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Research Article

PREPARATION AND EVALUATION OF PULSATILE DRUG DELIVERY OF FLUVASTATIN SODIUM

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ABSTRACT

Objective: In the present study, an attempt was made to develop the pulsatile drug delivery of fluvastatin sodium to reduce plasma cholesterol levels and to prevent cardiovascular diseases.

Method: Formaldehyde treated capsule bodies were used for the preparation of pulsincaps. It was sealed with a 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 4.5 kg/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.

Results: Optimized microsphere formulations 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. Drug–polymer interaction studies indicated no interaction in between the drug and the polymer.

Conclusion: Among all the formulations Fluvastatin sodium microspheres prepared with Eudragit RS100 in 1:3 ratio shown prolonged release for a period of 12 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.

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 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase at midnight [2]. Chronotherapy with HMG-CoA reductase inhibitors has suggested that evening dosing could be more effective than morning dosing [3]. 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 such as 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 antilipemic 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 hrs) and low bioavailability (24-29%) makes it an 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 bedtime (9 pm) and capable of releasing drug after predetermine time delay (5 hrs) and can be characterized by proportioning drug concentration in the early morning hours when free cholesterol levels are more prevalent.

MATERIALS AND METHOD

Materials

Fluvastatin sodium was a gratis sample obtained from Ranbaxy Lab. Ltd. (India). Eudragit RS-100 was obtained from Rohm GmbH and 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 25 ml of 15% v/v formaldehyde was taken into a bottom of desiccators, and a pinch of potassium permanganate was added to it to generate formalin vapors. The reaction was carried out for 12 hrs. After which the bodies were removed and dried at 50°C for 30 minutes to ensure completion of the reaction between gelatin and formaldehyde vapor. 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 an airtight container.

Preparation of hydrogel plug (HP)

Plug for sealing the capsule body was prepared by compressing equal amount of Karaya gum/Kondagogu gum/Xanthum gum/Guar gum and lactose using 7 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

Table 1: Formulation list of fluvastatin sodium microspheres prepared

Surfactants used	Eudragit RS100	
	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	1:1
	F-6	1:1.5
	F-7	1:2
	F-8	1:3

microspheres characteristics were investigated by changing polymer: Drug ratio weighed amount of Lovastatin and polymer Eudragit RS100 in 1:1 ratio were dissolved in 10 ml of acetone. The homogeneous drug and polymer organic 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 1 hrs. 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. In the case 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 40 mg of fluvastatin sodium into the formaldehyde-treated bodies by hand filling. The capsules containing the microspheres were then plugged with optimized HP. 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 HP

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

Drug content uniformity

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

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

Dissolution studies were carried out by USP XXIII dissolution test apparatus (paddle method). The capsule was tied to paddle with a cotton thread so that the capsule should be immersed completely in dissolution media but not float. To simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4, and 6.8 were sequentially used referred to as sequential pH change method. When performing experiments, the pH 1.2 medium was first used for 2 hrs (since the average gastric emptying time is 2 hrs), and then removed and the fresh pH 7.4 phosphate buffer saline was added. After 3 hrs (average small intestinal transit time is 3 hrs), the medium was removed and colonic fluid pH 6.8 buffer was added for subsequent hours. 900 ml of the dissolution medium was used at each time. The rotation speed was 100 rpm and temperature was maintained at 37±0.5°C. 5 ml 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 infrared (IR) spectra for the formulation, pure drugs, and excipients were recorded on JASCO Fourier transform IR (FTIR) spectrophotometer using KBr pellet technique at the resolution rate of 4 cm^{-1}. Spectrum was integrated into transmittance mode at the wave number range 380-4368 cm^{-1}.

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 an HP. When the pulsing cap was swallowed, the water-soluble cap dissolves in the gastric juice and the exposed HP 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 hrs were used for the preparation of pulsincaps. It was sealed with a 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. As aggregates, these microspheres were also non-sticky and free flowing. 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 132.55 and 178.46 μm. 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, and 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 sorbitan monooleate, 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.

Evaluation of the HP

HPs were evaluated for hardness, friability, weight variation, and lag time, and the results were shown in Table 3. The formulations fitted with the various HPs HP1, HP2, HP3, and HP4 shown 0.01%, 5.23%, 13.23%, and 17.45% of drug release, respectively, at the end of the 5^{th} hr. It was observed that 100 mg HP (Karaya gum and lactose in 1:1

ratio) having 4.5 kg/cm^2 hardness was satisfactory to retard the drug release in small intestinal fluid and to eject out the plugin colonic fluid and releasing the microspheres into the colonic fluid. This suggested that the lag time could also be adjusted and influenced by the plug composition.

