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IN-SITU GEL FOR OPHTHALMIC DELIVERY: A REVIEW

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KEYWORDS:

In-situ gel, sustained release, ophthalmic solution, phase transition, biodegradable polymer.

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ABSTRACT

Eye is the most interesting and challenging organ faced by the pharmaceutical researchers. The conventional ophthalmic dosage forms are account for 90% of currently accessible ophthalmic formulations. The major problem occurs in ophthalmic delivery is quick precorneal drug loss. To improve ophthalmic drug bioavailability, recently there are considerable efforts directed towards controlled and sustained drug delivery in modern pharmaceutical design and an intensive research have been undertaken in achieving much better formulation which not only extend the contact time of the vehicle at the ocular surface, but at the same time slow down the removal of the drug. So, to overcome these problems, ophthalmic *in situ* gels were developed. In situ forming gels are solution upon installation and undergoes phase transition in the ocular cul-de-sac to form viscoelastic gel and these gel gives a response to environmental changes (pH, temperature, ion exchange). The aim of this article is to present a basics and advances on in situ gel for ophthalmic delivery. Various biodegradable polymers are used in in situ gelling like carbopol, pluronics, alginate, gelrite etc. Commercial formulations of in situ polymeric systems are Regel Depot Technology, Cytoryn and Timoptic-Xe.

INTRODUCTION:

The eye is a unique organ because of its anatomy and physiology so main aim of researchers is the attainment of effective drug concentration at the intended site of action for a sufficient period of time to elicit the response. Various problems met leading to poor bioavailability of the ocular drugs are viz.,[1, 2] binding by the mucus proteins, drainage through eye, lacrimation and tear turnover, limited corneal area and trilaminate structure of eye, metabolism and high ocular shear rate ranging from 0.03 s-1 during inter-blinking periods to 4250 - 28500 s-1 during blinking.[3,4] These problems can be overcome by the use of an in situ gel system that are instilled as solution into the eye and undergo a sol- gel transition in the eye.

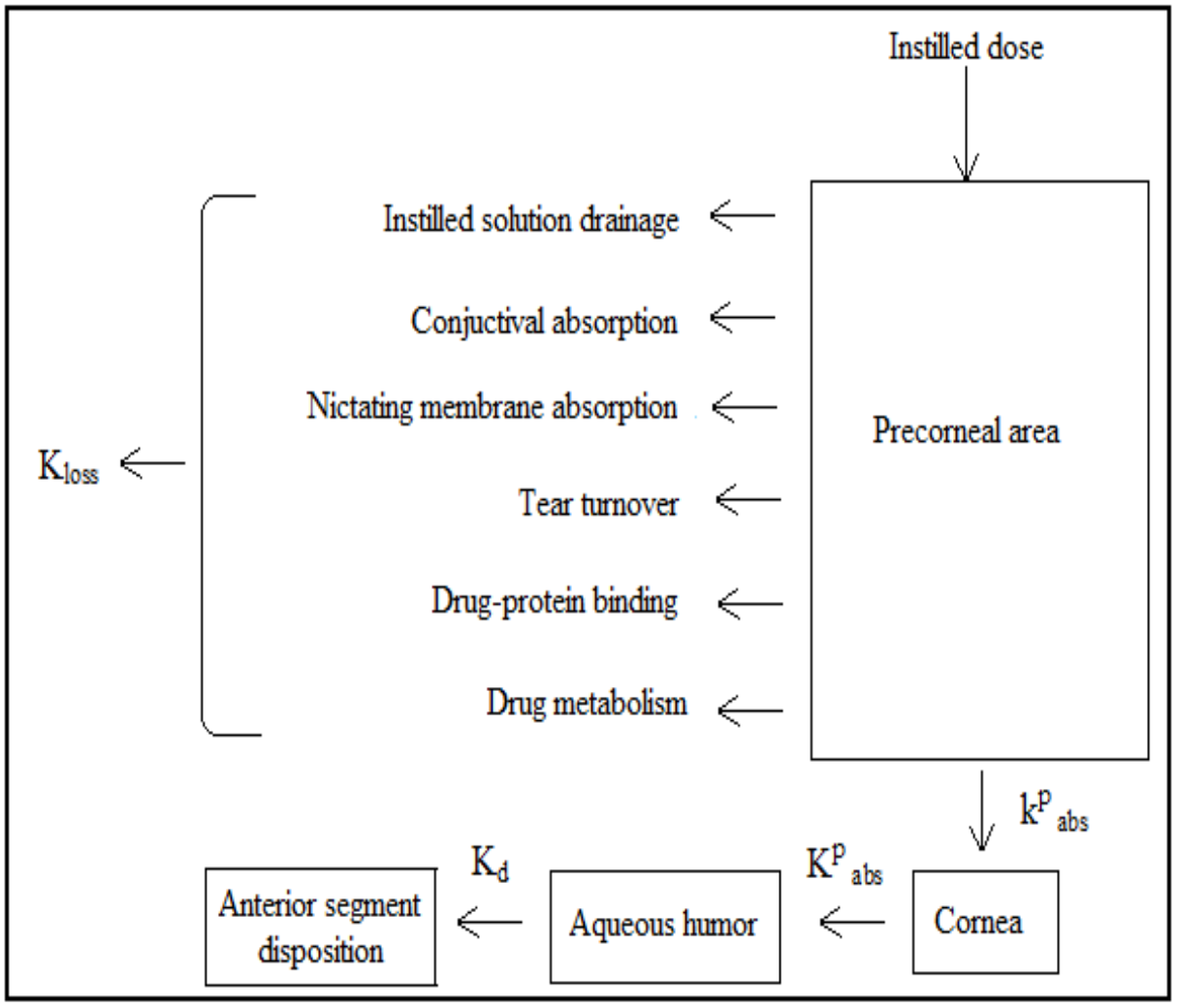


Fig. 1: Model depicting precorneal and ocular drug movement from topical instilled dose.

IN-SITU GELLING SYSTEM- The growth of in situ gel systems has gain more attention over the past few years. In the past few years, increasing number of in situ gel forming systems have been investigated. In-situ gel defined as polymer solution which can be administered as liquid & undergoes a phase transition to gel upon exposure to physiological environment of eye.[5] The gelation in eye can be done by three different ways: by change in temperature, pH, ionic change.[6-8]

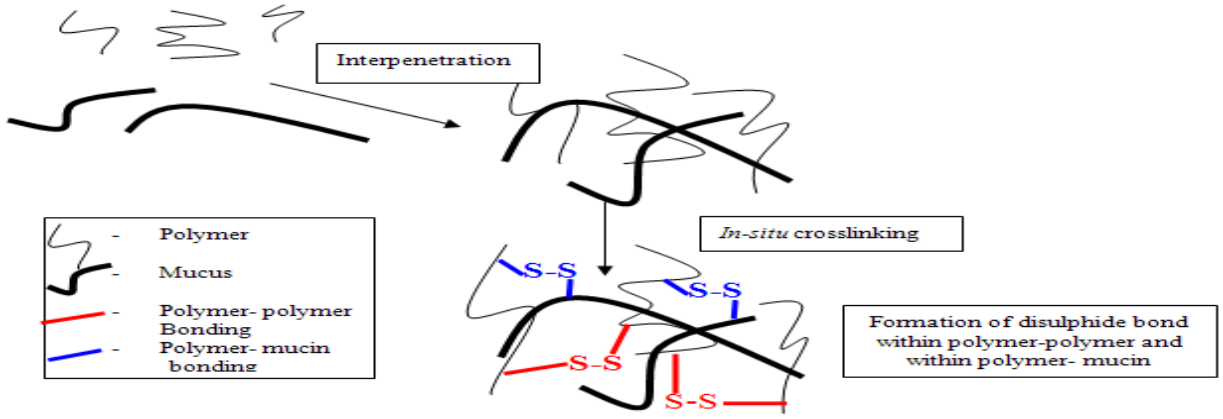


Fig. 2: In-situ crosslinking after instillation.

