Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 3, 2013

Research Article

FORMULATION AND EVALUATION OF OCTREOTIDE ACETATE LOADED PLGA MICROSPHERES

L. MARY SONY PHILO[1], SUMIT SHAH[2], A.V BADARINATH[1], C.GOPINATH[1]

[1]Annamacharya college of pharmacy, Rajampet, Kadapa, [2]Celon Laboratories Limited, Hyderabad, Andhra Pradesh, India.
Email: Sonyphilo5@gmail.Com

Received: 15 Jun 2013, Revised and Accepted: 09 Jul 2013

ABSTRACT

Objective: The objective of the present work was to formulate and evaluate controlled release Octreotide acetate microspheres for subcutaneous administration for treating symptoms associated with metastatic carcinoid and vasoactive intestinal peptide tumors (VIP-secreting tumors) and acromegaly effectively and also to improve patient compliance with fewer side effects. Octreotide is a long acting cyclic octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. It inhibits growth hormone, glucagon, and leutinizing hormone response to Gonadotropin releasing hormone, serotonin gastrin, vasoactive intestinal peptide, motilin and pancreatic polypeptide.

Method: Different formulations were prepared by following solvent evaporation technique (double emulsion) using biodegradable poly (Lactide-co-Glycolide) acid and evaluated for percentage yield, entrapment efficiency, surface morphology (SEM), particle size analysis, in-vitro drug release and stability studies.

Results: The prepared microspheres were white, free flowing and spherical in shape. The mean Particle size of the microspheres was found in the range of 26 to 206µm. The drug-loaded microspheres showed 70-86% of entrapment and release was extended up to 6 to 8 h releasing 93% of the total drug from the microspheres. The infrared spectra showed stable character of octreotide in the drug-loaded microspheres and revealed the absence of drug-polymer interactions. Scanning electron microscopy study revealed that the microspheres were spherical and porous in nature.

Conclusion: The octreotide is uniformly distributed within the microspheres which are made of a biodegradable D,L-lactic and glycolic acids copolymer. The optimized formulations of Octreotide acetate microspheres with controlled release were attempted for a release upto atleast one month.

Keywords: Bioavailability, Controlled release, Microspheres, Octreotide acetate, PLGA polymer, SEM, Subcutaneous.

INTRODUCTION

Many proteins currently being developed are aimed at chronic conditions where therapy may be required over months or years. Alternative administration by frequent injections to keep the protein drug at effective concentrations is tedious, expensive, and has poor patient compliance. Therefore, development of sustained release injectable dosage forms becomes necessary to improve the efficacy of peptide drugs and eliminate the need for frequent administration [1].

Octreotide is a long acting cyclic octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. It inhibits GH, glucagon, LH response to GnRH ,serotonin gastrin, vasoactive intestinal peptide ,motilin and pancreatic polypeptide. Octreotide is used to treat the symptoms associated with metastatic carcinoid and vasoactive intestinal peptide tumors (VIP-secreting tumors). Octreotide normalizes the growth hormone levels in acromegaly patients. The goal of treatment in acromegaly is to reduce GH and IGF levels to normal. Octreotide can be used in patients who had an inadequate response to surgery or in those for whom surgical resection is not an option. It may also be used in patients who have received radiation and have had an inadequate therapeutic response.

Marketed formulation of octreotide is available as Sandostatin® LAR Depot (octreotide acetate for injectable suspension) which is a somatostatin analogue. Its effects in the body are similar to those of the naturally occurring hormone, except that Sandostatin® LAR Depot remains active much longer than somatostatin.Sandostatin® LAR Depot binds to the somatostatin receptors, and thereby suppresses the release of the peptides and amines from the tumor and inhibits their action, suppressing the severe diarrhoea and flushing associated with this disease. Rapid gastrointestinal (GI) transit leads to accumulation of fluid in the colon and profuse volumes of watery stool Sandostatin® slows GI transit and increases fluid absorption by over 300%.

The aim of the present research was to formulate and evaluate octreotide acetate microspheres for subcutaneous administration for period on one month controlled release delivery system. Biodegradable microspheres were shown to improve the bioavailability of peptides by protecting them from physical degradation and proteolysis in body fluids. Poly (D,L-lactide) (PLA) and poly (D,L-lactide-co-glycolide) (PLGA) are the most widely used and well-characterized materials for the preparation of biodegradable microspheres.

