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FORMULATION AND CHARECTERIZATION OF *IN SITU* IMPALNT OF OCTREOTIDE ACETATE

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ABSTRACT

Octreotide is the acetate salt of a cyclic octapeptide. It is a long-acting octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. The peptide drugs after oral and parental administration the poor bioavailability in the blood due to their short biological half –lives caused by their metabolic instability. so that these peptide drugs are formulated by polymeric drug delivery systems such as micro particles or implants, has been proposed enabling their sustained release after a residence time in the polymer which protects the peptide against enzymatic and hydrolytic influences of biological media. In the present study, In situ implants of Octreotide acetate were prepared by polymer precipitation method. PLGA is dissolved in hydrophilic solvents such as Dimethyl sulphoxide, N-Methyl 2-pyrrolidone, PEG-200 and Triacetin until the formation of a clear solution. Different formulations were prepared using different concentration of polymer i.e. 5to35 % w/w. The characterization of implant was carried out by Determination of PLGA 5050 polymer ratio by NMR, Determination of molecular weight of polymer by GPC, Viscosity of polymer solution, Sterility test, In-vitro drug release, Release kinetics and Scanning electron microscopy. The Maximum percent of drug release with minimum Initial Burst release was found in formulation (F3) with NMP as solvent. Effect of gamma irradiation on molecular weight of polymer, viscosity of polymer solution, monomer ratio of lactide and glycolide and in-vitro release after gamma radiation was studied with F3 formulation.

Keywords: Octreotide acetate, *In situ* implant, PLGA, Sustained release, Parenteral depot

INTRODUCTION

Octreotide is the acetate salt of a cyclic octapeptide. It is a long-acting octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. Octreotide has been used to treat the symptoms associated with metastatic carcinoid tumors (flushing and diarrhea), and Vasoactive Intestinal Peptide (VIP) secreting adenomas (watery diarrhea), Octreotide substantially reduces and in many cases can normalize growth hormone and/or IGF-I (somatomedin C) levels in patients with acromegaly. The peptide drugs after oral and parental administration the poor bioavailability in the blood due to their short biological half –lives caused by their metabolic instability. so that these peptide drugs are formulated by polymeric drug delivery systems such as micro particles or implants, has been proposed enabling their sustained release after a residence time in the polymer which protects the peptide against enzymatic and hydrolytic influences of biological media [1]. Polymeric drug delivery systems are attractive alternatives to control the release of drug substances to obtain defined blood levels over a specified time. These delivery systems are limited by patient and physician acceptance related to ease of administration, reliable kinetic profiles, or product costs. The marketed preparation of Octreotide acetate in the form of microspheres is available on the name of SANDOSTATIN LAR depot manufactured by Novartis. It is available with three different strengths i.e. 10 mg, 20 mg & 30 mg for 1 month duration therapy [2].In-situ implants are advantageous over the microspheres and implants,

microspheres manufacturing process is often complex and difficult control. As a result, there are often questions involving costs and batch-to-batch product uniformity. In solid implants, they require surgical implantation or the use of large troches to administer the product so it is less patient and physician acceptance related to ease of administration. So that *insitu* implants have patient and physician acceptance related to ease of administration, reliable kinetic profiles, or product costs [3]. 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) *insitu* solidifying organ gels. Of these, *in situ* polymer precipitation systems have become commercially available so far. In polymer precipitation method, a biodegradable polymer dissolved in a biocompatible carrier. This Injectable implant system comprised of a water insoluble bio degradable polymer, such as Poly (DL-Lactide), Poly (DL-Lactide-co-glycolide) and Poly (DL-Lactide-co-caprolactone) Dissolved in a water miscible, physiologically compatible solvent. Upon injection in to an aqueous environment, the solvent diffuses in to the surrounding aqueous environment while water diffuses into the polymer matrix. Since the polymer is water in soluble, it precipitates up on contact with the water and results in a solid polymeric implant. Solvents which have been used in this approach include N-methyl-2- pyrrolidone, PEG-200, Dimethyl sulfoxide (DMSO), and Triacetin [4].

MATERIALS AND METHODS

Materials: Octreotide was obtained from Hanghou think chemical co, Ltd. China, poly (Lactide -co-glycolide) PLGA 50:50 (RG504) was obtained from Evonik, Germany. All solvents were HPLC grade and were obtained from Merck chemicals, Mumbai.

Preformulation study: General Procedure for the Preparation of calibration curve by UV: A stock solution of (1mg/ml) of standard drug was prepared, later required dilutions were made with a phosphate buffer pH 7.4. To a series of 10ml volumetric flasks aliquots standard solutions were taken and the volume was made up using a phosphate buffer pH 7.4. The absorbance of these solutions was measured at respective wave length of maximum absorbance, using 1cm quartz cuvette in UV- Visible spectrophotometer. Absorbance values were plotted against respective concentration to obtain standard calibration curve.

Solubility studies of Octreotide acetate: The solubility of Octreotide acetate was determined in different solvents such as Dimethyl sulphoxide, N-Methyl 2-pyrrolidone, PEG-200 and Triacetin. This was accomplished by adding 50 mg of Octreotide acetate in each 1ml of solvent.

