

FORMULATION AND DEVELOPMENT OF *IN SITU* IMPLANTS OF CYTARABINESANTHOSH KUMAR.J^{*1,2}, VORUGANTI SANTOSH², RAJU.TUMMA², ARAVIND G², RAVINDRA BABU D.S²¹Priyadarshini College of Pharmacy, Ghatkesar, Hyderabad, A.P, India, ²Celon Laboratories Pvt. Ltd, Pragathi nagar, Hyderabad, A.P, India. Email: santhoshjg@gmail.com

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ABSTRACT

The present study deals with the formulation and evaluation of cytarabine in situ implants. Cytarabine is a synthetic pyrimidine nucleoside. Cytarabine is most commonly used to treat acute myeloid leukaemia. The purpose of this research is to minimize the frequency of doses and toxicity and to improve the therapeutic efficacy by formulating cytarabine an subcutaneous in situ implants for at least 30 days. Cytarabine in situ implants were prepared by polymer precipitation method using two different grades of polymer PLGA with three different concentrations. This system is prepared by dissolving biodegradable polymer (PLGA 50:50, PLGA 75:25) in dimethyl sulfoxide (DMSO). Then drug was added to it, the polymer drug solution is injected in to the aqueous buffer from which the solvent dissipates in to buffer and forms a solid implant. Implants were characterized by drug entrapment efficiency, in vitro drug release studies. Based on burst phase release studies optimized formulations were selected. The optimized formulations were evaluated for surface morphology, sterility test, accelerated stability studies and DSC studies for determining physical state of cytarabine in formulation. From our studies it was observed that, the drug entrapment efficiency increased and the burst release decreased with increase in the polymer concentrations. The formulations with 33.3% polymer concentration exhibited moderate burst release (25.4% and 19.8%) and sustained release for 28 days (90.9% and 90%). The SEM configurations showed polymer precipitation and the cross linking of the polymer with porous surface. FTIR studies showed no drug polymer interactions. DSC studies revealed that cytarabine was distributed as amorphous form in implants. Release kinetics was calculated for optimized formulations and the formulation followed zero order kinetics with fickain diffusion.

Keywords: *In situ* implants, Cytarabine, PLGA, Sustained release, Parenteral depot.

INTRODUCTION

Polymeric drug delivery systems are attractive alternatives to control the release of drug substances to obtain defined blood levels over a specified time. The patients suffer from some disease conditions such as heart disorders, osteoporosis, tumors, and often benefit from such long term drug delivery systems due to improved patient compliance¹. Injectable *in situ* forming implants are classified in to five categories, according to their mechanism of depot formation: (1) thermoplastic pastes, (2) *in situ* cross linked systems, (3) *in situ* polymer precipitation, (4) thermally induced gelling systems, (5) *in situ* solidifying organ gels. Of these, *in situ* polymer precipitation systems have become commercially available so far ². The *in situ* forming implant systems have several advantages compared to traditional pre formed implants systems. Due to their injectable nature, implant placement is less invasive and painful for the patient thereby improving comfort and compliance. Additionally the manufacturing process required for fabrication is relatively mild. Currently, only two FDA approved products are on market utilizing this type of system, Eligard and Atridox. Eligard, using the atrigel delivery system and marketed by Sanofi-aventis in the US (Medigene in Europe), is a subcutaneously injected implant that release the leuprolid acetate over a period of 3 months to suppress testosterone levels for prostate cancer treatment ³. Atridox is another ISFI system that also uses the Atrigel delivery systems to deliver the antibiotic agent, Doxycyclin to sub-gingival space to treat periodontal disease⁴. Some disadvantages of *in situ* implants are high burst release, potential solvent toxicity and high viscosity of the polymeric solution which may lead to problems during administration⁵.

MATERIALS AND METHODS

Cytarabine was obtained from Shang Hai Hengrui International trading co, Ltd. China, poly (Lactide -co-glycolide) PLGA-75:25 (RG755S) and PLGA 50:50 (RG504) was obtained from Evonik roehm gmbh, Germany. All solvents were HPLC grade and were obtained from Merck chemicals, Mumbai.

Solubility studies of cytarabine

The solubility of cytarabine was determined in the solvents Dimethyl sulphoxide, N-Methyl pyrrolidine, Ethanol, ethanol+propyleneglycol, Dichloromethylene, acetone, Ethanol+PEG-

400. This was accomplished by adding excess drug cytarabine to each solvent of interest and allowing the mixtures to shake for 24 hours. All of the mixtures were then centrifuged, and a known volume of supernatant was removed from each mixture. The solubility's were determined by using UV-Visible spectrophotometer (PerkinElmer's) at 272 nm.

Table 1: Solubility of cytarabine in different solvents

Drug	Solvent	Quantity (mg/ml)
Cytarabine	DMSO (Dimethyl sulphoxide)	25.01
Cytarabine	NMP(N-Methyl pyrrolidine)	14.3
Cytarabine	Ethanol	10
Cytarabine	Ethanol+propylene glycol	20
Cytarabine	Ethanol+PEG400	16.6
Cytarabine	DCM(Dichloro methylene)	-
Cytarabine	Acetone	-

