INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

FLOATING MICROSPHERES OF CEFPODOXIME PROXETIL: FORMULATION AND OPTIMIZATION BY FACTORIAL DESIGN

AV. Pande, UA. Nimbalkar, MV. Dhoka* and PA. Sonawane

AISSMS College of Pharmacy, Kennedy Road, Near RTO, Pune, Maharashtra, India.

*Corresponding Author: madhura1777@yahoo.com

ABSTRACT

The objective of this study was to develop and evaluate floating microspheres of Cefpodoxime Proxetil for prolongation of gastric residence time. The microspheres were prepared by solvent evaporation (o/w emulsion) method using Eudragit S100. A 3^2 full factorial design has been applied. The variables conc. of ES100 and stirring speed was studied at three levels and arranged in 3^2 factorial design to study their influence on percentage drug release, percentage entrapment efficiency, and particle size. The physical characteristics of floating microspheres were evaluated using FTIR, DSC, and scanning electron microscopy.

Keywords: Floating microspheres, Solvent diffusion-evaporation method, Factorial design.

INTRODUCTION

Oral floating drug delivery system is one of the various approaches to improve residence time of drug in GIT[1,2]. Single-unit formulations are associated with non consistent drug release profile, absorption and dose dumping. Therefore multiple unit floating formulations are prepared. Their drug release profile and absorption is more predictable way and the risk of dose dumping is reduced[3].

Cefpodoxime Proxetil (CP) is 3rd generation broad spectrum β-lactam cephalosporin class of antibiotic administered orally having absorption in upper GIT (stomach and duodenum).It has the better solubility in acidic pH. Among the various reasons for its low bioavailability, poor solubility and premature conversion of Cefpodoxime Proxetil to Cefpodoxime by intestinal esterase enzyme are important. Floating dosage form of CP will offer better bioavailability as drug will remain in absorption window for long duration and it will also inhibit premature conversion of Cefpodoxime Proxetil to Cefpodoxime by intestinal esterase enzyme. Hence CP has all the ideal characteristics required for gastroretentive drug delivery system[4 5].

In the present work, floating multiparticulate drug delivery system is prepared and evaluated. The formulation is optimized by using factorial design with the help of design expert software.

MATERIALS AND METHODS

Cefpodoxime Proxetil (CP) was obtained as a gift sample from Maxim Pharmaceutical Ltd. (Pune, India). Eudragit S100 (ES100) was supplied by Degussa Pvt. Ltd. (Goa, India); all solvents used were of analytical grade and were used as obtained.

Preparation of floating microspheres

Floating microspheres with a central hollow cavity were prepared by using a solvent diffusion-evaporation technique.[5]

Weighed quantities of CP, ES100were dissolved in mixture of Ethanol and Dichloromethane and Isopropyl alcohol (1:1:1 ratio) using magnetic stirrer and sonicated for

10 min. This solution was poured into 100ml distilled water containing 0.05%w / v polyvinyl alcohol maintained at room temperature. The resultant emulsion was stirred with a propeller stirrer for 3hrs to allow the volatile solvent to evaporate. The microspheres formed were filtered, washed with water and dried overnight at room temperature. Formulation composition of A_1-A_9 batches are shown in Table I.

Experimental design and Statistical analysis

From the preliminary trials in the present study a 3^2 full factorial design was employed to study the effect of independent variables, i.e. stirring speed (X_1) and conc of ES100 (X_2) on dependent variables like % drug release (Y_1), Entrapment efficiency (Y_2) and Partical size (Y_3) (Table II). The fitted equations (full models) relating the responses i.e. Y_1, Y_2, Y_3 to the transformed factor were shown Table III, IV, V. The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative[6].

Validation of optimized model

Four optimum formulations were selected by feasibility grid search, performed over the entire experimental domain, to validate the chosen experimental design and polynomial equations[7]. The criterion for selection of optimum was primarily based on the highest possible values of drug entrapment efficiency (%), release of drug after 12 hrs, and smaller size of particle size for the floating microspheres formulations. The resultant experimental data of response properties were subsequently compared with predicted values (Table VI).

