Review Article

Ocular Drug Delivery System – A Review Based on Ocular *In-situ* Gels

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ABSTRACT

Drug transport by conventional ophthalmic formulation is restricted due to some static (different layers of eye i.e. cornea, sclera, retina) and dynamic barriers (blood aqueous & blood retinal barrier) and also by rapid precorneal elimination of drug, resulting in poor bioavailability and therapeutic response. The unique anatomy and physiology of eye challenges the pharmaceutical researchers for the ocular delivery of drugs and makes it more interesting field of research. This interest has been sparked by development of ocular *insitu* gels in which liquid gel phase transition upon administration into eye occurs due to temperature, pH or ion induced change. This novel drug delivery system prolongs residence time of drug in the eye thus decreases dosing frequency, improves bioavailability and prevents invasive treatment. Thus in this present update, the article discusses about the anatomy and physiology of eye, advantages, preparation methods and evaluation tests of ocular in-situ gels. This article also focuses on the past research work on ocular *insitu* gels and present available marketed formulations.

Keywords: In-situ gels, marketed products, past research work on *Insitu* gels, preparation methods, temperature induced in-situ gelling.

Received 24 March 2017 Received in revised form 12 May 2017 Accepted 14 May 2017

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INTRODUCTION

Eye is unique and vital organ. Today, topical ophthalmic application is considered the preferred way to achieve therapeutic levels of drug agents used to treat ocular diseases, such as conjunctivitis, keratitis, Blepharitis, mycotic keratitis, acrimonious keratitis. The conventional preparations for this route fall into several categories: solutions, suspensions, semisolids, and others. Bioavailability, particularly for ocular solutions, ranges from 1 to 10 % of the total administered dose. This is due to the rapid precorneal clearance kinetics resulting from reflex tearing and blinking, where half-life times of instilled isotonic solutions approximate only 15 seconds in the human.[1] The eye drop dosage form is easy to instill but suffers from the inherent drawback that the majority of the medication it contains is immediately diluted in the tear film as soon as the eye drop solution is instilled into the cul-de-sac and is rapidly drained away from the precorneal cavity by constant tear flow, a process that proceeds more intensively in inflamed than in the normal eyes, and by lachrymal-nasal drainage.[2] Therefore, only a very small fraction of the instilled dose is absorbed into the target tissues, and relatively concentrated solution is required for instillation to achieve an adequate level of therapeutic effect. The frequent periodic instillation of eye drops becomes necessary to maintain a continuous sustained level of medication. This gives the eye a massive and unpredictable dose of medication, and unfortunately, the higher the drug concentration in the eye drop solution, the greater the amount of drug lost through lachrymal-nasal drainage system. Subsequent absorption of this drained drug, if it is high enough, may result in undesirable systemic side effects. [3]

In situ gels are made from polymers that exhibit phase transition due to physicochemical change in the environment. They can be conveniently dropped as a solution into the conjunctival

sac in the eye. Upon contact with the lachrymal fluid, the polymer changes its conformation to form a gel. This delivery system has the ease of administration similar to an ophthalmic solution and has a long retention time because of the gel formation. Several polymers have been used for preparing in-situ gels. [4].

1.1 ANATOMY AND PHYSIOLOGY OF HUMAN EYE: [5-8]

The eye is most important organ of human body. The cornea, lens and vitreous body are transparent media with no blood vessels. Oxygen and nutrients are transported to these non vascular tissues by the aqueous humor. The aqueous humor has a high oxygen tension and about the same osmotic pressure as blood. The cornea also derives part of its oxygen need from the atmosphere and is richly supplied with free Nerve endings. The transparent cornea is continued posterior into opaque white sclera, which consists of tough fibrous tissue. Both cornea and sclera withstand the intra ocular tension constantly maintain in the eye. The eye is constantly cleansed and lubricated by the lachrymal apparatus. Which consist of four structures lachrymal glands, lachrymal canals, lachrymal sac

