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FORMULATION AND IN-VITRO CHARACTERIZATION OF FLOATING MICROSPHERES OF LAMIVUDINE

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Abstract:

Objective: The investigation was concerned with the formulation and In-vitro characterization of floating microspheres of lamivudine by employing different concentrations of HPMC K100M in order to increase the gastric residence time of lamivudine and reduced dosage frequency. The drug excepient compatibility studies were performed by FTIR &DSC. The gastro retentive floating microspheres were prepared by ionotropic gelation method. The prepared microspheres were evaluated for SEM analysis, in-vitro buoyancy studies and *in-vitro* dissolution studies. The compatibility of the drug with excepient was confirmed by FTIR &DSC study. The *in-vitro* drug release studies revealed that the drug release was sustained up to 14hrs for formulation F5 containing HPMC K100M as release retardant polymer at a concentration of 1:2 to the drug. Using Higuchi's model and the Korsmeyer equation, the drug release mechanism from the floating microspheres was found to be Anomalous (non-Fickian) diffusion. The gastro retentive floating microspheres of lamivudine were successfully formulated and evaluated employing HPMC K100M as release retardant polymer. A slow and spread over drug release up to14 hrs was observed with the formulation F5 with HPMC K100M at 1:2 concentrations to the drug. Thus current investigation was successful in extending the drug release and reducing the dosing frequency.

Keywords: Lamivudine, Floating microspheres, HPMC K100M, Floating lag time & *in-vitro* dissolution studies.

Introduction:

Lamivudine is a BCS Class I active anti-retroviral agent which belongs to non-nucleoside reverse transcriptase inhibitor. It is generally prescribed in the dose of 100-150 mg twice a day, and is well absorbed in the upper gastrointestinal tract with a short biological half life of 3-7 hrs.[1]Lamuvidine should be given frequently to maintain desired therapeutic activity.

The major problems associated with conventional drug delivery systems are to maintain drug concentration within the therapeutic effective concentration level success of an oral drug delivery system is depends on its degree of absorption through GIT. Floating drug delivery system is one of the methods to increase the gastric residence time. Flotation of the dosage form improves the absorption of the drug .the drugs which act locally in the stomach and the drugs which are poorly soluble in intestine (due to alkaline pH are the suitable candidates for formulation of floating drug delivery by increasing gastric residence time this results in improving of bio availability[2]. Microspheres can be defined as solid, approximately spherical particles ranging from 1-1000 micrometers containing dispersed drug in either solution or micro crystalline form hallow microspheres are considered as one of the most promising buoyant systems. The present work was aimed at the formulation of lamivudine floating microspheres using polymer in 5 different concentrations, in order it helps for ease of administration to decrease the dosing frequency, by sustaining the drug in the gastric pH and helps to release the drug up to>10hrs and characterization of the prepared formulations .

Materials and Methods:

Materials

Lamivudine is a gift sample from Hetero labs, Hyderabad. HPMC K100M was supplied by Colorcon Asia Pvt. Ltd., Mumbai, India. Sodium bicarbonate, Sodium alginate, Sodium bicarbonate were procured from SD Fine Chemicals, Mumbai, India.

Methods

Calibration curve of lamivudine microspheres:

Preparation of 0.1N HCL:

8.5 ml of HCL was dissolved in distilled water and volume was made up to 1000 ml to make 0.1N HCl.

Preparation of stock solution:

100 mg of drug was taken and transferred to a100 ml volumetric flask and methanol was added. Calibration curve to dissolve the drug and final volume was made up to 100 ml by use of 0.1N HCl then different concentrated solutions were prepared calibration curve was shown in figure No.1.

Drug Excepient Compatibility Studies:

The drug and excepient compatibility studies were performed by using FTIR studies and DSC and it was confirmed there is no chemical interaction between the drug and excipients, which were shown in Fig. No: 2-6.

Preparation of Floating Microspheres of Lamivudine:

Floating microspheres were prepared by ionotropic gelation method. Weigh the required quantities of drug ,sodium Alginate, sodium bicarbonate and HPMC K100M.sodium Alginate was dispersed in sufficient amount of water and stir until it gets completely dissolved and slowly add the drug substance along with the sodium bicarbonate and HPMC K100M to the dispersion by maintain the temperature between 40-50^{0}c. A 10% Calcium chloride solution was prepared and it act as a cross linking agent and placed on a magnetic stirrer.

