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REVIEW ARTICLE

INSITU GELLING SYSTEM: A REVIEW

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ABSTRACT

The purpose of writing this review on *insitu* gel was to complete the recent literature with special focus on *insitu* gelling system to increase the residence time of drug at the target site. *Insitu* gells are of particular interest for drugs that have very less half life and are readily dissolved. Recent developments of insitu gelling system are its use as vehicles for the delivery of drugs for both local treatment and systemic effects. The main interest in recent development of *insitu* gelling system has been shown in advantages of *insitu* polymeric delivery system such as ease of administration, reduced frequency of administration, improved patient compliance and comfort. This review summerises the studies to evaluate the performance and application of these systems. This review article is prepaired to discuss formulation factors used during development of *insitu* drug delivery system, relevant biodegrable polymers for sol-gel transition, characterization, evaluation parameter and commercial formulations of *insitu* polymeric system.

Keywords: Residence time, systemic effects, patient compliance evaluation.

INTRODUCTION

Insitu gel forming polymeric formulations are drug delivery systems that are in sol or suspension form before administration in the body, once administered undergoes gelation *insitu* to form a gel. *Insitu* gel forming systems have been widely investigated as vehicles for sustained drug delivery. [1]

PRINCIPLE OF INSITU GEL

Formulation of *insitu* gel system involves the use of gelling agent which can form a stable sol/suspension system to contain the dispersed drug and other excipients. The gelling of this sol/suspension system is to be achieved in gastric environment, triggered by ionic complexation due to change in pH. The formulation adopted is a gellan gum or sodium alginate solution containing calcium chloride and sodium citrate, which complexes the free Calcium ions and releases them only in the acidic enviorment of stomach. Gellan gum or sodium alginate acts as gelling agent and can produce textures in the final product that vary from hard, non elastic, brittle gels to fluid gels. [2] The free calcium ions gets entrapped in polymeric chains of gellan gum or sodium alginate thereby causing crosslinking of polymer chains to form matrix structure. This gelation involves the formation of double helical junction zones followed by re-aggregation of double helical segments to form a three dimensional network by complexation with cations and hydrogen bonding with water.[3]

ADVANTAGES OF INSITU GEL

1. Poor bioavailability problem of conventional ophthalmic solution can be overcome by use of gel that are instilled as drops into eyes.
2. It increases the contact time of drug at the site of maximum absorption.
3. Reduced frequency of administeration.
4. Reduced systemic absorption of drug drained through the nasolacrimal duct may result in some undesirable side effects.
5. It provides advantages such as the delivery of drugs with narrow absorption windows in the small intestinal region.
6. The GRDDS are advantageous for drugs absorbed through the stomach e.g. ferrous salts and for drugs meant for local action in the stomach and treatment of peptic ulcer disease e.g. antacids.

SUITABLE DRUG CANDIDATES FOR INSITU GEL

1. Narrow absorption window in GI tract, e.g., riboflavin and levodopa

2. Primarily absorbed from stomach and upper part of GItract, e.g., cinnerazine.

3. Drugs that act locally in the stomach, e.g., antacids and misoprostol

4. Drugs that degrade in the colon, e.g. ranitidine HCl and metronidazole

5. Drugs that disturb normal colonic bacteria, e.g., amoxicillin trihydrate

IDEAL CHARACTERISTICS OF POLYMERS

1. It should be biocompatible.
2. It should be capable of adherence to mucus.
3. It should have pseudo plastic behavior.
4. It should have good tolerance and optical activity.
5. I should influence the tear behavior.
6. The polymer should be capable of decrease the viscosity with increasing shear rate offering lowered viscosity during blinking and stability of tear film during fixation.

POLYMERS USED IN INSITU GELLING SYSTEM

1. Gellan gum: It is anionic deacetylated exocellular polysaccharide secreated by Pseudomonas elodea. It has tendency of gelation which is temperature dependent or cation induced. The formulation consisted of gellan solution with calcium chloride and sodium citrate complex. When administered orally, the calcium ions are released in acidic environment of stomach leading to gelation of gellan thus forming a gel in situ. In situ gelling gellan formulation as vehicle for oral delivery of theophylline is reported. [4]

2. Xyloglucan: Xyloglucan is a polysaccharide derived from tamarind seeds and is composed of a (1-4)-β-D-glucan backbonechain, which has (1-6)-α-D xylose branches that are partially substituted by (1-2)-β-D-galactoxylose. 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 solution20. Xyloglucan gels have potentially been used for oral, intraperitoneal, ocular and rectal drug delivery.[5]

3. 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-Lglucuronic acid blocks of the alginate chain. Alginic acid can be chosen as a vehicle for ophthalmic formulations, since it exhibits favorable biological properties such as biodegradability and nontoxicity. A prolonged precorneal residence of formulations containing alginic acid was looked for, not only based on its ability to gel in the eye, but also because of its mucoadhesive properties.[6]

4. 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 6.226. 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.[7]