Evaluation of microspheres

Prepared microspheres were evaluated for particle size, drug content, % yield, floating ability, entrapment efficiency, surface morphology, FTIR studies, DSC studies and in vitro drug release study.

The size was measured using an optical microscope with the help of a calibrated ocular micrometer[7].

The drug content was determined spectrophotometrically at 263 nm using methanol as solvent.

The percentage drug entrapment efficiency of microspheres was calculated as follows[8]

% Entrapment efficiency = (Calculated drug concentration / Theoretical drug concentration) x 100.

The yield was calculated by dividing the measured weight of microspheres by the total weight of all non-volatile components[8].

Floating behavior of hollow microspheres was studied in a USP dissolution test apparatus (Type II) by spreading the microspheres (100mg) on a 0.1mol L^{-1} HCl containing 0.02% Tween 80 as a surfactant and stirred with 100 rpm for 12 hrs. Buoyancy percentage was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microspheres[8] (Table VII).

The external and internal morphology of the microspheres were studied by scanning electron microscopy (SEM).

An IR spectrum of combination of pure drug (CP) and physical mixture with excipient (eudragit S100) was studied.

The DSC thermogram of microsphere was recorded using Differential scanning calorimeter (DSC 823 Mettler Toledo, Japan).

In Vitro Drug Release Study was carried out by using USP II (Paddle type) apparatus, 900ml of 0.1N HCl as medium with 100 rpm rotation speed maintained at 37°C. Sampling was done for every one hour till 12 hrs and analyzed at 263 nm by UV Spectrophotometer after suitable dilutions[9]. Cumulative percentage drug release was calculated using PCP Disso v2.08 Software (Poona College of Pharmacy, Pune) [10].

RESULTS AND DISCUSSION

Experimental design and Statistical analysis

Factorial design enables all factors to be varied simultaneously, allowing quantification of the effects caused by independent variables and interactions between them. In this study, a 3^2 full factorial experimental design was used to optimize the formulation of microspheres. Initial studies were undertaken to decide the

excipients and their levels in the experimental design.

Data Analysis

Various computations for the current optimization study using Response Surface Methodology (RSM) were carried out, employing the Design Expert Software (Version 7.1.4, Stat-Ease Inc., Minneapolis, MN). Statistical second-order model including interaction and polynomial terms were generated for all the response variables using multiple linear regression analysis (MLRA). The general form of the model is represented as in equation.

$$Y = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_1X_2+\beta_4X_1^2+\beta_5X_2^2$$

Where β_0, the intercept, is arithmetic average of all quantities outcomes of 9 runs, β_1 to β_8 are the coefficient computed from the observed experimental values of Y, and X_1 and X_2 are the coded levels of the independent variable(s).

Regression analysis

The coefficients of the polynomial equations were generated using multiple linear regression analysis (MLRA) for % drug release, % entrapment efficiency, particle size. The coefficients (β_0 to β_3) were calculated with β_0 as the intercept.

All the data of Summary output of regression analysis for effect of X1 & X2 on Y_1, Y_2 and Y_3 respectively are enlisted in Table III, IV, V.

Concerning Y_1, the equation of multiple linear regression analysis is as per below:

$$Y_1 = 90.99+1.97*X_1-5.76*X_2+0.43*X_1*X_2-0.013*X_1*X_1+0.8*X_2*X_2$$

The Y_1 for all batches A_1- A_9 shows good correlation co-efficient of 0.9899. From table VIII, Variable X1 has p value 0.0117 and variable X2 has p value0.0005 ($p<0.05$). As both variables have p value less than 0.05, both significantly affect the release profile. The equation suggests that factor X_1 has positive effect on % drug release. As stirring speed increases % drug release also increases. X_2 had negative effect on the % drug release that leads to decrement in the % drug release as concentration of ES100 increases. The 3-D plots shows that as the concentration of ES100 increase the % drug release from the microsphere get decreased significantly and as the stirring speed increases the % drug release get increased from the microsphere.(Figure I)

