Review on approaches and Evaluation of *In-Situ* ocular Drug delivery system

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**Abstract**

Eye is the most complex and precious organ of the body due to its immediate pre-corneal elimination of dosage form. In order to overcome this, researchers developed a new system; *in-situ* gel forming system. This formulation undergoes phase transition in the eye to form gel, thus prolonging the precorneal contact time which will result in improved ocular bioavailability. There are various novel ocular drug delivery systems such as *In-situ* gel, dendrimers, niosomes, nanoparticulate system, collagen shield, ocular iontophoresis suspension and ocusert etc. This system consists of polymer or mixture of polymers which exhibit sol-gel transition due to physicochemical parameters (temperature, ion exchange & pH) of the body.

**Keywords:** *In-situ* gel, Novel ocular delivery, Temperature, pH triggered

**INTRODUCTION**

The physiology, biochemistry and anatomy of the eye make this organ extremely impermeable to foreign substances. A major disadvantage of conventional ocular drug delivery system is that optimal concentration of drug won’t reach in required site of action. In order to treat eye disorders, the choice of administration of drugs is through topical instillation through eye drops [1].

The attempt for the development of *in-situ* gel systems had started from past few years. There are large number of inventions and many patents in the field of *in-situ* gelling system. There are several advantages for *in-situ* gel forming polymeric delivery systems such as ease of administration and reduced frequency of administration, improved patient compliance and comfort, and improved pre-corneal retention time. The one of the most important advantage of *in-situ* gel formulations is drugs which are directly given for local action into the eye for the treatment of allergic conditions. The *in-situ* gel drug delivery system will be suitable for the
delivery of required dose and also to prolong contact time of drug in contact with mucosal layer of eye. These problems generally occurred in the case of liquid dosage and semi-solid formulations. *In-situ* gel formation is characterized by using polymer or mixture of polymers in definite proportion. When the formulation is instilled into eyes the gel may form due to one or more combination of different stimuli like pH change, temperature modulation, solvent exchange and ion exchange mechanism. This formed gel will lead to deliver the drug in proposed manner [2].

The main objective of *in situ* gelling system is to attain the proposed quantity of drug in eye. This is the challenging thing which is overcome by new technology over conventional dosage form.

The various problem associated with poor bioavailability of ocular delivery are;

- Drainage of instilled solution
- Binding by lachrymal proteins
- Tear turn over
- Limited corneal area and poor corneal metabolism
- Tear evaporation
- Non-product absorption

To overcome these limitations, researchers had developed novel dosage form in ocular drug delivery. The main objective of the improvement is to maintain the drug in the eye cavity for a longer period of time. Successful results have been obtained especially in geriatric patients with collagen shields and inserts, in spite of disadvantages like poor patient compliance and losing the device without noticing it [3].

The most acceptable dosage form is one which can be administered in fewer doses in eye drops without creating any vision problems. Recently, researchers are well focused on ocular delivery systems and technologies in which drugs can be administered as an eye drop. Mostly lots of progress has been made in ophthalmic gel preparations in the development of *in-situ* gelling systems, these are the formulation which are in solution from when it instill into the eye it will convert into gel due to the phase transition. Thus, it will improve the pre-corneal residence time of the drug enhancing the ocular bioavailability. As a result, sustained release and enhanced patient compliance is achieved.

These are the six main properties of ophthalmic preparations: Sterility, Preservation, Particle Limitations, pH, Stability, and Eye comfort. The main requirement is sterility, it is very important to make sure that medications applied to the eye should be sterile. If there are any abrasions in the eye, it is very easy for microorganisms to penetrate that area and cause an eye infection. Infection of *Pseudomonas aeruginosa* can lead to rapid onset of blindness (2-3 days) as a result of exposure to contaminated ophthalmics. It is possible to sterilize products using either autoclaving (for heat stable drugs) or membrane filtration with a 0.22 micrometer
filter (for heat-labile drugs). Membrane filtration is very effective because it sterilizes the solution as well as removes particulate matter. Anti-bacterial preservatives are required in multi-dose containers for ophthalmic products. Preservatives help prevent the growth of bacteria and microorganisms, and prevent contamination. Not all drugs have USP particle test requirements, so must check the monograph for the limits specified. pH is another property of ophthalmic products. The tears have a pH of 7.4, so the ideal ophthalmic product should have a pH of 7.4 [3,4].

