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Ocular *in situ* gel: An overview

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ABSTRACT

Eye is the most sensitive organ of the body. Designing of ocular drug delivery system is the most challenging field for pharmaceutical scientists as less than 5% of administered drug enters the eye due to the complicated anatomical structure of the eye, small absorptive surface and low transparency of the cornea, lipophilicity of corneal epithelium, pre corneal loss (due to nasolacrimal drainage), bonding of the drug with proteins contained in tear fluid, blinking, low capacity of conjunctival sac, that restricts the entry of drug molecule at the site of action and ultimately leads to poor ocular therapy. To improve ophthalmic drug bioavailability, there are considerable efforts directed towards newer drug delivery systems for ophthalmic administration. These novel drug delivery systems offer manifold advantages over conventional systems as they increase the efficiency of drug delivery by improving the release profile and also reduce drug toxicity. A lot of research going on in this area proves the fact that *in situ* gelling systems can be beneficial in the ocular drug delivery. *In situ* gel forming systems are drug delivery systems that are in solution form before administration in the body but once administered, undergo in situ gelation, to form a gel triggered by external stimulus such as temperature, pH etc. This review is to Specify a brief summary about *in situ* gels, various approaches for in situ gelling systems, different types of polymers used in *in situ* gels, their mechanisms of gel formation and evaluation of polymeric *in situ* gel.

Keywords: *in situ* gel, polymers, Temperature induced *in situ* gel system, pH induced *in situ* gel system, Ion activated systems.

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1. INTRODUCTION

The ocular drug delivery system is considered as crucial and challenging as human eye is an isolated organ where the delivery of drug is quite difficult. Moreover, the conventional ophthalmic formulations exhibit a short pre-corneal residence time and poor bioavailability due to rapid and extensive elimination of drugs from pre-corneal lachrymal fluid by solution drainage, lachrymation, and non-productive absorption by conjunctiva [1]. In order to surpass the drawbacks associated with the conventional ophthalmic formulations, various attempts have been made towards the development of stable sustained release *in situ* gels. Newer research in ophthalmic drug delivery systems is directed towards incorporation of several drug delivery technologies, that includes to build up systems which not only extend the contact time of the vehicle at the ocular surface, but which at the same time slow down the elimination of the drug. In situ gel system is formulated as liquid preparation suitable to be instilled into eyes which upon exposure to the physiologic environment changes to gel, thus increasing the precorneal residence time of the delivery system, and enhances the ocular bioavailability of the drug [2] .The formation of gels depends on factors like change in a specific physico-chemical parameter (pH, temperature, ion-sensitive) by which the drug gets released in a sustained and controlled manner. There are various novel dosage forms like *in situ* gel, nanosuspension, nanoparticulate system, liposomes, niosomes, dendrimers, ocular iontophoresis, collagen shield, minidisc, ocular film, implants, ocuserts etc [3]. Development of ocular drug delivery systems has always been challenging because of the drawbacks with ocular route like non-productive absorption, impermeability of drugs to cornea, drainage, induced lachrymation and tear turn over. Topical application of drugs to the eye is the well established route of administration for the treatment of various ocular diseases like dryness, conjunctivitis, keratitis, eye flu etc. New approaches have been investigated for delivery of drugs to the eye by making use of polymers that pays a key role in delivery of drugs to the pre and intra ocular tissues [4]. Such persistent attempts have resulted into achieving the increase in bioavailability and extending the duration of therapeutic action of ocular drug. Smart polymeric systems have proved to be promising means of delivering the drugs. These polymers undergo sol-gel transition after administered. They are in solution phase before administration, but gels under physiological condition. The ocular bioavailability of the drugs can be improved by prolonging their residence time in the cul-de-sac and by increasing their corneal permeability [5].

This review demonstrates a brief summary about *in situ* gels, various approaches for in situ gelling systems, also different types of polymers, their mechanisms of gel formation and evaluation of polymeric *in situ* gel.

2. CLASSIFICATION OF OPHTHALMIC DRUG DELIVERY SYSTEMS

I. Conventional delivery systems

- Eye drops
- Ointments and Gels
- Ocuserts and Lacrisert

II. Drug delivery to anterior segment

- Contact lens
- Cal du sac inserts
- Subconjuctival/ Episcleral implants

III. Drug delivery to posterior segment

- Intravitreal implants (e.g, Duraser Technology system, Novadu Technology, I- vatio TA, NT-501)
- Injectable Particulate Systems (RETAAC, Cortiject, Visudyne**)**

IV. Physical devices

- Iontophoresis
- Micro- electromechanical intra ocular drug delivery devices

V. Vesicular system

- Liposomes
- Niosomes
- Discomes
- Pharmacosomes

VI. Controlled delivery systems

- *In situ gel* systems/ Phase transition systems
- Iontophoresis
- Dendrimer
- Contact lens
- Collagen shield
- Microemulsion
- Nanosuspensions
- Microneedle

VII. Particulates

- Nanoparticles
- Microparticles

VIII. Advanced delivery systems

- Cell encapsulation
- Gene therapy
- Stem cell therapy
- Protein and Peptide therapy
- Scleral plug therapy
- siRNA therapy
- Oligonucleotide therapy
- Aptamer

3. FATE OF FORMULATION ADMINISTERED THROUGH EYE [6]

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

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

4. IN SITU GELLING SYSTEM

In situ gel forming systems are drug delivery systems that are in solution form before administration in the body but once administered, undergo gelation in situ, to form a gel triggered by external stimulus such as temperature, pH etc and release the drug in sustained or controlled manner. This novel concept of producing in situ gel 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). In situ 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 [8]. Both natural as well as synthetic polymers can be used for the fabrication of *in situ* gels [9].