Method of preparation of implants

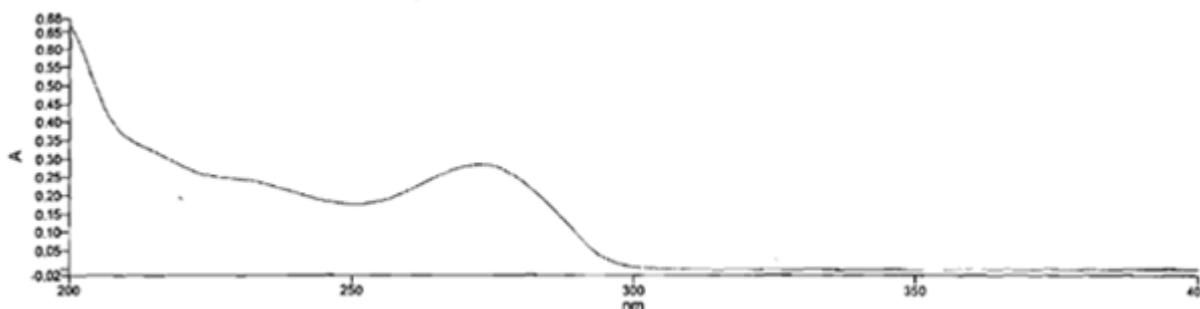
In situ implants of cytarabine were prepared by polymer precipitation method. PLGA is dissolved in organic phase containing dimethylsulfoxide (DMSO) in glass vials until the formation of a clear solution. Different formulations were prepared using 27-33% of polymer. To this 50 mg of cytarabine was added. The polymer-drug solution was stirred vigorously (for 2 hrs) until clear solution was formed. This solution is filled in pre filled syringes (PFS), gradually injected in to 7.4 pH phosphate buffer at 37°C with 18 gauge needle for the formation of implants. Within 5-10 minutes solid round implants was formed. Formed implants are evaluated.

Determination of λ max of cytarabine

The UV absorption spectrum of cytarabine in, 7.4 pH shown in fig1. A solution of cytarabine containing concentration 10 μ g/ml was prepared 7.4pH buffer and UV spectrum was taken using a PerkinElmer's double beam spectrophotometer and scanned between 200 to 400 nm. The maxima obtained in the graph were considered as λ max for the drug cytarabine. The compounds exhibited maximum at 272nm in 7.4pH buffer.

Table 2: Formulation of cytarabine in situ implants by using two different grades of polymers

S. No.	Test parameters	F1	F2	F3	F4	F5	F6
1	cytarabine	50mg	50mg	50mg	50mg	50mg	50mg
2	PLGA50:50	300mg	350mg	400mg	-	-	-
3	PLGA75:75	-	-	-	300mg	350mg	400mg
4	DMSO	800mg	800mg	800mg	800mg	800mg	800mg
5	Drug/polymer ratio	1:6	1:7	1:8	1:6	1:7	1:8
6	Polymer concentration (% w/w)	27.2	30.4	33.3	27.2	30.4	33.3
7	Weight of implants	1150mg	1200mg	1250mg	1150mg	1200mg	1250mg
8.	Injection volume	0.8ml	0.8ml	0.8ml	0.8ml	0.8ml	0.8ml

Fig. 1: Cytarabine λ_{max} in 7.4pH phosphate buffer

Characterization of implants

Drug entrapment efficiency

The amount of drug entrapped by was estimated by dissolving the implant in DCM and water in 1:1 ratio, under vigorous shaking for 3hours then the resultant solution is centrifuged, both layers was separated, cytarabine was soluble in water but not in DCM. The drug content in aqueous solution was analyzed spectrophotometrically by using UV-VIS spectrophotometer at 272.2nm with further dilutions against appropriate blank. The amount of the drug entrapped in the implant was calculated using the formula⁷

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Amount of drug actually present} \times 100}{\text{Theoretical drug load expected}}$$

Scanning electron microscopy

Scanning electron microscopy is used to determine surface morphology, texture and to examine the morphology of fractured and sectioned surface. SEM is most commonly used method characterizing the drug delivery systems. SEM studies were carried out by using (Hitachi -s-3700N).dry implants were placed on electron microscope brass stub coated with an ion sputter. Picture of cytarabine implants were taken by random scanning of stub.

In-vitro drug release

In vitro drug release studies were performed using modified diffusion apparatus using dialysis membrane. In situ implants were placed in to conical vials open on one side and closed with dialysis membrane on other side. The formulations were placed in to 50 ml 7.4 pH phosphate buffers at 37°C. At 1, 2, 7, 14, 21, and 28th day time intervals, 5 ml sample were withdrawn and replaced with fresh medium and withdrawn samples analyzed for drug content by uv-visible spectrophotometer at 272.2nm. After every one week the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium. The obtained data were fitted in to mathematical equation (zero order, first order, highuchi model) in order to describe the kinetics and mechanism of drug release from the implant formulations.

Sterility test

In situ implants were sealed in pre filled syringes and irradiated at ambient temperatures (~30. 8°C) γ -irradiation was performed

using a commercial ⁶⁰Co source to a dose of 20.6 kGy for four days. Irradiated in situ implants are inoculate in fluid thioglycolate medium, at 20-25°C for 15 days. Drug is sterilized by dissolving the drug in water which is filled in prefilled syringe and lyophilized⁸.

Stability studies

To assess the physical and chemical stability of the in situ implants, stability studies were conducted for 1 month under different storage conditions mentioned in ICH guidelines. The sample containing optimized formulation were placed in vials and stored at 40°C/75%RH. After 90 days the formulations was checked for physical appearance and drug content.

Differential Scanning Calorimetry

Differential scanning calorimetry is used to determine drug excipient compatibility studies, and also used to observe more phase changes, such as glass transitions, crystallization, amorphous forms of drugs and polymers. The physical state of cytarabine, PLGA, physical mixture of cytarabine, PLGA and formulation was analysed by differential scanning calorimeter (SHIMADZU DSC-60). The thermo grams of cytarabine, PLGA, physical mixture of cytarabine, PLGA and formulation were obtained at scanning rate of 10°C/min conducted over 25-300°C⁹.

FTIR studies

FTIR spectra obtained for cytarabine, polymer, physical mixture and Formulation presented in the fig. The characteristic peaks of cytarabine were compared with the peaks obtained for physical mixture of cytarabine and formulation.

In vitro release kinetics

The plots of cumulative percentage drug release v/s. time, cumulative percent drug retained v/s. root time, log cumulative percent drug retained v/s. time and log cumulative percent drug release v/s. log time were drawn. The slopes and the regression coefficient of determinations (r^2) were calculated¹⁰.

RESULTS AND DISCUSSION

Solubility studies

The solubility of cytarabine in different of solvents was carried out and it reveals that it is freely soluble in DMSO, and slightly soluble in NMP and ethanol. But practically soluble in water at 20°C.