MATERIALS AND METHODS

Materials

Octreotide acetate was purchased from Hemmo pharmaceutical PVD (Mumbai). Poly (D, L-Lactic-co-glycolic acid) 50:50 (PLGA 50:50) Resomer®RG 504 and Resomer®RG 504H were supplied by Evonik/Boehringer-Ingelheim (Germany). Poly (vinyl alcohol) (PVA) (MW 22000, 88% hydrolyzed) and ethyl acetate were supplied by Merck (India). Dichloromethane, Tween 80, Sodium chloride, Potassium chloride, di-sodium hydrogen phosphate, Potassium dehydrogenate phosphate and sodium azide were obtained from J. T. Baker (India).

Preparation of octreotide acetate microspheres

Octreotide acetate-loaded microspheres were prepared by a double emulsion-solvent evaporation technique. Briefly, 500 mg PLGA 5050 was dissolved in 5 mL dichloromethane (oil phase). An aqueous solution containing 50 mg of octreotide acetate in 1 ml of phosphate buffer pH 7.4 was prepared separately (inner aqueous phase or W1). The first aqueous (W1) phase was emulsified into the oil phase (containing PLGA), using a high-speed homogenizer (T18 basic, IKA, Germany) at 2-8 °C using different speeds and time durations to form water in oil primary emulsion. This primary emulsion was added in to 100 ml of external aqueous phase containing 1 % PVA solution to form secondary emulsion at 6000 rpm speed for 3 mins at 2-8°C

temperature. The wet microspheres were then stirred at 1000 rpm for 2 hrs at 2-8°C to permit evaporation of DCM and solidification of microspheres. The wet microspheres obtained were collected by centrifugation followed by filtration and Lyophillization [2,3,4,5].

Different formulation variables like volume of DCM (F1, F2 & F3), volume of PVA solution (F2, F4 & F5), Volume of inner aqueous phase (F2, F6 & F7) and effect of solvents (F2 & F8) were carried out as below mentioned Table 1:

Table 1: Formulation variable of octreotide acetate microspheres

Compositions	Formulation variables							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
octreotide acetate (mg)	50	50	50	50	50	50	50	50
Phosphate buffer pH 7.4	1	1	1	1	1	**0.5**	**1.5**	1
PLGA 5050 (mg)	500	500	500	500	500	500	500	500
DCM (ml)	**2.5**	**5**	**7.5**	5	5	5	5	5
Ethyl acetate (ml)	-	-	-	-	-	-	-	**5**
1 % PVA (ml)	100	100	100	**50**	**150**	100	100	100
1° Homogenization speed (rpm)	10000	10000	10000	10000	10000	10000	10000	10000
1° Homogenization time (mins)	1	1	1	1	1	1	1	1
1° Homogenization temperature (°C)	5	5	5	5	5	5	5	5
2° Homogenization speed (rpm)	6000	6000	6000	6000	6000	6000	6000	6000
2° Homogenization time (mins)	3	3	3	3	3	3	3	3
2° Homogenization temperature (°C)	5	5	5	5	5	5	5	5
Stirring speed (rpm)	1000	1000	1000	1000	1000	1000	1000	1000
Stirring time (hrs)	2	2	2	2	2	2	2	2
Stirring temperature (°C)	5	5	5	5	5	5	5	5

Different process variables like sped of primary homogenization (F9, F10 & F11), time of primary homogenization (F12 & F13) and effect of temperature for solidification of microspheres (F14 & F15) were carried out as below mentioned table 2:

Table 2: Process variable of octreotide acetate microspheres

Compositions	Process variables						
	F9	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**
Octreotide acetate (mg)	50	50	50	50	50	50	50
Phosphate buffer pH 7.4	1	1	1	1	1	1	1
PLGA 5050 (mg)	500	500	500	500	500	500	500
DCM (ml)	5	5	5	5	5	5	5
1 % PVA (ml)	100	100	100	100	100	100	100
1° Homogenization speed (rpm)	**6000**	**10000**	**14000**	10000	10000	10000	10000
1° Homogenization time (mins)	1	1	1	**3**	**5**	1	1
1° Homogenization temperature (°C)	5	5	5	5	5	5	5
2° Homogenization speed (rpm)	6000	6000	6000	6000	6000	6000	6000
2° Homogenization time (mins)	3	3	3	3	3	3	3
2° Homogenization temperature (°C)	5	5	5	5	5	5	5
Stirring speed (rpm)	1000	1000	1000	1000	1000	1000	1000
Stirring time (hrs)	2	2	2	2	2	2	2
Stirring temperature (°C)	5	5	5	5	5	**25**	**40**

