Review Article

Ocular in Situ Gels: Development, Evaluation and Advancements

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Abstract: The ocular drug delivery system is considered as crucial and challenging as the delivery of drug is quite difficult. Moreover, the conventional ophthalmic formulations exhibit a short pre-corneal residence time and poor bioavailability. Various attempts have been made towards the development of stable sustained release in-situ gels. Newer research in ophthalmic drug delivery systems is directed towards a incorporation of several drug delivery technologies, that includes to build up systems which is not only extend the contact time of the vehicle at the ocular surface, but which at the same time slow down the removal of the drug. This is a review on ocular in situ gels, delivery systems, evaluation and application.

Keywords: Insitu gel, Novel ocular drug delivery system, pH-triggered in situ system, Ion-activated in situ system, Temperature evident in situ system, Sol to gel.

INTRODUCTION
The ocular drug delivery system is considered as crucial and challenging as human eye is an isolated organ where the delivery of drug is quite difficult. Moreover, the conventional ophthalmic formulations exhibit a short pre-corneal residence time and poor bioavailability due to rapid and extensive elimination of drugs from pre-corneal lachrymal fluid by solution drainage, lachrymation, and non-productive absorption by conjunctiva [1]. In order to surpass the drawbacks associated with the conventional ophthalmic formulations, various attempts have been made towards the development of stable sustained release in-situ gels. Newer research in ophthalmic drug delivery systems is directed towards a incorporation of several drug delivery technologies, that includes to build up systems which is not only extend the contact time of the vehicle at the ocular surface, but which at the same time slow down the removal of the drug. In situ gel system is formulated as liquid preparation suitable to be instilled into eyes which upon exposure to the physiologic environment changes to gel results in in-situ gel, thus increasing the precorneal residence time of the delivery system, and enhances the ocular bioavailability of the drug [2]. The formation of gels depends on factors like change in a specific physico-chemical parameter (pH, temperature, ion-sensitive) by which the drug gets released in a sustained and controlled manner. These systems were evaluated for drug content, clarity, pH, gelling capacity, viscosity, in vitro drug release studies, texture analysis, sterility testing, isotonicity evaluation, accelerated studies and irritancy test. FT-IR spectroscopy was used to know drug and polymer incompatibilities [3]. There are various new dosage forms like insitu gel, collagen shield, minidisc, ocular film, osusert, nanosuspension, nanoparticulate system, liposomes, niosomes, dendirmer, ocular iontophoresis etc.

Development of ophthalmic drug delivery systems has always been challenging because of the drawbacks with ocular route like non-productive absorption, drainage, induced lacrimation, tear turn over, impermeability of drugs to cornea. Topical application of drugs to the eye is the well established route of administration for the treatment of various eye diseases like dryness, conjunctivitis, keratitis, eye flu etc. New approaches have been investigated for delivery of drugs to the eye by making use of polymers that pays a key role in delivery of drugs to the pre and intra ocular tissues [4]. Such persistent attempts have resulted into achieving the increase in bioavailability and extending the duration of therapeutic action of ocular drug.

Smart polymeric systems have proved to be promising means of delivering the drugs. These polymers undergo sol-gel transition after administered. They are in solution phase before administration, but gels under physiological condition. The ocular bioavailability of the drugs can be improved by prolonging their residence time in the cul-de-sac and by increasing their corneal permeability. [5]. There are various physical and chemical stimuli leading to in situ
gel formation viz. temperature, pH, electric field, magnetic field and light. Stimuli responsive polymer mimics biological system in a crude way where an external stimulus (pH and temperature) result in a change in the properties of the formulation. Both natural and synthetic polymers can be used for the production of in situ gels. So, in situ gels are administered by oral, ocular, rectal, vaginal, injectable and intra-peritoneal routes [6, 7].

This review demonstrates a brief summary about in situ gels, various approaches for in situ gelling systems. Also different types of smart polymers, their mechanisms of gel formation from the sol forms and evaluation of polymeric in situ gel

Anatomy and function of the eye [8]

An eye is a spherical structure with a wall made up of three layers: the outer part sclera, the middle parts choroid layer, ciliary body and iris and the inner section nervous tissue layer retina. The sclera is tough fibrous coating that protecting the inner tissues of the eye which is white except for the transparent area at the front, that is cornea allows light to enter to the eye. The choroid layer, situated in the sclera, contains many blood vessels that modified at front of the eye as pigmented iris the colored part of the eye (blue, green, brown, hazel, or grey).

