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An Overview of *In Situ* gelling system

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Abstract

Conventional liquid formulation for drug delivery shows low bioavailability because of rapid gastric emptying time from stomach/ duodenum or rapid precorneal drug loss from eye etc. Hence to improve the bioavailability of drug, *In situ* forming polymeric drug delivery system could be a better option. Intially they are in sol form before administered in the body, but once administered; undergo gelation *in situ* to form a gel. The controlled release of drug molecules via *In situ* forming system has a number of advantages, such as ease of administration, less complicated fabrication, less stressful manufacturing condition for sensitive drug molecules. The constant drug release may be achieved via various polymers (gellan gum, alginates, pectin, xyloglucans, PEO-PPO-PEO, poly (acrylic acid), poly (N-isopropyl acrylamide), chitosan) obeys Fick's law of diffusion which can be better explained by "egg box model". The possible mechanism from which above polymers forms *In situ* gel are: solvent exchange, UV- irradiation, pH change, osmotic pressure change, ionic cross linkage and temperature changes. These polymeric formulations possibly are administered by oral, rectal, ocular, vaginal, injectable and intraperitoneal routes. This article deals with the detail review of polymeric systems, their evaluation, biomedical applications, mechanism of polymer gelation, factor affecting polymers gelation, commercial formulation and their limitations.

Key-Words: In situ gel, Polymer gelation, Gellan gum

Introduction

In spite of various impediments in the bioavailability of orally delivered drugs, oral dosage forms, both solid and liquid, occupy a centre stage in the therapeutic regimen of diseases. Conventional liquid formulations for oral delivery show low bioavailability because of variable gastric emptying time (GET) the physiological state of the subject and the design of the formulation. Due to short residence time of solution in stomach, incomplete drug release from the system take place, which further leads to the poor oral bioavailability. Hence, desired pharmacological effect will not produce. Formulation design of liquid dosage forms has a vital significance in altered bioavailability studies. Thus, there is a need to increase the bioavailability of oral liquid dosage form to get the desired bioavailability and pharmacological effect. Over the past 30 years greater attention has been focused on development of controlled and sustained drug delivery systems. Amongst the extensive research has been carried in designing of polymeric drug delivery systems. The development of *In situ* gel systems has received considerable attention over the past few years (1).

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In situ gel forming liquid oral controlled release formulation is a new technology in the field of controlled drug delivery system. *In situ* gel forming drug delivery systems are in principle capable of releasing drug molecule in a sustained manner affording relatively constant plasma profiles. These hydrogels are liquid at room temperature but undergo gelation when in contact with body fluids or change in pH see figure 1. These have a characteristic property of temperature dependent, pH dependent and cation induced gelation. Compared to conventional controlled release formulations, *In situ* forming drug delivery systems possess potential advantages like simple manufacturing processes and ease of administration. These systems under go sol to gel transition once administered and the drug release take place from the gel matrix at a controlled fashion. The basic objective of this system is to maintain therapeutic level of the drug in body, extend the duration of drug action, reduces the dosing frequency and minimizing the adverse effect.

Sol to gel conversion occur within the body due to various physical and chemical stimuli's like temperature modulation, solvent exchange, pH change, presence of ions and light rays (2-4).

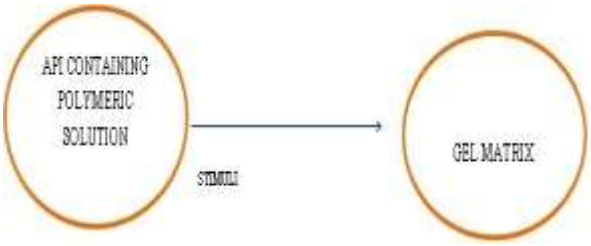
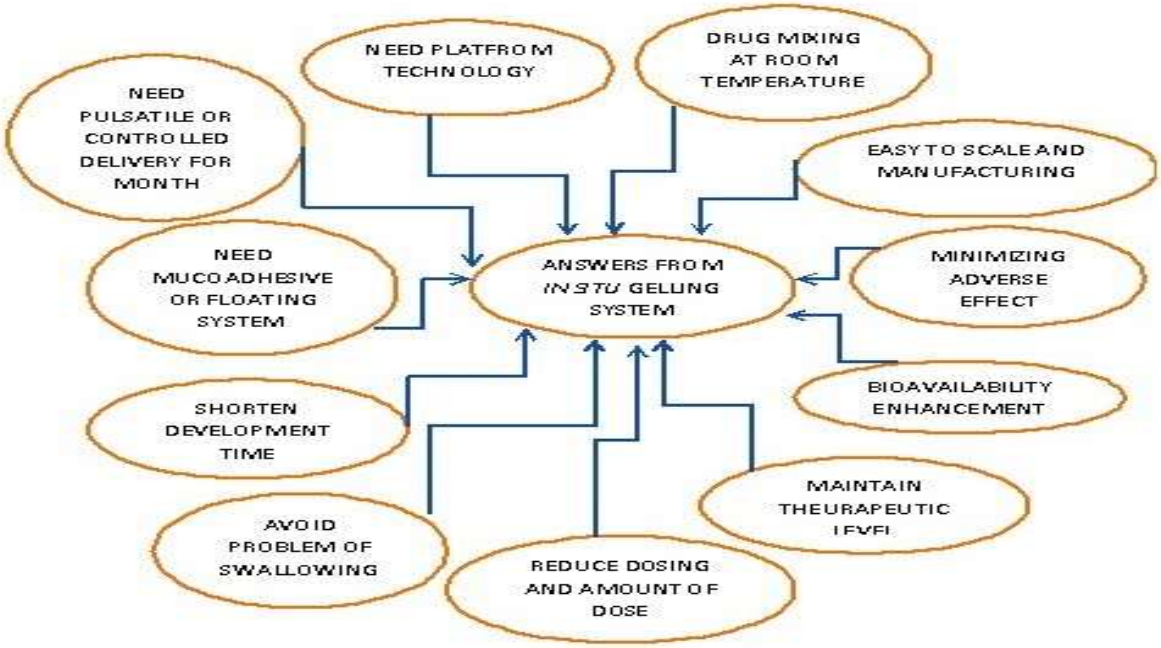


Fig 1: *In situ* gel formation

In situ gel forming systems that do not require organic solvent or co-polymerization agent have gained huge popularity (5). The concepts of *In situ* gel forming devices (ISFD) come in the pharmaceutical field in the early 1980's as parenteral controlled release dosage form. In last decades, these systems were popular due to its vital advantages in oral controlled drug delivery as compare to traditional parenteral drug delivery system.

Polymer gels are produced by cross linking of polymer chains by the formulation of either covalent bond (chemical cross linking) or non-covalent bonds. Non covalent bonds for example, can be hydrogen bonds or ion bridges, the latter being common in the gelation of polyelectrolyte (6).



