

An Overview of In Situ Gel Forming Implants: Current Approach Towards Alternative Drug Delivery System

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ABSTRACT: Usually, researchers find the difficulties for the development of novel drug delivery systems by implementing the novel technologies. Also there are lots of challenges to make these systems site specific for effective drug delivery for extended period of time. A novel biodegradable, injectable polymeric system namely *in-situ* forming implants (ISFIs) looks very promising drug delivery system. In-situ gel formation of drug delivery systems can be defined as a liquid formulation generating a solid or semisolid depot after administration. It is a blend of drug and biodegradable polymers dissolved or suspended in pharmaceutically acceptable water-miscible organic solvents. These are generally liquid formulations that form semi-solid or solid depots after injection at the injection site due to phase separation and provide a prolonged release over weeks to few months duration. After subcutaneous injection of ISFI the organic solvent dissipates into the surrounding tissue as water penetrates in, this leads to phase separation and precipitation of the polymer forming a depot (gel) at the injection site. The drug entrapped in the depot is then slowly released out into the surrounding body fluid due to the degradation of the polymers and finally reaches the systemic circulation. These high-value formulations ensure patient compliance; will contribute in the design and development of biotechnologically based products. The present review mainly focuses on advantages, disadvantages, classification, composition, methods and evaluation of *in-situ* gelling implants.

Keywords: *In-situ* forming implants, Biodegradable polymers, Semi-solid depot, Injectable polymeric system, Phase separation, Alternative drug delivery system, Quality control of parenterals, Depot system.

INTRODUCTION: Although most of the controlled drug delivery systems are designed for subcutaneous, transdermal, or intramuscular uses, others can also deliver drugs in to blood stream. This type of approach to drug delivery has become quite appealing for a number of classes of drugs, particularly those that cannot be given via oral route¹. Also many new biotechnology-based drug and compounds are not suitable to be administered via the oral route. Thus parenteral drug delivery has received significant research interest in last two decades². Parenteral administration of drug molecule is advantageous for easy access to systemic circulation with rapid drug absorption. This rapid drug absorption is unfortunately also accompanied by a rapid decline in the drug levels in the systemic circulation. In chronic conditions, parenteral formulations results in to quick decline of drug levels in systemic circulation which is not advantageous and needs to take multiple injections for years or even lifetime. For the effective treatment it is often

desirable to maintain systemic drug levels within the therapeutically effective concentration range for as long as treatment calls for. For this purpose new injectable drug delivery system has been developed which is called as parenteral depot formulation or *in-situ* forming parenteral system, also known as *in-situ* forming implant (ISFI)³.

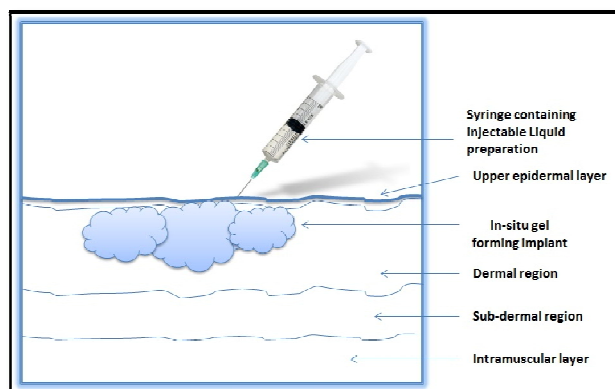


Figure 1: In-situ gel forming implantable system.

In-situ is a Latin word which means in position. In-situ gel formation of drug delivery systems can be defined as a liquid formulation generating a solid or semisolid depot after administration⁴ as shown in Fig.1. This new concept of producing a gel in-situ was suggested for the first time in the early 1980s⁵. It shows prolonged release even for more than weeks to little month duration⁶.

