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REVIEW ARTICLE

In Situ Gelling System: Smart Carriers for Ophthalmic Drug Delivery

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ABSTRACT

Eye is unique and vital organ. It is considered as window of the soul. It suffer from various diseases are treated by topical drug delivery in the form of solutions, suspensions and ointment. These conventional dosage forms suffer from the problems of poor ocular bioavailability because of dilution low residence time, blurred vision, undesirable side effects arising due to systemic absorption of the drug through naso-lacrimal drainage. To overcome this disadvantages along with consideration of anatomy physiology and biochemistry of eye researchers in ophthalmic drug delivery systems is directed towards a amalgamation of several drug delivery systems, that include to build up systems which not only prolong the contact time of the vehicle at the ocular surface but also slow down the removal of the drug so *in situ* gel is one of the smart carrier for the sustained and controlled ocular drug delivery. *In situ* forming ophthalmic hydrogels are liquid upon instillation undergoes phase transition in the ocular cul-de-sac to form visco elastic gel and this provides a response to environmental changes like temperature, ionic strength, ultra violet irradiation or pH. Due to these delivery system reduces disadvantages associated with conventional dosage form and thus serves as best alternative to conventional ophthalmic drops. In this article, an attempt has been made to highlight the reason behind poor bioavailability, concept and importance of *in situ* gel along with mechanism of gelation with different approaches as well as evaluation parameters.

KEYWORDS

Poor bioavailability, *In-Situ* Gel, Phase Transition Systems, Evaluation Parameter

INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The anatomy, physiology and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage[1,2]. The conventional ocular drug delivery systems like solutions, suspensions and ointment show drawbacks such as increased precorneal elimination, reduced drug concentration and blurred vision. Low absorption results are shorter duration of action due to this high frequency of eye drop instillation required[3]. It is associated with patient noncompliance. Bioavailability, particularly for ocular solutions ranges from 1%-10% of total administered dose[4,5,6]. Inclusion of excess drug in formulation in an attempt to overcome bioavailability problems is potentially dangerous if drug solution drained from eye is systemically absorbed from nasolacrimal duct[8,9,10,11,12,13].The short pre-corneal contact time combined with corneal impermeability results in low bioavailability its result is frequent dosing is needed[14,15,16,17]. The repeated administration of such drugs can cause ocular burning, discomfort,

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photo toxicity and photophobia, tissue necrosis. To avoid all problems related to conventional dosage form researchers in ophthalmic drug delivery systems is directed towards a amalgamation of several drug delivery systems, that include to build up systems which not only prolong the contact time of the vehicle at the ocular surface, but also slow down the removal of the drug so *in situ* gel is one of the smart carrier for the sustained and controlled ocular drug delivery. Which is liquid during instillation undergoes phase transition in the ocular cul-de-sac to form visco elastic gel therefore increase precorneal residence time of drug so reduced dosing frequency, decreases the systemic side effects and pronounced effect with lower doses of the drug. So increase patient compliance[1,4,18].

Overview of Anatomy and Physiology of Human Eye[19,20]

The human eye is one of the most vital organs in the body which provides vision. After the skin, eye is the most easily accessible site for topical administration of drugs. It contains Sclera which protects the inner layer of eye and also providing integrity to it which help in defining shape and length of the eye.

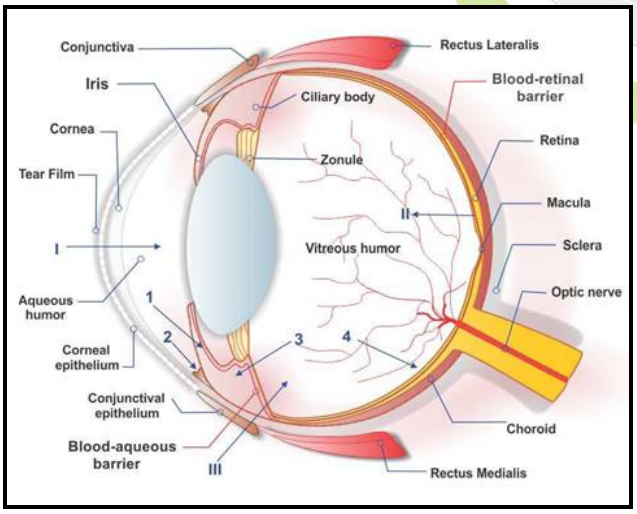

Figure 1: Anatomy of eye[21]

Cornea is a non-vascular structure gets the necessary nutrients from the capillaries. cornea made up of three principle layers Epithelium (hydrophobic in nature and make it barrier to hydrophilic drugs) stroma (main barrier for the lipophilic drugs) Endothelium (It consist of Na + /k +-ATPase pump which depends on the concentration of bicarbonate ion maintains the balance between passive movement of water into the stroma and the active movement of fluid out of it which is responsible for maintaining corneal transparency and thickness). Choroid it contains blood vessels and pigment that absorbs excess light and so prevents blurred vision. Ciliary Body secrets aqueous humor and alters shape of lens for near and far vision. Iris It regulates the amount of light entering the eye by altering the diameter of pupil. Retina is receptor of vision. The function of the retina is not just to be the screen onto which an image may be formed but also to collect the information contained in that image and transmit it to the brain in a suitable form for use by the body. Conjunctiva it protects exposed part of the eye.