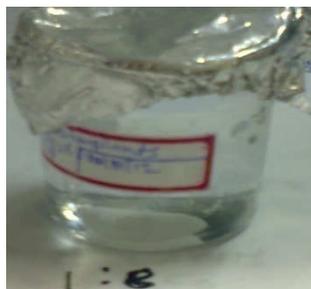
Formulation optimization

In situ implants of cytarabine were prepared by using polymer precipitation method. Formulations were prepared using two different grades of polymer with (PLGA 75:25, PLGA 50:50) different concentrations of polymers with a solvent DMSO. The compositions and ratios of in situ implants were listed in table 2. Formulations before injection in to buffer are clear and transparent. Upon injection of polymer solutions in to the phosphate buffer medium, the polymer solidified as the solvent dissipated in to aqueous medium and formed implants⁶. Out of 6 formulations based on burst

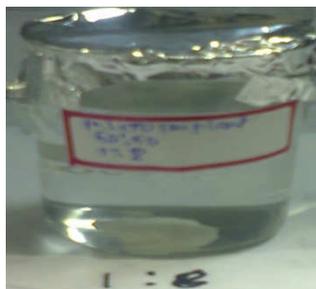
release, 2 formulations were selected as optimized formulations for further evaluations.

Drug entrapment efficiency

The entrapment efficiency of various formulations was studied. Drug loading percentage in the range of 80-94% was observed for F1, F2, F3, F4, F5, and F6. With increase in the drug to polymer ratio, the percentage drug encapsulated was also found to increase. In F6 as the polymer concentration is higher, 94% drug entrapment was observed.



(a) Cytarabine implant with PLGA 50:50



(b) Cytarabine implant with PLGA 75:25



(c) Cytarabine implant formation after injection in to buffer

Fig. 2:

Table 3: Drug entrapment efficiency of different formulations

S. No.	Formulations	% Entrapment efficiency
1	F1	84
2	F2	86.6
3	F3	95.6
4	F4	92.2
5	F5	98.2
6	F6	98.2

Scanning electron microscopy

The Morphology and surface appearance of in situ implants were examined by using SEM. The SEM photographs of F3 and F6 formulation showed that the implants have porous surface. The SEM images were shown in Figure no.

In vitro drug release

In vitro drug release studies were performed using dialysis membrane with 7.4 pH phosphate buffer. Comparison of in vitro

release studies of various formulations are shown in fig. As the polymer concentration is decreased, more burst release is seen. Formulation with 33.3%(F3&F6) showed a sustained release of drug for 28 days. On 28th day 90% of drug release was achieved indicating that more sustained drug levels are possible in period of two months. More prominent burst release was observed in case of F1, F2, F4 and F5 formulations. Only 20-25% burst release was observed for F3 and F6 formulations. From the above results, F3 and F6 were found to be the most suitable formulations and hence were optimized for the conduct of further studies.