MECHANISM OF GELATION- Gelation occurs via the cross linking of polymer network that can be achieved by covalent bond formation (chemical cross linking) or non covalent bond formation (physical cross linking).[9] This system known by their low viscosity solution that undergoes phase transition in cul-de-sac to form viscoelastic gel due to conformational changes in polymer on response to physiological environment.[10] The rate of in situ gel formation is important because between instillation in eye & before a strong gel is formed; the solution or weak gel is produced by the fluid mechanism of eye.[11]

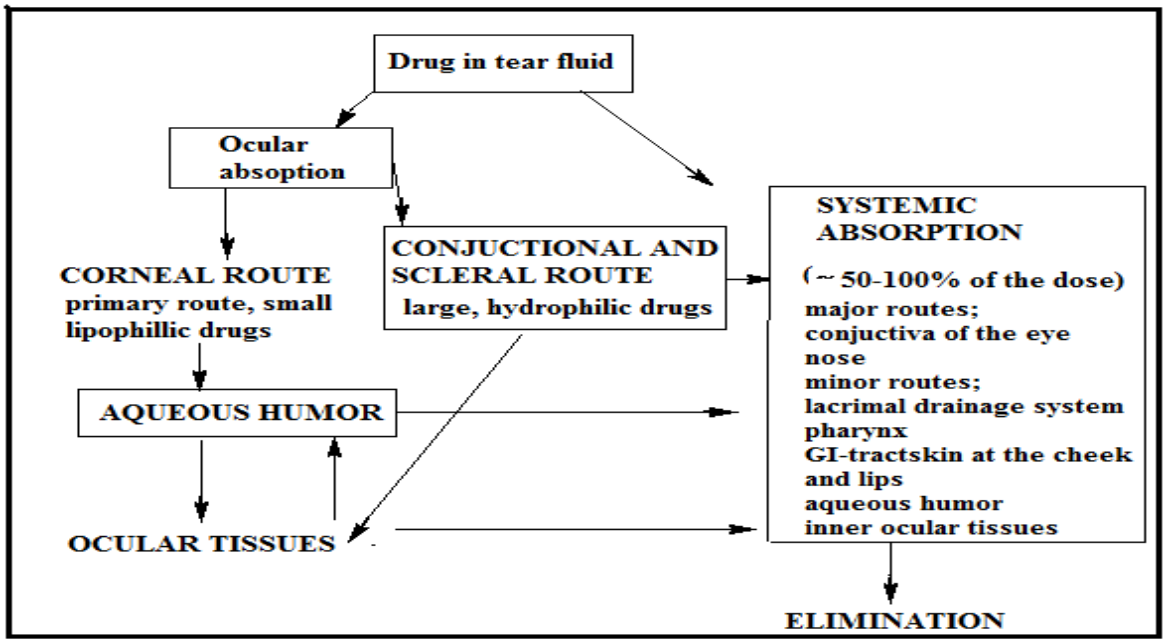


FIG. 3: Fate of ophthalmic drug delivery systems.

ADVANTAGE OF IN SITU GEL FOR OCULAR DELIVERY:-

- The benefit of administrating accurate & reproducible quantities compared to already formed gel.[12]
- ease of administration & reduced frequency of administration hence improved patient compliance & comfort.[13]

- Poor bioavailability & therapeutic response shown by conventional ophthalmic solution due to rapid precorneal elimination of drug may be overcome by use of in situ gel system that are instilled as drops into eye &undergoes a sol-gel transition from instilled dose.[14]
- Liquid dosage form that can prolong drug release & gel remain in contact with cornea of eye for extended period of time as compared to eye drop.[15]
- Reduced drug drained through the nasolacrimal duct may lead to some undesirable side effects as compared to other formulation.[16,17]
- To find a way to the protective barriers like drainage, lacrimation and conjunctival absorption.
- Generally more comfortable than ocular inserts as of blurred vision.

To provide site specific targeting within the ocular globe so as to prevent the loss to other ocular tissues.

CHARACTERISTICS REQUIRED FOR OCULAR DRUG DELIVERY SYSTEM [18, 19]

A good corneal permeation.

A extend contact time with corneal tissue.

Ease of installation for the patient.

A non-irritant and comfortable form (the viscous solution should not provoke lachrymation and reflex blinking).

Appropriate viscosity and concentration of viscolyzer.

WAYS OF GELATION- Following are the triggering mechanisms by which we can achieve *in-situ* gelation:-

1.Temperature dependent gelation*:* These *in-situ* gels are able to swell or deswell as a result of change in the temperature of the surrounding environment. This type of formulation is liquid at room temperature (20°-25°c) which undergoes gelation in contact with eye temperature (35-37°c).[20]

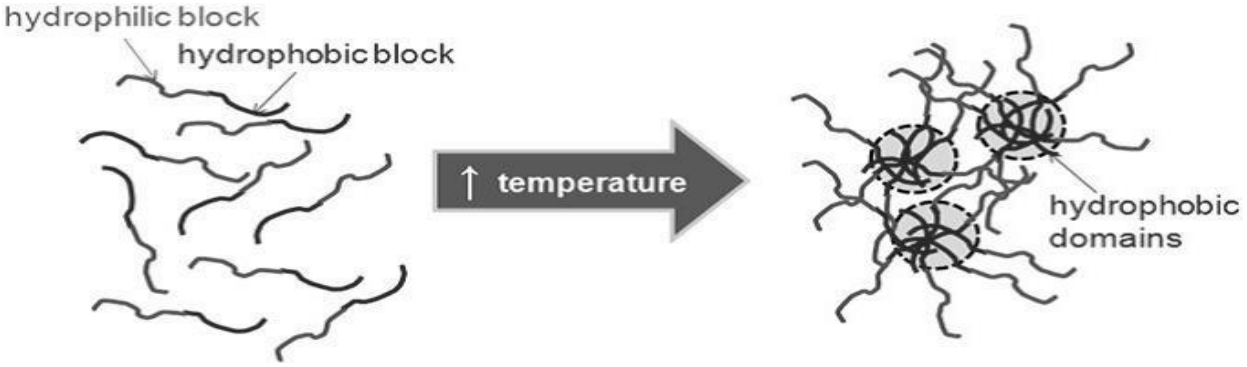


Fig. 4: Mechanism of temperature sensitive system.

2. pH triggered systems: In case of pH sensitive *in-situ* gels, Sol to gel transition takes place when pH is raised from 4.2 to 7.4 (eye pH). the pH-sensitive polymer contains pendent acidic or basic groups that either accept or release protons in response to changes in environmental pH .The polymers with a large number of ionizable groups are known as polyelectrolytes. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups.[21] At higher pH polymer forms hydrogen bonds with mucin which leads to formulation of hydrogen network.