Various literature of *In situ* gel has been reported for various clinical disorders like glycemia, rhythmic heart disease, carcinoma etc. For these clinical problems, *In situ* gel system was developed in such a way that control drug release can be obtained by incorporating a biosensor or smart polymer in the gel structure which response to the environmental stimulation (2). Gels containing these biosensors will undergo reversible sol-gel phase transition upon minute changes in environmental condition (7). Various drawbacks of liquid dosage forms that were overcome by *In situ* gel system were diagrammatically represented in Figure 2.

Fig. 2: Answers from *In situ* gelling system

Apporaches of In -Situ Gel drug delivery

In situ gelling system is gaining importance for various drug deliveries. The flexibility of these system permits its delivery via various routes such as oral, nasal, rectal, ocular and parental. Different polymers used for phase transition from sol to gel have different mechanism of action and varies with different physiological conditions, listed in (table 1.). Some broad approaches for sol-gel conversion are mentioned below:-

- The *In situ* gel formation can also be possible by the use of natural (eg. Gelatine) and synthetic polymers (poly (ethylene oxide)-poly(propylene oxide)- poly(ethylene oxide)), Which after administering into the body get converted to gel form in response to the physiological stimuli of temperature and pH (8-11).

- *In vitro* gel formation is initiated by polymerization reaction in presence of multifunctional monomers. These monomers were incorporated in growing polymeric chains which lead to formation of three dimensional covalently cross linked networks (12). The example of this type of system is the *In situ* gel of cyanoglycolate adhesive (13).

- *In situ* gel formation can also be triggered by the physical changes in biomaterials such as solvent exchange. This approach consists of dissolving a water- insoluble polymer in a water-miscible biocompatible solvent. Upon contact with body fluids, the solvent diffuses out of the polymer while water permeates the liquid polymer matrix. Due to its insolubility in water, the polymer precipitates, resulting in the formation of a solid polymeric implant. (14-18).

- *In situ* gel formation can also be initiated by the cross linking of soluble linear polymer or macromonomers. Typically cross linking was initiated by chemicals such as glutaraldehyde (cross linking agent) (19, 20) or by chemical reaction such as enzymatic reactions and also by photo initiated polymerization (21).

Table 1: Polymers used for different type of *In situ* based system

SYSTEM	MECHANISM	POLYMERS USED
TEMPERATURE SENSITIVE SYSTEM	LCST*	Poly(N-isopropylacrylamide) (PNIPAAm), (PEO-PPO-PEO).
	UCST**	poly (acrylic acid) (PAA), Poly (acrylamide)(PAAm).
	THERMOREVERSIBLE	PEO-POLY (L-Lactic acid)-PEO.
pH SENSITIVE SYSTEM poly electrolytes	Weakly acidic	Poly (acrylic acid)
	Weakly basic	poly(N, N'-diethylaminoethylmethaacrylate)(PDEAEM)
ELECTRICAL SIGNAL SENSITIVE	Pulsatile current	poly (2-acrylamido-2-methyl propane sulfonic acid-co-n-butyl methacrylate)
LIGHT SENSITIVE SYSTEM (heat)	Ultra violet light sensitive	-
	Visible light sensitive	Poly(N-isopropylacrylamide)
PRESSURE SENSITIVE SYSTM	Osmosis	Myverol 18-99
	Diffusion	N-methyl pyrrolidone
CHEMICAL STUMULI BASED SYSTEM	Ions and pH dependent	Gellan gum, Alginates, Pectins

*LCST lower critical solution temperature, **UCST upper critical solution temperature

In situ gels are solution which after delivered in body they form gel or get solidified within the desired tissue, organ or body cavity. From last few years the popularity of *In situ* gel was increasing in various pharmaceutical fields such as dug delivery, cell encapsulation, tissue repairing etc... *In situ* gelling system is useful for various pharmaceutical and non-pharmaceutical purposes (eg... jam, jelly).

Mali and Hajare formulated the occular *In situ* activated gel forming system and according to them *In situ* gel forming system are those which can deliver drug in a solution form, create little to no problem of vision and need be dose no more frequently once or twice daily. When they exposed to physiological condition they will shift to a gel phase (22).

According to rathore *In situ* activated gel forming system seems to be favoured as they can be administered in drop form and produce appreciably less inconvenience with vision. Moreover, they produce better sustained release properties then drops (23).

Advantages of *In Situ* gelling systems

The controlled release of drug molecule via *In situ* forming system has a number of advantages such as:-

- Ease of administration.
- Less complicated fabrication.
- Less stressful manufacturing condition for sensitive drug molecule.
- Prolonged gastric retention time that improves bioavailability.
- Reduced dose dumping.
- Improves solubility of drug that is less soluble in a high pH environment.
- It is conveniently dropped as a solution into the conjuctival sac, enhancing patient compliance and minimizing interference with blinking of eyes.
- With the help of *In situ* implants, delivery of drugs was extended from several days to months.
- It should be used for site specific drug delivery by employing various external physical, chemical and biological stimuli.

- Gastric retention may further extended to longer period of time via floating drug delivery system (24) or via muco adhesion.
- The novelty of *In situ* gelling system is in its versatility (Platform technology) of sustaining the release of both hydrophobic and hydrophilic drug (25).
- *In situ* gel has advantage of targeting the drug molecule to the specific cells producing various enzyme, protein etc. With the help of various polymers.

Limitations of *In Situ* gel

These systems may prove to be of little benefit for drugs having solubility or stability problem in the gastric environment or those that are irritant to the gastric mucosa. Drugs that are well absorbed along the entire GIT and those that undergo extensive first pass metabolism may not be suitable for formulating as *in situ* gelling systems as the slow emptying limits the systemic bioavailability.

The gelling time should be slow enough to allow the system to reach the stomach and fast enough to form gel before expulsion from the stomach. Syneresis, observed in gellan gum and alginate gels may result in concentration of gels along with an increase in the drug concentration as was found in the case of theophylline (26). The next limiting factor is related to the stability of the gels as formulations. The natural polymer solution is highly prone to bacterial and fungal contamination and carries a high risk of changes in the properties if stored beyond 6 months.

Another route proposed like parenteral delivery where polymer complexes have ability to undergo sol to gel transformation in response to change in temperature, pH, and solvent concentration can lead to an *In situ* forming delivery system (17). Sometime this route is painful because thermoplastic copolymer has higher melting point above 60°C so at the time of injection it may cause scar formation or tissue necrosis.

The *In situ* formulation of non-steroidal anti-inflammatory drugs (NSAIDs) has the drawback of gastric irritancy and first pass metabolism thus the topical delivery of (NSAIDs) has been explored as a potential method of avoiding the first pass effects and the gastric irritation. (27). Some depot forming polymers such as polaxamer 238, polaxamer 407 displayed muscular irritancy or toxicity comparable to that of traditional intra muscular (IM) vehicles, such as saline and peanut oil (28).The contribution of various researchers for *In situ* gelling system is listed in table 2.

