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Research Article

Novel Polymeric *in Situ* Gel Forming System for Ophthalmic Drug Delivery

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ABSTRACT

In the present update controlled and sustained drug delivery has become the standard in modern Pharmaceutical design and an intensive research have been undertaken in achieving much better drug product effectiveness, reliability and safety. This interest has been sparked by the advantages shown by *in situ* forming polymeric delivery systems such as ease of administration and reduced frequency of administration, improved patient compliance and comfort because of sustained and prolonged action in comparison to conventional drug delivery systems. The formation of gels depends on factors like temperature modulation, pH change, presence of ions and ultra violet irradiation, from which the drug gets released in a sustained and controlled manner. Various biodegradable polymers that are used for the formulation of *in situ* gels include chitosan, pluronics, xyloglucans, hydroxy propyl methyl cellulose, carbopol, gelrite, gellan gum, hyaluronic acid and alginates. Mainly *in situ* gels are administered by oral, ocular, rectal, vaginal, injectable and intraperitoneal routes. From a manufacturing point of view, the production of such systems is less complex and thus lowers the investment and manufacturing cost. The present study focused on the polymers used in the preparation of *in situ* gelling system for the ophthalmic drug delivery.

Keywords: *In situ* gel, Biodegradable polymers, chitosan, carbopol, hydroxy propyl methyl cellulose, gellan gum.

INTRODUCTION

Eye is a unique and very valuable organ. This is considered a window hinge. We enjoy it and look at the world body. But there are many eye diseases that can affect the eye and even loss of vision as well [1]. Ophthalmic drug delivery is one of the most interesting and challenging drug delivery for the pharmaceutical scientists. The anatomy, physiology and biochemistry of the eye render this organ exquisitely impervious to foreign substances [2]. Most conventional ophthalmic dosage forms are simplistic. It is usual that water-soluble drugs are delivered through topical administration in an aqueous solution, and water-insoluble drugs are administered topically as an ointment or aqueous suspension. The major deficiencies of these conventional dosage forms include poor ocular drug bioavailability, pulse-drug entry after topical administration, systemic exposure because of nasolacrimal duct drainage, and a lack of effective systems for drug delivery to the posterior segment of ocular tissue [3].

Eye drops are conventional ophthalmic drug delivery system often result in poor bioavailability and therapeutic response, because of the high tear fluid turnover, rapid precorneal elimination of drug [4] and non-productive absorption [5]. As a result, frequent dosing is usually needed in order to avoid the rapid dilution [6]. When a drug solution is instilled into the eye, the effective tear drainage (0.5-2.2 µL/min) and blinking action of eye result in 10-fold reduction in the drug concentration within 4-20 minutes. The limited permeability of the cornea contributes to the low absorption of ocular drugs [7,8]. So only 1-10 % of topically applied drug is absorbed and major part of drug drained into nose and gut. This in turn leads to extensive systemic absorption and result in unwanted side effects [9,10].

So as to increase the bioavailability and duration of the drug action various ophthalmic vehicles, such as viscous solutions, ointments, gels, polymeric inserts and suspensions have been used [11]. Several studies [8,12-14] revealed that corneal contact time has been increased to varying degree, but consequently installation of highly concentrated solutions and frequent administration is required, blurred vision from use of ointments and dosage heterogeneity of suspensions leads to poor patient compliance.

The anatomy of the eye: The eye is a delicate organ held in position in the orbital cavity by various ligaments and muscles. The structure of eye can be divided in two segments, namely anterior and posterior segments. Anterior segment comprises of two chambers, anterior (between the cornea and iris) and posterior (between iris and lens) chambers. Anterior segment tissues are cornea, pupil, aqueous humor, iris- ciliary body and lens while posterior segments tissues are sclera, choroid, retinal pigment epithelium (RPE), neural retina and vitreous humor. The conjunctiva is a protective layer which covers

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Table 1 Benefits and constraints of conventional dosage forms of ophthalmic drug delivery [11,12,15]

S. No.	Dosage form	Benefits	Constraints
1	Solution	Convenient	Rapid precorneal elimination. Loss of drug by drainage. No sustained action.
2	Suspension	Patient compliance. Best for drugs with slow dissolution.	Drug properties decide performance. Loss of drug from suspension.
3	Emulsion	Prolonged release of drug from the vehicle.	Blurred vision. Patient non-compliance. Possible oil entrapment.
4	Ointment	Flexible in drug choice. Improve the drug stability. Resistance to lacrimal drainage.	Sticking of eyelids. Blurred vision. Poor patient compliance.
5	Gels	Comfortable. Less blurred vision.	Matted eyelids after use. No rate control on diffusion.
6.	Erodible inserts	Sophisticated and effective system. Flexibility in drug type and dissolution rate. Need only to be introduced into eye and not to be removed.	Patient discomfort. Requires patient insertion with hospitalization. Movement of system around eye can cause abrasion.
7	Non-erodible inserts	Controlled rate of release of drug. Flexibility for delivery type of drug selected.	Patient discomfort. Irritation in eye can need sudden removal.

eyeball. It consists of a thin mucous membrane layer inside of the eyelids and anterior sclera Fig. 1.

Cornea is a major barrier for the traditional topical drug delivery in the treatment of the anterior segment diseases such as glaucoma, keratitis and bacterial and viral infections.[16] Cornea is a transparent, avascular and highly innervated tissue. It mainly consists five layers Fig. 2. Corneal epithelium (5-6 layers of columnar cells), bowman's layer (a homogenous non-cellular layer), stroma (thickest layer of cornea composed of collagen fibers and 90% water), descemet's membrane (thick membrane separating stroma and endothelium) and endothelium (single cell layer with pinocytotic vesicles)

The eye is constantly cleansed and lubricated by the lachrymal apparatus, which consists of four structures; lachrymal glands, lachrymal canals, lachrymal sac, Naso-lachrymal duct Fig. 3. 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 eyeball and is swept up by the blinking action of the eyelids. Muscles associated with the blinking reflex compress the lachrymal sac. When these muscles relax, the sac expands, pulling the lachrymal fluid from the edges of the lids, along the lachrymal canals, into the lachrymal sacs. Gravitational force, in turn, moves the fluid down the naso-lachrymal duct into the inferior meatus of the nose. Thus the eyeball is continually irrigated by a gentle stream of lachrymal fluid that prevents it from becoming dry and inflamed. The amount of lachrymal fluid renewed by the frequent involuntary blinking movements normally is just sufficient to keep pace with its disappearance from conjunctiva. However, an excessive formation and secretion of lachrymal fluid, or lacrimation, can occur when foreign bodies or other irritants get into the eye, when a bright light is shone into the eye, or in emotional stress.

