IJPSR (2013), Vol. 4, Issue 3 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 November, 2012; received in revised form, 12 January, 2013; accepted, 15 February, 2013

FORMULATION AND EVALUATION OF BSA LOADED PLGA MICROPARTICLES

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Keywords:

Microspheres, Bovine Serum Albumin, PLGA 50:50, Double emulsion solvent evaporation, SEM, DSC, Homogenization

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ABSTRACT: Protein drug delivery has emerged to be an important area of research in the field of novel drug delivery technology. The objective of the study was to prepare poly (D, L-lactide-co-glycolide) (PLGA) microspheres containing bovine serum albumin (BSA) as a model drug and to evaluate the various physicochemical characteristics of the formulations, namely morphology, particle size, FTIR, DSC, BSA encapsulation efficiency and in-vitro BSA release profile. BSA-loaded microspheres were prepared by double emulsion solvent evaporation method with different BSA: PLGA ratios and at different speeds of homogenization keeping the amount of BSA constant in all the formulations. Out of those 1:10 was selected as a optimized (drug: polymer) for BSA loaded PLGA microspheres, there after internal parameters like volume of inner aqueous phase(2ml), volume of DCM(10ml), concentration of polymer (9.09%),speed of homogenization, were selected as a optimized formulation parameters. Accelerated stability testing was performed with the optimized formulations for a period of eight weeks. The mean particle size and encapsulation efficiency of the microspheres were found to decrease as the speed of homogenization increased. And the same were found to increase simultaneously with increase in the amount of polymer. The in vitro release study showed a slow and steady release pattern of BSA. Accelerated stability studies indicated that formulations here stable during the period of study. Thus, a sustained release formulation of protein loaded PLGA microspheres was developed.

INTRODUCTION: Microspheres have played a vital role in the development of controlled/sustained release drug delivery systems. Microspheres have been of particular interest from the pharmaceutical point of view providing the possibility to achieve sustained and controlled drug release [1]. In a very short time, since their emergence, the field of controlled delivery of proteins has grown immensely. Because of their relatively large size, they have low transdermal bio-availabilities. Oral bioavailability is generally poor since they are poorly absorbed and easily degraded by proteolytic enzymes in the gastrointestinal tract ocular and nasal delivery is also unfavorable due to degradation by enzymes present in tissues and nasal mucosa, thus parenteral delivery is currently most demanding and suitable for delivery of such molecules. In systemic delivery of proteins, biodegradable microspheres as parenteral depot formulation occupy an important place because of several aspects like protection of sensitive proteins from degradation, prolonged or modified release, pulsatile release patterns. The main objective in developing controlled release protein injectables is avoidance of regular invasive doses which in turn provide patient

compliance, comfort as well as control over blood levels. BSA is a water-soluble protein, which is chosen for loading in to microspheres, development of microspheres containing BSA successfully gives a way for further development of other proteicious drugs, which have similar properties of BSA [2]. Among the various tentative for improving the administration of proteins, the microencapsulations into biodegradable polymers [3] represent a practical and promising approach. On the other hand, biodegradable microspheres can be injected [3] subcutaneously or intramuscularly and release the entrapped proteins for extended periods of time [4].

TABLE 1: COMMERCIAL BIODEGRADABLE DRUG PRODUCTS

Product	Drug	Company	Delivery technology	Polymeric carrier
Decapeptyl SR	Triptorelin	Ipsen	Microparticles	PLGA
Nutropin Depot	Somatropin	Genetech	Microparticles	PLGA
Risperdal Consta	Risperidone	Janssen	Microparticles	PLGA
Sandostatin LAR	Octreotide	Novaris	Microparticles	PLGA
Trelstar Depot	Triptorelin	Watson Pharma	Microparticles	PLGA
Trelstar LA	Triptorelin	Watson Pharma	Microparticles	PLGA
Vivitrol	Naltrexone	Cephalon	Microparticles	PLGA

MATERIALS AND METHODS: Materials: BSA was obtained from Celon Laboratories (Hyderabad), PLGA was obtained from Evonik Roehm Gmbh (Germany), Poly vinyl alcohol obtained from S.D. Fine chemicals (Mumbai). All solvents were HPLC grade and were obtained from Merck chemicals, Mumbai.

Preparation of BSA Microspheres: Double emulsion (W/O/W) solvent evaporation method was employed in the preparation of BSA loaded PLGA microspheres. This method for preparation of microsphere was reported to overcome the problem of low encapsulation efficiency of water soluble drug prepared by conventional water/oil emulsion solvent evaporation method. Polymer [Poly (L-lactic glycolic acid) (PLGA)] is dissolved in organic phase DCM (Dichloro methane).

In this organic phase, aqueous drug solution is emulsified using high speed homogenizer (IKA) operating around 10000 rpm for about 5 minutes to prepare water[1] /oil (w^1/o) primary emulsion. This primary emulsion is added to external aqueous phase containing surfactant (poly vinyl alcohol is used to prepare $w^1/o/w^2$ emulsion) at homogenizer speed around 8000 rpm for 3 minutes and then shifted to mechanical stirrer which was stirred at 1000 rpm for 1 hour at 2-8^oC then next 2 hrs at room temperature to permit evaporation of DCM.

The microspheres obtained is collected by centrifugation, filtration and then dried. Formed microspheres are evaluated [5].

