REVIEW

Approaches to develop PLGA based *in situ* gelling system with low initial burst

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Abstract: *In situ* gelling systems have gained much interest owing to their successful application in the preparation of controlled drug delivery and tissue engineering. The commonly used polymer for these systems is the biocompatible and biodegradable polymer of Poly (lactic-co-glycolic acid) (PLGA) that is available in the market as implants, microparticles and *in situ* implant. A polymeric solution is prepared by mixing the polymer with a biocompatible solvent which may be water miscible such as N-methyl pyrrolidone (NMP), 2-pyrrolidone and Dimethyl sulfoxide (DMSO) or partially water miscible solvents such as triacetin, benzyl benzoate, ethyl acetate, triethyl citrate and benzyl alcohol. Upon injection of this polymeric solution into buffer or physiological fluid, the system solidifies and the administered drug releases in a controlled manner. The major drawback of these systems is their high initial burst that characterized by release of a noticeable amount of the administered drug during the first release stage that usually results in drug toxicity and tissue irritation. This review focuses on presenting the different strategies utilized to decrease the initial burst from PLGA *in situ* gelling system.

Keywords: *In situ* gelling system, Drug delivery, PLGA, initial burst.

INTRODUCTION

Biocompatible *in situ* gelling systems have been sparked in the last decades for the purpose of drug delivery or injectable tissue engineering (Oh and Lee, 2007; Wang *et al*., 2010; Ko *et al*., 2013). Other important applications include; cell transplantation, three dimensional cell culturing, in vaccine and the orthopedic and dental administrations have also been reported (Hatefi and Amsden., 2002; Packhaeuser *et al*., 2004; Gutowska *et al*., 2001; Joo *et al*., 2009; Feng *et al*., 2006; Jiang *et al*., 2005). Pharmaceutically, these systems are liquid or syringeable semi-solids that congeal upon administration into the body due to one of the following mechanisms:

Thermoplastic pastes; that are injected into the body as molten which solidify upon cooling to body temperature (Bezwada, 1995).

In situ crosslinking; where polymer crosslinking is formed due to temperature change (thermosets), absorption of photons (photo-irradiation), ionic interaction between the anionic polymer and small cation, or presence of enzyme (Hatefi and Amsden., 2002).

In situ polymer precipitation; in which solvent exchange (Shah *et al*., 1993, Eliaz and Kost, 2000), temperature change (Jeong *et al*., 200, Paavola *et al*., 1998), or pH change (Siegel and Firestone, 1988, Tanaka, 1980) is the triggering factor.

Among the aforementioned mechanisms, *in situ* polymer precipitation based on the process of solvent removal or exchange has developed into commercially available products (Kempe and Mader, 2012). Leuprolide acetate, Doxycycline hyclate, Bupivacaine, Risperidone and Paclitaxel are common examples for drugs commercially available or in clinical trials as *in situ* forming implants (Kempe and Mader, 2012).

The biodegradable copolymers of lactide and glycolide namely; poly (lactide-co-glycolide) (PLGA) has been permitted for parenteral use by regulatory authorities all over the world (Fredenberg *et al*., 2011). The reason for the great interest and the wide spread use of these types of polymers is their biocompatibility, biodegradability, and mechanical strength (Jain, 2000). Jalil and Nixon mentioned that PLGA polymer degrades into the biocompatible lactic and glycolic acids (Jalil and Nixon., 1990; Tice and Tabibi., 1991; Wu., 1995; Kitchell and Wise., 1985; Cohen *et al*., 1994). Both acids are eliminated from the body as carbon dioxide and water after they have entered the tricarboxylic acid cycle (Jalil and Nixon., 1990; Tice and Tabibi., 1991; Wu., 1995; Tice and Cowsar., 1984). Glycolic acid may also excrete unchanged in the kidney (Wu, 1995). Different PLGA formulations containing a variety of drug classes for drug delivery use have been approved by the FDA. Among these are; microspheres, microcapsules, nanoparticles, pellets, implants, films, cylinders and foams (Arshady., 1991; Yeh *et al*., 1996; Desai *et al*., 2010; Sharma *et al*., 2007; Klose *et al*., 2008; Dong *et al*., 2006; Banu *et al*.,

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2008; Desai *et al*., 2008; Houchin and Topp., 2009). Common PLGA depot formulations available in the market include;

Lupron Depot®, Nutropin Depot®, Suprecur® MP, Decapeptyl®, Sandostatin LAR® Depot, Somatuline® LA, Trelstar™ Depot which are available as PLGA microparticles

Profact® Depot and Zoladex® available as PLGA implants.

