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In-situ Forming Parenteral Drug Delivery: A New-fangled Loom In Therapeutics.

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ABSTRACT

The parenteral route is the most common and effective route of administration for the drugs that have poor bioavailability and narrow therapeutic window. 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 with high degree of effectiveness, reliability and safety. This interest has also been sparked in parenterals; by the advantages offered by *in-situ* forming parenteral drug delivery systems such as ease of administration, reduced frequency of administration, decreased body dosage and thus reduced undesirable side effects, localized delivery for a site specific action, improved patient compliance and comfort. The *in-situ* formation of drug delivery system depends on factors like temperature modulation, pH change, presence of ions and ultraviolet radiations; from which drug get released in a sustained and controlled manner. Utilization of many biodegradable and biocompatable polymers in formulation of these systems, overcome the possibilities of adverse reactions. From manufacturing point of view even, these systems are cost effective in comparison with conventional parenteral drug delivery systems. Hence, *in-situ* forming parenteral drug delivery systems seems promising, as they offers a range of advantages over conventional parenteral delivery systems, and may escort to a new path in therapeutics in near future.

Keywords: Controlled Parenteral Drug Delivery, Parenteral Depots, Polymeric Conjugates, Bioerodible Systems, Implants, Targeted Drug Delivery.

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INTRODUCTION

Parenteral Drug Delivery System

The parenteral route is the most common and effective route of administration for the drugs that have poor bioavailability and drugs with narrow therapeutic window. As in the case of mucosal and transdermal drug delivery, where systemic bioavailability of a drug is always limited by its permeability across a permeation barrier (epithelial membrane or stratum corneum) and in oral drug delivery where the systemic bioavailability of a drug is often subjected to variable gastrointestinal transit time and biotransformation in the liver by 'first pass metabolism'. Parenteral drug delivery by intravenous, subcutaneous or intramuscular route can gain easy access to systemic circulation with rapid drug absorption however the disadvantage of this system is that, such rapid absorption is accompanied with rapid decrease in plasma concentration of the drug. In chronic conditions, multiple injections have to be administered for years together, resulting in a poor patient compliance to parenteral route. On the other hand the drugs such as cytokinins used in tissue regeneration therapy have very low half life of few hours or even few minutes, which is too less to exert their biological effect and thus the plasma level of such drugs should be maintained within therapeutic range to achieve the effective treatment. Use of continuous intravenous infusion may serve to maintain such levels but it has health hazards and therefore needs hospitalization of patient[1]. For this reason, drug delivery technology that can reduce the total number of injections throughout the treatment period will be truly advantageous not only in terms of compliance, but also for optimization of dosage regimen. Such reduction in frequency of drug dosing can be achieved, by the use of specific formulation technologies that guarantee the release of the active drug substance in a slow and predictable manner.

The development of new injectable drug delivery system called *Parenteral Depot* or *Parenteral Implant* has received considerable attention over past few years[2]. The increased interest is due to the advantages of this delivery system as ease of application, decreased body dosage and thus reduced undesirable side effects, localized delivery for a site specific action as compared to conventional parenteral delivery[3].

Types of Prolonged Release Parenteral Delivery Systems

There are two types of systems used for prolonged release parenteral drug delivery which are as follows:

1) Parenteral Depot System.

2) Polymeric Drug Delivery Systems.

1) Parenteral Depot System

These are long acting parenteral drug formulations designed, ideally to provide slow constant, sustained and prolonged action.

Approaches Used in Depot Formulation

1. Use of low aqueous soluble salt.

2. Use of largest particle with Crystallinity.

3. The suspension of the drug particle in vegetable oil and especially of gels with substances such as aluminum monostearate produces prolonged absorption rates.

Types of Depot

1) In one type of depot formulation which is referred to as "*dissolution controlled*' the rate of drug absorption controlled by the slow dissolution of drug particles, with subsequent release to tissue fluid surrounding the bolus of product in tissue.

2) The formulation is prepared by binding of drug molecule to adsorbents. Only the free portions in equilibrium with that, which is bound, can be absorbed. As drug is absorbed, a shift in equilibrium is established, and the drug is slowly released from the bound state to free state, e.g. binding of vaccines to aluminum hydroxide gel to provide a sustained release.

3) ***Encapsulation Type***- In this bio-absorbable or biodegradable macro-molecules such as gelatin, phospholipids and long chain fatty acids become a diffusion barrier and by the biodegradation of the barrier macromolecules the rate of release and hence absorption of drug is controlled.

4) ***Esterification Type***- In this esters of drug that are biodegradable are synthesized. The esterified drug is deposited at the site of injection in tissue to form a reservoir of drug. The rate of drug absorption is controlled by the partitioning of the drug ester from the reservoir to tissue fluid and by the rates at which the drug ester regenerates the active drug molecule. Often these esters are dissolved or suspended in a vehicle, which further delays the release.

2) Polymeric Drug Delivery Systems

Many classes of cross-linked polymer gels display phase transition characteristics i.e. abrupt change in swollen volume in response to small environmental changes like pH, light, temperature, intensity, electric field, ionic strength and even specific stimuli like glucose concentration. Drugs containing charged hydrogel networks have been recognized as useful matrices for delivering drugs, because their change in volume in response to external stimuli consequently deliver drug solution. Such hydrogels have been applied in glucose sensitive

insulin releasing devices; an osmotic insulin pump and site specific delivery in the gastrointestinal tract.

Types of Polymeric Drug Delivery Devices

The polymeric devices are generally classified into following categories:

1) Diffusion Controlled Devices:

- Monolithic Devices
- Reservoir Devices

2) Solvent Controlled Devices:

- Osmotically Controlled Devices
- Swelling Controlled Devices

3) Chemically Controlled Devices:

- Bioerodible System
- Drug-polymer Conjugates

Based on the above systems various dosage forms like emulsions, liposomes, biodegradable microspheres, micelles, suspension, solid lipid nanoparticles have been formulated. These initial dosage forms have demonstrated some success in few applications, but there are certain limitations to these dosage forms and hence there is need to improve the formulations of parenteral drug delivery[1].

