

In vitro Release Kinetics of Progesterone from Biodegradable *In situ* Implant System

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ABSTRACT: *In situ* implants containing Progesterone (PRG) were prepared by using biodegradable Poly (DL-lactide-co-glycolide) polymer. Dimethyl sulfoxide (DMSO) was used as an aprotic solvent in this implant formulation. This system was prepared by dissolving a water insoluble and biodegradable polymer (PLGA) in a biocompatible organic solvent (DMSO) and then the drug progesterone was added to the polymer solution to produce the drug solution. When the PLGA-PRG solution (0.5 ml) was injected subcutaneously into rat (weight 130g), the solvent dissipated into the surrounding tissue leading phase separation and subsequent coagulation of the polymer & drug to form an implant *in situ*. The implants were removed from the rat after one hour and stored in freezing condition. The digital photographs of the *in-situ* formed implants obtained after 1hour shows the evidence of the formation of the implants. Two formulations of implants were made. One contained 10% of progesterone and the other 20%. *In vitro* dissolution studies of progesterone was performed at static condition in ethanol-water mixture (30:70) at 37°C for 30 days. The implants of 20% progesterone loading showed about 65% release and the implants of 10% loading showed 56% release within 30 days. The release mechanism from these implants resembles closely to Higuchian pattern and first order. The release rate was found faster from the implants with higher drug loading of 20% progesterone, compared to implants of 10% drug loading.

Key words: Biodegradable, Implant, Biocompatible, Sustained Release

INTRODUCTION

Biodegradable polymers may be defined as synthetic or natural polymers which are biodegradable *in vivo*, either enzymatically or non-enzymatically, to produce nontoxic by-products. These can be further metabolized or excreted via normal physiological pathways.¹ These polymers have become increasingly important in the development of controlled release systems. Currently a number of biodegradable polymers are being evaluated as carriers for the controlled release of low molecular weight drugs, polymer of these kinds have both active & passive role.

They may simply serve as a matrix from which the drug can diffuse or dissolve & once the drug is depleted, the polymer degrades to harmless products, which can be eliminated from the body.^{2,3} Alternatively the polymer may have an active role in drug release in that its rate of degradation or erosion controls the rate at which drug is released from the system.

The trend in drug delivery technology has geared toward biodegradable polymer excipients requiring no follow up surgical removal once the drug supply is depleted. The most widely investigated and advanced polymers in regard to available toxicological and clinical data are the aliphatic polyesters based on lactic and glycolic acids.⁴ The family of homo- and copolymers derived from these monomers has

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received considerable attention since about 1973 as excipients for drug delivery. Features such as biocompatibility, predictability of biodegradation kinetics, ease of fabrication, and regulatory approval in commercial sutures applications have attracted investigators to lactic/ glycolic polymers.⁵

Among the first reports of polylactic acid used in controlled release were those of Boswell, Yolles, Wise, Sinclair and Beck. These research teams were seeking delivery systems for such agents as narcotic antagonists, contraceptive hormones and other conventional drug compounds. Biodegradable polymeric implant material provides substantially continuous release of bioactive agent during *in vivo* use. Bioactive agent is initially released in amounts that are less than degradation rate of polymer, thereby promoting migration of cells into material. Later larger amounts of bioactive agent are released, thereby promoting differentiation of cells.⁶

Implants are used as depot formulations either to limit high drug concentrations to the immediate area surrounding the pathology or to provide sustained drug release for systemic therapy. Clinically, implant systems have been used in situations where chronic therapy is indicated, such as hormone replacement therapy e.g. Human growth hormone releasing peptide-1 (GHRP-1), chemical castration in the treatment of prostate cancer and in the targeted delivery antibiotics.⁷

MATERIALS AND METHODS

Chemicals. Progesterone, Poly (Lactide-co-glycolide) 50:50, Dimethylsulfoxide (DMSO), Acetonitrile, Ethanol and Distilled Water.

Animal. Albino Rats were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) Dhaka. All rats were weighed approximately 130 g & with in the age range of 2-3 months.

