Current Pharma Research
ISSN-2230-7842
CODEN-CPRUE6
www.jcpronline.in/

Research Article

Formulation and Pharmacodynamic Evaluation of Fluoxetine HCl Mucoadhesive Microsphere.

[1] *Madhuri T. Deshmukh, [2]Shrinivas K. Mohite*

[1]Department of Pharmaceutics, Rajarambapu College of Pharmacy, Kasegaon-415404, Maharashtra, India,

[2]Department of Pharmaceutical Chemistry, Rajarambapu College of Pharmacy, Kasegaon-415404, Maharashtra, India.

Received 28 May 2018; received in revised form 21 June 2018; accepted 22 June 2018

***Corresponding author E-mail address:** madhurideshmukh9@yahoo.com*

ABSTRACT
The aim of this research was to formulate and pharmacodynamicaly evaluate Fluoxetine Hcl microsphere microspheres to enhance bioavailability and antidepressant activity. Fluoxetine HCl microsphere was prepared by using Eudragit Rs polymer Microsphere by using emulsion solvent evaporation Fluoxetine Hydrochloride comes under category of selective inhibitor of serotonin reuptake type of drug used for treating depression. It is practically soluble in water having to BCS class I and 72%. Bioavailability Fluoxetine HCl microspheres were evaluated for entrapment efficiencies*, in-vitro* release, *in-vitro* mucoadhesive, *in-vivo* FTIR, DSC, X-ray diffraction studies and stability study. Formulation F9 microspheres batch was found to be optimized and followed zero-order release kinetic.The optimized formulation was mucoadhesive in nature and increased antidepressant activity. Stability studies was carried out for F9 at a temperature of 40±2°C/ RH 75±5% formulation revealed that the drug behavior was within permissible limits.

KEYWORDS
Fluoxetine, Eudragit RS, Microsphere, emulsion solvent evaporation, gastro retentive delivery.

1. INTRODUCTION

Conventional dosage form is less successful because of its less residence time in G.I. fluid. Hence mucoadhesive microsphere drug delivery systems are used to enhance the residence time at the site of application, therapeutically effective plasma drug concentration reducing the dosing frequency, minimize fluctuations, effective absorption, enhanced bioavailability of the drugs, maximum utilization of drugs and better patience compliance .[1-2]

Main focus of this research is to prepare sustain microspheres of Fluoxetine Hcl which provides slow release of drug in gastrointestinal tract. Fluoxetine Hcl is effective against the negative symptoms of schizophrenia. Fluoxetine Hcl practically soluble in water belongs to BCS class I and 72%. bioavailability. Fluoxetine Hcl is extensively metabolized in liver. The drug has a moderate elimination half-life implying that once daily therapy is adequate for treatment of schizophrenic conditions**.** Hence the objective of the present work was to formulate the mucoadhesive microsphere of Fluoxetine using Eudragit RS polymer to improve residence time, reduced dosing frequency and increases bioavailability in the treatment of depression. [3-6]

2. MATERIALS AND METHODS

2.1. Materials

Fluoxetine was obtained from Enaltec Lab Private Ltd, Mumbai, Eudragit RS 100 gift sample from Lobachem Mumbai, Carbopol and Span 20 was purchased from Nulife Pune.

2.2. Preparation of microsphere[7-8]

The microspheres Of Fluoxetine Hcl were prepared using emulsion solvent evaporation method. Solution of Eudragit RS formed in acetone (4-8% w/v) to drug (200 mg) was added to formed solution. Carbopol 974P and HPMC K4M were added in 1:1 ratio and the solution was stirred for 1 hour. Formed solution was poured in liquid paraffin (300 ml) containing 0.75% span 80 previously cooled at 5^0 C for 1 hour. The emulsion was stirred for 40 min (500-1000 RPM) filtered and washed with n-hexane and dried. Different batches of various ratios of drug, polymer combination and combination of liquid paraffin were successfully prepared.

2.3. Optimization of microsphere formulations

Optimization was done by 3^2 factorial design using Design expert (Version 9.2; Stat-Ease Inc., Minneapolis, Minnesota, USA). The optimal levels of variables were determined by 3^2 factorial design. The significant factors selected were concentration of Eudragit RS 100 and RPM examining 9 runs. The dependant variables selected were entrapment efficiency, % mucoadhesion, % drug release. Microsphere prepared by using emulsification solvent evaporation method.

