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Research Article

DEVELOPMENT AND CHARACTERIZATION OF LOVASTATIN LOADED MICROSPHERES

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Abstract: In this study, the use of biodegradable polymers for microencapsulation of Lovastatin using solvent evaporation technique was investigated. Ethyl Cellulose and Eudragit L100 was selected as a retardant polymer because of its advantages over other biodegradable polymers. Eight different batches of microspheres were prepared by varying the concentration of polymer. The microspheres were characterized for drug content, percentage yield and encapsulation efficiency, particle size analysis and surface morphology. Microsphere prepared with low drug content produces higher percentage yield and encapsulation efficiency values. It was observed the increase in concentration of the polymer, increases the mean particle size of the microspheres. The effect of polymer concentration on the *in vitro* release of Lovastatin from the microspheres was also studied. It can be seen that by increasing the polymer concentration, decreases the rate of drug release from the microspheres dramatically. The kinetics of drug release from F1 microspheres predominantly follows Higuchi pattern followed by zero order. The release kinetics of F3 and F4 predominantly follows zero order followed by Higuchi and then first order.

Keywords: Lovastatin, Microspheres, Biodegradable polymers, Solvent evaporation.

1. Introduction

Microspheres can be defined as solid approximately spherical and polymeric particles ranging in size from 1 to 100 µm. They are made from polymeric, waxy or other protective materials such as natural, semi synthetic and synthetic polymers and are used as drug carrier matrices for drug delivery. Lovastatin is a hypolipidemic class of drug. It is a water insoluble drug and is a HMG-CoA reductase inhibitor. It is widely used in the treatment of hyperlipidemia and dyslipidemia. The half life of the drug is 1.1- 1.7 hrs, usual dose is 20 mg. Thus to achieve maximum therapeutic effect with a low risk of adverse effects and to improve patient compliance a controlled release formulations of Lovastatin is required[1]. Though statins have been associated with adverse side effects including hepatic toxicity and myopathy, the use of a controlled drug delivery system may eliminate them [2].

2. Objectives

The purpose of the present work was to prepare and evaluate oral controlled release microparticulate drug delivery system of Lovastatin using ethyl cellulose and Eudragit L100 by solvent Evaporation method with high entrapment efficiency and extended release. To optimize the various processes and formulation parameters such as drug-polymer ratio and stirring speed for maximizing the entrapment and prolong release. To evaluate the drug content, *in vitro* drug release, drug-polymer interactions, micromeritic properties, shape and surface morphology.

3. Material Used

Lovastatin was procured as a gift sample from Artemis Biotech, Mumbai, India. Eudragit was purchased from Vikram Thermo Ltd, Ahmedabad. Ethyl cellulose, Tween 80, ethanol and Dichloromethane purchased from S.D Fine Chemicals, Mumbai. All reagents used were of analytical-reagent grade.

4. Preparation of microspheres by Solvent Evaporation Method

Lovastatin loaded microspheres were prepared by solvent evaporation technique. Ethyl cellulose and Eudragit L100 was dissolved in a mixture of methanol and dichloromethane (1:1) at room temperature. Lovastatin was added to above solution and then it was stirred on a magnetic stirrer to form a homogenous solution. Then the above solution was poured into 100 ml of water containing 0.01% tween 80 maintained at room temperature. The mixture was stirred for three hour. The microspheres were separated by filtration and then dried at room temperature. The formulation plan described in table 1.

TABLE 1: FORMULATION PLAN FOR THE MICROSPHRES

Materials	F1	F2	F3	F4	F5	F6
Lovastatin (mg)	100	100	100	100	100	100
Ethyl Cellulose (mg)	100	200	300	400	500	600
EudragitL100 (mg)	100	200	300	400	500	600
Solvent Ratio (DCM: Methanol v/v)	1:1	1:1	1:1	1:1	1:1	1:1
Tween 80 (%)	0.01	0.01	0.01	0.01	0.01	0.01

5. Characterization of Microspheres

The microspheres were characterized by their micromeritics properties such as

i) **Determination of Percentage Yield (w/w):** The dried microspheres were weighed over the electronic digital balance and the percentage yield (w/w) was determined by using formula as shown in Eq. 1

$$\%\text{yield} = \frac{\text{Total amount of dried microspheres}}{\text{Total wieght of raw material}} \times 100 \quad \ldots\ldots\ldots\ldots (\text{Eq. } 1)$$

ii) **Determination of Flow Properties:** The flow properties of prepared microspheres were determined by various tests such as angle of repose, carr's index and hausner's ratio[3].