5. 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. These 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 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.[8]

6. 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. The main advantage of using pectin for these formulations is that it is water soluble, so organic solvents are not necessary in the formulation. Divalent cations present in the stomach, carry out the transition of pectin to gel state when it is administered orally. Calcium ions in the complexed form may be included in the formulation for the induction of pectin gelation. Sodium citrate may be added to the pectin solution to form a complex with most of calcium ions added in the formulation. By this means, the formulation may be maintained in a fluid state (sol), until the breakdown of the complex in the acidic environment of the stomach, where release of calcium ions causes gelation to occur. The quantities of calcium and citrate ions may be optimized to maintain the fluidity of the formulation before administration and resulting in gelation, when the formulation is administered in stomach. The potential of an orally administered in situ gelling pectin formulation for the sustained delivery of Paracetamol has been reported.[9]

7. Pleuronics: Poloxamers or pluronic (marketed by BASF Corporation) are the series of commercially available difunctional triblock copolymers of non-ionic nature. They comprise of a central block of relatively hydrophobic polypropylene oxide surrounded on both sides by the blocks of relatively hydrophilic poly ethylene oxide. Due to the PEO/PPO ration of 2:1, when these molecules are immersed into the aqueous solvents, they form micellar structures above critical micellar concentration. . 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. 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.

SYNTHETIC POLYMERS

Synthetic polymers are popular choice mainly for parenteral preparations. The trend in drug delivery technology has been towards biodegradable polymers, requiring no follow up surgical removal, once the drug supply is depleted. Aliphatic polyesters such as poly (lactic acid), poly (glycolic acid), poly (lactide-coglycolide), poly (decalactone), poly ε-caprolactone have been the subject of the most extensive recent investigations. 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 poly(lactidecoglycolide), poly (decalactone), poly ε-caprolactone have been the subject of the most extensive recent investigations.[10]

DIFFERENT APPROACHES FOR IN SITU GEL DRUG DELIVERY

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

1. Physiological stimuli (eg temperature and pH)

2. Physical changes in biomaterials (eg diffusion of solvent and swelling)

3. Chemical reactions (eg, enzymetic, chemical and photo initiated polymerization)

INSITU GEL FORMATION BY PHYSIOLOGICAL STIMULI

Temperature 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 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. Negative temperature-sensitive hydrogels have a lower critical solution temperature (LCST) and contract upon heating above the LCST. Polymers with low critical temperature (LCST) transition between ambient and physiologic temperature is used for this purpose. One of the most extensively investigated polymers that exhibit useful LCST transition is poly (Nisopropylacrylamide) (PNIPAAm). A positive temperature sensitive hydrogel has an upper critical solution temperature (UCST), such hydrogel contracts upon cooling below the UCST. Polymer networks of poly (acrylic acid) (PAA) and polyacrylamide (PAAm) or poly (acrylamide-co-butyl methacrylate) have positive temperature dependence of swelling. The most commonly used thermoreversible gels are these prepared from poly (ethylene oxide)-b-poly (propylene oxide)-bpoly (ethylene oxide) (Pluronics®, Tetronics®, poloxamer.) Polymer solution is a free flowing liquid at ambient temperature and gels at body temperature.[11]

pH triggered systems- Another formation of in situ gel based on physiologic stimuli is formation of gel is induced by pH changes. All the pH-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH .The polymers with a large number of ionizable groups are known as polyelectrolytes. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups. The most of anionic pH-sensitive polymers are based on PAA (Carbopol®, carbomer) or its derivatives. Likewise polyvinylacetal diethylaminoacetate (AEA) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition43. 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 factor 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 7.444. Mixtures of poly (methacrylic acid) (PMA) and poly (ethylene glycol) (PEG) also has been used as a pH sensitive system to achieve gelation.[12]

In situ formation based on physical mechanism

Swelling

In situ formation may also occur when material absorbs water from surrounding environment and expand to occur desired space. One such substance is myverol 18-99 (glycerol mono-oleate), which is polar lipid that swells in water to form lyotropic liquid crystalline phase structures. It has some Bioadhesive properties and can be degraded in vivo by enzymatic action.[13]

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Diffusion

This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. Nmethyl pyrrolidone (NMP) has been shown to be useful solvent for such system.[14]

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. . Gellan gum commercially available as Gelrite® is an anionic polysaccharide that undergoes in situ gelling in the presence of mono- and divalent cations, including Ca2+, Mg2+, K+ and Na+.. Gelation of the low-methoxy pectins can be caused by divalent cations, especially Ca2+. Likewise, alginic acid undergoes gelation in presence of divalent/polyvalent cations e. g. Ca2+ due to the interaction with glucuronic acid block in alginate chains.[15]

