Review Article

Smart *In situ* Gels for Glaucoma- An Overview

Sandeep DS*, Narayana Charyulu R, Anoop Narayanan V
Department of Pharmaceutics, NGSM Institute of Pharmaceutical Sciences,
Nitte (Deemed to be university) Paneer, Deralakatte, Mangalore, Karnataka, India.
***Corresponding author's E-mail:** sandypharama@gmail.com

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ABSTRACT

Glaucoma is a group of progressive optic neuropathies characterized by degeneration of retinal ganglion cells and resulting changes in optic nerve head. It occurs usually by an increase in intraocular pressure than a normal eye can tolerate. Chronic glaucoma with open-angle is a major problem of public health and it is the second leading cause of blindness in the word. Treatment of glaucoma begins with the use of various drugs like prostaglandins, carbonic anhydrase inhibitors, β- blockers, etc which are available in conventional eye drops. These eye drops require frequent instillation and cause lachrymal drainage, low corneal permeability leading to poor availability. One of the best possible alternatives to overcome demerits of eye drops is ophthalmic in situ gels which are developed in recent years by significant efforts. Ophthalmic in situ gelling solutions were developed by pH-triggered, temperature triggered and ion activated mechanisms using different polymers. These formulations undergo phase transaction from sol to gel upon administration into the eye and make the drug to retain for longer periods. Since these formulations become gelling solutions as they instilled into eyes, they also referred as smart gels. This article reviews the concept of development of ophthalmic in situ gels for treatment of glaucoma.

Keywords: Glaucoma, Eye drops, Ocular drug delivery, Ophthalmic in situ gels.

INTRODUCTION

Eye is the most important and sensitive organ; in fact, it is the window of our soul. The eye is unique organ from the anatomical and physiological point of view. The eye has special attributes that allow local drug delivery and non-invasive clinical assessment of disease but also makes understanding disease pathogenesis and ophthalmic drug delivery challenges. In most cases, ocular therapy requires administration of drugs into the cul-de-sac. Because many parts of the eye are relatively inaccessible to systemically administered drugs, the drugs may require delivery to treat the precorneal region for such infections as conjunctivitis and blepharitis or to provide intra-ocular treatment *via* the cornea for diseases such as glaucoma.[1]

A cross-section of the eye shown in Figure 1. The internal structures of the eye and blood supply are illustrated in the same. The cornea, lens, and vitreous body are all transparent media with no blood vessels; oxygen and nutrients are transported to these nonvascular tissues by the aqueous humor. The aqueous humor has a high oxygen tension and about the same osmotic pressure as blood.[2]

The cornea is covered by a thin epithelial layer, continuous with the conjunctiva at the cornea sclerotic junction; the main bulk of the cornea is formed of criss-crossing layers of collagen and is bounded by elastic laminae on both front and back.[2] A layer of endothelium covers its posterior surface. The cornea is richly supplied with free nerve endings. The transparent cornea is continued posteriorly into the opaque white sclera, which consists of tough fibrous tissue. Both cornea and sclera withstand the intra-ocular tension constantly maintained in the eye.[3]

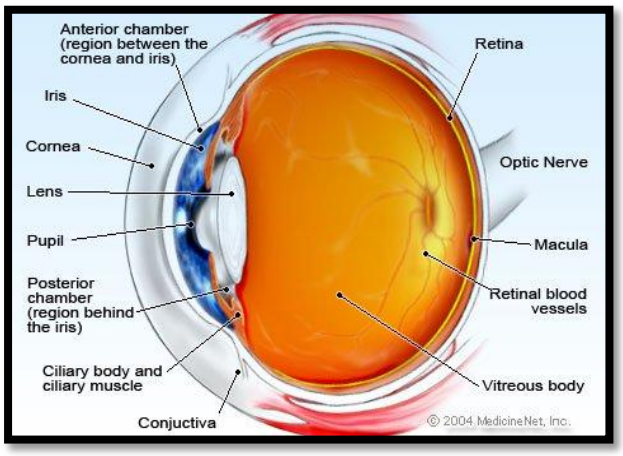

Figure 1: Anatomy of Human Eye

The lachrymal fluid in humans has a normal volume of 7µl and is an isotonic aqueous solution of bicarbonate and sodium chloride (pH 7.4) that serves to dilute irritants or to wash the foreign bodies out of the conjunctival sac.