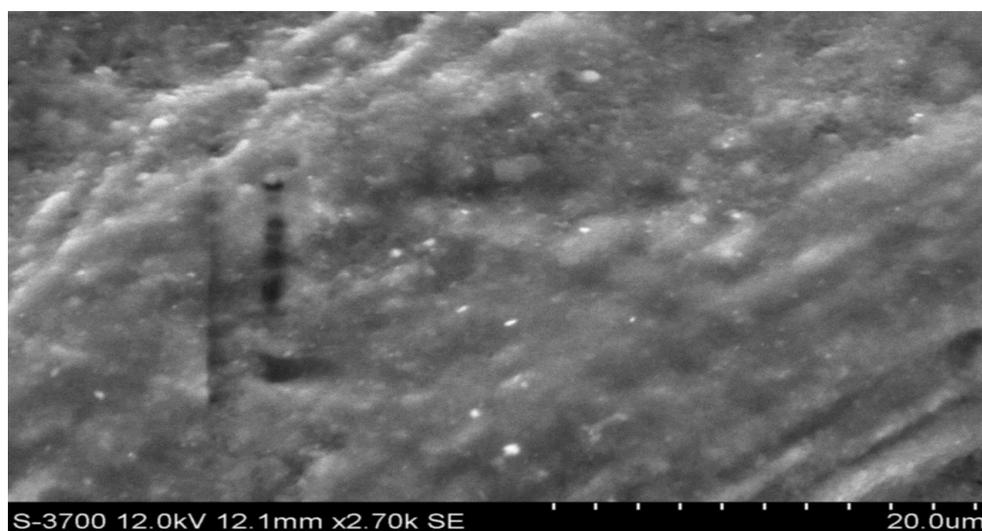


Fig. 3: SEM Image of Optimized F3 Formulation

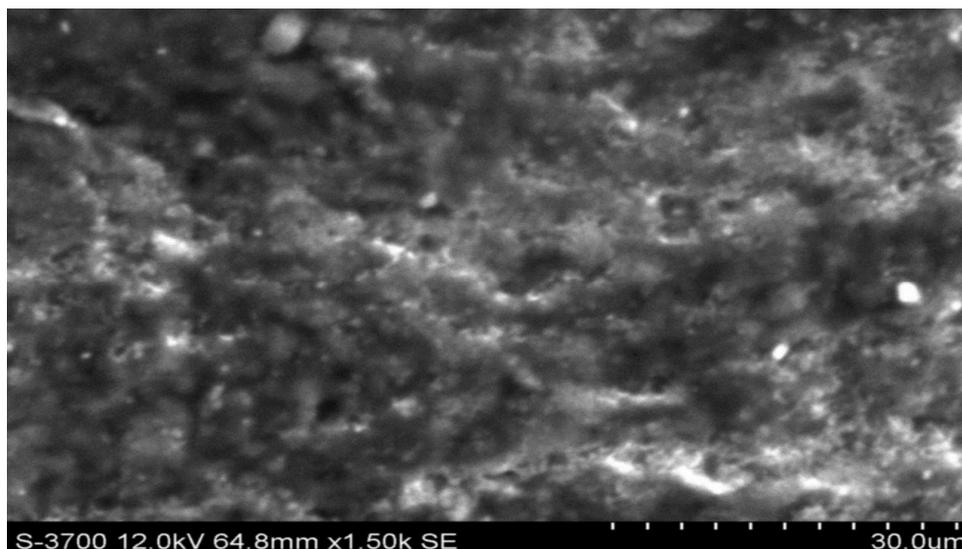


Fig. 4: SEM Image of Optimized F6 Formulation

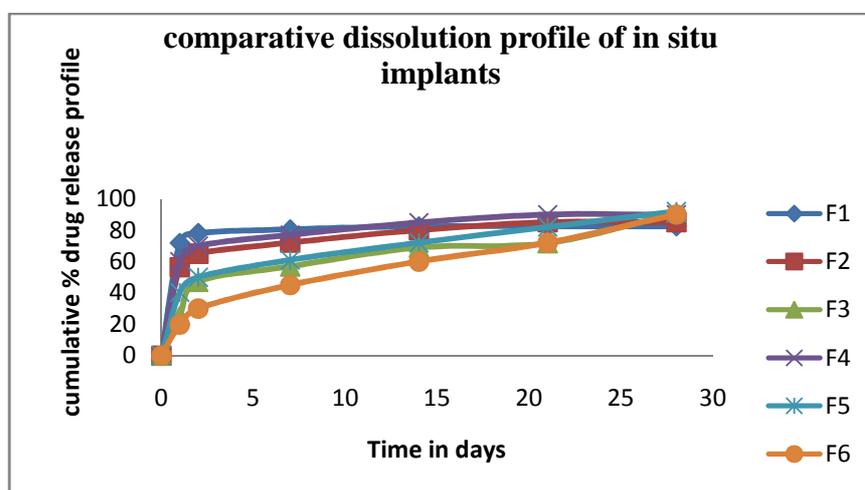


Fig. 5: In vitro drug release profile of cytarabine implants

Sterility test

Sterilization of in situ implants was performed by using γ radiations. After exposure to γ radiations slight colour change was observed. These implants were placed in thioglycolate medium for 15 days, there is no bacterial growth was observed. It indicates that implants pass the sterility test.

Stability studies

Accelerated stability studies of cytarabine in situ implants (f6) at temperature 40°C/75%RH as per ICH guidelines was studied for 90 days. The physical appearance of the formulation was a clear white to light gold color and it was observed that there was no colour change indicating physical stability. The drug content was analyzed and data is presented in table no. From the data, it is observed that there was negligible change in the drug content indicating chemical stability.

Differential Scanning Calorimetry (DSC)

In order to confirm the physical state of cytarabine in in situ implants DSC of cytarabine, physical mixture of cytarabine and polymer, cytarabine implants were carried out and were shown in figure. The DSC trace of cytarabine showed a sharp endothermic peak 213.12. The physical mixture of cytarabine and polymer showed same thermal behavior 213.16, as the individual component, indicating that there was no interaction between drug and polymer in solid state. The reported melting point range of cytarabine is between 211°C-213°C, thus indicating there is no change of cytarabine in pure state, physical mixture of drug and polymer. The absence of endothermic peak of the cytarabine at 213.12 in the DSC of the cytarabine implants suggests that the cytarabine existed in an amorphous or disordered crystalline phase as a molecular dispersion in polymeric matrix.

Table 4: Sterility testing of optimized formulations of cytarabine implants

Formulation	Radiations	Intensity of radiations	Exposure duration	Inoculation medium	Incubation time	Result
F3	γ rays	20.6 kGy	4days	thioglycolate medium	15days	No bacterial growth
F6	γ rays	20.6 kGy	4days	thioglycolate medium	15days	No bacterial growth

Table 5: Stability testing of implants

S. No.	Test	Justification	Initial	1 Month	2 Months	3 Months
1.	Description	A clear white to light gold color				
2.	pH	Between 7.00 and 9.50	7.86	7.83	7.80	7.78
3.	Assay of F6 formulation	95.00-105.00%	98.6%	98.1%	97.8%	98.2%
4.	Assay of F3 formulation	95.00-105.00%	98.3%	98.3%	98.2%	98.2%