Enzymatic cross-linking

In situ formation catalysed by natural enzymes has not been investigated widely but seems to have some advantages over chemical and photochemical approaches. For example, an enzymatic process operates efficiently under physiologic conditions without need for potentially harmful chemicals such as monomers and initiators. Intelligent stimuli-responsive delivery systems using hydrogels that can release insulin have been investigated. Cationic pH-sensitive polymers containing immobilized insulin and glucose oxidase can swell in response to blood glucose level releasing the entrapped insulin in a pulsatile fashion. Adjusting the amount of enzyme also provides a convenient mechanism for controlling the rate of gel formation, which allows the mixtures to be injected before gel formation.[16]

Photo-polymerisation

Photo-polymerisation is commonly used for in situ formation of biomaterials**.** A solution of monomers or reactive macromer and initiator can be injected into a tissues site and the application of electromagnetic radiation used to form gel. Acrylate or similar polymerizable functional groups are typically used as the polymerizable groups on the individual monomers and macromers because they rapidly undergo photo-polymerisation in the presence of suitable photo-initiator. 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. Photopolymerizable systems when introduced to the desired site via injection get photo cured 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.[17-18]

APPLICABILITY OF IN SITU POLYMERIC DRUG DELIVERY SYSTEM

1. Ocular drug delivery system

In ocular delivery system natural polymers like gallan gum, alginic acid & xyloglucan are most commonly used. For local ophthalmic delivery system various compoundslike antimicrobial agent, anti-inflammatory agent & autonomic drugs are used to relieve intra ocular tension in glaucoma. Conventional delivery system often result in poor availability & therapeutic response because high tear fluid turn over & dynamics which cause rapid elimination of the drug from the eye so, the overcome the bioavailability problem ophthalmic in-situ gel was developed. To improve the bioavailability viscosity enhancers such as Hydroxy Propyl Methyl Cellulose, Carboxy Methyl Cellulose, Carbomers, Poly Vinyl alcohol used to increase the viscosity of formulation in order to prolong the precorneal residence time & improve the bioavailability, ease to manufacture. Penetration enhancer such as preservatives, chelating agent, surfactants are used to enhance corneal drug penetration.[19]

2**. Nasal drug delivery system**

In nasal in-situ gel system gallan gum & xanthan gum are used as in-situ gel forming polymers Momethasone furoatewas evaluated for it‘s efficacy for the treatment of allergic rhinitis. Animal study were conducted using allergic rhinitis model & effect of in-situ gel on antigen induced nasal symptoms in sensitizes rats was observed. In-situ gel was found to inhibit the increase in nasal symptoms are compared to marketed preparation nosonex (Momethasone furoate suspension 0.05%).

3**. Rectal drug delivery system**

The rectal route may be used to deliver many types of drugs that are formulated as liquid, semisolid (ointments, creams and foams) and solid dosage forms (suppositories).

Conventional suppositories often cause discomfort during insertion. In addition, suppositories are unable to be sufficiently retained at a specific position in the rectum, sometimes they can migrate up-wards to the colon that makes them possible for drug to undergo the first-pass effect. Choi et al. developed novel in situ gelling liquid suppositories with gelation temperature at 30–36°C. Poloxamer 407 and/ or poloxamer 188 were used to confer the temperature-sensitive gelation property.In-situ gel possesses a potential application for rectal & vaginal route .Miyazaki et al. investigated the use of

xyloglucan based thermo reversible gel for rectal drug delivery of Indomethacin. Administration of indomethacin loaded xyloglucan based system to rabbit indicated broad drug absorption & a longer drug residence time as compared to that resulting after administration of commercial suppository. For better

therapeutic efficacy &patient compliance, mucoadhesive, thermo sensitive, prolonged release vaginal gel incorporating Clotrimazole-β-cyclodextrin complex formulated for treatment of vaginitis.[20]

4. **Vaginal drug delivery system**

The vagina, in addition to being an important organ of reproductive tract, serves as a potential route for drug administration. Formulations based on a thermo-plastic graft copolymer that undergo in situ gelation have been developed to provide the prolonged release of active ingredients such as nonoxynol-9, progestins, estrogens, peptides and proteins62. Chang et al. have recently reported a mucoadhesive thermo-sensitive gel (combination of poloxamers and polycarbophil), which exhibited, increased and prolonged antifungal activity of clotrimazole in comparison with conventional PEG-based formulation.

5. Injectable drug delivery system

One of the most obvious ways to provide sustained release medication is to place the drug in delivery system and inject or implant the system into the body tissue. Thermoreversible gels mainly prepared from poloxamers are predominantly used. The suitability of poloxamer gel alone or with the addition of hydroxypropylmethylcellulose (HPMC), sodium carboxymethylcellulose (CMC) or dextran was studied for epidural administration of drugs in vitro. The compact gel depot acted as the rate limiting step and significantly prolonged the dural permeation of drugs in comparison with control solutions. J. M. Barichello et al. evaluated Pluronic F127 gels, which contained either insulin or insulin-PLGA nanoparticles with conclusion, that these formulations could be useful for the preparation of a controlled delivery system. Likewise, poloxamer gels were tested for intramuscular and subcutaneous administration of human growth hormone or with the aim to develop a long acting single dose injection of lidocaine. g Injectable drug delivery system, cross linking of hydrazide modified by aluronic acid with aldehyde modified version of cellulose derivatives such as carboxy methyl cellulose, methyl cellulose, hydroxy propyl methyl cellulose are used. These in-situ forming gel were used for preventing postoperative peritoneal adhesion thus avoiding pelvic pain, bowel obstruction & infertility. For a better therapeutic efficacy & patient compliance, mucoadhesive, thermo sensitive, prolonged release vaginal gel incorporating Clotrimazole-β-cyclodextrin complex was formulated for treatment of virginities.[21]