The dispersion was taken into the syringe and drops wise it is add to the Calcium chloride solution by using #24 gauge needles and it causes the gelation of poured droplets and it leads to formation of microspheres. The prepared microspheres were allowed to stand in the calcium chloride solution for up to 30 min for curation, after that the prepared microspheres were filtered by using Whattman filter paper and they are dried at 50^{0}c in an hot air oven [4].

The prepared floating microspheres composition were showed in table no.1

Table no.1: Formula for preparation of lamivudine floating microspheres

Ingredients	**Formulations**				
	F1 (mg)	**F2 (mg)**	**F3 (mg)**	**F4 (mg)**	**F5 (mg)**
Lamivudine	100	100	100	100	100
HPMC K100M	100	125	150	180	200

Sodium Alginate	100	100	100	100	100
Sodium bicarbonate	200	200	200	200	200

Micromeretic Properties of Microspheres:

The prepared microspheres were evaluated for angle of repose, Bulk density, Tapped Density, Carr's Index, Hausner's Ratio table no.2

Table No.2 Micromeritic properties of microspheres:

Formulation	Angle of repose (θ)	Bulk density (g/ml)	Tapped density (g/cc)	Carr's index (%)	Hausner's ratio
F1	32.3±06	0.15±15	0.13±18	10.04±15	1.12±21
F2	34.03±30	0.16±12	0.12±26	11.4±20	1.13±26
F3	31.02±20	0.02±21	0.18±81	9.08±26	1.10±10
F4	37.22±26	0.21±12	0.06±60	15.06±60	1.21±12
F5	29.06±60	0.13±04	0.15±30	8.48±84	1.07±26

SD n=3

Evaluation of Microspheres:

Particle Size Analysis:

The particle size of microspheres was determined by using SEM analysis in which 100 particles were measured using light microscope [5].

SEM Analysis:

The samples for the SEM analysis were prepared by sprinkling the microspheres on one side of the double adhesive stub. The stub was then coated with fine gold dust. The microspheres were then observed with the scanning electron microscope. Microspheres size was shown in the figure 7& 8.

Estimation of drug content:

The drug content in each formulation was determined by triturating 100mg microspheres and powder equivalent to average weight was added in 100ml of 0.1N HCL buffer solution, followed by stirring. The solution was filtered,

diluted suitably and the absorbance of resultant solution was measured under UV spectroscopy at 282 nm using 0.1N HCL buffer solution as a blank [6].The results are revealed in table no.3.

Encapsulation efficiency (EE)

Drug loaded microcapsules (100 mg) were powdered and suspended in water and then sonicated for about 20 minutes. It was shaken for another 20 minutes for the complete extraction of drug from the microcapsules. The mixture was filtered through a 0.45 µm membrane filter. Drug content was determined by UV visible spectrophotometer at 282 nm. The percent entrapment was calculated using the following equation and the results are revealed in table no.3.

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Percentage Yield of Microspheres:

Microspheres recovered at the end of preparation were weighed and the yield was calculated as a percentage of the total amounts of polymer and drug added during the preparation of microspheres [7].The results are revealed in table no.3.

$$\%\text{Percent yield} = \frac{\text{The amount of microspheres obtained (gms)}}{\text{The theoretical amount (g)}} \times 100$$

Table no.3: Evaluation of prepared microspheres

SD n=3

Formulation code	Percentage yield (%)	Drug content (%)	Entrapment efficiency	% Moisture loss
F1	82.5±26	94.06±12	88.06±24	3.67±85
F2	87.23±12	95.26±07	90.13±26	3.97±27
F3	73.24±06	96.72±26	85.32±12	2.92±12
F4	79.52±16	96.12±26	89.15±21	4.23±65
F5	94.7±17	99.20±15	92.35±16	1.21±51

Percentage Moisture content

The Lamivudine loaded microspheres of different polymers were evaluated for percentage of moisture loss which sharing an idea about its hydrophilic nature. The Microspheres weighed initially and kept in desiccators containing calcium chloride at 37°C for 24 hours. When no further change in weight of sample was observed, the final weight was noted down and the results are mentioned in table no.3.