Solubility studies of Polymer: The solubility of PLGA 50:50 was determined from 5 % *w/w* to 50 % *w/w* in different solvents such as Dimethyl sulphoxide, N-Methyl 2-pyrrolidone, PEG-200 and Triacetin.

Method of preparation of implants: In situ implants of Octreotide acetate were prepared by polymer precipitation method. PLGA is dissolved in hydrophilic solvents such as Dimethyl sulphoxide, N-Methyl 2-pyrrolidone, PEG-200 and Triacetin until the formation of a clear solution. Different formulations were prepared using different concentration of polymer i.e. 5-35% w/w. Drug solution is prepared by dissolving 10 mg of drug in 0.5 ml of acetate buffer having pH 4.0 and filled in to 2 ml clear USP type II glass vial for Lyophillization**.** Polymer solution was added in to 10 mg of lyophilized Octreotide acetate, mixed thoroughly with 1 ml tuberculin syringe until it formed clear solution and injected in to 1 ml of phosphate buffer saline (pH 7.4) using 18 gauge needle for the formation of in-situ implants. After 30 minutes solid in-situ implant was formed and added another 50 ml of phosphate buffer saline (pH 7.4) [5, 6].

Characterization of implants

Determination of PLGA 5050 polymer ratio by NMR: ^{1}H NMR analysis has been done to determine the co-monomer ratio of lactide and glycolide polymer [7].

Determination of molecular weight of polymer by GPC: Gel permeation chromatography (GPC) is a type of size exclusion chromatography that separates analytes on the basis of size. Gel permeation chromatography is conducted almost exclusively in chromatography columns. GPC is often used to determine the relative molecular weight of polymer samples as well as the distribution of molecular weights. Samples are dissolved in an appropriate solvent, in the case of GPC these tend to be organic solvents and after filtering the solution it is injected on to a column [8, 9].

Viscosity: Viscosity of different formulations was determined using Brookfield viscometer 2000^{+} (Brookfield engineering laboratories, USA) with spindle no.6 at 50 rpm at temperature 25±1°C [10,11].

Scanning electron microscopy: The Morphology and surface appearance of *in situ* implants were examined by using SEM (Using Hitachi-S-3700N).

Scanning electron microscopy was carried out to study the morphological characteristics of Octreotide acetate PLGA *In-situ* implant. The samples for the SEM analysis were prepared by placing the implant on one side of adhesive stub. Then the implant was coated with gold (100A°) before microscopy. Finally the morphology of the implant was observed with the scanning electron microscopy [3].

In-vitro drug release: In vitro drug release studies were performed by injecting the formulation in to 50 ml 7.4 pH phosphate buffers at 37°C. At 1, 3, 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 210nm. After every one week the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium [12, 13].

Sterility test: Polymer solution was filled in 1 ml tuberculin syringes and irradiated at ambient temperatures**.** γ -irradiation was performed using a commercial Co 60 source to a dose of 25 Kgray for two days. Irradiated Polymer solution was inoculated in fluid thio-glycolate medium, at 20-25°c for 15 days. Drug solution is sterilized by dissolving the drug in water and by filtering with 0.2µ filter which is filled in 2 ml clear glass USP type II vials and lyophilized [1, 5]**.**

Stability studies: To assess the physical and chemical stability of the in situ implants, stability studies were conducted for 3 month at 25°C/60%RH mentioned in ICH guidelines. After 90 days the formulations was checked for physical appearance and dissolution study.

RESULTS AND DISCUSSIONS

Standard calibration curve of Octreotide acetate in UV spectrophotometer: The UV absorbance's of Octreotide acetate standard solution in the range of 10-50 µg/ml of drug in buffer, pH7.4 showed linearity at λ max 210nm. The linearity was plotted for absorbance against concentration with R2 value 0.9995 and with the slope equation y = 0.0179x-0.003. The absorbance values and standard curve were shown in Figure 1.

Solubility study of Octreotide acetate: The solubility of Octreotide acetate in different of solvents was carried out and it reveals that it is soluble in Dimethyl sulphoxide, N-Methyl 2-pyrrolidone, PEG-200 and Triacetin.

Solubility study of PLGA Polymer: The solubility of Polymer in different solvents was carried out and results were shown in below mentioned table 1. It was observed that the different solvents having different % solubility in polymer. Maximum % solubility was obtained with NMP i.e. 50% w/w.

Formulation optimization: Four different hydrophilic solvents were selected for optimization study. Table 2 containing two solvents i.e. NMP & DMSO and table 3 containing another two solvents i.e. PEG 200 & Triacetin.

Viscosity: Viscosity of polymer solution for different formulations is given in table 4. As the concentration (%w/w) of polymer solution increased, the viscosity of polymer solution in each solvent was increased. For in-vitro release study, maximum % w/w polymer solution or highest viscosity of polymer solution in different solvents were selected.

In-vitro drug release: The *in vitro* dissolution profile of four different formulations i.e. formulation F3, F6, F9 & F12 was performed. The dissolution was carried out for a period of 28 days in saline phosphate buffer (pH 7.4) at 37°C.

The cumulative percent release of F3, F6, F9 &F12 formulations at various time intervals was calculated. The cumulative percent drug release in F3, F6, F9 & F12 formulations was plotted against time in figure 2. The Maximum percent of drug release with minimum Initial Burst release was found in F3 formulation i.e. formulation with NMP as solvent.

