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PREPARATION AND EVALUATION OF LAMIVUDINE LOADED MICROSPHERES

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ABSTRACT

The present study is planned to prepare microsphere for sustained release of lamivudine. Microspheres are prepared using polymers such as Eudragit (RS100 & RL100) and magnesium stearate as the droplet stabilizer in order to get drops of uniform size by employing solvent evaporation method using an acetone and paraffin systems. Fourier Transform Infrared Spectroscopy (FTIR), X-ray powder diffractiometry and electron microscopy characterized the Microspheres after their preparation for their particle size determination, percentage yield and percentage drug entrapment efficacy. The *in vitro* release studies were performed in acidic buffer (pH1.2) and phosphate buffer (pH6.8). The prepared microsphere were white, free flowing and spherical in shape. The drug-loaded microsphere showed 70-90% of entrapment and release was extended up to 8 h. The infrared spectra showed stable character of lamivudine in drug-loaded microsphere and revealed the absence of drug : polymer interactions. X-ray diffraction pattern showed that there was decrease in crystallinity of the drug. Scanning electron microscopy studies revealed that microsphere were spherical in nature. The best fit of release kinetics was achieved with zero order. From the result of various parameters like yield value, % drug entrapment efficiency, microscopic evaluation, in vitro drug release studies and various kinetic model study, FS2 was selected as the best formulation among all prepared formulations.

KEYWORDS: Lamivudine, Eudragit RS 100, Eudragit RL 100, Ethyl cellulose, SLS, Magnesium stearate, Microsphere, Solvent evaporation method.

INTRODUCTION

AIDS is a collection of symptoms and infections resulting from the specific damage to the immune system caused by the human immunodeficiency virus (HIV). The late stage of the condition leaves individuals prone to opportunistic infections and tumours. Although treatments for AIDS and HIV exist to slow the virus's progression, there is no known cure. HIV is transmitted through direct contact of a mucous membrane or the blood stream with a body fluid containing HIV, such as blood, semen, vaginal fluid, pre seminal fluid and breast milk. Antiretroviral treatment reduces both the mortality and the morbidity of HIV infection.[1]

Lamivudine is an active antiretroviral drug belonging to non-nucleosides reverse transcriptase inhibitor. Lamivudine treatment has gained immense popularity in the AIDS treatment in the present era. Dosage and duration of Lamivudine therapy should be individualized according to requirement and response of the patient. The daily recommended dose is 150 mg twice a day. Lamivudine is rapidly absorbed after oral administration with an absolute bioavailability of 86% ± 16%, peak serum concentration of Lamivudine (Cmax) of 1.5 ± 0.5 mcg/mL and mean elimination half-life (t½) of 5 to 7 hours, thus necessitating frequent administration to maintain constant therapeutic drug levels. The oral administration of Lamivudine exhibits side effects in GIT as well as in CNS. Thrombocytopenia, paresthesias, anorexia, nausea, abdominal cramps, depressive disorders, cough and skin rashes etc., have been reported as possible adverse reactions.[2]

Controlled release (CR) preparations helps to maintain the blood levels of the active ingredient for a prolonged period of time and to achieve maximum therapeutic effect with simultaneous minimization of adverse effects. Therefore, the objective of the present work is to provide a long acting pharmaceutical composition containing Lamivudine in a modified micro particulate drug delivery, which possess many advantages such as high bioavailability, rapid kinetic of absorption as well as avoidance of hepatic first pass effect and improvement of patient compliance. Microspheres is one of the approach in delivering the therapeutic substances to target the site of action. It could provide a larger surface area by small spherical particles with the range of 1µm-1000µm.[3] They are spherical free flowing particles consisting of proteins or synthetic polymers, which are biodegradable in nature. It is rapidly absorbed after oral administration

with an Absolute bioavailability of 85%, Peak serum concentration of 1.5 ± 0.5mcg/ml and mean elimination half-life of 5 to 7 hours, metabolized by liver but hepatic metabolism is low (5-10%) and excreted primarily unchanged in urine.[4]

MATERIALS AND METHODS

Table 1: List of materials.

Sl. No.	Materials	Source
1.	Lamivudine	Apotex Pvt. Ltd.
2.	Eudragit RS 100	Rohm pharma, Germany
3.	Eudragit RL 100	Rohm pharma, Germany
4.	Magnesium stearate	Sisco research laboratories Pvt. Ltd.
5.	Liquid paraffin	Fischer inorganics & aromatics Ltd.
6.	Acetone	SD fine chemicals Ltd.
7.	Petroleum ether	SD fine chemicals Ltd.
8.	n-hexane	SD fine chemicals Ltd.
9.	Sodium hydroxide pellets	Karnataka fine chem, Bangalore.

Preparation of microsphere[5,6]

Lamivudine Microsphere was prepared by the solvent evaporation method. In this method a combination of the polymers (Eudragit RS100, Eudragit RL100) in different ratios were dissolved in acetone in a beaker with the magnetic stirrer at 800 rpm. The drug particles were dispersed in liquid paraffin (50% heavy+50% light) containing 1% w/w Magnesium stearate. The polymer solution was added slowly to the drug dispersion by means of a burette. The mixture was agitated at room temperature (25˚c) using mechanical stirrer. Stirring was continued for 3h until the acetone evaporates completely. n-hexane or petroleum ether was added to the system for hardening of the microspheres and to accelerate settling. The prepared microspheres were filtered by using Vacuum filter. The obtained microspheres were washed repeatedly with n-hexane until free from oil. The collected microspheres were dried at room temperature for 24 hours.

