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Newer Trends in *In Situ* Gelling Systems for Controlled Ocular Drug Delivery

Abstract

In situ -gelling systems has the great potential in the wide areas of drug delivery. The application areas of such systems are broad and these systems can be formulated not only as gels but also as *in situ* gelling nanospheres, microspheres and liposomes. To overcome the drawbacks associated with conventional drug delivery systems and take advantages of both solutions and gels, such as accurate dosing, ease of administration of the former and longer precorneal residence of the latter, a newer concept of *in situ* gelling drug delivery systems was came in light for the first time in the early 1980s. *In situ* gelling systems are the polymeric solutions which can be administered in solution form and immediately undergoes sol-gel phase transition in to a viscoelastic gel upon exposure to physiological conditions (e.g. pH, temperature and ionic concentration) or application of external triggers. *In situ* gelling systems seems to have wide applications in ocular therapy. A lot of research is going on in this area proves the fact that *in situ* gelling systems can be advantageous in the ocular drug delivery. In the present review we will discuss recent researches involving application of *in situ* gelling polymers, their applications in the various domains of drug delivery and marketed formulations based on *in situ* gelling systems.

Keywords: *In situ* gelation; Ocular drug delivery; pH sensitive gels; Temperature sensitive gels; Ion sensitive gels

Review Article

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Abbreviations: HPMC: Hydroxypropyl Methylcellulose; MC: Methylcellulose; PLGA: Poly (Lactide-co-Glycolide); CAP: Cellulose Acetate Phthalate; LCST: Low Critical Solution Temperature; UCST: Upper Critical Solution Temperature; PPO: Polypropylene Oxide; PEO: Polyethylene Oxide; MTA: Methozolamide; PNIPAAm: Poly (N-Isopropylacrylamide); GC: Guluronic Acid; HEC: Hydroxyethyl Cellulose; IOP: Intraocular Pressure; PLA: Polylactic Acid; PCL: Polycaprolactone; PEG: Polyethylene Glycol; PCL: Polycaprolactone; PBCs: Pentablock Copolymers; CPEX: Ciprofloxacin; HPBCD: Hydroxyl-Propyl Beta Cyclodextrin; SBECD: Sulfobutyl Ether Beta Cyclodextrin; TA: Triamcinolone Acetonide; RTG: Reverse Thermal Gel; ACZ: Acetazolamide; NTX: Naltrexone Hydrochloride; CMC: Carboxymethyl Cellulose Sodium; ALG: Alginate; TEC: Triethyl Citrate; FLZ: Fluconazole; HGH: Human Growth Hormone

Introduction

Ocular drugs are mostly applied locally to the surface of the eye as eye drops for treatment of either the external ocular infections such as conjunctivitis, blepharitis, keratitis sicca, or intraocular diseases such as glaucoma, proliferative vitreoretinopathy, endophthalmitis, recurrent uveitis, acute retinal necrosis and cytomegalovirus retinitis etc [1]. However, due to efficient protective mechanisms of the eye (e.g. lachrymal secretion, blinking reflex) and systemic absorption in the conjunctiva, major part of the drug is rapidly eliminated from the ocular surface and only a small fraction of drug is absorbed into the eye, which results in poor bioavailability of the drugs. This needs frequent dosing of eye drops, which causes pulse kinetics of the drugs in the eye [2].

Other ocular dosage forms include viscous solutions, suspensions, ointments, hydrogels etc [3]. Viscous solutions are beneficial over eye drops but may have the risk of obstruction of the puncti and canaliculi [4]. Suspensions based ocular drug delivery formulations are generally used for administering poorly soluble drugs, but the grittiness and irritation on application due to suspended particles limits their application and also it suffers high variability in efficiency [5]. The variability in efficiency in case of ocularly applied suspensions may be due to the non-uniformity of the doses. Ointments are not used greatly because of the risk of uneven volume administration, greasiness and blurred vision [4-6]. The drawbacks like inaccurate and irreproducible drug administration, crusting of eyelids, and lachrymation associated with the preformed hydrogels, limiting their application as ocular drug delivery systems [7]. Clearly the existing ocular delivery systems are primitive and inefficient to treat severe ocular diseases, thus cannot be used extensively for ocular drug delivery.

In ocular therapy, a liquid dosage form is preferred from the point of patient acceptability. Thus an ideal ocular drug delivery system is the one, which can be administered in drop form without causing any problem in normal vision, can produce sustained drug release and does not require frequent dosing. The main advantage of such a system is the opportunity of accurate and reproducible administration of drugs in reproducible manner, and increased drug bioavailability by prolonged retention [1].

In situ gelling systems can fulfill these criteria successfully as they can be retained at the ocular surface for longer duration and thus can increase the residence time of the drug at the site

of action, resulting in enhanced drug bioavailability and lesser patient incompliance as compared to conventional ocular drug delivery system [7-10]. A wide variety of drug molecules and materials of therapeutic advantages such as antibiotics (Ofloxacin, Ciprofloxacin, Gatifloxacin), beta blockers (Timolol, Carteolol), NSAIDs (Ketorolac Tromethamine, Indomethacin), Pilocarpine hydrochloride, Puerarin, Recombinant rhEGF, Antivirals (Acyclovir) has been delivered through *in situ* gelling systems, which shows the importance of *in situ* gelling formulations as the future drug delivery systems [7-9,11-25].

Additionally, the pseudoplastic flow character of *in situ* gel forming polymers helps in minimizing in interference with blinking of the eyelids. The drug can be dissolved or dispersed in *in situ* gelling polymer solution, which upon instillation into the eye undergo phase transition in the conjunctival cul-de-sac to form viscoelastic gels due to conformational changes of polymers in response to the physiological conditions [9,18,26] (Table 1).

Table 1: Marketed Products of *In situ* Polymeric System [26].

Name of Marketed Product	Drugs Used in Formulation	Manufacturing Company
Timoptic-XE®	Timolol maleate	Merck and Co.Inc.
Regel Depot-Technology	Human Growth Hormone	Macromed
Cytoryn	Interleukin-2(IL-2)	Macromed
Azasite	Azithromycin	InSite Vision
Pilopine HS	Pilocarpine hydrochloride	Alcon Laboratories Inc.
Akten™	Lidocaine hydrochloride	Akten
Virgan	Ganciclovir	Spectrum Thea Pharmaceuticals

Advantages of *in situ* gelling ocular drug delivery systems

Conventional ocular delivery system (i.e. eye drops) shows poor drug bioavailability and lesser therapeutic response because of high tear turnover and thus rapid precorneal elimination of drug. Therefore high frequency of eye drops instillation is required which results in patients' incompliance. Inclusion of excess drug in formulation is attempted to overcome the bioavailability problem, but that may be absorbed systematically from nasolacrimal duct and thus may be potentially dangerous [27]. To overcome the problems associated with conventional ocular delivery, *in situ* gelling system are better alternative. The main advantage of *in situ* forming gels is sustained and controlled drug delivery and less or no blurred vision as is the case with ointments. Other advantages of *in situ* gelling systems over eye drops and ointments are increased drug bioavailability due to increased precorneal contact, improved patient compliance because of reduced dosing frequency, requirement of lesser concentration of drug, minimal chances of nasolacrimal drainage of drug thus reduced wastage and lesser systemic side effects. Further the *in situ* gelling systems may be more comfortable than insoluble or soluble insertion [27-29].

Ideal requirements for *in situ* gelling systems for ophthalmic applications

An ideal *in situ* gelling drug delivery formulation should comply following requirements [27-29]:

Gelation (sol-gel phase transition): The system should be presented in form of solution and form a gel under physiological conditions or presence of the trigger for gelation. In other words the formulation should start gelation rapidly after administration to avoid precorneal drainage.

Sustained drug release: The system should sustain drug release for prolonged durations to produce optimal bioavailability with minimal side effects.

Optimal pH: The pH of the system should not be highly acidic/alkaline, as it may cause irritation or damage to the tissues.

Clarity: In case of ocularly applied *in situ* gels, the formulation should be clear, transparent and colorless. It should not hinder the normal vision. Any impurity like particles should not be present, as it may cause irritation to the ocular tissues.

Rheological properties: The main prerequisites of an *in situ* gelling system are viscosity and gel strength. The formulation should have an optimum viscosity, allowing easy administration and undergo a rapid sol-to-gel transition. Additionally, the gel formed should preserve its integrity without dissolving or eroding over a prolonged period of time. *In situ* gelling systems generally show pseudoplastic flow characteristics. Since the shear rate and the tissue movement is very high in some parts of the body thus the viscoelastic fluids with a viscosity that is high under conditions of low shear rate and low under conditions of high shear are preferred. The two main prerequisites of an *in situ* gelling system are viscosity and gel strength. The formulation should have an optimum viscosity that will allow easy administration as a solution which would undergo a rapid sol-to-gel transition.

Sterility: It should be sterile to prevent any possible damage to tissues at the site of application because of microbes.

Stability: The formulation should be stable and should not degrade or deteriorate on storage during its shelf-life.

Drug content: The system should contain required amount of the active ingredients without any chemical degradation or interaction with the polymers or other excipients in any undesirable manner.