The lachrymal fluid in humans has a normal volume of 7µl and is an isotonic aqueous solution of bicarbonate and sodium chloride (pH 7.4) that serves to dilute irritants or to wash the foreign bodies out of the conjunctival sac. It contains lysozyme, whose bactericidal activity reduces the bacterial count in the conjunctival 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.

The aqueous humor in humans has a volume of approximately 300µl that fills the anterior chamber of the eye. Aqueous humor is secreted by the ciliary's processes and flows out of the anterior chamber at a turnover rate of approximately 1% /min .The drainage system has recently been defined at the sinus venous sclera, of low blood pressure. This drainage is an unspecific mechanical process different from the production of aqueous humor. The rate of drainage is comparable to the rate of production, thus maintaining a constant intra-ocular tension of 25-30 mm/Hg in humans.

This intra-ocular pressure remains fairly constant even when the arterial pressure widely fluctuates. It rises slightly when the external ocular muscles contract and on winking. It is known that the focusing mechanism of the eye depends upon the existence of a fairly constant intra-ocular tension. If the tension is too high, as in glaucoma the ciliary's muscles may not be able to bring about accommodation. The high intra-ocular pressure may also cause the restriction of the retinal circulation with resultant damage to the retina.

On the other hand, an excessive reduction in the intra-ocular tension may slacken the suspensory ligaments of the lens and allows the latter to bulge. One of the major problems encountered with the topical delivery of ophthalmic drugs is the rapid and extensive precorneal loss caused by the drainage and high tear fluid turnover. Lacrimation and blinking are actually efficient protective mechanisms, which keep the eye free of foreign substances, but they prevent efficient ocular residence time of conventional eye drops is limited to a few minutes and the ocular absorption of a topically applied drug is reduced to approximately 1-10%. Furthermore, drug uptake occurs as a massive pulse entry, followed by a rapid decline. The drug is mainly absorbed systemically via conjunctiva and nasal mucosa, which may result in some undesirable side effects [17-20].

The precorneal tear film (thickness 3-10 μm; volume ~10 μL) is composed of the following Layers as shown in Fig. 4. The superficial lipid layer (thickness 100 nm), which spreads over the aqueous layer during eye opening. It contains such lipids as triglycerides, phospholipids, sterols, sterol esters, fatty acids, and helps tear fluid to maintain its normal osmolality by limiting evaporation. The aqueous layer, which contains inorganic salts, glucose, urea, retinol, ascorbic acid, proteins, lipocalins, immunoglobulins, lysozyme, lactoferrin and glycoproteins. The tear fluid has an osmolality of 310-350 mOsm/kg and a mean pH of 7.4. Its buffering ability is determined by bicarbonate ions, proteins and mucins. The viscosity is ~3 mPas, with a non-Newtonian rheological behaviour. The surface tension is ~44 mN/m. The mucus layer, which forms a gel with viscoelastic properties. Mucins improve the spreading of the tear film and enhance its stability and cohesion. The mucus gel entraps bacteria, cell debris and foreign bodies forming bundles of thick fibers, which are conveyed by blinking to the inner canthus and expelled onto the skin. Mucus, which is charged negatively, can bind positively charged substances [21].

Routes of ocular drug delivery

Topical administration: It is the most commonly used route of drug administration for the treatment of anterior segment complications. Posterior segment drug delivery via topical route suffers from drug loss in the precorneal area and anterior segment, drug elimination from the anterior chamber by the canal of Schlemn or via absorption through iris-ciliary's body. Enzymatic metabolism in the anterior chamber limits the entry of intact drug into the posterior segment tissues. Limited success has been achieved with topical administration in the area of posterior segment drug delivery. However, topical administration is not a feasible approach for delivering therapeutic agents in the treatment of retinal diseases.

Systemic administration: Due to the presence of BRB, systemic administration has achieved a limited success to deliver drugs to the vitreo-retinal tissues. Only 1-2% of plasma drug concentration is achieved in the vitreous humor and therefore requires frequent administration to maintain therapeutic drug level. This route of administration may also result in non-specific binding of drug to other tissues and cause systemic cytotoxicity.

Intravitreal administration: Intravitreal administrations of therapeutic agents by direct injection into the mid vitreous region and sustain and controlled released Intravitreal implants have become a mainstay treatment option of posterior segment diseases. Various therapeutic agents, such as antiviral agents - ganciclovir, acyclovir, cidofovir and foscarnet and antibiotics-cephalexin, gentamicin and cefazolin have been evaluated to design the effective treatment of CMV retinitis and endophthalmitis. Longer retention time and higher vitreous concentration of ganciclovir was obtained following this route of administration. However, patient noncompliance, pain and discomfort are major obstacles to the clinical application.

Scleral administration: Due to its large surface area, easy accessibility and relatively high permeability to macromolecules, the sclera recently has become a potential vector for posterior segment drug delivery. Scleral drug delivery has been attempted by different ways, such as scleral plugs and implants, Subconjunctival injection, subtenon injection etc. Sustained release transscleral device has also been studied to overcome complications related to the intravitreal injection. However, scleral plugs and implants suffer from disadvantages such as patient discomfort and surgical risks. Subconjunctival administration of various therapeutic agents has recently gained a special attention due to the lower surgical risks and patients compliance. Subconjunctival administration of therapeutic agents could also result in higher aqueous and plasma concentration as compared to peribulbular administration. Transscleral administration of drugs offers a promising therapeutic approach for the treatment of various posterior segment diseases [16].

Barriers for ocular drug delivery: Ocular tissue are protected from exogenous toxic substances in the environment or blood stream by a variety of mechanisms, notably, tear secretion continuously flushing its surface, an impermeable surface epithelium and a transport system actively cleaning the retina of agents potentially able to disturb the visual process. However, the same protective mechanisms may cause sub therapeutic drug levels at the intended site.