The aqueous humor in humans has a volume of approximately 300µl that fills the anterior chamber of the eye. Aqueous humor is secreted by the ciliary's processes and flows out of the

anterior chamber at a turnover rate of approximately 1% / min.The drainage system has recently been defined at the sinus venous sclera, of low blood pressure.[4]

Glaucoma is a disease with a characteristic of the higher level of intraocular pressure (IOP) which might progressively hurt visibility. The average IOP of the population is 15.5 ± 2.57 mmHg. If people whose IOP is 20.5 mmHg or higher could be suspected of having glaucoma and IOP over 24 mmHg is a definite case of glaucoma. Although intraocular pressure (IOP) is often elevated, which is considered the greatest risk factor for glaucoma, vision loss and progressive neuropathy can occur without elevations in IOP. Other risk factors for the development of glaucoma include advanced age, family history, and black race.[1,5–7]

Glaucoma has been called the silent thief of sight, because the loss of vision often occurs gradually over a long period of time, once vision lost cannot be normally recovered and so treatment is aimed at preventing further loss. Glaucoma affects one in 200 people aged fifty and younger and one in 10 over the age of eighty. If the condition is detected early, it is possible to arrest development or slow the progression of medical and surgical means. Untreated glaucoma can lead to permanent damage to optic nerve and results in visual field loss, which over time can progress to blindness.[8]

The occurrence of glaucoma and optic nerve damage is shown in Figure 2 and 3.

Treatment of glaucoma initially begins with eye drops followed by surgery. If there is a good candidate for glaucoma eye drops then the patent may be prescribed more than one type to achieve the best IOP control.[9]

A wide range of drugs are available for the treatment of glaucoma in conventional eye drops. A list of drugs with different brands, their composition, manufacturing company and volume in bottle size is shown in below picture table as Figure 4.

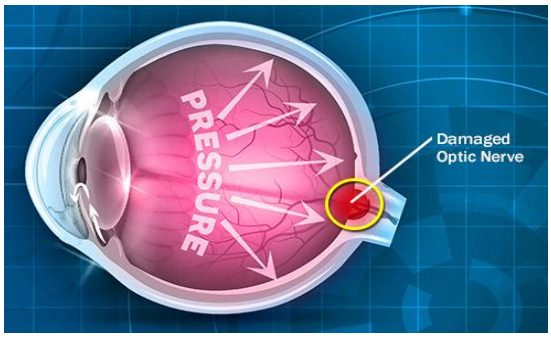

Figure 2: Occurrence of Glaucoma

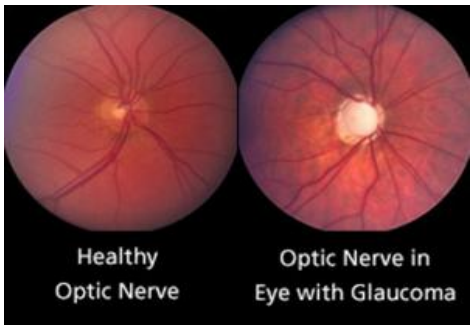

Figure 3: Optic nerve damage

Some of the commercially available eye drops of glaucoma drugs are shown in below Figure 5.