Table 2: Contribution Table

POLYMER	MODEL DRUG	CONCLUSION REMARKS	REFERENCE
Poloxamer 407	Recombinant human growth hormone	Controlled release of human growth hormone following intramuscular or subcutaneous administration.	29
Physically cross linked dextran	Recombinant human interlukin-2	Release of drug of over period of 5 days with excellent biodegradability.	31
Poloxamer 407	Insulin	Subcutaneous delivery of peptides and proteins having short half-lives.	32
Xyloglucan	Pilocarpine	In vitro release of pilocarpine from gel follow root-time kinetics over a period of 6 h.	33
Human serum albumin and tartaric acid derivatives	Doxorubicin	Sustained release of drug for approx 100 hrs.	34
Gellan gum	Theophylline	3 to 5 times enhancement in bioavailability then conventional market formulation.	35
Low Methoxy Pectin	Paracetamol Ambroxol	pH<3 suitable for gelation, very Weak gel formed at pH-3 resulting in poor suistained release characteristic.	36, 37
Chitosan and Glycerylmonooleate	LidocaineHCl Cimitidine Ketoprofen Dexamethasone	Mucoadhasive property of the gel evaluated. Usefull via oral as well as parenteral routes.	25
Poly lactic acid	Testosterone	A controlled zero order in vitro release	38

		was observed.	
Pheniramine maleate and albumin	Polyacrylic acid and poly methacrylic acid	Sustained delivery of pheniramine for 2 days and of albumin FITC for 5 days.	17
Clotrimazole	Poloxamer 188	Prolonged antifungal effects using an *In situ* gelling and mucoadhesive vaginal gel.	39, 40

Formulation methodology

In situ gelling system was prepared by very efficient method. It is flexible for both hydrophilic and lipophillic drugs (25). It can be formulated with different polymers, and by incorporating different excipients it can be used as novel system such as floating system (24), mucoadhesive system (41) which can give the controlled release or site specific drug delivery (42). They can be formulated by dissolving different polymers (gellan gum, sodium alginate, pectin etc.) concentration in deionized water containing different concentration of sodium citrate (complex forming agent) at 90°C with continuous stirring. Then this polymeric solution was allow to cool below 40°C and then drug, calcium chloride (source of Ca^{++} ions) and other excipient like calcium carbonate(floating agent) was added.(43, 26, 24, 44).

The solvent exchange approach for *In situ* gel / implant formation consist of dissolving a water insoluble polymer in a water miscible, biocompatible solvent. Upon contact with body fluids, the solvent diffuses out of the polymer while water permeates in the liquid polymer matrix. Due to its insolubility in water, the polymer precipitates, resulting formation of an *In situ* gel (5).

Mechanisms for polymer gelation

In *In situ* gelling systems, polymers are used for sol to gel conversion and their mechanism of gelation is more or less similar. This system may be administered via one or different routes and the mechanisms of gelation may vary with the routes of administration. The detailed mechanisms for polymer gelation are discussed below:-

Sol – Gel conversion based on physiological stimuli

Temperature sensitive system

Some polymers undergo abrupt changes in solubility in response to environmental temperature. The ideal system should be in solution form which is free flowing, injectable liquid at the ambient temperature; and it should form gel at body temperature with minimal syneresis.

In situ gelling system should be formulated in such a way that it should be able to recognize the small temperature difference between oral cavity or appendages at the surface of skin and physiologic or ambient body temperature. So that effective gelation would be possible at proper site of action.Temperature has a vital role in sol-gel conversion, different polymers behaves in a different way on the application of temperature.

Some polymer undergoes abrupt changes in solubility in response to increase in environmental temperature (Lower critical solution temperature, LCST) eg... Negative temperature sensitive gel, they have a LCST and contract upon heating above the LCST (45, 46). This phase separation is governed by balance of hydrophilic and hydrophobic moieties on the polymer chain and the free energy of mixing (47). Free energy varies with enthalpy, entropy and temperature.

$\Delta G = \Delta H - T\Delta S$

As the positive enthalpy term (ΔH) is smaller than entropy term (ΔS), an increase in temperature results in a larger $T\Delta S$ making ΔG negative and favouring polymer chain association (5, 48).

Some molecular interaction depends on temperature such as hydrogen bond formation and hydrophobic effects they contribute for phase separation. At, LCST hydrogen bonding between polymer and water becomes unfavourable as compare to polymer-polymer and water-water interaction. At the temperature above LCST sudden transition occurs, so that the solvated polymer quickly changes into hydrophobic structure shown in figure 3. (50, 51). Eg.:-Poly(N-isopropylacrylamide)(PNIPAAm), poly(ethyleneoxide)-poly(propyleneoxide)- poly (ethyleneoxide) (PEO-PPO-PEO).

HEATING ABOVE LCST

Fig. 3: *In situ* gel formation above LCST

Some polymer form positive temperature sensitive gel which has an upper critical solution temperature (UCST). Such solution contracts upon cooling below the UCST to form gel (figure 4.).

COLLING BELOW UCST

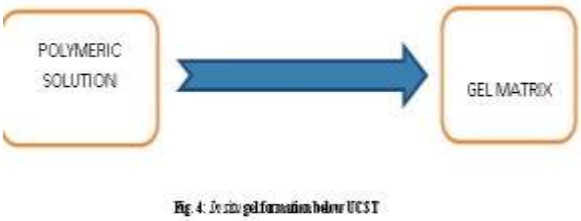


Fig. 4: *In situ* gel formation below UCST

The melted polymer solution was injected in the body that form depot upon cooling to body temperature. The requirement of polymers for such system include low melting, glass transition temperature in the range of 25 -65°C and intrinsic viscosity in the range of 0.05 – 0.8 dl/g. At the time of application the temperature of polymer solution should be above 37°C but below 65°C.This polymeric solution behaves like viscous fluid which solidify to highly viscous depot at the body temperature but it is very painful for the patient and the high temperature increases the chances of necrosis and scar tissue formation at the site of injection.eg poly (acrylic acid) (PAA), Poly (acrylamide) (PAAm), Poly (acrylamide-co-butyl methacrylate) (2).

Despite of this, some polymer form thermoreversible gel, and the most commonly used thermoreversible gel are pluronics® and tetronics®. For parenteral application of thermoreversible gel, they should be biodegradable. So that for enhancement of biodegradability PPO segment of PEO-PPO-PEO was replaced by poly (L-lactic acid) segment (52-54).

pH sensitive system

Polymers of this system contain acidic or basic pendant groups which either accept or release the proton in response to changes in environmental pH. Some polymers were known as "polyelectrolytes" because they contain large number of ionisable groups (2). Swelling of polymer increases as the external pH increase in case of weakly acidic (anionic) groups eg: - poly (acrylic acid), but decreases if polymer contain weakly basic (cationic) groups eg :- poly(N, N'-diethylaminoethyl methaacrylate)(PDEAEM).