This includes the blend of drug and biodegradable polymers dissolved or suspended in pharmaceutically acceptable water-miscible organic solvent respectively⁸. After subcutaneous injection of ISFI the organic solvent dissipates into the surrounding tissue as water penetrates in, this leads to phase separation and precipitation of the polymer forming a depot (gel) at the injection site⁶. As a result of phase separation by solvent exchange, the water miscible organic solvent diffuses into the surrounding aqueous medium while the aqueous body fluid penetrates into the organic phase and slowly releases the drug entrapped in the depot into the surrounding body. The release of drug is due to degradation of the biodegradable polymer⁹.

Gelation occurs via the cross-linking of polymer chains that can be achieved by covalent bond formation (chemical cross-linking) or non-covalent bond formation (physical crosslinking). The mechanisms of solidification include phase separation induced by pH, solvent exchange or temperature, solubility change, and physical or chemical cross linking¹⁰⁻¹⁴. In this review we will focus on advantages and disadvantages of ISFI, their classification, composition, general methods of preparation and different evaluation parameters. Table 1 shows the advantages and disadvantages of ISFI.

Table 1: Advantages and disadvantage of ISFI¹⁵⁻¹⁹

Advantages	Disadvantage
<ul style="list-style-type: none"> • Ease to administer • Simple to manufacture • Economic • Less invasive and painful as compared to implants • Dose reduction. • Enhanced patient compliance by reducing the frequency of application. • Local or systemic site specific and prolonged release drug delivery. • No surgery required 	<ul style="list-style-type: none"> • The initial rapid drug release prior to solidification of the polymer. • Toxicity because of organic solvents used. • High viscosity of the polymeric solution which may lead to problems during administration.

Classification of in-situ forming parenteral implants: Depot formulation may be classified as follows:

On the basis of process used for controlled drug release: Based on the process and possible mechanism of drug release, Table 2 shows various classification of in-situ forming parenteral implants on the basis of process used for controlled drug release¹⁵⁻²³.

Table 2: Classification on the basis of process used for controlled drug release¹⁵⁻²³

Sr. No.	Type	Factors controlling drug release
1.	Dissolution controlled depot formulation	Rate of dissolution of drug particles in the formulation or in tissue fluid surrounding the formulation
2.	Adsorption type depot preparation	Concentration of unbound drug available for absorption
3.	Encapsulation type depot preparation	Rate of permeation across permeation barrier and rate of biodegradation of barrier macromolecules
4.	Esterification type depot preparations	Rate of interfacial partitioning of drug esters from reservoir to tissue fluids and rate of bioconversion of drug ester to regenerate active drug

On the basis of mechanism of depot formation²⁴⁻²⁶: Fig.2 illustrates different types of in-situ implants and Table 3 describes their properties in short.

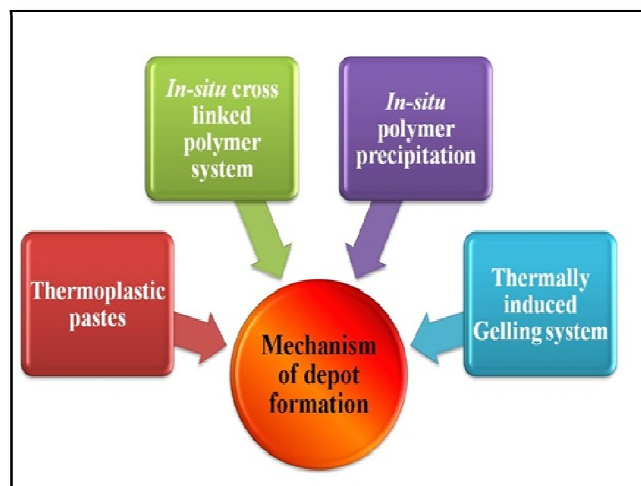


Figure 2: Mechanism of In-situ forming implant

Thermoplastic pastes: Thermoplastic pastes are the semisolid polymers, which when injected are in the melt form and solidifies to form depot on cooling at body temperature²⁷⁻²⁹. The requirements for such ISFD is that the polymer must have low melting or glass transition temperatures in the range of 25 to 65°C and an intrinsic viscosity in the range of 0.05 to 0.8 dl/g³⁰. Below the viscosity threshold of 0.05 dl/g there is no

delayed diffusion, whereas above 0.8 dl/g the ISFD was no longer injectable using a needle³¹. At injection temperatures above 37°C but below 65°C these polymers behave like viscous fluids which solidify to highly viscous depots. 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. Intratumoral injection of Taxole or application of the paste within tumor resection sites are examples for the TP approach³².