Table 2: Formula for Lamivudine loaded microsphere.

Sl. No.	Formulation code	Microsphere ingredients and process parameter						
		Lamivudine (mg)	Eudragit RS 100(mg)	Eudragit RL 100(mg)	RS 100 : RL1100	Magnesium stearate (mg)	Liquid paraffin (Heavy:Light)	Speed (rpm)
1.	FS1	300	300	-	-	100	50:50	1100
2.	FS2	300	600	-	-	100	50:50	1100
3.	FS3	300	900	-	-	100	50:50	1100
4.	FL1	300	-	300	-	100	50:50	1100
5.	FL2	300	-	600	-	100:100	50:50	1100
6.	FL3	300	-	900	-	100	50:50	1100
7.	FS1L1	300	-	-	1:1	100	50:50	1100
8.	FS2L2	300	-	-	1:2	100	50:50	1100
9.	FS3L3	300	-	-	1:3	100	50:50	1100

Evaluation of Microsphere

Bulk Density (Db)[7]

Where, the bulk density of the formulated ingredients was evaluated using a bulk density apparatus. It is the ratio of the total mass of the powder to the bulk volume of the powder. It was measured by pouring the weighed powder into a measuring cylinder and the volume was noted. It is expressed in gm/cc and is given by

$$Db = M/V$$

Where,
M –Mass of the powder.
V –Bulk volume of the powder

Tapped Density (Dt)[7]

It is the ratio of total mass of the powder to the tapped volume of powder. The tapped volume was measured by tapping the powder to constant volume. It is expressed in gm/cc and is given by

$$Dt = M/Vt$$

Where,
M –Mass of the powder
Vt –Tapped volume of the powder.

Compressibility index (Carr's Index)[7]

Carr's index measures the propensity of granule to be compressed and the flow ability of granule. Carr's index and Hausner's ratio were calculated using

$$I = Dt – Db / Dt X 100$$

Where,
Dt – Tapped density of the powder
Db – Bulk density of the powder.

Angle of Repose[7]

The frictional forces in a loose powder can be measured by the angle of repose. This is the maximum angle possible between the surface of a pile of powder and the horizontal plane. Sufficient quantities of Lamivudine microspheres powder were passed through funnel from a particular height (2 cm) on to a flat surface until it formed a heap, which touched the tip of a funnel. The

height and radius of the heap were measured. The angle of repose was calculated using the formula.

Angle of Repose (Θ) = tan-1 (h/r)

Where, h = Height of the heap

r = Radius of the circle formed by the granule heap

Microsphere characterization

Particle size distribution analysis[8]

Formulations of the microspheres were analysed for particle size by optical microscope. The instrument was calibrated and found that 1unit of eyepiece micrometer was equal to 7.5 µm. 300 microspheres sizes were calculated under 10X magnification.

Percentage drug entrapment efficiency[9,10]

Microspheres equivalent to 100 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1 N Hydrochloric acid repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1 N Hydrochloric acid. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically (UV 1700, Shimadzu, Japan) at 293 nm against appropriate blank. The amount of drug entrapped in the microspheres was calculated by the following formula.

$$\% \text{ Entrapment} = \frac{\text{Actual content}}{\text{Theoretical content}} \text{ X } 100$$

In vitro Drug Release Study[9,11]

Apparatus: Dissolution test apparatus (USP XXXIII)

Method : USP type-1 apparatus (basket)

Speed : 50 rpm

Temperature : 37±0.50C

Dissolution medium : (1) pH 1.2 buffer : 900ml

(2) pH 6.8 buffer : 900 ml

Procedure

Accurately weighed microspheres (equivalent to 300 mg of Lamivudine) were taken for dissolution studies in USP dissolution apparatus (basket type). Aliquots of sample were withdrawn at predetermined intervals of time and analysed for drug released by measuring the absorbance at 271nm (phosphate buffer pH 6.8 and Hydrochloric acid buffer pH 1.2 were used as dissolution mediums).The volume withdrawn at each time intervals replaced with the same amount of fresh dissolution medium.

FTIR Study

Drug-polymer interactions were studied by FTIR spectroscopy. IR spectra for drug, drug loaded Eudragit RS 100 microspheres, and Eudragit RL 100 microspheres recorded in a Fourier transform infrared (FTIR) spectrophotometer (FTIR-8400 S, Shimadzu, Japan) with KBr pellets. The scanning range was 40-4000 cm^{-1}.

Differential Scanning Calorimetry

DSC scans of about 10mg, accurately weighed Lamivudine, drug loaded Eudragit RS 100, RL 100, were performed by using an automatic thermal analyser system (DSC 60, SHIMADZU, JAPAN) with TDS tread line software. Sealed aluminium-lead pans were used in the experiments for all the samples. All the samples were run at a scanning rate of 10 °C/min from 50-350 °C.