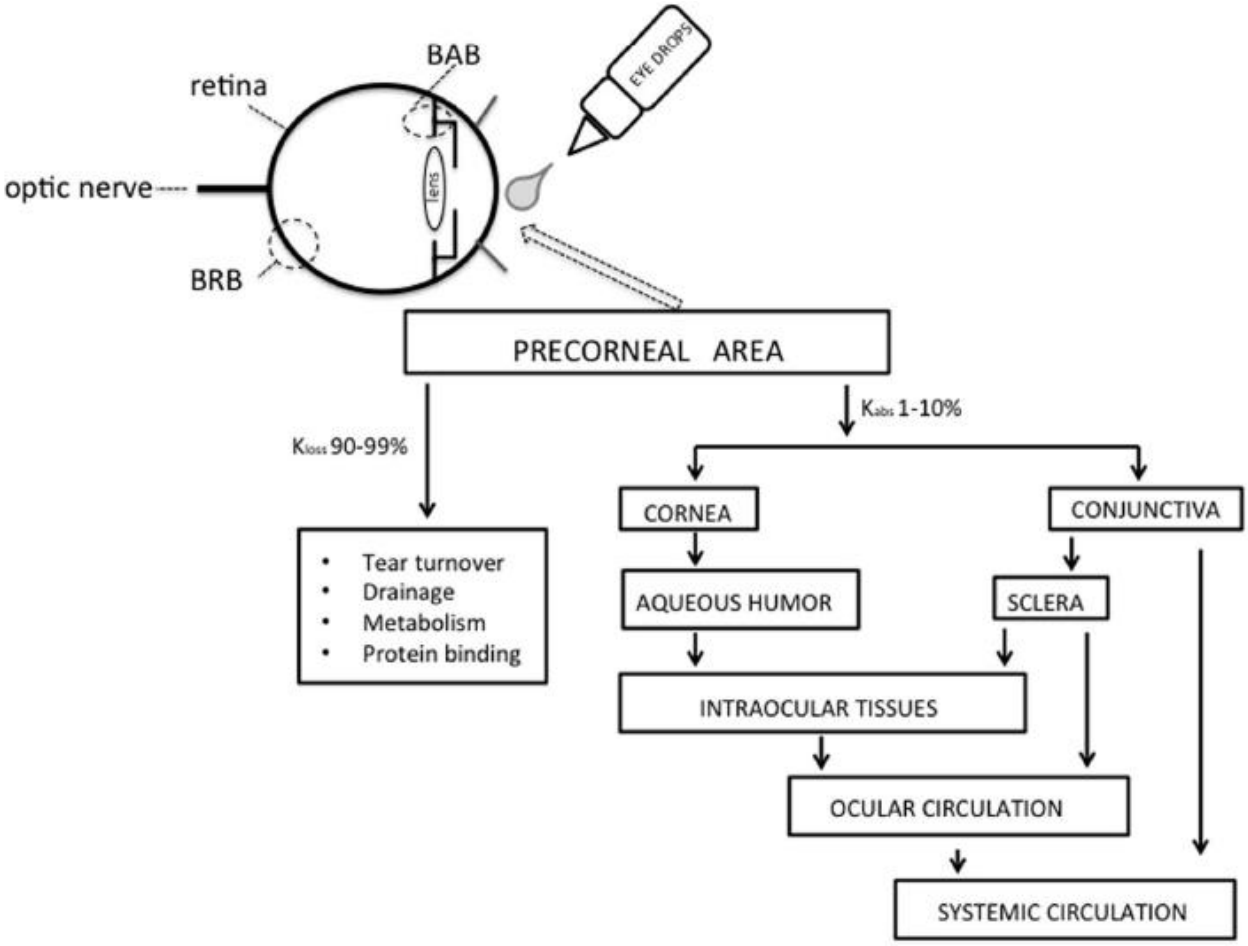

Figure 1: Model depicting precorneal and ocular drug movement from topical instilled dose[7]. (BAB: blood-aqueousbarrier, BRB: blood-retinalbarrier)

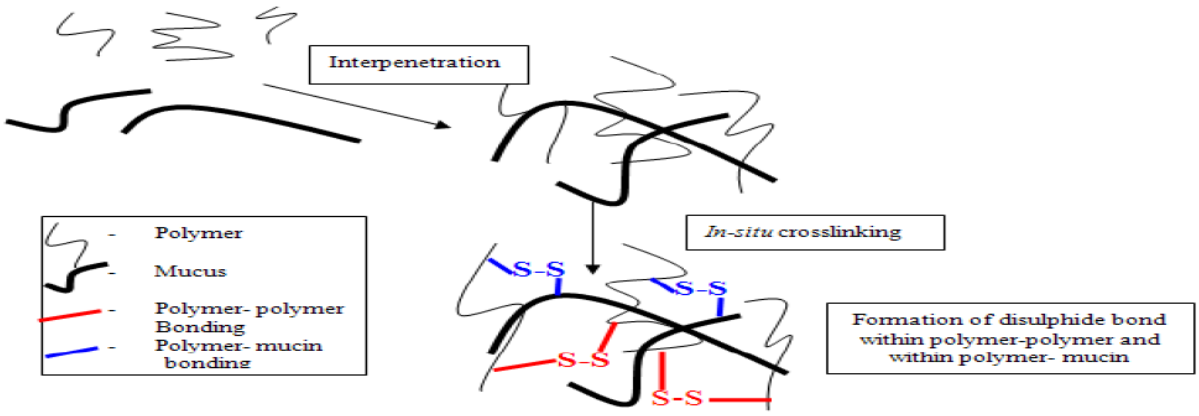

Figure 2: *In situ* cross linking after instillation.

