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Research Article

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Formulation and evaluation of pulsatile drug delivery of fluvastatin sodium

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ABSTRACT

In the present study, an attempt was made to develop the pulsatile drug delivery of Fluvastatin sodiumto reduce plasma cholesterol levels and to prevent cardiovascular diseases.Formaldehyde treated Capsule bodies were used for the preparation of pulsincaps. It was sealed with unhardened cap of the capsule. The microspheres were prepared by emulsion solvent evaporation technique. Hydrogel plug (karaya gum and lactose in 1:1 ratio) having 4.5kg/cm^{2}hardness and 100 mg weight was placed in the capsule opening and found that it was satisfactory to retard the drug release in small intestinal fluid and to eject out the plug in colonic fluid and releasing the microspheres into colonic fluid after a lag time criterion of 5 hours.The sealed capsules were completely coated by dip coating method with 5% cellulose acetate phthalate to prevent variable gastric emptying. Optimized microsphere formulations were selected based on dissolution studies. Dissolution studies of pulsatile capsule device in media with different p^H (1.2, 7.4 and6.8) showed that drug release in colon could be modulated by optimizing the concentration of polymers in the microspheres. Drug–polymer interaction studies indicated no interaction in between the drug and the polymer.Among all the formulations Fluvastatin sodium microspheres prepared with Ethyl cellulose in 1:3 ratio,span 80 as surfactant shown prolonged release for a period of 11 hours.The obtained results showed the capability of the system in delaying drug release for a programmable period of time and to deliver the drug in the early morning hours when cholesterol synthesis are more prevalent.

Keywords: Fluvastatin sodium, Pulsatile, Hydrogel Plug,Lactose

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INTRODUCTION

Chronopharmaceutics is intended to deliver drugs at a time that preferably counterparts the biological requisite of a specified disease treatment or prevention [1]. Cholesterol biosynthesis follows a circadian rhythm. 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. This is due to the higher activity HMG-CoA reductase at midnight[2].Chronotherapy with HMG-CoA reductase inhibitors have suggested that evening dosing could be more effective than morning dosing[3]. The activity of HMG-CoA reductase has circadian rhythm city, 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[4].

Fluvastatin sodium is an antilipidemic agent that competitively inhibits HMG-CoA reductase. It belongs to a class of medications called statins and is used to reduce plasma cholesterol levels and prevent cardiovascular diseases[5]. Its short biological half life (3 hours) and low bioavailability(24%-29%) makes it appropriate candidate for pulsatile drug delivery system.Hence the objective of the present work is to formulate a pulsatile drug delivery of Fluvastatin sodium which can be taken before bed time (9 pm) and capable of releasing drug after predetermine time delay(5 hours) and can be characterized by proportioning drug concentration in the early morning hours when free cholesterol levels are more prevalent.

EXPERIMENTAL SECTION

Materials and methods

Materials: Fluvastatin sodium was a gratis sample obtained from Ranbaxy Lab. Ltd. (India).Ethyl cellulose was obtained from Rohm GmbH & Co. KG. (Darmstadt, Germany).Karaya gum,Kondagogu gum,Xanthum gum and Guar gum purchased from Yarrow chem. Products, Mumbai. All reagents used were of analytical-reagent grade.

Preparation of Cross-Linked Gelatin Capsules

The '0' sized hard gelatin capsules (approximately 100 in number) were taken. The bodies of the capsules were then placed on wire mesh, which was kept in a desiccator. An aliquot of 25ml of 15% v/v formaldehyde was taken into a bottom of desiccator and a pinch of potassium permanganate was added to it to generate formalin vapours. The reaction was carried out for 12 hours. After which the bodies were removed and dried at 50^0C 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 [6] .These capsule bodies were capped with untreated caps and stored in air tight container.

Preparation of Hydrogel Plug

Plug for sealing the capsule body was prepared by compressing equal amount of karaya gum/kondagogu gum/xanthum gum/guar gum and lactose using7 mm punches and dies on rotary tablet press[7].

