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OPHTHALMIC INSITU GEL - AN OVERVIEW

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ABSTRACT

Occular drug delivery is one of the most interesting and challenging endeavor facing the pharmaceutical scientist. The poor bioavailability of conventional ophthalmic formulations (solutions, suspensions, and ointments) is due to rapid precorneal drug loss (through dilution and drainage from the eye) high variability in efficiency, and blurred vision etc. so there was a need for developing advanced drug delivery system. There are some static and dynamic barriers which affects the bioavailability of drug. To overcome the conventional drug therapy drawbacks the polymeric systems are in solution form before administering in the body, but once administered these systems undergo gelation. In situ gels are the liquid preparations which upon instillation undergo phase transition in cul-de-sac of the eye to form a viscous gel and this occurs due to the environmental changes in the eye. This novel drug delivery system promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with mucosa. The primary requirement of a successful control release product focuses on increasing patient compliance, good stability and biocompatibility characteristics which make the in situ gel dosage forms very reliable. This review is to specify the basic anatomy and physiology of human eye, various approaches used for formulation of in-situ gels and polymers used in the formulation of in situ gels. These systems are evaluated for drug content, clarity, pH, gelling capacity, viscosity, in vitro drug release studies, texture analysis, sterility testing, isotonicity evaluation, accelerated studies and irritancy test. FT-IR spectroscopy is used to know drug and polymer incompatibilities.

KEYWORDS: In situ gel, in situ gelling polymers, pH sensitive, temperature sensitive, sterility testing.

INTRODUCTION

Major problem in ocular therapeutics is the attainment of optimal drug concentration at the site of action, which is compromised mainly due to precorneal loss resulting in only a small fraction of the drug being ocularly absorbed. The effective dose administered may be altered by increasing the retention time of medication into the eye by using in situ gel-forming systems. Ophthalmic drug delivery is an extremely interesting and highly challenging endeavor.[1,2] The anatomy, physiology, and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage.[3] Ophthalmic ointments ensure superior drug bioavailability by increasing the contact time, minimizing the dilution by tears, and resisting nasolacrimal drainage. Major disadvantage of ointment, providing blurred vision, due to this it could be used either night time or for treatment on the outside and edges of the eyelids. Suspension as ophthalmic delivery systems relies on the assumption that particles may persist in conjunctival sac. Precorneal drug loss can be minimal, such as retarding drainage by using diffusion-controlled, nonerodible polymeric insert. The major disadvantage of inserts is the lack of patient acceptance owing to difficult administration. The development of newer, more sensitive diagnostic techniques and therapeutic agents render urgency to the development of more successful ocular delivery systems. The primitive ophthalmic solution, suspension, and ointment dosage forms are clearly no longer sufficient to combat these diseases, and current research and development efforts to design better therapeutic systems are the primary focus of this research work. The aim of the present investigation is to formulate an in situ gel using novel gum system. In situ gel solution increases the residence time and also sustain the release mechanism of the drug.

EYE

The eye is one of the most complex and important sensory organs in the human body. It provides the ability to see in both bright and dim light, focusing on objects both near and far. Its three types of cone cells are able to distinguish millions of distinct colors and produce the

high quality images that the body relies on for many daily activities.[4]

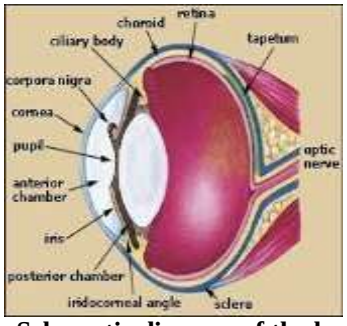

Figure 1: Schematic diagram of the human eye.

Eye Anatomy

The eyes are a pair of spherical organs located within the orbits of the skull. Each eye is roughly 1 inch (2.5 cm) in diameter and fills most of the space within the orbit. Three distinct tissue layers; the fibrous, vascular, and nervous tunics make up the wall of the eye and surround its gel-filled center. The fibrous tunic forms the outermost layer of the eye and is instrumental in protecting its delicate inner tissues.

