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IN SITU GEL FORMING A NOVEL APPROACH FOR SUSTAINED OPHTHALMIC DRUG DELIVERY

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Abstract: In the development of ophthalmic products, drug delivery is one of the most challenging and difficult fields for investigators. The conventional formulations such as solutions, suspensions, ointments, etc. shows some constraints such as increased precorneal elimination, high variability in efficiency and blurred vision, respectively, which reduce their bioavailability. In situ activated gel- forming systems are liquid upon instillation and undergo phase transition in the ocular cul-de-sac to form a viscoelastic gel in response to environmental changes such as change in temperature, pH and osmolarity. In the past few years, an impressive number of novel temperature, pH, and ion induced in situ gel-forming systems have been reported for sustain ophthalmic drug delivery. This review includes investigation of various temperature, pH and ion induced in situ-forming polymeric systems used to achieve prolonged contact time of drugs with the cornea and increase their bioavailability.

Keywords: Ophthalmic, in-situ, osmalirity, precorneal.

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INTRODUCTION

Eye is most interesting organ due to its drug disposition characteristics. Generally, topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy1. A significant challenge to the formulator is to circumvent (by pass) the protective barriers of the eye without causing permanent tissue damage. Development of new, sensitive diagnostic techniques and novel therapeutic agents provide ocular delivery systems with high therapeutic efficacy. Conventional ophthalmic formulations like solution, suspension, and ointment have many disadvantages which result into poor bioavailability of drug in the ocular cavity. The specific aim of designing a therapeutic system is to produce an optimal concentration of a drug at the active site for the required duration2. Ocular disposition and elimination of a therapeutic agent is dependent upon its physicochemical properties as well as the relevant ocular anatomy and physiology. A successful design of a drug delivery system, therefore, requires an integrated knowledge of the drug molecule and the constraints offered by the ocular route of administration3. The various techniques that have been attempted to gain the bioavailability and the duration of the therapeutic action of ocular drugs can be divided into 2 categories. The first is based on the use of sustained drug delivery systems, which produce the controlled and continuous delivery of ophthalmic drugs. The second involves increase corneal drug absorption and reduce precorneal drug loss. Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolong period of time. Consequently it is imperative to optimize ophthalmic drug delivery; one of the way to do so is by addition of polymers of various grades, development of *in situ* gel or colloidal suspension or using erodible or non erodible insert to prolong the precorneal drug retention[4].

1.1 THE ANATOMY OF THE EYE

The human eye, elegant in its detail and design, represents a gateway to the process we call vision. The eyeball is spherical in shape and about 1 inch across. It houses many structures that work together to facilitate sight. The human eye is comprised of layers and internal structures, each of which performs distinct functions. The detailed description of each eye part is given below.
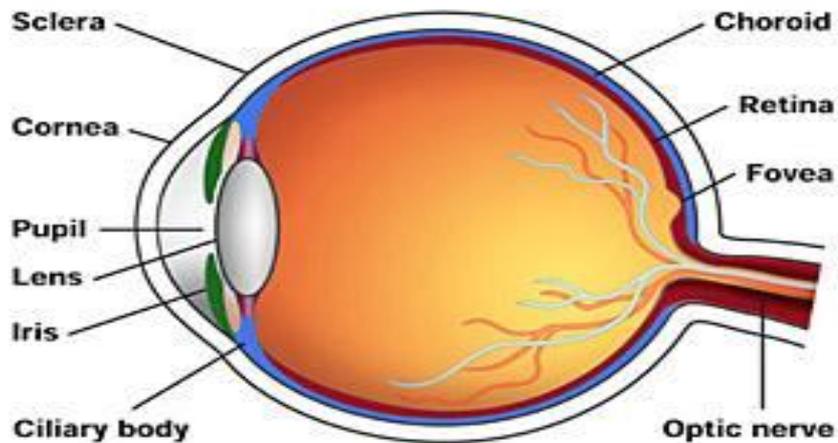
Figure 1.1 Structure of Eye-Ball

Sclera

The sclera (white portion of the eye) is the tough white sheath that forms the outer- layer of the ball. It is a firm fibrous membrane that maintains the shape of the eye as an approximately globe shape. It is much thicker towards the back/posterior aspect of the eye than towards the front/anterior of the eye.

Conjunctiva

The conjunctiva is a thin transparent mucous epithelial barrier, lines the inside of the eyelids, and covers the anterior one-third of the eyeball. The respective portion of conjunctiva is referred to as the palpebral and bulbar conjunctiva. The conjunctiva is composed of two layers: one is outer epithelium and second is underlying stroma (substantia propria). The exposed surface of the eye contains conjunctiva and cornea and is covered with the tear film. The conjunctiva produce tear film by way of secreting substantial electrolytes, fluid, and mucins.