Scanning Electron microscopy (SEM)

Scanning electron microscopy was used to examine the surface morphology of microspheres. Dried microspheres were mounted on to stubs by using double-sided adhesive tape. The microspheres were coated with gold and observed under scanning electron microscope (Joel, JSM-5600 LV, Japan) for surface characteristics.

X-ray powder Diffractiometry (XRRD)

The powder X-ray diffraction study was carried out at Solid State Structural Chemistry Unit, IISc, Bangalore, to characterize the polymorphic forms of Lamivudine, Lamivudine loaded Eudragit RS 100 microspheres, Lamivudine loaded Eudragit RL 100 microsphere.

Stability studies[12]

The microspheres of the optimized formulations (FS2) were placed in a screw capped glass container and stored at ambient humidity conditions, at various temperatures like 25 ± 2 °C (60 ± 5RH), 30 ± 2 °C (65 ± 5RH) and 40 ± 2 °C (75 ± 5RH) for a period of 90 days. The samples were analysed for physical appearance and for the drug content at regular interval of 30 days.

RESULTS

Physical characteristics of microspheres

Table 3: Percentage production yield, mean particle size and percentage entrapment efficacy of formulation FS1 – FS3L3.

Formulation	% Yield	Mean Paricle Size (µM)	% Entrapment Efficiency
FS1	73.76±2.623	525.3±7.623	82.23±1.213
FS2	84.96±1.863	535.3±5.671	95.01±2.711
FS3	78.96±2.45	511.6±9.131	91.14 ±1.939
FL1	78.96±2.30	388.8±4.567	73.5 ±5.91
FL2	70.01±0.76	420.4±5.8995	79.33±3.101
FL3	74.4±1.99	427.3±5.1223	80.25±1.586
FS1L1	72.89±8.11	347.5±7.7854	80.38±0.8459
FS2L2	75.09±3.11	366±9.9472	83.21±1.78
FS3L3	80.76±0.11	380.4±10.3632	91.5±.21

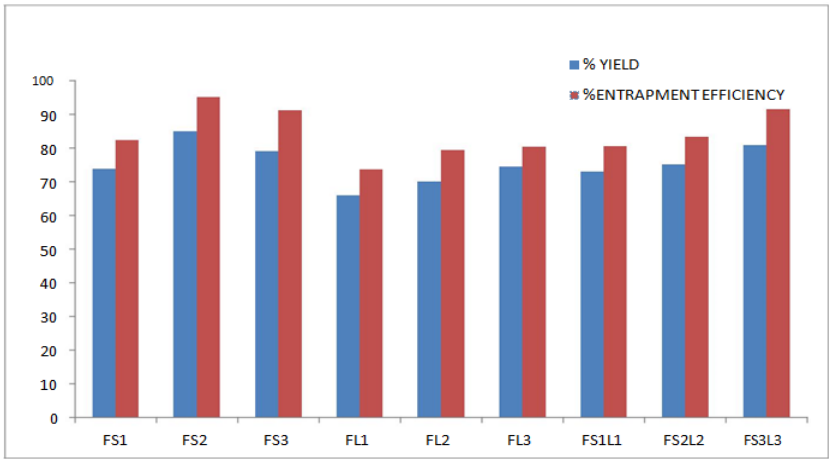

Figure 1: Yield of preparation and encapsulation efficiency data (n=3) formulation FS1-FS3L3.

Table 4: Evaluation of microspheres for Bulk density, Tapped density, compressibility index, Angle of repose.

Formulation	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility index (%)	Angle of repose
FS1	0.373	0.432	13.65	27.01
FS2	0.361	0.429	15.85	24.835
FS3	0.384	0.444	13.5	31.61
FL1	0.381	0.431	11.60	28.35
FL2	0.372	0.423	11.05	28.29
FL3	0.4	0.454	11.89	33.1
FS1L1	0.398	0.455	12.52	28.47
FS2L2	0.363	0.421	13.77	25.02
FS3L3	0.392	0.444	11.71	31.75

In vitro release studies

Table 5: In vitro release studies of formulations S1, S2 and S3.

Time (hrs)	% Cumulative drug release			Dissolution media
	FS1	FS2	FS3	
0	0	0	0	-
1	28.78±1.381	23.31±1.013	22.16 ±1.00	HCL(1.2)
2	55.13±2.132	26.03±0.992	25.45±3.114	
3	66.48±0.771	37.21±1.171	33.39±2.222	PBS(6.8)
4	70.42±1.224	40.75±0.300	37.39±0.100	
5	81.39±1.152	42.65±0.111	41.17±1.151	
6	89.13±2.005	52.41±1.132	46.64±2.183	
7	92.72±1.191	68.197±0.222	58.09±3.324	
8	97.51±0.990	80.48±1.432	68.18±0.321	