Evaluation of Microspheres:

Percentage yield: The prepared microspheres were collected and weighted. The actual weight of obtained microspheres divided by the total amount of all material that was used for the preparation of the microspheres multiplied by 100 gives the % yield of microspheres (equation) [7]:

% Yield = Actual weight of product/ Total weight of excipients and drug × 100

Drug entrapment efficiency: The amount of drug entrapped was estimated by dissolving the 100mg of microspheres in DCM and water in 3:1 ratio ,under vigorous shaking for 1hr, the resultant solution is centrifuged, both layers were 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 272.7nm with further dilutions against appropriate blank. The amount of the drug entrapped in the microspheres was calculated using the formula [6]:

Encapsulation efficiency=

$$\frac{\text{Actual weight of drug in sample}}{\text{Theoretical weight of drug in sample}} \times 100$$

Scanning Electron Microscopy: Microspheres were observed and photographed with scanning electron microscopy (SEM) (Using Hitachi-S-3700N). Scanning electron microscopy was carried out to study the morphological characteristics of cytarabine PLA microspheres. The samples for the SEM analysis were prepared by sprinkling the microspheres on one side of adhesive stub. Then the microspheres were coated with gold (100A°) before microscopy. Finally the morphology of the microspheres was observed with the scanning electron microscopy [7].

Particle Size Analysis: Determination of average particle size of cytarabine microspheres was very important character. It was carried out by using Malvern instruments, Startech Labs Pvt. Ltd.

***In-vitro* Drug Release:** An in vitro release method using a regenerated cellulose membrane dialysis apparatus (Float-a-Lyzer) was suitable for studying in vitro release of cytarabine-loaded biodegradable microspheres. Microspheres suspension containing known amount of drug was placed in Float-a-Lyzer. The Float-a-Lyzer was placed in beaker containing 50ml 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 and sink conditions. The samples analyzed for drug content by UV-Vis Spectrophotometer at 272.7nm. After every one week the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium.

***In-vitro* Drug Release Kinetic Study:** In order to describe the kinetics of the release process of drug in the different formulations, zero order (Qt = Q0+K0t), First order (lnQt = lnQ0+K1t), Higuchi KHt1/2) and Korsmeyer- Peppas (Qt/Q8= Ktn) models were fitted to the dissolution data of all formulations using linear regression analysis. A value of n=0.5indicates case-I (Fickian) diffusion or square root of time kinetics, 0.5<n<1anomalous (non-Fickian) diffusion, n=1 Case-II transport and n>1 Super Case-II transport [8].

Stability studies: To assess the physical and chemical stability of the microspheres, stability studies were conducted for 2 months under various storage conditions mentioned in ICH guidelines. The sample containing optimized formulation were placed in vials and stored at 40±2°C/75±5% RH. After 60 days the formulations were checked for physical appearance and drug content.

TABLE 2: THE FORMULATION COMPOSITION AND RATIOS OF BSA MICROSPHERES

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
BSA, mg	100	100	100	100	100	100	100	100
Volume of water,ml	2	2	4	6	2	2	2	2
PLGA, mg	500	1000	1000	1000	1000	1000	1000	1000
Conc. of polymer, (%w/w)	4.76	9.09	9.09	9.09	9.09	6.25	9.09	9.09
Mol. Wt. of polymer, KD	50	50	50	50	50	50	50	30
Vol of dcm consumed	10	10	10	10	10	15	10	10
Drug: Polymer	1:5	1:10	1:10	1:10	1:10	1:10	1:10	1:10
Water: Organic	1:5	1:2.5	1:2.5	1:1.6	1:5	1:7.5	1:5	1:5
Vol. of 0.5% PVA consumed, ml	50	100	100	100	100	100	150	100

RESULTS AND DISCUSSION:

Standard calibration curve of BSA in UV Spectro photometer: The UV absorbance's of BSA standard solution in the range of 10-50 µg/ml of drug in buffer, pH 7.4 showed linearity at λ max 279nm. The linearity was plotted for absorbance against concentration with R2 value 0.9991 and with the slope equation y=0.0006x-0.0033. The absorbance values and standard curve shown in **Figure 1**.

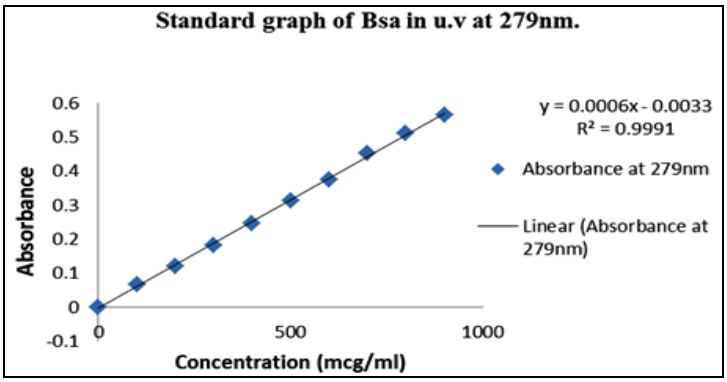

FIGURE 1: STANDARD GRAPH OF BSA IN PHOSPHATE BUFFER OF pH 7.4

Preformulation Studies:

Compatibility Studies: The compatibility between the drug and the selected polymer and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-polymer mixture, which confirmed the absence of any chemical interaction between the drug, polymer and other excipients. The results are shown in **Table 3** and **Figure 2, 3, 4**.

