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Ophthalmic pH Sensitive In-Situ Gel: A Review

Garge Lajri [1]* and Saudagar Ravindranath [2]

[1]* Department of Pharmaceutical Quality Assurance Techniques, R.G.Sapkal College of Pharmacy, Anjaneri, Nashik-422213, India

[2] Department of Pharmaceutical Chemistry, R.G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India

ABSTRACT

Ocular Drug Delivery has been a major challenging and interesting field for the pharmaceutical scientist due to unique anatomy and physiology of eye. The eye suffers from various problems like glaucoma, dry eye syndrome, keratitis, endopthalmitis, trachoma and conjunctivitis. To achieve the effective ocular therapy within the eye. An adequate amount of active ingredients must be delivered and maintained at the site of action. The conventional therapy exhibits the poor bioavailability due to the rapid precorneal drug loss. There are some static and dynamic barriers which also affect the bioavailability of drug. There are considerable efforts which are directed towards newer drug system for ocular administration to overcome the drawbacks of conventional therapy. The use of in situ overcomes the problems when drop instilled into the eye it undergoes sol-gel transition and transition form in cul-de-sac. This review involves in-situ gel system which includes thermally triggered system, pH triggered system and ion cross linking system. It involves detailed method of preparation and evaluation parameters of pH triggered system.

Keywords: Ocular Drug Delivery, in-situ gel, pH sensitive, temperature sensitive, ion sensitive.

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***Address for Correspondence:**

Lajri Garge, Department of Pharmaceutical Quality Assurance Techniques, R.G.Sapkal College of Pharmacy, Anjaneri, Nashik-422213, India.

INTRODUCTION

The eye is a sensory organ that converts light to an electric signal that is treated and interpreted by the brain.[1] Eye is vital and delicate organ of the body and delivering drug into eye is most complicated task as it offers the numerous protective mechanisms that are present in the eye to shield it from various foreign particles.2 Ophthalmic delivery system is a challenging area for the formulation chemist due to unique anatomy and physiology of the eye. The anatomy and physiology of the eye render this organ delicately impervious to foreign substances. The challenge to the formulator is to avoid the protective barriers of the eye without causing permanent tissue damage.[3] There are many eye diseases which can be affected to the eye and also eye vision. Therefore marketed ophthalmic dosage formulations are classified as conventional and non-conventional drug delivery systems. There are most commonly existing ophthalmic preparations such as drops and ointments about 70% of the eye dosage formulations are in market. But these formulation when instilled into eye they are rapidly drained away from the ocular surface due to blinking, tear flow and lacrimal nasal drainage of the eye. Only a small amount of drug is available for its therapeutic effect ensuing in frequent dosing application to the eye. So to overcome these problems newer pharmaceutical ophthalmic formulation such as in-situ gel, nanoparticle, liposome, nano suspension, micro emulsion, intophoresis and ocular inserts have been established in last three decades to increase the bioavailability of the drug in a sustained and controlled manner.[4]

ANATOMY OF EYE

The human eye is divided into two important segments i.e. anterior segment which involves the cornea, conjunctiva, iris, pupil, ciliary body, anterior chamber, aqueous humor, lens and trabecular meshwork and the posterior segment includes vitreous humor, sclera, retina, choroid, macula and optic nerve. The outermost membrane of the eye is cornea it is a clear, transparent, thin vascular tissue that is composed of five layers: epithelium, bowmans's layer, stroma, Descemet's membrane and endothelium. Aqueous humor consists of clear liquid that fills both the posterior and anterior chambers of the eye. It is major source of nutrition for the cornea.[5] The iris is a thin circular contractile curtain located in front of the lens but behind the cornea it is a diaphragm of variable size whose function is to adjust the size of the pupil to regulate the amount of light admitted into the eye and adjust with the help of iris sphincter and dilator

muscle. The ciliary muscle is a ring of smooth muscles in the eye's middle layer that controls space for observing objects at varying distances. The lens is a transparent biconvex structure encased in a thin transparent covering shape of the lens. It is a flexible unit consisting of layers of tissue enclosed in a capsule. It is deferred from the ciliary muscles by very thin fibers called the zonules. The conjunctiva is a mucous membrane that begins at the edge of the cornea and lines the inside surface of the eyelids and sclera upto limbus. It provides protection to the eyes by secreting mucus that prevents entry of microorganisms and lubricating the eyes. The sclera is protective outer layer of the eye referred to as the "white of the eye" and it maintains the shape of the eye. It acts as a principal protection to the internal organs. The sclera is juxtaposed by a highly vascularized tissue known as the choroid which is sandwiched between the retina and sclera. The choroid is a thin highly vascular membrane that is dark brown in colour and contains a pigment that absorbs excess light and so prevents blurred vision it is the second layer of the eye and lies between the sclera and the retina. It contains the blood vessels that provide nourishment to the outer layers of the retina. The retina is a multiple layers and complex structure that consists of vascular glial and neural cells and nerve fibers. It is located at the back of the human eye. It is a light-sensitive structure which contains photosensitive cells that capture light rays and convert them into electrical impulses. These impulses travel along the optic nerve to the brain, where they are converted into images. The vitreous humour is smaller section in front which contains water like transparent thin-jelly-like substance that is distributed between retina and lens.[5,8,9]