Table No. 1: Formulation code

Ingredient	**Formulation Code**								
	F1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	**F 9**
Drug(Fluoxetine)	1	1	1	1	1	1	1	1	**1**
Polymer (Eudragit)	1	1	1	3	3	3	5	5	**5**

Speed (r.p.m.)	500	1000	500	500	1000	750	750	750	**1000**
Liquid paraffin	**300**	**300**	**300**	**300**	**300**	**300**	**300**	**300**	**300**

2.4. Factorial Design: [9]

3^2 factorial design was used for mathematical modeling and analysis of responses where the amounts of Polymer(X_1) and speed (X_2) were selected as the independent factors. The levels of the two factors were selected and on the basis of the preliminary studies carried out before implementing the experimental design. A statistical model was used to evaluate the responses which involve polynomial terms.

$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1 + b_{22}X_2^2$

Where Y is the dependent variable, b0 is the arithmetic mean response of the 9 runs, and b_1 is the estimated coefficient for the factor X_1. The main effects ($X_1$and X_2) represent the average result of changing one factor at a time and (X_1X_2) represent interaction factor

2.5. Drug entrapment efficiency: [10]

Microspheres (50 mg) were powdered and suspended in 50 ml of 0.1 N Hcl followed by 30 min. sonication. The solution was kept undisturbed for 24 hours; and filtered. The filtrate recovered was examined spectrophotometrically at 254 nm and entrapment efficiency was calculated by the following formula:

$$\text{Entrapment Efficiency} = \frac{\text{Practical Drug Content}}{\text{Therotical Drug Content}} \times 100$$

2.6. Morphology of microsphere: [11]

The external and internal morphology of the microspheres were studied by using scanning electron microscopy in Pune University (Physics Department). The sample was loaded on copper sample holder and sputter coated with platinum.

2.7. In-vitro wash off test: [12]

The *in-vitro* wash off test was carried out to evaluate the mucoadhesive potential of the microspheres. In brief, a 1cm by 1cm rat mucosa was cut and tied onto glass slide by thread. Around 100 microspheres were spread on the wet mucosa and the prepared slide was hung onto one of the grooves of the USP tablet disintegrating test apparatus filled with 0.1 N Hcl giving regular up and down movements for 60 minutes. At the end of 60 min, numbers of microspheres still adhering to the intestinal mucosa were counted.

% Mucoadhesion = (Wa-Wl) X 100 / Wa

Where, Wa = weight of microspheres applied; Wl = weight of microspheres leached out.

2.8. In- Vitro Release Profile of Fluoxetine from microspheres:

In-vitro drug Release Studies Release of Fluoxetine from the microspheres was studied in phosphate buffer of pH 7.4 (900 ml) using a dissolution rate test apparatus with a rotating paddle stirrer at 50 rpm and 37 ± 1°C. A sample of microspheres equivalent to 10 mg of Fluoxetine was used in each test. Samples of dissolution fluid were withdrawn at different time intervals and were assayed at 254 nm for Fluoxetine content using a Shimadzu UV- 1700 double beam spectrophotometer (Shimadzu Corporation, Japan). From this percentage drug release was calculated.

2.9. Release kinetic studies: [13]

The rate and the mechanism of release of fluoxetine from the prepared mucoadhesive microspheres were analyzed by fitting the dissolution data into various kinetic models like zero order; first order, Higuchi's model and coefficient of correlation (r) values were calculated for the linear curves by regression analysis of the above plots.

2.10. Fourier transforms infrared spectroscopy (FTIR) studies: [14]

FTIR spectra for pure Fluoxetine and Fluoxetine microspheres were determined to check the interaction between drug and excipient. .FTIR spectra of pure drug and microsphere were recorded using KBr disc using FTIR spectrophotometer (Jasco-4100s, Japan).

2.11. Differential scanning calorimeter (DSC) studies: [15]

The thermal behavior of pure Fluoxetine and Fluoxetine microspheres were studied using a DSC Perkin Elmer DSC at a heating rate of 10°C/minutes. Samples were accurately weighed into aluminum pans and then sealed. The measurements were performed at a heating range of 30-250°C under nitrogen atmospheres**.**

2.12. In-vivo Study: [16-19]

The approval of the Institutional Animal Ethics Committee was obtained before starting the study. The protocol of study was approved by RDCOP/ IAEC/ Approval / 2016 – 17/ 02 dated 8.8.2016**r**espectively.*In vivo* antidepressant activity of the Fluoxetine Hydrochloride microsphere was carried out. The study for optimized formulation was performed on normal healthy Wistar albino rats of either sex weighing 150 to 250 g each were used for the study. *In-vivo* efficiency of the prepared batch was assessed in healthy normal Wistar rats by measuring the antidepressant effect produced after oral administration. To carry out the study the animals were divided into groups such as normal, standard and microsphere treated. The animals were provided with housing in polypropylene cages having artificial lighting to simulate the day-night cycles, and access to food and water *ad libitum*. As per the test protocol, the forced swimming test and tail suspension test were performed.

