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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 ultra violet irradiation, from which the drug gets released in a sustained and controlled manner. Various biodegradable polymers that are used for the formulation of in situ gels include 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[1]. *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[2]. 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[3]. 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[4]. 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. Also,

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 p^H), 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[5]. 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 faccilated 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 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 alower 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[6]. 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[7]. 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[8]. 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 into desired body cavity[9].

p^H triggered systems -

Another formation of *in situ* gel based on physiologic stimuli is formation of gel is induced by p^H changes[5]. 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[7]. The most of anionic pH-sensitive polymers are based on PAA (Carbopol®, carbomer) or its derivatives[10]. Likewise polyvinylacetal diethylaminoacetate (AEA) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition[11]. 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 p^H 7.4.The author found that at concentrations high enough to cause gelation, however, the low p^H 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 p^H responsive polymer mixtures that was sol at p^H 4 and gel at p^H 7.4[12]. Mixtures of poly(methacrylic acid) (PMA) and poly (ethylene glycol) (PEG) also has been used as a p^H sensitive system to achieve gelation[13].

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[14]. 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 action[15].

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[16].

***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 ones[17] .While k-carrageenan forms rigid, brittle gels in reply of small amount of K^+, i-carrageenan forms elastic gels mainly in the presence of Ca^{2+}. Gellan gum commercially available as Gelrite® is an anionic polysaccharide that undergoes *in situ* gelling in the presence of mono- and divalent cations, including Ca^{2+}, Mg^{2+}, 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. Ca^{2+} due to the interaction with guluronic acid block in alginate chains[18].

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 formation[19].

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[5].Acrylate or similar polymerizable functional groups are typically used as the polymerizable groups on the individual monomeers amd 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[20]. 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 al[21].

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 delivery[22]. 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[23].

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[4].

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[26]. 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[27]. Xyloglucan gels have potentially been used for oral, intraperitoneal, ocular and rectal drug delivery[28,29].

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 [30]. 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 [31]. 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*[32]. *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 trivalent metal ions by a cooperative process involving consecutive glucuronic residues in the α-L-glucuronic acid blocks of the alginate chain [33]. 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 [34].

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[35].

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[36]. 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[37].

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- 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-HPMC system) was designed and developed by Ismail et al. for plasmid DNA delivery[38].

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[39].

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 ™ 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[40].

Pluronic F-127 was used as an *in situ* gel forming polymer together with mucoadhesive polymers such as Carbopol 934 and hydroxylpropylmethylcellulose 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[41].

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 ε-caprolactone have been the subject of the most extensive recent investigations.

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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(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[43].

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 (photopolymerizablesystems) 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 *in situ* 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[44]. 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-lactide-co-glycolide) and poly(DL-lactide-co-Ξ-caprolactone) form solvent-removal precipitating polymeric systems[45].

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[46],

Various types of penetration enhancers have been evaluated for organic drugs including surfactants, bile salts, chelators, fatty acid salts, phospholipids, glycyrrhetenic 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 gentamycin (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[47].

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^H of tissue fluid, and mucocilliary clearance (as in case of nasal administrations). While formulation factors include clarity, p^H, gelation temperature, viscosity, osmolarity, spreadability[3].

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[4]. 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[30].

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 deliver[48]. Drug release from these *in situ* gels is prolonged due to longer 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[49]. 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[50].

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 rhinitis[51]. 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^{o}. 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 route[52].

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 vaginitis[25]. In addition, a significant reduction of drug C_{max} was observed after administration of *in situ* polymeric system thus indicating the avoidance of adverse effects of indomethacin on nervous system[26].

Injectable-Drug Delivery Systems

The development of injectable in-situ forming drug delivery systems has received a considerable interest over the last decade. A novel, injectable, thermosensitive *in situ* gelling hydrogel was developed for tumor treatment. This hydrogel consisted of drug loaded chitosan solution neutralized with β-glycerophosphate. Local delivery of paclitaxel from the formulation injected intratumorally was investigated using EMT-6 tumors implanted subcutaneously on albino mice. Ito et al. designed and synthesized injectable hydrogels that are formed *in situ* by cross-linking of hydrazide modified hyaluronic acid with aldehyde modified versions of cellulose derivatives such as carboxymethylcellulose, hydroxypropylmethylcellulose and methylcellulose.These *in situ* forming gels were used for preventing postoperative peritoneal adhesions thus avoiding pelvic pain, bowel obstructions and infertility 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 vaginitis[53].

