Review Article

INSITU GEL: A NOVEL SYSTEM FOR OCULAR DRUG DELIVERY

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ABSTRACT

Eye is the most vital organ of the body. To achieve effective ocular therapy, an adequate amount of active ingredients must be delivered and maintain at the site of action within the eye. The anatomical structure and the protective physiological process of the eye exert a formidable defense against ophthalmic drug delivery, leads to poor precorneal drug loss results in poor ocular by availability and ultimately poor ocular therapy. To improve ophthalmic drug bioavailability, there are considerable efforts directed towards newer drug delivery systems for ophthalmic administration. Since Conventional delivery systems often result in poor bioavailability and therapeutic response because of high tear fluids turn over and dynamics cause rapid elimination of the drug from the eyes. Newer research in ophthalmic drug delivery systems is directed towards a amalgamation 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. There are various new dosage forms like *Insitu* gel, collagen shield, minidisc, ocular film, ocusert, nanosuspension, nanoparticulate system, liposomes, niosomes, dendrimers, ocular iontophoresis etc. So, to overcome bioavailability problems, ophthalmic in situ gels were developed. This review deals with the study of a novel in-situ gel approach as a means to localize and prolong drug activity at its site of action. These are solutions, instilled as drops into the eye and undergo a sol to gel transition in the cul-de-sac, improved ocular bioavailability by increasing the duration of contact with corneal tissue, thereby reducing the frequency of administration required in case of conventional ophthalmic solutions, thus optimizing ocular therapy.

Keywords: In situ gels, pH-triggered *Insitu* system, Ion-activated *Insitu* system, Temperature evident *Insitu* system.

INTRODUCTION

Eye is most interesting organ due to its drug disposition characteristics. Generally, topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy[1]. A significant challenge to the formulator is to circumvent (bypass) the protective barriers of the eye without causing permanent tissue damage[2].

Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy. Conventional ophthalmic formulations like solution, suspension, and ointment have many disadvantages (Table 1) which result into poor bioavailability of drug in the ocular cavity.

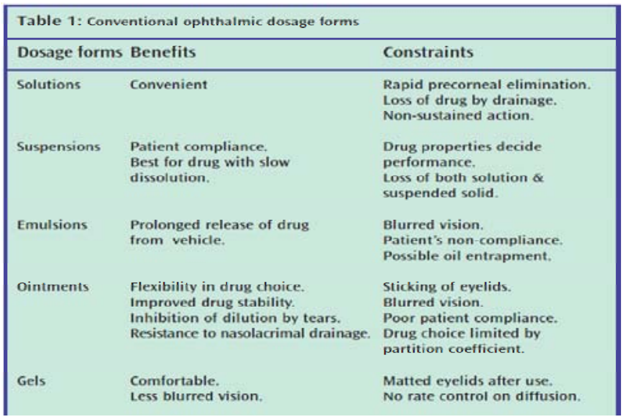

Table 1: Conventional ophthalmic dosage forms

Dosage forms	Benefits	Constraints
Solutions	Convenient	Rapid precorneal elimination. Loss of drug by drainage. Non-sustained action.
Suspensions	Patient compliance. Best for drug with slow dissolution.	Drug properties decide performance. Loss of both solution & suspended solid.
Emulsions	Prolonged release of drug from vehicle.	Blurred vision. Patient's non-compliance. Possible oil entrapment.
Ointments	Flexibility in drug choice. Improved drug stability. Inhibition of dilution by tears. Resistance to nasolacrimal drainage.	Sticking of eyelids. Blurred vision. Poor patient compliance. Drug choice limited by partition coefficient.
Gels	Comfortable. Less blurred vision.	Matted eyelids after use. No rate control on diffusion.