Group I - Normal group

Group II - Standard group

Group III –Microsphere treated formulation

2.13. Forced swim test

For the FST, mice of the either sex were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm) containing 19 cm of water at 25°C±1°C. Treatment was given 60 minutes prior to study as described by study design all animals were forced to swim for 6 minutes and the duration of immobility was observed and measured during the final 4 minutes interval of the test. Each mice was judged to the immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect.

2.14. Tail suspension test:

The tail-suspension test is a mouse behavioral test useful in the screening of potential antidepressant drugs, and assessing of other manipulations that are expected to affect depression related behaviors. Mice are suspended by their tails with tape, in such a position that it cannot escape or hold on to nearby surfaces. During this test, typically six minutes in duration, the

resulting escape oriented behaviors are quantified. The tail-suspension test is a valuable tool in drug discovery for high-throughput screening of prospective antidepressant compounds

2.15. Stability study: [20]

Stability studies were carried out for Fluoxetine microsphere as per ICH guidelines. The best mucoadhesive microspheres formulation (F9) was sealed in high-density polyethylene bottles and stored at 25±2°C/60±5%, 40±2°C/75±5% relative humidity (RH) for 90 days. The samples (F9) were evaluated for entrapment efficiency and percentage mucoadhesion.

3. RESULTS AND DISCUSSION

The Fluoxetine microspheres were prepared using emulsion solvent evaporation technique. The formula optimization was done by 3^2 factorial design. The significant factors selected were concentration of polymer and speed. The dependant variables selected were entrapment efficiency, % mucoadhesion and % drug release. The model was analysed for fitting into appo. Mathematical model and evaluated statistically for ANOVA. The response surface analysis was carried out employing the 3D response surfaces.

3.1. Process yield

The production yields of microspheres prepared by emulsion-solvent evaporation method were found to be between 79 to 91.31% as shown in Table 3.The maximum percentage yield was found of F9 batch and was noted to be 92.35 % among all the batches.

Table 2: Physicochemical properties of Fluoxetine mucoadhesive microspheres*:*

Formulation code	Process yield (%)
F1	88.08 ± 0.03559
F2	91.1 ± 0.294392
F3	92.69 ± 0.149666
F4	88.06 ± 0.037417
F5	87.31 ± 0.029439
F6	90.13 ± 0.008165
F7	79.91± 0.029439
F8	81.83± 0.008165
F9	84.08 ± 0.029439

3.2. SEM of microspheres:

The morphology of the mucoadhesive microspheres of best formulation F9 was examined by SEM. SEM photographs revealed that Fluoxetine microsphere were discrete and rough surface.

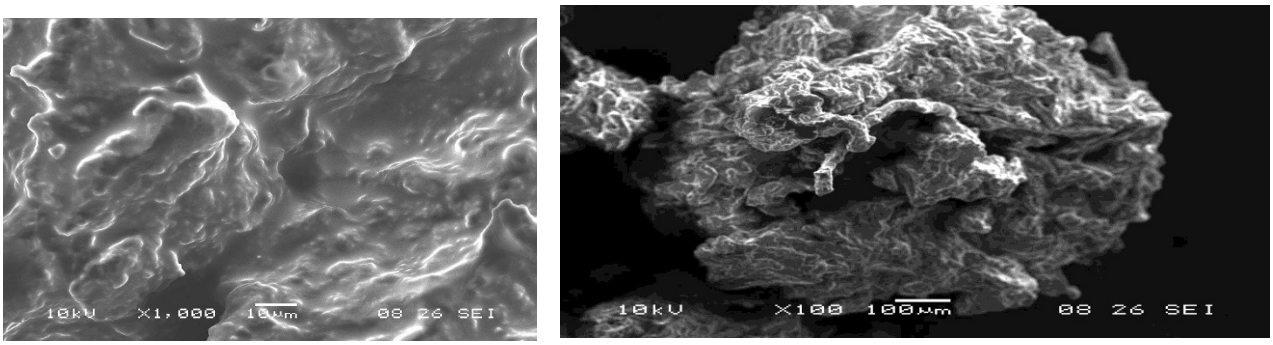

Figure 1: SEM of mucoadhesive microspheres of optimized batch: a) × 35 b) × 500

3.3. Mucoadhesive test:

The study of *in-vitro* wash off test revealed that all the batches of prepared microspheres had good bioadhesive property ranging from 78 % to 91%. On increasing the polymer concentration, the bioadhesive property of the microspheres also increased.

3.4. In-vitro Drug release studies:

The *in-vitro* drug release studies were carried out for Fluoxetine microspheres in phosphate buffer (pH 7.4) over 6 hr. The drug release of Fluoxetine microsphere ranging from 83 % to 96 %. drug release.