The eye can tolerate a pH of 3.5 to 10.5 due to the buffer capacity of the tears. The best buffers to use are 1.9% boric acid buffer or a phosphate buffer. A low buffer capacity is chosen to allow tear fluid to adjust the pH to neutrality after application to the eye. Consequently, too high a buffer capacity may overwhelm the tear fluid and prevent acclimation to physiological pH. Many ophthalmic products are alkaloid weak bases because they are more bioavailable at alkaline pH (unionized drug form) but are more soluble at acidic pH (ionized salt form). Ophthalmic dosage forms also require tonicity adjusters for the comfort of the eye.

Tear fluid is isotonic with blood and other tissues, so the drug solutions need to be isotonic to reduce tearing and irritation. Irritation of the eye leads to increased tearing which washes away the drug and leads to less being absorbed. 0.9% sodium chloride and 1.9% boric acid are isotonic with the tears. Typically, the eye can tolerate solutions with tonicity values ranging from equivalents of 0.5% to 1.6% sodium chloride without discomfort. There are even some therapeutic drug concentrations that are hypertonic, but they are applied in such small amounts that the eye can tolerate them. Antioxidants, chelating agents, and surfactants are all used to stabilize ophthalmic products. Lastly adjuvants are added to provide lubrication and protection against drying and cracking. Emollients provide oil to the tears to prevent them from evaporating. This is particularly important in patients with dry eyes. Demulcents have the ability to hold water and keep the membranes of the eyes in a hydrated form. All of these properties of ophthalmic are very important to the sterility, stability, and comfort of the product once applied to the eye [4,5].

**Anatomy of eye**

Anatomical view of human eye
The eye is a slightly asymmetrical globe, about an inch in diameter. The front part of the eye (the part you see in the mirror) includes:

- The iris
- The cornea
- The pupil
- The sclera
- The conjunctiva
Just behind the iris and pupil lies the lens, which helps to focus light on the back of the eye. 80% of the eye is filled with a clear gel called as vitreous. Light passes through the pupil and the lens then it will reaches back of the eye. The inner part of the eye is protected by special light-sensing cells are together known as retina. The retina transforms light into electrical impulses. Behind the eye, the optic nerve conveys these impulses to the brain. The macula is a small extra-sensitive area which is present in retina that gives central vision. Which is located in the center of the retina and contains the fovea, a small depression or pit at the middle of the macula that gives the clear vision.

**Eye Conditions**

- **Age-related macular degeneration**: A loss of central vision.
- **Amblyopia (lazy eye)**: One eye sees better than the other as a result of not using the other eye during childhood. The weaker eye may or may not “wander.” The weaker eye is called the "lazy eye."
- **Astigmatism**: A defect that causes an inability to properly focus light onto the retina. Astigmatism causes blurry vision that can be corrected with glasses, contact lenses, or, in some cases, surgery.
- **Black eye**: Swelling and discoloration (bruise) around the eye as a result of injury to the face.
- **Blepharitis**: Inflammation of the eyelids near the eyelashes. Blepharitis is a common cause of itching or a feeling of grit in the eyes.
- **Cataract**: A clouding of the natural internal lens of the eye, which can cause blurred vision.
- **Chalazion**: An oil-making gland gets blocked and swells into a bump.
- **Conjunctivitis**: Also known as "pinkeye," conjunctivitis is an infection or inflammation of the conjunctiva, the clear layer that covers the front of the eye. It is usually caused by allergies, a virus, or a bacterial infection.
- **Corneal abrasion**: A scratch on the clear part of the front of the eye. Pain, light sensitivity, or a feeling of grit in the eye is the usual symptoms.
- **Diabetic retinopathy**: High blood sugar damages blood vessels in the eye. Eventually, weakened blood vessels may start leaking or overgrow the retina, threatening vision.
- **Diplopia (double vision)**: Seeing double can be caused by many serious conditions. Diplopia requires immediate medical attention.
- **Dry eye**: Either the eyes don’t produce enough tears, or the tears are of poor quality. Dry eye can be caused by medical problems such as lupus, scleroderma, and Sjogren's syndrome.
- **Glaucoma**: Progressive loss of vision usually associated with increased pressure inside the eye. Peripheral vision is lost first, often going undetected for years.
- **Hyperopia (farsightedness)**: Inability to see near objects clearly. The eye is “too short” for the lens, or certain eye muscles have weakened with age.
Hyphema: Bleeding into the front of the eye, between the cornea and the iris. Hyphema is usually caused by trauma.

Keratitis: Inflammation or infection of the cornea. Keratitis typically occurs after germs enter a corneal abrasion.

Myopia (nearsightedness): Inability to see clearly at a distance. The eye is “too long” for the lens, so light isn’t focused properly on the retina.