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 tissues[54].

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[26].

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[26].

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[55].

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. Thermo-gravimetric 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[55].

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[28]. 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[56].

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 ×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[57].

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 *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[58].

COMMERCIAL FORMULATIONS OF IN-SITU POLYMERIC SYSTEMS AT A GLANCE

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[59].

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 hGH-deficiency[60].

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[61].

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

1) Peppas N, Langer R. New challenges in biomaterials. Science1994; 263:171520.
2) Zhidong L ,Jaiwei L, Shufang N, Hui L, Pingtian D, Weisan P.Study of an Pharm alginate-HPMC based *in situ* gelling ophthalmic delivery system for gatifloxacin. Int J. 2006; 315:12-7.
3) Sarasija S, Shyamala B. Nasal Drug Delivery: An Overview, Indian J Pharm.Sci. 2005, 67(1): 19-25.
4) Wataru K, Yasuhiro K, Miyazaki S, Attwood D. *In situ* gelling pectin formulations for oral sustained delivery of paracetamol. Drug Develop Ind Pharm 2004;30:593-9.
5) Marsha Ritter Jones, MS, Philip B. Massersmith, In-situ forming biomaterials,Oral Maxillofacial Surg Clin N Am 14 (2002):29-38
6) Peppas NA, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm 2000;
7) Qiu Y, Park K. Environment-sensitive hydrogels for drug delivery. Adv Drug Deliv Rev 2001;53:321-39.
8) Bromberg LE, Ron ES. Temperature-responsive gels and thermogelling polymer matrices for protein and peptide delivery. Adv Drug Deliv Rev 1998;31:197-221.
9) Cappello J, Crissman JW, Crissman M, Ferrari FA, Textor G, Wallis O, et al. In-situ self-assembling protein polymer gel systems for administration, delivery, and release of drugs. J Control Release 1998;53:105-17.
10) Soppimath KS, Aminabhavi TM, Dave AM, Kumbar SG, Rudzinski WE. Stimulus-responsive "smart" hydrogels as novel drug delivery systems. Drug Dev Ind Pharm 2002;28: 957-74.
11) Aikawa K, Mitsutake A, Uda H, Tanaka S, Shimamura H, Aramaki Y, et al. Drug release from pH-response polyvinylacetal diethyl aminoacetate hydrogel, and application to nasal delivery. Int J Pharm 1998;168:181-8.
12) Kumar S, Himmelstein K. Modification of in-situ gel behaviour of Carbopol solutions by hydroxypropylmethylcellulose.J.Pharm.Sci.1995;84:344-8.
13) Alexandridis P,Lindman B,Amphiphilicblock polymers. Amsterdam:Elsvier;2000
14) Esposito E, Carratto V & et al.Comparative analysis of tetracycline containing dental gels; poloxomers and mono-glycerides based formulation . Int.J.Pharm.1996;142:9-23.
15) Geraghaty P,Attwood D, et al.An investigation of parameters influencing the Bioadhesive properties of Myverol 18-99/ water gels.Biomaterials 1997, 18:63-7.
16) Motto F, Gailloud P, et al., In-vitro assessment of new embolic liquids prepared from preformed polymers and water miscible solvents aneurysm treatment. Biomaterials 2000,21:803-11
17) Bhardwaj TR, Kanwar M, Lal R, Gupta A. Natural gums and modified natural gums as sustained-release carriers. Drug Devel Ind Pharm 2000;26:1025-38.
18) Guo J-H, Skinner GW, Harcum WW, Barnum PE. Pharmaceutical applications of naturally occurring

water-soluble polymers. Pharm Sci & Technol Today 1998;1:254-61.

19) Podual K, Doyle III FJ, Peppas NA. Dynamic behavior of glucose oxidase-containing microparticles of poly(ethylene)- grafted cationic hydrogels in an environment of changing pH. Biomaterials 2000;21:1439-50.

20) Burkoth AK, Anseth KS. A review of photocrosslinked polyanhydrides: *In situ* forming degradable networks. Biomaterials 2000;21:2395-404.

