced in-situ-forming systems has been prepared for sustained ophthalmic drug delivery. Now a days in situ gel have been used as vehicles for the delivery of drugs for both local treatment and systemic effects. The present article describes the formulation and evaluation of an In-situ ophthalmic drug delivery system based on the concept of pH triggered in-situ gelation, temperature dependent in-situ gelation and ion activated in-situ gelation.

Keywords: conventional ophthalmic formulations, precorneal drug loss, in-situ forming gels, in-situ gelation, temperature dependent in-situ gelation and ion activated in-situ gelation

1. INTRODUCTION
In clinical practice the anterior segment of the eye (cornea, conjunctiva, sclera, anterior uvea) can be treated with topical ocular eye drops which are the most commonly used dosage form in ocular drug treatment. The eye drops are rapidly drained from the ocular surface and,
therefore, the time for drug absorption is only a few minutes and bioavailability is very low, typically less than 5%. Bioavailability and duration of activity may be increased modestly by prolonged action dosage forms, but they have not gained wide acceptance by the patients. Even from the modified formulations the ocular drug absorption is limited by the corneal and conjunctival epithelial barriers of the eye. Drugs are commonly applied to the ocular system for a localized action on the surface or in the interior of the eye. Ophthalmic drug delivery is still one of the most challenging areas facing the scientists today. The biggest challenge is that of circumvention of the protective barriers of the eye without causing permanent tissue damage. Ophthalmic preparations are defined in the USP as sterile dosage forms essentially free from foreign particles, suitably compounded and packaged for instillation into the eye. Ophthalmic dosage forms are similar to parenteral dosage forms in their requirement for as well as consideration for osmotic pressure, tonicity, preservation, tissue compatibility, the avoidance of pyrogen in intraocular dosage forms, particulate matter and suitable packaging. Traditional topical therapeutic dosage forms are restricted to solution, suspension and ointment but with advances in material science, the range of ophthalmic dosage form has expanded significantly to include gels either preformed or spontaneous gels responsive to the ocular environment, and ocular inserts, both forms reducing dosage frequency. Hydrogel used in ophthalmology are generally classified into two distinct groups: preformed gels and *in-situ* activated gel-forming systems. Preformed gels include systems that are administered as viscous preparations on the eye; they are structured before application. *In-situ* gels refers to polymeric system that are topically applied as solutions or suspensions and that shift from a sol to a gel phase as they are exposed to ocular surface physiological conditions like pH (for pseudo latexes), temperature (for poloxamer hydrogel) or the presence of ions (for sodium alginate). [1, 2, 3, 4]

### 1.1. ANATOMY OF THE EYE

![Anatomy of human eye](image)

*Fig1: Anatomy of human eye.*
The human eye is the essential sense organ of the body. Eye is able to refract light and produce a focused image that can stimulate nervous system and enable the ability to see. The Structure of the eye and different Parts of the Eye:

**Aqueous Humour:** It is a jelly-like substance located in the anterior chamber of the eye.

**Choroid:** The choroid layer is located behind the retina and absorbs unused radiation.

**Ciliary Muscle:** The ciliary muscle is a ring-shaped muscle attached to the iris. It is important because contraction and relaxation of the ciliary muscle controls the shape of the lens.

**Cornea:** Cornea is a clear transparent epithelial membrane. Light rays pass through the cornea to reach the retina. The cornea is convex anteriorly and it is involved in refracting (bending) light rays to focus them on the retina.

**Fovea:** The fovea is a small depression (approx. 1.5 mm in diameter) in the retina. This is the part of the retina in which high-resolution vision of fine detail is possible.

**Hyaloid:** The hyaloid diaphragm divides the aqueous humour from the vitreous humour.

**Iris:** The iris is the visible coloured part of the eye and extends anteriorly from the ciliary body, lying behind the cornea and in front of the lens. It divides the anterior segment of the eye into anterior and posterior chambers which contain aqueous fluid secreted by the ciliary body. The iris is supplied by parasympathetic and sympathetic nerves. Parasympathetic stimulation constricts the pupil and sympathetic stimulation dilates it.

**Lens:** The lens of the eye is a flexible unit that consists of layers of tissue enclosed in a tough capsule. It is suspended from the ciliary muscles by the zonules fibers.

**Optic Nerve:** The optic nerve is the second cranial nerve and is responsible for vision. Each nerve contains approximately one million fibres transmitting information from the rod and cone cells of the retina.

**Papilla:** The papilla is also known as the "blind spot" and is located at the position from which the optic nerve leaves the retina. [2]
Pupil: The pupil is the aperture through which light and hence the images we see and "perceive" enters the eye. This is formed by the iris. As the size of the iris increases (or decreases) the size of the pupil decreases (or increases) correspondingly.