Emulsions which are widely used in parenteral preparations are not much used as long acting formulations due to the stability problem. The possibilities of dispersion break down or abrupt dissolution into the body fluids has made emulsions a poor choice as long acting formulations[4]. Liposomes like emulsions are not the promising dosage forms for a long acting formulation as local retention of liposome-entrapped drugs is likely to be longer than that of free drugs, but it may not always be long enough to maintain local therapeutic drug levels, due in part to rapid clearance by macrophages and other cells[5]. Other problem, such as stability issues, sterilization problems and often low drug entrapment limits the utility of liposomes[6]. Microspheres are easy to deliver to the site of action but they have several inherent disadvantages. These include the need for reconstitution before semi-solid injection, a relatively complicated manufacturing procedure to produce a sterile, stable and reproducible product, and the possibility of microsphere migration from the site of injection[7]. Micelles, which are also prone to migration, suffer from the fact that there are a large number of variables which influence micelle properties. Controlling factors like core block length and corona outer shell length, which significantly influences drug loading and size distribution, at the same time is almost impossible. Furthermore,

the stability of micelles is highly dependent on their critical micelle concentration (CMC), which is the minimum polymer concentration required for micelle formation. Lower the value of the CMC, the greater the thermodynamic stability of micelles in dilute solutions. Once diluted below the CMC, micelles begin to disassemble into single chains[8]. Thus with considering the all above limitation an alternative delivery system called i*n-situ* forming parenteral drug delivery system has been developed.

In-situ Forming Parenteral Drug Delivery Systems

In-situ forming parenteral drug delivery systems is the liquid formulation made up of biodegradable polymers, which can be injected via syringe into body where they form a semi-solid depot and retard the release of drug from the depot[9].

The field of *in-situ* forming implants has grown exponentially in recent years, in parallel to the development of new protein therapeutics caused by the explosion of genomic information and the final mapping of the human genome[10]. A large number of peptides and proteins may become candidates for therapeutic application. The understanding of protein functions in the etiopathology of currently incurable diseases could lead to new therapeutic approaches. Hence, the development of new injectable drug delivery systems which protect proteins against denaturation in body fluids and allow sustained release profiles are in great demand.

These innovative systems, which are easier to administer and better accepted by several patients than existing delivery solutions, are prepared by dissolving biodegradable polymers in biocompatible organic solvents. Drug is added to the polymer solution, where it forms a homogenous solution or a suspension depending on its solubility. The drug suspension or solution is injected subcutaneously forming an ‘‘*in-situ* implant’’, which slowly releases the drug over time. Atrix Laboratories has pioneered this approach[11], and researchers at ALZA Corporation[12] have refined the system to minimize the so called ‘‘burst effect’’, or rapid release of drug in the first 24 h.

Polymers Used For _In-situ_ Forming Parenteral Drug Delivery System[13]

A) Natural:

1. Cellulose Derivatives- HPMC, methyl cellulose, ethyl (hydroxyethyl cellulose).
2. Xyloglucan- a polysaccharide derived from tamarind seed.
3. Starch.
4. Dextran.
5. Gelatin.
6. Carageenan.

B) Synthetic:

1. Poloxamer systems- Poloxamer (Pluronics), poloxamer or poly (acrylic acid) copolymer.
2. Poly ethyl-poly (alkyl cynoacrylates).
3. Poly amides- Nylon 6-10-cynoacrylates, poly butyl-nylon 6-6, poly acrylamides, poly amino acid, poly urethane.
4. Aliphatic polyesters- Poly (lactic acid), Poly lactide-co glycolide, poly glycolic acid, poly caprolactone, poly dihydroxy butyrate, poly hydroxy butyrate, covalently cross linked protein, cross linked ampipathic block co-polymer.
5. Poly phosphazenes.
6. Poly orthoesters.
7. Polyanhydrides.

Classification

There are four main classes of *in-situ* forming parenteral drug delivery system viz.

1) *In-situ* crosslinked systems,

2) *In-situ* polymer precipitation,

3) Thermoplastic pastes, and

4) In-situ solidifying organogels.

1) *In-situ* Crosslinked Systems

Crosslinked polymer networks can be formed *in-situ* in a variety of ways, forming solid polymer systems or gels. Means of accomplishing this end include free radical reactions initiated by heat (thermosets) or absorption of photons, or ionic interactions between small cations and polymer anions.

A) Thermosets

Thermoset polymers can flow and be molded when initially constituted, but after heating, they set into their final shape. This process is often called ‘‘curing’’ and involves the formation of covalent crosslinks between polymer chains to form a macro molecular network. Reheating a cured polymer only degrades the polymer[14]. This curing is usually initiated chemically upon addition of heat. Unfortunately, there have not been many articles written regarding the application of chemically initiated thermoset systems for the delivery of pharmaceutically active agents into the body. This may be due to the limitations and adverse effects associated with it. In particular, the reaction conditions for *in-vivo* applications are quite stringent, including a narrow range of physiologically acceptable temperatures, requirement for nontoxic monomers and/or solvents, moist and oxygen-rich environments, the need for rapid processing, and clinically

suitable rates of polymerization[15].

Dunn *et al*. used biodegradable copolymers of D,L-lactide or L-lactide with ε-caprolactone to prepare a thermosetting system for prosthetic implants and slow release drug delivery systems. This system is liquid outside the body and is capable of being injected via a syringe and needle and once inside the body, it cures. The multifunctional polymers in their thermosetting system were first synthesized via copolymerization of D,L-lactide or L-lactide with ε-caprolactone using a multifunctional polyol initiator and a catalyst (e.g. peroxides) to form polyol terminated liquid prepolymers. This pre-polymer was then converted to an acrylic ester terminated prepolymer. Curing the liquid acrylic terminated pre-polymer is initiated by the addition of either benzoyl peroxide or N,N-dimethyl-p-toluidine, prior to injection into the body. After introduction of the initiator, the polymer system is injected and polymer solidification occurs. The estimated time of reaction is between 5 to 30 min[16,17].

The advantage of using this system is its facile syringeability. There are a couple of disadvantages accompanied with this system, which have limited its application. When a bioactive agent (e.g. flurbiprofen) was incorporated into this system, a burst in drug release during the first hour was observed. This burst was due to the lag time for solidification of the polymer. While the cross-linking reaction inside the body is in process and the polymer is in liquid form, the drug can diffuse out of the system more rapidly, thereby causing the burst. This high concentration of drug at the site of reaction may result in the appearance of side effects of the drug. Furthermore, the heat released upon curing (up to 94^0C have been reported for poly(methyl methacrylate) used as a prosthetic bone cement) due to the exothermic nature of the crosslinking reaction, can cause necrosis to the surrounding tissues[18-20]. Additionally, introduction of free radical producing agents such as benzoyl peroxide into the body may induce tumor promotion[21].