Preparation of microspheres

All the microspheres formulations were prepared by emulsion solvent evaporation technique[8]and the composition was shown in table 1. 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 Ethyl cellulose in 1:1 ratio were dissolved in 10ml of acetone. The homogeneous drug and polymer organic solution was then slowly added in a thin stream to 100ml 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. The microspheres finally air dried over a period of 12 hrs and stored in a desiccator.Incase of 1:1.5, 1:2 and 1:3 core:coat ratios, the corresponding polymer get varied respectively.

Designing of Pulsincap

The Pulsincap was designed by filling the microspheres equivalent to 40mg of Fluvastatin sodium into the formaldehyde treated bodies by hand filling.The capsules containing the microspheres were then plugged with optimized hydrogel plug. The joint of the capsule body and cap was sealed with a small amount of the 5% ethyl cellulose ethanolic solution[9]. The sealed capsules were completely coated by dip coating method with 5% cellulose acetate phthalate in 5:5 (v/v) mixture of acetone: ethanol plasticized with n-dibutyl phthalate (0.75%), to prevent variable gastric emptying. Coating was repeated until an 8–12% increase in weight is obtained. Percentage weight gain of the capsules before and after coating was determined.

Physicochemical Characterization of Hydrogel Plug

Hydrogel Plugs were studied for hardness, friability, weight variation and lag time[10] .

Drug content uniformity

Then encapsulated microspheres equivalent to 40mg of Fluvastatin sodium were taken into mortar and grounded with the help of pestle. The grounded power mixture was dissolved in 6.8 p^H buffer, filtered and estimated spectrophotometrically at 304 nm[11].

***In vitro* release profile of pulsatile capsule**

Dissolution studies were carried out by using USP XXIII dissolution test apparatus (paddle method). Capsule was tied to paddle with a cotton thread so that the capsule should be immersed completely in dissolution media but not float. In order to simulate the pH changes along the GI tract, three dissolution media with p^H 1.2, 7.4 and 6.8 were sequentially used referred to as sequential p^H change method. When performing experiments, the pH 1.2 medium was first used for 2 hours (since the average gastric emptying time in stomach is 2 hrs), then removed and the fresh pH 7.4 phosphate buffer saline (PBS) was added. After 3 hours (average small intestinal transit time is 3 hours), the medium was removed and colonic fluid p^H 6.8 buffer was added for subsequent hours. Nine hundred milliliters of the dissolution medium was used at each time. Rotation speed was 100 rpm and temperature was maintained at 37±0.5^{o}C. Five milliliters of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed at 304 nm, by UV absorption spectroscopy and the cumulative percentage release was calculated over the sampling times[12].

IR spectral studies

The IR Spectra for the formulation, pure drugs and excipients were recorded on JASCO FT-Infra Red Spectrophotometer using KBr pellet technique at the resolution rate of 4 cm^{-1}. Spectrum was integrated in transmittance mode at the wave number range 380 to 4368 cm^{-1}.

Differential scanning calorimetry (DSC) studies: The pure drug and optimized formulation were subjected to differential scanning calorimeter equipped with an intra cooler (NETZSCH, Japan.). Indium/zinc standards were used to calibrate the DSC temperature and enthalpy scale. The sample were sealed in aluminum pans and heated at a constant rate 20°C/min over a temperature range of 20-250°C. An inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 ml/min.

RESULTS AND DISCUSSION

Strategy of the Pulsincap dosage form: Pulsincap dosage form was a capsule which consists of a water insoluble body and a water soluble cap. The microspheres were sealed within the capsule body by means of a hydrogel plug. When the pulsing cap was swallowed, the water soluble cap dissolves in the gastric juice and the exposed hydrogel plug begins to swell.At predetermined time after ingestion, the swollen plug was ejected out and the encapsulated drug formulation was then released into the colon, where it is dissolved and then absorbed into blood stream. In the present study, capsule bodies which were hardened with formaldehyde treatment for 12 hours were used for the preparation of pulsing caps. It was sealed with unhardened cap of the capsule. The microspheres were prepared by emulsion solvent evaporation technique .The method employed gave discrete, spherical, non-sticky and free flowing microspheres. The formation of a stable emulsion in the early stages is important if discrete microspheres are to be isolated. An optimal concentration of emulsifier is required to produce the finest stable dispersion. Below optimal concentration the dispersed globules/droplets tend to fuse and produce larger globules because of insufficient lowering in interfacial tension, while above the optimal concentration no significant decrease in particle size is observed, because a high amount of emulsifying agent increases the viscosity of the dispersion medium. The optimal concentration of surfactant was found to be 1.0%. Microscopic examination of the formulations revealed that the microspheres were spherical and appeared as aggregates or discrete particles.