Optic neuritis: The optic nerve becomes inflamed, usually from an overactive immune system. Painful vision loss in one eye typically results.

Pterygium: A thickened conjunctival mass usually on the inner part of the eyeball. It may cover a part of the cornea, causing vision problems.

Retinal detachment: The retina comes loose from the back of the eye. Trauma and diabetes are common causes of this problem, which often requires urgent surgical repair.

Retinitis: Inflammation or infection of the retina. Retinitis may be a long-term genetic condition or result from an infection.

Scotoma: A blind or dark spot in the visual field.

Strabismus: The eyes do not point in the same direction. The brain may then favor one eye, causing decreased vision (amblyopia) in the other eye.

Stye: Bacteria infect the skin on the edge of the eyelid, creating a tender red bump.

Uveitis (iritis): The colored part of the eye becomes inflamed or infected. An overactive immune system, bacteria, or viruses can be responsible.

Eye Tests

Tonometry: A test that measures pressure in the eye, called intraocular pressure. Tonometry is used to check for glaucoma.

Slit lamp examination: A physician or optometrist shines a vertical slit of light across your eye while examining through a microscope. This general exam can detect many eye problems.

Fundoscopic exam: Dilating drops first widen the pupil. By shining bright light in the back of the eye, the examiner can view the retina.

Refraction: If vision is impaired, a series of lenses are placed before the eyes to determine the right corrective lens prescription.

Visual acuity test: Reading ever-smaller-sized letters across the room identifies distance vision problems. Reading up-close can identify problems with near vision.

Fluorescein angiography: A fluorescent dye is used to take a sequence of retinal images.

Regular adult eye exam: This collection of tests may include the ones mentioned above plus others, such as eye movement.
Eye Treatments

- **Contact lenses and glasses**: Glasses or contact lenses correct refractive errors such as nearsightedness, farsightedness, and astigmatism.
- **LASIK (laser assisted in situ keratomileusis)**: A doctor creates a thin flap in the cornea with a precise cutting device or a laser, following which, an excimer laser reshapes the cornea, improving nearsightedness, excessive farsightedness, and astigmatism.
- **Radial keratotomy (RK)**: A series of small incisions are made in the cornea to correct nearsightedness. Radial keratotomy is rarely used today.
- **Photorefractive keratectomy (PRK)**: A doctor rubs off the surface cells from the cornea, then uses a laser to improve nearsightedness. The corneal cells grow back and the eye heals very much like a corneal abrasion.
- **LASEK (laser epithelial keratomileusis)**: Similar to PRK, in which a flap is cut into the corneal substance. Instead of a surgical flap, though, the topmost layer of cornea cells is retracted or removed after which a laser is used to reshape the cornea.
- **Artificial tears**: Eye drops with similar composition to natural tears, used to treat dry or irritated eyes.
- **Cyclosporine eye drops (Restasis)**: Dry eye is often associated with microscopic inflammation, and anti-inflammatory eye drops (like cyclosporine) can often help.
- **Laser photocoagulation**: A doctor uses a laser to treat parts of the retina with poor circulation or to treat abnormal blood vessels directly. Laser photocoagulation is most often done for diabetic retinopathy but can also be used for sealing retinal tears.
- **Cataract surgery**: The cloudy cataract is removed from the lens and replaced by a manmade lens [5,6].

**TYPES OF CONVENTIONAL DOSAGE FORMS**

**Solutions**

Ophthalmic solutions are sterile, isotonic, which may be aqueous or oily preparation including emulsion & suspension of one or more active ingredients meant for instil into the eye, were the drug will absorbed or adsorbed into the eye to produce intended action. They may contain recipients which regulate osmotic pressure, pH, and viscosity of the preparations, some times which may or may not use preservative also.

**Ointment**

These are semisolid dosage forms which are meant for external use, generally it contains solid or semisolid hydrocarbon base of melting or softening point which resembles to human body temperature. After applying the ointment to the eye, it will converts into small drops, which remains for a long duration of time in conjunctival sac, thus increasing drug’s bioavailability. Eye ointments have few disadvantages such as blurring of vision and sometimes have irritating effects in eye, because of which they are mainly applied night-time, although they are safe and well tolerated.
Gels

Gel formation is an extreme case of viscosity enhancement through the use of viscosity enhancers. Instead of giving multiple doses in case of solutions the dosing interval can be reduced in case of gels. Cellulose acetate phthalate dispersion constituted a microreservoir system of high viscosity. Poloxamer 407 is used as an ophthalmic vehicle for pilocarpine delivery and found that the gel formation enhances the activity of pilocarpine. Timolol maleate form thermo gelling drug delivery system composed of cellulose ether ethylhydroxylethylcellulose. The effect of Flurbiprofen, which is a NSAID, formulated in Pluronic F-127 and carbopol 940. Gelrite is a polysaccharide which is also known as gellan gum. It forms a clear gel in the presence of mono or divalent cations. The high viscosity of the gel, however, results in blurred vision and malted eyelids which substantially decreases patient acceptability. Sterilization is another drawback for large scale production.

