Research Article *ISSN: 2277-8713*
Yashavantsinh Chavda, IJPRBS, 2013; Volume 2(3): 89-106 *IJPRBS*

ISSN: 2277-8713
IJPRBS
A Path for horizing your innovative work
INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

PREPARATION AND EVALUATION OF LAMIVUDINE MICROSPHERES WITH EUDRAGIT® POLYMERS BY SOLVENT EVAPORATION METHOD FOR LYMPHATIC SYSTEM

YASHAVANTSINH CHAVDA[1*], BHAVIN BHIMANI[1], Dr. UPENDRA PATEL[1], Dr. RAGIN SHAH[1], GHANSHYAM PATEL[2], DHIREN DASLANIYA[2]

1. Department of Pharmaceutics, Arihant School of Pharmacy and BRI, Adalaj, Gandhinagar.
2. Department of Pharmacy, JJT University, Jhunjunu, Rajasthan.

IJPRBS-QR CODE *PAPER-QR CODE*

Accepted Date:
30/04/2013
Publish Date:
27/06/2013
Keywords
Anti-ritroviral,
% Drug encapsulation efficiency,
Independent variables,
Dependent variable.

Corresponding Author
Mr. Yashavantsinh Chavda

Abstract

To formulate and evaluate a lamivudine microspheres using a combination of eudragit RS 100 and eudragit RL 100 polymers. Lamivudine is an anti-retroviral drug which is used in the treatment of AIDS disease. The lamivudine microspheres were prepared by the solvent evaporation method using different concentration of the eudragit polymers. The effect of polymer ratio on % drug encapsulation efficiency was investigated using 3^2 full factorial designs. The parameters determined were bulk density, tape density, angle of repose, particle size, drug content, % drug encapsulation efficiency & *in vitro* dissolution. Concentration of eudragit RS 100 (X_1) & RL 100 (X_2) was used as an independent variables and % drug encapsulation efficiency (Y) used as a dependent variable. Particle size of microspheres affected by stirring speed and concentration of the eudragit polymers. As the stirring speed increased, the particle size decreased and as the concentration of eudragit increases, the particle size also increased. Larger microspheres showed greater drug loading and smaller microspheres showed a faster drug release. A surface plots are also presented to graphically represent the effect of independent variables on the % drug encapsulation efficiency. The validity of generated mathematical model was tested by preparing checkpoint formulation. Formulation F3 showing higher drug content and drug release profile as compaired other formulation. So, F3 formulation was taken as the optimized formulation.

INTRODUCTION

Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers having a particle size ranging from 1-1000 µm. Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 µm to 1000 µm). Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres and ceramic microspheres are commercially available. Solid and hollow microspheres vary widely in density and, therefore, are used for different applications. Hollow microspheres are typically used as additives to lower the density of a material. Solid microspheres have numerous applications depending on what material they are constructed of and what size they are. Glass microspheres are primarily used as filler for weight reduction, retro-reflector for highway safety, additive for cosmetics and adhesives, with limited applications in medical technology. Ceramic microspheres are used primarily as grinding media. Microspheres vary widely in quality, sphericity, uniformity of particle and particle size distribution.

Eudragit RL 100 and Eudragit RS 100 colourless, clear to cloudy granules with a faint amine like odour. Eudragit RL and Eudragit RS, also referred to as ammoniomethacrylate copolymers in the USP32–NF27 monograph, are copolymers synthesized from acrylic acid and methacrylic acid esters, with Eudragit RL (Type A) having 10% of functional quaternary ammonium groups and Eudragit RS (Type B) having 5% of functional quaternary ammonium groups. The ammonium groups are present as salts and give rise to pH-independent permeability of the polymers. Both polymers are water-insoluble, and films prepared from Eudragit RL are freely permeable to water, whereas, films prepared from Eudragit RS are only slightly permeable to water. Solvent-free granules (Eudragit RL 100 and Eudragit RS 100) contain ≥97% of the dried weight content of the polymer. 1 g of the substances dissolves in 7 g aqueous methanol, ethanol and isopropyl alcohol (containing approx. 3 % water), as well as in acetone, ethyl acetate and methylene chloride to give clear to cloudy solutions. The substances are practically insoluble in

petroleum ether, 1 N sodium hydroxide and water.

