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DESIGN, DEVELOPMENT AND CHARECTERISATION OF FLUVASTATIN SODIUM PULSATILE CAPSULE

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ABSTRACT

The objective of the present study, was to develop a pulsatile capsule of Fluvastatin sodium to reduce plasma cholesterol levels. The microspheres formulations of Fluvastatin sodium were prepared by emulsion solvent evaporation process by using polymer: drug ratio. Weighed amount of drug (Fluvastatin sodium) and polymer (Eudragit RL100) in 1:1 ratio was dissolved in 10 ml of acetone. The Pulsi cap was similar in appearance to a hard gelatin capsule, but the main body was water insoluble. Fluvastatin sodium microspheres were placed into the formaldehyde treated bodies by hand filling. The capsules containing the microspheres were then plugged with prepared hydrogel plug. The joint of the capsule body and cap was sealed with a small amount of the 5% ethyl cellulose ethanolic solution. The sealed capsules were completely coated by dip coating method with 5% cellulose acetate phthalate in 8:2 (v/v) mixture of acetone: ethanol plasticized with dibutyl phthalate (0.75%), to prevent variable gastric emptying.

Results: Optimized microsphere formulation(F20) were selected based on dissolution studies. Dissolution studies of pulsatile capsule device in media with different pH (1.2, 7.4 and 6.8) showed that drug release in colon could be modulated by optimizing the concentration of polymers in the microspheres. Conclusion: Among all the formulations Fluvastatin sodium microspheres prepared (F20) with Eudragit RS100 in 1:3 ratio shown prolonged release for a period of 12 hours. The obtained results showed the good stability and capability of the system in delaying drug release for a

programmable period and to deliver the drug in the early morning hours when cholesterol synthesis is more prevalent.

Keywords: Fluvastatin sodium, pulsatile, hydrogel plug

INTRODUCTION [1-10]

Pulsatile drug delivery system is the one type of drug delivery system, where the delivery device is capable of releasing drug after predetermined time-delay (i.e., lag time). Pulsatile systems are designed in a manner that the drug is available at the site of action at the right time in the right amount. These systems are beneficial for drugs having high first-pass effect, drugs administered for diseases that follow chrono pharmacological behavior, drugs having specific absorption site in gastro intestinal tract, targeting to colon and cases where night time dosing is required.

A circadian rhythm occurs during hepatic cholesterol synthesis. The cholesterol synthesis is generally higher during the night than during daylight and diurnal synthesis may represent up to 30%–40% of daily cholesterol synthesis. The activity of HMG-CoA reductase has circadian rhythmicity, as it is highest at night. Studies with relationship of concentrations of mevalonic acid in plasma and circadian rhythm to cholesterol synthesis rates in man had suggested that the mevalonic acid concentrations were observed when the patients were at rest, at least 5 hours after the last meal.

[11-13] Fluvastatin is an antilipidemic agent that competitively inhibits hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in cholesterol biosynthesis. Fluvastatin belongs to a class of medications called statins and is used to reduce plasma cholesterol levels and prevent cardiovascular disease3. Its short biological Half-life (3 hours) and low bioavailability (24%-29%) due to extensive first pass metabolism makes it suitable candidate for Pulsatile drug delivery system.

Chronotherapy refers to the administration of drugs at a certain time of the day when efficacy is highest and side-effects are lowest. Fluvastatin is a hypolipidemic drug belonging to the class of pharmaceuticals called "statins". These drugs inhibit the HMG-CoA reductase which catalysis the early and rate limiting step in the biosynthesis of cholesterol. The activity of HMG-CoA reductase has circadian rhythmicity, as it is highest at night. The free cholesterol levels have been reported to be lowest at 2 p.m. to 6 p.m. and peak at 6 a.m. Some marketed

preparations like Lescol, Mevacor, Prachol and Zocor showed that evening dosing frequency of these medications is more effective than morning dosing. On the basis of those studies (market preparations) it is recommended that HMG-CoA reductase inhibitors can be administered between the evening meal and bedtime **[5]**.

The objective of the present work is to formulate a pulsatile drug delivery of Fluvastatin which can be taken before bed time (9 pm) and capable of releasing drug after predetermine time delay (5 hours) and can characterized by proportioning drug concentration in the early morning hours when free cholesterol levels are more prevalent.

