International Journal of Advanced Research and Review

www.ijarr.in

A NOVEL APPROACH TO DELIVER THERAPEUTIC AGENTS USING IN SITU FORMING IMPLANT BASED ON SOLVENT INDUCED PHASE SEPARATION TECHNIQUE FOR LONG TERM CONTROLLED RELEASE.

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ABSTRACT

Biodegradable injectable in situ forming drug delivery systems represent an attractive alternative to microspheres and conventional implants as parenteral depot systems. In situ forming Implant concept includes biodegradable polymers dissolved in or diluted with water miscible, physiological compatible organic Solvents. Upon injection through i.m. or S.c. solvent dissipates into the physiological space & due to insolubility of polymer in physiological fluid it gets precipitated out & forms adepot with entrapped drug inside it. Drug is released from these depots either by diffusion or erosion of it. ISFI are capable to deliver them for few weeks to several months. The desired drug release profile can be obtained by modifying polymer concentration, combination of hydrophilic & hydrophobic biocompatible solvents & by using additives. Various therapeutic agents which include synthetic drugs, hormones & other protein drugs are successfully formulated& patented with this technology. Here in this review article these topics are discussed briefly.

Keywords: In situ Implant, Biodegradable polymers, PLGA, Long term release, Solvent exchange technique

INTRODUCTION

The parenteral route of administration is the most effective route for the delivery of the active pharmaceutical substances with narrow therapeutic index, poor bioavailability especially for those drugs, prescribed to unconscious patients.[1] This route of administration i.e. subcutaneous, intramuscular, intravenous, intradermal and intraarterial etc. also possess good absorption characteristics and provide good bioavailability of drugs because the therapeutic agents given by this route bypass the long gastro intestinal route to be get absorbed into the blood & there are not chances of first pass metabolism due to direct absorption into the systemic route. [2]

Some chronic conditions need drug delivery over prolong period of time. Conventional drug delivery systems such as oral/transmucosal systems can deliver it only up to few hours. That is not enough because in that case patient has to take medications daily until the condition get cured or in some incurable conditions for lifetime. During this treatment routine it is not necessary that patient will take drugs on regular basis. In some of cases medication non-adherence is noticed such as patient forgets to take medications, taking medications on irregular timings, presence of any psychotic condition etc. results in fluctuations in the plasma drug concentration that leads to

delay in the therapy. To overcome these problems various novel drug delivery systems are developed. Among them all parenteral controlled drug release formulations are the most promising for long termed controlled delivery of therapeutic agents.

In conventional parenteral drug delivery systems, to maintain effective therapeutic drug concentration in blood it requires frequent injections which ultimately lead to patient discomfort. In parenteral drug delivery field major invention to reduce the frequency of dosing, for targeted drug delivery, to sustain drug release up to longer period various novel technologies have been developed[3]such as **A**. **Injectables** that include Solutions, Colloidal Dispersions, Microspheres, Microcapsules, Nanoparticles, Niosomes, Liposomes, Resealed erythrocytes, Polymeric Micelle, In situ forming implants, **B. Solid Implants,C. Infusion Devices** such as Osmotic Pumps (Alzet) , Vapor Pressure Powered Pumps,Battery Powered etc. [1],[4]

In situ forming Implants

Biodegradable injectable in situ forming drug delivery systems represent an attractive alternative to microspheres and conventional implants as parenteral depot systems. These Biodegradable in situ Implants effectively solve the difficulties of injecting microspheres into the biological system using large syringes, Invasive procedures to remove conventional Implant after its application etc. [5] 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. Drug is released from this depots either by diffusion or erosion of it. [6]

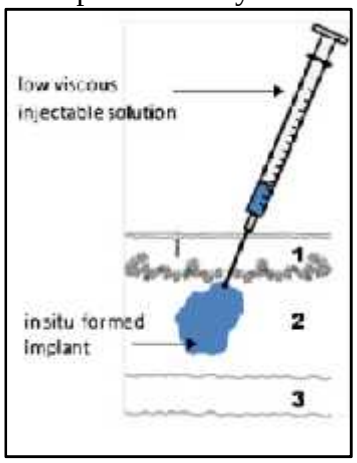

Figure: 1 In situ forming Implant Schematic

The gelation/depot formation occurs with different stimuli/mechanisms. The various strategies that have been used to prepare in situ forming systems [7]