B) Photocrosslinked Gels

Most of the *in-situ* forming gels are formed by using chemical crosslinking to increase their mechanical strengths. This chemical crosslinking is achieved by the formation of ionic interactions between the carboxyl groups of the polymer and polyvalent cations such as calcium, copper, and aluminum. Although chemical crosslinking methods are very popular for this purpose, plenty of room for their improvement still exists. For instant this crosslinking is affected by changes in the surrounding aqueous phase, such as changes in pH, temperature, or polymer concentration, because the ionic bond is weak and unstable by nature. Appropriate characteristics, such as rigid gel strength and high water capacity are required for the gel to act as

a retarding matrix. However, adjusting all of the characteristics to the appropriate levels with the conventional method is difficult. To overcome the shortcomings of the conventional method, the application of photopolymerization to the preparation of *in-situ* gels is very useful. Photopolymerization, which is triggered by light irradiation (Figure. 1), has recently been gaining great attention in medical fields, because it allows the rapid conversion of a monomer or macromer solution to a gel or solid under physiological conditions. Various gels for use in drug delivery systems have been prepared with this technique using mono, di or multifunctional vinylated monomers or macromers, such as 2-hydroxyethyl methacrylate (HEMA), poly(ethylene glycol) dimethacrylate, poly(ethylenence glycol) diacrylate, methacrylated sebacic acid, chitosan-introduced azide and lactose moieties and styrenated gelatin[22].

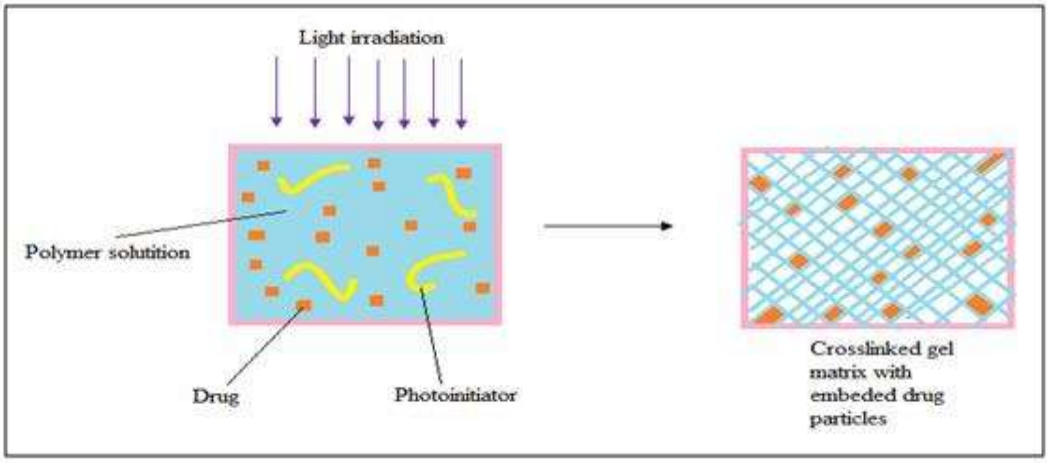

Figure 1: Formation of photocrosslinked gel.

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[15]. This approach has many advantages. Photoinitiated reactions provide rapid polymerization rates at physiological temperatures. Further, because the initial materials are liquid solutions or moldable putties, the systems are easily placed in complex shaped volumes and subsequently reacted to form a polymer of exactly the required dimensions. 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 brittleness of the polymer[23].

Numerous medical applications benefit from the ability to form polymers *in-situ* using photopolymerization. For example, dentist's photopolymerize dimethacrylate monomers in the presence of silica particle fillers in tooth caries to render tooth-colored composite restorations as alternatives to mercury amalgam fillings. In addition to these long-standing applications, recent advances in polymer chemistry and processing have led to many new applications for forming biomaterials *in-situ* using photopolymerization such as focal uses photoinitiated polymerizations to *in-situ* seal air leaks associated with lung surgery and fluid leaks from the dura after neurosurgery with degradable hydrogels. The uniqueness to their approach relates to a two part process that first stains the tissue with photoinitiator molecules and subsequently introduces a reactive macromer that is polymerized from the tissue interface in a controlled and adherent fashion. Hence, drug-loaded solutions can be injected subcutaneously and upon gelation allow for the sustained delivery of active agents[24].

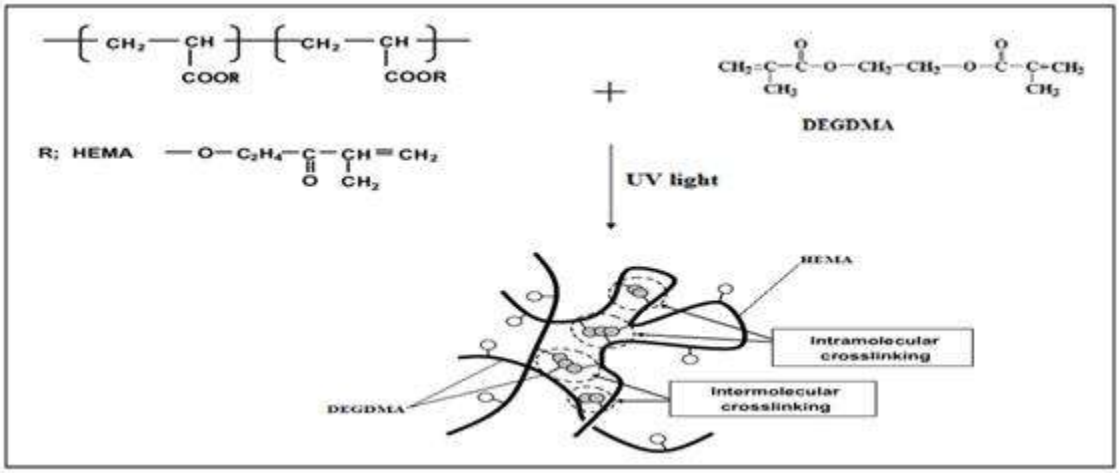

Figure 2: Photocrosslinking of HEMA and DEGDMA.