$$\% \text{ of moisture loss} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

In vitro drug release:

The USP dissolution rate testing apparatus was employed to study the in-vitro drug release of lamivudine microspheres of different ratios (F1, F2, F3,F4 and F5) using 0.1N HCL buffer Solution as a dissolution medium.100mg equivalent of lamivudine containing microspheres was taken a dissolution test was being carried out at 50 rpm maintained at $37^{o}C \pm 0.5^{o}C$. 5ml of samples were withdrawn at specific time interval of 30 min, 1,2,3,4,5,6..up to 14 hrs respectively. The sample volume was replaced by an equal volume of fresh medium [8]. The concentration was determined under UV spectroscopy at 282nm. The percentage of drug release at various time intervals was calculated and their percentage drug release was shown in fig no.:9

***In vitro* drug release kinetics**

In order to study the exact mechanism of drug release from the microsphere, drug release data was analyzed according to Zero order :$Q = k_o t$[12], First order : $\ln (1-Q) = - K_1 t$[13], Higuchi square root : $Q=K_2 t^{½}$ [14], Korsmeyer Peppas model[9].The criteria for selecting the most appropriate model was chosen on the basis of goodness offit test the values are revealed in table no.4 & 5.

Table no.: 4 Different kinetic equations for formulations (F1-F5).

Order kinetics	Formulations				
	F1	F2	F3	F4	F5
Zero order	0.946	0.981	0.976	0.936	0.944
First order	0.934	0.796	0.942	0.762	0.936
Higuchi's	0.956	0.897	0.965	0.953	0.953

model					
Peppas model	0.618	0.715	0.764	0.663	0.721

Table no. : 5 Kinetic values obtained from different plots of F5 formulation.

parameters	ZERO ORDER	FIRST ORDER	HIGUCHIS ORDER	PEPPAS ORDER
	% CDR Vs T	Log % Remain Vs T	%CDR Vs √T	Log CDR s Log T
Slope	7.543X	-0.127x	23.73X	1.188x + 0.733
R 2	0.944	0.936	0.953	0.721

Figure captions:

1. Fig no.1: Calibration curve of Lamivudine
2. Fig no.2: FT-IR of Lamivudine
3. Fig no.3: FT-IR of HPMC K 100M
4. Fig no.4: FT-IR of formulation F5
5. Fig no.5: DSC of pure Lamivudine
6. Fig no.6: DSC of formulation F5
7. Fig no.7: SEM of microspheres
8. Fig no.8: SEM of microspheres
9. Fig no.9: *In vitro* drug release studies of floating microspheres of Lamivudine (F1-F5)
10. Fig no.10: *in-vitro* release profile according to zero order kinetics for F5 formulation
11. Fig no.11: *in-vitro* release profile according to first order kinetics for F5 formulation
12. Fig no.12: *in-vitro* release profile according to Higuchi's release model for F5 formulation
13. Fig no.13: *in-vitro* release profile according to Peppas release model for F5 formulation

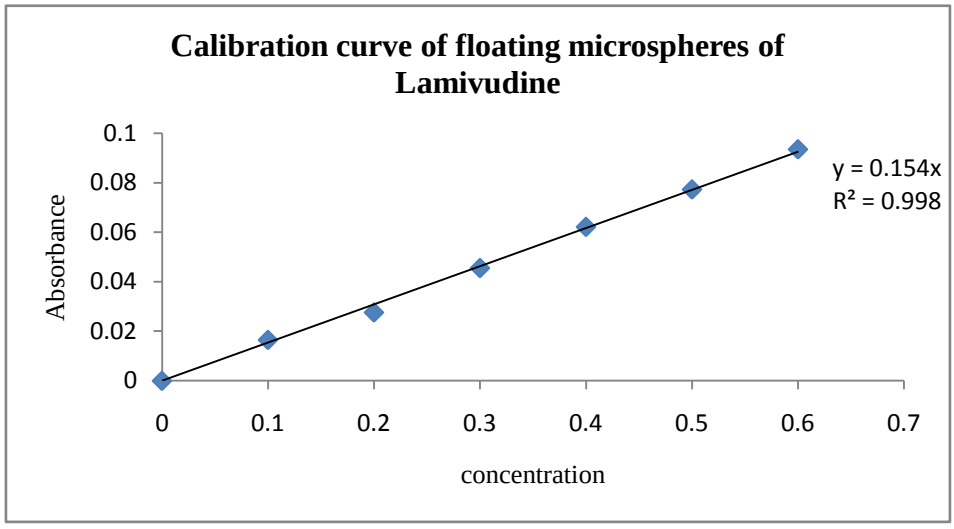


Fig 1: Calibration curve of Lamivudine.