In vitro release kinetics: The plots of cumulative percentage drug release v/s. time cumulative percent drug retained v/s. root time percent drug retained v/s. time and log cumulative percent drug release v/s. log time were drawn and represented graphically as shown in figure 3-6. The slopes and the regression co-efficient of determinations (r2) were listed in Table 5. 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 korsemeyer-peppas model was found to be in the range of less than0.45 for prepared *in situ* implants indicating Fickian diffusion of Octreotide acetate from implants. From above viscosity results, release profile and release kinetics, we can concluded that formulation with NMP was given desired release profile i.e. more than 90% release after 28 days with less than 20% release after one day and more than 1500 cps viscosity. So for further characterization, formulation F3 was selected as an optimized formulation.

Effect of gamma irradiation on molecular weight of polymer, viscosity of polymer solution, monomer ratio of lactide and glycolide and in-vitro release

Determination of molecular weight of polymer by GPC: Gel permeation chromatography (GPC) is a type of size exclusion chromatography that separates

analytes on the basis of size. Formulation F3 was studied for molecular weight determination purpose. Initial molecular weight of polymer solution was 58,549 Daltons (Figure 7) and it was significantly reduced after gamma irradiation i.e. 33,251 Daltons (Figure 8) [13].

Determination of polymer ratio by NMR: ^{1}H NMR analysis has been done to determine the co-monomer ratio of lactide and glycolide polymer with formulation containing NMP as solvent (F3). Co-monomer ratios (Figure 9) were determinate by integrating the methane group of the lactide unit at 4.75 ppm (1.9287) and for the methylene group of the glycolide unit at 5.25 ppm (1.0). These integral values, LA and GA respectively, were converted into co-monomer ration, R LA and R GA using the following equations:

R LA = ILA + IGA / ILA = 1.9287 + 1 / 1 = 1.9287

RGA = IGA + ILA / IGA = 1 + 1.9287 / 1.9287 = 2

So, from ^{1}H NMR spectrum and co-monomer ratio, we can conclude that PLGA polymer has 50% lactide and 50% glycolide.

Determination of viscosity for polymer solution: Viscosity of formulation F3, after and before gamma irradiation was determined using Brookfield viscometer 2000^{+} (Brookfield engineering laboratories, USA) with spindle no.6 at 50 rpm at temperature 25±1°c. Viscosity of F3 formulation before gamma irradiation was 2000 cps and it was reduce significantly after gamma irradiation i.e. 1700cps.

***In-vitro* drug release study:** The *in vitro* dissolution profile of F3 formulation (Figure 10) after γ irradiations was performed. The dissolution was carried out for a period of 28 days in 7.4 pH saline phosphate buffer. It was observed that initial burst release after gamma irradiation was increased from 17% to 21% and more than 90% of drug was released within 28 days.

Sterility test: Sterilization of polymer solution for formulation F3 was performed by using γ irradiation sterilization method. After exposure to γ irradiations slight color change was observed i.e. from dark yellowish to light yellowish. This solution was placed in thio-glycolate medium for 15 days and it was observed that there was no bacterial growth. It indicates that the polymer solution was sterile after γ irradiation.

Scanning electron microscopy: Scanning electron microscopy was carried out to study the morphological characteristics of Octreotide acetate PLGA *In-situ* implant. Surface morphology of solid implant during dissolution i.e. after 1 day (Figure 11) and after 15 days (Figure 12) was studied. As the polymer was degraded during time, the porosity of implant was increased and release also increased.

Stability studies: Accelerated stability studies of Octreotide acetate Implant at temperature 25±2°C/60±5% RH as per ICH guidelines were studied for 90 days. The assays and appearance of samples were determined as a function of the storage time. The physical appearance of polymer solution was not change significantly and dissolution study was found to be 25 % release after 1 day.

CONCLUSIONS

From the executed experimental results, it could be concluded that attempts were made to prepare Octreotide acetate *Insitu*-implant for controlled release by polymer precipitation method using PLGA 50-50 polymer. The selection of hydrophilic solvent such as Dimethyl sulphoxide, N-Methyl 2-pyrrolidone, PEG-200 and Triacetin, concentration of polymer, were found to have played a predominant role in the preparation. The Maximum percent of drug release with minimum Initial Burst release was found with NMP as optimized formulation. Molecular weight of polymer solution in NMP was significantly decreased after gamma irradiation. From the experimental results it is evident that the controlled release Insitu implant of Octreotide acetate can be successfully formulated for subcutaneous administration in the treatment of patients with carcinoid tumors, Acromegaly.

ACKNOWLEDGEMENT

The authors are grateful to Director, Indian Institute of Chemical Technology (IICT), Secunderabad for providing analytical facilities.