TOPICAL GLAUCOMA DRUGS

BRAND NAME	GENERIC NAME	MANUFACTURER	CONCENTRATION	BOTTLE SIZE
Beta Blockers				
Betagan	levobunolol hydrochloride	Allergan, and generic	0.25% 0.5%	5ml, 10ml 5ml, 10ml, 15ml
Betimol	timolol hemihydrate	Akorn	0.25% 0.5%	5ml 5ml, 10ml, 15ml
Betoptic-S	betaxolol hydrochloride	Alcon	0.25%	5ml, 10ml, 15ml
Istalol	timolol maleate	Bausch + Lomb	0.5%	2.5ml, 5ml
Timoptic	timolol maleate	Valeant Ophthalmics, and generic	0.25% 0.5%	5ml, 10ml, 15ml 5ml, 10ml, 15ml
Timoptic (preservative-free)	timolol maleate	Valeant Ophthalmics	0.25% 0.5%	unit-dose unit-dose
Timoptic-XE	timolol maleate	Valeant Ophthalmics, and generic	0.25% 0.5%	2.5ml, 5ml 2.5ml, 5ml
Prostaglandin Analogs				
Bimatoprost	bimatoprost	generic	0.03%	2.5ml, 5ml, 7.5ml
Lumigan	bimatoprost	Allergan	0.01%	2.5ml, 5ml, 7.5ml
Travatan Z	travoprost	Alcon	0.004%	2.5ml, 5ml
Travoprost	travoprost	generic	0.004%	2.5ml, 5ml
Xalatan	latanoprost	Pfizer, + generic	0.005%	2.5ml
Zioptan	tafluprost	Akorn	0.0015%	unit-dose
Alpha Agonists				
Alphagan P	brimonidine	Allergan	0.1%, 0.15%	5ml, 10ml, 15ml
Brimonidine	brimonidine	generic	0.15%, 0.2%	5ml, 10ml, 15ml
Carbonic Anhydrase Inhibitors				
Azopt	brinzolamide	Alcon	1%	5ml, 10ml, 15ml
Trusopt	dorzolamide	Merck, + generic	2%	5ml, 10ml
Combination Glaucoma Medications				
Combigan	brimonidine/timolol	Allergan	0.2%/0.5%	5ml, 10ml
Cosopt	dorzolamide/timolol	Akorn, + generic	2%/0.5%	5ml, 10ml
Cosopt PF	dorzolamide/timolol	Akorn	2%/0.5%	unit-dose
Simbrinza	brinzolamide/brimonidine	Alcon	1%/0.2%	8ml

Figure 4: Anti glaucoma eye drops of different drugs with brand names

Figure 5: Eye drops of anti glaucoma drugs available in the market

These conventional eye drops have some following disadvantages

- Poor bioavailability, because of rapid precorneal elimination, conjunctival absorption drainage by gravity and normal tear turnover.
- Frequent instillation of medication leads to poor patient compliance.
- Systemic absorption of drug and additives through nasolachrymal duct may result in undesirable effect.
- Amount of drug delivered during application may vary. The drop size of ocular medication is not uniform and dose delivered is generally not correct.[10,11,12]

In order to overcome these demerits, one of the best possible ocular drug delivery system is the development of smart ophthalmic *in situ* gelling solutions which are liquid preparations upon instillation undergoing a phase transition in the ocular cul-de-sac to form a viscoelastic gel and this provides a response to environmental changes.

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 the 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.[13]

In situ gel forming ophthalmic drug delivery systems prepared from polymers that exhibit reversible phase transitions (sol–gel–sol) and pseudoplastic behavior to minimize interference with blinking. Such a system can be formulated as a liquid dosage form suitable to be administered by instillation into the eye which, upon exposure to physiological conditions, changes to the gel phase, thus increasing the pre-corneal residence time of the delivery system.

Advantages of ophthalmic *in situ* gels over conventional dosage forms

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 the drug by increasing the corneal contact time. This can be achieved by effective adherence to the 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 the therapeutic performance of the drug.
7. To provide better housing of delivery system.[14]

Mechanism of *in situ* gels

***In situ* formation based on physical mechanism**

Swelling

In situ formation may also occur when a material absorbs water from surrounding environment and expand to desired space. One such substance is myverol (glycerol mono-oleate), which is polar 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.[15]

Diffusion

This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of the polymer matrix. N-methyl pyrrolidone (NMP) has been shown to be useful solvent for such system.[15]

***In situ* formation based on chemical reactions mechanism**

Chemical reactions that result *in situ* gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes, and photo-initiated processes.[16]

Approaches of ophthalmic *in situ* gels formulation

There are mainly 3 approaches in the development of ophthalmic *in situ* smart gels which are as follows

1. Temperature-triggered systems

2. pH-triggered systems

3. Ion activated systems

Temperature triggered systems

Temperature-sensitive *in situ* gels are probably the most commonly studied class of environment-sensitive polymer systems in drug delivery research 9**.** The use of biomaterial whose transitions from sol-gel is triggered by an increase in temperature; is an attractive way to approach *in situ* gels formation for ophthalmic drug delivery. Three main strategies exist in designing of the thermo-responsive sol-gel polymeric system. For convenience, temperature-sensitive *in situ* gels are classified into negatively thermo-sensitive, positively thermo-sensitive, and thermally reversible gels. Negative temperature-sensitive *in situ* gels 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 are used for this purpose. The formulation is liquid at room temperature (20-25°C) which undergoes gelation in contact with body fluid (35-37°C).[16]

Examples: Pluronic (Poloxamer), Cellulose derivatives, Polymethacrylates.

pH triggered systems

In these, the formation of the 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 *in situ* gel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if the polymer contains weakly basic (cationic) groups.

