Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 2, 2013

Review Article

IN SITU HYDROGEL: DIFFERENT APPROACHES TO OCULAR DRUG DELIVERY

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Received: 07 Feb 2013, Revised and Accepted: 25 Mar 2013

ABSTRACT

Ophthalmic drug delivery is one of the more interesting and challenging endeavor facing the pharmaceutical scientist. The conventional ophthalmic drug delivery systems like solution, suspensions and ointment's show drawbacks such as increased pre-corneal elimination, high variability in efficiency and blurred vision respectively.

To overcome these drawbacks there are considerable efforts directed towards newer drug delivery system for ophthalmic administration. Newer research in ophthalmic drug delivery system is directed towards amalgamation of several drug delivery technologies, that includes to build up systems which is not only extend the contact time of the vehicle at the ocular surface, increase corneal absorption of drug, but at the same time slow down the removal of the drug..

In situ forming ophthalmic hydro-gels 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. The factors considered during the formulation of in situ Hydrogel like temperature modulation, pH change, and presence of ion. The choice of a particular in situ Hydrogel depends on its intrinsic properties and envisaged therapeutic use. In situ forming ophthalmic gelling system provides ease of administration and reduces frequency of administration, improve patient compliance and comfort.

Keywords: In-situ gel, Temperature triggered, Ion triggered, P^H triggered.

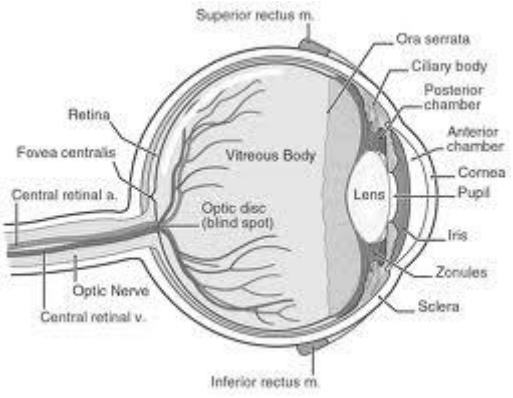
INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging for 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. The development of newer, more sensitive diagnostic techniques and therapeutic agents render urgency to the development of more successful ocular delivery system[1].

There are most commonly available ophthalmic preparations such as drops and ointments about 70% of the eye dosage formulations in market. But these preparations when instilled into eye they are rapidly drained away from the ocular surface due to blinking tear flow and lachrymal nasal Drainage of the eye. Only a small amount of drug is available for its therapeutic effect resulting in frequent dosing application to the eye. So overcome to these problems newer Pharmaceutical ophthalmic formulation such as in-situ gel, nanoparticle, liposome, nanosuspension, microemulsion, intophoresis and ocular inserts have been developed in last three decades increase the bioavailability of the drug as a sustained and controlled manner[2].

Anatomy and function of eye

The eye is a spherical structure with a wall made up of three layers; the outer part sclera, the middle parts choroid layer, Ciliary body and iris and the inner section nervous tissue layer retina[2]. 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 aqueous humor has a high oxygen tension and about the same osmotic pressure as blood**[1].**



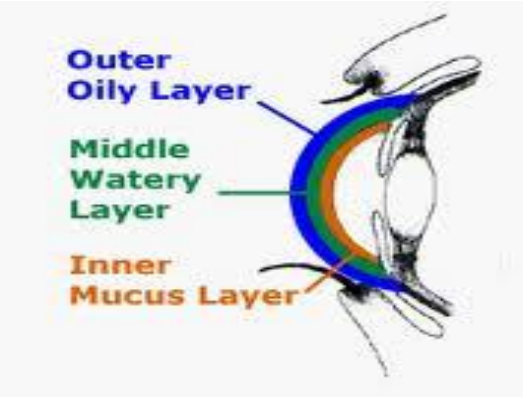


Fig. 1: Anatomy of eye Fig. 2: Anatomy of cornea

In figure 2. Anatomy of cornea, the cornea is covered by a thin epithelial layer continuous with the conjunctiva at the cornea – sclerotic junction, its posterior surface is covered by a layer of Endothelium.The eye is constantly cleansed and lubricated by the lachrymal apparatus which consist of four structure**[3]**

1. Lachrymal glands
2. Lachrymal canals
3. Lachrymal sac
4. Naso lachrymal duct

Lachrymal fluid in human has a normal volume of 7 µl and it is an isotonic aqueous solution of sodium chloride (p^H-7.4).The rate of blinking varies widely from one person to another, but approximately 20 blinking per minute. During each blink movement the eyelid are closed for a short period of about 0.3 sec.The aqueous humor in humans has a volume of approximately 300µl that fills in the anterior chamber of the eye (in fort of the lens).The aqueous humor is secreted by the ciliary process and flows out of the anterior chamber at a turnover rate of approximately 1%/min.In clinical practice the anterior segment of the eye(cornea, conjunctiva, sclera) can be treated with topical ocular eye drops, the most commonly used dosage form in ocular drug treatment. Unfortunately 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%[4].