Eligard® available as *in situ* forming implant.

Diffusion and degradation/erosion are the main two pathways related to the process of drug release from PLGA drug delivery systems (DDSs). D'Souza *et al*., reported that the release from PLGA is initially diffusion-controlled followed by a final stage of degradation/erosion controlled (D'Souza *et al*., 2005). Wang *et al*., illustrated also a two-phase release profile for Metoclopramide and its salt (monohydrochloride) from PLGA/benzyl benzoate solutions following injection into buffer; an early diffusion, followed by erosion (Wang *et al*., 2004). The release profile (shape of the release) for PLGA DDSs is usually tri-phasic, some-times bi-phasic and rare to be mono-phasic (Fredenberg *et al*., 2011). For PLGA *in situ* gelling systems, the most common profile is the tri-phasic one in which there is an initial burst release phase followed by a slow release phase and finally a rapid release phase. Ahmed *et al*., demonstrated a tri-phasic release pattern for haloperidol *in situ* gel prepared with PLGA (Ahmed *et al*., 2012). Ibrahim *et al*., reported the same behavior for meloxicam in *situ* implants prepared using PLGA that was dissolved in N-methyl pyrrolidone (NMP) (Ibrahim *et al*., 2013).

Initial burst is the major disadvantage of the polymeric solutions that solidify in the body (Kranz *et al*., 2001). It is a high release rate of the drug or the administered material that is noticed at the beginning of the process. Drug toxicity or tissue irritation are the major drawbacks associated with phenomenon (Huang and Brazel, 2003; Lu and Anseth, 1998; Jeong *et al*., 2000; Shively *et al*., 1995). The major causes for this behavior include; the rapid release of drug adsorbed on the polymeric surface (Pekarek *et al*., 1994), unequal distribution of the drug within the polymeric network (Mallapragada *et al*., 1997; Kishida *et al*., 1998) and/or rapid diffusion of the drug to the surrounding medium prior to the solidification process (Ahmed *et al*., 2012). Several attempts have been conducted to overcome this drawback, among the factors that should considered are; the type of solvent, the lactide-to-glycolide (L-G) ratio of the polymer, the concentration and molecular weight (viscosity) of the polymer, incorporation of plasticizer or surfactant, *in situ* microparticles technique and formation of *in situ* PLGA microglobules. Table 1 illustrates some commonly used techniques and examples of *in situ* gelling system with low initial drug release rate based PLGA.

In this review, the different technique utilized to produce biocompatible *in situ* gelling system based on PLGA with low initial burst will be stressed. The article will provide an overview on the exact mechanism of these techniques and evidences from the previously published articles in the same field.

PLGA in situ implant based hydrophobic solvents

Solvents commonly used for dissolving PLGA can be classified into two main categories; water miscible solvents and partially water miscible ones. Common examples for the former include NMP, dimethyl sulfoxide (DMSO), propylene glycol, acetone, tetrahydrofuran (THF), 2-pyrrolidone (Dunn *et al*., 1997), glycofurol (Eliaz and Kost, 2000) or low molecular weight PEG (Dittgen *et al*., 1998). While the later include benzyl benzoate, ethyl benzoate, ethyl acetate (Lambert and Peck, 1995), triacetin (Brodbeck *et al*., 1999), triethyl citrate (Shah *et al*., 1993) or benzyl alcohol (Kang and Singh, 2005). Among the aforementioned, NMP is most frequently used due to its solvating power. Strickley reported the use of NMP, DMSO and PEG 400 in many commercial injectable products (Strickley, 2004) while 2-pyrrolidone has been used in veterinary injectable products (Dong *et al*., 2006). Royals *et al* reported the biocompatibility of NMP/PLGA and DMSO/PLGA solutions after they have administered to rhesus monkey (Royals *et al*., 1999).