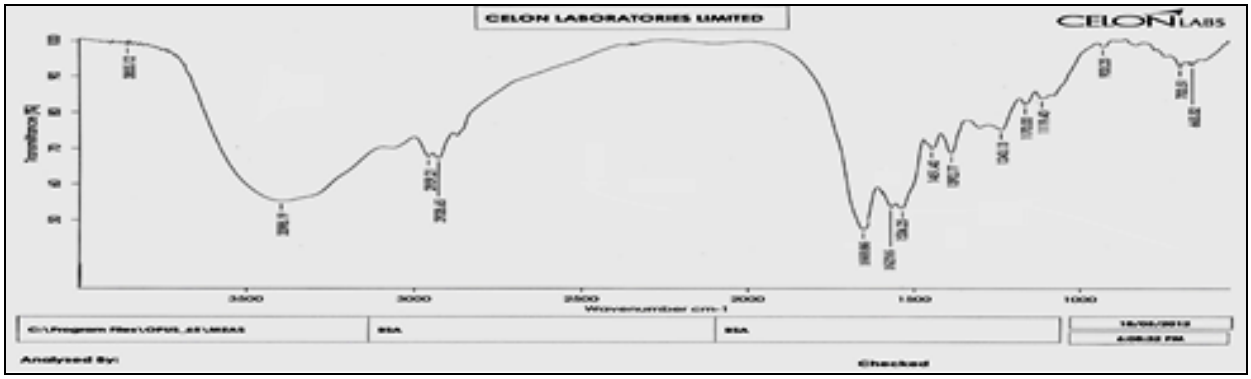

FIGURE 2: FTIR OF BSA

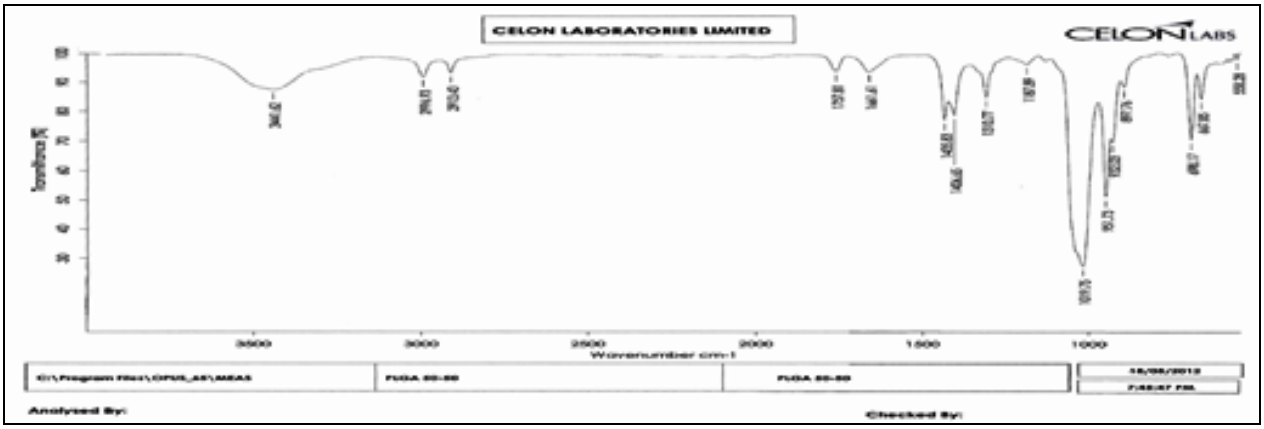

FIGURE 3: FTIR OF PLGA

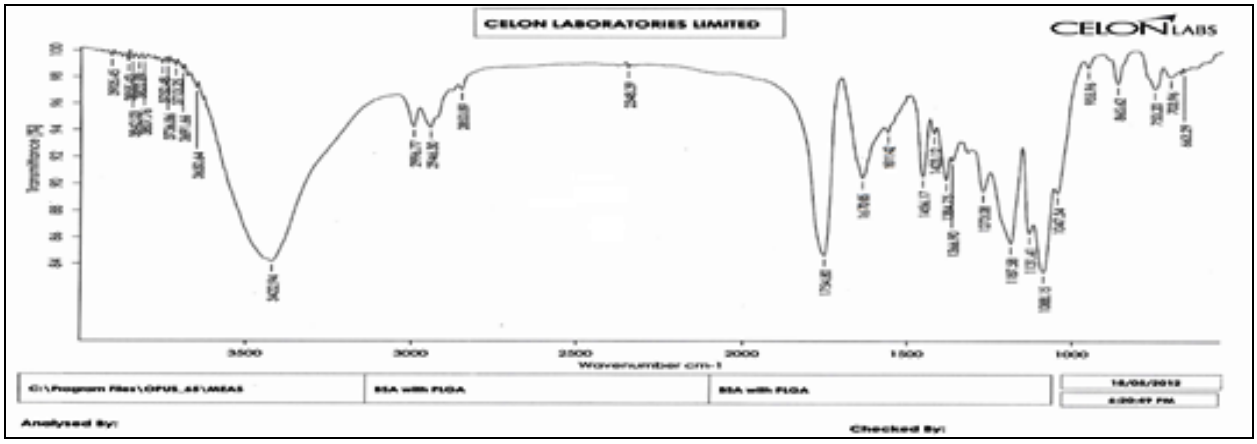

FIGURE 4: FTIR OF BSA WITH PLGA FORMULATION

TABLE 3: INTERPRETATION OF FTIR SPECTRUMS OF BSA, PLGA, BSA WITH PLGA.