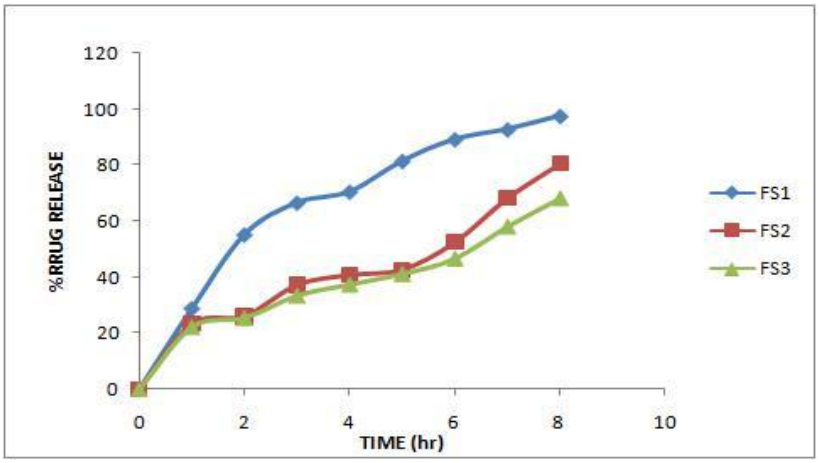

Figure 2: In vitro release profile of Lamivudine (n=3) from FS1, FS2 and FS3.

Table 6: In vitro release studies of formulations L1, L2 and L3.

Time (hrs)	% Cumulative drug release			Dissolution media
	FL1	FL2	FL3	
0	0	0	0	-
1	36.46±1.111	47.29±0.121	48.43±1.975	HCL(1.2)
2	46.07±0.121	50.12±0.231	71.49±0.453	
3	48.59±2.110	56.66±1.321	74.21±0.101	PBS(6.8)
4	58.76±1.000	66.91±1.231	79.72±1.176	
5	67.82±0.143	79.08±2.311	85.98±0.487	
6	80.93±1.007	91.07±1.213	91.64±1.257	
7	87.03±2.001	94.64±0.121	94.03±1.587	
8	93.17±1.612	97.00±0.123	96.66±1.358	

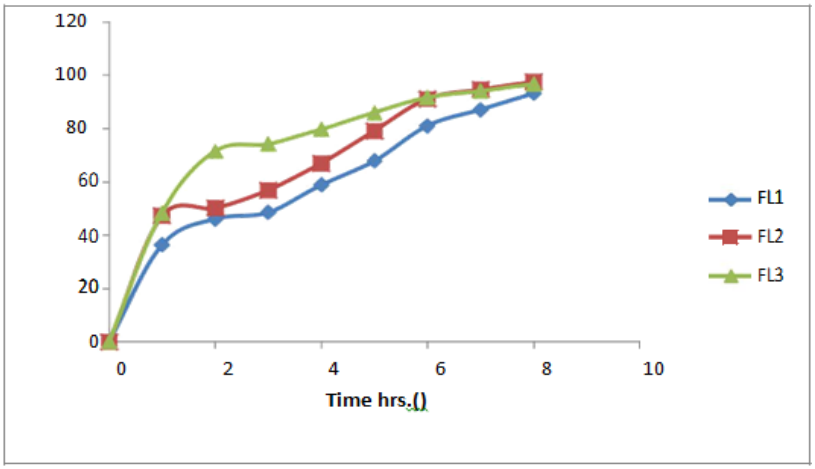

Figure 3: In vitro release profile of Lamivudine (n=3) from FL1, FL2 and FL3.

Table 7: In vitro release studies of formulationsFS1L1, FS2L2 and FS3L3.

Time (hrs)	% Cumulative drug release			Dissolution media
	FS1L1	FS2L2	FS3L3	
0	0	0	0	0
1	8.26±O.001	11.00±0.114	22.09±0.542	HCL (1.2)
2	12.73±1.901	15.72±1.140	28.50±1.048	
3	14.50±1.302	19.00±2.110	37.11±0.469	PBS (6.8)
4	19.11±0.012	24.50±1.111	45.45±0.578	
5	25.00±1.265	30.77±0.111	53.90±1.258	
6	28.98±1.214	37.11±1.100	60.11±0.147	
7	35.00±0.000	43.50±0.457	67.81±0.254	
8	40.00±1.247	49.99±0.489	75.05±1.165	

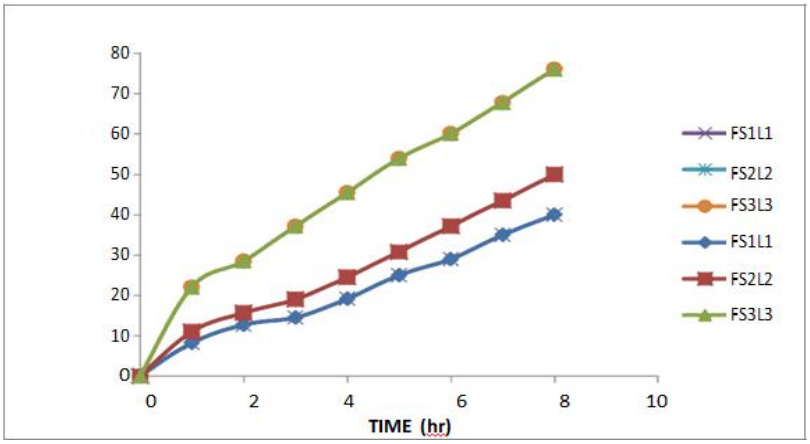

Figure 4: In vitro release pro file of Lamivudine (n=3) from FS1L1, FS2L2, FS3L3.