The clear transparent bulge cornea situated at the front of the eye that conveys images to the back of the nervous system. The adult cornea has a radius of approximately 7-8mm that is a vascular tissue to which provides nutrient and oxygen are supplied via lachrymal fluid and aqueous humor as well as from blood vessels of the junction between the cornea and sclera. The cornea is made of five layers as epithelium, bowman’s layer, stroma, descemet’s membrane and endothelium which are main pathways of the drug permeation to eye.

The main barrier of drug absorption into the eye is the corneal epithelium, in comparison to many other epithelial tissues (intestinal, nasal, bronchial, and tracheal) that is relatively impermeable. The epithelium is squamous stratified, (5-6 layer of cells) with thickness of around 50-100 μm. The basal cells are packed with a tight junction forming not only an effective barrier to dust particle and most microorganisms, and also for drug absorption. The transcellular or paracellular pathway is the main pathway to penetrate drug across the corneal epithelium. The lipophilic drugs choose the transcellular route whereas the hydrophilic one chooses paracellular pathway for penetration (passive or altered diffusion through intercellular spaces of the cells).

Ocular absorption of drug

The common method of ocular drug delivery is topical administration of ophthalmic dosage formulation drops into the lower cul-de-sac. Eye drops outflow quickly due to the eye blinking reflux, and the precorneal region returns to maintain resident volume of around 7μl. The available concentration of drug in precorneal fluid provides the driving force for passive transport of drug across the cornea. However, the epithelium is the predominant rate limiting barrier for hydrophilic drugs and where as stroma is rate limiting step for most of the lipophilic drugs.
The molecules up to 20,000 Da can cross the conjunctiva, while the molecules up to 5,000 Da can cross the cornea. The human conjunctiva shows 2-30 times more permeable for drugs than cornea and also loss of drug by this route is a major path for drug clearance. A thin fluid layer is covering the exposed part of the eye called as precorneal tear film. The film thickness is about 3–10 mm depending on the measurement method with the resident volume approximately 10 μl. The osmolality of the tear fluid is approx. 310–350 mOsm/kg in normal eyes and is maintained by the monovalent and divalent inorganic ions present in fluid such as Na+, K+, Cl-, HCO3-, and proteins. The mean pH of normal tears is about 7.4. Diurnal patterns change the pH of tear, which is a general shift from acid to alkaline during the day.

The buffer capacity of the tears fluid is determined by bicarbonate ions, proteins, and mucin [2]. Tears exhibit a non-Newtonian rheological behavior with viscosity is about 3 mPas. The mean surface tension of tear film value is approximately 44 mN/m [9].

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OCULAR SUSTAINED DRUG DELIVERY SYSTEMS:

In the novel drug delivery system various approaches like In situ gelling, use of mucoadhesive polymers, polymer coated Nanoparticles and Liposomal formulations are used. These delivery systems delay the elimination of active ingredient from eye and also improve corneal penetration of drug molecule.

I. Liposomes:

Liposomes are biocompatible and biodegradable lipid vesicles made up of natural lipids and about 25–10 000 nm in diameter. They are having an intimate contact with the corneal and conjunctival surfaces which is desirable for drugs that are poorly absorbed, the drugs with low partition coefficient, poor solubility or those with medium to high molecular weights and thus increases the probability of ocular drug absorption. The corneal epithelium is thinly coated with negatively charged mucin to which the positive charged surface of the liposomes may bind. Roonal Jain et al.; formulated and evaluated soft contact lenses coated with ciprofloxacin entrapped in liposomes. Ciprofloxacin released from the liposomes coated on contact lens inhibited the Staphylococcus aureus and Pseudomonas aeruginosa. Approximately 40% of the Ciprofloxacin was retained up to three months [10].

II. Niosomes:

Niosomes are non-ionic surfactant vesicles that have potential applications in the delivery of hydrophobic or amphiphilic drugs. Niosomes are developed as they are chemically stable as compared to liposomes, non toxic and do not require special handling techniques. Ghada Abdelbary et al; investigated the feasibility of using non-ionic surfactant vesicles (niosomes) as carriers for the ophthalmic controlled delivery of a water soluble local antibiotic; gentamicin sulphate. They showed a substantial change in the release rate and an alteration in the % EE of gentamicin sulphate from niosomal formulations upon varying type of surfactant, cholesterol content [11].