The basic action for the swelling of polymer is ionization. The cationic polyelectrolytes such as PDEAEM cross linked at low pH due to ionization and thus form gel, whereas polyanions such as PAA dissolve or swell more at high pH and form gel.

The pendant acidic or basic groups in polyelectrolytes undergo ionization just like acidic or basic group of monoacids or monobases.

Firestone reported that the swelling of polyelecrolytes is mainly due to the electrostatic repulsion among charges present on the polymer chain and the extent of swelling is influenced by the presence of comonomer such as 2-hydroxyethyl methacrylate, methyl methacrylate and malic anhydride they act as a counter ion that reduces electrostatic repulsion. Electrostatic repulsion can also further be reduced by the change in pH and ionic strength. Different comonomer behaves in different way leading to different pH sensitive behaviour to form a gel (2, 55).

Sol gel conversion based on physical stimuli

Electrical signal sensitive system

Electro sensitive gels have been applied in control drug delivery system. These gels were usually made up of polyelectrolytes, as are pH sensitive gels. They undergo shrinking or swelling in the presence of an applied electric field. When potential is applied, hydrated H^+ ions migrated towards the cathode resulting in loss of water at the anode site. At the same time, electrostatic attraction of negatively charged group towards the anode surface creates a uniaxial stress along the gel axis; mostly at the anode site for eg. Gels made of poly (2-acrylamido-2-methyl propane sulfonic acid-co-n-butyl methacrylate) were able to release endrophonium chloride and hydrocortisone in pulsatile manner using electric current (56). Control pulsatile delivery was achieved by varying the intensity of electric stimulation in distilled or deionized water.

Light sensitive gels

They have potential application in ophthalmic drug delivery system. Light sensitive gels have advantages over other systems because use of temperature sensitive gel is rate limited to thermal diffusion, while pH sensitive gel can be limited by H^+ ion diffusion. Light sensitive gel can be separated into UV sensitive and visible light sensitive gel.

The UV sensitive gels were synthesized by introducing leuco derivative (light sensitive agent) molecule into the polymer network (57). At fix temperature, the gel discontinuously swelled in response to UV irradiation but shrinks when the UV light was removed. The UV light induced swelling was due to an increase in osmotic pressure within the gel it is because of the appearance of cynide ions formed by leuco derivative on irradiation of UV.

Visible light sensitive gel prepared by light sensitive chromophore (eg tri sodium salt of copper chlorophylline) to poly (N-iso propylacrylamide) polymer (58). On irradiation of visible light,

chromophore absorbed light which dissipated locally as heat which increases the temperature of polymer. The increased temperature alters swelling of poly (N-iso-propyl acrylamide) polymer to form temperature sensitive gel. The increment of temperature is propotional to light intensity and the chromophore concentration. The potential application of visible light responsive gel for temporal drug delivery was also reported (59).

For this type of system, long wavelength UV and visible light were used. Short UV rays have limited penetration to tissue and are harmful for skin. A ketone, such as 2, 2 dimethoxy-2-phenyl acetophenone is used for UV, whereas camphroquinone and ethyl eosin initiator used for visible light (60). They can be designed both for immediate and sustained drug delivery system. Sawhney et al reported the photopolymerizable biodegradable gel as a tissue contacting material and a controlled release carrier (61).

Pressure sensitive system

In this system *In situ* gel was formed by the absorption of water from the outer surrounding medium. Eg;- myverol 18-99 is polar lipid that swells in presence of water to form lytropic crystalline *In situ* gel structure (62, 63). It is also called as osmosis.

Whereas in diffusion, solvent from polymer diffuses out to the surrounding tissue which results in precipitation or solidification of polymer matrix. Eg: - N-methyl pyrrolidone (64).

In situ gel based on sound and magnetic field has also been reported by bouralis et al (65).

Sol gel conversion based on chemical stimuli

Various polymers undergo precipitation of inorganic solids via enzymatic process such as pH and ions from biological fluids and form *In situ* gel.

The solution of polymers such as alginate, gellan, pectin etc. contains divalent ions. At the formulation time, sodium citrate was added in the prepration, which initially form complex with polymer. This complex was break down in highly acidic environment of the stomach to release free divalent ions (Ca^{++}). Whereas, gelation involves the formation of double helical junction zone followed by aggregation of double helical segments to form three dimensional networks by complexation with cations and hydrogen bonding with water (66-69).

Gelation is better explained by the egg box model that explained chain-chain interaction (70). When calcium ions are added to a sodium alginate solution, such as aligment of the G-blocks occurs, and the calcium ions are bound between the two chains like egg in egg box.

Sol gel conversion based on the biochemical stumuli

They formed gels due to change in blood glucose level (22, 65), or due to specific antigen (71) or due to thrombin (72). These are self-regulated system. They require the sensing ability and an automatic shut off mechanism.When glucose is oxidized to gluconic acid with the help of enzyme called glucose oxidase, the change in environmental pH in the body may occur. If we use pH sensitive polymer (for insulin release) in this system then pulsatile drug release was achieved.If system made up of polycations, such as PDEAEM, lowering of pH results the swelling of membrane due to ionization of PDEAEM and swelling may cause release of insulin (73).

If gel made up of polyanions then it was grafted with porous filter and immobilized with glucose oxidase. The grafted polyanions chains are expanded at pH 7 due to electro static repulsion among the charges on the polymer chains. When pH gets lowered due to conversion of glucose to gluconic acid by glucose oxidase, the chain gets collapse due to the protonation of the carboxyl groups of the polymer (74). An antigen antibody semi interpenetrating network was prepared by grafting an antigen and corresponding antibody to different polymer network (71). Gel was formed in the presence of free antigen that competes with the polymer bound antigen. Thrombin induced infection responsive gel was prepared by attaching gentamycin chemically to the polymer backbone through peptide linkers that can be enzymatically degraded by thrombin (72). Different mechanism of gelation's shown diagrammatically in figure 5.

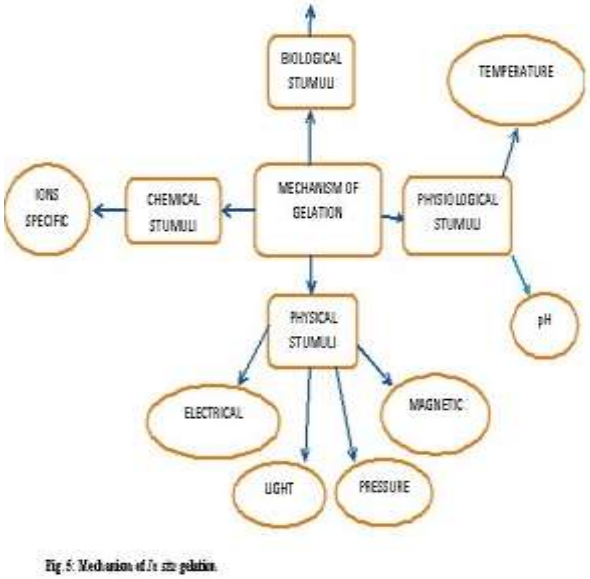


Fig. 5: Mechanism of *In situ* gelation

Based on the above mechanisms some widely used polymers for *in situ* gelation were discussed below:-

Gellan gum

Gellan gum is a water soluble polysaccharide produce by *pseudomaonas elodea* (75). Various brand name of gellan gum are applied gel, Phytagel or Gelrite™ or Kelcogel™. The repeating unit of the gellan gum is a tetrassacharide which consist of two residues of L-rhamnose and D-glucouronic acid. The tetrassacharide has the following structure:-

((D-Glc(β1→4)D-GlcA(β1→4)D-Glc(β1→4)L-Rha(α1→3))$_n$.