The specific aim of designing a therapeutic system is to achieve an optimal concentration of a drug at the active site for the appropriate duration.[2] Ocular disposition and elimination of a therapeutic agent is dependent upon its physicochemical properties as well as the relevant ocular anatomy and physiology. A successful design of a drug delivery system, therefore, requires an integrated knowledge of the drug molecule and the constraints offered by the ocular route of administration.[3]

The various approaches that have been attempted to increase the bioavailability and the duration of the therapeutic action of ocular drugs can be divided into two categories. The first one is based on the use of sustained drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs. The second involves maximizing corneal drug absorption and minimizing precorneal drug loss.[3]

Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolong period of time. Consequently it is imperative to optimize ophthalmic drug delivery; one of the ways to do so is by addition of polymers of various grades, development of in situ gel or colloidal suspension or using erodible or non erodible insert to prolong the precorneal drug retention.[4]

Physiological barriers to diffusion and productive absorption of topically applied drug exist in the precorneal and corneal spaces. The precorneal constrains responsible for poor ocular bioavailability of conventional ophthalmic dosage forms are solution drainage, lacrimation, tear dilution, tear turnover and conjuctival absorption (Fig. 1). Drug solution drainage away from the precorneal area has been shown to be the most significant factor in reducing the contact time of the drug with the cornea and consequently ocular bioavailability of topical dosage forms. The instilled dose leaves the precorneal area within 2 minutes of instillation in humans. In rabbits the process of drainage, generally takes 5-10 minutes. However, most of the drugs are rapidly lost through nasolacrimal drainage immediately following dosing. Both the conjuctival and nasal mucosa has been indicated as the main potential sites for systematic absorption of topically applied drugs. Tears dilute the drug remaining in the cul-de-sac, which reduces the transcorneal flux of the drug. The drug entity, pH, tonicity of the dosage forms as well as formulation adjuvants can stimulate tear production.

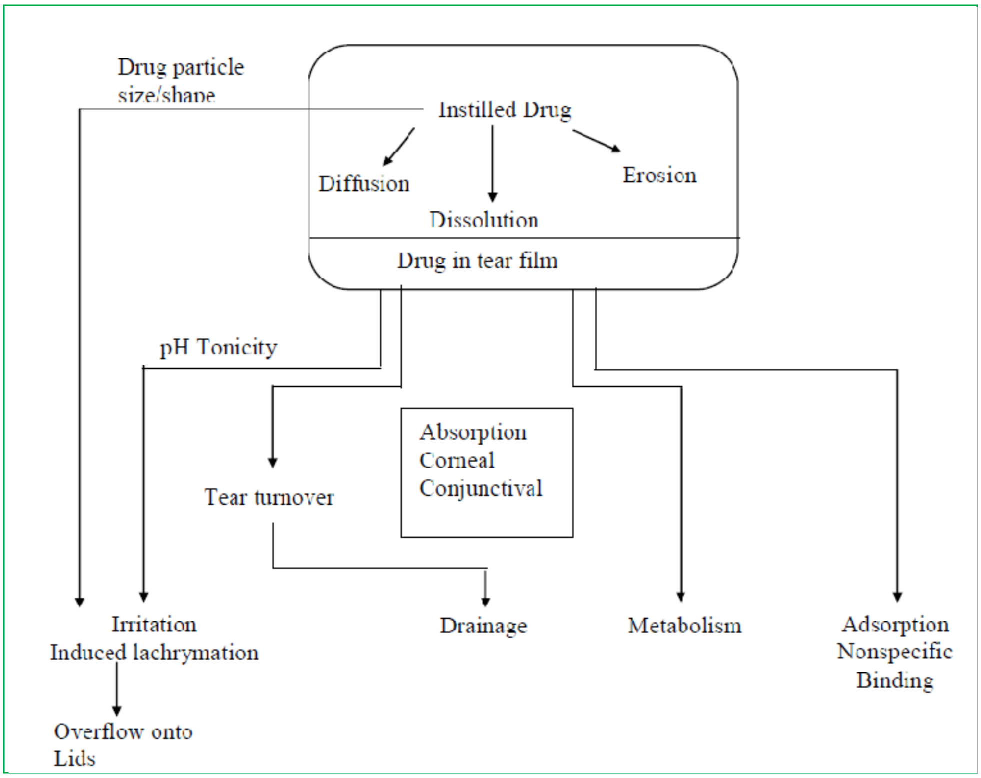

Figure 1: Absorption mechanism of conventional eye drops

Topical application of ophthalmic drugs is further made inefficient by tear turnover, which is about 16% in human. Due to these factors typically less than 1% of the drug reaches the aqueous humor. Metabolism in the precorneal area has been shown to account for further loss of the drug. The low fraction of the applied dose further undergoes rapid elimination from the intraocular tissues and loss through the canal of schlemn (or) via absorption through the ciliary body (or) suprachoroid into episcleral space. Binding of drug to protein also contributes to the loss of drugs through the precorneal parallel elimination loss pathway.