Cornea

The cornea is a located at the front of the eye. Surface of the adult cornea has a radius of approximately 8 mm. It has an important optical function as it refracts light entering the eye which then passes through the pupil and onto the lens (which then focuses the light onto the retina). The cornea, a non-vascular structure (does not contain any blood vessels) gets the necessary nutrients from the capillaries that terminate in loops at its circumference. It is

supplied by many nerves derived from the ciliary nerves. These enter the laminated tissue of the cornea. It is therefore extremely sensitive. 5-9
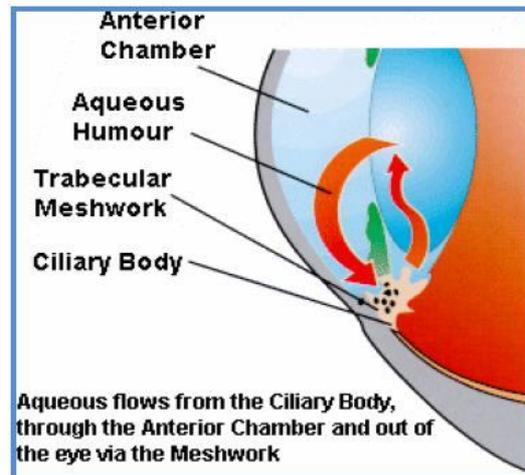
Aqueous humor

Figure 1.2. Pathway of Aqueous Humor

The aqueous humor is a jelly-like substance located in the outer/front chamber of the eye. It is a watery fluid that fills the "anterior chamber of the eye" which is located immediately behind the cornea and in front of the lens. The aqueous humor is very slightly alkaline salt solution that includes tiny quantities of sodium and chloride ions. It is continuously produced, mainly by the ciliary processes, flows from the posterior chamber through the pupil into the anterior chamber, and exits via the trabecular route at the angle and the uveoscleral route.

Schlemm's canal (canal of Schlemm or the scleral venous sinus), is a circular channel that collects aqueous humour from the anterior chamber and via the anterior ciliary veins delivers it into the bloodstream. In human, the rate of aqueous humor turnover is approximately 1% - 1.5% of the anterior chamber volume per minute. The rate of aqueous formation is approximately 2.5 µl/min. Aqueous humor consists of pressure dependent and pressure independent pathways. The pressure dependent outflow refers to the trabecular meshwork-schlemm‟s canal-venous system, while pressure independent outflow refers to any non trabecular outflow and is called as uveoscleral outflow.10

Pupil

Pupil generally appears to be the dark "centre" of the eye, but can be more accurately described as the circular aperture in the centre of the iris through which light passes into the

eye. The pupil size (and therefore the amount of light that is admitted into the eye) is controlled by the pupillary reflex (also known as the "light reflex").

Iris

The iris is a thin circular contractile curtain 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 of the pupil to regulate the amount of light admitted into the eye. It is the coloured part of the eye (shades may vary individually like blue, green, brown, hazel, or grey).

Ciliary Muscle

The ciliary muscle is in the eye"s middle layer it is ring of striated smooth muscles that controls accommodation for viewing objects at varying distances and regulates the flow of aqueous humour into schlemm"s canal. Contraction and relaxation of the ciliary muscle alters the curvature of the lens. This process may be described simply as the balance existing at any time between two states. Ciliary Muscle relaxed **(**This enables the eye to focus on distant objects**)** and Ciliary Muscle contracted (This enables the eye to focus on near objects).

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 (which first refracted by the cornea). The lens focuses light into an image on the retina. It is able to do this because the shape of the lens is changed according to the distance from the eye of the object(s) the person is looking at. This adjustment of shape of the lens is called accommodation and is achieved by the contraction and relaxation of the ciliary muscles.

Vitreous Humour

The vitreous humour (also known as the vitreous body) is located in the large area that occupies approximately 80% of each eye in the human body. The vitreous humour is a perfectly transparent thin-jelly-like substance that fills the chamber behind the lens of the eye. It is an albuminous fluid enclosed in a delicate transparent membrane called the hyaloid membrane.

Retina

The retina is located at the back side of the human eye. The retina may be described as the "screen" on which an image is formed by light that has passed into the eye via the cornea, aqueous humour, pupil, lens, and finally the vitreous humour before reaching the retina. The

function of the retina is not just to be the screen onto which an image may be formed but also to collect the information contained in that image and transmit it to the brain in a suitable form for use by the body. The retinal "screen" is therefore a light-sensitive structure lining the interior of the eye. It contains photosensitive cells (called rods and cones) and their associated nerve fibers that convert the light they detect into nerve impulses that are then sent onto the brain along the optic nerve.

Macula

The center of the retina is called the macula it contains a high concentration of photoreceptor cells which convert light into nerve signals. Because of the high concentration of photoreceptors, we are able to see fine details such as newsprint with the macula. The fovea at the very center of the macula, the site of our sharpest vision.
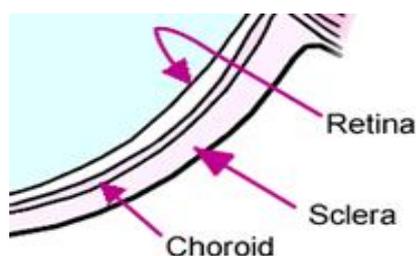
Choroid

Figure 1.3. Posterior view of eye

The choroid layer is located behind the retina and absorbs unused radiation and nourishes the outerportions of the retina. It is a thin, highly vascular (i.e. it contains blood vessels) membrane that is dark brown in colour and contains a pigment that absorbs excess light and so prevents blurred vision (due to too much light on the retina). The choroid has one of the highest blood flows in the body. The choroid is loosely attached to the inner surface of the sclera by the lamina fusa.