Lamivudine is a nucleoside reverse transcriptase inhibitors (NRTIs) and primarily used in the treatment of one of the most common chronic disease of the planet, AIDS. It has short biological half-life 5-7 h. It has 80-87% bioavailability. The daily dose of drug is 150 mg twice a day or 300 mg once a day.

Currently available marketed dosage forms does not deliver drugs specific to lymph. From this dosage forms low concentration of drug reaches in lymphatic system. Even the most potent antiviral therapy, which practically reduces viral load from the peripheral blood to undectable levels, but latent virus, lurks in lymphatic system. So by developing drug-loaded microspheres which will be up-taken by the GALT (Gut Associated Lymphoid Tissues) can thus result in enhanced drug concentrations in lymphatics.

MATERIALS AND METHODS

Materials

Lamivudine was obtained from Ranbaxy, Malaysia SDN. BHD as a gift sample. Eudragit® RS 100 and Eudragit® RL 100 was obtained from Evonik Degussa, Mumbai, India. Acetone, methanol and light liquid paraffin were obtained from S.D. fine chemicals, Mumbai, India used as dispersing agent. Span-80 were of analytical grade. Magnesium stearate and n- hexane were of analytical grade and used as droplet stabilizer and washing agent respectively.

Methods

Lamivudine microspheres were prepared by solvent evaporation method. In this method a combination of Eudragit RS100 and Eudragit RL100 (in different ratios) was dissolved in a mixture of solvents containing acetone (10.0 ml) and methanol (15.0 ml) in a 100 ml of beaker with the help of magnetic stirrer. After complete dissolved, this solution was added with drug (1000 mg). Magnesium stearate (50 mg) as dispersing agent, was dispersed in drug and polymer solution with the help of sonicator. Resulting dispersion was poured in another 250 ml beaker, containing mixture of light liquid paraffin (100 ml) and span-80 (1% v/v), with continued stirring at 500, 1000 & 1500 rpm. Stirring was continued for 3 h until solvents evaporated completely. After evaporation of solvents, formed

microspheres were filtered and residue was washed 4-5 times in 50 ml petroleum ether, each. Microspheres were dried at room temperature for 24 h.

Factorial design

A full factorial 3^2 design was used for optimization procedure. It is suitable for investigating the quadratic response surfaces and for constructing a second-order polynomial model, thus enabling optimization of the microspheres. Mathematical modeling, evaluation of the ability to fit to the model and response surface modeling were performed with employing sigma plot software (Version 11.0). The studied factors (independent variables) were concentration of Eudragit RS 100 (*X*1) and concentration of Eudragit RL 100 (*X*2). Preliminary studies provided a setting of the levels for each formulation variable. The response (dependent variables) studied was % Drug encapsulation efficiency *(Y)*. Table 1 summarizes the independent and dependent variables along with their levels. The resulted formulations (testing runs) are listed in Table 2. The factorial formulations were coded as F1 to F9. A statistical model incorporating interactive and polynomial term was used to evaluate the response

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta 11X1^2 + \beta 22X2^2$$

Where, Y is the dependent variables, β_0 is the arithmetic mean response of the nine runs, and β_1 is the estimated coefficient for the factor β_1. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity.

Drug entrapment efficacy

Microspheres were crushed in a glass mortor and pestle, and powdered. A content equivalent to 30mg of lamivudine was transferred into 100 ml volumetric flask. The content was dissolved by using phosphate buffer pH 6.8 and made up to 100 ml. From the above solutions, 5 ml was diluted to 50 ml using the phosphate buffer pH 6.8. The resulting solution was filtered, and the filtrate was analysed for the drug content. The entrapment efficacy

was calculated using the following formula,

$$Drug\ Entrapment\ Efficacy = \frac{Actual\ drug\ content}{Theoretical\ drug\ content} \times 100$$

Percentage yield

% yield determined by following equation,

$$\%Yield = \frac{Practicle\ yield}{Theoretical\ yield} \times 100$$

Particle size determination by microscopy

The particle size of the microspheres was determined using an optical microscopy method. Approximately 300 microspheres were taken on a glass slide and the particle size measured using a calibrated optical microscope under regular polarized light.