As the conventional dosage forms are less bioavailable in case of ocular preparations the newer trend has came ie, Ophthalmic gels. This review attempts to discuss the various approaches, Mechanisms and evaluations of ocular preparations [7].

METHOD OF PREPARATION OF IN-SITU OCULAR GEL

Methods

Cold Method

HEC was dissolved in water was added to Pluronic solution (prepared by dispersing Pluronics over the distilled water with continuous stirring (500rpm) and it is kept in refrigerator (4 degree) until the Pluronics dissolves (Magnetic stirrer) (24Hour)

Mixed both solution @300 rpm on magnetic stirrer, 500mg drug solution was prepared by dissolving in water was prepared and added to above solution.

0.02% Benzalkonium chloride solution was added to above solution as preservative and the pH was adjusted to 7.4 using 0.5M NaOH, which is then sterilized in Autoclave @ 121 degrees for 20 minute [7].

IN-SITU OPHTHALMIC GEL SYSTEM

This is a system were the formulation behave like solution form, which changes the behavior to gel when it is instilled into the eye. Few mechanisms proposed are:

In-situ gel formation based on physical mechanism

Swelling

In-situ formation may also occur when material absorbs water from surrounding environment and expand to occupy desired space. Substance like glycerol which is polar polymerizable rapidly undergo photo-polymerisation in the presence of suitable photoinitiator. Generally, long wavelength ultraviolet and visible wavelengths are used. Short wavelength ultraviolet is
not used often because it has limited penetration of tissue and biologically harmful. A ketone, such as 2,2 dimethoxy-2-phenyl acetoephone, is used as the initiator for ultraviolet photopolymerization, where as camphor quinone and ethyl eosin initiators are used in visible light systems. These systems can be designed for degradation by chemical or enzymatic processes or can be designed for long term persistence in-vivo Photopolymerizable systems. When it is introduced to the desired site via injection it gets photocured in-situ with the help of fiber optic cables and then releases the drug for prolonged period of time. The photo-reactions provide rapid polymerization rates at physiological temperature. Further, the systems are formed in complex shaped volumes leading to an implant formation. A photopolymerizable, biodegradable hydrogel is a tissue contacting material and controlled release carrier.

**In-situ formation based on chemical reactions**

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

**Ionic cross linking**

In Ionic cross linking polymer undergo phase transition in different ions due to this gels are formed. Mostly the polysaccharides are from the ion-sensitive ones, i-carrageenan forms elastic gels mainly in the presence of Ca^{2+} and K-carrageenan forms brittle and rigid gels in presence of small amount of K+. Gellan gum is a polymer which expressed in the name of Gelrite is an anionic polysaccharide which is widely used, that undergoes in-situ gelling in the presence of mono- and divalent cations, including K^+ Ca^{2+}, a Na^+ and Mg^{2+}, Gelation of the low-methoxy pectins can be caused by divalent cations, especially Ca^{2+}. Likewise, alginic acid undergoes gelation in presence of divalent/polyvalent cations e+g. Ca^{2+} due to the interaction with glucoronic acid block in alginate chains.

**Enzymatic cross-linking**

In case of enzymatic cross linking mostly gels are formed by chemical and photochemical and it may also forms by catalytical reactions, even though in-situ formation catalysed by natural enzymes has not been investigated widely but seems to have some advantages over chemical and photochemical approaches. For example, an enzymatic process operates efficiently under physiologic conditions without need for potentially harmful chemicals such as monomers and initiators. Intelligent stimuli-responsive delivery systems using hydrogels that can release insulin have been investigated. Cationic pH-sensitive polymers containing immobilized insulin and glucose oxidize can swell in response to blood glucose level releasing the entrapped insulin in a pulsatile fashion. Adjusting the amount of enzyme also provides a convenient mechanism for controlling the rate of gel formation, which allows the mixtures to be injected before gel formation [8,9].
APPROACHES IN IN-SITU GEL DRUG DELIVERY