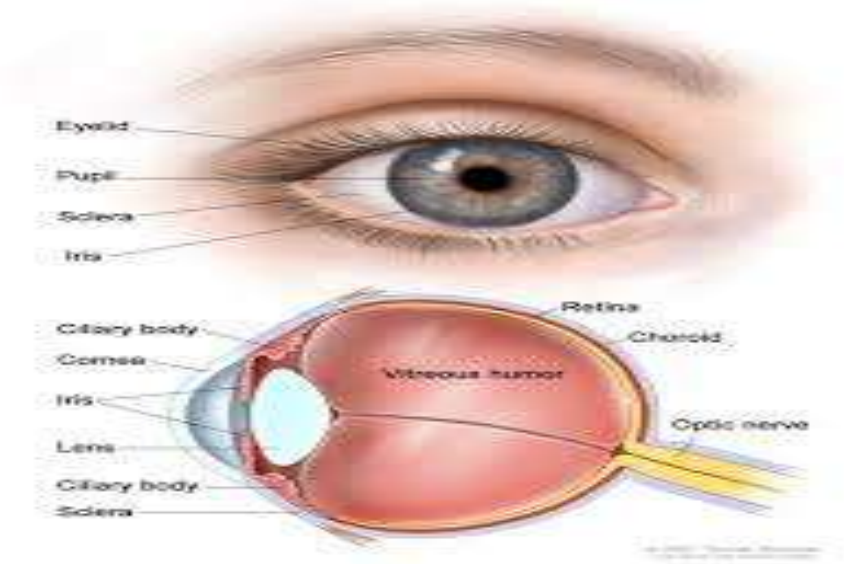


Figure 1: Structure of Eye Ball

and naso lachrymal duct. The lachrymal fluid secreted by the lachrymal glands is emptied on the surface of the conjunctiva of the upper eyelid at a turnover rate of 16% per min. It washes over the eye ball and is swept up by the blinking action of the eyelids. Thus the eye ball is continually irrigated by a gentle stream of lachrymal fluid that prevents it from becoming dry and inflamed. The lachrymal fluid in humans has a normal volume of 7µL and is an isotonic aqueous solution of bicarbonate and sodium chloride that serves to dilute irritants or to wash the foreign bodies out of the conjuctival sac. It contains lysozyme, whose anti bactericidal activity reduces the bacterial count in the conjuctival sac. The rate of blinking varies widely from one person to another, with an average of approximately 20 blinking movements per min. During each blink movement the eyelids are closed for a short period of about 0.3 sec. [5] The eye ball is an organ protected from exogenous substances and external stress by various barriers, therefore, therapeutic drugs must be transported across several protective barriers regard less of which administration route is utilized such as eye drops, intravitreal injection and implant. The human eye is challenging subject for topical administration of the drugs. The basis of this can be found in the anatomical arrangements of surface tissue and in permeability of the cornea. The protective operation of eyelids and lachrymal system is such that there is rapid removal of material instilled into eyes unless the

material is suitably small in volume and chemically and physiologically compatible with surface tissues [6]. The eye is referred to as a globe is actually two spheres one set in the other, the front sphere is the smaller of the two and is bordered anteriorly by the cornea where as the larger posterior sphere is an opaque fibrous shell encased by the sclera. The combined weight of both spheres has been given as 6.77-7.5g, with a volume approximately 6.5ml. The circumference of the eye is about 75mm, along with the rest of the orbital contents; the eye is located within the bony orbital cavity of the head. The eye is spherical structure with of three layers, the outer part sclera, the middle parts choroid layer, ciliary body and the inner section nerves tissue layer retina. The sclera is though fibrous coating that protecting the inner tissues of eye which is white except for the transparent area at the front, and the cornea allows light to enter the eye. The choroid layer, situated in the sclera contains many blood vessels that modified at front of the eye as pigmented iris the colored part of the eye. The aqueous humor is very slightly alkaline salt solution [7]. Pupil generally appears to be the dark centre of the eye, but can be more accurately described as the circular apertures in the centre of the iris through which passes into the eye. The iris is a thin circular contractilecurtoin located in front of the lens but behind the cornea. The iris is a diaphragm of variable size whose function is to adjust the size at the pupil to regulate the amount of light admitted into the eye. The ciliary muscle is a ring of striated smooth muscles in the eye s middle layer that controls accommodation for viewing objects at varying distance and regulates the flow of aqueous humor into schlemm's canal. The conjunctiva is a thin transparent mucous epithelial barrier, lines the inside of the eyelids. The conjunctiva is composed of two layers: an outer epithelium and its underlying stroma conjunctiva contribute to the formation of the tear film by way of secreting substantial electrolytes, fluid, and mucins. The cornea is a strong clear bulge located at the front of the eye. It has an important optical function as it refracts light entering the eye which then passes through the pupil and onto the lens. The lens is a transparent structure enclosed in a thin transparent capsule. It is located behind the pupil of the eye and encircled by the ciliary muscles. It helps to refract light travelling through the eye. The lens focuses light into an image on the retina. Oxygen and nutrients are transported by Aqueous humor as is non vascular. The vitreous humor is located in the large area that occupies approximately 80% of each eye in the human body. The vitreous humor is a perfectly transparent thin jelly-like substance that fills the chamber behind the lens of the eye, non vascular structure to which oxygen and nutrients are transported by aqueous humor. The retina is located at the back of the human eye. The retinal "screen" is therefore a light-sensitive structure lining the interior of the eye. It contains photosensitive cells and their associated nerve fibres that convert the light they detect into nerve impulses that are then sent onto the brain along the optic nerve. [8]

1.2 NASOLACHRYMAL DRAINAGE OR SYSTEMIC DRUG ABSORPTION: [9, 10]

Most of the administered drug is lost through nasolacrimal drainage immediately after dosing. The drainage allows drug to be systemically absorbed across the nasal mucosa and the gastrointestinal tract leading to multifarious effects.