1. **Solidifying organogels. (Triggered by solubility change):** These are semi-solid systems consisting of a three-dimensional network of self-assembled gelator fibers and a continuous liquid organic phase. The gels are prepared by dissolving the gelator, in concentrations up to 15 wt.%, in the heated solvent. Upon cooling the solubility of the gelator decreases. The gelator self-assembles into aggregates, which form the network by intermolecular physical interactions.[9]

2. **Cross-linked systems.**

   A. **Photo initiated cross-linked systems:** In this kind of systems initial materials are liquid solutions containing polymer, drug & other additives, which can be easily injected. Afterward the rapid polymerization at physiological temperatures using

photosensitive initiators (e.g. eosin dyes) and ultraviolet (UV) or visible light forms the polymer matrix of the required dimension. Polymers used in this kind of system are biodegradable modified meth(acrylate) such as poly(N-(2-hydroxypropyl) methacrylamide lactate), some block polymers etc.[7]

B. **Chemical Cross-linked systems:** chemical cross-linking includes the formation of polymer networks via covalent links using Crosslinking agents like benzoyl peroxide, dialdehydes (e.g. glyoxal and glutaraldehyde), oxalic acid etc. [7],[8]

C. **Physical cross-linked systems:**The physical cross-linking of polymers leads to the formation of a covalent network structure. They are formed by inter- and intramolecular bonding via hydrogen bonds or various charge interactions, such as ionic interaction or polyelectrolyte complex (PEC) interactions. [7]

3. **Phase separation systems:**Hereby polymers undergo abrupt changes in their solubility in response to changes in their environmental temperature, pH or by solvent removal.

A. **pH induced gelling:** Sol-to-gel transitions induced by changes in environmental pH are related to polymers containing ionizable functional groups. Below the pH 5 they remain as free flowing liquid but at physiological pH (7.4) get converted into gels/depots. eg. System containing Poly(methacrylic acid) (PMA)& PEG which form water-insoluble interpolymeric complexes (IPCs) at pH<5.8.[10]

B. **Solvent exchange:** In this approach biodegradable polymers such as PLGA, PLA, PCL are dissolved in highly water miscible solvent. These polymers are insoluble in water. So when the this polymeric solution is injected via i.m. or s.c. route the organic solvent readily dissipates into the physiological fluid & the polymer comes in contact with physiological fluid & depot formation occurs at site due to insolubility of polymer in water (Physiological fluid). [11]

C. **Temperature induced gelling:** Here the gelling is bring by temperature change. Below the physiological temperature (38^oC) it remains as liquid but when injected gelation occurs due to temperature change. (at lower critical solution temperature (LCST)) [12]

Phase separation by Solvent exchange [11]

This concept, conceived by Dunn et al., employs biodegradable carriers dissolved in or diluted with water miscible, physiological compatible organic Solvents. Prior to injection the drug is added and forms an injectable solution or dispersion. After subcutaneous injection of the formulation into the body the organic solvent dissipates into the surrounding tissue as aqueous body fluids penetrate into the implant.This leads to phase separation and precipitation of the polymer, forming a depot at the injection site. The active pharmaceutical ingredient (API) gets entrapped within the matrix as it solidifies and is released by diffusion processes or as the implant biodegrades