Table 3: *In-vitro* drug release of Fluoxetine Hcl microsphere

Formulation code	*In-vitro* Dissolution (%)
F1	93.33± 0.014142
F2	86.57± 0.057155
F3	83.33 ± 0.021602
F4	91.66 ± 0.029439
F5	89.22 ± 0.021602
F6	88.45 ± 0.043205
F7	96.66 ± 0.045461
F8	93.41 ± 0.029439
F9	92.88 ± 0.064807

Table 4: ANOVA output of the 3^2 design for optimization of microspheres

Sr. No.	Outcomes	Entrapment Efficiency (%)	% mucoadhesion after 1 h (%)	Drug Release
1	F value	21.78	43.20	16.15
2	P value	0.0145	0.0054	0.0223

3	R^2 value	0.9976	0.9935	0.9902
4	Adequate Precision	28.59	22.77	25.84

3.5. FTIR studies:

FTIR spectrum of pure drug and mucoadhesive microsphere of drug and polymers were studied. (Fig.8.4). It was observed that Fluoxetine showed characteristic peak at 3329 cm^{-1} for – NH group whereas Eudragit showed –CO group at 1672cm^{-1}. However shift in – CO and –NH peaks of polymer and drug to 1650 $cm^{-1}$and 3350cm^{-1}suggested possibility of H – bonding.

3.6. DSC studies:

The melting point of Fluoxetine at 162°Cwas observed and the same for Eudragit was observed at 121°C in the form of broad end other. However the thermogram of microspheres showed a distinct broad end other akin to Eudragit but at somewhat lower temperature of 87C. This might be the result of uniform distribution of fluoxetine in microspheres of Eudragit carrier and the amorphisation of crystal structure of the same leading to a reduction in melting temperature

3.7. Stability studies:

Stability studies for the optimized microsphere were carried out at a temperature of 40±2°C/ RH 75±5% for a period of 90 days. Formulation was evaluated for physical appearance and drug content. There was no any significant change in physical appearance and drug content during stability studies. Hence, it was concluded that the F9 batch of tablet have good stability during their shelf life.

3.8. Factorial equation and response surface plot:

A 3^2 full factorial design was constructed using design expert (Version 9.2; Stat-Ease Inc., Minneapolis, Minnesota, USA) for mathematical modeling and analysis of responses where the amounts of Polymer(X_1) and speed (X_2) were selected as the independent factors. The levels of the two factors were selected on the basis of the preliminary studies carried out before implementing the experimental design

The polynomial equations generated are as follow:

$$\% \text{ mucoadhesion} = + 87.30 + 2.81\ X1 - 0.43X2 + 0.47X1X2 + 0.41X1^2 + 3.02\ X2^2 \quad (1)$$

$$\% \text{ Drug content} = 88.23 + 1.81\ X1 - 4.34\ X2 - 0.11\ X1X2 + 0.41\ X1^2 - 2.22\ X2^2 \quad (2)$$

$$\% \text{ Drug release} = +8.90 - 2.83X1 + 3.29X2 + 1.56X1X2 + 1.32X1^2 + 1.25X2^2 \quad (3)$$

Where X1 = conc. of polymer and X2 = speed

All the polynomial equations were found to be statistically significant determined using as per provision of design expert software.Equation can draw conclusion after considering magnitude of coeficient and mathematical sign carried.

3.9. Percent drug mucoadhesion:

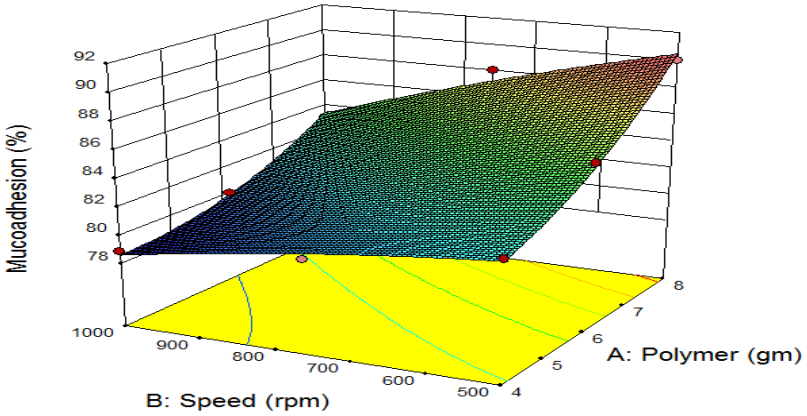

Figure 2: Percent drug mucoadhesion3D graph

Factorial equation and response surface plot for mucoadhesion:
% Mucoadhesion after 1 hour = +87.30 +2.81X1 -0.43X2 +0.47X1X2 +0.41$X1^2$ +3.02$X2^2$
WhereX1= Conc. Of polymer and X2= RPM

Percent Drug content:

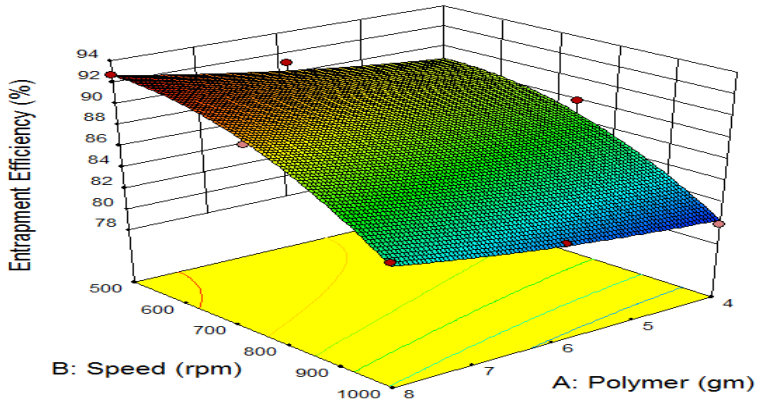

Figure 3: Percent drug content 3D graph

Factorial equation and response surface plot for drug release:
Drug release= 88.23 + 1.81 X1 – 4.34 X2 – 0.11 X1X2 + 0.41 $X1^2$- 2.22 $X2^2$equation (2)
Where X1 = Conc. of polymer and X2= RPM

Percent drug release:

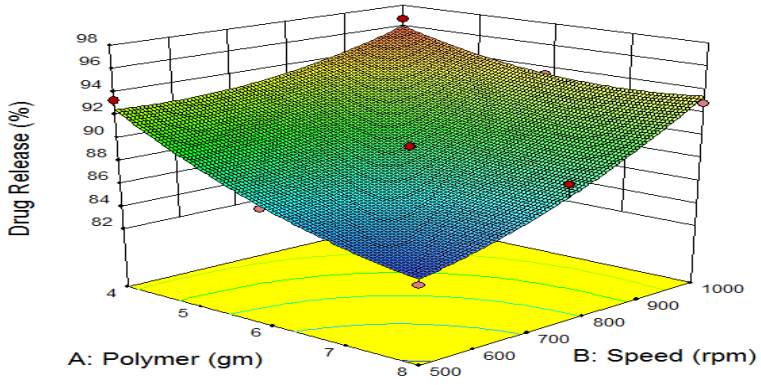

Figure 4: Percent drug release 3D graph

Factorial equation and response surface plot for drug release:

Drug release= +8.90 -2.83X1 +3.29X2 +1.56X1X2 +1.32$X1^2$ +1.25$X2^2$

Where X1 = Conc. of polymer and X2= RPM

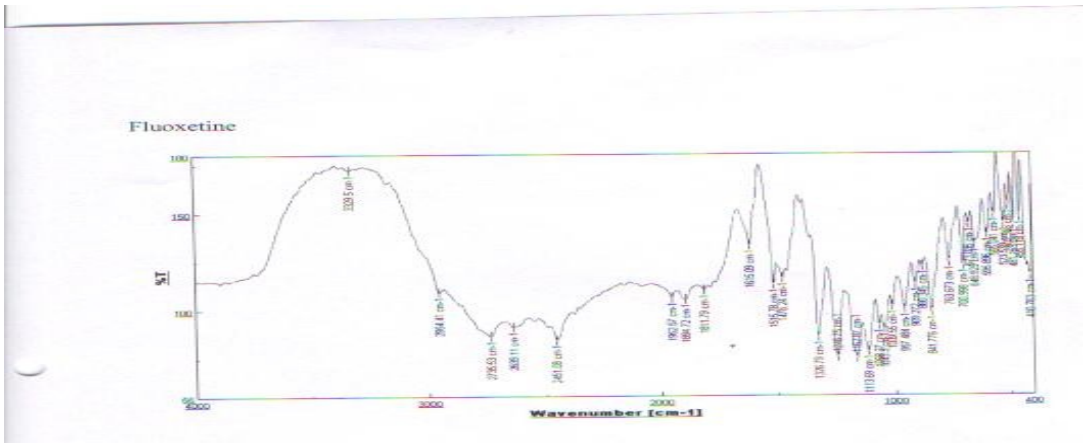

Figure 5: FTIR of Pure Fluoxetine

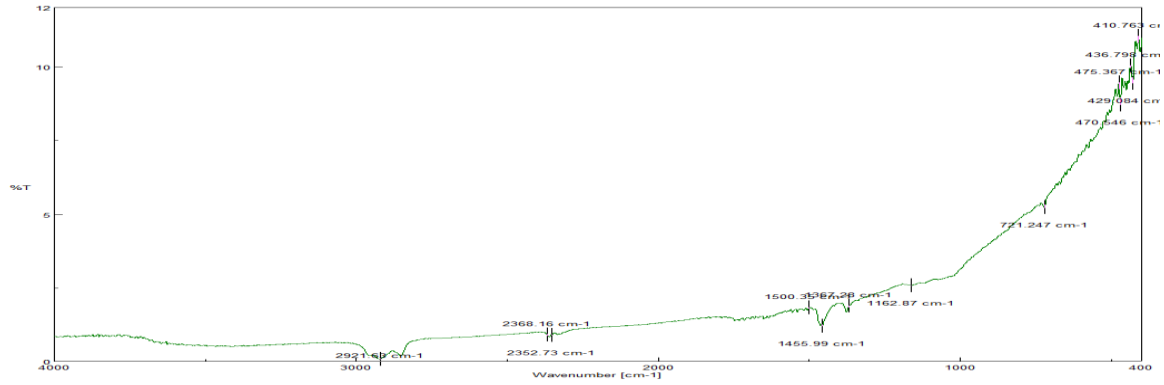

Figure 6: FTIR of Fluoxetine HCl microsphere Eudragit RS polymer

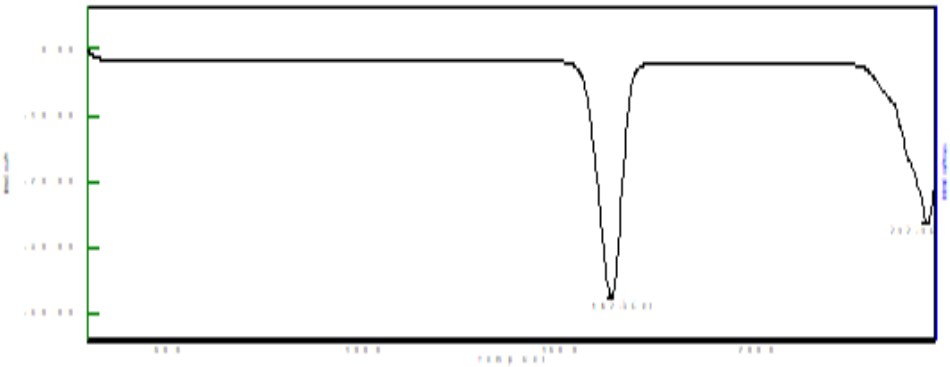

Figure 7 : DSC of Fluoxetine

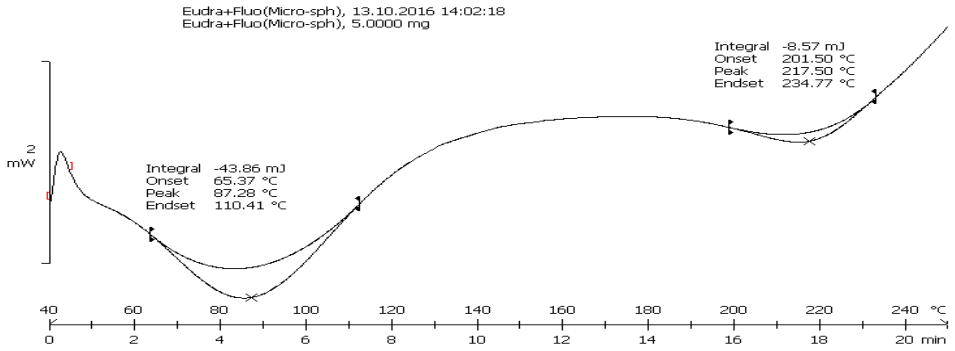

Figure 8: DSC of Fluoxetine microsphere containing Eudragit RS polymer

Table 5: Stability studies of microsphere

Sr. no	Duration	Drug Content (%)	% mucoadhesion	% drug release
1	0	78 ±4.76 %	93.05±3.59	78 ±4.76 %
2	1 month	76 ±3276 %	94.8±3.59	76 ±3276 %
3	2 month	77 ±2.66 %	91.3±3.59	77 ±2.66 %
4	3 month	78 ±1.96 %	93.08±3.59	78 ±1.96 %

3.10. Effect of acute treatments of Fluoxetine API, Fluoxetine-Eudragit microsperes in F.S.T.:
Animals from group I (normal), submitted to a 5 min FST in second session after 24 h of first habituation session on day 1, showed increased duration of immobility and decreased swimming duration indicating induction of depression like state in animals. Animals from group II (Standard); group III (Microsphere formulation);showed significant decrease in duration of immobility and increase in swimming duration on day 1 as compared to group I (normal) as shown in table 7.