Anteriorly the fibrous tunic consists of the cornea, a clear layer of dense regular fibrous connective tissue. The cornea forms a circular window allowing light to enter the eye while blocking foreign material from entering. Extending from the cornea to cover the sides and posterior of the eye is the sclera. The sclera is a thick, white layer of dense irregular connective tissue that acts like a tough yet flexible shell for the eye.[5] A thin layer of mucous membrane known as the conjunctiva covers the anterior surface of the sclera and the inside of the eyelids as depicted in figure 1. The conjunctiva secretes mucus to lubricate the surface of the eye and contains blood vessels that support the tissues of the sclera. Deep to the fibrous tunic is the vascular tunic that provides blood supply to the eye. It has three major parts: the choroid, ciliary body, and iris.

The choroid is a layer of connective tissues lining the inside of the sclera and providing blood flow to the sclera and retina. It also contains a high concentration of melanin, giving it a black color and helping it to absorb light in the eye. The ciliary body is a widened ring of tissue at the anterior edge of the choroid. It contains the ciliary muscles and the ciliary bodies. The ciliary muscles pull on the zonular fibers and the lens of the eye to focus light, while the ciliary bodies produce aqueous humor. The iris is a ring of pigmented smooth muscle extending from the anterior edge of the ciliary body and surrounding the pupil. Movement of the smooth muscle tissue in the iris adjusts the size of the pupil, the circular hole in the center of the iris. The lens is a large, clear mass of protein fibers found just posterior to the iris. It is connected to the ciliary body by many tiny fibers known as zonular fibers. The lens is extremely flexible and changes its shape when the ciliary body pulls on its edges.

The innermost layer of the eye is formed by the nervous tunic, or retina. It is a thin, delicate layer of nervous tissue that is loosely connected to the choroid. Millions of photoreceptor cells, bipolar cells, and ganglion cells are distributed throughout the retina to detect light and transmit visual information to the brain. The macula lutea is the region of central vision, which contains the fovea. The fovea is an important region of the retina, as it contains the highest concentration of photoreceptors in the eye. Nervous tissue in the retina is connected to the brain through the optic nerve, which passes through a hole in the choroid and sclera in the posterior of the eye. Where the optic nerve forms there are no photoreceptors, resulting in a blind spot known as the optic disk.

The lens divides the interior of the eye into two regions namely the anterior chamber and the vitreous chamber. The vitreous humor, a clear, gel-like mass of water and proteins, fills the vitreous chamber. It gives shape to the eye, holds the retina in place against the choroid, and allows light to pass easily through the eye. The anterior chamber is filled with aqueous humor, a clear liquid produced by the ciliary body. Aqueous humor plays a number of roles in the eye, including inflating the eye through intraocular pressure; providing a clear medium for light to pass through the eye; and nourishing the cells of the cornea and lens.

Physiology of the Eye

The overall function of the eye is to act like a biological camera - it absorbs light and translates images into nerve signals to conduct to the brain. Light entering the eye first passes through the cornea, where it is refracted to begin the process of focusing. It next passes through the pupil, where the contraction of muscles in the iris controls the size of the pupil and the amount of light entering the eye.[6] Light passes through the lens, where it is further refracted to focus on the retina. Contractions of the ciliary muscles pull on the zonular fibers and the lens, allowing the lens to accommodate vision at varying differences. To view objects that are close to the eye, the ciliary muscles relax and permit the lens to assume a wide shape. The wide shape of the lens allows it to refract the light to a high degree to focus on the retina. For distant objects, the ciliary muscles pull on the lens to flatten it, reducing the amount of refraction and focusing distant light on the retina.

Once light has passed through the lens, it continues through the vitreous humor and passes through the retina. Photoreceptor cells in the retina are specialized to detect light and produce nerve signals in response to light. Rods are the more numerous and sensitive of the photoreceptors and are specialized for seeing in low-light situations. They produce grayscale images in low light, but are overwhelmed by light during the day or in a normally lit room at night. Cones, on the other hand, are

specialized for detecting light in brighter conditions and are able to differentiate colors. The three types of cone cells, red, green, and blue, are able to detect specific colors, or wavelengths, of light. The combination of the three types of cone cells produces all of the colors that the human eye can detect. Once the photoreceptors have detected light, the cells produce an action potential that is conducted to bipolar cells and ganglion cells in the retina. These cells transmit the signal into the optic nerve, where it travels to the brain to be processed.