Brodbeck *et al*., explained the role of solvent properties on the dynamics of polymer precipitation and *in vitro* release of chicken egg white lysozyme protein. The release of this protein from the NMP-PLGA based system exhibited a distinct initial burst while, depots of PLGA in triacetin or ethyl benzoate (with low solvent /water affinity) showed lower initial burst. They attributed the lower initial burst behavior for triacetin and ethyl benzoate to the slower phase inversion process that produce implant characterized by a less porous, more fluid, two-phase structure. This implant releases protein more uniformly (Brodbeck *et al*., 1999). The same interpretation was also reported by Wang *et al.* for Metoclopramide release prepared with PLGA in different solvents. PLGA/NMP system showed the fastest release followed by triacetin which migrated into buffer phase more slowly and finally benzyl benzoate due to its limited water solubility (Wang *et al*., 2004). Ahmed *et al*., studied the release of haloperidol in four different solvents; NMP and DMSO (water miscible), triacetin and ethyl acetate (partially water miscible). The initial haloperidol release was higher with DMSO followed by NMP then ethyl acetate and finally triacetin as indicated in fig. 1 and 2. They infer that solvents type is among the formulation factors that had a marked effect on haloperidol initial burst and attributed this behavior to the slow phase inversion rate (Ahmed *et al*., 2012).

Table 1: Approaches to decrease the drug initial release rate from PLGA based *in situ* gelling system

Technique	Example	Reference
Implant based on hydrophobic solvent	Insulin/PLGA in benzyl benzoate and benzyl alcohol	Dhawan *et al*. (2011)
	Metoclopramide/PLGAs in benzyl benzoate	Wang *et al*. (2004)
	Haloperidol/PLGA in triacetin and ethyl acetate	Ahmed *et al*. (2012)
	Chicken egg white lysozyme protein /PLGA in triacetin or ethyl benzoate	Brodbeck *et al*. (1999)
PLGA lactide-to-glycolide ratio	Leuprolide acetate/PLGA (75:25)/NMP	Sartor, (2003); Dunn *et al*., (2003)
	Rosiglitazone/PLGA (65:35, 75:25, 85:15)/NMP or triacetin	Madan *et al*., (2009)
	Fluorescein/PLGA (50:50, 75:25)/NMP	Patel *et al*. (2010)
Polymer concentration and molecular weight	Meloxicam/PLGA (0.3, 0.5, 0.7 dl/g)	Ibrahim *et al*. (2013)
	Haloperidol/PLGA (20, 30, 40% wt)	Ahmed *et al* (2012)
	Fluorescein/PLGA (0.2,0.3, 0.4, and 0.45 dL/g)	Patel *et al*. (2010)
Incorporation of plasticizer or surfactant	Meloxicam/PLGA/PEG 400	Ibrahim *et al*. (2013)
	Aspirin/PLGA/PEG 400	Tang and Singh. (2008)
	Hen egg protein/PDLA/Pluronic	DesNoyer and McHugh, (2003)
	Fluorescein/PLGA/Pluronic P85	Patel *et al*. (2010)
In situ microparticles (ISM) and microglobules	Diltiazem hydrochloride/PLGA ISM	Kranz and Bodmeier (2007)
	Bupivacaine hydrochloride/PLGA ISM	Kranz and Bodmeier (2008)
	Cytochrome c/PLGA microglobules (premicrospheres' or `embryonic microspheres)	Jain *et al*. (2000)

It has been reported also that the release of protein from PLGA based *in situ* gelling system could be modified by changing the injectable depots aqueous miscibility (Graham *et al*., 1999). By reducing the solvent/nonsolvent affinity of the prepared PLGA solutions, the rate of phase inversion is slowed and a more uniform release is obtained. Typical solvents for this behavior include triacetin, benzyl benzoate, ethyl benzoate, (Brodbeck *et al*., 1999), triethyl citrate (Shah *et al*., 1993) or benzyl alcohol (Kang and Singh, 2005). The process of polymer solidification could take from hours to days in these slow phase inverting systems. Morphological characterization of such systems revealed that these depots possess a smaller pore size and are more or less homogeneously dense. The main drawback of these solutions is their viscosities that make it difficult to inject without previous warm-up to 37 °C (Kempe and Mader, 2012).

PLGA lactide-to-glycolide ratio

It has been stated that, the choice of PLGA may be considered as the key factor in the process of modifying the drug release from PLGA based *in situ* gelling system (Ahmed *et al*., 2012). The ratio of the lactide-to-glycolide in PLGA may be of 50:50, 65:35, 75:25, and 85:15. As the ratio of the lactic acid increases the hydrophobicity of the polymer is increased as lactic is more hydrophobic that glycolic acid, consequently PLGA will absorb less water and degrade more slowly (Jain, 2000).