A novel approach to immobilize non-uniform initial drug concentration profiles in multilaminated matrix devices utilizing photopolymerization techniques were investigated by Lu *et al.* (Figure. 2). Solution polymerization of 2-hydroxyethyl methacrylate (HEMA) and diethylene glycol dimethacrylate (DEGDMA) in the presence of a model compound, acid orange 8 (AO8), was conducted using UV light and photoinitiators to construct a laminated matrix device. In this process, each layer was polymerized with a different AO8 concentration to form a non-uniform initial concentration profile in the matrix devices. The AO8 diffusion coefficients measured in this work were used in a concurrently developed model to predict the effects of non-uniform AO8 concentration profiles on AO8 release patterns. The release data predicted by the

model agreed well with the experimentally determined data. The results indicated that a zero-order release pattern can be approximated by employing a suitable non-uniform initial drug concentration profile[25]. Thus, to achieve prolonged release, this delivery system is best suited for large drug molecules.

C) Ion-mediated Gelation

Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive one. 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 gulcuronic acid block in alginate chains[26]. Alginates are natural polymers, which have been widely investigated for drug delivery. They can be used directly as a drug carrier or as a carrier of another delivery system such as liposomes.

Cui *et al*. used thermally sensitive Ca-loaded vesicles, capable of releasing Ca^{+2} when heated to body temperature, along with sodium alginate to form a fluid suspension that gels at 37^0C (**Figure. 3**). 1,2-bis(palmitoyl)-glycero-3-phosphocoline (DPPC) and 1,2-bis(myristoyl)-glycero-3-phophocoline (DMPC) were used to prepare both Ca-loaded and drug loaded phospholipid vesicles. The molar ratio of DPPC: DMPC was adjusted to 9:1 to bring the melting point of the liposomes below body temperature. It is well known that the permeability of the phospholipid bilayers is strongly temperature dependent. At temperatures below the lipid chain melting transition, phospholipid bilayers are relatively impermeable to multivalent ions. However, phospholipid permeability has been shown to be several orders of magnitude higher at the melting temperature. The addition of drug-filled liposomes to the formulation resulted in a hydrogel that released entrapped drug (metronidazole) in a controlled manner. Drug release was characterized by a rapid burst-type release followed by a slower controlled release of drug from the hydrogel matrix. Metronidazole was released more rapidly from the 15% DMPC liposome than from the pure DPPC liposome due to the difference in bilayer permeability of the two compositions at the experimental temperature (37^0C)[27,28].

This approach clearly improved the half-life of the liposomes and proved to be advantageous for certain local delivery applications in which *in-situ* gelation is required. The disadvantages of

using this system are a short shelf life due to the slow leakage of Ca^{2+} from the liposomes and a large amount of drug released in the initial release burst[29,30].

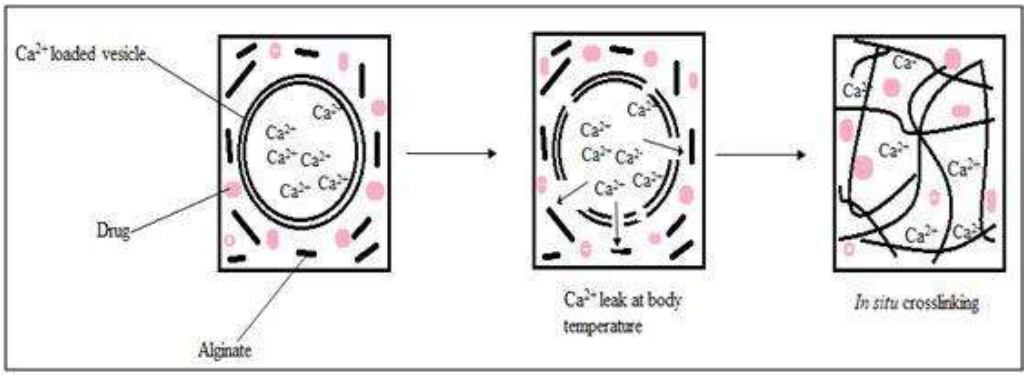

Figure. 3: *In-situ* gelation of alginates mediated by Ca^{2+} ions released from Ca^{2+} loaded vesicles.

Westhaus and Messersmith, introduced thermally triggered Ca^{2+} release from liposomes to form calcium alginate hydrogels, and a protein-based system in which triggered release of calcium activates transglutaminase enzyme-catalyzed cross-linking of proteins. The fundamentals of this system are the same as the Cui *et al*. hydrogel system mentioned above and has the same problem of calcium leakage out of the liposomes and hence, a short shelf life[31].

Despite these applications, there are two important factors, which have limited the use of calcium alginate for drug delivery purposes. The first factor is their potential immunogenicity and the second is the long time required for their *in-vivo* degradation. For example, cytotoxicity and the non-biodegradable nature of calcium alginate wound dressings induce a chronic foreign body reaction[32].

2) *In-situ* Polymer Precipitation

Solutions that undergo sol-gel transformations when they meet physiological conditions may serve as an *in-situ* gelling drug delivery system; that is an injectable parenteral formulation which transforms into a gel drug delivery system in physiologic conditions.

A) Solvent-removal Precipitation

This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. Formation of *in-situ* gelling complexes using poly-ethylene glycol (PEG) and polymethacrylic acid (PMA) or polyacrylic acid (PAA) as an injectable drug delivery system is one possible way (**Figure. 4**). At low pH the aqueous solubility of the complex is minimal, but a clear viscous solution is obtained when a small amount of ethanol is added. When this solution containing a model drug is injected into the physiological environment, gelation is initiated by a membrane formed around the injected liquid

caused by ethanol diffusion and bulk fluid infusion; further diffusion over time caused the entire gel to form. As time progresses, the complex will dissociate into its constituent water-soluble polymers, releasing the entrapped drug from the gel. These polymers are then expected to be renally excreted because of their low molecular weight and high water solubility[33].

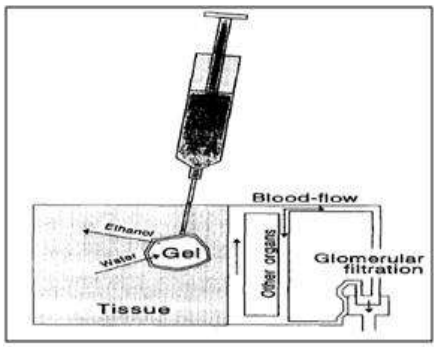

Figure. 4: Formation of *in-situ* gelling complex by solvent removal precipitation.

