



Preparation and Evaluation of Pulsatile Drug Delivery of Lovastatin

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ABSTRACT

In the present study, an attempt was made to develop the pulsatile drug delivery of Lovastatin to the colon. Formaldehyde treated Capsule bodies were used for the preparation of pulsatile caps. It was sealed with unhardened cap of the capsule. The microspheres were prepared by emulsion solvent evaporation technique. Optimized microsphere formulations were selected based on dissolution studies. Hydrogel plug (karaya gum and lactose in 1:1 ratio) having 4.5kg/cm² 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. 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. Among all the formulations Lovastatin 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: Lovastatin; Hyperlipidemia; Pulsatile; Microspheres; Hydrogel Plug; Solvent evaporation

INTRODUCTION

Pulsatile Drug Delivery System is useful in the diseases in which the drug release is timed to match rhythms of the disease so as to optimize the therapeutic effect and minimize the side effects. Circadian rhythm occurs during hepatic cholesterol synthesis which is higher during the night than daylight and diurnal synthesis represent up to 30-40% of daily cholesterol synthesis¹. However the maximal production occurs in the early morning. So there is a constant need for new delivery systems that can provide increased therapeutic benefits to the patients by delivering drug at the right time, right place & in right amounts to coincide with circadian rhythm of body². Lovastatin is a cholesterol-lowering agent and is structurally similar to the HMG, a substituent of the endogenous substrate of HMG-CoA reductase. Lovastatin lowers hepatic cholesterol synthesis by competitively inhibiting HMG-CoA reductase, the enzyme that catalyzes the rate-limiting step in the cholesterol biosynthesis pathway via the mevalonic acid pathway³. Due to its short biological half life (5.3 hours) and low bioavailability (5%), it requires frequent administration⁴. Studies with relationship of concentrations of mevalonic acid in plasma and circadian rhythm to cholesterol synthesis rates in man had revealed that the mevalonic acid concentrations were detected when the patients were at rest, at least 5 hours after the dinner time.

Hence pulsatile drug delivery system of Lovastatin were developed, which can be targeted to colon in therapeutic

point of view for the treatment of hyperlipidemia, to deliver the drug at such a time when it was needed to modulate the drug level in synchrony with the circadian rhythm of cholesterol synthesis to reduce the dosing frequency, to avoid the drug loss due to the hepatic metabolism and to improve patient compliance. The pulsatile drug delivery of Lovastatin can be taken before bed time (10 pm) and capable of releasing drug at 3.00 am by proportioning drug concentration in the early morning hours when free cholesterol levels are more prevalent⁵. The intentionally delaying the drug absorption for a specified time period of 5 hours(lag time) was controlled by hydrogel plug which will be taken at bed time with a programmed start of drug release early in morning hours.

MATERIALS AND METHODS

Lovastatin was a gratis sample obtained from Ranbaxy Lab. Ltd. (India). Eudragit RS-100 were 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 one 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°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 formaldehyde⁵. These capsule bodies were capped with untreated caps and stored in a 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 using 7mm punches and dies on rotary tablet press⁷.

Preparation of Microspheres

All the microspheres formulations were prepared by emulsion solvent evaporation technique⁸ 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 Lovastatin and polymer Eudragit RS100 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.

Table 1: List of Lovastatin 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

Designing of Pulsin cap

The Pulsin cap was designed by filling the microspheres equivalent to 20mg of Lovastatin 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 solution⁹. 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-dibutylphthalate (0.75%), to prevent variable gastric emptying. Coating was repeated until an

8–12% increase in weight is obtained. % weight gain of the capsules before and after coating was determined¹⁰.

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 20mg of Lovastatin were taken into mortar and grounded with the help of pestle. The grounded power mixture was dissolved in 6.8 pH buffer, filtered and estimated spectrophotometrically at 239 nm¹¹.

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 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 hours (since the average gastric emptying time 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 pH 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°C. Five milliliters of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed at 239 nm, by UV absorption spectroscopy and the cumulative percentage release was calculated over the sampling times¹².

IR Spectral Studies

The IR Spectra for the formulation, pure drugs and excipients were recorded on JASCO FT-Infra Red Spectrophotometer using KBr pellet technique¹³ at the resolution rate of 4 cm⁻¹. Spectrum was integrated in transmittance mode at the wave number range 380 to 4368 cm⁻¹.