Ocular tolerance: The polymers should be biocompatible and well tolerated with the ocular tissues. It should not produce any damage to tissues in the form of irritation, redness, swelling or any undesirable adverse effects etc.

Reproducibility: The system should show the same properties on repeated preparation and large scale production. Ideally an *in situ* gelling system should be a free flowing liquid to allow reproducible administration.

Isotonicity: The formulation should be isotonic to prevent tissue damage or irritation of eye.

Adhesiveness: The polymer should be capable to adhere to the precorneal surface of the eye.5.

Classification of *In Situ* Gelling Polymers

Polymers used for formulating *in situ* gelling systems can be classified on the basis of their origin or mechanism of gelation.

According to their origin

Depending on the origin *in situ* gelling systems can be divided into two types:

Natural: examples include chitosan, alginic acid, xyloglucan, gellan gum, sodium hyaluronate, pectin.

Synthetic/semi synthetic: e.g. hydroxypropyl methylcellulose (HPMC), methylcellulose (MC), cellulose acetate phthalate (CAP), Carbopol, Pluronics, poly(lactide-co-glycolide) (PLGA).

According to physiological mechanisms causing gelation of polymers

A variety of mechanisms and approaches present at the site of application can be utilized for the formation of *in situ* gelling drug delivery systems. On the basis of the triggers involved in the phase transition from sol-to-gel phase *in situ* gelling systems can be categorized into following three types [11,18]:

pH triggered *in situ* gelling polymers: 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 [18]. 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]. Figure 1 shows the *in situ* gelling phenomena by pH modification of the system.

Pseudolatexes (e. g. cellulose acetate phthalate latex): Pseudolatexes are artificial latexes prepared in aqueous medium by dispersion of a pre-existing polymer. They ensures the physical stability of the latex and the drug molecules sensitive to aqueous environment as they can be lyophilized and obtained in easily redispersible powder form. Another advantage of pseudolatexes is that, their production doesn't require use of organic solvents. Cellulose acetate phthalate latex (CAP latex) is a pseudolatex *in situ* gelling system. CAP latex remains free flowing solution at acidic pH (pH 4.2) and transform into the gel at neutral pH (pH 7.2), and also remains stable at relatively low pH [5].

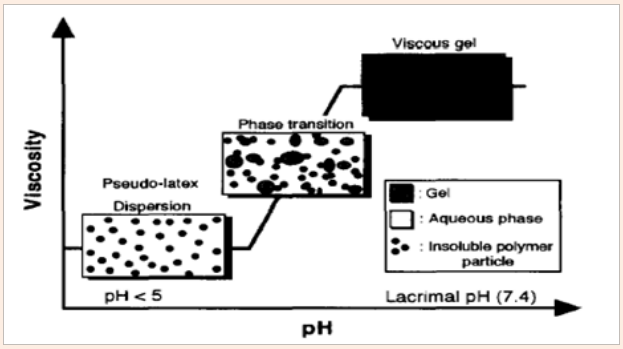

Figure 1: Scheme of pH triggered *in situ* gelling system.

Polyacrylic acid (Carbopol): Polyacrylic acid commercially known as Carbopol is a widely used polymer in ophthalmology to enhance precorneal retention undergoes sol to gel transition in aqueous solution as the pH is raised above its pKa of about 5.5. It also exhibits very good mucoadhesive properties as compared to other polymers like cellulose derivatives, and polyvinyl alcohol [5]. Srividya et al. [11], developed a pH-triggered *in situ* gelling system based on polyacrylic acid (Carbopol 940) for ocular drug delivery of ofloxacin. To enhance the viscosity of the system they added HPMC in the formulation. The formulation was observed therapeutically efficacious, stable and non-irritant in Draize eye test in female albino rabbits and showed sustained drug release of ofloxacin over duration of 8 hrs. The formulations were shear thinning and observed to have pseudoplastic rheology. The formulations showed antimicrobial efficacy against *Pseudomonas aeruginosa* and *Staphylococcus aureus* in agar diffusion test using cup plate technique [11].

Nanjwade et al. [17], formulated and evaluated an ophthalmic delivery system for ketorolac tromethamine based on the concept of pH-triggered *in situ* gelation using polyacrylic acid (Carbopol® 934) as the gelling agent with HPMC (Methocel K4M) as viscosity enhancer. The formulation was observed to be therapeutically efficacious, stable, non-irritant, and provided *in vitro* sustained drug release over a period of 8 hrs. The study suggested that the developed formulation is a viable alternative to conventional eye drops due to its ability to enhance bioavailability through its longer precorneal residence time and ability to produce sustained drug release [17].

A pH-triggered *in situ* gelling ophthalmic delivery system of an antiglaucoma drug, timolol maleate was developed by Gupta et al. [31]. They overcome the risk of systemic side effect due to absorption of drug into systemic circulation of patient. Polyacrylic acid (Carbopol) was used as the gelling agent in combination with chitosan (as viscosity enhancer). The 0.4% w/v Carbopol/0.5%

w/v chitosan based *in situ* gelling system was in liquid state at room temperature and at the pH of formulation i.e. pH 6.0, and underwent rapid transition into the viscous gel phase at pH 7.4 of the lacrimal fluid. Carbopol-chitosan based formulation was observed to be therapeutically efficacious and showed a fickian release behaviour over 24 h periods as compared to the Glucomol® (a 0.25% timolol maleate ophthalmic solution), 0.4% w/v Carbopol solution as well as liposomal formulation. The study suggests that the developed system is a viable alternative to conventional eye drops and can also prevent the rapid drainage as in case of liposomes [31].

In situ ophthalmic gel of fluroquinolone antibiotic ciprofloxacin hydrochloride using polyacrylic acid (Carbopol® 980NF) as pH sensitive phase transition polymer was prepared by Jain et al. [15]. HPMC (Methocel® K100LV) was added as a release retardant, and ion exchange resin as a complexing agent. To avoid incompatibility between drug and polyacrylic acid, ciprofloxacin hydrochloride was complexed with ion exchange resin. The formulation was observed to be stable and nonirritant to rabbit's eye and showed 98% *in vitro* drug release over a period of 24 hours [15].

Temperature triggered *in situ* gelling polymers: Temperature triggered *in situ* gelling polymers remains liquid at room temperature (20-25˚C) and undergoes gelation at physiological temperature (35-37˚C) [5]. 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 transform in to gel on increase of the surrounding temperature. There is gradual desolvation of the polymer and increased micellar aggregation (entanglement of the polymeric network) [20,27]. 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 [18]. Figure 2 shows the *in situ* gelation of the temperature triggered polymers.

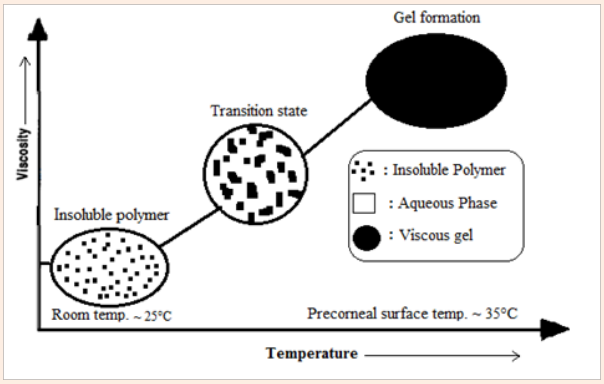

Figure 2: Schematic of temperature triggered *in situ* gelling system.

Following are some examples of temperature triggered *in situ* gelling polymeric systems:

Poloxamers: Poloxamers, commercially known as Pluronic®, are the thermoreversible polymers commonly used for formation of thermosensitive *in situ* gelling systems. Poloxamers are ABA-type triblock copolymers composed of polyethylene oxide (PEO) (A) and polypropylene oxide (PPO) units (B). Polypropylene oxide units are hydrophobic in nature which is surrounded by hydrophilic units of ethylene oxide. These polymers are available in liquid, semisolid, and solid forms. Several molecular weights (1100 to 14,000) of Poloxamers are available, depending on the distribution and weight ratio (from 1:9 to 8:2) of ethylene oxide-propylene oxide units, resulting in different gelation characteristics [5,32].

Pluronic F127 is most commonly used Poloxamer polymer in pharmaceuticals, due to its colorless and transparent gels forming character [5]. It consists of polyoxyethylene units (70%) and polyoxypropylene blocks (30%). Upon heating from 4°C to 23°C or more, aqueous solution of Pluronic F127 at a concentration of ≥15%, transformed to a semisolid gel from a low viscosity solution, but again converts into solution upon cooling, i.e. shows reversible gelation. The occurrence of thermogelation is characterized by a sol-gel transition temperature, i.e. a temperature, below which polymer solution remains fluid allowing a comfortable and accurate drug administration, and above that it converts into a gel [9]. Poor mechanical strength, faster erosion and non-biodegradability are the main drawbacks of Poloxamer polymers gels. However, the polymers can be made biodegradable by synthesizing new polymers by linking together a few (usually 3) Poloxamer 407 'monomers' via degradable carbonate linkage, which upon hydrolysis under physiological conditions degrade into soluble Poloxamer 407 units and carbonate. Interestingly, the dissolution time of the gel can be increased by optimizing polymer concentrations and total polymer concentration can be decreased upto two-fold by oligomerization [5].