Table 1: Solubility of PLGA Polymer in different solvents

Type of solvent	Maximum % Solubility (w/w)
NMP	50
DMSO	30
PEG-200	20
Triacetin	15

Table 2: Formulation with NMP & DMSO

Test parameters	F1	F2	F3	F4	F5	F6
Octreotide acetate (mg)	10	10	10	10	10	10
PLGA 50:50 (mg)	100	100	100	100	100	100
NMP (mg)	300	230	185	-	-	-
DMSO (mg)	-	-	-	380	300	230
Polymer concentration (% w/w)	25	30	35	20	25	30
Injection volume (ml)	0.3	0.3	0.3	0.3	0.3	0.3

Table 3: Formulation with PEG 200 & Triacetin

Test parameters	F7	F8	F9	F10	F11	F12
Octreotide acetate (mg)	10	10	10	10	10	10
PLGA 50:50 (mg)	100	100	100	100	100	100
PEG 200 (mg)	850	530	380	-	-	-
Triacetin (mg)	-	-	-	1700	850	530
Polymer concentration (% w/w)	10	15	20	5	10	15
Injection volume (ml)	0.3	0.3	0.3	0.3	0.3	0.3

Table 4: Viscosity of different formulations

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Viscosity (cps)	1400	1700	2000	1200	1400	1600	1300	1500	1700	1000	1200	1400

Table 5: Release rate profile of Formulations F3, F6, F9 & F12

Type of Formulation	Zero order (R^2)	First-order (R^2)	Higuchi (R^2)	Korsmeyer – Peppas (n)
F3	0.874	0.991	0.975	0.514
F6	0.695	0.915	0.883	0.327
F9	0.628	0.812	0.847	0.268
F12	0.461	0.470	0.659	0.179

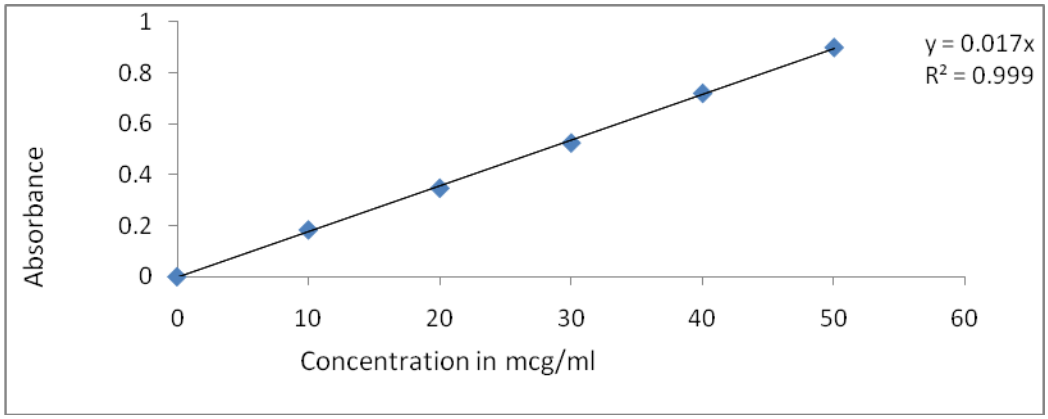


Figure 1: Standard graph of Octreotide acetate in acetate buffer pH 4.0.

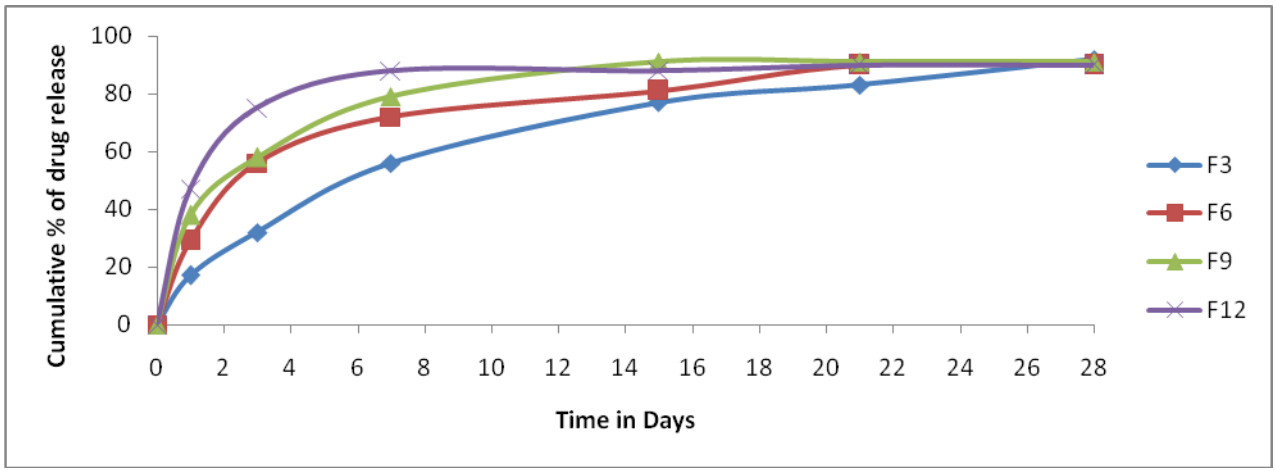


Figure 2: *In- vitro* release studies for optimized formulations – F3, F6, F9 & F12