Functional groups	Assement Peak of pure drug (cm^{-1})	Assement Peak of Microspheres (cm^{-1})	Range of groups (cm^{-1})
C-H(alkyl) Stretching	2928.65	2853.89	2850-3000
C=O(aromatic ketone) Stretching	1669.86	1670.65	1665-1685
COOH (unsaturated carboxylic acid)	1629.6	1611.42	1600-1690
O-H(Carboxylic acid)	3398. 28	3422.96	3200-3600

Formulation optimization: The Microspheres were prepared by double emulsion technique using homogenizer (IKA). Formulations was optimized for *in vitro* release profile , particle size and entrapment efficiency. The drug polymer ratio was 1:10 for optimized formulation, PVA concentration was 0.5%, aqueous phase volume was 2ml and DCM volume was 10ml. The formulation containing BSA kept at constant strength was prepared with different ratios of DCM, poly l-lactic acid, PVA, water and all other parameters like temperature and rpm were optimized.

Evaluation of Microspheres:

Percentage yield and Entrapment efficiency of BSA microspheres: The percentage yield and encapsulation efficiency were determined for all the formulations from F1to F8 it was in the ranges from, percentage yield (58.3% - 69.9%) and encapsulation efficiency (75.4%-80.1%).

Among those compositions, 3 Formulations are selected as optimized batches for further evaluation based on in vitro dissolution profile and entrapment efficiency.

TABLE 4: PERCENTAGE YIELD AND ENTRAPMENT EFFICIENCY OF BSA MICROSPHERES

S. no.	Batches	Percentage yield (%)	Entrapment efficiency (%)
1	F1	58.3	75.4
2	F2	68.5	78.3
3	F3	69.4	82.7
4	F4	64.7	80.8
5	F5	68.8	87.4
6	F6	69.8	85.6
7	F7	66.7	86.9
8	F8	69.9	80.1

Scanning Electron Microscopy: SEM micrographs and typical surface morphology of the microspheres are given in **Fig. 5-6** for F5, F6 formulations. It was observed that microspheres were spherical with smooth surface.

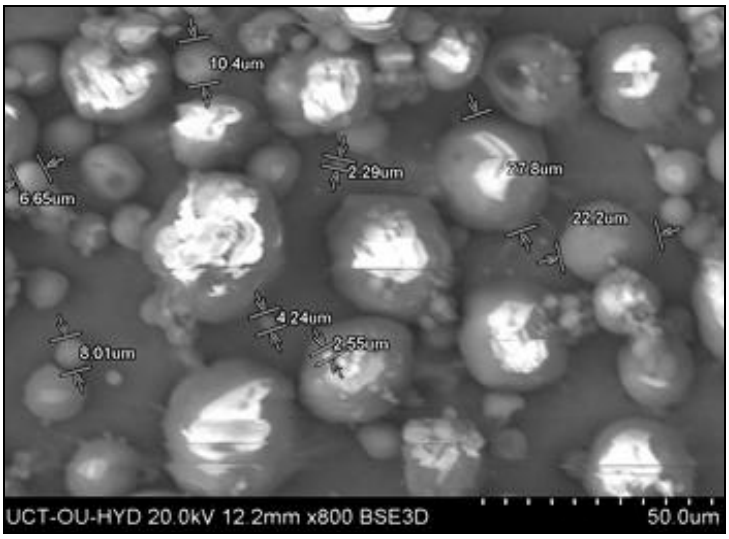

FIGURE 5: SEM PHOTOGRAPHY OF MICROSPHERES FOR F5 FORMULATION

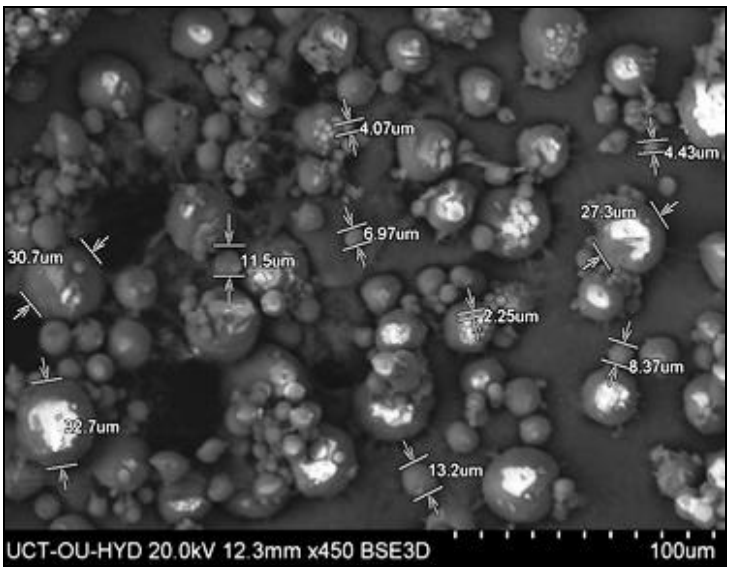

FIGURE 6: SEM PHOTOGRAPHY OF MICROSPHERES FOR F6 FORMULATION

Particle Size Distribution: The particle size distribution was analyzed for F5, F6, F7 formulations of BSA microspheres by wet method. The particle size was optimum in F5 Formulation, When compared to F6 and F7, The results were shown in **Table 5 and Figure 7, 8, 9**.