Physiology of Eye[22]

Three types of fluids are present in eye Tears (Function of Tear Lubrication, Nourishment, Provide oxygen, protection) Aqueous humour (responsible for the maintenance of shape of the eye ball, It maintains the intraocular pressure 12-20 mm Hg).Vitreous humour (It maintains the shape of eyeball and keeps retina attached to choroid) The cornea, lens and vitreous body are all transparent media with no blood vessels; oxygen and nutrient are transported to this non vascular tissue by aqueous humor. The lachrymal fluid 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. 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. It contains lysozyme, whose bactericidal activity reduces the bacterial count in the conjunctival sac. The rate of blinking varies widely from one person to another, with an average of approximately 20 blinking movements

per min. During each blink movement the eyelids are closed for a short period of about 0.3 sec[14].

Routes of Ocular Drug Delivery

There are several possible routes of drug delivery into the ocular tissues. The selection of the route of administration depends primarily on the target tissue.

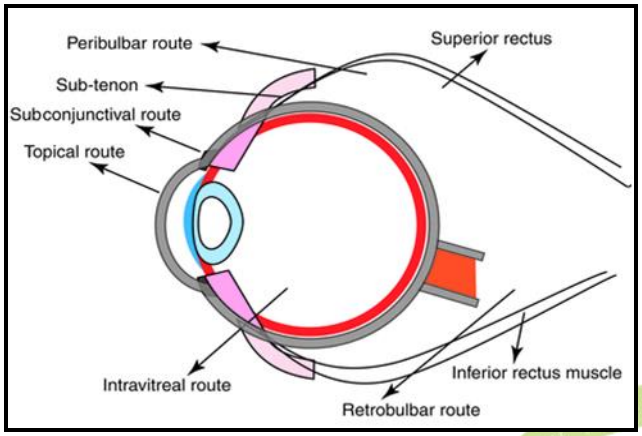

Figure 2: Different Routes of administered for Ocular Drug Delivery[23]

Drug Movement and Sites of Absorption for Ocular Delivery

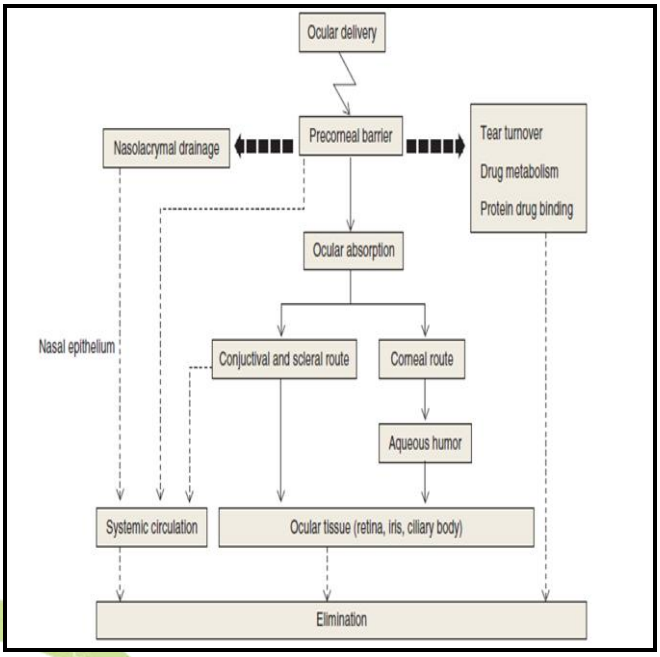

Figure 3: Schematic model indicate the drug movement and barriers in ocular delivery[18]

Table 1: Routes of Administration for Ocular Drug Delivery[24]

Route	Dosage Forms	Advantages	Disadvantages
Topical	Solutions, suspensions, ointments, gels etc.	Ease of administration	Poor bioavailability, suitable only for anterior segment, blurring vision
Sub conjunctival	Injectables	Delivery of large molecular size drugs, sustained release of drug	Patient non compliance, suitable for only water soluble drugs
Retrobulbar	Injectables (used for anesthetization)	-	Perforation of globe, patient non- compliance
Peribulbular	Injectables (used for anesthetization)	Avoidance of perforation of globe	Non-compliance in pediatrics patients and patient with mental disorders.
Intraviteral	Injectables	Sustained delivery of drug to posterior segment of the eye	Patient non compliance

The topical delivery through ocular route has been extensively used for the local treatment of eye pathologies. Poor bioavailability pertinent to conventional eye drops is attributed to physiological constraints such as limited area of absorption, lipophilic temperament of the corneal epithelium and a series of elimination factors such as nasolacrimal drainage, tear turnover and tear evaporation that reduce the contact time of medication with the corneal surface. A schematic model, depicting the transportation and fate of drug molecule with the challenges is shown in fig.3[18].

Table 2: Routes of Absorption of Drugs in Eye[21,24]

Target site	Salient features
Cornea	Bowman's capsule is lipophilic, allows diffusion of small lipophilic molecules. Stroma is hydrophilic, allows diffusion of hydrophilic and larger molecules.
Conjunctiva	Main barrier for drug absorption, allows absorption of hydrophilic and large molecules. Absorption of peptides is less due to enzymatic degradation.
Sclera	Some drugs (β-blockers) diffuse readily. Tran's scleral iontophoresis is used for intravitreal administration.
Aqueous Humor	Drugs absorbed through cornea discharge through aqueous humor into systemic routes.
Vitreous Humor	Drugs absorbed through sclera and conjunctiva discharge through vitreous humor into systemic routes.