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. Anyway, most of small molecular weight drug dose is absorbed into systemic circulation rapidly in few minutes. This contrasts the low ocular bioavailability of less than 5%. Drug absorption into the systemic circulation decreases the drug concentration in lacrimal fluid

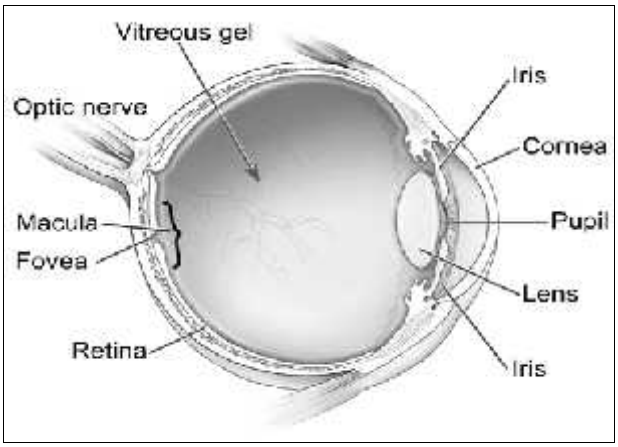

Fig. 1. Anatomy of the eye [16]

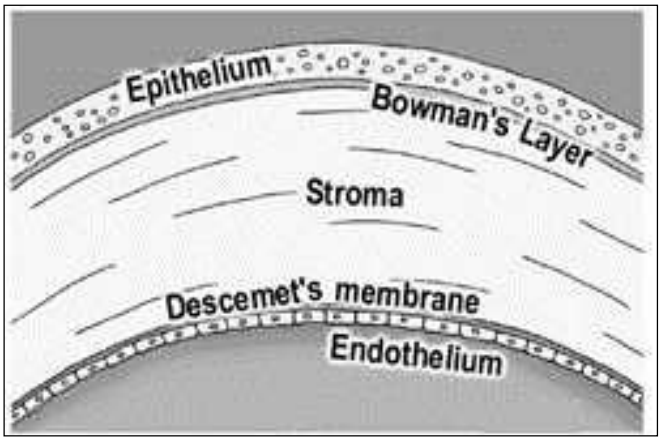

Fig. 2. Cross section through the cornea [2]

extensively. Therefore, constant drug release from solid delivery system to the tear fluid may lead only to ocular bioavailability of about 10%, since most of the drug is cleared by the local systemic absorption anyway.

Lacrimal fluid eye barrier: Corneal epithelium limits drug absorption from the lacrimal fluid into the eye. The corneal barrier is formed upon maturation of the epithelial cells. They migrate from the limbal region towards the centre of the cornea and to the apical surface. The most apical 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. Despite the tightness of the corneal epithelial layer, transcorneal permeation is the main route of drug entrance from the lacrimal fluid to the aqueous humor. 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. Drug absorption across the bulbar conjunctiva has gained increasing attention recently, since conjunctiva is also fairly permeable to the hydrophilic and large molecules. Therefore, it may serve as a route of absorption for larger bio-organic compounds such as proteins and peptides. Clinically used drugs are generally small and fairly lipophilic. Thus, the corneal route is currently dominating. In both membranes, cornea and conjunctiva, principles of passive diffusion have been extensively investigated, but the role of active transporters is only sparsely studied.

Blood ocular barrier: The eye is protected from the xenobiotic 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. This barrier prevents the access of plasma albumin into the aqueous humor, and limits also the access of hydrophilic drugs from plasma into the aqueous humor. Inflammation may disrupt the integrity of this barrier causing the unlimited drug distribution to the anterior chamber. In fact, the permeability of this barrier is poorly characterised. The posterior barrier between blood stream and eye is comprises 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. Despite its high blood flow the choroidal blood flow constitutes only a minor fraction of the entire blood flow in the body. Therefore, without specific targeting systems only a minute fraction of the intravenous or oral drug dose gains access to the retina and choroid. Unlike blood brain barrier, the blood-eye barriers have not been characterised in terms of drug transporter and metabolic enzyme expression. From the pharmacokinetic perspective plenty of basic research is needed before the nature of blood-eye barriers is understood [1].

Transcorneal penetration

Corneal barrier: The corneal epithelium is the main barrier to the drug absorption into the eye compared to many other epithelium tissues (intestinal, nasal, bronchial and tracheal) corneal epithelium is relatively impermeable, but it is more permeable compared to the stratum corneum of the skin. A tight junction serves as a selective barrier for small molecules and they completely prevent the diffusion of macromolecules via the paracellular route [8].

Non-corneal absorption: Its common knowledge that the ocular bioavailability of drugs applied topically as eye drops is very poor. The absorption of drug in the eye is severely limited by some protective mechanisms that ensure the proper functioning of the eye Fig. 5.

Physicochemical drug properties: Drug penetrates across the corneal epithelium via transcellular or paracellular pathway. Lipophilic drugs prefer the transcellular route while the hydrophilic drugs penetrate primarily through the paracellular pathway which in involves passive or altered diffusion through intercellular spaces. Physicochemical drug properties such as lipophilicity, solubility, molecular size and shape, charge and degree of ionization affect the route and rate of permeation in cornea [8,22].

In situ hydrogels: Hydrogels are three-dimensional, hydrophilic, polymeric network capable of imbibing large amount of water or biological fluids, while remaining insoluble in aqueous solutions due to chemical or

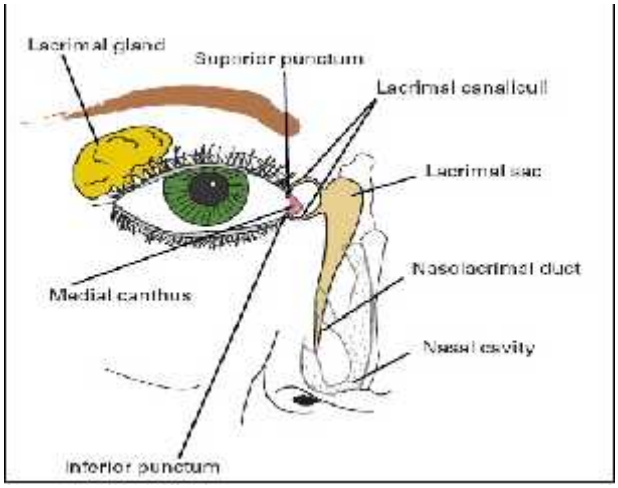

Fig. 3. Naso-lachrymal drainage system [17]

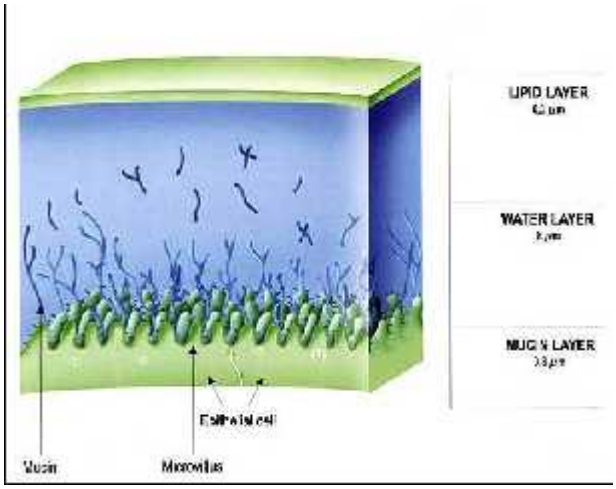

Fig. 4. Structure of tear film in the human eye [18]

physical cross-linking of individual polymer chains. They resemble natural living tissue more than any other class of synthetic biomaterials due to their high water content. Furthermore, the high water content of the materials contributes to their biocompatibility. Hydrogels show minimal tendency to adsorb proteins from body fluids because of their low interfacial tension.