Concerning Y_2, the equation of multiple linear regression analysis is as per below:

$$Y_2 = 77.55-3.89*X_1+7.21*X_2-1.97*X_1*X_2-0.73*X_1*X_1+2.22*X_2*X_2$$

The Y2 for all batches A_1- A_9 shows good correlation co-efficient of 0.9984. From table VIII, Variable X1 has p value 0.0003 and variable X2 has p value0.0001 ($p<0.05$). Hence both significantly affect the release profile.

The equation suggests that factor X_1 has negative effect on % entrapment efficiency. As level of X_1 decreases, % entrapment efficiency also decreases. X_2 had positive effect on the % entrapment efficiency that leads to increment in the % entrapment efficiency as levels of X_2 increases. The 3-D plots shows that as the concentration of ES100 increase the % entrapment efficiency increases significantly and as the stirring speed increases the % entrapment efficiency decreases. (Figure II)

Concerning Y_3, the equation of multiple linear regression analysis is as per below:

$$Y_3 = 186.67- 44.17*X_1+25.83*X_2-11.25*X_1*X_2 - 22.5*X_1*X_1-2.5*X_2*X_2$$

The Y_3 for all batches A_1- A_9 shows good correlation co-efficient of 0.9936. From table VIII, Variable X1 has p value 0.0017 and variable X2 has p value0.0004 ($p<0.05$). thereby significantly affecting the release profile. The equation suggests that factor X_1 has negaitive effect on particle size. As level of X_1 increases particle size decreases. X_2 had positive effect on the particle size that leads to increment in the particle size as levels of X_2 increases. The 3-D plots shows that as the concentration of ES100 increase the Particle Size get increased significantly and as the stirring speed increases the Particle Size get decreased. (Figure III)

Validation of Optimum Floating Microspheres Formulations

For all 4 checkpoint formulations, the results were found to be within limits. Table VI lists the checkpoints, the predicted and experimental values of all the response variables, and the percentage error in prognosis. Linear correlation plots between the observed and predicted values of drug entrapment efficiency, % drug rel_{12hrs}, and particle size, demonstrated higher values of R^2, indicating excellent fitting of model. Upon comparison of the observed responses with that of the anticipated responses, the prediction error varied between -1.04 and 2.55%. Thus, the low magnitudes of error as well as the significant values of R^2 in the current study indicate a high prognostic ability of floating microspheres formulations of CP.

Evaluation of Microspheres

The mean particle size of the microspheres was found to increase with increasing ES100 concentration and was in the range 150±3.3 µm to 285 ±9.8 µm . Also the sizes of the resulting microspheres were decreased with increasing speed of stirring. The drug entrapment efficiency (EE) of microspheres varied from 69.96 to 92.14% (Table VII). A result demonstrated that higher drug solubility in the solvent system and increased concentration of ES100 increases the entrapment of the drug. Stirring speed had a negative effect on entrapment efficiency. The percentage yield of microspheres varied from 68.14 to 87.1% (Table VII). The result indicates that higher the speed and polymer conc, better is the % yield. The purpose of preparing floating microspheres was to extend the gastric residence time of a drug. The microspheres containing ES100 showed good floating ability range from74.94 to 90.73 (Table VIII) due to insolubility of ES100 polymers in the gastric fluid (pH 1.2). The results also showed a tendency that the larger the particle size, the longer floating time. The SEM view of the microspheres showed a hollow spherical structure with a smooth surface morphology and exhibited a range of sizes within each batch. The shell of the microspheres also showed some porous structure which indicates leaching of the drug during the dissolution without gelation of the polymeric surface. (Figure IV). The spectral observations indicated that the principal IR absorption peaks observed in the spectra of CP were close to those in the spectra of the CP microspheres and physical mixture. IR spectrums of the microspheres indicate that there is no strong interaction between the drug and the polymers (Figure V). DSC has been one of the most widely used calorimetric techniques to characterize the physical state of drug in the polymeric matrix. The DSC thermogram of CP exhibited a single sharp endothermic peak at 89° corresponding to its melting transition temperature. The thermograms of the ES100 based microsphere showed no such characteristic peak, indicating that the drug was uniformly dispersed at the molecular level in microspheres (Figure VI)