Retina: The retina may be described as the "screen" on which an image is formed by light that has passed into the eye via the cornea, aqueous humour, pupil, lens, then the hyaloid and finally the vitreous humour before reaching the retina. The retina contains photosensitive elements (called rods and cones) that convert the light they detect into nerve impulses that are then sent onto the brain along the optic nerve. [5]

Sclera: The sclera is a tough white sheath around the outside of the eye-ball. It consists of a membrane that maintains the shape of the eye and gives the attachment to the extrinsic muscle of the eye.

Vitreous Humour: The vitreous humour (vitreous body) is a jelly-like substance. [6]

Advantages of Ocular Drug Delivery Systems
Various advantages of ocular drug delivery system are given below. [5]
Easy convenience and needle free drug application without the need of trained personnel assistance for the application, self medication, thus improving patient compliances compared to parenteral routes.

Good penetration of hydrophilic, low molecular weight drugs can be obtained through the eye. Rapid absorption and fast onset of action because of large absorption surface area and high vascularisation.

Ocular administration of suitable drug would therefore be effective in emergency therapy as an alternative to other administration routes.
Avoidance of hepatic first pass metabolism and thus potential for dose reduction compared to oral delivery.

Disadvantages
Various disadvantages of ocular drug delivery system are given below.
The physiological restriction is the limited permeability of cornea resulting into low absorption of ophthalmic drugs.
A major portion of the administered dose drains into the lacrimal duct and thus can cause unwanted systemic side effects.

The rapid elimination of the drug through the eye blinking and tear flow results in a short duration of the therapeutic effect resulting in a frequent dosing regimen.

1.2. EYE DISEASES

Various eye diseases are [6, 7]

**Corneal ulcer**

Corneal ulcer is local necrosis of corneal tissue, usually associated with corneal infection (*keratitis*) following trauma or infection spread from the conjunctiva or eyelids. The most common infecting microbes are *staphylococci, streptococci, pneumococci and herpes simplex viruses*. Extensive ulceration and healing by fibrosis cause opacity of the cornea and irreversible loss of sight.

**Trachoma**

Trachoma is a chronic inflammatory condition caused by *chlamydia trachomatis* in which fibrous tissue from in conjunctiva and cornea, leading to eyelid deformity and possibly blindness. It occurs mostly where there is poor hygiene.

**Stye**

Stye is an acute and painful pyogenic infection of sebaceous or meibomian glands of the eyelid margin. A crop of styes may occur due to localized spread to adjacent glands. Infection of meibomian (tarsal) glands may block their ducts, leading to cyst formation which may damage the cornea. The most common infecting organism is *staphylococcus aureus*.

**Blepharitis**

Blepharitis is microbial or allergic inflammation of eyelid margins. The most common causes are *staphylococcal* infection, seborrhea (excessive sebaceous gland secretion) or allergy to dandruff. If ulceration occurs, healing by fibrosis may distort the eyelid margins, preventing complete closures of the eye. This may lead to drying of the eye, conjunctivitis and possibly corneal ulceration.

**Bacterial conjunctivitis**

Conjunctivitis is commonly called "Pink Eye" in North America, and "Madras eye" in India. It is an inflammation of the conjunctiva (the outermost layer of the eye and the inner surface...
of the eyelids), most commonly due to an allergic reaction or an infection (usually bacterial, but sometimes viral). Redness (Hyperemia), irritation (Chemosis) and watering (Epiphora) of the eyes are symptoms common to all forms of conjunctivitis.

Acute allergic conjunctivitis is typically itchy and often involves some lid swelling. Chronic allergy often causes just itch or irritation. Viral conjunctivitis is often associated with an infection of the upper respiratory tract, a common cold, and/or a sore throat. Its symptoms include watery discharge and variable itch. The infection usually begins with one eye, but may spread easily to the other.

**Dry eye syndrome**

Dry eye syndrome is the condition of inadequate wetting of the ocular surface.

**Glaucoma**

Glaucoma is the buildup of pressure in the anterior and posterior chambers of the choroid layer that occurs when the aqueous humor fails to drain properly.

**Endophthalmitis**

It is severe form of intraocular inflammation (purulent uveitis) involving ocular cavities & inner coats of eyeball. Causative organisms include *Streptococci, E.coli, Pseudomonas*, etc.

