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IN SITU GEL FORMING INJECTABLE DRUG DELIVERY SYSTEM

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ABSTRACT

Recently, controlled and sustained drug delivery has become the standard in modern pharmaceutical design and an intensive research have been undertaken in achieving much better drug product effectiveness, reliability and safety. This interest has been sparked by the advantages shown by *in situ* gel forming drug delivery systems such as ease of administration and reduced frequency of administration, improved patient compliance and comfort. The formation of gels depends on factors like temperature modulation, pH change, presence of ions and ultra violet irradiation, from which the drug gets released in a sustained and controlled manner. Various biodegradable polymers that are used for the formulation of *in situ* gels include gellan gum, alginic acid, xyloglucan, pectin, chitosan, poly(DL lactic acid), poly(DL-lactide-co-glycolide) and poly-caprolactone. Mainly *in situ* gels are administered by oral, ocular, rectal, vaginal, injectable and intraperitoneal routes. *In situ* gel forming injectable drug delivery system is the ability to inject a drug incorporated into a polymer to a localized site and have the polymer form a semi-solid gel drug depot has a number of advantages. Among these advantages is ease of application and localized, prolonged drug delivery. Biodegradable injectable *in situ* gel forming drug delivery systems represent an attractive alternative to microspheres, liposomes and emulsion as parenteral depot systems. For these reasons a large number of *in situ* gelling polymeric delivery systems have been developed and investigated for use in delivering a wide variety of drugs. The various strategies that have been used to prepare *in situ* gelling systems and outline their advantages and disadvantages as localized drug delivery systems. From a manufacturing point of view, the production of such devices is less complex and thus lowers the investment and manufacturing cost.

INTRODUCTION: *In situ* is a Latin phrase which translated literally as "In position ". *In situ* gel is drug delivery systems that are in sol form before administration in the body, but once administered, undergo gelation *in situ*, to form a gel. Administration route for *in situ* gel oral, ocular, rectal, vaginal, injectable and intraperitoneal routes.

Ease of administration and reduced frequency of administration, improved patient compliance and comfort. Deliverance of accurate dose, i*n situ* gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. Smart polymeric systems represent promising means of delivering the drugs. These polymers undergo sol-gel transition, once

administered. Various natural and synthetic polymers are used for formulation development of *in situ* forming drug delivery system. *In situ* gel formulations offers an interesting alternative for achieving systemic drug effects of parenteral routes, which can be inconvenient or oral route, which can result in unacceptably low bioavailability and passes the hepatic first-pass metabolism, in particular of proteins and peptides. This is novel drug delivery system [1, 2].

Approaches of *In situ* Gel Drug Delivery: There are four broadly defined mechanisms used for triggering the *in situ* gel formation of biomaterials:

1) Physiological stimuli (e.g., temperature and pH)
2) Physical changes in biomaterials (e.g., solvent exchange and swelling)
3) Chemical reactions (e.g., enzymatic, chemical and photo-initiated polymerization).

1. ***In situ* formation based on Physiological Stimuli:**

a. **Thermally trigged system:** Temperature-sensitive hydrogels are probably the most commonly studied class of environment-sensitive polymer systems in drug delivery research. The use of biomaterial whose transitions from sol-gel is triggered by increase in temperature is an attractive way to approach in-situ formation. The ideal critical temperature range for such system is ambient and physiologic temperature, such that clinical manipulation is facilitated and no external source of heat other than that of body is required for trigger gelation.

A useful system should be tailorable to account for small differences in local temperature, such as might be encountered in appendages at the surface of skin or in the oral cavity. Three main strategies are exists in engineering of 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 (N-isopropylacrylamide) (PNIPAAm). PNIPAAm is a water soluble polymer at its low LCST, but hydrophobic above LCST, which result on precipitation of PNIPAAm from the solution at the LCST. Pluronics are poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPOPEO) triblock co-polymer that are fluid at low temperature, but forms thermo responsible gel when heated as a consequences of a disorder-order transitionin micelle packing which makes these polymers suitable for *in situ* gelation [3]. A positive temperaturesensitive 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 [4].

The most commonly used thermoreversible gels are these prepared from poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (Pluronics®, Tetronics®, poloxamer). Polymer solution is a free flowing liquid at ambient temperature and gels at body temperature [5]. A novel “protein polymers” ProLastins, which undergo an irreversible sol gel transition. When injected as a solution into the body, the material forms a firm, stable gel within minutes. It remains at the site of injection providing absorption times from less than one week to many months. Such a system would be easy to administer into desired body cavity [6].