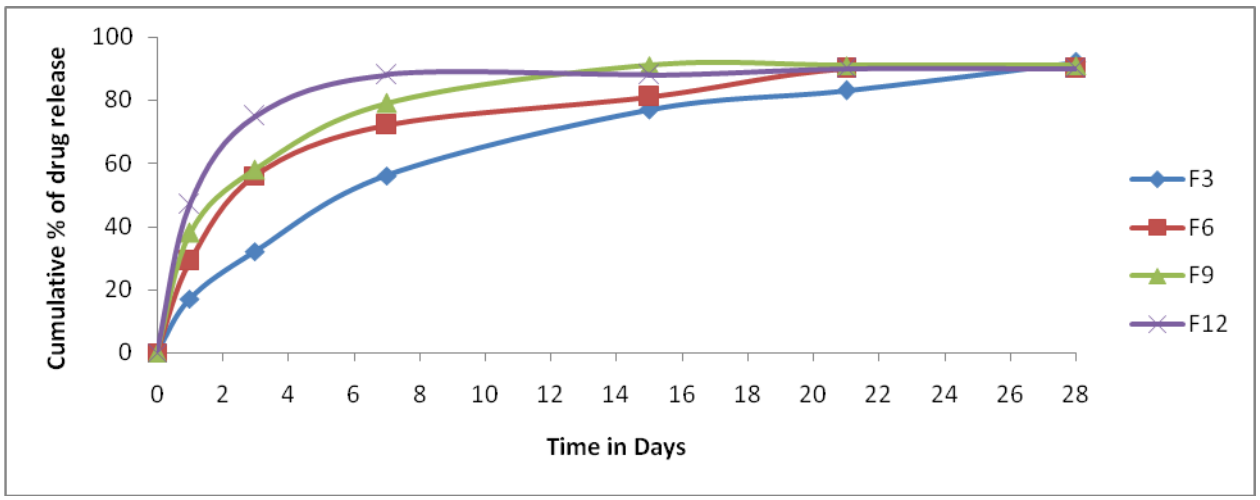


Figure 3: Zero order release studies for optimized formulations F3, F6, F9 & F12

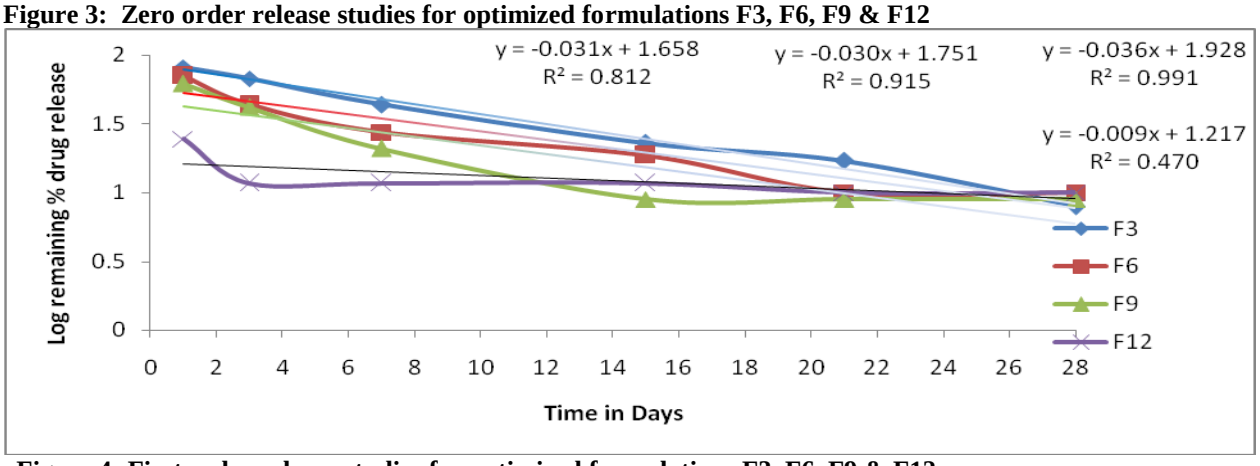


Figure 4: First order release studies for optimized formulations F3, F6, F9 & F12

