ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
Review Article
July 2017 Vol.:9, Issue:4

A Novel Approach In-Situ Gel for Sustained Drug Delivery: A Review

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
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Submission: 7 July 2017
Accepted: 12 July 2017
Published: 25 July 2017

Keywords: Sustained release, biodegradable polymer, mucosal absorption, thermal triggered, pH triggered system

ABSTRACT

Sustained drug delivery has become the standard in modern Pharmaceutical design and an intensive research has 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 polycaprolactone. 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. Present review emphases mechanism of *in-situ* gel formation, polymers used for *in-situ* gel, methods of preparation and applicability in different drug delivery system.

INTRODUCTION

Over the past 30 years, greater attention has been focused on development of controlled and sustained drug delivery systems. The goal in designing these systems is to reduce the frequency of dosing or to increase effectiveness of the drug by localization at the site of the action, decreasing the dose required or providing uniform drug delivery. Polymers have historically been the keys to the great majority in drug delivery systems.

Gel

Gels are an intermediate state of matter containing both solid and liquid components. The solid component comprises a three dimensional network of inter connected molecule or aggregates which immobilizes the liquid continuous phase. Gels may also be classified based on the nature of the bonds involved in the three-dimensional solid network. Chemical gels arise when strong covalent bonds hold the network together and physical gels when hydrogen bonds and electrostatic and van der Waals interaction maintain the gel network.[1]

Hydrogels

Hydrogels are polymeric networks that can absorb and retain large amounts of water and biological fluids and swell, still maintaining their three-dimensional structure. These polymeric networks contain hydrophilic domains that are hydrated in an aqueous environment, thereby creating the hydrogel structure. The term *network* indicates the presence of cross-links, which helps avoid the dissolution of the hydrophilic polymer in an aqueous medium.

Hydrogels have many advantages over other drug delivery systems such as good mechanical and optical properties and biocompatibility. The degradation products of hydrogels are usually non-toxic or have lower toxicity. Lower interfacial tension between the surface of the hydrogel and the physiological fluid helps to minimize protein adsorption and cell adhesion on the hydrogel's surface. The soft rubbery nature of hydrogels also can minimize mechanical irritation when used as *in-vivo* implants Hydrocolloids can be defined as polymers endowed with the ability to swell in water or aqueous solvent and induce a liquid to gel transition. Gels are at the upper limit of viscous preparations, and they are formed when high molecular weight polymers or high polymer concentrations are incorporated in the formulations. In addition, the ability of hydrogels to release an entrapped drug in an aqueous medium and to

regulate the release of such drug by control of swelling and by cross linking makes them particularly suitable for controlled release applications. Hydrogels can be applied for the release of both hydrophilic and hydrophobic drugs and charged solutes.

Currently, two groups of hydro gels are distinguished, namely preformed and in situ forming gels. Preformed hydro gels can be defined as simple viscous solutions, which do not undergo any modifications after administration. *In-situ* gels can be defined as formulations, applied as solutions, sols or suspensions that undergo gelation after instillation due to physicochemical changes inherent to the stomach.[2]