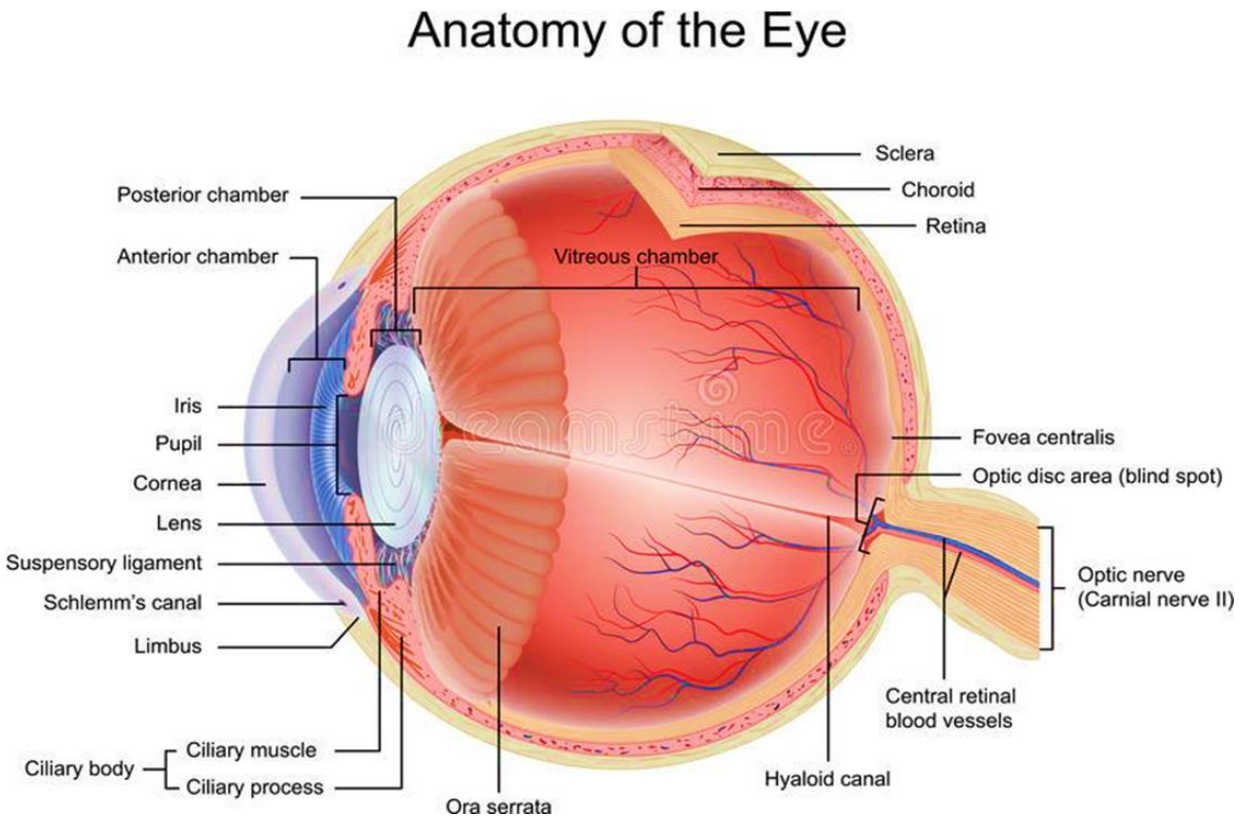

Figure 1: Anatomy of the human eye

MODE OF DRUG ADMINISTRATION TO EYE

A successful design of a drug delivery system therefore requires a combined knowledge of the drug molecule. There are some possible routes of drug delivery into the ocular tissues. The selection of the route of administration of drug depends primarily on the target tissue. A significant challenge to the formulator is to bypass the protective barriers of the eye without causing permanent tissue damage. Ophthalmic drug delivery is used only for the treatment of local conditions of the eye and cannot be used as a entry of a drug into the systemic circulation. Conventional ophthalmic formulations like solution, suspension and ointment have several disadvantages which effect into poor bioavailability of drug in the ocular cavity. Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the front area of the eye for prolong period of time.[1,2] The bioavailability of traditional ocular drug delivery systems such as eye drops is very poor because the eye is protected by a series of complex defense mechanisms that make it tough to achieve an effective drug concentration within the target area of the eye. The anatomy physiology of the eye is one of the most complex and unique systems in the human body. Lachrymation, effective drainage by the nasolacrimal system the inner and outer blood-retinal barrier the impermeability of the cornea and inability of absorption by other non-corneal structures cause the eye to be impervious to foreign substances. While these innate barriers are advantageous for delaying the invasion of undesired molecules pathogens and particulates they pose significant challenges to delivering ocular drugs.[10] Some routes of administration of the drug to the are given below. (Figure 2)