- **Angle of repose:** Angle of Repose was determined using funnel method. The powder was poured through a funnel that can be raised vertically until a specified cone height (h) was obtained. Radius of the heap (r) was measured and angle of repose (θ) was calculated using the formula as shown in Eq. 2

$$\tan\theta = \frac{h}{r}$$

$$\text{Therefore; } \theta = \tan^{-1}\left(\frac{h}{r}\right) \quad \ldots\ldots\ldots (\text{Eq. } 2)$$

Where, θ is angle of repose, h is height of cone, r is radius of cone.

- **Bulk Density**

Bulk density denotes the total density of the material. It includes the true volume of interparticle spaces and intraparticle pores. The packing of particles is mainly responsible for bulk. Bulk density was measured using the formula as shown in Eq. 3

$$\text{Bulk Density} = \frac{\text{Weight of the powder}}{\text{bulk volume of the powder}} \quad \ldots\ldots\ldots\ldots (\text{Eq. } 3)$$

- **Tapped density**

A weighed quantity of powder blend was introduced into 10 ml measuring cylinder. After that the initial volume was noted and the cylinder was allowed to fall onto a hard surface from the height of 2.5 cm at second intervals. Tapping was continued until no change in volume was noted. Tapped density was measured using the formula as shown in Eq. 4

$$\text{Tapped Density} = \frac{\text{Mass of Microspheres}}{\text{Volume of Microspheres}} \quad \ldots\ldots\ldots (\text{Eq. } 4)$$

- **Carr's index (% compressibility index):** It is simple tests that evaluate the flow ability of a powder by comparing the poured density and tapped density of the powder and the rate at which was packed down. It was determined by taking small quantity of microspheres samples in 10ml measuring cylinder. The height of the sample was measured before and after tapping indicates poured and tapped density. The compressibility index (I) was calculated using formula shown in Eq. 5,

$$\text{I} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}} \times 100 \quad \ldots\ldots\ldots\ldots (\text{Eq. } 5)$$

The values below 15% indicates powder which usually gives rise to good flow characteristics, whereas above 25% indicates poor flowability.

- **Hausner ratio:** Hausner's ratio was calculated using formula as shown in Eq. 6

$$\text{Hausner ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}} \quad \ldots\ldots\ldots\ldots (\text{Eq. } 6)$$

Lower hausner ratio (<1.25) Indicates better flow properties than higher ones (>1.25).

iii) **Determination of drug content:** 10 mg accurately weighed portion of microspheres were taken in a clean 100 ml volumetric flask and dissolved in about 2ml of acetone and the volume was made up to the mark with buffer pH 6.8. After filtration and dilution, samples were analyzed spectrophotometrically and the amounts of drug encapsulated in the microspheres were calculated. The drug content of each sample was determined.

iv) **Determination of Entrapment Efficiency:** The entrapment efficiency was determined by the formula as shown in Eq. 7,

$$\text{Entrapment Efficency} = \frac{\text{Weight of drug in microspheres}}{\text{Weight of drug added}} \times 100 \quad \ldots\ldots (\text{Eq. } 7)$$

v) **Determination of Particle Size of microspheres:** The particle size of the microspheres was determined by using an Optical microscope. The microspheres were examined by optical microscope. The freshly prepared microspheres were examined on an optical microscope and size of the microspheres was measured by using a pre-calibrated ocular micrometer and stage micrometer. About 100-200 particles of each formulation were observed and counted.

vi) **Determination of Surface Morphology:** The surface characteristics of microspheres were studied by scanning electron microscope (Hitachi S- 3400N). Sample of microspheres was mounted on stub and coated with layer of gold using a sputter coater. The samples were scanned at 5kV voltage and photographed at magnification ratio of 10X.

vii) ***In-vtro* drug release study**

Drug release studies were carried out USP dissolution apparatus type I basket type rotating at 100 rpm in pH 6.8 phosphate buffer as dissolution medium (900 ml) maintained at 37 ± 0.5°C. at specific time intervals, up to 12 hours, aliquots were withdrawn and analyze d at 238 nm spectrophotometrically (Shimadzu 1700) against pH 6.8 Phosphate buffer as blank. The withdrawn volume was replaced with an equal volume of fresh pH 6.8 phosphate buffer to maintain sink conditions. All experiments were performed in triplicate.