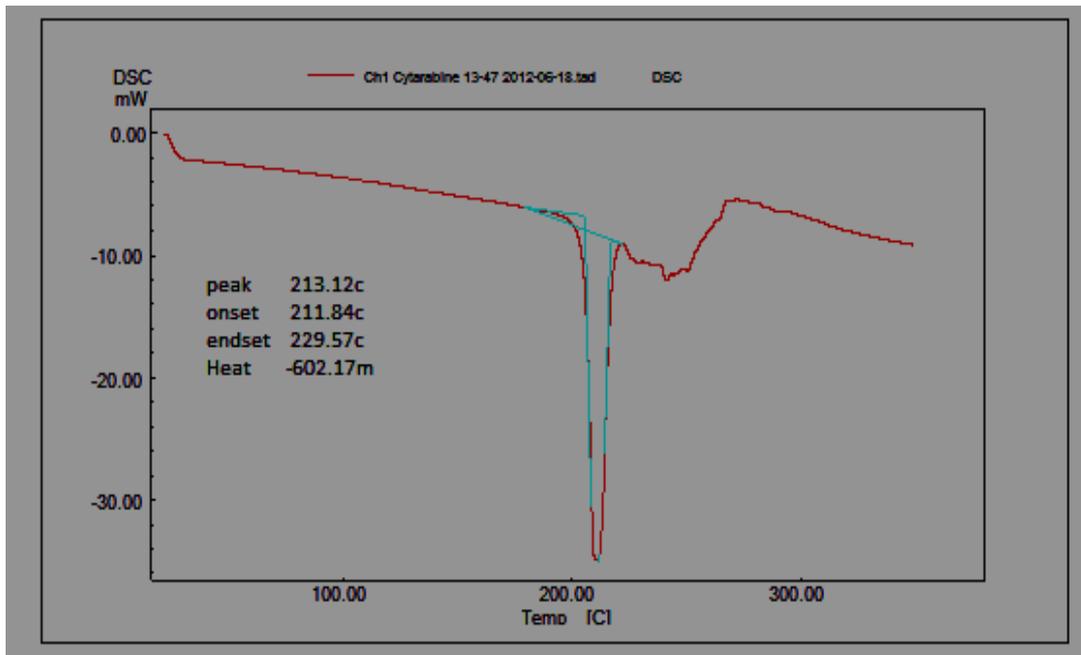


Fig. 6: DSC peak of cytarabine

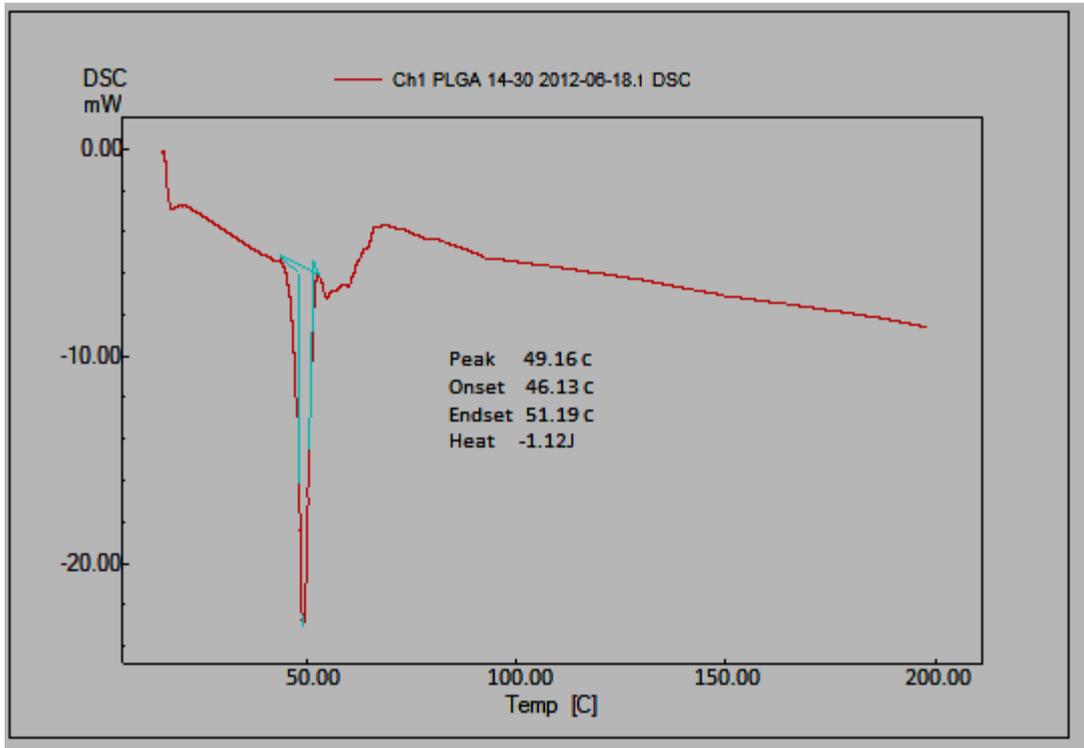


Fig. 7: DSC peak of polymer PLGA75:25

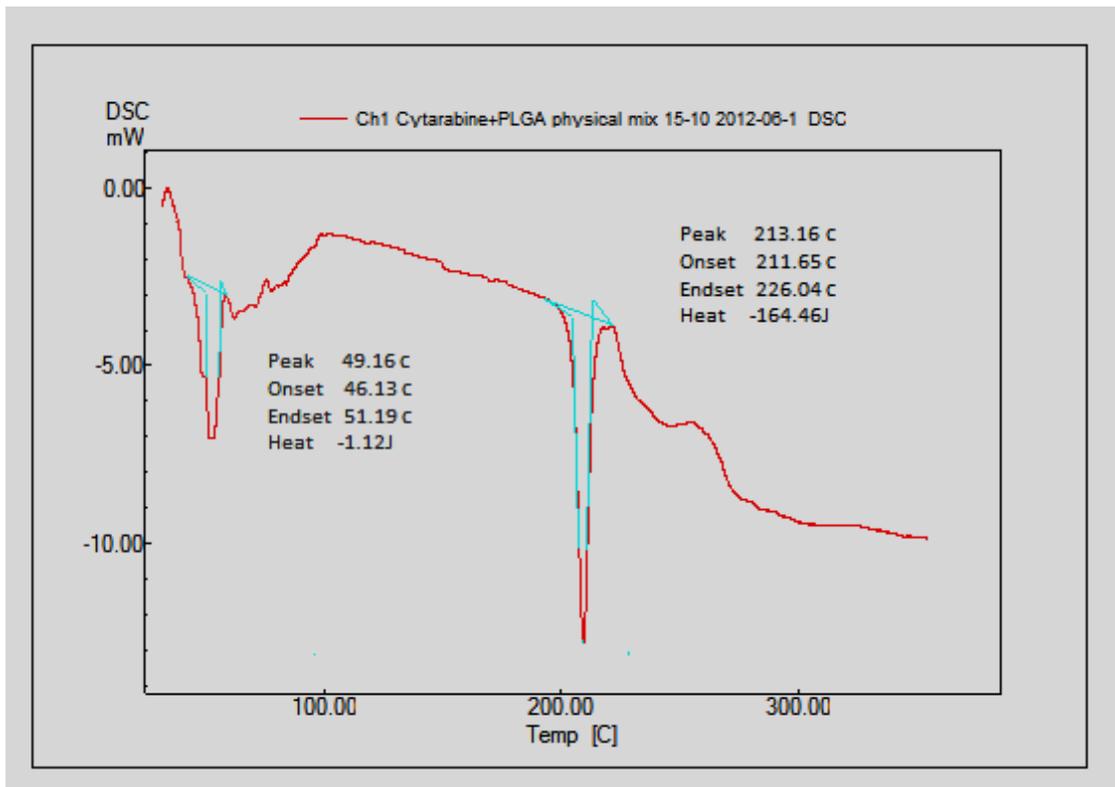


Fig. 8: DSC peak of physical mixture

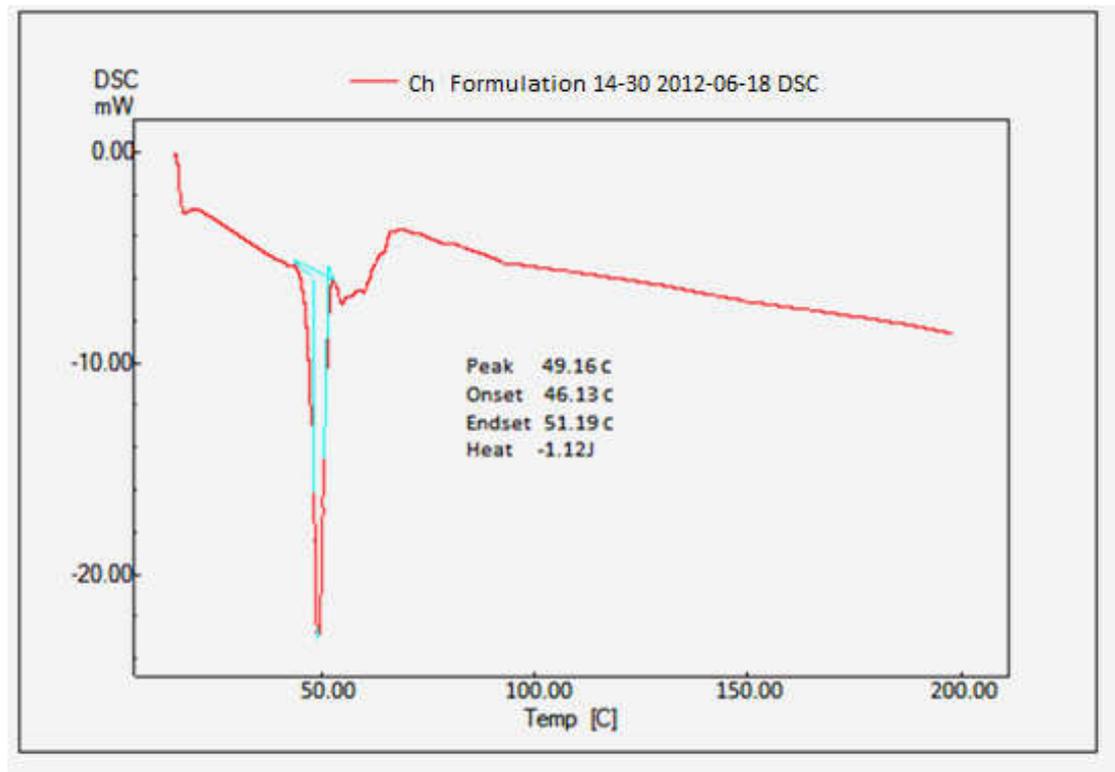


Fig. 9: DSC peak of formulation

FTIR Studies

FTIR spectra obtained for cytarabine, physical mixture and Formulation presented in the fig10-12. The characteristic peaks of cytarabine were compared with the peaks obtained for physical mixture of cytarabine, formulation and were given in figure 10-12.

The characteristics peaks found in cytarabine, physical mixture and formulations, hence it appears there was no chemical interaction between cytarabine and polymer and it can be concluded that the characteristics bands of cytarabine were not affected after successful load formulation of implants.

In vitro release kinetics

The plots of cumulative percentage drug release v/s. time, cumulative percent drug retained v/s. root time, log cumulative percent drug retained v/s. time and log cumulative percent drug release v/s. log time were drawn and represented graphically as shown in fig13-16. The slopes and the regression co-efficient of

determinations (r²) were listed in Table 6. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent 'n' values of korsmeyer-peppas model was found to be in the range of less than 0.45 for prepared *in situ* implants indicating Fickian diffusion of cytarabine from implants.