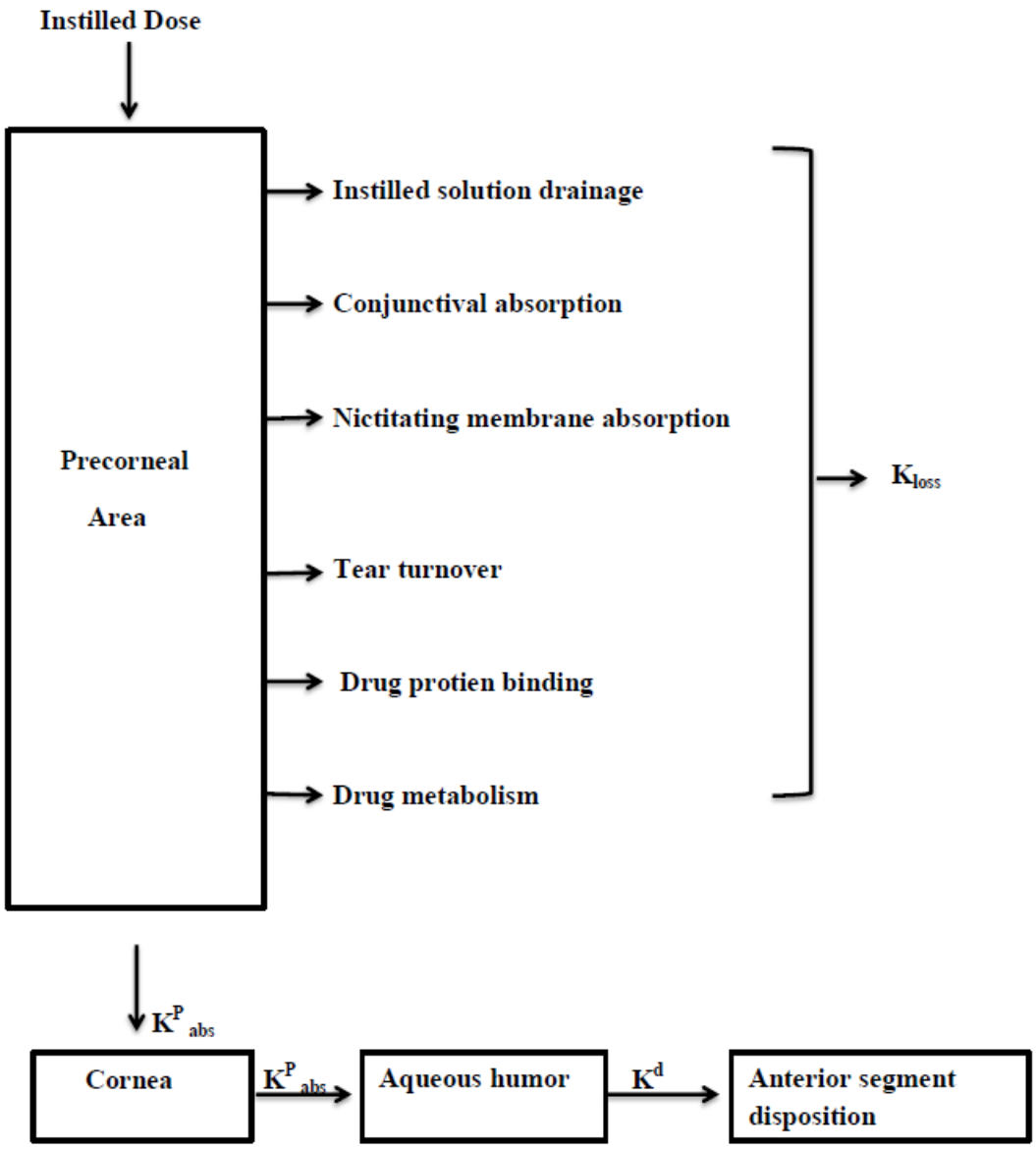

Figure 2: Routes of Administration of drug into Eye

1. Topical Administration

Topical ocular drug administration is skilled by eye drops but they have only a short contact time on the eye surface. The contact and thereby duration of drug action can be prolonged by formulation design that is by gels, gellifying formulations, ointments and insert. It is the most commonly used route of drug administration for most of the drugs that are topically applied. The site of action is typically different layers of the cornea, conjunctiva, sclera and the other tissues of the anterior segment such as the iris and ciliary body. Upon administration precorneal factors and structural barriers negatively affect the bioavailability of topical formulations. Precorneal factors comprise solution drainage, blinking, tear film, tear turn over and induced lacrimation. Viscosity is another factor that can regulate nonproductive absorption and ocular absorption. Increasing viscosity may decrease drainage rate, prolong precorneal residence time and increase ocular absorption.[7,10]

2. Oral Administration

Oral delivery alone or in combination with topical delivery has been studied for different reasons. Topical delivery failed to produce therapeutic concentrations in the posterior segment. Also oral delivery was studied as a patient preferred route to treat chronic retinal diseases as compared to the parenteral route. However limited availability too many of the targeted ocular tissues bounds the utility of oral administration which requires high dosage to achieve important therapeutic efficacy. Such doses can result in systemic side effects. Hence parameters such as safety and toxicity need to be measured when trying to obtain a therapeutic response in the eye upon oral administration.[7]