PLAN OF WORK

1. To carry out the preformulation studies for the selected drugs.
2. To prepare the microspheres for the selected drugs with the synthetic polymers by solvent evaporation technique.
3. Optimization of best drug: polymers ratio for the prolonged release by carrying out the dissolution studies.
4. To prepare an impermeable capsule body.
5. To prepare the hydrogel plugs by using the natural or synthetic polymers.
6. Optimization of best hydrogel plug by carrying out the evaluation test for hardness, friability, weight variation, lag time and Swelling Index.
7. Development of Pulsatile drug delivery systems by filling microspheres in "0" size capsule.
8. Evaluation of the dosage forms for their physicochemical parameters
9. To carry out the *in-vitro* release study in three dissolution media viz pH 1.2, 7.4 and 6.8.
10. To carry out the stability studies as per ICH guidelines for the optimized formulation.
11. To carry out the x-ray studies for evaluating the gastric emptying and colon retention time
in rabbits.
12. To carry out the *in vivo* pharmacokinetic studies using rabbits.

MATERIALS AND EQUIPMENTS [14]

METHODOLOGY [15-24]

Formulation of Fluvastatin sodium microspheres:

All the microspheres formulations were prepared by emulsion solvent evaporation technique **[22]** and the composition was shown in table 6.17 & 6.18. The effect of various formulation and processing factors on microspheres characteristics were investigated by changing polymer: drug ratio. Weighed amount of Fluvastatin sodium and polymer (Eudragit FS30/ Eudragit RL100) in 1:1 ratio was dissolved

in 10 ml of acetone. The homogeneous drug and polymer solution was then slowly added in a thin stream to 100 ml of liquid paraffin containing 1% surfactant (tween 80/span 80) with constant stirring for 1h. The resulting microspheres were separated by filtration and washed with petroleum ether 3-4 times. The microspheres finally air dried over a period of 12 hrs and stored in a desiccate over fused calcium chloride. In case of 1:1.5, 1:2 and 1:3 core: coat ratios, the corresponding polymer get varied respectively.

Preparation of Cross-Linked Gelatin Capsules [25-30]:

The '0' sized hard gelatin capsules, about 100 in number were taken. The bodies of the capsules were then positioned on a wire mesh. 25 ml of 15% v/v formaldehyde was taken into a desiccator and a pinch of potassium permanganate was added to it to produce formalin vapours. The reaction was done for period of 12 hours. After which the bodies were removed and dried at 50^{o}C for 30 minutes to ensure completion of reaction between gelatin and formaldehyde vapour. The bodies were dried at room temperature to facilitate removal of residual formaldehyde **(Table 4)**.

Preparation of Hydrogel Plug:

Plug for sealing the capsule body was prepared by compressing equal amount of HPMC K100, Carbapol, Na CMC and Methyl Cellulose and lactose using 7mm punches and dies on rotary tablet press **[18]**. The composition of hydrogel plugs was tabulated in 6.12 **(Table 5)**.

Table 1: List of materials used

S.no	Name of the product	Name of the supplier
1	Fluvastatin sodium	Aurobindo Pharma limited; Hyderabad
2	Eudragit FS30D	Evonik India Private Limited.; Mumbai
3	Eudragit RL100	Evonik India Private Limited.; Mumbai
4	Lactose	S. D. Fine-Chem Ltd.; Mumbai
5	Carbopol	S. D. Fine-Chem Ltd.; Mumbai
6	Sodium carboxy methyl cellulose	S. D. Fine-Chem Ltd.; Mumbai
7	HPMC K 100	S. D. Fine-Chem Ltd.; Mumbai
8	Methyl Cellulose	S. D. Fine-Chem Ltd.; Mumbai
9	Sodium Hydroxide	S. D. Fine-Chem Ltd.; Mumbai
10	Potassium dihydrogen ortho phosphate	S. D. Fine-Chem Ltd.; Mumbai
11	Acetone	S. D. Fine-Chem Ltd.; Mumbai
12	Formaldehyde	S. D. Fine-Chem Ltd.; Mumbai
13	Liquid paraffin	S. D. Fine-Chem Ltd.; Mumbai
14	Tween 80	S. D. Fine-Chem Ltd.; Mumbai
15	Span 80	S. D. Fine-Chem Ltd.; Mumbai
16	Petroleum ether	S. D. Fine-Chem Ltd.; Mumbai
17	Cellulose acetate phthalate	S. D. Fine-Chem Ltd.; Mumbai
18	n-dibutyl phthalate	S. D. Fine-Chem Ltd.; Mumbai
19	Ethanol	S. D. Fine-Chem Ltd.; Mumbai

Table 2: List of equipment used

S.no	Name of the equipment	Name of the supplier
1	Electronic balance	Shimadzu Corporation, Japan.
2	pH meter	Digisun Electronics, Mumbai.
3	Dissolution apparatus	Lab India Disso 2000, Chennai.
4	UV/VIS Spectrophotometer	Techcomp, UV 2300
5	Spray dryer	Labultima, Model: LU 222 Advanced
6	Freeze dryer	Modulyod 230
7	Compression machine	Rimek, 10 stationary punching machine
8	Infrared spectrophotometer	Shimadzu Corp, Japan
9	Differential scanning colorimeter	DSC Q20 V24.2 Build 107
10	X-Ray diffraction	Philips PW 1710.
11	Scanning electron microscopy	Model JSM 840A, Jeol, Japan.