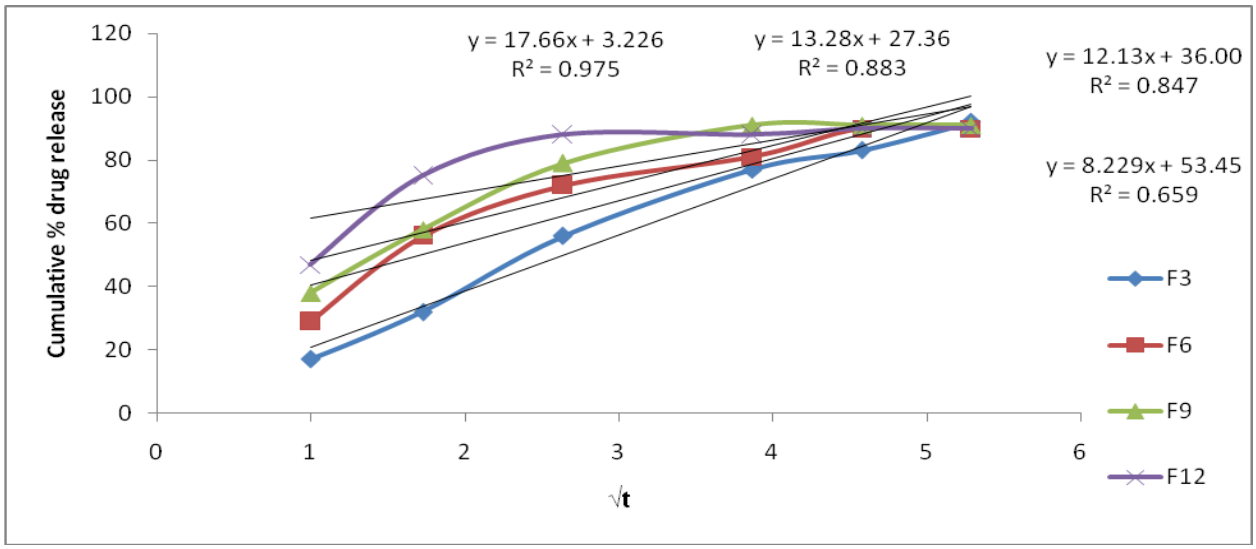


Figure 5: Higuchi's order plot for optimized formulations F3, F6, F9 & F12

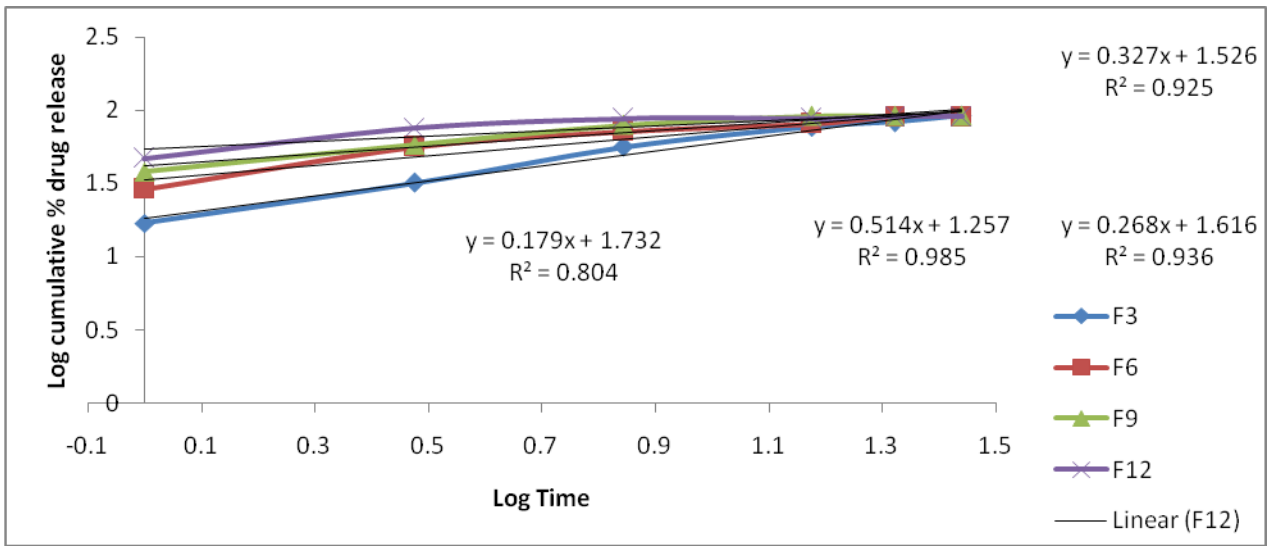
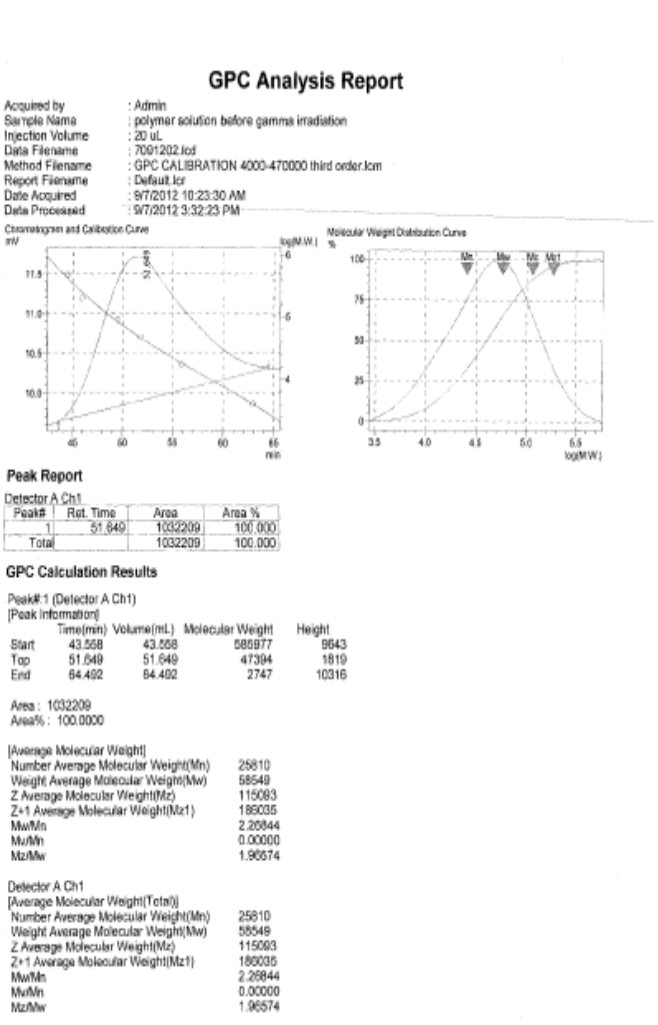


