AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

In-Situ Gelling System: A Novel Approach for Ocular Drug Delivery

Gourav Rajoria*, Arushi Gupta
1 Department of Pharmaceutics, ISF College of Pharmacy, Moga, Punjab, 142001 India

ABSTRACT

Eye, which is the most vital organ of the body suffer from various eye problems like glaucoma, endopthalmitis, dry eye syndrome, trachoma, keratitis, conjunctivitis etc. Most ocular diseases are treated by topical drug application in the form of solutions, suspensions and ointment. These conventional dosage forms suffer from the problems of poor ocular bioavailability because of dilution and rapid drainage. Prolonged drug delivery can be achieved by various new dosage forms like *in-situ* gel, collagen shield, minidisc, ocular film, ocusert, nanosuspension, nanoparticulate system, liposomes, niosomes, dendrimers, ocular iontophoresis etc. The most successful of these is the *in-situ* forming ophthalmic drug delivery systems prepared from polymers that exhibit reversible liquid-gel phase transition .The aim of this article is to present a concise review of *in-situ* gelling system to overcome all above problems. This review also summarizes various temperature, pH, and ion induced *in-situ* forming polymeric systems used to achieve prolonged contact time of drugs with the cornea and increase their bioavailability.

Keywords: Ophthalmic Solution, *In-Situ*, Hydrogel, Liquid-gel transition.

*Corresponding Author Email: Gouravceutics@gmail.com
Received 22 May 2012, Accepted 14 June 2012

Please cite this article in press as: Rajoria G *et al.*, *In-Situ* Gelling System: A Novel Approach for Ocular Drug Delivery. American Journal of PharmTech Research 2012.

INTRODUCTION

In the development of ocular drug delivery system lot of complications and difficulties are found. The conventional drug delivery such as suspension, ointment, solution show some drawbacks like increase pre-corneal drainage, blurred vision, low bioavailability low residence time. Various problems encountered in poor bioavailability of the eye installed drugs are:

- Binding by the lachrymal proteins,
- Drainage of the instilled solutions,
- Lachrimation and tear turnover,
- Limited corneal area and poor corneal metabolism,
- Non-productive absorption/adsorption,

For the therapeutic treatment of most ocular problems, topical administration clearly seems to be the preferred route, because in case of systemic administration of drugs, only a very small fraction of their total dose reach the eye from the general circulatory system.[1] Even for this fraction, distribution to the inside of the eye is further hindered by the blood-retinal barrier (BRB).

Consequently, there is a window of only ~5 to 7 minutes for any topically introduced drug to be absorbed and in many cases, not more than 2% of the medication introduced to the eye is actually be absorbed [2]. The main biological barrier to penetration of the medication is represented by the cornea. The human cornea is composed of five tissue types with three of them, the epithelium, the endothelium and the inner stroma, being the main barriers to absorption. The rest is washed away and absorbed through the nasolacrimal duct and the mucosal membranes of the nasal, oropharyngeal, and gastrointestinal tract. Figure (1) shows the pre-corneal and intraocular drug movement from topical dosing.

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid pre-corneal elimination of the drug may be overcome by the use of a gel system that are instilled as drops into the eye and undergo a sol-gel transition in the *cul-de-sac.* This new system developed is called *in-situ* gelling system [3]. This system shows various advantages like:

- Like Improved Patient Compliance
- Reduce Dose Frequency
- Increase Bioavailability
- Sustain And Controlled Delivery.

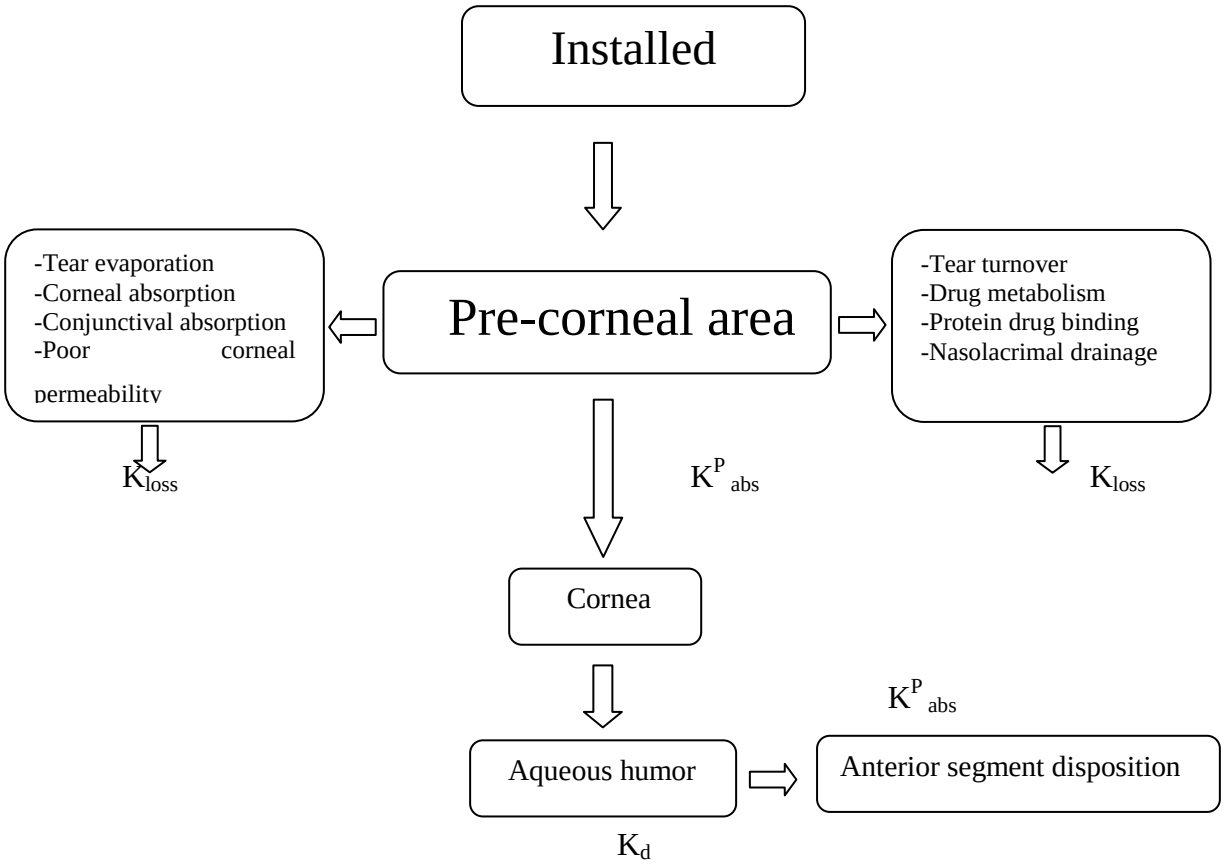


Figure 1:- Model depicting pre-corneal and intraocular drug movement from topical dosing.

The *in-situ* gelling occurs due to different stimuli ion activation (sodium alginate), temperature change (poloxamer, chitosan), pH change (carbopol), environmental change, solvent exchange.

From the early 1970's natural and synthetic polymers began to be used for controlled release formulations. Various natural and synthetic polymers are used for formulation development of *in-situ* forming drug delivery systems which release the drug as they themselves degrade and are sometimes finally absorbed within the body. Use of biodegradable and water soluble polymers for the *in-situ* gel formulations can make them more acceptable and excellent drug delivery systems[4].

***In-Situ* Gelling System**

In-situ forming hydrogels are refer to polymer solution which can be administered as liquid upon instillation and undergo phase transition in the ocular *cul-de-sac* to form viscoelastic gel and this provides a response to environmental changes. Gelation can be triggered by temperature, pH, ions; solvent induced and may be UV induced. Three methods have been employed to cause phase transition on the surface: change in temperature, pH, and electrolyte composition[5]. *In-situ* hydrogels are providing such 'sensor' properties and can undergo reversible sol-gel phase

transitions upon changes in the environmental condition[6]. It is widely accepted that increasing the viscosity of a drug formulation in the pre-corneal region will lead to increased bioavailability, due to slower drainage rate from the cornea[7]. Moreover, the efficacy of ophthalmic hydrogels is mostly based on an increase of ocular residence time via enhanced viscosity and mucoadhesive properties. Since resulted swollen hydrogel is aqueous based, it is very comfortable in the human eye. *In-situ* gels are preferred since they are conveniently dropped in the eye as a solution, where undergo transition into a gel.

Ideally, an *in-situ* gelling system should be a low viscous, free flowing liquid to allow for reproducible administration to the eye as drops, and the gel formed following phase transition should be strong enough to with stand the shear forces in the *cul-de-sac* and demonstrated long residence times in the eye. In order to increase the effectiveness of the drug a dosage form should be chosen which increases the contact time of the drug in the eye. This may then prolonged residence time of the gel formed *in-situ* along with its ability to release drugs in sustained manner will assist in enhancing the bioavailability, reduce systemic absorption and reduce the need for frequent administration leading to improved patient compliance[8]. Different polymers used for this *in-situ* gelling system according to their sensitivity for example- sodium alginate, gelrite, carbopol, poloxamer.

Table 1:- Classification of In-Situ Gelling Systems:-

Sr.no	In-situ gelling systems	Polymers used
1.	Temperature dependent systems	chitosan, pluronics, tetronics, xyloglucans, hydroxypropylmethyl cellulose or hypromellose (HPMC).
2.	pH-triggered systems	Cellulose acetate phthalate (CAP) latex, carbopol, polymethacrilic acid(PMMA), polyethylene glycol (PEG), pseudolatexes.
3.	Ion-activated systems (osmotically induced gelation):	gelrite, gellan, hyaluronic acid, alginates.

THERMOREVERSIBLE HYDROGELS

In thermo sensitive systems gelling of solution is triggered by change in temperature. Sustained drug delivery can be providing by the use of temperature sensitive polymers that change from solution to gel at the temperature of the eye[9] (37ºC). These preparations are liquid at room temperature (20ºC-25ºC) and become gel at body temperature (35ºC-37ºC) due to change in temperature. These temperature sensitive gels are classified into three types i.e., negative temperature sensitive, positively thermo sensitive and thermally reversible gels[10]. Negative temperature-sensitive hydrogels have a lower critical solution temperature (LCST) and contract

upon heating above the LCST i.e. Copolymers of (N-isopropylacrylamide) (NIAAm) show an on-off drug release with on at a low temperature and off at high temperature allowing pulsatile drug release. LCST systems are mainly relevant for controlled release of drugs, and of proteins in particular. Thermosensitive polymers may be fixed on liposome membranes; in that case liposomes exhibit control of their content release[11]. A positive temperature-sensitive hydrogel has an upper critical solution temperature (UCST), such hydrogel contracts upon cooling below the UCST. Polymer networks of poly (acrylic acid) (PAA) and polyacrylamide (PAAm) or poly (acryl amide-co-butyl methacrylate) have positive temperature dependence of swelling[12]. Figure (2) showing the mechanism of temperature sensitive gel formation.