Table 3: In-situ forming parenteral drug delivery system²⁴⁻²⁶.

Feature	Thermoplastic pastes	Thermogelling system	Polymer precipitation
Injection	Semisolid paste	Aqueous sol	Organic solution
Depot formation	Solidification	Sol-gel	Phase separation
Polymer	POE	ABA and BAB	PLGA
Drug loading	Dry powder	Aqueous solution	Organic solution
Protein stability	High	Medium	Medium
Drug burst	Low	Medium	High
Release	Surface erosion	Poor diffusion	Low perfusion/bulk erosion
Local tolerance	High	High	Low
Injection pain	Low	Low	High

In-situ cross linked polymer system: The formation of a cross-linked polymer network is advantageous, because of the possibility to control the diffusion of hydrophilic macromolecules³⁰. This system helps to release peptides and proteins over a prolonged period of time. The polymers used in this system must contain double bonds and free radical-initiation. These two factors are detrimental to living tissue and further to the encapsulated drug. Thus, protection of the bioactive agents during the cross-linking reaction is necessary. This could be achieved by encapsulation into fast degrading gelatin micro-particles³².

In-situ polymer precipitation: The concept of ISFD based on polymer precipitation was first developed by Dunn and co-workers in 1990. This system consists of a water-insoluble and biodegradable polymer which is dissolved in a biocompatible organic solvent to which

a drug is added forming a solution or suspension after mixing. When this formulation is injected into the body the water miscible organic solvent dissipates and water penetrates into the organic phase. This leads to phase separation and precipitation of the polymer forming a depot at the site of injection. This method has been developed by ARTIX Laboratories and is designated as the Atrigel technology³¹⁻³³.

Thermally induced Gelling system: Temperature affects the solubility of numerous polymers. The prototype of a thermo sensitive polymer is poly (N-isopropyl acryl amide), poly-NIPAAm, which exhibits a rather sharp lower critical solution temperature, of approximately 32°C. Unfortunately, poly-NIPAAm is not suitable for biomedical applications due to its well-known cytotoxicity. Moreover, Poly-NIPAAm is non-biodegradable. Triblock poly (ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) copolymers, PEO–PPO–PEO, known as poloxamers or Pluronics, have shown gelation at body temperature when highly concentrated polymer solutions 15% (w/w) were injected. These concentrations of a surfactant, however, lead to notable cytotoxicity and, furthermore, they increase the plasma cholesterol and triglycerol levels in rats after intraperitoneal injection^{27-28, 31}.

In-situ solidifying organogels: Organogels are composed of water insoluble amphiphilic lipids, which swell in water and forms lyotropic liquid crystals. Examples of amphiphilic liquid are glycerol mono-oleate, glycerol mono-palmitostearate, glycerol mono-linoleate, sorbitonmono-stearate (SMS). It also contains different gelation modifiers i.e. Polysorbate 20 and 80 in various organic solvents and oils. These compounds forms cubic liquid crystals phase upon injection into aqueous medium which is gel like and highly viscous²⁷.

pH-responsive system: This is more feasible approach for targeting the anticancer drugs, since anti-tumor, since tumor extracellular microenvironments, and endosomal and lysosomal compartments are more acidic than blood and normal tissues. Polymers, hydrogels, micelles, liposomes and inorganic solids have been reported as carriers in pH-responsive drug delivery systems. In a very recent report, mesoporous silica nanoparticles were developed for cancer-targeted drug delivery in vivo after functionalization. The pH- responsive systems based on mesoporous silica materials usually involve on/off capping or gating (by functional groups, polyelectrolytes and ring-shaped compounds) or host–guest interactions (electrostatic, covalent bonding and coordination bonding).