TABLE 1: POLYMERS AND THEIR PHASE TRANSITION TEMPERATURE

Polymer	Phase Transition temperature in aqueous solution
LCST behavior	
PNIPAM	30-34°c
Poly(N,N-diethyl arylamide)	32-34°c
Poly(methyl vinyl ether)	37°c
Poly (N-vinyl caprolactam)	30-50°c(a)
PEO-b-PPO	20-85°c
Poly (GVGVP)	28-30°c
UCST behavior	
PAAm/PAAc	25°c

Strongly dependent on molecular weight and concentration

b. **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 diethyl aminoacetate (AEA) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition [7]. Drug formulated in liquid solutions have several limitations, including limited bioavailability and propensity to be easily removed by tear fluid. A poly (acrylic acid) (PAA) solution that would be gel at pH 7.4 for minimize this factor and maximize this drug delivery.

At concentrations high enough to cause gelation, however, the low pH of PAA solution would cause damage to surface of eye before being neutralized by the lacrimal fluid. This problem was solved by partially by combining PAA with HPMC, a viscous enhancing polymer, which resulted in pH responsive polymer mixtures that was sol at pH 4 and gel at pH 7.4. Mixtures of poly (methacrylic acid) (PMA) and poly (ethylene glycol) (PEG) also has been used as a pH sensitive system to achieve gelation.[8]

2. ***In situ* formation based on Physical mechanism-**

a. **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 [9]. Lipid that a swell in water to form lyotropic liquid crystalline phase structures. It has some bioadhesive properties and can be degraded *in vivo* by enzymatic action.

b. **Diffusion:** This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. N- methyl pyrrolidone (NMP) has been shown to be useful solvent for such system [10].

3. ***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.

a. **Ionic cross-linking:** Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones. While k-carrageenan forms rigid, brittle gels in reply of small amount of K^+, i-carrageenan forms elastic gels mainly in the presence of Ca^{2+}. Gellan gum commercially available as Gelrite® is an anionic polysaccharide that undergoes *in situ* gelling in the presence of mono- and divalent cations, including Ca^{2+}, Mg^{2+}, K^+ and Na^+. Gelation of the low-methoxy pectins can be caused by divalent cations, especially Ca^{2+}. Likewise, alginic acid undergoes gelation in presence of divalent/polyvalent cations e. g. Ca^{2+} due to the interaction with guluronic acid block in alginate chains [8].

b. **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 [9].

c. **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 amd macromers because they rapidly undergo photo polymerisation in the presence of suitable photoinitiator. Typically long wavelength ultraviolet and visible wavelengths are used. Short wavelength ultraviolet is not used often because it has limited penetration of tissue and biologically harmful.

A ketone, such as 2,2 dimethoxy-2-phenyl acetophenone, is often used as the initiator for ultraviolet photo- polymerization, whereas camphorquinone and ethyl eosin initiators are often used in visible light systems. These systems can be designed readily to be degraded by chemical or enzymatic processes or can be designed for long term persistence in vivo. Photopolymerizable systems when introduced to the desired site via injection get photocured *in situ* with the help of fiber optic cables and then release the drug for prolonged period of time. The photo-reactions provide rapid polymerization rates at physiological temperature. Furthermore, the systems are easily placed in complex shaped volumes leading to an implant formation [8].

Classifications of *In-situ* Polymeric Systems:

Natural polymers- Alginicacid, pectin, chitosan, dextran, gellan gum, chitosan, carboxymethyl chitin.

Synthetic polymers- Aliphatic polyesters such as poly(lactic acid), poly(glycolic acid), poly(D,L-lactide)-PEG-poly(D,L-lactide), polyε-caprolactone, poly(N-isopropyl acrylamide) [3, 11].

Parenteral Controlled Drug Delivery Systems: Parenteral drug delivery refers to administration by injection which takes the drug directly into the tissue fluid or blood without having to cross the intestinal mucosa. The limitations of oral route are circumvented. Action is faster and surer (valuable in emergency). Gastric irritation and vomiting is not provoked. It can be employed even in unconscious, uncooperative or vomitose patient. There are no chances of interference by food or digestive juices. Liver is also bypassed by this route. But this route specifically requires that the drug delivery system should be sterile, besides being invasive and painful, assistance of other person often being required (though self injection is possible, e.g. insulin by diabetics), there are chances of local injury and being more risky.

Once administered, the action is difficult to revert back in case of side effects or toxicity. The different parenteral routes are subcutaneous, Intravenous, Intramuscular, Intra dermal and Intraperitonial etc.

- Subcutaneous route is limited to well absorbed water soluble drugs like insulin and dose volume is limited to 0.5 to 1.5ml
- Deep intramuscular aoute is for polymeric systems and volume size is restricted to 2ml
- Intravenous route is useful for administration of liposomes, nanoparticles and polypeptides. A disadvantage of i. v. route is that the system may be taken up by reticuloendothelial system.
- Intraperitoneal route is important in targeting of antineoplastics to the lymphatic systems.