The nasolachrymal drainage system consists of three parts:

I. **The secretary system:** It consists of basic secretors that are stimulated by blinking and temperature change due to tear evaporation and reflex secretors that have an efferent parasympathetic nerve supply and secrete in response to physical or emotional stimulation.
II. **The distributive system:** It consists of the eyelids and the tear meniscus around the lid edges of the open eye, which spread tears over the ocular surface by blinking, thus preventing dry areas from developing.
III. **The excretory system:** The excretory part of the nasolachrymal drainage system consists of - lachrymal puncta, the superior, inferior and common canaliculi; the lachrymal sac; and the nasolachrymal duct. In humans, the two

puncta are the openings of the lachrymal canaliculi and are situated on an elevated area known as the lachrymal papilla. It is thought that the tears are largely absorbed by the mucous membrane that lines the ducts and the lachrymal sac; only small amount reaches the nasal passages.

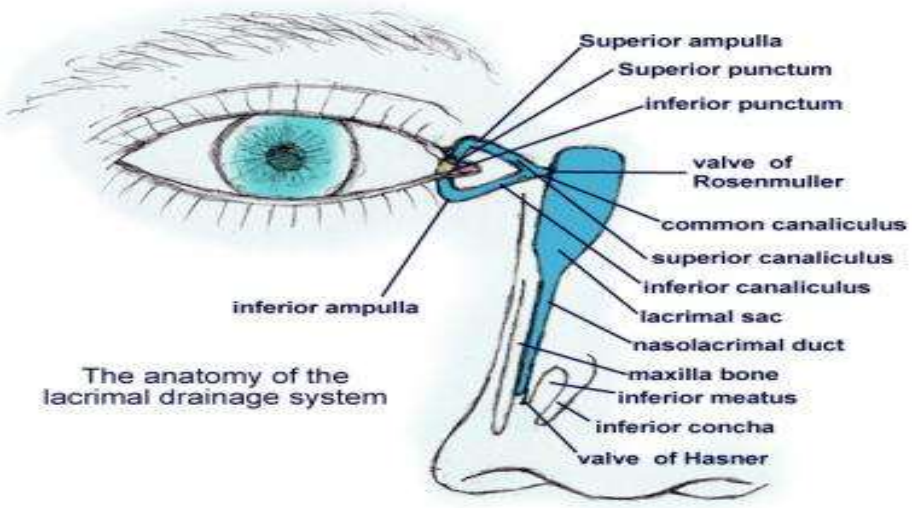


Figure 2: The Anatomy of the Nasolacrimal Drainage System

The cul-de-sac of the eye normally holds around 7-9µl. If care is taken not to blink, the normal tear flow rate is 1µl /min and pH is maintained at 6.5-7.6.

1.3 OCULAR ABSORPTION OF DRUGS: [22] Any drug remaining on the **ocular** surface for a sufficient period of time can be absorbed into the anterior chamber via either the corneal or the conjunctival – scleral route. **Corneal absorption** is considered to be the major penetration pathway for topically applied drugs.