Optic nerve

The optic nerve (a bundle of over 1 million nerve fibers) is responsible for transmitting nerve signals from the eye to the brain. These nerve signals contain information on an image for processing by the brain. The front surface of the optic nerve, which is visible on the retina, is called the optic disk.

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 a short contact time on the eye surface. The contact, and thereby duration of drug action, can be prolonged by formulation design (e.g. 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.

BARRIERS FOR OCULAR DELIVERY

Drug loss from the ocular surface

After instillation, the flow of lacrimal fluid removes instilled compounds from the surface of the eye. Even though the lacrimal turnover rate is only about 1 µl/min the excess volume of the instilled fluid is flown to the nasolacrimal duct rapidly in a couple of minutes. Another source 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.

Lacrimal fluid-eye barriers

Corneal epithelium control drug absorption from the lacrimal fluid into the eye. The corneal epithelial cells form tight junctions that limit the paracellular drug permeation. Therefore, lipophilic drugs have typically at least an order of magnitude higher permeability in the cornea

than the hydrophilic drugs. In general, the conjunctiva is leakier epithelium than the cornea and its surface area is also nearly 20 times greater than that of the cornea.

Blood-ocular barriers

The eye is protected from the xenobiotics in the blood stream by blood-ocular barriers. These barriers have two parts: blood-aqueous barrier and blood-retina barrier. The anterior blood-eye barrier is composed of the endothelial cells in the uvea (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 plasma albumin into the aqueous humor, and also control the access of hydrophilic drugs from plasma into the aqueous humor. The posterior barrier between blood stream and eye is comprised of retinal pigment epithelium (RPE) and the tight walls of retinal capillaries. Unlike retinal capillaries the vasculature of the choroid has extensive blood flow and leaky walls. Drugs easily gain access to the choroidal extravascular space, but thereafter distribution into the retina is limited by the RPE and retinal endothelia. 11

Table 1.1: Barriers for the Ocular delivery

Parameters	Conjuctivita	Cornea	Sclera
Surface Area	17.65±2.12 cm2	1.04±0.12	16-17
Thickness	-	0.57mm	0.4-.0.5 mm
Structural Composition	Mucous membrane	5 layers	Collagen fibers
	Epithelium	Epithelium	Water
	Vasculature	Bowman"s	Proteoglycans
		membrane	Mono polysaccharides
		Stomata	Elastic fibres
		Endothelium	fibroblast

MECHANISM OF OCULAR DRUG ABSORPTION

Drugs administered by instillation must penetrate the eye and do so primarily through the cornea followed by the non-corneal routes. These non-corneal routes involve drug diffusion across the conjunctiva and sclera and appear to be particularly important for drugs which are poorly absorbed across the cornea.12
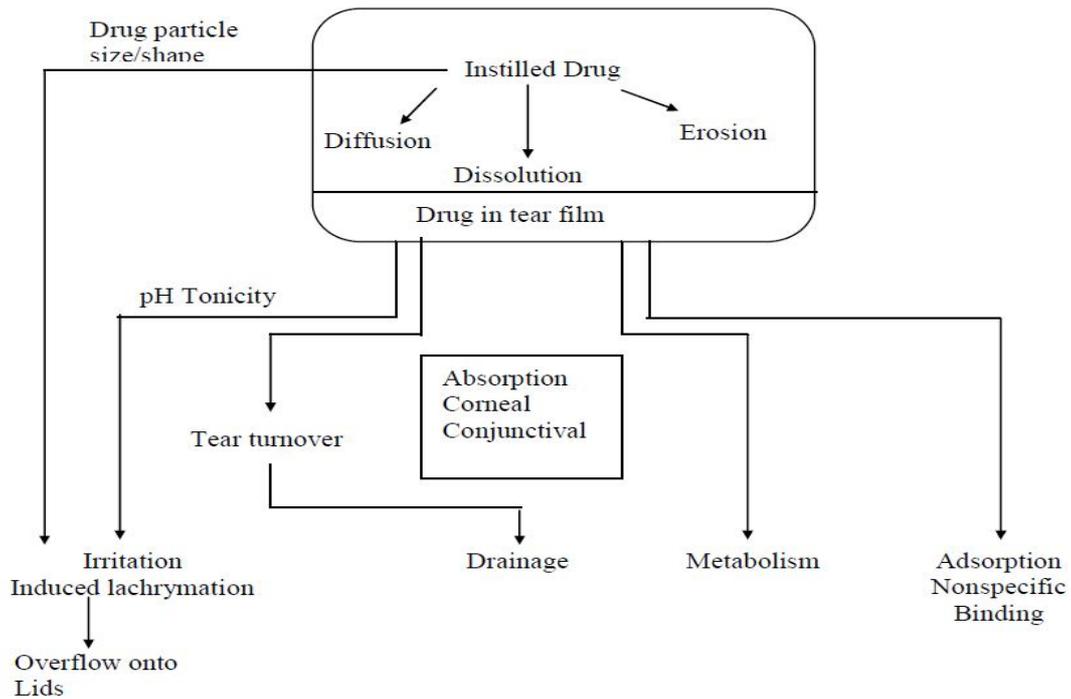