It is observed from the formula of the tetrasacchride; that the units are connected by (α1→3) glycosidic bond. Gellan gum is an anionic deacetylated exocellular polysaccharide (35). It has a tendency of gelation which is temperature dependant and cationic induced (76). Gellan gum forms a coaxial triangular three fold double helix structure (pitch 56.4Å) from two left handed chains coiled around each other with acetate residues on the periphery and the glyceryl groups stabilizing the interchain association. Hydrogen bond formed between the hydroxyl methyl of 4- linked glucosyl unit of one chain and carboxylate groups of other. These gelation involves the formation of double helical junction zones followed by aggregation of the double helical segments to form a three dimensional network by complexation with cations and hydrogen bonding with water (67, 69). When administered orally, the calcium ions from calcium chloride were released in the acidic environment of stomach leading to gelation of gellan gum thus forming *in situ* gel.

Alginates

Alginates are widely used natural polysaccharide polymers isolated from brown seaweed (phaeophyceae). They are used because of their non-toxicity, biocompatibility and biodegradability (77). 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 (78, 30, 79). Gelation of dilute solutions of sodium alginate occurs on addition of Di and trivalent metal ions by co-operative process involving consecutive G- residues in the α-L glucuronic acid blocks of the alginate chain in a manner described by the "egg box" model (66). Alginates can also be used as a vehicle for ophthalmic formulation, since it exhibits favourable biological properties such as biodegradability (77). The homogeneous blocks of of alginic acid are separated by blocks made of random or alternating units of mannuronic and glucuronic acid (80). They undergo proton catalyzed hydrolysis which depends on time, pH and temperature.

Chitosan

Chitosan is a biodegradable, thermosensetive, polycationic polymer obtained by alkaline deacetylation of chitin a natural component of shrimb, crab shell and cell wall of bacteria and mushroom (30, 81, 82). Chitosan is metabolized by lysozymes and it has moderate immune stimulating effects with low allergenic property (83). Chitosan is stable crystalline and typically not soluble in water. Chitosan accumulate positive charge in an acidic aqueous media, which protonates amino group and thereby overcoming the associative forces between chains. Chitosan remains in solution form upto pH 6.2, and by exceeding pH 6.2 it will form a hydrated gel like precipitate. (81). The gel formed due to removal of repulsive interchain electrostatic forces (amino group) by neutralization of amino group, which allows extensive hydrogen bonding and hydrophobic interaction (1, 84). When pH of acidic chitosan solution was changed to alkaline pH it may lose its charge and form viscous gels (85).

Pectin

Pectin are natual polysaccharide found in plant cell walls, they are linear connected by α-(1-4)-D-galacturonic acid residues, which have carbonyl groups. High methoxy gelation and low methoxy gelation are two gelation characteristics of pectin. It has been suggested by Oakenfull that hydrogen bonding and hydrophobic interactions are important forces in the aggregation of pectin molecules (86, 87). Gel is formed by hydrogen bonding between free carboxyl groups on the pectin molecules and also between the hydroxyl groups of neighbouring molecules. When acid is added, the carboxyl ions are converted to mostly unionised carboxylic acid groups. This decrease in the number of negative charges that not only lowers the attraction forces between pectin and water molecules, but also lowers the repulsive forces between pectin molecules. Sugar also further decreases the hydration of the pectin by competing for water. These conditions further decrease the ability of pectin to stay in the dispersed state and hence forms gel.

The rate at which gel formation takes place is also affected by the degree of esterification (DE). The higher DE causes more rapid setting. Rapid-set pectins (i.e. pectin with a DE of above 72%) also gel at lower soluble solids and higher levels than slow-set pectins (i.e. pectin with a DE of 58-65%).

LM-pectins (low methoxy) require the presence of divalent cations (usually calcium) for proper gel formation. The mechanism of LM-pectin gelation is based mainly on the well-known 'egg-box' model (70). Furthermore, amidation increases or improves the

gelling ability of LM-pectin: amidated pectins; need less calcium ions to form gel and are less prone to precipitation at high calcium levels (86, 88).

Xyloglucan

Xyloglucan polysaccharide derived from tamarind seeds is composed of a (1-4)-β-D-glucan backbone chain which has (1-6)-a-D-xylose branches that are partially substituted by (1-2)-b- D-galactoxylose (89, 33). The xyloglucan derived from tamarind seeds is composed of three units of xyloglucan oligomers with heptasaccharide, octasaccharide and nonasaccharide, which differ in the number of galactose side-chains. When xyloglucan derived from tamarind seed is partially degraded by β-galactosidase, the resultant product exhibits thermally reversible gelation and the sol to gel transition is temperature dependent with the degree of galactose elimination (90). xyloglucan forms gel at much lower concentration. Such gelation does not occur with native xyloglucan. Xyloglucan gels have potentially been used for oral, intraperitoneal, ocular and rectal drug delivery (89, 91, 92). Xyloglucan form thermally reversible gel at body temperature. Chilled solution of xyloglucan is administered to slower the gelation time (minutes) of polymer.

Xyloglucan has broad application in drug delivery system since its gelation does not require the presence of H + ions and its use is not restricted by the nature of the drug but gellan gum containing formulation have drawback with certain drug salts that may cause gelation before administration or in vitro gelation (33).

Carrageenan

They are marine hydrocolloids obtained by extraction from some membranes of the class Rhodophyceae. It is a polysaccharide made up of repeatative sequence of disaccharide β-D galactopyranose linked 1, 3 called A-residue and α-D galactopyranose residue linked through positions 1, 4 called B-residue. The gelling of carrageenan is caused by helix formation and this can only be possible with the repeat structure where B residue is in 1-C-4 conformation. Carrageenan is of three type ie.. κ-, ι- and λ carrageenan they consist of sulphated esters of d-galactose and 3, 6- anhydro-d-galactose; copolymers linked with A-1, 3 and B 1, 4 in the polymer.