Advanced drug delivery technology that can reduce the total number of injection throughout the drug therapy period will be truly advantageous not only in terms of compliance, but also for potential to improve the quality of the therapy. Such reduction in frequency of drug dosing is achieved, in practice, by the use of specific formulation technologies that guarantee that the release of the active drug substance happens in a slow and predictable manner.

Implantable parenteral system has advantages over injectable controlled-release formulations are-

1. More effective and more prolonged action
2. A significantly small dose is sufficient.

Following are types of implants;

1. *In situ* forming implants (*In situ* Depot-forming systems)

   a. *In situ* precipitating implants (ISI)

   b. *In-situ* microparticle implants

   c. *In situ* gels

   d. *In situ* cross-linked gels

2. **Solid implants**: These solid implants are cylindrical, monolithic devices of mm or cm dimensions, implanted by minor surgical incisions or injected through a large bore needle into the s.c. or i.m. tissue this disadvantage is overcome by using injectable *in situ* gels.

Injectable *In situ* Gel forming Drug Delivery System: The *in situ* gel forming system is a proprietary delivery system that can be used for both parenteral and site specific drug delivery. *In situ* gel forming system was initially developed by Dunn and co-workers at Southern Research Institute in Birmingham, Alabama in 1987 [12].

Biodegradable injectable *in situ* gel forming drug delivery systems represent an attractive alternative to microspheres, liposomes and emulsion as parenteral depot systems. Because liposome's are versatile carriers for both hydrophilic and lipophilic drug molecules but suffer from several disadvantages like, high production cost, leakage of drug, short half life and low solubility. On the other hand microspheres injected into the body using conventional needles and syringes. Thus, they have been the most widely accepted biodegradable polymesr system for parenteral uses.

However, the manufacturing processes for microspheres are often complex and difficult to control. As a result,this involving costs and batch-to-batch product uniformity. Emulsions are used extensively in parenteral products but usually not in long acting formulations because of the stability problems accompanying this dosage form. The possibility of dispersion break down or dissolution in the surrounding body fluid has made emulsions a poor choice for long acting formulations.[8] It consists of biodegradable polymers dissolved in a biocompatible carrier. When the liquid polymer system is placed in the body using standard needles and syringes, it solidifies upon contact with aqueous body fluids to form solid implant. If a drug is incorporated into the polymer solution, it becomes entrapped within polymer matrix as it solidifies. Drug release occurs over time as polymer biodegrades. Biodegradable polymers used in these systems are Polyhydroxyacids, polyanhydrides, polyorthoesters, polyesteramides and others [12].

The development of injectable in-situ forming drug delivery systems has received considerable interest over the last decade. A novel, injectable, thermosensitive *in situ* gelling hydrogel was developed for tumor treatment. This hydrogel consisted of drug loaded chitosan solution neutralized with β-glycerophosphate. Local delivery of paclitaxel from the formulation injected intratumorally was investigated using EMT-6 tumors implanted subcutaneously on albino mice. Ito *et al*., designed and synthesized injectable hydrogels that are formed *in situ* by cross-linking of hydrazide modified hyaluronic acid with aldehyde modified versions of cellulose derivatives such as carboxymethylcellulose, hydroxypropylmethyl cellulose and methylcellulose. These *in situ* forming gels were used for preventing postoperative peritoneal adhesions thus avoiding pelvic pain, bowel obstructions and infertility For a better therapeutic efficacy and patient compliance, mucoadhesive, thermosensitive, prolonged release vaginal gel incorporating clotrimazole-β-cyclodextrin complex was formulated for the treatment of vaginitis [13].

This system serves many advantages over conventional methods of drug administration.

1. Ease of administration
2. Reduced dosing frequency
3. Deliverance of accurate dose
4. Prolonged delivery periods
5. Decreased body drug concentration
6. Improved patient compliance and comfort.
7. Its production is less complex and thus lowers the investment and manufacturing cost [8].

Approaches of injectable *In situ* Gel forming Drug Delivery System:

1. Photocrosslinked gels
2. Thermally induced sol-gel transitions
3. pH dependent gels
4. *In situ* solidifying organogels

1. **Photocrosslinked gels:** Photopolymerizable, degradable biomaterials would provide many advantages over chemically initiated thermoset systems. In this approach, prepolymers are introduced to the desired site via injection and photocured *in situ* with fiber optic cables then release the drug for prolonged period of time.This approach has many advantages. Photoinitiated reactions provide rapid polymerization at physiological temperature.Furthermore, the systems are easily placed in complex shaped volumes leading to an implant formation [14].