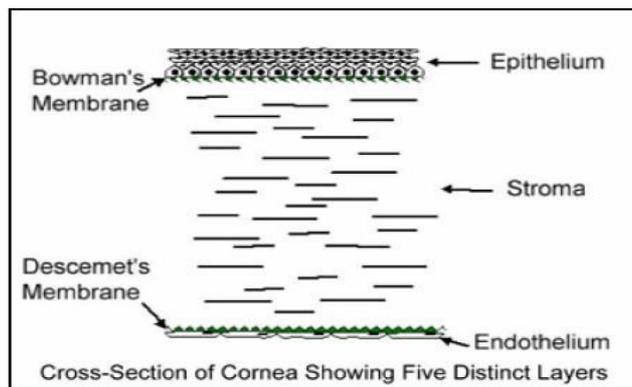**Figure 1.4. Ocular Drug Absorption**

Corneal permeation

Figure 1.5. Corneal Membrane Depicting Various Barrieirs to drug Absorption

The permeation of drugs across the corneal membrane occurs from the precorneal space. Thus, the mixing and the kinetic behavior of drug disposition in tears have a direct bearing on efficiency of drug absorption into the inner eye. The productive absorption of most ophthalmic drugs results from diffusional process across corneal membrane. The efficiency of absorption process is a function of rate and extent at which the transport processes occur. The flux of any drug molecule across the biological membrane depends on the physicochemical properties of the permeating molecule and its interaction with the membrane. The extent to which the transport or absorption process occurs is also function of physiological mechanism of precorneal fluid drainage or turnover. In terms of transcorneal drug permeation, the cornea can be considered to consist of three primary layers (epithelium, stroma and endothelium). The epithelium and endothelium contain on the order of a 100 fold greater amount of lipid material than the stroma. Consequently, depending on the physicochemical properties of a diffusing drug, the resistance offered by the individual layers varies greatly. Epithelium, being lipodal, represents a diffusional barrier offering high resistance to ionic or other aqueous soluble or polar species. In contrast, compounds with relatively low polarity encounter a greater diffusional resistance in the hydrophilic stroma layer. This frequently cited concept of drug permeation across the corneal membrane is referred to as "differential solubility concept".

Non-corneal permeation

Primary mechanism of drug permeation is the sclera is likely to be diffusion across the intercellular aqueous media in the case of structurally similar corneal stroma. Therefore the possibility of partitioning mechanism cannot be eliminated. Although like cornea, the conjunctiva is composed of an epithelial layer covering an underlying stroma, the conjunctival epithelium offers substantially less resistance than does the corneal epithelium.

EYE INFECTIONS

Eyes can get infections from bacteria, fungi or viruses. Eye infections can occur in different parts of the eye and can affect just one eye or both. Common eye infections are Conjunctivitis, Corneal ulcers & Endophthalmitis.

Conjunctivitis

Conjunctivitis is swelling (inflammation) or infection of the membrane lining the eyelids (conjunctiva).It is characterized by cellular infiltration and exudation. Staphylococcus aureus is the most common cause of bacterial conjunctivitis and blepharo-conjunctivitis.Conjunctivitis can be classified as (1) Infective – Acute, Subacute & Chronic (2) Allergic conjunctivitis.

Corneal ulcers/ Keratitis

Inflammation of cornea (Keratitis) is characterized by corneal oedema, cellular infiltration & ciliary congestion. Being the most anterior part of eyeball, cornea is exposed to atmosphere & hence prone to get infected easily. Bacterial corneal ulcers are the most commonly caused by virulent organism. Common bacteria associated with corneal ulceration are Staphylococcus aureus, Pseudomonas pyocyanea, E.coli. Proteus etc.

Reasons for poor ocular bioavailability

- Drug-Protein interaction
- Drug metabolism
- Drainage
- Induced Lacrimation
- Evaporation of tears
- Corneal Absorption
- Conjunctival absorption
- Normal tear turnover

Nasolacrymal drainage system
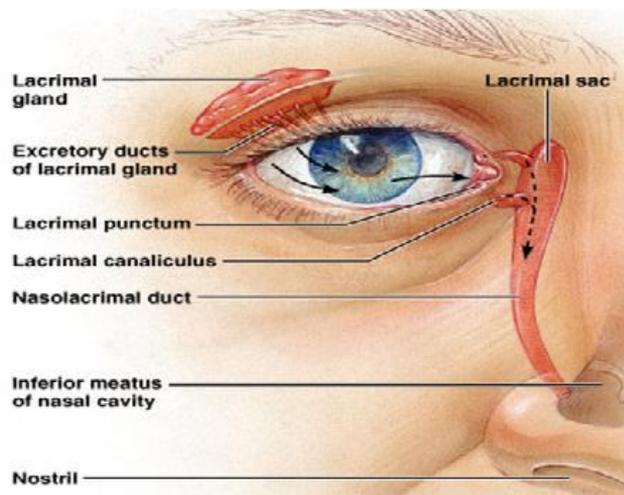
Figure 1.6. Nasaolachrymal Drainage Apparatus

The naso lachrymal drainage system consists of three parts: the secretory system, the distributive system and the excretory system. The secretory system 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. The distributive system 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. The excretory part of the nasolachrymal drainage system consists of: the 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 tears are largely absorbed by the mucous membrane that lines the ducts and the lachrymal sac; only a small amount reaches the nasal passage.[13-18].