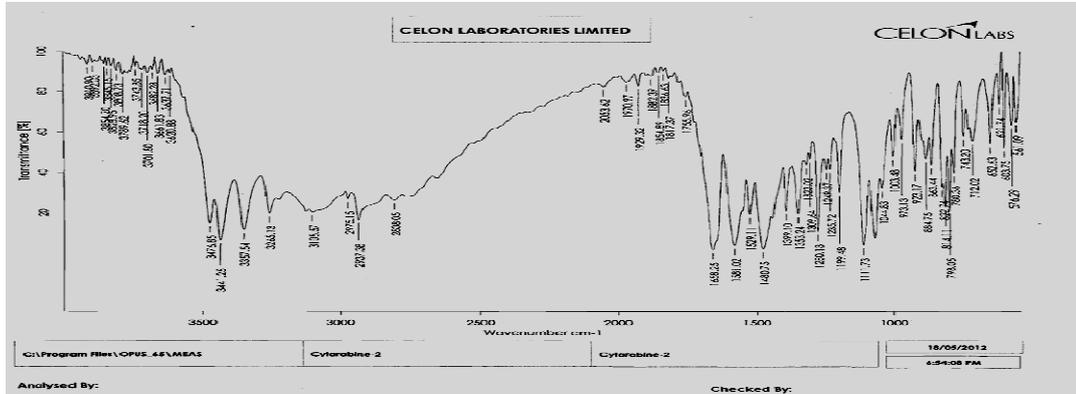


Fig. 10: FTIR spectra of cytarabine

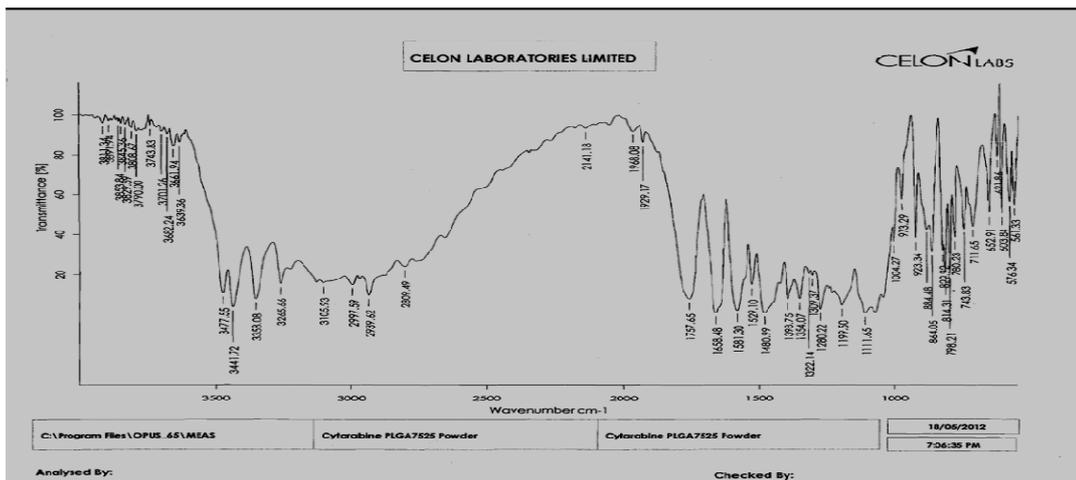


Fig. 11: FTIR spectra of cytarabine and PLGA physical mixture

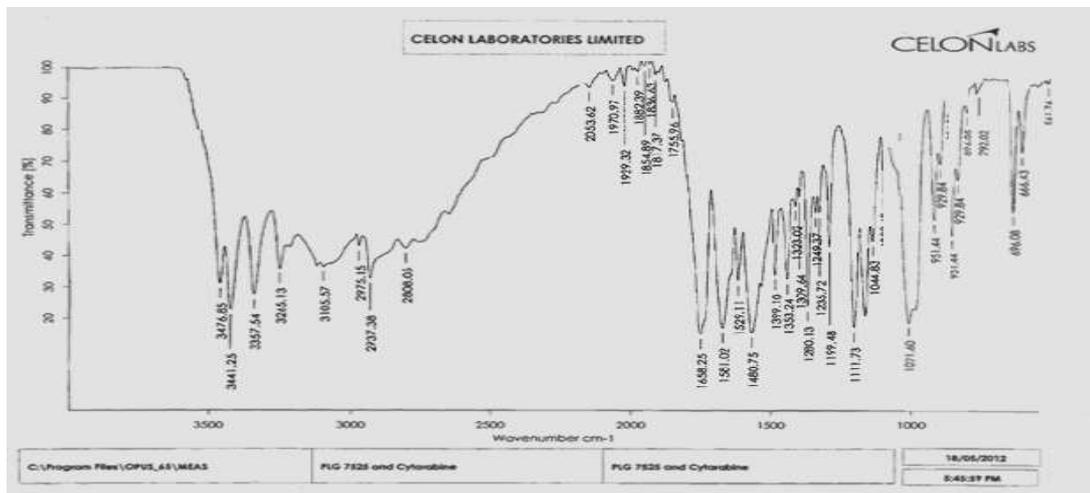


Fig. 12: FTIR spectra of cytarabine Formulation

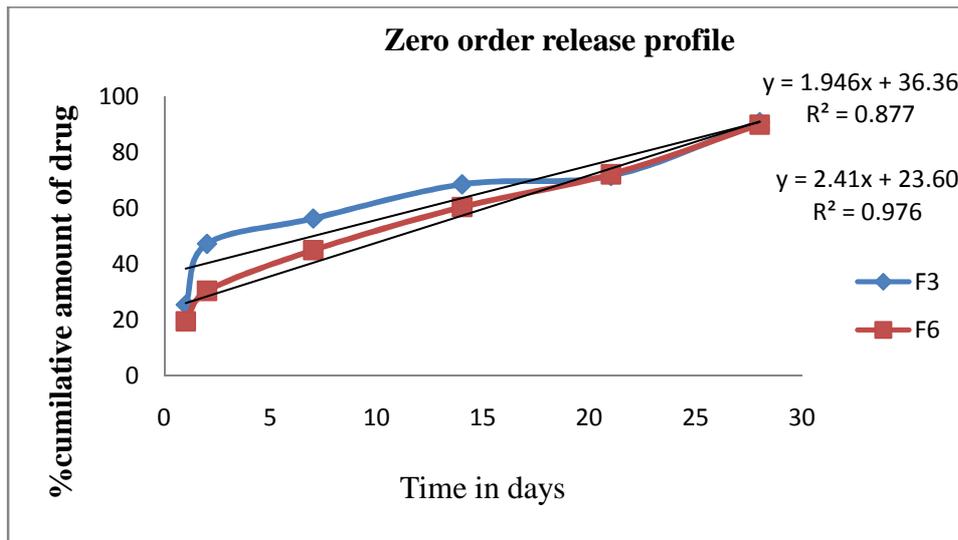


Fig. 13: Comparison of zero order release profile for optimized formulations of in situ implants F3, F6

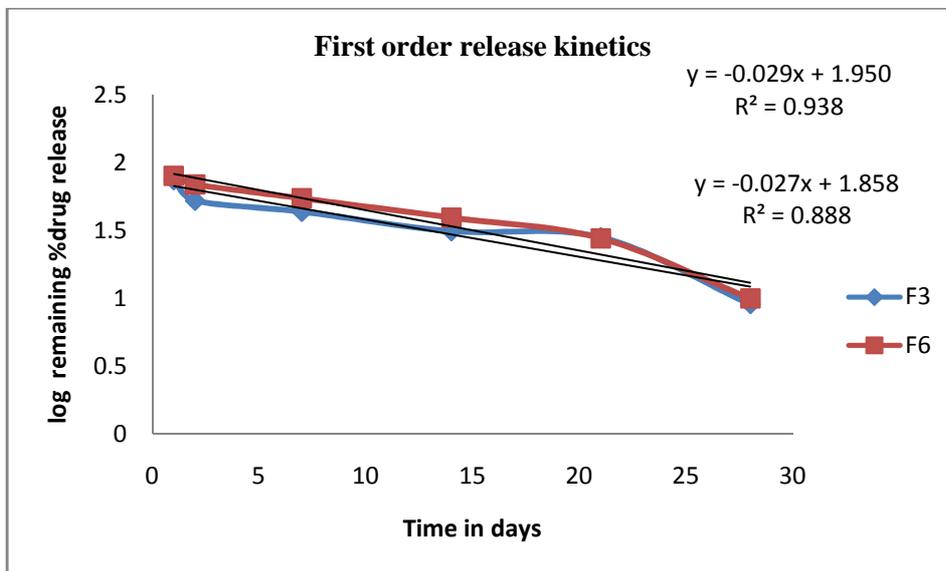


Fig. 14: Comparison of First order release kinetics for optimized formulations of in situ implants F3, F6