5. ADVANTAGES OF IN SITU GELS [10]

- Less blurred vision as compared to ointment.
- Decreased nasolacrimal drainage of the drug which may cause 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.
- Improved local bioavailability due to increased precorneal residence time and absorption.
- Its production is less complex and thus lowers the investment and manufacturing cost.

6. MECHANISM OF *IN SITU* GELS

The mechanism of in situ gels is based on following mechanisms:

6.1 Based on physical mechanism

6.1.1 Swelling:

In this method of *In situ* gel formation material absorbs water from surrounding environment and expand to desired space. For example glycerol mono-oleate, which is polar lipid swells in water to form lyotropic liquid crystalline phase structures. It has some bioadhesive properties and can be degraded *in vivo* by enzymatic action [11].

6.1.2 Diffusion:

This method involves the diffusion of solvent from polymer solution into surrounding tissue which results in precipitation or solidification of polymer matrix. N-methyl pyrrolidone (NMP) has been shown to be useful solvent for such system [12].

6.2 Based on chemical reaction mechanism

Chemical reactions that results *in situ* gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes, and photo-initiated processes [13].

7. POLYMERS USED IN THE FORMULATION OF *IN SITU* GELS

7.1 Definition

A polymer is a macromolecule composed of repeating structural units and these subunits are connected by covalent chemical bonds [14]

7.2 Ideal characteristics of polymers [15]

The polymers used for in-situ gelling systems should have following characteristics:

It should be biocompatible.

It should be capable of adherence to mucus.

The polymer should be capable of decreasing viscosity with increasing shear rate there by offering lowered viscosity during blinking and stability of tear film during fixation.

It should have pseudo plastic behaviour.

It should be tolerable.

It should have good optical activity.

It should influence the tear behaviour.

7.3 Polymers used in *in-situ* gels

7.3.1 Carbopol

It is a pH sensitive polymer. It is also called as carbomer, acrylic acid polymer, etc. [16]

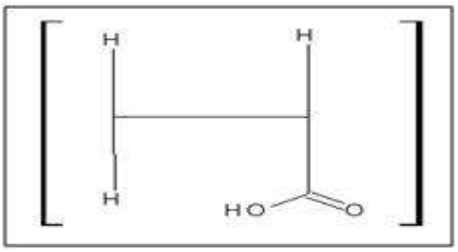

Figure 3: Structure of Carbopol [17]

7.3.1.1 Properties of Carbopol [17]

1) Carbopol is a high molecular weight, cross linked polyacrylic acid derivative and has strongest mucoadhesive property.

2) It is a water soluble vinyl polymer.

3) It shows sol to gel transition, in aqueous solution, when the pH is raised above its pKa value of about 5.5.

4) As the concentration of carbopol increases, its acidic nature may cause irritation to eye. Addition of cellulose will reduce polymer concentration and will also improve gelling property.

Table 1: Various grades of carbopol [17]

Grade	Cross linking density
Carbopol 934	Lowest
Carbopol 940	Highest
Carbopol 981	Intermediate

Table 2: Uses of Carbopol [16]

Use	Concentration (%)
Gelling agent	0.5-2.0
Emulsifying agent	0.1-0.5
Suspending agent	0.5-1.0

7.3.1.2 Mechanism

Mucoadhesive property of carbopol is due to four mechanisms of interaction between mucin and poly (acrylic acid)-electrostatic interaction, hydrogen bonding, hydrophobic interaction and inter diffusion.[18] Carbopol molecule is tightly coiled acidic molecule. Once dispersed in water, carboxylic group of the molecule partially dissociates to form flexible coil. Being a pH sensitive polymer, increase in solution pH results swelling of polymer. In acidic medium, it is in collapsed state due to hydrogen bonding, as the pH increases, electrostatic repulsion occur between the anionic groups, results gel swelling. The gelling effect is activated in two stages:dispersion and hydration of carbopol, neutralizing the solution by addition of sodium hydroxide,Triethanolamine, or potassium hydroxide [17].

7.3.2 Poloxamer

It is a temperature sensitive polymer. It is commercially called as Pluronic.

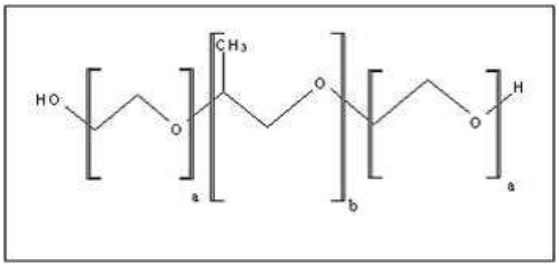

Figure 4: Structure of poloxamer

7.3.2.1 Properties of Poloxamer

1) It is a water soluble tri-block copolymer consisting of two polyethylene oxide (PEO) and polypropylene oxide (PPO) core in an ABA configuration.