Further, the ability of molecules of different sizes to diffuse into (drug loading) and out (drug release) of hydrogels allows the possible use of dry or swollen polymeric networks as drug delivery systems for oral, nasal, buccal, rectal, vaginal, ocular and parenteral routes of administration. These are polymers endowed with an ability to swell in water or aqueous solvents and induce a liquid–gel transition. Both natural and synthetic polymers can be used for the production of hydrogels. Cross-linking of the polymers can be achieved by various chemical and physical crosslinking methods [24-26]. Generally two groups of hydrogels are distinguished:-

Preformed Hydrogels

In situ forming gels

Performed hydrogels: Preformed hydrogels can be defined as simple viscous solutions which do not undergo any modifications after administration. The use of preformed hydrogels still has drawbacks that can limit their interest for ophthalmic drug delivery or as tear substitutes. They do not allow accurate and reproducible administration of quantities of drugs and, after administration; they often produce blurred vision, crusting of eyelids, and lachrymation.

In situ forming gels: *In situ* hydrogels can be instilled as eye drops and undergo an immediate gelation when in contact with the eye. *In situ* forming hydrogels are liquid upon instillation and undergo phase transition in the ocular cul-de-sac to form viscoelastic gel and this provides a response to environmental changes. Three methods have been employed to cause phase transition on the surface: change in temperature, pH, and electrolyte composition.

Increase in solution viscosity by using polymers improves retention of product on the corneal surface. More recently, the approach to improve precorneal retention is based on the use of mucoadhesive polymers. The principle for use of bioadhesive vehicles relies on their ability to interact with the mucin-coating layer present at the eye surface. The polymers chosen to prepare ophthalmic hydrogels should meet some specific rheological characteristics. It is generally well accepted that the instillation of a formulation should influence tear behaviour as little as possible. Because tears gave pseudo-plastic behaviour, pseudo-plastic vehicles would be more suitable as compare to Newtonian formulations, which have a constant viscosity independent of the shear rate, whereas pseudo plastic solution exhibit decreased viscosity with increasing shear rate, thereby offering lowered viscosity during blinking and stability of the tear film during fixation [18,27].

Drug Release from hydrogels: As discussed in the previous sections, hydrogels have a unique combination of characteristics that make them useful in drug delivery applications. Due to their hydrophilicity, hydrogels can imbibe large amounts of water (90% w/w). Therefore, the molecule release mechanisms from hydrogels are very different from hydrophobic polymers, as shown in Fig. 6. 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.

In situ forming gelling system: *In situ* gelling system for the ophthalmic delivery system prepared from polymers that exhibit reversible phase transition (sol-gel-sol) and pseudo plastic behaviour to minimize interference with blinking of eye [7,13,29]. Gelation can be triggered by temperature, pH, ions; solvent induced and may be UV induced. But three major methods have been employed to cause phase transition on the surface: change in temperature, pH, and electrolyte composition [30]. There are three broadly defined mechanisms used for triggering the *in situ* gel formation of biomaterials in ophthalmic delivery:

Thermally triggered system: Gelling of the solution is triggered by change in temperature, thus sustaining the

Table 2 Comparison of various routes of ocular drug administration [16]

Route	Advantages	Disadvantages/Risks
Topical	Patient compliance Ease of administration	Sub therapeutic drug level Precorneal loss Conjunctival absorption
Systemic	Control the spread of infection to other tissues	Need of frequent administration Systemic toxicity Sub therapeutic ocular drug level
Intravitreal injection	Therapeutic drug level No or low systemic toxicity	Patient non-compliance Vision threatening surgical risks
Intravitreal implants	Sustain therapeutic level	Removal of implant Vision threatening surgical risks
Scleral plugs and implants	Lower surgical risks Sustain therapeutic level	Patient discomfort Repeated operation
Subconjunctival injections	Relatively non-invasive Sustain therapeutic level	Systemic absorption Risk of ocular infection

drug release. This can be achieved by using a polymer that is a solution at room temperature (<25°C) and a gel at body temperature. Thermosetting polymer poloxamer has been used for increasing contact time, increases elasticity of the gel and decreases the sol-gel transition temperature.

pH triggered system: Gelling of the solution is triggered by a change in pH. At pH 4.4 the formulation is a free-running solution which undergoes coagulation when the pH is raised by the tear fluid to pH 7.4. The pH change of about 2.8 units after instillation of the formulation (pH 4.4) into the tear film leads to an almost instantaneous transformation of the highly fluid latex into a viscous gel. Cellulose acetate phthalate latex, cross-linked polyacrylic acid and derivatives of carbomers are used.

Ionic triggered system: Gelling of the solution can also be triggered by a change in ionic strength. It is assumed that the rate of gelation depend on the osmotic gradient across the surface of the gel. It is therefore likely that the osmolality of the solution might have an influence on the rate of the sol gel transition occurring in the eye. 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 sodium, Calcium and Magnesium cations are particularly suited to initiate gelation of the polymer when instilled as a liquid solution in the conjunctival cul-de-sac [28,29,31].