After light has passed through the retina, it is absorbed by the choroid. The choroid prevents excess light from remaining within the eye and forming afterimages. High intensity lights can overcome the absorptive effects of the choroid, resulting in the "red eye" seen in pictures[7].

ROUTES OF OCULAR DRUG DELIVERY

There are several possible routes of drug delivery into the ocular tissues. The selection of the route of administration depends primarily on the target tissue.

- **Topical route**

Typically topical ocular drug administration is accomplished by eye drops, but they have only short contact time on the eye surface. The contact, and thereby duration of drug action, can be prolonged by formulation design (e.g.m gels, gelifying formulations, ointments, and inserts).

- **Subconjunctival administration**

Traditionally subconjunctival injections have been used to deliver drugs at increased levels to the uvea. Currently this mode of drug delivery has gained new momentum for various reasons. The progress in materials sciences and pharmaceutical formulation have provided new exciting possibilities to develop controlled release formulations to deliver drugs to the posterior segment and to guide the healing process after surgery.

- **Intravitreal administration**

Direct drug administration into the vitreous offers distinct advantage of more straightforward access to the vitreous and retina. It should be noted; however that delivery from the vitreous to the choroid is more complicated due to the hindrance by the RPE (Retinal Pigment Epithelium) barrier. Small molecules are able to diffuse rapidly in the vitreous but the mobility of large molecules, particularly positively charged, is restricted.

Factors Affecting Corneal Penetration of Drugs

While treating anterior segment of the eye in ophthalmological practice, topical medications are the most commonly prescribed medications. The current issue has three studies related to the efficacy or side effects of topical medications used in the management of various anterior and posterior segment diseases.[8] The intraocular bioavailability is very low due to very rapid drainage of drug from the ocular surface and only few minutes are available for the drug to be absorbed. In most cases the topically administered ocular drugs do not reach the posterior segment of the eye like retina, vitreous and choroid and these can be treated by using intravenous or intra-vitreal routes of drug administration. Only 1-7% of dose of the drug can reaches into the aqueous humor because corneal epithelium can effectively limit the drug delivery into the eye. The penetration of drugs through cornea is very important clinically because it is the major determinant of the efficacy of drug topically applied to the eye.

Ocular preparations in majority are formulated in an aqueous vehicle and the bioavailability of drugs from aqueous based products is mainly affected by the factors which are categorized into the following three groups.

- Physiological factors.
- Physicochemical factors.
- Formulation factors.

Physiological factors

Physiological factors include some precorneal factors and membrane factors. Pre-corneal factors like tears secretions turn over, ocular drainage of the instilled drug, non-corneal absorption (Conjunctival absorption), protein binding and corneal absorption rate are the contributing factors in the net pre-corneal drug loss. These factors collectively lead to corneal contact time of the drug 2-4 minutes in human for an instilled solution. Normal tears volume is only 7 µl and tears wash out at the rate of 16% per minute. The pre-corneal area can hold approximately 30 µl including tears when eye is not blinking. When drug is instilled, the excess of volume is spilled out or drain through the nasolacrimal apparatus. Normally tears contain 0.7% of protein and this level increases during inflammation or infection. As tears are replaced quickly, so they remove both free and bound from of drug. Conjunctiva has 17 times greater surface area & higher permeability as compared to cornea[9]. The absorption of drugs through the tissues other than cornea is considered as nonproductive absorption (fig 2). Thicknesses, porosity, tortuosity of the cornea, surface area available for absorption and lipophilicity / Hydrophilicity balance are the major membrane factors contributing in the ocular absorption of drugs. The lipophilic drugs have greater penetration through cornea as compared to hydrophilic drugs.