III. Implants:

For chronic ocular diseases like cytomegalovirus (CMV) retinitis, implants are effective drug delivery system. Earlier non biodegradable polymers were used but they needed surgical procedures for insertion and removal. Presently biodegradable polymers such as Poly Lactic Acid (PLA) are safe and effective to deliver drugs in the vitreous cavity and show no toxic signs. Intravitreal implants of Fluocinolone acetonide were developed for the treatment of posterior segment and reported to control the ocular inflammation of retina [12].
IV. Dendrimers:
Dendrimers are large and complex molecules with well defined chemical structure. Dendrimers can successfully used for different routes of drug administration and have reported to have better water-solubility, bioavailability and biocompatibility. The residence time was longer for the solutions containing dendrimers with carboxylic and hydroxyl surface groups. Vandamme et al.; [7] developed and evaluated poly (amidoamine) dendrimers containing fluorescein for controlled ocular drug delivery and determined the influence of size, molecular weight and number of amine, carboxylate and hydroxyl surface groups in several series of dendrimers. The residence time was longer for the solutions containing dendrimers with carboxylic and hydroxyl surface groups [13].

V. Micro emulsion:
Micro emulsion is dispersion of water and oil stabilized using surfactant and co-surfactant to reduce interfacial tension and usually characterized by small droplet size100 nm, higher thermodynamic stability and clear appearance. Selection of aqueous phase, organic phase and surfactant/co-surfactant systems are critical parameters which can affect stability of the system. Vandamme et al.; reported optimization of these components results in significant improvement in solubility of the drug molecule e.g. Indomethacin, Chloramphenicol for eye diseases [14].

VI. Nanosuspensions:
Nanosuspensions have emerged as a promising strategy for the efficient delivery of hydrophobic drugs because they enhanced not only the rate and extent of ophthalmic drug absorption but also the intensity of drug action with significant extended duration of drug effect. For commercial preparation of nanosuspensions, techniques like media milling and high-pressure homogenization have been used. Pingatello et al.; formulated nanosuspension of Fluribuprofen using Eudragit RS 100. The higher drug level in the aqueous humor was reported using Eudragit RS 100 nanosuspensions for the ophthalmic controlled delivery of ibuprofen [15].

IN SITU FORMING GELS:
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.

Advantages of in-situ gels [16]:
- Increased accurate dosing. To overcome the side effects of pulsed dosing produced by conventional systems. 
- To provide sustained and controlled drug delivery.
- To increase the ocular bioavailability of drug by increasing the corneal contact time. This can be achieved by effective adherence to corneal surface.
- To circumvent the protective barriers like drainage, lacrimation and conjunctival absorption.
- To provide comfort, better compliance to the patient and to improve therapeutic performance of drug.
- To provide better housing of delivery system.

Mechanism of Sol-Gel Formulation:
In situ forming hydrogels are liquid preparations upon instillation undergo phase transition in the ocular cul-de-sac to form viscoelastic gel and this provides a response to environmental changes.

In situ gel forming ophthalmic drug delivery systems prepared from polymers that exhibit reversible phase transitions (sol–gel–sol) and pseudo plastic behavior to minimize interference with blinking. Such a system can be formulated as a liquid dosage form suitable to be administered by instillation into the eye which, upon exposure to physiological conditions, changes to the gel phase, thus increasing the pre-corneal residence time of the delivery system.

The vast majority of the In situ forming drug delivery systems reported is based on polymeric materials which forms gel matrices upon administration. Polymers that have been investigated includes [1] polysaccharides like alginate, gellan and xylategcan, [2] polyesters like PLA and PLGA, [3] polyethers like PEG-PPG-PEG (Poloxamers) or [4] mixed polyesters and polyether’s such as PEG-PLGA-PEG.

Advantages over Conventional Eye Preparations:
Eye drops that are conventional ophthalmic delivery systems often result in poor bioavailability and therapeutic response, because of high tear fluid turnover and dynamics cause rapid precorneal elimination of the drug. A high frequency of eye drop instillation is associated with patient non-compliance. Inclusion of excess drug in the formulation is an attempt to overcome bioavailability problem is potentially dangerous if the drug solution drained from the eye is systemically absorbed from the naso lacrimal duct. Various ophthalmic vehicles such as inserts, ointments, Suspensions, and aqueous gels have been developed in order to lengthen the residence time of instilled dose and enhance the ophthalmic bioavailability. These ocular drug delivery systems however have not been used extensively because of some drawbacks such as blurred vision from ointments or low patient compliance from inserts [16].