Table 3: List of Fluvastatin sodium Microspheres Prepared

Surfactants Used	Polymers Used			
	Eudragit RL100		Eudragit FS30D	
	Formulation Code	Core: Coat	Formulation Code	Core: Coat
SPAN 80	F17	1:1	F25	1:1
	F18	1:1.5	F26	1:1.5
	F19	1:2	F27	1:2
	F20	1:3	F28	1:3
TWEEN 80	F21	1:1	F29	1:1
	F22	1:1.5	F30	1:1.5
	F23	1:2	F31	1:2
	F24	1:3	F32	1:3

Table 4: Physical characteristics of empty gelatin capsules with or without Formalin treatment

Type of capsules	Length (mm)		External diameter (mm)		Thickness (mm)		Weight of empty capsule (mg)
	Cap	Body	Cap	Body	Cap	Body	
Untreated	10.7±0.1	17.7±0.2	7.3±0.2	6.7±0.1	0.2±0.1	0.2±0.1	93.7±2.4
Formalin Treated	-	18.1±0.2	-	7.0±0.1		0.2±0.1	94.5±2.1

Table 5: Composition of Hydrogel Plugs for Fluvastatin sodium

Hydrogel Plug Code	Composition (1:1)	Quantity (mg)
HP1	Carbopol: lactose	100
HP2	Na CMC: lactose	100
HP3	HPMC K 100: lactose	100
HP4	Methyl Cellulose: lactose	100

Evaluation characteristics of hydrogel plugs:

Table 6: Evaluation characteristics of hydrogel plugs

Hydrogel Plug Code	Weight (mg)	Thickness (mm)	Hardness (kg/cm^2)	Lag time (hrs)
HP1	100±1.3	3.45± 0.11	4.8±0.03	5±0.01
HP2	100±1.2	3.42±0.13	4.6±0.02	4.5±0.02
HP3	100±1.4	3.41±0.07	4.3±0.04	4.1±0.02
HP4	100±1.1	3.42±0.09	4.1±0.01	3.0±0.01

Characterization of Microspheres [24]

Table 7: Flow Properties of Fluvastatin sodium microspheres prepared Eudragit RL100 in different ratios by employing Span 80 as surfactant (n=3)

Formulation	Angle of repose (θ)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's Index (%)	Hausner's Ratio
F17	27.64	0.867±0.08	1.057±0.08	15.86±0.04	1.20±0.03
F18	25.73	0.886±0.04	1.063±0.04	14.18±0.02	1.20±0.06
F19	23.44	0.897±0.03	1.058±0.07	13.13±0.06	1.18±0.04
F20	20.39	0.913±0.07	1.059±0.02	12.32±0.04	1.16±0.07

Table 8: Percentage yield, mean particle size, drug content and % entrapment efficiency of Fluvastatin sodium microspheres prepared with Eudragit RL100 in different ratios by employing Span 80 as surfactant(n=3)

Formulation	% Yield	Average Particle Size(µm)	Drug content	% Entrapment Efficiency
F17	91.68	132.55±0.07	44.96±0.02	89.92±0.09
F18	93.89	146.36±0.05	37.94±0.04	94.85±0.06
F19	95.13	154.44±0.03	31.82±0.05	96.42±0.04
F20	96.47	168.47±0.06	24.46±0.03	97.84±0.07

Table 9: *In-vitro* Dissolution data of pulsatile device consisting of Fluvastatin sodium microspheres prepared with Eudragit RS100in different ratios by employing Span 80 as surfactant (n=3)