Leuprolide acetate was prepared as *in situ* forming implant system utilizing 45% PLGA 75/25 in N-methyl-2-pyrrolidone (NMP) [Sartor, 2003; Ravivarapu *et al*., 2000; Chu *et al*., 2002; Dunn *et al*., 2003]. The prepared leuprolide system maintained an effective suppression of serum testosterone in dogs below the medical castrate level for approximately 91 days without high initial burst. Madan *et al*., studied the release of Rosiglitazone from *in situ* gel formulation with different vehicles (NMP and triacetin), PLGA concentrations, and L-G ratios (65:35, 75:25, 85:15), they illustrated that the ratio 85:15 showed more sustained release with comparatively less burst effect (Maden *et al*., 2009). Patel *et al* reported similar finding for the release profile of fluorescein (model drug) from in *situ* forming implant consisting of poly(D,L-lactide-co-glycolide), dissolved in 1-methyl-2-pyrrolidinone (NMP). They studied the effect of different formulation components on drug release profile and indicated that PLGA with a lactide to glycolide ratio of 75:25 released drug at a slower rate compared to PLGA with 50:50 L/G ratio formulation which could be concluded that the ratio of the polymer subunit composition also affecting the drug release from these systems (Patel *et al*., 2010).

Polymer concentration and Molecular weight (viscosity)

It is previously stated that polymer concentrations for *in situ* forming implant formulations could be in the range 10-80 wt. % and the viscosity of the prepared polymeric solutions is greatly affected by the polymer concentration in solution and its molecular weight (McHugh, 2005). High polymer concentrations usually in the range (40 wt. %-50 wt. %) results in decreased the drug initial release

but the viscosity should be considered since the injectability could be impaired (Kempe and Mader, 2012; Kranz *et al* 2001).

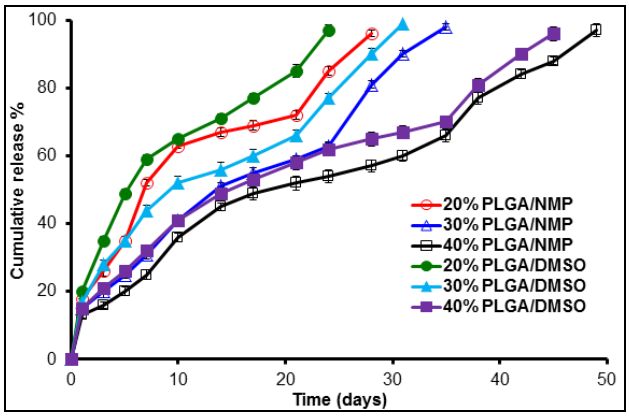

Fig. 1: Haloperidol *in vitro* release from PLGA solutions containing different concentration of PLGA in NMP and DMSO (Ahmed *et al*., 2012).

Lambert and Peck studied the release of bovine serum albumin from PLGA solution in which a smaller burst effect was obtained when both high molecular weight PLGA (75-115 000 Da) and polymer loading in the solvent were used (Lambert and Peck, 1995). Ahmed *et al* have studied the release of haloperidol from a polymeric solution containing 20, 30 and 40% PLGA (50:50 L-G ratio, molecular weight (MW) 60,000–70,000 Da, intrinsic viscosity 0.5 dL/g) in four different organic solvents namely; NMP, DMSO, triacetin and ethyl acetate. The initial release of the drug decreased as the polymer concentration was increased. Fig. 1and 2 show the release of the drug from *in situ* implant containing increasing concentration of the polymer in four different organic solvents.

The effect of different PLGA molecular weight (intrinsic viscosity 0.3, 0.5 and 0.7 dl/g) on the initial burst and cumulative release of meloxicam have been studied by Ibrahim *et al*. They mentioned that the polymer viscosity has a great impact on meloxicam initial burst, as the intrinsic viscosity of the polymer increases the hydrophilicity decreases and so, PLGA grades that have low intrinsic viscosity (low molecular weight) will have greater water solubility owing to the rapid uptake of water while high molecular weight ones will possess an opposite effect that account for their low initial burst (Ibrahim *et al*., 2013; Bodmer *et al*., 1992). Possible explanation for the effect of PLGA molecular weight could be illustrated by photo-imaging of the implant using scanning electron microscopy (SEM) after injection into phosphate buffer (fig. 3). Implants with low molecular weight (intrinsic viscosity 0.3) polymer shows multiple pores, which account for the rapid solvent exchange while this pores decreased in number and size with high molecular weight (intrinsic viscosity 0.7) polymer. This effect was also illustrated by Patel *et al* who stated that SEM micrographs of implant cross sections prepared with lower MW PLGA were more porous than their corresponding higher Mw PLGA implants (Patel *et al*., 2010).