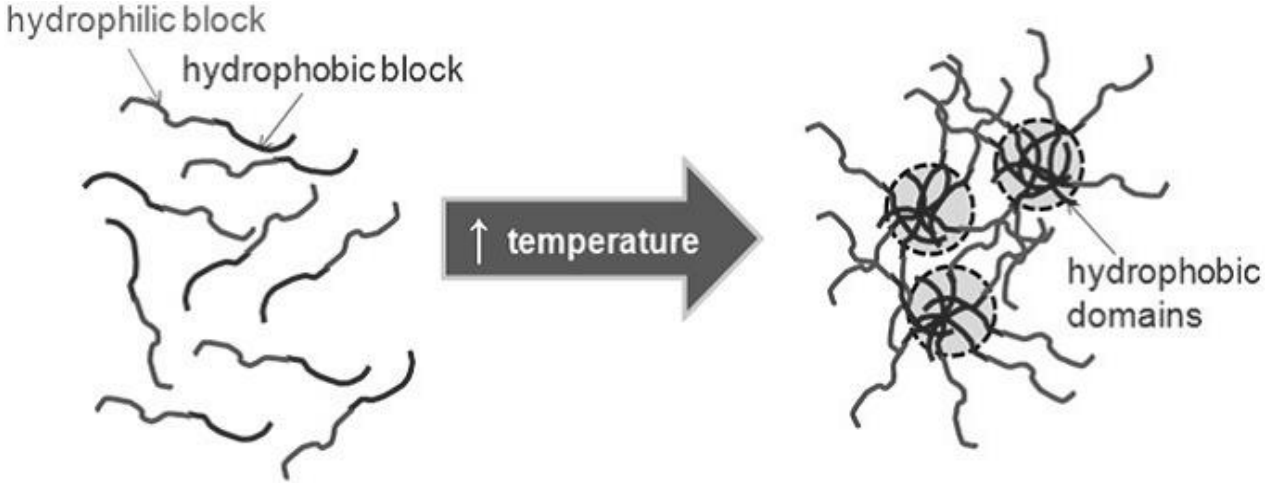


Figure 2:- Mechanism of temperature sensitive system.

Polymers used in temperature sensitive system:-

Poloxamers

Poloxamers are a broad group of compounds that were introduced in the early 1950s as food additives and for pharmaceutical preparations. These water-soluble surfactants are triblock co-polymers prepared from poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide) commercially available as Pluronic®**.** These are the most commonly used thermosetting polymers and could be applicable for the development of effective ophthalmic drug delivery[13]. Figure (3) shows the general structure of the pluronics.

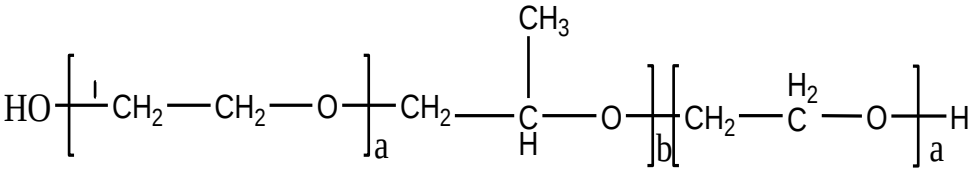


Figure 3: - The general structure for the pluronics.

Depending upon the ratio and the distribution along with the chain of the hydrophilic and hydrophobic subunits, several molecular weights are available having different gelling properties.

Table 2:- Different grades of Poloxamer

Poloxamer	Pluronic ®	A	B	Content of Oxyethylene(%)	Molecular Weight
124	L 44 NF	12	20	44.8-48.6	2200
188	F 68 NF	80	27	79.9-83.7	8400
237	F 87 NF	64	37	70.5-74.3	7959
338	F 108 NF	141	44	81.4-84.9	14600
407	F 127 NF	101	56	71.5-74.9	12600

Pluronics F127, which gives colourless and transparent gel so commonly used in the pharmaceutical industries. Pluronic F 127 is no more damaging to the mouse or rabbit cornea than a physiological saline. The Poloxamers are reported to be well tolerated and non-toxic even though large amounts (25-30%) of polymers are required to obtained a suitable gel. At concentrations of 20% w/v and higher aqueous solutions of Poloxamer-407 remain as a liquid at low temperatures [<15ºC] and yield a highly viscous semisolid gel upon instillation into the cul-de-sac[14]. At low temperatures, the poloxamer forms micellar subunits in solution, and swelling gives rise to large micellar subunits and the creation of cross-linked networks. The result of this phenomenon is a sharp increase in viscosity upon heating.

Three principal mechanisms have been proposed to explain the liquid-gel phase transition after an increase in temperature, including: -

1. Gradual desolvation of the polymer.
2. Increased micellar aggregation.
3. The increased entanglement of the polymeric network.

In this way we sustain our formulation with the help of temperature sensitive method[15].

Desai S.D. and Blanchard J. 1997 developed formulations of 1% pilocarpine hydrochloride containing PF127 alone or with one of the following additives present poly (ethyleneglycol) 4600 (PEG), poly (vinylpyrrolidone) 10000 (PVP), poly (vinylalcohol) 10000 (PVA), methylcellulose 15cP (MC), and hydroxypropyl methyl cellulose 80−120cP (HPMC) and observed that the PEG- and PVP- containing PF127 formulations of pilocarpine Hcl released the drug at a significantly faster rate than the control PF127 formulation, which had no additive present. The PF127 formulations of pilocarpine HCl containing MC or HPMC exhibited the slowest dissolution rates and released the drug slowest[16].

Bochot A. et al., 1998 characterized a new ocular delivery system based on a dispersion of peggylated liposomes in a thermosensitive gel and showed that the thermosensitivity of PF127 was maintained after introducing the liposomes into the gel[17].

Hong-Ru Lin and K.C. Sung, 2000 developed and characterized a series of carbopol- and pluronic based solutions as the *in situ* gelling vehicles for ophthalmic drug delivery. The results demonstrated that the carbopol/pluronic mixture can be used as an *in situ* gelling vehicle to enhance the ocular bioavailability[18].

Desai Suketu D. and Blanchard J, 2000 were prepared a biodegradable polyisobutylcyanoacrylate (PIBCA) colloidal particulate system of pilocarpine and incorporate it into a PF127 based gel delivery system and evaluated its ability to prolong the release of pilocarpine. They concluded that the formulation which contained 1% pilocarpine incorporated into a PF127 gel containing 5% MC increases contact time and bioavailability of pilocarpine[19].

Carmignani C. et al., 2002 evaluate some solubilizing agents Tyloxapol (TY) and Cremophor EL (CR) and one polymer, Pluronic P85 for the preparation of 1.0% tropicamide ophthalmic solutions adjusted at physiologically compatible pH, potentially showing increased eye tolerance, activity, stability compared with standard commercial eye drops[20].

El-Kamel A.H, 2002 developed PF127 based formulations of TM and obtained slowest drug release from 15% P F127 formulations containing 3% methyl cellulose. In vivo study showed that the ocular bioavailability of TM, measured in albino rabbits, increased by 2.5 and 2.4 fold for 25% PF127 gel formulation and 15% PF127 containing 3% MC, respectively, compared with 0.5% TM aqueous solution[21].

Yoo M.K. et al, 2005 studied release of ciprofloxacin from Chondroitin 6-Sulfate Graft-poloxamer. In vitro for ophthalmic drug delivery and concluded that the gelation temperature of C6S-g-Poloxamer copolymer was dependent on the concentration of the graft copolymer and the content of C6S. Ciprofloxacin release behaviour in vitro, as well as the adhesion and morphology of human lens cells (B3), was affected by the introduction of C6S into poloxamer[22].

Qi Hongyi et al., 2006 optimized and developed a thermosensitive ophthalmic gel of puerarin was with 21.0% (w/v) PF127 and 5.0% (w/v) PF68 as the gel matrix, HPCD as the solubilizing agent, NaCl as the isotonicity agent, and BC as bacterial inhibitor. This *in situ* gelling formulation was a free flowing liquid below the room temperature and could convert to a gel that had an eligible gel strength and bioadhesive force after instilled into conjunctival sac[23].

Dumortier G. et al., 2006 developed a thermogelling ophthalmic formulation of cysteine and optimized formulations combined either cysteine (2%) / PF127 (16.5%) or cysteine (2%) /

PF127 (20%) / PF68 (5%) and were characterized by an adequate temperature of gelification (TG) (25.9°C and 26.9°C, respectively), an important gel strength (5.1daN and 5.3daN, respectively) and a drastic increase in the apparent viscosity between 24°C and 32°C (multiplication factor of 78 and 77-fold, respectively). Cysteine addition produced only slight but significant decrease in temperature of gelling and increase in gel strength[24].

Gupta H et al., 2007 developed a temperature and pH triggered novel *in situ* gel system using poloxamer and chitosan of TM[25]. Developed formulation was clear, isotonic solution that converted into gel at temperatures above 35º C and pH 6.9–7.0.

Ma Wen-di et al., 2008 were studied pluronic-g-poly (acrylic acid) copolymers as a temperature-responsive *in situ* gelling vehicle for an ophthalmic drug delivery system. In vivo experimental results, along with the rheological and in vitro drug release studies, demonstrated that *in situ* gels containing pluronic-g-PAA copolymer may significantly prolong the pre-corneal resident time, and may further improve ocular drug bioavailability[26].

Vehanen K et al., 2008 investigated the use of poloxamers in peribulbar injection for controlled drug delivery and concluded that poloxamer was well tolerated in peribulbar injections and did not cause acute toxicity at the site of injection[27].