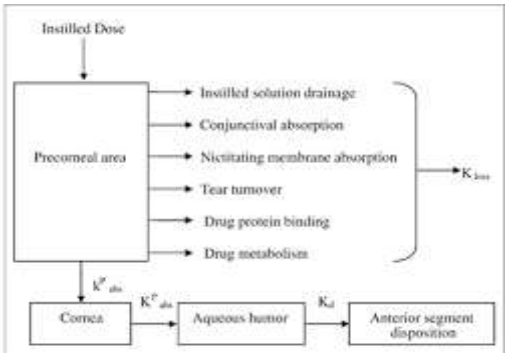

Figure 2: Schematic representation of corneal absorption.

Physicochemical factors

Partition coefficient, solubility, ionization constant and molecular weight are the main physicochemical factors contributing in the ocular penetration of drugs. Partition coefficient is the parameter used for the penetration of drugs through different biological membranes. The corneal permeability of any drug depends upon its lipophilic characters (Partition coefficient). The maximum penetration of a drug is the multiplicative factor of permeability coefficient and tears solubility. The concentration of poorly soluble drug in the pre-corneal tears film may be limited which can result in low corneal absorption. The ionization constant (pKa) of a drug is important factor for its corneal penetration. The extant of ionization can influence the diffusion of drugs across the membranes. Most of the drugs are week acids or weeks bases and are partially ionized at physiological pH. The ionized form of drug is poorly lipid soluble (Limited corneal penetration) and if this proportion is high then it is difficult for a drug to achieve therapeutic concentration. Molecular weight is a less critical factor because ophthalmic preparations have very low and narrow molecular weight range. Drugs having molecular weight greater than 500 Da offer poor corneal penetration and vice versa.

Formulation factors

Concentration, particle size, shape & dissolution rate, pH & tonicity and viscosity are the formulation factors that can affect the corneal penetration of the drugs. By increasing the solution concentration, corneal penetration can be enhanced. Particle size & shape is mostly concerned with the use of ophthalmic suspensions. Drug particles can be deposited at the outer surface of the eye which can cause irritation & abrasion upon movement through eye lids while blinking. Increase in particle size can leads to poor corneal penetration. Irregular particles or edged particles can cause more irritation as compared to spherical particles. Concentration, size & shape of the particles together can determine the irritation potential of the suspended particles.

The human tears pH ranges from 7.14-7.28 and possess relatively weak buffer capacity. The hypotonic solution can increase the corneal permeability while instillation of hypertonic solution can decrease the permeability of the corneal epithelium. The hypotonic solution can create an osmotic gradient between the tears film and surrounding tissues. The corneal epithelium has greater tolerability to large variations in the pH and tonicity. It is generally believed that by increasing the viscosity of the ophthalmic solution, corneal penetration can be increased because it can increase the contact time of the drug with corneal epithelium. The most commonly used viscosity improving agents in ophthalmic preparations are hydroxy propyl methyl cellulose (HPMC) and poly vinyl alcohol (PVA) etc. Penetration enhancers (actin), cytoskeleton inhibitors (cytochalasin B), surfactants (benzalkonium chloride, sodium lauryl sulphate), chelators (EDTA) and preservatives (Benzalkonium chloride, organomercurials) etc. can be used in the ophthalmic preparations to enhance the corneal penetration by one or another mechanism.[10]

Advantages of In Situ forming gel

- Less blurred vision as compared to ointment.
- Decreased nasolacrimal drainage of the drug which may causes 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 dosing frequency compared to preformed gel.
- Reduced number/frequency of applications hence improved patient compliance and comfort.
- More comfortable than insoluble or soluble insertion.
- Increased bioavailability due to increased precorneal residence time and absorption.
- Avoidance of hepatic first pass.

MECHANISM OF INSITU GEL FORMATION

In situ gel formation is based on physical and chemical reactions mechanisms.[13]

Physical mechanisms include swelling and diffusion. In swelling mechanism in situ formation may also occur when material absorbs water from surrounding environment and expand to 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. It has some bioadhesive properties and can be degraded in vivo by enzymatic action. Diffusion. 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) has been shown to be useful solvent for such system.[12]

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

Approaches of in-situ gelling system

pH triggered systems

In this system change in pH causes formation of gel (fig. 3). In this approach, pH responsive or pH sensitive polymers are used. pH sensitive polymers have acidic or alkaline ionisable functional groups which are called as polyelectrolytes.[14] The polyelectrolytes those are present in the formulation causes increase in external pH that leads to the swelling of hydrogel that leads to the formation of in-situ gel. Suitable polymers for pH triggered systems are the polymers that are having anionic groups. Some of them are cellulose acetate

phthalate (CAP), Carbomer and its derivatives, Polyethylene glycol (PEG), Pseudo latexes and poly methacrilic acid (PMC) etc.