The barriers

I) Drug loss from the ocular surface

After instillation, the flow of lacrimal fluid removes instilled compounds from the surface of the eye. routes of drug kinetics refer to following processes: 1) transcorneal permeation from the lacrimal fluid into the anterior chamber, 2) non-corneal drug permeation across the conjunctiva and sclera into the anterior uvea, 3) drug distribution from the blood stream via blood-aqueous barrier into the anterior chamber, 4) elimination of drug from the anterior chamber by the aqueous humor turnover to the trabecular meshwork and Sclemm's canal, 5) drug elimination from the aqueous humor into the systemic circulation across the blood-aqueous barrier, 6) drug distributionfrom the blood into the posterior eye across the blood-retina barrier,7) intravitreal drug administration, 8) drug elimination from the vitreous via posterior route across the blood-retina barrier, and 9)drug elimination from the vitreous via anterior route to the posterior chamber. though the lacrimal turnover rate is only about 1 µl/min the excess volume of the instilled fluid is flown to the nasolacrimal duct rapidly in a couple of minutes [33]·Another source of non-productive drug removal is its systemic absorption instead of ocular absorption. Systemic absorption may take place either directly from the conjunctival sac via local blood capillaries or after the solution flow to the nasal cavity [32,33]. Anyway, most of small molecular weight drug dose is absorbed into systemic circulation rapidly in few minutes. This contrasts the low ocular bioavailability of less than 5% [33]·Drug absorption into the systemic circulation decreases the drug concentration in lacrimal fluid extensively. Therefore, constant drug release from solid delivery system to the tear fluid may lead only to ocular bioavailability of about 10%, since most of the drug is cleared by the local systemic absorption anyway [36]·

II) Lacrimal fluid-eye barriers

Corneal epithelium limits drug absorption from the lacrimal fluid into the eye [31]. The corneal barrier is formed upon maturation of the epithelial cells. They migrate from the limbal region towards the center of the cornea and to the apical surface. The most apical corneal epithelial cells form tight junctions that limit the paracellular drug permeation [34]. Therefore, lipophilic drugs have typically at least an order of magnitude higher permeability in the cornea than the hydrophilic drugs [37].Despite the tightness of the corneal epithelial layer, transcorneal permeation is the main route of drug entrance from the lacrimal fluid to the aqueous humor.In general, the conjunctiva is more leaky epithelium than the cornea and its surface area is also nearly 20 times greater than that of the cornea [38,39]· Drug absorption across the bulbar conjunctiva has gained increasing attention recently, since conjunctiva is also fairly permeable tothe hydrophilic and large molecules [40]. Therefore, it may serve as a route of absorption for larger bio-organic compounds such as proteins and peptides. Clinically used drugs are generally small and fairly lipophilic. Thus, the corneal route is currently dominating. In both membranes, cornea and conjunctiva, principles of passive diffusion have been extensively investigated, but the role of active transporters is only sparsely studied.

III) Blood-ocular barriers

The eye is protected from the xenobiotics in the blood stream by blood-ocular barriers. These barriers have two parts: blood-aqueous barrier and blood-retina barrier the anterior blood-eye barrier is composed of the endothelial cells in the uvea. This barrier prevents the access of plasma albumin into the aqueous humor, and limits also the access of hydrophilic drugs from plasma into the aqueous humor. Inflammation may disrupt the integrity of this barrier causing the unlimited drug distribution to the anterior chamber. In fact, the permeability of this barrier is poorly characterised. The posterior barrier between blood stream and eyeis comprised of retinal pigment epithelium (RPE) andthe tight walls of retinal capillaries [31,34]. Unlike retinal capillaries the vasculature of the choroid has extensive blood flow and leaky walls. Drugs easily gain access to the choroidal extravascular space, but thereafter distribution into the retina is limited by the RPE and retinal endothelia. Despite its high blood flow the choroidal blood flow constitutes only a minor fraction of the entire blood flow in the body. Therefore, without specific targeting systems only a minute fraction of the intravenous or oral drug dose gains access to the retina and choroid. Unlike blood brain barrier, the blood-eye barriers have not been characterised in terms of drug transporter and metabolic enzyme expression.

Classification Of Ocular Drug Delivery System:

Ocular drug deliveries consist of following types of dosage forms:

1). Semisolid -Gel, Ointment

2). Solid -Ocular Inserts.

3). Liquid – Solution, Suspension

4). Intraocular –Implant, Injections

Types of Ocular Gel:

I. Organogel
II. Hydrogel

Table 2: General classification of gel [5]

Class	Description	Examples	Advantage	Limitation
Organo gel	Hydrocarbon type Animal/vegetable fat, Hydrophilic	Aluminium Stearate, Carbowax®.	1) Template vehicle. 2) Chemical Stability. 3) Process Benefits.	1)Greasy property. 2) If impurity present then no gelling.
Hydrogel	Organic Hydrogel Natural /synthetic gum	Sodium CMC,PF127®, Veegum®.	1) Biodegradable. 2)Environmental sensitive. 3) Biocompatible.	1)Expensive 2)Difficulty in sterilization.

Advantages of Ocular In Situ Hydrogel:

I. Reduced dose concentration.
II. Reduced dosing frequency.
III. Improved patient acceptability.
IV. Generally more comfortable than insoluble or soluble insertion.
V. Increased bioavailability due to-
   a. Increased precorneal residence time.
   b. Decreased nasolacrimal drainage of the drug.

VI. Chances of undesirable side effects arising due to systemic absorption of the drug through naso-lacrimal duct are reduced[6].
VII. Easy to manufacture and hence less complex process and reduces cost of Manufacturing[7].

Limitation of Ocular in Situ Hydrogel:

I. Blurred vision.
II. Matted eyelids.
III. Limited values in terms of improvement of bioavailability[8].

OCULAR IN SITU HYDROGEL

A more desirable dosage form is one that can deliver drug in a solution form, create little to no problem of vision and need be dosed no more frequently than once or twice daily. In situ activated gel forming systems are those which are when exposed to physiological conditions will shift to a gel phase. This new concept of producing a gel in situ was suggested for the first time in the early 1980s. Gelation occurs via the cross-linking of polymer chains that can be achieved by covalent bond formation (chemical cross-linking) or non-covalent bond formation (physical cross-linking)[9][10].