The physiological barriers to topical corneal absorption force the clinician to recommend frequent doses of drug at extremely high concentrations. This pulsed type of dosing is represented with many side effects. Frequent local instillations of antiglaucoma agents, antibiotics, antivirals and sulfonamides provide an unusually high drug and preservative concentrations at the epithelial surface resulting in ocular cytopathologies[5].

The conventional ocular delivery systems are used ubiquitously in today's ocular disease management are solutions, suspensions, these are sterile, contain a preservatives, is isotonic, has a pH of 7.4 for patient comfort and has limited shelf life after opening the container. Eye drops provide a pulse entry of the drug, followed by a rapid decline in drug concentration, the kinetics, of which approximately to the first order. To overcome these problems, it is the consensus of most clinicians that a solution or suspension form of a drug delivery system is preferred by the patient provided that extended duration can be accomplished with this forms.[5]

The first approach made towards research in the field of improving the ocular contact time of solutions utilizes the incorporation of polymers into an aqueous medium such as polyvinyl alcohol (PVA), polyvinyl pyrolidine (PVP), methylcellulose (MC), carboxy methyl cellulose (CMC), and hydroxy propyl cellulose (HPC). The increased solution viscosity reduced the solution drainage. Increasing the solution viscosity of pilocarpine solution from 1 to 100 cps through the incorporation of methylcellulose reduced the solution drainage rate constant 10 times while only a 2-fold increase in pilocarpine concentration in the aqueous humor was obtained. An optimal viscosity of 12-15 cps has been suggested for ocular drug absorption by Paton and Robinson.

Natural polymers namely sodium haluronate and chondroition sulfate are being investigated as viscosity inducing agents. Prolonged residence time with an extended duration of action for 1% pilocarpine has been observed with 0.2-0.3% sodium hyaluronate solutions. In considering approach of increasing solution viscosity to enhance ocular drug absorption the lipophilicity of the drug should be taken into account. The results to date suggest that increasing solution viscosity has limited utility in causing marked improvement in the amount of drug absorbed.[5]

INSITU GELLING SYSTEMS[6]

This novel drug delivery system promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with mucosa, that problems generally encountered in semisolid dosage forms.

In situ-gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. Smart polymeric systems represent promising means of delivering the drugs; these polymers undergo sol-gel transition, once administered. From the early 1970's natural and synthetic polymers began to be investigated for controlled release formulations. The advantages of using biodegradable polymers in clinical applications are apparent. Various natural and synthetic polymers are used for formulation development of *in situ* forming drug delivery systems.

Approaches of In Situ Gel Drug Delivery[6]:

There are four broadly defined mechanisms used for triggering the *in situ* gel formation of biomaterials:

- Physiological stimuli (e.g., temperature and pH),
- Physical changes in biomaterials (e.g., solvent exchange and swelling),
- Chemical reactions (e.g., enzymatic, chemical and photo-initiated polymerization).

1. *In situ* formation based on physiological stimuli

Thermally trigged system

Temperature-sensitive hydrogels are probably the most commonly studied class of environment-sensitive polymer systems in drug delivery research. The use of a biomaterial whose transitions from sol-gel is triggered by increase in temperature is an attractive way to approach in-situ formation. The ideal critical temperature range for such system is ambient and physiologic temperature, such that clinical manipulation is facilitated and no external source of heat other than that of body is required for trigger gelation. A useful system should be tolerable to account for small differences in local temperature, such as might be encountered in appendages at the surface of skin or in the oral cavity.