Evaluation of Octreotide acetate microspheres

Determination of percentage yield

Microspheres were weighed and the yield of microspheres was calculated using the formula:

Percentage yield = Practical yield (gm) / Theoretical yield × 100

Determination of drug entrapment efficiency (EE)

The amount of drug entrapped was estimated by dispersing 50 mg of microspheres in DCM and water in 3:1 ratio, under vigorous shaking for 1hr, the resultant solution was centrifuged. Both layers were separated. As the octreotide acetate was soluble in water but not in DCM, the drug content in aqueous solution was analyzed by using HPLC at 220 nm with further dilutions against appropriate blank.

The amount of the drug entrapped in the microspheres was calculated using the formula:

% EE = Actual weight of drug in sample/ Theoretical weight of drug in sample x 100.

Particle size analysis

The mean diameter of microspheres was determined by laser diffractometer (Mastersizer X, Malvern Instrument, UK). Microparticles were suspended in 0.3% aqueous solution of Tween 80 and sonicated for 15 s prior to particle size determination.

Scanning electron microscopy (SEM)

The morphology of micro particles was examined by scanning electron microscopy (MW2300, Cam Scan-England). Samples were mounted on metal stubs and sputter-coated with gold for 4 min prior to examination under.

***In-vitro* drug release**

The in-vitro drug release from the microspheres was carried out by using a regenerated cellulose membrane dialysis apparatus Float-A-lyzer.2ml of microspheres suspension containing known amount of drug was placed in the Float-A-lyzer and this was placed in 250 ml of PBS (pH 7.4), maintained at 37°C and stirred with the help of a magnetic stirrer. Aliquots (2ml) of release medium were withdrawn at different time intervals and the sample was replaced with fresh PBS (pH 7.4) to maintain constant volume. The samples were analyzed for drug content by HPLC at 220nm. Upon completion of one week, the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium.

Stability studies

To assess the physical and chemical stability of the microspheres, stability studies were conducted for 3 months under various storage

conditions mentioned in ICH guidelines. The optimized formulation was placed in vials and stored at 25±20c/ 60±5% RH. After 90 days the formulations were checked for physical appearance and drug content.

RESULTS AND DISCUSSIONS

Summarized results of formulation and process variable are given in below Table 3:

Table 3: Results of formulation variables of octreotide acetate microspheres

Batches	Percentage Yield	Entrapment Efficiency%	Rounded Mean Particle Size
F1	71.4%	65	86
F2	70.5%	77	83
F3	65.6%	66	47
F4	50.6%	57	56
F5	53.9%	62	67
F6	72.8%	84	91
F7	73.7%	83	98
F8	72.2%	82	74
F9	72.8%	84	72
F10	**76.5%**	**87**	**83**
F11	71.4%	74	61
F12	60.2%	67	53

Effect of DCM volume\Polymer concentration

As the volume of DCM increased or decreased the polymer concentration, the viscosity of polymer was decreased. Encapsulation efficiency of octreotide microspheres was increased with increasing polymer concentration and the particle size was decreased. No significant difference was observed between F1 & F2 formulation in terms of particle size, encapsulation efficiency and initial burst release but there was significant difference between F2 & F3 formulation in term of particle size and encapsulation efficiency. The contribution of a high polymer concentration to the encapsulation efficiency can be interpreted in two ways. First, when highly concentrated, the polymer precipitates faster on the surface of the dispersed phase and prevents drug diffusion across the phase boundary. Second, the high concentration increases viscosity of the solution and delays the drug diffusion within the polymer droplets. In-vitro release of the microspheres with high polymer concentration or lower DCM volume was lower than the higher volume of DCM [6].