All the gelling type of carrageenan ie.. κ, β and ι carrageenan contain 3, 6 anhydro bridge on the β- unit which helps in formation of crosslinked networks and gels by initiating the sugar to flip from the 4-C-1 conformation. κ- Carrageenan has poor freeze thaw stability because it forms a firm clear, brittle gel. But ι-carrageenan forms junction zones in soft elastic gels with having good freeze thaw stability. λ- Carrageenan is non-gelling due to lack of 1-C-4 conformation. Thus, 3, 6 anhydro links allows the galactose residue to revert to their 4-C-1 conformation. So double helix structure was not formed which is required for gelling (93). It is used as gelling and thickening agent (94).

Pluronics® (Poloxamer)

They are tri block copolymer composed of PEO (a) and PPO (b) units. They consist of more than 30 different non-ionic surface active agents. Chemically they are oxirane, methyl polymer with oxirane or α- hydro-ω-hydroxypoly (oxyethylene) a block copolymer. They are available in various grades based on its molecular weight and physical form. The grades assigned for pluronics are F, P and L for flakes, paste and liquids respectively. In pluronics central PPO (hydrophobic) block is surrounded by PEO (hydrophilic) blocks (95). When this molecule immersed into the aqueous solvents, they form micellar structure above critical micellar concentration and also at the body temperature due to PPO block dehydration (96, 97). With increasing temperature, the micellization becomes more important, and at a definite point, micelles come into contact and further did not move. In addition the formation of highly ordered cubic crystalline structure may be the driving force for gel formation (98, 99). Pluronics F-127 used with mucoadhesive polymers such as carbopol 934 and HPMC to ensure long residence time at the application site.

Synthetic polymers

They should prepare properly under GMP condition from monomer unit, so that when it will administered it will not produce any type of inflammation and adverse effect. Synthetic polymer was used in different type of systems like thermoplastic, photopolymerizable, thermosetting, thermosensitive systems etc. they are biefly explained below:-

The delivery of thermoplastic triblock copolymer paste (poly (D, L-lactide)-block-poly (ethylene glycol)-block-poly (D, L-lactide)) is very painfull. Because, the melting point of these polymer is more than 60°C and on cooling to the body temperature it may form gel. So that at the time of injection, temperature of paste is at least 60°C which may cause scar formation or tissue necrosis (100). Synthetic polymers are also used in the photopolymerizable system that remains in the solution form but by irradiation of the light source they form gel. They need a macromer (PEG-oligoglycolyl-acrylate), a photosensitive initiator (eosine dye) and a light source for gel formation. These systems can be used to release water soluble drugs and enzymes at a controlled rate.

When sol to gel transition occurs in a thermosetting system it is called as curing. Thermosetting system used biodegradable copolymers of DL-lactide or L-lactide with ε-caprolactone for prosthetic implant and slower the release of drug delivery rate (101).

Poly (NIPAAM), poly (N-isopropyl acrylamide) is an example of thermosensitive polymer used for *In situ* gel formation. It has lower critical solution temperature phase separation at about 32°C (102). They are triblock copolymers consisting of poly (oxyethylene) and poly (oxypropylene) units that undergo solubility changes with changes in environmental temperature.

Drug release kinetics from gels

The *In situ* gelling system should be designed in such a way, so that its control drug release was achieved throughout its residence time and these may enhance bioavailability of the formulation (1). Most gels that are used in pharmaceutical application consist of typically 1% polymer and 99% water. For low molecular weight drug the resistance produced by polymer network was very less to diffuse out of the gel. The drug release from polymeric gel matrix may involve the penetration of water in gel and simultaneous release via diffusion, as governed by Fick's law (103). The drug release from this system depends on water amount and its swelling properties (104). It has been reported that the drug release from gels were linear after a short lag period that indicates the diffusion controlled release of drug (66, 35, 105). Different *in situ* gelling systems with their polymers used are listed in (table 3.)

Table 3: Different *In situ* gelling systems with their polymers

Systems	Polymers
pH Triggered System	Cellulose acetate phthalate(CAP) latex, carbopol, poly methacrylic acid(PMMA), polyethylene glycol(PEG), pseudolatexes.
Temperature dependent system	chitosan, pluronics, tetronics, xyloglucans, hydroxyl propyl methyl cellulose or hypromellose(HPMC).
Ion activated systems	gelrite, gellan, hyaluronic acid, alginates.
UV induced system	poly (N-iso-propyl acrylamide gel).

Routes of administration of *In Situ* gelling system

Various natural and synthetic polymers are used for formulation of *In situ* forming drug delivery system. Depending on the routes of the administration this *In situ* polymeric system may be classified as below:-

***In situ* gel delivery via oral routes**

Various natural polymers such as pectin, xyloglucan and gellan gum were reported for *In situ* forming oral drug delivery system. Miyazaki *et al.* developed *In situ* gelling gellan formulation for oral administration of 1% w/v aq. solution of gellan gum to the rats and rabbits and evaluated as sustained release vehicles. The *In vitro* release of theophylline from the rigid gellan gels followed root time kinetics over a period of 6 hr (35).

Ganapati *et al.*, developed a floating *In situ* gelling liquid formulation for control drug delivery of ranitidine (H_2 blocker) by using sodium alginate as a *In situ* gelling polymer (77).

***In situ* gel delivery via occular routes**

Ocular route is preffered for various compounds like antimicrobial, anti-inflammatory and autonomic drugs and also to overcome bioavialibility problems (106). Miyazaki *et al.*, formulated *In situ* gels for ocular delivery of xyloglucan, these polymeric systems were observed to show a significant mitotic response for a period of 4 hr when instilled in to the lower cul de sac of rabbit eye (33).

Besides these polymers, alginic acid can also be used for ophthalmic drug delivery because of its bioavailabilty and non-toxicity. Alginic acids have additional property of mucoadhesion along with long residence time (107, 108).

***In situ* gel delivery via rectal and vaginal routes**

For better therapeutic efficacy and patient compliance, a muco adhesive thermosensitive, prolonged release vaginal gel incorporating clotrimazole-β-cyclodextrin complex was formulated for the treatment of vaginitis (109). Pluronics along with Carbopol and HPMC is used to ensure the prolong drug release vagina. *In vitro* drug release shows that the antimycotic efficacy of formulation for longer period of time.

***In situ* gel delivery via injectables**

Injectables of *In situ* forming gel have received considerable interest in the past decade. Various natural and synthetic polymers were used for injectable

deliveries of *In situ* gel. Basic criteria for selection of polymer is to deliver from parentral route are that it should be biodegradable, nontoxic and doesn't produce any anaphylactic reaction or inflammation at the site of application. Drug deliveries via this route is act as implant in the body and slowly release the drug from hours to months depending on the polymer used for drug delivery.Various polymers, like thermosetting, thermoplastic, precipitating polymeric system, photopolymerizable systems etc. were used to deliver the drug effectively through this route. Biotechnological advancement causes the development of the labile macromolecular therapeutical agents that require a complex formulation for their efficient administration (110). Vegitable oil and biocompatible hydrophilic solvent form a injectable *In situ* forming organogel when mixed with N-stearoyl L-alanine (m) ethyl esters. Leuprolide loaded organogel when given subcutaneously may release the drug for 14 days to 25 days.