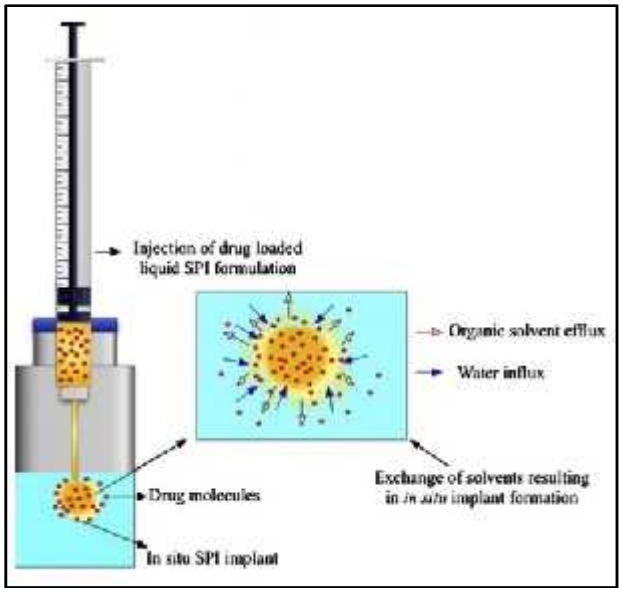

Figure 2: Mechanism of Phase separation

The controlled release of bioactive macromolecules via in situ forming systems has a number of advantages, such as [15]

- Ease of administration (Elimination of any invasive procedures.)
- Less complicated fabrication,
- less stressful manufacturing conditions for sensitive drug molecules
- Long term release of drug in controlled manner which reduces frequency of dose administration as well as increases the patient compliance
- Stable drug plasma level can be achieved which results in a better therapeutic outcome.

Components of In situ forming Implants

Biodegradable Implants

A wide number of polymers for their potential to form SPI-based drug delivery systems have been investigated [16],[17],[18]. Polymer selection of Biodegradable Polymers should consider chemical stability and physical stability, Biocompatibility, safety, ease of availability etc. In ISFI system synthetic polymers have been used widely because of their major characteristics of water insolubility that results in precipitation at the injection site & formation of solid depot.
Ideal characteristic of a polymer to be used to from SPI system include:[15]

(1) Biodegradability and biocompatibility. (2) Approval in drug delivery systems for parenteral administration by FDA and European Medicine Agency (EMA). (3) Well define methods of production for hydrophilic or hydrophobic small molecules or macromolecules in various carrier based drug delivery system. (4) Drug protection from degradation. (5) Sustained release. (6) Modification of PLGA for better interaction with biological materials. (7) Possibility to target specific organs or cells. (8) Glass transition temperature is above physiological temperature of 37°C, which imparts a moderately rigid chain configuration and therefore the mechanical strength at ambient temperatures. (9) Availability in different grades (ratio and molecular weight) provide wide range of physicochemical and degradation characteristic and thus provide different release

profiles. (10) The polymer should be water insoluble to allow the coagulation of polymer and formation of depot

Recently biodegradable non-polymeric carrier has also been used for SPI based drug delivery such as sucrose acetate isobutyrate (SAIB) made through the esterification of sucrose with acetic anhydride and isobutyric anhydride, which produces a clear, very viscous liquid with a high molecular weight. SAIB formulations are injected into a tissue, the solvent diffuses out leaving a matrix that is both adhesive and viscous. The advantages associated with this system, are the low cost of materials in comparison to PLGA and the simplicity of manufacturing. There is however an appreciable burst release that occurs due to the lag-period between injection and formation of the implant

Biodegradable Polymers used in In situ Forming Implant (ISFI) formulation: [13],[17]

Table 1: List of Biodegradable Polymers used in ISFI

Sr. No	Polymer	Degradation time
1	PLGA (Poly(D,L-lactide-co glycolide)	
	50:50	1-2 month
	85:15	5-6 month
	75:25	4-5 month
	65:35	3-4 month
2	PLA [poly(lactic acid)]	18-24 month
3	PCL [poly(capronolactone)]	>24 month
4	PPF [poly(propylene fumarate)]	2-3 month

Solvents

An ideal solvent used in ISFI should have properties such as water miscibility, biocompatibility and organic in nature, should efficiently dissolve the polymer, and be miscible with water and bodily fluids readily[11].The viscosity of solvent plays an important role in the syrigability criteria.For example, highly viscous solvents, combined with around 30% of polymer as well as drug, could pose difficulty when injecting via conventional needles. Therefore overall viscosity should be within the range that is syringeable.