In vitro dissolution studies (Figure VII) indicates that formulation F_1-F_3 showed 96.07 to 99.19% $rel_{12hrs.}$these formulation releases 90% drug i.e T_{90} within $10_{hrs.}$ The behavior of these formulations may be due to fewer polymers available to retard the drug release, also showing less entrapment efficiency as compared to the other formulations. The ES100 concentration was increased to achieve further retardation of drug release. For Formulations F_4-F_9, the drug release was 83.43 to 93.29% drug release in 12 hrs. It was observed that as the concentration of ES100 was increased the % cumulative drug release decreased. Kinetic treatment of the dissolution data indicate that Hixon Crowel was best fit model for formulations A_1-A_3 which indicate that the release rate is limited by the drug particles dissolution rate and not by the diffusion. For formulation A_4-$A_{9,}$ Marix was

the best fit model which indicates that drug release via a diffusion mechanism.

CONCLUSION

From the results it can be concluded that Eudragit S100 can be used to formulate an efficient floating microsphere for CP with good percentage floating ability and practical yield. Floating microspheres also showed porous nature, as revealed by the scanning electron microscopic studies which is helpful for getting required dissolution.

The application of 3^2 factorial designs demonstrates a useful tool for optimization of CP microsphere. The results of multiple regression analysis led to a statistical model that described adequately the influence of the selected variables at different levels on the chosen response.

Using the contour plot, data from statistical design one can select suitable composition of formulation to obtain microsphere with appropriate particle size, % drug release, % entrapment efficiency on the application of the system. Thus, the current study attained the successful design, development and optimization of floating microspheres formulation.

Table I: Formulation of microspheres using f^3 factorial design

Ingredients	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9
CP (gm)	1	1	1	1	1	1	1	1	1
ES100 (gm)	0.5	0.5	0.5	1	1	1	1.5	1.5	1.5
Solvent ratio	1:1:1	1:1:1	1:1:1	1:1:1	1:1:1	1:1:1	1:1:1	1:1:1	1:1:1

CP: Cefpodoxime Proxetil, ES100: Eudragit S100,
Solvent ratio: Ethanol: Dichloromethane: Isopropyl Alcohol)

Table II: Effect of Independent variable on dependent variable by 3^2 full factorial design

Formulation	Independent variable		Dependent variable		
	X_1	X_2	Y_1	Y_2	Y_3
A_1	300	0.5	96.07	74.12	210
A_2	500	0.5	97.36	72.12	160.5
A_3	700	0.5	99.19	69.98	150
A_4	300	1	89.43	80.34	260
A_5	500	1	90.23	77.74	185
A_6	700	1	93.29	73.12	160
A_7	300	1.5	83.12	92.12	285
A_8	500	1.5	86.98	87.23	210
A_9	700	1.5	87.96	80.12	180

X_1 – stirring speed (rpm) X_2 - conc of ES100 (gm)
Y_1 = Rel_{12hrs} (%), Y_2 = Entrapment efficiency (%), Y_3 = Partical size (µm)