2) Polypropylene oxide is the hydrophobic central part which is surrounded on both sides by hydrophilic Polyethylene oxide.

3) It has good thermal setting property and increased drug residence time.

4) It gives colourless, transparent gel [17].

5) Concentrated aqueous solutions of Poloxamer form thermoreversible gels [18].

Table 3: Various grades of poloxamer [17]

Poloxamer	Molecular weight
124	2200
188	8400
237	7959
338	14600
407	12600

7.3.2.2 Uses of Poloxamer

a) Gelling Agent.

b) Emulsifying Agent.

c) Solubilizing Agent.

7.3.2.3 Mechanism

At room temperature (25ºC), poloxamer behaves as viscous liquid and is transformed to transparent gel when temperature increases (37ºC). At low temperature, it forms small micellar subunit in solution and increase in temperature results increase in viscosity which leads to swelling to form large micellar cross linked network.

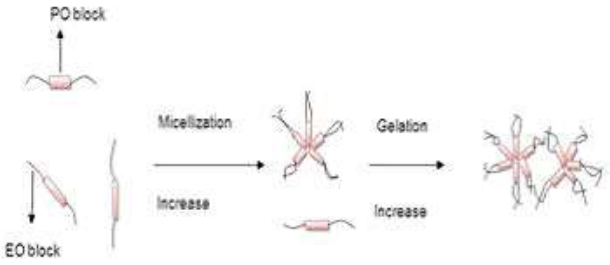

Figure 5: Gelling mechanism of Poloxamer

7.3.3 Gellan Gum

It is an ion-sensitive polymer. It is also known as Gelrite®(trade name).

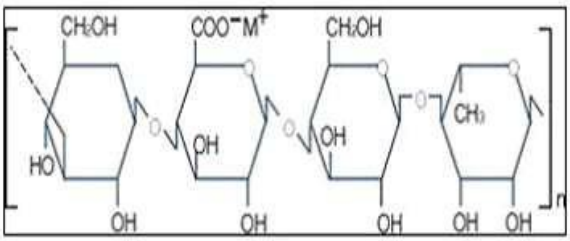

Figure 6: Structure of Gellan gum

7.3.3.1 Properties of Gellan Gum

1) Gellan gum is a linear,anionic heteropolysaccharide secreted by the microbe Sphingomonas elodea [17,18].

2) The backbone of the polymer consists of glucose,glucoronic acid and rhamnose in the molar ratio 2:1:1.These are linked together to give a tetrasaccharide repeat unit.

3) Gelrite is deacetylated gellan gum, obtained by treating gellan gum with alkali to remove the acetyl group in the molecule.

4) Upon instillation, gelrite forms gel due to the presence of calcium ions.

5) The gelation involves the formation of double helical junction zones followed by aggregation of double helical segment to form three dimensional networks by complexaton with cations and hydrogen bonding with water.

6) It is widely used in ophthalmology because of its thixotropy,thermo plasticity and pseudo plasticity.

7.3.3.2 Uses of Gellan Gum

a) Thickening Agent.

b) Gelling Agent.

c) Stabilizing Agent.

7.3.3.3 Mechanism

Gellan gum produce a cation induced *in situ* gelation (Ca2+, Mg 2+, K+, Na+) due to the cross linking between negatively charged helices and mono or divalent cations (Na+, Ca+, Mg+). Divalent ions are superior in promoting gelation as compared to monovalent cations. Gelation prolongs the residence time of drug at absorption site and bioavailability of the drug is increased.

7.3.4 Sodium Alginate

It is an ion-sensitive polymer. It is also known as algin, alginic acid, sodium salt, E401, Kelcosol,Keltone,Protanal,sodium polymannuronate [16].

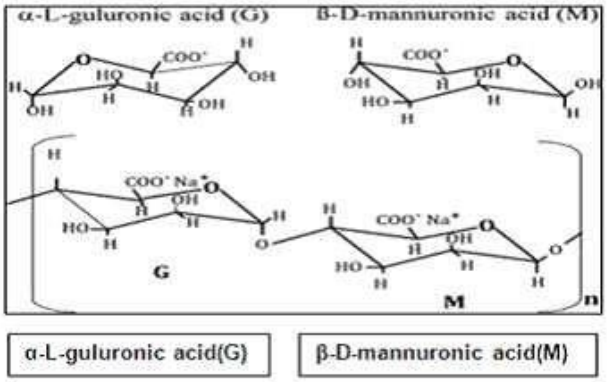

Figure 7: Structure of Sodium Alginate

7.3.4.1 Properties of Sodium Alginate [17]

1) Sodium alginate is a gum extracted from brown algae.

2) It is a salt of alginic acid.

3) It is a linear block polysaccharide consisting of two types of monomers-β-D-Mannuronic acid and α-L-glucouronic acid residues joined by 1,4-glycosidic linkages.

4) It exhibits good mucoadhesive property due to presence of carboxylic group.

5) It is biodegradable and non-toxic.

6) It has high molecular weight of 20 to 600 kDa. [14]

7.3.4.2 Uses of Sodium Alginate [16]

a) Thickening Agent.

b) Suspending Agent.