21) Sawhney AS, Pathak CP, Hubbell JA, Hill JL, Desai NP. Photopolymerizable biodegradable hydrogels as tissue contacting materials and controlled release carriers.US Patent 5410016. 1995.

22) Dumitriu S, Vidal PF, Chornet E. Hydrogels based on polysaccharides. In: Dumitriu.S, editor. Polysaccharides in medical applications. New York: Marcel Dekker Inc; 1996. p. 125-242.

23) Ni Y, Kenneth MY. In-situ gel formation of pectin. 2004. United States Patent 6777000.

24) Miyazaki S, Kawasaki N. Comparison of *in situ* gelling formulations for the oral delivery of cimetidine. Int J Pharm 2001;220:161-8.

25) Bilensoy E, Rouf MA, Imran V, Murat S, Hincal AA. Mucoadhesive thermosensitive prolonged release vaginal gel for clotrimazole: β-cyclodextrin complex. AAPS Pharm Sci Tech 2006;7:38.

26) Miyazaki S, Suisha F, Kawasaki N. Thermally reversible xyloglucan gels as vehicles for rectal drug delivery. J Control Rel 1998;56:75-83.

27) Kawasaki N, Ohkura R, Miyazaki S, Uno Y, Sugimoto S, Attwood D. Thermally reversible xyloglucan gels as vehicles for oral drug delivery. Int J Pharm 1999;181:227-34.

28) Suisha F, Kawasaki N, Miyazaki S, Shirakawa M, Yamotoya K, Sasaki M, *et al*. Xyloglucan gels as sustained release vehicles for intraperitoneal administration of mitomycin C. Int J Pharm 1998;172:27-32.

29) Miyazaki S, Suzuki S, Kawasaki N, Endo K, Takahashi A, Attwood D. *In situ* gelling xyloglucan formulations for sustained release ocular delivery of pilocarpine hydrochloride. Int J Pharm 2001;229:29-36.

30) Miyazaki S, Hirotatsu A, Kawasaki N, Wataru K, Attwood D. *In situ* gelling gellan formulations as vehicles for oral drug delivery. J Control Rel 1999;60:287-95.

31) Crescenzi V, Dentini M, Coviello T. Solutions and gelling properties of microbial polysaccharides of industrial interest: The case of gellan. In: Dawes EA, editor. Novel biodegradable microbial polymers. Dordrecht: Kluwer Academic Publishers; 199:227-84.

32) Grasdalen H, Smidsroed O. Gelation of gellan gum. Carbohydrate Polymers 1987;7:371-93.

33) Sechoy O, Tissie G, Sebastian C, Maurin F, Driot JY, Trinquand C. A new long acting ophthalmic formulation of carteolol containing Alginic acid. Int J Pharm 2000;207:109-16.

34) Smart JD, Kellaway IW, Worthington HE. An *in vivo* investigation of mucosa adhesive materials for use in controlled drug delivery. J Pharm Pharmacol 1984;36:259-99.

35) Al-Shamklani A, Bhakoo M, Tuboku MA, Duncan R. Evaluation of the biological properties of alginates and gellan and xanthan gum. Proc Int Symp Control Release Bioact Mater 1991;18:213-4.

36) Hatefi A, Amsden B. Biodegradable injectable *in situ* forming drug delivery systems. J Control Release 2002;80:9-28.

37) Chenite A, Chaput C, Wang D, Combes C, Buschmann MD, Hoemann CD *et al*. Novel injectable solution of chitosan form biodegradable gels *in situ*. Biomaterials 2000;21:2155-61.

38) Ismail FA, Napaporn J, Hughes JA, Brazean GA. *In situ* gel formulation for gene delivery: release and myotoxicity studies. Pharm Dev Technol 2000;5:391-7.

39) Schmolka IR: Artificial skin, Preparation and properties of pluronic F127 gels for the treatment of burns. J. Biomed. Mater. Res. 6:571-582, 1972.

40) Kabanov A, Batraoka E, Alakhov V: Pluronic block copolymers as novel polymer therapeutics for oral and gene delivery. J. Control. Rel. 82:189-212, 2002.

41) Alexandridis P, Hatton TA. Poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) block copolymer surfactants in aqueous solutions and interfaces: thermodynamics, structure, dynamics and modeling. Colloid Surfaces 1995; A96:146.