Haglund *et al*. investigated the physical properties of such a system with respect to its potential as a parenteral drug delivery system. An insoluble polymer complex of PMA 10% (MW 15000), obtained by precipitation from its sodium salt in acidic pH using 5 M hydrochloric acid with PEG 20% (MW 18500), resulted in an aqueous medium. The complex was dissolved in 75% hydro-alcoholic solvent containing ethanol to obtain a stock solution with a total polymer content of ~20%. This solution was used for subsequent experiments. The system was stable below pH <5.7, the complex was insoluble in water but dissolved in a hydroalcoholic solvent to yield a clear viscous solution. Upon injection, the diffusion of ethanol from the liquid transformed the system into a gel upon contact with physiological conditions. The gel disappeared from the site with time due to dissociation of the complex. Water soluble and low molecular weight; the dissociated components were eliminated by glomerular filtration. Using a concentration of 50% ethanol as the cosolvent, the system was injected by syringe[33].

Dunn *et al*. introduced an *in-vivo* setting system made of biodegradable polymers[34]. This injectable implant system is comprised of a water insoluble biodegradable polymer, such as poly(DL-lactide), poly(DL-lactide-co-glycolide) and poly(DL-lactide-co-ε-caprolactone), dissolved in a water miscible, physiologically compatible solvent. Upon injection into an aqueous environment, the solvent diffuses into the surrounding aqueous environment while water diffuses into the polymer matrix. Since the polymer is water insoluble, it precipitates upon contact with the water and results in a solid polymeric implant. Solvents which have been used in this approach include N-methyl-2-pyrrolidone (NMP), propylene glycol, acetone, dimethyl sulfoxide (DMSO), tetrahydrofuran, 2-pyrrolidone and triacetin, but the most preferred are NMP

and DMSO because of their pharmaceutical precedence[35]. Due to the number of disadvantages inherent in this system, it has not been extensively investigated or endorsed by fellow pharmaceutical scientists. One of the problems is the possibility of a burst in drug release especially during the first few hours after injection into the body. Since this injectable implant system is administered as a liquid, it is reasonable to assume that there is a lag between the injection and the formation of the solid implant. During this lag time the initial burst of drug may exceed the plasma concentration achieved using conventional implant systems. This initial burst of drug has been linked to tissue irritation and sometimes to systemic toxicity. Due to this unwanted phenomenon, the use of this system has been limited only to drugs with a wide therapeutic index. In order to control the burst effect four factors have been examined the concentration of polymer, the solvent used[9], and the addition of a surfactant[36]. All of these parameters influence the rate of precipitation of the polymer.

The modification of *in-situ* polymer precipitation by solvent removal technique is *in-situ* forming microspheres or microparticles (ISM). These systems consist of an internal drug containing polymer-solvent phase (polymer phase) emulsified into an external phase (e.g. an oil phase). Upon injection of this emulsion, the internal polymer phase releases the drug in a controlled release fashion. Solvents for the polymers are for example NMP, DMSO and 2-pyrrolidone, which are able to form highly concentrated polymer solutions. Peanut oil, oil for injection, can be used as a biocompatible external oil phase. The ISM systems have a significantly reduced myotoxicity and lowered viscosity (the viscosity is primarily controlled by the external oil phase and not by the internal polymer phase). Therefore, these systems are easier to inject when compared to the viscous *in-situ* implant. Kranz *et al.* investigated the *in-vitro* drug (diltiazem hydrochloride and buserelin acetate) release from different *in-situ* forming biodegradable drug delivery systems, namely polymer solutions (*in-situ* implants) and *in-situ* microparticle (ISM) systems. The drug release from ISM systems (poly(D,L-lactide) (PLA) or poly(D,L-lactide-co-glycolide) (PLGA) solution dispersed into an external oil phase) was investigated as a function of the type of solvent and polymer, polymer concentration and internal polymer phase:external oil phase ratio and was compared to the drug release from *in-situ* implant systems and microparticles prepared by conventional methods (solvent evaporation or film grinding). Upon contact with the release medium, the internal polymer phase of the ISM system solidified and formed microparticles. The initial drug release from ISM systems decreased with increasing polymer concentration and decreasing polymer phase: external oil phase ratio. The type of biocompatible solvent also affected the drug release. It decreased in the rank order

DMSO>NMP>2-pyrrolidone. In contrast to the release of the low molecular weight diltiazem hydrochloride, the peptide release (buserelin acetate) was strongly dependent on the polymer degradation/erosion. One advantage of the ISM system when compared to *in-situ* implant systems was the significantly reduced burst effect because of the presence of an external oil phase. ISM systems resulted in drug release profiles comparable to the drug release of microparticles prepared by the solvent evaporation method. Therefore, the ISM systems are an attractive alternative to existing complicated microencapsulation methods[37].

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 diethylaminoacetate (AEA) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition. Drug formulated in liquid solutions have several limitations, including limited bioavailability and propensity to be easily removed by tear fluid. Kumar and Himmelstein sought to minimize these factors and maximize the drug delivery by making a poly(acrylic acid) (PAA) solution that would be gel at pH 7.4. They found that at concentrations enough to cause gelation, the low pH of PAA solution would cause damage to surface of eye before being neutralized by the lacrimal fluid. This problem was solved partially by combining PAA with HPMC, a viscous polymer, which resulted in pH responsive polymer mixtures that was solution at pH 4 and gel at pH 7.4. Mixtures of poly(methacrylic acid) (PMA) and poly (ethylene glycol) (PEG) also have been used as a pH sensitive system to achieve gelation[26].

C) Thermally induced Sol-Gel Transitions

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 facilated and no external source of heat other than that of body is required for trigger gelation[26].