6**. Dermal and transdermal drug delivery system**

Thermally reversible gel of Pluronic F127 was evaluated as vehicle for the percutaneous administration of Indomethacin. In-vivo studies suggest that 20% w/w aqueous gel may be of practical use as a base for topical administration of the drug. Poloxamer 407 gel was found suitable for transdermal delivery of insulin73. The combination of chemical enhancers and iontophoresis resulted in synergistic enhancement of insulin permeation.

7. Oral drug delivery system

For the oral in situ gel delivery system pectin, xyloglucan & gellan gum natural polymers are used. Pectin formulation for sustained delivery of paracetamol has been reported. Advantages of pectin is water soluble so, no need to add organic solvent. Cross-linked dextran hydrogels with a faster swelling under high pH conditions, likewise other polysaccharides such as amide pectins, guar gum and insulin were investigated in order to develop a potential colon-specific drug delivery system. W. Kubo et al. developed the formulations of gellan and sodium alginate both containing complexed calcium ions that undergo gelation by releasing of these ions in the acidic environment of the stomach. Oral delivery of paracetamol was studied. Hydrogels made of varying proportions of PAA derivatives and crosslinked PEG allowed preparing silicone microspheres, which released prednisolone in the gastric medium or showed gastroprotective property.[22]

EVALUATION AND CHARACTERIZATION OF IN SITU GELLING SYSTEM

1. Clarity

The clarity of formulated solution is determined by visual inspection under black & white Background.

2. **Texture analysis**

The consistency, firmness &cohesiveness of in situ gel are assessed by using texture profile analyzer which mainly indicated gel strength & easiness in administration in vivo higher value of adhesiveness of gel are needed to maintain an intimate contact with mucus surface.

3. **pH of gel**

pH can be determined formulation is taken in beaker & 1ml NaOH added drop wise with continuous stirring. pH is checked by using pH meter.

4**. 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.

5. **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.

6**. Gelling capacity**

In-situ gel is mix with simulated tear fluid (in the proportion of 25:7 i.e. application volume 25µl & normal volume of tear fluid in eye is 7µl) to find out gelling

capacity of ophthalmic product. The gelation assessed visually by noting the time for & time taken for dissolution of the formed gel.

7. **Rheological studies**

The viscosity measured by using Brookfield viscometer, cone & plate viscometer. In-situ gel formulation is placed in sample tube. Formulation should have viscosity 5-1000mPas, before gelling & after ion gel activation by eye will have viscosity of from about 50-50,000 mPas.

8. **Isotonicity evaluation**

Isotonicity is important characteristics of ophthalmic preparation. Isotonicity is maintained to prevent tissue damage or irritation of eye. All ophthalmic preparation are subjected to isotonicity testing, science they exhibited good release characteristics & gelling capacity & the requite velocity. Formulation mixed with few drops of blood & observed under microscope at 45x magnification & compared with standard marketed ophthalmic formulation.

9**. Swelling studies**

Swelling studies are conducted with a cell, equipped with thermo jacket to maintain a constant temperature .The cell contains artificial tear fluid (composition – 0.67g Nacl, 0.20g NaHCo3, 0.008g Cacl2.2H2o & distilled water q.s to 100g)80. swelling medium equilibrating at 370c one milliliter of formulated solution is placed in dialysis bag & put into the swelling medium. At specific time interval the bag is removed from the medium & weight is recorded. The swelling of the polymer gel as a function of time is determined by using the following relationship.

$$\% St = (Wt - W0)\ 100/W0$$

St = Swelling at time‘t’.

W0=Initial weight of gelling solution.

Wt=Final weight of gel.

10**. Statistical analysis**

Analysis of variance (ANOVA) is used the testing the difference between calculated parameters using SPSS statistical package. Statistical difference yielding $P \leq 0.05$ is considered. Duncan multiple comparison is applied when necessary to identify which of the individual formulations are significantly different.

11. **High performance liquid chromatography**

The HPLC system is used in reversed phase mode. Analysis is performed on a Nova pack C18 packed column (150 mm length X 3.9 mm i.d).

12. **Fourier transformer infra red**

The possibility of drug excipient interaction is investigated by FTIR studies. The FTIR graph of pure drug & combination of drug with excipient are recorded by using KBR pellets.