The progress that has been made in gel technology is in the development of a droppable gel. Insitu gel-forming systems can be described as low viscosity solutions that undergo phase transition in the conjunctival cul-de-sac to form viscoelastic gels due to conformational changes of polymers in response to the physiological environment. The rate of *in situ* gel formation is important because between instillation in the eye and before a strong gel is formed; the solution or weak gel is produced by the fluid mechanism of the eye **[10]**.

Gel forming ophthalmic solution have been developed and approved by FDA for Timolol Maleate this is used to reduce elevated intra ocular pressure (IOP) in the management of Glaucoma. Timolol maleate ophthalmic solution initially developed, require a twice a day dosage for most patient. With in situ gel IOP lowering capacity extended from 12 to 24 hrs and thus requires only once a dosing. This extended duration of efficacy was demonstrated for both gels forming product in controlled clinical trial. The first gel forming solution, TIMOLOL®XE uses the polysaccharide gellan gum and reported in situ in response to the higher ionic strength of tear fluid (U.S Patent4, 861,460), alternative ion sensitive system have been patented, The second product (Timolol maleate) uses the polysaccharide xanthum gum as the gelling agent and is reported to gel in contact with tear fluid, At least in part due to presence of tear protein Lysozyme (U.S. patent 6, 174,524)**[11]**.

Mechanism Of In Situ Gel Formation

Sol to gel phase transition ocular in Hydrogel due to:

I. Physical stimuli: It includes change in temperature, electric field, light.
II. Chemical stimuli: It includes change in pH and ion activation from biological fluid.
III. Biochemical stimuli: It includes change in glucose level.

Physical, Chemical and Toxicological properties of Hydrogel:

I) Factors affecting swelling of Hydrogel

I) Cross linking Ratio

The cross linking ratio is one of the most important factor that affect the swelling of Hydrogel. It is defined as the ratio of moles of cross linking agent to the moles of polymer repeating units. The higher the crosslinking ratio, the more cross linking agent is incorporated in the Hydrogel structure. Highly cross linked Hydrogel have a tighter structure, and will swell less compared to the same hydrogels with lower cross linking ratios. Cross linking hinders the mobility of the polymer chain, hence lowering the swelling ratio**[12]**.

II) Swelling Ratio

The chemical structure of the polymer may also affect the swelling ratio of the hydrogels. Hydrogels containing hydrophilic groups swell to a higher degree compared to those containing hydrophobic groups. Hydrophobic groups collapse in the presence of water, thus minimizing their exposure to the water molecule. As a result, the hydrogels will swell much less compared to hydrogels containing hydrophilic groups. Swelling of environmentally-sensitive Hydrogel can be affected by specific stimuli. Swelling of temperature-sensitive Hydrogel can be affected by changes in the temperature of the swelling media. There are many other specific stimuli that can affect the swelling of other environmentally-responsive Hydrogel**[12].**

II) Dynamics of swelling

The swelling kinetics of hydrogels can be classified as diffusion-controlled (Fickian) and relaxation-controlled (non-Fickian) swelling. When water diffusion into the Hydrogel occurs much faster than the relaxation of the polymer chains, the swelling kinetics is diffusion-controlled**[12].**

III) Mechanical properties

Mechanical properties of hydrogels are very important for pharmaceutical applications. For example, the integrity of the drug delivery device during the life time of the application is very important to obtain FDA approval, unless the device is designed as a biodegradable system. A drug delivery system designed to protect a sensitive therapeutic agent, such as protein, must maintain its integrity to be able to protect the protein until it is released out of the system. Changing the degree of cross linking has been utilized to achieve the desired mechanical property of the Hydrogel. Increasing the degree of cross linking of the system will result in a stronger gel. However, a higher degree of cross linking creates a more brittle structure. Hence, there is an optimum degree of cross linking to achieve a relatively strong and yet elastic Hydrogel. Copolymerization has also been utilized to achieve the desired mechanical properties of hydrogels. Incorporating a co-monomer that will contribute to H-bonding can increase the strength of the Hydrogel.[12]

IV) Cytotoxicity and in vivo toxicity

Cell culture methods, also known as cytotoxicity tests, can be used to evaluate the toxicity of hydrogels. Three common assays to evaluate the toxicity of hydrogels include extract dilution, direct contact and agar diffusion. Most of the problems with toxicity associated with hydrogel carriers are the untreated monomers, oligomers and initiators that leach out during application. Therefore, an understanding the toxicity of the various monomers used as the building blocks of the Hydrogel is very important**[12]**.

VARIOUS APPROACHES OF INSITU GELATION

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid pre corneal elimination of the drug may be overcome by the use of in situ gel-forming systems that are instilled as drops into the eye and undergo a sol–gel transition in the cul-de-sac.