The preparations of the pH responsive systems on a large scale are often very costly due to the complex processes³⁴.

Table 4: Composition of in-situ implants^{10, 13,28,29,35-38}

Sr. No.	Content	Desired characteristics	Example
1.	Active Pharmaceutical Ingredient	--	Peptide Drugs, Protein Drugs, Desensitizing Agents, Antigens, Vaccines ,Anti-Infective , Antibiotics, Antimicrobials, Anti-allergic, Steroidal Anti-Inflammatory Agents, Decongestants, Anticholinergics, Sympathomimetics, Sedatives, Hypnotics, Psychic Energizers, Tranquilizers, Androgenic Steroids, Estrogens, Progestational Agents, Humoral Agents, Prostaglandins, Analgesics, Antispasmodics, Antimalarials, Antihistamines, Cardioactive Agents, Nonsteroidal ,Anti-Inflammatory Agents, Antiparkinsonian Agents, Antihypertensive Agents, B-Adrenergic Blocking Agents, Nutritional Agents, and The Benzophenanthridine Alkaloids.
2.	Solvent Hydrophobic	Nontoxic, water miscible, biocompatible, able to form concentrated polymer solution	Triacetate, Ethyl acetate, Benzyl benzoate
	Hydrophilic		N-methyl-2-Pyrrolidone, Dimethyl sulfoxide, Propylene glycol, Furox
3.	Polymer Thermoplastic system	Low degree of crystallisation ,more hydrophobic, soluble in biocompatible solvent	Acrylonitrile butadiene styrene (ABS), acrylic, polyester, polypropylene, polystyrene, cellulose acetate, Teflon, nylon, Polylactic acids, Polybenzimidazole, Polycarbonate, Polyether sulfone , Polyether ether ketone, Polyether amide, Polyvinyl chloride
	Thermosetting system	Low molecular weight, liquid at room temperature	Polyester resins, polyurethanes, polyurea, polyurea /polyurethane hybrid, Bakelite, Diallyl phthalate
4.	Additive Hydrophilic additive	--	Mannitol , Polyvinyl Pyrrolidone
	Hydrophobic additive		Glycerol Monostearate, Ethyl Heptanoate, Stearic Acid, Ethyl Heptanoate, Methyl Heptanoate And Ethyl Non-oate.

Composition of In-situ implants: In the design of ISFI, polymers play a vital role. Both biodegradable and non-biodegradable polymers are used which upon injection at the tissue site convert in to semi-solid or solid depot and releases the drug in slow manner for extended period of time imparting effective delivery. Table 4 illustrates the composition of in-situ implants along with their examples used in the design of ISFIs for various drugs³⁵⁻³⁸.

General Method of In-situ gel Preparation: The following Fig.3 illustrates the general method of preparing the in-situ gelling solution.

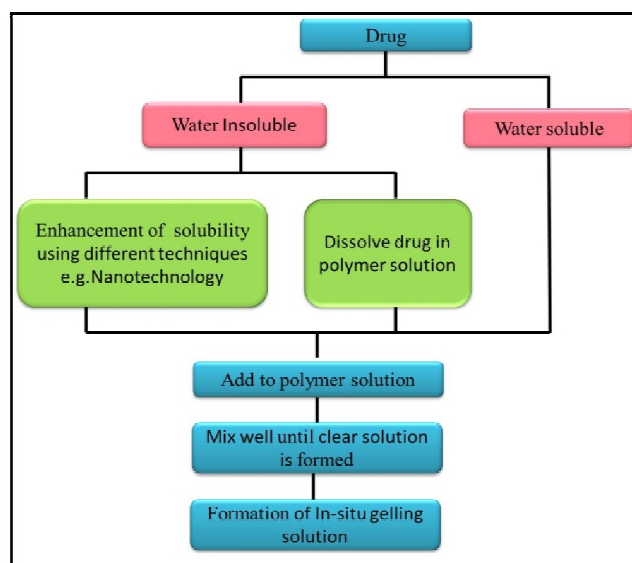


Figure 3: General method of preparing the in-situ gelling solution.