Effect of PVA volume

An increase in the volume of PVA solution resulted in an increase the encapsulation efficiency and particle size of octreotide loaded microspheres. Formulation F3 has shown more particle size than formulation F4. The increase in the particle size was attributed to a reduction in agitation that occurred because of a decrease in mixing efficiency associated with higher volumes. A reduction in mixing efficiency probably produced as increase in the size of the emulsion droplets during the preparative process, which would result in the formulation of large microparticles [7]. As a result of increased particle size, there is as associated increase in particle volume, which enables more octrotide to be incorporated into the microparticles.

Effect of solvent

Microspheres prepared with DCM (F6) were given higher entrapment efficiency and higher particle size than microsphere prepared with ethyl acetate (F8). Because, boiling point of DCM (40°C) is lower than ethyl acetate (77°C) so that solidification of microspheres was faster with lower boiling point. Because of longer time of solidification, entrapment efficiency was decreased.

Effect of primary homogenization speed

The particle size of microspheres was decreased with increase in speed of primary homogenization. Formulation F9 having 72 µm particle size as compare to formulation F11 having 61 µm. As the homogenization speed increases, the shear stress increases and the established balance between tangential stresses at the droplet interface impacted by the homogenizer and interfacial tension is going to be altered. The larger tangential stress leads to a reduction in droplet size, while the homogenization speed affects the relative viscosity of the emulsion. Typically, the viscosity reduction at a higher rotational speed is responsible for a decrease in particle size [8,9,10,11].

Effect of primary homogenization time

The particle size of microspheres was decreased with increase in time of primary homogenization. Formulation F10 having 83 µm particle size as compare to formulation F12 having 53 µm. Mechanism follows the same as mentioned above parameter.

Effect of temperature on solidification of microspheres

The microspheres prepared at 2-8°C and 40°C (F2 & F8) has shown higher entrapment efficiency and lower initial burst release than microspheres prepared at 25°C (F7). Microspheres prepared at 40°C solidified rapidly, forming a dense thin skin, indicating high drug encapsulation efficiency. Although faster skin formulation may reduce drug loss, the increase in solubility of drug at higher temperature and faster mass transfer may also increase the amount of octreotide leaving the dispersed phase during formation. The microspheres fabricated at lower temperature solidify slower, the lower solubility and mass transfer of octreotide at that temperature probably compensate for the relative ease of diffusing through the softer and less dense skin. The activity also remains fairly intact at this lower temperature. The resultant encapsulation efficiency for microspheres prepared at higher and lower temperature is almost similar. Initial burst release of microspheres prepared at higher temperature is slightly higher than microspheres prepared at 5°C because higher porosity of microspheres [12].

From above results of all parameters, it was observed that the formulation F10 was given desired particle size, entrapment efficiency and initial burst release.

Characterization of octreotide loaded microspheres

Formulation F10 was chosen for characterization of octreotide loaded PLGA microspheres.

Mean particle size distribution

The mean diameter of microspheres was determined by laser diffractometer (Mastersizer X, Malvern Instrument, UK). Microparticles were suspended in 0.3% aqueous solution of Tween 80 and sonicated for 15 s prior to particle size determination. The mean particle size of formulation F10 was shown below in Fig 1.

Scanning electron microscopy (SEM)

The morphology of microparticles was examined by scanning electron microscopy (MW2300, Cam Scan-England). Samples were mounted on metal stubs and sputter-coated with gold for 4 min prior to examination under. The SEM picture showed in Fig 2 that the shape of the microspheres was spherical and smooth surface with less porosity. SEM picture was compared with formulation F7 and it was observed that porosity of microspheres was more as inner aqueous phase volume increased.

***In-vitro* drug release**

The in-vitro drug release from the microspheres was carried out by using a regenerated cellulose membrane dialysis apparatus Float-A-lyzer [12,14,15]. 2ml of microspheres suspension containing known amount of drug (50 mg microspheres) was placed in the Float-A-lyzer and this was placed in 250ml of PBS (pH 7.4), maintained at 37°C and stirred with the help of a magnetic stirrer. Aliquots (2ml) of release medium were withdrawn at different time intervals and the sample was replaced with fresh PBS (pH 7.4) to maintain constant volume. The samples were analyzed for drug content by HPLC at 220nm. Upon completion of one week, the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium. Initial burst release means release of drug within 24 hrs and F10 has shown 18% IBR[13,14,15].

The cumulative percent release of F7, F9 & F10 formulations at various time intervals was calculated. The cumulative percent drug release in F7, F9 & F10 formulations was plotted against time in figure 4.