13**. Thermal analysis**

Thermo gravimetric analysis can be conducted for in situ forming polymeric system to quantitative the percentage of water in hydrogel. Different scanning calorimetry is used to observed, if there are many changes in thermograms as compared with pure ingredients used thus indicating the interaction.

14**. In vitro drug release studies**

In vitro release study of in situ gel solution is carried out by using Franz diffusion cell. The formulation is placed in donor compartment & freshly prepared simulated tear fluid in receptor compartment. Between receptor & donor compartment dialysis membrane is placed (0.22 µm pore size). The whole assembly is placed on thermostatically controlled magnetic stirrer. The temperature of the medium is maintained at 37ºc± 0.5ºc.

1ml sample is withdrawn at predetermined time interval of 1hr for 6hrs the sample volume of fresh medium is replaced. The withdrawn sample is diluted to 10ml in volumetric flask with respective solvent & analyzed by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using an equation generated from standard calibration curve. The percentage cumulative drug release (% CDR) calculated. The obtained data is further subjected to curve fitting for drug release data. The best fit model is checked for Krosmeyers peppas & Fickinian diffusion mechanism of their kinetics.

15**. Ocular irritancy studies**

Ocular irritancy studies are performed on male albino rabbits, weighing 1-2 kg. The modified Draize technique is used for ocular irritation potential of ophthalmic products. The formulation is placed in lower cul-de-sac & irritancy is tested at time interval of 1hr, 2hrs, 48hrs, 72hrs, & 1 week after administration. The rabbits are observed periodically for redness, swelling, & watering of eyes.

16. **Sterility testing**

Sterility testing is carried out as per the IP 1996. The formulation is incubating for not less than 14 days at 300-350c in the fluid thioglycolate medium to find the growth of bacteria & at 200-250 c in Soya bean casein digest medium to find the growth of fungi in formulation.

17**. Accelerated stability studies**

Formulation is replaced in amber colored vials & sealed with aluminum foil for the short term accelerated stability study at 40± 20 c & 75 ±5% RH as per International Conference of Harmonization (ICH) State Guidelines. Sample is analyzed at every month for clarity, pH, gelling capacity, drug content, rheological evaluation & in vitro dissolution.

18**. Histopathological studies**

Two mucosa tissue pieces (3 cm2) 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

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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.

19. **Antimicrobial activity**

Antimicrobial efficacy studies are carried out to ascertain the biological activity of sol-gel-system against microorganisms. This is determined in agar diffusion medium employing cup plate techniques. The microbial growth of bacteria is measured by conc of

Standard preparation of antibiotic and carried out the microbial assay serial dilution method is employed.[23]

RECENT ADVANCEMENTS

One of the challenges facing todays pharmaceutical industry centreson 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 biodegrable polymers are used for formulation of insitu gels, but there are fabrication problems, difficult process ability, and use of organic solvents for their preparation (especially for synthetic polymer based systems), burst effect and irreproducible drug release kinetics. Natural polymers satisfy the characteristics of an ideal polymer but batch to batch reproducibility is difficult therefore synthetic polymers are used. The recent advancement of biotechnologies has lead to the development of liable 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 biocompatible hydrophilic solvent led to the formation of injectable, insitu forming organ gel. Following subcutaneous injection, leuprolide-loaded organ gel degraded and gradually released leuprolide for 14 to 25d.

The gastroretentive drug delivery system of famotidine was prepared, by employing two different grades of methocel K 100 and K15 M by effervescent technique; these grades of methocel were evaluated for their gel forming properties. Sodium bicarbonate was incorporated as a gas generating agent. The floating tablets were evaluated for uniformity of weight, hardness, friability, drug content, invitro buoyancy and dissolution studies. [24]

The gastro retentive controlled release drug delivery system of verapamil Hcl was prepared in an effort to increase the gatric residence time of the dosage form and to control the drug release. Hydroxypropyl methyl cellulose, carbopol and xanthan gum were incorporated for gel forming properties. Buoyancy was achieved by adding an effervescent mixture of sodium bicarbonate and anhydrous citric acid. Invitro release studies were performed and drug release kinetics was evaluated by the linear regression method. Optimized intragastric floating tablet showed no significant change in physical appearance, drug content after storage at 75% relative humidity for 3 months.

Formulation, evaluation and optimization of stomach specific in situ gel of clarithromycin and metronidazole benzoate was studied by using sodium alginate as polymer and calcium carbonate was used as a cross linking agent .This study reported that oral administration of aqueous solutions containing sodium alginate resulted in formation of In situ gel ,such formulations are homogenous liquid when administered orally and become gel at contact site .stability study of check point batch after three month showed no change in in-vitro drug release profile, % assay and evaluation parameters .it was concluded that by adopting a systemic formulation approach ,an optimum point could be reached in the shortest time with minimum effort.[25]

Novel Floating In situ Gelling System of Levofloxacin Hemihydrate was developed and evaluated by using varying concentrations of gellan gum and sodium alginate in deionized water containing sodium citrate. a stomach specific in situ gel of levofloxacin hemihydrate could be prepared using floating mechanism to increase the residence time of the drug in stomach and thereby increasing the absorption. This study shows the feasibility of in vitro gel forming from aqueous solution of sodium alginate and gellan gum containing Calcium ions in a complex form. it is concluded that levofloxacin hemihydrate could be targeted to stomach and be released slowly over a period of time.