Table III: Summary output of regression analysis for effect of X1 & X2 on Y_1

Regression statistics for Y_1		
F value	58.87	
Predicted R square	0.9044	
R Square	0.9899	
Adjusted R square	0.9731	
Standard error	0.34%	
Observations	9	
Coefficients:		
Coefficient	Coefficient value	P-value
β_0	90.99	0.0034
β_1	1.97	0.0117
β_2	-5.76	0.0005
β_3	0.43	0.3972
Equation		
$Y_1 = 90.99+1.97*X_1-5.76*X_2+0.43*X_1*X_2-0.013*X_1*X_1+0.8*X_2*X_2$		

Table IV: Summary output of regression analysis for effect of X1 & X2 on Y_2

Regression statistics for Y_2		
F value	378.69	
Predicted R square	0.9816	
R Square	0.9984	
Adjusted R square	0.9958	
Standard error	0.02%	
Observations	9	
Coefficients:		
Coefficient	Coefficient value	P-value
β_0	77.55	0.0002
β_1	-3.89	0.0003
β_2	7.21	0.0001
β_3	-1.97	0.0037
Equation		
$Y_2 = 77.55-3.89*X_1+7.21*X_2-1.97*X_1*X_2-0.73*X_1*X_1+2.22*X_2*X_2$		

Table V: Summary output of regression analysis for effect of X1 & X2 on Y_3

Regression statistics for Y_3		
F Value	93.68	
Predicted R square	0.9241	
R Square	0.9936	
Adjusted R square	0.9830	
Standard error	0.17%	
Observations	9	
Coefficients:		
Coefficient	Coefficient value	P-value
β_0	186.67	0.0017
β_1	-44.17	0.0004
β_2	25.83	0.0019
β_3	-11.25	0.0341
Equation		
$Y_3 = 186.67- 44.17*X_1+25.83*X_2-11.25*X_1*X_2 - 22.5*X_1*X_1-2.5*X_2*X_2$		

Table VI: Comparison of experimental results with predicted responses of ES 100 based floating microsphere formulations

Batch Code	Stirring Speed (rpm)	ES100 Conc. (gm)	Response	Predicted Value	Experimental Value	% Error
S_5	**620**	**1.1**	EE(%)	76.24	75.5	-0.9801
			Rel $_{12h}$(%)	91.09	92.02	1.0106
			Particle size (µm)	171.984	176.5	2.5586
S_6	620	1.2	EE(%)	77.71	77.1	-0.7911
			Rel $_{12h}$(%)	90.09	91.03	1.0326
			Particle size (µm)	175.5	180	2.5
S_7	640	1.1	EE(%)	75.18	74.4	-1.0483
			Rel $_{12h}$(%)	91.50	92.01	0.5542
			Particle size (µm)	169	173.4	2.5374
S_8	640	1.2	EE(%)	76.57	75.7	1.1492
			Rel $_{12h}$(%)	90.51	91.08	0.6258
			Particle size (µm)	172.06	178.2	3.4455

TABLE VII: Evaluation Parameters, % yield and floating ability for ES100 based Floating Microspheres

Batch Code	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9
% Yield	68.14	70.12	74.56	77.45	79.34	80.31	81.00	83.98	87.1
Floating Ability After 12h	84.32	80.63	74.23	87.23	81.51	79.26	90.73	89.34	84.5