Evaluation of the microspheres

All the formulations offered good flow properties.The particle size of the microspheres ranged between 132.55and 178.46μm. The use of the surfactant permits the remarkable reduction in the size of the microspheres as the result of decrease in the interfacial tension. All formulations had a narrow particle size distribution.The mean particle size of microspheres was influenced by the type of surfactant used and polymer proportion in the formulation. The mean size increased with increasing polymer concentration.It would appear that increasing polymer concentration produced a significant increase in viscosity of the internal phase, thus leading to an increase of emulsion droplet size and finally a higher microspheres size.Microspheres were developed with 1:1, 1:1.5, 1:2, 1:3 ratios of core:coat to determine the affect of coating material concentration on the release rate of Fluvastatin sodium. These microspheres were characterized for Drug content and % Encapsulation Efficiency. The results are given in Table 2. The technique also showed good entrapment efficiency. Two types of surfactants used have an influence on the particle size distribution of the microspheres. The hydrophobic surfactant Span 80 (Sorbitan monooleate, HLB 4.3) is found to produce smaller particle size microspheres compared to hydrophilic surfactant Tween 80 (Polyoxyethylene 20

sorbitanmonooleate, HLB 14.9). Span 80 is oil soluble and produces a stable emulsion when the dispersion medium is oil. This may explain why smaller particle sizes are obtained with span 80.

Table:1- Formulation list of Fluvastatin sodium microspheres Prepared

Surfactants Used	Ethyl cellulose	
	Formulation Code	Core: Coat
SPAN 80	F-1	1:1
	F-2	1:1.5
	F-3	1:2
	F-4	1:3
TWEEN 80	F-5	1
	F-6	1:1.5
	F-7	1:2
	F-8	1:3

Table 2: Evaluation data of Fluvastatin sodium microspheres prepared with Ethyl cellulose in different ratios by employing different surfactants(Mean±S.D)

Formulation	Angle of Repose	Bulk Density (g/cm^3)	Carr's Index	Hausner's Ratio	Average Particle Size (µm)	Drug Content	% Encapsulation Efficiency
F-1	27.64±0.04	0.514±0.06	15.87±0.08	1.188±0.08	139.44±0.06	46.56±0.02	93.12±0.05
F-2	26.93±0.03	0.519±0.05	15.49±0.07	1.183±0.09	156.47±0.02	37.16±0.06	92.90±0.04
F-3	26.10±0.05	0.521±0.07	15.42±0.09	1.182±0.09	168.39±0.08	31.25±0.07	94.39±0.03
F-4	26.75±0.09	0.531±0.08	15.31±0.07	1.181±0.07	179.33±0.09	23.47±0.09	93.88±0.09
F-5	26.52±0.02	0.514±0.05	15.87±0.06	1.188±0.06	135.13±0.02	44.13±0.08	88.26±0.04
F-6	26.14±0.03	0.522±0.04	15.67±0.05	1.185±0.09	157.24±0.04	37.34±0.06	93.35±0.06
F-7	25.71±0.07	0.524±0.03	15.48±0.09	1.183±0.09	168.49±0.09	32.23±0.09	97.66±0.08
F-8	26.71±0.06	0.527±0.02	15.27±0.08	1.180±0.03	179.18±0.01	23.41±0.06	93.64±0.03

**Each sample was analyzed in triplicate (n=3)*

Table - 3 Evaluation characteristics of hydrogel plugs prepared with various natural polymers(Mean±S.D)

Hydrogel Plug code	Composition (1:1)	Weight (mg)	Thickness (mm)	Hardness (kg/cm2)	Lag time (hours)
HP1	Karayagum:lactose	100±1.3	3.45± 0.11	4.8±0.03	5±0.01
HP2	Kondagogugum:lactose	100±1.2	3.42±0.13	4.6±0.02	4.5±0.02
HP3	Xanthan gum:lactose	100±1.4	3.41±0.07	4.3±0.04	4±0.02
HP4	Guargum: lactose	100±1.1	3.42±0.09	4.1±0.01	3.5±0.01