7.3.4.3 Mechanism [17]

The monomers of alginate (β-D-mannuronic acid (M) and α-L- glucuronic acid (G) are arranged as M-M block or G-G block with alternating sequence (M-G) block. Upon interaction of G block of polymer with calcium moieties, formation of homogenous gel takes place. Mechanical strength and porosity of hydrogel depends on G: M ratio, type of cross linker used and concentration of alginate solution.

7.3.5 Chitosan

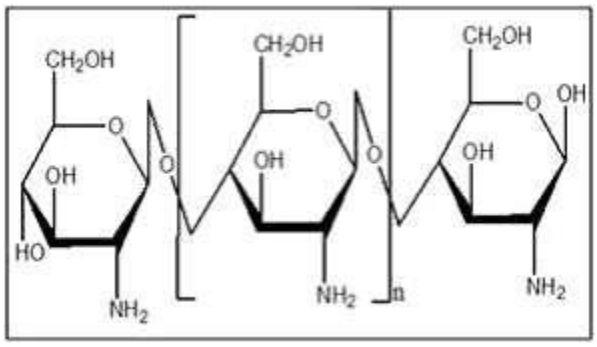

Figure 8: Structure of Chitosan

7.3.5.1 Properties of Chitosan

1) Chitosan is a cationic polysaccharide which consists of copolymers of glucosamine and N-acetyl glucosamine, these are natural polymer obtained by deacetylation of chitin [17].

2) Chitosan has mucoadhesive property due to electrostatic interactions between positively charged amino group and negatively charged mucin [17].

3) It is a polycationic polymer and also called as ophthalmic vehicle [19].

4) It is biodegradable, biocompatible and non-toxic polymer [19].

5) It exhibits pseudoplastic and viscoelastic behaviour [19].

6) It has good antibacterial and bioadhesive property [17,19].

7) It has ability to convert into hydrogel at ocular pH [20].

7.3.5.2 Mechanism

The mucoadhesive property of chitosan is due to the formation of ionic interaction between the positively charged amino groups of chitosan and negatively charged sialic acid residues of mucin. It is used as viscosity ehancing agent in artificial tear formulations because of its bioadhesive, hydrophilic and good spreading properties.

7.3.6 Hydroxy Propyl Methyl Cellulose (HPMC)

It is a temperature sensitive polymer. It is also known as Hypromellose, Methocel etc [16].

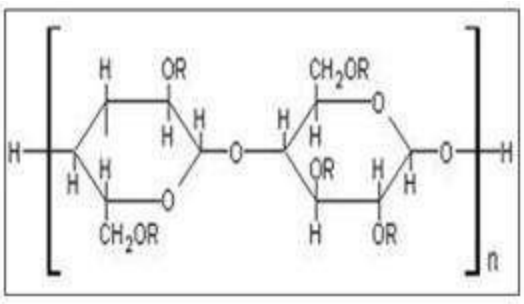

Figure 9: Structure of HPMC [17]

7.3.6.1 Properties of HPMC

1) It is water soluble cellulose ether [21].

2) Widespread acceptance of HPMC due to [22]:

a) Solubility characteristics of the polymer in organic and aqueous solvent system.

b) Non-interference with drug availability.

c) Flexibility and absence of taste and odour.

d) Stability in the presence of heat, light, air or reasonable levels of moisture.

3) It is composed of glucan chain with repeating β-(1,4)-D-glucopyranose unit [17].

4) It increases its viscosity when temperature increases [17].

5) At low concentrations (1-10 wt.%) aqueous solutions of HPMC are liquid at low temperature but gel upon heating [23].

6) It shows phase transition between 75ºC and 90ºC.These phase transition temperatures can be lowered by chemical or physical modifications [23].

7) By reducing the hydroxyl propyl molar substitution of HPMC, its transition temperature can be lowered to 40^0C.

7.3.6.2 Uses of HPMC [16]

a) Thickening Agent.

b) Suspending Agent.

c) Stabilizing Agent.

Table 4: Commercial grades of HPMC [23]

Polymer	Grade	Viscosity mPas)
HPMC	Methocel	
	A4MP	4000
	A15-LV	15
	A15CP	1500
	A4CP	400

7.3.6.3 Mechanism [23]

Gelation of 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 37ºC.

8. APPROACHES FOR IN SITU GELLING SYSTEM: [24]

The various approaches for *in situ* gelling system are:

- Temperature induced *in situ* gel systems
- pH induced *in situ* gel systems
- Ion activated systems

8.1 Temperature triggered *in situ* gelling system:

In drug delivery research temperature sensitive in situ gels are probably the most commonly studied class of

environment- sensitive polymer systems. In this gelling system polymers are liquid at room temperature (20-25°C) and undergoes gelation at physiological temperature (35-37°C) [25]. An ideal temperature triggered gelling polymer solution should remain liquid below its low critical solution temperature (LCST) and up to its upper critical solution temperature (UCST) and should transform into gel on increase of the surrounding temperature. There is gradual desolvation of the polymer and increased micellar aggregation (entanglement of the polymeric network) [26,27]. For an optimum temperature triggered *in situ* gelling solution, the phase transition temperature should be more than room temperature (25°C) so that it can be easily administered to eye and gelled at precorneal temperature (35°C) without having any effect of tear fluid dilution even at concentration as low as 5% w/v [28]. Figure 10 shows the *in situ* gelation of the temperature triggered polymers.