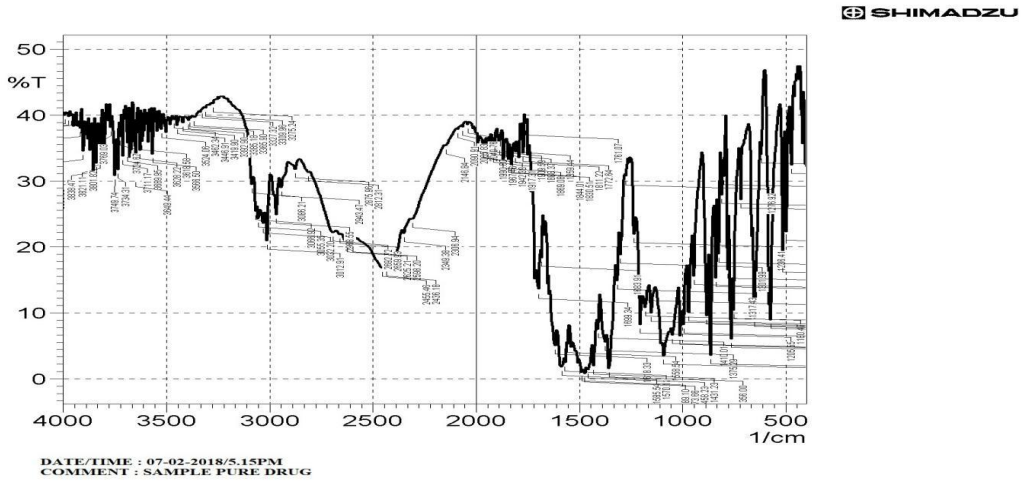


FIG:2 FT-IR of Lamivudine.

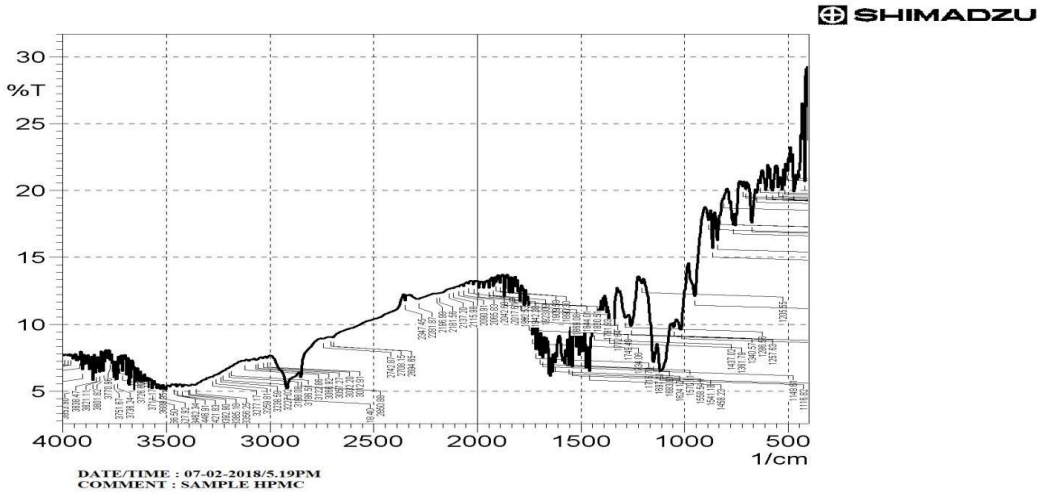


FIG:3 FT-IR of HPMC K 100M.