Preparation of Injection for the Implant. Polymer was weighed in an analytical balance (College Instrument Company, England) into glass vials. Polymer was dissolved completely in the

weighed amount of DMSO. Progesterone was weighed and dissolved in the polymer solution thoroughly for 30 minutes according to the Table 1.

Table 1. The Formulation for the Preparation of Biodegradable Implants.

Ingredients	Formulation 1	Formulation 2
Progesterone	100 mg	50 mg
Poly (lactic-co-glycolic acid) (PLGA)	500 mg	500 mg
Dimethyl Sulfoxide (DMSO)	1.50 ml	1.25 ml

Formation of *in situ* implant. Rat was injected with 0.5ml of injection formulation for each implant bilaterally under the skin. By this way 4 implants from two different formulations were collected after one hour of injection by sacrificing the rat.

Dissolution study procedure. *In-vitro* dissolution studies of Progesterone implants were carried in static condition. The four implants formed *in-vivo* were placed in four different 50ml conical flask. Then 25ml ethanol-water (30:70) was added in each conical flask. Flasks were capped by using rubber cork at room temperature. 5ml ethanol-water samples were withdrawn at a pre-determined rate-using pipette & then replaced with 5 ml of fresh ethanol-water mixture. The release of drug content from the implants was determined spectrophotometrically at 240 nm.

RESULTS AND DISCUSSION

Poly DL-lactide-co-glycolide (DL-PLGA) polymer was used for the formation of *in-situ* implants containing Progesterone (PRG). Polymer and drug both were dissolved in an aprotic solvent, dimethyl sulfoxide (DMSO) and then injected into rat subcutaneously. Two formulations of implants were made, having drug loading of 10% and 20% of progesterone. Aprotic solvent was quickly dissipated from the injection site, thus precipitating the PLGA polymer and the drug together to form a solid *in-situ* implant. A schematic diagram has been shown in the Figure 1.

Albino rats (weight 130 g) were used in this experiment. Ether was used as anesthesia before injections were made. Just after one hour of injection, rats were opened through abdomen and implants were taken out and dried in desiccators overnight.

Finally it was stored in a refrigerator until used. Digital photographs of the process are shown in the Figure 2 and the photographs of the implants after removal are shown in the Figure 3.

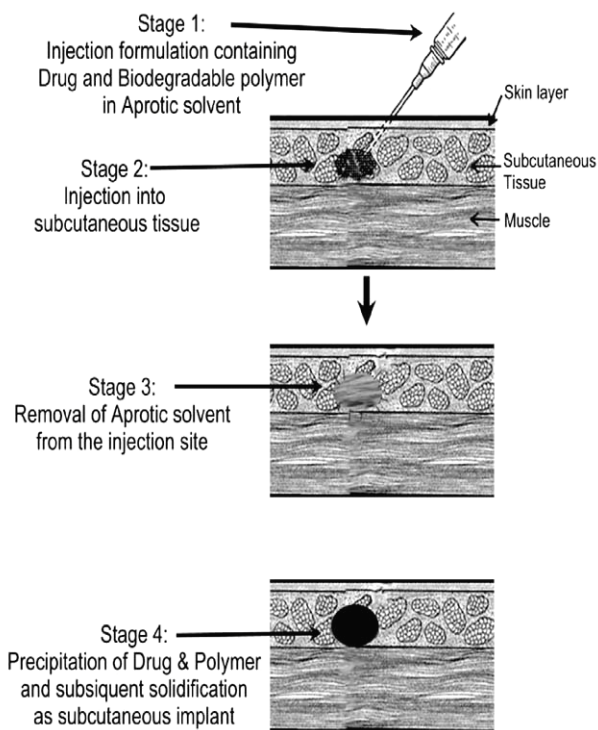


Figure 1. Schematic diagram of the formation stages of in-situ implants.