***In vitro* drug release Study**

The prepared microspheres were subjected to *in vitro* drug release sequentially in three different suitable dissolution media. USP type I (basket type) dissolution apparatus was used. The dissolution medium for the first 2 hr was 900 ml of 0.1 N HCl (pH 1.2) and continued in phosphate buffer pH 6.8 for the next 3 hr and following 7 hr in phosphate buffer pH 7.4. The temperature of dissolution medium was maintained at 37 ± 0.5 °C and the basket was rotated at 50 rpm. An aliquot of 5 ml was withdrawn at predetermined time intervals and replaced with an equal volume of the fresh dissolution medium to maintain sink conditions. The samples were analyzed at 272 nm, for the percentage drug release using an UV-Visible double beam spectrophotometer. The release study was performed in triplicates.

Scanning electron microscopy (SEM) study

Scanning electron photomicrographs of drug-loaded eudragit microspheres were taken. A small amount of microspheres was spread on aluminium stub. Afterwards, the stub containing the sample was placed in the scanning electron microscopy (SEM) chamber. A scanning electron photomicrograph was taken at the acceleration voltage of 15 KV, chamber pressure of 0.6 mm Hg.

Statistical analysis of data

Statistical analysis of the factorial design formulations was performed by multiple regression analysis using Microsoft Excel

2007. Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis. 3D response plots were constructed using sigma plot software. Two random check points covering the entire range of experimental domain were carried out to determine the validity of the model generated. Subsequently, the resultant experimental data of the response properties were quantitatively compared with those of the predicted values. Predicted values were compared with the resulting experimental values and the percentage bias was calculated. The composition of checkpoint formulations is shown in Table 3. The checkpoint formulations were coded as CPT10 and CPT11 respectively.

Check point formulation was prepared similar to the factorial formulations and evaluated for *in vitro* dissolution study. From the result of experimental data of % Drug encapsulation efficiency of check point the polynomial equation was validated.

Stability study of optimized formulation

The optimized formulation (F3) was monitored up to 1 month at short term stability conditions of temperature and relative humidity (40 ± 2^0C /75% ± 5%RH). The microspheres were sealed in aluminium foil and kept in humidity chamber. Samples were withdrawn after one month and characterized for drug content and *in vitro* drug release.

RESULTS AND DISCUSSION

For, optimization of drug:polymer ratio 3^2 full factorial design was applied. F1 to F9 batch taken. Drug content (9.423±0.102) and % drug encapsulation efficiency (93.33±1.20) which was higher as compaired to other formulations. From result it was found that F3 formulation having good result as compaired to other batches. So, F3 formulation was optimized.

All batches showed different release behaviour with % drug encapsulation efficiency. Figure 1 show that the cumulative % drug releases from formulations F1 to F9. As the concentration of eudragit RL 100 increases the release rate of drug also increases. The result indicate that the eudragit RL 100 having 10% ammonium quaternary compound while eudragit RS 100 having 5% ammonium quaternary compound. F3 batch shows that the

cumulative % drug release (95.93±3.025) was higher than the other batches. Formulation F3 provides a desired drug release hence it was selected as a optimized formulation.

Release profile followed Higuchi model (R^2=0.924). It appears that mechanism of drug release from microspheres was diffusion controlled.

SEM of optimized formulation (F3)

SEM study shows that particles made of Eudragit RL100 and RS100 were spherical and not aggregated. The surface of the drug-loaded microspheres manifested the presence of drug particles, clearly visible from outside at high magnification (Figure 2). However, according to the type and concentration of the polymer, there were no significant differences in morphology of the microspheres.