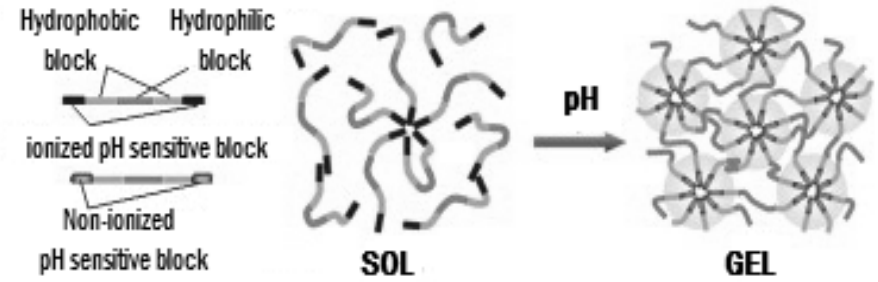


Fig. 5: Mechanism of pH sensitive system.

3. Ion-activated systems (osmotically induced gelation)*:* In this case, gelation of the instilled solution is triggered by alteration in the ionic strength.[22] It is found that the rate of gelation depend on the osmotic gradient across the surface of the gel. The aqueous polymer solution forms a clear gel in the presence of the mono (na+) or divalent cations (ca+) typically found in the tear fluids. The electrolyte of the tear fluid and especially Na+, Ca2+ and Mg2+ cations are responsible for gelation of the polymer solution when instilled as a liquid solution in the conjunctival cul-de-sac.[25]

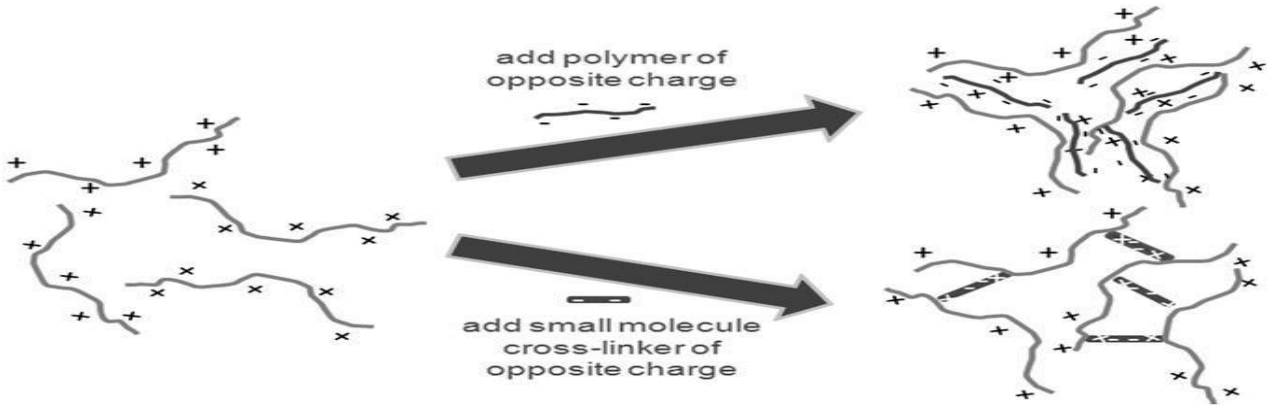


Fig. 6: Mechanism showing Ion activated systems.

IDEAL CHARACTERISTICS OF POLYMERS [23]

A polymer used to in situ gels should have following characteristics:

It should be biocompatible. It should be capable of adhere to mucus. It should have pseudo plastic behaviour. It should have good tolerance & optical activity. It should influence the tear behaviour. The polymer should be capable of decrease the viscosity with increasing shear rate there by offering lowered viscosity during blinking & stability of the tear film during fixation.

Poloxamer (Pluronic): Poloxamer is the tri-block copolymer of Polyethylene oxide–Polypropylene oxide Polyethylene oxide. Poloxamer is made of a central hydrophobic part (polyoxypropylene) surrounded by hydrophilic part (polyethylene oxide). The poloxamer triblock copolymers are available in many grades differ in molecular weights and physical forms. They can be classified in different grades, as F for flakes, P for paste, L for liquid. Pluronics or Poloxamers also undergo in situ gelation by temperature change. They also undergo changes in solubility with change in temperature. Pluronic F-127 is a very commonly used Poloxamer for ophthalmic preparations that converts into a colorless and transparent gel at temperature above 350C.

Mechanism: The mechanism involving the sol-to-gel transformation, after increase in temperature there is gradual desolvation of the polymer and increased micellar aggregation (entanglement of the polymeric network). The micelles formation takes place due to the polyoxypropylene block dehydration, at definite point micelles come in contact and no longer move. Micelles composed of central Hydrophobic part (polyoxypropylene) surrounded by Hydrophilic part (Ethylene oxide).

Drawback: Poloxamer has weak mechanical strength which leads to rapid erosion. Therefore blend of Poloxamer with the other polymer is used.[24-29]

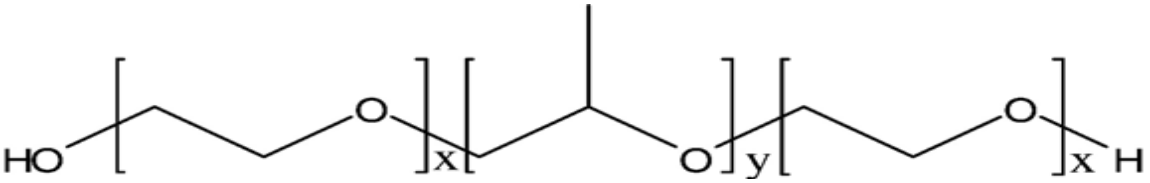


Fig. 7: PEO-PPO-PEO (Poloxamer).

Chitosan: Chitosan is a biodegradable, polycationic, thermosensitive polymer obtained by alkaline deacetylation of chitin, a natural component of shrimp and crab shell. It consists of copolymers of glucosamine and N-acetylglucosamine. The primary amino group responsible for easy chemical modification of chitosan and salt formation with acids. At acidic pH, the amino groups are protonated, which enhances solubility, whereas chitosan is insoluble at alkaline and neutral pH.[30-32] Chitosan is a biocompatible pH dependent cationic polymer, which dissolved in aqueous solutions upto a pH of 6.2.[33] Neutralization of chitosan aqueous solution to a pH exceeding 6.2 leads to the formation of a hydrated gel. The pH gelling, polysaccharides solution are changed into thermally sensitive pH dependent gel forming aqueous solutions, without any chemical modification or cross linking network by addition of polyol salts bearing a single anionic head such as glycerol, sorbitol, fructose or glucose phosphate salts to chitosan solution[34]. Chitosan also act as a penetration enhancer that increases transcorneal permeation of the drug by opening the tight junctions between epithelial cells [35-38] or by intracellular routes[34] or some investigators also proposed the mechanism underlying this permeation enhancing effect seems to be based on the positive charges in the polymer network which interact with the cell membrane resulting in a

structural recombining of tight junction-associated proteins.[39] Besides this, chitosan has ability to convert into hydrogel at ocular pH (pH 7.4) which makes it best suited choice for the development of in situ gelation.[40-42]

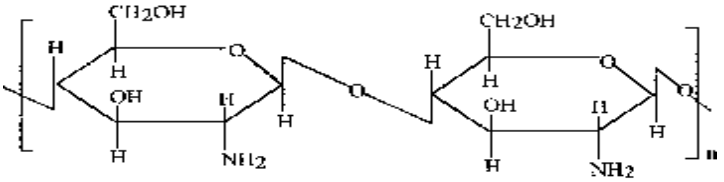


Fig. 8: The structure of chitosan

Cellulose acetate hydrogen phthalate (CAP): CAP is the only polymer which provides buffering that is low enough to gel effectively in the cul-de-sac of the eye. The pH change of 2.8 units after instillation of the native formulation (pH 4.4) into the tear fluid leads to an almost instantaneous transformation of the fluid into a viscous gel.[43-45] The gelation capacity of CAP latexes has been visualized in vitro by scanning electron microscopy and in vivo in rabbits by incorporating crystal voilet in ophthalmic formulations. The efficacy of a preparation has been evaluated by measuring pharmacological responses and precorneal residence time by γ scintigraphy.