***In situ* gel delivery via nasal route**

Various polymers like gellan gum, xanthum gum, carbopol etc were used to deliver the drug via nasal delivery in the form of *In situ* gel. Park *et al.*, developed a intranasal *In situ* gel containing plasmid DNA as a new route of delivery for therapeutic genes and DNA vaccines. To improve the intranasal absorption of plasmid DNA, they designed delivery systems composed of *In situ* gelling and mucoadhesive polymers. Poloxamers (Pol) were used to provide *In situ* gelling property. Polycarbophil (PC) or polyethylene oxide (PEO) was also used as mucoadhesive polymers (111).

Factors affecting *In Situ* gelling system

There are various formulations and physiological factors which affect formulation efficacy such as viscosity, drug release, *in situ* gel formation and stability of the preparation, they were discussed below.

Concentration of polymers

For *In situ* gelation polymers like gellan gum, chitosan, pectin, sodium alginate has been used. When we increase the polymer concentration the gel strength is also increased due to increasing chain interaction and this forms the brittle gel. Whereas by lowering the polymer concentration, gels were formed softer and these may result the burst release of active ingredients and failure to achieve sustained effect. The concentration of polymer should be optimized in such a way that, high concentration may produce gel of acceptable gel strength and an enough low concentration which may form gel of acceptable viscosity for ease of swallowing (112).

It is reported that all concentration of polymers used showed shear thinning behaviour, the effect being most pronounced at higher concentration and sols showed a marked increase in viscosity with high concentration of vehicle. The gelation temperatures of the formulations decreased by addition of increasing concentrations of Carbopol (ie, from 29°C for 18% PF127 to 23.9°C for 18% PF127, 0.5% Carbopol)(113).

Chemical structure of polymer

The conversion of polymer to gel is also depends on the chemical structure of polymer. For example it has been suggested by Oakenfull that hydrogen bonding and hydrophobic interaction are important forces in the aggregation of pectin molecules. Gel formation is caused by hydrogen bonding between free carboxyl groups on the pectin molecules and also between the hydroxyl groups of neighbouring molecules (87).

Amide group in the polymer i.e. amidation increases or improves the gelling ability of polymer as in case of LM-pectin: amidated pectin needs less calcium ions for gel formation and is also less prone to precipitation at high calcium levels (88).

Temperature

In situ gel forming polymer which is based on temperature is also called as temperature sensitive polymer. They have disadvantage of *In vitro* gelation, if proper temperature condition is not maintained or if not stored at specific condition. They undergo sol to gel conversion specifically at the body temperature. Temperature may possibly affect the stability of the sol state of polymer and sometime premature gelation may occur.

In case of pectin, gel strength increases with increasing Ca^{++} ions concentration but reduces with increment in temperature and acidity (pH<3).

Viscosity of solution

Viscosity of solution should be good enough so that fluidity of formulation will maintained properly and will easily pour out from the bottle or pass through the syringes. If viscosity of the solution will not maintain properly, the problem of swallowing may occur.

pH

Drug with pH dependent solubility are not suitable candidate for *In situ* gel formation but it is desirable for those drugs; that are having increased solubility in an acidic atmosphere. pH plays vital role for the formation of the gel at the various site of body. At specific pH polymers form gel by cross linking of the polymeric chain with divalent ions (43). Divalent ions were present in the formulation as a complex with sodium citrate. The complex breaks down in the acidic environment of the stomach and releases the divalent ions, which are available for complexation with the

polymer chain eg. Solubility of chitosan in acidic medium is also dependent on its molecular weight (114). Acidic solution of chitosan when subjected to alkaline pH loses this charge and forms viscous gels. So that chitosan solution should be stable at acidic pH.

In case of pectin formulation very weak gels were formed at pH 3.0 resulting in poor sustained release characteristics compared with those formed at pH 1.2. Visual observations showed *In situ* gelation of 1.5 % (w/v) pectin formulation under conditions of both high (pH 1.0-1.6) and low gastric acidity (pH 3.3-3.6) (115).

Ionic concentration and types of Ions

It is obsereved that divalent cations such as Ca^{++} are known to produce stronger gellan gum gels than monovalent ions such as K^+ (67, 116, 117). Calcium chloride was incorporated with sodium citrate in oral formulation as a source of Ca^{++} ions to form a calcium citrate complex. Sometime gelation may occur at room temperature due to excess of calcium ions. If monovalent ions are used in presence of divalent ions, then concentration of monovalent ions for effective gelation should be high enough to maintain gel strength, fluidity and drug release (118, 119).

It is found that K^+ ion is more efficient gel promoter than Na^+ ion (98). It is due to the smaller size of the hydrated K^+ ion, as compared to hydrated Na^+ ions (116). The strength of gel formed by calcium ions increased with increasing the polyguluronate content, whereas polymannuronate sequence remains soluble in Ca^{++} ions (68).

Evaluation and characterization of *In Situ* gelling system

Viscosity of the Solution

The rheological property of the solution needs considerable attention, as it is decisive in determining palatability of the preparation and patient acceptance. The viscosity of sols has usually been measured at 20^0C using a cone and plate viscometer (Brookfield) with cone angle 1^0 34^- using a 1 ml aliquot of sample (112).

Measurement of Gel Strength

Gel strength is a very significant parameter as it governs the rate of release of drug from the gel. The gel strength will be measured at 37°C using a rheometer by the method described (120), cylindrical gels of 1-2% w/v polymer prepared by placing a 30 ml sample of the solution in to a cellular tube, immersing the tube in 50 ml of pH 1.2 simulated gastric fluid and allowing to equilibrate for 24 hr. The cylindrical gels (15 mm diameter and 15 mm height) were placed in the rheometer and raised at a rate of 60 mm min^{-1} so pushing a probe slowly through the gel. The changes in the load on the probe were measured as a function of the depth of immersion of the probe below the gel surface (121).

***In vitro* gelation study**

The gelation of solution can be observed in gelation cell. The cells are cylindrical reservoir capable of holding 3 ml of the gelation solution (simulated gastric fluid of pH 1.2, without enzymes). Within the cells a 250 µl transparent plastic cup is located at the bottom to hold the gel sample in place, after its formation. 100 µl of the formulation is placed in the cavity of the cup using a micropipette, and 2 ml of the gelation solution (SGF) is added in the reservoir. Formation of gel in reservoir can be observed by visual examination (122).

Determination of Drug Loading

Drug loading can be determine by adding one ml of solution in 50 ml of buffer or appropriate solvent and sonicated for 10-15 min. The solution is filtered through a nylon syringe filter (0.45 um) and the concentration of the drug in the solution can be measured either spectrophotometrically or by HPLC.