The strength of solvent and its affinity for water determine the nature of phase inversion and implant formation. For example, solvent that has a high water affinity exhibits fast forming phase inversion (fPI) such as NMP and DMSO. Because they have ability to readily dissipate into the physiological fluid. Hydrophobic solvents exhibit slow forming phase inversion (sPI) such as triacetin and ethylbenzoate because of low water miscibility. The notable differences are seen in the morphology of both fPI & sPI. The fPI shows the more porous structure whereas sPI shows rigid & less porous structure because of slow solidification rate. These parameters also affect on the drug release &polymer degradation. [11],[14]

Commonly used biocompatible solvents in ISFI systems [11], [14]

Table 2: List of Biocompatible Solvents used in ISFI

Sr. No	Solvent	Nature	Water solubility	LD_{50} (mg/kg)
1	**N-Methyl 2-Pyrrolidone (NMP)**	Hydrophilic	Completely miscible	IV, mice: 155 IP, mice: 3050 Oral, rat: 3914 Dermal, rabbit: 8000
2	**Dimethyl Sulphoxide (DMSO)**	Hydrophilic	Completely miscible	IV, rat: 5360 IP, rat: 8200 SCU, rat: 12000 Oral, rat: 14500 Dermal, rat: 40000
3	**Triacetin**	Hydrophobic	61.2 g/L at 20 °C	IM 1740 mg/kg
4	**Benzyl Benzoate (BB)**	Hydrophobic	15.4 mg/L at 20 °C	Oral, rabbit: 1680 Dermal, rabbit: 4000
5	**Benzyl Alcohol (BA)**	Hydrophilic	33 g/L at 20 °C	IV, mice: 480 Oral, rabbit: 1040 Dermal, rabbit: 2000
6	**Ethyl Acetate**	Hydrophilic	NA	Dermal, rabbit:3600 mg/kg/day
7	**PEG 400**	Hydrophilic	Completely miscible	IV, rat: 7313 IP, rat: 9708
8	**Propylene carbonate (PC)**	Hydrophobic	NA	Oral, rat: 29100 Dermal, rabbit:20001

IP: Intra peritoneal, SCU: Subcutaneous, IV: Intravenous, IM: Intramuscular

Factors affecting on Drug release from In Situ Forming Implants

1. **Polymer related factors [15]**

A. Effect of Monomer Ratio and Crystallinity

Glycolic acid is more hydrophilic than lactic acid. Hence more the glycolic acid faster will be the degradation and more will be the rate of drug release. Hydrolysis occurs fast in amorphous forms than crystalline ones shows rapid drug release. Eg. Miyajima et al. over a decade ago, examined how the crystallinity ofPoly(L-lactic acid) (PL(L)A) influenced the release of papaverine fromrods. The results showed that an increase in crystallinity would resultin a faster release rate due to the microporous structure associatedwith a crystalline polymer. Initially the PL(L)A was amorphous butonce immersed into the aqueous medium, the polymer became semi-crystalline and therefore microporous. The emergence of micropores resulted in drug release occurring via water filled channels and therefore afaster release profile.[19]

B. Molecular Weight

Polymer molecular weight (M_W) is an extremely important property that can affect a number of physico-chemical and mechanical properties such as solubility, viscosity, diffusivity, glass transition temperature and modulus. It is well known

that the greater the MW of the polymer, the greater will be the reduction in drug release. The availability of polymers in a vast range of M_Ws also makes it easier to modify drug release rate from SPI implants, as addition of external additives can be avoided. Astaneh et al. studied the effect of varying PLGA MWon the leuprolide acetate release profile of from SPI implants. The results showed that theSPI systemprepared with the highest MWPLGA (i.e. 48 kDa) had a significantlylower initial release within the first 24 h compared to the lowerpolymer MWimplant (i.e. 12 and/or 34 kDa). But there is also a problem with increasing molecular weight of increased viscosity which present problem while injecting (Syringeability), so it is important to choose required grade of PLGA while considering resultant viscosity of formulation.[20],[21]