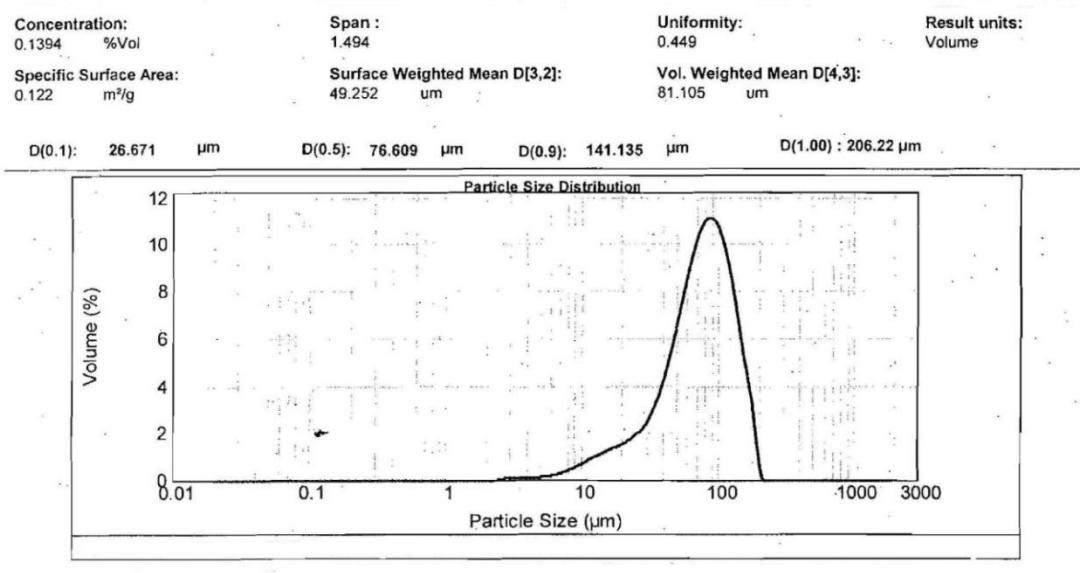

Fig. 1: Mean Particle size of formulation F10

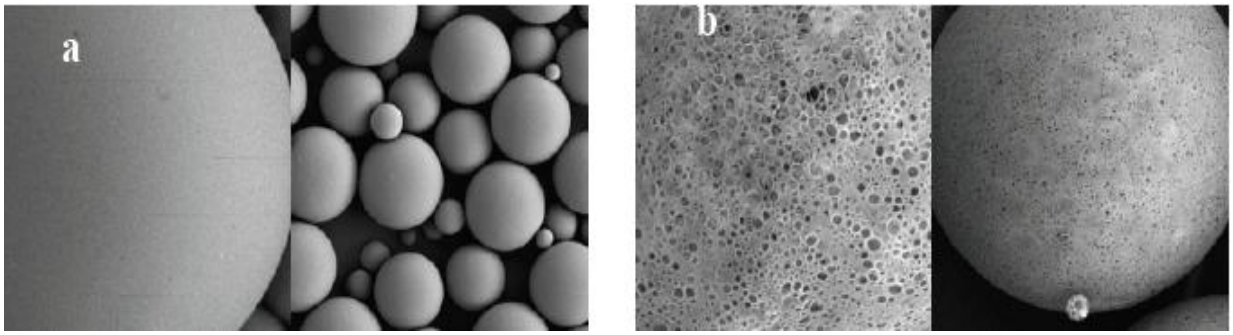

Fig. 2: a) SEM picture of formulation F10. b) SEM picture of formulation F7.