Ophthalmic Drug Delivery System

Topical delivery of eye-drops into the lower cul-de-sac is the most common method for the administration of therapeutic agents in the treatment of ocular diseases. However, one of the major problems encountered with solutions is the rapid and extensive elimination of drugs from the pre-corneal area by solution drainage and lachrymation. Consequently , the overall absorption of a topically applied drug is limited to 1-10%. Such disadvantages have led to other approaches being investigated. Some common methods to prolong pre-corneal residence time include use of hydrogels, liposomes, inserts, micro and nano - carrier systems. In comparison with traditional formulations, these systems have the following advantages.[19]

- Increased contact time
- Prolonged drug released
- Reduced systemic side effects
- Reduced number of applications
- Better patient compliance

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of the drug may be overcome by the use of a gel system that are instilled as drops into the eye and undergo a sol-gel transition from the instilled dose. These are the characteristics required to optimize ocular drug delivery systems.

➢ A good corneal penetration.

➢ A prolonged contact time with corneal tissue.

➢ Simplicity of installation for the patient.

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

➤ Appropriate rheological properties and concentration of viscolyzer.

Initial attempts to overcome the poor bioavailability of topically instilled drugs typically involved the use of ointments based on mixtures of white petrolatum and mineral oils and suspensions. Ointments ensure superior drug bioavailability by increasing the contact time with the eye, minimizing the dilution by tears, and resisting nasolachrymal drainage because these vehicles have the major disadvantage of providing blurred vision, they are nowadays mainly used for either night time administration or for treatment on the outside and edges of the eyelids. Use of suspensions as ophthalmic delivery systems relies on the assumption that particles may persist in the conjunctival sac. The efficiency of suspensions has shown high variability, which occurred as a result of inadequate dosing, probably mainly due to the lack of patients compliance in adequately shaking the suspension before administration.

These disadvantages have led to other approaches being investigated. One of the common methods to optimize prolonged precorneal residence time is to use hydrogels, liposomes, micro and nano carrier systems in comparison with traditional formulations.

Even though various drug delivery systems mentioned above offer a numerous advantages over conventional drug therapy but still they are not devoid of pitfalls, including:

- Poor patient compliance and difficulty of insertion as in ocular inserts.
- Tissue irritation and damage caused by penetration enhancers and collagen shields.
- Toxicity caused by insertion of foreign substances like albumin and poly butyl cyano acrylate, as in case of nanoparticles and microspheres.
- Change in pharmacokinetic and pharmacodynamics of the drug as caused by altering the chemical structure of the drug (prodrug approach) 21-26

IN-SITU GEL

The use of preformed hydrogels still has drawbacks that can limit their interest for ophthalmic drug delivery. They do not allow accurate and reproducible administration of quantities of drug. They often produce blurred vision, crusting of eyelids and lachrymation upon administration. Distinguishing from preformed hydrogels, *in situ* forming gels are formulations, applied as a solution, which undergoes gelation after instillation due to physicochemical changes inherent to the biological fluids. In this way, the polymers, which show sol-gel phase transition and thus trigger drug release in response to external stimuli, are the most investigated. In-situ hydrogels are providing such „sensor" properties and can undergo reversible sol-gel phase transitions upon changes in the environmental condition. These "intelligent" or "smart" polymers play important role in drug delivery since they may dictate not only where a drug is delivered, but also when and with which interval it is released.

The liquid to semisolid phase change can be triggered by:

1. Increased temperature

2. Increased pH

3. Ionic strength of the tear film

A polymer used to prepare *in situ* gels should have following characteristics.

1. It should be biocompatible.

2. It should be capable of adherence to mucus.

3. It should have pseudoplastic behaviour.

4. It should have good tolerance and optical clarity.

5. It should influence the tear behaviour.

6. The polymer should be capable of decreasing the viscosity with increasing shear rate there by offering lowered viscosity during blinking.27

TEMPERATURE INDUCED *IN SITU* GEL

These *in situ* gels are liquid at room temperature (20–25°C) but due to an increase in temperature undergo gelation when in contact with body fluids (35– 37°C), Different thermal setting gels have been described, including for example Poloxamers, cellulose derivatives, and xyloglucan. Poloxamers, commercially available as Pluronic®, are the most commonly used thermal setting polymers in ophthalmology. The poloxamers (Fig. 1.7) consist of more than 30 different non-ionic surface active agents. These polymers are ABA-type triblock copolymers composed of polyethylene oxide (PEO) (A) and polypropylene oxide (PPO) units (B). Pluronic F-127, which gives colorless and transparent gels, is the most commonly used polymer in pharmaceutical technology. Concentrated aqueous solutions of poloxamer form thermo reversible gels. The gelation mechanism of poloxamer solutions has been investigated extensively, but is still being debated.
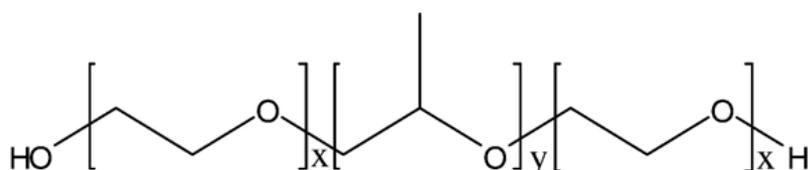