Various approaches are used

I. Temperature triggered in situ Hydrogel
II. pH triggered in situ Hydrogel
III. Ion activated in situ Hydrogel

I) TEMPERATURE TRIGGERED IN SITU HYDROGEL

Temperature-sensitive hydrogels are probably the most commonly studied class of environment-sensitive polymer systems in drug delivery research. The use of biomaterial whose transitions from sol-gel is triggered by increase in temperature is an attractive way to approach in-situ formation The ideal critical temperature range for such system is ambient and physiologic temperature, such that clinical manipulation is facilitate and no external source of heat other than that of body is required for trigger gelation **[7].**

Three main strategies are exists in engineering of thermo responsive sol-gel polymeric system. Temperature-Sensitive Hydrogel Are Classified Into:[7]

i. Negatively Thermo Sensitive Hydrogel
ii. Positively Thermo Sensitive Hydrogel
iii. Thermally Reversible Hydrogel

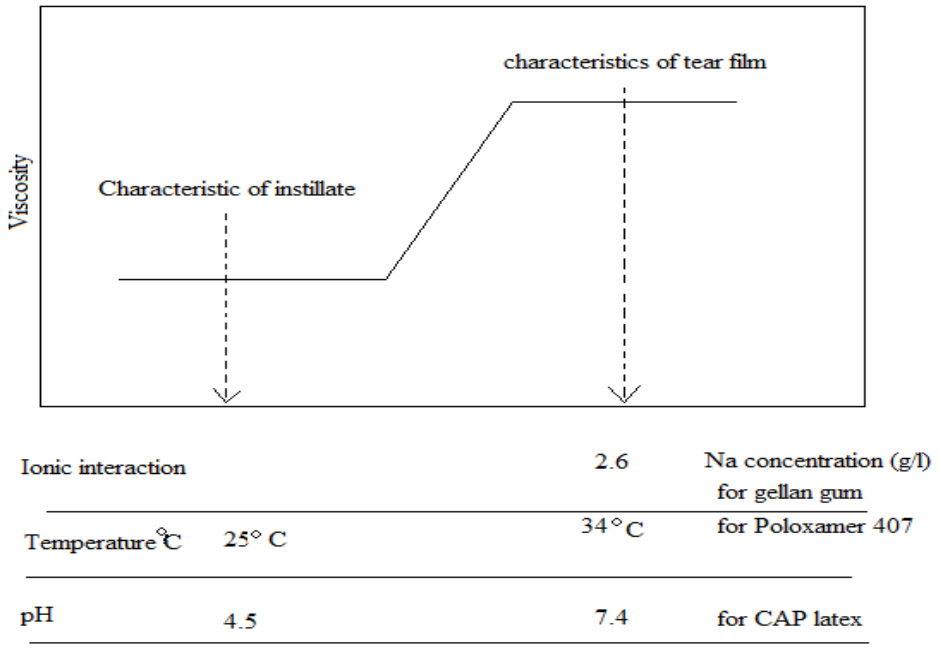


Fig. 3: Schematic representation of the viscosity change on the ocular surface when using ophthalmic in situ gelling systems[13].

i) Negative temperature-sensitive hydrogels

Negative temperature-sensitive hydrogel 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 (Nisopropylacrylamide) (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 co-polymer that are fluid at low temperature, but forms thermo responsible gel when heated as a consequences of a disorder-order transition in micelle packing which makes these polymers suitable for *in situ* gelation.

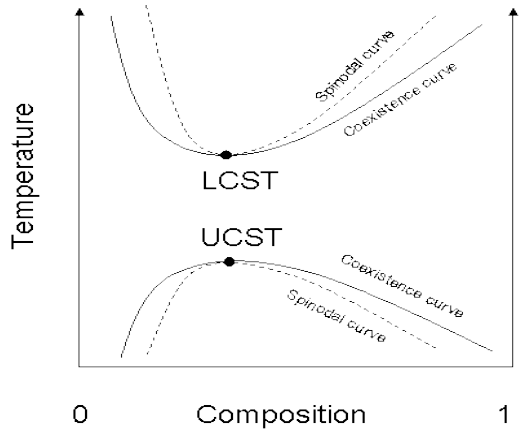


Fig. 4: A plot of typical thermo sensitive polymer solution behavior[41].

ii) Positive temperature sensitive hydrogel

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 (acryl amide-co-butyl methacrylate) have positive temperature dependence of swelling**[14]**.The most commonly used thermo reversible 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. Polymer solution is a free flowing liquid at ambient temperature and gels at body Temperature

iii) Thermally reversible hydrogels

Thermo reversible gels can be prepared with naturally occurring polymers. Most natural polymer aqueous solutions form a gel phase when their temperature is lowered. Classic examples of natural polymers exhibiting a sol–gel transition include gelatin and carrageenan, Chitosan. At elevated temperatures; these polymers adopt a random coil conformation in solution. Upon cooling, a continuous network is formed by partial helix formation**[12]**.

Thermo reversible gels, there remains an important drawback associated with their use; the risk of gelation before administration by increase in ambient temperature during packing or storage**[7].**

Polymer use in temperature triggered in situ hydrogel

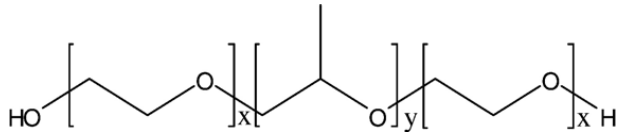
I. Poloxamer (e.g. pluronic F127®,poloxomer-407)

II. Cellulose derivative: Methyl cellulose(MC), Hydroxy propyl methyl cellulose(HPMC),

a. Ethyl (hydroxyethyl) cellulose

III. Xyloglucan

I) Poloxamer



Structure of PEO-PPO-PEO (Poloxamer).[16]

Poloxamer is a nonionic surfactant composed of polyoxyethylene-polyoxypropylene Copolymers in a concentration ranging from 20-30%. At low concentrations (10-4–10-5 %) 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**[15]**. Micellization occurs in dilute solutions of block copolymers in selected solvents above the Critical micellar

concentration, at a given temperature. Due to the PEO/PPO ration of 2:1, when these molecules are immersed into the aqueous solvents, they form micellar structures above critical micellar concentration **[7]**.