Evaluation of in-situ implants: ISFI are characterized by performing following tests:

In-vitro drug release: Dialysis membrane can be used to study in-vitro drug release. In-situ implants are placed in conical vials open on one side and closed with dialysis membrane on other side. The formulations were placed in 50 ml water for injection at 37°C. At different time intervals, 5 ml samples were withdrawn and replaced with fresh medium and the withdrawn samples analyzed for drug content by UV-visible spectrophotometer. After every one week the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium. The obtained data was fitted into mathematical equation (zero order, first order, Higuchi model) in order to describe the kinetics and mechanism of drug release from the implant formulations³⁹.

Other method is to use cylindrical mould made of poly-tetra-fluoro-ethylene with defined geometry (2.5 mm in diameter and 5 mm in depth) to study in vitro drug release kinetics from injectable ISD systems. The mould is immersed in the release medium in a glass jar and drug is allowed to diffuse out from top surface into the release medium in controlled manner. This is effective method to identify an accelerated release condition.

Clarity test: It helps to visually access the appearance of clear, transparent liquids, for clarity and presence of undesirable components such as suspended matter, free water (or oil) and particulates. Clarity test apparatus can be used for this purpose. In this test, the formulated liquid is observed against black and white background in presence of light to detect the presence of undesirable components⁴⁰.

Viscosity and rheology: This is an important parameter for the in situ gels to be evaluated. These properties of in situ forming drug delivery systems can be assessed by using Brookfield rheometer or some other type of viscometers such as Ostwald's viscometer or Cone and Plate viscometer. The viscosity of the formulation should be such that no difficulties are faced during its administration to patient^[41].

Estimation of drug uniformity: It helps to determine the uniform drug release from the formulation at predetermined time interval. In this test formulations containing 1 mg drug dissolved in appropriate solvent, make up the volume upto 10 ml with solvent in 10ml volumetric flask and then filter and record the absorbance by using UV-spectrophotometer. Concentrations of drug were calculated from the standard calibration curve prepared in solvent⁴¹.

Texture analysis: It helps to determine the firmness, consistency and cohesiveness of formulation, 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 surface like tissues^{33 41}.

In-vitro diffusion studies: It helps to determine the drug release profile of drug from its formulation. Franz diffusion cell is used to determine the in-vitro diffusion in which cellophane membrane is sandwiched securely between donor and receptor compartment with the epidermis site facing the donor compartment. The receptor compartment is filled with buffer solution, which is continuously stirred and thermostated at 37°C ±1°C throughout the experiment. Before starting the experiment the donor cell was sealed with paraffin film and covered with alumi-

nium foil to prevent exposure to light. At predetermined time interval 5ml of aliquots are withdrawn and are replaced with an equal volume of fresh buffer to ensure sink condition and drug content can be determined by spectrophotometrically. Higuchi's equation ($Q = Kt^{1/2}$) and Korsmeyer-Peppas Equation are used to know precisely the mechanism of drug release from the injectable in situ gels⁴¹.

Gel strength: Gel strength is a measure of the ability of a colloidal dispersion to develop and retain a gel form. It is the force expressed in grams, necessary to depress by 4mm the surface of gel with standard 0.5 inch diameter cylinder probe. Rheometer is used for this purpose. 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. The probe is slowly pushed through gel and change in load on the probe can be measured as a function of depth of immersion of probe below the gel surface^{27,41}.

Sol-Gel Transition temperature and Gel time: This test is performed for the in situ gel forming systems containing thermo-reversible 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 above⁴².