Figure 1.7. PEO-PPO-PEO (Poloxamer)

Ultrasonic velocity, light-scattering and small-angle neutron scattering measurements of aqueous poloxamer solutions have clearly indicated a micellar mode of association. Micelle formation occurs at the critical micellization temperature as a result of PPO block dehydration. With increasing temperature, micellization becomes more important, and at a definite point, micelles come into contact and no longer move. In addition, the formation of highly ordered structures, such as cubic crystalline phase, has been proposed as the driving force for gel formation, but this hypothesis has been questioned recently.

Thermo reversible gels can be prepared with naturally occurring polymers. Most natural polymer aqueous solutions form a gel phase when their temperature is lowered. Classic examples of natural polymers exhibiting a sol–gel transition include gelatin and carrageenan. At elevated temperatures, these polymers adopt a random coil conformation in solution. Upon cooling, a continuous network is formed by partial helix formation29.

Some cellulose derivatives are an exception to this gelation mechanism. At low concentrations (1–10% wt), their aqueous solutions are liquid at low temperature, but gel upon heating. Methylcellulose (Fig. 1.8a) and hydroxyl propyl methylcellulose (HPMC) (Fig. 1.8b) are typical examples of such polymers.
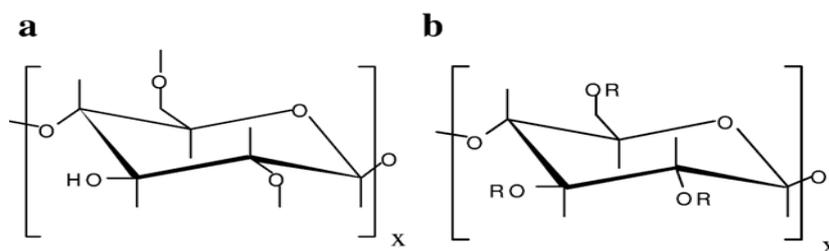

Figure 1.8. Structure of (a) Methylcellulose (MC) (b)Hydroxypropyl methylcellulose (HPMC)

Methylcellulose solutions transform into opaque gels between 40 and 50°C, whereas HPMC shows phase transition between 75 and 90°C. These phase transition temperatures can be lowered by chemical or physical modifications. For example, NaCl decreases the transition temperature of methylcellulose solutions to 32–34°C. Similarly, by reducing the hydroxypropyl molar substitution of HPMC, its transition temperature can be lowered to 40 °C30.

pH INDUCED IN SITU GEL

Pseudolatexes, Carbomers and various grades of Chitosan are used to prepare pH induced in situ gelling system.

Pseudolatexes can be described as artificial latexes prepared by the dispersion of a pre-existing polymer in an aqueous medium. In situ gelling pseudolatexes for ophthalmic use can be

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described as aqueous colloidal dispersions of polymer, which become viscous gels after instillation in the conjunctival cul-desac due to modification of the pH. Two principal methods are commonly used to prepare ophthalmic pseudolatexes, the solvent evaporation process and the salting out process. Some prerequisites necessary for an optimal formulation of ophthalmic pseudolatex are listed below

1. Solubility of the polymer selected in organic solvents as well as insolubility in water.

2. Existence on the macromolecule of ionizable groups, which can react with the electrolytes of the lachrymal fluid.

3. Use of a high molecular weight polymer.

4. Rapid coagulation process after instillation to avoid precorneal drainage of the instilled formulation before the phenomenon of gelation appears.

5. Compatibility of the different components of the colloidal dispersion with precorneal tissues.

Another polymer which shows pH induced gelation is Carbomer. Cross-linked poly (acrylic acid) of high molecular weight, commercially available as Carbopol®, is widely used in ophthalmology to enhance precorneal retention to the eye.
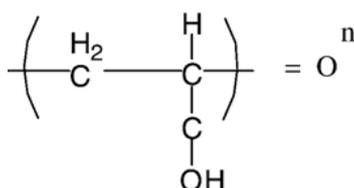

Figure 1.9. The structure of carbomer

Four mechanisms of interaction between mucin and poly (acrylic acid) have been described: electrostatic interaction, hydrogen bonding, hydrophobic interaction, and interdiffusion. As the concentration of Carbopol increases in the vehicle, its acidic nature may cause stimulation to the eye tissues. In order to reduce the total polymer content and improve the gelling properties, ocular drug delivery systems based on a combination of Carbopol and methylcellulose and combination of Carbopol and hydroxypropylmethylcellulose have been developed. For both systems it was found that a reduction in the Carbopol concentration without compromising the in situ gelling properties as well as overall rheological behaviors can be achieved by adding a suitable viscosity enhancing polymer.24

ION INDUCED IN SITU GEL

Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones. While k-carrageenan forms rigid, brittle gels in reply of small amount of K+, carrageenan forms elastic gels mainly in the presence of Ca2+. Gellan gum commercially available as Gelrite® is an anionic polysaccharide that undergoes 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, especially Ca2+. Likewise, alginic acid undergoes gelation in presence of divalent/polyvalent cations.