Polymers used in *in situ* gelling systems

Chitosan: Chitosan is a biodegradable, thermosensitive, polycationic polymer obtained by alkaline deacetylation of chitin, a natural component of shrimp and crab shells.[28] chitosan is a highly basic polysaccharide, so it can form poly-oxy salts, films, chelate metal ions and optical structures. It is soluble in dilute acids such as acetic acid, formic acid. It has the characteristic of forming hydrogels that are highly swollen hydrophilic polymer networks, capable of absorbing large amounts of water, such that they have become widely used in controlled release systems [33,34]. Chitosan exhibits several favourable properties such as biodegradability and biocompatibility. It also has mucoadhesive properties due to its positive charge at neutral pH that enables an ionic interaction with the negative charges of sialic acid residues of mucus, which is soluble in water up to pH 6.2. Basification of chitosan aqueous solutions above this pH leads to the formation of a hydrated gel-like precipitate [20,28,35]. Gratieri et al. prepared a poloxamer/chitosan *in situ* forming gel for prolonging retention time for ocular delivery. They used thermosetting polymer poly (ethylene oxide)–poly (propylene oxide)–poly (ethylene oxide) (PEO–PPO–PEO, poloxamer), with a mucoadhesive agent (chitosan). The rheology, texture and mucoadhesive profiling was done along with the retention time of the formulation in the human volunteers. The results showed that the chitosan improved the mechanical strength and texture of poloxamer. Which showed that poloxamer: chitosan in 16:1 %w/w was able to withstand low shearing forces, with high adhesiveness at the site of administration [6]. While ur-rehman et al. prepared Chitosan *in situ* gelling system for improved drug loading and retention of metoprolol in the poloxamer 407 gel. They prepared Novel poloxamer gels containing CT–TPP complex formed in situ during the administration were prepared by mixing poloxamer–CT and poloxamer–TPP solutions. The studies showed that the ionotropic gelation of the natural polymer chitosan with TPP in *in situ* gelling system was suitable method to enhance the drug delivery application of poloxamer based Thermoreversible gelling system [36]. Varshosaz et al. Designed thermosensitive chitosan/poloxamer *in situ* gel for ocular delivery of ciprofloxacin. Mixtures of solutions of Pluronic (10-25% w/w) with chitosan (0.1-0.3% w/w) of different molecular weights were prepared. Ciprofloxacin release was determined using a membraneless dissolution model in artificial tear solution up to 8 hours. The rheological behaviour of solutions in response to dilution or temperature changes and also the phase change temperature (PCT) were determined using a Cup & Bob viscometer. It was liquid in non-physiologic conditions (pH 4 and 25ºC) and transferred to the gel form upon physiologic conditions (pH 7.4 and 37ºC). The developed formulation is a viable alternative to conventional eye drop by virtue of its ability to enhanced and longer antibacterial effect through its longer precorneal residence time and ability to sustain drug release [37].

Pluronics: Poloxamer or pluronic is block copolymer consisting of non-ionic poly (oxyethylene) and poly

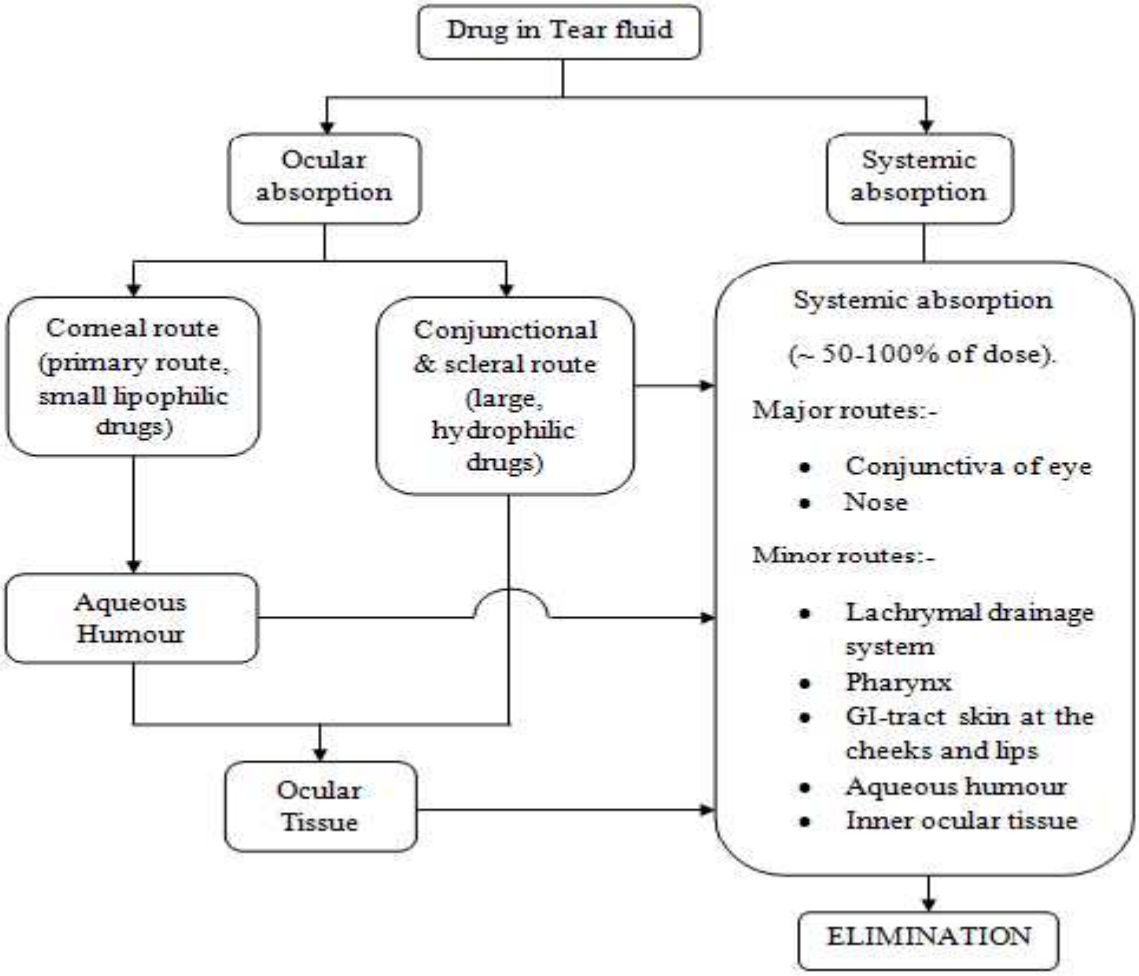

Fig. 5. Precorneal barriers limiting penetration of topically administered drug [23]