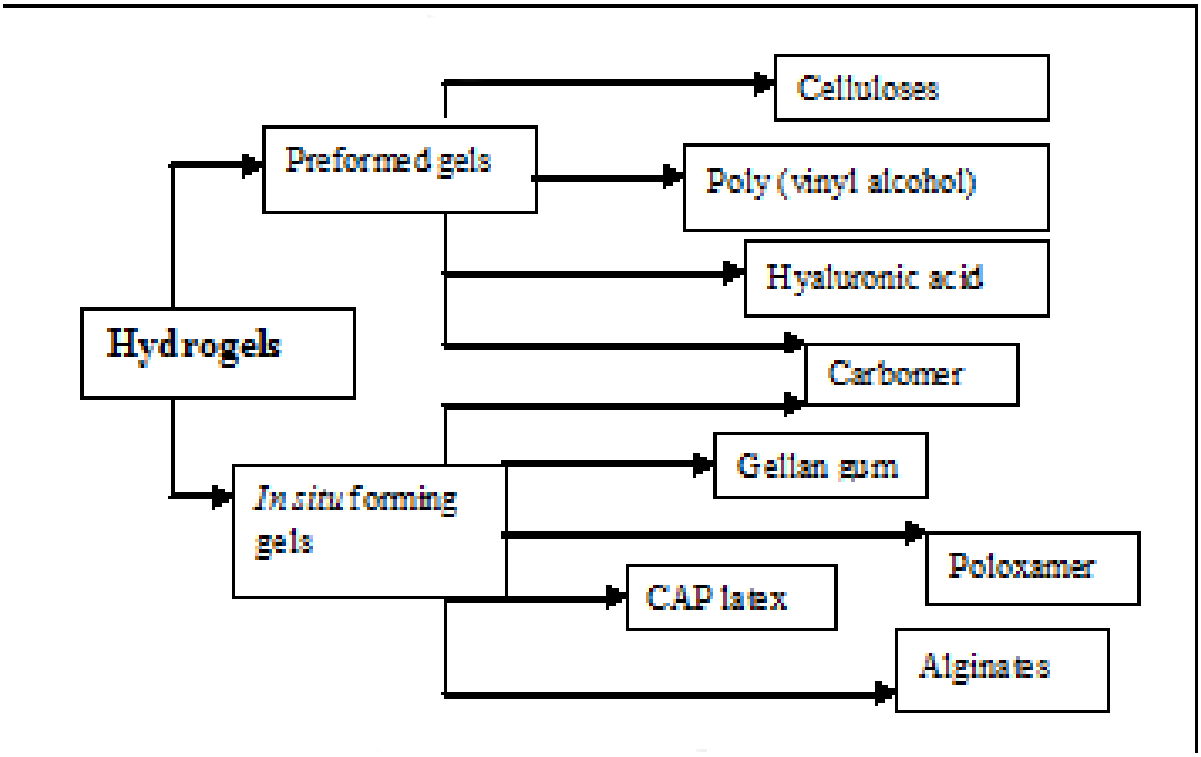

Figure 1: Classification of hydrogels.

***In-Situ* Gel Delivery Systems**

In-situ gelation is a process of gel formation at the site of application after the composition or formulation has been applied to the site. In the field of human and animal medicine, the sites of application refers to various injection sites, topical application sites, surgical sites, and others where the agents are brought into contact with tissues or body fluids. As a drug delivery agent, the *in-situ* gel has an advantage related to the gel or polymer network being formed *in-situ* providing sustained release of the drug agent. At the same time, it permits the drug to be delivered in a liquid form. *In-situ* is a Latin phrase meaning *in the place.*

Distinguishing from preformed hydrogels, *in-situ* forming gels are formulations, applied as a solution, which undergoes gelation after instillation due to physicochemical changes inherent to the biological fluids. In this way, the polymers, which show sol-gel phase transition and thus trigger drug release in response to external stimuli, are the most investigated. *In-situ* hydrogels are providing such 'sensor' properties and can undergo reversible sol-gel phase transitions upon changes in the environmental condition. These "intelligent" or "smart" polymers play important role in drug delivery since they may dictate not only where a drug is delivered, but also when and with which interval it is released [3].

Advantages of *in-situ* forming gel:

1) Ease of administration

2) Improved local bioavailability

3) Reduced dose concentration Reduced dosing frequency

4) Improved patient compliance and comfort

5) Its production is less complex and thus lowers the investment and manufacturing cost.