6 RESULTS AND DISCUSSION

The present research was executed in two phases. In the first phase, preformulation study was carried out to characterize the drug and to study drug-excipient compatibility. In the second phase, microspheres were prepared and evaluated certain parameters to optimize the polymer and its concentration.

6.1 DRUG EXCIPIENT INTERACTION STUDY

Drug and polymers were initially analyzed for any physical incompatibility whereby the samples were stored in vials at 45˚C for a period of 2 weeks. FTIR technique has also been used here to study the physical and chemical interactions between drug and excipients used. On FTIR analysis, it was observed that the drug sample was pure and there was no

chemical interaction between Lovastatin and the polymers used. No significant shifts in the peak corresponding to the drug or the polymers were observed. Hence, the drug and the polymers can be successfully incorporated in the formulation of microspheres. The FTIR of drug and polymer shown in figure 1, 2, 3, 4.

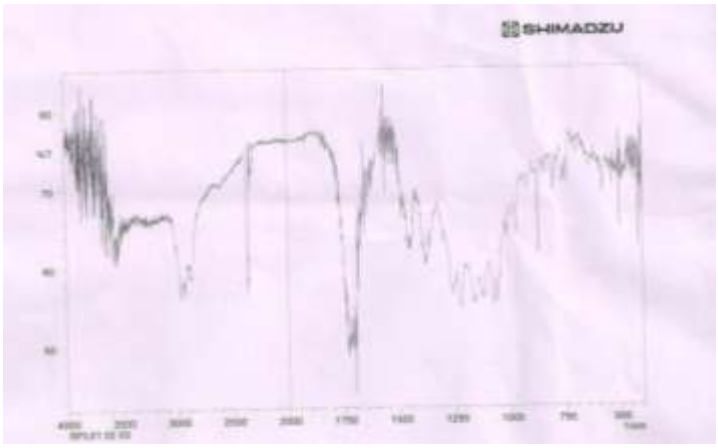

Figure 1: IR spectra of Lovastatin

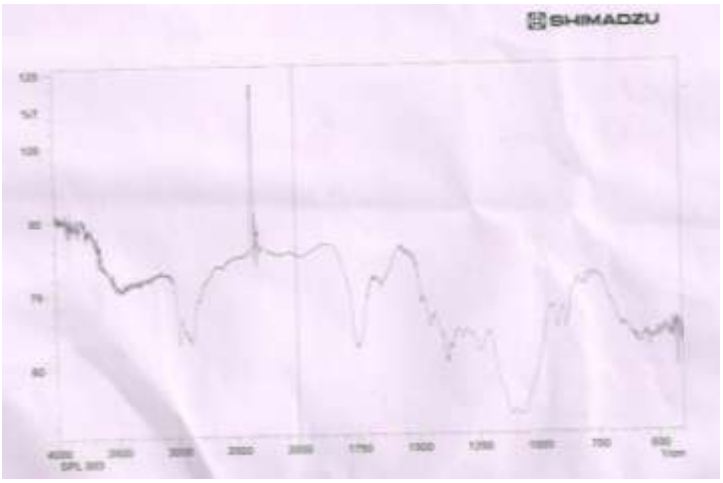

Figure 2: IR spectra of Ethyl cellulose

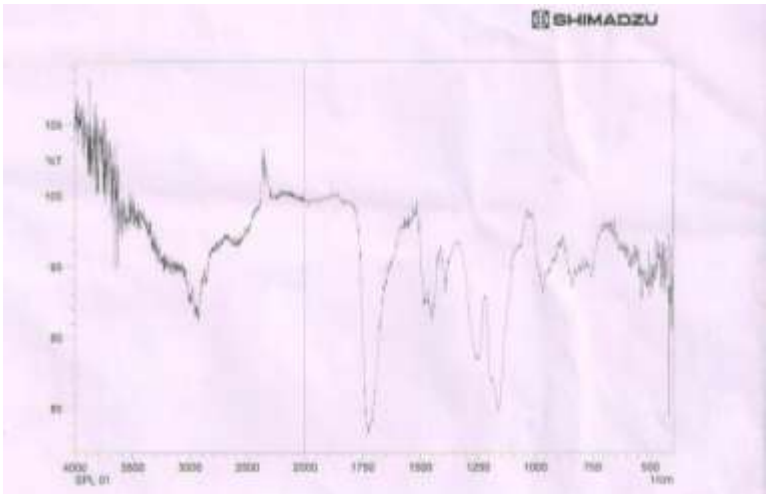

Figure 3: IR spectra Eudragit L100

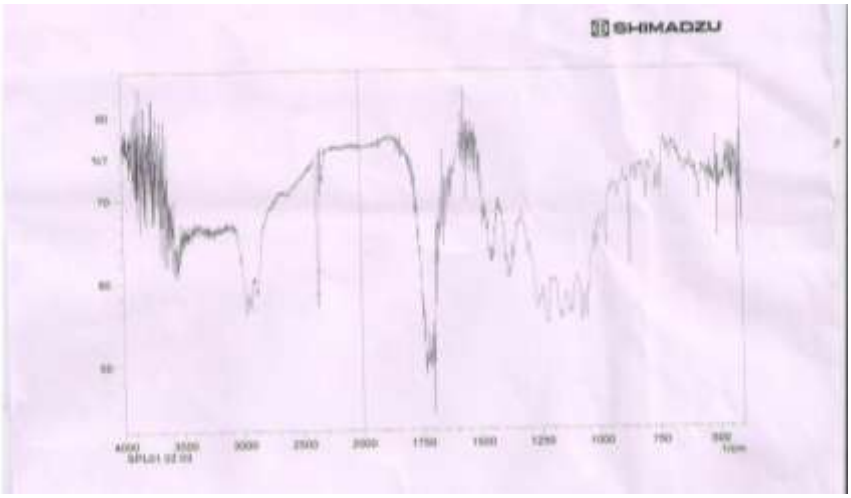

Figure 4: IR Spectra of Lovastatin +Ethyl Cellulose+Eudragit L100

6.2 EVALUATION OF MICROSPHERES

1 Micromeritic properties