(oxypropylene) units. Poloxamer undergo thermal gelation or sol-gel transition in the 25-35ºC temperature range. Below transition temperature, poloxamer solutions allow a comfortable and precise delivery by the patient in cul-de-sac, where thermo gelation occurs. Pluronic F-127 was used as an *in situ* gel forming polymer together with mucoadhesive polymers such as Carbopol 934 and hydroxy propyl methylcellulose to ensure long residence time at the application site. Due to inherent surface active properties, poloxamers were employed as solubilizer and also proposed as artificial tears. Pluronic® F-127 is no more damaging to the mouse or rabbit cornea than a physiological saline solution [20,28,35]. Asasutjarit et al. optimized and evaluated thermoresponsive *in situ* gelling system for the ophthalmic delivery of diclofenac sodium. Pluronic F127 based thermoresponsive polymer was used by cold method and the physicochemical properties were investigated i.e., pH, flow ability, sol–gel transition temperature, gelling capacity and rheological properties. *In-vivo* ophthalmic absorption was performed in rabbits. The optimised formulation exhibited sol-gel transition at 32.6±1.1ºC with pseudoplastic flow behaviour [12]. While Shastri et al. developed the thermoresponsive mucoadhesive ophthalmic *in situ* hydrogel for the ophthalmic drug delivery of moxifloxacin. Pluronic F127 and Gelrite were used as thermoresponsive polymers. Gelation temperature, gel strength, bioadhesion force, viscosity and *in vitro* drug release after 1 and 10 hours were selected as dependent variables. Pluronic F68 loading with Pluronic F127 was found to have a significant effect on gelation temperature of the formulation and to be of importance for gel formation at temperatures 33–36 °C. Gelrite loading showed a positive effect on bioadhesion force and gel strength and was also found helpful in controlling the release rate of the drug [38]. Gupta et al. developed a dual-drug delivery system based on *in situ gel* forming nanosuspension of forskolin to enhance antiglucoma efficacy. Forskolin nanocrystals have been successfully manufactured and stabilized by poloxamer 407. These nanocrystals have been characterized in terms of particle size by scanning electron microscopy and dynamic light scattering. The *in situ* platform was developed using polycarbophil/poloxamer 407. The formulation was stable over a period of 6 months at room temperature. In vitro release experiments indicated that the optimized platform was able to prolong and control forskolin release for more than 5 h [39].

Xyloglucans: Xyloglucan is a polysaccharide derived from tamarind seeds and is composed of a (1-4)-β-D-

glucan backbone chain, which has (1-6)-α-D xylose branches that are partially substituted by (1-2)-β-D-galactoxylose**.** When Xyloglucan is partially degraded by β-galactosidase, the resultant product exhibits thermally reversible gelation by the lateral stacking of the rod like chains. The sol-gel transition temperature varies with the degree of galactose elimination. It forms thermally reversible gels on warming to body temperature. Its potential application in oral delivery exploits the proposed slow gelation time (several minutes) that would allow *in situ* gelation in the stomach following the oral administration of chilled xyloglucan solution. Xyloglucan gels have potentially been used for oral, intraperitoneal, ocular and rectal drug delivery [28].

Cellulose derivatives: Mucoadhesive properties, Physical combinations of methylcellulose (a thermally induced gelling material) and carbomer (pH-induced gelling polymer), which are able to achieve a desired viscosity at lower polymer concentration, were investigated *in situ* thermo gelling system consisting of ethyl-(hydroxyethyl) cellulose and a charged surfactant. Another approach was the formulation of pseudo latex, an *in situ* gelling system based on cellulose acetate hydrogen phthalate (CAP). CAP is the only polymer known to have a buffer capacity which is low enough to gel effectively in the cul-de-sac of the eye. The pH change of 2.8 units after instillation of the native formulation (pH 4.4) into the tear film leads to an almost instantaneous transformation of the highly fluid latex into a viscous gel. The high CAP concentration (30%) and the low pH of the preparation can elicit discomfort in some patients. Also, the formation of macromolecular complexes help in prolonged activity and increased bioavailability by using a sparingly soluble drug complex in combination with mucoadhesive polysaccharide hydrogels [20]. Vijaya and Goud, developed the ion-activated *in situ* gelling system for azithromycin using different concentration (1-5% w/v) and different proportions of the hydrocolloids hydroxypropyl methyl cellulose and sodium caboxymethy cellulose along with the pectin in minute concentrations. Formulations were evaluated for pH, antimicrobial efficacy and drug release. The *in-vitro* release was temporally controlled for more than 4 hours. From the above study it was concluded that pectin based *in situ* gels can be successfully used to prolong the duration of action of azithromycin [40].

Carbopol: 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. Carbopol® 934 is a synthetic polymer composed of 62% of carboxyl groups with a high molecular weight (approximately 3×10^6) formed by repeating units of acrylic acid, cross-linked with either allylsucrose or allylethers of pentaerythritol. Carbopol is a well-known pH dependent polymer, which stays in solution form at acidic pH but forms a low viscosity gel at alkaline pH. Carbopol offers the advantage of exhibiting excellent mucoadhesive properties when compared with other polymers (e.g. cellulose derivatives and polyvinyl alcohol). As the concentration of Carbopol increases in the vehicle, its acidic nature may cause stimulation to the eye tissues. So HPMC is used in combination with carbopol to impart the viscosity to carbopol solution, while reducing the acidity of the solution. Various water soluble polymers such as carbopol system, hydroxy propyl methylcellulose system, poly-(methacrylic acid) poly-(ethylene glycol) come under the category of pH-induced *in situ* precipitating polymeric systems [28,35]. Reddy et al. formulated pH-triggered *in situ* gelling system for prulifloxacin using carbopol 940 in combination with hydroxypropyl methyl cellulose which acted as viscosity enhancing agent. The developed formulation was stable, non-irritant and provided sustained release over 8-hours period and it is a viable alternative to conventional eye drops [41]. While ramchandra et al. developed the pH-triggered *in situ* gelling system for the ophthalmic delivery of ciprofloxacin by using polyacrylic acid (carbopol 934) in combination with hydroxypropyl methyl cellulose which acted as viscosifying agent. The developed formulation was efficacious, stable, non-irritant and provided sustained release over 8-hours period and it was a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through it longer precorneal residence time and ability to produce sustained release [42]. Kanoujia et al. formulated a pH-triggered *in situ* gelling ocular system for gatifloxacin using carbopol 940 along with HPMC K15M. The prepared formulation was evaluated for pH, clarity, drug content, gelling capacity, and bioadhesive strength and *in-vitro* drug release. The gel provided sustained drug release over an 8 hour period. The developed formulation can be used as an *in-situ* gelling vehicle to enhance ocular bioavailability and the reduction in the frequency of instillation thereby resulting in better patient compliance [43]. Basaran and Bozkir, developed the thermosensitive and pH-triggered *in situ* ophthalmic gelling system for ciprofloxacin hydrochloride with reduced pre-corneal elimination in order to improve the bioavailability and therapeutic response. Hydroxypropyl-β-cyclodextrin was used in order to increase the stability of ciprofloxacin hydrochloride. Carbopol 934 and 940 and poloxamer 407 were used as pH and thermosensitive polymers. Formulations were successfully prepared which were liquid at room temperature and exhibited viscosity increase and gelation at ophthalmic temperature. As a result of antimicrobial efficacy and *in-vitro* release experiments, the developed formulations were found therapeutically efficient and provided sustained release of the drug over an 8 h period [44]. While Wu et al. developed the pH-triggered *in situ* gelling vehicle for ophthalmic delivery of puerarin. Carbopol 980NF was used as the gelling agent is combination with HPMC E4M. The optimum concentration of carbopol 980NF and HPMC E4M for the *in situ* gel forming delivery system were 0.1% w/v and 0.4% w/v respectively. When these two vehicles were combined, an *in situ* gel that had the appropriate gel strength and gelling capacity under physiological condition. This combined solution could flow freely under non-physiological condition and