**1.3. CONVENTIONAL DOSAGE FORM [5, 8, 9]**

**1.3.1. Eye Drops [5, 8]**

Eye drops are saline-containing drops used as a route to administer medication in the eye. Depending on the condition being treated, they may contain steroids, antihistamines, sympathomimetics, beta receptor blockers, parasympathomimetics, parasympatholytics, prostaglandins, non-steroidal anti-inflammatory drugs (NSAIDs) or topical anesthetics. Eye drops sometimes do not have medications in them and are only lubricating and tear-replacing solutions. Eye drops have less of a risk of side effects than do oral medicines, and such risk can be minimized by occluding the lacrimal punctum, (i.e. pressing on the inner corner of the eye) for a short while after instilling drops.

**1.3.2. Viscous solutions [9]**

To prolong precorneal residence time and to improve bioavailability, attempts were made to increase the viscosity of the formulation. The viscosity enhancers used were hydrophilic polymers such as cellulose, polyalcohol and polyacrylic acid. Sodium carboxy methyl
cellulose is one of the most important mucoadhesion polymers having good adhesive strength. Carbomer were used in liquid and semisolid formulations as suspending or viscosity increasing agents. Polycarbophil is water insoluble cross linked polyacrylic acid helps in the retention of the drug delivery system in the eye due to the formation of hydrogel bonds and mucoadhesive strength. Hyaluronic acid offers a biocompatible and biodegradable matrix for fabrication of ocular sustained release dosage forms. Films and microspheres were also prepared from hyaluronic acid. Polysaccharide such as xanthan gum was found to increase the viscosity. Today, hydrophilic polymers continue to be used in formulation of numerous ophthalmic products for bioadhesion rather than viscosity enhancement. Viscosity vehicles increases the contact time and no marked sustaining effect is seen.

1.3.3. Gels [5]

Gels consist of high molecular weight hydrophilic, cross-linked polymers or co-polymers that form a three dimensional network in water. These gels have been shown to combine significantly longer residence time in the cul-de-sac with increased drug bioavailability. So the dosing frequency can be decreased to once a day. Poloxamer 407 is used as viscosity enhancer. Gelrite is a polysaccharide (gellen gum), which forms a clear gel in the presence of mono or divalent cation. The high viscosity of the gel, however, results in blurring of vision and un lubricated eyelids which substantially reduce patient acceptability. Sterilization is another drawback for large-scale production.

1.3.4. Ointment [8]

Ointments are semisolid preparations intended for external application. They are usually formulated using mixtures of semisolid and solid hydrocarbons (paraffins) which have a melting or softening point close to body temperature and are non-irritating to the eye. Ointments may be either simple bases, where the ointments forms one continuous phase, or compound bases where a two-phased system (e.g. an emulsion) is employed. The medical agent is added to the base either as a solution or as a finely micronized powder. Upon instillation in the eye, ointment break up in to small droplets and remain as a depot of drug in the cul-de-sac for extended periods. Ointments are therefore useful in improving drug bioavailability and in sustaining drug release. Although safe and well tolerated by the eye, ointments suffer with relatively poor patient compliance due to blurring of vision and occasional irritation. For this reason they are often used as a means of nighttime medication.
Limitation of conventional dosage form [9]

1. The conventional liquid ophthalmic formulation is eliminated from the precorneal area immediately upon instillation because of lacrimal secretion and nasolacrimal drainage.
2. Only a small fraction of the drug being ocularly absorbed. Only 10% drug Concentrations available at the site of actions.
3. In vivo resident experiments showed the drug resident time and the total resident amount in rabbit’s conjunctivae sac were 2.0 to 5.0 folds less in conventional than in situ gel.
4. Some conventional ophthalmic preparation such as gels, ointment, and viscous preparation were reported to blurred vision.
5. These preparations have no bioadhesive property.

2. IN-SITU FORMING GELS

The new concept of producing a gel in situ (eg. in the cul-de-sac of the eye) was suggested for the first time in the early 1980s. It is widely accepted that increasing the viscosity of a drug formulation in the precorneal region will leads to an increased bioavailability, due to slower drainage from the cornea. 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-gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and ionic cross linking. [9, 10]

Advantages of in-situ forming gels are [9]

1. Increased accurate dosing. To overcome the side effects of pulsed dosing produced by conventional systems.
2. To provide sustained and controlled drug delivery.
3. To increase the ocular bioavailability of drug by increasing the corneal contact time. This can be achieved by effective adherence to corneal surface.
4. To provide targeting within the ocular globe so as to prevent the loss to other ocular tissues.
5. To circumvent the protective barriers like drainage, lacrimation and conjunctival absorption.
6. To provide comfort, better compliance to the patient and to improve therapeutic performance of drug.
7. To provide better housing of delivery system.
2.1. Approaches of in-Situ Gel Drug Delivery [9, 10, 11, 12]

There are different approaches reported for in-situ gels. An in-situ gelling system should be a low viscous, free flowing liquid to allow reproducible administration to the eye as drops, and the gel formed following phase transition should be strong enough to withstand 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 the residence time of the gel formed in-situ along with its ability to release drugs in sustained manner which assist in enhancing the bioavailability, reduce systemic absorption and reduce the need for frequent administration leading to improved patient compliance.