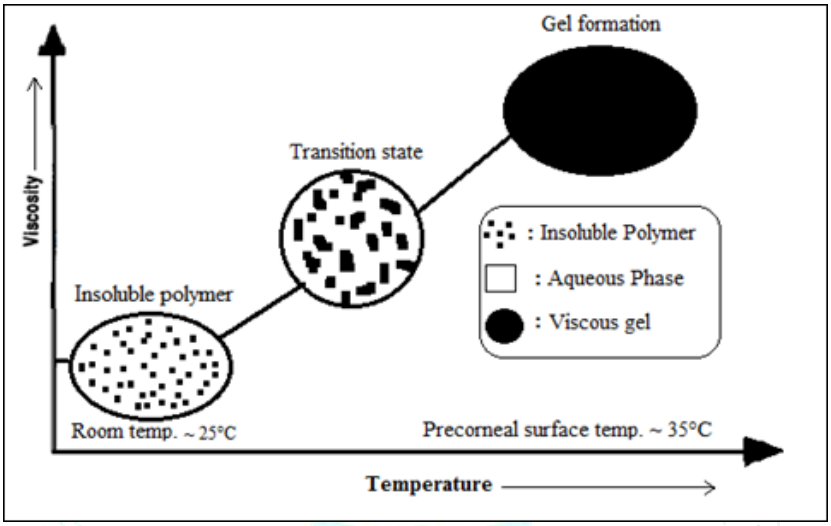

Figure 10: Schematic of temperature triggered *in situ* gelling system [29]

8.2 pH triggered in situ gelling system: pH triggered in situ gelling systems are solutions, which upon exposure to the pH of the lachrymal fluid converts into the gel phase e.g. such as cellulose acetate phthalate and Carbopol [28]. The pH sensitive polymers contain either weakly acidic or basic groups along the backbone of the polymer, these either release proton or accept free proton in response to change in pH. At specific pH there is Electrostatic, hydrophobic interaction and Hydrogen bonding takes place, hence leads to inter-diffusion and a conformational change in the polymer results in its swelling. Hence sol to gel transition is pH triggered [30]. Fig. 11 shows the *in situ* gelling phenomena by pH modification of the system.

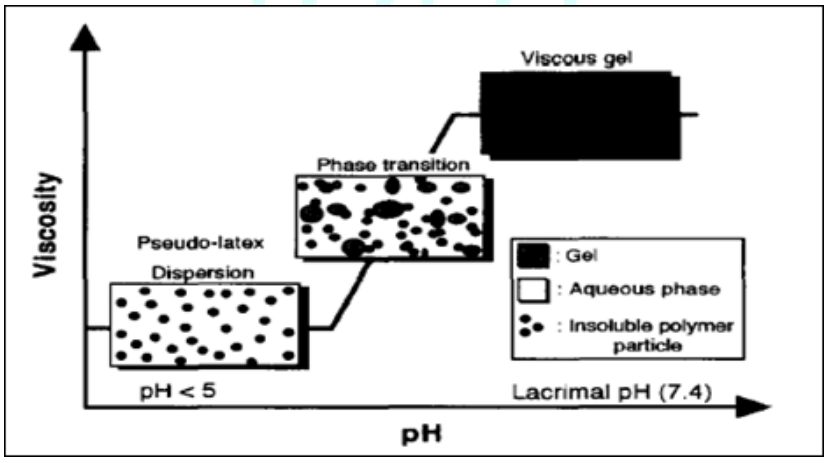

Figure 11: pH triggered in situ gelling system [29]

8.3 Ion triggered *in situ* gelling system: In ion triggered in situ gelling system solution viscosity increases upon exposure to ionic concentration of the tear fluids [28]. It is also called osmotically induced gelation. Ion sensitive polymers are able to crosslink with cations (monovalent, divalant) present in lacrimal fluid on ocular surface and enhance the retention time of drug [30].

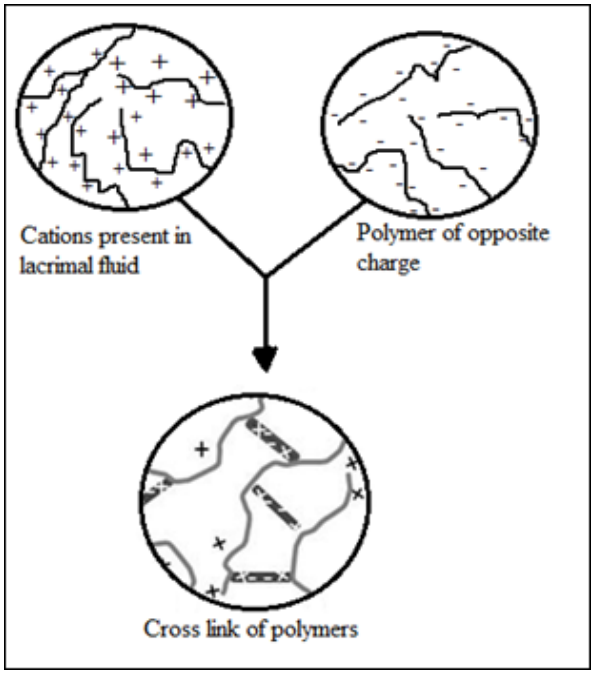

Figure 12: Schematic of mechanism of ion triggered *in situ* gelling system [29]