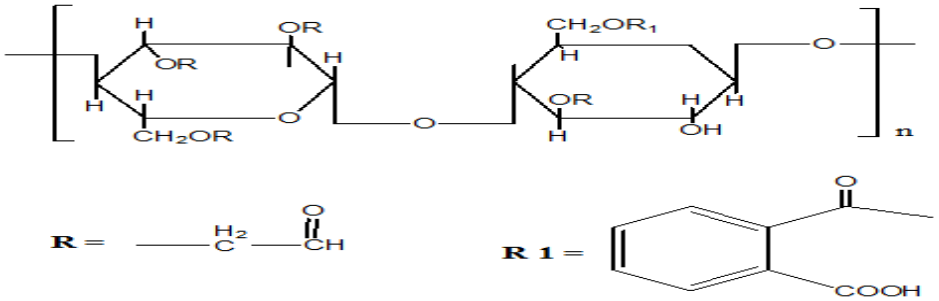


Fig. 9: Structure of cellulose acetate phthalate.

Carbopol: Carbopol is a polyacrylic acid (PAA) polymer, which changed to gel as the pH is raised from 4.0 to 7.4.[46] Carbopol remains in solution form at acidic pH but transform into a low viscosity gel at alkaline pH. HPMC is used in combination with carbopol which enhance viscosity of carbopol solution, while reducing the acidity of the solution. Comparing different types of poly (acrylic acid) (Carbopol 940-934-941and 910) [47] concluded that Carbopol 940 showed superior appearance and clarity.

Mechanism: At specific pH there is hydrophobic, electrostatic interaction and hydrogen bonding takes place, hence leads to interdiffusion. The phase transition for carbopol solution was mediated by the raise of pH from 4.0 to 7.4 which is due to ionization of Carbopol polymer. At pH 7.4, the mutual repulsion of ionized carboxyl groups may produce more stretched carbopol network and those carboxyl groups may also form stable hydrogen bonds with water molecules through hydrophilic interactions. [25, 48, 49] On the other hand, the hydrophobic nature of carbopol backbone may form hydrophobic interchain aggregation; this cross-linking phenomenon may result in

transformation of viscous gel at pH 7.4 environment.[50]

Drawback: As concentration increases, acidic nature may cause lacrimation, hence combination of polymers are used.

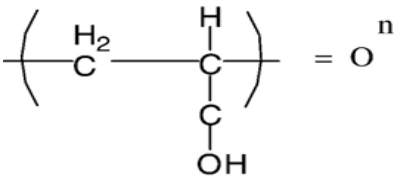


Fig. 10: The structure of carbomer.

Alginates: It consist of (1- 4) linked α-L-guluronic acid and β- D-mannuronic acid. The formulations containing alginic acid choose for, not only because of its gelling ability in the eye but also of its mucoadhesive properties.[51, 52]

Mechanism: Alginate is a copolymer with two types of monomers used, β- D-mannuronic acid (M) and α-L-guluronic acid (G), arranged as homopolymeric blocks of M-M blocks or G-G blocks together with blocks of alternating sequence (M-G). The polymer forms 3- dimensional ionotropic hydrogel matrices, mostly by the interaction of calcium ions with G moieties which leads the formation of inhomogeneous gel. The characteristic properties of these hydrogels, such as mechanical strength and porosity, are dependent upon the G:M ratios, concentration and viscosity of the initial alginate solution and type of ionic cross-linker (bi- or poly- valent cations) etc.[53, 54] Alginate with a high G content will improve the gelling properties and reduce the total polymer to be introduced into the eyes.

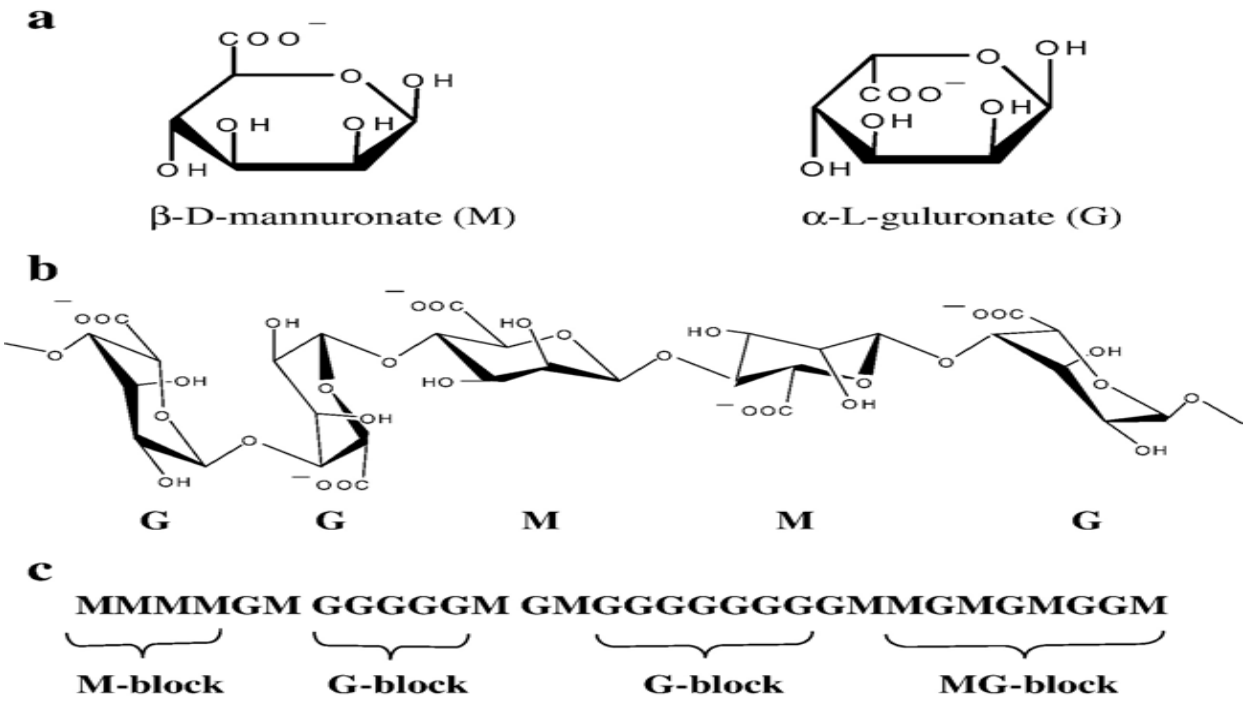
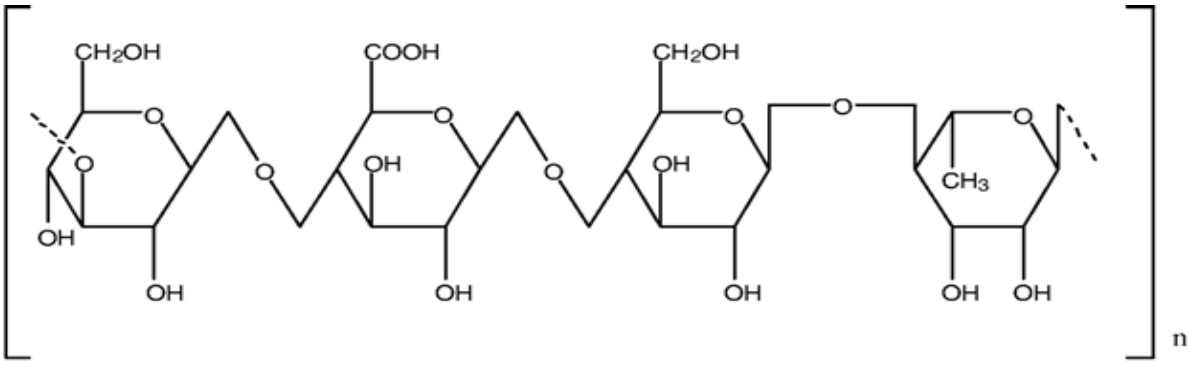