Statistical analysis of the data and validation of the model

The statistical analysis of the factorial design formulations was performed by multiple linear regression analysis carried out in Microsoft Excel 2007. The % drug encapsulation efficiency values for the 9 formulations (F1 to F9) showed a wide variation; the results are shown in Table 4. The data clearly indicate that the values of % drug encapsulation efficiency are strongly dependent on the independent variables. The fitted full model equation relating the response Y (% drug encapsulation efficiency) to the transformed factor are shown in following equation,

$$\boldsymbol{Y=}\ 81.94 + 8.36X_1 - 4.16X_2 - 0.59X_1X_2 + 0.74X_1^2 - 2.45X_2^2$$

The P value for X_1, X_2 and X_2^2 was found to be 0.0001, 0.0003, 0.0070 respectively (Table 6.) which is less than 0.05. Thus X_1, X_2, and X_2^2 has significant effect on dependent variable (Y) while other term X_1X_2, X_1^2 were rendered insignificant having P value greater than 0.05.

So, the reduced model equation is as follows:

$$\boldsymbol{Y}= 81.94 + (8.36X1) - (4.16X2) - (2.45X2X2)$$

The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e., positive or negative). Table 6. Shows the results of the analysis of variance (ANOVA), which was performed to identify insignificant factors. The high values of correlation coefficient for drug encapsulation

efficiency indicate a good fit, i.e., good agreement between the dependent and independent variables. The significance test for regression coefficients was performed by applying the student F test. A coefficient is significant if the calculated F value is greater than the critical value of F.

Figure 3 and 4 showed the response surface plot and Counter plot of concentration of eudragit RS 100 (X_1) and concentration of eudragit RL 100 (X_2) versus % drug encapsulation efficiency respectively. The plot was drawn using Sigma Plot Software 11.0 demonstration version. The data demonstrate that both X_1 and X_2 affect the % drug encapsulation efficiency. It can say that the drug encapsulation efficiency and drug release profile may be changed by appropriate selection of the X_1 and X_2 levels. The area in counter plot (Figure 4.) shows if we selected X_1 and X_2 in this range we get the desired % drug encapsulation efficiency of lamivudine microspheres.

Validation of statistical model

To validate evolved model formulation CPT10 and CPT11 (check point formulation) were prepared. Dependent parameter i.e. % drug encapsulation efficiency was determined and compared with predicted values as shown in Table 7. The results obtained with check point formulations are very close to predicted values (Table 7). Thus, we can conclude that the statistical model is mathematically valid**.**

STABILITY STUDY OF OPTIMIZED FORMULATION (FORMULATION-F3)

Short term stability studies were performed at temp of 40±2^{0}C / 75±5% RH over a period of one month (30 days) of lamivudine microspheres. Optimized formulation were packed in amber coloured rubber stopper vials & kept in stability chamber maintained at 40±2^{0}C / 75±5% RH. Samples were taken at one month interval. At the end of one month period, dissolution test was performed to determine the drug release profile. Results are given in Table 9 and in Figure 5. Dissolution profiles before and after storage are nearly overlapable. The change in the drug release pattern i.e. dissolution profile was not significantly different from the one month previous dissolution profile. The developed dosage form passes stability study carried out for 30 days at 40±20^{o}C / 75±5% RH.

CONCLUSION.

Lamivudine was successfully formulated as sustained release microspheres to deliver drug up to 12 hrs. Eudragit RS 100 and Eudragit RL 100 were used for preparation of lamivudine microspheres. Eudragit polymers were used for sustained release formulation. Eudragit RS 100 & Eudragit RL 100 are pH independent polymers and water insoluble polymers but permeable. Particle size of microspheres affected by stirring speed and concentration of the eudragit polymers. As the stirring speed increased, the particle size decreased and as the concentration of eudragit increases, the particle size also increased. Larger microspheres showed greater drug loading and smaller microspheres showed a faster drug release. In 3^2 full factorial design the F3 batch was selected because it has higher drug content, % drug encapsulation efficiency and drug release rate as compaired to other batches. Formulation F3 was seen to be stable after one month of stability study.