Figure 6: Korsmeyer –Peppa's model for optimized formulations F3, F6, F9 & F12



GPC Analysis Report

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Sample Name : polymer solution before gamma irradiation
Injection Volume : 20 uL
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Method Filename : GPC CALIBRATION 4000-470000 third order.lcm
Report Filename : Default.lcr
Date Acquired : 9/7/2012 10:23:30 AM
Data Processed : 9/7/2012 3:32:23 PM

Chromatogram and Calibration Curve

Molecular Weight Distribution Curve

Peak Report

Detector A Ch1

Peak#	Ret. Time	Area	Area %
1	51.649	1032209	100.000
Total		1032209	100.000

GPC Calculation Results

Peak#1 (Detector A Ch1)
[Peak Information]

	Time(min)	Volume(mL)	Molecular Weight	Height
Start	43.558	43.558	586977	9643
Top	51.649	51.649	47394	1819
End	64.492	64.492	2747	10316

Area : 1032209
Area% : 100.0000

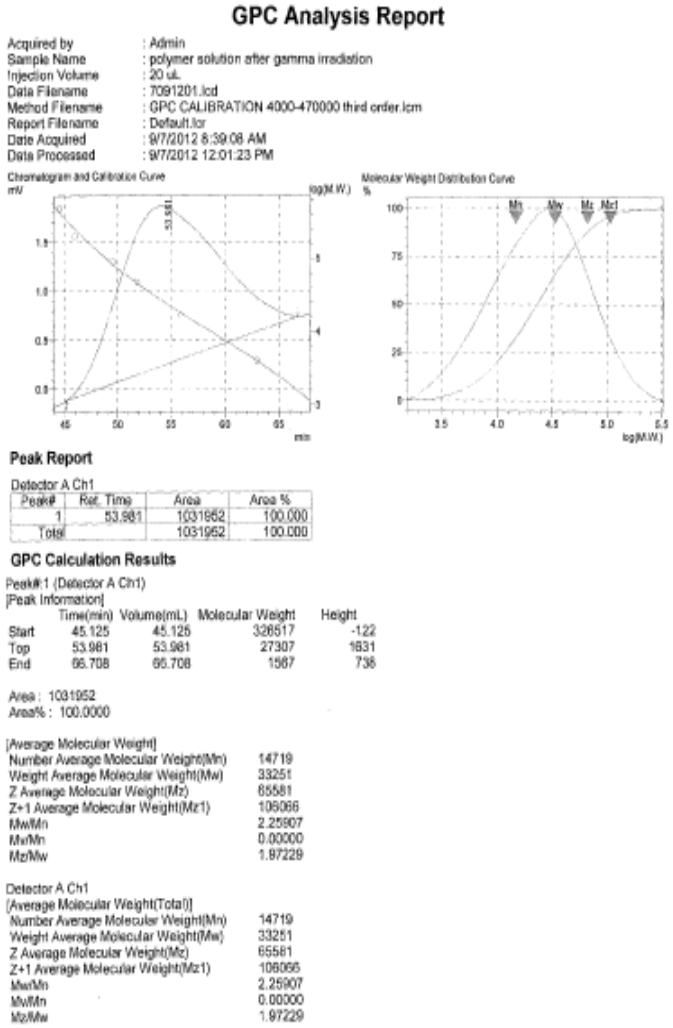
[Average Molecular Weight]

Number Average Molecular Weight(Mn)	25810
Weight Average Molecular Weight(Mw)	58549
Z Average Molecular Weight(Mz)	115083
Z+1 Average Molecular Weight(Mz1)	180035
Mw/Mn	2.26844
Mv/Mn	0.00000
Mz/Mw	1.96574

Detector A Ch1
[Average Molecular Weight(Total)]

Number Average Molecular Weight(Mn)	25810
Weight Average Molecular Weight(Mw)	58549
Z Average Molecular Weight(Mz)	115083
Z+1 Average Molecular Weight(Mz1)	180035
Mw/Mn	2.26844
Mv/Mn	0.00000
Mz/Mw	1.96574

Figure 7: Before gamma irradiation



GPC Analysis Report

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Report Filename : Default.lcr
Date Acquired : 9/7/2012 8:39:08 AM
Data Processed : 9/7/2012 12:01:23 PM

Chromatogram and Calibration Curve

Molecular Weight Distribution Curve

Peak Report

Detector A Ch1

Peak#	Ret. Time	Area	Area %
1	53.981	1031952	100.000
Total		1031952	100.000

GPC Calculation Results

Peak#1 (Detector A Ch1)
[Peak Information]

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Top	53.981	53.981	27307	1831
End	66.708	66.708	1567	736

Area : 1031952
Area% : 100.0000

[Average Molecular Weight]

Number Average Molecular Weight(Mn)	14719
Weight Average Molecular Weight(Mw)	33251
Z Average Molecular Weight(Mz)	65581
Z+1 Average Molecular Weight(Mz1)	106066
Mw/Mn	2.25907
Mv/Mn	0.00000
Mz/Mw	1.97229

Detector A Ch1
[Average Molecular Weight(Total)]

Number Average Molecular Weight(Mn)	14719
Weight Average Molecular Weight(Mw)	33251
Z Average Molecular Weight(Mz)	65581
Z+1 Average Molecular Weight(Mz1)	106066
Mw/Mn	2.25907
Mv/Mn	0.00000
Mz/Mw	1.97229