Some polymers undergo abrupt changes in solubility in response to increases in environmental temperature (lower critical solution temperature, LCST). This phase separation is generally viewed as a phenomenon governed by the balance of hydrophilic and hydrophobic moieties on the polymer chain and the free energy of mixing. The temperature dependence of certain molecular interactions, such as hydrogen bonds and hydrophobic effects, contribute to phase separation. At the LCST, hydrogen bonding between the polymer and water becomes unfavorable, compared to polymer-polymer and water-water interactions, and an abrupt transition occurs as the solvated macromolecule quickly dehydrates and changes to a more hydrophobic structure. Alternatively, some amphiphilic polymers that self-assemble in solution, show micelle packing and gel formation because of polymer-polymer interactions when temperature is increased. The ideal system would be a solution that is a free flowing, injectable liquid at ambient temperature. It should then gel at body temperature with minimal syneresis. Moreover, loading with drugs or cells should be achieved by simple mixing. When administered parenterally, these systems should exhibit a pH close to neutrality and should be bioresorbable[13]. Poly(N-isopropyl acrylamide) i.e. poly NIPAAM is an example of a thermosensitive polymer (**Figure. 5**). It exhibits the phenomenon of lower critical solution temperature (LCST) phase separation. Poly NIPAAM shows a very well defined LCST at about 32^0C, which can be shifted to body temperature by formulating poly NIPAAM based gels with salts and surfactants[38,39]. However, acrylamide based polymers with quaternary ammonium in their structure, in general, are not suitable for implantation purposes due to cell toxicity[40]. The observation that acryl amide-based polymers activate platelets on contact with blood, along with the poorly understood metabolism of poly NIPAAM and its non degradability, make it difficult to win FDA approval[41,42].

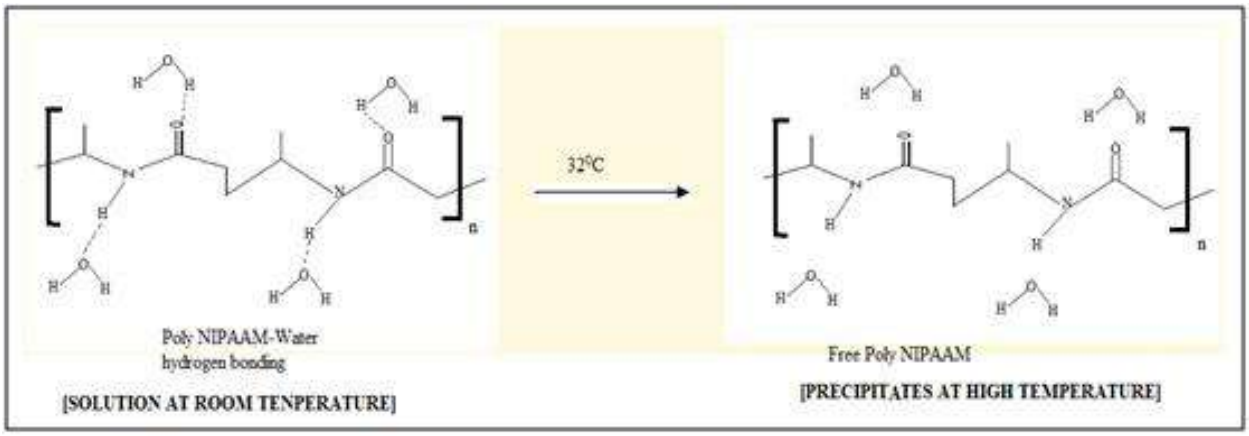

Figure. 5: Poly NIPAAM solution behavior at various temperature.

Therefore, the vast majority of the drug delivery systems which employ LCST, use block copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) simply because of FDA approval.

No organic solvent is required for this system but it bears the problem common to sol-gel systems, which is the high initial burst effect. When a polymer system goes through the sol-gel process, it shrinks and its volume changes dramatically. This phenomenon can exude significant amounts of the encapsulated bioactive agent out of the hydrogel and create an initial burst.

Jeong B *et al*. investigated an aqueous solution of newly developed low-molecular-weight PEG-PLGA-PEG triblock copolymers with a specific composition is a free flowing sol at room temperature but becomes a gel at body temperature. Two model drugs, ketoprofen and spironolactone, which have different hydrophobicities, were released from the PEG-PLGA-PEG triblock copolymer hydrogel formed *in-situ* by injecting the solutions into a 37^0C aqueous environment. Ketoprofen (a model hydrophilic drug) was released over 2 weeks with a first-order release profile, while spironolactone (a model hydrophobic drug) was released over 2 months with S-shaped release profile. The release profiles were simulated by models considering degradation and diffusion, and were better described by a model assuming a core-shell structure of the gel[43].

Chenite *et al*. developed a novel hydrogel system composed of neutral solutions of chitosan[44]. Chitosan is obtained by alkaline deacetylation of chitin, a natural component of shrimp or crab shells. Chitosan is a biocompatible pH-dependent cationic polymer, which remains dissolved in aqueous solution up to a pH of 6.2. Neutralization of chitosan aqueous solutions to a pH exceeding 6.2 leads to the formation of a hydrated gel-like precipitate. In this study, pH-gelling cationic polysaccharide solutions were transformed into thermally sensitive pH-dependent gel-forming aqueous solutions, without any chemical modification or crosslinking. This was done by addition of polyol salts bearing a single anionic head, such as glycerol, sorbitol, fructose or glucose-phosphate salts to chitosan aqueous solutions. This system was examined for delivery of biologically active growth factors *in-vivo* as well as encapsulation of living chondrocytes for tissue engineering. Although this transformation has solved the non-degradability problem of chitosan and can be considered as an advantage for this system, there is a lack of data presented regarding the volume change of the hydrogel and release profile data for the growth factor. Therefore, its suitability as a drug delivery vehicle requires further examination.

Other thermally sensitive polymer systems have also been developed. For example, the concept of stereo complex formation was exploited recently to form a novel hydrogel, based on self-

assembling of entantiomeric lactic acid oligomers grafted to dextran[45]. L- and D-lactic acid oligomers were coupled to dextran, yielding dex-(L)lactate and dex-(D)lactate, respectively. Upon dissolving each product in water separately and mixing the solutions, a hydrogel formed at room temperature. Although no drug delivery applications have been demonstrated to date, this approach can be manipulated for delivering pharmaceutically active agents into the body without the need for crosslinking agents and organic solvents. In two reports, one by Petka WA *et al.*, 1998 and the other by Wang C *et al.*, 1999, protein domains were used to form hydrogels. Petka *et al*. used recombinant DNA methods to create artificial proteins that undergo reversible gelation in response to changes in pH or temperature[46]. Wang *et al*., reported a hybrid hydrogel system assembled from water-soluble synthetic polymers and a well-defined protein-folding motif, the coiled coil[47]. These hydrogels undergo temperature-induced collapse owing to the cooperative conformational transition of the coiled-coil protein domain. Such new systems are still in the development stage and need more experimental studies.