Mechanism of gelation

The gelation mechanism of Poloxamer (Fig.5. Schematic illustration of micellar phases formed by the Pluronics® with increasing temperature.[16]) solutions has been investigated extensively, but is still being debated. Ultrasonic velocity, light-scattering and small angle neutron scattering measurements of aqueous Poloxamer solutions have clearly indicated a micellar mode of association. 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, but this hypothesis has been questioned recently. 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. The Poloxamers are reported to be well tolerated and non-toxic even though large amounts (25-30%) of polymers are required to obtained a suitable gel[16].

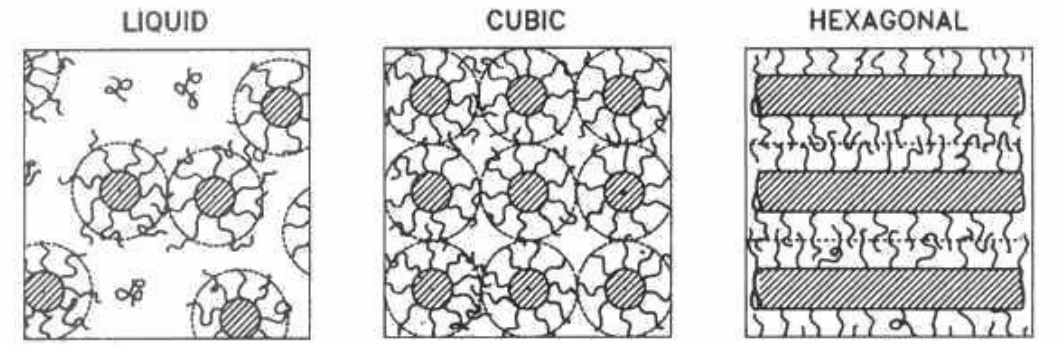


Fig. 5: Schematic illustration of micellar phases formed by the Pluronics® with increasing temperature.[16]

(Pluronic® F127) was found to gel at a concentration of 20 wt. % at 25 °C, which is less than that of the other members of the Poloxamer series. At room temperature (25 °C), the solution behaves as a mobile viscous liquid, which is transformed into a semisolid transparent gel at body temperature (37 °C)[14].

Potential drawbacks

Potential drawback of Poloxamer gels include their weak mechanical strength, rapid erosion (i.e. dissolution from the surface), and the non biodegradability of PEO-PPO-PEO, which prevents the use of high molecular weight polymers that cannot be eliminated by renal excretion. To circumvent the biodegradability issue, new polymers were synthesized by linking together a few (usually 3) Poloxamer 407 'monomers' via degradable carbonate linkage.As the carbonate linkages were hydrolyzed under physiological conditions, the hydrogel degraded into soluble Poloxamer 407 units and carbonate. Variation of the polymer concentration enabled modification of the gels dissolution time (25–80 days).

Various poloxamer grades are available some of them which used in ophthalmic drug delivery system as follows: Table-1 poloxamer grades

Table 1: Poloxamer grades

Nonproprietary name	Commercial grade	HLB value	pH of 2.5% w/v aqueous solution
Poloxamer 184	L-64	12–18	5–7.5
Poloxamer 185	P-65	12–18	6–7.4
Poloxamer 407	F-127	18–23	6–7.4

As Gelling agent concentration 15–50%; AS In situ gelling agent-13-14%

ii) Cellulose derivatives

Cellulose derivatives also cause gelation E.g.: Methylcellulose and Hydroxyl propyl methylcellulose(HPMC) are typical examples of such polymers. At low concentrations (1–10 wt. %), their aqueous solutions are liquid at low temperature, but gel upon heating.

Methylcellulose solutions transform into opaque gels between 40 and 50 °C, whereas HPMC shows phase transition between 75 and 90 °C. These phase transition temperatures can be lowered by chemical or physical modifications. For example, NaCl decreases the transition temperature of methylcellulose solutions to 32–34 °C.

Similarly, by reducing the hydroxyl propyl molar substitution of HPMC, its transition temperature can be lowered to 40 °C. Gelation of methylcellulose or HPMC solutions is primarily caused by the hydrophobic interaction between molecules containing methoxy substitution. At low temperatures, the macromolecules are hydrated, and there is little polymer– polymer interaction other than simple entanglement. As the temperature is raised, the polymers gradually lose their water of hydration, which is reflected by a decline in relative viscosity.

Eventually, when sufficient but not complete dehydration of the polymer occurs, polymer–polymer associations take place, and the system approaches an infinite network structure, as reflected experimentally by a sharp rise in relative viscosity. This sol–gel transformation has been exploited to design in situ gelling systems. These systems exhibited low viscosity at 23 °C and formed soft gels at 37 °**[6]**

iii) Xyloglucan

When xyloglucan is partially degraded by β-galactosidase, the resultant product exhibits thermally reversible gelation in dilute aqueous solutions. Such behavior does not occur with native xyloglucan. Gelation is only possible when the galactose removal ratio exceeds 35 %[17].

The transition temperature is inversely related to polymer concentration[18] and the galactose removal ratio[17]. For example, the sol–gel transition of xyloglucan was shown to decrease from 40 to 5 °C when the galactose removal ratio increased from 35 to 58%. Xyloglucan formulations were assessed for ocular delivery of pilocarpine; using Poloxamer 407 as a positive thermosensitive control.