**Each sample was analyzed in triplicate (n=3)*

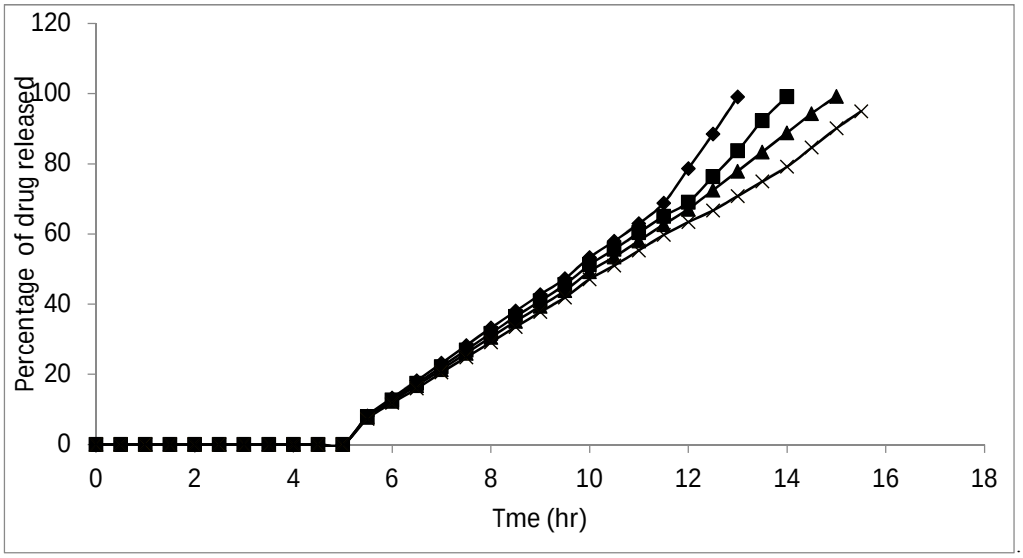
Table-4 In-vitro dissolution kinetics parameters of Fluvastatin sodium microspheres prepared with ethylcellulose in different ratios by employing different surfactants

Formulation	Correlation coefficient				Release kinetics			Diffusion Exponent value(n)
	Zero order	First order	Higuchi	Peppas	K_o (mg/hr)	T_{50} (hr)	T_{90} (hr)	
F1	0.9926	0.7407	0.9089	0.9941	4.44	4.5	8	0.8882
F2	0.9968	0.7705	0.9167	0.9960	4.16	4.8	8.6	0.8804
F3	0.9990	0.7991	0.9278	0.9971	3.92	5.2	9.2	0.8750
F4	0.9983	0.8085	0.9352	0.9983	3.63	5.5	10	0.8651
F5	0.9862	0.7245	0.8952	0.9902	4.87	4.1	7.4	0.8956
F6	0.9940	0.7326	0.9117	0.9948	4.34	4.6	8.3	0.8848
F7	0.9975	0.7683	0.9178	0.9946	4.16	4.8	8.7	0.8778
F8	0.9979	0.7709	0.9315	0.9968	3.70	5.4	9.7	0.8500