The major challenge of ocular delivery is associated with the elimination of instilled dose by a number of elimination factors, which can be resolved effectively by formulation engineering. These desired formulations should be a free-flowing liquid at room temperature to allow easily reproducible administration into the eye as a drop and should undergo *in situ* phase transition to form a strong gel that is capable of withstanding for prolonged period of time at delivery site. This would result in achieving maximum drug bioavailability is the only solution to this problem.

Barriers for Ocular Delivery[1,21]

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 drained via the nasolacrimal duct rapidly in a couple of minutes. Another source of non-productive drug removal is its systemic absorption instead of ocular absorption.

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 Para cellular 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.

Blood-Ocular Barriers

The eye is protected from the xenobiotic in the blood stream by blood-ocular barriers. These barriers have two parts, the anterior blood-aqueous barrier is composed of the endothelial cells in the uvea. 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. Unlike retinal capillaries the vasculature of the choroid has extensive blood flow and leaky walls. Drugs easily gain access to the choroidal extra vascular space, but thereafter distribution into the retina is limited by the RPE and retinal endothelia.

Factor Attributing to Poor Bioavailability of an Ophthalmic Formulation[1,4, 26,27,28,30]

- Binding by the lachrymal proteins
- Drainage of the instilled solutions
- Lachrimation and tear turnover
- Limited corneal area and poor corneal penetration
- Non-productive absorption/adsorption;
- Tear evaporation and permeability;
- Formulation Factor pH, pKa of drug viscosity of formulation

Characteristics Required to Optimize Drug Delivery Systems[4,14,25,26,29]

- Good corneal penetration
- Prolonged contact time with corneal tissue
- Simplicity of installation for the patient
- Non- irritative and comfortable form
- Minimum protein binding
- Sterile, Isotonic , pH adjustment

***In-Situ* Gelling System**

The concept of producing *in situ*-gel in the cul-de-sac of the eye was suggested for the first time in the early 1980s.the disadvantages associated with conventional system are reduces by use of bioadhesive and phase transition systems. Biadhesive are either polymeric solutions or micro-particle suspensions. They are retained in the cul-de-sac by adhesive bonds established with the mucin (mucin having negative charge and polymer eg. Chitosan having positive charge) or the epithelium. Phase transition systems which are instilled in a liquid form and shift to the gel or solid phase once in the cul-de-sac[34]. Among these, the *in situ* gel forming formulations seem to be a promising tool[26,31,32].

"In-situ gelling systems are viscous, mucoadhesive, polymer-based liquids that exhibit sol-to-gel phase transition with its favourable residence time on the ocular surface due to change in a specific physico-chemical parameter like temperature, ionic strength, ultra violet irradiation or pH" [1,26,35,36,37]. These intelligent or smart polymers play important role in drug delivery since they may dictate not only where a drug is delivered, but also when and with which interval it is released.

Advantages of *In-Situ* Ocular Drug Delivery Systems [1,2,4,14,18,25,26,29,30]

- It overcome the side effects of pulsed dosing produced by conventional systems and reduce frequency of dosing
- Less blurred vision as compared to ointment.
- Decreased nasolacrimal drainage of the drug which may causes undesirable side effects
- Due to systemic absorption (i.e. reduced systemic side effects).
- The possibility of administering accurate and reproducible quantities, in contrast to already gelled formulations and moreover promoting precorneal retention
- Sustained, Prolonged drug release and maintaining relatively constant plasma profile.
- Reduced frequency of applications hence improved patient compliance and comfort.
- Generally more comfortable than insoluble or soluble insertion.
- Increased bioavailability due to Increased precorneal residence time and absorption
- Easy scale-up and sterilization
- Ease of system engineering in a combinatory approach (by choosing polymers with multiple function penetration enhancer/ mucoadhesion /*in situ* gel property).

Requirement of Ideal System[1,14,26]

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 culdesac and demonstrated long residence times in the eye 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.

Approaches for *In Situ* Gelling Polymeric Drug Delivery System

Physiological Stimuli Approach

a) Temperature Sensitive[1,4,18,25,26]

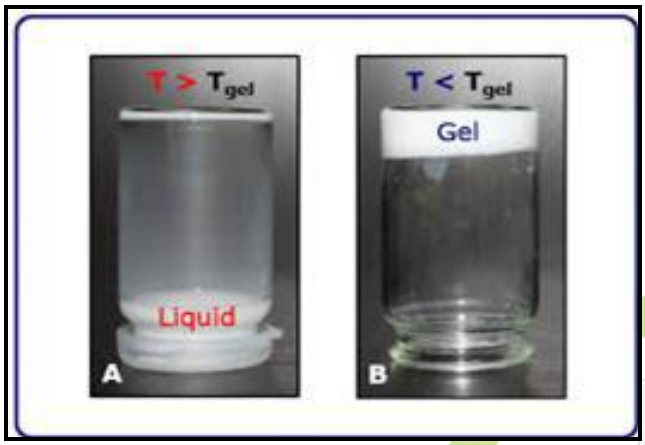

Figure 4: Temperature induced In-Situ gelling system[38]

The use of biomaterial whose transitions from sol-gel is triggered by increase in temperature is an attractive way to approach *in-situ* gel formation. This type of formulation is liquid at room temperature (20°-25°c) which undergoes gelation in contact with eye temperature (35-37°c) Three main strategies exists in engineering of thermo responsive sol-gel polymeric system[42].