Mayol L. et al., 2008 studied influence of hyaluronic acid (HA) on the gelation properties of poloxamers and concluded that the addition of low molecular weight HA into poloxamers blends as a useful tool to engineer thermosensitive and mucoadhesive polymeric platforms for sustained drug delivery[28].

Mansour M et al., 2008 developed poloxamer-based *in situ* gelling formulations of ciprofloxacin hydrochloride (HCl) using different concentrations of PF127 and PF68. Which Showed optimum release and mucoadhesion properties and improved ocular bioavailability as evidenced by an enhanced therapeutic response compared with the marketed conventional eye drops[29].

Cao F. et al., 2010 developed new method for ophthalmic delivery of azithromycin by poloxamer/carbopol - based *in situ* gelling system. Addition of carbopol 974P (CP 974P) to the gelling systems increased the solubility of azithromycin by salt effect and enhanced the mucoadhesive property of the systems[30].

Gratieri T et al., 2010 investigated *in situ* forming gel comprised of the combination of a thermosetting polymer, poly (ethylene oxide) – poly (propylene oxide) – poly (ethylene oxide) (PEO–PPO–PEO, poloxamer), with a mucoadhesive agent (chitosan) [31].

Ammar HO. et al., 2010 developed dorzolamide hydrochloride *in situ* gel nanoemulsion for ocular delivery and concluded the enhanced efficacy of dorzolamide HCl[32].

Asasutjarita. R.et al., Optimization and evaluation of thermoresponsive diclofenac sodium ophthalmic in situ gels. In this research poloxamer/carbopol used, poloxamer increase the viscosity and carbopol increase the solubility of the drug diclofenac sodium by salt effect and enhanced the mucoadhesive property of the systems[33].

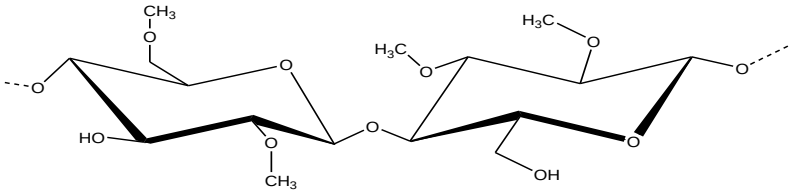
Varshosaz Jet al., Designing of a Thermo sensitive Chitosan/Poloxamer *In Situ* Gel for Ocular Delivery of Ciprofloxacin. *In situ* gel-forming eye-drop using 15% Pluronic F127 as the gelling agent and 0.1% low molecular weight of chitosan as a viscosity enhancing agent[34].

Qian, Y et al., developed *in situ* gelling ophthalmic drug delivery system for methazolamide. In vitro release studies demonstrated a diffusion-controlled release of methazolamide from the poloxamer solutions over a period of 10 hours. In vivo evaluation indicated that the poloxamer solutions had a better ability to retain drug than methazolamide eye drops[35].

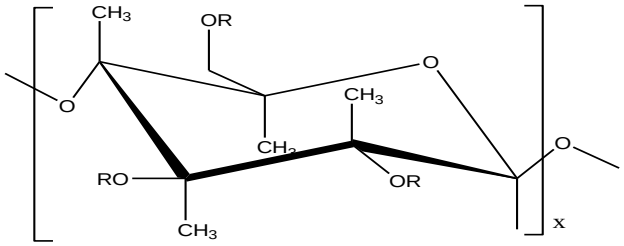
Polysaccharides:-

Cellulose derivatives

Thermo reversible gels can be prepared with naturally occurring polymers. Most of natural polymer aqueous solutions form a gel phase when their temperature is lowered. Some examples of natural polymers exhibiting a sol–gel transition include gelatin and carrageenan. At elevated temperatures, these polymers adopt a random coil conformation in solution. Upon cooling, a continuous network is formed by partial helix formation[36]. Some cellulose derivatives are an exception to this gelation mechanism. At low concentrations (1–10wt. %), their aqueous solutions are liquid at low temperature, but gel upon heating. MC and HPMC are typical examples of such polymers.



Methylcellulose



R = CH_3 or $CH_2CH(CH_3)OH$ or H

Hydroxypropylmethylcellulose (HPMC)

Figure 4:- Schematic structure of methyl cellulose and hydroxyl methyl cellulose.

MC solutions transform into opaque gels between 40 and 50°C, where as HPMC shows phase transition between 75 and 90°C. These phase transition temperatures can be lowered by chemical or physical modifications[37, 38]. For example, NaCl decreases the transition temperature of MC solutions to 32–34°C. Similarly, by reducing the hydroxyl propyl molar substitution of HPMC, its transition temperature can be lowered to ~40°C[39]. Tate et al evaluated MC based constructs as potential tissue engineering scaffolds for the repair of brain defects[40].

Xyloglucan

Xyloglucan is hemicelluloses which is obtained from cell wall of all vascular plants. (in dicotyledonous plants), it is the most abundant hemicellulose in the primary cell wall. Xyloglucan binds to the surface of cellulose micro fibrils and may link them together. It is the target of xyloglucan endo transglycosylase which cuts and ligates xyloglucans, as a means of integrating new xyloglucans into the cell wall. It is also thought to be the target of alpha-expansion, which promotes cell wall enlargement.

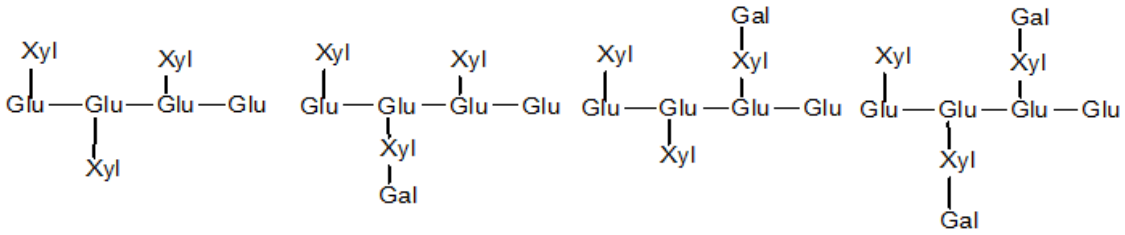


Figure 5:- Structure of repeating units of xyloglucan.

Xyloglucan (figure: 5) is highly water soluble and cannot form ordered crystalline microfibrils as cellulose. Xyloglucan is partially degraded by β-galactosidase, the resultant product exhibits thermally reversible gelation in dilute aqueous solutions[39]. Such behavior does not occur with native Xyloglucan. Gelation is only possible when the galactose removal ratio exceeds ~35%[41]. The transition temperature is inversely related to polymer concentration and the galactose removal ratio[42]. Xyloglucan formulations were assessed for ocular delivery of pilocarpine; using Poloxamer 407 as a positive thermosensitive control. The 1.5 wt. % xyloglucan formulation enhanced the miotic response to a degree similar to that of a 25wt. % Poloxamer 407 gel[43].

Chitosan

Chitosan is a natural polymer obtained by deacetylation of chitin. Chitin is the second most abundant polysaccharides in nature after cellulose. The main commercial sources of chitin are the shell wastes of shrimp, crab, lobster, krill, and squid. It is a biologically safe, non-toxic, biocompatible, and biodegradable polysaccharide. Being a bioadhesive polymer and having antibacterial activity, chitosan is a good candidate for site-specific drug delivery[44]. Chitosan is a

linear polysaccharide consisting of (1-4)-linked 2-amino-2-deoxy-b-D-glucopyranose. Chitosan have the reactive amino groups, reactive hydroxyl groups and chelates many transitional metal ions[45]. Figure (6) shows the various groups of chitosan.

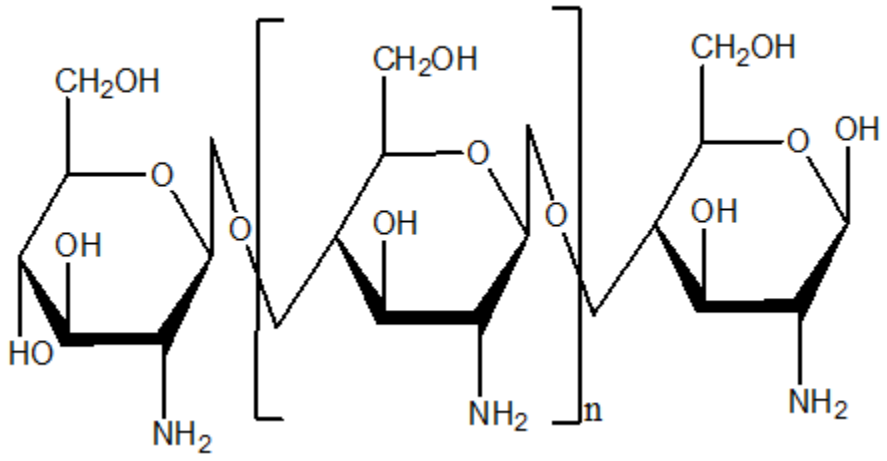


Figure 6:- Structure of chitosan

Chitosan derivatives (N-trimethyl chitosan, mono-N-carboxy methyl chitosan) are effective and safe absorption enhancers to improve mucosal (nasal, peroral) delivery of hydrophilic macromolecules such as peptide and protein drugs and heparins[46].

Felt O. et al., 1999 evaluated the mucoadhesive polysaccharide chitosan as a potential component in ophthalmic gels for enabling increased precorneal drug residence times and observed the 3-fold increase of corneal residence time was achieved in the presence of chitosan when compared to Tobrex®[47].

Ruel-Garie´py E et al., 2000 investigated the physical properties of a chitosan: glycerophosphate (GP) thermosensitive solution which gels at 37°C and evaluate the in vitro release profiles of different model compounds and results indicated that the chitosan:GP thermo sensitive solutions gel rapidly at body temperature, can remain in the sol state at 4°C and can sustain the delivery of macromolecules[48].

D.N. Mishra and R.M. Gilhotra., 2008 designed and characterized the bioadhesive in-situ gelling ocular inserts of gatifloxacin sesquihydrate and concluded that cumulative drug released from the formulation ranged from 95-99% within 8-12h. Thus sustained the drug release for the longest period of time (12h) [49].

Gupta H. et al., 2007 developed ion- and pH-activated novel *in-situ* gel system for sustained ocular drug delivery of Timolol maleate. Chitosan in combination with gellan gum were used as gelling agent and concluded that the developed formulation was found to be enhanced

transcorneal drug permeation, and prolonged the retention at corneal site. It was also found suitable for sustained topical drug delivery to eyes and can prove as better alternative to conventional eye drops for the better drug therapy of glaucoma and other ocular disorders[25].