Evaluation of the Hydrogel Plug

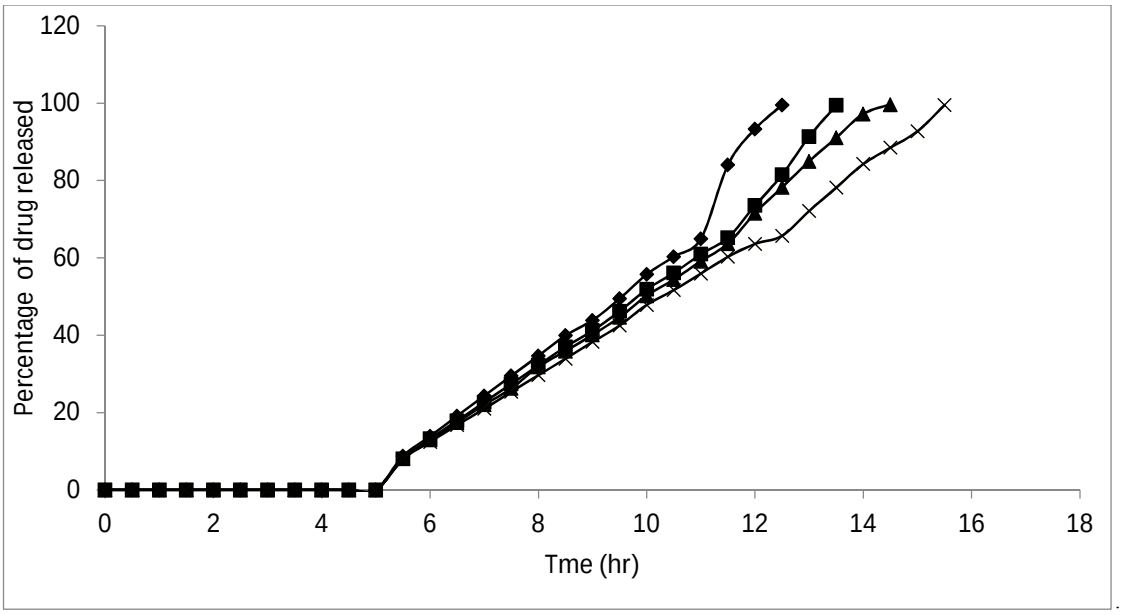
Hydrogel Plugs were evaluated for hardness, friability, weight variation and lag time and the results were shown in Table 3. The formulations fitted with the various hydrogel plugs HP1,HP2, HP3, HP4 shown 0.01% , 5.23% , 13.23 % and 17.45 % of drug release respectively at the end of 5th hour . It was observed that 100 mg hydrogel plug (karaya gum and lactose in 1:1 ratio) having 4.5kg/cm^2 hardness was satisfactory to retard the drug release in small intestinal fluid and to eject out the plug in colonic fluid and releasing the microspheres in to colonic fluid. This suggested that the lag time could also be adjusted and influenced by the plug composition**.**

Figure 1: Comparative *In-vitro* drug release profiles plot of fluvastatin sodium from microspheres prepared with Ethyl cellulose in different ratios by employing span 80 as surfactant



F1(-■-)**Fluvastatin sodium microspheres prepared with Ethyl cellulose in 1:1 ratio**
F2(-♦-)**Fluvastatin sodium microspheres prepared with Ethyl cellulose in 1:1.5 ratio**
F3(-▲-)**Fluvastatin sodium microspheres prepared with Ethyl cellulose in 1:2ratio**
F4(×-) **Fluvastatin sodium microspheres prepared with Ethyl cellulose in 1:3 ratio**

Figure 2: Comparative *In-vitro* drug release profiles plot of fluvastatin sodium from microspheres prepared with Ethyl cellulose in different ratios by employing Tween 80 as surfactant



F5(-■-)**Fluvastatin sodium microspheres prepared with Ethyl cellulose in 1:1 ratio**
F6(-♦-)**Fluvastatin sodium microspheres prepared with Ethyl cellulose in 1:1.5 ratio**
F7(-▲-)**Fluvastatin sodium microspheres prepared with Ethyl cellulose in 1:2ratio**
F8(-×-) **Fluvastatin sodium microspheres prepared with Ethyl cellulose in 1:3 ratio**

Dissolution studies of Pulsin caps

During dissolution studies, it was observed that, the enteric coat of the cellulose acetate phthalate was intact for 2 hours in pH 1.2, but dissolved in intestinal pH, leaving the soluble cap of capsule, which also dissolved in pH 7.4,

then the exposed polymer plug absorbed the surrounding fluid, swelled and released the drug through the swollen microspheres. After complete wetting of the plug, it formed a soft mass, which was then easily ejected out of the capsule body; releasing the microspheres into simulated colonic fluid (pH 6.8 phosphate buffer). From the *In-vitro* release studies of device, it was observed that with all formulation, there was absolutely no drug release in simulated gastric fluid (acidic pH 1.2) for 2 hours and in simulated intestinal fluid (pH 7.4 phosphate buffer). Burst effect was found in colonic medium (pH 6.8 phosphate buffer).