3. Systematic Administration

Following systemic administration the blood-aqueous barrier and blood-retinal barrier are the major barriers for the anterior segment and posterior segment of the ocular drug delivery. Due to the presence of blood retinal barrier systemic administration has achieved a limited success to deliver drugs to the vitreo-retinal tissues. This route of administration may also result in non-specific binding of drug to other tissues and cause systemic cytotoxicity. Only 1-2% of plasma drug concentration is achieved in the vitreous

humor and therefore requires frequent administration to maintain therapeutic drug level. Even though it is ideal to deliver the drug to the retina through systemic administration it is still a challenge because of the blood-retina barrier which strictly regulates drug permeation from blood to the retina. Hence specific intravenous targeting systems are needed to conveyance molecules through the choroid into deeper layers of the retina.[10]

4. Intravitreal Administration

The entry of drug straight into the vitreous and retina can be achieved by administrating drug directly into the vitreous. Small molecules are able to diffuse rapidly in the vitreous but the movement of large molecules particularly positively charged is restricted. The delivery from the vitreous to the choroid is more complicated due to the hindrance by the RPE (Retinal Pigment Epithelium) barrier. Distinct other routes intravitreal injection carries higher drug concentrations to the vitreous and retina. Drug distribution in the vitreous is nonuniform .This is also depends on the pathophysiological condition and molecular weight of the administered drug. Longer retention time and higher vitreous concentration of drugs was obtained following this route of administration.[1,2,10]

THE BARRIERS

I. Drug Loss from the Ocular Surface

After instillation the flow of lacrimal fluid removes instilled compounds from the surface of the eye. Routes of drug kinetics refer to following processes:

1) Transcorneal permeation into the anterior chamber from lachrymal fluid.

2) Non-corneal drug permeation through the conjunctiva and sclera into the anterior uvea.

3) Drug distribution from the blood stream via blood-aqueous barrier into the anterior chamber.

4) Elimination of drug from the anterior chamber to the aqueous humor turnover to the trabecular meshwork and Sclemm's canal.

5) Drug removal from the aqueous humor into the systemic circulation across the blood-aqueous barrier.

6) Drug distribution from the blood into the posterior eye across the blood-retina barrier and intravitreal drug administration.

7) Drug elimination from the vitreous via posterior route across the blood-retina barrier.

8) Drug elimination from the vitreous via anterior route to the posterior chamber.

Though the lacrimal turnover rate is only about 1 µl/min the excess volume of the instilled fluid goes to the nasolacrimal duct rapidly in a couple of minutes. Another cause of non-productive drug removal is its systemic absorption instead of ocular absorption. Systemic absorption may take place either directly from the conjunctival sac via local blood capillaries or after the solution flow to the nasal cavity.[13]

II. Lacrimal Fluid Barriers

Corneal epithelium confines drug absorption from the lacrimal fluid into the eye. Mature epithelial cells forms corneal barrier from the limbal region they migrate towards the center of the cornea and to the apical surface. The corneal epithelial cells form tight connections that limit the paracellular drug permeation. Therefore lipophilic drugs have normally at least an order of magnitude higher permeability in the cornea than the hydrophilic drugs.[1,2] Despite the tightness of the corneal epithelial layer transcorneal permeation is the key route of drug entrance from the lacrimal fluid to the aqueous humor. In general the conjunctiva is more permeable epithelium than the cornea and its surface area is also nearly 20 times greater than that of the cornea. Absorption of the drug across the bulbar conjunctiva has gained increasing attention recently, since conjunctiva is fairly permeable to the hydrophilic and large molecules. Therefore, for larger bio-organic compounds such as proteins and peptides it may serve as a route of absorption.[2]

III. Blood- Occular Barriers

The eye is protected from the xenobiotics in the blood stream by blood-ocular barriers. These barriers have two types: blood-aqueous barrier and blood-retina barrier. The anterior blood-eye barrier is composed of the endothelial cells in the uvea it is the middle layer of the eye beneath the the sclera. It consists of the iris, ciliary body and choroid. This barrier prevents the access of hydrophilic drugs and plasma albumin into the aqueous humor. The posterior barrier between blood stream and eye is involved of retinal pigment epithelium (RPE) and the tight walls of retinal capillaries. Inflammation may interrupt the integrity of this barrier causing the unlimited drug delivery to the anterior chamber. In fact the permeability of this barrier is poorly categorized. Unlike retinal capillaries the vasculature of the choroid has wide blood flow and leaky walls. Drugs easily gain access to the choroidal extravascular space but after that distribution into the retina is limited by the RPE and retinal endothelia. Despite its high blood flow the choroidal blood flow establishes only a minor fraction of the entire blood flow in the body. Unlike blood brain barrier the blood-eye barriers have not been considered in terms of drug transporter and metabolic enzyme expression.[1,2,13]

Classification of Ocular Drug Delivery System:

Ocular drug deliveries consist of following types of dosage forms:

1). Semisolid -Gel, Ointment.