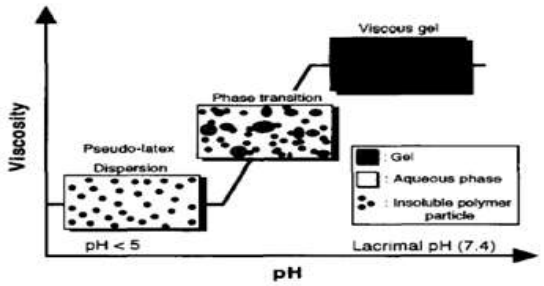

Figure 3: Mechanism of pH triggered systems.

Temperature triggered in situ gel

Temperature induced in-situ gelling system: Temperature induced systems are most widely used systems in in-situ gelling formulations. In this type of systems, no external heat other than body temperature is required to cause gelation. There are three types of temperature induced systems. Some of them are, negatively thermo sensitive type (poly(N-isopropylacrylamide), positively thermo sensitive type (polyacrylic acid) and thermally reversible type (poloxamer, pluronics, Tetronics). In temperature induced gelling system, temperature responsive polymers or thermo responsive polymers are used that exhibit a drastic and discontinuous change in their physical properties with temperature (fig 4). This type of polymers belongs to the category of stimuli responsive materials that change their properties continuously with environmental conditions.[15] These polymers exhibit a miscibility gap at high or low temperatures an upper or lower critical solution temperature exists. The range at which the solution exists at upper critical solution temperature is 0^{o} -100^{o}C. In this approach, the solution is liquid at room temperature and when reaches the body fluid due to exposure to body temperature it converts into gel. As the body cannot maintain upper critical solution temperature, lower critical solution temperature suitable polymers are used that undergo polymer-polymer interaction that causes sudden change in polymer solubility. As the solution is in liquid form, at lower critical solution temperature the hydrogen bonding between polymer and water cause an abrupt changes and leads to the formation of gel.

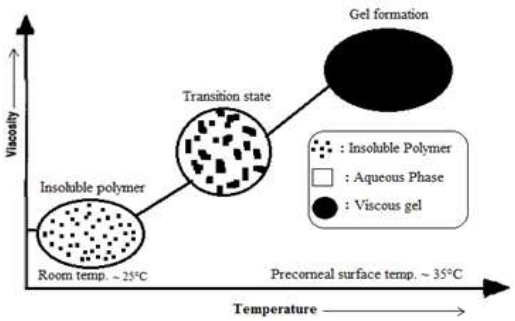

Figure 4: Mechanism of temperature triggered systems.

Ion activated in situ gelation

In this method, gelling of the solution instilled is triggered by change in the ionic strength. It is assumed 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 or divalent cations typically found in the tear fluids. The electrolyte of the tear fluid and especially Na+, Ca2+ and Mg2+ cations are particularly suited to initiate gelation of the polymer when instilled as a liquid solution in the conjunctival cul-de-sac.[16] The polymers which show osmotically induced gelation are gelrite or gellan gum, hyaluronic acid and alginates.

EVALUATION OF OPHTHALMIC INSITU GEL

These formulations are evaluated for clarity, pH, gelling capacity, drug content, rheological study, in vitro diffusion study, isotonicity, in vivo ocular testing in rabbits and accelerated stability studies.

Test for Clarity

The formulations were observed for general appearance i.e. color, odour and for the presence of suspended particulate matter. The clarity of the preparation was checked using against black and white background.

Determination of pH

The pH of all formulations was recorded using a calibrated digital pH meter immediately after preparation. The pH of in situ gel solution should be 7.4 for all the formulations. The formulation should have an optimum viscosity that will allow for easy instillation into the eye as a liquid (drops), which would undergo a rapid sol-to-gel transition (triggered by pH, temperature or ion exchange).[17]

Gelling capacity

The gelling capacity is determined by placing a drop of the formulation in a vial containing 2 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling is noted.

