In-Situ gel: New trends in Controlled and Sustained Drug Delivery System

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Abstract: Recently, controlled and sustained drug delivery has become the standard in modern Pharmaceutical design and an intensive research have been undertaken in achieving much better drug product effectiveness, reliability and safety. This interest has been sparked by the advantages shown by in situ forming polymeric delivery systems such as ease of administration and reduced frequency of administration, improved patient compliance and comfort. The formation of gels depends on factors like temperature modulation, pH change, presence of ions and ultraviolet irradiation, from which the drug gets released in a sustained and controlled manner. Various biodegradable polymers that are used for the formulation of in situ gels include gellan gum, alginic acid, xyloglucan, pectin, chitosan, poly(DL-lactic acid), poly(DL-lactide-co-glycolide) and poly-caprolactone. Mainly in situ gels are administered by oral, ocular, rectal, vaginal, injectable and intraperitoneal routes. The in situ gel forming polymeric formulations offer several advantages like sustained and prolonged action in comparison to conventional drug delivery systems. From a manufacturing point of view, the production of such devices is less complex and thus lowers the investment and manufacturing cost.

Keywords: Biodegradable polymers, controlled release, in situ gels, poly (lactic-co-glycolic acid), sustained release.

INTRODUCTION

Over the past 30 years greater attention has been focused on development of controlled and sustained drug delivery systems. Amongst the extensive research has been carried in designing of polymeric drug delivery systems. The development of in situ gel systems has received considerable attention over the past few years. In the past few years, increasing number of in situ gel forming systems have been investigated and many patents for their use in various biomedical applications including drug delivery have been reported. 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. In situ gel formulations offers an interesting alternative for achieving systemic drug effects of parenteral routes, which can be inconvenient or oral route, which can result in unacceptably low bioavailability and passes the hepatic first-pass metabolism, in particular of proteins and peptides. 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 semi-solid 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. This review attempts to discuss the newer developments and strategies for this drug delivery including physiological factors, physiochemical factors and formulation factors to be considered in the development of in-situ drug delivery system.
different types of smart polymers, their mechanisms of
gel formation from the sol forms, evaluation and
characterization of in situ polymeric formulations are
discussed.

APPROACHES OF IN SITU GEL DRUG
DELIVERY
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).

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
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 tailorable 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
thermoreponsive 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 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-isopropylacrylamide) (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-PPO-PEO) 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. Cappello et al. developed novel “protein
polymers” 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
to 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 (caticonic) groups. The
most of anionic pH-sensitive polymers are based on
PAA (Carbopol®, carbomer) or its derivatives. Likewise polyvinylacetal diethylaminoacetate (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. Kumar and Himmelstein sought 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. The author found that at concentrations high
eough 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 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.

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
lipid that swells in water to form lyotropic liquid crystalline phase structures. It has some Bioadhesive properties and can be degraded invivo by enzymatic action15.

**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 system16.

**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 crosslinking**
Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones17. 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 guluronic acid block in alginate chains18.

**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 be injected before gel formation19.

**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, whereas 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 vivo20. Photopolymerizable systems when introduced to the desired site via injection get photocured in situ 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. A photopolymerizable, biodegradable hydrogel as a tissue contacting material and controlled release carrier is reported by Sawhney et al21.

**CLASSIFICATIONS OF IN SITU POLYMERIC SYSTEMS**

**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 delivery22. 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 gelation23.

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-galactopyranose. 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.

**Gellangum**

Gellan gum (commercially available as Gelrite™ or Kelcogel™) 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 divalent 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 favorable 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 polyelectrolytes or polylysine. Chitosan aqueous solution is used as a vehicle for ophthalmic delivery. Various water soluble polymers such as carbopol system- hydroxypropylmethylcellulose 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. A pH induced in-situ precipitating polymeric system (an aqueous solution of carbopol) was used to formulate poly (methacrylic acid) copolymers for the delivery of indomethacin. The pH-induced in-situ precipitation of poly (methacrylic acid) copolymers was used to formulate poly (methacrylic acid) copolymers for the delivery of indomethacin. The pH-induced in-situ precipitation of poly (methacrylic acid) copolymers was used to formulate poly (methacrylic acid) copolymers for the delivery of indomethacin. The pH-induced in-situ precipitation of poly (methacrylic acid) copolymers was used to formulate poly (methacrylic acid) copolymers for the delivery of indomethacin.
carbopol-HPMC system) was designed and developed by Ismail et al. for plasmid DNA delivery\(^3\).  

**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 polyethylene oxide\(^3\).  

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 \(\alpha\)-Hydro-o-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.  