Ganapati rohith et al studied Floating drug delivery of a locally acting H2-antagonist An approach using an in situ gelling liquid formulation by using sodium alginate and calcium carbonate .By observing various evaluation parameters for the studied formulations, it can be stated that incorporation of sodium citrate and calcium chloride in the formulation is required to control and sustained the drug release from in situ gel.

Formulation and Evaluation of Floating Oral In Situ Gelling System of Amoxicillin was done by using sodium alginate, calcium chloride, sodium citrate ,HPMC K100, and sodium bicarbonate from which concluded that the floating in situ gel system of amoxicillin with increase gastric residence time can be formulated using sodium alginate as a gelling polymer and HPMC K100 as a thickening agent the prepared formulation can provide a site specific delivery of amoxicillin with zero order release kinetics.[26] A method for producing in situ gelation of poloxamer and mucoadhesive polymer chitosan was developed by utilizing the property of poloxamer solution to convert in to gel at physiological temperatures and of chitosan to undergo ion responsive gelation in presence of Sodium tripolyphosphate. Differential scanning calorimetry and tube inversion techniques were used to study micellization and gelation of the poloxamer 407 in presence of chitosan. Mixture of poloxamer and tripolyphosphate was found to reduce the critical micellization temperature and critical gelation

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temperature of poloxamer solution in water. Poloxamer gel, so formed in situ, after the addition of chitosan and tripolyphosphate had shown decline in the dissolution rate and release characteristics of metoprolol, doxycycline and flufenamic acid. In addition to that, variation in the composition of both polymer components and tripolyphosphate had also shown the possibility to control the pH of system so that, it would enhance the solubility profile of drug.

A hydrogel was developed which had shown the promising results in the spinal cord injury,when injected through 40 ìm needle in the solution phase, which converted in to gel inside the target tissue. Formulation was prepared by polycondensation, using two FDA approved polymer viz. Polyacrylic acid (Carbomer 947P) and Agarose, a common polysaccharide. Solution was injected in spinal cord of mouse and in situ gel formation was confirmed by magnetic resonance imaging that showed the presence of polymeric network at injection site. Hydrogel, so produced, had provided enough data to be considered as a new biocompatible tool that can be used as a local reservoir for in situ delivery of drugs.

The textural characteristics like syneresis, opacity and fracture characteristics of gellan, agar and their mixed gels were studied on application of uniaxial compression. Increase in the concentration of colloids had shown to improve the opacity but reduces the syneresis. Syneresis was found to be at lower side, as 1.5% for mixed gel having 2% each of gellan and agar while opacity was found to be 30%, with even more values for opacity for the agar gel. Stress and energy for compression had improved the level of gelling agents.

Formulation of pH sensitive hydrogels by morpholine derivatives was done by utilizing homopolymers and copolymers of N-ethylmorpholine methacrylamide and N, N-dimethylacrylamide applied in the form of matrices for ibuprofen release, which prevent the damage of mucous membrane due to drug. Hydrogel so formed were studied for the release rate at temperature 37 C and different pH values 2, 5 and 7 respectively. Dissolution of the ibuprofen from different formulation at different pH was studied. Results had shown that hydrogel were able to prevent crystallization of the ibuprofen at all pH.

A pH sensitive insitu hydrogel based on the macro monomer synthesized by heat initiated free radical polymerization of methoxy poly(ethylene glycol)-poly(caprolactone)-acryloyl chloride, poly(ethylene glycol)-methyl ether methacrylate and methacrylic acid. Macro monomer and hydrogels were characterized by utilizing NMR and FT-IR techniques. Other profile for the macro monomer produced was also studied like morphology, swelling behavior, in vitro drug release etc and toxicity profile of the macro monomer. Hydrogel that showed the sharp changes in the different pH values were selected as most promising candidate for oral drug delivery of dexamethasone in the inflammatory bowel disease. [27]

Examination of the cytocompatibility of amphiphilic, thermoresponsive and chemically cross linkable macromer forming an in situ hydrogel was done, via in vitro studies. Macromers were synthesized by pentaerythritol diacrylate monostearate, N-iso propylacrylamide, acrylamide and hydroxyethyl acrylate using different molar ratios and changing molecular weights. The lower critical solution temperature was evaluated to determine the cytocompatibility with the fibroblast cell of rat. Cell viabilities of over 80, were observed after the incubation of cell for 24 hour, with molecular weight in range 1500-3000 daltons. Chemical modification of the macromers had also shown the time and dose dependent effect on cell viability. The data obtained had depicted that chemically modified macromers form a less cytotoxic physical gel, while phase separation increased the cytotoxicity.