TABLE 5: PARTICLE SIZE DISTRIBUTION OF BSA MICROSPHERES

S. no	Batches	Mean Particle size (µm)
1	F5	73.752
2	F6	105.786
3	F7	45.437

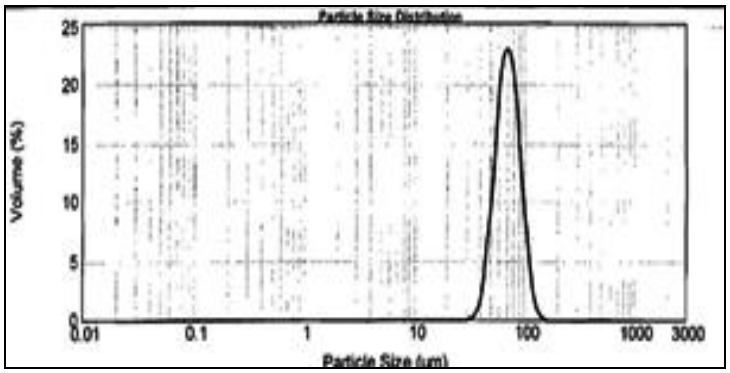

FIGURE 7: PARTICLE SIZE DISTRIBUTION OF BSA MICROSPHERES FOR F5 FORMULATION

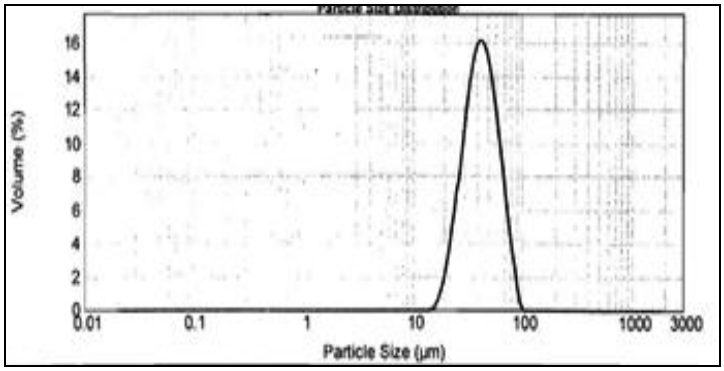

FIGURE 8: PARTICLE SIZE DISTRIBUTION OF BSA MICROSPHERES FOR F6 FORMULATION

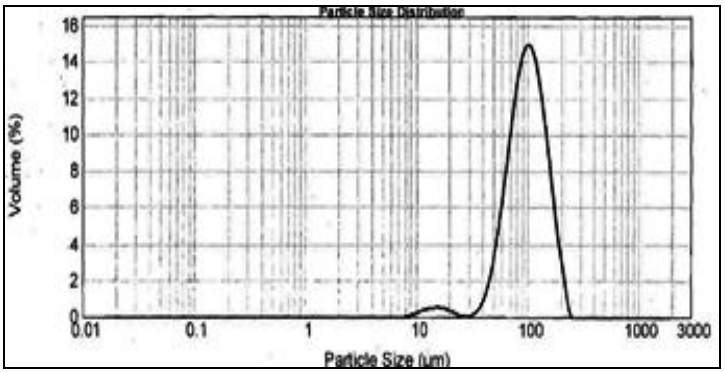

FIGURE 9: PARTICLE SIZE DISTRIBUTION OF BSA MICROSPHERES FOR F7 FORMULATION

***In-vitro* cumulative % Drug Release Profile:** The *in vitro* dissolution profile of prepared formulations was determined by modified dissolution apparatus method. The dissolution was carried out for a period of 30 days in 7.4 pH phosphate buffer. The cumulative percent release of F1-F8 formulations at various time intervals was calculated and tabulated in **Table 6** for F5 formulation 86.8% drug release was achieved on 30th day.

TABLE 6: *IN VITRO* CUMULATIVE % DRUG RELEASE DATA OF BSA MICROSPHERES

Sl. no	Days	% Cumulative Drug Release							
		F1	F2	F3	F4	F5	F6	F7	F8
1	0	0	0	0	0	0	0	0	0
2	1	41.6	14.6	10.88	12.73	14.7	15.4	14.9	45.6
3	5	52.3	19.5	16.87	15.28	21.23	20.7	21.3	55.8
4	10	65.6	29.6	27.72	29.16	33.39	34.6	35.7	63.9
5	15	72.4	37.6	35.68	41.99	44.29	43.9	44.8	68.8
6	20	74.6	57.8	56.03	64.65	55.69	57.4	53.8	73.4
7	25	74.6	78.3	78.93	75.8	81.27	82.5	85.8	73.4
8	30	74.6	78.3	82.4	76.5	86.8	84.5	85.8	73.4

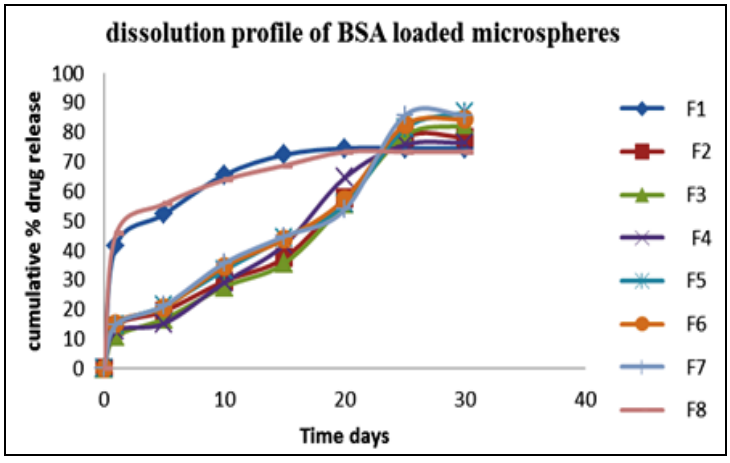

FIGURE 10 : COMPARISON OF CUMULATIVE % DRUG RELEASE OF ALL FORMULATIONS

Kinetic Profiles: The release kinetics of F5, F6, F7 formulations was studied. All formulations follow Zero order release kinetics and follow non- fickian diffusion when it applied to the Korsmeyer-Peppa's Model for mechanism of drug release. F5 formulation has better kinetic results when compared to F6 and F7 formulations. The results are shown in **Figure 11-14**.

