

Formulation and Evaluation of Gastroretentive Floating Microspheres loaded with Lamivudine

Shikhar R. Karosiya*, Vaishali M. Vaidya, Nitin S. Bhajipale, Rahul S. Radke

Department of Pharmaceutics, SGSPS Institute of Pharmacy, Akola, (MS) India - 444004

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Abstract



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Some drugs give poor absorption because of a narrow absorption window in the gastrointestinal tract (GIT). To improve the absorption of such drugs in GIT, gastro retentive drug delivery techniques play a vital role. Lamivudine is an antiviral medicine. The floating gastroretentive microspheres were prepared to attain an extended therapeutic action of Lamivudine. Floating microspheres were formulated to prolong the gastric retention and enhancement of bioavailability of the drug candidate. The floating microspheres were formulated using guar gum and xanthan gum as natural polymers. The proposed floating system was evaluated by preliminary evaluation parameters, micrometric investigation as well as Particle size investigation, Percentage yield of microspheres, Drug entrapment efficiency, In-vitro drug release studies, and their kinetics. The formulated microsphere is evaluated in the form of its stable nature. The formulated microsphere was found to be smooth, spherical, and slightly aggregated containing guar gum whereas xanthan gum containing microsphere was found porous, irregular, grossly, and discrete globular. Formulated microsphere gives a good percentage of drug entrapment and practical yields. The floating microsphere prolonged the drug release by up to 12 hrs. The IR spectroscopy of the prepared formulation was shown that the chemical structure of the Lamivudine was unchanged. The prepared lamivudine microspheres presented gastro retentive and extended-release properties.

Keywords: Lamivudine; Microspheres; Narrow absorption window; Floating system

*Address for Correspondence:

Shikhar R. Karosiya, Department of Pharmaceutics, SGSPS Institute of Pharmacy, Akola, (MS) India - 444004

INTRODUCTION:

Floating microspheres are part of gastro-retentive drug delivery schemes. Floating microspheres is a non-effervescent methodology¹⁻². Gastro-retentive floating Microspheres are categorized in low-density methods that have satisfactory buoyancy to float above gastric fluids and stay in the stomach for an elongated period³⁻⁴. As the microsphere floats above gastric matters, the drug is released gradually at a preferred rate causing improved gastric retaining along with a reduction of fluctuations in plasma drug concentration⁵⁻⁷.

Lamivudine is a cytidine analog that acts as an antiretroviral drug. It can obstruct both types 1 HIV reverse transcriptase and type 2 HIV reverse transcriptase. Lamivudine also obstructs reverse transcriptase of Hepatitis B⁸⁻¹⁰. Lamivudine is chemically 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3 oxathiolan-5-yl]-1,2 dihydropyrimidin-2-one (Figure1) with chemical formula C₈H₁₁N₃O₃S¹¹⁻¹².

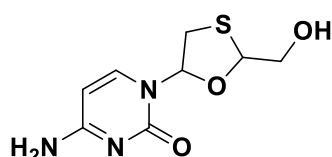


Figure 1: Structure of Lamivudine

As per the literature assessment, different dosages for Lamivudine were available¹³⁻¹⁸. Also, some floating systems were reported for Lamivudine¹⁹. The present research intends to formulate and evaluate natural polymer-based microspheres to improve therapeutic action as well as the bioavailability of Lamivudine.

MATERIAL AND METHODS:

Materials

Lamivudine standard was obtained as a gift sample from Cipla. Pvt Ltd Pune, Xanthan gum from Yarrow chem products, Mumbai, and Guar gum from Agro Gums. Ahmedabad, Gujarat, Also the other excipients for microsphere preparation from S D Fine Chem Limited, Mumbai. All additional chemicals and reagents used were of pharmaceutical ranking.

Methods

Floating Gastro retentive Microsphere preparation²⁰

The Floating Gastro retentive Microsphere was prepared by solvent evaporation using several proportions of the drug: natural gum such as 1:1.10, 1:1.20, and 1:1.30. In preparation, gum was dissolved separately in a sufficient quantity of water for 2 hours. In the resultant flowable mass 100mg drug was introduced with 10mL of methylene chloride and mixed to get drug-gum dispersion. This dispersion was acidified with 0.5 mL concentrated sulphuric acid to an obtained clear solution.

Then the solution was stirred at 300 rpm for one hour. The mixture was then transferred speedily into liquid paraffin solution containing span 80, after that in the stirred solution add 1.2% w/v dichloromethane and 0.15 % w/v of glutaraldehyde with continued heating for 2.5 hours.

Collecting the microspheres and co-precipitate were separated from the Whatman 2 filter paper by filtration, oven-dried (80°C), and kept in a desiccator until a constant weight was obtained. Six formulations of microspheres were prepared by the above stated technique and labeled as B1-B6 (**Table.1**).

Table 1: Composition of Lamivudine gastroretentive Microspheres

Batches	Lamivudine(mg)	Guar Gum(mg)	Xanthan Gum (mg)	Liquid Paraffin(ml)	Span 80
B1	100mg	10	-	200	0.5
B2	100mg	20	-	200	0.5
B3	100mg	30	-	200	0.5
B4	100mg	-	10	200	0.5
B5	100mg	-	20	200	0.5
B6	100mg	-	30	200	0.5

Evaluation of Floating Gastro retentive system [20]

Preformulation Testing

Preformulation testing is the primary step in the rational development of dosage forms of a drug substance. In the current research, preformulation studies were done by performing different studies such as description, melting point, and solubility behavior of drug by taking a few amounts of sample.