Fig.11: Structural characteristics of alginates: (a) alginate monomers (b) chain conformation and (c) block distribution.

Gelrite(Gellan gum): Gellan gum is anionic heteropolysaccharide with a tetrasaccharide repeating unit of one α-L rhamnose, one β- D-glucuronic acid and two β-D-glucuronic acid residues. The polysaccharide can be formed by aerobic fermentation and then isolated from the fermentation broth by alcohol precipitation. It shows gelation by ionic crosslinking. It shows a clear gel in the presence of mono- or divalent cations. The electrolytes of the tear fluid, especially Na+, Ca2+ and Mg2+ cations are responsible to initiate gelation of the polymer when instilled as an aqueous solution into the cul-de-sac.

Mechanism- There is cross linking of negatively charged cations(Na+, ca2+ and Mg2+). Once gelled, the formulation resists the natural drainage process from the precorneal area which increases the residence at the site of drug absorption and, subsequently, the bioavailability of the drug. The rate of *in-situ* gel formation is important because between instillation in the eye, and before a strong gel is formed; the solution or weak gel is prone to elimination by the fluid mechanics of the eye. It is assumed that the rate at which electrolytes from the tear fluid are adsorbed by the polymer depends on the osmotic gradient across the surface of the gel. As a result, the osmolality of the solutions may have an influence on the sol–gel transition rate. The hypotonic samples were non-irritating, whereas isotonic and hypertonic solutions caused an increase in lachrymation and blurred vision. The high tolerance of the hypotonic samples is due to the rapid formation of a gel residing in the conjunctival sac, thus avoiding any solution spreading over the sensitive cornea. When instilling hypotonic GelriteR solutions, the gels remain in the human eye for 20 h.[55, 9]



[→3)-β-D-Glcp-(1→4)- β- D -GlcpA-(1→4)- β- D - Glcp- (1→4)- α- L-Rhap-(1→]n

Fig. 12: The structure of deacetylated gellan gum.

Xanthan gum- xanthum gum is a high molecular weight anionic polysaccharide produced by the fermentation of the gram-negative bacterium *Xanthomonas campestris.* Xanthan gum has a cellulosic chain of D-glucose linked β-1, 4. For every alternate glucose there is a side chain consisting of β-D-mannose-(1,4)-β-D-glucuronic acid- (1,2)-α-D-mannose.

The terminal mannose moiety may carry pyruvate residues linked to the 4-and 6-positions. The internal mannose unit is acetylated at C-6. The degree of substitution for pyruvate usually varies between 30 and 40% whereas it is as high as 60–70% for acetate. The anionic character of this polymer is due to the presence of both pyruvic acid and glucuronic acid groups in the side chain**.** the drug release was the result of a complex interplay of osmotic forces, electrostatic interactions and water uptake between drug and polymer.

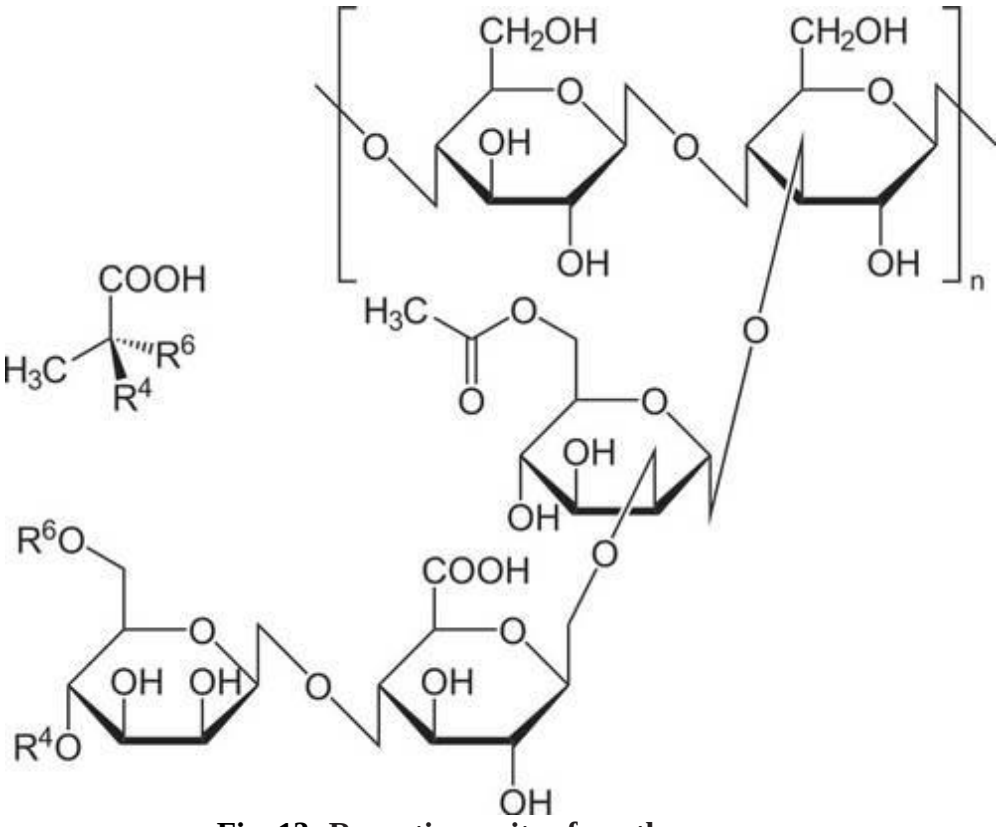


Fig. 13: Repeating units of xanthum gum.

EVALUATION PARAMETERS: following evaluation parameters followed for In-situ gel.for ocular delivery:

1. **Clarity:** The clarity of the formulations before and after gelling will be determined by visual inspection of the formulations under fluorescent light, alternatively against white and black backgrounds.[56, 57]
2. **pH:** The pH of the prepared in situ gelling system after addition of all the ingredients was measured using pH meter.[58]
3. **Gelling capacity:** The gelling ability of the prepared formulations was determined either visually or by SEM.