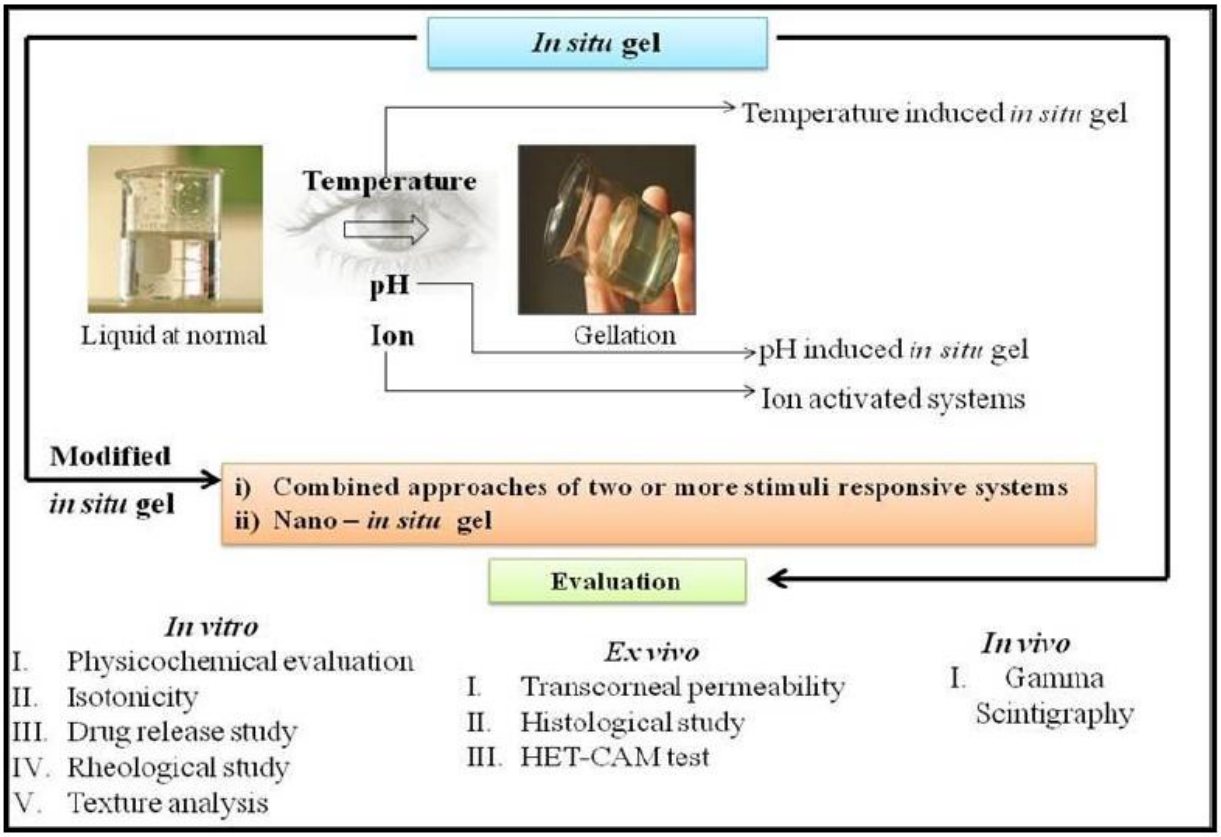

Figure 13: Overview on *in situ* gel

9. Evaluation of Ocular *in situ* gel

Ocular *in situ* gel can be tested for various parameters in order to ensure that prepared formulation satisfy safety guidelines for ocular drug delivery system (ODDS).

9.1 Visual appearance and clarity Visual appearance and clarity of prepared *in situ* formulation is checked for presence of any particulate matter under fluorescent light against a white and black back ground [31].

9.2 pH pH affects both solubility as well as stability of drug in ophthalmic formulations. It should be such that the formulation will remain stable at that pH at the same time there would no irritation to the patient upon administration. It is measured by digital pH meter [32].

9.3 Gelling capacity Gelling capacity of formulation is determined by placing a drop of the formulation in a vial containing 2.0 ml of freshly prepared simulated tear fluid and time taken for its gelling is noted [33].

9.4 Isotonicity Isotonicity is important characteristics of ophthalmic formulation which has to be maintained to prevent any tissue damage or irritation to the eye. It refers to the osmotic pressure exerted by salts in aqueous solution. Ophthalmic formulation must possess osmotic pressure within the range of 290-310 mOsmol/kg. Tonicity is measured by using osmometer [34,35].

9.5 *In vitro* drug release study

In vitro drug release study is done by using Franz diffusion cell. In receptor compartment freshly prepared artificial tear fluid (ATF) is placed. Dialysis membrane is placed in between receptor and donor compartments. Whole assembly is kept on the thermostatically controlled magnetic stirrer to simulate in vivo conditions and temperature of medium is maintained at 37°C ± 0.5°C. Medium is continuously stirred at 20 rpm. 1ml of formulation is placed in donor compartment. Sample (0.5ml) is withdrawn at predetermined time interval and same is replaced by ATF. Samples are analysed either on UV spectrophotometer or HPLC [36, 37].

9.6 Rheological studies Brookfield viscometer is mainly used for determination of Viscosity of ophthalmic *in situ* gels. Viscosity is measured before and after gelation by increasing angular velocity gradually from 0.5 to 100 rpm [38].