The 1.5 wt. % xyloglucan formulation enhanced the miotic response to a degree similar to that of a 25 wt. % Poloxamer 407 gel [19]. Xyloglucan is approved for use as a food additive. However, its relatively low transition temperature (22–27 °C) makes handling at room temperature problematic.

Table 2: Commercial grades of cellulose and relates substance

Cellulose-related substance	Grade	Viscosity (mPa s)	pH
Hydoxypropyl Methyl cellulose	Methocel A4MP	4000	5.5–8.0 for a 1% w/v aqueous solution
	A15-LV	15	
	A15CP	1500	
	A4CP	400	
	K-Series(generally retardant for release as viscosity increases)		
Ethyl cellulose	Ethocel, Aqualon		
	Ethocel Std 4 Premium	3.0–5.5	
	N-7	5.6–8.0	

As gelling agent 1-3%

P^H TRIGGERED IN SITU HYDROGEL

All the p^H-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental p^H.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. All the p^H-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental p^H.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**[23]**.Gelling of the solution is triggered by a change in p^H. At pH 4.4 the formulation is a free-running solution which undergoes coagulation when the p^H is raised by the tear fluid to p^H 7.4. The p^H change of about 2.8 units after instillation of the formulation (p^H 4.4) into the tear film leads to an almost instantaneous transformation of the highly fluid latex into a viscous gel**[22]**.

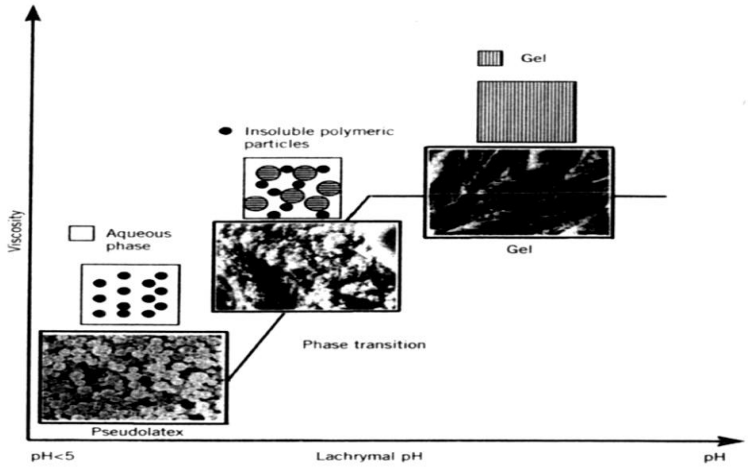


Fig. 6: Schematic representation of p^H dependent in situ gels[20].

Polymer use in P^H triggered in situ gel

I. Polyacrylicacid(carbopol940)
II. Pseudo latex e.g. (CAP Latex) cellulose acetate phthalate latex, carbomer.

I) Polyacrylicacid(carbopol940)

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.Carbopol® 934 is a synthetic polymer composed of 62% of carboxyl groups with a high molecular weight (approximately3×106) formed by repeating units of acrylic acid, cross-linked with either ally sucrose or allylethers of pentaerythritol.Carbopol offers the advantage of exhibiting excellent mucoadhesive properties when compared with other polymers. (E.g. Cellulose derivatives and Polyvinyl Alcohol). 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 p^{Ka} 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 p^H 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].

II) Pseudo Latex

PSEUDO LATEX coagulates within a few seconds when placed in the cul-de-sac since the lacrymal fluid has a $p^H$of 7.2. The p^H of the un-gelled formulation is 4.4 and is therefore sufficient to keep the dispersion in a stable form. The P^H change of 2.8 units after instillation due to the surrounding tear fluid leads to an almost instantaneous transformation of the highly fluid latex into a viscous gel. The partially gelified polymeric dispersion shows the surface of the latex particles starting to dissolve. They cannot be washed out of the cul-de-sac by the lachrymal fluid and they form a micro reservoir in situ with a high viscosity[4].

Eg.Cap Latex

First preliminary investigations of p^Hsensitive nano-particulate systems (latex) for ophthalmic administration began in the early

1980s. The choice of this polymer was determined by the compatibility of the polymer with the active compound, the ability of the CAP latex to be a free-running solution at p^H 4.2 and a gel at 7.2, and finally, the latex stability at relatively low pH which is a prerequisite to ensuring the stability of pilocarpine. The gelation capacity of CAP latexes has been visualized in vitro by scanning electron microscopy and in vivo in rabbits by incorporating methylene blue in ophthalmic formulations. The efficacy of a preparation based on pseudo latex has been evaluated by measuring pharmacological responses and precorneal residence time by γ scintillography. This technique has clearly demonstrated the superiority of CAP latex over a solution to prolong the corneal residence time of pilocarpine. Finally, it is important to note that irritation tests on Rabbits including examination of the cornea, the iris and the conjunctiva have demonstrated that the investigated pseudo latexes did not induce irritation. However, a sensation of discomfort seems to be unavoidable after the coagulation of the solution in the cul-de-sac as is the case for any semisolid Preparation[6].

ION ACTIVATED IN SITU HYDROGEL

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. The aqueous polymer solution forms a clear gel in the presence of the mono or divalent cations typically found in the tear fluids. The electrolyte of the tear fluid and especially Na, Ca and Mg cations are particularly suited to initiate gelation of the polymer when instilled as a liquid solution in the conjunctival cul-de-sac [42].