The results of all eight formulations are shown in Table no. 2, which were evaluated for variables parameters such as bulk density, tapped density, Carr's compressibility index, and Hausner's ratio. The Carr' compressibility index for formulation F1, was found in the range of 10-19 which indicates the 5-15 which indicates the excellent flow properties, formulations F2, F3, F4, F5 was found in the range of 12-16 which indicates good flow properties, formulation F6 was found in the range of 18-21 which indicates fair to passable flow properties.

Table 2: Micromeritic properties of Lovastatin Microspheres

Formulation	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr' compressibility index	Hausner's Ratio	Angle of Repose (◦)
F1	0.421±0.05	0.471±0.08	10.6±0.78	1.11±0.29	18°35±1.25
F2	0.435±0.04	0.499±0.06	12.82±0.32	1.14±0.21	20°42±1.83
F3	0.460±0.08	0.540±0.11	14.81±0.28	1.17±0.54	23°70±3.35
F4	0.491±0.09	0.588±0.07	16.49±0.62	1.19±0.49	24°55±2.45
F5	0.650±0.06	0.650±0.06	17.07±0.73	1.20±0.08	27°66±4.21
F6	0.735±0.10	0.735±0.10	19.86±0.82	1.25±0.40	30°72±3.26

2 Particle Size Determination

The mean particle size of the microspheres for F1 to F6 in the range of which indicates that with the increase in polymer concentration, the particle size of microspheres increases. This may be because of viscosity of the polymer solution which increases as the polymer concentration increases which in turn decreases the stirring efficiency[4]. As the stirring rate is kept constant for all batches, it was found to be insufficient to break the particles into smaller size at higher polymer concentration. The particle size F1 to F6 formulations as shown in table 3.