Edsman et al. [33], studied the gel and the sol-gel transition of a temperature sensitive *in situ* gelling polymer, Poloxamer 407 by rheological measurements. However the contact time of the Poloxamer in human eye was observed to be increased with increasing concentration of Poloxamer, but the maximum contact time observed was about 1hr. The sol-gel phase transition temperature increased with decreasing concentration, and all preparations showed a transition temperature below 35˚C, however at higher concentrations preparations formed gels at room temperature itself with increased gel strengths. Elasticity of the gels increased with increasing concentration of Poloxamer but the dependence on concentration was very small. The study concluded that *in situ* gels based on Poloxamer could not be considered promising because of tear dilution effect and dependence of sol-gel transition temperature on concentration of Poloxamer [33].

Methozolamide (MTA) containing thermosensitive *in situ* gel has been prepared using Poloxamer analogs by Qian et al. [34] for the treatment of glaucoma. MTA loaded (0.15% w/v) optimized formulation contained 21% w/w Poloxamer 407 and 10% w/w Poloxamer P188 and was examined for gelation temperature, rheological properties, *in vitro* release and *in vivo* evaluation.

Sustained diffusion controlled drug release over a period of 10 hrs was observed in the *in vitro* drug release study. The study concluded that the *in situ* gelling system has more significant ocular delivery than conventional MTA eye drops [34].

A thermosetting gel with a suitable phase transition temperature by combining Pluronic analogs (21% Pluronic F127 and 10% Pluronic F68) and investigated the effect of a mucoadhesive polysaccharide sodium hyaluronate, on the ocular retention of the gel. The formulation was observed to be free flowing liquid below 25°C and formed a firm gel under physiological conditions and showed highest viscosity before and after tear dilution. Gamma scintigraphic study demonstrated at least three fold longer ocular retention of the 99mTc-DTPA labeled gel as compared to the phosphate buffered solution. Addition of sodium hyaluronate resulted in decreased gel strength thus no further increase in the ocular retention. The Pluronics based *in situ* gelling formulation is advantageous as it could be more readily administrated and showed a prolonged ocular residence time [35].

Nanjawade et al. [5], suggested that thermogelation occurs from the interaction between different molecules of Pluronics. Micellar mode of association of aqueous Poloxamer solutions has been clearly indicated by ultrasonic velocity, light-scattering and small-angle neutron scattering measurements. PPO blocks from micelles by dehydration at critical micelle temperature, which become more significant on increasing the temperature and finally, at a specific temperature these micelles associate with each other to form gel. Thus, the possible mechanism of gelation of Poloxamer solution with increasing temperature may be the packing of micelles and micelle entanglements. Another hypothesis suggests that upon increasing the temperature the hydration spheres around the hydrophobic units modifies, and thus induces the interactions between these molecules of Pluronics. Additionally intramolecular hydrogen bonding has also been proposed for gelation [5].

A low viscosity aqueous solution of Poloxamer 407 (P407), at a concentration of ≥18% w/w (a 7:3 ratio of PEO and PPO), is converted to a gel under the ambient temperature, but this gelation ability is lost on dilution with tear fluid, because of lower concentration of P407. Thus to ensure a good gel formation under physiological conditions 25% w/w P407 can be used [8]. The viscosity of Poloxamers solutions (20-30% w/w) increases when temperature is raised to the eye temperature (33-34°C) from a critical temperature (16°C) [1].

The increased activity of pilocarpine in Poloxamer 407 gels as compared to the solution is reviewed by Nanjawade et al. [5]. Poloxamers have also been evaluated for the treatment of dry eye, because of their protective and mucomimetic action. The mucomimetic character of Poloxamers is due to their propylene oxide and ethylene oxide chains simulating mucin action by adsorption of the tears films on the ocular epithelium [5].

The effect of hyaluronic acid (HA) on the gelation properties of Poloxamers has been studied by Mayol et al. [36], for developing a thermosensitive and mucoadhesive polymeric system for ocular drug delivery. The addition of HA in the Poloxamers blends delayed the gelation temperature by few degree Celsius and at specific concentration of Poloxamer/HA it is possible to get a thermoreversible gel with a gelation temperature close to body temperature. The gel showed a prolonged and controlled release of acylovir for more than 6 hrs. The study suggested that the addition of low molecular weight HA into Poloxamers blends results in making the thermosensitive and mucoadhesive polymeric systems for sustained drug delivery [36] (Figure 3).

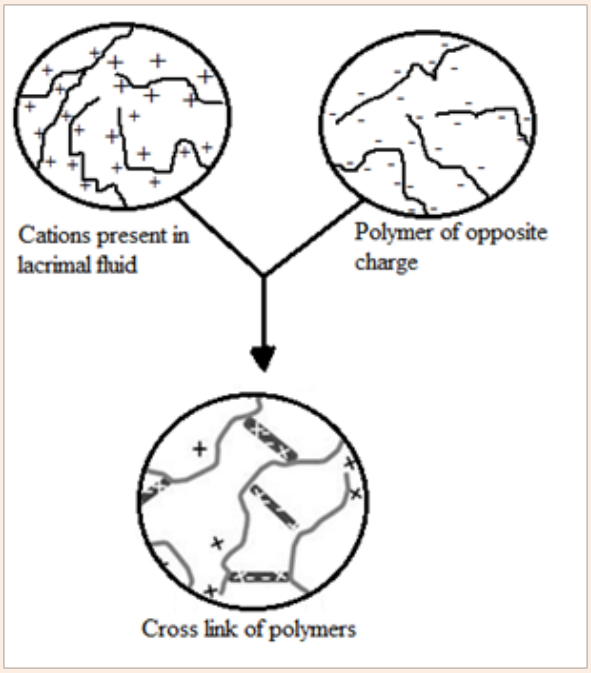

Figure 3: Schematic of mechanism of ion triggered *in situ* gelling systems.

Poloxamines: These are commonly known as Tetronics (tetra functional block copolymers of ethylene and propylene oxide) [37,38]. Kang et al. [39] tested the *in situ* gelling thermosensitive injectable hydrogel based on Tetronic-oligolactide copolymer (made of Tetronic® 1307 and Pure L-lactide) in a rat hemisection traumatic spinal cord injury model. The copolymer showed prompt gelation (within 10sec) after being injected into lesion cavities [39].

Cellulose derivatives: Ethyl (hydroxyl ethyl) cellulose, methylcellulose and HPMC are some of the cellulose derivatives which are being used as *in situ* gelling polymers. Aqueous solutions of ethyl (hydroxyethyl) cellulose (EHEC) exhibit thermosensitive gelation. On addition of sodium dodecyl sulphate or cetyl triammonium bromide, EHEC (1%-4% w/w) solutions undergoes sol-to-gel phase transition upon heating to 30-40°C and forms stiff and clear gels [5].

Some cellulose derivatives remain liquid at low temperature and become gel upon heating, for example aqueous solutions of methylcellulose and HPMC undergoes phase transition into gels between 40-50°C and 75-90°C respectively. However,

phase transition temperatures of methylcellulose and HPMC are higher than the physiological temperatures, but can be lowered by making chemical or physical changes in the polymers. For example, addition of NaCl in methylcellulose or lowering the hydroxypropyl molar substitution of HPMC, the phase transition temperatures can be reduced to 32-34°C and 40°C, respectively in these polymers. Hydrophobic interaction between molecules with methoxy groups causes gelation of methylcellulose or HPMC solutions. Polymer-polymer interaction occurs between macromolecules due to hydration at lower temperature. The water of hydration is lost gradually on increasing the temperature resulting in lower viscosity. At the transition where enough dehydration of the polymers takes place, they start associating and the viscosity starts increasing showing a network structure formation. This phenomenon of gel formation from a solution has been successfully used in development of *in situ* gelling formulations [5].

Bain et al. [40], studied methylcellulose based *in situ* fast gelling vehicle for ophthalmic drug delivery. The gelation temperature of 1% methylcellulose solution was decreased from 59°C to the physiological temperature i.e. 37°C by addition of fructose and sodium citrate tribasic dihydrate (SC) in different proportions. The 1% methylcellulose gel temperature was reduced from 59 to 54°C on varying the fructose concentration from 2 to 10%. To further reduce the gelation temperature of the methylcellulose, SC was added in the methylcellulose (1%) and fructose (10%) system in varying concentrations and the temperature was reduced till 32 °C with the variation of SC concentration from 1 to 5%. Sustained release of Ketorolac Tromethamine was observed from the developed formulation corresponding to gelation temperature 32°C over a period of 9 hrs [40].

Xyloglucan: Xyloglucan, a polysaccharide obtained from tamarind seed and approved for use as food additive. Partially degraded xyloglucan by β-galactosidase to > 35% galactose removal ratio exhibits thermally reversible gelation in dilute aqueous solutions. The sol-gel transition temperature of xyloglucan varies with degree of galactose elimination and polymer concentration and related inversely, for example, on increasing the galactose removal ratio from 35 to 58% the sol-gel transition of xyloglucan was observed to be decreased from 40 to 5°C. Xyloglucan forms gels by the lateral stacking of rod like chains. The 1.5% w/w xyloglucan based *in situ* gelling formulation showed similar miotic response as shown by 25% w/w Pluronic F127 gel [28].