Mehra GR. et al., 2010 studied in situ gelling solution of pilocarpine based on alginate along with novel bioadhesive tamarind gum and widely used bioadhesive, chitosan and found that the tamarind gum based formulation released about 25 % drug in initial hour and about 80% of the drug was released during the study of 12 h[50].

pH Sensitive Hydrogels

Gelling of the solution is triggered by a change in the pH. Cellulose acetate phthalate (CAP) latex, cross linked acrylic, and derivatives such as Carbomer are used for this method[51]. Cellulose acetate derivatives are the only polymer known to have a buffer capacity that is low enough to gel effectively in the cul-de-sac of the eye. The pH change of about 2.8 units after instillation of the native formulation (pH 4.4) into the tear film leads to an almost instantaneous transformation of the highly fluid latex into viscous gel[6]. Gel is formed at eye pH i.e, (7.4).different polymers are used in the production of pH sensitive formulation like carbopol. Cellulose acetate phthalate latex, poly acrylic acid.

Principle mechanism for pH sensitive gel:-

All the pH-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH. The polymers with a large number of ionizable groups are known as polyelectrolytes. Swelling (figure: 7) of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups[52].

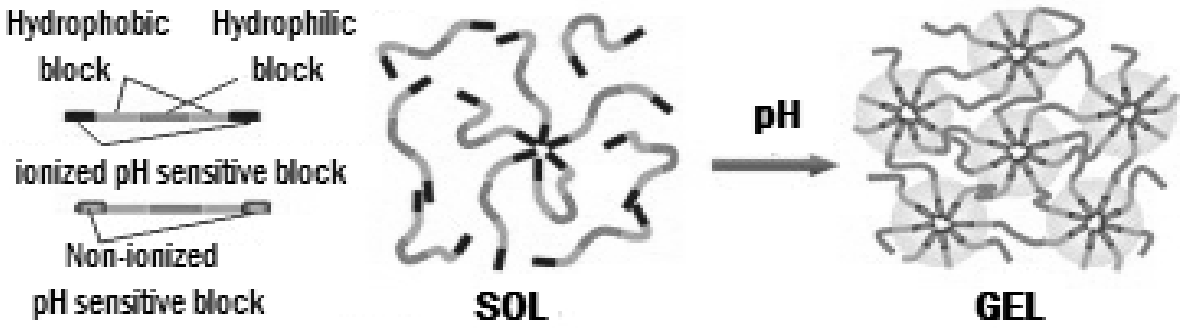


Figure 7:- pH sensitive system.

Polymers used for pH sensitive system:-

Cellulose acetate phthalate latex (CAP-latex)

First preliminary investigations of pH sensitive nanoparticulate systems (latex) for ophthalmic administration began in the early 1980s. The choice of this polymer was determined by the compatibility of the polymer with the active compound, the ability of the CAP latex to be a free running solution at pH 4.2 and a gel at 7.2, and finally[52], but the low pH of the preparation can elicit discomfort in some patients. The gelation capacity of CAP latexes has been visualized in vitro by scanning electron microscopy and in vivo in rabbits by incorporating methylene blue in ophthalmic formulations. The efficacy of a preparation based on pseudolatex has been evaluated by measuring pharmacological responses and precorneal residence time by γ scintigraphy. This technique has clearly demonstrated the superiority of CAP latex over a solution to prolong the corneal residence time of pilocarpine[6]. Figure (8) represent the structure of the cap.

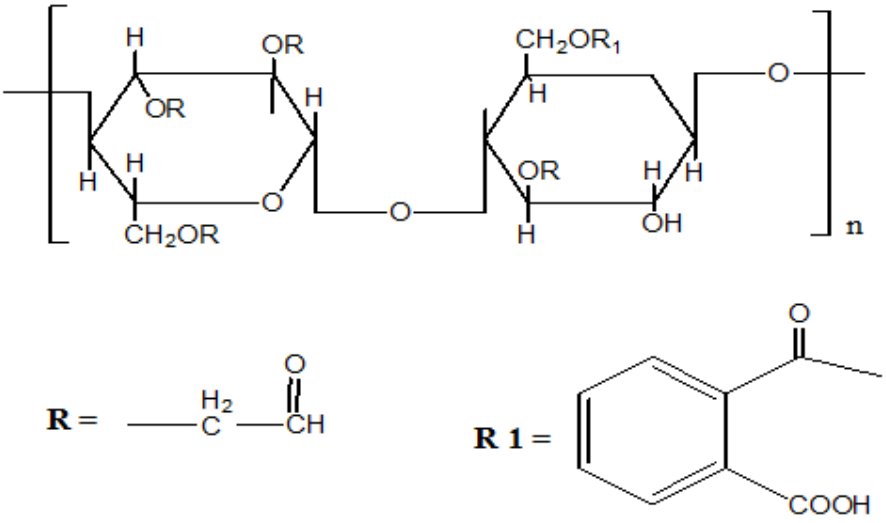


Figure 8:- Structure of cellulose acetate phthalate.

Carbomer

Cross-linked poly (acrylic acid) of high molecular weight, commercially available as Carbopol® (figure: 9), is widely used in ophthalmology to enhance pre-corneal retention to the eye[47]. Carbopol® 934 is a synthetic polymer composed of 62% of carboxyl groups with a high molecular weight (approximately 3×106) formed by repeating units of acrylic acid, cross-linked with either allyl sucrose or allyl ethers of pentaerythritol. Carbopol offers the advantage of exhibiting excellent mucoadhesive properties when compared with other polymers (e.g. cellulose derivatives, and polyvinylalcohol). The mechanisms involved in the mucoadhesion ability of Carbopol have been investigated previously. Four mechanisms of interaction between mucin and poly (acrylic acid) have been described: electrostatic interaction, hydrogen bonding, hydrophobic interaction, and inter diffusion. These mechanisms can be explained by the similar features of the

mucus network and the cross-linked poly (acrylic acid): macromolecular expanded network, negative charges, and significant hydration in aqueous media and significant number of carboxyl groups[53]. The efficacy of Carbopol in enhancing pre-corneal residence time has been extensively studied by incorporating tracers such as sodium fluorescein[54] or active compounds such as pilocarpine or prednisolone. As the concentration of Carbopol increases in the vehicle, its acidic nature may cause stimulation to the eye tissues. In order to reduce the total polymer content and improve the gelling properties, an ocular drug delivery system based on a combination of Carbopol and methylcellulose has been developed[55].

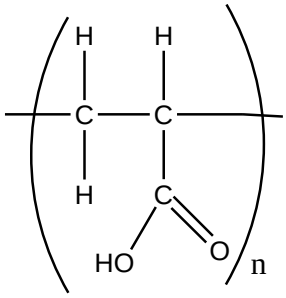


Figure 9:- Schematic structure of Carbopol

Hydroxypropyl methylcellulose and methylcellulose were combined with carbopol to increase the viscosity of the gels and to reduce the concentration of the incorporated carbopol. Controlled release in situ gels consisting of carbopol and cellulose derivatives showed an increase in viscosity, gelling capacity.

Carbopol is a polyacrylic acid (PAA) polymer, which shows a sol to gel transition in aqueous solution as the pH is raised above its pKa of about 5.5[13]. The rheological properties of this system were investigated and sol to gel transition occurred primarily by an increase in pH due to the presence of Carbopol[55]. Also developed a similar delivery system by a combination of carbopol and hydroxypropyl methylcellulose[56]. For both systems it was found that a reduction in the carbopol concentration without compromising the in situ gelling properties as well as over all rheological behaviours can be achieved by adding a suitable viscosity enhancing polymer.

Srividya B.et al., 2001developed pH triggered in situ gelling system for sustained ophthalmic delivery of ofloxacin and concluded that the developed formulation was therapeutically efficacious, stable, non-irritant and provided sustained release of the drug over an 8-h period[57].

Wu Chunjie et al., 2007 developed a carbopol/HPMC – based pH activated in situ gelling ophthalmic system for puerarin and also studied the effect of hydroxypropyl-β-cyclodextrin (HP-β-CD) on the aqueous solubility and in vitro corneal permeation of puerarin. The studies

concluded that combined polymer systems performed better in retaining puerarin than puerarin eye drops[58].

Al-Kassas R.S. and El-Khatib M.M, 2009 designed controlled release ophthalmic delivery systems for ciprofloxacin. The antimicrobial efficiency of the selected formulation against gram-positive and gram-negative organisms including Echerichia coli, Staphylococcus strains and Pseudomonas aeruginosa confirmed that the designed formulation has prolonged the antimicrobial effect of ciprofoxacin and retained its properties against bacteria[59].

Nanjwade B.K. et al., 2009 developed a novel pH-triggered in situ gel for sustained ophthalmic delivery of ketorolac tromethamine. The developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to produce sustained drug release[60].

Gupta H. et al., 2010 developed ion- and pH-activated novel *in-situ* gel system for sustained ocular drug delivery of Timolol maleate. Chitosan in combination with gellan gum were used as gelling agent and concluded that the developed formulation was found to be non-irritant, enhanced transcorneal drug permeation, and prolonged the retention at corneal site[25].

Pandey A, et al, 2010 Development and Optimization of Levobunolol Hydrochloride *In-situ* Gel for Glaucoma Treatment. Carbopol provide phase change from liquid to gel and HMPC provide Mucoadhesive strength and increase viscosity of formulation[61].

Mohanambal E, et al, 2011 Formulation and Evaluation of pH-triggered *in-situ* Gelling System of Levofloxacin. The levofloxain *in-situ* gelling system formulated by using poly acrylic acid (Carbopol 940) in combination with hydroxyl propyl methyl cellulose (HPMC) which acted as viscosity enhancing agent. The developed formulation was stable, non-irritant and provided sustained release over 8-hour period and it is a viable alternative to conventional eye drops[62].

Ion-Sensitive Hydrogels

In ion activated system gelling of solution is triggered by cations present in the present in the eye tear fluid like Na+, Ca++ and Mg++. Generally anionic polymers are used in the formation of ion sensitive drug delivery system[52, 63]. Polymers like sodium alginate, gelrite, tamarind gum, gellen gum are used in these formulations.