Figure 3: FTIR spectrum of Fluvastatin sodium

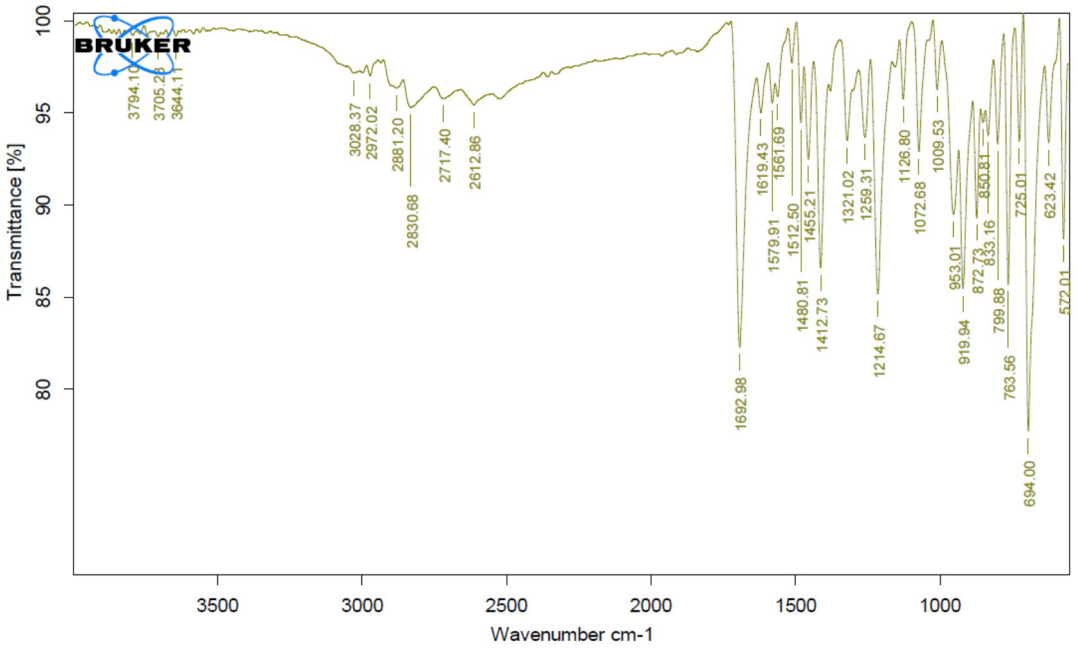


Figure 4 : FTIR spectrum of optimized formulation of Fluvastatin sodium