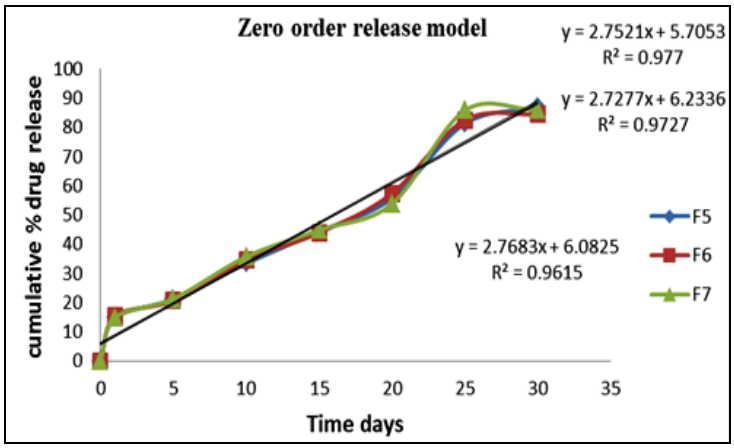

FIGURE 11: COMPARISION OF ZERO ORDER RELEASE STUDIES FOR OPTIMIZED FORMULATIONS F5, F6, F7.

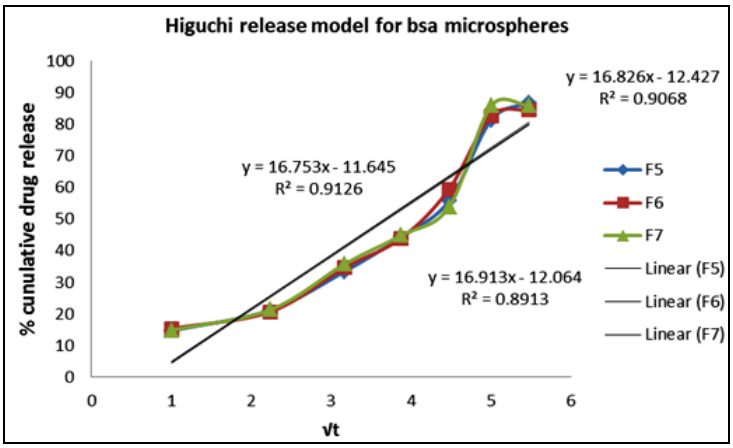

FIGURE 13: COMPARISION OF HIGUCHI'S MODEL RELEASE STUDIES FOR OPTIMIZED FORMULATIONS F5, F6, F7.

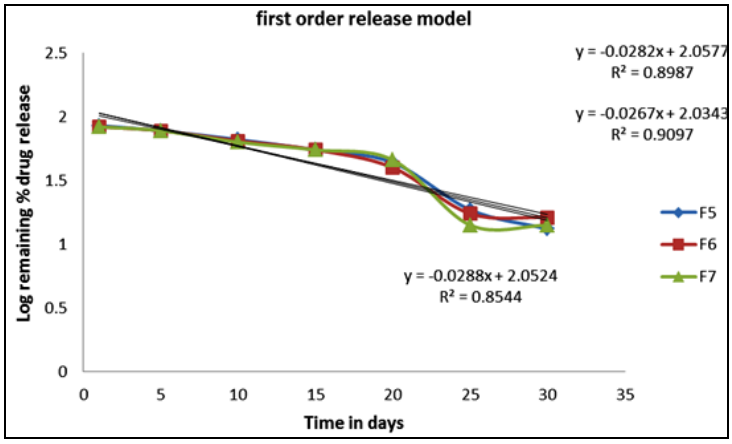

FIGURE 12: COMPARISION OF FIRST ORDER RELEASE STUDIES FOR OPTIMIZED FORMULATIONS F5, F6, F7

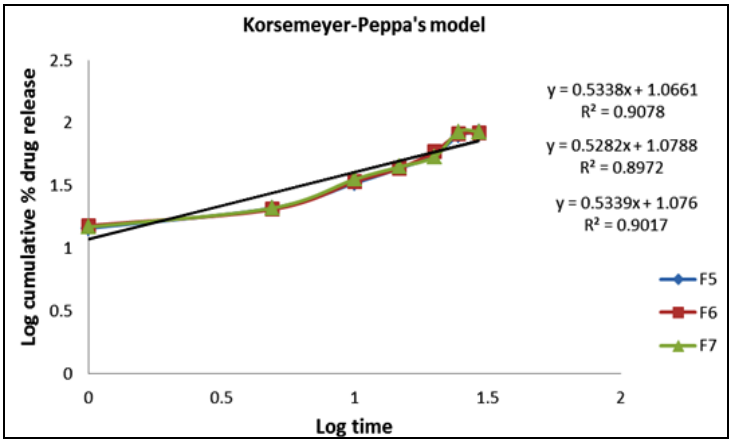

FIGURE 14: COMPARISON OF KORSEMEYER- PEPPAS MODEL RELEASE STUDIES FOR OPTIMIZED FORMULATIONS F5, F6, F7

TABLE 7: REGRESSION COEFFICIENT (R^2) VALUES OF DIFFERENT KINETIC MODELS AND DIFFUSION EXPONENT (n) OF PEPPAS MODEL FOR BSA MICROSPHERES

S. no.	Type of Formulation	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Korsmeyer-Peppas	
					(R^2)	n
1	F5	0.977	0.8987	0.9068	0.9078	0.5338
2	F6	0.972	0.9097	0.9126	0.8972	0.5282
3	F7	0.961	0.8544	0.8913	0.9017	0.5339

Stability test: The stability of the BSA microspheres was evaluated after storage at 40°C/75%%RH and room temperature for 60 days. The assays of the samples were determined as a function of the storage time. The microspheres stored at 40°C were found to be stable for duration of 60 daysdays (**table 8**).