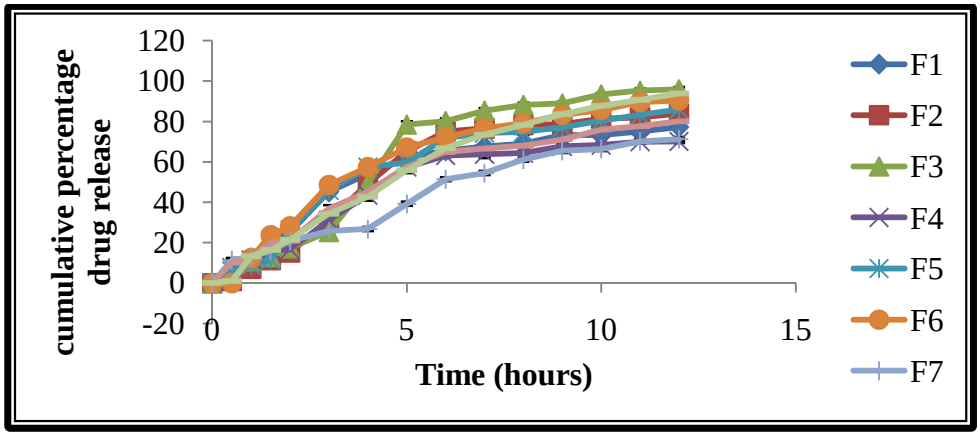

Figure 1. *In vitro* drug release studies of formulations (F1 to F9)

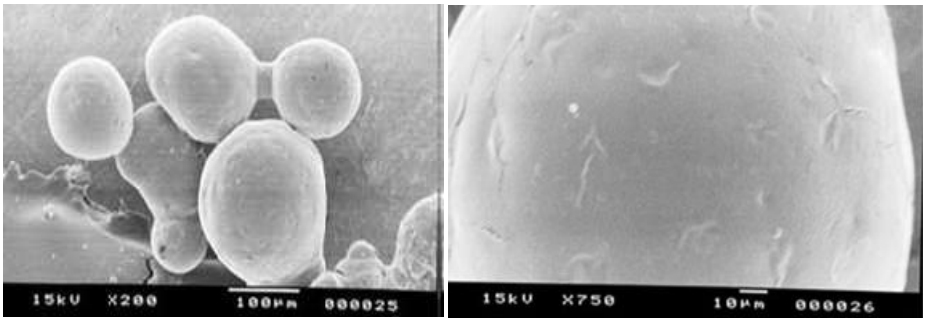

Figure 2. Scanning electrone micrographs of optimized formulation

3D surface plot

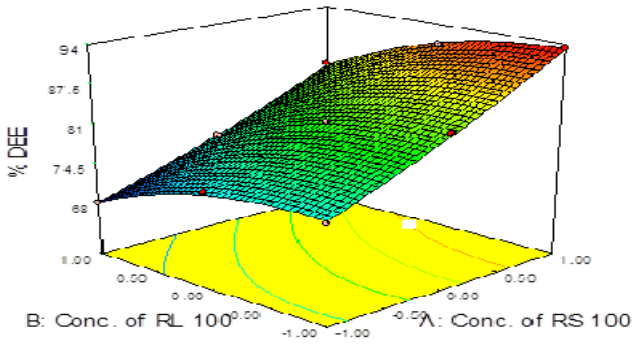

Figure 3. Response surface plot showing the influence of Concentration of Eudragit RS 100 and concentration of Eudragit RL 100 on response Y