The most of anionic pH-sensitive polymers are based on PAA (Carbopol, carbomer) or its derivatives. Sol to gel transition when pH rises from 4.2 to 7.4; at higher pH polymer forms hydrogen bonds with mucin which leads to the formation of *in situ* gel. The formulation with pH-triggered *in situ* gel is therapeutically efficacious, stable, non-irritant and provides sustained release of the drug for a longer period of time than conventional eye drops.

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Examples: Cellulose acetate phthalate, Polyacrylic acid (Carbopol), Polycarbophils.

Ion activated systems

Ion activated gelling system is triggered by cations present in eye tear fluid like Na^{+}, Ca^{+2} and Mg^{+2}. Generally, anionic polymers are used in the formation of ion sensitive drug delivery system. Polymers like sodium alginate, gelrite, tamarind gum, gellen gum are used in combination with other polymers like MC and HPMC to increase the effect. They provide sustained release of drug by providing mucoadhesiveness. This system based on the mechanism of ionic interaction of ions of polymer and divalent ions of tear fluid. As soon as anionic polymers come in contact with cationic ions they convert into a gel. The concentration of Na^{+} in human tear is 2.6 g/l is particularly suitable to cause gelation of material when formulation administered topically.[17]

Examples: Gellan gum (Gelrite R), Sodium Alginate.

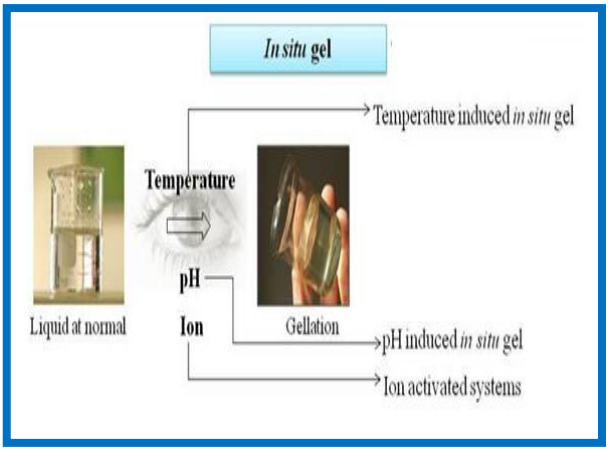

Figure 6: Overview of ophthalmic *In situ* gelling mechanisms

Evaluation parameters for ophthalmic *in situ* gels

Ocular *in situ* gel can be tested for various parameters in order to ensure that prepared formulation satisfies safety guidelines for Ocular drug delivery systems.

Drug-polymer compatibility study and thermal analysis

Drug-polymer compatibility study should be performed with Fourier Transform Infra Red (FTIR) spectroscopy. During gelation process, the nature of the interacting forces can be evaluated using the technique by employing KBr pellet method. Thermo gravimetric Analysis (TGA) can be conducted for *in situ* forming polymeric system to quantify the percentage of water in the hydrogel. Differential Scanning calorimetry (DSC) conducted to observe if there are any changes in thermo grams as compared with pure active ingredients used for gelation.[18]

General appearance and clarity

Visual appearance and clarity of prepared *in situ* formulation is checked for the presence of any particulate matter under fluorescent light against a white and black background.[19]

pH

pH affects both solubility as well as the stability of the drug in ophthalmic formulations. It should be such that the formulation will remain stable at that pH at the same time there would no irritation to the patient upon administration. It is measured by digital pH meter. The pH of ophthalmic *in situ* gelling formulations should be in the range of 7-7.4 which is the desired pH range of eye.[20]

Viscosity and Rheology

This is an important parameter for the *in situ* gels, to be evaluated. Viscosity and rheological properties of *in situ* forming drug delivery systems may be assessed using Brookfield rheometer or some other type of viscometers such as Ostwald's viscometer. The viscosity of these formulations should be such that no difficulties are envisaged during their administration by the patient, especially during parenteral and ocular administration.[21,22]

Gelation Temperature

This parameter is necessary for the ophthalmic *in situ* gels prepared by temperature triggered mechanism using poloxamers as temperature dependent polymers.