Three main strategies are exists in engineering of thermoresponsive sol-gel polymeric system. For convenience, temperature-sensitive hydrogels are classified into negatively thermosensitive, positively thermosensitive, and thermally reversible gels (1, 3). Negative temperature-sensitive hydrogels have a lower critical solution temperature (LCST) and contract upon heating above the LCST. Polymers with low critical temperature (LCST) transition between ambient and physiologic temperature is used for this purpose. One of the most extensively investigated polymers that exhibit useful LCST transition is poly(N-isopropyl acrylamide) (PNIPAAm). PNIPAAm is a water soluble polymer at its low LCST, but hydrophobic above LCST, which result on precipitation of PNIPAAm from the solution at the LCST. Pluronics are poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPOPEO) triblock co-polymer that are fluid at low temperature, but forms thermo responsible gel when heated as a consequences of a disorder-order transition in micelle packing which makes these polymers suitable for *in situ* gelation. A positive temperature sensitive hydrogel has an upper critical solution temperature (UCST), such hydrogel contracts upon cooling below the UCST. Polymer networks of poly(acrylic acid) (PAA) and polyacrylamide (PAAm) or poly(acrylamide-co-butyl methacrylate) have positive temperature dependence of swelling. The most commonly used thermoreversible gels are these prepared from poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (Pluronics®, Tetronics®, poloxamer). Polymer solution is a free flowing liquid at ambient temperature and gels at body temperature. Novel “protein polymers” called as ProLastins, which undergo an irreversible sol gel transition, when injected as a solution into the body, the material forms a firm, stable gel within minutes. It remains at the site of injection providing absorption times from less than one week to many months. Such a system would be easy to administer into desired body cavity.

pH triggered systems

Another formation of *in situ* gel based on physiologic stimuli is formation of gel is induced by pH changes. All the pH-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH. The polymers with a large number of ionizable groups are known as polyelectrolytes. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups. The most of anionic pH-sensitive polymers are based on PAA (Carbopol®, carbomer) or its derivatives. Likewise poly vinyl acetal diethyl amino acetate (AEA) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition. Drug formulated in liquid solutions have several limitations including limited bioavailability and propensity to be easily removed by tear fluid. To minimize this factors and maximize this drug delivery by making a poly(acrylic acid) (PAA) solution that would be gel at pH 7.4, by that we found that at concentrations high enough to cause gelation, however, the low pH of PAA solution would cause damage to surface of eye before being neutralized by the lacrimal fluid. This problem was solved by partially by combining PAA with HPMC, a viscous enhancing polymer, which resulted in pH responsive polymer mixtures that was sol at pH 4 and gel at pH 7.4. Mixtures of poly(methacrylic acid) (PMA) and poly(ethylene glycol) (PEG) also has been used as a pH sensitive system to achieve gelation.

2. *In situ formation based on physical mechanism*

Swelling

In situ formation may also occur when material absorbs water from surrounding environment and expand to occur desired space. One such substance is myverol 18-99 (glycerol mono-oleate), which is polar 1400 lipid that swells in water to form lyotropic liquid crystalline phase structures. It has some Bioadhesive properties and can be degraded *in vivo* by enzymatic action.

Diffusion

This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. N-methyl-pyrrolidone (NMP) has been shown to be useful solvent for such system.

3. *In situ formation based on chemical reactions*

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

Ionic cross linking

Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones .While k-carrageenan forms rigid, brittle gels in reply of small amount of K+, i-carrageenan forms elastic gels mainly in the presence of Ca2+. Gellan gum commercially available as Gelrite® is an anionic polysaccharide that undergoes *in situ* gelling in the presence of mono- and divalent cations, including Ca2+, Mg2+, K+ and Na+. Gelation of the low-methoxy pectins can be caused by divalent cations, especially Ca2+. Likewise, alginic acid undergoes gelation in presence of divalent/polyvalent cations e. g. Ca2+ due to the interaction with glucuronic acid block in alginate chains.

Enzymatic cross-linking

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 oxidase 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.

Photo-polymerisation

Photo-polymerisation is commonly used for *in situ* formation of biomaterials. A solution of monomers or reactive macromer and initiator can be injected into a tissues site and the application of electromagnetic radiation used to form gel. Acrylate or similar polymerizable functional groups are typically used as the polymerizable groups on the individual monomers and macromers because they rapidly undergo photo-polymerisation in the presence of suitable photoinitiator. Typically 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 acetophenone, is often used as the initiator for ultraviolet photo- polymerization, where as camphorquinone and ethyl eosin initiators are often used in visible light systems. These systems can be designed readily to be degraded by chemical or enzymatic processes or can be designed for long term persistence in vivo. Photo polymerizable systems when introduced to the desired site via injection get photocured *insitu* gel with the help of fiber optic cables and then release the drug for prolonged period of time. The photo-reactions provide rapid polymerization rates at physiological temperature. Furthermore, the systems are easily placed in complex shaped volumes leading to an implant formation.