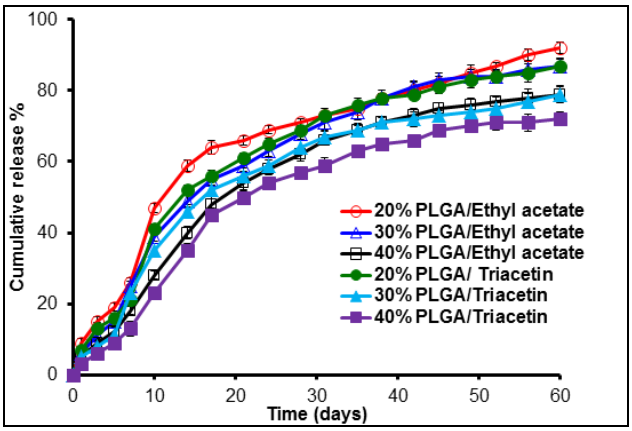

Fig. 2: Haloperidol *in vitro* release from PLGA solutions containing different concentration of PLGA in Ethyl acetate and Triacetin (Ahmed *et al*., 2012).

Incorporation of plasticizer or surfactant

The incorporation of PEG 400 has been reported to decrease the drug initial burst possibly by its plasticizing effect on the PLGA matrix system (Tan *et al*., 2004). This effect was recently illustrated by Ibrahim *et al*, who develop and optimize *in situ* implant formulation of meloxicam and studied the effect of incorporation of 10-30% PEG 400 with NMP (polymeric solvent) on the initial burst and cumulative release (Ibrahim *et al*., 2013). They concluded that the burst effect was influenced by the solvent mixture (NMP and PEG 400). Another possible explanation for the effect of PEG is its solubilizing power that allows uniform distribution of the drug particles inside the PLGA matrix and prevents adsorption of any drug particles at the surface.

Another evidence for the effect of PEG is that demonstrated by Chandrashekar *et al*., who reported decrease in leuprolide acetate initial burst (release during the first 24h) from 50% to 34% with the incorporation of 10% PEG to the polymeric solution of PLGA dissolved in DMSO (Chandrashekar *et al*., 2000). Tang and Singh also verified that the addition of PEG400 to PLGA *in situ* gel forming system significantly decreased the initial burst of aspirin from 36.9±1.9% to 30.9±1.2% (Tang and Singh. 2008).

The incorporation of biocompatible surfactants such as Tweens, Spans, Chremophores or Pluronics could have a positive effect on the release profile and duration of activity. Elias-Al-Mamun *et al* illustrated the effect of incorporation of biocompatible excipients such as Tween 20, Tween 60, Span 20, Span 80, Chremophore EL, or Chremophore RH 40 on the *in vitro* release of tamsulosin

from biodegradable PLGA *in situ* implants. They stated that it was clearly observed that the studied surfactants lower the release rate of tamsulosin but prolong its activity (Elias-Al-Mamun *et al*., 2009). Patel *et al* studied the effect of incorporation up to 5% Pluronic P85 (P85, Mw: 4600 Da) on the release profile of sodium fluorescein (low Mw mock drug molecule) *in situ* forming implant formulation prepared with PLGA dissolved in NMP. They verified that Pluronic P85 concentration showed minimal effect on sodium fluorescein release during the first hour. However, after 1 hour and up to 4 day time of the release (during the intermediate release stage), Pluronic P85 concentration in the range of 1-2.5% appears to lower the drug initial burst. Exactly at the end of the 4 day time point, *in situ* forming implants with 1% and 2.5% P85 released about 33.6% and 28.2% respectively of their drug, than the corresponding formulations without any Pluronic P85, 38.2%. They also stated that increasing the concentration of Pluronic P85 beyond 5% reversed any lowering of the drug burst release. DesNoyer and McHugh studied the effect of variations in Pluronic concentration and molecular weigh on the protein release from PLGA/NMP solutions. They used Pluronic L101 and L121 (fairly hydrophobic), the only difference between them being the higher molecular weight of the L121, in this study. They indicated that, the Pluronic molecules preferably direct themselves in such a way that the hydrophobic PPO parts is inserted in the polymer matrix while the hydrophilic PEO parts are extended in the surrounding aqueous phase the effect that leads to segregation of the Pluronic molecules and formation of a phase boundary. This effect was more obvious with the higher molecular weight Pluronics with overall reduction in the burst effect (Patel *et al*., 2010)