Contour plot

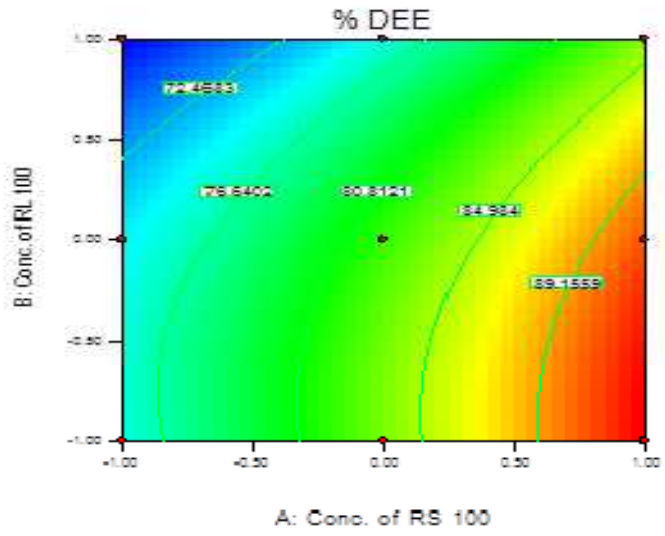

Figure 4. Contour plot showing relationship between concentration of eudragit RS 100 and concentration of eudragit RL 100 on % drug encapsulation efficiency.

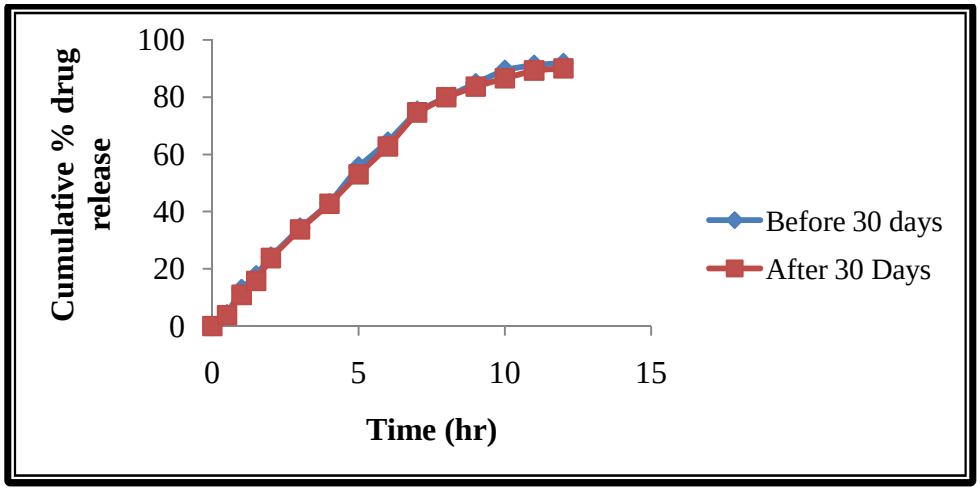

Figure 5. Comparison of *in vitro* dissolution profile of formulation- F3 before and after (30th day) stability study stored at 40°C

Table 1

Coding of variable.

Coded value	X_1 (concentration of Eudragit RS 100)	X_2 (concentration of Eudragit RL100)
-1	1.4	0.4
0	1.5	0.5
1	1.6	0.6

Table 2

Formulation layout for factorial formulation.

Batches	Coded value		Actual value	
	X_1	X_2	X1 (concentration of Eudragit RS 100)	X2 (concentration of Eudragit RL100)
F1	-1	-1	1.4	0.4
F2	-1	0	1.4	0.5
F3	-1	1	1.4	0.6
F4	0	-1	1.5	0.4
F5	0	0	1.5	0.5
F6	0	1	1.5	0.6
F7	1	-1	1.6	0.4
F8	1	0	1.6	0.5
F9	1	1	1.6	0.6

Table 3

Independent variables in checkpoint formulation.

Formulation	Coded value		Actual value	
	X_1	X_2	X1 (concentration of Eudragit RS 100)	X2 (concentration of Eudragit RL100)
CPT10	-0.5	+0.5	1.45	0.55
CPT11	+0.5	-0.5	1.55	0.45

Table 4

Drug content, entrapment efficiency, Percentage yield, and particle size of lamivudine microspheres.