Temperature sensitive hydrogels are classified into:

i) Negative Temperature Sensitive Hydrogels

Negative temperature-sensitive hydrogels have a lower critical solution temperature (LCST) and contract upon heating above the LCST. Eg. (Nisopropylacrylamide) (PNIPAAm).

ii) Positive Temperature Sensitive Hydrogels

Positive sensitive hydrogel has an upper critical solution temperature (UCST), such hydrogel contracts upon cooling below the UCST.

Eg. poly (acrylic acid) (PAA) and polyacrylamide .

iii) Thermally Reversible Hydrogels

The most commonly used thermoreversible gels are these prepared from poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (Pluronics®, Tetronics®, poloxamer). It can also prepared with naturally occurring polymers. Most natural polymer aqueous solutions form a gel phase when their temperature is lowered.

Poloxamer

The Poloxamers are well tolerated, non-toxic and nonionic surfactant composed of polyoxyethylene-polyoxypropylene Copolymers. Suitable gel was found at a concentration of 20%.As Gelling agent concentration 15–50%, AS *In situ* gelling agent 13-14%[37].

Mechanism of gelation

The gelation mechanism of Poloxamer solutions has been investigated, Ultrasonic velocity, light-scattering and small angle neutron scattering measurements of aqueous Poloxamer solutions have clearly indicated a micellar mode of association. Poloxamer is in a concentration ranging from 20-30%. At low concentrations they form Monomolecular micelles, but higher concentrations result in multi molecular aggregates consisting of a hydrophobic central core with their hydrophilic poly oxy ethylene chains facing the external medium. Micelle formation occurs at the critical micellization temperature as a result of PPO block dehydration. With increasing temperature, micellization becomes more important, and at a definite point, micelles come into contact and no longer move. In addition, the formation of highly ordered structures, such as cubic crystalline phase, has been proposed as the driving force for gel formation. Thus, packing of micelles and micelle entanglements may be possible mechanisms of Poloxamer solution gelation with increased temperature. Furthermore, it has suggested that intra molecular hydrogen bonds might promote gelation[1].

pH Triggered In Situ Hydrogel[1,4]

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. Gelling of the solution is triggered by a change in pH. At pH 4.4 the formulation is a free-running solution which undergoes coagulation when the pH is raised by the tear fluid to pH 7.4. The pH change of about 2.8 units after instillation of the formulation (pH 4.4) into the tear film leads to an almost instantaneous transformation of the highly fluid latex into a viscous gel[22].

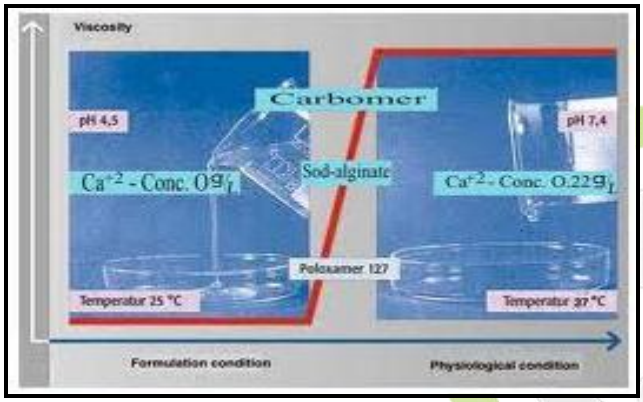

Figure 5: pH induced *In-situ* gelling system[38]

1) Polyacrylicacid (carbopol)

Cross-linked poly (acrylic acid) of high molecular weight, commercially available as Carbopol®, is widely used in ophthalmology to enhance precorneal retention to the eye. As the concentration of Carbopol increases in the vehicle, its acidic nature may cause stimulation to the eye tissues. In order to reduce the total polymer content and improve the gelling properties, an ocular drug delivery system based on a combination of Carbopol and methylcellulose has been developed.

Mechanism of Gelation

Carbopol is a poly acrylic acid (PAA) polymer, which shows a sol to gel transition in aqueous solution as the pH is raised above its pKa of about Methylcellulose, a viscosity enhancing polymer, exhibits a sol to gel transition in aqueous solution in the range of 50–55 °C. The rheological properties of this system were investigated and sol to gel transition occurred primarily by an increase in pH due to the presence of Carbopol. The temperature-mediated effect occurred only at very low shear rates. They have also developed a similar delivery system by a combination of Carbopol and hydroxyl propyl methyl cellulose. For both systems it was found that a reduction in the Carbopol concentration without compromising the *in situ* gelling properties as well as overall rheological behaviors can be achieved by adding a suitable viscosity enhancing polymer[6].

2) Physical Change In Biomaterial Approach[1,4,14,25,26,38]

a) Swelling Mechanism

In-situ formation may also occur when material absorbs water from surrounding environment and expand into 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.

b) Diffusion Mechanism

This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. N-methylpyrrolidone (NMP), dimethyl sulfoxide (DMSO), tertahydrofuran, 2-pyrrolidone and triacetin has been shown to be useful solvents for such system.

3) Chemical Reaction Approach[25,26]

a) Ionic Cross Linking

Gelling of the solution triggered by a change in ionic strength, It is assumed that the rate of gelation depend on the osmotic gradient across the surface of the gel. It is therefore likely that the osmolality of the solution might have an influence on the rate of the sol to gel transition occurring in the eye.

Gellan Gum

Gellan gum (Gelerite) is a linear, anionic hetero polysaccharide secreted by the microbes Sphingomonas elodea Gelerite has been granted regulatory approval as pharmaceutical excipient.