FTIR study

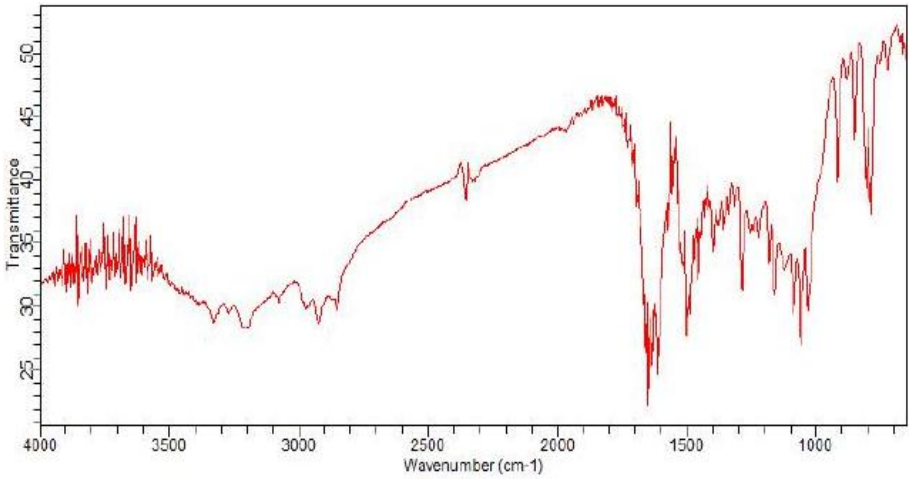

Figure 5: FTIR spectra of pure lamivudine.

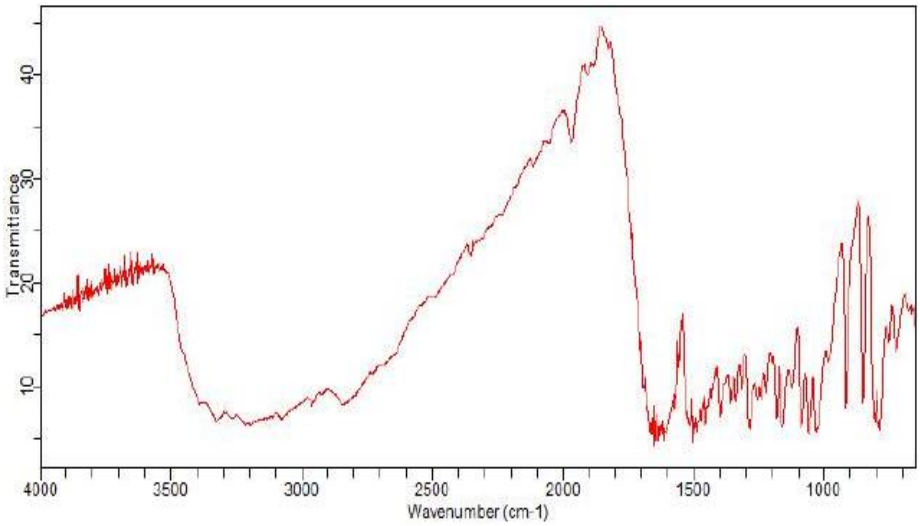

Figure 6: FTIR spectra of formulation FS2.

X-Ray powder diffractiometry (XRD)

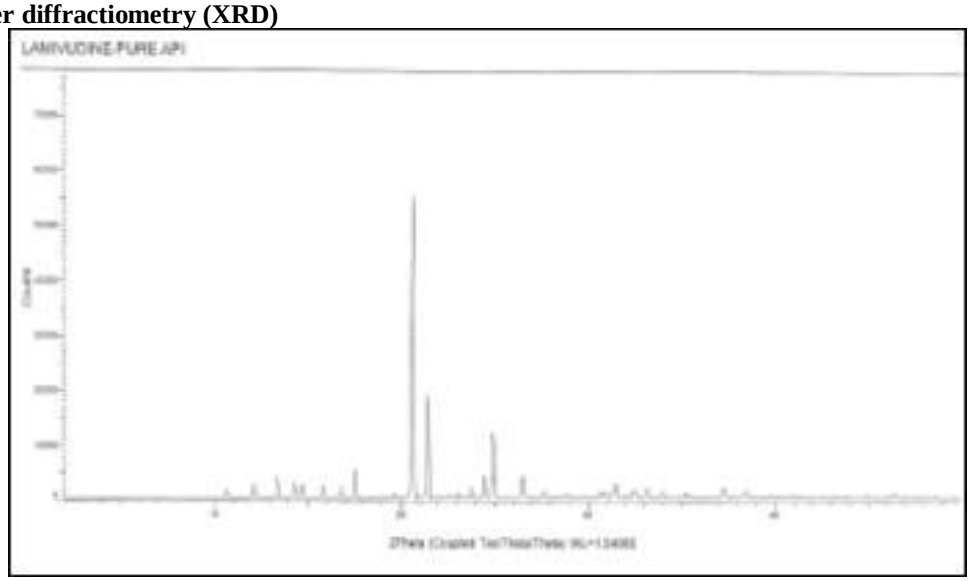

Figure 7: X-ray powder diffraction of Lamivudine.