Gellan gum (Gelrite) is a linear, anionic hetero polysaccharide secreted by the microbe Sphingomonas elodea (formerly known as Pseudomonas elodea). The polysaccharide can be produced by aerobic fermentation and by alcohol precipitation they isolated from the fermentation broth . The polymer backbone consists of glucose, glucuronic acid, and rhamnose in the molar ratio 2:1:1. These are linked together to give a tetra saccharide repeat unit .The native polysaccharide is partially esterified with L-glycerate and acetate, but the commercial product Gelrite has been completely deesterified by alkali treatment. Gelrite (deacetylated gellan gum) is one of the most interesting in situ gelling polymers that has been tested since it seems to perform very well in humans. Gelrite has been granted regulatory approval as pharmaceutical excipient and is marketed by Merck in a controlled-release glaucoma formulation called Blocarden Depot (Timoptic). As a low viscosity solution formulations with the Gelrite can be administered to ocular mucosa. On contact with cations in tear fluid the formulation will form a clear gel. This is caused by cross linking of the negatively charged polysaccharide helices by monovalent and divalent cations (Na+, K+, Ca+). Several models have been presented to explain gellan gum gelation.

Mechanism involved in sol to gel transistion by gelrite is as follows, in an ion free aqueous medium, Gelrite forms double helices at room temperature. This solution has a viscosity near to that of water and the helices are only weakly associated with each other (by van der Waals attraction). When gel-promoting cations are present, some of the helices associate into cation-mediated aggregates, which produce cross-linking of the polymer. On heating the polysaccharide in an ion free environment, the polysaccharide becomes a disordered coil. However, on heating a sample with cations present, the non aggregated helices melt out first, and the aggregated helices melt out at a higher temperature in a second transition. The divalent ions such as magnesium or calcium were superior to monovalent cations in promoting the gelation of the polysaccharide.

However the concentration (2.6 g/L) of sodium in tears is quite sufficient to produce the gelation. Corneal contact time of formulations based on gellan gum has been investigated using

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two main methods, which are fluorometry and γ- scintigraphy. Both techniques have demonstrated improved residence times with Gelrite when compared with saline or various commercial solutions. Gelrite has also provided corneal residence time superior to those of other hydrogel preparation.[28]

DRUG RELEASE FROM HYDROGELS:

As discussed in the previous sections, hydrogel are useful in drug delivery applications because hydrogel have a unique combination of characteristics. Due to their hydrophilicity, hydrogels can imbibe large amounts of water (N90 wt.%). Therefore, the molecule release mechanisms from hydrogels are very different from hydrophobic polymers. Both simple and sophisticated models have been previously developed to predict the release of an active agent from a hydrogel device as a function of time. These models are based on the rate limiting step for controlled release and are therefore categorized as diffusion, swelling & chemically controlled mechanism.

1. Diffusion-Controlled Release Systems

There are two types of diffusion controlled release systems: reservoir devices and matrix devices. In each case the release of the drug occurs by diffusion through the hydrogel mesh or the water-filled pores.

1. Reservoir Systems: A reservoir delivery system consists of a drug core enclosed in a hydrogel membrane, usually in the form of capsules, cylinders, spheres or slabs. In order to maintain a constant release rate the drug concentration difference must remain constant. This is achieved by concentrating the drug in the centre of the device.

2. Matrix Systems: In matrix systems the drug is dispersed throughout the hy- drogel lying within the three-dimensional structure of the polymer. Matrix tablets are constructed through a compression of a mixture of drug and poly- mer powders. Drug release occurs through the macromolecular mesh or water- filled pores. Note that the release rate is here proportional to the square root of time initially rather than the constant time-independent rate available with reservoir systems.

2. Swelling-Controlled Release System

In swelling-controlled release systems the drug is dispersed within a glassy polymer as in a matrix device. Once the polymer comes into contact with water or another biofluid it begins to swell. The glass transition temperature of the polymer is lowered allowing a relaxation of molecular chains so that the drug can now diffuse out of the swollen rubbery area of the polymer. This is also known as Case II transport and is characterised by constant, i.e. time-

independent, release kinetics. In some cases a combination of swelling controlled release as well as diffusion occurs, this is known as anomalous transport.

3. Chemically-Controlled Release Systems

In the case of release with a chemical reaction drugs and/or products of polymer degradation can react with the released medium inside its pores. The released medium molecules diffuse to hydrogel medium pores. During the contact with drugs or products of polymer degradation they undergo a chemical reaction. This reaction can be reversible or irreversible, simple or complex and slow or fast. Then, the products of chemical reactions undergo interior and exterior processes of diffusion.