TABLE 8: STABILITY TEST DATA FOR OPTIMIZED FORMULATIONS F5, F6, F7

Formulation code	Characters	Effect of stability on Assay at 40oc				
		0 days	15 days	30 days	40 days	60 days
F5	Description (color)	White to off-white	White to off-white	White to off-white	White to off-white	White to off-white
	Assay (%)	87.4±0.65	87.6±0.47	87.5±0.72	87.9±1.0	87.1±0.65
F6	Description (color)	White to off-white	White to off-white	White to off-white	White to off-white	White to off-white
	Assay (%)	85.6±0.96	85.9±0.92	85.7±0.62	85.4±0.58	85.3±0.53
F7	Description (color)	White to off-white	White to off-white	White to off-white	White to off-white	White to off-white
	Assay (%)	86.9±0.9	86.5±0.65	86.8±0.69	86.1±0.86	86.4±0.74

Differential Scanning Calorimetry (DSC): In order to confirm the physical state of BSA in microspheres, DSC of BSA, PLGA, physical mixture of BSA and polymer, BSA microspheres formulations were carried out and were shown in graph .The DSC trace of BSA showed a sharp endothermic peak 68.6^{o}C. the physical mixture of BSA and polymer showed same thermal behavior 69.1^{o}C, as the individual component, indicating that there was no interaction between drug and polymer in solid state.

The reported melting point range of BSA is between 68^{o}C-70^{o}C, thus indicating there is no change of BSA in pure state, physical mixture of drug and polymer. . The absence of endothermic peak of the BSA at 68.6 ^{o}C in the DSC of the BSA microspheres suggests that the BSA existed in an amorphous or disordered crystalline phase as a molecular dispersion in polymeric matrix[10].

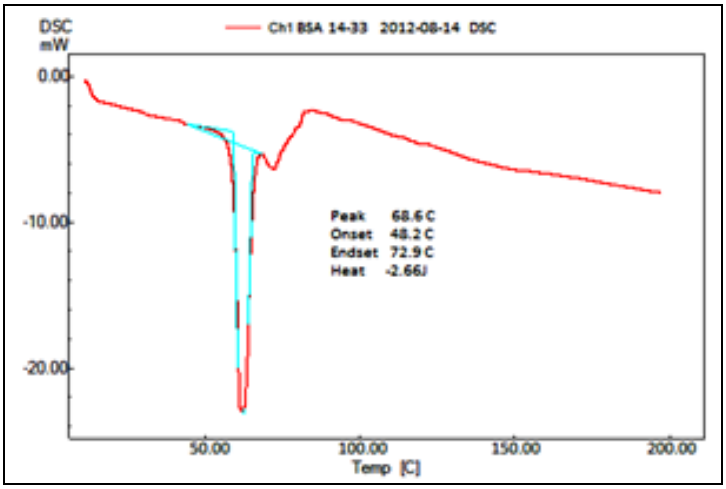

FIGURE 15: DSC PEAK OF BSA.