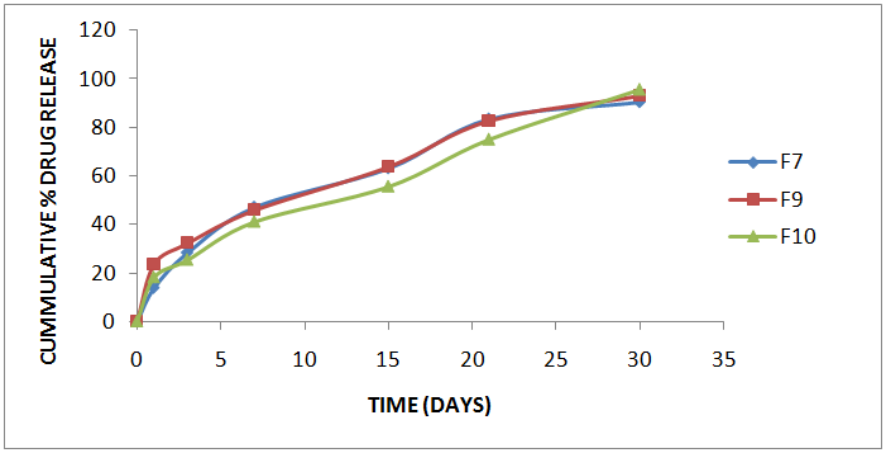

Fig. 4: Comparison of in-vitro drug release profile of Octreotide acetate from the formulation F7, F9 and F10

Release Kinetics

The release kinetics of F7, F9 & F10 formulations was studied. All formulations follow Higuchi release kinetics and follow Anomalous (non-Fickian) diffusion when it applied to the Korsmeyer-Peppa's Model for mechanism of drug release. F10 formulation has better kinetic results when compared to F7 and F9 formulations. The results are shown in Figure 5, 6, 7 & 8 and in Table 5.

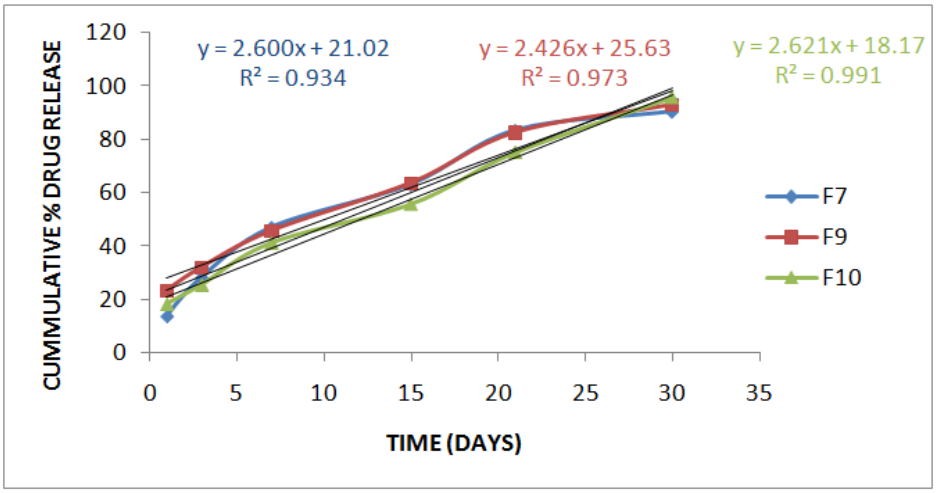

Fig. 5: Comparison of Zero order Release profiles of optimized formulations F7, F9 and F10

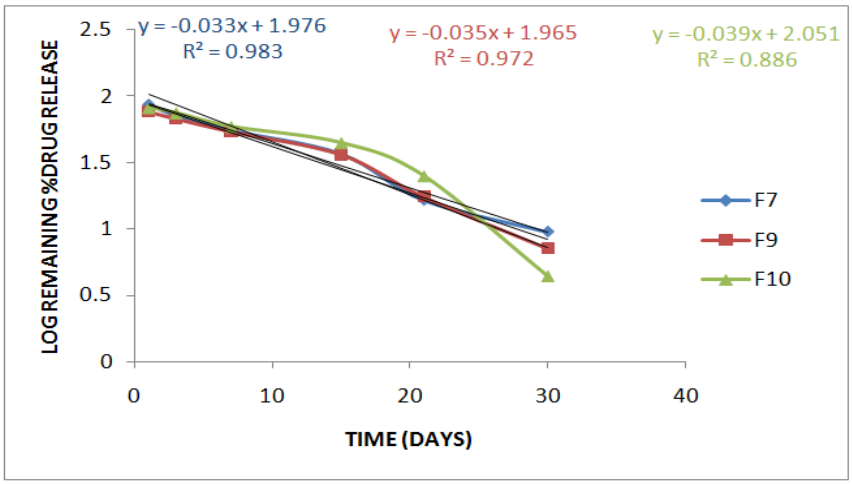

Fig. 6: Comparison of First order Release profiles of optimized formulations F7, F9, F10