Time (hrs)	Percentage of drug release			
	F17	F18	F19	F20
0.5	0	0	0	0
1	0	0	0	0
1.5	0	0	0	0
2	0	0	0	0
2.5	0	0	0	0
3	0	0	0	0
3.5	0	0	0	0
4	0	0	0	0
4.5	0	0	0	0
5	0	0	0	0
5.5	7.82±0.08	4.56±0.06	3.48±0.11	2.94±0.15
6	13.56±0.11	8.93±0.09	7.57±0.15	7.02±0.08
6.5	19.34±0.06	14.41±0.11	12.22±0.06	11.68±0.15
7	24.6±0.09	19.64±0.08	17.45±0.11	15.81±0.11
7.5	29.89±0.15	25.45±0.12	22.16±0.08	17.25±0.06
8	35.75±0.13	31.29±0.15	28.25±0.09	21.42±0.11
8.5	41.38±0.12	36.89±0.09	34.64±0.06	25.61±0.09
9	47.03±0.09	42.52±0.11	40.8±0.08	29.82±0.11
9.5	52.71±0.07	47.63±0.08	45.1±0.09	34.05±0.08
10	57.88±0.11	53.05±0.09	49.91±0.15	38.31±0.06
10.5	64.71±0.08	60.12±0.13	53.21±0.12	42.58±0.11
11	70.21±0.13	65.63±0.09	60.55±0.08	46.88±0.06
11.5	75.75±0.12	71.38±0.08	70.19±0.06	51.21±0.15
12	81.85±0.09	77.46±0.06	74.95±0.11	55.55±0.09
12.5	87.17±0.08	82.22±0.13	79.65±0.08	59.92±0.15
13	93.34±0.11	89.17±0.09	83.65±0.13	64.31±0.08
13.5	99.26±0.06	95.35±0.08	87.36±0.12	68.73±0.11
14	--	99.38±0.11	91.83±0.08	73.16±0.06
14.5	--	--	95.87±0.11	77.62±0.15
15	--	--	99.61±0.08	82.1±0.09
15.5	--	--	--	86.61±0.11
16	--	--	--	91.13±0.08
16.5	--	--	--	95.41±0.09
17	--	--	--	99.98±0.12

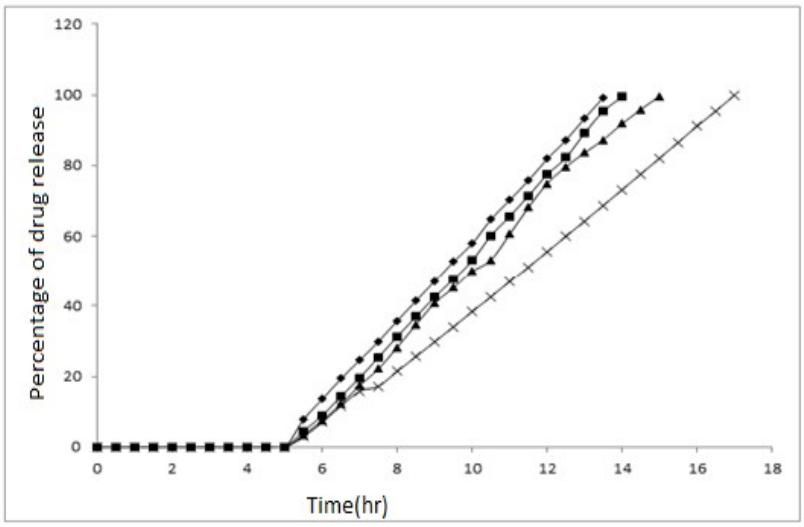

Figure 1: Comparative *In-vitro* drug release profile plot of pulsatile device consisting of Fluvastatin sodium microspheres prepared with Eudragit RL100 in different ratios by employing Span 80 as surfactant

F17(-■-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:1 ratio**
F18(-♦-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:1.5 ratio**
F19(-▲-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:2ratio**
F20(-×-) Fluvastatin **sodium microspheres prepared with Eudragit RL100 in 1:3 ratio**

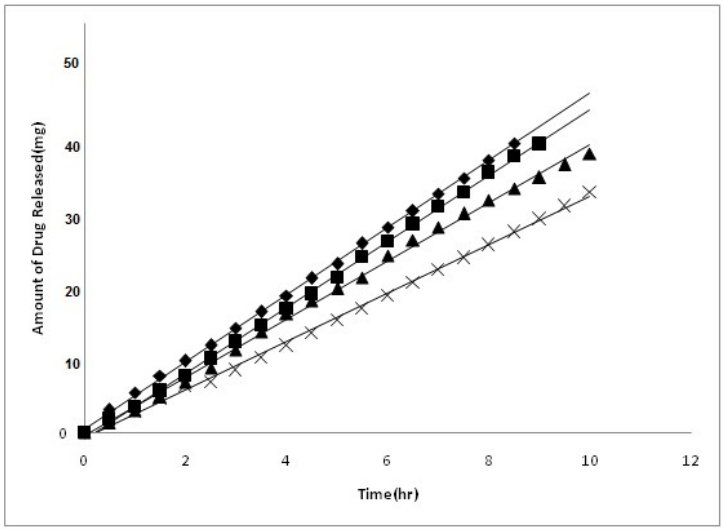

Figure 2: Comparative Zero order plot of pulsatile device consisting of Fluvastatin sodium microspheres prepared with Eudragit RL100 in different ratios by employing Span 80 as surfactant