Pluronic or Poloxamers also undergo \textit{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 \(^3\) F 127. 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.\(^4\)  

Pluronic F-127 was used as an \textit{in situ} gel forming polymer together with mucoadhesive polymers such as Carbopol 934 and hydroxypropylmethylcellulose 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.\(^5\)  

**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-co-glycolide), poly (decalactone), poly \(\varepsilon\)-caprolactone have been the subject of the most extensive recent investigations\(^6\).  

Various other polymers like triblock polymer systems composed of poly(D,L-lactide)-block-poly(ethylene glycol)-block-poly(DL-lactide), blends of low molecular weight poly(D,L-lactide) and poly(\(\varepsilon\)-caprolactone) are also in use. These polymers are mainly used for the injectable \textit{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.\(^6\)  

Another type of synthetic polymeric system includes the \textit{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 \(\varepsilon\)-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 \textit{in situ} precipitating polymeric systems, the polymer precipitation from solution may lead to gel formation \textit{in situ} and this precipitation can be induced by change in temperature (thermosensitive systems), solvent removal or by change in pH.\(^7\) An important example of thermosensitive polymer is poly-(N-isopropyl acrylamide), [poly (NIPAAM)], which is used for the formation of \textit{in situ} gels. It has lower critical solution temperature phase separation at about 32. The polymers such as poly(DL-lactide), poly(DL-lactide-co-glycolide) and poly(DL-lactide-co-\(\varepsilon\)-caprolactone) form solvent-removal precipitating polymeric systems.\(^8\)  

**ENHANCEMENT OF MUCOSAL ABSORPTION**  
Unlike the most small drug molecules, some drugs and peptides do not cross the mucosal membrane efficiently. As a result the systemic bioavailability in simple solution formulation is very low. The low
mucosal absorption can be attributed to poor membrane permeability due to molecular size, lack of lipophilicity or enzymatic degradation. To overcome these problems of poor membrane permeability most frequent used approach is the use of absorption enhancers. It is possible to greatly improve the mucosal absorption of polar drugs by administrating in combination with an absorption enhancer that promote transport of drug across the mucosal membranes (in case of oral or nasal or ocular or rectal or vaginal tissue). They act by one or combination of the following mechanisms:

1. Alteration of properties of mucosa layer,
2. Opening tight junctions between epithelial cells,
3. Reversed micelle formation between membranes,
4. Increasing the membrane fluidity by

Various types of penetration enhancers have been evaluated for organic drugs including surfactants, bile salts, chelators, fatty acid salts, phospholipids, glycyrrhetinic acid derivatives, cyclodextrins and glycols. Polyoxyethylene-9-lauryl ether (BL-9) in saline solution improves the nasal absorption of hydralazine in both in-situ and in vivo nasal absorption studies in rats. The nasal absorption of gentamicin (60 mg/ml in saline solution) in humans has observed to increase by incorporation of 1% sodium glycocholate and peak serum levels were achieved in 30-60. Most peptides and proteins show insufficient nasal bioavailability. Number of approaches has been described to improve their systemic bioavailability. Insulin is poorly absorbed from nasal mucosa. Many compounds of different chemical structure have been investigated to promote transnasal insulin absorption. The STDHF enhanced the effects of absorption enhancers on intranasal insulin delivery in rats, rabbits and sheep. Among medium chain fatty acids, sodium caprylate (1%) exhibit the strongest promoting effect. The fatty acids show higher hemolytic activity than glycocholate. The compound carbenoxolone, glycerrhetenic acid salt has structures similar to triterpenes and show promoting effect similar to bile acids and saponins.

**FORMULATION DESIGN**

The design of in-situ gel formulation depends on the physicochemical properties of the drug molecule, the diseased condition for which treatment is required, the patient population and the marketing preference. Physico-chemical factors include molecular weight, lipophilicity and molecular charge; an anatomical and physiological factor includes membrane transport, p<sub>41</sub> of tissue fluid, and mucocilliary clearance (as in case of nasal administrations). While formulation factors include clarity, p<sub>41</sub>, gelation temperature, viscosity, osmolarity, spreadability.

**APPLICABILITY OF IN SITU POLYMERIC DRUG DELIVERY SYSTEM**

Depending on the route of administration, these in situ polymeric systems may be classified as illustrated in following section

**Oral-delivery**

Pectin, xyloglucan and gellan gum are the natural polymers used for in situ forming oral drug delivery systems. The potential of an orally administered in situ gelling pectin formulation for the sustained delivery of paracetamol has been reported. The main advantage of using pectin for these formulations is that it is water soluble, so organic solvents are not necessary in the formulation. In situ gelling gellan formulation as vehicle for oral delivery of theophylline is reported. 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. An increased bioavailability with sustained drug release profile of theophylline in rats and rabbits was observed from gellan formulations as compared to the commercial sustained release liquid dosage form.