By visual inspection- The gelling capacity is determined by pouring a drop of the solution in a vial containing 2 ml of simulated tear fluid which should be freshly prepared and equilibrated at 370C, and both the time of gelation and the time taken for the gel formed to dissolve will to be noted.

The composition of the artificial tear fluid: Sodium chloride 0.670g, Sodium bicarbonate 0.200g, Calcium chloride dihydrate 0.008g, and purified water q.s. 100 g. physiological pH (7.4±0.2) was adjusted by adding the required amount of 0.1 N HCL.[4]

By SEM- SEM studies the surface morphology of the formulations at solution state and at gel state. By SEM image we can study compact and loose surface morphology of In-situ gel which helps in finding the gelation time of in situ-gel.[59]

4. **Viscosity and Rheological studies**:

Viscosity of the instilled formulation is an important factor in determining the residence time of drug in the eye. Rheology of formulation need to be determined before and after gelation by using either the Brookfield's viscometer (RVT model) or Cone and plate geometry viscometer (Brookfield RVCP DV-III). The formulation before gelling should have viscosity from 5 to 1000 mpas. After ion gel activation in the eyes it will have viscosity of about 50-50,000 mpas. The samples are analysed both at room temperature at 25 °c and thermo stated at 37 °c ± 0.5 °c by a circulating bath connected to viscometer adaptor prior to each measurement.[60, 61] Also rheological study needs to be performed for formulations with and without drug to analyze the effect of addition of drug on rheological behavior of polymer blend.[56] Angular velocity run from 10-100 rpm.[62] The hierarchy of shear rates was reversed and the average of two readings was used to calculate viscosity.

5. **Drug content:**

It is determined by taking 1ml of the formulation and diluting it to 100ml with distilled water. 1 ml was withdrawn and further diluted to 10 ml with distilled water. Concentration was determined at 200-400mm by using UV visible spectroscopy.[63]

6. **Isotonicity Evaluation:**

Isotonicity is important characteristic of the ophthalmic dosage forms. Isotonicity has to be maintained to prevent tissue damage or irritation of eye. All ophthalmic dosage forms are subjected to isotonicity testing, since they exhibited good release characteristics, optimum 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.[64,2,58]

7. **In Vitro Drug Release Profile: can be studied by either of the following method:**

By using dialysis tube: This study is performed in the Dialysis tube containing 1 ml of the formulation, which is then suspended in beaker at 37 ± 0.50C containing 100 ml artificial simulated tear fluid (pH 7.4) under continuous stirring at 20 RPM to stimulate the blinking effect

dialysis membrane (0.22 µm pore size), previously soaked overnight in simulated tear fluid is mounted by tied and sandwiched between the donor and receiver compartment.

Aliquots of 1 ml withdrawn at different time intervals and equal volumes of fresh media added to replace the withdrawn samples. Withdrawn samples analyze by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using an equation generated from standard calibration curve. The percentage cumulative drug release (% CDR) calculated. The obtained data is further subjected to curve fitting for drug release data.[65]

By using franz diffusion cell- In vitro release studies can also be carried out by using bi-chambered donor receiver compartment model (Franz diffusion cell). In this method 1ml of solution spread uniformly on a dialysis membrane, which is then contacted with receptor medium which is stirred continuously at 20 rpm to simulate blinking action of eyelids membrane (0.22 µm pore size), previously soaked overnight in simulated tear fluid is mounted by tied and sandwiched between the donor and receiver compartment. Aliquots of 1 ml withdrawn at different time intervals and equal volumes of fresh media added to replace the withdrawn samples. Withdrawn samples analyze by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using an equation generated from standard calibration curve. The percentage cumulative drug release (% CDR) calculated. The obtained data is further subjected to curve fitting for drug release data.[65]

8. In vivo Scintigraphy Studies:

Gamma scintigraphy is a well-established technique for in vivo evaluation of ophthalmic retention time. In this study the rabbits of either sex weighing 2–3 kg are used. The radiolabeled drug solution will then mixed with other formulation ingredients and required concentration of polymers. Gamma camera is autotuned to detect the radiation of radiolabeled material. Rabbits need to be anesthetized, then they are positioned under gamma camera and 25 µl of the radiolabeled drug solution and in-situ gel formulation containing radiolabeled drug instilled onto the left corneal surface of the rabbits (two groups). Recording will be conducted 5 sec after instillation and continued for 20 min using 128 ×128 pixel matrix. Individual 68 frames (68×16 sec) will be captured by dynamic imaging process. Region of interest (ROI) will be selected on the one frame of the image and time-activity curve will be plotted to calculate the rate of drainage from eye. A single whole body static image will also be taken after 2 hr of instillation of drug solution and formulation to see whether the drug entering in the blood or not.[66]

1. Antimicrobial activity:

Antimicrobial efficacy studies are carried out to ascertain the biological activity of sol-gel-system against microorganisms.This was determined by the agar diffusion test employing “Cup plate

technique". Sterile solutions of drug (standard solution) and the developed formulations were diluted at different concentration (test solutions) these solutions were poured in to cups bored into sterile nutrient agar previously seeded with test organisms (Pseudomonas aeruginosa and Staphylococcus aureus). After allowing diffusion of the solutions for 2 hours, the agar plates were incubated at 37°C for 24 hrs. The zone of inhibition (ZOI) measured around each cup was compared with that of control. The entire operation except the incubation was carried out in a laminar airflow unit. Both positive and negative controls were maintained during the study.[67]

Methods: The procedure employed in microbial assay may be divided into two broad classifications. Disc diffusion methodand Turbidimetric method (or) Serial dilution method

Disc Diffusion Method: Filter paper discs of 6mm diameter were impregnated with optimum amount of drug. The discs may be dried in incubator and stored in refrigerator. Liquid culture of the bacteria in broth was flooded on a solid medium in a plate (muller Hinton agar (or) nutrient agar) and excess was thrown away. Alternatively, the culture plate may sub cultured by the bacterial culture by using swab. The medicated discs were then placed on the plate and incubated overnight the zone of inhibition around the disc is noted.

Turbidimetric method or serial dilution method: In the turbidimetric assay of drug, the potency of the drug is based on inhibition of microbial growth as indicated by measurement of the turbidity (transmittance) of a suspension of suitable microorganism in a fluid medium to which have been added graded amounts of the test compounds changes in transmittance produced by known concentration of reference material which were compared with results.