F17(-■-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:1 ratio**
F18(-♦-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:1.5 ratio**
F19(-▲-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:2ratio**
F20(-×-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:3 ratio**

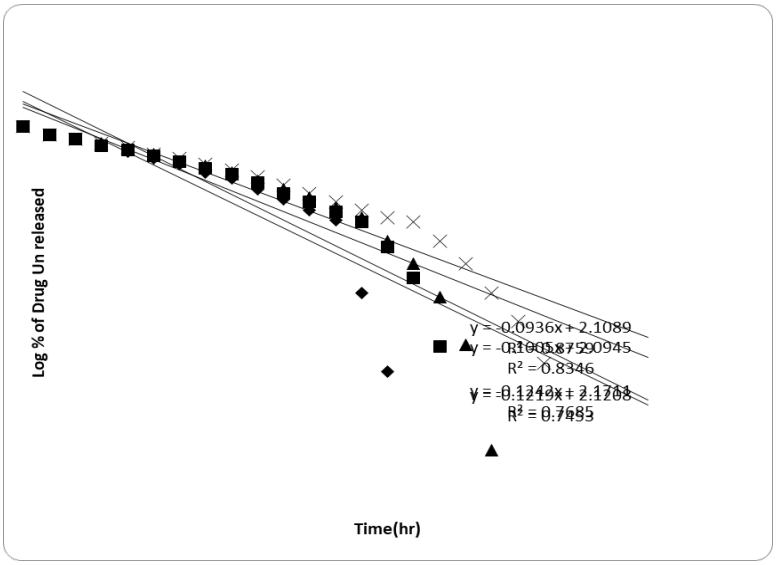

Figure 3: Comparative first order plot of pulsatile device consisting of Fluvastatin sodium microspheres prepared with Eudragit RL100 in different ratios by employing Span 80 as surfactant

F17(-■-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:1 ratio**
F18(-♦-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:1.5 ratio**
F19(-▲-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:2ratio**
F20(-×-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:3 ratio**

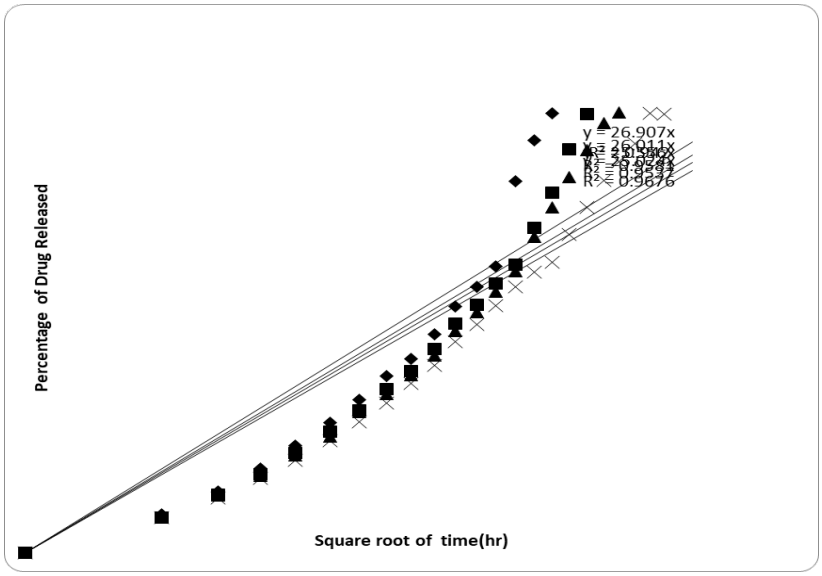

Figure 4: Comparative Higuchi plots of pulsatile device consisting of Fluvastatin sodium microspheres prepared with Eudragit RS100in different ratios by employing Span 80 as surfactant

F17(-■-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:1 ratio**
F18(-♦-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:1.5 ratio**
F19(-▲-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:2ratio**
F20(-×-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:3 ratio**