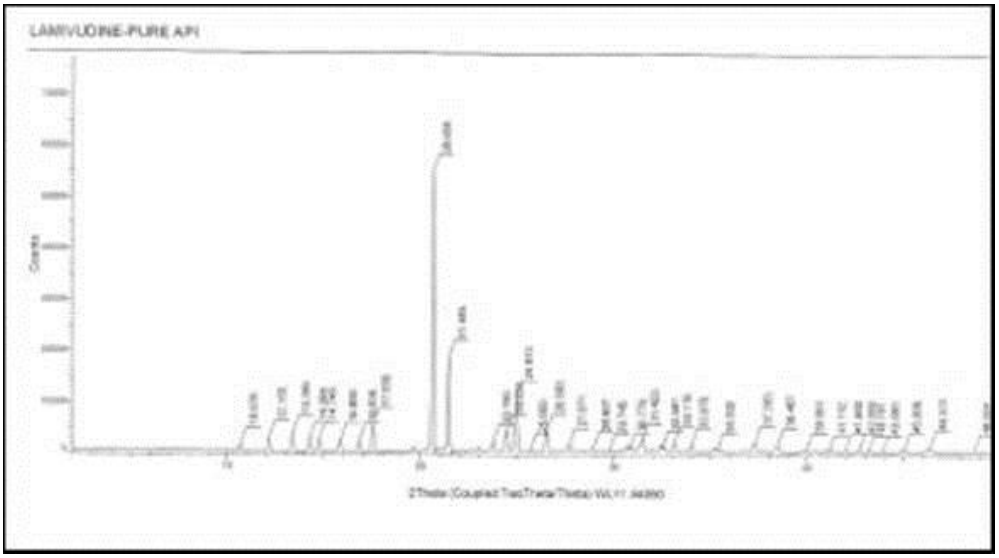

Figure 8: X-ray powder diffraction of Lamivudine loaded with Eudragit RS100.

Scanning electron microscopy (SEM)

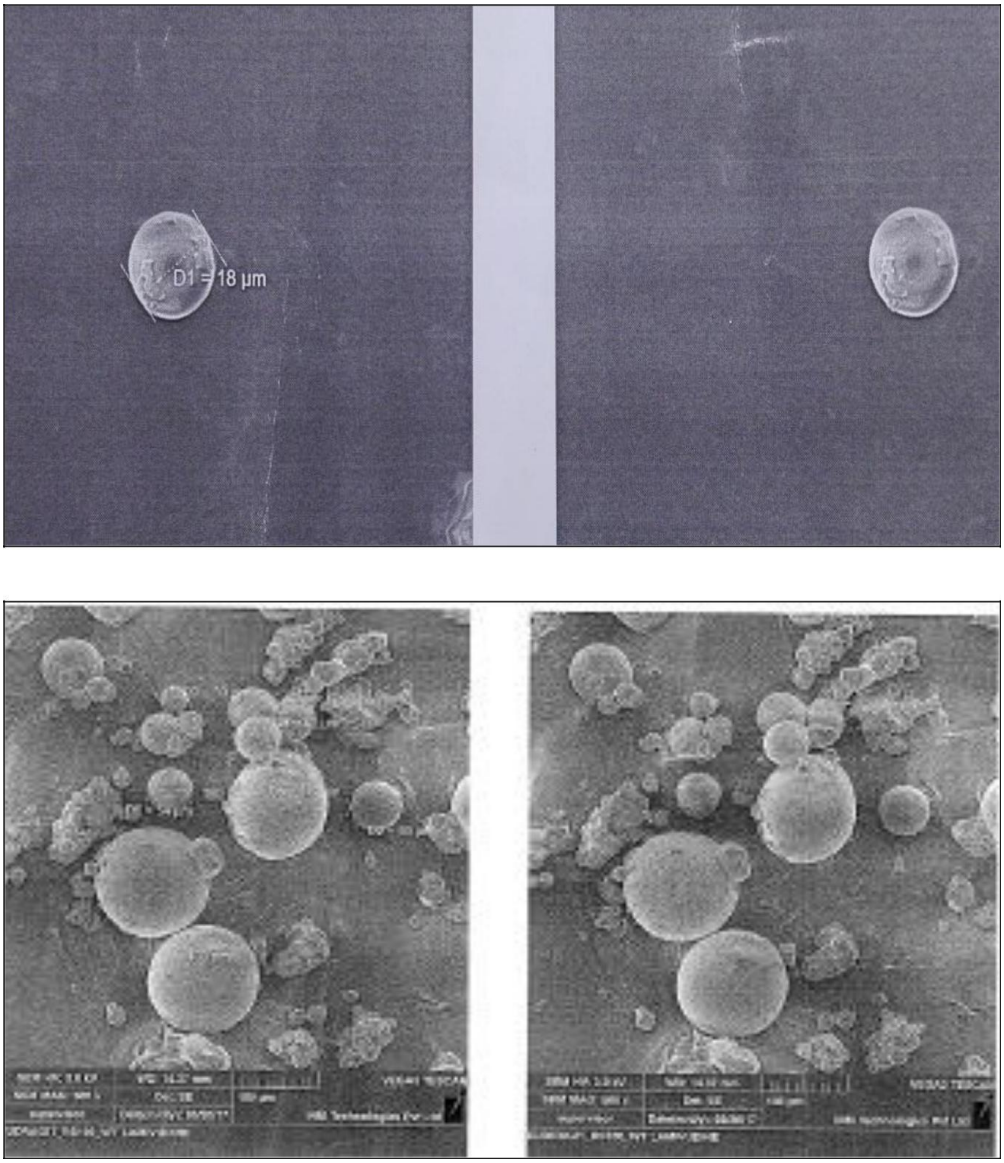

Figure 9: SEM photograph of optimized formulation (FS2).