   A photopolymerizable biodegradable hydrogel as a tissue contacting material and controlled release carrier. This system consisted of a macromer with at least two free radical-polymerizable regions (PEG-oligoglycolylacrylates), a photosensitive initiator (eosin dye) and a light source (ultraviolet or visible light). By exposing the mixture of macromers and photoinitiator to the light source, the macromer undergoes rapid crosslinking and forms a network. These networks can be used to entrap water-soluble drugs and enzymes and deliver them at a controlled rate.

   Use of an argon laser as a light source offers a greater depth and degree of polymerization, less time is required and an enhancement of the physical properties of the polymer is realized. These advantages are offset by reports that the increased polymerization caused by the laser results in increased shrinkage and brittlness of the polymer.

   As an example of the drug delivery capabilities of this approach, the delivery of various proteins from a photopolymerized PEG–PLA hydrogel is illustrated in Fig.1. Release of the proteins from these hydrogels was relatively rapid, with completion achieved within 5 days. The release rate was dependent on protein molecular weight, decreasing as molecular weight increased. The release was diffusionally controlled for molecules below a critical molecular weight. For the larger immunoglobulin G, release required the degradation of the hydrogel structure to afford larger openings within the gel allow for diffusion. Thus, to achieve prolonged release, this delivery system is best suited for large drug molecules [8].

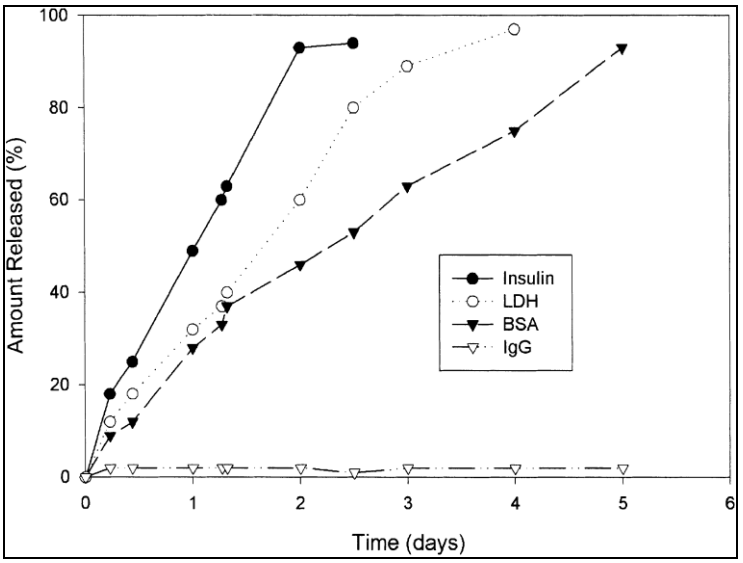

FIG. 1: PROTEIN RELEASE FROM PHOTOCROSSLINKED BIODEGRADABLE HYDROGEL

2. **Thermally-induced Sol-Gel Transitions:** Many polymers undergo abrupt changes in solubility in response to changes in environmental temperature. This physical characteristic has been employed to form drug depots by using polymer systems, which undergo a sol–gel transition upon injection into the body.

   Poly(*N*-isopropylacrylamide) [poly(NIPAAM)] isan example of a thermosensitive polymer. It exhibitsthe phenomenon of lower critical solution temperature (LCST) phase separation. Reviews of polyNIPAAM and its gel applications are numerous. PolyNIPAAM shows a very well defined LCST at about 32^{o}C, which can be shifted to body temperature by formulating polyNIPAAM based gels with salts and surfactants. Although numerous poly(*N*-alkylacrylamides) and polymers numerous poly(*N*-alkylacrylamides) and polymers with respect to the sharpness of its almost

discontinuous transition, which is usually observed only with ionizable polymers. These features make poly(NIPAAM) an interesting potential material for use in *in situ* setting dug delivery. However, acryl-amide based polymers with quaternary ammonium in their structure, in general, are not suitable for implantation purposes due to cell toxicity. The observation that acrylamide-based polymers activate platelets on contact with blood, along with the poorly understood metabolism of polyNIPAAM and its non-degradability, make it difficult to win FDA approval. Therefore, the vast majority of the drug delivery systems which employ LCST, use block copolymers of poly(ethyleneoxide) (PEO) and poly(propylene oxide) (PPO) simply because of FDA approval.