Table 3: Mean Particle Size Determination

Formulation code	Mean particle size (µm)
F1	22.5
F2	34.5
F3	45.6
F4	47.1
F5	56.2
F6	67.3

3. Surface Morphology

The Scanning electron microscopy (SEM) was used to determine the shape and surface morphology of microspheres. SEM images of the formulation F1 as shown in figure 5, 6 revealed that the microspheres were spherical in shape. The surface topography reveals that the microspheres were porous which may be due to rapid escape of the volatile solvents during formulation.

Figure 5: Scanning Electron Microscopy of Lovastatin Microspheres (F1)

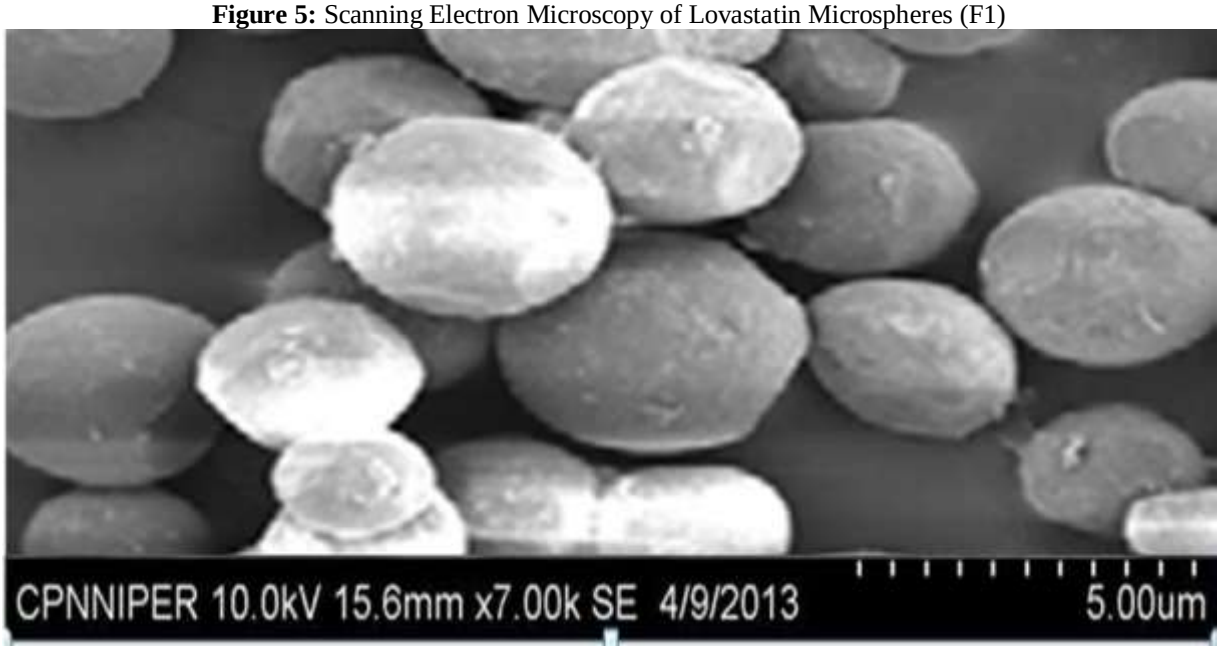

Figure 6: Scanning Electron Microscopy of Lovastatin Microsphers (F1)

4. Percentage yield

The percentage yields of different formulations F1 to F6 were calculated and the yield was found to be in the range of 68.6-84.3%. The loss of material during preparation of microspheres may be due to process parameters as well as during filtration of microspheres. Percentage yield of all the batches is shown in table no. 4

Table 4: Percentage practical yield of Lovastatin Microspheres

Formulation code	Total amount of the ingredients (mg)	Practical yield (mg)	% Practical yield
F1	300	253	84.3
F2	500	413	82.6
F3	700	582	83.1
F4	900	618	68.6
F5	1100	776	70.5
F6	1300	964	74.2

5. Estimation of drug content and Encapsulation efficiency

The drug content was found to be in the range of 22.33-22.69 % for formulations F1 to F6. The microspheres of batch F1 showed highest drug content of 22.33 %, while lowest drug content was observed in batch F6 i.e., 22.69 %. The percentage encapsulation efficiency of Lovastatin microspheres for formulations F1 to F6 was found to be in the range of 82.77% to 89.47 %. From the results it was seen that as the polymer concentration increased, viscosity of the dispersed phase also gets increased, encapsulation efficiency increased. Generally encapsulation efficiency of a drug depends on the solubility of the drug in the organic solvent and continuous phase. But, an increase in the concentration of polymer in a fixed volume of organic solvent also results in an increase in encapsulation efficiency[5]. The percentage encapsulation efficiency and percentage drug content of different formulations is shown in table 5.