Various other polymers like methylcellulose (MC), hydroxyl propyl methyl cellulose (HPMC) are used in combination of these polymers to increase the effect. They provide sustain release of drug by providing mucoadhesivenes.

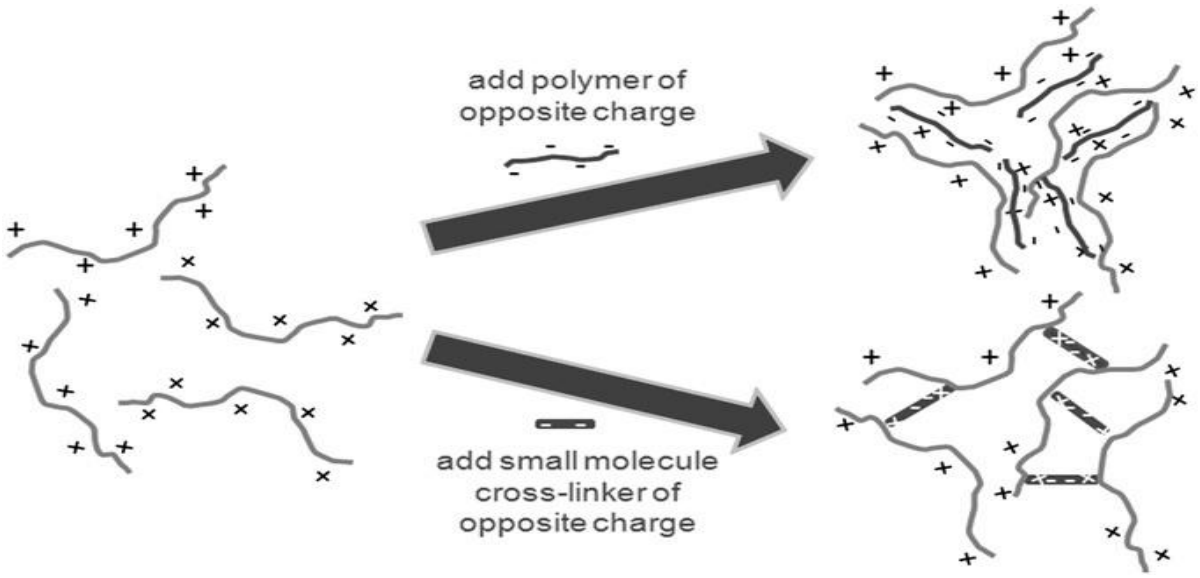


Figure 10:- Mechanism showing Ion activated system.

This system based on the mechanism (figure 10) of ionic interaction of ions of polymer and divalent ions of tear fluid. When anionic polymers come in contact with cationic ions they convert into gel viscosity of solution increases to an extent[64].

Gelrite

Gellan gum (figure: 12) is a linear, anionic hetero polysaccharide secreted by the microbe Sphingomonaselodea (formerly known as Pseudomonaselodea). Gelrite is deacetylated gellan gum which gels upon instillation in the eye due to the presence of cations[65]. The polysaccharide can be produced by aerobic fermentation and then isolated from the fermentation broth by alcohol precipitation. The polymer back bone consists of glucose, glucuronic acid, and rhamnose in the molar ratio 2:1:1[66, 67]. It has the tendency of gelation which is temperature dependent or cations induced .This gelation involves the formation of double helical junction zones followed by aggregation of the double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water[68].

Gelrite has been granted regulatory approval as pharmaceutical excipient and is marketed by Merck in a controlled release glaucoma formulation called Blocarden® Depot (Timoptic®). Formulations with the Gelrite can be administered to ocular mucosa as a low viscosity solution. On contact with cations in tear fluid the formulation will form a clear gel. This is caused by cross linking of the negatively charged polysaccharide helices by monovalent and divalent cations (Na+, K+, Ca+). In anion free aqueous medium, The divalent ions such as magnesium or calcium were superior to monovalent cations in promoting the gelation of the polysaccharide. However the concentration of sodium in tears (2.6g/L) is quite sufficient to induce the gelation. Gelrite has

also provided corneal residence time's superior to those of other hydrogel preparations based on polymers such as cellulosic derivatives or xanthan gum. The rheological properties of gellan gum such as thixotropy, pseudo plasticity, and thermo plasticity are further advantages for its use in ophthalmology[13]. The most important gel-promoting ion in vivo is Na^{+69}.

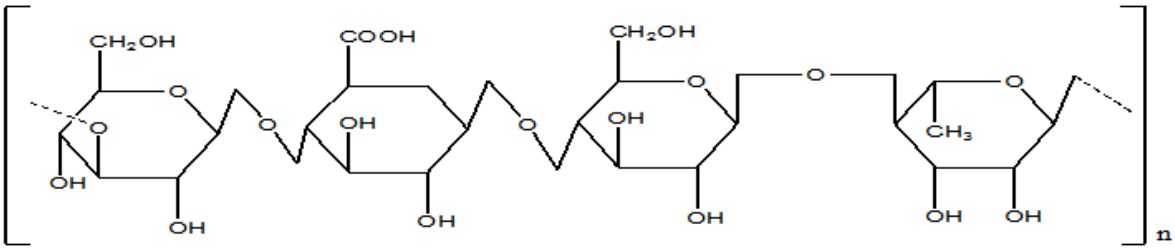


Figure 11:- Schematic structure of gellan gum.

Balasubramaniam J. and Pandit J.K. 2003 developed ion - activated in situ gelling systems for sustained ophthalmic delivery of ciprofloxacin Hcl and concluded that the formulated systems provided sustained release of the drug over an 8-hr period in vitro[71].

Sultana Y et al., 2006 ion-activated, Gelrite based in Situ Ophthalmic Gels of Pefloxacin Mesylate and compared with conventional eye drops and concluded that the system was capable for effective and controlled management of conjunctivitis for 12hr[72].

Liu Y. et al., 2010 investigated in Situ Gelling Gelrite/Alginate Formulations as Vehicles for ophthalmic drug delivery and found that the optimum concentration of Gelrite solution for the in situ gel forming delivery systems was 0.3% (w/w) and that for alginate solution was 1.4% (w/w). The mixture of 0.2% Gelrite and 0.6% alginate solutions showed a significant enhancement in gel strength at physiological condition[73].

Vodithala S. et al., 2010 developed ion activated ocular in situ gels of ketorolac tromethamine using Gelrite as a polymer and concluded that the developed formulations showed sustained release of drug for upto 6hrs. The formulations were found to be non-irritating with no ocular dam-age[74].

Rajas N, J et al., 2011. In situ ophthalmic gels a developing trend. Levofloxacin hemihydrate which is a broad spectrum anti bacterial agent used in the treatment of ocular infections was successfully formulated as in situ gel using Gelrite as polymer. The formulated systems provided sustained release of the drug for more than 8hr period. The developed formulation is a viable alternative to conventional eye drop due to its ability to enhance bioavailability through its longer pre-corneal residence time and ability to sustain release of the drug[53].

Geethalakshmi A, et al., 2012. Sustained ocular delivery of brimonidine tartrate using ion activated in situ gelling system. Gelrite is used as gelling agent in the preparation of brimonidine tartrate to enhance the viscosity of formulation. Also provide sustain release of drug in the eye[74].

Rupenthal ID, et al., 2011 Jun. Comparison of ion-activated in situ gelling systems for ocular drug delivery. Part 2: Precorneal retention and in vivo pharmacodynamic study. Various polymers used like gellan gum, xanthan gum and carrageenan in the formulation of ion activated system which provide sustained release of drug[75].

Alginates

Alginates (figure: 13) consist of (1→4) linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues of widely varying composition and sequence. By partial acid hydrolysis, alginate was separated into three fractions. Alginate with a high guluronic acid content will improve the gelling properties and reduce the total polymer to be introduced into the eye. The alginate forms 3-dimensional ionotropic hydrogel matrices, generally by the preferential interaction of calcium ions with the G moieties resulting in the formation of in homogeneous gel[52]. The characteristic properties of these hydrogels, such as mechanical strength and porosity, are dependent upon the G: M ratios, type of ionic cross linker (bio or polyvalent cations), concentration and viscosity of the initial alginate solution. Alginates were approved by the regulatory authorities such as the Food and Drug Administration, for human use as wound dressing material and as food additives[13].

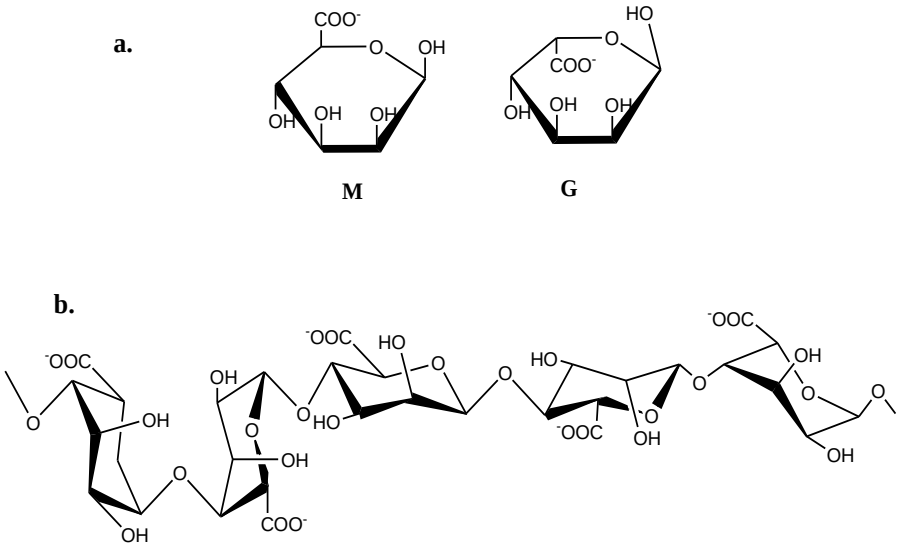


Figure 12:- schematic structure of (a) Alginate monomers (b) Chain conformation

Cohen S. et al., 1997 developed a novel in situ forming ophthalmic drug delivery system from alginates undergoing gelation in the eye and concluded that Pilocarpine is released slowly from

alginate gels, over a period of 24h, and the release occurs mostly via diffusion from the gels. Dissolution of the hydrogels in the releasing media was negligible for the first 12h of incubation at 37^0C[76].