Differential scanning

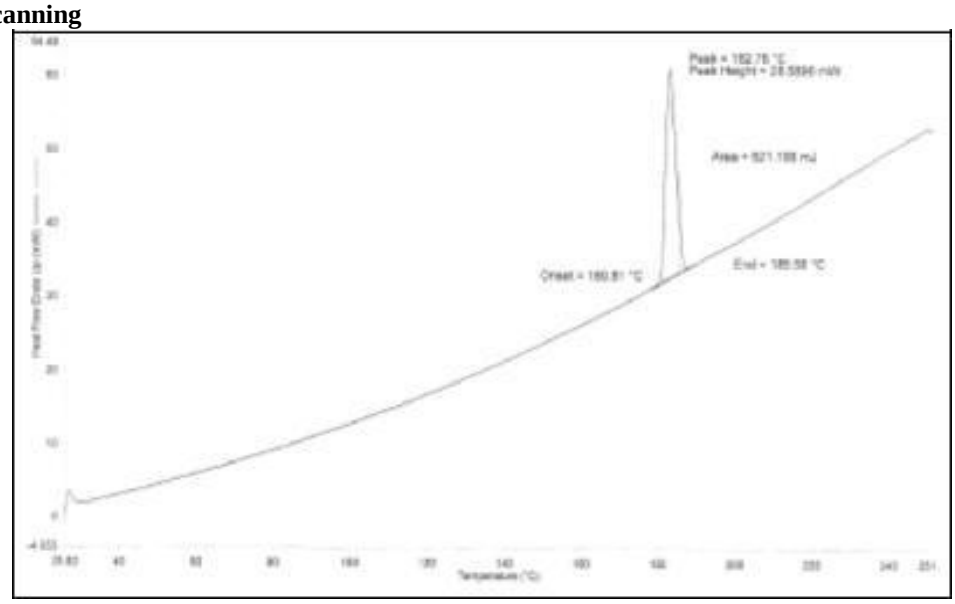

Figure 10: DSC thermogram of Lamivudine.

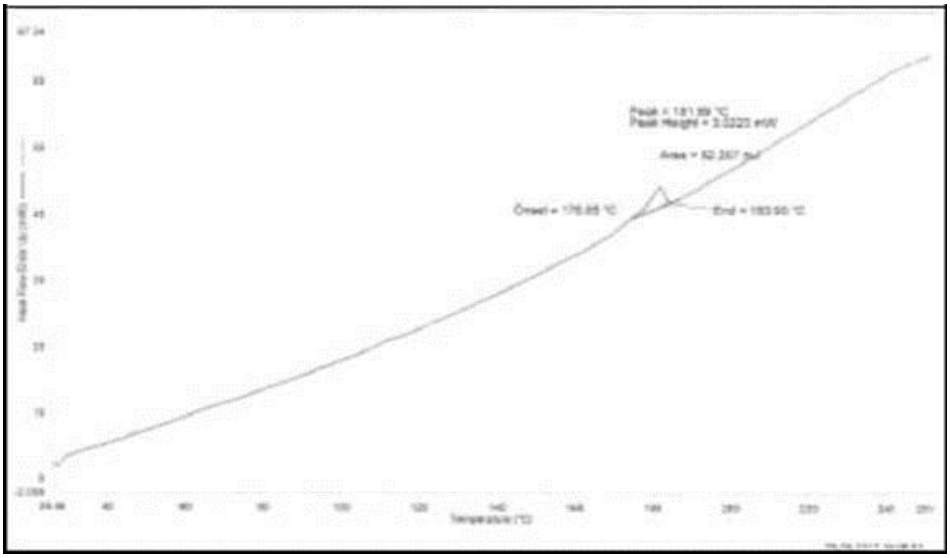

Figure 11: DSC thermogram of optimized formulation (FS2).

Stability studies

Table 8: Stability studies of FS 2 formulation.

Sampling intervals (days)	Storage conditions					
	25 ± 2 °C / 60 ± 5RH		30 ± 2 °C / 65 ± 5RH		40 ± 2 °C / 75 ± 5RH	
	Physical appearance	Drug content* (mg)	Physical appearance	Drug content* (mg)	Physical appearance	Drug content* (mg)
0	No change	96.22	No change	96.09	No change	98.43
30	No change	99.05	No change	98.43	No change	96.09
60	No change	93.75	No change	91.40	No change	99.05
90	No change	96.22	No change	98.43	No change	96.22

* indicates average of two readings.