Miyazaki et al. [7], evaluated enzyme degraded xyloglucan polysaccharide as sustained release thermosensitive *in situ* gelling system for ophthalmic drug delivery of pilocarpine. Three different formulations containing 1, 1.5 and 2% w/w concentration of xyloglucan were compared to 25% Pluronic F 127 solution. Xyloglucan formulations showed higher viscosity than Pluronic solution at 5°C. The viscosity was increased on increasing the concentration and formulations showed shear thinning behavior at higher concentrations. Results of rheological characterization suggests, that similar gel strengths can be achieved using much lower concentrations of xyloglucan (2%w/w) as that of 25% Pluronic solutions, with the added advantage of more viscous sols that can prevent leakage of solution from the precorneal area. The gels showed sustained drug release of pilocarpine for over a period of 6 hrs and the drug release from all the gels followed higuchi square root of time kinetics. Miotic effect of pilocarpine was tested in rabbit's eye and for up to 4 hrs significant miotic effect was observed with all the formulations. 1.5% w/w concentration of xyloglucan formulation showed similar miotic response as that of 25% Pluronic formulation. The duration of miotic effect was increased with increasing concentration of xyloglucan. Xyloglucan used in this study has 44% of galactose removal exhibited a thermally reversible transition from sol to gel at temperatures of between 22°C to 27°C [7].

Poly (N-isopropylacrylamide): The thermoreversible phase transition temperature of poly (N-isopropylacrylamide) (PNIPAAm), a well-known thermosensitive polymer is 32°C. Because of its phase transition temperature closer to human body surface temperature, this *in situ* gel forming polymer has been utilized in enhancing ocular absorption of tilisolol [20].

In order to reduce the concentration of temperature triggered *in situ* gelling polymer Pluronic F127 for ocular delivery of timolol maleate, El-Kamel [9], investigated addition of methylcellulose, HPMC, CMC as viscosity enhancing agents. The effect of various isotonicity agents (sodium chloride, Manntol, Sorbitol, Propylene glycol, and Glycerol) and thickening agents on viscosity and ability of the formulations to administer timolol was also evaluated. NaCl increased the viscosity by increasing the CMC (critical micelle concentration) and CMt (critical micelle temperature) of the Pluronic F127 gel resulting in closer packing of Pluronic F127 micelles and gel formation at lower temperatures. Mannitol and sorbitol also increased the viscosity by enhancing the H-bonding; however propylene glycol and glycerol did not show much effect on the viscosity. All the formulations showed pseudoplastic flow character at ocular temperature. Formulations containing thickening agents showed higher viscosity in the following order PF 15%- MC 3%>PF 15%-HPMC 2%>PF 15%-CMC 2.5%> PF-15% (control). Viscosity was increased with increasing the concentrations of additives. Interestingly, the viscosity of 3% methylcellulose containing formulation (PF 15%-MC 3%) was comparable to 25% Pluronic F127 formulation. Gelation of Pluronic occurs by H-bonding of oxygen atom of ether group with water. Thus the addition of compounds containing hydroxyl groups, which augment H-bonding, resulting in increased viscosity and strength of the gel.

Drug release was observed to be decreased with increasing concentration of Pluronic, which may be due to increased number and size of micelles and decreased number and dimensions of water channels, resulting in enhanced cross-linking between neighboring micelles leading to higher viscosity and decreased drug release. Drug release was observed in the following order: PF 15% > PF 15%-CMC 2.5% ≥ PF 15%-HPMC 2% > PF 15%- MC 3% ≥ PF 25%. The slowest drug release from PF 15%-MC 3% may be due to presence of hydrophobic regions in both Pluronic and methylcellulose and inverted thermal behavior i.e. gelation on heating and melt on cooling. The amount of drug in aqueous humor was observed to be higher for *in situ* gel formulations than plain timolol solution. Finally, the formulation containing PF 15%-methylcellulose 3% was best suited as *in situ* gelling system for ocular delivery of timolol maleate [9].

Ion triggered *in situ* gelling polymers: These include polymers whose solution viscosity increases upon exposure to ionic concentration of the tear fluids [18]. 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].

Ion triggered *in situ* gelling polymeric systems are discussed below:

Gellan gum: Gellan gum is commercially known as Gelrite®. Deacetylated gellan gum is an anionic extracellular polysaccharide secreted by *Pseudomonas elodea*. When formulated in aqueous solution, it forms clear gels in the presence of the mono or divalent cations present in the lachrymal fluids [1]. It is one of the most commonly used *in situ* gelling polymers. It has been approved as pharmaceutical excipient. Various mechanisms have been suggested to explain the gelation of gellan gum. In the solution Gelrite® molecules remains weakly associated with each other by vanderwaals bonds and forms double helices at room temperature. When Gelrite® solution comes in contact with the cations, some of the helices forms aggregates mediated by cations, resulting in cross linking of the polymer. Just to mention here that gelation can be induced more effectively in presence of divalent ions such as magnesium or calcium than the monovalent cations.

Gelrite® forms a low viscosity solution, which upon administration to eye transforms in to clear gel by interaction/crosslinking of negatively charged polysaccharide helices of Gelrite® with monovalent and divalent cations (Na^+, K^+, Ca^+) present in the tear fluid. Fluorometry and γ-scintigraphy techniques have confirmed enhanced corneal contact times of gellan gum based formulations as compared to saline or various eye drops formulations. A controlled-release glaucoma formulation consist of Gelrite® is marketed by Merck under the brand name Blocarden® Depot (Timoptic®) [41].

Balasubramaniam et al. [13], formulated an ion-activated *in situ* gelling ophthalmic delivery system containing fluoroquinolone antibiotic ciprofloxacin hydrochloride using gellan gum alone and in combinations with sodium alginate as gelling agents. The developed formulations showed *in vitro* sustained release of the drug over a period of 8 hrs. However, the combined formulation i.e. gellan and sodium alginate both, did not offer any advantage over the formulations based on gellan alone. The formulations were observed to be therapeutically efficacious as it successfully inhibited the growth of *S. aureus* and *P. aeruginosa* microorganisms for 24 hrs [13].

In another study Balasubramaniam et al. [13], developed an ophthalmic delivery system of the NSAID indomethacin, using gellan gum as ion activated *in situ* gelling system. The formulations showed *in vitro* sustained release of the drug over 8 hrs and were therapeutically efficacious in a uveitis induced rabbit eye model [13]. In both cases the formulations were devoid of any deleterious effect on the ocular tissues of rabbit's eyes. Further, no leakage of the gelled material and no unwanted irritation were observed from any part of the eye from the formulations on the ocular tissues. The authors suggested that these formulations can be viewed as a viable alternative to conventional eye drops because of their ability to enhance precorneal residence time and thereby ocular bioavailability. The ease of administration coupled with the ability to provide sustained release of the drug is another advantage, as it could probably result in less frequent administration, thus enhances patient compliance.

Alginic acid/sodium alginate: Sodium alginate is a natural hydrophilic polysaccharide approved by FDA for human use as wound dressing material and as food additives consist of (1→4) linked β-D-mannuronic acid (M) and α-L guluronic acid (G) units of varying composition and sequence. A high guluronic acid content shows good gelation and thus reduces the total amount of alginate. On interaction of G-units with calcium ions present in the ocular fluid, alginate forms inhomogeneous 3-dimensional ionotropic gel. The physicochemical properties of the gels, e.g. mechanical strength and porosity, depends on the concentration and viscosity of the alginate solution, G: M ratios, type of ionic crosslinker (bi or poly-valent cations) etc.

Sodium alginate forms three dimensional hydrogel and the high G content alginate forms a low viscosity, free-flowing liquid at concentrations suitable for gel formation in the lacrimal fluid [16]. Alginate transforms into stable gel upon exposure to divalent cations, which is not easily eroded by tear fluid.

Sechoy et al. [22], compared alginic acid and hydroxyethylcellulose (HEC) as *in situ* gelling system for prolonged delivery of carteolol. However, the viscosity of both alginic acid and HEC based formations was almost similar but the alginic acid showed slower *in vitro* drug diffusion of carteolol and better adhesive properties with mucin coated corneal surface as compared to HEC. The slower diffusion of carteolol is may be due to an ionic interaction between carteolol and alginic acid. Maximal decrease in IOP was obtained 1 hr after instillation for both 1% carteolol-alginic acid and 1% carteolol solutions, but carteolol-alginic acid formulations significantly extended and showed greater pressure reducing effect as compared to plain carteolol solutions. Interestingly, the pharmacokinetic profile of carteolol was similar in aqueous humor, iris: ciliary body and plasma for both treatments. However, higher carteolol concentrations were observed in aqueous humor for alginic acid formulations. Almost 50% increase in the $AUC_{0\rightarrow8}$ hr was observed due to presence of alginic acid in the formulation. No change was observed in carteolol concentration of iris: ciliary body after 15 days administration for both formulations. Carteolol-alginic acid eye drops showed excellent ocular tolerance without any irritation or histopathological changes. The peak plasma concentration of carteolol was lower for alginic acid formulations as compared to plain carteolol solution. The alginic acid based *in situ* gelling systems are able to reduce the frequency of carteolol dosing from twice to only once a day [22].