3) Thermoplastic Pastes

Thermoplastic pastes are polymer systems, which are injected into the body as a melt and form a semi-solid upon cooling to body temperature. They are characterized as having a low melting point, ranging from 25 to 65^0C, and an intrinsic viscosity from 0.05 to 0.8 dl/g, measured at 25^0C[48]. It has been reported that an intrinsic viscosity below 0.05 dl/g gives inappropriate release profile to a drug, and a carrier copolymer with an intrinsic viscosity above 0.8 dl/g may be too viscous to be easily administered through a needle[49]. The facile injectability of these systems, when heated slightly above their melting point, is due to their low molecular weight and low *Tg* (glass transition temperature). These polymeric systems flow easily when pushed or stretched by a load, usually at elevated temperatures. They mostly hold their shape at room temperature and can be formed into different shapes by applying heat[50]. Bioerodible thermoplastic pastes could be prepared from monomers such as D,L-lactide, glycolide, ε-caprolactone, trimethylene carbonate, dioxanone and ortho esters[49,51] polymers and copolymers of these monomers have been extensively used in a number of biomedical areas, from carriers of pharmaceutical compounds[52], to surgical sutures[53]. They therefore have a demonstrated track record of biocompatibility and thus are attractive starting points for new material development.

Drugs are incorporated into the molten polymer by mixing, without the application of solvents. Thermoplastic pastes (TP) allow local drug delivery at sites of surgical interventions for the delivery of antibiotic or cytotoxic agents. Alternatively, they can be used to generate a subcutaneous drug reservoir from which diffusion occurs into the systemic circulation.

Walter *et al*. placed a Taxol™ loaded poly (bis(p-carboxyphenoxy)propane-sebacic acid) implant beside brain tumors or within tumor resection sites and demonstrated the effectiveness of the method in rats after surgery[54]. In an effort to develop a means of avoiding surgery and circumventing the invasiveness of Walter's method, Zhang *et al*. developed a thermoplastic triblock polymer system composed of poly(D,L-lactide)-*block*-poly(ethylene glycol)-*block*-poly(D,L-lactide) and blends of low-molecular weight poly(D,L-lactide) and poly(ε-caprolactone) for the local delivery of Taxol™. Both polymeric systems were capable of releasing Taxol™ for a long period of time (greater than 60 days), although at a very low rate[55]. The advantages of using this system over systemic administration of Taxol™ are reduced side effects due to the local delivery of Taxol™ to the tumor site. There are some noteworthy disadvantages associated with this polymeric system. The melting points of these polymeric pastes are greater than 60^0C; therefore the temperature of the paste at the time of injection should be at least 60^0C. This temperature can be very painful for a patient and increases the chance of necrosis and scar tissue formation at the site of injection[56]. The second disadvantage is the very slow rate of drug release (40% drug mass released after 60 days when the block copolymer was used and 35% drug mass released after 30 days when the blend of PDLLA and PCL was used). This slow rate of release, which had a significant impact on the efficacy of the polymeric paste formulation to inhibit the tumor growth, may be due to the high molecular weight of PCL, the high degree of crystallinity in the synthesized polymer (PCL) or the affinity of the drug for the polymer versus the aqueous phase.

In another approach Winternitz *et al*. added methoxy(polyethylene glycol) (MePEG) in amounts up to 30% to the poly(ε-caprolactone) paste, which brought down the melting point from 55 to around 50^0C and increased the crystallinity of the polymer from 42 to 51%[52]. Taxol™ showed a biphasic *in-vitro* release profile composed of a burst phase during the first couple of days followed by a much slower release rate. Nevertheless, this delivery system, with slight changes with respect to polymer composition, was tested in human prostate LNCaP tumors grown subcutaneous in castrated athymic male mice and promising results were obtained[57].

Thermoplastic injectable implants have even been used for delivery of pharmaceutically active agents into the eye[58]. An injectable implant system was developed by Davis *et al*. made of copolymers of PCL and poly(ethylene glycol) which was capable of being injected through a 25 gauge needle when heated to 50^0C. This invention avoids the hazards of eye surgery to insert the drug delivery device and also avoids possible intraocular chemical reactions. The only problem that this system bears is the temperature of the paste at the time of injection (50^0C), which

appears to be too high for the eye environment. It is claimed that *in-vivo* compatibility and degradation life times in the eye can be ascertained by injecting the sterilized paste into both the anterior chamber and vitreous cavity of laboratory rabbits' eyes. Absence of experimental data or examples, regarding the *in-vivo* compatibility of this system, emphasizes the necessity for further studies.

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 deliveries are primarily glycerol esters of fatty acids, for example glycerol monooleate, glycerol monopalmitostearate and glycerol monolinoleate which are waxes at room temperature. These compounds form a cubic liquid crystal phase upon injection[59] 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. This gel forming nature has been used to form drug depot systems for the delivery of both water soluble and water insoluble drugs. For example, Ericsson *et al*.[60] used a glycerol monooleate system to deliver somatostatin subcutaneously in rabbits while Yim *et al*. developed a formulation for interferon-α composed primarily of aluminum monostearate and peanut oil[61], and Gao and co-workers demonstrated the use of a glycerol palmitostearate (Precirol) system to deliver the lipophilic drugs levonorgestrel and ethinyl estradiol. The equilibrium water content of the organogel formed is typically approximately 35%, which therefore produces relatively short release duration for hydrophilic drugs. For the somatostatin example given above, somatostatin release lasted for only 6 h. Much more sustained release can be achieved using a lipophilic drug. In the work of Gao *et al.*, *in-vitro* release of levonorgestrel was observed for up to 14 days, while *in-vivo* studies of levonorgestrel in the organogel injected subcutaneously into rabbits demonstrated an estrus blockage for up to 40 days[62,63].