Method for preparation of *in-situ* gel:

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 triggered 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 tailor able to account for small differences in local temperatures, 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 thermo-responsive sol-gel polymeric system. For convenience, temperature-sensitive hydrogels are classified into negatively thermo-sensitive, positively thermo-sensitive, and thermally reversible gels[4-6]. 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-isopropyl acrylamide) (PNIPAAm). PNIPAAm is a water soluble polymer at its low LCST, but hydrophobic above LCST, which results on precipitation of PNIPAAm from the solution at the LCST.[7,8]Pluronics are poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPOPEO) triblock copolymer 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[9]. 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[10]. The most commonly used thermo-reversible 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[11]. 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[12].

pH triggered systems:

Another formation of *in-situ* gel based on physiologic stimuli is formation of gel is induced by pH changes[8]. 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[10]. The most of anionic pH-sensitive polymers are based on PAA (Carbopol®, carbomer) or its derivatives[13]. Likewise polyvinylacetal diethylamino acetate (AEA) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition[14]. 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 enough to cause gelation, however, the low pH of PAA solution would cause damage to surface of eye before being neutralized by the lacrimal fluid. This problem was solved by partially by combining PAA with HPMC, a viscous enhancing polymer, which resulted in pH responsive polymer mixtures that was sol at pH 4 and gel at pH 7.4[15]. Mixtures of poly (methacrylic acid) (PMA) and poly (ethylene glycol) (PEG) also has been used as a pH sensitive system to achieve gelation[16].

***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[17]. 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 bio-adhesive properties and can be degraded *in-vivo* by enzymatic action[18].

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[19].

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

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

Ionic cross-linking

Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones[20].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 chains[21].

Enzymatic cross-linking

In-situ formation catalyzed by natural enzymes has not been investigated widely but seems to have some advantages over chemical and photochemical approaches. For example, an enzymatic process operates efficiently under physiologic conditions without need for potentially harmful chemicals such as monomers and initiators. Intelligent stimuli-responsive delivery systems using hydrogels that can release insulin have been investigated. Cationic pH-sensitive polymers containing immobilized insulin and glucose oxidase can swell in response to blood glucose level releasing the entrapped insulin in a pulsatile fashion. Adjusting the amount of enzyme also provides a convenient mechanism for controlling the rate of gel formation, which allows the mixtures to be injected before gel formation[22].

Photopolymerization

Photopolymerization is commonly used for *in-situ* formation of biomaterials. A solution of monomers or tissues site and the application of electromagnetic radiation used to form gel[8].Acrylate or similar reactive macromer and initiator can be injected into 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 photopolymerization, where as camphor quinone 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[23]. 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 are reported by Sawhney et al[24].

Polymers used in *in-situ* gelling systems

Materials that exhibit sol to gel transition in aqueous solution at temperatures between ambient and body temperature is of interest in the development of sustained release vehicles with *in-situ* gelation properties.

A polymer used to prepare *in-situ* gels should have following characteristics:

1. It should be biocompatible.

2. It should be capable of adherence to mucus.

3. It should have pseudoplastic behaviour.

4. It should have good tolerance and optical clarity.

5. It should influence the tear behaviour.

6. The polymer should be capable of decreasing the viscosity with increasing shear rate there by offering lowered viscosity during blinking and stability of the tear film during fixation.

Polymers capable of *in-situ* gelation include Poloxamer, Pluronics, various copolymers such as PEO-PLLA and PEG-PLGA-PEG, cellulose acetophalate latex, Pectin, Gelrite, Gellan gum, Alginate, Carbopol, chitin and Matrigel. The gel formation is induced by temperature change (Poloxamer, Pluronics, PEO-PLLA diblock copolymer, PEG-PLGA-PEG triblock copolymer, and Matrigel), pH change (cellulose acetophalate latex and Carbopol), or reaction with mono- or di-valent cations (Gelrite). Some of the most important polymers used as *in-situ* gelling agents are described here.