TABLE 1: THE PROPERTIES OF PLURONICS (L, P, F-LIQUID, PASTES, FLAKES)

Copolymer	Composition	Mppo	PEO wt.%	HLB
L64	$EO_{13}PO_{30}EO_{13}$	1740	50	12-18
P103	$EO_{17}PO_{60}EO_{17}$	3465	30	7-12
P104	$EO_{27}PO_{61}EO_{27}$	3540	40	12-18
F127	$EO_{100}PO_{65}EO_{100}$	3780	70	18-23

Triblock PEO–PPO–PEO copolymers (Pluronics or Poloxamers) are available in a variety of lengths and are of particular interest, as their gelation phenomena have been extensively studied. The properties of some Pluronic copolymers frequently used in drug delivery studies are collected in **Table 1**. It is significant to note that, although most of the Pluronics listed in Table 1 have a LCST well above normal body temperature, they do exhibit gelation at body temperature in concentrated solutions. However, application of concentrated polymer solutions. (16 wt.%) in drug delivery may be disadvantageous as it changes the osmolality of the formulation, kinetics of gelation, and causes discomfort in ophthalmic applications due to vision blurring and crusting.

Since F127 has been reported to be the least toxic of the commercially available Pluronics, it has been used most extensively in drug delivery studies. One other reason for the popularity of Pluronics is its inhibitory effect on P-glycoprotein. Certain Pluronics, e.g., P85, strikingly increases the cytotoxicity of drugs such as daunorubicin, against multi-drug cell over expressing P-glycoprotein. It appears that unimers of Pluronics are able to inhibit P-glycoprotein. The mechanism of inhibition is unclear, but it may be related to the changes at a membrane level induced by Pluronics. This may inhibit P-glycoprotein or enhance cellular uptake of drugs.The antitumor effect of Pluronic F-127 containing mitomycin C (MMC) on sarcoma180 ascites tumor mice was evaluated.

The Pluronic F-127 gels were evaluated as a sustained release vehicle for intraperitoneal administration of release vehicle for intraperitoneal administration of MMC. Tumor cell injections were made on day 0 and injections of MMC in 25% (w/w) Pluronic F-127 on day 1, both intraperitoneally. A prolongation of the life span of tumor-bearing mice following injection of therapeutic Pluronic F-127 was noted, and Pluronic F-127 containing MMC was therapeutically more active than free drug. MacroMed developed thermosensitive biodegradable polymers based on ABA and BAB triblock co-polymers, in which A denotes the hydrophobic polyester block and B denotes the hydrophilic poly(ethylene glycol) block.

Low molecular weight polymers of this polymer class are water-soluble and yield a temperature-dependent reversible gel–sol transition.[8] The aqueous polymer solution (sol) of PEG–PLA–PEG (Mr 5000-2040-5000) is loaded with drug at 45^{o}C and then injected into animals to form a gel at body temperature continuously releasing hydrophilic model substances, such as fluorescein isothiocyanate dextran (FITC-dextran), over 10–20 days [15].

The synthesis of PEG–PLA/PLGA–PEG is quite complex and careful control of the molecular weight is essential since this parameter affects thermosensitivity in a critical way. The phase diagram of PEG–PLA/PLGA–PEG is affected by numerous parameters and it should be noted that the LCST shifts are a function of block lengths and block composition.

Monomethoxy-PEG is polymerized with L-lactide or glycolide in toluene solution. The resulting AB diblock copolymers are then coupled using hexamethylene diisocyanate. The polymers are purified by fractional precipitation.Both ABA and BAB triblock copolymers have then been claimed by MacroMed as thermosensitive liquid drug carrier systems with gelation properties [8].

Sol-gel transitions occur around 30^0C at polymer concentrations of 15-23% (w/w) in aqueous solution. Below the LCST, the system behaves as a Newtonian fluid and changes to a visco-elastic state with a 4-fold increase in absolute value of viscosity after gelation. The mechanism by which thermoreversible gelation occurs is thought to be different in BAB and ABA triblock copolymers. In both cases micellar structures are obtained in the sol state as shown in **fig. 2**.