Cohen et al. [23], demonstrated *in situ* gelation of aqueous solution of sodium alginate in the eye, without the addition of external calcium ions or other bivalent/polyvalent cations. The % of guluronic acid in polymer backbone plays a major role in alginate gelation and drug release. Alginates with guluronic acid contents > 65% gelled instantaneously, whereas with low guluronic acid contents gelled slowly and forms weak gels. *In vitro* release of pilocarpine from the gel was observed for 24 hrs mainly via diffusion with negligible dissolution of the gel for first 12 hrs. Gel with high content of guluronic acid showed IOP reduction in rabbit eyes for longer duration (10 hrs) as compared to the pilocarpine nitrate solution (3 hrs). No significant difference in duration or extent was observed in the pressure reduction

from pilocarpine nitrate solution or the gel with low guluronic acid content. The study suggested that the alginates with high guluronic acid content can serve as *in situ* gelling ophthalmic drug delivery systems for overcoming the poor bioavailability of drugs caused by dilution and drainage of the eye drop solutions from the eye [23].

The rheology of ion triggered *in situ* gelling systems based on gelrite® for ocular drug delivery has been studied by Carlfors et al. [42]. The study suggested that rapid gelation in the eye is necessary for successful application of *in situ* gelling systems. Since sol-gel transition occurs on ion uptake by the polymers, which depends on the osmotic gradient across the surface of the gel, thus the osmolality of the formulations may have an influence on the ocular performance of the gels. To check the effect of osmolality glycerol was used as tonicity agent and the precorneal contact time was determined in human and rabbits eyes. The contact time of the gels was observed to be decreased with increasing osmolality. The results from the human contact time studies showed that the gelrite® gels maintained their integrity for several hours in comparison to *in situ* gels based on carbomer (pH triggered) and Poloxamer (temperature triggered) polymers. Interestingly the gels were much better retained in the human eyes as compared to that of rabbit's eyes. The ocular residence time of gels is depend on the rheological properties and viscosity [21,22,42]. The gels showed high elasticity, which was concentration independent at 0.6% gelrite® and above, suggesting longer precorneal contact times. Up to 1% concentration of gelrite® solutions showed pseudoplastic behavior at different shear rates, suggesting that the formulations can be easily administered. The solutions were nonirritating; the reason for high tolerance may be the rapid gel formations in the conjunctival area without affecting sensitive cornea [42].

Ion activated *in situ* gelation of sodium alginate in combination of HPMC for sustained drug delivery gatifloxacin has been developed by Liu et al. To improve patient compliance, amount of alginate was decreased by addition of HPMC as viscosity enhancing agent. *In vitro* sustained drug release over 10 hrs was observed for formulation containing alginate and HPMC in combination. *In vivo* precorneal retention studies also indicated that the alginate-HPMC solution retained the drug better than the alginate or HPMC solutions alone. Excellent ocular tolerance was reported without any sign of irritation with all the formulations [16].

Combination of polymers having different gelation mechanisms: To reduce the amount of polymers required for gelation and to get better gels with improved gelling properties combination of two or more polymers with different gelation mechanism can be used for developing *in situ* drug delivery system. In such an attempt researchers developed a combination of thermosensitive polymers, methylcellulose or HPMC and pH triggered polymer Carbopol. The former polymers exhibited thermal gelation and the latter pH dependent gelation. The final formulation formed an easy flowing formulation, which reversibly gelled with a sol-gel transition between 25˚C and 37˚C as well as with a pH increase from 4.0 to 7.4. Further, the concentration of the *in situ* gelling solution and strength of the gel formed should be such that it can neutralize the dilution effect caused by tear fluid on application [18]. Therefore high concentrations of polymers are required in most of the *in situ* gelling systems. For example, 25% (w/v) Pluronics and 30% (w/v) CAP, concentration is required to form firm gel upon administration in the eye [10]. As the concentration of Carbopol increases in the vehicle, its acidic nature may cause stimulation to the eye tissue. More examples of such systems are discussed in following paragraphs.

Stimulation to the ocular tissues may occur on increasing the concentration of Carbopol in the formulation, thus to reduce its concentration and to achieve better gelation combination of Carbopol and methylcellulose has been used as ocular drug delivery system [44].

Gratieri et al. [44], developed a thermosetting polymer, Poloxamer and mucoadhesive agent chitosan based *in situ* gelling system with improved mechanical and mucoadhesive properties for prolonged ocular retention and treatment of ocular diseases. Chitosan was observed to improve the mechanical strength and texture properties of Poloxamer formulations and also confers mucoadhesive properties in a concentration dependent manner. Poloxamer-chitosan (16:1) formulation showed optimum gelation temperature 32˚C and four fold increased retention in the human eyes as compared to the conventional solution suggesting the increased retention of the gel at the corneal surface [44].

A combination of pH and ion triggered polymers based *in situ* gelling systems has been prepared by blending three different polymers namely Carbopol 940, sodium alginate and guar gum in various concentrations and checked the gel forming capacity and compatibility of the blend. Guar gum is a viscosity enhancing polymer, which also shows sol-gel transition on changing the pH of the solution. Sodium alginate served as ion triggered *in situ* gelling polymer. The formulations were used for ocular drug delivery of timolol maleate. Formulations showed an increase in the viscosity upon exposure to physiological conditions (pH, temperature and ionic concentrations) as present in the eye. The formulations exhibited pseudoplastic or newtonian flow behavior in rheological measurements. In *ex vivo* release studies of timlolol maleate from hydrogels through excised bovine cornea using a modified franz diffusion cell, drug release was observed to be extended upto 5 times than the conventional eye drops formulations. Results suggested that the formulations can be administered in the eye as drops to form hydrogels, which could withstand the sheer force in the cul-de-sac [21].

A novel copolymer, i.e. poly (N-isopropylacrylamide)-chitosan (PNIPAAm-CS) containing timolol maleate based thermosensitive *in situ* gel for ocular drug delivery has been investigated by Cao et al. [20]. The LCST of copolymer was 32°C, i.e. close to the surface temperature of the eye. The C_{max} of timolol maleate in aqueous humor of the rabbit's eye was 2 folds higher from PNIPAAm-CS as compared to the conventional eye drop, and the AUC was also greater for the PNIPAAm-CS gel. The PNIPAAm-CS gel-forming solution of timolol maleate also showed a stronger capacity to reduce the intraocular pressure (IOP) over a period of 12 hrs as

compared to the conventional eye drop of same concentration. Further in the MTT test PNIPAAm-CS gel showed a very little cytotoxicity in the concentration range of 0.5-400 µg/ml. The study suggests that the PNIPAAm-CS can improve the bioavailability, efficacy, and compliance of some eye drugs and thus is a potential material for *in situ* gelation in ocular drug delivery [20].

The suitable compositions of chitosan-PluronicF 127 solution to achieve good gel forming properties and drug release behavior for enhancing the low bioavailability and ocular residence time of ciprofloxacin has been investigated by Varshosaz et al. [12]. In this investigation, various compositions of Pluronic F127 (10-25% w/w) with different molecular weights chitosan (0.1-0.3% w/w) solutions were prepared. The formulation consisted of 15% Pluronic F127 and 0.1% low molecular weight chitosan, was suggested as a suitable ophthalmic preparation for sustained release of ciprofloxacin because of highest release efficiency and an acceptable mean release time. The drug release from the gel followed Higuchi model and Fickian mechanism. It was liquid in non-physiologic conditions (pH 4.0 and 25ºC) and transformed in to the gel form in physiologic conditions (pH 7.4 and 37ºC). The phase transition temperature of this *in situ* gel did not change upon dilution and the zone of inhibition of *Pseudomonas aeruginosa* and *Staphylococcus aureus* was significantly greater for the formulation as compared to the marketed eye drop formulation of ciprofloxacin in the agar diffusion test [12].

Gupta et al. [45], developed temperature and pH-triggered *in situ* ocular delivery system of timolol maleate using Pluronic F127 and chitosan as thermosensitive and pH-sensitive polymers, respectively. The formulation was a clear, isotonic solution that converted into the gel above 35°C and pH 6.9-7.0. The formulation showed significantly higher drug transport across corneal membrane and increased ocular retention time. The study suggested that the developed system is a viable alternative to conventional eye drops for the treatment of glaucoma and various other ocular diseases [45].