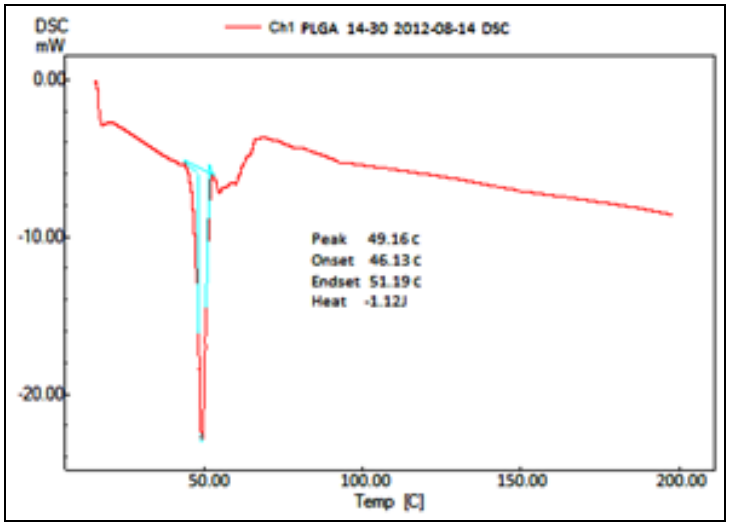

FIGURE 16: DSC PEAK OF POLYMER PLGA 50:50

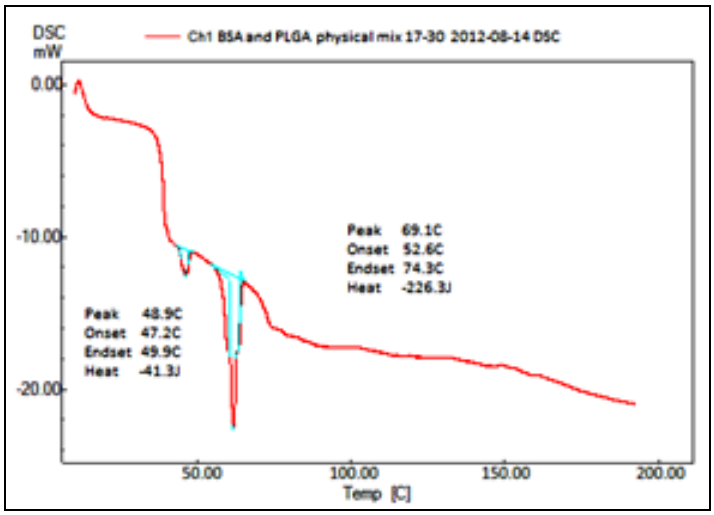

FIGURE 17: DSC PEAK OF PHYSICAL MIXTURE

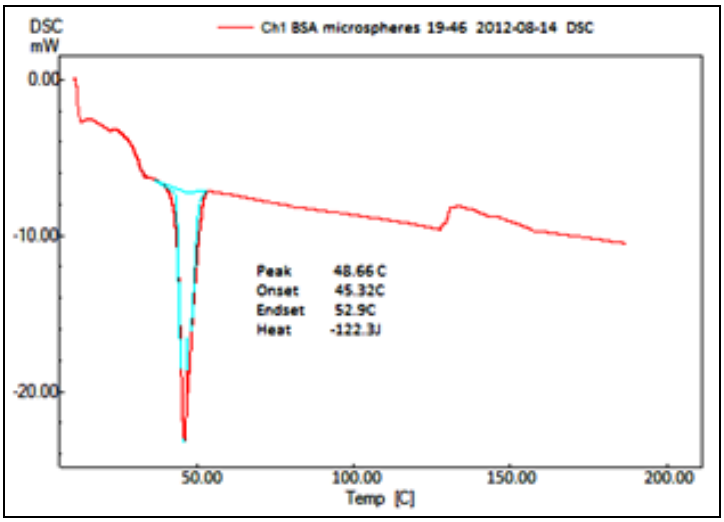

FIGURE 18: DSC PEAK OF FORMULATION

CONCLUSION: The conclusions drawn from the present investigation are given below:

- Preformulation studies like solubility, melting point, and uv analysis of BSA complied with IP standards.
- The FTIR spectra revealed that, there was no interaction between polymer and BSA , All the excipients used were compatible with the BSA.
- Surface smoothness of the BSA microspheres was increased by increasing the polymer concentration, which was confirmed by SEM.
- As the drug to polymer ratio was increased, the mean particle size of BSA microspheres was also increased. The BSA microspheres with normal frequency distribution were obtained.

- Entrapment efficiency increase with increase in the polymer concentration From the results it can be concluded that as the drug to polymer ratio decrease, initial burst release will increases.
- 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 less than 0.45 for the BSA microspheres indicating Fickian diffusion of drug from microspheres.
- Accelerated stability studies indicating that BSA microspheres are stable on storage.
- The DSC data indicates that there is no inter action between drug and polymer and it also indicates that BSA still present in lattice structure in physical mixture where as it was completely amorphous in microspheres.
- From the study, it is evident that promising sustained release microspheres of BSA may be developed by double emulsion solvent evaporation method by using polymers like PLGA.

ACKNOWLEDGEMENTS: The authors acknowledge Celon Laboratories, Hyderabad, India, for gift sample of BSA, PLGA. The authors also thank Aravind. G, Manager (FRD), Celon Labs, Mahesh Kumar, Summit Shah, Senior Research Associate (FRD), Celon Labs, Hyderabad.

REFERENCES:

1. Chein YM, editor, Novel drug delivery systems, 2nd edition, New York; Marcel, Dekker Inc, 1992; 50: 1-2.
2. Joseph R. Robinson, Vincent H. L. Lee, *Controlled Drug Delivery fundamental & applications*, Second Edition, Revised and Expanded, Marcel Dekker, INC. New York, 581-590.
3. Singh, U.V., Bisht, K.S., Rao, S., Uma Devi, P., Udupa, N., Reduced toxicity andenhanced antitumor efficacy of plumbagin using poly (lactic-co-glycolic) biodegradable injectable implant. Indian Journal of Pharmacology 1997, 29, 168-172.
4. Lawrence K. Fung, W. Mark Saltzman, Polymeric implants for cancer chemotherapy, Advanced Drug Delivery Reviews 26 (1997) 209–230.
5. Yodthong Baimark, Mangkorn Srisa-Ard And Prasong Srihanam, Preparation of Bovine Serum Albumin Hollow Microparticles by the Water-in-Oil Emulsion Solvent Diffusion
6. Nighute AB and Bhise SB "preparation and evaluation of rifabutin loaded polymeric microspheres " *Research J. Pharm. and Tech.* 2009; 2(2): 371-374
7. Freiberg, XX. Zhu: polymer microspheres for controlled drug release *International Journal of Pharmaceutics* 2004; 282; 1-18.
8. Siepmann, J.; Kranz, H.; Bodmeier, R.; Peppas, N.A. HPMC-matrices for controlled drug delivery: a new model combining diffusion, swelling, and dissolution mechanisms and predicting the release kinetics. Pharm. Res. 16, 1748–1756, (1999).
9. AngelaHG, Patrick DP. Characterization of biodegradable PLGA polymers and microspheres .J. Pharm and Biomed Anal 1995; 13:747-60.

How to cite this article:
Voruganti S, Padman JSC: Formulation and Evaluation of BSA Loaded PLGA Microparticles. *Int J Pharm Sci Res*. 2013; 4(3); 1013-1021.