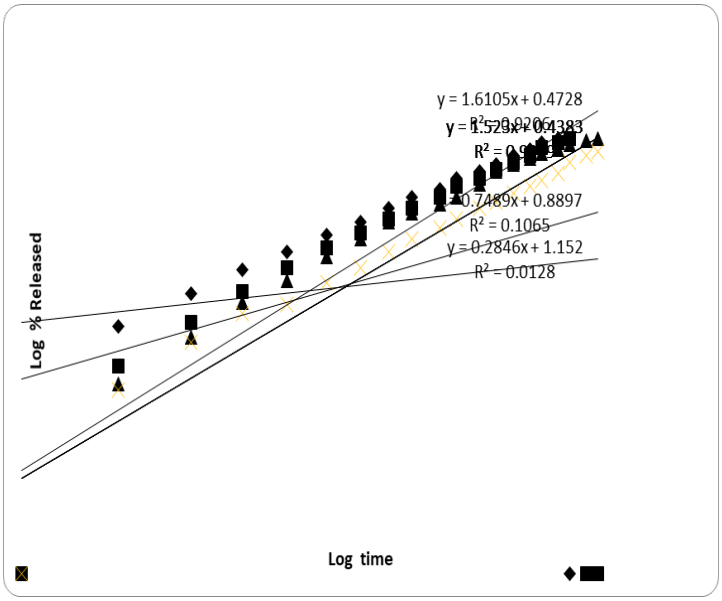

Figure 5: Comparative Peppas plots of pulsatile device consisting of Fluvastatin sodium microspheres prepared with Eudragit RS100in different ratios by employing Span 80 as surfactant

F17(-■-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:1 ratio**
F18(-♦-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:1.5 ratio**
F19(-▲-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:2ratio**
F20(-×-) **Fluvastatin sodium microspheres prepared with Eudragit RL100 in 1:3 ratio**

Table 10: *In-vitro* dissolution kinetics parameters of pulsatile device consisting of Fluvastatin sodium microspheres prepared with Eudragit RL100 in different ratios by employing Span 80 as surfactant

Formulation	Correlation coefficient (R^2)				Release kinetics			Diffusion Exponent value(n)
	Zero order	First order	Higuchi	Peppas	K_o (mg/hr)	T_{50} (hr)	T_{90} (hr)	
F17	0.9996	0.8056	0.9303	0.9990	4.72	4.23	7.62	0.9112
F18	0.9989	0.7971	0.9107	0.9998	4.4	4.5	8.18	1.082
F19	0.9986	0.8073	0.9214	0.9985	3.84	5.1	9.2	1.108
F20	0.9983	0.6380	0.9052	0.9991	3.28	6.09	10.97	1.182

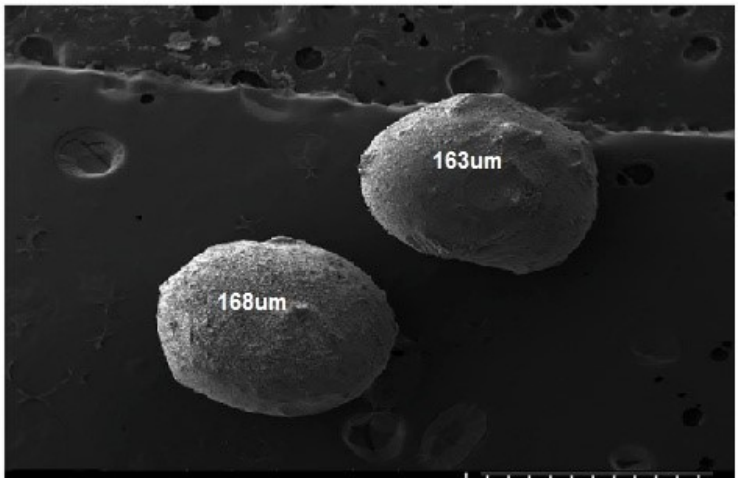

Figure 6: Scanning Electron Microscope Photograph of Fluvastatin sodium microspheres