1.2 EVALUATION TECHNIQUES[29-31]

Physical appearance

The clarity of formulated solution and gel was determined by visual inspection under black and white background.

pH

The pH was measured of in situ solutions using a calibrated digital pH meter at 25°C. All measurements of pH were made in triplicate.

Determination of drug content

Drug containing preparation were shaken for few minute and 100 µg/L stock solution of final preparation using artificial tear fluid pH 7.4. From that 10µg/L solution was prepared and measure at suitable nm.

In vitro Gelling Capacity

The gelling capacity was determined by freshly prepared drop of system in a vial containing 2ml of freshly prepared artificial tear fluid (pH 7.4) and equilibrated at 37°C. The visual assessment of gel formation was carried out. Time required for gelation as well as time taken for the formed gel to dissolve were also noted. Different grades were allotted as the gel integrity, weight, and rate of formation of gel with respect to time. The grades were given as no gelation (-), gelation after few minutes and remains few h (+), gelation immediate and remains few h (++), and gelation immediate and remain extended time (+++). Rheological behavior

The prepared formulations were evaluated for viscosity in order to identify the compositions that best suit for use as in situ gelling systems. The viscosity of the systems was measured using

Brookfield viscometer (LV DVII +PRO model) at 12 rpm for the purpose of comparative evaluation at non physiological condition. Effect of stress on Viscosity

The rheological studies of samples were carried out with Brookfield Viscometer (LV DVII +PRO model). The formulations (pH 5.0, RT 25 0C) were poured into sample adapter of the Brookfield Viscometer and angular velocity was increased gradually from 0.5 to 12 RPM. The hierarchy of the angular velocity was reversed. The average of the two readings was used to calculate the viscosity. The formulation was then poured into an ointment jar and the pH raised to 7.4 (37 0C) by adding freshly prepared ATF. The viscosity measured at both the pH for individual formulation.

In-vitro drug release study44

The In-vitro release studies of levofloxacin from the formulation were studied through the cellophane membrane using a modified USP XXIII dissolution apparatus. The dissolution medium used was freshly prepared artificial tear fluid (pH 7.4). A cellophane membrane previously soaked overnight in the dissolution medium was tied to one end of the specifically designed glass cylinder (open at both end of 5 cm diameter). One ml of the formulation (equivalent to 5 mg of levofloxacin) was accurately pipetted into this assembly. The glass cylinder was suspended in 100 ml of dissolution medium at 37 ± 0.5 °C, so that the membrane just touches the receptor medium surface. A Teflon TM coated magnetic bar continuously stirred the receiving medium at 50 rpm to avoid diffusion layer effects. A sample was placed evenly on the surface of the membrane in the donor compartment. Aliquots, each of 5 ml volume were withdrawn at hourly interval and replaced by an equal volume of dissolution medium to maintain the sink condition. The aliquots were diluted with dissolution medium and analysed by UV Spectrophotometer at suitable nm.

Antimicrobial efficacy studies42

Antimicrobial efficacy was determined by agar diffusion test employing cup plate technique. The microbiological studies ascertained the biological activity of the optimised formulation and marketed eye drops against microorganism Pseudomonas arugenosa and Staphyllococcus aurious as test microorganism. A layer of nutrient agar (20 ml) seeded with the test micro organism was allowed to solidify in Petri dish. Cups were made on the solidified agar layer with the help of sterile borer with 4 mm diameter. Marketed sterile formulation and developed formulation diluted suitably to 5 and 50 µg/ml solution and were poured into cups of agar plates. After allowing diffusion of solution for two hours, the agar plates were incubated at 37°C for 2 hrs. The zone of inhibition (ZOI) was measured around each cup and was compared with the marketed formulation. The entire operation except the incubation was carried out in a laminar air flow unit. Each solution was tested in triplicate.

Stability Studies42

The selected sterile formulation was stored at 400C and Humidity 75±5 RH for 3 months and formulation was finally evaluated for drug content, viscosity, pH and in vitro drug release. After 1 months of storage the formulation evaluated for and drug.

1.3. APPLICATION OF IN SITU OPHTHALMIC DRUG DELIEVERY SYSTEM22-28

a) Sustained drug delivery
b) Site-specific drug delivery
c) Local action in stomach
d) Reduce dose frequency
e) Ease of administration and better patient compliance
f) Site-specific drug delivery

1.4 FUTURE POTENTIAL:

In situ gel approach is suitable for treatment of various bacterial infection in eye. Release the drug continuously in controlled manner. Reduced the fluctuations in the plasma level of the drug. Also enhancing the bio-availability and release the drug in controlled manner. And large numbers of companies are focusing on this type technique. And recent publication says in situ ophthalmic dosage form offers various future potential.

1.5 CONCLUSION:

Higher residence time is required to treat various opthalmic infection the conventional eye drops have very short residence time. The in situ gel produce gel in physiological condition so increase drug residence time The in situ gel enhancing the bio-availability and release the drug in controlled manner. This approach is suitable for the sever eye infection And large numbers of companies are focusing on this type technique.

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