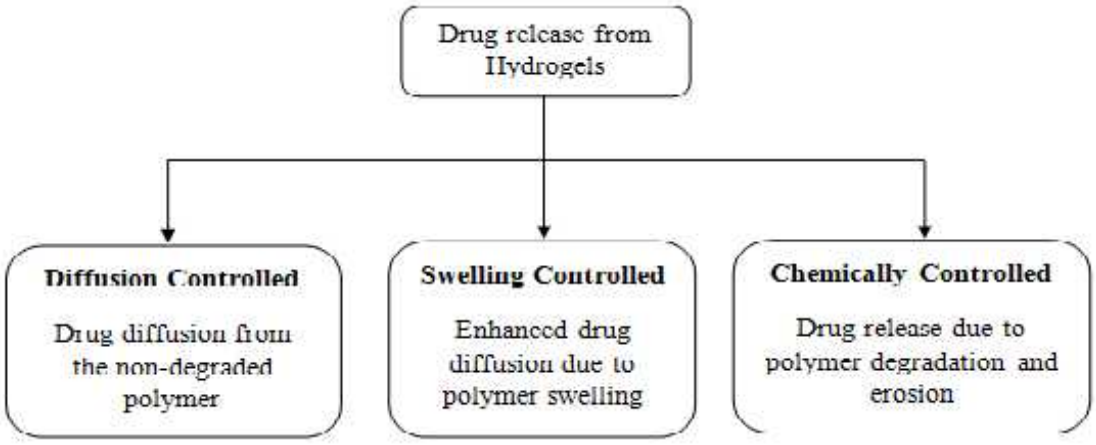

Fig. 6. Drug release from hydrogels [28]

showed the character of pseudo-plastic fluid under physiological conditions. Both *in-vitro* release studies and *in-vivo* pharmacokinetics studies showed that the formulation 1 released 89.8% drug after 6 hours while the formulation 2 showed some delayed released and released 80.7% drug after 6 hours indicated that the combined polymer systems performed better in retaining puerarin than puerarin eye drops. Carbopol 980NF/HPMC E4M can thus be a viable alternative to conventional puerarin eye drops and showed better patient compliance, but also prolonged the precorneal residence time thus higher bioavailability [45].

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 then isolated from the fermentation broth by alcohol precipitation. 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 repeated unit. The native polysaccharide is partially esterified with L-glycerate and acetate, but the commercial product Gelrite has been completely de-esterified 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. Formulations with the Gelrite can be administered to ocular mucosa as a low viscosity solution. 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+, Ca2+). Gellan gum produces temperature dependent or cations induced *in situ* gelling. Once gelled, the formulation resists the natural drainage process from the precorneal area. Residence at the site of drug absorption is prolonged and, subsequently, the bioavailability of the drug is increased [20,28,35]. Rupenthal et al. performed the comparison between the ion-activated *in situ* gelling system and conventional eye drops for physicochemical characterisation and *in-vitro* release studies. They compared a number of anionic polysaccharides (gellan gum, xanthan gum, carrageenan and alginate) to an uncharged (HPMC) and a positively charged (chitosan) polymer system with emphasis on the gelling behaviour, rheological and textural properties, gel microstructure, contact angle and in vitro release characteristics. All systems exhibited physically entangled polymer networks that were able to disentangle upon shear stress and significantly prolonged the in vitro release of a model hydrophilic drug compared to a solution. While systems based on HPMC and chitosan showed no structural changes upon addition of cations, formulations based on gellan gum and carrageenan demonstrated a remarkable increase in viscosity, pseudoplasticity and hardness upon addition of Ca2+ and K+ respectively. This renders them favourable for ocular use as they would gel once in contact with the cations of the tear fluid, thus reducing nasolacrimal drainage [46]. Zhu et al. designed the microemulsion *in situ* electrolyte triggered gelling system for ophthalmic delivery of lipophilic drug (cyclosporine A). Cyclosporine A loaded microemulsion was prepared using castor oil, Solutol HS 15 (surfactant), glycerol and water. This microemulsion was then dispersed in a Kelcogel® solution to form the final microemulsion *in situ* electrolyte-triggered gelling system. *In-vitro* the viscosity of the cyclosporin A microemulsion Kelcogel® system increased dramatically on dilution with artificial tear fluid and exhibited pseudo-plastic rheology. *In-vivo* studies revealed that the $AUC_{0\text{-}32h}$ of corneal cyclosporine A for the microemulsion Kelcogel® system which was approximately 3-fold greater than for a cyclosporine A emulsion [47]. While Carlfors et al. studied the rheology of Gelrite® *in situ* gels. A complementary *in-vivo* study for determining precorneal contact times in humans and in rabbits was performed. The elastic moduli of the gels increased with increasing concentration of electrolytes. At physiological concentration of the electrolytes, the elasticity of the gels was independent of Gelrite® concentration. The human contact times increased up to 20 hours with decreased osmolality of the formulations. The results indicate that a

Table 3 Classification of *in situ* Polymeric systems [30,32]

S. No.	*In situ* gelling system	Polymer used
1	Temperature dependent system	Chitosan, Pluronics, Tetronics, Xyloglucans, Hydroxypropylmethyl cellulose or Hypromellose (HPMC).
2	pH-triggered system	Cellulose Acetate Phthalate (CAP) latex, Carbopol, Polymethacrilic acid (PMMA), Polyethylene glycol (PEG), Pseudo latexes.
3	Ion-activated system	Gelrite, Gellan gum, Hyaluronic acid, Alginates.

high rate of the sol/gel transition results in long contact times. Rheological characterization is a powerful tool for the evaluation of gels for use in ophthalmic drug delivery [48].