Table 6: Forced Swimmimg test

TIME	Normal		Standard		Eudragit	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
15 m	181.3333	±4.565	202.3333	±18.075	181.1667	±3.736
30 m	191.1667	±3.070	238.8333	±9.516	207.500	±7.548
60 m	186.3333	±3.040	233.3333	±11.164	215.8333	±9.610

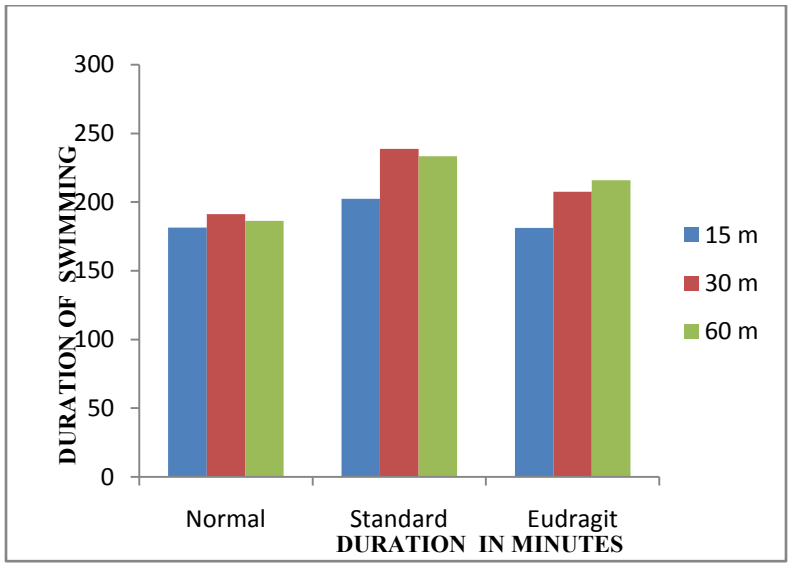

Figure 9: Effect of swimming time in Fluoxetine Hcl microsphere

3.11. Effect of acute treatments of Fluoxetine API, Fluoxetine-Eudragit microsperes in immobility time

As compared to the animals in the normal group, the immobility time was significantly reduced in all treated animals and an improved activity was seen in the animals treated with Eudragit RS formulation.

Table 7: Immobility Time

Treatment	Observations	
	Immobility Time (Before)	**Immobility Time (After)**
Normal(Saline)	133.33±1.571	134.17±1.585
Std (Fluoxetine)	137.50±1.935	53.33±1.663
T-1 (Eudragit RS)	145.83±1.114	62.5±1.758

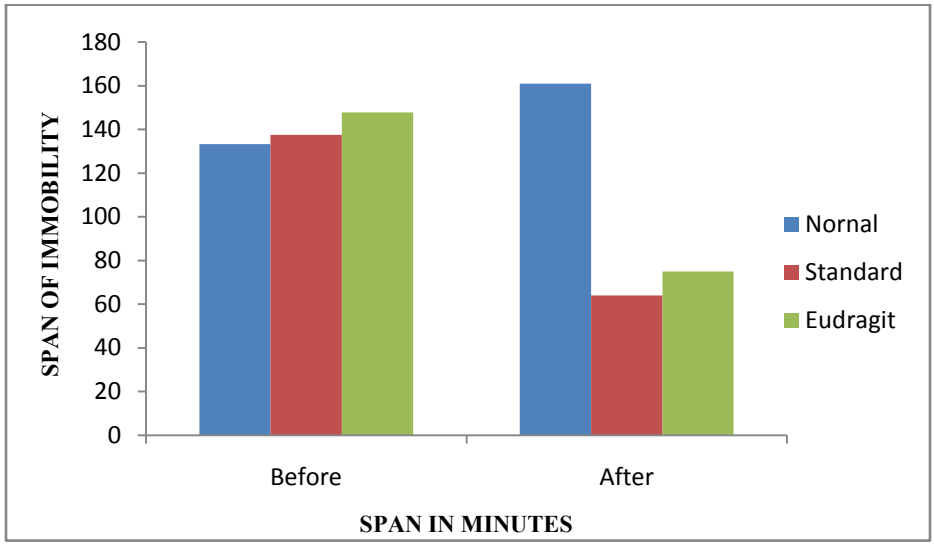

Figure 10: Effect of immobility time in Fluoxetine Hcl microsphere

4. CONCLUSION

FLU is soluble in water having only 72% oral bioavailability. Fluoxetine undergoes extensive hepatic metabolism. Hence mucoadhesive microspheres of Fluoxetine were prepared to enhance the bioavailability and to prepare sustain release action in gastrointestinal tract. Microsphere formulations prepared by using Eudragit Rs polymer were prepared by using emulsion solvent evaporation method. The significant factors selected were concentration of polymer and RPM. The dependant variables selected such as entrapment efficiency, % mucoadhesion and % drug release. It was found that on increasing the polymer concentration, the drug content, mucoadhesion and drug release of the microspheres also increased. In case in-vivo study such as F.S.T. and T.S.T showed significant decrease in duration of immobility and increase in swimming duration as compared to normal group and duration of immobility was significantly decreased as compared to normal group.