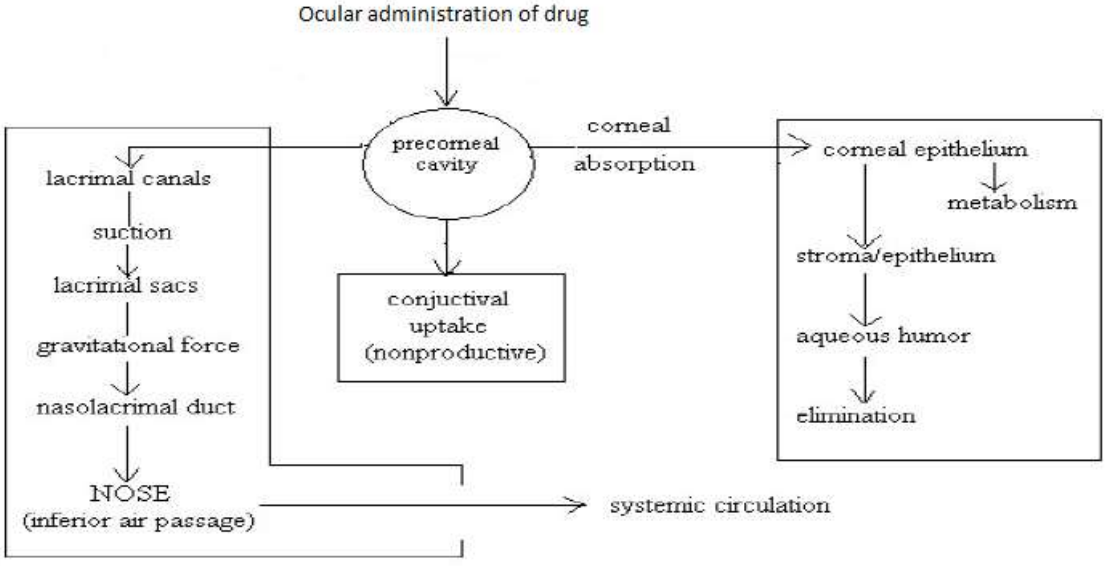


Figure 3: Flow Chart for Ocular Absorption of Drugs

1.4 OCULAR IN SITU GELLING SYSTEM: [11-15**]**

DEFINITION: In situ activated gel forming systems are those which can deliver drug in a solution form and when exposed to physiological conditions will shift to a gel phase.

MECHANISM:

Gelation occurs via the cross-linking of polymer chains that can be achieved by covalent bond formation (chemical cross-linking) or non-covalent bond formation (physical cross-linking). In situ gel-forming systems can be described as low viscosity solutions that undergo phase transition in the conjunctiva cul-de-sac to form viscoelastic gels due to conformational changes of polymers in response to the physiological environment. The rate of in situ gel formation is important because between instillation in the eye and before a strong gel is formed; the solution or weak gel is produced by the fluid mechanism of the eye. [11] Both natural and synthetic polymers can be used for the production of in situ gels. [12]

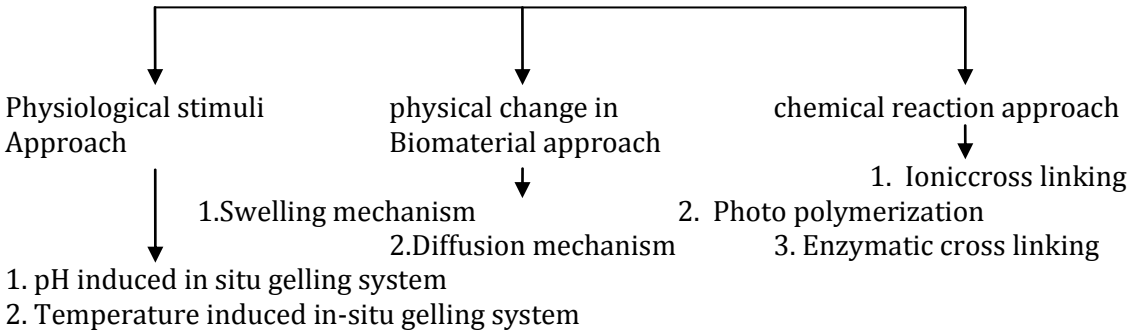
1.4.1 ADVANTAGES OF IN-SITU FORMING GEL: [12]

- Less blurred vision as compared to ointment.
- Decreased nasaolachrymal drainage of the drug which may cause undesirable side effects due to systemic absorption (i.e. reduced systemic side effects).
- The possibility of administering accurate and reproducible quantities, in contrast to already gelled formulations and moreover promoting precorneal retention.
- Sustained, Prolonged drug release and maintaining relatively constant plasma profile.
- Reduced number/frequency of applications hence improved patient compliance and comfort.
- Generally more comfortable than insoluble or soluble insertion.
- Increased bioavailability due to increased precorneal residence time and absorption.

1.4.2 GENERAL USE OF POLYMERS IN OCULAR IN-SITU GELLING SYSTEM: [15]

Carbopol
Gellan gum
Cabromer
Polyacrylic acid
Chitosan
Xanthan gum
Methyl cellulose
Xyloglucan
Poloxamer
Sodium alginate
HPMC

1.4.3 PREPARATION METHODS OF OCULAR IN SITU GELS: Ocular in situ gels



(1) Physiological stimuli approach [14] or Stimuli-responsive in situ gel systems:

a. Temperature induced in-situ gelling system.
I. Negative temperature-sensitive hydro gels
II. Positive temperature-sensitive hydro gels
b. pH induced in-situ gelling systems.

(2) Physical change in biomaterial approach:

a. Swelling mechanism.
b. Diffusion mechanism (or) solvent exchange mechanism.