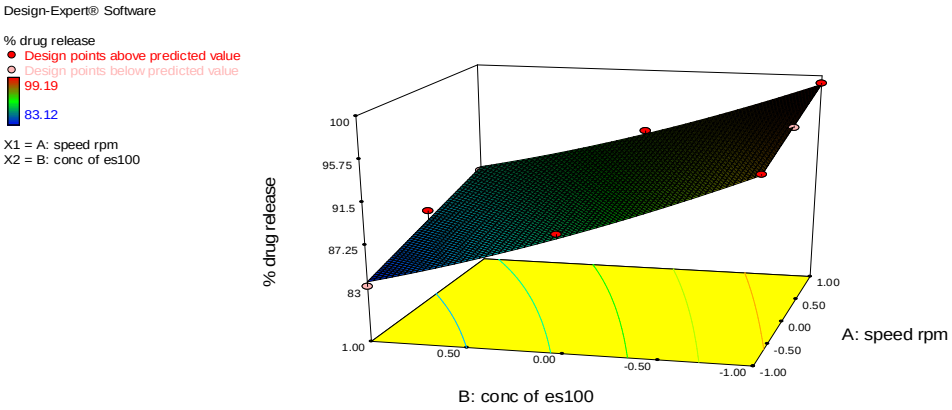

Fig. I: Three dimensional response surface plots for % Drug Release

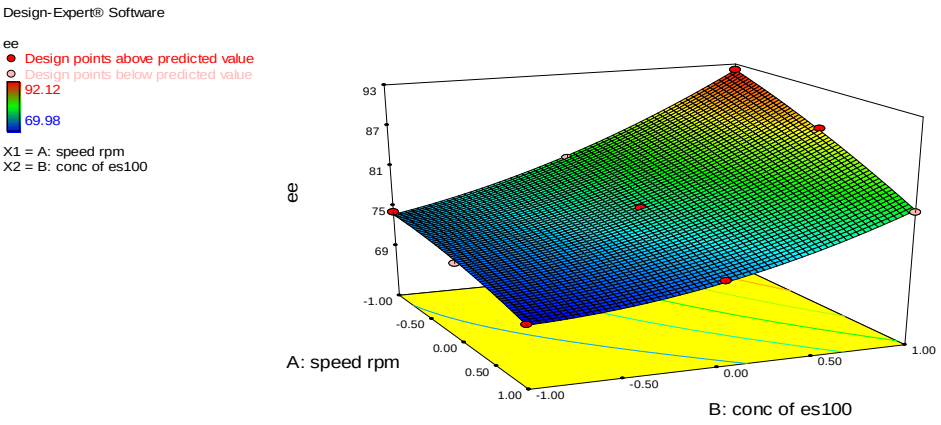

Fig. II: Three dimensional response surface plots for % entrapment efficiency

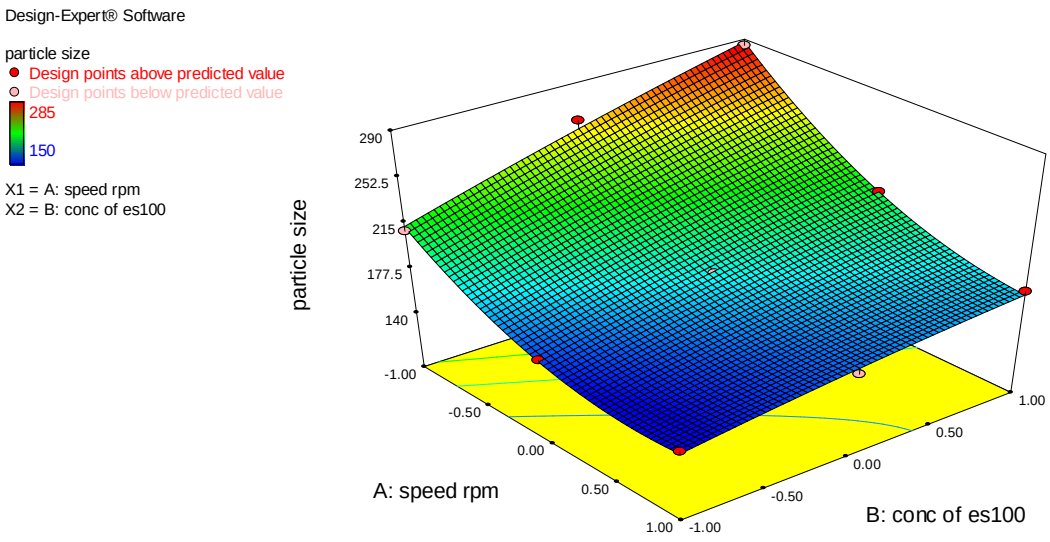

Fig. III: Three dimensional response surface plots for particle size