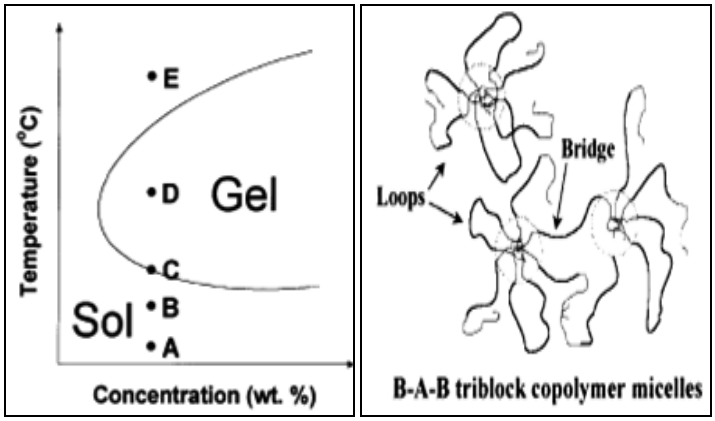

FIG. 2: POSSIBLE MICELLAR CHAIN TOPOLOGIES OF PEG–PLGA ABA- AND BAB TRIBLOCK COPOLYMERS IN WATER

The gelation is driven by entropy, as elevated temperatures decrease the hydration of the PEG chains. This results in reduced water–polymer hydrogen bonding and thus, a more hydrophobic character. Hydrophobic A-blocks consisting of PLA or PLGA will associate leading to a core-corona structure with dangling PEG chains in the case of BAB polymers and PEG loops in the case of ABA polymers.

Gelation of the triblock copolymers is the result of dense micelle packing and phase mixing of the corona (PEG) with the core leading to chain entanglement in the ABA type or micellar bridging in the BAB type. The latter mechanism could lead to irreversible aggregation. The physicochemical properties of these thermosensitive gel systems are anything but straightforward.

The ratio of hydrophilic (PEG) and hydrophobic (PLGA/PLA) segments, block-length, hydrophobicity (PCL. PLA. PLGA), polydispersity and stereo-regularity (amorphous/semi-crystalline) of the hydrophobic A-block are critical factors. Slight changes can have drastic effects on micellar properties. Another aspect deserves some consideration, namely the loading of the gels with hydrophilic drugs. At the sol–gel transition state, the system's volume will contract leading to the expulsion of the aqueous phase in which proteins are dissolved. This effect causes some initial drug burst and only those proteins associated with or dissolved in the lipophilic core do not experience this push-out effect. It is interesting to note that ABA polymers were investigated more intensively than the BAB type, probably because they are more easily produced in a one-step synthesis [3].

3. **pH induced-Gel:** 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.

   The water-soluble polymers such as hydroxyl propyl methyl cellulose (HPMC) carbopol system and polymethacrylic acid (PMA)–PEG for plasmid DNA delivery. Carbopol is a pH dependent polymer, which forms a low viscosity gel in alkaline environment (e.g., pH 7.4) and stays in solution in acidic pH. The addition of HPMC, a viscosity inducing agent, to carbopol reduces the carbopol concentration and hence the solution acidity while preserving the viscosity of the *in situ* gelling system. This system gels upon an increase in pH when injected.

4. ***In situ* Solidifying Organogels:** Organogels or oleaginous gels are composed of water-insoluble amphiphilic lipids, which swell in water and form various types of lyotropic liquid crystals.

The nature of the liquid crystalline phase formed depends on the structural properties of the lipid, temperature, nature of the drug incorporated, and the amount of water in the system. The amphiphilic lipids examined to date for drug delivery are primarily glycerol esters of fatty acids, for example glycerol monooleate, glycerol monopal-mitostearate, and glycerol monolinoleate which are waxes at room temperature. These compounds form a cubic liquid crystal phase upon injection into an aqueous medium. The cubic phase consists of a three-dimensional lipid bilayer separated by water channels. This liquid crystalline structure is gel-like and highly viscous [8].

In general, sorbitan monostearate organogels have a very short half-life at the injection site. This may be attributed to the diffusion of water molecules within the gelled structure which results in the subsequent disruption of the networked structure due to the emulsification of the gel surface. The same group has also reported the development of a sorbitan monostearate based organogels which has shown sustained delivery of a model antigen and radiolabelled bovine serum albumin after intra-muscular administration of the same in mice. The results indicated the probable use of the formulation as depot. L alanine based injectable *in situ* forming organogels may be used for the delivery of labile macromolecular bioactive agents.

These *in situ* forming organogels may be used for sustained delivery of bioactive agents after the same is being administered within the body. Various L-alanine derivates, viz. N-stearoyl l-alanine (m) ethyl esters, may be used to immobilize vegetable and synthetic oil in the presence of a hydrophilic solvent. These gels are thermoreversible in nature. The gel-to-sol transition of the L-alanine based organogels was dependent on the concentration of the gelator and the nature of the solvent [16, 17].

Subcutaneously injected *in situ* forming organogels prepared from L-alanine derivatives in safflower oil were used in the long term delivery of leuprolide, a LHRH agonist used in prostate cancer 76. The gels were shown to slowly degrade and release the therapeutic peptide for a period of 14 to 25days.

The histopathological examination of injected site indicated biocompatibility of the l-alanine organogels. A polymer of stearyl acrylate by free radical polymerization using ethylene glycol dimethacrylate as a crosslinking agent. The crosslinking reaction was carried out in oleyl alcohol, a plant derived oil. The organogel, so developed, were thermosensitive in nature which allowed release of the incorporated bioactive agent when the temperature was above 40 ^{o}C while the release was ceased when the temperature fell below 36^{o}C [18].