2). Solid -Ocular Inserts.

3). Liquid – Solution, Suspension.

4). Intraocular –Implant, Injections.

Types of Ocular Gel

I. Organogel

II. Hydrogel

Advantages of Ocular In-situ Hydrogel

I. Reduced dose concentration.

II. Reduced dosing frequency.

III. Improved patient acceptability.

IV. Generally more comfortable than insoluble or soluble insertion.

V. Increased bioavailability due to-

a. Increased precorneal residence time.

b. Decreased nasolacrimal drainage of the drug

VI. Chances of undesirable side effects arising due to systemic absorption of the drug through naso-lacrimal duct are reduced.

VII. Easy to manufacture and hence less complex process and reduces cost of Manufacturing.[6,11]

Disadvantages of Ocular In-situ Hydrogel

I. Blurred vision.

II. Difficulty in self-insertion.

III. Matted eyelids.

IV. Limited values in terms of improvement of bioavailability.[16,19]

Classification of In Situ Gel

1. Based on physical stimuli.

a. Thermally Triggered System.

b. pH Triggered System.

2. Based on physical mechanism

a. Swelling.

b. Diffusion.

3. Based on chemical reaction

a. Ion Cross Linking.

b. Enzymatic Cross Linking.

c. Photo- Polymerisation.

In-Situ formation based on physiological stimuli

a. Thermally Trigged System

Temperature-sensitive hydrogels are possibly the most commonly studied class of environment-sensitive polymer systems in drug delivery research. The use of biochemical whose transitions from sol-gel is triggered by increase in temperature is a smart way to approach in-situ formation. In this system gelling of the solution is triggered by change in temperature thus satisfying the drug release. These hydrogels are liquid at room temperature (20–25 °C) and undergo gelation when in contact with body fluids (35– 37 °C) due to an increase in temperature. The ideal critical temperature range for such system is ambient and physiologic temperature such that clinical manipulation is facilitate and no external source of heat other than that of body is required for trigger gelation. A useful system should be tolerable to account for small differences in local temperature such as might be encountered in additions at the surface of skin or in the oral cavity. Three main strategies are occurs in engineering of thermo responsive sol-gel polymeric system. The temperature-sensitive hydrogels are classified into negatively thermo sensitive, positively thermo sensitive and thermally reversible gels.[2,3,13]Negative temperature-sensitive hydrogel have a lower critical solution temperature (LCST) and contract upon heating below the LCST. Polymers with low critical temperature (LCST) transition between ambient and physiologic temperature is used for this resolve. One of the most extensively investigated polymers that exhibit useful LCST transition is poly (N-isopropyl acrylamide) (PNIPAAm). A positive temperature sensitive hydrogel has 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 required of swelling. The most commonly used thermo reversible gels are these prepared from poly (ethylene oxide)-*b*-poly (propylene oxide)-*b*-poly (ethylene oxide) (Pluronics®, Tetronics®, poloxamer). Polymer solution is a free flowing liquid at require temperature and gels at body temperature. Novel "protein polymers" called as ProLastins which undergo an irreversible sol gel transition when injected as a solution into the body the material forms a firm stable gel within minutes. It remains at the site of injection providing absorption times from less than one week to many months. Such a system would be easy to administer into desired body cavity. [13]

b. pH Triggered Systems

Another formation of in situ gel based on physiologic stimuli is formation of gel is made by pH changes. In this system gelling of the solution is triggered by a change in pH. At pH 4.4 the formulation is a free flowing solution which undergoes coagulation when the pH is raised by the tear fluid to pH 7.4. The pH change of about 2.8 units after instillation of the formulation pH4.4 into the tear film leads to an almost rapid transformation of the highly fluid latex into a viscous gel. All the pH-sensitive polymers contain dependant acidic or basic groups that accept or release protons in response to changes in environmental pH. The polymers with a great number of ionizable groups are known as polyelectrolytes. Swelling of hydrogel increases as the exterior pH increases in case of weakly acidic (anionic) groups but decreases if polymer contains weakly basic (cationic) groups. The most of anionic pH sensitive polymers are based on PAA (Carbopol®, carbomer) or its derivatives. Likewise poly vinyl acetal diethyl amino acetate (AEA) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition. Drug formulated in liquid solutions have several limitations including bioavailability and partiality to be easily distant by tear fluid. To minimize this factors and exploit this drug delivery by making a poly(acrylic acid) (PAA) solution that would be gel at pH 7.4 by that we found that at concentrations high to cause gelation however the low pH of PAA solution would cause damage to surface of eye before being neutralized by the lacrimal fluid. This problem was solved by partially by combining PAA with HPMC a viscous enhancing polymer which resulted in pH responsive polymer mixtures that was solution at pH 4 and gel at pH 7.4. Mixtures of poly (methacrylic acid) (PMA) and poly (ethylene glycol) (PEG) also has been used as a pH sensitive system to attain gelation.[2,13]