Gellan gum

Gellan gum commercially available as linear, anionic deacetylated exocellular polysaccharide secreted by the microbe *Sphingomonas paucimobilis* (formerly known as *Pseudomonas elodea*) with a tetrasaccharide repeating unit of one α-L-rhamnose, one β-D-glucuronic acid and two β-D-glucose. It has the characteristic property of temperature dependent and cation-induced gelation involving 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 sol-gel transition process is induced by the presence of monovalent or divalent ions such as Na+ and Ca2+. Some other parameters influence the phase transition e.g. the concentration of polysaccharide, the temp of the preparation, and the nature and the concentration of cations. It was determined that divalent ions such as magnesium or calcium were superior to monovalent cations in promoting the gelation of the polysaccharide. Because of its ability to form strong clear gels at physiological ion concentration, deacetylated gellan gum has been widely investigated for use as an in situ gelling agents in ocular formulations. It has been reported to provide a significantly prolonged corneal contact time in comparison with conventional solutions and is currently marketed in the controlled-release timolol formulation Blocadren Depot (Timoptic-XEw). It has also been suggested that gellan gum is a promising polymer for use in nasal formulations.

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[23]. 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 polysaccharide 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[24].

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- hydroxypropyl methylcellulose 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 were carried out. A sustained release of indomethacin was observed for a period of 8h *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[25]**.**

Sodium Alginate

Sodium alginate is a salt of Alginic acid - a linear block copolymer polysaccharide consisting of β-D-mannuronic acid and α-L-glucuronic acid residues joined by 1,4-glycosidic linkages.[26](Fig. 2). Aqueous solutions of alginates form firm gels on addition of di- and trivalent metal ions. The thermal properties of water insoluble alginate films containing di and trivalent cations. The results indicated that the alginates form compact structures when the ionic radii of the cation are lower. Changes in the film structure during ionic exchange were studied on the basis of its glass transition temperature (Tg) and heat capacity using differential scanning calorimetry (DSC).[27]Sodium alginate has been employed in the preparation of gels for the delivery of biomolecules such as drugs, peptides and proteins.[28]Gastro retentive *in-situ* gelling liquid formulation for controlled delivery of ranitidine using sodium alginate (low, medium and high viscosity grades), calcium carbonate (source of cations) and ranitidine. Prepared formulations were evaluated for viscosity, buoyancy lag time and buoyancy duration, drug content and *in-vitro* drug release. Formulation variables such as concentration of sodium alginate, calcium carbonate and drug significantly affected the formulation viscosity, floating behavior and *in-vitro* drug release. Analysis of the release pattern showed that the drug release from *in-situ* gel followed a diffusion mechanism[29]. It exhibits favorable biological properties such as biodegradability and nontoxicity[30] and mucoadhesive properties.[31-32]

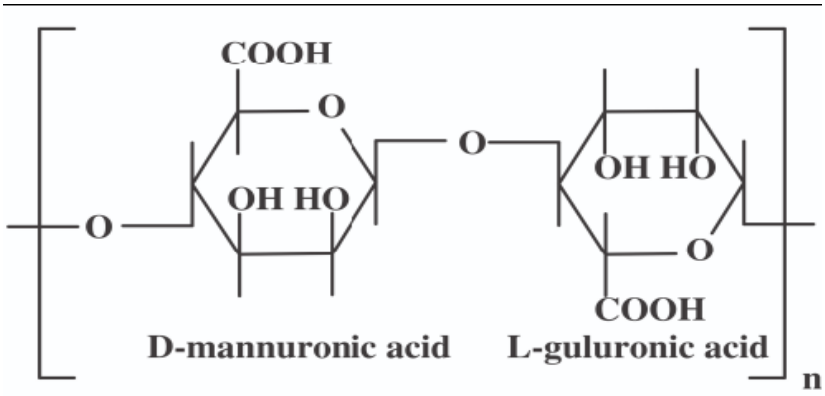

Fig. 2: Structure of Alginic acid

Pluronic F 127

A compound which has received considerable attention is the polyoxyethylene/polyoxypropylene/ polyoxyethylene tri-block copolymer Pluronic F127 (Poloxamer 407) the thermo reversible gelation of which was demonstrated by an author. Gels of Pluronic F127 have been explored for application in ophthalmic, topical, nasal, rectal, subcutaneous, intraperitoneal administration. There are, however, inherent problems associated with tri-block copolymers polyoxyethylene and polyoxypropylene; commercial samples are subject to formulation to formulation variability and laboratory synthesis is complicated by the so called transfer reaction which results in the presence of di block impurities. These problems may be avoided through the use of block copolymers in which oxy-butylenes is substituted for oxy-propylene as the hydrophobe, which can be tailor made to have the necessary sol-gel transition between ambient and body temperatures to confer *in-situ* gelation characteristics.