CLASSIFICATIONS OF IN SITU POLYMERIC SYSTEMS[6]

Pectin

Pectins are a family of polysaccharides, in which the polymer backbone mainly comprises α-(1-4)-D-galacturonic acid residues. Low methoxypectins (degree of esterification <50%) readily form gels in aqueous solution in the presence of free calcium ions, which crosslink the galacturonic acid chains in a manner described by egg-box model . Although the gelation of pectin will occur in the presence of H + ions, a source of divalent ions, generally calcium ions is required to produce the gels that are suitable as vehicles for drug delivery. The main advantage of using pectin for these formulations is that it is water soluble, so organic solvents are not necessary in the formulation. Divalent cations present in the stomach, carry out the transition of pectin to gel state when it is administered orally. Calcium ions in the complexed form may be included in the formulation for the induction of pectin gelation. Sodium citrate may be added to the pectin solution to form a complex with most of calcium ions added in the formulation. By this means, the formulation may be maintained in a fluid state (sol), until the breakdown of the complex in the acidic environment of the stomach, where release of calcium ions causes gelation to occur. The quantities of calcium and citrate ions may be optimized to maintain the fluidity of the formulation before administration and resulting in gelation, when the formulation is administered in stomach. The potential of an orally administered *in situ* gelling pectin formulation for the sustained delivery of Paracetamol has been reported.

Xyloglucan

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.

Gellan gum

Gellan gum (commercially available as Gelrite TM or Kelcogel TM) is an anionic deacetylated exocellular polysaccharide secreted by Pseudomonas elodea with a tetrasaccharide repeating unit of one α-L-rhamnose, one β-D-glucuronic acid and two β-D-glucuronic acid residues. It has the tendency of gelation which is temperature dependent or cations induced. This gelation involves the formation of double helical junction zones followed by aggregation of the double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water. The formulation consisted of gellan solution with calcium chloride and sodium citrate complex. When administered orally, the calcium ions are released in acidic environment of stomach leading to gelation of gellan thus forming a gel *in situ*. *In situ* gelling gellan formulation as vehicle for oral delivery of theophylline is reported.

Alginic acid

Alginic acid is a linear block copolymer polysaccharide consisting of β-D-mannuronic acid and α-L-glucuronic acid residues joined by 1,4-glycosidic linkages . The proportion of each block and the arrangement of blocks along the molecule vary depending on the algal source. Dilute aqueous solutions of alginates form firm gels on addition of di- and tri- valent metal ions by a cooperative process involving consecutive glucuronic residues in the α-L-glucuronic acid blocks of the alginate chain. Alginic acid can be chosen as a vehicle for ophthalmic formulations, since it exhibits favourable biological properties such as biodegradability and nontoxicity. A prolonged precorneal residence of formulations containing alginic acid was looked for, not only based on its ability to gel in the eye, but also because of its mucoadhesive properties.

Xanthum gum

Xanthan gum is a high molecular weight extra cellular polysaccharide produced by the fermentation of the gram-negative bacterium Xanthomonas campestris. The primary structure of this naturally produced cellulose derivative contains a cellulosic backbone (β- D-glucose residues) and a trisaccharide side chain of β-D-mannose-β-D-glucuronicacid-α-D-mannose attached with alternate glucose residues of the main chain. The anionic character of this polymer is due to the presence of both glucuronicacid and pyruvic acid groups in the side chain.

Chitosan

Chitosan is a biodegradable, thermosensitive, polycationic polymer obtained by alkaline deacetylation of chitin, a natural component of shrimp and crab shell. Chitosan is a biocompatible pH dependent cationic polymer, which remains dissolved in aqueous solutions up to a pH of 6.2. Neutralization of chitosan aqueous solution to a pH exceeding 6.2 leads to the formation of a hydrated gel like precipitate. The pH gelling cationic polysaccharides solution are transformed into thermally sensitive pH dependent gel forming aqueous solutions, without any chemical modification or cross linking by addition of polyol salts bearing a single anionic head such as glycerol, sorbitol, fructose or glucose phosphate salts to chitosan aqueous solution.