Polmers Use In Ion Activated In Situ Gelation:

I. Gellerite gellan gum,

II. Alginates.

I) Gellan Gum

Gellan gum (Gelerite) is a linear, anionic hetero polysaccharide secreted by the microbe Sphingomonas elodea (formerly known as Pseudomonas elodea). The polysaccharide can be produced by aerobic fermentation and then isolated from the fermentation broth by alcohol precipitation. The polymer backbone consists of glucose, glucuronic acid, and rhamnose in the molar ratio 2:1:1.These are linked together to give a tetra-saccharide repeat unit The native polysaccharide is partially esterified with L-glycerate and acetate, but the commercial product **Gelerite** has been completely de-esterified by alkali treatment.

Gelerite® (deacetylated gellan gum) is one of the most interesting in situ gelling polymers that has been tested since it seems to perform very well in humans. Gelerite has been granted regulatory approval as pharmaceutical excipient and is marketed by Merck in a controlled-release glaucoma formulation called Blocarden® Depot (Timoptic®). [6]

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.

The divalent ions such as magnesium or calcium were superior to monovalent cations in promoting the gelation However the concentration of sodium in tears (2.6 g/L) is quite sufficient to induce the gelation.Corneal contact time of formulations based on gellan gum has been investigated using two main methods, which are fluorometry and γ-scintigraphy**[6]**

II) Alginate

Alginate with a high guluronic acid content will improve the gelling properties and reduce the total polymer to be introduced into the eye. The alginate forms 3-dimensional ionotropic hydrogel matrices, generally by the preferential interaction of calcium ions with the G moieties resulting in the formation of in homogeneous gel. The characteristic properties of these hydrogels, such as mechanical strength and porosity, are dependent upon the G: M ratios, type of ionic cross linker (bio or poly-valent cations), concentration and viscosity of the initial alginate solution. Calcium-cross linked alginate gels show good mechanical properties even when prepare from relatively low solution concentrations of the polymer, 0.5%w/v, and they can physically entrap a whole array of molecules, and sustain their release. Alginates of a pharmaceutical grade and which comply with all the quality requirements in the European and US Pharmacopoeias can be obtained from several manufacturers. Moreover, alginates were approved by the regulatory authorities such as the Food and Drug Administration, for human use as wound dressing material and as food additives **[6]**.

Sodium alginate, the sodium salt of Alginic acid, is a natural hydrophilic polysaccharide containing two types of monomers, b-D-mannuronic acid (M) and a-L-guluronic acid (G).The polymer forms three-dimensional hydrogel matrices and the high G content alginate forms a low viscosity, free-flowing liquid at concentrations suitable for gel formation in the lachrymal fluid. Sodium Alginate was chosen as a vehicle for ophthalmic formulations since it exhibits several favorable biological properties such as biodegradability and non-toxicity. A prolonged precorneal residence of formulations containing Alginic acid was looked for, not only based on its ability to gel in the eye but also because of its mucoadhesive properties**[30].**

Formulation approach for the drug with polymer

Table 3: Formulation approach

	Drug	Polymer	System
1.	Ofloxacin[21]	Polyacrylic Acid(Carbopol 940)With HPMC (Methocel E5m0lv)	P^H Triggered In Situ Gelling System
2.	Aceclofenac[22]	Carbopol940 With HPMC	P^H Triggered In Situ Gelling System
3.	Moxifloxacin[23]	Poloxomer®F127, Poloxomer®F68, Carbopol 934NF, MC E461.	P^H Triggered In Situ Gelling System
4.	Leofloxacin[24]	Carbopol 940, HPMC.	P^H Triggered In Situ Gelling System
5.	Ketorolac	Gelerite®	Ion Activated In Situ Gelling System
6.	Tromomethamine[25]		

7.	Diclofenac[26]	Sodium Alginate, HPMC,HPC,HEC, Carbopol.	P^H Triggered In Situ Gelling System
8.	Fluconazole[27]	Chitosan(Deacetylated), Poloxomer407.	Temperature Triggered In Situ Gelling System
9.	Baicalin[28]	Carbopol 974p(0.3%W/V) HPMC E4M(0.6%W/V)	P^H Triggered In Situ Gelling System
10.	Cartelol[29]	Alginic Acid, HEC.	Ion Activated In Situ Gelling System
11.	Indomethacin[29]	Alginate Gel.	Ion Activated In Situ Gelling System, Long Acting.
12.	Ofloxacin[29]	Sodium Alginate.	Ion Activated In Situ Gelling System,
13.	Ciprofloxacin[30]	Chitosan, Poloxamer.	Temperature Triggered In Situ Gelling System

PATENT AVAILABLE

Patent available on in situ ocular hydrogel:

Table 4: Patent available

S. No.	Patent no.	Petent name	Comment
1.	US2011/0003816 A1	Ophthalmic composition Halder *et al*.	Ophthalmic composition of beta blocker and polymeric vehicle.
2.	US2011/0028477 A1	Stable ophthalmic formulation Aleo *et al*.	Stable formulation suitable for treatment of glaucoma and ocular hypertension
3.	US2011/0082221 A1	In situ gelling system as a sustained delivery for front of eye. Haug *et al*.	Ophthalmic formulation used as vehicle for sustained delivery using alginate and excipient.
4.	US2010/0069482 A1	Gel useful for delivery of ophthalmic drug. Longo *et al*.	Gel formulation containing a buffer and polymer for treating the diseases of eye.
5.	US2010/0267664 A1	Ophthalmic composition containing, polysaccharide-borate gelling system. Asgharian *et al*.	Composition useful as artificial tears and lubricant and also useful for topical delivery in eye.
6.	US2010/0234336A1	Ophthalmic compositions Xia *et al*.	An aqueous ophthalmic composition containing carboxy containing poly anionic polymer to maintain viscosity.
7.	US2010/0216700A1	Methods of treating ocular disorders. Li *et al*.	Treatment of ocular disorders using emulsions and molecular dispersions in form of gel for hydrophobic drug.
8.	US2008/0132444 A1	Ocular agent delivery system Li *et al*.	Novel ocular drug delivery agent containing emulsion emulsions and gel using hydrophobic agent.
9.	US2006/0094643 A1	Composition of hyaluronic acid and method of use Svirkin *et al*.	Composition use for the treatment of dryness of eye.
10.	US2005/0129771 A1	Ophthalmic composition containing a polysaccharide /borate gelling system Asgharian *et al*.	Composition form the gel /partial gel upon instillation in to eye.
11.	US2004/0137069 A1	Piperazine ophthalmic gel Takruri *et al*.	Aq.gel for ophthalmic formulation as a vehicle for treatment of myopia.
12.	US67030339 B2/2004	reversible gelling system for ocular drug delivery, Xia *et al*.	Ophthalmic aq.gel containing propylene oxide and ethylene oxide and hpmc.
13.	US2003/0077302	Diclofenamide suspension gel,claus herz *et al*.	It is an ophthalmic gel for treating glaucoma.
14.	US6511660B1/2003	Ophthalmic drug delivery formulation and method for preparing same Lin *et al*.	Novel ophthalmic drug delivery formations containing mixture of carbopol and pluronic.
15.	US6583124B2/2003	Ophthalmic composition containing galactomannarn polymers and borate. Asgharian *et al*.	This composition containing gelling amount of galactomannarn polymers and borate.
16.	US5492937/1996	Gel forming liquid carrier composition Bogentoft *et al.*	A carrier composition is liquid at or below room temperature form a high viscosity layer or gel at body temperature
17.	US005371108A/1994	Dry eye treatment process and solution Korb et al.	Applying gel of oil and wax over the eye, then disperse in aqueous phase.
18.	US32969/1989	Injectable viscoelastic ophthalmic gel Trager *et al.*	For ophthalmic surgical and treatment procedure.
19.	US4738851/1988	Controlled release ophthalmic gel formulation Schoenwald *et al*.	Controlled release gel contain sodium carboxy methyl cellulose and colloidal magnesium aluminum silicate

EVALUATION OF OCULAR IN SITU HYDROGEL

The insitu gel formulations is evaluated for clarity, pH measurement, gelling capacity, drug content, rheological study, invitro diffusion study, isotonicity, *in vivo* ocular testing in rabbits and accelerated stability studies. The pH of In-situ gel solution should be 7.4 for all the formulations. The formulation should have an optimum viscosity that will allow for easy instillation into the eye as a liquid (drops), which would undergo a rapid sol-to-gel transition (triggered by pH, temperature or ion exchange).[43]

1. Physical parameters:

The In-situ gel solution is tested for clarity, pH, gelling capacity, and drug content estimation[43].

2. Gelling capacity

The gelling capacity is determined by placing a drop of the formulation in a vial containing 2.0 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling is noted.[43].

3. Rheological studies

The viscosity measurements can be calculated using Brookfield viscometer, Cone and Plate Viscometer. [43]

4. In vitro drug release studies

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. [43].

5. Texture analysis

The consistency, firmness and cohesiveness of *insitu* 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 mucus Surface[43].

6. Isotonicity evaluation

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].

7. Ocular irritancy test

The Draize irritancy test 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 eye is normally 100µl placed into the lower cul de sac with observation of the various criteria made at a designed required time interval of1hr, 24hrs, 48 hrs, 72hrs, and 1week after administration. Three rabbits (male) weighing 1.5 to 2kg 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.[43].

8. Accelerated stability studies

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. The storage conditions and the lengths of studies chosen should be sufficient to cover storage, shipment, and subsequent use. [43].

Table 5: Stability condition

General case Study	Storage condition	Minimum time period covered by data at submission
Long term*	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH	12 months
Intermediate**	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

*It is up to the applicant to decide whether long term stability studies are performed at 25 ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH.

**If 30°C ± 2°C/65% RH ± 5% RH is the long-term condition, there is no intermediate condition.

Drug substances intended for storage in a refrigerator Study:

Table 6: Stability condition for drug substance intended for storage in a refrigerator

Drug substances intended for storage in a refrigerator Study	Storage condition	Minimum time period covered by data at submission
Long term	5°C ± 3°C	12 months
Accelerated	25°C ± 2°C/60% RH ± 5% RH	6 months

It is considered unnecessary to continue to test a drug substance through 6 months when a significant change has occurred within the first 3 months.[44]

9. Sterility studies

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 method[43].

10. Sol-Gel transition temperature and gelling time

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[7].

CONCLUSION

Ophthalmic drug delivery system is burgeoning field in which most of the researchers are taking challenges to combat various problems related to this delivery. Steady advancement in the understanding of principles and processes governing ocular drug absorption and disposition and continuing technological advances have surely brought some improvements in the efficacy of ophthalmic delivery systems. The primary requirement of a successful controlled release product focuses on increasing patient compliance which the *insitu* gels offer. Exploitation of polymeric *insitu* gels for controlled release of various drugs provides a number of advantages over conventional Dosage forms. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the in situ gel dosage forms very reliable. Use of biodegradable and water soluble Polymers for the insitu gel formulations can make them more acceptable and excellent drug delivery systems

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