9.7 Texture analysis The consistency, firmness, and cohesiveness of *in situ* gel are assessed by using texture profile analyzer. This mainly indicates gel strength and easiness in administration. Texture analysis provides information on hardness, compressibility and adhesiveness which can be correlated with various parameters like ease of removal from container, good spreadability on corneal surface and adherence to mucous layer in order to prolong residence time [39].

9.8 Transcorneal permeability study

Transcorneal permeability of drug is evaluated by using goat eye cornea. The fresh whole eyeball of goat is obtained from local butcher's shop and transported in laboratory in normal saline solution (4°C). Cornea is then carefully excised along with 2-4 mm of surrounding sclera tissue and wash with saline solution. Excise cornea is place in between donor and receptors compartment of Franz diffusion cell in such a way that epithelial surface face the donor compartment. Receptor compartment is filled with freshly prepared artificial tear fluid (ATF). Whole assembly is placed on thermostatically controlled magnetic stirrer, temperature (37°C ± 0.5°C) as well as stirring rate (20 rpm) is maintained. 1ml of prepared formulation is placed in donor compartment. Samples (0.5ml) are withdrawn at predetermined time interval of 1hr to 5hr and same volume is replaced by ATF. Samples are then diluted upto 10ml and analysed on either UV spectrophotometer or HPLC [40, 41].

9.9 Ocular irritation study As there is ban on Draize study in many countries ocular irritation study of *in situ* formulation can be performed by one of the following method.

9.9.1 Histological study To evaluate effect of *in situ* formulation on corneal structure and study the irritation potential, corneas are removed from the eyes of freshly sacrificed goat and incubated at 37°C for 5 hrs in formulation. Sodium dodecylsulfate (SDS) solution in phosphate buffer saline (PBS) 0.1% (w/w) is used as the positive control. After incubation, corneas are washed with PBS and immediately fixed in formalin (8%, w/w). Tissues are dehydrated in an alcohol gradient, placed in melted paraffin and solidified in block form. Cross sections are cut, stained with haematoxylin and eosin (H&E). Cross sections are observed microscopically for any modifications [42].

9.9.2 Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) HET-CAM test is performed by incubating the eggs for 10 days at 37°C and relative humidity of about 70% with automatic turning once per hour. After incubation period, a portion of each egg shell is removed and a drop of water is placed onto the air sack membrane to avoid capillary damage during its removal. The CAM is then carefully exposed to 0.1 ml or 0.1 gm of test substances, which is washed-off with normal saline solution after 30 sec of exposure. Simultaneously, CAM is exposed to saline solution (negative control) and 1% SDS solution (positive control). Each CAM is observed microscopically after 5 minutes for haemorrhage, lysis and coagualtion. An irritation score (IS) is calculated for each CAM by using following formula; $IS = 301 - Hemorrh\,age$
$300\times5 + (301 - Lysis)300\times7 + 301 - Coagulation300\times9$
Irritation score is given according to following scheme; 0 = no reaction; 1 = slight reaction; 2 = moderate reaction; 3= severe reaction [43, 44].

9.10 *In vivo* Scintigraphy Studies Gamma scintigraphy is a well-established technique for in vivo evaluation of ophthalmic retention time. Although the rabbit is the commonly recommended animal model for evaluation of ophthalmic formulations, but human volunteers are preferred for this study due to physiological differences between rabbits and humans, especially the blinking rate [45].

9.11 Accelerated stability study A stability study for *in situ* formulation is carried out as per ICH guidelines to determine the physical stability of the formulation under accelerated storage conditions. Formulation is subjected to elevated temperatures and humidity conditions of 25±1°C/ 60%RH, 30±1°C/ 65%RH and 40 ± 2°C/ 75 ± 5 % RH. Samples are withdrawn at the end of 0, 30, 60 and 90 days and then evaluated for active drug content [46].

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

Table 5: Marketed Products of ophthalmic *In situ* gels [47]

Product Name	Drug Used	Mfg. Company
Timoptic-XE	Timolol maleate	Merck and Co.Inc
Cytoryn	Interleukin-2(IL-2)	Macromed
Azasite	Azithromycin	InSite Vision
AktenTM	Lidocaine hydrochloride	Akten
Virgan	Ganciclovir	Spectrum Thea Pharmaceuticals
Pilopine HS	Pilocarpine hydrochloride	Alcon Laboratories Inc.

CONCLUSION

Ocular drug delivery system is burgeoning field in which most of the researchers are taking challenges to combat various problems associated 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. *In situ* gelling systems are promising ocular delivery systems because they can overcome the drawbacks associated with conventional ocular dosage forms thus in the recent years ophthalmic *in situ* gelling drug delivery systems have drawn much attention of researchers. They are easy to administer with improved patient compliance. The principal advantages of these systems are the possibility of administering accurate and reproducible quantities of drugs, increased precorneal contact time, prolonged drug release, drug delivery to deeper tissues, and reduced frequency of administration. Further, drug loaded nanoparticles, liposomes or other colloidal drug carriers can also be incorporated in these systems to obtain sustained drug delivery in a much improved and effective manner. Future use of biodegradable and water soluble polymers for the in situ gel formulations can make them more acceptable and excellent drug delivery systems. Moreover, in situ gels have ease of commercialization which adds advantage from industrial point of view.

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