Table 5: Drug Content and Entrapment Efficiency

Formulation code	Dug content (mg/ml)	Percentage Entrapment Efficiency (%w/w)
F1	22.33±0.85	82.79±0.52
F2	22.40±0.68	84.77±0.34
F3	22.47±0.07	86.01±1.19
F4	22.55±0.21	87.85±0.06
F5	22.62±0.26	88.09±1.29
F6	22.69±0.34	89.47±1.02

6. *In vitro* drug release

Dissolution studies on all ten formulations of Lovastatin microspheres were carried out using a USP XXIII type I basket type apparatus. Table showed the *in vitro* drug released by microspheres after 11 hours for formulations F1 to F6, increase in the polymer concentration decreases the release rate. The increase in polymer ratio leads to increase of polymer matrix into the microspheres which leads to increased diffusional pathlength. This may decrease the overall drug release from polymer matrix. Further microspheres prepared at lower polymer concentration have large surface area exposed to dissolution medium and hence showed maximum drug release. Maximum drug released for F1 to F6 was 91.01 ±03%.

Table 6: *In-Vitro* Drug Release Data of Various Formulations

Time (hrs)	% Drug Released					
	Formulation Batches					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	**0**
1	15.28±22	13.91±01	13.41±32	14.42±31	11.41±55	12.41±52
2	24.51±67	26.41±62	18.01±24	19.69±62	22.26±09	18.49±64
3	33.41±82	34.28±34	30.22±45	27.01±89	26.71±27	22.69±87
4	42.07±56	48.11±51	39.20±92	38.21±56	34.56±11	34.51±24
5	57.41±32	52.01±43	51.22±67	49.36±75	49.41±82	52.12±56
6	63.22±43	60.01±62	62.34±14	54.64±42	56.05±53	55.02±21
7	68.61±78	69.45±24	68.07±03	66.78±34	62.51±52	60.55±29
8	78.92±12	76.55±78	73.34±06	72.25±76	70.12±32	68.56±19
9	87.67±56	83.56±89	82.42±05	79.12±63	76.14±24	74.34±54
10	91.12±64	88.41±34	87.21±18	86.11±54	84.12±72	81.12±21
11	91.01±03	88.37±25	87.11±78	86.43±09	84.35±11	81.06±42

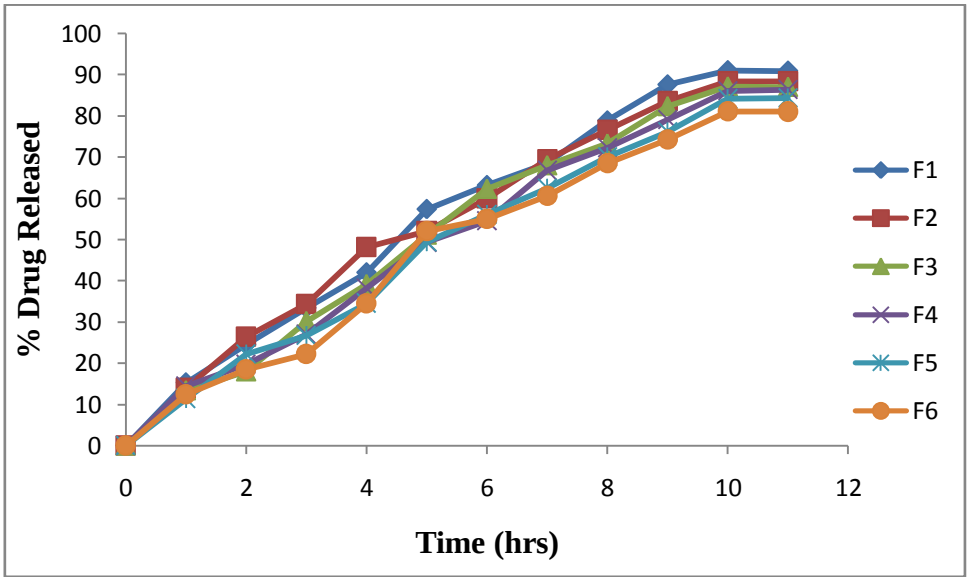

Figure 7: Drug Release of Formulations

Release kinetics of the selected formulation (F1)