Dissolution studies of pulsincaps

During dissolution studies, it was observed that the enteric coat of the cellulose acetate phthalate was intact for 2 hrs in pH 1.2, but dissolved in intestinal pH, leaving the soluble cap of capsule, which also dissolved in pH 7.4, and 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 the simulated colonic fluid (pH 6.8 phosphate buffer). From the *in-vitro* release studies of the device, it was observed that with all formulation, there was absolutely no drug release in simulated gastric fluid (acidic pH 1.2) for 2 hrs and in simulated intestinal fluid (pH 7.4 phosphate buffer). Burst effect was found in colonic medium (pH 6.8 phosphate buffer).

In-vitro release profiles in the colonic medium were found to have very good controlling efficacy. Pulsincaps loaded with microspheres prepared with fluvastatin sodium and Eudragit RS100 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 8.5 hrs (5-13.5th hr), 9 hrs (5-14th hr), 10 hrs (5-15th hr), and 12 hrs (5-17th hr), respectively, and are shown in Fig. 1.

Pulsincaps loaded with microspheres prepared with fluvastatin sodium and Eudragit RS100 in 1:1, 1:1.5, 1:2, and 1:3 ratios by employing Tween 80 as surfactant shown controlled drug release for a period of 8 hrs (5-13th hr), 8.5 hrs (5-13.5th hr), 9 hrs (5-14th hr), and 11 hrs (5-16th hr), respectively, and are shown in Fig. 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 the case of hydrophilic Tween 80. This is due to the hydrophilic nature of the surfactant. Drug release was found to be slower in the case of microspheres prepared with Span 80.

The correlation coefficient values for dissolution kinetics data were shown in 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 0.8918 to 1.182 indicating super case-II transport diffusion mechanism.

Drug and excipient compatibility studies

The FTIR spectrum of fluvastatin sodium pure drug (Fig. 3a) showed characteristic peaks at wave numbers were 1013.16 cm^{-1}, 3688.12 cm^{-1}, 1214.96 cm^{-1} and 1481.32 cm^{-1} denoting stretching vibration of C-F stretching, O-H stretching, C-O stretching, and CH_3 deformations, respectively. The FTIR spectrum (Fig. 3b) of optimized formulation (F4) showed characteristic peaks at wave numbers were 1009.53 cm^{-1}, 3644.11 cm^{-1} ,1214.67 cm^{-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 the optimized formulation. There was no change or shifting of characteristic peaks in drug loaded microspheres

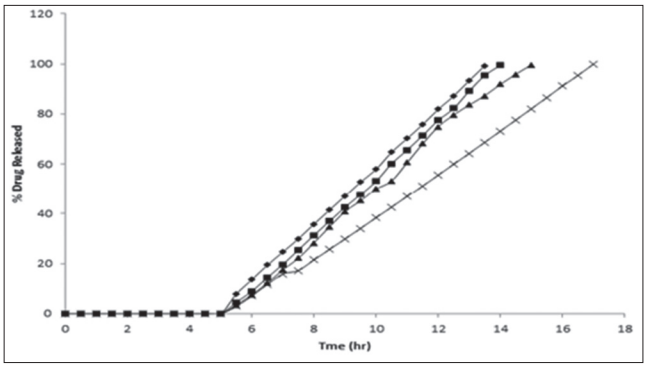

Fig. 1: Comparative *in-vitro* drug release profiles plot of fluvastatin sodium from microspheres prepared with Eudragit RS100 in different ratios by employing Span 80 as surfactant. (-■-)F_1– Fluvastatin sodium from microspheres prepared with Eudragit RS100 in 1:1 ratio and by using span 80 as a surfactant, (-♦-)F_2- Fluvastatin sodium from microspheres prepared with Eudragit RS100 in 1:1.5 ratio and by using span 80 as a surfactant, (-▲-)F_3- Fluvastatin sodium from microspheres prepared with Eudragit RS100 in 1:2 ratio and by using span 80 as a surfactant, (-×-)F_4- Fluvastatin sodium from microspheres prepared with Eudragit RS100 in 1:3 ratio and by using span 80 as a surfactant

Table 2: Evolutional data of fluvastatin sodium microspheres prepared with Eudragit RS100 in different ratios by employing different surfactants (mean±SD)

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±0.12	0.867±0.08	15.86±0.04	1.22±0.03	132.55±0.07	44.96±0.02	89.92±0.09
F-2	25.73±0.08	0.886±0.05	14.18±0.02	1.20±0.06	146.36±0.05	37.94±0.04	94.85±0.06
F-3	23.44±0.06	0.897±0.03	13.13±0.06	1.18±0.04	154.44±0.03	31.82±0.05	96.42±0.04
F-4	20.39±0.09	0.913±0.07	12.32±0.04	1.16±0.07	168.47±0.06	24.46±0.03	97.84±0.07
F-5	28.26±0.11	0.783±0.04	15.72±0.07	1.22±0.05	140.53±0.02	44.38±0.07	88.76±0.02
F-6	26.45±0.05	0.799±0.05	14.36±0.05	1.20±0.02	156.33±0.04	37.66±0.04	94.15±0.03
F-7	24.87±0.07	0.914±0.02	13.74±0.06	1.18±0.04	164.46±0.07	31.46±0.08	95.33±0.05
F-8	22.66±0.13	0.928±0.08	12.71±0.05	1.16±0.03	178.46±0.02	24.13±0.04	96.52±0.06