Limitations [8]:

TABLE 3: LIMITATIONS OF INJECTABLE *IN SITU* GEL DRUG DELIVERY

Drug Delivery system	Common problems
Photocrosslinked gels	Shrinkage and brittleness of the polymers due to high crosslinking
Thermally induced sol–gel transition	Burst in drug release
pH dependent gels	Burst in drug release
Organogels	Lack of toxicity data and Phase separation

Sterilization and Packaging: Those system is a viscous polymer solution so poses a difficulty in pouring in vials and aspirate into syringes at the time of use. Therefore, the products are filled into plastic syringes and packaged with foil-lined material to protect from moisture. Atrix Laboratories has developed custom-made equipment to fill a variety of plastic syringes with the polymer solutions within narrow fill volumes.

As the drug and polymer are in solution, degradation of both components and reactions between the two may occur somewhat faster with some formulations than in a dry, solid state. With these products, the drug and polymer solution are maintained in separate syringes until use. At the time of use the two syringes are coupled together and the contents are mixed thoroughly by moving the materials back and forth between the two syringes. The homogeneous solution or mixture is drawn into one syringe, the two syringes are decoupled, and a needle is attached for injection. This type of product provides for the maximum stability of the drug as well as the polymer. It also allows the drug to be sterilized by gamma irradiation in a dry state where it is often more stable.

Specific syringe configurations have been developed that enable the two syringes to be connected directly together using luer lock fittings, ensure that when the needle is attached to the syringe with the product, it remains in place during the injection.

Loading of drug into plastic syringes can be done by different ways. One of these techniques is powder filling, where precise control of fill weight is necessary. The equipment for powder filing has been custom designed and fabricated. Second is when the quantity of drug is too small to precisely fill the syringes or if the flow characteristics are not satisfactory, then the drug can be dissolved in water, sterile-filtered, and filled into plastic syringes where the drug can be lyophilized to a dry powder.

Filling the polymer into the syringes first involves simply loading the solvent and polymer into a sterile plastic container and placing it on a roll mixer. The polymer solution is then transferred from the plastic container to the syringe-filling equipment where it is loaded into individual syringes. The plastic container can then be discarded and the need for thorough cleaning is eliminated. The filled syringes are capped and placed into foil-lined packages to prevent moisture absorption. The drug is either powder-filled or lyophilized into syringes. If the drug is stable to gamma irradiation, then terminal sterilization is done by this method. If the drug is not stable to gamma irradiation, then the lyophilization is carried out under aseptic conditions, and the polymer solution is sterilized by gamma irradiation [19].

Evaluation and characterizations of *In situ* Gel System:
In situ gels may be evaluated and characterized for the following parameters;

1. **Clarity:** The clarity of formulated solutions determined by visual inspection under black and white background**.**

2. **Texture analysis:** The firmness, consistency and cohesiveness of formulation are assessed using texture analyzer which mainly indicates the syringeability of sol so the formulation can be easily administered in-vivo. Higher values of adhesiveness of gels are needed to maintain an intimate contact with surfaces like tissues.

3. **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.

4. **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.

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

6. **Fourier transform infra-red spectroscopy and thermal analysis:** During gelation process, the nature of interacting forces can be evaluated using this technique by employing potassium bromide pellet method. Thermogravimetric analysis can be conducted for *in situ* forming polymeric systems to quantitate the percentage of water in hydrogel. Differential scanning calorimetry is used to observe if there are any changes in thermograms as compared with the pure ingredients used thus indicating the interactions**.**

7. ***In-vitro* drug release studies:** For injectable *in situ* gels, the formulation is placed into vials containing receptor media and placed on a shaker water bath at required temperature and oscillations rate. Samples are withdrawn periodically and analysed for drug release using analytical technique [3].

Commercial formulations of *In-situ* Polymeric Systems:

1. **Regel-depot-technology:** Regel is one of the Macromed's proprietary drug delivery system and based on triblock copolymer, composed of poly (lactide-co-glycolide)-poly (ethylene glycol)-poly(lactide-co-glycolide). It is a family of thermally reversible gelling polymers developed for parenteral delivery that offers a range of gelation temperature, degradation rates and release characteristics as a function of molecular weight, degree of hydrophobicity and polymer concentration. Following injection, the physical properties of polymer undergo a reversible phase change resulting in formation of a water insoluble, biodegradable gel depot.

2. **Oncogel**- 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.

3. **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 [8].