Method of preparation (pH triggered method)

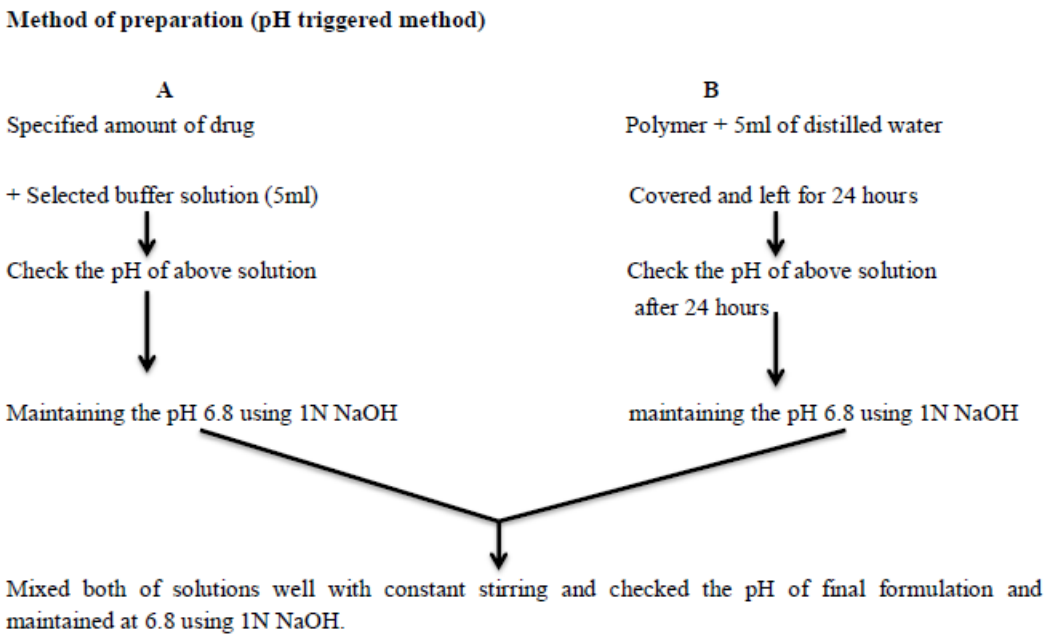

Figure 3: Method of Preparation

In Situ formation based on physical mechanism

a. Swelling

In situ gel formation material absorbs water from surrounding environment and expands to desired space. One such substance is myverol 18-99 (glycerol mono-oleate) which is polar lipid that swells in water to form lyotropic liquid crystalline phase structures. It has some bioadhesive properties and can be degraded invivo by enzymatic action.[21,23]

b. Diffusion

This method involves the diffusion of solvent from polymer solution into adjacent tissue and results in precipitation of polymer matrix. N methyl pyrrolidone (NMP) has been shown to be a suitable solvent for such system.[14,21]

In Situ formation based on chemical reaction

Chemical reactions that results in situ gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes and photo-initiated processes.[20]

a. Ionic Cross Linking

Polymers may undergo phase transition in existence of several ions. Some of the polysaccharides fall into the class of ion-sensitive ones.[20] While k-carrageenan forms rigid, fragile gels in account of small amount of K+, i-carrageenan forms flexible gels mainly in the presence of Ca2+. Gellan gum economically available as Gelrite® is an anionic polysaccharide that goes through in situ gelling in the presence of mono and divalent cations, including Ca2+, Mg2+, K+ and Na+. Gelation of the low methoxy pectins can be caused by divalent cations that are Ca2+. Likewise alginic acid goes through gelation in presence of divalent/polyvalent cations. Example Ca2+ due to the contact with guluronic acid block in alginate chains.[21]

b. Enzymatic Cross Linking

In Situ formation catalysed by natural enzymes has not been considered widely but appears to have some advantages over chemical and photochemical approaches. For example, an enzymatic process works efficiently under physiologic conditions without need for potentially harmful chemicals such as monomers and initiators. Intelligent stimuli-responsive delivery systems using hydrogels that can release insulin which have been investigated. Cationic pH-sensitive polymers containing immobilized insulin and glucose oxidase can swell in response to blood glucose level releasing the entrapped insulin in a pulsatile fashion. Adjusting the amount of enzyme also provides a appropriate mechanism for controlling the rate of gel formation which allows the mixtures to be injected before gel formation.[20,21]

c. Photo – Polymerization

Photo-polymerisation is commonly used for in situ formation of biomaterials. A solution of reactive macromer and initiator can be injected into a tissues site and the use of electromagnetic radiation to form gel. Acrylate or related polymerizable functional groups are normally used as the individual monomers and macromers because they rapidly undergo photo-polymerisation in the existence of suitable photoinitiator. Typically long ultraviolet and visible wavelengths are used. Short wavelength ultraviolet is not used often because it has partial penetration of tissue and biologically harmful. A ketone such as 2,2 dimethoxy-2-phenyl acetophenone, is often used as the initiator for ultraviolet photo-polymerization while camphorquinone and ethyl eosin initiators are often used in visible light systems. These systems can be designed readily to be degraded by chemical or enzymatic processes for long term persistence in vivo. Photo polymerizable systems when introduced to the desired site through injection get photo cured in situ with the help of fiber optic cables and then release the drug for prolonged period of time. The photo-reactions provide fast polymerization rates at physiological temperature. Furthermore, the systems are easily located in complex shaped volumes leading to an implant formation.[13,21]