Batch	Drug content* (mg)	Drug encapsulation efficiency* (%)	Yield* (%)	Particle size* (µm)
F1	6.219±0.251	75.04±3.84	68.5±2.5	269±4.68
F2	8.563±0.365	84.03±4.12	84.0±3.9	382±5.92
F3	9.423±0.102	93.33±1.20	93.3±1.0	139±3.51
F4	6.178±0.541	74.84±4.35	75.3±3.4	247±2.31
F5	8.903±0.212	81.65±2.69	81.5±2.8	329±3.06
F6	5.089±0.354	90.78±5.49	75.5±4.7	187±4.65
F7	3.748±0.158	68.15±2.09	90.8±1.6	574±3.28
F8	8.045±0.209	75.23±1.08	84.3±2.7	647±5.17
F9	8.439±0.509	84.08±3.84	74.5±3.7	391±4.96

***Values are mean ± SD (n=3)**

Table 5

Release kinetic profile of formulation F3 batch

Formulation code	R^2			
	Zero-order	First-order	Higuchi	K-peppas
F3	0.7234	0.9804	0.9972	0.9169

Table 6

Regression statistics for Y

Regression Statistics for Y	
Multiple R	0.9993
R Squre	0.9986
Adjusted R Squre	0.9964
Standard Error	0.4884
Observations	9

Coefficients	P-value
β_0 = 81.94	0.0002
β_1 = 8.36	0.0001
β_2 = -4.16	0.0003
β_{11} = 0.74	0.1401
β_{22} = -2.45	0.0070
β_{12} = -0.59	0.1100

Table 7

Calculations for testing the model in portions.

	DF	SS	MS	F	R2	
Regression						
FM	5	537.21	107.46	450.98	0.9986	Fcal= 5.392
RM	3	534.74	178.24	271.31	0.9923	
Error						Fcri= 9.552
FM	3	0.7148	0.2382	-	-	
RM	5	3.2848	0.6569	-	-	DF= (2,3)

DF: degree of freedom, SS: sum of squares, MS: mean of squares, F: Fischer's ratio,

R2: regression coefficient, FM: full model, RM: reduced model.

Table 8

The experimental and predicted values for response Y

Formulation	% Drug encapsulation efficiency (Y)		
	Experimental value	Predicted value	% Bias
CPT 10	83.09	77.61	6.59
CPT 11	88.69	81.03	8.63

Table 9

Evaluation after short term stability study

Formulation	Drug content* (mg)	% Drug encapsulation efficiency*
F3	9.209±0.304	92.89±2.08

***Values are mean ± SD (n=3)**

REFERENCES

1. Kataria S, Middha A, Sandhu P, Bilandi A and Kapoor B, "Microspheres- A Review." *International Journal of Research In Pharmacy And Chemistry,* **2011**, 1(4), 1184-1198.

2. Nath B, Nath LK and Kumar P, "Preparation and *In vitro* Dissolution profile of Zidovudine loaded microspheres made of Eudragit RS100, RL100 and their combinations." *Acta Polonia Pharmaceutica-Drug Research*, **2011**, 68(3), 409-415.

3. Sahoo SK, Mallick AA, Barik BB and Senapati PC, "Formulation and In vitro Evaluation of Eudragit® microspheres of Stavudine" *Tropical Journal of Pharmaceutical Research*, **2005**, 4(1), 369-375.

4. Kannan K, Karar PK and Manavalan R, "Formulation and Evaluation of sustained release microspheres of Acetazolamide by solvent evaporation technique." *Journal of Pharmaceutical Sciences & Research* **2009**, 1(1), 36-39.

5. Verma A, Tripathi A, Saraf SA, and Saraf S, "Fabrication and evaluation of sustained release microspheres of Ketorolac tromethamine." *International Journal of Pharmacy & Pharmaceutical Sciences* **2010**, 2(4), 44-48.

6. Madhavi B, Kusum B, Srikanth V, Vinod KR and Banji D, "Formulation and evaluation of Venlafaxine HCL microspheres." *Hygeia Journal For Drugs & Medicines,* **2011**, 3(1), 64-70.

7. Chauhan MK and Kawadkar J, "Intra-articular delivery of genipin cross-linked chitosan microspheres of Flubriprofen: Preparation, Characterization, *in vitro* and *in vivo* studies." *European Journal of Pharmaceutics and Biopharmaceutics,* 2012, 81, 563-572.