Carbopol

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. 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 methyl cellulose system, poly(methacrylic acid)-poly(ethylene glycol) come under the category of pH-induced in-situ precipitating polymeric systems. Based on this concept, the formulation and evaluation of an ophthalmic delivery system for indomethacin for the treatment of uveitis was carried out. A sustained release of indomethacin was observed for a period of 8 h *in vitro* thus considering this system as an excellent candidate for ocular delivery.

Pluronic F-127

Poloxamers or pluronic (marketed by BASF Corporation) are the series of commercially available difunctional triblock copolymers of non-ionic nature. They comprise of a central block of relatively hydrophobic polypropylene oxide surrounded on both sides by the blocks of relatively hydrophilic poly ethylene oxide. Due to the PEO/PPO ration of 2:1, when these molecules are immersed into the aqueous solvents, they form micellar structures above critical micellar concentration. They are regarded as PEO-PPO-PEO copolymers. Chemically they are Oxirane, methyl-, polymer with oxirane or α-Hydro-ω-hydroxypoly(oxyethylene)$_a$ poly(oxypropylene)$_b$ poly(oxyethylene)$_a$ block copolymer. The pluronic triblock copolymers are available in various grades differing in molecular weights and physical forms. Depending upon the physical designation for the grades are assigned, as F for flakes, P for paste, L for liquid. Pluronics or Poloxamers also undergo *in situ* gelation by temperature change. They are triblock copolymers consisting of poly(oxyethylene) and poly(oxypropylene) units that undergo changes in solubility with change in environment temperature. Pluronic™ F127. A 25-40% aqueous solution of this material will gel at about body temperature, and drug release from such a gel occurs over a period of up to one week. Pluronic F-127 was used as an *in situ* gel forming polymer together with mucoadhesive polymers such as Carbopol 934 and hydroxy propyl methyl cellulose to ensure long residence time at the application site. Controlled release of drug was achieved in-vitro indicating antimycotic efficacy of developed formulation for a longer period of time.

Synthetic polymers

Synthetic polymers are popular choice mainly for parenteral preparations. The trend in drug delivery technology has been towards biodegradable polymers, requiring no follow up surgical removal, once the drug supply is depleted. Aliphatic polyesters such as poly(lactic acid), poly(glycolic acid), poly(lactide-coglycolide), poly(decalactone), poly-ε-caprolactone have been the subject of the most extensive recent investigations.

Various other polymers like triblock polymer systems composed of poly(D,L-lactide)-block poly(ethylene glycol), block poly(D,L-lactide), blends of low molecular weight poly(D,L-lactide) and poly(ε-caprolactone) are also in use. These polymers are mainly used for the injectable *in situ* formulations. The feasibility of lactide/glycolide polymers as excipients for the controlled release of bioactive agents is well proven. These materials have been subjected to extensive animal and human trials without evidence of any harmful side effects. When properly prepared under GMP conditions from purified monomers, the polymers exhibit no evidence of inflammatory response or other adverse effects upon implantation. Another type of synthetic polymeric system includes the *in situ* cross linked system, where the polymers form cross linked networks by means of free radical reactions that may occur by means of light (photopolymerizable systems) or heat (thermosetting systems). Thermosetting systems are in the sol form when initially constituted, but upon heating, they set into their final shape. This sol-gel transition is known as curing. But if this cured polymer is heated further, it may lead to degradation of the polymer. Curing mainly involves the formation of covalent cross links between polymer chains to form a macromolecular network. Dunn et al. designed a thermosetting system using biodegradable copolymers of DL-lactide or L-lactide with ε-caprolactone for prosthetic implant and slow release drug delivery systems. This system is liquid outside the body and is capable of being injected by a syringe and needle and once inside the body, it gels. In *insitu* precipitating polymeric systems, the polymer precipitation from solution may lead to gel formation *in situ* and this precipitation can be induced by change in temperature (thermosensitive systems), solvent removal or by change in pH. An important example of thermosensitive polymer is poly-(N-isopropyl acrylamide), [poly (NIPAAM)], which is used for the formation of *in situ* gels. It has lower critical solution temperature phase separation at about 32. The polymers such as poly(DL-lactide), poly(DL-lactideco-glycolide) and poly(DL-lactide-co-Ξ-caprolactone) form solvent-removal precipitating polymeric systems.