(3) Chemical reaction approach or Chemically induced in situ gelling system

a. Ionic cross linking.
b. Photo-polymerization.
c. Enzymatic cross-linking.

(1) Physiological stimuli approach (or) stimuli-responsive in-situgel systems:

Stimuli responsive polymers are defined as polymers that undergo relatively large and

abrupt physical or chemical changes in response to small external changes in the environmental conditions. [15]

(A) Temperature induced in situ gelling system:

Temperature is the most widely used stimulus in environmentally responsive polymer systems as it is easy to control whenever needed and it is applicable to both in vitro and in vivo systems. The ideal critical temperature for this system is ambient and physiologic temperature.[12] In these systems, gelling of the solution is triggered by change in temperature, thus sustaining the drug release.[12]. These hydro gels are liquid at room temperature (20-25ºC) and undergo gelation when in contact with body fluids (35-37ºC) due to change in temperature. [12,13]. Temperature sensitive gels are of three types: positive temperature sensitive gel, negative temperature sensitive gel and thermally reversible gel. Negative temperature sensitive gels have Lower Critical Solution Temperature (LCST), such gels contracts on heating above LCST. Positive temperature sensitive gel has Upper Critical Solution Temperature (UCST), such gels contracts on cooling below UCST. [13]

Principle:

In this system, the sol-gel phase transition due to increase in temperature occurs mainly by three mechanisms, Desolvation of the polymer, increased micellar aggregation and increased entanglement of polymeric network. As the temperature increases polymeric chain degrades, which leads to the formation of hydrophobic domain and phase transition (liquid to hydro gel) occurs. [13]

(B) pH induced in-situ gel systems:

Polymers containing acidic or alkaline functional groups that respond to changes in pH are called pH sensitive polymers. [20]. In this system, sol to gel transition takes place when pH is raised from 4.2 to 7.4 (eye pH). At higher pH, polymer forms hydrogen bonds with mucin which leads to hydrogel formation.[12,13]

Principle:

All the pH sensitive polymers contain pendant acidic or basic groups that can either accept or release protons in response to changes in environmental pHs The polymers with a large number of ion stable groups are known as polyelectrolytes. Swelling of polymer increases as the external pH increases in the case of weakly acidic(anionic) groups, also known as polyacids, but decreases if polymer contains weakly basic(cationic) groups termed as polybases.[12,13].

(2) Physical change in biomaterial approach:

(A) Swelling mechanism:

In this approach, in-situ gel is formed when material absorbs water from surrounding environment and expands to occupy desired space. One such substance is myverol (glycerol mono-oleate), which is polar lipid that swells in water to form lyotropic liquid crystalline phase structures.

(B) Diffusion mechanism:

This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. N-methyl pyrrolidone (NMP), dimethylsulfoxide(DMSO), tetrahydrofuran, 2 pyrrolidone and triacetin have been shown to be useful solvents for such system.

(3) Chemical reaction approach:

(A) Ionic cross linking:

Certain ion sensitive polysaccharides undergo phase transition in presence of various ions such as K+, Ca^{2+}, Mg^{2+}, Na+. These polysaccharides fall into the class of ionsensitive ones.[12].

Principle:

Gelation occurs by ionic interaction of polymer and divalent ions of tear fluid. When anionic polymers come in contact with cationic ions, it converts to form gel.

(B) Photo-polymerization:

A solution of monomers or reactive macromer and initiator can be injected into a tissue site and to this site electromagnetic radiation is applied due to which the gel is formed. Acrylate or similar polymerizable functional groups are typically used as the polymerizable groups on the individual monomers and macromers because they rapidly undergo photo polymerization in the presence of suitable photo initiate or Photopolymerizable systems when introduced to the desired site via injection get photocured in situ with the help of fiber

optic cables and then release the drug for prolonged period of time.

(C) Enzymatic cross-linking:

This approach is still under investigation as natural enzymes are responsible for forming the in-situ gel. But it is estimated that this method will be more advantageous than chemical and photochemical approaches. For example, intelligent stimuli-responsive delivery systems using hydro gels that can release insulin have been investigated. [12]

1.5 EVALUATION TESTS: [16-22]

Test for clarity and appearance:

Clarity is one of the most important characteristic features of ophthalmic preparations. Clarity is evaluated by visual observation against a black and white background.

Determination of pH:

It is the most important parameter involved in ophthalmic formulations. Ophthalmic formulations should have a pH ranging between 5-7.4.

Gelling capacity test:

The gelling capacity was determined by placing a drop of the formulation in a vial containing 2ml of freshly prepared simulated tear fluid and equilibrated at 37^0c and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve.