In Situ Gelling Polymers

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

Ideal Characterstics of Polymers

1. It should be biocompatible.

2. It should be capable of adherence to mucus and non irritating.

3. It should have pseudo plastic behaviour.

4. It should influence the tear behaviour.

5. 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. [13,23]

POLYMERS USED AS IN SITU GELLING AGENTS ARE

1. Gellan gum

2. Alginic gum

3. Carbomer

4. Pluronic F127

5. Xylogulan

6. Pectin

7. Xanthum Gum

8. Chitosan

Carbomer

Carbomer is a high molecular weight, cross linked polyacrylic acid derivative and has strong mucoadhesive property. It is aqueous soluble vinyl polymer. In aqueous solution it shows sol to gel transition when the pH is raised above its pKa of about 5.515. As the concentration of carbomer increases its acidic nature may lead to irritation to eye. Addition of cellulose will reduce polymer concentration and will improve gelling property. Different grades of carbomer are present in market which includes carbopol 934(lowest cross linking density), carbopol 940 (highest cross linking density) and carbopol 981 (intermediate cross linking density). Carbopol is used as gelling, emulsifying and suspending agents. Carbopol are in compatible with cationic polymers, strong acids, high level of electrolytes and they swell in water 1000 times when exposed to water to form gelation pH of 4-6 do not dissolve in water.[13]

EVALUATION PARAMETERS

1. Physical Appearance:

Physical appearance of formulation was visually observed.

2. pH:

pH affects both solubility as well as stability of drug in ophthalmic formulations. It should be such that the formulation will remain stable at that pH. The pH of the prepared in situ gelling system after addition of all the ingredients will be measured using pH meter.[13]

3. Clarity:

The clarity of the formulations before and after gelling will be determined by visual inspection of prepared in situ formulation is checked in presence of any particulate matter under fluorescent light against a white and black background. [12,13]

3. Gelling Capacity Test:

Gelling capacity of prepared formulation is determine by placing the drop of formulation into a test tube containing 2.0 ml of pH 7.4 freshly prepared simulated tear fluid (STF) equilibrated at 35±1◦C and visually observed. The time taken for gelling was noted.[29]

4. 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 200-400nm by using UV visible spectroscopy.[13]

5. Viscosity:

Viscosity measures the resistance of a solution to flow when a stress is applied. It is an important factor in determining residence time of drug in the eye. The solutions were allowed to gel in the STF and then viscosity measurements can be calculated by using Brookfield viscometer. The In situ gel formulation was placed in sampler tube. The angular velocity ran from 10-100 rpm. Viscosity of the formulations increases with increases in the polymer concentration. The hierarchy of shear rate was reversed and average of two readings was used to calculate the viscosity. The samples are analysed at room temperature at 25 °C.[26]

6. *In-vitro* Release Studies:

In vitro drug release study of In-situ gel solution was carried by using Franz diffusion cell. Formulation placed in donor compartment and freshly prepared stimulated tear fluid in the receptor compartment. Between donor and receptor dialysis membrane is placed .Then whole assembly is placed in thermostatically controlled magnetic stirrer. The temperature of medium was maintained at 37 °c ±0.5 °c. 1 ml of sample is withdrawn at predetermine time interval of 1 hour to 6 hour and same volume of fresh is replaced. The withdrawn sample is diluted to 10 ml of volumetric flask with respective solvent and analysed by UV spectrophotometer.[30]

7. Accelerated Stability Studies:

A stability study for in situ formulation is carried out as per ICH guidelines to determine the physical stability of the formulation under accelerated storage conditions. All the formulation were analyzed for visual appearance, clarity, pH and drug remaining for 6 weeks of stability studies.[23]

REFERENCES

1. Pandya TP, Modasiya MK, Patel VM, Ophthalmic In-Situ Gelling System, International Journal of Pharmacy and Life Sciences, 2011; 2(5): 730-738.
2. Jain S, Jain P, Mishra M, Pathak A, A Review on Triggered Gel for Ocular Drug Delivery System, International Journal of Pharmaceutical and Biological Archieves, 2014; 5(4):19-24.
3. Tinu TS, Litha T, Kumar AB, Polymers used in Ophthalmic In Situ Gelling System, International Journal of Pharmaceutical Sciences Review and Research, 2013; 20(1):176-183.
4. Sharma Jitendra PK, Banik A, Dixit S, A New Trend: Ocular Drug Delivery System, An International Journal of Pharmaceutical Science, 2011; 2(3):1-20.
5. Yumei WU et al., Research Progress of in-situ gelling Ophthalmic Drug Delivery System, Asian Journal of Pharmaceutical Science, 2018; 22-40.
6. Gambhire S, Bhalerao K, Singh S, In Situ Hydrogel: Different Approach to Ocular Drug Delivery, International Journal of Pharmacy and Pharmaceutical Science, 2013; 5(2):27-36.
7. Saini N, Kumar D, An Insight to Ophthalmic Drug Delivery System, International Journal of Pharmaceutical Studies and Research, 2012; 3(2):09-13.