4. **Marketed products:** A number of marketed products based on atigel technology are enlisted in table 2. These products have been approved by FDA [19].

TABLE 2: MARKETED PRODUCTS BASED ON ATRIGEL TECHNOLOGY

Marketed Product	Active ingredient	Use
Atridox	8.5% Doxycycline	Periodontal treatment product with sub gingival Delivery
Atrisorb	---	GTR barrier product without any drug for guided tissue regeneration of periodontal tissue
Atrisorb D	4%Doxycycline	For periodontal tissue regeneration
Eligard	Leuprolide acetate	1-, 3-, and 4-month products for treatment of prostate cancer
Lupron depot	Leuprolide acetate	2 and 4 month preparation for treatment of advanced prostate cancer
Sandostatin	Octreotide acetate	Acromegaly

Applicatons:

1. Scherlund *et al*., evaluated the EHEC/surfactant system for the local delivery of anesthetic agents to the periodontal pocket. By incorporating small amounts of lidocaine and prilocaine into the solution without affecting gelation behavior. These formulations showed sustained drug release over a minimum of 60 min, making them interesting for short-term pain control.

2. Intraperitoneal administration of mitomycin C in a 1.5-wt% xyloglucan gel to rats resulted in a broad concentration–time profile, as opposed to a narrow peak and rapid disappearance from the peritoneal fluid and plasma when the drug was given as a solution.

3. Intratumoral injection of the paclitaxel-loaded hydrogel was as efficacious as four intravenous injections of the commercially available Taxol formulation in inhibiting the growth of EMT-6 cancer cells, and proved to be less toxic [19].

4. Amphiphilic derivatives of l-alanine were demonstrated to form gels *in situ* when admixed with vegetable oils and low amounts of organic solvents approved for parenteral use and have good biocompatibility following subcutaneous administration and degraded over several weeks, a potent agonist of luteinizing hormone-releasing hormone used in the palliative treatment of hormonally-dependent prostate cancer, endometriosis, and precocious puberty [20].

5. Poloxamer temperature-sensitive solutions for drug delivery applications: some examples since 1997 [19].

Poloxamer	Conc. (wt%)	Drug	Objective of the study
407	36	Recombinant human growth hormone	Controlled release of human growth hormone following intramuscular or subcutaneous administration.
407	20 or 30	Insulin	Subcutaneous delivery of peptides and proteins having short half-lives.
407	25	Vancomycin	Prolonged residence time of vancomycin in a body site with a high infection risk.
407	25	Ibuprofen	Controlled release of ibuprofen for epidural analgesia.
407	20	Paclitaxel	Intratumoral administration of paclitaxel.
407	25	Deslorelin or GnRH	Intramuscular sustained release of deslorelin and GnRH to induce the release of luteinizing hormone and formation of luteal tissue in cattle.
407	25,30 and35	Ceftiofur	Sustained release gel formulation of ceftiofur for treating foot infections in cattle.
407 and/or, 188 and additives	15, 15 and 20	Acetaminophen	Increased bioavailability using an *in situ* gelling and mucoadhesive liquid suppository.
407 and 188 and additives	15	Propranolol	Increased bioavailability using an *in situ* gelling and mucoadhesive liquid suppository.
407, 188	15, 15 and 20	Clotrimazole	Prolonged antifungal effects using an *in situ* gelling and mucoadhesive vaginal gel.
407 and thickening agents	15, 20 or 25	Timolol maleate	Enhanced ocular bioavailability of timolol maleate.
407, 188	21	None	Development of a thermosetting gel with a suitable phase transition temperature for ocular delivery.
407		Piroxicam	Enhanced efficacy of piroxicam following percutaneous absorption.
	46	Fentanyl	Poloxamer gels as release vehicles for percutaneous administration of fentanyl.

CONCLUSION:

- The primary requirement of a injectable *in situ* gel sustained and prolonged release product focuses on increasing patient compliance and a number of advantages over conventional dosage forms.
- Use of biodegradable and water soluble polymers for the *in situ* gel formulations can make them more acceptable and excellent drug delivery systems.
- These delivery systems have unique challenges associated with their development that are related to drug stability, drug release kinetics and the conditions under which the system is

delivered to the body. The continuous advances in biotechnology and drug development will produce more pharmaceutically active agents that will be difficult to administer by conventional means, and an increased demand for controlled or site-specific delivery system is anticipated.

FUTURE SCOPE:

- Injectable *in situ* gel appears to provide efficacious products with significant advantages over other existing delivery systems.
- It includes certain improvements such as lower the initial drug burst; use of new polymers and solvents in long-term drug release and tissue compatibility.
- If these modifications implemented successfully then these will surely increase its uniqueness and its applicability to a wide variety of drug delivery product

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