Mechanism of Gelation

Formulations with the Gelerite can be administered to ocular mucosa as low viscosity solution. On contact with cations in tear fluid the Formulation will form a clear gel. This is caused by cross linking of the negatively charged polysaccharide helices by monovalent and divalent cations (Na+, K+, Ca+). In an ion free aqueous medium, Gelrite forms double helices at room temperature. This Solution has a viscosity close to that of water and the helices are only weakly associated with each other (by van der Waals attraction). When gel-promoting cations are present, some of the helices associate into cation-mediated aggregates, which cross-link the polymer. On heating the polysaccharide in an ion free environment, the polysaccharide becomes a disordered coil. However, on heating a sample with cations present, the non aggregated helices melt out first, and the aggregated helices melt out at a higher temperature in a second transition. However the concentration of sodium in tears (2.6 g/L) is quite sufficient to induce the gelation[1,41].

b) Photo-Polymerization

A solution of monomers or reactive macromer and initiator can be injected into a tissues site and 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 photo polymerization in the presence of suitable photo initiator. Photopolymerizable systems when introduced to the desired site via injection get photocured *in situ* with the help of fiber optic cables and then release the drug for prolonged period of time.

c) Enzymatic Cross-Linking

In- situ formation catalyzed 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 hydro gels that can release insulin have been investigated.

Ideal Characteristics of Polymer Used in *In Situ* Gel[26]

- It should be biocompatible,
- It should be capable of adherence to mucus,
- pseudo plastic behavior,
- good tolerance and optical clarity
- It should be capable of decreasing the viscosity with increasing shear rate

Evaluation of Ocular *In Situ* Hydrogel

1. Physical parameters[4,26,39]

A. Clarity

The Clarity of formulated solution is determined by visual inspection under black and white background.

B. pH of Gel

The pH of each of prepared ophthalmic formulation was recorded using previously calibrated digital pH meter. Formulation was taken in a beaker and 0.1M NaOH was added drop wise with continuous stirring. pH was checked using pH meter.

C. Viscosity

Viscosity can be calculated by using Brookfield viscometer, cone and plate viscometer. The In-situ gel formulation was placed in sampler tube. The samples are analyzed 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.

D. Gelling Capacity

Mix in-situ gel with simulated tear fluid (in the proportion of 25:7 application volume 25µl and volume of tear fluid in eye is 7 µl) to find out gelling capacity of ophthalmic product. The gelation may be assessed visually by noting the time for and time taken for dissolution of the formed gel.

2. Texture Analysis[4,26]

The firmness, consistency and cohesiveness of hydrogels are assessed using texture analyzer which mainly indicates the syringeability of sol so the formulation can be easily administered in vivo. Higher values of adhesiveness of gels are needed to maintain an intimate contact with surface like tissues.

3. Drug Content[26]

Is calculated using equation generated from standard calibration curve? The % cumulative drug release is calculated. The 1 ml of formulation was dissolved in 100ml of artificial tear fluid. The whole system was stirred on magnetic stirrer for 4-5 hr. From this solution the sample should be withdrawn and analyzed for UV for Drug content.

5. In vitro Drug Release Studies[1,4,26]

In vitro release study of *Insitu* gel solution is carried out by using Franz diffusion cell. The formulation placed in donor compartment and freshly prepared simulated tear fluid in receptor compartment. Between donor and receptor compartment dialysis membrane is placed (0.22µm pore size). The whole assembly is placed on the thermostatically controlled magnetic stirrer. The temperature of the medium is maintained at 37°C ± 0.5°C. 1ml of sample is withdrawn at predetermined time interval of 1hr for 6 hrs and same volume of fresh medium is replaced. The withdrawn samples are diluted in a volumetric flask with respective solvent to specific volume and analyze by UV spectrophotometer at respective nm using reagent blank. The drug content is calculated using the equation generated from standard calibration curve then the % cumulative drug release (%CDR) is calculated. The data obtained is further subjected to curve fitting for drug release data. The best fit model is checked for Krosmeyers peppas & Fickinian diffusion mechanism for their kinetics.

6. Sol-gel Transition Temperature and Gelling Time[38]

For *in situ* gel forming systems incorporating thermo reversible polymers, the sol-gel transition temperature may be defined as that temperature at which the phase transition of sol meniscus is first noted when kept in a sample tube at a specific temperature and then heated at a specified rate. Gel formation is indicated by a lack of movement of meniscus on tilting the tube. Gelling time is the time for first detection of gelation as defined above

7. Sol-Gel Transition pH

For *in situ* gel forming systems incorporating pH sensitive polymers, the sol-gel transition pH may be defined as that pH at which the phase transition of sol meniscus is first noted when Formulation take in a beaker and 0.1M NaOH was added drop wise with continuous stirring. pH was checked using pH meter. Gel formation is indicated by a lack of movement of meniscus on tilting the beaker. Gelling pH is the pH for first detection of gelation

8. Interaction Study[4,26]

It was performed with Fourier Transform Infra Red (FTIR) spectroscopy. During gelation process the nature of interacting forces can be evaluated using the technique by employing Kbr Press Pellet method. Thermo Gravimetric Analysis (TGA) can be conducted for in- situ forming polymeric system to quantitate the percentage of water in hydrogel. Differential Scanning Calorimetry (DSC) conducted to observe if there are any changes in thermograms.

9. Antimicrobial Activity[4,7,29]

Antimicrobial efficacy studies are carried out to ascertain the biological activity of sol-gel-system against microorganisms. This is determined in agar diffusion medium employing 'Cup Plate Techniques'. The microbial growth of bacteria is measured by conc. of antibiotic compared with that produced by known conc. of standard preparation of antibiotic. Carried out by microbial assay serial dilution method is employed.