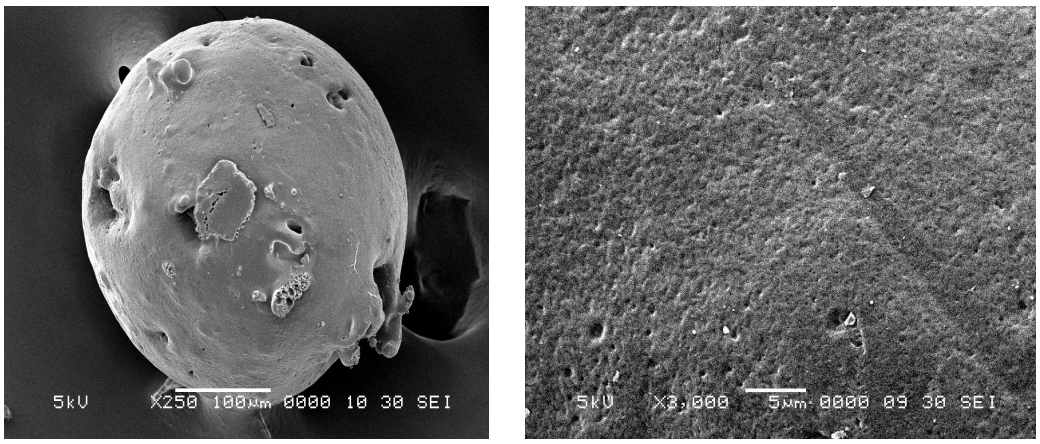

Fig. IV: Scanning electron microphotographs of floating microspheres showing spherical structure and porous nature

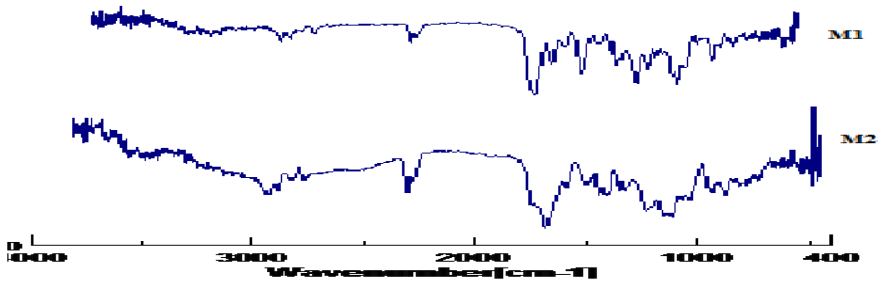

Fig. V: Infra red spectrum of CP (M1) and ES100 based microspheres (M2)

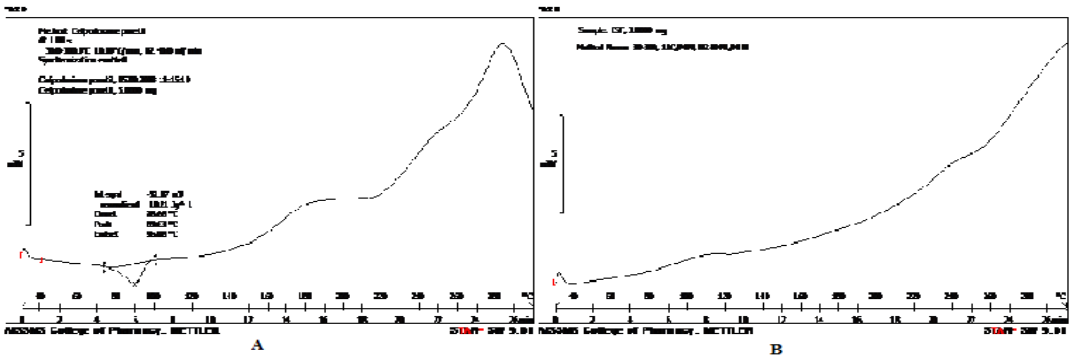

Fig. VI: DSC thermogram of CP (A), ES100 Microsphere (B)