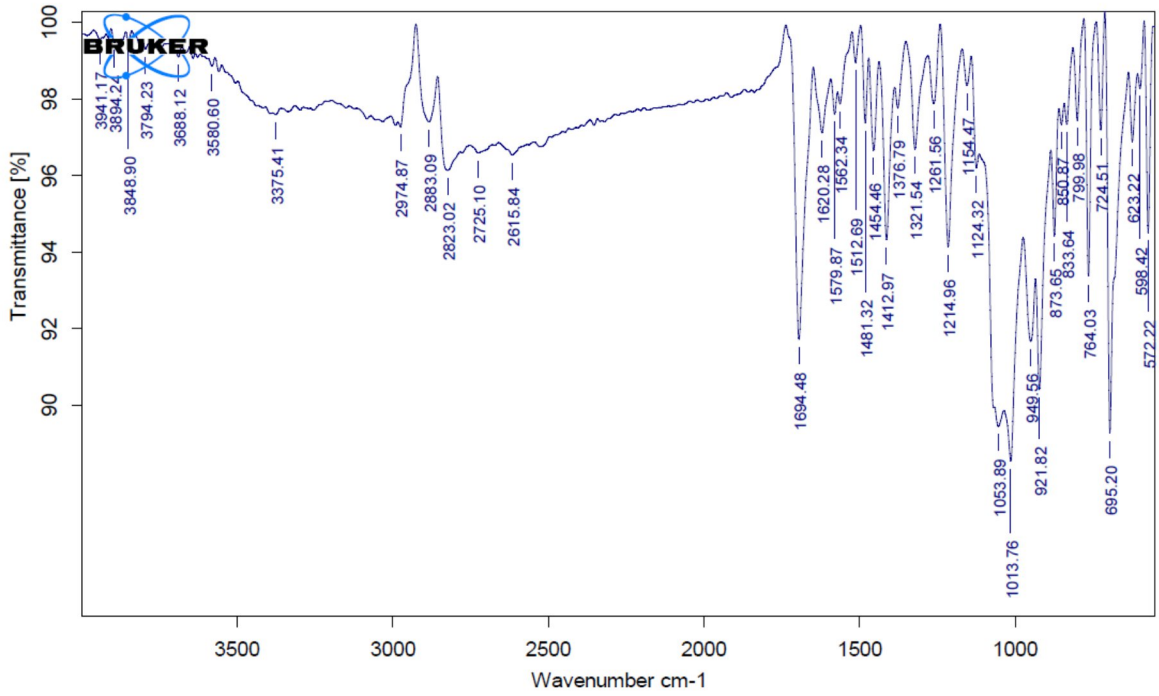


Figure 5: DSC thermo gram of Fluvastatin sodium

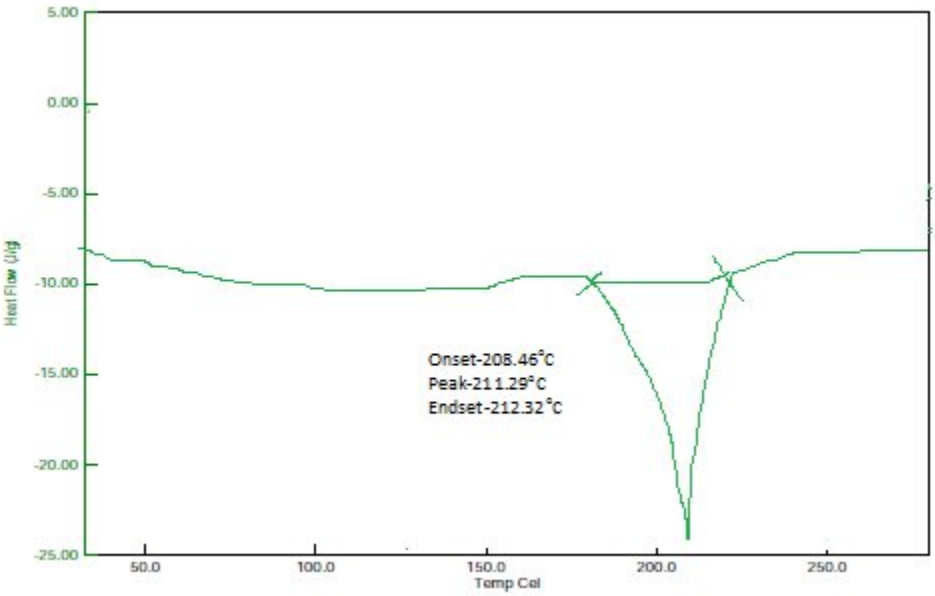
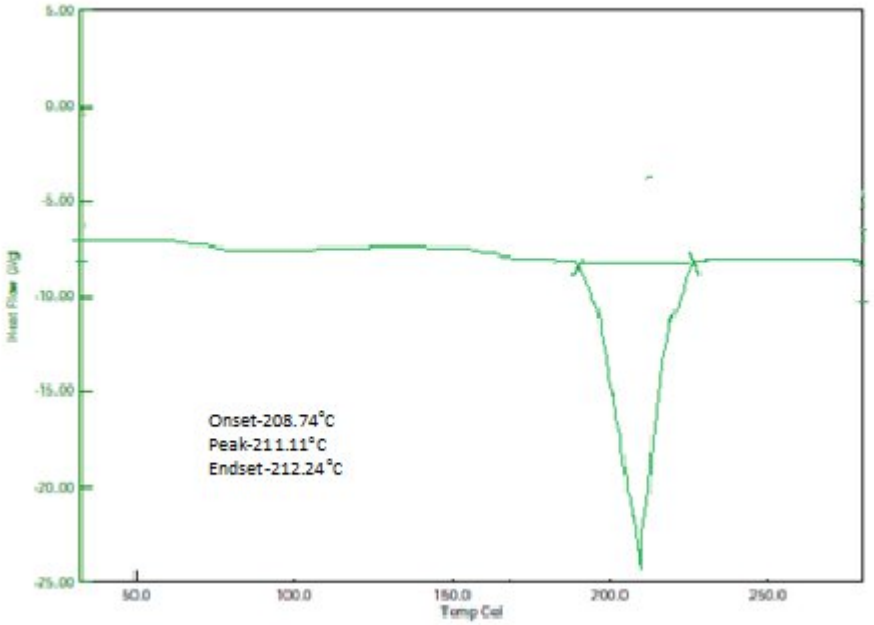