DISCUSSION

Method

The purpose of the present study was to formulate microspheres for an antiviral drug, Lamivudine. The microspheres were prepared by solvent evaporation method using polymers like Eudragit RS 100, Eudragit RL 100. Microencapsulated techniques have mostly been used for lipophilic and hydrophilic drugs showed low loading efficiency. In present study liquid paraffin and acetone system were used for the preparation of microspheres. Magnesium stearate was used as droplet

stabilizer to prevent droplet coalescence in the oil medium and n-hexane was added as a non- solvent to the processing medium to solidify the microspheres.

To achieve the optimization of the formulation different weight ratios of each polymer were used to encapsulate the same weight of drug, weights of polymers were 300mg, 600mg and 900 were used.

Weight of drug was 300mg. Further increase in weight of polymer causes the increase in viscosity of the polymeric solution and also causes the film or debris formation of polymers in the container. Thus totally 3 batches were prepared for each polymer, which were named as FS1, FS2, FS3, FL1, FL2, FL3, FS1L1, FS2L2, FS3L3 for Eudragit RS 100, Eudragit RL 100 Respectively.

It was observed that when the speed of stirrer was below 500 rpm, there was no formation of spherical microspheres. Whereas, at speed of above 1500 rpm, the resulting high turbulence caused frothing and adhesion to the wall of container. The desired spherical microspheres were obtained at stirring speeds of 1000-1200 rpm.

All the formulations were subjected for production yield, particle size, drug entrapment, in vitro drug release and FTIR, XRD, and SEM analysis, DSC analysis.

The production yield for different formulations was found acceptable. The higher yield was obtained for the formulation FS2. It was observed that particle size was increased with the increase in polymer concentration for each of different polymer.

The entrapment efficiency of Lamivudine in the microspheres was greater than 70%.The highest entrapment efficiency was found for the formulation FS2. It was observed that if the polymer concentration increased, entrapment efficiency was also increased.

The release profile was studied for 8 hours. The better drug release was obtained for formulation FS1 that was 97.5% respectively. The FS1 showed much prolonged release compared to other formulations. FS2 was considered as the optimized formulation because of higher entrapment and higher yield as compare to other formulations.

From the result of various parameters like yield value, % drug entrapment efficiency, microscopic evaluation, in vitro drug release studies and various kinetic model study, FS2 was selected as the best formulation among all prepared formulations. However dissolution studies reports shows that the FS1 formulation is giving higher drug release, but it doesn`t give better yield and drug entrapment efficiency as compared to FS2.

The drug polymer interaction study of the pure drug and best formulation FS2, were carried out by FTIR. As shown in figure 5&6 it revealed that there were no significant difference in the IR spectra of pure drug Lamivudine and drug loaded microspheres. The results suggest that the drug was stable during the encapsulation process.

The X-ray powder diffraction patterns (Figure 10) of pure drug and Eudragit RS 100 loaded microspheres containing Lamivudine revealed that the intensity of the peaks for the pure drug was sharp. However, when it was incorporated into the polymer matrix, the drug peak shows a slight loss in sharpness due to decreased crystallinity of the pure drug. Lamivudine loaded with Eudragit RS100 formulation were subjected to microscopic evaluation under trinocular microscope. All the prepared microspheres were found to be spherical in shape.

The SEM analyses (Figure 9) of FS2 were carried out. From this study, it was observed that surfaces of all microspheres were rough and drug crystals were observed on the surface of microspheres.

In the present investigation, DSC thermogram of pure drug, drug loaded microspheres of formulations FS2 were taken. As shown in figure 7 the thermogram of pure Lamivudine shows melting point at 180.81°C. Drug loaded Eudragit RS 100 microspheres (Formulation FS2) showed a broad small peak at 176.85 °C as shown in figure 8, indicating the presence of drug in crystalline form. The reduction of height and sharpness of the peak was due to the presence of polymer in the microspheres.

Stability studies of the formulation were carried out as per the ICH guidelines. The optimized formulation i.e. (FS2) were subjected to stability studies at 25°C, 30°C, and 40°C at ambient humidity for a period of 90 days. The physical stability was assessed by the appearance and the chemical stability by change in the drug content. The results showed that the formulations were stable at the end of 90th day.

CONCLUSION

The present study was attempted to develop microsphere for Lamivudine using two different polymers. It was prepared by solvent evaporation technique. The prepared microspheres were subjected to various parameters like: percentage yield, particle size and drug entrapment, in vitro drug release study, FTIR analysis, XRD analysis, SEM analysis, DSC analysis, stability studies. Among evaluation FS2 were give higher percentage yield and entrapment efficiency. All prepared formulations were subjected for in vitro drug release study for 8 hrs. In which FS1 gave prolonged and complete release compare to all other formulations. Optimized formulation FS2 were subject for analysis which showed that there were no drug polymer interaction. FS2 further subjected for DSC that showed pure drug gave sharp peak at melting point of drug & in the formulation gave peak near the pure drug melting point of drug but loss of sharpness and reduction of height. FS2 formulation was

subjected for SEM and stability studies by storing at various ICH Storage condition for 90 days. It shows better storage at 25˚ C ± 2˚/60%RH. The sample was analysed for its drug content and physical appearance at an interval of 30 days

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