D.N. Mishra and R.M. Gilhotra. 2008 designed and characterized the bioadhesive *in-situ* gelling ocular inserts of gatifloxacin sesquihydrate and concluded that cumulative drug released from the formulation ranged from 95-99% within 8-12h. The formulation with 2% sodium alginate and 1% chitosan, sustained the drug release for the longest period of time (12h). Zero-order release of the drug was from optimized the formulation with 2% sodium alginate and 1% chitosan[77].

Abraham S. et al., 2009 developed sustained ophthalmic delivery of ofloxacin from an ion-activated in situ gelling system and concluded that the alginate/HPC solution retained the drug better than the alginate or HPC solutions alone and drug release over a period of 8 hours[78].

Mehra G.R. et al., 2010 developed Pilocarpine *in-situ* gelling solution based on alginate along with novel bioadhesive tamarind gum, widely used bioadhesive, chitosan and alginate as a polymer and concluded that the formulation showed release about 25 % drug in initial hour and about 80 % of the drug was released during the study of 12 h[79].

Preetha JP et al., 2010 developed an in situ gelling ophthalmic formulation of diclofenac sodium and concluded that the formulation having 1.5% sodium alginate and 0.75% of HEC shows better drug release when contacted with STF solution at 8 hrs study period and shows antimicrobial, antibacterial and antifungal efficacy with selected microorganisms[80].

Liu Z. et al., 2006. Developed alginate/HPMC –based in situ gelling ophthalmic delivery system for gatifloxacin and demonstrated that the alginate/HPMC mixture can be used as an in situ gelling vehicle to enhance ocular bioavailability and patient compliance[81].

Gaonkar GV et al., Oct-Nov. 2010.Formulation Development And Evaluation Of Long Acting Ophthalmic *In-Situ* Gelling Systems Of Ketorolac Tromethamine And Ofloxacin: A Research. Aqueous solutions of Sodium Alginate, Xanthan Gum and Carbopol 971P coupled with added viscolizers are attractive *in-situ* gel forming systems, promising controlled ocular drug delivery of Ketorolac Tromethamine and Ofloxacin[82].

Liu Y. et al., 2010 investigated in Situ Gelling Gelrite/Alginate Formulations as Vehicles for ophthalmic drug delivery and found that the optimum concentration of Gelrite solution for the in situ gel forming delivery systems was 0.3% (w/w) and that for alginate solution was 1.4% (w/w). The mixture of 0.2% Gelrite and 0.6% alginate solutions showed a significant enhancement in gel strength at physiological condition[72].

DRUG RELEASE FROM HYDROGELS:

Hydrogels have a unique combination of characteristics that make them useful in drug delivery applications. Due to their hydrophilicity, hydrogels can imbibe large amounts of water. Therefore, the molecule release mechanisms from hydrogels are very different from hydrophobic polymers. Both simple and sophisticated models have been previously developed to predict the release of an active agent from a hydrogel device as a function of time. These models are based on the rate limiting step for controlled release and are therefore categorized as diffusion, swelling & chemically controlled mechanism[63] (figure 14).

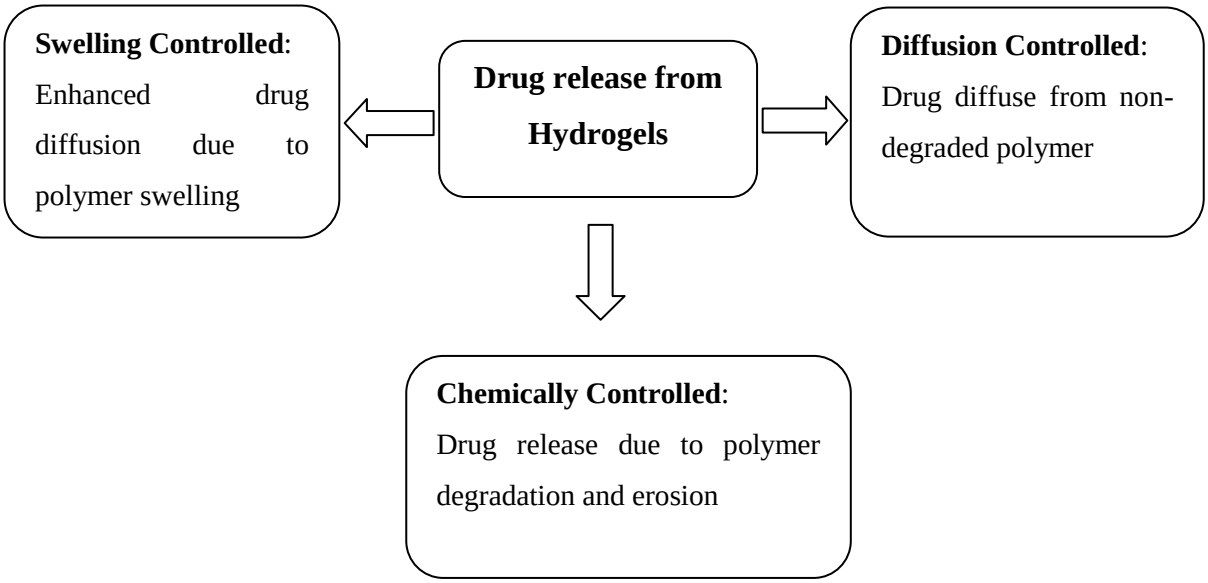


Figure 13:- Drug release from hydrogel

EVALUATIONS OF *IN-SITU* GEL SYSTEM

The prepared *in-situ* gel formulations were evaluated for clarity, pH measurement, gelling capacity, drug content, rheological study, *in vitro* diffusion study, isotonicity, antibacterial activity, *in-vivo* ocular testing in rabbits and accelerated stability studies. The formulation should have an optimum viscosity that will allow for easy instillation into the eye as a liquid (drops), which would undergo a rapid sol-to-gel transition (triggered by pH, temperature or ion exchange).

Texture analysis

The firmness, consistency, and cohesiveness of hydrogels are evaluate by using texture analyser which mainly indicates the syringeability of sol so can formulation can easily be administered *in-vivo*. Higher values of adhesiveness are needed to maintain the intimate contact with the tissues[83].

Physical parameters

The formulated *in-situ* gel solution is tested for clarity, pH, gelling capacity, and drug content estimation.

Gelling capacity

The gelling capacity of the prepared formulation is determined by placing a drop of the formulation in a vial containing 2.0 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling is noted[84, 85, 86].

Rheological studies

The viscosity measurements can be calculated using Brookfield viscometer, Cone and Plate viscometer. The *in-situ* gel formulations were placed in the sampler tube. From the literature it was evident that, the formulation before gelling should have a viscosity of 5 to 1000 mPas. And after ion gel activation by the eye, will have a viscosity of from about 50- 50,000 mPas. The samples are analyzed both at room temperature at 25°C and thermo stated at 37°C ± 0.5°C by a circulating bath connected to the viscometer adaptor prior to each measurement. The angular velocity of the spindle was increased 20, 30, 50, 60, 100, 200 and the viscosity of the formulation is measured. All the formulations exhibited Newtonian and pseudoplastic flow characteristics before and after gelling in the simulated tear fluid respectively[83, 87, 88].

In vitro drug release studies

In vitro release study of *in-situ* gel solution was carried out by using Franz diffusion cell. The formulation placed in donor compartment and freshly prepared simulated tear fluid in receptor compartment. Between donor and receptor compartment dialysis membrane is placed (0.22µm pore size). The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37°C ± 0.5°C. 1ml of sample is withdrawn at predetermined time interval of 1hr for 6 hrs and same volume of fresh medium is replaced. The withdrawn samples are diluted to 10ml in a volumetric flask with respective solvent and analyzed by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using the equation generated from standard calibration curve. The % cumulative drug release (%CDR) calculated**.** The data obtained is further subjected to curve fitting for drug release data. The best fit model is checked for Krosmeyers Peppas and Fickinian diffusion mechanism for their kinetic[89, 90].

Isotonicity Evaluation

Isotonicity is important characteristic of the ophthalmic preparations. Isotonicity has to be maintained to prevent tissue damage or irritation of eye. All ophthalmic preparations are

subjected to isotonicity testing, since they exhibited good release characteristics and gelling capacity and the requisite viscosity. Formulations are mixed with few drops of blood and observed under microscope at 45X magnification and compared with standard marketed ophthalmic formulation[1, 91].

Antibacterial activity

The microbiological growth of bacteria is measured by concentration of antibiotics and this has to be compared with that produced by known concentration of standard preparation of antibiotic. To carryout microbiological assay serial dilution method is employed[1, 92].

Ocular irritancy test

The Draize irritancy test was designed for the ocular irritation potential of the ophthalmic product prior to marketing. According to the Draize test, the amount of substance applied to the eye is normally 100µl placed into the lower *cul-de-sac* with observation of the various criteria made at a designed required time interval of 1hr, 24hrs, 48 hrs, 72hrs, and 1week after administration. Three rabbits (male) weighing 1.5 to 2kg are used for the study. The sterile formulation is instilled twice a day for a period of 7 days, and a cross-over study is carried out (a 3 day washing period with saline was carried out before the cross-over study). Rabbits are observed periodically for redness, swelling, watering of the eye[92, 93, 94].

Accelerated stability studies

Formulations are placed in ambient colour vials and sealed with aluminium foil for a short term accelerated stability study at 40±2 °C and 75±5% RH as per International Conference on Harmonization (ICH) states Guidelines. Samples are analyzed every month for clarity, pH, gelling capacity, drug content, rheological evaluation, and *in vitro* dissolution[91].

CONCLUSION

The complications in eye formulation are mainly due to specific anatomical and physiological features of eye. The development of *in-situ* stimuli activated gel-forming systems for ophthalmic drug delivery provides simplest and best gel-forming systems. It is an ideal system that maintains effective level of drug for the longer duration following a single application and offers the primary requirement of a successful controlled release product that increases patient compliance. Moreover, various polymers used in this system provide advantage over conventional drug delivery system. This system is preferred over other systems for ocular delivery because it can be administered in drop form and creates significantly fewer problems with vision as well as have

sustained release. In the recent era of technology, combinatorial approach seems to be a focus of research in the development of safe and efficient ophthalmic drug delivery systems.