10. Gel-strength[7,26,38]

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.

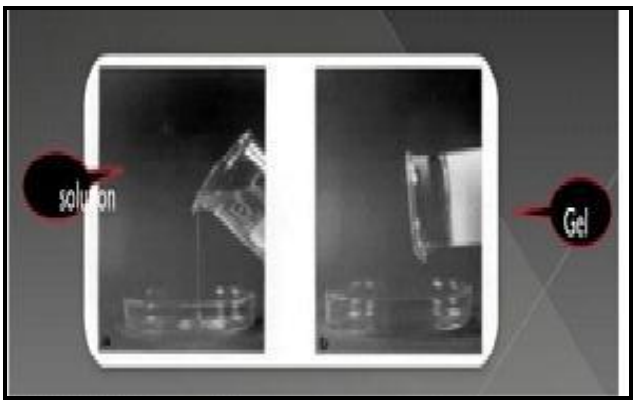

Figure 6: Mechanism of determination of gelling strength[38]

11. Swelling Studies[7,39]

Swelling studies are conducted with a cell, equipped with thermo jacket to maintain a constant temperature. The cell contains artificial tear fluid Swelling medium equilibrating at 370c 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.

$$[\% St = (Wt - W0)\ 100/W0]$$

Where, St = Swelling at time't.W0=Initial weight of gelling solution.

Wt= Final weight of gel.

12. Viscosity and Rheology[29]

This is an important parameter for the *in situ* gels, to be evaluated. The viscosity and rheological properties of the polymeric formulations, either in solution or in gel form were determined with Brookfield rheometer or some other type of viscometers such as Ostwald's viscometer. The viscosity of these formulations should be such that no difficulties should encounter during their administration by the patient, especially during parenteral and ocular administration**.**

13. Draize Irritancy Test[7,26,39]

It is designed for the ocular irritation potential of the ophthalmic product prior to marketing. According to the Draize test, the amount of substance applied to the eyes is normally 25µl placed into the lower cul-de-sac with observation of the various criteria made at a designed required time interval of 1 hr, 24hrs, 45 hrs, 72 hrs and 1 week after administration. Three rabbits (male) weighing 1.5 to 2 kg are used for the study. The sterile formulation is instilled 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). Rabbits are observed periodically for redness, swelling, watering of the eye.

14. Sterility Testing[38,40]

The test for sterility is an important aspect for ophthalmic preparations. The test for sterility is intended for detecting the presence of viable forms of bacteria, fungi and yeast in or on sterilized preparations is carried out according to pharmacopoeial (1996) was followed for the sterility testing of eye drops. Sterility testing was carried out by incubating formulations for not less than 14 days at 30 to 35 °C in the fluid thioglycolate medium to find the growth of bacteria and at 20 to 25°C in the soyabean-casein digest medium to find the growth of fungi in the formulations.

15. Isotonicity Evaluation[4,26]

Isotonicity is important characteristic of the ophthalmic preparations. Isotonicity should be maintained to prevent tissue damage or irritation of eye. All ophthalmic preparations undergo isotonicity testing, Formulations mixed with few drops of blood and observed under microscope at 45X magnification and compared with standard marketed ophthalmic formulation[43].

16. Accelerated Stability Studies[1]

Formulations are placed in ambient colour vials and sealed with aluminium foil for a short term accelerated stability study at 40±2 °C and 75±5%

RH as per International Conference on Harmonization (ICH) states Guidelines. Samples are analyzed every month for Clarity, pH, gelling capacity, drug content, rheological evaluation, and *in vitro* dissolution.

17. Statistical Analysis[4]

The results obtained from the experiments of mucoadhesive strength and release studies were analysed statistically using multivariate tests. A statistically significant difference was conducted by using various SPSS software and difference was considered to be significant at $P<0.05$.

Principle Applications of In-Situ Gelling System[12,26]

a) In situ Forming Drug Delivery System for Oral Administration

The pH –sensitive hydrogel have a potential use in site specific delivery of drugs to specific regions of the GI tract, hydro gels made of varying proportions of PAA derivatives and cross linked PEG allowed preparing silicone microspheres, which released prednisolone in the gastric medium. Guar gum and insulin use for colon- specific drug delivery system. Pectin, xyloglucan and gellan gum are the natural polymers used for *in situ* forming oral drug delivery systems. The potential of an orally administered *in situ* gelling pectin formulation for the sustained delivery of Paracetamol has been reported.

b) In situ Forming Drug Delivery System for Ocular Administration

The In-situ ophthalmic gels provide number of advantages over conventional dosage forms like sustained and prolonged release of drug, good stability, biocompatibility, ease of installation increase patient compliance, bioavailability and therapeutic response. For *in situ* gel based ocular delivery polymers such as poloxamer, carbomer, gellan gum, alginic acid and xyloglucan are most commonly used polymers along with viscosity enhancer such as Hydroxy propyl methyl cellulose, caboxy methyl cellulose, poly vinyl alcohol. Local ophthalmic drug delivery has been used for various compounds such as antimicrobial agents, anti-inflammatory agents and autonomic drugs used to relieve intraocular tension in glaucoma.[16]

c) In Situ Forming Drug Delivery System for Nasal Administration

Gellan gum and Xanthan gum were used as *in situ* gel forming polymers. Animal studies were conducted using an allergic rhinitis model and the effect of *in situ* gel on antigen induced nasal symptoms in sensitized rats was observed. *In situ*-gel was found to inhibit the increase in nasal symptoms are compared to marketed nosonex (Momethasone furoate suspension 0.05%)

d) In Situ Forming Drug Delivery System for Rectal and Vaginal Administration

In situ gels possess a potential application for drug delivery by rectal and vaginal route. Conventional suppositories often cause discomfort during insertion along with it enable to be sufficiently retained at a specific position in the rectum sometime they can migrate up-wards to the colon that makes them possible for drug to undergo the first-pass effect. One of the thermo sensitive gel developed using polymer such as poloxamer and polycarbophil which exhibit increased and prolonged antifungal activity of clotrimazol compared with conventional PEG-based formulation.