C. Influence of End Group

Polymers having ester end group were relatively hydrophobic in nature than acid end group results in slow drug release.In a study conducted by Chhabra et al. the aimwas to determine theinfluence of carboxylic acid and ester groups at the end of PLA/PLGApolymers on the release of lysozyme. It was seen that the end groupsof the polymer chains had a significant effect on the type of solventthat would be required for optimal solubility of the polymer. The polymers consisting of acid end groups were more hydrophilic than esterend group polymers therefore a solvent of a hydrophilic nature wasrequired for a solution to be formed successfully. Consequently, themore hydrophobic polymers were not soluble in hydrophilic solventsand required more hydrophobic solvents. Upon investigating the release of lysozyme fromsystems produced using the polymer variations,they found that those systems that contained polymer with carboxylicacid groups resulted in a greatly reduced burst release of approximately4% compared to 20–30% for the other formulations containing polymerswith ester end groups. This reduction in burst release was attributed tothe theory that the carboxylic acid end groups of the polymer have theability to form chemical linkages with amino acid residues of the lysozymethat contain hydroxyl groups.[22]

D. Effect of Polymer Concentration[23], [24],[29]

The increase in polymer concentration reduces the rate of phase inversion of SPI (slow phase inverting) implants, which in turn results in a reduction in drug release as well as increase in viscosity of system.

2. Solvent [11],[25],[26],[29]

The ideal solvent or solvent blend for in situ systems needs to possess suitable properties in terms of water affinity, viscosity, ability to dissolve the polymer and last but not least, safety. More hydrophilic solvents results in the formation of fast forming implant which is more porous & shows more rapid drug release with initial burst release whereas hydrophobic solvent shows slower implant formation with least drug release. Hence combinations of both are used to achieve optimum drug release profile.

3. Additives[11], [27], [28], [29]

Use of additives which are hydrophilic in nature generally results in rapid liquid-liquid demixing and generated higher bursts eg. PVA, mannitol. Addition of amphiphilic or hydrophobic additives reduces the burst while also modifying the morphology of the

system, with a transition towards a sponge structure. For example Glycerol monostearate, ethyl heptanoate, stearic acid, ethyl heptanoate, methyl heptanoate and ethyl nonoate

Tarek Ahmedet. al.reviewed various approaches to decrease the drug initial release rate from PLGA based *in situ* Implant system which are given below.

Table 3: Approaches for reduction of burst release

TECHNIQUE USED	EXAMPLE
Implant based on hydrophobic solvent	Insulin/PLGA in benzyl benzoate and benzyl alcohol Metoclopramide/PLGAs in benzyl benzoate Haloperidol/PLGA in triacetin and ethyl acetate Chicken egg white lysozyme protein /PLGA in triacetin or ethyl benzoate
PLGA lactide-to-glycolide ratio	Leuprolide acetate/PLGA (75:25)/NMP Rosiglitazone/PLGA (65:35, 75:25, 85:15)/NMP or triacetin Fluorescein/PLGA (50:50, 75:25)/NMP
***In situ* microparticles (ISM) and microglobules**	Diltiazem hydrochloride/PLGA ISM Bupivacaine hydrochloride/PLGA ISM Cytochrome c/PLGA microglobules (premicrospheres' or `embryonic microspheres)
Incorporation of plasticizer or surfactant	Meloxicam/PLGA/PEG 400 Aspirin/PLGA/PEG 400 Hen egg protein/PDLA/Pluronic Fluorescein/PLGA/Pluronic P85
Polymer concentration and molecular weight	Meloxicam/PLGA (0.3, 0.5, 0.7 dl/g) Haloperidol/PLGA (20, 30, 40% wt) Fluorescein/PLGA (0.2,0.3, 0.4, and 0.45 dL/g)

Sterilization

In situ implants are given by parenteral route so it must be sterile. There are various methods of sterilization of it such as terminal filtration, sterilization by gamma radiation& plasma sterilization which is currently under evaluation.[30]Among them all Gamma irradiation is the currently only accepted method for terminal sterilization. The major disadvantage of terminal gamma sterilization is radiolytic degradation of incorporated drug and polymer matrix. It has been reported that biodegradable polymers undergo chain incision and cross linking after exposure to gamma irradiation. Mainly there is decrease in molecular weight of polymer so the formulation should be formulated using polymer.[15]