Depending upon the method employed to cause sol to gel phase transition on the ocular surface; various approaches for the preparation of in-situ gel are available these are-

2.1.1. Temperature dependant system

2.1.2. pH dependant system

2.1.3. Ion activated system

2.1.1. Temperature dependant system

The use of a material 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 to 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-PPOPEO) triblock
copolymer that are fluid at low temperature, but forms thermo responsible gel when heated as a consequence 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)-b-poly(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.

2.1.2. pH dependant system

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 pH sensitive polymers are based on PAA (Carbopol, carborer) or its derivatives. Likewise polyvinyl acetal diethyl 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.
2.1.3. Ion activated system
In this the phase transition from gel to sol is triggered by change in ionic strength. The most common polymer used for ion sensitive in-situ gel is gellan gum i.e. gelrite which is linear anionic heteropolysaccharide secreted by microbes Sphingomonas elodea (formerly known as Pseudomonas elodea). The gelrite consist of glucose, glucouronic acid and rhamnose in molar ratio 2:1:1. The gelrite when comes in contact with the cations present in tear fluid changes in to viscous clear gel. This is caused due to cross linking of negatively charged helices by monovalent or divalent cations like Na\(^{+}\), Ca\(^{2+}\), K\(^{+}\). The mechanism of gelrite is like in ion free solution it forms double helices at room temperature and has low viscosity as that of water but when gel forming cations like calcium magnesium are present then they form cation mediated cross linking in the polymer the cations which are divalent like calcium or magnesium are more superior to the monovalent cations like sodium in the promotion of gelation of the polysaccharides.

2.2. EVALUATION OF IN SITU GELLING SYSTEM
In-situ gels are evaluated & characterized by the following parameters-

2.2.1. Appearance
Clarity is one of the most important characteristic features of ophthalmic preparations. Formulations are evaluated for clarity by visual observation against a black and white background. [13, 14]

2.2.2. pH of Gel
pH of the ophthalmic formulation is determined by using pH meter. The pH meter is calibrated before each use with standard pH 4, 7, 9.2 buffer solutions. The pH meter electrode is immersed in formulation and pH is recorded. [15]

2.2.3. Gelation temperature
This test is carried out for the thermo sensitive gels. A 20 ml transparent vial containing a magnetic bar and 10g of thermo sensitive sol is placed in a low temperature cryostatic water bath. A digital thermometer is immersed in the sol. The sol is heated at the rate of 1\(^{\circ}\) C/min with a continuous stirring with the magnetic bar. When the magnetic bar stops moving due to gelation, the temperature is determined as a gelation temperature. [16, 19]
2.2.4. Gelling ability
The gelling ability is determined by placing a drop of the system in a vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 37°C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve.[17, 18]

2.2.5. Drug Content
The drug content is determined by diluting 1 ml of the formulation to 50 ml freshly prepared simulated tear fluid (pH 7.4). The formed gel is completely crushed with the help of a glass rod, followed by vigorous shaking until the formed gel completely dispersed to give a clear solution. The volume is adjusted to 100 ml with simulated tear fluid. The solution is filtered through a membrane filter and drug concentration is then determined by using UV-Vis spectrophotometer at suitable wavelength. [19]

2.2.6. Rheological characterization
The viscosity is measured by using Brookfield viscometer, cone & plate viscometer. In-situ gel formulation is placed in sample tube. Formulation should have viscosity 5-1000 mPas, before gelling & after formation of gel will have viscosity from about 50-50,000 mPas. [13, 19, 20]

2.2.7. Isotonicity evaluation
Isotonicity is an important characteristic of ophthalmic preparation. Isotonicity is maintained to prevent tissue damage or irritation of eye. All ophthalmic preparations are subjected to isotonicity testing. For isotonicity testing formulation is mixed with few drops of blood & observed under microscope at 45 x magnification & compared with standard marketed ophthalmic formulation. [13, 21, 22]

2.2.8. Swelling studies
Swelling studies are conducted with a cell, equipped with thermo jacket to maintain a constant temperature. The cell contains artificial tear fluid (composition – 0.67g NaCl, 0.20g NaHCO₃, 0.008g CaCl₂·2H₂O & distilled water q.s to 100ml). Swelling medium is equilibrating at 37°C one milliliter of formulated solution is placed in dialysis bag & put into the swelling medium. At specific time interval the bag is removed from the medium & weight is recorded. The swelling of the polymer gel as a function of time is determined by using the following relationship. [13]
% St = (Wt – W0) 100/W0

Where,
St = Swelling at time ‘t’.
W0=Initial weight of gelling solution.
Wt=Final weight of gel.