**Ocular- Delivery**

For in situ gels based ocular delivery, natural polymers such as gellan gum, alginic acid and xyloglucan are most commonly used polymers. Local ophthalmic drug delivery has been used for various compounds such as antimicrobial agents, antiinflammatory agents and autonomic drugs used to relieve intraocular tension in glaucoma. Conventional delivery systems often result in poor bioavailability and therapeutic response because high tear fluids turn over and dynamics cause rapid elimination of the drug from the eye. So, to overcome bioavailability problems, ophthalmic in situ gels were developed. Much of the interest in the pharmaceutical application of gellan gum has concentrated on its application for ophthalmic drug delivery. Drug release from these in situ gels is prolonged due to lower precorneal contact times of the viscous gels compared with conventional eye drops. Miyazaki et al. attempted to formulate in situ gels for ocular delivery using Xyloglucan (1.5% w/w) as the natural polymer. These in situ forming polymeric systems were observed to show a significant mitotic response for a period of 4h when instilled into lower cul-de-sac of rabbit eye. 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 with the water-soluble Carbopol system has been reported.
Nasal -Drug Delivery Systems

An in-situ gel system for nasal delivery of mometasone furoate was developed and evaluated for its efficacy for the treatment of allergic rhinitis41. Gellan gum and xanthan gum were used as in situ gel forming polymers. Animal studies were conducted using an allergic rhinitis model and the effect of in situ gel on antigen induced nasal symptoms in sensitized rats was observed. In-situ gel was found to inhibit the increase in nasal symptoms as compared to marketed formulation nasonex (mometasone furoate suspension 0.05%). Intact ciliated respiratory epithelium and normal goblet cell appearance indicated from histopathology of rat nasal cavity proved that these formulations were safe for nasal administration. Wu et al. designed a new thermosensitive hydrogel by simply mixing N-[2-hydroxy-3-methyltrimethylammonium]propyl]chitosan chloride and poly (ethylene glycol) with a small amount of α-β-glycerophosphate; for nasal delivery of insulin. The formulation was in solution form at room temperature that transformed to a gel form when kept at 37°C. Animal experiments demonstrated hydrogel formulation to decrease the blood-glucose concentration by 40-50% of the initial values for 4-5 h after administration with no apparent cytotoxicity. Therefore, these types of systems are suitable for protein and peptide drug delivery through nasal route52.

Rectal and Vaginal -Delivery

In situ gels also possess a potential application for drug delivery by rectal and vaginal route. Miyazaki et al. investigated the use of xyloglucan based thermoreversible gels for rectal drug delivery of indomethacin. Administration of indomethacin loaded xyloglucan based systems to rabbits indicated broad drug absorption peak and a longer drug residence time as compared to that resulting after the administration of commercial suppository. For a better therapeutic efficacy and patient compliance, mucoadhesive, thermosensitive, prolonged release vaginal gel incorporating clotrimazole-β-cyclodextrin complex was formulated for the treatment of vaginitis53.

EVALUATION AND CHARACTERIZATIONS OF IN SITU GEL SYSTEM

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 tissues54.

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 above26.

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 surface26.

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.\(^5\)

**Fourier transform infra-red spectroscopy and thermal analysis**

During gelation process, the nature of interacting forces can be evaluated using this technique by employing potassium bromide pellet method. Thermogravimetric analysis can be conducted for in situ forming polymeric systems to quantitate the percentage of water in hydrogel. Differential scanning calorimetry is used to observe if there are any changes in thermograms as compared with the pure ingredients used thus indicating the interactions.\(^5\)

**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.\(^24\) 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.\(^25\) 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.\(^6\)

**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 hematoxylin and eosin. The sections under microscope were photographed at original magnification \(\times 100\). 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 ultrastructure of mucosa morphology could be seen and the epithelial cells appeared mostly unchanged.\(^5\)

**RECENT ADVANCES**

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 processability, 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. The formulation is made up of vegetable oil and a biocompatible hydrophilic solvent used to lead to the formation of injectable, in situ forming organogel. Following subcutaneous injection, leuprolide-loaded organogel degraded and gradually released leuprolide for 14 to 25d.\(^5\)

**COMMERCIAL FORMULATIONS OF IN-SITU POLYMERIC SYSTEMS AT A GLANCE**

**Timoptic-XE**

It is a timolol maleate opthalmic 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.\(^5\)

**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 Regmel. 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 Regmel drug delivery system for treatment of patients with hGH-deficiency.

Cytorny
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 Regmel 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. Cytorny enhances the immunological response by safely delivering four times the maximum tolerated dose allowed by conventional IL-2 therapy. Cytorny also activates the systemic antitumor immunity. Regmel 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.

CONCLUSION
In 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. Use of biodegradable and water soluble polymers for the in situ gel formulations can make them more acceptable and excellent drug delivery systems.

REFERENCES
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