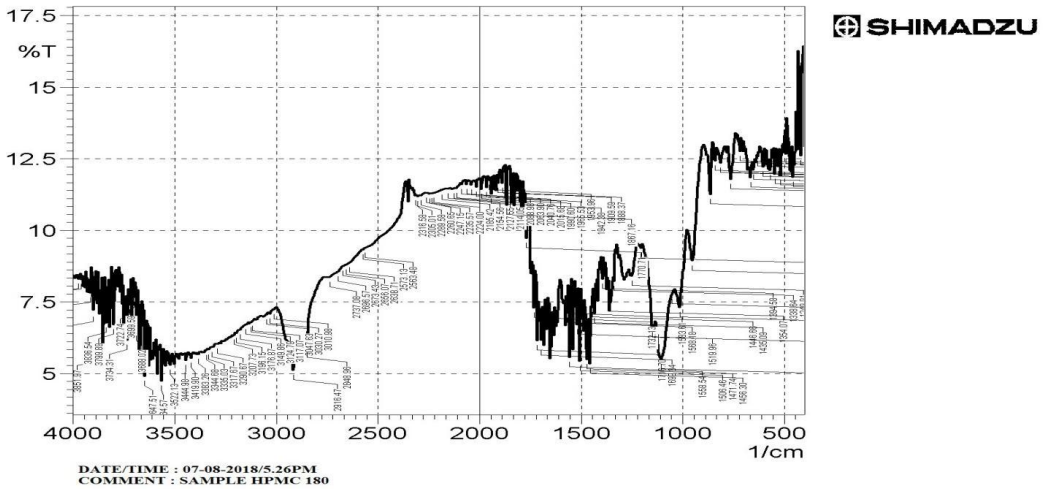
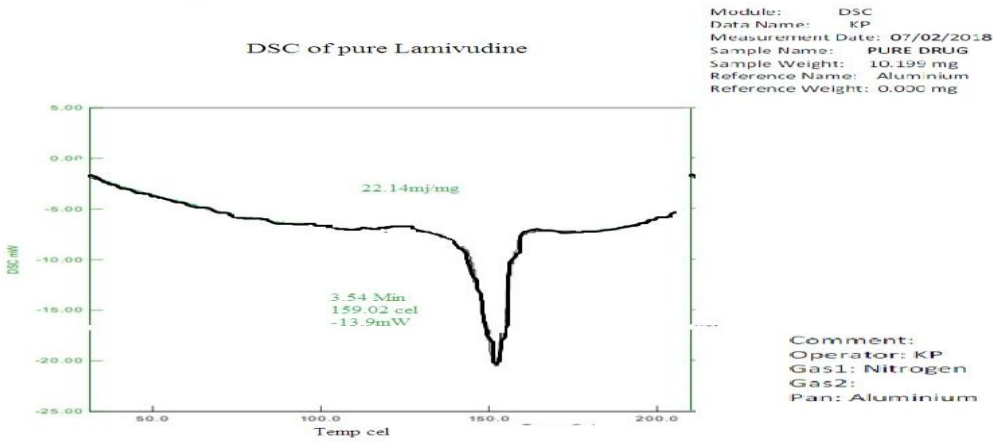
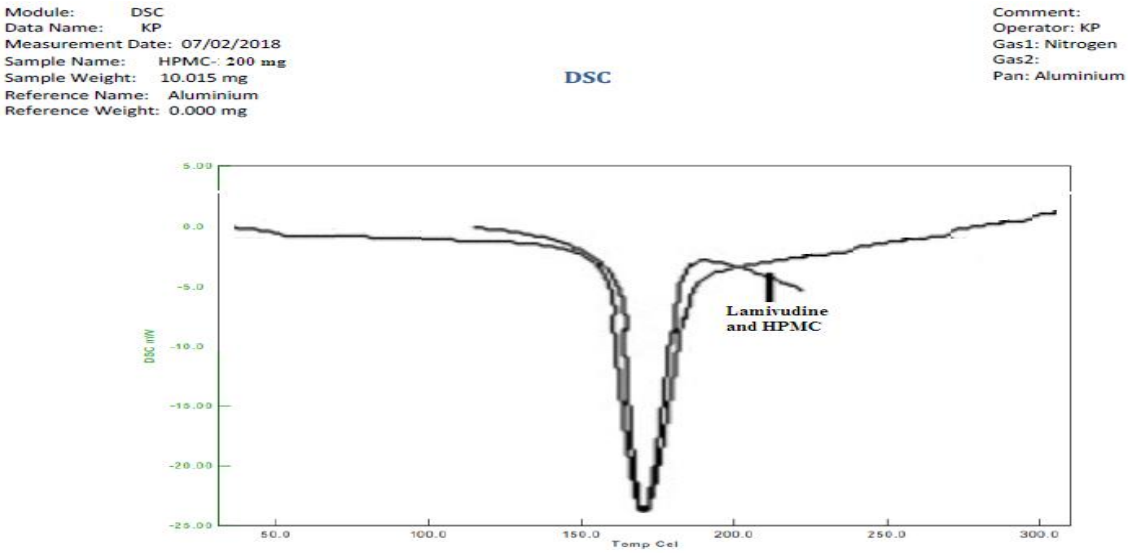