REFERENCES

1. Rathore KS. In-situ Gelling Ophthalmic Drug Delivery System: An Overview Int J Pharm Pharma Sci 2010; 2(4).
2. Gaudana R, Jwala J, Boddu HS, Sai K, Mitra AK. Recent Perspectives in Ocular Drug Delivery. Pharma Res 2009; 26(5).
3. Shastri DH, Patel LD. A Novel Alternative to Ocular Drug Delivery System: Hydrogel. Int J Pharma Res 2010; 2(1).
4. Jigar V. A Review on Novel in Situ Polymeric Drug Delivery System. Int J Pharma Res Dev 2011; 3 (5):53-59.
5. Pandya et al., Ophthalmic In-Situ Gelling System. Int J Pharm Life Sci 2011; 2 (5): 730-738.
6. Shastri, DH, Patel LD. A Novel Alternative to Ocular Drug Delivery System: Hydrogel. Int J Pharm Res 2010; 2(1): 1-13.
7. Miller S, Donovan M. Effect of Poloxamer 407 Gel on the Miotic Activity of Pilocarpine Nitrate in Rabbits. Int J Pharm 1982; 12:147-152.
8. Indu PK, Manjit S, Meenakshi K. Formulation and Evaluation of Ophthalmic Preparations of Acetazolamide. Int J Pharm 2000; 199: 119–127.
9. Wei G, Xu H, Ding PT, Li S M, Zheng J M J, Thermosetting Gels With Modulated Gelation Temperature For Ophthalmic Use. The Rheological and Gamma Scintigraphic Studies. J Controlled Rel 2002; 83:64-65.
10. Qiu Y, Park K, Environment-Sensitive Hydrogels for Drug Delivery. Advanced Drug Delivery Rev 2001; 53:321-39.
11. Bromberg LE, Ron ES, Temperature Responsive Gels and Thermo Gelling Polymer Matrices for Protein and Peptide Delivery. Advanced Drug Delivery Rev 1998; 31:197-221.
12. Kono K. Thermosensitive Polymer-Modified Liposomes. Advanced Drug Delivery Rev 2001; 53:307.
13. Nanjwade BK, Manvi FV, Manjappa AS. In Situ -Forming Hydrogels for Sustained Ophthalmic Drug Delivery. J Controlled Rel 2007; 122: 119-134.

14. Chen-Chow PC, Frank SG. In Vitro Release of Lidocaine from Pluronic F 127. Int J Pharma Sci 1981; 8: 89-99.
15. Shastri DH, Patel LD. Novel Alternative to Ocular Drug Delivery System: Hydrogel. Int J Pharma Res 2010; 2(1).
16. Desai SD, Blanchard J. Pluronic F127 - Based Ocular Delivery System Containing Biodegradable Polyisobutylcyanoacrylate Nanocapsules Of Pilocarpine. Drug Delivery 2000; 7: 201-207.
17. Bochot A, Fattal E, Gulik A, Couarraze G, Couvreur P. Liposomes Dispersed Within A Thermosensitive Gel: A New Dosage Form For Ocular Delivery Of Oligonucleotides. Pharm Res 1998; 15(9):1364-9.
18. Hong-Ru Lin, Sung KC. Carbopol/pluronic phase change solutions for ophthalmic drug delivery Journal of Controlled Release 2000; 69(3): 379–388.
19. Desai SD, Blanchard J, Pluronic F127 - Based Ocular Delivery System Containing Biodegradable Polyisobutylcyanoacrylate Nanocapsules Of Pilocarpine. Drug Delivery 2000; 7: 201-207.
20. Carmignani C, Rossi S, Saettone MF, Burgalassi S. Ophthalmic Vehicles Containing Polymer Solubilized Tropicamide: ‘‘In Vitro/In Vivo’’ Evaluation. Drug Dev Ind Pharm 2002; 28(1): 101–105.
21. El-Kamel AH. In Vitro and In Vivo Evaluation of Pluronic F127 - Based Ocular Delivery System for Timolol Maleate. Int J Pharm 2002; 241: 47-55.
22. Yoo MK, Cho KY, Song HH, Cho YJ, Kwon JW, Kim MK, Lee JH, Wee WR, Cho CS. Release Of Ciprofloxacin From Chondroitin 6-Sulfate - Graft - Poloxamer Hydrogel In Vitro For Ophthalmic Drug Delivery. Drug Dev Ind Pharm 2005; 31: 455–463.
23. Qi H, Li, Li., Huang C, Li W, Wu C. Optimization And Physicochemical Characterization Of Thermosensitive Poloxamer Gel Containing Puerarin For Ophthalmic Use. Chem Pharm Bull 2006; 54(11): 1500-1507.
24. Dumortier G, El Kateb N, Sahli M, Kedjar S, Boulliat A, Chaumeil J C. Development Of A Thermo gelling Ophthalmic Formulation Of Cysteine. Drug Dev Ind Pharm 2006; 32:63–72.
25. Gupta H, Jain S, Mathur R, Mishra P, Mishra AK. Sustained Ocular Drug Delivery from A Temperature and PH Triggered Novel In-Situ Gel System. Drug Delivery 2007; 14:507–515.

26. Wen-Di Ma, Xu H, Nie, Shu-Fang, Pan, Wei-San., Temperature-Responsive, Pluronic-G-Poly(Acrylic Acid) Copolymers In Situ Gels For Ophthalmic Drug Delivery: Rheology, In Vitro Drug Release, And In Vivo Resident Property. Drug Dev Ind Pharm 2008; 34:258–266.
27. Vehanen K, Hornof M, Urtti A, Uusitalo H. Peribulbar Poloxamer for Ocular Drug Delivery. Acta Ophthalmologica 2008; 86:91–96.
28. Mayol L, Quaglia F, Borzacchiello A, Ambrosio L, Rotonda MI. A Novel Poloxamers/Hyaluronic Acid In Situ Forming Hydrogel For Drug Delivery: Rheological, Mucoadhesive And In Vitro Release Properties. Eur J Pharma Biopharm 2008; 70:199-206.
29. Mansour M, Mansour S, Nahed D. Ocular Poloxamer-Based Ciprofloxacin Hydrochloride In Situ Forming Gels. Drug Dev Ind Pharm 2008; 34:744–752.
30. Cao F, Zhang X, Ping Q. New Method for Ophthalmic Delivery of Azithromycin by Poloxamer/Carbopol-Based In Situ Gelling System. Drug Delivery 2010; 17(7): 500–507.
31. Gratieri T, Gelfuso GM, Rocha EM, Sarmento VH, Freitas O De, Lopez R F V. A Poloxamer/Chitosan in Situ Forming Gel with Prolonged Retention Time for Ocular Delivery. Eur J Pharm Biopharm 2010; 75: 186–193.
32. Ammar HO, Salama HA, Ghorab M, Mahmoud AA, Development of Dorzolamide Hydrochloride In-Situ Gel Nanoemulsion for Ocular Delivery. Drug Dev Ind Pharm 2010; 1–10.
33. Asasutjarita R, Thanasanchokpibullb S, Fuongfuchatc, A, Veeranondhad S, Optimization and Evaluation of Thermoresponsive Diclofenac Sodium Ophthalmic In-Situ Gels Int J Pharm 2011; 411. 128–135.
34. Varshosaz J, Tabbakhian M, Salmani Z, Designing Of A Thermosensitive Chitosan/Poloxamer In-Situ Gel For Ocular Delivery Of Ciprofloxacin The Open Drug Delivery J 2008; 2, 61-70.
35. Qian Y, Et Al., Preparation And Evaluation Of In-Situ Gelling Ophthalmic Drug Delivery System For Methazolamide. Drug Dev Ind Pharm 2010; 36(11): 1340-1347.
36. Ruel-Gariepy E, Leroux JC. In-Situ Forming Hydrogels — Review Of Temperature Sensitive Systems. Eur J Pharm Biopharm 2004; 58: 409-26.
37. Sarkar N. Thermal Gelation Properties of Methyl and Hydroxypropyl Methylcellulose. J Appl Poly Sci 1979; 24: 1073–87.

38. Heymann E. Studies on Sol–Gel Transformations, I. The Inverse Sol–Gel Transformation of Methylcellulose in Water, Trans. Faraday Soc 1935; 31:846–64.
39. Nanjwade BK, Manvi FV, Manjappa AS. In-Situ Forming Hydrogels for Sustained Ophthalmic Drug Delivery. J Controlled Rel 2007; 122:119-34.
40. Tate MC, Shear DA, Homan SW. Biocompatibility Of Methylcellulose-Based Constructs Designed For Intracerebral Gelation Following Experimental Traumatic Brain Injury. Biomaterials 2001; 22:1113-23.
41. Shirakawa M, Yamatoya K, Nishinari K. Tailoring Of Xyloglucan Properties Using an Enzyme. Food Hydrocolloids 1998; 12:25-28.
42. Shastri D, Pandya H, Parikh RK, Patel CN, Smart Hydrogels in Controlled Drug Delivery. Pharma Times 2006; 38 (11): 13-18.
43. Miyazaki S, Suzuki S, Kawasaki N, Endo K, Takahashi A, Attwood D. In-Situ Gelling Xyloglucan Formulations For Sustained Release Ocular Delivery Of Pilocarpine Hydrochloride. Int J Pharm 2001; 229:29–36.
44. Patel V, Patel M, Patel R, Chitosan: A Unique Pharmaceutical Excipient. Drug Delivery Technology 2005; 5(6).
45. Dutta PK, Dutta J, Tripathi, Chitin and Chitosan: Chemistry, Properties and Applications. J Scientific Ind Res 2004; 63: 20-31.
46. Van Der Lubben, IM, Verhoef, JC, Borchard G, Junginger, HE. Chitosan and Its Derivatives in Mucosal Drug and Vaccine Delivery. Eur J Pharm Sci 2001; 14: 201–207.
47. O Felt, Baeyens V, Zignani M, Buri P, Gurny R. Mucosal Drug Delivery Ocular—Encyclopedia Of Controlled Drug Delivery, Vol.2, University Of Geneva, Geneva, Switzerland 1999; 605–622
48. Ruel-Garie´Py, E, Cheniteb, A, Chaput, C, Guirguisa, S, Leroux, JC. Characterization of Thermosensitive Chitosan Gels for the Sustained Delivery of Drugs. Int J Pharm 2000; 203: 89–98.
49. Mishra DN, Gilhotra RM. Design And Characterization Of Bioadhesive In-Situ Gelling Ocular Inserts Of Gatifloxacin Sesquihydrate. Daru 2008; 16(1): 1-8.
50. Mehra, GR, Manish, Rashi MS, Neeraj G, Mishra DN. Enhancement Of Miotic Potential Of Pilocarpine By Tamarind Gum Based In-Situ Gelling Ocular Dosage Form. Acta Pharmaceutica Sci 2010; 52: 145-154.
51. Lin HR Sung KC. Carbopol/Pluronic Phase Change Solutions for Ophthalmic Drug Delivery. J Controlled Rel 2000; 69: 379–388.