In situ microparticles and microglobules

Bodmeier; Kranz and Bodmeier developed in 1997 and 1998 respectively a novel polymeric based *in situ* forming microparticle (ISM) system as an attempt to control the release of drug from such systems (Bodmeier, 1997; Kranz and Bodmeier, 1998). The ISM system consists of an inner drug polymer-solvent phase which is emulsified into an outer phase usually oil. This emulsion when injected into buffer or gets in contact with physiological fluids, the internal polymeric phase solidifies and microparticles are formed spontaneously. The ISM offers may advantages over its corresponding polymeric *in situ* forming implant such as; little myotoxicity, better syringability and injectability (since viscosity is highly dependent on the outer oil phase and not on the polymeric phase) and lower initial burst effect (Jain *et al*., 1998; Kranz and Bodmeier, 1998; Kranz and Bodmeier, 2007). The process of ISM system preparation is quite simple compared to the conventional methods for the preparation of microparticles (Kranz and Bodmeier, 2007).

Kranz and Bodmeier studied the release of diltiazem hydrochloride and buserelin acetate from two different *in situ* forming systems namely; *in situ* implants (ISI) and *in situ* microparticles. Either poly(d,l-lactide) (PLA) or poly(d,l-lactide-*co*-glycolide) (PLGA) in DMSO, NMP or 2-pyrrolidone was used to form polymeric solutions that were used as *in situ* implants. The ISM systems were prepared by utilizing the previously described polymeric solutions that were emulsifying into peanut oil at different polymeric solution to oil phase ratios. The release of both drugs from the *in situ* implant systems showed an initial high burst release compared to the release from the ISM system. They concluded that the ISM system significantly reduced both drug initial burst effect when compared to the *in situ* implant systems (polymer solutions) and attributed that effect to the presence of an outer oil phase which had made a partial barrier between the inner polymer solution and the outer aqueous medium. Another possible mechanism for the lower initial drugs burst was the less porous surface of the ISM compared to the ISI system (Kranz and Bodmeier, 2007). Another comparative study between both systems (ISI and ISM) was conducted on bupivacaine hydrochloride utilizing poly(d,l-lactide) (PLA) as a biocompatible polymer which was dissolved in various organic solvents to prepare ISI while the ISM was prepared using peanut oil as external phase at different polymer phase to oil phase ratios as previously described. A reduced initial bupivacaine hydrochloride release was also exhibited from the ISM compared to the ISI and they also attributed this behaviour to presence of external oil phase and the less porous surface of the ISM (Kranz and Bodmeier, 2008). Ahmed *et al* also reported the same results for haloperidol *in vitro* and *in vivo* release from ISM and ISI systems (Ahmed *et al*., 2012).

Jain *et al*. (2000) developed a novel in situ method for the preparation of injectable stable dispersion of PLGA microglobules (premicrospheres or embryonic microspheres). The preparation made up of two oil phases. The oil phase I consists of a mixture of PLGA/ triacetin/drug/PEG 400/tween 80. This mixture is added dropwise to oil phase II which composed of miglyol 812 and span 80 and homogenized to produce rubbery injectable dispersion of PLGA microglobules. The produced embryonic or pre-microspheres harden, shrink, were able to entrapping the drug and form true microspheres *in situ* within 17 minutes. One major advantage of this system is its ability to control the release of cytochrome c from few days to weeks. The burst of the drug was less than 30% (within the first 24 hrs) of the total drug load and they attributed the major amount to the unencapsulated drug (Jain *et al*., 2000). We expected that the formulation and processing factors of this method could be optimized to give lower initial drug release.