FIG:4 FT-IR of formulation F5.



DSC of pure Lamivudine.



DSC of formulation F5.

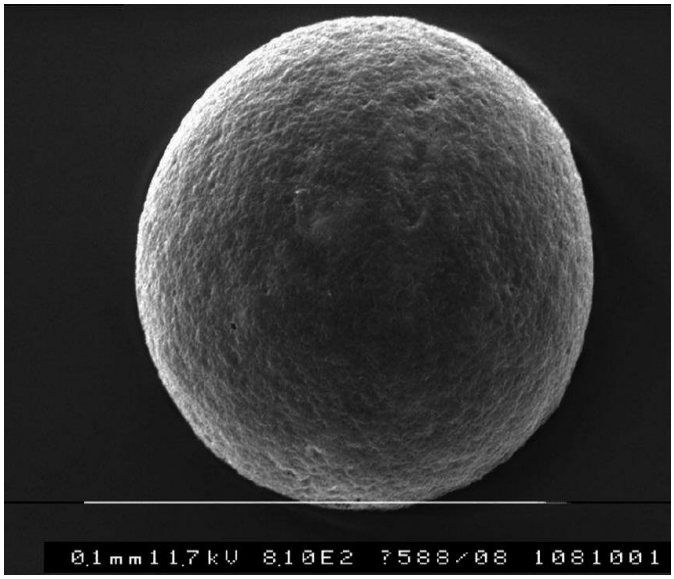


Fig no.:7 SEM of microspheres

Fig no.: 8 SEM of microspheres

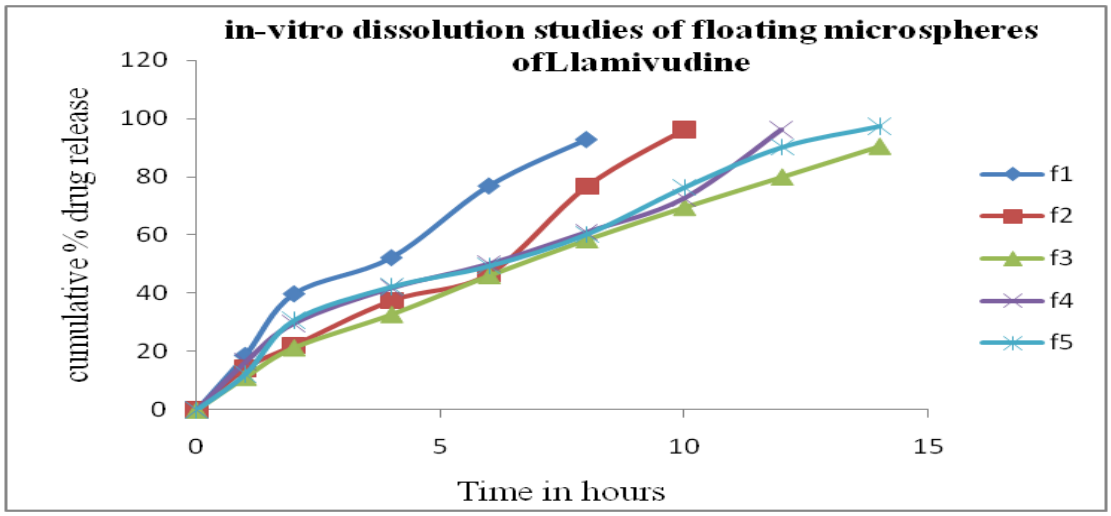


Fig no.: 9 In vitro drug release studies of floating microspheres of lamivudine (F1-F5).