EVALUATION AND CHARACTERIZATIONS OF IN SITU GEL SYSTEM[6-7]

In situ gels may be evaluated and characterized for the following parameters;

Clarity

The clarity of formulated solutions determined by visual inspection under black and white background.

Texture analysis

The firmness, consistency and cohesiveness of formulation are assessed using texture analyzer which mainly indicates the syringeability of sol so the formulation can be easily administered in-vivo. Higher values of adhesiveness of gels are needed to maintain an intimate contact with surfaces like tissues.

Sol-Gel transition temperature and gelling time

For in situ gel forming systems incorporating thermoreversible polymers, the sol-gel transition temperature may be defined as that temperature at which the phase transition of sol meniscus is first noted when kept in a sample tube at a specific temperature and then heated at a specified rate. Gel formation is indicated by a lack of movement of meniscus on tilting the tube. Gelling time is the time for first detection of gelation as defined above.

Gel-Strength

This parameter can be evaluated using a rheometer. Depending on the mechanism of the gelling of gelling agent used, a specified amount of gel is prepared in a beaker, from the sol form. This gel containing beaker is raised at a certain rate, so pushing a probe slowly through the gel. The changes in the load on the probe can be measured as a function of depth of immersion of the probe below the gel surface.

Viscosity and rheology

This is an important parameter for the *in situ* gels, to be evaluated. The viscosity and rheological properties of the polymeric formulations, either in solution or in gel made with artificial tissue fluid (depending upon the route of administrations) instead of 5% mannitol, were determined with Brookfield rheometer or some other type of viscometers such as Ostwald's viscometer. The viscosity of these formulations should be such that no difficulties are envisaged during their administration by the patient, especially during parenteral and ocular administration.

In-vitro drug release studies

For the *in situ* gel formulations to be administered by oral, ocular or rectal routes, the drug release studies are carried out by using the plastic dialysis cell. The cell is made up of two half cells, donor compartment and a receptor compartment. Both half cells are separated with the help of cellulose membrane. The sol form of the formulation is placed in the donor compartment. The assembled cell is then shaken horizontally in an incubator. The total volume of the receptor solution can be removed at intervals and replaced with the fresh media. This receptor solution is analyzed for the drug release using analytical technique. For injectable *in situ* gels, the formulation is placed into vials containing receptor media and placed on a shaker water bath at required temperature and oscillations rate. Samples are withdrawn periodically and analyzed.

Histopathological studies

Two mucosa tissue pieces (3 cm^2) were mounted on in vitro diffusion cells. One mucosa was used as control (0.6 mL water) and the other was processed with 0.6 mL of optimized organogel (conditions similar to in vitro diffusion). The mucosa tissues were fixed in 10% neutral carbonate formalin (24 hours), and the vertical sections were dehydrated using graded solutions of ethanol. The subdivided tissues were stained with haematoxylin and eosin. The sections under microscope were photographed at original magnification ×100. The microscopic observations indicate that the organogel has no significant effect on the microscopic structure of the mucosa. The surface epithelium lining and the granular cellular structure of the nasal mucosa were totally intact. No major changes in the ultra structure of mucosa morphology could be seen and the epithelial cells appeared mostly unchanged.

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. Formulations are mixed with few drops of blood and observed under microscope at 45X magnification and compared with standard marketed ophthalmic formulation[7].

Drug polymer interaction study and thermal analysis

Interaction 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 quantitate the percentage of water in hydrogel. Differential Scanning calorimetry (DSC) conducted to observe if there are any changes in thermograms as compared with pure active ingredients used for gelation[7].

Antibacterial activity

The microbiological growth of bacteria is measured byconcentration of antibiotics and this has to be compared with that produced by known concentration of standard preparation of antibiotic. To carryout microbiological assay serial dilution method is employed[7].

Ocular irritancy test

The Draize irritancy test was 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[7].

Accelerated stability studies

Formulations are placed in ambient colour vials and sealed with aluminium foil for a short term accelerated stability study at 40±2°C and 75±5% RH as per International Conference on Harmonization (ICH) states Guidelines. Samples are analyzed every month for clarity, pH, gelling capacity, drug content, rheological evaluation, and *in vitro* dissolution[7].