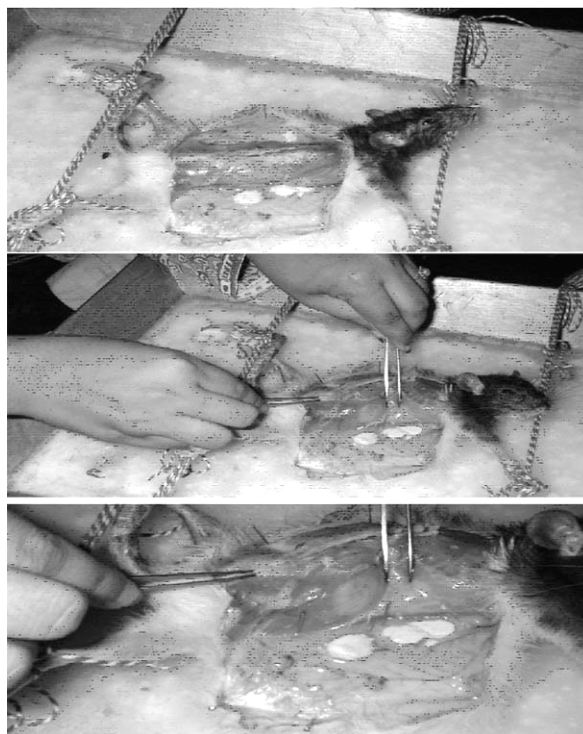


Figure 2. Stages of *in situ* implants formation in rat

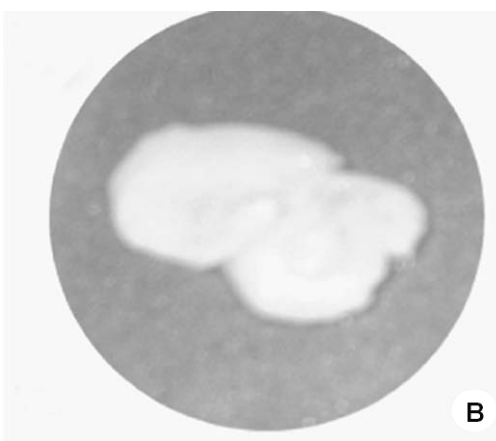
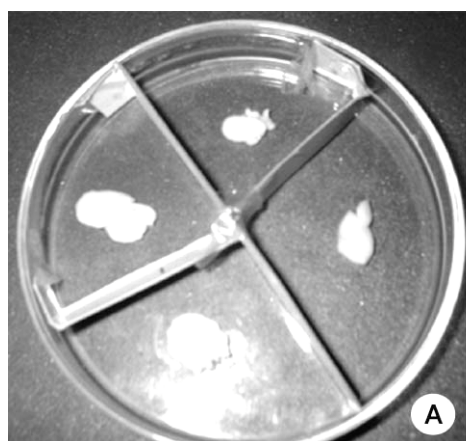


Figure 3. Digital photographs of four implants (A) and a close view of an implant (B).

In vitro dissolution studies were performed to observe the *in-situ* formation of the solid implants and release of progesterone (PRG) from these implants. As these biodegradable implants are *in-situ*

in the body system, there has been a poor hydrodynamic activity in the tissue sites compared with the stomach environment. Here four 50ml conical flasks were used to study dissolution in static

condition at 37°C. Since progesterone is insoluble in water, ethanol-water (30:70) combination was used as a dissolution medium. 5ml of the dissolution medium was withdrawn everyday from the conical flask to analyze the progesterone content and a fresh 5 ml of the medium was replaced. Total experiment was conducted for 30 days.

The implants of 20% progesterone loading (formulation 1) showed about 65% release and the implants of 10% loading (formulation 2) showed 56% release within 30 days. The release data of formulation 1 and 2 was plotted in two different models, viz, Zero order and Higuchi shown in the Figures 4 and 5, respectively.