Alginic acid: Alginic acid is insoluble in water, but sodium alginate produces clear gels. Alginate is a natural block copolymer containing two types of monomers: β-D mannuronic acid and α-L-guluronic acid residues joined by 1,4-glycosidic linkage. Alginate is a well-known polysaccharide widely used due to its gelling properties in aqueous solutions related to the interactions between the carboxylic acid moieties and bivalent counter ions, such as calcium, lead, and copper; it is also possible to obtain an alginic acid gel by lowering the environmental pH value. Dilute aqueous solutions of alginates form firm gels on addition of di and trivalent metal ions by a cooperative process involving consecutive guluronic residues in the α-L-guluronic acid blocks of the alginate chain. Ionotropic hydrogels are formed by interaction of calcium ions with guluronic acid monomers. Consequently, *in situ* gelling could occur because of the ionic strength of the tear fluid. Alginate has also been proposed in the field of pharmaceutics for its *in situ* gelation properties, particularly for the application of alginate gels for ocular drug delivery, since this dosage form is so effective as compared to solutions [20,28,35]. Mandal et al. prepared and evaluated the ionically triggered *in situ* gelling system for the ophthalmic delivery of moxifloxacin hydrochloride using sodium alginate, a novel ophthalmic gel forming mucoadhesive polymer along with the Hydroxypropyl methyl cellulose as viscosifying agent. The formulation was evaluated for the clarity, pH measurement, gelling capacity, drug content, rheological study, *in-vitro* diffusion study, antibacterial activity etc. The developed formulation exhibited sustained release of drug from the formulation over a period of 10 hours. Thus proved that these *in situ* gelling systems containing gums may be a valuable alternative to the conventional systems [49]. Liu et al. develop an ion-activated *in situ* gelling vehicle for ophthalmic delivery of matrine. The rheological properties of polymer solutions, including Gelrite, alginate, and Gelrite/alginate solution, were evaluated. In addition, the effect of formulation characteristics on *in vitro* release and *in vivo* precorneal drug kinetic of matrine was investigated. It was found that the optimum concentration of Gelrite solution for the *in situ* gel-forming delivery systems was 0.3% (*w*/*w*) and that for alginate solution was 1.4% (*w*/*w*). The mixture of 0.2% Gelrite and 0.6% alginate solutions showed a significant enhancement in gel strength at physiological condition. Both the *in vitro* release and *in vivo* pharmacological studies indicated that the Gelrite/alginate solution had the better ability to retain drug than the Gelrite or alginate solutions alone [50]. While Liu et al. developed and evaluated the alginate/HPMC based *in situ* gelling system for ophthalmic delivery system for Gatifloxacin. Alginate (Kelton®) was used as the gelling agent in combination with HPMC (Methocel E50LV) which acted as a viscosity-enhancing agent. The rheological behaviours of all formulations were not affected by the incorporation of gatifloxacin. Both *in-vitro* release studies and *in-vivo* pre-corneal retention studies indicated that the alginate/HPMC solution retained the drug better than the alginate or HPMC E50LV solutions alone. These results demonstrated that the alginate/HPMC mixture can be used as an *in situ* gelling vehicle to enhance ocular bioavailability and patient compliance [7]. Cohan et al. prepared ion-activated *in situ* gel forming system for the ophthalmic drug delivery of pilocarpine nitrate using Sodium Alginate as polymer. The aqueous solution of Sodium Alginate became gel in the eye, without addition of external calcium ions and other bivalent/polyvalent cations. *In-vitro* studies indicated that pilocarpine is released slowly from alginate gels, over a period of 24 h, and the release occurs mostly via diffusion from the gels. Dissolution of the hydrogels in the releasing media was negligible for the first 12 h of incubation at 37ºC. The overall results of the study indicated that the *in situ* gelling alginate system was an excellent drug carrier for the prolonged ophthalmic delivery of pilocarpine. There alginate is an ideal excipient for the eye drops [13].

Hyaluronic acid: Hyaluronan is beneficial to dry eye patients due to its rheological properties which are similar to those of mucus, its water retention capacity and its protective role at the corneal/conjunctival epithelium. Hyaluronic acid and carbomer hydrogels have no cytotoxicity, but possess antioxidant properties. Hyaluronic acid reduces the cytotoxicity of Benzlkonium chloride due to ionic attractions, entrapment in the sponge-like domain of the hyaluronan network or a shift of the binding equilibrium away from the interaction with cell membranes. Hyaluronan and carbomer hydrogels could, therefore, be interesting to use not only in dry eyes, but also in ocular surface disorders involving oxidative stress [20]. Mayol et al. reported the thermosensitive Poloxamer/hyaluronic acid *in situ* forming hydrogel for the ophthalmic drug delivery of acyclovir. They studied the effect of hyaluronic acid on the gelation temperature of poloxamer blend. The gelation temperature, viscoelastic properties and mucoadhesive force of the system were investigated. The

studies showed that there was rheological synergism between poloxamer/hyaluronic acid which lead to change of the flow behaviour from a quite Newtonian one to a pseudoplastic one. *In-vitro* release studies showed that optimised formulation was able to prolong and control acyclovir release for more than 6 hours [51]. While Cho et al. developed the grafting of poloxamer onto the hyaluronic acid for application of tissue engineering oriented ophthalmic drug delivery system for ciprofloxacin. Graft copolymers were prepared by coupling mono amine-terminated poloxamer (MATP) with hyaluronic acid (HA) backbone using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxyl succinimide (NHS) as coupling agents. The gelation temperature of graft copolymers was dependent on the content of HA and the concentration of poloxamer. A sustained ciprofloxacin release *in-vitro* was dependent on the content of HA. The results of this study indicate that the bioadhesive, thermally gelling and tissue regeneration properties of these graft copolymers will be expected to be an excellent drug carrier for the prolonged delivery to surface of the eye [52].

CONCLUSION

The primary requirement of a successful controlled release product focus on the sustained release of the drug, therapeutic efficacy of the system and better patient compliance with reduced local side effects which the *in situ* gels offer most. The polymeric *in situ* gelling system has number of advantages over conventional dosage forms. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the *in situ* gel dosage form very reliable. While the use of biodegradable and water soluble polymers for the *in situ* gel formulations can make them more acceptable and excellent drug delivery systems with reduced manufacturing cost.

Conflict of Interest: The authors declare that there is no conflict of interests regarding the publication of this article.

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