The results obtained from *in-vitro* release studies were plotted in different kinetic models. The mathematical models are used to evaluate the kinetics and mechanism of drug release from the formulation. The model that best fits the release data is selected based on the correlation coefficient (r^2) value in various models. The model that gives high r value is considered as the best fit of the release data [6]. Mathematical models are

- Zero order release model (Cumulative percent drug released vs. time)
- First order release model (Log cumulative percent drug retained vs. time)

Zero order release Equation

It describes the systems where the drug release rate is independent of its concentration of the dissolved substance. This ideal delivery is particularly important in certain classes of medicines intended, for example for antibiotic delivery, heart and blood pressure maintenance, pain control and antidepressants. The zero order release is expressed in Eq. no.8

$Q_t = Q_o + K_o t$……..(Eq.8)

Where Qo = Initial amount of drug

Q = Cumulative amount of drug release

K_o = Zero order release constant

t = time in hours

First order release equation

First order release equation describes that release is concentration dependent. This model has also been used to describe absorption and/or eliminating of drug. The first order release is expressed in Eq 9.

Log Q_t = log Q_o + k_t / 2.303……(Eq. 9)

Where Q_o = initinal amount of drug

K = first order release constant

T = time in hours

A graph is plotted between the time taken on x-axis and the log cumulative percentage of drug remaining to be released on y-axis and it gives a straight line

A graph is plotted between the time taken on x- axis and it gives in a straight line.

Table 7: *In-Vitro* release profile of formulation F1

Time (sec)	%CDR	% Drug Retained	Log % Drug Retained
0	0	100	2
1	15.28	84.72	1.927986
2	24.51	75.49	1.877889
3	33.41	66.59	1.823409
4	42.07	57.98	1.763278
5	57.41	42.59	1.629308
6	63.22	36.78	1.565612
7	68.61	31.39	1.496791
8	78.92	21.08	1.323871
9	87.67	12.33	1.090963
10	91.12	8.88	0.948413
11	91.01	8.99	0.95376