Temperature triggered in-situ gel
In temperature triggered method, transmission of sol-to-gel occurs when it comes in contact with tear fluid. The system is designed to use polaxomer and pluronis as polymer for ophthalmic drug delivery using in-situ gel formation. The Bioadhesive gelling properties of these polymers are expected to an excellent drug carrier for prolonged drug delivery to the surface of the eye. Other example, Polaxomer-407, chitosan, tetronics, xyloglucans, HPMC are the polymers with a viscosity that increases when its temperature is raised to the eye temperature.

pH-triggered in-situ gel
In pH triggered system the main polymer used for the preparation is Carbopol 940 which is used as gelling agent in combination with HPMC which is a viscosity enhancer. The formulation with pH triggered in-situ gel is therapeutically stable, non-irritant and efficient and it will show a delaying in release of drug for prolonged period of time than conventional eye drops. Another example is cellulose acetate phthalate which may form gel by phase transition system where there is a change in pH. Other examples - polyethylene glycol, pseudo latexes.

Ion activated system
Gellan gum is the most important polymer which form gel depend on Ion activated system. Gellan gum is an anionic Exo-cellular polysaccharide which is water soluble, and undergoes cation-induced gelation. Which is available as Gelrite. The solution to gel transition process is induced by the presence of mono-valent or divalent ions such as Na+ and Ca+, some other parameters influence the phase transition such as the concentration of polysaccharide, the temperature of the preparation, and the nature and the concentration of cations [10].

EVALUATION OF IN SITU GELLING SYSTEM

Drug-polymer interaction study and thermal analysis
Compatibility study can be performed with Fourier Transform Infra Red (FTIR) spectroscopy. During gelation process the nature of the interacting forces can be evaluated using the technique by employing KBr pellet method. Thermo gravimetric Analysis (TGA) can be conducted for in situ forming polymeric system to analyze the percentage of water in hydrogel. Differential Scanning calorimetry (DSC) conducted to observe if there are any changes in thermo grams as compared with pure active ingredients used for gelation.

Physical evaluation & pH
The appearance of the formulation should be determined visually such as clarity, transparency and pH was measured using pH meter. The pH of ophthalmic formulation should be such that the formulation should be stable at that pH and there should not be any irritation to the patient upon administration.
Gelling capacity
The gelling capacity was measured by visual method. 2ml sample was placed in a vial containing 5ml of freshly prepared artificial tear fluid and it is visually assessed for the gel formation. The time taken for gel formation is noted.

Texture analysis
The cohesiveness, firmness and consistency of in-situ gel are checked by the help of texture profile analyzer which mainly reflects gel strength and ease of administration. High degree of adhesiveness of gels is required to maintain a better contact with mucous membrane of the eye.

Isotonicity evaluation
Isotonicity is the one of the most important test to be conducted in case of ophthalmic preparations, if the preparations are not isotonic to that of eye secretions, it will lead to eye irritation intern it will result in eye damage. Isotonicity test is conducted by adding few drops of blood into 3-4 drops of formulation which is observered under microscope (45X) which is then compared with marketed product.

Antibacterial activity
The microbiological growth of bacteria is measured by concentration of antibiotics and is compared with the product that produced known concentration of standard preparation of Antibiotic. To carryout microbiological assay serial dilution method is employed.

Ocular irritancy test
The Draize irritancy test is the important test to be done for every ophthalmic formulation prior to marketing of the formulation. normally 100μl of the formulation will be instill into the eye which will reach into the cul-de-sac the irritancy is checked by various criteria for a time intervals 1st hr, 24th hr, 48th hrs, 72nd hrs, and 1week after administration. Three rabbits (male) weighing 1.5 to 2kg are used for the study. The sterile formulation is instilled twice a day for a period of 7 days, and a cross over study is carried out (a 3 day washing period with saline was carried out before the cross over study). Rabbits are observed periodically for watering of the eye, swelling, and redness.

Accelerated stability studies
In order to conduct accelerated stability study formulations are filled in glass vials which is then sealed with aluminum foil, the temperature and relative humidity is maintained at 40±2 °C and 75±5% RH respectively as per International Conference on Harmonization (ICH) states Guidelines. After keeping the formulations which are checked every month for gelling capacity, pH, drug content, rheological evaluation, clarity and in-vitro dissolution [10,11].
CONCLUSION
The conventional dosage forms for ocular drug delivery is the challenging task for researchers now a day’s. In order to overcome several disadvantages related to conventional dosage form polymeric in-situ gels are developed, for prolonging the release of drug from the formulation by formation of gels. This provides other advantages over conventional dosage forms such as good stability, biocompatibility and use of biodegradable polymers make the ocular in-situ gelling system more preferable for treatments of ocular diseases.

REFERENCE