RESULTS AND DISCUSSION

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



pulsincaps. 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. 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.

All the formulations offered good flow properties. The particle size of the microspheres ranged between 140.48 and 179.41 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, 1:3 ratios of core:coat to determine the affect of coating material concentration on the release rate of Lovastatin. 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.

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 0.4%, 7.14%, 15.63 % and 18.21 % 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/cm² 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 into colonic fluid. This suggested that the lag time could also be adjusted and influenced by the plug composition.

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).

In-vitro release profiles in colonic medium were found to have very good sustaining efficacy. Pulsin caps loaded with microspheres prepared with Lovastatin and Eudragit RS100 in 1:1, 1:1.5, 1:2 and 1:3 ratios by employing span 80 as surfactant shown sustained drug release for a period of 6 hours (5th hour to 11th hour), 7.5 hours (5th hour to 12.5th hour), 9.5 hours (5th hour to 14.5th hour) and 12 hours (5th hour to 17th hour) respectively and are shown in Figure 1.

Pulsin caps loaded with microspheres prepared with Lovastatin and Eudragit RS100 in 1:1, 1:1.5, 1:2 and 1:3 ratios by employing Tween 80 as surfactant shown sustained drug release for a period of 5.5 hours (5th hour to 10.5th hour), 7 hours (5th hour to 12th hour), 9 hours (5th hour to 14th hour) and 11.5 hours (5th hour to 16.5th 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 1.0091 to 1.0545 indicating supercase-II transport diffusion mechanism.

The FTIR spectrum of Lovastatin pure drug (Figure 3) showed characteristic peaks at wave numbers were 3441.53 cm⁻¹, 2933.58 cm⁻¹, 1705.84 cm⁻¹, 1277.66 cm⁻¹ and 1067.17 cm⁻¹ denoting stretching vibration of O-H stretching, C-H asymmetric stretching, carbonyl



stretching, C-O-C asymmetric bending and C-O-C symmetric bending respectively. The FTIR spectrum (Figure 4) of optimized formulation (F4) showed characteristic peaks at wave numbers were 3442.23 cm⁻¹, 2934.57 cm⁻¹, 1706.38 cm⁻¹, 1278.10 cm⁻¹, 1066.19 cm⁻¹ denoting stretching vibration of O-H stretching, CH asymmetric stretching, carbonyl stretching, C-O-C asymmetric bending and C-O-C symmetric bending 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.

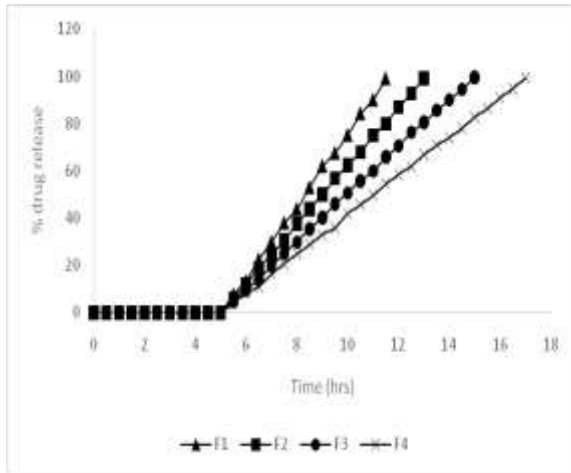


Figure 1: Comparative *In-vitro* drug release profiles plot of lovastatin from microspheres prepared with Eudragit RS100 in different ratios by employing span 80 as surfactant

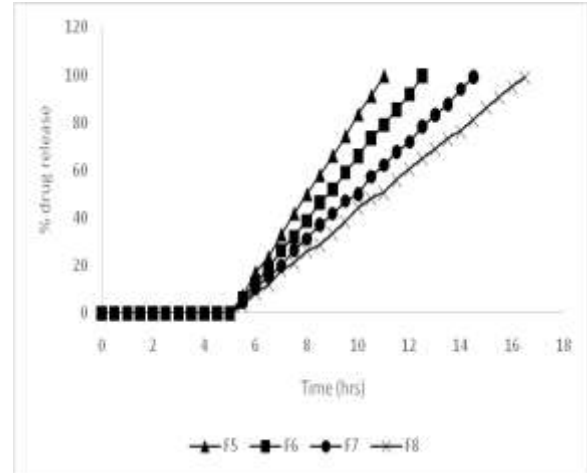


Figure 2: Comparative *In-vitro* drug release profiles plot of lovastatin from microspheres prepared with Eudragit RS100 in different ratios by employing Tween 80 as surfactant