Another factor that could play a role in the release of drugs from in situ implant system is the drug lipophilicity. Deadman *et al*., studied the effect of drug lipophilicity on its release profile from different controlled release vehicles such as, PLGA micro particles and in situ

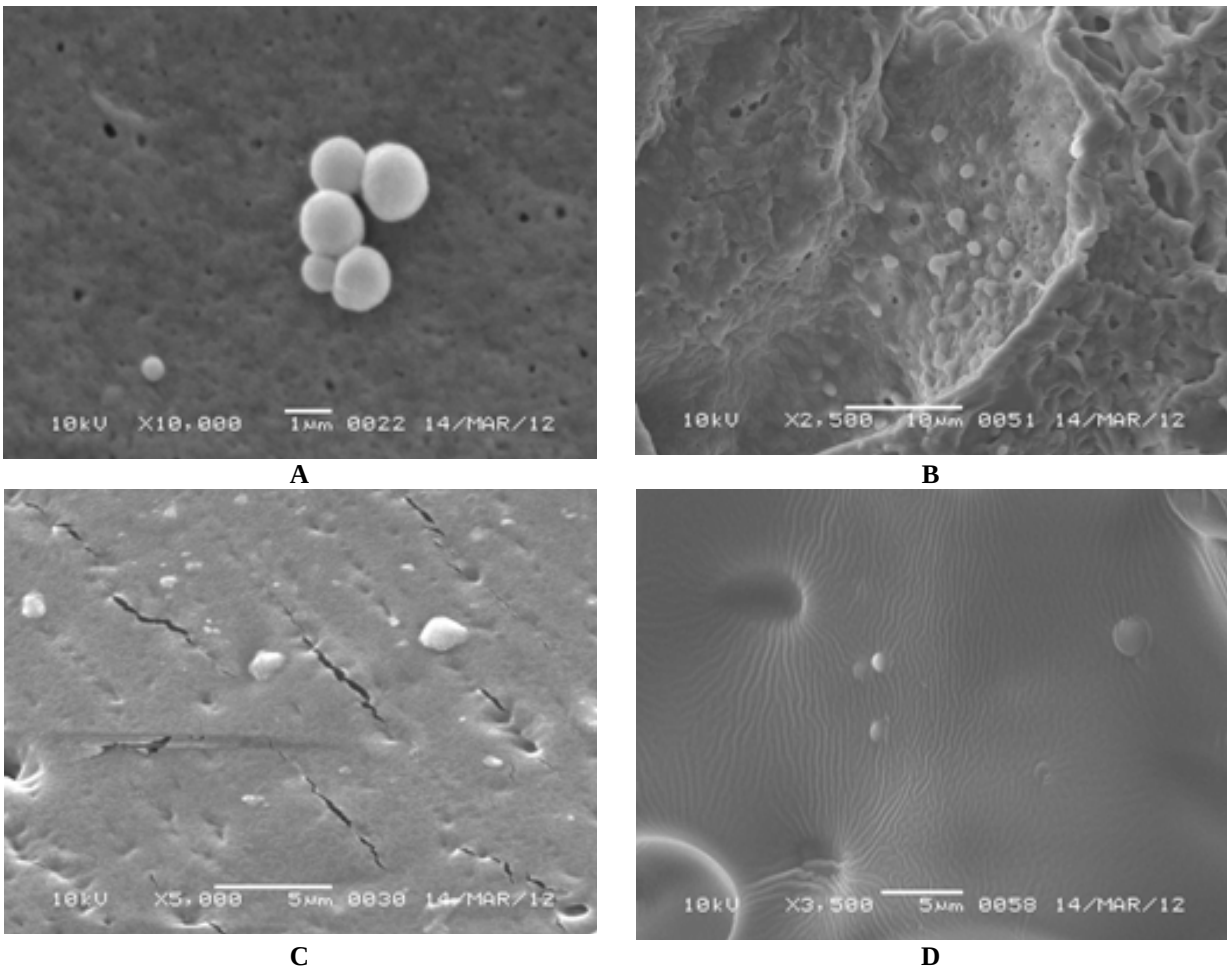

Fig. 3: Scanning electron microscope imaging for PLGA (0.3 dl/g) implant surface (A) and after a cut section (B); for PLGA (0.7 dl/g) implant surface (C) and after a cut section (D)

forming depots. They reported that, although there was minor effect of drug lipophilicity on the *in vitro* studies that effect was obvious *in vivo* which attributed to the interactions between the formulation and the biological tissue.

CONCLUSION

The demand to prepare proficient inject able biocompatible controlled release systems is increasing. PLGA based *in situ* gelling system could fulfill this requirement as this system provides efficient tool for the delivery of micro and macromolecules. Several attempts have been investigated to lower the initial burst associated with this system. The processing and formulation factors and a combination of two more techniques could be optimized to produce the drug release.

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