Ex vivo drug release studies: Goat corneas are used to examine the permeation across the corneal membrane. The cornea is carefully removed along with a 5-6 mm of surrounding scleral tissue and washed with cold saline. The washed corneas are kept in cold freshly prepared solution of tear buffer of pH 7.4. The study is carried out by using Franz-diffusion cell in such a way that the cornea side is continuously remained in an intimate contact with formulation in the donor compartment. The receptor compartment is filled with STF pH 7.4 at 34˚C ± 0.5˚C. The receptor medium is stirred on a magnetic stirrer. The samples are withdrawn at different time intervals and analyzed for drug content. Receptor phase is replenished with an equal volume of STF (pH 7.4) at each time interval.[68]

Ocular irritation studies: Ocular irritancy studies were performed on male albino rabbits, weighing 1-2 kg. The studies were carried out with the guidelines of Council for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

The Draize-irritancy test was generally performed for the ocular irritation potential of the ophthalmic product prior to marketing. Mostly, left eye of each rabbit was used for test while the right eye was served as control. According to the Draize test, the amount of solution applied to the eye is normally 100µl is placed into the lower cul-de-sac. After dosing, the lids were held together for few seconds in order to avoid loss of the dosage form by lacrmation. The observation of the redness, swelling and irritation was done at time interval of 1hr, 24hrs, 48 hrs, 72hrs, and 1week after administration. The sterile formulation is administered 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).[69,62,70,71]

Sterility- All ophthalmic preparations should be sterile therefore the test for sterility is very important evaluation parameter. The sterility test was performed according to Indian Pharmacopoeia1996. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically transferred to fluid thioglycolate medium (20 ml) and soyabean-casein digest medium (20 ml) separately. The liquid was mixed with the media. The inoculated media were incubated for not less than 14 days at 30°C to 35°C in the case of fluid thioglycolate medium and 20°C to 25°C in the case of soyabean-casein digest media.[72]

Accelerated stability studies: Formulations are placed in ambient coloured vials and sealed with aluminium foil for a short terms accelerated stability study at 25°C to 28°C ambient temperature (temperature in the working area), 4±1°C (refrigerated temperature) and 37±2°C (temperature in the incubator) as per International Conference on Harmonization (ICH) states guidelines. Samples are analysed every month for clarity, pH, gelling capacity, drug content, rheological evaluation, and in vitro dissolution.[58]

Drug Polymer interaction study: Interaction studies can be performed in three ways one is by using UV, second is by taking IR spectra and third is by using DSC instrument.

In first method by UV the solutions of Polymer and drug prepared separately and in combinations and are autoclaved. The ultraviolet spectra taken before and after autoclaving using double beam ultraviolet visible spectrophotometer. Compare both the spectra for any possible change in solution content due to interactions between different ingredients.[66]

In the second method the IR spectra was taken by using FTIR spectrophotometer. The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi on KBr-press and the spectra was scanned in the wave number range of 6000-400 cm-1. The FTIR graph of pure drug and combination of drug with excipient are recorded, then compared [73].

In the third method DSC scan is runned for individual component and the mixture for the interaction study. [59] The interaction studies were carried out to check any possible physiochemical interaction among the formulation ingredients.

If UV spectra, IR spectra and DSC graph of the ingredients before and after mixing found to be identical and no additional peak emerged or existent peak shifted that confirms the formulation ingredients were compatible to each other and no physicochemical reactions taking place.

GLOBAL OPHRHALMIC MARKET- According to the forecast of some of the market research companies, the $25 billion global ophthalmic drug and devices market is expected to grow $36 billion by 2014. Scrip business insight which have estimated the market at $16.2 billion in 2010, expects growth to top $21 billion. The current annual growth rate is estimated at 4.6 percent. The USA is the largest market accounting for 40 percent of the total market and five European union state accounts for 18 percent of the global market. Glaucoma was the largest segment in market with share of 35 percent. There are around 109 compounds in developed pipelines. According to some data from the market 4914 ophthalmlogy clinical trials are being conducted worldwide and 102 in india. Currently marketed products of the in situ gelling formulations for ocular delivery are greater interest for pharma industry. One of the landmark product is timoptic-XE® in 0.25 and0.50% strengths by merck and company.

FUTURE PROSPECT

• To find the other triggering factors for the *in-situ* gel formation.

• To increase the proportion of the drug that cross the corneal membrane by reducing the rapid elimination.

• To search for the penetration enhancers devoid of side effects.

• To target the drug in the posterior chamber through non-invasive route.

APPLICABILITY OF IN SITU POLYMERIC DRUG DELIVERY SYSTEM:-

ORAL DRUG DELIVERY SYSTEM:- The pH-sensitive hydrogels have a potential use in site-specific delivery of drugs to specific regions of the GI tract. Hydrogels made of varying the proportions of PAA derivatives and crosslinked PEG allowed preparing silicone microspheres, which released prednisolone in the gastric medium or showed gastroprotective property.[74] Cross-linked dextran hydrogels with a faster swelling under high pH conditions, likewise other polysaccharides such as amidaded pectins, guar gum and inulin were investigated in order to develop a potential colon-specific drug delivery system. W. Kubo et al.[75] developed the formulations of gellan and sodium alginate both containing complexed calcium ions that undergo gelation by releasing of these ions in the acidic environment of the stomach.

Oral delivery of paracetamol was studied. For the oral in situ gel delivery system pectin, xyloglucan & gellan gum natural polymers are used. Pectin formulation for sustained delivery of paracetamol has been reported.[76] Advantages of pectin is water soluble so, no need to add organic solvent.

OCULAR DRUG DELIVERY SYSTEM:- In ocular delivery system natural polymers like gallen gum, alginic acid & xyloglucan are most commonly used. For local ophthalmic delivery system various compounds like anti-inflammatory agent, antimicrobial agent & autonomic drugs are used to relieve intra ocular tension in glaucoma. Conventional delivery system often results in poor availability & therapeutic response because high tear fluid turn over & dynamics which cause rapid elimination of the drug from the eye so, to overcome the bioavailability problem ophthalmic in-situ gel were developed.[77] To improve the bioavailability viscosity enhancers such as Hydroxy Propyl Methyl Cellulose, Carboxy Methyl Cellulose, Carbomers , Poly Vinyl alcohol used to increase the viscosity of formulation in order to prolong the precorneal residence time & improve the bioavailability , ease to manufacture.[33] Penetration enhancer such as preservatives , chelating agent , surfactants are used to enhance corneal drug penetration .[78]

NASAL DRUG DELIVERY SYSTEM :- In nasal in-situ gel system gellan gum & xanthan gum are used as in-situ gel forming polymers Momethasone furoate was evaluated for its efficacy for the treatment of allergic rhinitis.[79] Animal study were conducted using allergic rhinitis model & effect of in-situ gel on antigen induced nasal symptoms in sensitizes rats was observed . In-situ gel was found to inhibit the increase in nasal symptoms are compared to marketed preparation nosonex (Momethasone furoate suspension 0.05%).

RECTAL DRUG DELIVERY SYSTEM:- The rectal route may be used to deliver many types of drugs that are formulated as liquid, semisolid (ointments, creams and foams) and solid dosage forms (suppositories). Conventional suppositories often cause discomfort during insertion. In addition, suppositories are unable to be sufficiently retained at a specific position in the rectum, sometimes they can migrate up-wards to the colon that makes them possible for drug to undergo the first-pass effect. Choi et al.[80] developed novel in situ gelling liquid suppositories with gelation temperature at 30–36°C. Poloxamer 407 and/ or poloxamer 188 were used to confer the temperature-sensitive gelation property. In-situ gel possesses a potential application for rectal & vaginal route .Miyazaki et al. investigated the use of xyloglucan based thermo reversible gel for rectal drug delivery of Indomethacin. Administration of Indomethacin loaded xyloglucan based system to rabbit indicated broad drug absorption & a longer drug residence time as compared to that resulting after administration of commercial suppository. For better therapeutic efficacy & patient compliance, mucoadhesive, thermo sensitive, prolonged release vaginal gel incorporating

Clotrimazole-β-cyclodextrin complex formulated for treatment of vaginitis.[81]

VAGINAL DRUG DELIVERY SYSTEM:- The vagina, in addition to being an important organ of reproductive tract, serves as a potential route for drug administration. Formulations based on a thermo-plastic graft copolymer that undergo in situ gelation have been developed to provide the prolonged release of active ingredients such as nonoxynol-9, progestins, estrogens, peptides and proteins. [82] Chang et al.[83] have recently reported a mucoadhesive thermo-sensitive gel (combination of poloxamers and polycar-bophil), which exhibited, increased and prolonged antifungal activity of clotrimazole in comparison with conventional PEG-based formulation.