Xyloglucan

An alternative polymer is the polysaccharide xyloglucan, which also exhibits sol-gel transition in the required temperature region, and which has the additional advantage of recognized nontoxicity and lower gelation concentration. Xyloglucan polysaccharide derived from tamarind seed 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. The tamarind seed xyloglucan is composed of 3 units of xyloglucan oligomers with a heptasaccharide, octasaccharide and monosaccharide, which differ in a number of galactose side chains. When xyloglucan derived from tamarind seed is partially degraded by β- galactosidase, the resultant

product exhibits thermally reversible gelation, the sol to gel transition temperature varying with degree of galactose elimination. Such gelation does not occur with native xyloglucan. In their study, they have used a xyloglucan sample with a percentage of galactose removal of 44 %, which exhibited a transition from sol to gel at temp between 22-27^{o}C. In dilute aqueous solution over the concentration range 1-2 % w/w the gelation is thermally reversible, i.e. the gels revert to their sol phase on cooling below the gelation temperature. The same authors have previously reported the potential use of xyloglucan gels for rectal and intraperitoneal drug delivery, and we now consider the possible application of in situ gelling xyloglucan formulations for oral administration. Its potential application in oral delivery exploits the slow gelation time (several minutes) that, it is proposed, would allow in situ gelation in the stomach following the oral administration of chilled xyloglucan solutions.

Pectin

Pectins are a family of polysaccharides, in which the polymer backbone mainly comprises α-(1-4)-D-galacturonic acid residues. Low methoxy pectins (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[33]. 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 complex form may be included in the formulation for the induction of pectin gelation[34].

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.

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-glucuronic acid-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 glucuronic acid and pyruvic acid groups in the side chain [35].

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 insimple 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 frequently 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 promotes 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[36],

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 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[37].

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[38].

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, anti-inflammatory 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

turnover 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[39]. 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[40]. 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[41].

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[42]. 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 4-5hrs after administration with no apparent cytotoxicity. Therefore, these types of systems are suitable for protein and peptide drug delivery through nasal route [43].

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[44]. 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[45].

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 crosslinking 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[46].

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 syringe ability 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[47].

Sol-Gel transition temperature and gelling time

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

Gel-Strength

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

Viscosity and Rheology

This is an important parameter for the *in-situ* gels, to be evaluated. The viscosity and rheological properties of the polymeric formulations, either in solution or in gel made with artificial tissue fluid (depending upon the route of administrations) instead of 5% mannitol, were determined with Brookfield rheometer or some other type of viscometers such as Ostwald's viscometer. The viscosity of these formulations should be such that no difficulties are envisaged during their administration by the patient, especially during parenteral and ocular administration[48].

Fourier transform infrared 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[48].

***In-vitro* drug release studies**

For the *in-situ* gel formulations to be administered by oral, ocular or rectal routes, the drug release studies are carried out by using the plastic dialysis cell. The cell is made up of two half cells, donor compartment and a receptor compartment. Both half cells are separated with the help of cellulose membrane. The sol form of the formulation is placed in the donor compartment.The assembled cell is then shaken horizontally in an incubator. The total volume of the receptor solution can be removed at intervals and replaced with the fresh media. This receptor solution is analyzed for the drug release using analytical technique. For injectable *in-situ* gels, the formulation is placed into vials containing receptor media and placed on a shaker water bath at required temperature and oscillations rate. Samples are withdrawn periodically and analyzed[49].

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[50].

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.

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