The sol-gel phase transition temperature (gelation temperature) is determined for formulations by taking 2 ml of refrigerated sample to a test tube sealed with a parafilm. Then these test tubes were placed in the water bath to heat. The temperature is increased in steps of 10°C/minute. Gel formation is indicated by a lack of movement of the meniscus on tilting the tube. The temperature was allowed to increase with constant rate until the gel again comes in liquid form to measure sol temperature.[23,24]

Gel strength

This parameter can be evaluated using a rheometer. Depending on the mechanism of the gelling of gelling agent used, a specified amount of gel is prepared in a beaker, from the sol form. This gel containing beaker is raised at a certain rate, so pushing a probe slowly through the gel. The changes in the load on the probe can be measured as a function of depth of immersion of the probe below the gel surface.[25]

Gelling Capacity

The gelling capacity was determined by placing one drop of the formulation in a vial containing 2 ml of freshly prepared artificial tear fluid and observing the time required to form gelation of formulation and also time taken for the gel re-dissolve; the composition of artificial tear fluid used is a composition of NaCl- 0.670 g, sodium bicarbonate- 0.200 g, calcium chloride- $2H_2O$ 0.008 g, in 100 ml of purified water. The grading of gelling capacity is based on the time taken to form gel and time taken to dissolve the gel in simulated tear fluid. Formulations with good gelling capacity should show gelation remaining for longer periods once gelation occurs.[26]

Texture analysis

The consistency, firmness, and cohesiveness of *in situ* gel are assessed by using texture profile analyzer which mainly indicated gel strength and easiness in administration *in vivo*. Higher values of adhesiveness of gels are needed to maintain an intimate contact with the mucous surface.

Texture analysis provides information on mechanical properties of samples, namely hardness, compressibility, and adhesiveness. These properties can be directly correlated with sensory parameters *in vivo* and, therefore, are valuable in the development of a product with desirable attributes that contribute to patient acceptability and compliance. A formulation designed for ophthalmic use should be, for example, easily removed from the package, present a good spreadability on the corneal surface and adhere to the mucous layer without disintegrating, in order to prolong retention time.[27]

Isotonicity Evaluation

Isotonicity is an important characteristic of the ophthalmic preparations. Isotonicity has to be maintained to prevent tissue damage or irritation of the eye. All ophthalmic preparations are subjected to isotonicity testing, since they exhibited good release characteristics and gelling capacity and the requisite viscosity. Formulations are mixed with few drops of blood and observed under a microscope at 45X magnification and compared with standard marketed ophthalmic formulation.[28]

***In vitro* drug release study**

In vitro drug release study is done by using Franz diffusion cell. In receptor compartment freshly prepared simulated tear fluid is placed. The dialysis membrane is placed in between receptor and donor compartments. The whole assembly is kept on the thermostatically controlled magnetic stirrer to simulate *in vivo* conditions and temperature of the medium is maintained at 37°C ± 0.5°C. Medium is continuously stirred at 20 rpm. 1ml of the formulation is placed in donor compartment. Sample (0.5ml) is withdrawn at a predetermined time interval and same is replaced by ATF. Samples are analyzed either on UV spectrophotometer or HPLC.[29,30]

***Ex vivo* drug release studies**

Goat corneas are used to study 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 the Franz-diffusion cell in such a way that corneum side continuously remains in an intimate contact with the 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.[31]

Sterility testing

Sterility testing is performed by aseptically transferring 2ml of the formulation into 20ml thioglycolate medium

and soya bean - casein digest medium in two separate test tubes. The inoculated media are incubated at 30 to 35˚C (thioglycolate medium) and 20 to 25˚C (soya bean - casein digest medium) for 14 days and observed for turbidity and microbial growth.[32]

Drug Content

Tests for drug content will be carried out for all the prepared gel formulations. 1 ml of the formulation is taken in 50 ml volumetric flask, dissolved in phosphate buffer pH 7.4 with gentle stirring and final volume was adjusted to obtain concentration 25 µg/ ml respectively. The absorbance was measured at measure wavelength of particular drug using phosphate buffer pH 7.4 as blank using UV spectrophotometer.[33]

Antimicrobial efficacy testing

This test is performed with antibacterial formulations to exhibit antimicrobial resistance against microbes. Antimicrobial efficacy test is determined using agar diffusion method. The sterile nutrient agar media previously seeded with test organisms *(Pseudomonas aeruginosa and Staphylococcus aureus)* is placed in Petri plates and 0.5 ml of sterile standard solution and formulation is placed into the bored cups of the media. The plates are incubated at 37°C for 24 h and the zone of inhibition (ZOI) is measured and compared with the control drug.[34]

Ocular irritation study

As there is a ban on Draize study in many countries ocular irritation study of *in situ* formulations can be performed by one of the following methods.