52. Rajas NJ, Kavitha K, Gounder T, Mani T. In-Situ Ophthalmic Gels A Developing Trend. Int J Pharm Sci Rev and Res 2011; Volume 7, Issue 1.
53. Leung SS, Robinson JR. The Contribution Of Anionic Polymer Structural Features To Mucoadhesion. J Controlled Rel 1988; 5:223–231.
54. Chrai SS, Robinson JR. Ocular Evaluation of Methylcellulose Vehicle in Albino Rabbits. J Pharm Sci. 1974; 63: 1218–1222.
55. Kumar S, Haglund BO, Himmelstein KJ. In-Situ Forming Gels For Ophthalmic Drug Delivery, J Ocular Pharmacology 1994; 10(1): 47–56.
56. Kumar S, Himmelstein KJ, Modification Of In-Situ Gelling Behavior Of Carbopol Solutions By Hydroxypropyl Methylcellulose, J Pharm Sci 1995; 84(3): 344–348.
57. Srividya. B, Cardoza, Rita M, Amin, PD. Sustained Ophthalmic Delivery of Ofloxacin from a pH Triggered In-Situ Gelling System. J Controlled Release 2001; 73: 205–211.
58. Wu, Chunjie, QI, Hongyi, Chen, Wenwen, Huang, Chunyan, Su, Cheng, LI, Wenmin., Hou, Shixiyang. Preparationn and Evaluation of a Carbopol/HPMC- Based In-Situ Gelling Ophthalmic System For Puerarin. Yakugaku Zasshi 2007; 127(1): 183-191.
59. Al-Kassas RS, And El-Khatib MM. Ophthalmic Controlled Release In-Situ Gelling Systems For Ciprofoxacin Based On Polymeric Carriers. Drug Delivery 2009; 16(3): 145–152.
60. Nanjwade BK, Manjappa AS, Murthy RSR, Yuvaraj DPA Novel Ph-Triggered In-Situ Gel For Sustained Ophthalmic Delivery of Ketorolac Tromethamine. Asian J Pharm Sci 2009; 4(3): 189-199.
61. Pandey A, Mali. PY, Patel DK, Ramesh R. Development and Optimization of Levobunolol Hydrochloride In-Situ Gel for Glaucoma Treatment. Int J Pharm & Bio Arch 2010; 1(2): 134 – 139.
62. Mohanambal E, Arun K, Abdul Hasan Sathali A. Formulation and Evaluation of pH-Triggered In-Situ Gelling System Of Levofloxacin. Ind J Pharm Edu Res 2011; Vol 45/ Issue 1.
63. Pandya, TP, Modasiya MK, Patel. VM. Ophthalmic In-Situ Gelling System. Int J Pharm & Life Sci 2011; 2(5).
64. Bhaskaran S, Lakshmi PK, Harish CG. Topical Ocular Drug Delivery: A Review. Ind J Pharm Sci 2005; 64(4):404-408.
65. Carlfors J, Edsman K, Petersson R, Jornving, K. Rheological Evaluation Of Gelrite In-Situ Gels For Ophthalmic Use. Eur J Pharm Sci 1998; 6: 113-119

66. Miyanzaki S, Hirotatsu A, Kawasaki N, Wataru K, Attwood D. In-situ Gelling Gellan Formulations as Vehicles For Ocular Drug Delivery J Control Rel 1999; 60. 287.
67. Crescenzi V, Dewtini M, Coviello T. Solution and Gelling Properties of Microbial Polysaccharides of Industrial Interest. The Case of Gellan in Dawes EA, Editor Nowel Biodegradable Microbial Polymers, Dordrecht: Kluwer Academic Publishers 1990; 227-84.
68. Nirmal HB, Bakliwal SR, Pawar SP. In-Situ Gel: New Trends in Controlled and Sustained Drug Delivery System. Int J Pharmtech Res 2010; Vol.2, No.2, Pp 1398-1408.
69. Paulsson, M, Hagerstrom, H, Edsman, K. Rheological Studies of the gelation of Deacetylated Gellan Gum (Gelrite) In Physiological Conditions. Eur J Pharm Sci 1999; 9: 99–105.
70. Balasubramaniam J, Kant S, Pandit JK. In Vitro And In Vivo Evaluation Of The Gelrite® Gellan Gum-Based Ocular Delivery System For Indomethacin. Acta Pharm 2003; 53:251–261.
71. Sultana Y, Aqil M, Ali A. Ion-Activated, Gelrite Based In-Situ Ophthalmic Gels of Pefloxacin Mesylate: Comparison with Conventional Eye Drops. Drug Delivery 2006; 13:215–219.
72. Liu Y, Liu J., Zhang X, Zhang R, Huang Y, Wu C. 2010; In-Situ Gelling Gelrite/Alginate Formulations As Vehicles For Ophthalmic Drug Delivery. Amer Assoc Pharm Sci 11(2): 610-620.
73. Vodithala, S, Khatry S, Shastri N, Sadanandam M. 2010; Formulation and evaluation of ion activated ocular gels of Ketorolac Tromethamine. Int J Cur Pharm Res 2(3): 33-38.
74. Geethalakshmi A, Karki R, Jha SK, Venkatesh DP, Nikunj B. Sustained Ocular Delivery Of Brimonidine Tartrate Using Ion Activated In Situ Gelling System. Current Drug Delivery 2012; 9(2):197-204
75. Rupenthal ID, Green CR, Alany RG. Comparison of Ion-Activated In-Situ Gelling Systems for Ocular Drug Delivery. Part 2: Precorneal Retention and In Vivo Pharmacodynamic Study. Int J Pharm 2011; 15; 411(1-2):78-85.
76. Cohen S, Lobel E, Trevgoda A, Peled Y. A Novel In-Situ Forming Ophthalmic Drug Delivery System from Alginates Undergoing Gelation in the Eye. J Controlled Rel 1997; 44:201–208.
77. Mishra DN, Gilhotra R.M. Design and Characterization of Bioadhesive In-Situ Gelling Ocular Inserts Of Gatifloxacin Sesquihydrate. Daru 2008; 16(1):1-8.

78. Abraham S, Furtado S, Bharath S, , Basavaraj BV, Deveswaran R, Madhavan V. Sustained Ophthalmic Delivery Of Ofloxacin From An Ion-Activated In-Situ Gelling System. Pak J Pharm Sci 2009; 22(2), 175-179.
79. Mehra, GR, Manish M, Rashi S, Neeraj G, DN Mishra. Enhancement of Miotic Potential of Pilocarpine by Tamarind Gum Based In-Situ Gelling Ocular Dosage Form. Acta Pharma Sci 2010; 52: 145-154.
80. Preetha JP, Karthika K, Rekha NR, Elshafie K. Formulation and Evaluation of In-Situ Ophthalmic Gels of Diclofenac Sodium. J Chem Pharm Res 2010; 2(3):528-535.
81. Liu Z, Li J, Nie S, Liu H, Ding P, Pan W. Study Of An Alginate/HPMC-Based In-Situ Gelling Ophthalmic Delivery System For Gatifloxacin. Int J Pharm 2006; 315 12–17.
82. Gaonkar GV, Gude RS. Formulation Development And Evaluation Of Long Acting Ophthalmic In-Situ Gelling Systems Of Ketorolac Tromethamine And Ofloxacin: A Research Int J Drug Formul & Res 2010; 1(Ii):185-200.
83. Sautou-Miranda V, Labret F, Grand-Boyer A, Gellis C, Chopineau J. Impact Of Deep-Freezing On The Stability Of 25 Mg/Ml Vancomycin Ophthalmic Solutions. Int J Pharm 2002; 234: 205– 207.
84. Mitan R, Gokulgandhi Jolly R, Parikh, Megha B, Dharmesh MM. A pH Triggered In-situ Gel Forming Ophthalmic Drug Delivery System for Tropicamide. Drug Deliv Technol 2007; 5: 44–49.
85. Pandit D, Bharathi, A, Srinatha, Ridhurkar, Singh S. Long Acting Ophthalmic Formulation of Indomethacin: Evaluation Of Alginate Gel Systems. Indian J Pharm Sci 2007; 69: 37–40.
86. Rathore KS. In-situ Gelling Ophthalmic Drug Delivery System: An Overview. Int J Pharm Pharm Sci 2010; 2(4).
87. Indu PK, Manjit S, Meenakshi K. Formulation and Evaluation of Ophthalmic Preparations of Acetazolamide. Int J Pharm 2000; 199:119–127.
88. Kashyap N, Vishwnath B, Sharma G. Design and Evaluation Of Biodegradable, Biosensitive In-Situ Gelling System For Pulsatile Delivery Of Insulin. Biomaterials 2007; 28: 2051-60.
89. Gupta S, Vyas SP. Carbopol/Chitosan Based Ph Triggered In-Situ Gelling System For Ocular Delivery Of Timolol Maleate. Scientia Pharmaceutica 2010; 78: 959-976.
90. Edsman K, Carlfors J, Petersson R. Rheological Evaluation Of Poloxamer As An In-Situ Gel For Ophthalmic Use. Eur J Pharma Sci 1997; 6:105–112.

91. Doijad RC, Manvi FV, Malleswara Rao VSN, Prajakta, Alsae. Sustained Ophthalmic Delivery of Gatifloxacin from In-situ Gelling System. Indian J Pharm Sci 2006; 68: 814–818.
92. Draize J, Woodward G, Calvery O. Methods For The Study Of Irritation And Toxicity Of Substance Applied Topically To The Skin And Mucous Membrane. J Pharmacol Exp Ther 1994; 82: 377–390.
93. Rathore KS, Nema RK. An Insight in to Ophthalmic Drug Delivery System, Int J Pharma Sci Drug Res 2009; 1(1): 1-5.
94. Rathore KS, Nema RK. Management of Glaucoma: A Review. Int J PharmTech Res 2009; 1(3): 863-869.