e) In Situ Forming Drug Delivery System for Parenteral Administration

Controlled parenteral systems used in drug delivery are implants, microspheres and manufacturing process, high production cost and drug leakage. Injectable *in situ* gel forming drug delivery system represents an attractive alternative to microspheres and implants as parenteral depot systems and has following advantages over conventional parenteral system less invasive technique, Direct delivery to a target area, Biodegradable and biocompatible Economical.

f) *In Situ* Forming Drug Delivery System for Dermal and Transdermal Administration

Thermally reversible gel of pluronics F127 was evaluated as vehicle for the percutaneous administration of indomethasine. Poloxamer 407

Marketed Product of *In-Situ* Gel[25]

Brand Name	Active Ingredient	Mechanism of Gelation	Gelling Agents	Target Indication
Cytron (Rathi.,2000)	Interlukin-2	Thermo responsive	poly (lactide-coglycolide)-poly(ethylene glycol)-poly(lactide-coglycolide	anti-tumer immunity
Virgan (Aman kant et al.,2011)	Ganciclovir	pH induced	Carbopol 974	corneal ulcers
Betoptic S® (Kuno and Shinobu Fuji., 2011)	Betaxolol	Ion induced	Ambelrite®IRP-69	glaucoma
Azasite (Kuno and Shinobu Fuji., 2011)	Azithromycin	pH induced	Polycarbophil	Bacterial conjuctivitis

gel was found suitable for transdermal delivery of insulin. For insulin permeation combination of chemical enhancer and iontophoresis shows synergistic effect.

CONCLUSION

In situ gel administered in the form of solution reduces problem of blured vision, accurate dose and reproducible quantity and due to phase transition system Increased precorneal residence time, decreased nasolacrimal drainage of drug. So improve bioavailability reduced dosing frequency and improved patient compliance this is the primary requirement for successful delivery system. Use of natural, biodegradable and water soluble polymers for the formulations make it more acceptable and excellent drug delivery systems with good stability and biocompatibility. Advantage from industrial point of view easy to manufacture and hence less complex process and reduces manufacturing cost and commercially available formulation.

REFERENCES

1. Gambhire, S., Bhalerao, K. & Singh, S. (2013). *In situ* hydrogel: Different approaches to ocular drug delivery. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(2), 27–36.
2. Deepak, S. & Singh, T. R. (2013). In-situ gel system for ophthalmic preparation. *Innovare Journal of Health Science*, 1(1), 6-15
3. Mansour, M., Mansour, S., Nahed, D. (2008). Ocular Poloxamer-Based Ciprofloxacin Hydrochloride. *Informa Health Care*, 34, 744–752.
4. Rathore K. S. (2010). *Insitu* Gelling Ophthalmic Drug Delivery System: An Overview. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2(4), 30-34.
5. Wu, H., Liu, z., Li, N., Jiaveri, Li. (2011). Design and evaluation of baicalin-containing *in situ* pH-triggered gelling system for

sustained ophthalmic drug delivery. *International Journal of Pharmaceutics*, 410, 31–40.