In situ gelling formulations based on more than one gelation triggering mechanism by optimizing Carbopol (pH sensitive polymer) and Pluronic (temperature sensitive polymer) concentrations in various ratios for ocular drug delivery of pilocarpine hydrochloride was developed by Lin and Sung, 2000. Carbopol and Pluronic solutions at concentrations equal to or less than 0.2% (w/w) and 13% (w/w), respectively remains free flowing liquids on exposure to physiological conditions (pH 7.4 and 37°C), but form gels even in nonphysiological conditions at concentrations more than 0.5% and 15%, respectively. However, 0.3-0.4% (w/w) Carbopol solution and 14% (w/w) Pluronic solution remains liquid and form gels in physiological conditions. Accordingly, 0.3% and 14% (w/w) concentrations of Carbopol and Pluronic were selected for preparation of *in situ* gelling formulations. Formulations showed pseudoplastic and newtonian flow behavior. All the polymers showed higher shear stress at physiological conditions as compared to nonphysiological conditions, showing the sol-gel transition. Pluronic solution showed only slight increase in shear stress which may be due to lesser concentration of Pluronic solution taken in order to keep free flowing characteristics of the formulation. At physiological conditions, shear stress of Carbopol-Pluronic system was much greater than that of Carbopol or Pluronic solutions alone. The reason for this may be the hydrogen bonding between carboxyl groups of Carbopol with ether group of Pluronic leading to formation of stronger gel. Addition of pilocarpine did not affect the rheological behavior of the solutions significantly. *In vitro* drug release kinetics suggested that release occurred primarily by diffusion and Carbopol-Pluronic system had better ability to sustain the release of the drug as compared to individual polymers [10].

Qi et al. [8], formulated mucoadhesive *in situ* gelling ophthalmic drug delivery systems containing puerarin based on thermosensitive (Poloxamer 407 and 188) and pH sensitive polymers (Carbopol). Addition of Carbopol enhanced the mucoadhesion force without affecting the rheological properties of the gel. Poloxamer 407 and 188 were used as thermosensitive polymers and Carbopol 1342 P NF was used as pH sensitive polymer. The combined solutions formed gels under physiological conditions and showed better ability to retain the drug at ocular surface for relatively longer time as compared to the Poloxamer analogs or Carbopol alone [8].

Carbopol solution in physiological and nonphysiological conditions showed pseudoplastic and newtonian flow behavior, respectively, whereas, Pluronic solutions in both physiological and nonphysiological conditions showed Newtonian flow behavior. The Carbopol-Pluronic combined system showed pseudoplastic flow behaviour at physiological conditions [46].

A copolymer Pluronic F127-g-poly (acrylic acid) was used as *in situ* gelling vehicle to prolong the resident time and improved the bioavailability of the ocularly applied drugs. The drug release rates were observed to be decreased on increasing the concentration of copolymer solution and molar ratio of acrylic acid/Pluronic and the gel dissolution majorly influenced the release rate of the drug. The gel showed 5 and 2.6 folds increase in drug resident time and the total resident amount in rabbit's conjunctival sac as compared to the eye drops, suggesting the bioadhesive character of the gel. The authors suggested that the Pluronic-g- poly (acrylic acid) copolymer can improve drug bioavailability and thus can serve as a promising *in situ* gelling vehicle for ophthalmic drug delivery system [18].

A high concentration (20 to 25%w/v) of thermo-reversible gelling polymer Pluronic F127 is required for *in situ* gelation but it causes irritation to the eye. Thus to reduce the concentration of Pluronic F127, Shastri et al. [47], combined it with polymers like HPMC as a viscosity increasing agent or with polymers such as Carbopol 940, xanthan gum, and sodium alginate (high glucuronic acid content) for pH and cation-triggered sol-gel transition, respectively. The study suggested that by combining Pluronic F127 with other *in situ* gelling or viscosity enhancing polymers, not only the concentration of Pluronic F127 can be reduced from 25% to 15% w/v but also the individual polymer concentrations (i.e. carbomer and sodium alginate) can also be reduced without compromising the *in vitro* gelation capacity as well as overall rheology of the system [47].

Kumar et al. [48], developed an *in situ* gelling ocular drug delivery system based on combination of methylcellulose or HPMC and Carbopol to reduce the total polymer content of the formulation. The concept was to induce gelation both by pH and temperature changes. In this formulation methylcellulose or HPMC were taken as temperature triggered *in situ* gelling polymers and Carbopol served as pH triggered *in situ* gelling polymer. Methylcellulose is a viscosity enhancing polymer, shows a sol to gel transition in aqueous solution at temperature around 50-55°C [48]. The formulation developed was an easy flowing solution which can be reversibly gelled in a sol-gel transition with an increase in pH from 4.0 to physiological pH 7.4 and at a temperature between 25 and 37°C. A probable mechanism of pH induced gelation is with an increase in pH, the buffering capacity of tear fluid transforms the polyacrylic acid solution in to a gel, whereas thermal gelation may be induced by decreased degree of hydration of methylcellulose, along with a conformational alteration of the polymer structure with a temperature rise [1]. On investigation of rheological properties of the formulation it was found that the sol-gel transition occurred primarily by an increase in pH due to the presence of Carbopol, whereas the temperature induced gelation was occurred only at very low shear rates. It is worth to mention here that the *in situ* gelling properties and rheological behaviors can be achieved for above discussed combination polymers based *in situ* gelling systems at a reduced Carbopol concentration by addition of a suitable viscosity enhancing polymer [10].

In 2004 Lin et al. [25], prepared a series of sodium alginate and Pluronic F127 based solutions as the *in situ* gelling vehicles for ophthalmic delivery of pilocarpine. The formulation showed that the optimum concentration of sodium alginate solution for the *in situ* gelation was 2% w/w and that for Pluronic F127 it was 14% (w/w). Whereas, the mixture of 0.1% sodium alginate and 14% Pluronic F127 solutions was free flowing at pH 4.0 and 25°C and showed a significant increase in gel strength in the physiological condition; this gel mixture was also found to flow freely under nonphysiological conditions. From the results of various studies it was observed that the alginate-Pluronic F127 solution retained pilocarpine better than the sodium alginate or Pluronic solutions alone [25].

In situ thermosensitive hydrogel of chitosan and disodium α-D-Glucose l-phosphate (DGP) containing levocetrizine dihydrochloride for anti-allergic conjunctivitis has been evaluated by Chen et al. [49]. They examined gelation time, gelation temperature, and morphology for hydrogel formation. Modulation in concentration of chitosan, DGP and drug affected the sol-gel phase transition behavior. Optimized formulation had prolonged ocular residence time and provided sustained release with initial rapid release. Formulation showed high corneal permeation of the drug with excellent ocular tolerance without irritancy in *in vivo* study as compared to the conventional eye drop. The study suggests that the formulation is a promising ophthalmic delivery system [49].

For treatment of glaucoma Li et al. [50], developed and evaluated the brinzolamide drug-resin loaded thermosensitive *in situ* gelling system. In this formulation drug combined with ion exchange resin produced a new compound but not a physical mixture which prolonged the drug retention time at the ocular surface. The formulation was prepared with Poloxamer F127 (as gelling agent) in combination with Carbopol 934P (as viscosity enhancing agent) by cold method. The formulation was stable; nonirritant to rabbit's eye, exhibited sol-gel transition at 33.2±1.1°C and showed sustained release over a period of 8 hrs. The formulation significantly increased the bioavailability of drug and considered as safe for ocular drug delivery [50].

Patel et al. [51], developed a biodegradable, injectable *in situ* thermosensitive hydrogel as depot for sustained delivery of protein therapeutics in posterior eye segment for treatment of neovascular diseases. Triblock (TB) polycaprolactone-polyethylene glycol-polycaprolactone [(PCL-PEG-PCL), BAB] and pentablock copolymers (PBCs) polylactic acid (PLA) [(PLA-PCL-PEG-PCL-PLA), CBABC] and [(PEG-PCL-PLA-PCL-PEG), ABCBA] were synthesized. Polymers were examined for their thermosensitive behavior, molecular weight, hydrophobicity and block arrangement on polymer crystallinity, sol-gel transition, micelle size, viscosity, *in vitro* drug release. PBCs have faster rate of degradation relative to TB due to the presence of PLA block in PBCs which significantly reduces the crystallinity. PBCs also showed sustained release of IgG for more than 20 days as compared to TB. PBCs exhibited excellent cell viability and biocompatibility on human retinal pigment epithelial cell line and mouse macrophage cells. Since PBCs showed biodegradability, biocompatibility, thermosensitivity, ease of handling, hence it can be considered as potential biomaterial for sustained delivery of drugs not only for the posterior eye segment but also for anterior segment disorders [51].

Sparfloxacin loaded *in situ* gelling system containing sodium alginate as ion sensitive polymer and methylcellulose as viscosity enhancing agent was formulated by Khan et al. [52]. Optimized formulation was in solution form at pH 4.7 and converted to gel form at pH 7.4 in tear fluid. Results indicated sustained release of drug over a period of 24hrs. The formulation was found to be nonirritant, showed significant antimicrobial effect and good ocular tolerance with enhanced corneal permeation. Also it was stable for longer period of time with shelf life of 2.28 years [52].

In Mohan et al. [53] described the formulation and evaluation of a combined pH-triggered, thermoreversible and ion activated *in situ* gelling ophthalmic delivery system of a fluroquinolone antibacterial agent, ciprofloxacin. Polyacrylic acid (Carbopol 940), Pluronic F127 and gellan gum were used for pH-triggered *in situ* gelation, thermoreversible gelation and ion activated system, respectively. HPMC was added with Carbopol as viscosity enhancer and in combination of Pluronic F127 for reducing the concentration of Pluronic F127. Gelrite® was used for cation induced gelation (0.6%). The Gelrite® formulation showed long duration of release followed by combination of Carbopol, HPMC and Pluronic F127 and HPMC [53].