Pyrogen test: In this test a pyrogenic substance or the formulation to be tested is injected into the vein of a rabbit, whether an elevation of temperature occurs within a period of 3 hours or not is observed. Recently, an in-vitro test method has been developed utilizing the gelling property of pyrogenic endotoxins from gram-negative bacteria, a firm gel is formed within 60 min of the lysate of the amoebocytes of limulus Polyphemus (the horseshoe crab). In the presence when incubated at 37°C. The limulus amoebocyte lysate (LAL) test has been found to be 5 to 10 times more sensitive than the rabbit test³³.

Optical Microscopy: It helps to study the shape and surface morphology of in-situ implants studied. In this test the dry implants on optical microscope brass stab and observed. These studies were carried out on initial day, 10th day and 30th day so as to observe the degradation of polymer.

Gelling temperature: Gelling temperature is the maximum temperature at which gel is formed. In this test appropriate solvent is selected on basis of polymer used and warmed on a water bath to 37°C. Formula-

tion is then introduced into 1ml of solvent with the help of syringe and changes in consistency of formulations is inspected visually at interval of five minutes and results are noted as positive and negative for gel formation. The formation of gel is determined by flow or no-flow criterion over 30 seconds when the vial is tilted at an angle of 90°C⁴³.

Determination of drug content: The vials containing the formulation are shaken for 1-2 minutes. Then transfer 1ml of the formulation to 15ml centrifuge tube with a micropipette. To this add 10ml of Methanol to completely precipitate the polymer. Then centrifuge the contents of the tube at 1500rpm for 15 minutes. From tube collect about 0.1ml of the clear supernatant and dilute it with methanol in 10 ml volumetric flask and the final volume was made up with methanol and estimate the drug content by using UV-spectrophotometer.

Analytical evaluation: Different analytical techniques like electron paramagnetic resonance spectroscopy (EPRS), Fourier Transform Infrared spectroscopy (FTIR), Differential Scanning Calorimeter (DSC) can be used to examine the ISFI. EPRS helps to understand the exact mechanism of implant formation and drug release in-vitro and in-vivo.

FTIR determines the drug-excipient compatibility, nature of interacting forces. KBr pellet method can be used to record FTIR spectra for drug and formulation. Also, the intersection between different ingredients in the formulation can be determined by using DSC technique. This is done by comparing the thermograms of prepared formulation with that of pure ingredient⁴⁴.

Pharmacokinetic study: Pharmacokinetic studies can be performed by using different animals like rats and rabbits that are fasted overnight. This study helps to determine the in-vivo drug release from the administered dose of the drug from the ISFI. The formulation is injected to animals either intramuscular or subcutaneous route by using needle. Then collect the blood samples (0.5ml) at different time intervals. Then these collected samples were deproteinised by using acetonitrile and then analyzed by using different analytical techniques like UV-spectroscopy, mass spectroscopy or ultra-performance liquid chromatography mass spectroscopy to obtain the data about plasma drug concentration of drug at different time interval. From obtained plasma drug concentration different pharmacokinetic parameters such as maximum plasma concentration, maximum time to reach maximum plasma concentration (t_{max}) and mean residence time can be determined⁴⁵⁻⁵⁴.

Mashayekhi et al. used Female Sprague-Dawley rats to perform in-vivo studies leuprolide acetate release from an in-situ forming PLGA system⁵⁵. Tarek et al. used Male New Zealand white rabbits to perform in-vivo studies of atorvastatin biodegradable in situ gel⁵⁶.

CONCLUSION: From overall discussion it is concluded that the in-situ forming implants (ISFI) are the alternative drug delivery system to the frequently administered parental injections. This will decrease the dosing frequency, ultimately convenient to the patients, which may improve the quality of life of patients suffering from cancer and for chronic therapy. The review article has cleared all the ideas regarding materials used for preparation of these systems and various evaluation parameters to be tested for making effective in-situ forming implants.

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