9. Kawashima Y and Takeuchi H, "Design of pH-sensitive microspheres for the colonic delivery of the immunosuppressive drug Tacrolimus." *European Journal of Pharmaceutics and Biopharmaceutics*, **2004**, 58, 37-43.

10. Bodmeier R and Herrmann J, "Biodegradable, Somatostatin acetate containing microspheres prepared by various aqueous and non-aqueous solvent evaporation methods." *European Journal of Pharmaceutics and Biopharmaceutics*, **1998**, 45, 75-82.

11. Patel K and Patel V, "Preparation and Characterization of Tramadol HCL microspheres." *International Journal of Drug Development & Research (IJDDR),* **2010**, 2(3), 605-611.

12. Khanam Z and Alam MdI, "Fabrication and evaluation of Propranolol HCl loaded microspheres by ionic-gelation technique." *Scholars Research Library* **2012**, 4(3), 815-820.

13. Kaza R and Pravallika P, "Formulation and evaluation of controlled release microspheres of acyclovir sodium." *International Journal of Innovative Pharmaceutical Research* **2011**, 2(1), 98-101.

14. Kishorbapu A and Bhanuteja A, "Formulation and evaluation of double walled microspheres loaded with pantoprazole." *International Journal of Research in Pharmacy and Chemistry* **2011**, 1(4), 770-779.

15. Dey S and Malgope A, "Formulation and optimization of sustained release stavudine microspheres using response surface methodology." *International Scholarly Research Network* **2011**, 1-7.

16. Bhanupriya B and Sivabalan M, "Preparation and charactrisation of tramadol microspheres for post-operative pain." *International Journal of Pharmaceutical & Biological Archives* **2011**, 2(3), 938-944

17. Dey S, Roy N and Mazumdar B, "Effect of formulation and process variables on the characteristics of microspheres of antiviral drug (stavudine) prepared by oil-in-oil solvent evaporation technique." *International Journal of Pharmacy and Pharmaceutical Sciences* **2010**, 2(2), 52-59.

18. Phalguna Y and Gudas G, "HPMC microspheres of zidovudine for sustained release." *International Journal of Pharmacy and Pharmaceutical Sciences* 2010, 2(4), 41-43.

19. Palanisamy M and Khanam J, "Cellulose-based matrix microspheres of prednisolone inclusion complex: preparation and characterization." *AAPS PharmSciTech* **2011**, 12(1), 388-400.

20. Lucinda-silva M and Baccarin A, "Ethylcelullose microspheres containing sodium diclofenac: development and characterization." *Acta Farm. Bonaerense* **2006**, 25(3), 401-404.

21. Dey S and Mazumder B, " Comparative study of stavudine microspheres prepared using ethylcellulose alone and in combination with eudragit® rs100." *Malaysian Journal of Pharmaceutical Sciences* **2010**, 8(2), 45-57.

22. Keshari A and Dash V, "Formulation and evaluation of zidovudine loaded chitosan microspheres for controlled release." *International Journal of Drug Development & Research* **2012**, 4(1), 96-105.

23. Nayak B and Nayak U, "Lamivudine loaded microspheres for oral use: design, development and establishment of *invivo-invitro* correlation." *Asian Journal of Pharmaceutical and Clinical Research* **2009**, 2(1), 55-60.

24. Surendiran S and Yuvaraj V, "preparation and evaluation of ibuprofen micro spheres by using co-acervation phase separation technique." *International Journal of ChemTech Research* **2010**, 2(2), 1214-1219.

25. Sathiyasunder R and Murugesan A, "Formulation development and evaluation of carprofen microspheres." *International Journal of ChemTech Research* **2010**, 2(3), 1674-1676.

26. Gowardipe S and Jaganathan K, "Formulation And *In Vitro* Evaluation Of Lamivudine and Zidovudine Controlled Release Bilayer Matrix Tablets." *International Journal of Pharma World Research*, **2012**, 3(1), 1-17.

27. Patel J, Dhingani A and Dabhi M, "Design and development of sustained release microspheres of Quetiapine fumarate using 3^2 full factorial design." *Journal Of Pharmacy Research*, **2010**, 3(12), 2871-2875.