Drug Content Uniformity:

Drug content of in-situ gelling formulations was determined by accurately dissolving (1mL) measured quantity of formulation in 100mL simulated tear fluid. The formulation is shaken for 2-3min to completely dissolve, until it gives a clear gel solution. The solution is filtered through Millipore membrane filter (0.45μm) and drug content is analyzed at respective nm. [18].

Viscosity:

It is the important factor to determine the residence time of drug in the eye by considering the viscosity of the instilled formulation. For the prepared sample solutions viscosity measurement is carried out at 37°C at various angular velocities by using Brookfield viscometer. A typical run involved changing the angular velocity from 1rpm to 100 rpm at a controlled ramp speed can be carried out. The device registers viscosity values in every 20 sec.

In vitro drug permeation studies: [30]

a) 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 ± 0.5^0C containing 100 ml artificial simulated tear fluid (pH 7.4) under continuous stirring at 20 RPM to simulate 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.

b) 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.

Ex vivo drug release studies: [30]

Drug permeation experiments can also be carried out using freshly excised goat

cornea. Goat whole eyeballs were transported from the local butcher shop to the laboratory in cold (4°C) normal saline within 1hr of slaughtering of the animal. The cornea was carefully excised along with 2 to 4mm of surrounding sclera tissue and was washed with cold normal saline till the washing was free from proteins. Freshly excised cornea was fixed between clamped donor and receptor compartments in such a way that its epithelial surface faced the donor compartment. Then the samples are withdrawn from receptor compartment and analysed at respective nanometer.

Release kinetic studies:

To study the drug release kinetics, data obtained from in vitro permeation studies were fitted in various kinetic models: zero order as the cumulative percent of drug permeated versus time, first order as the log cumulative percentage of drug remaining versus time, and Higuchi's model as the cumulative percent drug permeated versus square root of time. The release mechanism from in-situ gel was determined by fitting the data into the Korsmeyer-Peppas model as the log cumulative percentage, and the exponent "n" was calculated from the slope of the straight line.

Antifungal Studies:

The antifungal efficiency and prolonged effect of selected sustained release in situ gel formulations containing antifungal drugs is carried out on Candida albicans and Asperigillus fumigates species using nutrient agar medium. By using diffusion method, test organisms previously seeded (10CFU/ml) in the nutrient agar medium [19]. The aliquot test samples poured into Petri dish containing nutrient agar medium using micropipette. The plates left for 30min and incubated at 25°C for 24hr. The diameters of zone of inhibition for Candida albicans and Asperigillus fumigates were measured after 24hrs and 120hrs respectively.

Stability of InSitu Gel:

Stability studies were carried out on in situ gelling formulations according to ICH guidelines [20]. All formulations stored in closed amber glass bottles and placed in humidity chamber with a relative humidity of 75±5% and temperatures of 40±2°C or at room temperature. Samples withdrawn at time points 0, 3 weeks, 6 weeks, 3 months, and 6 months and analyzed for drug concentration. The formulations evaluated at periodic intervals for pH, clarity, and drug content. The degradation rate constant is determined from the plot of logarithm of the remaining drug versus time.

Sterility Testing:

Sterility testing is performed for aerobic and anaerobic bacteria and fungi by using fluid Thioglycolate and soybean Casein digest medium respectively as per the Indian Pharmacopoeia. Formulations taken into laminar airflow and passed through a membrane filter of 0.45µm with the help of vacuum pump. After filtration, the filter paper is removed from funnel and cut into two halves. One half is dropped in bacterial media (fluid Thioglycolate) and other is dropped in fungal media (soybean casein digest). Both media are kept for incubation at 37°C for 7 days and observed for any microbial growth. The sterility test results are compared with positive and negative controls. [21]

Ocular irritancy test:

The draize technique is 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, 24hr, 48hr, 72hr and 1week after administration. Three male rabbits weighing 1.5-2 kg are used for the present study. The sterile formulations are instilled twice a day for a period of 7 days and a cross over study is carried out with a 3 days washing period with saline before the cross over study. Rabbits are observed periodically for redness, swelling, watering of eye. [22]