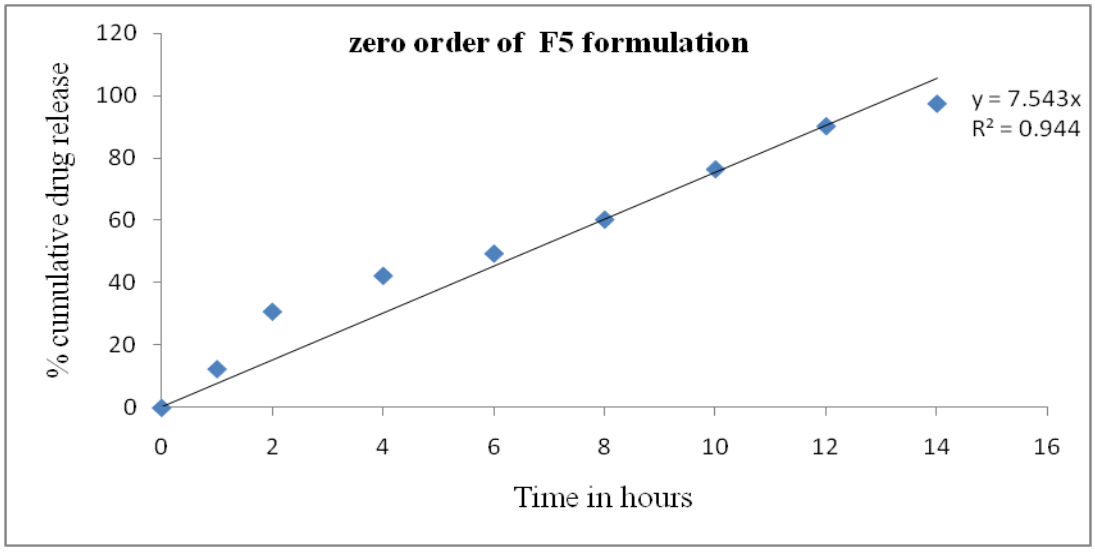


Fig.No:10. in-vitro release profile according to zero order kinetics for F5 formulation.

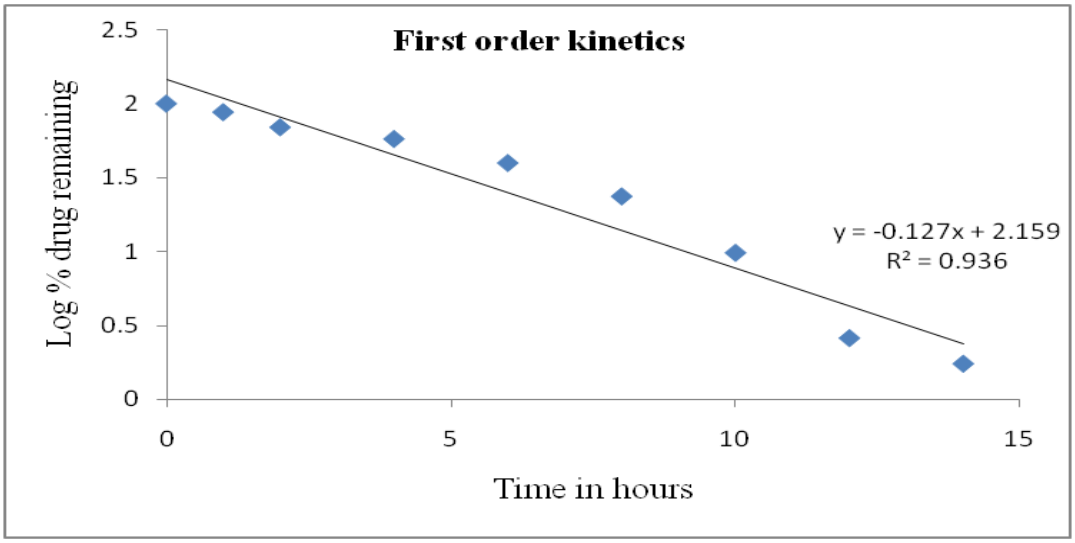


Fig.No:11.in-vitro release profile according to first order kinetics for F5 formulation.