INJECTABLE DRUG DELIVERY SYSTEM:- One of the most obvious ways to provide sustained- release medication is to place the drug in delivery system and inject or implant the system into the body tissue. Thermoreversible gels mainly prepared from poloxamers are predominantly used.[84] The suitability of poloxamer gel alone or with the addition of hydroxypropylmethylcellulose (HPMC), sodium carboxymethylcellulose (CMC) or dextran was studied for epidural administration of drugs in vitro.[85] The compact gel depot acted as the ratelimiting step and significantly prolonged the dural permeation of drugs in comparison with control solutions. J. M. Barichello et al.[86] evaluated Pluronic F127 gels, which contained either insulin or insulin-PLGA nanoparticles with conclusion, that these formulations could be useful for the preparation of a controlled delivery system. Likewise, poloxamer gels were tested for intramuscular and subcutaneous administration of human growth hormone [87] or with the aim to develop a long acting single dose injection of lidocaine.[84] J. R. DesNoyer and A. J. McHugh [88] invented a new class of injectable controlled release depots of protein which consisted of blends of Pluronics with poly (D, L-lactide)/1-methyl-2- pyrrolidone solutions. Some other thermosensitive hydrogels may also be used for parenteral administration. ReGel ® (triblock copolymer PLGAPEG- PLGA) was used as a drug delivery carrier for the continuous release of human insulin [89]. Steady amounts of insulin secretion from the Re- Gel ® formulations upto day 15 of the subcutaneous injections were achieved. B. Jeong et al.[90] reported the synthesis of a biodegradable poly (ethylene oxide) and poly (L-lactic acid) hydrogel, which exists in a form of sol at an elevated temperature (around 45°C) and forms a gel after subcutaneous injection and subsequent rapid cooling to body temperature. In-situ forming Injectable drug delivery system , cross linking of hydrazide modified by aluronic acid with aldehyde modified version of cellulose derivatives such as carboxy methyl cellulose , methyl cellulose, hydroxy propyl methyl cellulose are used. These in-situ forming gel were used for preventing postoperative peritoneal adhesion thus avoiding pelvic pain, bowel obstruction & infertility. For a better therapeutic efficacy & patient compliance, mucoadhesive , thermo sensitive , prolonged release vaginal gel incorporating Clotrimazole-β-

cyclodextrin complex was formulated for treatment of virginities.[91]

Dermal And Transedermal Drug Delivery:- Thermally reversible gel of Pluronic F127 was evaluated as vehicle for the percutaneous administration of Indomethacin.[92] In-vivo studies suggest that 20% w/w aqueous gel may be of practical use as a base for topical administration of the drug. Poloxamer 407 gel was found suitable for transdermal delivery of insulin.[93] The combination of chemical enhancers and iontophoresis resulted in synergistic enhancement of insulin permeation.

Marketed Products of In Situ Polymeric System:

Timoptic-XE- It is a timolol maleate ophthalmic gel formulation of Merck and Co. Inc., supplied as a sterile, isotonic, buffered, aqueous gel forming solution of timolol maleate. This formulation is available in two dosage strengths 0.25% and 0.5% in market. The pH of the solution is approximately 7.0, and the osmolarity is 260-330 mOsm. Each ml of Timoptic-XE 0.25% contains 2.5 mg of timolol (3.4 mg of timolol maleate). Inactive ingredients include gellan gum, tromethamine, mannitol, and water for injection and the preservative used is benzododecinium bromide 0.012%. Timoptic-XE, when applied topically on the eye, reduces the elevated, as well as normal intraocular pressure, whether or not accompanied by glaucoma.[94]

Regel depot-technology- Regel is one of the Macromed's proprietary drug delivery system and based on triblock copolymer, composed of poly (lactide-co-glycolide)-poly(ethylene glycol)poly(lactide-co-glycolide). It is a family of thermally reversible gelling polymers developed for parenteral delivery that offers a range of gelation temperature, degradation rates and release characteristics as a function of molecular weight, degree of hydrophobicity and polymer concentration. Following injection, the physical properties of polymer undergo a reversible phase change resulting in formation of a water insoluble, biodegradable gel depot. Oncogel is a frozen formulation of paclitaxel in Regel. It is a free flowing liquid below room temperature which upon injection forms a gel in-situ in response to body temperature. hGHD-1 is a novel injectable depot formulation of human growth hormone (hGH) utilizing Macromed's Regel drug delivery system for treatment of patients with hGH deficiency.[95]

Cytoryn- This 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. It is a free flowing liquid below room temperature that instantly forms a gel depot upon injection from which the drug is released in a controlled manner. Cytoryn enhances the immunological response by safely delivering four times the maximum tolerated dose allowed by conventional IL-2 therapy. Cytoryn also activates the systemic antitumor immunity. Regel system stabilizes and releases IL-2 in its bioactive form. The release of drugs is controlled by the rate of diffusion from

and degradation of the depot.[96]

AzaSite- AzaSite is a marketed product of InSite Vision which has been approved in april 2007. AzaSite is a topical ophthalmic solution of azithromycin formulated in DuraSite (polycarbophil, edetate disodium, sodium chloride). AzaSite is supplied as a sterile aqueous ophthalmic formulation designed for topical administration. The recommended initial dose of the drug is instill 1 drop in the affected eye(s) twice daily, eight to twelve hours apart for the first two days and then instill 1 drop in the affected eye (s) once daily for the next five days.

Pilopine HS- Pilopine HS is a marketed product of Alcon Laboratories Inc. Pilopine HS (pilocarpine hydrochloride ophthalmic gel) 4% 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.

Akten™ - Akten™ is an HPMC-based gel of lidocaine hydrochloride for ocular surface anesthesia. Akten™ contains 35 mg of lidocaine hydrochloride per mL as the active ingredient. Akten™ also contains Hypromellose, Sodium Chloride, and Purified Water as inactive ingredients. The pH may be adjusted to 5.5 to 7.5 with Hydrochloric Acid and/or Sodium Hydroxide. The recommended dose of Akten™ is 2 drops applied to the ocular surface in the area of the planned procedure. Akten™ may be reapplied to maintain anesthetic effect.

Virgan- Vigran is an ophthalmic antiviral that is indicated for the treatment of acute herpetic keratitis. The recommended dosing regimen for Virgan is 1 drop in the affected eye 5 times per day (approximately every 3 hours while awake) until the corneal ulcer heals, and then 1 drop 3 times per day for 7 days. Virgan (ganciclovir) contains carbomer 974. The carbomers are polyacrylic acid derivatives that impart high viscosity to their aqueous solutions at neutral pH (above their pKa values) due to ionization and hydration of the carboxyl groups.

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.

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