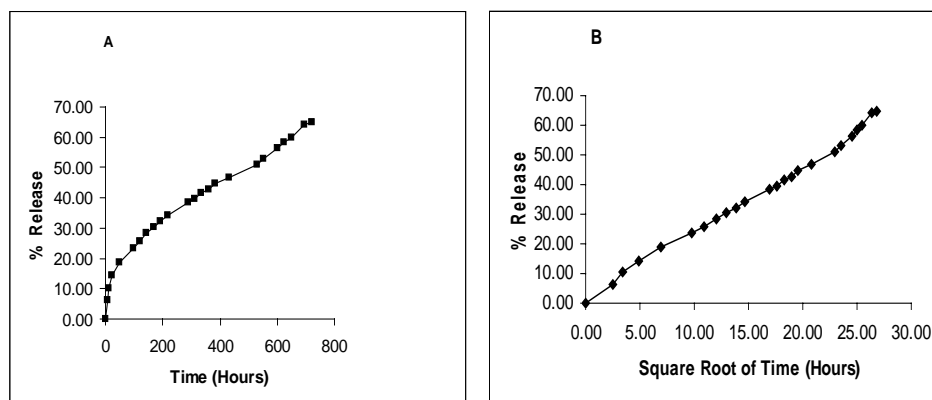


Figure 4. *In-vitro* percent release of Progesterone from DL-PLGA Implants formed *in-situ* in rats; (A): Zero Order Plot, (B): Higuchi Plot, of Formula 1(Dug Loading 20%).

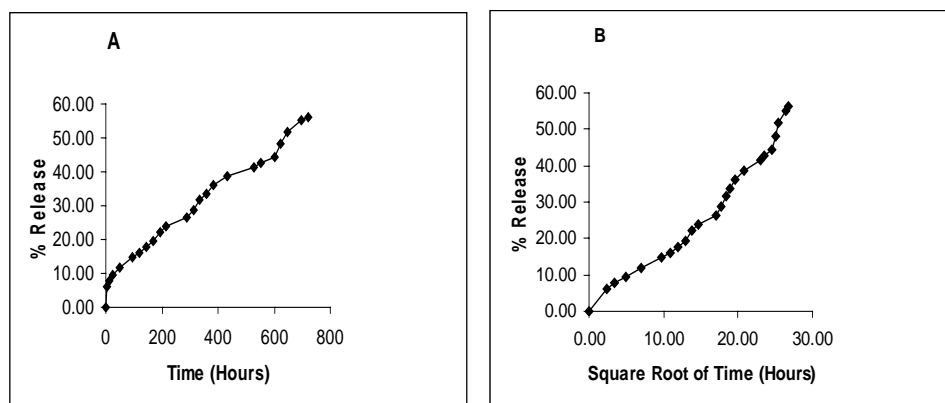


Figure 5. *In-vitro* percent release of Progesterone from DL-PLGA Implants formed *in situ* in rats; (A): Zero Order Plot, (B): Higuchi Plot, of Formula 2 (Dug Loading 10%).

Table 2. *In vitro* release rates and correlation coefficients of progesterone from DL-PLGA implants formed *in situ* in rats.

	Implant Formula 1		Implant Formula 2	
	Release Rate	Correlation Coefficient	Release Rate	Correlation Coefficient
Zero order Plot	0.075 %/hour	0.9465	0.0678 %/hour	0.9822
Higuchi Plot	2.2648 %/hour ^{1/2}	0.9938	1.9811 %/hour ^{1/2}	0.9647

The correlation coefficients values of the trend lines of the graphs showed that formulation 1 best fits in Higuchian pattern and the formulation 2 fits in first order pattern. However, it is very difficult at this stage to explain in details the actual mechanism of

release since, the polymer degradation starts during the dissolution period. The values of the correlation coefficients are shown in the Table 2.

The release rate of progesterone was also calculated from the trend lines of the graphs for both

formulation 1 and 2. These graphs clearly indicate that the release rate of progesterone was higher from the formulation 1 having 20% drug loading than the formulation 2 of 10% drug loading. This is obvious phenomenon that the higher loaded matrices release faster due to higher pore formation and high flux due to faster saturation at the diffusion layer.

CONCLUSION

The *in vitro* release study of progesterone from the *in situ* formed biodegradable implants was carried out on the static condition using ethanol-water as dissolution medium & actual percentage of drug was monitored for 30 days. This showed a sustained release of drugs over this period. Thus the evaluated dosage form may serve as novel drug delivery system.

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