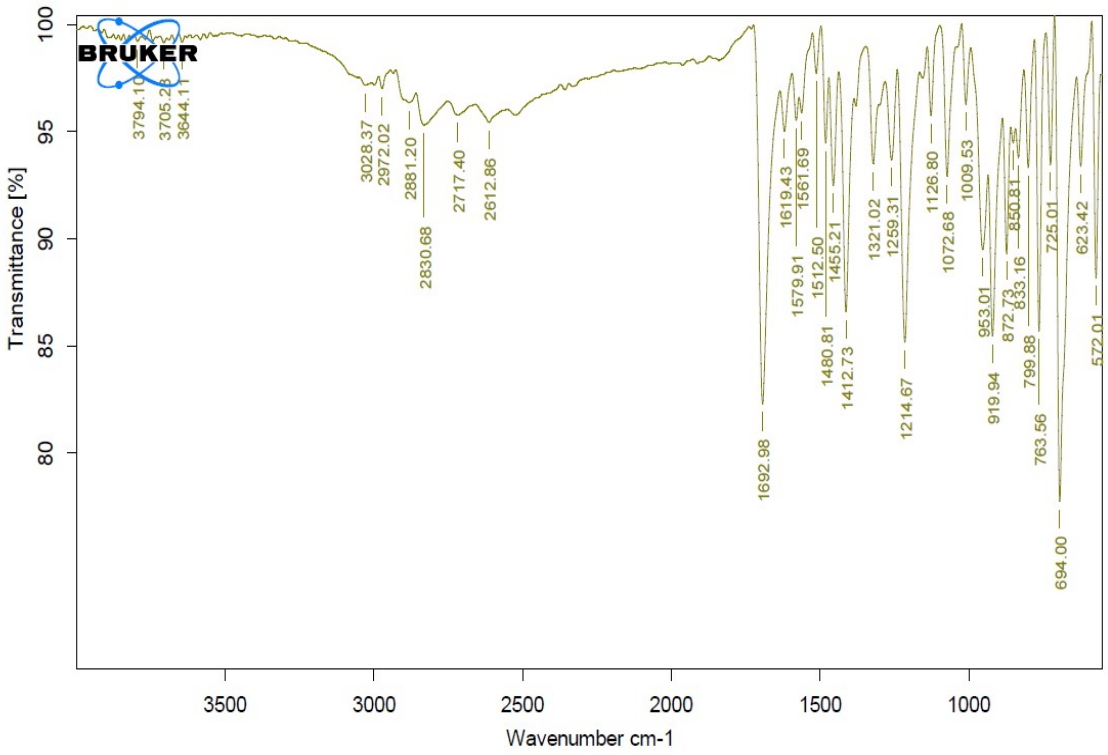

Figure 7: FTIR spectrum of optimized formulation (F20) of Fluvastatin sodium

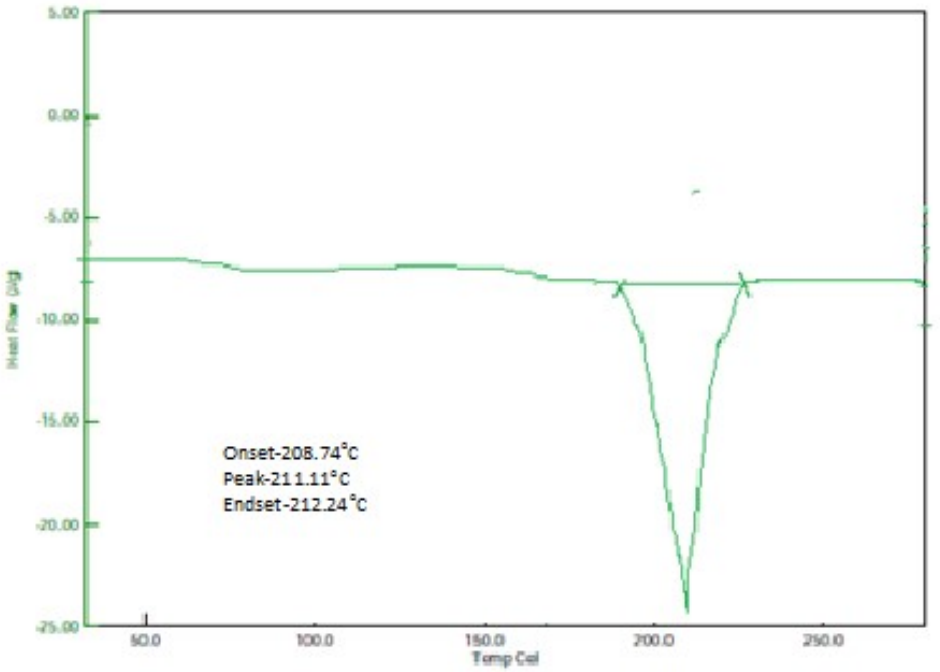

Figure 8: DSC thermo gram of optimized formulation (F20) Fluvastatin sodium

Stability Studies [32, 33]

Table 11: Drug Content, Encapsulation Efficiency and Dissolution Kinetics of Fluvastatin sodium Microspheres stored at 25±2^0 C/60±5% RH and 40±2^0 C/75±5% RH