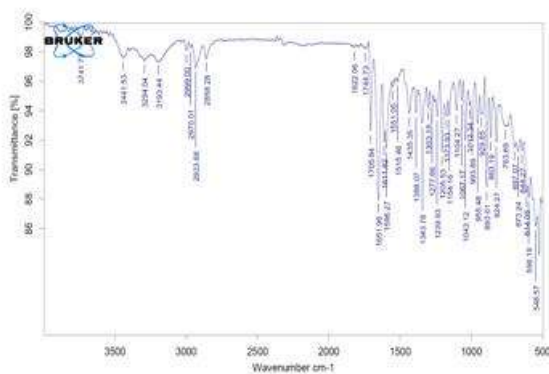


Figure 3: FTIR spectrum of pure Lovastatin

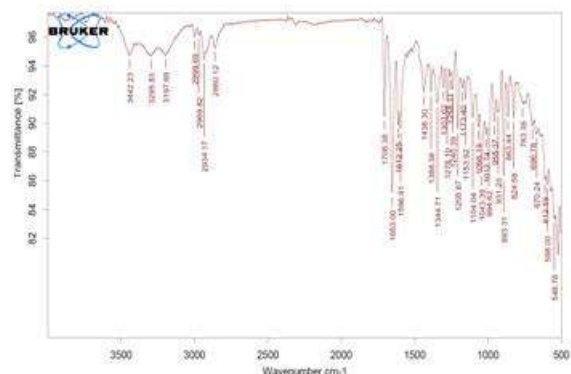


Figure 4: FTIR spectrum of optimized formulation

Table 2: Evaluation data of Lovastatin microspheres prepared with Eudragit RS100 in different ratios by employing different surfactants.

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	27.19	0.905	15.7	1.17	140.46	46.38	92.76
F-2	25.13	0.912	14.8	1.16	156.12	37.45	93.62
F-3	22.84	0.928	13.6	1.15	167.88	31.35	94.05
F-4	21.17	0.941	12.8	1.14	179.43	24.06	96.24
F-5	27.67	0.770	15.81	1.20	147.84	45.80	91.60
F-6	25.11	0.784	14.61	1.18	163.17	36.93	92.32
F-7	23.55	0.796	13.91	1.16	172.83	31.21	93.63
F-8	22.16	0.812	12.86	1.14	189.65	23.77	95.08

Table 3: Evaluation characteristics of hydrogel plugs prepared with various natural polymers

Hydrogel Plug Code	Composition (1:1)	Weight (mg)	Thickness (mm)	Hardness (kg/cm ²)	Lag time (hours)
HP1	Karaya gum: lactose	100 ± 1.4	3.45 ± 0.12	4.8	5
HP2	Kondagogu gum: lactose	100 ± 1.1	3.42 ± 0.16	4.6	4.5
HP3	Xanthan gum: lactose	100 ± 1.5	3.41 ± 0.23	4.3	4
HP4	Guargum: lactose	100 ± 1.2	3.42 ± 0.17	4.1	3.5

Table 4: *In-vitro* dissolution kinetics parameters of Lovastatin 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	0.9999	0.9433	0.9877	0.9999	3.03	3.3	5.9	1.0091
F2	0.9999	0.9340	0.9879	0.9999	2.5	4.0	7.2	1.0544
F3	0.9998	0.9254	0.9877	0.9998	2	5.0	9.0	1.0248
F4	0.9997	0.9289	0.9862	0.9995	1.65	6.0	10.9	1.0168
F5	0.9998	0.9405	0.9883	0.9992	3.33	3.0	5.4	1.0212
F6	0.9999	0.9396	0.9865	0.9999	2.63	3.8	6.8	1.0181
F7	0.9997	0.9266	0.9860	0.9995	2.08	4.8	8.7	1.0188
F8	0.9996	0.9233	0.9864	0.9993	1.72	5.8	10.5	1.0245

CONCLUSION

Among all the formulations Lovastatin 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 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 hours and sustained release for a period of 12 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 is more prevalent.

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