Miscellaneous Approaches of *In Situ* Gelling Systems

Various novel drug delivery systems are used for sustained drug delivery by *in situ* gelling system like use of mucoadhesive polymers, polymer coated Nanoparticles and Liposomal formulations are used. These delivery systems delay the elimination of active ingredient from eye and also improve corneal penetration of drug molecule.

Liposome incorporated *in situ* gel

Active ingredients encapsulated in lipid vesicles like liposome allow not only improved solubility and transport of drug through cornea, but is also a tool for prolong and controlled delivery of drug. Gel and liposome based formulations containing ciprofloxacin (CPFX) were developed by Budai et al. [25], to minimize tear driven dilution in the conjunctival sac. The bioadhesive polymer such as poly (vinyl alcohol) and polymethacrylic acid derivatives were used for gel preparation and lecithin and α-L-dipalmithoyl-phosphatidylcholine provided the encapsulating agent for drug into liposome. Encapsulation of the CPFX into liposomes prolonged the *in vitro* release of the antibacterial agent from liposomal vesicles and by use of liposomal formulation, higher drug concentration can be achieved at the site of action along with prolonged contact time, thus ocular bioavailability can be improved [54].

Yu et al. [55], developed timolol maleate (TM) liposomes incorporated ion sensitive deacetylated gellan gum gel. Liposomes were prepared by reverse evaporation coupled with pH gradient technique (REVPR) and were found to be round and uniform shape in TEM. Timolol maleate loaded liposomes incorporated in *in situ* gel showed longer retention time on corneal surface, nonirritant to ocular tissue, 1.93 folds increase in permeability coefficient and quickly reduced the intraocular pressure as compared to timolol maleate eye drops. The optimized formulation was prepared with TM 0.25 wt%, soyabean phosphatidylcholine 2.0 wt%, cholesterol 0.75 wt%, deacetylated gellan gum 4.0 wt%. Encapsulation efficiency of liposomes was found to be 47% and particle size was 136 nm with no charge. After administration of eye drops, biggest efficacy occurred after 30 min and diminished after 240 min. Formulation showed longer retention time than commercial drops, TM liposomes, and TM gel. The study suggested that the TM liposome incorporated *in situ* gels had great potential for ocular delivery with extended retention time; enhance bioavailability and corneal permeability [55].

Cyclodextrin incorporated *in situ* gel

Fernandez F et al. [56], formulated the inclusion complex of fluconazole in hydroxyl-propyl beta cyclodextrin (HPBCD) and in sulfobutyl ether beta cyclodextrin (SBECD) then incorporated in ion sensitive gellan gum and κ-carrageenan *in situ* gels. HPBCD and SBECD showed low cell cytotoxicity in keratocytes as assessed by the label free xCELLigence system for real time monitoring. At the ocular surface lacrimal fluid causes phase transition resulting in *in situ* gelation, which further resulted in controlled drug release [56].

Micelles incorporated *in situ* gel

Famili et al. [57], developed triamcinolone acetonide (TA) micelles incorporated in reverse thermal gel (RTG) as an injectable ocular drug delivery system for sustained release of drug for upto one year. The RTG chemistry exhibits a novel [poly (nisopropylacrylamide), PNIPAAm] polymer coupled to a [poly (serinol hexamethylene urea), PSHU] backbone, for complete physiological clearance of system during degradation. PEGylated polyurethane triblock copolymer based micelle system was fabricated through a filter extrusion method with favorable TA encapsulation capacity. Then micelles were incorporated in RTG. RTG and micelles combined system was well tolerated by rats after intravitreal injection. Results indicated the good biocompatibility, degradability, ease of administration and long term TA release profile than current conventional corticosteroid preparations [57].

Nanoparticles incorporated *in situ* gel

Recently, nanoparticles have been employed to address issues related to topical formulations. These represent promising drug carrier for targeting ocular tissues by remaining at the site of application (cul-de-sac) and providing prolonged release of active ingredient by particle degradation or erosion, drug diffusion or a combination of both, depending on the biodegradable or inert nature of the polymer. The acetazolamide (ACZ) containing pH triggered polymeric nanoparticulate *in situ* gel (NP-ISG) to enhance the permeation across ocular barriers and enhance the residence time on ocular surface was developed by Singh et al. [58]. Optimized nanoparticles were prepared by nanoprecipitation method and dispersed in Carbopol 934P for the formulation of nanoparticulate *in situ* gels (NP-ISG1-NP-ISG5). *Ex vivo* transcorneal permeation study showed higher permeation of ACZ from NP-ISG5 and nanoparticles as compared to the eye drops and ACZ suspension. Optimized formulation i.e. NP-ISG5 exhibited prolonged residence time, sustained release for up to 8hrs, higher permeation, nonirritant property and significantly decreased intra ocular pressure than eye drops. Novel nanotechnology based developed ocular *in situ* gel formulation could be considered as safe, efficacious and patient complient for management of glaucoma [58].

Lou et al. [59], optimized and evaluated the curcumin loaded albumin nanoparticles encapsulated in thermosensitive *in situ* gel (CurBSANPsGel). Nanoparticles were prepared through desolvation method and gels were prepared through cold method. Varying concentration of Pluronic F127 and Pluronic F68 on sol-gel transition temperature were evaluated by the central composite design and response surface method. The optimized CurBSANPsGel formulation was composed of Pluronic F127 (26%w/w) and Pluronic F68 (4%w/w) as gelling matrix. The optimized formulation was transformed into a semisolid gel above 34.2°C at ocular surface and showed sustained release effect. *In vivo* study showed that formulation can be considered as safe and increase the bioavailability of curcumin in the aqueous humor [59].

In situ hydrogels

Since the development of hydrogel in 1960s, numerous studies have been done on adapting the hydrogels as biomaterials. Among these studies, the biodegradable hydrogels driven from natural polymers that were susceptible to enzymatic degradation have gained considerable attentions. By using natural polymer, Xu et al. [60], developed *in situ* gelling injectable polysaccharides cross linked hydrogel for Avastin®. Hydrogel was prepared by simple mixing of glycol chitosan and oxidized alginate aqueous solution. *In vitro* degradation study showed that the oxidized alginate in hydrogel, controlled the rate of degradation and increase in concentration of oxidized alginate, decreased the release of

avastin from hydrogel. *In vitro* release study showed sustained release over a period of 3 days with an initial burst release for 4hrs. SDS-PAGE analysis showed that structure stability of avastin released from hydrogel at examined time points was same as native avastin. Results indicated that developed formulation could be potential ocular drug delivery system for avastin with controlled degradation and drug release rate [60].

Luo et al. [61], developed the diclofenac sodium loaded thermosensitive PEG-PCL-PEG (PECE) hydrogel by synthesizing PECE block polymers by coupling MPEG-PCL copolymer using IPDI reagent and sol-gel transition as a function of temperature was monitored by a rheometer. The optimized formulation containing PECE (30%w/v) aqueous solution exhibited sol-gel transition at 35˚C. *In vitro* release profile showed sustained release of encapsulated diclofenac sodium from hydrogel over a period of 7 days. 0.1% w/v drug loaded hydrogel was observed to be nontoxic to HCEC and L929 cells after 24hrs culturing by MTT assay. *In vivo* eye irritation test indicated that the instillation of 30%w/v PECE hydrogel or 0.1% w/v drug loaded PECE hydrogel to rabbit's eye did not show eye irritation within 72hrs. 1.6 fold increment in $AUC_{0\text{-}48h}$ of 0.1% w/v diclofenac sodium loaded PECE hydrogel as compared to commercial 0.1% w/v diclofenac sodium eye drop in *In vivo* study. The study suggested that the optimized formulation can be used as an alternative to conventional eye drops [61].

In 2014, Yu et al. [62] developed covalently cross linked injectable *in situ* PEG hydrogel for sustained delivery of Avastin® to treat corneal neovascularization. PEG hydrogel was prepared through thiol-maleimide reaction utilizing 4 arms PEG-Mal and 4 arm PEG-SH. Hydrogels with different gelling time, pore size, swelling ratio and mechanical property were obtained by manipulating the concentration of 4 arms PEG-SH. *In vitro* release study showed sustained release of Avastin® from PEG hydrogel over a period of 14 days and had no apparent cytotoxicity on L929 cells after 7 days of incubation. LIVE/DEAD assay also indicated normal morphology of encapsulated L929 cells and cells were well spread, grew in PEG hydrogels after incubation of 3, 5, and 7 days. The results suggested that the formulation could be suitable for sustained release of Avastin® to inhibit corneal neovascularization [62].