Histological study

To evaluate the effect of *in situ* formulations on the corneal structure and study the irritation potential, corneas are removed from the eyes of freshly sacrificed goat and incubated at 37°C for 5 hrs in the formulation. Sodium dodecylsulfate (SDS) solution in phosphate buffer saline (PBS) 0.1% (w/w) is used as the positive control. After incubation, corneas are washed with PBS and immediately fixed in formalin (8%, w/w). Tissues are dehydrated in an alcohol gradient, placed in melted paraffin and solidified in block form. Cross sections are cut, stained with haematoxylin and eosin (H&E). Cross sections are observed microscopically for any modifications.

Hen's Egg Test-Chorioallantoic Membrane (HET-CAM)

HET-CAM test is performed by incubating the eggs for 10 days at 37°C and relative humidity of about 70% with automatic turning once per hour. After the incubation period, a portion of each egg shell is removed and a drop of water is placed onto the air sack membrane to avoid capillary damage during its removal. The CAM is then carefully exposed to 0.1 ml or 0.1 gm of test substances, which is washed-off with normal saline solution after 30 sec of exposure. Simultaneously, CAM is exposed to a saline solution (negative control) and 1% SDS solution (positive control). Each CAM is observed microscopically after 5 minutes for hemorrhage, lysis and coagualtion.[35,36]

Accelerated Stability Studies

Formulated gel preparations are kept at different temperature conditions like 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) for 6 weeks. The following parameters of the gel such as color, consistency, drug content and degradation rate constant (K) are studied. To assess the shelf life, the samples are subjected to stability studies. Selected sterilized formulations are stored at 4±1°C (refrigerated temperature), 37±1°C (ambient temperature) and 45±1°C (extreme temperature) for a period of 3 months and analyzed at intervals of 7, 14, 28, 42, 60 and 90 days. The formulations are evaluated at periodic intervals for drug content (by UV Spectrophotometer), clarity, pH, sol-gel transition, rheology, *in vitro* drug release and sterility.[37]

Release mechanisms:

To examine the mechanism of drug release from formulated *in situ* gels, *in vitro* and *ex vivo* permeability data were fitted to zero order, first order, Higuchi release model, and Korsmeyer and Peppa's model and the model with higher correlation coefficient is considered to be the best model to know its release mechanism. [38]

CONCLUSION

Glaucoma is a worldwide leading cause of irreversible vision loss. Even though drugs available for glaucoma in conventional eye drops have certain demerits like precorneal elimination, lachrymal drainage which leads to poor bioavailability, hence there is need to develop a formulation approach to overcome demerits of conventional eye drops. Ophthalmic *in situ* gelling systems offer better drug bioavailability since they undergo a transition from sol to gel upon administration into cul-de-sac thereby they retain for longer periods in the eye to enhance the drug retention time and improve the drug contact time with ocular tissue. However, these systems have proved their effectiveness theoretically and much more efforts yet required producing such systems on a commercial scale. *In situ* gel system is the more idyllic approach for the treatment of glaucoma. Several promising *in vitro* and *in vivo* results have been reported so far with different types of *in situ* gelling systems. In future, more study is required for reconnoitering *in situ* formulations in the treatment of various ocular chronic diseases. This article provides valuable information regarding the formulation development, approaches and its evaluation process details as a ready reference for the research scientists who are involved in ophthalmic *in situ* gelling systems. Recent studies reveal that ophthalmic *in situ* gels have great potentials, being able to use for treatment of glaucoma.

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Corresponding author's Biography: Mr. Sandeep DS

Mr. Sandeep DS is working as Assistant professor at NGSM Institute of Phramceuticlal Sciences in the department of Pharmaceutics, at Nitte (Deemed to be university). Currently he is carrying out his doctoral research in the area of formulation development of ophthalmic *in situ* gels for Glaucoma.