Measurement of drug release rate from gels

The release rate of drugs were measured by using a plastic dialysis cell as described by Miyazaki et al., 1984. The capacity of each half-cell was 4 ml and the surface area of the membranes was 2.67 cm^2 . The gel prepared in buffer and loaded with a known weight of drug was formed in the donor compartment, and an equal volume of the pH 1.2 simulated gastric fluid was placed in the receptor compartment. The gel donor phase and the aqueous receptor phase were separated by a cellulose membrane (Viskase Sales, size 36: 32). The assembled cell was shaken horizontally at the rate of 60 strokes min^{-1} in an incubator. The release medium was replaced by pH 6.8 simulated intestinal fluids after 1 h to simulate passage through the gastrointestinal tract. The total volume of the receptor solution was removed at intervals throughout the release period and replaced by fresh release medium. The concentrations of drug were determined spectrophotometrically at respective wavelengths of drugs (123).

Texture analysis

The consistency, firmness and cohesiveness of *In situ* gel are assessed by using texture profile analyzer which mainly indicated gel strength and easiness of administration *in vivo*. Higher values of adhesiveness of gels are needed to maintain an intimate contact with mucus surface (124).

Measurement of Diffusion Coefficient

The diffusion coefficient of membrane of gel can be measured by method described by Ganguly *et al.*, (25) The polymer solution in 0.33 M citric acid is stored

overnight for complete hydration. Membranes of different thickness are prepared by casting the solution on glass plates using appropriate spacer (0.5-1 mm) and heating at 60°C for 24 hr in an oven. A varnier calliper is used to measure the thickness of the membrane. The membrane is used in side-by-side diffusion cell with a 3-cm^3 volume and 1.13-cm^2 surface area for diffusion studies. A saturated solution of the drug is placed in the donor compartment and the whole assembly is equilibrated at 37±0.2^{0}C. 10 µl of sample is collected from the receiver side and the drug concentration can be measured spectrophotometrically.

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**.**

Fourier transforms infra-red spectroscopy and thermal analysis

During gelation process, the nature of interacting forces can be evaluated using this technique by employing potassium bromide pellet method. Thermo gravimetric analysis can be conducted for *Insitu* forming polymeric systems to quantitate the percentage of water in hydrogel. Differential scanning calorimetry is used to observe if there are any changes in thermo grams as compared with the pure ingredients used thus indicating the interactions (125).

Histopathological studies

This study was carried out by using 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 (126).

Marketed formulation

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 (127).

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 drug 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 GH) utilizing Macromed' Regel drug delivery system for treatment of patients with hGH- deficiency (128).

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 respons by safely delivering four times the maximum tolerated dose allowed by conventional IL- 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 (129).

Application of *In Situ* gel in various fields

In situ gelling system is widely used for sustaining the drug release by using various polymers like gellan gum, sodium alginate, pectin etc. Various different excipients were used with *In situ* gel forming polymer which helps in extending the time period of drug delivery by further sustaining the release by floating, mucoadhesion or by matrix formation in the polymer chain. *In situ* gelling system has numerous uses in various field like biotechnology, tissue engineering, gene delivery, contraception, site specific or targeted drug delivery etc. In the current scenario various researches has been going on to exploit its maximum potential by various other ways which are enlisted below:-

***In situ* gelling system in tissue engineering**

Tate *et al.,* (130) evaluated methylcellulose-based constructs as potential tissue engineering scaffolds for the repair of brain defects. These systems exhibited low viscosity at 23°C and formed soft gels intra cerebrally at 37°C. The gels were biocompatible both in the presence of cultured cells and in the injured rat brain.

The chitosan/GP system was also evaluated as a tool for cartilage repair. Hoemann *et al.,* (131) reported that primary calf chondrocytes could proliferate in solidified chitosan/GP solutions both *In vitro* and *In vivo*. Mechanical testing of 3-week aged *In vitro* implants demonstrated the initiation of functional matrix deposition. After injection into bone defects in rabbits, the chitosan/GP solution adhered to both bone and cartilage. Later, a hybrid implant, made of the chitosan/GP solution and whole blood, was developed to improve cartilage healing (132).

***In situ* gelling system in local or site specific drug delivery**

Gariepy *et al.,* developed thermosensitive *In situ* gel for the local administration of paclitaxel, to prevent local tumor recurrence (49) *In vitro* release profiles demonstrated controlled drug delivery for over 1 month. In mice, intratumoral injection of the paclitaxel-loaded hydrogel was as efficacious as four intravenous injections of the commercially available Taxol®. Formulation is inhibiting the growth of EMT-6 cancer cells, and proved to be less toxic.

Poloxamer 407 containing temperature sensitive system for cosmetic and medical uses

In situ gel containing poloxamer 407 is a temperature sensitive system which is used in the design of various medical, pharmaceutical, and cosmetic systems. Early studies evaluated poloxamer 407 thermosensitive solutions for the treatment of burns (95), topical administration of anticancer agents, and sustained delivery of drugs after extravascular parenteral injection (133). After parenteral injection, poloxamer gels can prolong drug release compared to solutions, but the delivery period rarely exceeds a few days (134). This characteristic makes poloxamer gels interesting for short-term therapies like pain management (135), infection treatment (134, 136) and fertility control (137). Besides injectables, other administration routes have been evaluated, such as rectal (138, 139), vaginal (39, 40), transdermal (140, 141) and ophthalmic (142, 143). Poloxamer formulations generally increased drug residence time at application sites, resulting in improved bioavailability and efficacy.

***In situ* gelling systems in ophthalmic diseases**

Gupta *et al.,* developed an *In situ* gel-forming system of timolol maleate based on the concept of both temperature and pH-triggered *In situ* gel that are instilled as drops into the eye and undergo a sol to gel transition in the cul-de-sac. Pluronic F-127 (a thermosensitive polymer) in combination with chitosan (pH-sensitive polymer also acts as permeation enhancer) was used as gelling agent. Developed formulation was converted into gel at temperatures above 35°C and pH 6.9–7.0. The developed system is suitable alternative to conventional eye drops for the treatment of glaucoma and various other occular diseases (144).

Conclusion

In situ gelling systems present a novel and interesting approach to obtain sustained or prolonged release of drugs. The main aim of the system is to maintain the desired therapeutic level of drug over an extended period of time, decrease the dose and minimizing the adverse effect associated with it. The system was reported as reliable candidate, as it prolonged the release of both hydrophilic and hydrophobic drugs, provide excellent stability and promising biocompatibility. This approach is a successful redesign of conventional liquid preparations because it enhances the *in-vivo* drug retention time at the desired site of action and finally may enhance the bioavailability of drug. Extended residence time of the system is an attribute of its rheological and mucoadhesive properties, which is basically affected by various formulation and physiological factors such as pH, temperature, concentration of polymers etc. For site specific drug delivery various stimuli responsive *In situ* gelling system has been reported and on the basis of these researches formation of gel and release of drug at a specific site is based on various external and internal stimuli such as pressure, pH, temperature, and ions etc. The applications of natural polymers are favorable for *In situ* gel formation and these polymers

are found to be biocompatible, biodegradable and non-toxic in nature.

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