A thermoresponsive and bioadhesive, in situ gelling system of drug delivery was formulated for treatment of esophageal pain and inflammation. Bioadhesive polymer used was Metolose, a cellulose derivative with thermoresponsive property. Thermal gelation temperature for the polymer was around 65-66 C. Alteration of pH between 2 to 10 and presence of alcohols had not influenced the gelation temperature but using water soluble salts and changing the concentration of polymer in solution (2-3%w/w) thermal gelation point can be reduced. Effect on the thermal gelation point, in vitro liberation and penetration was studied. Study showed that thermal gelation point did not vary but liberation and penetration of drug was changed. Morphological studies of esophagus had also shown that the system did no have any irritant effect or tissue damage effect on esophageal mucosa on exposure even after 12 hour.

The evaluation of the gelation and release characteristics of different formulation was done containing release characteristics of physical mixtures of varying derivative pectin which was methoxylated to different concentration of xyloglucan and pectin where xyloglucan degree, i.e. high (31%) and low (9%) over a wide range of had the thermally reversible gelation and pectin had the pH varying from 1.2 to 5.0. A source of calcium in ion responsive gelation properties, in order to formulate complexed form was also added which released the Calcium in situ gelling vehicle for sustained drug delivery via oral route. Rheological studies showed that inclusion of pectin (0.75% w/w) had increase the gel strength of the 1.5% and 2.0% w/w xyloglucan and the resulting formulation produced. Plasma concentration studies in rats on oral administration the formulation had also shown the more sustained release and improved drug bioavailability was achieved by gel formed due to in situ gelation of this formulation, while the pectin (0.75%w/w) and xyloglucan(1.75%w/w) did not form any gel, under these condition. Visual observation of the gastric content of rat had shown that inclusion of pectin with xyloglucan had also reduced the erosion of gel formed

Formulation a floating in situ gelling system of clarithromycin was done using gellan gum as gelling polymer and calcium carbonate as gas generating floating agent. In situ gelling system was prepared by dissolving different concentration of the gellan gum in water and uniformly dispersing the sucralfate. Formulation variable especially, gellan gum and

sucralfate had shown significant effect on the in vitro release of drug. The resultant in situ gelling system in comparison to amoxicillin suspension had shown improved efficacy towards the clearance of the H. pylori. Prolonged gastric residence time, because of floating, had also shown to have improved efficacy of the drug towards peptic ulcer treatment.

Formulation of an intra gastric floating in situ gel system was done for controlled amoxicillin delivery in treatment of peptic ulcer disease caused by Helicobacter pylori (H. pylori). The system was prepared by, using different concentration of gellan gum in water containing citrate salt, to which varying drug and calcium carbonate (gas forming agent) was added. It had been shown, that concentration of calcium carbonate and gellan gum had significantly affected in vitro drug release. H. pylori clearance efficacy of the in situ gel forming system in reference to amoxicillin suspension had shown significant anti H. pylori effect when studied by polymerase chain reaction technique and microbial culture method. The results had also shown that in situ gel forming system of amoxicillin was more effective than the suspension due the prolonged gastro-retentive residence time.

Comparision of the gelation and drug release characteristics of different formulation containing derivatized pectin was done which was methoxylated to different degree, i.e. high (31%) and low (9%) over a wide range of pH varying from 1.2 to 5.0. A source of calcium in complex form was also added which released the Calcium on breaking under the acidic conditions. In presence of Ca ions dilute pectin solution (1.5%w/w) undergo gelation due to cross linking of the galacturonic chain, at lower pH, but the gelating tendency of the pectin was reduced at the higher pH due to lack of Ca ions released from complex. Gelation property of the formulation prepared by pectin with 9% degree of esterification was examined in presence of Ca (1.6mM) over pH range of 2.5 to 5.0 and compared to the pectin with 31% degree of esterification. Pectin with low degree of esterification was found to produce the better gelation in comparison to pectin with high degree of gelation. A sustained delivery of the ambroxol was also shown by the formulation containing with Pectin low degree of esterification in gastric acidity controlled rabbits at pH 5.5-5.7 and in situ gelation was confirmed by visual examination. So, Pectin with low degree of esterification had shown to be a potential candidate for producing in situ gelling system for sustained delivery of drugs at gastric pH.

The effect of the varying gastric pH over a pH range of 1-3 was done on gelation of the liquid formulation of pectin and in vitro and in vivo release of the paracetamol and ambroxol was studied from the resultant gels. Formulation was comprised of pectin with complexed Ca in aqueous medium. When such 2+ complex came in the acidic medium Ca were released 2+ which convert solution of pectin to gel. Gel (pectin 1-2%) suitable for the sustained delivery of paracetamol and ambroxol was formed at pH< 3 in vitro. While, very weak gels were produced at pH 3.0, leading to poor sustained release characteristics, in comparison to those, formed at pH 1.2, but no gelation at pH 3.5 was seen. Bioavailability for paracetamol and ambroxol from in situ formed gels were studied in gastric acidity controlled rabbits and visual observation for in situ gelation showed better gelation at both high and low gastric pH. Bioavailabilities for both drugs were found same for gels formed at low as well as high pH.