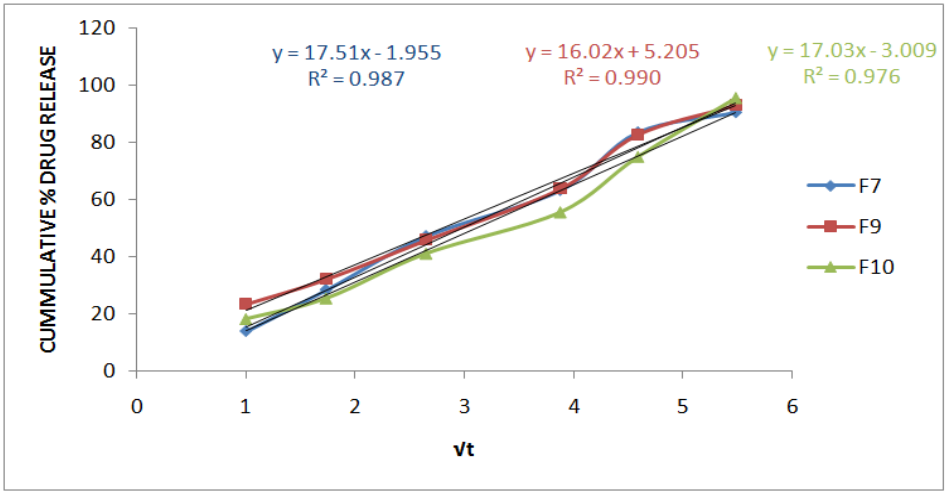

Fig. 7: Comparison of Higuchi's order plot for optimized formulations F7, F9 and F10

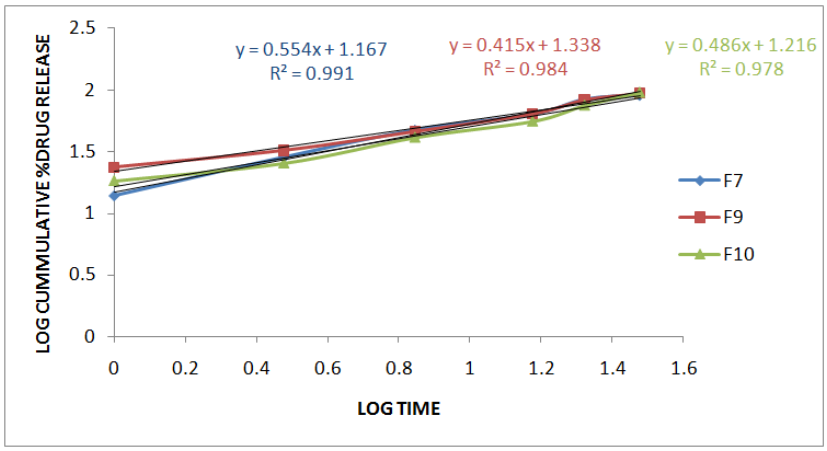

Fig. 8: Comparison of Korsmeyer-Peppas model for optimized formulations F7, F9 and F10

Table 4: Curve fitting data of drug release profiles for optimized formulations F7, F9 and F10

Formulation Code	Zero order (r^2)	First order (r^2)	Higuchi (r^2)	Korsmeyer Peppas (n)
F7	0.934	0.983	0.987	0.554
F9	0.973	0.972	0.990	0.415
F10	**0.991**	0.886	0.976	0.486

From the Table (4), r^2 value for formulation F10 was found to be more and was found to follow zero order kinetics, which states that the drug release is independent of concentration.

Stability studies

Accelerated stability studies of octreotide acetate microspheres at temperature 25±20C/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. There was no color change in the physical appearance, particle size was not change significantly and assay was found to be 95 % after 90 days. From the data, it is observed that there was negligible change in the drug content indicating chemical stability. The results of stability data has shown in below table 5.