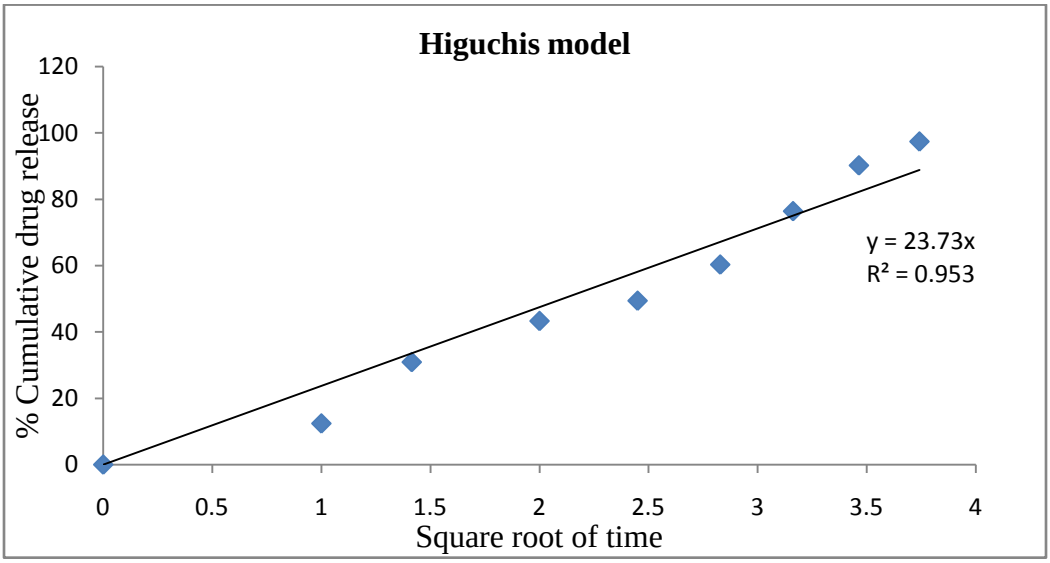


Fig.No:12.in-vitro release profile according to Higuchi's release model for F5 formulation.

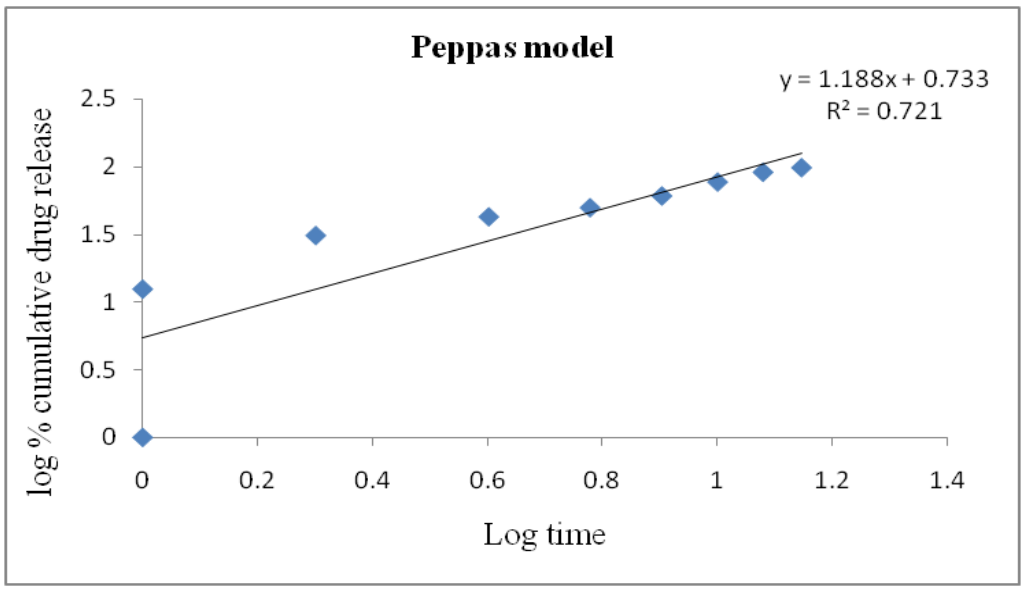


Fig.No:13.in-vitro release profile according to Peppas release model for F5 formulation.

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Conflict of Interest: The authors do not have any conflict of interest.

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