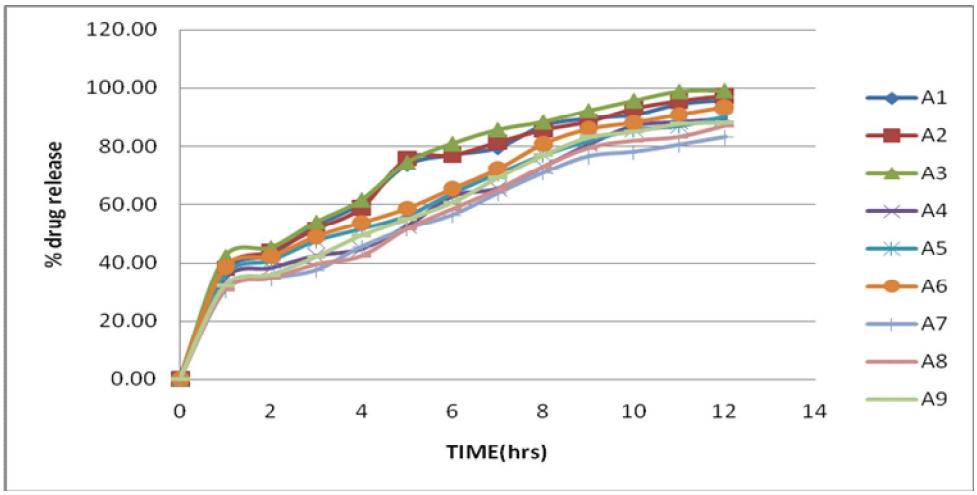

Fig. VII: Drug release profile of CP from formulations A_1 to A_9 (n=3)

REFERENCES

1. Arora S, Ali J, Ahuja A, Khar RK and Baboota S. Floating drug delivery system: A review. AAPS Pharm Sci Tech. 2005;6(47):43.
2. Deshpande AA, Rhodes CT, Shah NH and Malick AW. Controlled release drug delivery system for prolonged gastric residence: A Review. Drug Dev Ind Pharm. 1996;22(6):531-539.
3. Kawashima Y, Niwa T, Takeuchi H, Hino T, Itoh Y. Preparation of multiple unit hollow microspheres (microballoons) with acrylic resin containing tranilast and their drug release characteristics (in vitro) and floating behavior(in vivo). J Controlled Release. 1991; 16:279-290.
4. Kakumanu VK, Arora V and Bansal AK. Investigation on physicochemical and biological differences of Cefpodoxime Proxetil enantiomers. Eur J pharmaceutics and biopharmaceutics. 2006;64:255-259.
5. Frampton JE, Brogden RN, Langtry HD and Buckley MM. Cefpodoxime Proxetil: Review of its antibacterial activity, pharmacokinetic properties and therapeutic potential Drugs. 1992;44(5):889-917.
6. Prajapati BG, Patel BD and Patel RP. SR Release Matrix Tablet of Nicorandil: Formulation and Optimization by Factorial Design. PharmExpt. 2010;1:1-7.
7. Singh B and Ahuja N. Response surface optimization of drug delivery system. In Jain NK, editor. Progress in Controlled and Novel Drug Delivery System. New Delhi, India: CBS Publishers and Distributors. 2004:76-97, 470-509.
8. Srivastava AK, Ridhurkar DN and Wadhwa S. Floating microspheres of cimetidine: formulation, characterization and in vitro evaluation. Acta Pharma. 2005;55:277-285.
9. Soppimath KS, Kulkarni AR and Aminabhavi TM. Development of hollow microspheres as floating controlled release system for cardiovascular drugs: Preparation and release characteristics. Drug Dev Ind Pharm. 2001;27:507.
10. Ketkar A, Patil V and Paradkar A. PCP Disso v2.08 software.