6. Gupta, H., Jain, S., Mathur, R. (2007). Sustained ocular drug delivery from a temperature and pH triggered novel*in situ* gel system. *Informa Healthcare Drug Delivery*, 14, 507-15.
7. Zarikar, N., Kulkarni, A., Patel, R. (2013). Opthalmic in-situ drug delivery system: a review, *International Journal of Pharmaceutical Research and Development*, 5(05), 48-55
8. Al-kassas, R. S. & El-khatib, M. M. (2009). Ophthalmic controlled release *in situ* gelling systems for ciprofloxacin based on polymeric carriers. *Informa Healthcare*, 16(3).145–152.
9. Chaitanya, B. S., Dharmamoorthy, G., Kotha, S. B., Reddy, A. K., Siva, M. S. (2012). Formulation And Evaluation of Ph-Triggered *In situ* Gelling System of Prulifloxacin. *International Journal of Advanced Pharmaceutics*. 2(1), 28-34.
10. Liu, Y., Liu, J., Zhang, X., Zhang, R., Huang Y., Wu, C. (2010). *In situ* Gelling Gelrite/Alginate Formulations as Vehicles for Ophthalmic Drug Delivery. *AAPS Pharma SciTech*, 2, 810-820
11. Swarbrick, J., Boylan, J. C. *Encyclopedia of Pharmaceutical Technology*.vol II, 1st edition, New York: Marcel Dekker Inc: 1995, 43-70.
12. Patel, N., Shinde, G., Rajesh, K. Ophthalmic insitu gel, pharmagene, 1(4), 29-33.
13. Jain, N. K. (2001). *Advances in controlled and novel drug delivery*. (1999). CBS Publishers and Distributors pvt.
14. Kant, A., Reddy, S., Shankraiah. M. M., Venkatesh, J. S., Nagesh, K. (2011). *In-situ* Gelling System – A Overview *Pharmacologyonline*, 2, 28-44
15. Jain, S. P., Shah, S. P., Rajadhyaksha. N. S. (2008). *In situ* ophthalmic gel of ciprofloxacin hydrochloride for once a day sustained delivery. *Drug Development and Industrial Pharmacy*, 34, 445-52
16. Lokhande, U. R., Gaikwad, D. D. (2012). Design and development of pH triggered *in situ* gelling system of Ciprofloxacin. *International Research Journal of Pharmacy*, 3(5), 418-422.
17. Mali, M. N., Hajare, A. A. (2010). *In Situ* Gel-Forming Systems for Sustained Ocular Drug Delivery, *European Industrial Pharmacy*, 17-20.
18. Agrawal A. K., Das, M. Jain, S. (2012). *In situ* gel systems as "smart" carriers for sustained ocular drug delivery. *Informa Healthcare*, 383–402.
19. Tortora, G. S., Derridkson, B. (Edi 10) (2003). *Principal of Anatomy and Physiology*. (pp579-587) Published by Hoboken John Wiley and Sons.
20. Ross and Wilson. (Edi 12) (2014), *Anatomy and Physiology in Health Education and Illness* (pp 196-205) Published by churchill livingstone elsewire.
21. Patil, A. A novel ophthalmic drug delivery system:*in situ* gel. *International Journal of Pharmaceutical Sciences and Research*, 3(09), 2938–2946.
22. Guyton, A. C., Hall, J. E. (Edi 10) *Textbook of Medical Physiology* (pp578-588) Elsevier publication,
23. Aldrich, D. S. et al., (2013). Ophthalmic Preparations. *Stimuli to the revision process*, 39(5), 1–21.
24. Cohen, S. et al. (1997). A novel *in situ* forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. *Journal of Controlled Release*, 44, 201-208.
25. Prajapati, P. M. (2013) Droppable Gel (*In situ* Gel) A Smart Approach for Ocular Drug Delivery *Recent Advances and Innovations*, (5), 353–361.
26. Patil, R. N. & Kumar, R. S. (2014). *In situ* gelling system : novel approach for

ophthalmic drug delivery. *World Journal of Pharmacy and Pharmaceutical Science, 3*(7), 423–440.

27. Nagesh, C., Patil, M., Chandrasekhar, S., Sutar, R. (2012). A novel *in situ* gel for sustained ophthalmic delivery of Ciprofloxacin hydrochloride and Dexamethasone- design and characterization. *Scholar Research Library*, 4(3), 821-827.
28. Rathbone M. J., Lane, M. E. Modified Release Drug Delivery Technology. *Informa Healthcare*. 2(2), 171-181.
29. Gupta, A., Manocha, N. (2012). Formulation and Evaluation of In-Situ Ophthalmic Drug Delivery System. *International Journal of Pharmaceutical & Biological Archives, 3*(4), 715-718.
30. Jain, N. K. Pharmaceutical Product Development. CBS Publishers and Distributors pvt.ltd.
31. Srividya, B., Cardoza, R. M., Amin, P. D. (2001). Sustained ophthalmic delivery of ofloxacin from a pH triggered *in situ* gelling system. *Journal of Controlled Release, 73*, 205–211.
32. Pandya, T. P., Modasiya, M. K., Patel, V. M. (2011). Opthalmic *In-Situ* Gelling System. *International Journal of Pharmacy & Life Sciences, 2*(5), 55-64
33. Rozier, A., Mazuel, C., Grove, J. (1989). Gelrite: a novel, ion-activated, in-situgelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol. *International Journal of Pharmaceutics*, 57:163-168.
34. Ding, S. (1998). Recent developments in ophthalmic drug delivery. *Research Focus*, 1(8), 328-335.
35. Nisha, S., Deepak, K., & Solan, H. P. (2012). An insight to ophthalmic drug delivery system. *International Journal of Pharmaceutical Studies and Research*, 9-13.
36. Vodithala S, Khatry S, Shastri N, Sadanandam M. (2010). Development and Evaluation ofThermo reversible Ocular Gels of Ketorolac Tromethamine .*International Journal of Biopharmaceutics*; 1(1): 39-45.
37. Eve, R. G., Leroux, W. (2004). *In situ*-forming hydrogels—review of temperature-sensitivesystems. *European Journal of Pharmaceutics and Biopharmaceutics*, (58), 409–426.
38. More, P.K., (2015). Nasal in-situ gel : a novel approach for nasal drug, *World Jounal of Pharmaceutical Research*, 2015; 4(2), 686–708.
39. Nerkar, T., Gujarathi, N. (2013). *In situ* gel : novel approach in sustained and controlled drug delivery system. *Pharma science monitor, 4*(4), 1–18.
40. Aishwarya, J. Jadhav, Sheetal. B. Gondkar, Ravindra, B. Saudagar. (2014). A Review on nasal drug delivery system. *World Journal of Pharmacy and Pharmaceutical Sciences*, 3(8), 231-254.
41. Balasubramaniam, J., Kant, S., Pandit, J. K. (2003). *In vitro* and *in vivo* evaluation of the Gelrite gellan gum-based ocular delivery system for indomethacin. *Acta Pharm.* 53, 251-61
42. Klouda, L., & Mikos, A. G. (2008). Thermoresponsive hydrogels in biomedical applications. *European Journal of Pharmaceutics and Biopharmaceutics*, 68, 34-45.