In situ gelling ocular films/inserts

Ocular films or inserts are preparations with a solid or semi-solid consistency usually composed of a polymeric vehicle containing the drug, whose size and shape are especially designed for ophthalmic application. Abdelkader et al. [63], prepared preservative free naltrexone hydrochloride (NTX) loaded single dose *in situ* gelling ocular film with different amorphous biodegradable polymers for treatment of impaired corneal wound healing, severe dry eye. The films were characterized for physicochemical compatibility, moisture sorption, surface pH, mechanical properties, sterilisability, surface morphology, mucoadhesion, *in vitro* release, conjunctival irritation and accelerated stability at 40˚C/75% relative humidity for 3 months. Prepared formulation had 18 times more stability of NTX than solution form. Glycerin plasticised film formulation showed better mechanical properties than polyethylene glycol (PEG) 400 and triethylcitrate (TEC). Superior mucoadhesion was observed for formulation containing glycerin and PEG 400. Optimized formulation contained carboxymethylcellulose sodium (CMC) and sodium alginate (ALG) had minimal % moisture sorption, good mechanical properties, superior mucoadhesive property, *in vitro* release, excellent chemical stability and minimal conjunctival irritation. Results demonstrated the potential of ocular delivery of these novel formulations for corneal disorders [63].

Nanoemulsified *in situ* gels

Nanoemulsions (NE) as ophthalmic drug delivery systems have been widely used due to its intrinsic advantages such as sustained release of drug to the cornea, higher penetration into the deeper layers of the ocular structure and in aqueous humor through the cornea, as well as ease of sterilization. Ammar et al. [64], prepared and evaluated the dorzolamide hydrochloride containing nanoemulsified *in situ* gel for treatment of glaucoma with prolonged residence time and higher bioavailability. The optimized formulation contained triacetin (7.8%), poloxamer 407 (13.65%), poloxamer 188 (3.41%), miranol C2M (4.55%), and water (70.59%). *In vivo* study on albino rabbits showed better biological performance, faster onset of action, and prolonged effect as compared to drug solution, market preparation and *in situ* gels. This study showed superiority of NE drug delivery system over the eye drops of *in situ* gels for ocular use [64].

For ophthalmic delivery of fluconazole (FLZ), Pathak et al. [65], developed a novel pH triggered nanoemulsified *in situ* gel (NE-ISG) for ocular delivery with enhanced residence time and permeation across ocular barrier for fungal infection. Optimized nanoemulsion (NE4) was prepared by spontaneous emulsification then nanoemulsified sol (NE-ISG1-NE-ISG5) were prepared by dispersing it in Carbopol 934 solution. On the basis of gelation ability and residence time optimized formulation was selected. Significantly higher permeation of FLZ from NE-ISG and NE4 than commercial eye drops was proved by *ex-vivo* transcorneal permeation study. Developed formulation did not damage ocular tissue and were nonirritating to ocular surface showed by HET-CAM test. The results showed higher *in vitro* efficacy, safety and patient compliance with higher permeation and precorneal residence time for treatment of fungal infection [65].

Chitosan and its derivatives

Chitosan is a linear amino polysaccharide of glucosamine and N-acetylglucosamine obtained by partial N-deacetylation of chitin from naturally abundant crustacean shells [66]. It is usually characterized by an average degree of deacetylation. Aqueous acetic acid is generally used as a solvent for chitosan and on dissolution, amino groups of the chitosan become protonated making it positively charged [67]. It is biocompatible, biodegradable, mucoadhesive, easily available, nontoxic, inexpensive and organic solvents are not required for its solubilization [67]. The excellent muoadhesive properties of chitosan are due to the electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces [68]. Further, it has been shown to possess permeation enhancing effect across biological surfaces.

Horvat et al. [69], formulated sodium diclofenac containing mucoadhesive *in situ* gelling drug delivery system with thiolated

poly (aspartic acid) (ThioPASP). ThioPASP increased the residence time of drug on ocular surface by forming disulphide linkage with glycoproteins of mucin and showed strong mucoadhesion at lower concentrations 3%, 5% w/w and addition of small amount of oxidants also improved the mucoadhesion. The effect of thiol groups on the structure, swelling behavior and mucoadhesive character of the gel and drug release profile was studied. Sustained release was observed for up to 24 hrs in the release study performed using franz diffusion cell. The results showed the importance of thiol group and the formulation can be used as *in situ* gels for a once daily dose administration for ocular drug delivery [69].

Gupta et al. [70], formulated timolol maleate loaded chitosan/HPMC based polymer matrix for increasing the ocular retention. The ocular retention was studied on New Zealand rabbits by a noninvasive technique gamma scintigraphy. The formulation was observed to remain at the corneal surface for longer time duration and cleared at a slow rate. Further the formulation was found to be practically nonirritant by hen's egg chorioallantoic membrane test [70].

Characterization Parameter of Ocular *In Situ* Gel Formulation

In situ gelling systems can be evaluated for various characterization parameters such as clarity, pH measurement, gelling capacity, drug content, rheological study, *in vitro* diffusion study, isotonicity, *in vivo* ocular tolerance in rabbits and accelerated stability studies. These parameters are summarized below:

Physical appearance

The formulations should be observed for general appearance i.e. color, odour and for the presence of suspended particulate matter [29]. Preferably the gels should be transparent in appearance.

Gelling capacity

The gelling capacity of formulations can be observed by time taken for gel formation in a vial containing 2.0 ml of freshly prepared simulated tear fluid [28].

Determination of pH

The pH of formulations must be checked using pH meter immediately after preparation [29]. The pH of the gel formulation should preferably be near to ocular pH to avoid ocular irritation and enhance patient compatibility and tolerance.

Viscosity measurement or rheological studies

Viscosity and rheological properties of *in situ* forming gel can be measured by using Brookfield viscometer, Cone and Plate viscometer or some other type of viscometers such as Ostwald's viscometer. The viscosity of these formulations should be 5-1000 m Pas, before gelling and after formation of gel viscosity should have about 50-50,000 m Pas [25,27].

Texture analysis

The gel strength and adhesiveness of *in situ* gel is measured by texture profile analyzer [25]. This study is performed to check the ability of the gel to retain at the ocular surface for prolong duration.

Isotonicity measurement

Isotonicity is important characteristic of the all ophthalmic preparations. It should be maintained to prevent tissue damage and irritation of eye. For isotonicity testing of preparation, formulation is mixed with few drops of blood and observed under microscope and compared with standard ophthalmic preparation [27].

Drug polymer interaction study

Interaction between drug and polymer should be observed to check the compatibility between various ingredients of the formulation by suitable method such as Fourier Transform InfraRed (FTIR) spectroscopy analysis of their physical mixture [27].

In vitro drug release study

In vitro release study of *in situ* gelling system can be carried out using Franz diffusion cell to check the duration of drug release from the formulation [28].

Sterility testing

Eye is a sensitive organ; therefore sterility testing is an important parameter for all ophthalmic preparations. Sterility testing must be performed for aerobic and anaerobic bacteria and fungi by using suitable media under aseptic conditions [29].

Ocular irritancy test

Ocular irritancy of ophthalmic products can be observed by Draize irritancy test to check the ocular tolerance of the preparation. Rabbits are observed periodically for redness, swelling, watering of the eye [27].

Drug content

It is an important parameter to be measured as the formulation should contain the accurate amount of the drug as directed by the physician.

Accelerated stability studies: to check the shelf-life the formulation

Accelerated stability study should be performed as per International Conference on Harmonization (ICH) Guidelines [27].

Commercially Available Ocular *In Situ* Gelling Systems

An *in situ* forming gel Timoptic-XE®, containing timolol meleate in Gelrile® has been commercially available since 1994. This formulation is available in two dosage strengths 0.25% and 0.5% in market. Timoptic-XE®, when applied topically on the eye, reduces the elevated, as well as normal intraocular pressure, whether or not accompanied by glaucoma. Other systems under clinical assessment are DuraSite® and Smart Hydrogel™. DuraSite® uses polycarbophil, a cross-linked poly acrylic acid, to achieve the desired *in situ* gelling property. Drugs, such as diclofenac,

levobunolol and pilocarpine have been reformulated in Durasite® [71].

Other commercially available *in situ* gelling system based on parenteral delivery that offers a wide range of gellation are Regel: depot-technology and Cytoryn. Regel: Depot technology contains human growth hormone (hGH) utilizing Macromed's Regel drug delivery system for treatment of patients with hGHdeficiency. Cytoryn is one of the Macromed's products, which is a novel, peritumoral, injectable depot formulation of interleukin-2 (IL-2) for cancer immunotherapy using Regel drug delivery system [72]. AzaSite (azithromycin ophthalmic solution) 1% is for topical ophthalmic use indicated for the treatment of bacterial conjunctivitis caused by susceptible isolates of the following microorganisms: CDC coryneform group, *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus mitis* group, *Streptococcus pneumonia* (www.azasite.com) [73]. Pilopine HS (pilocarpine hydrochloride ophthalmic gel) is a sterile topical ophthalmic aqueous gel which contains more than 90% water and employs carbopol 940, a synthetic high molecular weight cross-linked polymer of acrylic acid, to impart a high viscosity. It is available in market as 4% aqueous gel used as miotic (parasympathomimetic) to control intraocular pressure. Akten is a opthalmic gel used as local anesthetic for ocular surface anesthesia during ophthalmologic procedures. Akten is available in market as 3.5% (35 mg/mL) Ophthalmic Gel (www.drugs.com) [74-76].

Conclusion

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 *in situ* gelling ophthalmic 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. Finally, these systems can be represented as unique and effective noninvasive ocular drug delivery systems as they can be administered in solution form and undergo gelation at the site of action.

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