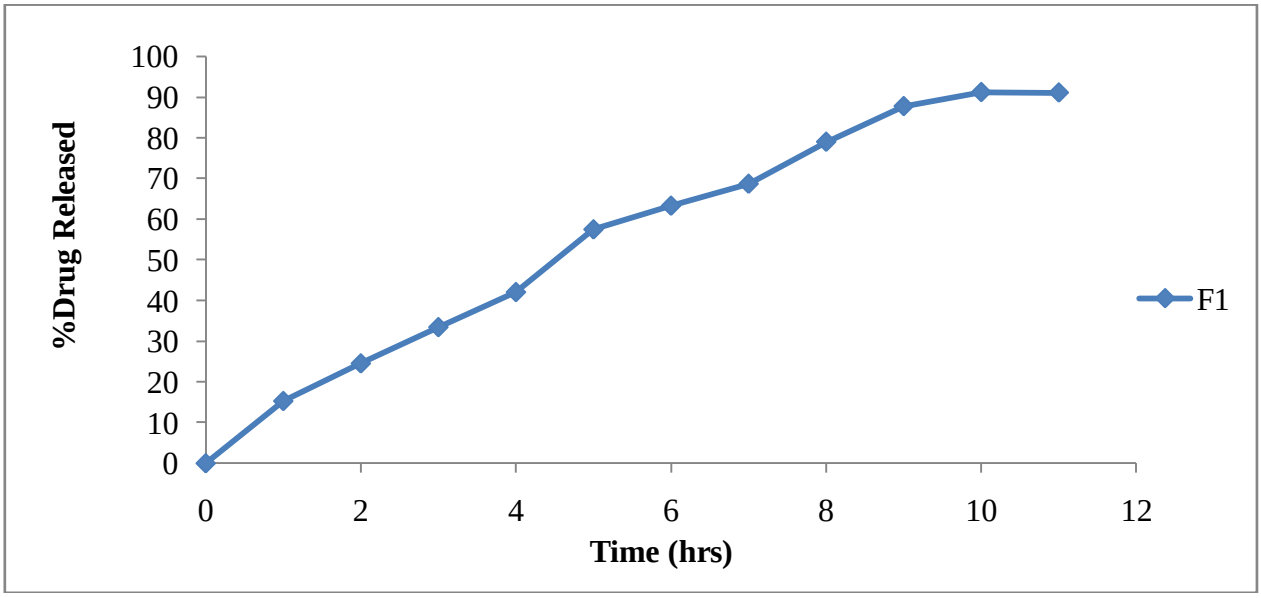

Figure 9: Zero order release profile of Formulation F1

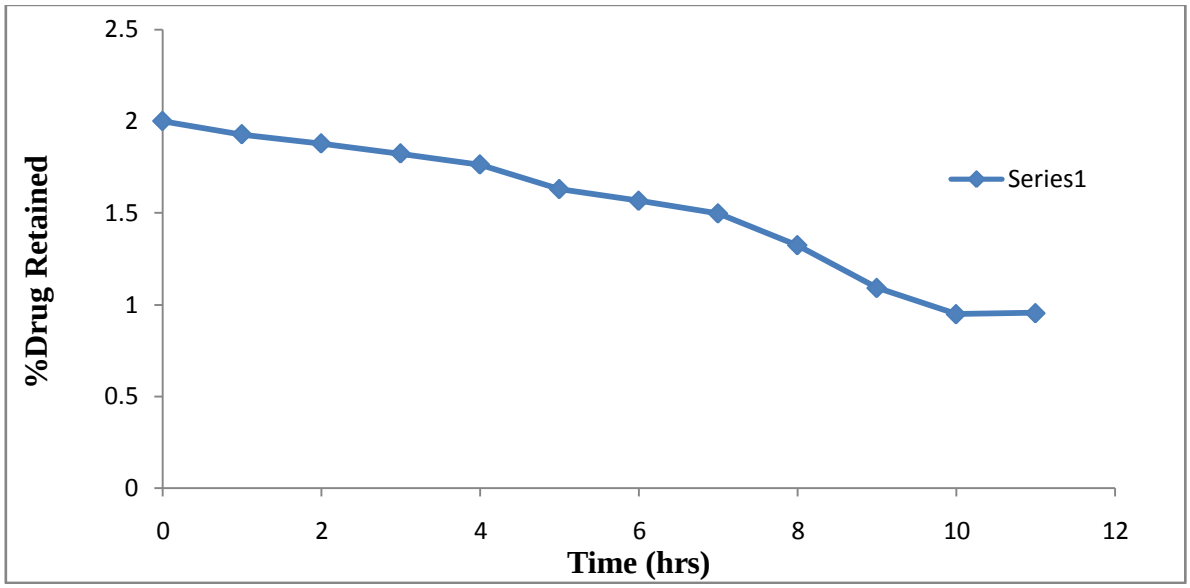

Figure 10: First order release profile of formulation F1

For zero order kinetics, data was plotted with time on x-axis and percent cumulative drug released (%CDR) on y-axis as shown in figure 9

For first order kinetics, data was plotted with time on x-axis and log cumulative percent retained on y-axis as shown in figure 10

From the figure 9 and figure 10, regression co-efficient (r^2) values were found to be 0.975 and 0.956 for zero order and first order model respectively. Since the r^2 value was high for zero order kinetics, it can be concluded that the formulation F1 best follows the second order kinetics.

CONCLUSION

Lovastatin loaded microspheres were prepared by solvent evaporation method using Eudragit L100 and Ethyl Cellulose. Polymer-drug ratio and stirring speed of the system were important to obtain spherical particles with smooth surfaces. The yields of preparation and encapsulation efficiencies were very high for all microspheres obtained. The prepared microspheres were free flowing and discrete. The FT-IR suggested no drug polymer interaction during the process. It was concluded that the Biodegradable Microspheres have potential advantages over conventional oral dosage form with improved patient compliances, convenience, bioavailability and rapid onset of action. The drug release was studied up to 11 hours and results indicate that release of drug decreases with increase in polymer concentration.

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