8. Hajare A, Mali S, Salunke S, Nadaf S, Bhatia N, Bagal P, Gaikwad S, Pawar K, A Rational Approach to Ocular Drug Delivery System: An Overview, World Journal of Pharmaceutical Science, 2014; 3(2):3324-3348.
9. Yerikala R, Kothapalli CB, Peddappi Reddigari JR, A Novel Approach on Ophthalmic Drug Delivery System, Journal of Drug Delivery and Therapeutics, 2017; 7(6):117-124.
10. Palani S, Joseph NM, Goda CC, Zachariah A, Ayenew Z, Ocular Drug Delivery: A Review, International Journal Pharmaceutical Science and Research, 2010; 1(3):1-11.
11. Hoare TR, Kohane DS, Hydrogels in drug delivery: Progress and Challenges, Polymers, 2008; 49: 1993-2007.
12. Sandeep DS, Charyulu NR, Narayanan AV, Smart In Situ Gels for Glaucoma-An Overview, International Journal of Pharmaceutical Science Research and Review, 2018; 50(1):94-100.
13. Majeed A., Khan N. A. Ocular in situ gel: An overview. Journal of Drug Delivery and Therapeutics, 2019; *9*(1):337-347.
14. Singh A, Negi D, Mishra N, Baldi A, Recent trends in Ocular Drug Delivery, Pharmaspire, 2018; 10(2):55-63.
15. Prausnitz MR, Noonan JS, Permeability of Cornea, Sclera and Conjunctivia: A Literature Analysis of Drug Delivery to Eye, Journal of Pharmaceutical Science, 1998; 87(12):1479-1488.
16. Ahmed EM, Hydrogel: Preparation, Characterization and application: A Review, Journal of Advanced Research, 2015; 6: 105-121.
17. Saini R, Saini S, Singh G, Dr. Banerjee A, In Situ Gels- A New Trends In Ophthalmic Drug Delivery System, International Journal of Pharmaceutical Science and Research, 2015; 6(5):886-890.
18. Nittur JR, Kunchu K, Teetha G, Tamizh M, In Situ Ophthalmic Gels: A Developing Trend, International Journal of Pharmaceutical Science Research and Review, 2011; 7(1):8-14.
19. Wadhwa K, Sharma C, Gosawmi M, Thakur N, In-Situ Ocular Gel-A Novel Approach Towards Ocular Drug Delivery, European Journal of Biomedical and Pharmaceutical Science, 2018; 5(6):237-244.
20. Agarwal KI, Mehta N, Namdev A, Gupta AK, In-Situ Gel Formation of Ocular Drug Delivery System an Overview, Asian Journal of Biomedical and Pharmaceutical Science, 2011; 1(4):01-07.
21. Nirmal HB, Bakliwal SR, Pawar SP, In-Situ gel: New Trends in Controlled and Sustained Drug Delivery System, International Journal of Pharma Tech Research, 2010; 2(2):1398-1408.
22. Shaikh A, Talat F, Shahi S, pH Triggered In-Situ Gel: Formulation Development and Evaluation of In Situ Ophthalmic Gel of Olopatadine Hydrochloride, International Journal of Pharmaceutical Science Review and Research, 2015; 35(1):180-185.
23. Majeed A, Khan NA, Ocular in situ gel: An Overview, Journal of Drug Delivery and Therapeutics, 2019; 9(1):337-347.
24. Noriyuki K, Shinobu F, Recent Advances in Ocular Drug Delivery Systems, Polymers, 2011; 3:193-221.
25. Aldrich DS, Bach CM, Brown W, Chambers W, Fleitman F, Hunt D et al., Ophthalmic Preparations, Stimuli to the Revision Process, 2013; 39(5):1-21.
26. Uddin MS, Mamun AA, Kabir MD, Setv JR, Zaman S, Begum Y et al., Quality Control Test for Ophthalmic Pharmaceuticals: Pharmacopoeial Standards and Specifications, Journal of Advances in Medical and Pharmaceutical Sciences, 2017; 14(2);1-17.
27. Arto Urtti ,Challenges and Obstacles of Ocular pharmacokinetics and drug delivery, Advances Drug Delivery Reviews, 2006; 58:1131-1135.
28. Patel PB, Shastri DH, Shelat PK, Shukla AK, Ophthalmic Drug Delivery System: Challenges and Approach, Systematic Reviews in Pharmacy, 2010; 1(2):113-120.
29. Gupta A, Manocha N, Formulation and Evaluation of In-Situ Ophthalmic Drug Delivery System, International Journal of Pharmaceutical and Biological Archives, 2012; 3(4):715-718.