2.2.9. Fourier transformer infra red
The possibility of drug excipient interaction is investigated by FTIR studies. The FTIR graph of pure drug & combination of drug with excipient are recorded by using KBr pellets. [13, 22]

2.2.10. Thermal analysis
Thermo gravimetric analysis can be conducted for in situ forming polymeric system to determine the percentage of water in hydrogel. Differential scanning calorimetry is used to observe, if there are any changes in thermograms as compared with pure ingredients used, thus indicating the interaction. [13]

2.2.11. In vitro Release Studies
In vitro release of drug from in-situ gelling formula is studied using a modified USP dissolution testing apparatus. The dissolution medium used is freshly prepared simulated tear fluid (pH 7.4). Cellulose membrane (Spectra/Por dialysis membrane, 12,000–14,000 MW cut off), previously soaked overnight in the dissolution medium, is tied to one end of specifically designed glass cylinder (open at both ends and of 2.0 cm diameter). An accurately weighed amount of the formulations (1ml) are transferred to the glass tubes. Then the glass cylinders are attached to the metallic driveshaft of the dissolution apparatus and suspended in 100 ml of dissolution medium maintained at temperature of 37±1°C. The shafts are allowed to rotate at a constant speed (50 rpm). At predetermined time intervals, aliquots are withdrawn and replaced by an equal volume of the receptor medium. The drug content in the withdrawn samples is determined by using UV-visible double beam spectrophotometer. [23, 24, 25]

2.2.12. Permeation studies across a sheep’s corneal membrane
To study permeation across sheep’s corneal membrane whole eyeballs of goat are procured from slaughter house and transported to laboratory in cold condition in normal saline maintained at 4°C. The corneas are 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 simulated tear fluid (pH 7.4). This membrane is sandwiched between donor and receptor chamber. Simulated tear fluid is used as a diffusion medium. The formulation to be tested is added to the donor chamber with the help of a micropipette. The donor surface of the membrane is constantly in contact with simulated tear fluid. A temperature of 37 ± 0.5°C is maintained throughout the study. A magnetic stirrer in the cell provided continuous agitation. At regular time intervals, 1 ml of sample is withdrawn and replaced with fresh simulated tear fluid in order to maintain sink conditions. The samples are appropriately diluted and the absorbance is measured at appropriate wavelength using a UV-VIS spectrophotometer. [15, 19, 26]

2.2.13. Ocular irritancy studies
Ocular irritancy studies are performed on male albino rabbits, weighing 1-2 kg. The modified Draize technique is used for ocular irritation potential of ophthalmic products. The formulation is placed in lower cul-de-sac & irritancy is tested at time interval of 1hr, 2hrs, 48hrs, 72hrs, & 1 week after administration. The rabbits are observed periodically for redness, swelling and watering of eyes. [7, 13]

2.2.14. Antimicrobial activity
Antimicrobial efficacy is determined by the agar diffusion test employing ‘Boar well method’. The formulations (test solutions) are poured in to wells bored into sterile nutrient agar previously seeded with test organisms after allowing diffusion of the solutions agar plates are incubated at 37°C for 24 hr. The zone of inhibition (ZOI) is obtained which is measured by an antibiotic zone finder. The entire operation except the incubation is carried out in laminar airflow unit. Each solution is tested in triplicate. Both positive and negative controls are maintained through the study. [16, 27]

2.2.15. Sterility testing
Sterility testing is carried out as per the IP 1996. The formulation is incubated for not less than 14 days at 30-35°C in the fluid thioglycolate medium to find the growth of bacteria & at 20-25°C in Soya bean casein digest medium to find the growth of fungi in formulation. [14, 27 28]

2.2.16. Accelerated stability studies
Formulation is placed in amber colored vials & sealed with aluminum foil for the short term
accelerated stability study at 40± 20°C & 75 ±5% RH as per International Conference of Harmonization (ICH) State Guidelines. Sample is analyzed at every month for clarity, pH, gelling capacity, drug content, rheological evaluation & in vitro dissolution. [29. 30]

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