Figure 8: After gamma irradiation

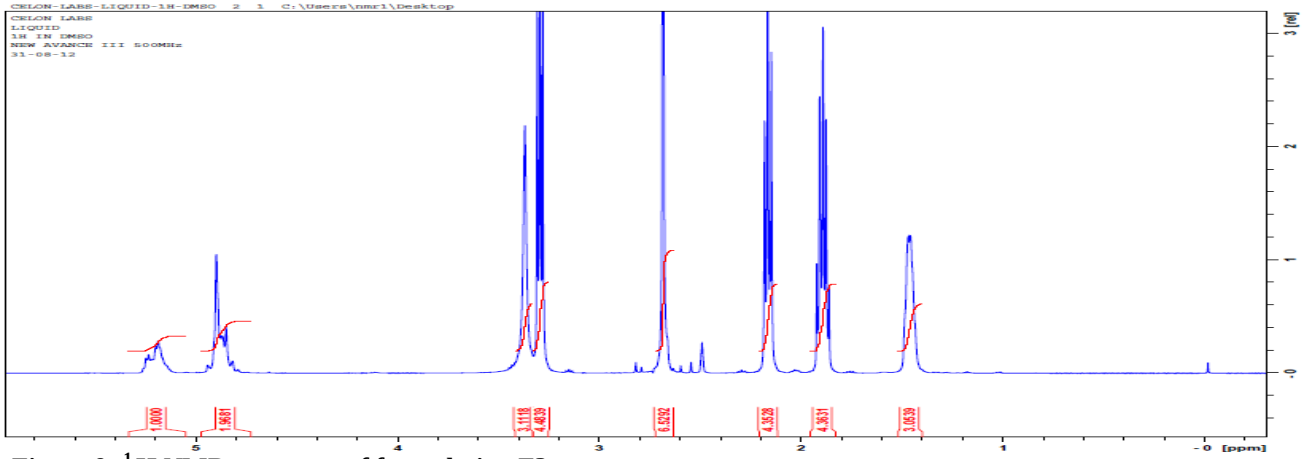

Figure 9: ^{1}H NMR spectrum of formulation F3

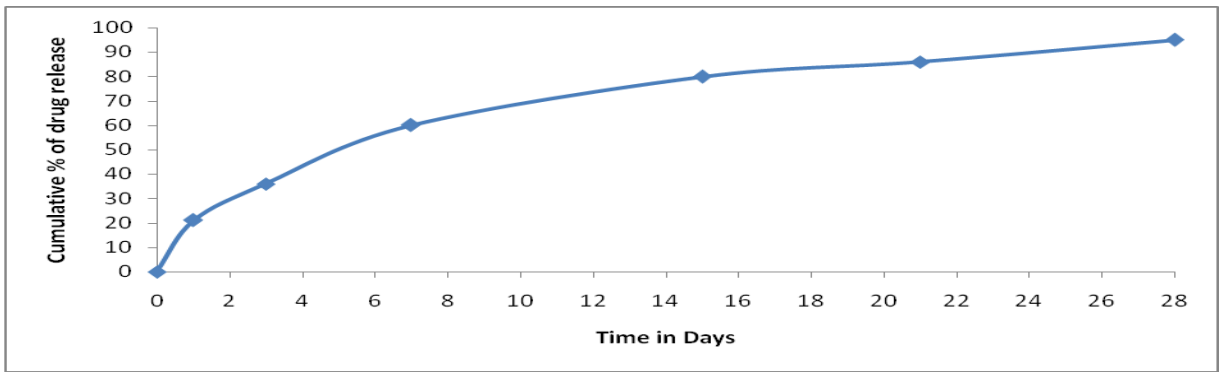

Figure 10: *In- vitro* release studies for optimized formulations – F3

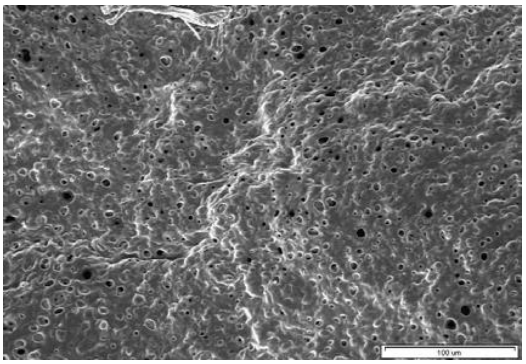

Figure 11: SEM of implant after 1 Day

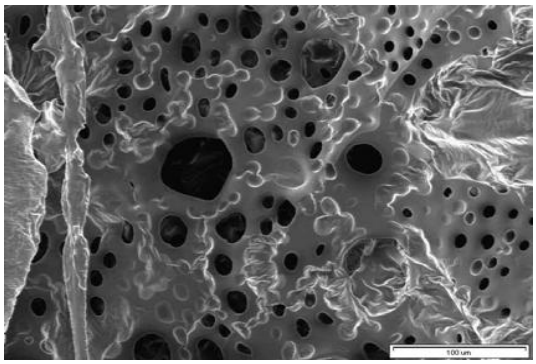

Figure 12: SEM of implant after 15 Days

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