Enhancing Ocular Bioavailability:
Gel systems are better retained in the eye than conventional eye drops and are better tolerated by
patients than inserts and ointments. Like ointments, gels are also difficult to administer for some patients. In this respect In situ gels are interesting since these are conveniently dropped as a solution into the conjunctival sac, where they undergo a transition into a gel with its favourable residence. The sol-gel-sol transition occurs as a result of chemical and physical change induced by the physiological environment. Liquid-gel phase transition dependent delivery system vary according to the particular polymer employed and their mechanism for triggering the transition to gel phase in the eye take advantage of change in temperature, pH, ion sensitivity or lysozymes upon contact with tear fluid.

In situ forming hydrogels can be classified as follows:

- Liquid-gel phase transition varies according to the particular polymer employed and their mechanism for triggering the transition to gel phase in the eye due to change in temperature, pH, ion sensitivity.

I. Temperature triggered:
Formulations are liquid at room temperature (20-25°C) and undergo gelation when comes in contact with application site (35-37°C), due to an increase in temperature. Temperature-sensitive hydrogels undergo a volume phase-transition or a sol–gel phase-transition at a critical temperature, namely, lower critical solution temperature (LCST) or upper critical solution temperature (UCST). The mechanism involving the sol-to-gel transformation is after increase in temperature there is gradual desolvation of the polymer and increased micellar aggregation (entanglement of the polymeric network). The micelles formation takes place due to the polyoxy propylene block dehydration, at definite point micelles come in contact and no longer move [17].

Examples: Pluronic (Poloxamer), Cellulose derivatives, Polymethacrylates.

II. PH triggered:
Formulations are polymeric dispersion in aqueous system which undergoes spontaneous gelation in response to change in pH after application at the target site. At specific pH there is Electrostatic, hydrophobic interaction and Hydrogen bonding takes place, hence leads to interdiffusion. The observed phase transition for carbopol solution was mediated by the variation of pH from 4.0 to 7.4 and can be attributed to ionization of Carbopol polymer [18].

Example: Cellulose acetate phthalate, Polyacrylic acid (Carbopol), Polycarboxphils

III. Ion activated:
In this type of In situ hydrogels, the sol-to-gel transition is induced by the presence of mono or divalent cations such as Na+, K+, Ca2+, and Mg2+ ions. The electrolytes of the tear fluid and especially Na+, Ca2+ and Mg2+cations are particularly suited to initiate gelation of the polymer when instilled as a liquid solution into the cul-de-sac. There is cross linking of negatively charged polysaccharide and cations [19].

Examples: Gellan gum (Gelrite R), Sodium Alginate.

EVALUATIONS OF INSITU GEL SYSTEM
Evaluation parameters for insitu gel formulations includes clarity, pH measurement, gelling capacity, drug content, rheological study, in vitro diffusion study, isotonicity, antibacterial activity, in vivo ocular testing in rabbits and accelerated stability studies. The formulation should have an optimum viscosity that will allow for easy instillation into the eye as a liquid (drops), which would undergo a rapid sol-to-gel transition (triggered by pH, temperature or ion exchange).

1. Physical parameters
Physical parameters to be tested for insitu gel solution are clarity, pH, gelling capacity, and drug content estimation.

2. Gelling capacity
The gelling capacity of the prepared formulation is determined by placing a drop of the formulation in a vial containing 2.0 ml of freshly prepared simulated tear fluid and visually observe.

3. Rheological studies
The viscosity measurements can be calculated using Brookfield viscometer, Cone and Plate viscometer. In-situ gel formulation is placed in sample tube. Formulation should have viscosity of 5-1000 m Pas, before gelling & after formation of gel should have viscosity from about 50-50,000 m Pas.

4. In vitro drug release studies
In vitro release study of insitu gel solution is carried out by using Franz diffusion cell. The best fit model is check for Krosmeyers Peppas and Fickinian diffusion mechanism for their kinetics [21].

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

6. Isotonicity evaluation
Isotonicity is important characteristic of the ophthalmic preparations. Isotonicity has to be maintained to prevent tissue damage or irritation of eye. All ophthalmic preparations are subjected to isotonicity testing, since they exhibited good release characteristics and gelling capacity and the requisite viscosity.

7. Drug-polymer interaction study and thermal analysis
Interaction study should 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 quantify 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.

8. **Antibacterial activity**
The microbiological growth of bacteria is measured by concentration of antibiotics and this has to be compared with that produced by known concentration of standard preparation of antibiotic.

9. **Ocular irritancy test**
The Draize irritancy test should designed for the ocular irritation potential of the ophthalmic product prior to marketing. According to the Draize test, the amount of substance applied to the eye is normally 100μl placed into the lower culdesac with observation of the various criteria made at a designed required time interval of 1hr, 24hrs, 48 hrs, 72hrs, 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 redness, swelling, watering of the eye [22].

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

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