42) Liu F, Wilson BC. Hyperthermia and photodynamic therapy. In: Tannock I, Hill RP, editors. Basic science of oncology. New York: McGraw-Hill; 1998. p. 443-53.

43) Dunn RL, English JP, Cowsar DR, Vanderbelt DD. Biodegradable *in situ* forming implants and methods for producing the same. US Patent 5340849 1994.

44) Siegel RA, Firestone BA. pH dependent equilibrium swelling properties of hydrophobic

poly electrolyte copolymer gels. Macromolecules 1988;21:3254-9.
45) Eliassaf J. Aqueous solution of poly-(N-isopropyl acrylamide). J App Polymer Sci 1978; 22:873-4.
46) Illum L., Fisher A.N, Jabbal-Gill.I, Davis S.S, Bioadhesive starch microspheres and absorption enhancing agents act synergistically to enhance the nasal absorption of polypeptides; Int. J. Pharm., 222 (2001) ,109-119.
47) Chein Y.w., Novel drug delivery systems, Marcel Dekker Inc.50 (2), 1982, 229- 260.
48) Mikkelson TJ. Ophthalmic drug delivery. Pharm Tech 1984;8:90-8.
49) Miyazaki S, Suzuki S, Kawasaki N, Endo K, Takahashi A, Attwood D. *In situ* gelling xyloglucan formulations for sustained release ocular delivery of pilocarpine hydrochloride. Int J Pharm 2001;229:29-36.
50) Schoenwald RD. Ocular drug delivery: Pharmacokinetic considerations. Clin Pharma cokinet 1990;18:255-69.
51) Cao S, Ren X, Zhang Q, Chen E, Xu F, Chen J, *et al*. *In situ* gel based on gellan gum as new carrier for nasal administration of mometasone furoate. Int J Pharm 2009;365:109-15.
52) Wu J, Wei W, Wang LY, Su ZG, Ma G. A thermosensitive hydrogel based on quaternized chitosan and poly (ethylene glycol) for nasal delivery system. Biomaterials 2007;28:2220-32.
53) Ito T, Yeo Y, Highley CB, Bellas E, Benitez CA, Kohane DS. The prevention of peritoneal adhesions by *in situ* cross-linking hydrogels of hyaluronic acid and cellulose derivatives. Biomaterials 2007;28:975-83.
54) Kashyap N, Viswanad B, Sharma G, Bhardwaj V, Ramarao P, Kumar MNV. Design and evaluation of biodegradable, biosensitive *in situ* gelling systems for pulsatile delivery of insulin. Biomaterials 2007; 28:2051-60.
55) Kashyap N, Viswanad B, Sharma G, Bhardwaj V, Ramarao P, Kumar MNV. Design and evaluation of biodegradable, biosensitive *in situ* gelling systems for pulsatile delivery of insulin. Biomaterials 2007;28:2051-60.
56) Chandrashekhar G, Udupa N. Biodegradable injectable implant system for long term drug delivery using poly (lactic-co-glycolic) acid copolymers. J Pharm Pharmacol 1998; 48:669-74.
57) Sambhaji Pisal,1 Vijay Shelke, et al., Effect of Organogel Components on In Vitro Nasal Delivery of Propranolol Hydrochloride, AAPS PharmSciTech 2004; 5 :4,63
58) Plourde F, Motulsky A, Couffin-Hoarau AC, Hoarau D, Ong H, Leroux JC. First report on the efficacy of l-alanine-based *in situ*-forming implants for the long-term parenteral delivery of drugs. J Control Release 2005;108:433-41.
59) Sterile ophthalmic gel forming solution, Timoptic-XE; , 0.25% and 0.5%, (Timolol maleate ophthalmic gel forming solution), Merck and Company Inc. NJ 08889: Whitehouse Station, USA.
60) Ramesh CR, Zentner GM, Jeong B. Macro med, Inc. Biodegradable low molecular weight triblock poly (lactide-co- glycolide) polyethylene glycol copolymers having reverse thermal gelation properties. US patent 6201072. 2001.
61) Rathi R, Zentner C, Gaylen M, Jeong B. Macro med, Inc. Biodegradable low molecular weight triblock poly (lactide-co-glycolide) polyethylene glycol copolymers having reverse thermal gelation properties. US patent 6117949. 2000.

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