1.6 PAST RESEARCH WORK ON OCULAR IN-SITU GELS: [23-34]

Table 1: Past Research work on Ocular *Insitu* Gels

Drug	Disease conditions	Gelation mechanism	References
Brimonidine tartrate	Glaucoma	Temperature dependent	23
Brimonidine tart rate	Glaucoma	thermo sensitive	24
Gatifloxacin	Keratitis	pH trigged	25
Gatifloxacin	Keratitis	Ion activated	27
Lomefloxacin Hcl	conjunctivitis	pH induced	26
Ofloxacin	Corneal ulcers, bacterial conjunctivitis	Ion activated	28
Ketorolac tromethamine	Ocular inflammation conditions	Ion activated	29
Levofloxacin	Kerato conjunctivitis	Ion activated	33
Ciprofloxacin	Bacterial keratitis	pH induced	34
Nor floxacin	Conjunctivitis	pH triggered	30
Voriconazole	Fungal keratitis	pH induced	31
Fluconazole	Acrimonious keratitis	Ion activated	32

1.7 CURRENT IN SITU GEL FORMULATIONS PRESENT IN MARKET: [7,12,14,15]

Table 2: Marketed Ocular *Insitu* Gels

Product name	Drug used	Mfg company
Tim optic-XE	Timolomalate	Merck and Co.Inc.
Cytoryn	Interleukin-2	Macro med
Azasite	Azithromycin	Insite vision
Pilopine HS	Pilocarpine Hcl	Alcon Lab.inc
Akten	Lidocaine Hcl	Akten
Virgan	Ganciclovir	SpectrumThea pharmaceuticals

1.9 CONCLUSION

In conclusion, the polymeric in-situ gels offer controlled release of various drugs improving bioavailability and patient compliance and provide no. of advantages over conventional dosage forms like eye drops. Good stability and biocompatibility make the in-situ gel dosage form very reliable. These systems are seem to be preferred as they can be administered in drop form and provide significantly less problems with vision and more over provide sustainity in drug release. The future use of water soluble, natural and biodegradable polymers for in-situ gel formulation can make ocular in-situ gel as excellent drug delivery systems. Further we should consider the most efficacious combination of optimal drugs dose and release pattern (sustained/pulsatile release responding to triggered) according to the pathophysiology and progressive coverage of targeted disease.

REFERENCES

1. Hill JM, Callaghan O, Hobden RJ, Kaufman E, Mitra AK. Ophthalmic drug delivery systems. New York, Marcel Dekker Inc; 1993. vol 58. p. 1-204.
2. Bourlais CL, Acar L, Zia H, Sado PA, Needhan S, Leverge R.(Ophthalmic drug delivery systems – Recent Advances). Progress in Retinal and Eye Research 1998; 17(1):33-58.
3. Chein YW, Novel drug delivery systems, 2nd ed. New York, Marcel Dekker Inc; 1993, vol 29. p. 269-300.
4. Gennaro AR, Remington. The science and practices of pharmacy, 21st ed. , Pennsylvania, Mark Publishing Company; 2000. vol 1. p. 852-870.
5. Singh Deepti Bhattacharya A, Sara UVS. In-situ gel for ophthalmic delivery: a review International Journal of Universal Pharmacy and Bio Sciences., 2013; 2(4): 542-567.
6. Martindale, The complete drug reference, 34th ed. Pharmaceutical press; 1996, p. 127-227.
7. Mitra AK. Ophthalmic drug delivery systems. 2nd ed., New York; Marcel Dekker, Inc : 2003.