Figure 6: DSC thermo gram of optimized formulation of Fluvastatin sodium



Pulsin caps loaded with microspheres prepared with Fluvastatin sodium and Ethyl cellulose in 1:1,1:1.5 , 1:2 and 1:3 ratios by employing span 80 as surfactant shown controlled drug release for a period of 8 hours (5^{th} hour to 13^{th} hour), 9 hours (5^{th} hour to 14^{th}hour) and 10 hours(5^{th} hour to 15^{th} hour), 11hours(5^{th} hour to 16^{th} hour) respectively and are shown in figure 1.

Pulsin caps loaded with microspheres prepared with Fluvastatin sodium and ethyl cellulose in 1:1,1:1.5,1:2 and 1:3 by employing Tween 80 as surfactant ratios shown controlled drug release for a period of 7.5 hours (5^{th} hour to 13^{th} hour), 8.5 hours (5^{th} hour to 14^{th}hour) and 9.5 hours(5^{th} hour to 15^{th} hour), 10.5 hours(5^{th} hour to 16^{th} hour) respectively and are shown in figure 2.

The type of surfactant taken also affects the in-vitro release behavior of the microspheres. In vitro release study shows that the rate of drug release was faster in case of hydrophilic tween 80. This is due to the hydrophilic nature of the surfactant. Drug release was found to be slower in case of microspheres prepared with span 80.

The correlation coefficient values for dissolution kinetics data was shown in the Table 4. These values clearly indicated that the drug release followed zero order kinetics and the mechanism of drug release was governed by Peppas - Korsmeyer model. The exponential coefficient(n) values were found to be in between 0.8500 to 0.8956 indicating that the drug release followed non-fickian diffusion mechanism. These results indicated that the release rate was found to decrease with increase in concentration of coating material applied.

Drug and excipient compatability studies

The FTIR spectrum of Fluvastatin sodium pure drug (Figure 3) showed characteristic peaks at wave numbers were 1013.16cm^{-1},3688.12 cm^{-1},1214.96$cm^{-1}$and 1481.32cm^{-1}denoting stretching vibration of C-F stretching, O-H stretching, C-O stretching and CH_3 deformations respectively.The FTIR spectrum (Figure 4) of optimized formulation showed characteristic peaks at wave numbers were 1009.53cm^{-1},3644.11cm^{-1},1214.67cm^{-1} and 1480.31 cm^{-1} denoting stretching vibration of C-F stretching, O-H stretching, C-O stretching and CH_3 deformations respectively. From the figures it was observed that similar peaks were also reported in optimized formulation. There was no change or shifting of characteristic peaks in drug loaded microspheres suggested that there was no significant drug polymer interaction which indicates the stable nature of the drug in optimized formulation. The Fluvastatin sodium thermal curve is characterized by a sharp endothermic peak at 211.29° C (Figure 5) corresponding to the melting point of the drugs and an identical peak (211.11° C) was also observed in the optimized formulation(Figure 6) . The thermo graphic result shows that the drugs retain its identity in the optimized formulation.

CONCLUSION

Among all the formulations Fluvastatin sodium microspheres prepared with Ethyl cellulose in 1:3 ratio shown prolonged release for a period of 11 hours.The obtained results showed the capability of the system in delaying drug release for a programmable period of time and the possibility of exploiting such delay to attain colon targeting. In accordance with the chronomodulated therapy of hepatic cholesterol synthesis, the lag time criterion of 5 hours and controlled release for a period of 11 hours was satisfied. The dosage form can be taken at bed time and will release the contents in the early morning hours when cholesterol synthesis are more prevalent.

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