Drug content

The drug content was determined by accurately placing 100 µl of formulations in a test tube and suitably diluted with simulated tear fluid (STF) to obtain a concentration of 10 µg/ml. By using UV-Visible spectrophotometer the drug concentration was determined.[18]

Rheological studies

Viscosity and rheological properties of in situ forming drug delivery systems can be assessed by using Brookfield rheometer or some other type of viscometers such as Ostwald's viscometer. The viscosity of these formulations should be such that no difficulties are envisaged during their administration by the patient, especially during parenteral and ocular administration.[19]

In vitro drug release studies

In vitro release study of in situ gel solution is carried out by using Franz diffusion cell. The formulation placed in donor compartment and freshly prepared simulated tear fluid in receptor compartment. Between donor and receptor compartment dialysis membrane is placed (0.22 µm pore size). The whole assembly is placed on the thermostatically controlled magnetic stirrer. The temperature of the medium is maintained at 37°C ± 0.5°C. 1ml of sample is withdrawn at predetermined time interval of 1hr for six hrs and same volume of fresh medium is replaced. The withdrawn samples are diluted in a volumetric flask with respective solvent to specific volume and analyze by UV spectrophotometer at respective nm using reagent blank. The drug content is calculated using the equation generated from standard calibration curve then the % cumulative drug release (% CDR) is calculated. The data obtained is further subjected to curve fitting for drug release data.[20]

Texture analysis

The consistency, firmness and cohesiveness of in situ gel are assessed by using texture profile analyzer which mainly indicated gel strength and easiness in administration in vivo. Higher values of adhesiveness of gels are needed to maintain an intimate contact with mucus surface.

Sterility Testing

Sterility testing was performed for aerobic and anaerobic bacteria and fungi by using fluid thioglycolate and soybean casein digest medium respectively as per the Indian Pharmacopoeia. The method used for sterility testing was direct inoculation method.10 ml culture was added to 100 ml of culture medium. Both medias were kept for incubation at 32^{o}C for 7 days and observed for any microbial growth.[21]

Isotonicity

Evaluate on Isotonicity is important characteristic of the ophthalmic preparations. Isotonicity should be maintained to prevent tissue damage or irritation of eye. All ophthalmic preparations undergo isotonicity testing, Formulations mixed with few drops of blood and observed under microscope at 45X magnification and compared with standard marketed ophthalmic formulation.

Ocular irritancy test

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

Accelerated stability studies

Formulations are placed in amphor colour vials and sealed with aluminum foil for a short term accelerated stability study at 40±2°C and 75±5% RH as per International Conference on Harmonization (ICH) states Guidelines. Samples are analyzed every month for clarity, pH, gelling capacity, drug content, rheological evaluation, and in vitro dissolution. The storage conditions and the lengths of studies chosen should be sufficient to cover storage, shipment, and subsequent use.

CONCLUSION

Development of ophthalmic drug delivery system has proved to be beneficial as compared to the conventional drug delivery. Likewise it is also challenging enough to establish successful ophthalmic drug delivery systems. However, the persistent attempts towards advancement in the understanding of principles and processes governing ocular drug absorption and disposition have led to the improvements in the efficacy of ophthalmic delivery systems. One such novel approach is development of in-situ ocular gels. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the in situ gel dosage forms very reliable. The evaluation of in-situ gels can be carried out based on the parameters like gelling capacity, rheological studies, in-vitro drug release studies, drug-polymer interaction study, thermal analysis, antibacterial activity and ocular irritancy test. Use of biodegradable and water soluble polymers for the insitu gel formulations can make them more acceptable and excellent drug delivery systems. Insitu activated gel-forming systems seemed to be favoured as they can be administered in drop form and produce appreciably less inconvenience with vision. Moreover, they provide better sustained release properties than drops. This type of dosage forms are used now a day in combat glaucoma, dry eye syndrome, sjogren's syndrome, ARMD, trachoma etc.

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