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

Table 3: Evaluation characteristics of hydrogel plugs prepared with various natural polymers (mean±SD)

Hydrogel plug code	Composition (1:1)	Weight (mg)	Thickness (mm)	Hardness (kg/cm^2)	Lag time (hrs)
HP1	Karaya gum:lactose	100±1.3	3.45±0.11	4.8±0.03	5±0.01
HP2	Kondagogu gum: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 Eudragit RS100 in different ratios by employing different surfactants

Formulation	Correlation coefficient				Release kinetics			Diffusion exponent value (n)
	Zero order	First order	Higuchi	Peppas	K_0 (mg/hr)	T_{50} (hr)	T_{90} (hr)	
F1	0.9996	0.8056	0.9303	0.9990	4.72	4.23	7.62	0.9112
F2	0.9989	0.7971	0.9107	0.9998	4.4	4.5	8.18	1.082
F3	0.9986	0.8073	0.9214	0.9985	3.84	5.1	9.2	1.108
F4	0.9983	0.6380	0.9052	0.9991	3.28	6.09	10.97	1.182
F5	0.9976	0.7964	0.9404	0.9975	4.96	4.03	7.25	0.8918
F6	0.9998	0.8044	0.9268	0.9995	4.64	4.31	7.75	0.9427
F7	0.9994	0.8068	0.9228	0.9928	4.08	4.9	8.82	1.0301
F8	0.9998	0.7640	0.9261	0.9993	3.60	5.5	10.0	1.1035

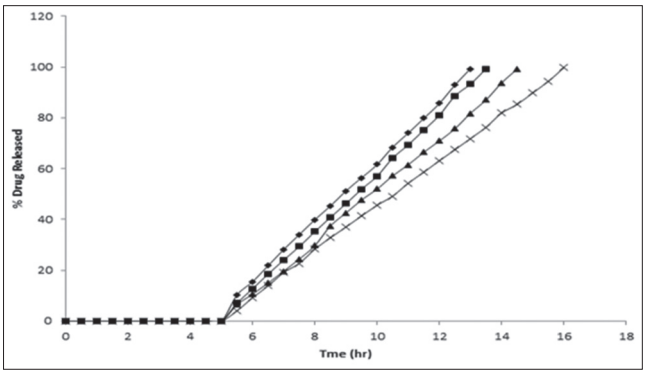

Fig. 2: Comparative *in-vitro* drug release profiles plot of fluvastatin sodium from microspheres prepared with Eudragit RS100 in different ratios by employing Tween 80 as surfactant. (-■-)F_5– Fluvastatin sodium from microspheres prepared with Eudragit RS100 in 1:1 ratio and by using Tween 80 as a surfactant, (-♦-)F_6- Fluvastatin sodium from microspheres prepared with Eudragit RS100 in 1:1.5 ratio and by using Tween 80 as a surfactant, (-▲-)F_7- Fluvastatin sodium from microspheres prepared with Eudragit RS100 in 1:2 ratio and by using Tween 80 as a surfactant, (-×-)F_8- Fluvastatin sodium from microspheres prepared with Eudragit RS100 in 1:3 ratio and by using Tween 80 as a surfactant

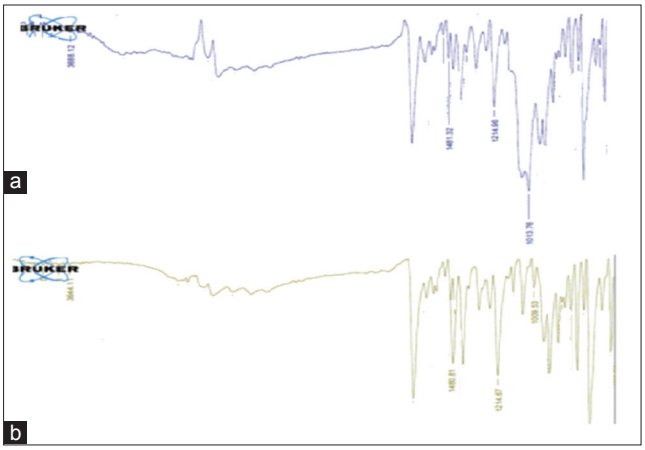

Fig. 3: Fourier transform infrared spectrum of (a) pure fluvastatin sodium, and (b) optimized formulation

suggested that there was no significant drug-polymer interaction which indicates the stable nature of the drug in the optimized formulation.

CONCLUSION

Among all the formulations, fluvastatin sodium microspheres prepared with Eudragit RS100 in 1:3 ratio shown prolonged release for a period of 12 hrs. 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 Chrono modulated therapy of hepatic cholesterol synthesis, the lag time criterion of 5 hrs and sustained release for a period of 12 hrs was satisfied. The dosage form can be taken at bedtime and will release the contents in the early morning hours when cholesterol synthesis is more prevalent.

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