The chitosan-glyceryl monostearate based in situ gelling system was prerared to produce sustained drug delivery and site specific targeting. The system was prepared by using 3%w/v chitosan and 3% w/v glyceryl monostearate in 0.33M citric acid solution. Formation of in situ gel was performed at biological pH and in vitro release for drugs was performed in Sorenson's phosphate buffer (pH 7.4). Gelation of the system was characterized by studying effect of cross linker, determination of diffusion coefficient and water uptake by thermogravimetric analysis (TGA). Mucoadhesive property of gel was also studied using EZ tester. Studies showed that cross linker had result in the decrease of the rate and extent of the drug release. Glyceryl monostearate had enhanced mucoadhesive property of chitosan to a great extent. This in situ gel system on the basis of study had been reported suitable for both oral as well as parentral sustained delivery of drugs.

Evaluation of the sustained delivery of paracetamol was done via two in situ gel forming formulations. Formulation was basically consists of aqueous solution of gellan gum (1.0% w/v) or sodium alginate (1.5%w/v) containing the calcium ion complex, which in acidic environment causes release of calcium ions. These calcium ions then caused the gelation of the gelling agents (gellan gum or sodium alginate). In vitro studies had demonstrated diffusion controlled release of paracetamol from in situ formed gels over a period of 6 hour. Bioavailability of paracetamol for in situ gel formulated was found to be similar to commercially available suspension of paracetamol.

The gelling property for oral delivery of the cimetidine was evaluated. Formulations prepared were dilute solution of the enzyme treated xyloglucan which form thermosensitive gel on body temperature along with gellan gum and sodium alginate. Complexed calcium ions were also added which on release in the acidic environment form gel on contact with the polymers gellan gum and sodium alginate. In vitro study for cimetidine release was conducted over a period of 6 hour. Plasma levels of cimetidine after oral administration to rabbits were compared with commercially available cimetidine/alginate suspension and in vivo release characteristics were found to be identical with commercial preparation.

COMMERCIAL FORMULATIONS OF IN-SITU POLYMERIC SYSTEMS AT A GLANCE

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

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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.

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.

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.[28]

CONCLUSION

In conclusion, the primary requirement of a successful controlled release product focuses on increasing patient compliance which the in situ gel offers. Exploitation of polymeric in- situ gel 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. The choice of a particular hydrogel depends on its intrinsic properties and investigated therapeutic use. For instance, the formation of a transparent gel is particularly important when ophthalmic application is considered. Non biodegradable gels could prove useful for administration routes other than parenteral. Polaxamer hydrogel perhaps stand for the most extensively studied systems. However, despite the clinical acceptance of poloxamer as solublizer and thickening agents, however, these systems may not be adapted for sustained release of hydrophilic, low molecular weight drugs because their large , water filled pores permit rapid diffusion . PEO/PLGA hydrogels are particularly attractive systems for pharmaceutical applications.

Development of an efficient gastro retentive dosage form for stomach specific drug delivery is a real challenge. So, in order to produce the desired gastro retention various approaches have been employed, out of which, floating drug delivery system has emerged as most promising technique. Floating *in situ* gelling system is one of the approaches of floating drug delivery system which undergo sol to gel transition in acidic stomach conditions and provide stomach specific release of drug for longer duration while being buoyant on the gastric fluid surface. Such systems provide the advantage of better absorption of drugs which are absorbed from the upper part of stomach. As the system remains in the stomach for longer duration local action of drug due to prolonged contact time to gastric mucosa is increased. This leads to less frequent dosing and improved efficiency of treatment. By understanding the floating and gel forming behavior of polymers we can look forward to improve the gastric retention and hence bioavailability improvement of various pharmacologically active agents similarly, good stability and better drug release than other conventional dosage forms make such system more reliable.

Dosage form with prolonged gastric retention and its compatibility with stomach physiology is the real challenge. So in order to achieve gastric retention various approaches have been done from several years. Out of which floating insitu drug delivery system is the most promising technique which undergo sol to gel transition in acidic medium of stomach and provide site specific release for longer duration of time by floating on the surface of gastric fluid, due to which its contact time with gastric mucosa is increased . This results in less frequent dosing and improves patient compliance. Insitu gels are not helpful for sustained drug delivery but also become convenient for pediatric and geriatric patients. Several biodegradable polymers are available with in situ gelling activity. By complete knowledge of floating behavior of biodegradable polymer we can look forward to improve gastric retention and hence bioavailability of pharmaceutical agents. In situ gel have good stability and biocompatibility characteristics and better drug release which make it more reliable dosages from over the conventional one.[30]

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