5. ACKNOWLEDGEMENT

The authors are thankful to Rajarambapu College of Pharmacy, Kasegaon 415 404, Maharashtra, India for their valuable support and permission to carry out the work.

6. REFERENCES

1. Bindu M.B., Zulkar N.K., Ramalingam R., Ravindernath A., Kusum B., Naga M.M., David B. Formulation and evaluation of mucoadhesive microspheres of venlafaxine hydrochloride. J. of Pharma. Res., 3, 11 (2010): 2597-2600.
2. Parmar V., Patel J., Jani G., Prajapati L., GagoriaJ.; First derivative spectrophotometric determination of Fluoxetine Hydrochloride and Olanzapine in tablets; Int. J. of Pharma. Sci. and Res., 2, 11 (2011): 2996-3001.

3. Thakkar V., Shaikh Y., Tejal S., Gandhi T.; Design and evaluation of sustained release Enteric coated dosage form of Fluoxetine Hydrochloride; Int. J. of Pharma. Sci. and Edu.Res., 4,46 (2012): 330-338.

4. Ali B., Rabba A, Fayed M, Say K, Anwer, Ansari M, Shdefat R., Gabr G.; Development and optimization of Fluoxetine orally disintegrating tablets using Box-Behnken design; Trop. J.of Pharma. Res.; 15, 4(2016): 669-677.

5. Hiemke C., Härtter S.; Pharmacokinetics of selective serotonin reuptake inhibitors; Pharmaco & Therapeu; 85 (2000):11–28.

6. Garud N., Garud A.; Preparation and invitro evaluation of Metformin microspheres using non-aqueous solvent evaporation technique; Trop. J. of Pharma. Res.; 11.4(2012):577-583.

7. Gaur P., Mishra S., Bajpai M.; Formulation and evaluation of controlledrelease of telmisartan microspheres: In vitro/in vivo study; J. of Food and Drug. Ana.; 22(2014):542 - 548.

8. Venkateswaramurthy N., Sambathkumar R., vijayabaskaran M., Perumal P.; Formulation and in-vitro evaluation of furazolidone mucoadhesive microspheres; Int.J.of Pharma. and Pharmaceut. Sci; 2, 3(2010):104 - 106.

9. Nappinai M., Sivaneswari S.; Formulation optimization and characterization of gastroretentive Cefpodoxime Proxetil mucoadhesive microspheres using 3 2 factorial design; J. of Pharma. Res.; 7 (2013):304-309.

10. Sonawane S., Bhalekar M., Shimpi S. Preparation and evaluation of microsphere of xyloglucan and its thiolated xyloglucan derivative. Int. J. of Bio. Macromolecules; 69 (2014): 499– 505.

11. Ararath D., Velmurugan S. Formulation and evaluation of Nevirapine mucoadhesive microspheres. Int. J. Pharm. Sci.; 7, 6 (2015) 342-348.

12. Galgette U., Chaudhari P.D. Preparation and evaluation of bioadhesive microspheres prepared by ion gelation method and effect of variables on quality of microspheres. Am. J. Pharm. Tech. Res.; 3, 4 (2013) 535-546.

13. Patil S., Choudhary B., Rathor A , Roy K.,Mahadik K.; Enhanced bioavailability oral and anticancer activity of novel curcumin loaded mixed miscelle in human lung cancer cells; Phytomedicine; 22(2015): 1103–1111.

14. Swain S., Behhera A., Dinda C., Patra C., Jammula S., Rao B. Formulation design, optimization and Pharmacodynamic evaluation of sustained release mucoadhesive microcapsule of Venlafaxine HCl. Ind. J. of Pharma. Sci.; 6 (2014) 354-363.

15. Hardenia S. , Jain A. , Patel R., Kaushal A.; Formulation and evaluation of mucoadhesive microspheres of Ciprofloxacin ; J.of Adv. Pharma Edu.& Res.; 14(2011): 214-224.

16. Jain J., Bhore P., Parmar J., Shaikh A ; Antidepressant activity of cordiadichotoma leaf extracts in animal behaviour models: a research; Int.J.of Drug Formulation & Res.; 1, 4(2010): 294-310.

17. Steru L., Chermat R., Thierry B. ;The tail suspension test: A new method for screening antidepressants in mice ; Psychopharmacol .; 85(1985):367- 370.

18. Nerkar P.,Gattani S.;In vivo, in vitro evaluation of linseed mucilage based buccal mucoadhesive microspheres of venlafaxine ; Drug Delivery; 18,2(2011): 111–121.

19. Uppsala P., Atchuta K., Patro S ., Murali B.;Asian J. of Pharma. And Clinical Res.; 8,5 (2015):287.

20. Stability testing of new drug substance and product, ICH Topic Q 1A (R2), February 2003.