Here is the list of various patents available for different formulation of PLGA

Table 4: List of PLGA Based Patents

SR NO.	TITLE	POLYMER USED	SOLVENT	DRUG	RELEASE DURATION
1	**Pharmaceutical composition of Tapentadol for parenteral Administration(US009629818B2)[31]**	PLGA 50:50	NMP	Tapentadol	5 days, 7 days, 15 days & 30 days.
2.	**Injectable flowable compositionComprising Buprenorphine (US20130210853A1)[32]**	PLGA 50:50	N-methyl 2-pyrrolidone, 2-pyrrolidone, N,N-dimethylformamide, dimethyl sulfoxide, propylene carbonate, caprolactam, tri acetin	Buprenorphine	4-6 Weeks
3	**In-situGelling Drug Delivery System[33]**	PLGA 70:30, 85:15, 50:50, 90:10	PEG 200-400	Morphine-Diclofenac co drug	
4	**Sustained Delivery Formulations of Risperidone Compounds[34]**	PLG 50:50, 75:25, PLGH 50:50, 65:35, 75:25, 85:15	NMP	Risperidone	
5	**Pharmaceutical composition Containing goserelin for in-situ Implant[35]**	PLGA 50:50 85:15 75:25	NMP	Goserlin acetate	One month & three month
6	**Injectable Depot Gel Composition and Method of Preparing the Composition[36]**	PLGA 50:50	Triacetin	Lysozyme	
7	**Injectable Depot Compositions and Uses there of[37]**	PLGA 50:50	BB, BB, Ethanol, propylene Glycol	Human Growth Hormone, Bupivacaine, Platelet Derived Growth Factor	
8	**Liquid Polymeric Compositions for Controlled Release of Bioactive Substances[38]**	PLGA 75:25	Triaceti, Glycerol formal	Ivermectin, Eprinomectin	

Table 5: PLA/PLGA based commercial formulation.[39-50]

PRODUCT NAME	DOSAGE FORM	MANUFACTURER	DRUG	RELEASE DURATION
Decapepty®	Microparticles	Ferring	Triptorelin acetate	1
Decapepty® SR	Microparticles	Ipsen-Beaufour	Triptorelin acetate	1,3,6
Zoladex®	Implant	AstraZeneca	Goserelin acetate	1,3
Lupron ® Depot	Microparticle	Takeda –Abbott	Leuprolide acetate	1,3,4
SandostatinLAR®	Microparticle	Novartis	Octreolide acetate	1
Nutropin®Depot	Microparticle	Genentech	Somatropin (hrGH)	1,2
Profact®Depot	Implants	Sanofi-Aventis	Buserelin Acetate	2,3
Superecur® MP	Microparticle	Sanofi-Aventis	Buserelin Acetate	1
Eligard®	Implants	Sanofi-Aventis	Leuprolide Acetate	1,3
Luprogel®	Liquid	MediGene AG	Leuprolide Acetate	1
Trelstar® Depot	Microparticle	Watson	Triptorelin pamoate	1,3
Trelstar ®LA	Microparticle	Watson-Debio	Triptorelin pamoate	1,3
Arestin®	Microparticle	OraPharma	Minocycline HCL	0.5
Atridox®	Implants	CollaGenex	Doxyclycline	0.25
Risperdal Consta	Microparticle	Johnson & Johnson	Risperidone	0.5
SIMARTshot B12	Microparticle	Stockgaurd	Vitamin B12	4,8
Vivitrol®	Microparticle	Alkemes	Naltrexone	1
Revalor- XS®	Implants	Intervet	Trenbolone	6
Ozurdex®	Implants	Allergan	Dexamethasone	1
Gliadel®	Implants	MGI Pharma	Carmustine	20

CONCLUSIONS

Solvent induced in situ forming Implants present superior advantages over other dosage forms. In situ implants are successfully prepared for various therapeutic agents which include synthetic

drugs, hormones & other protein drugs by using different biodegradable polymers, biocompatible solvents & other additives for the long term controlled release. ISFI are capable to deliver them for few weeks to several months. The desired drug release profile can be obtained by modifying polymer concentration, combination of hydrophilic & hydrophobic biocompatible solvents & by using additives. The studied patent also summarizes the composition & application of various marketed formulation based on this ISFI technology. In conclusion, ISFI are now days getting attention because of their advantage in flexibility of drug delivery, biodegradability, ease of preparation & improved patient compliance.

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