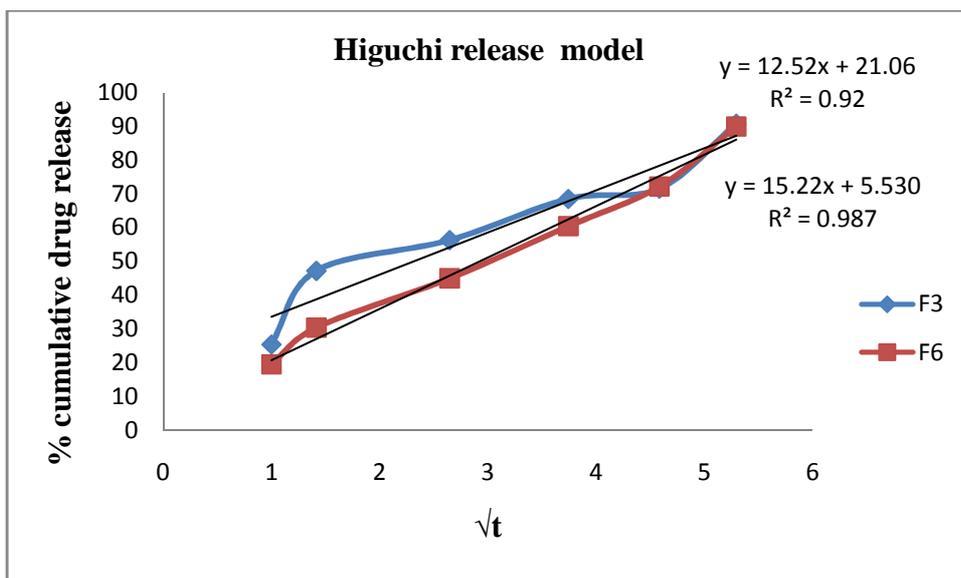


Fig. 15: Higuchi release model for optimized formulations of in situ implants F3, F6.

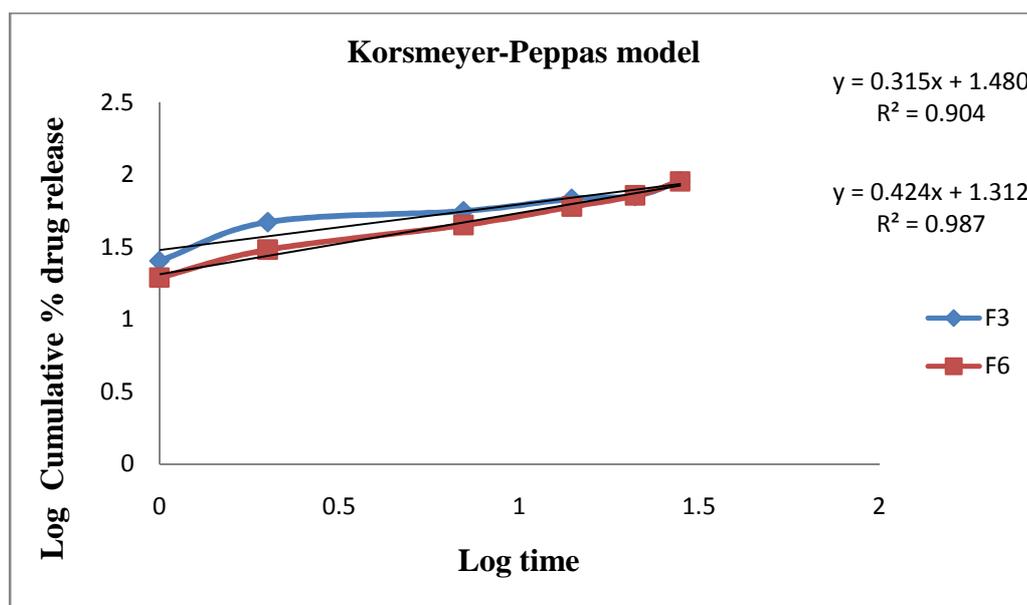


Fig. 16: Korsmeyer-Peppas model for optimized formulations of in situ implants F3, F6

Table 6: Regression coefficient (r^2) values of different kinetic models and diffusion exponent (n) of peppas model for insitu implants of cytarabine.

S. No.	Type of Formulation	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Korsmeyer Peppas	
					(R^2)	n
1	F3	0.965	0.938	0.920	0.904	0.315
2	F6	0.976	0.888	0.987	0.987	0.424

CONCLUSION

Pre formulation studies like solubility and Uv analysis of cytarabine were complied with IP standards. The FTIR spectra revealed that there were no inter actions between polymer and cytarabine. The PLGA polymer used was compatible with cytarabine. Entrapment efficiency increases with increase in polymer concentration. From the results it can be concluded that as the drug to polymer ratio decrease, initial burst release will increase. Surface morphology of in situ implants was observed, it is found to be porous surface, which was confirmed with SEM. In vitro drug release studies indicate that amount of drug release prolongs with increase in polymer concentration. The in vitro performance of cytarabine in situ implants prolonged and sustained release of drug. From Sterility studies it can be concluded that, implants are sterile after exposing to γ radiations. The co-efficient of determination indicated that the release data was best fitted with Zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent ' n ' values of Korsmeyer-Peppas model was found to be less than 0.45 for the cytarabine in situ implants indicating Fickian diffusion of drug from implants. Accelerated stability studies indicating that in situ implants are stable on storage. The DSC data indicates that there is no inter action between drug and polymer, and it also indicates that cytarabine still present in lattice structure in physical mixture where as it was completely amorphous in implants. From the study it is evident that promising sustained release in situ implants of cytarabine may be developed by polymer precipitation method by using polymer PLGA.

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