8. Kute PR, Gondkar SB, Saudagar RB. (Ophthalmic In-situ Gel: An Overview). World J. Pharm. Sci., 2015; 4(4): 549–568.
9. Aqil, Advances in Ophthalmic Drug Delivery System: Part I &II. 12th April 2005 (http://www.Pharmainfo. Net).
10. Vyas S P, Khar R K, Controlled Drug Delivery, Concepts and Advances. Vallabh Prakashan, pp. 382-407.
11. Mali Mahesh N, Hajare Ashok A. In-situ gel forming systems for sustained ocular drug delivery system. European Industrial Pharmacy., 2010; 5: 17-20.
12. Patil Rajeshwari N, Kumar Rachana S. In-situ gelling system: Novel approach for ophthalmic drug delivery. World Journal of Pharmacy and Pharmaceutical Sciences., 2014; 3(7): 423-440.
13. Tinu TS, Thomas LithaKumar Anil B. Polymers used in ophthalmic in-situ gelling system. International Journal of Pharmaceutical Sciences Review and Research.2013;20(1);176-183.
14. Kumar Lalit, Singh Ravindra Pal, Gupta Singh Stuti, KumarDhiraj. in-situ gel: A novel system for ocular drug delivery. International Journal of Pharmaceutical Sciences Review and Research.2011; 9(2): 83-91.
15. Patel Nisha, Shinde Gajanan, Rajesh KS. Ophthalmic In-situ gel. Pharmagene. 2014; 1(4): 29-33.
16. H. G. Choi, J. H. Jung, J. M. Ryu, S. J. Yoon, Y. K. Oh, and C. K. Kim, "Development of In situ-gelling and mucoadhesive acetaminophen liquid suppository," International Journal of Pharmaceutics,vol.165,no.1,pp.33–44,1998.
17. D.S.Singhare, S.Khan, and P.G.Yeole, "Temperature induced In situ gel of Lidocaine hydrochloride for periodontal anaesthsia," IndianDrugs, vol.42, no.8, pp.519–524, 2005.
18. D. Shastri, S. Prajapati, and L. Patel, "Thermo reversible mucoadhesive ophthalmic In situ hydro gel: Design and otimization using a combination of "Pharmaceutical applications of naturally occurring water soluble polymers," Pharmaceutical Science and Technology Today, vol.1, no.6, pp.254–261, 1998.
19. H. W. Bauer, W. M. M. Kirby, J. C. Slerris, and M. Truck, "Antibiotic susceptibility testing by a standardized single disc method," American Journal of Clinical Pathology, vol. 45, pp. 493–496, 1996.
20. "Q1A (R2): stability testing of new drug substances and products," in Proceedings of the International Conference on Harmonization(ICH'03), Geneva, Switzerland, 2003.
21. Controller of Publication. Indian Pharmacopoeia, Vol. 2, Ministry of Health and Family Welfare, Government of India, New Delhi, pp: A117.
22. Sharma Hemant K, Gupta Mukesh, Gupta Rakesh. pH sensitive in situ ocular gel: A review. International Journal of Scientific Research and Reviews. 2014; 3(2): 260-273.
23. Patel JM.,Shah KK, Patel MM.(Formulation and evaluation of temperature dependent
24. Ophthalmic gel forming solution of Brimonidine Tartrate. International Journal of Pharmaceutical and Chemical Sciences., 2012; 1(4): 1190-1199.
25. Patil Akash, Tagalpallewar Amol, Rasve Ganesh, Bendre Arvind, Khapekar Priyanka. Formulation and evaluation of thermosensitive in-situ gel of Brimonidine Tartrate. Journal of Pharmacy Research., 2012; 5(7): 3597-3601.
26. Kanoujia Jovita, Sonker Kanchan, Pandey Manisha, Kymonil Koshy M, Saraf Shubhini A. Formulation and characterization of a novel pH-triggered in-situ gelling ocular system containing Gatifloxacin. International Current Pharmaceutical Journal, 2012; 1(3): 43-49.
27. Shankar SJ, Kalikonda Anudeep. pH induced in-situ gel formulation of Lomefloxacin HCl. Universal Journal of Pharmacy, 2014; 3(1): 38-43.
28. Doijad RC, Manvi FV, MalleswaraRao VSN, Alase Prajakta. Sustained ophthalmic delivery of Gatifloxacin from in-situ gelling system. Indian Journal of Pharmaceutical Sciences., 2006; 68(6): 814-818.
29. Gonjari Indrajeet D, Kashikar Vrushali S. In situ gelling system of Ofloxacin- In vivo precorneal drainage study. Scholars Research Library, Der Pharmacia Lettre, 2012; 4(5): 1541-1546.
30. Vodithala Sirish, Khatry Sadhna, Shastri Nalini, Sadanandam.M. Formulation and evaluation of ion activated ocular gels of Ketorolac Tromethamine. International Journal of Current Pharmaceutical Research., 2010; 2(3): 33-38.
31. Priyanka D. Dabir, Dr. S. R. Shaha and Swati V. Deore, OPTHALMIC INSITU GEL: A REVIEW, European Journal of Pharmaceutical and Medical Research 2016, 3(6), 205 - 215.
32. https://www.hindawi.com/journals/bmri/ 2013/341218. Available online scholars research library.com.
33. www.ijpsi.org/Papers/Vol3(11)/F0311038043.pdf.
34. www.jpsbr.org/index_htm_files/7_JPSBR_12_RV098.pdf.

35.K.S. Rathore. In-situ gelling ophthalmic drug delivery system an over view. International journal of pharmacy and pharmaceutical sciences, vol 2, supple 4, 2010.
36. Ashwani mishra. A review on triggered gel for ocular drug delivery, International journal of pharmaceutical and biological archives, 2014, 5(4):19-25.
37.Suryawanshi sarika. Novel polymeric in situ gels for ophthalmic drug delivery system, International journal of research in pharmacy and science, 2012, 2(1), 67-83.
38. Sharma Hemant K. A review on PH sensitive in-situ ocular gel, IJSRR 2014, 3(2), 260-273.
39. Sarika Meshram, Santosh Thorat. Ocular in-situ gels development, evaluation and advancements, SAJP, 2015, 4(7):340-346.