Storage conditions	Time interval	Drug Content	Encapsulation Efficiency	Release Rate Constant (mg/hr) K_o	$t_{50\%}$	$t_{90\%}$
25±2^0 C/ 60±5% RH	1st month	24.40	97.60	3.28	6.09	10.97
	2nd month	24.38	97.52	3.28	6.09	10.97
	3rd month	24.35	97.40	3.28	6.09	10.97
40±2^0 C/ 75±5% RH	1st month	24.37	97.48	3.28	6.09	10.97
	2nd month	24.34	97.36	3.28	6.09	10.97
	3rd month	24.32	97.28	3.28	6.09	10.97

Tabl 12: Invitro dissolution data of Fluvastatin sodium Microspheres stored at 25±2^0 C/60±5% RH and 40±2^0 C/75±5% RH after stability studies

S. No	Time (h)	Initial	Percentage of Fluvastatin sodium Released ($\bar{x}$ ± sd)					
			25±2^0 C/60±5% RH			40±2^0 C/75±5% RH		
			1st month	2nd month	3rd month	1st month	2nd month	3rd month
1.	0	0	0	0	0	0	0	0
2.	5.5	2.94±0.15	2.84±0.09	2.76±0.13	2.67±0.06	2.72±0.13	2.64±0.08	2.55±0.05
3.	6	7.02±0.08	6.93±0.14	6.86±0.06	6.75±0.13	6.81±0.11	6.73±0.09	6.66±0.08
4.	6.5	11.68±0.15	11.57±0.10	11.48±0.08	11.40±0.09	11.45±0.14	11.39±0.11	11.13±0.05
5.	7	15.81±0.11	15.72±0.11	15.64±0.05	15.64±0.05	15.57±0.06	15.43±0.10	15.36±0.11
6.	7.5	17.25±0.06	17.16±0.15	17.07±0.09	16.98±0.09	17.06±0.05	16.97±0.11	16.85±0.10
7.	8	21.42±0.11	21.33±0.13	21.26±0.11	21.14±0.15	21.19±0.13	21.12±0.06	21.04±0.13
8.	8.5	25.61±0.09	25.52±0.09	24.53±0.13	25.33±0.09	25.39±0.09	25.32±0.08	25.52±0.11
9.	9	29.82±0.11	29.73±0.06	29.64±0.15	29.54±0.06	29.59±0.15	29.53±0.13	29.46±0.09
10.	9.5	34.05±0.08	33.96±0.08	33.87±0.06	33.77±0.08	33.82±0.13	33.74±0.09	33.65±0.08
11.	10	38.31±0.06	38.22±0.10	38.13±0.09	38.02±0.13	38.09±0.10	38.02±0.15	37.94±0.06
12.	10.5	42.58±0.11	42.49±0.08	42.40±0.05	42.30±0.06	42.37±0.05	42.31±0.10	42.27±0.09
13.	11	46.88±0.06	46.80±0.11	40.71±0.06	46.60±0.11	46.66±0.11	46.59±0.06	46.43±0.11
14.	11.5	51.21±0.15	51.12±0.14	51.03±0.05	50.92±0.10	50.98±0.13	50.91±0.08	50.83±0.10
15.	12	55.55±0.09	55.47±0.09	55.39±0.11	55.26±0.15	55.32±0.05	55.26±0.06	54.97±0.13
16.	12.5	59.92±0.15	59.84±0.13	59.76±0.08	59.63±0.09	59.69±0.15	59.61±0.13	59.54±0.15
17.	13	64.31±0.08	64.22±0.11	64.13±0.10	64.02±0.06	64.09±0.09	64.03±0.11	63.94±0.10
18.	13.5	68.73±0.11	68.64±0.14	68.54±0.13	68.45±0.11	68.51±0.06	68.44±0.08	68.37±0.11
19.	14	73.16±0.06	73.08±0.12	72.99±0.09	72.90±0.09	72.99±0.08	72.92±0.09	72.57±0.13
20.	14.5	77.62±0.15	77.54±0.15	77.43±0.06	77.32±0.06	77.37±0.09	77.31±0.11	77.43±0.15
21	15	82.1±0.09	82.07±0.13	81.91±0.08	81.80±0.08	81.86±0.05	81.80±0.09	81.73±0.09
22	15.5	86.61±0.11	86.52±0.10	86.44±0.15	86.31±0.13	86.38±0.06	86.32±0.10	86.26±0.10
23	16	91.13±0.08	91.07±0.11	90.95±0.10	90.83±0.10	90.89±0.10	90.83±0.13	90.76±0.13
24	16.5	95.41±0.09	95.33±0.13	95.25±0.09	95.16±0.14	95.21±0.15	95.16±0.09	95.08±0.15
25	17	99.98±0.12	99.90±0.10	99.82±0.11	99.72±0.13	99.79±0.09	99.73±0.06	99.66±0.08

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