Statistical analysis

The results obtained from the experiments of mucoadhesive strength and release studies were analysed statistically using multivariate tests. A statistically significant difference was conducted by using various SPSS software and difference was considered to be significant at $P<0.05$.[7]

RECENT ADVANCES[6]

One of the challenges facing today's pharmaceutical industry centers on coming up with efficient treatment options that are readily acceptable to physicians and patients. Delivery systems must also contribute to a better therapeutic outcome if they are going to provide viable alternatives to pharmaceuticals currently delivered by other routes. *In situ* gel formulations are one of the challenging drug delivery systems. Various biodegradable polymers are used for formulation of *in situ* gels, but there are fabrication problems, difficult process ability, and use of organic solvents for their preparation (especially for synthetic polymer based systems), burst effect and irreproducible drug release kinetics. Natural polymers satisfy the characteristics of an ideal polymer but batch to batch reproducibility is difficult therefore synthetic polymers are used. The recent advancement of biotechnologies has led to the development of labile macromolecular therapeutic agents that require complex formulations for their efficient administration *N*-stearoyl L-alanine(m)ethyl esters when mixed with a vegetable oil and a biocompatible hydrophilic solvent led to the formation of injectable, *in situ* forming organogel. Following subcutaneous injection, Leuprolide-loaded organogel degraded and gradually released Leuprolide for 14 to 25d.

Commercial formulations of in-situ polymeric systems at a glance[6]

Timoptic-XE

It is a timolol maleate ophthalmic gel formulation of Merck and Co. Inc., supplied as a sterile, isotonic, buffered, aqueous gel forming solution of timolol maleate. This formulation is available in two dosage strengths 0.25% and 0.5% in market. The pH of the solution is approximately 7.0, and the osmolarity is 260-330 mOsm. Each ml of Timoptic-XE 0.25% contains 2.5 mg of timolol (3.4 mg of timolol maleate). Inactive ingredients include gellan gum, tromethamine, mannitol, and water for injection and the preservative used is benzododecinium bromide 0.012%. Timoptic- XE, when applied topically on the eye, reduces the elevated, as well as normal intraocular pressure, whether or not accompanied by glaucoma[8].

Regel: depot-technology

Regel is one of the Macromed's proprietary drug delivery system and based on triblock copolymer, composed of poly (lactide-co-glycolide)-poly (ethylene glycol)-poly(lactide-co-glycolide). It is a family of thermally reversible gelling polymers developed for parenteral delivery that offers a range of gelation temperature, degradation rates and release characteristics as a function of molecular weight, degree of hydrophobicity and polymer concentration. Following injection, the physical properties of polymer undergo a reversible phase change resulting in formation of a water insoluble, biodegradable gel depot. Oncogel is a frozen formulation of paclitaxel in Regel. It is a free flowing liquid below room temperature which upon injection forms a gel in-situ in response to body temperature. hGHD-1 is a novel injectable depot formulation of human growth hormone (hGH) utilizing Macromed's Regel drug delivery system for treatment of patients with hGHdeficiency[9].

Cytoryn

This is one of the Macromed's products, which is a novel, peritumoral, injectable depot formulation of interleukin-2 (IL-2) for cancer immunotherapy using Regel drug delivery system. It is a free flowing liquid below room temperature that instantly forms a gel depot upon injection from which the drug is released in a controlled manner. Cytoryn enhances the immunological response by safely delivering four times the maximum tolerated dose allowed by conventional IL-2 therapy. Cytoryn also activates the systemic antitumor immunity. Regel system stabilizes and releases IL-2 in its bioactive form. The release of drugs is controlled by the rate of diffusion from and degradation of the depot[10].

CONCLUSION

The primary requirement of a successful controlled release product focuses on increasing patient compliance which the *in situ* gels offer. Exploitation of polymeric in-situ gels for controlled release of various drugs provides a 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 forms very reliable. These are easy to instill at the same time improves ocular bioavailability by increasing the duration of contact with corneal tissue, thereby reducing the frequency of administration required in case of conventional ophthalmic solutions, thus optimizing ocular therapy. Use of biodegradable and water soluble polymers for the *in situ* gel formulations can make them more acceptable and excellent drug delivery systems.

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