Although they can be formulated with a low concentration of water, the viscosity of the system is reduced by mixing with vegetable oils. Reducing the viscosity in this manner eases injectability and increases the release duration, particularly for lipophilic drugs. For example, Gao *et al*. found that incorporating glycolyzed apricot kernel oil (Labrafil 1944 CS) reduced the *in-vitro* release rate of levonorgestrel, from 36.2 to 19.9 mg/cm at day 14 for 0 and 20% oil incorporation, respectively. Lipophilic drug release from these organogels is also dependent

upon the solubility of the drug in the cubic phase. If the drug concentration exceeds its solubility in the cubic phase then drug particles will form. The presence of these solid particles has been demonstrated to produce zero-order release kinetics, with a rate which increases as particle size decreases. Another advantage of these systems is that they are biodegradable. Biodegradation occurs through the action of lipases and for the glycerol palmitostearate or Labrafil 1944 CS system, requires between 5-6 weeks[62,63].

Organogels thus are a promising injectable delivery system for lipophilic compounds. There are some disadvantages inherent to this approach. Purity of waxes and stability of oils are the major issues that need to be addressed. There are number of waxes such as carnauba wax, wool wax, spermaceti wax and esparto wax, used for cosmetic purposes but no for parenteral applications. Only beeswax is readily available in various purified grades. Oils usually need a stabilizer, antioxidant and preservative to increase their shelf life and stability. Moreover, the difference between the melting point of waxes and oils makes this system susceptible to phase separation. Labrafil and Precirol are a mixture of many different vegetable oils and glyceryl esters of fatty acids, respectively. Unfortunately, there is still concern over the purity and lack of toxicity data for these waxes and oils. Another drawback is the need to apply heat to mix the oil and wax phase. Temperatures of up to 60^0C for 30 min have been used[63,64]. Temperatures this high can easily reduce the potency of many drugs.

Subsequent **Table 1** summarizes the various classes, common problems associated with and polymers used in *in-situ* forming parenteral drug delivery systems.

Table. 1: Summary of various *in-situ* forming parenteral drug delivery systems.

Delivery System	Common Problem	Common Components
***1)In-situ* Cross Linked System**		
A) Thermosets	❖ Unacceptable level of heat released during reaction and peroxides as curing agent. ❖ Burst in drug release. ❖ Toxicity of un-reacted monomers.	❖ Oligomers of PLA, PDLLA and PCL, Polyols as initiator.
B) Photocrosslinked Gels	❖ Shrinkage and brittleness of the polymer due to high degree of crosslinking.	❖ PGA, PLA, PCL and PEG, initiators such as eosin dye. ❖ Light source (e.g., UV or laser).
C) Ion Mediated Gelation	❖ Low shelf life, burst in drug release. ❖ Long degradation time.	❖ Alginate with Ca^{2+} as gelling agent.

***2)In-situ* Polymer Precipitation**		
A) Solvent-removal Precipitation	❖ Burst in drug release. ❖ Application of organic solvents.	❖ PDLLA, PCL and PLA. ❖ Solvents such as DMSO or NMP.
B) Thermally Induced Sol–Gel Transition.	❖ Stability of oils and purity of waxes.	❖ NIPAAM, PEG, PLA, PLGA, Chitosan and Pluronics.
C) pH Triggered System	❖ Initial burst release.	❖ Carbopol with HPMC, PAA, Polyvinylacetal diethylaminoacetate.
3)Thermoplastic Pastes	❖ High temperature at the time of injection.	❖ PLA, PLGA and PCL. ❖ Alcohols as initiator.
4)Organogels	❖ Phase separation of waxes (e.g., Beeswax, Pericerol). ❖ Lack of toxicity data.	❖ Oils such as peanut oil and Labrafil.

Evaluation of *In-situ* Gel Systems

In-situ gels could be evaluated and characterized for the following parameters;

1) Clarity:

The clarity of formulated solutions is determined by visual inspection under black and white background[26].

2) Texture Analysis:

The firmness, consistency and cohesiveness of formulations 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[26].

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[26].

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[26].

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[26].

Commercial Formulations of *In-situ* Polymeric Systems at a Glance

Regel: Depot Technology:

Regel is one of the Macromed's proprietary drug delivery system and based on triblock copolymer, composed of poly(lactide-co-glycolide)-poly(ethylene glycol)-poly(lactide-co-glycolide). It is a family of thermally reversible gelling polymers developed for parenteral delivery that offers a range of gelation temperature, degradation rates and release characteristics as a function of molecular weight, degree of hydrophobicity and polymer concentration. Following injection, the physical properties of polymer undergo a reversible phase change resulting in formation of a water insoluble, biodegradable gel depot. Oncogel is a frozen formulation of paclitaxel in Regel. It is a free flowing liquid below room temperature which upon injection forms a gel *in-situ* in response to body temperature. hGHD-1 is a novel injectable depot formulation of human growth hormone (hGH) utilizing Macromed's Regel drug delivery system for treatment of patients with hGH- deficiency[26].

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[26].

A list of some marketed in-situ forming parenteral formulations[65] is given below in **Table. 2**:

Table. 2: List of some marketed *in-situ* forming parenteral drug delivery products.

Marketed Product	API	Use
Atridox	8.5% Doxycycline	Periodontal treatment product with sub-gingival delivery.
Atrisorb D	4% Doxycycline	For periodontal tissue regeneration.

Eligard	Leuprolide Acetate	1, 3 and 4 month preparation for treatment of prostate cancer.
Lupron Depot	Leuprolide Acetate	2 and 4 moth preparation for treatment of advanced prostate cancer.
Sandostatin	Octreotide Acetate	Against Acromegaly.

CONCLUSIONS

Parenteral route of administration is the most effective route for drugs with poor bioavailability and narrow therapeutic window; however the rapid elimination of drug and hence the peaks and valleys in plasma profile have lead to need of constant infusions which ultimately needs therapeutic monitoring and hospitalization of patient. Also in case of various chronic diseases, the higher frequency of dosing has resulted poor patient compliance to this rout. To overcome such problems various attempts have been made to prolong the drug release of drug from parenteral formulations. Among these conventional formulations such as emulsions, suspensions and some modern formulations like micelles and liposomes have shown some success; however there are several problems associated with these too. Therefore, to achieve the prolonged and predictable drug release from parenteral formulation a new delivery system, *in-situ* forming parenteral drug delivery systems or *in-situ* forming gels, has emerged. This system is a polymeric delivery system which comprises a solution of biodegradable polymer in which drug is dispersed; which after injection in body by intramuscular or subcutaneous route, and at physiological conditions forms a gel matrix, which retards the rapid release of drug. Thus, are promising and may lead to a new-fangled path of controlled, safe, efficient and targeted parenteral drug delivery in near future.

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