Table 5: Accelerated stability (25±2°C / 60±5% RH) data of octreotide acetate microspheres

Test Description	0 days White to almost white	15 days White to almost white	30 days White to almost white	45 days White to almost white	60 days White to almost white	75 days White to almost white	90 days White to almost white
Assay of F7 formulation	82.6%	81.7%	80.4%	79.5%	77.8%	76.3%	75.4%
Assay of F9 formulation	83.9%	83.4%	82.4%	81.7%	80.3%	79.6%	78.2%
Assay of F10 formulation	86.5%	86.3%	85.5%	84.4%	83.6%	83.3%	82.4%

CONCLUSION

In the present study, attempts were made to prepare octreotide acetate microspheres for controlled release by double emulsion solvent evaporation technique using PLGA 50-50 polymer. The selection of organic solvent, concentration of polymer, speed of primary homogenization and solidification temperature were found to have played a predominant role in the preparation. The formed microspheres were found to be uniform and spherical in shape. The optimized formulations exhibited 93% in vitro controlled release for one month. From the experimental results it is evident that the controlled release microspheres of octreotide acetate can be successfully formulated for subcutaneous administration in the treatment of patients with metastatic carcinoid and vasoactive intestinal peptide tumors (VIP-secreting tumors) and acromegaly.

REFERENCES

1. Kyonghee Park, Goo Young Jung, Myong-ki kim. Triptorelin acetate-loaded poly (lactide-co-glycolide) (PLGA) microspheres for controlled drug delivery. Macromolecula Research 2012;20:847-51.
2. N.Thejaswi, Prathima Srinivas, Sadanandam, Sumit Shah. Preparation and characterization of Goserelin acetate loaded microspheres. International Journal of pharmacy and pharmaceutical science 2013;5 suppl1:184-89.
3. Raju.T, Santhosh Kumar.J, Ravindra Baboon D.S, Arvind G. Formulation and Evaluation of Cytarabin PLA microspheres. International Journal of pharmacy and pharmaceutical science 2013;5suppl1:87-93.
4. Yi-Yan Y, Tai-Shung C, Xin-Lia B. Effect of preparation condition on morphology and release of biodegradable polymeric microspheres containing protein fabricated by double-emulsion method. Chemical Engineering Sci 2000;55:2223-26.
5. Rajesh P, Jolly RP, Iieena Soni, Kishor NK. Poly (D,L- Lactide-co-Glycolide) microspheres containing 5-Fluorouracil: Optimization of process parameters. AAPS 2003; 4:1-8.
6. Yoon You and Kiran park. Control of encapsulation efficiency and initial burst in polymeric microparticles systems. Arch Pharm. Res 2004;1:1-12.
7. Igartua M, Hernandez RM, Esquisabel A. Influence of formulation variables on the in-vitro release of albumin from biodegradable microparticulate systems. J. Microencapsulation 1997;14:349-56.
8. Sah HK, Toddywala R, Ch'in YW. Biodegradable microcapsules prepared by a w/o/w technique: effect of shear force to make a primary w/o emulsion on their morphology and protein release. J. Microencapsulation 1993;12:59-69.

9. Maia JL, Santana MHA, Re MI. The effect of some processing condition on the characteriscts of biodegradable microspheres obtained by an emulsion solvent evaporation process. Braz J Chem Eng 2004;21:1-18.
10. Jeyanthi R, Mehta RC, Thanoo BC. Effect of processing parameters on the properties of peptide-containing PLGA microspheres. J. Microencapsulation 1997;14:163-74.
11. Raouf G, Cecilia S, Johan C. Effect of preparative parameters on the characteristics of poly (D,L- lactide-co-glycolide) microspheres made by the double emulsion method. Int J pharm 1996;141:205-16.
12. Yi-Yan Y, Hui-Hui C, Tai-Shung C. Effect of preparation temperature on the characteriscts and release profile of PLGA microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. J Control Rel 2000;69:81-96.
13. Alexandra P, Katerina G, Kristina M. Poly lactide-co-glycolide microparticles as system for controlled release of protein preparation and characterization. Acta Pharma 2004; 54:215-29.
14. Mcgee JP, Singh M, Ohagan DT. The encapsulation of a model protein in poly (D,L-lactide-co-glycolide) microparticles of various sizes: an evaluation of process reproducibility. J. Microencapsulation 1997;14:197-210.
15. Laura C. Herrera, María V. Defain Tesoriero. In Vitro Release Testing of PLGA Microspheres with Franz Diffusion Cells. Centre of Research and Development in Chemistry, National Institute of Industrial Technology 2012;13:220-34.