Analytical methodology

Preparation of Calibration Curve

The calibration curve for lamivudine was prepared by taking 10 mg drug powder in a 100 mL phosphate buffer solution. The prepared solution was kept for sonication and the final solution was made with 6.8 phosphate buffer to get the solution of 1000 µg/ml. The drug solution was scanned (200-400 nm) against the reagent blank and the absorption spectrum are recorded at 270 nm. A graph was plotted by taking the concentration of the drug solutions on the x-axis and the corresponding absorbance values on the y-axis.

Drug Excipient Compatibility Study

IR spectroscopy was achieved on an FT-IR spectrophotometer. The pellets of drug sample and potassium bromide were prepared by compressing the powders at five tons for 5 min on KBr - press and the spectra were scanned in the range of 4000 - 450 cm⁻¹. FTIR study was carried out on Lamivudine and their physical mixture.

Micromeritic properties

The microspheres were characterized by their angle of repose bulk density, tapped density, compressibility index, and Hausner's ratio.

Determination of Particle size

The particle size determination was carried out by the laser particle counting method. In this technique, the formulation was kept in front of laser light, and after an essential number of runs; they were directed towards the detector. From this, the particle size range and the average mean particle size of the formulation were studied.

Surface morphology

The surface morphology of prepared floating microspheres was studied using scanning electron microscopy. The

microsphere preparations were scanned randomly, and photographs were taken at appropriate magnification.

Determination of Percentage yield

The % yield of the prepared microsphere was conducted by taking the weight of the drug and polymer used for the preparation of microspheres. The % yield was calculated by using the following formula:

$$\% \text{ Yield of Microsphere} = \text{Practical Yield} / \text{Theoretical yield} \times 100$$

Drug Loading and Drug Entrapment

Accurately weighed 100 mg of microspheres and smooth in a mortar, 100 ml of 0.1N HCl (pH 1.2) was added, and the aqueous suspension was then sonicated for the whole dissolution. From the above prepared suspension, an aliquot of 1 ml was taken and diluted to 10 ml. The mixture was then filtered and evaluated spectrophotometrically by Shimadzu UV spectrophotometer (Japan), at 270 nm for the estimation of free drug. The quantity of drug-loaded and entrapped in the microspheres was calculated by using formulas.

Buoyancy percentage

Microspheres (0.1 g) were spread over the surface of a dissolution apparatus (type II) filled with 900 mL 0.1 mol L⁻¹ HCl. The medium was agitated with a paddle rotating at 100 rpm for 12 h. The floating and the settled portions of the microspheres were recovered separately. The microspheres were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the Microspheres,

In vitro drug release Study

In-vitro drug release studies of floating microspheres were performed using USP type II dissolution apparatus in 900 ml of 0.1N HCl (pH 1.2) dissolution media at 50 rpm and 37°C. At each definite interval, 5 ml of the sample was withdrawn and was replaced by equal volumes of fresh dissolution medium on each occasion. The sample was evaluated by a UV spectrophotometer at 270 nm.

Release kinetics

The mechanism of drug release and release rate kinetics from the floating microspheres were studied by exposing in vitro drug release studies to different kinetics models, Higuchi matrix, Korsmeyer Peppas model, Zero order, and First order kinetics.

Stability study

A stability study was carried out for a period of up to 60 days for particular formulations. The selected formulations were examined for their physical appearance, drug entrapment, and *in-vitro* release study.

RESULT AND DISCUSSION

Six formulations batches (B1 to B6) of floating microspheres were prepared by using different ratios of Guar gum (B1-B3) and xanthan gum (B4-B6) respectively.

Table 2: Calibration data of drug at 270nm

Sr.no	Concentration((μ g/mL)	Absorbance
1	0	0
2	5	0.156
3	10	0.32
4	15	0.49
5	20	0.65
6	25	0.801
7	30	0.969
Slope		0.0323
R^2		0.999
Equation		$y = 0.0323x + 0.0009$

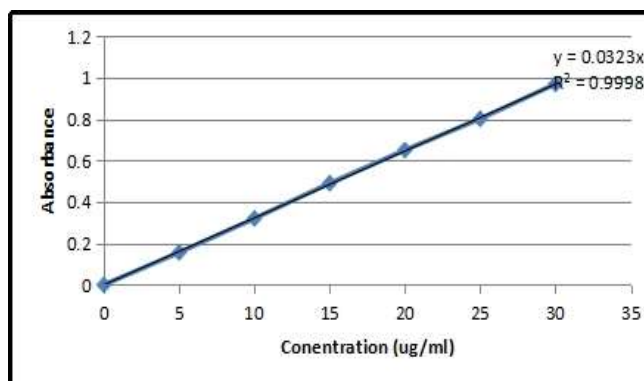


Figure 2: Calibration curve of Lamivudine in 6.8 Phosphate buffer at 270 nm

A preformulation study was done by performing different studies such as description, melting point, and solubility behavior of drug by taking a few about of sample of lamivudine it was found the given sample having off-white color along with characteristic odor and drug gives melting point at 161°C. Lamivudine was found to be highly soluble in phosphate buffer. The calibration curve for lamivudine was prepared in a 5-30 μ g/ml concentration range at the observed wavelength of 270nm. The calibration data is summarized in (Table 2 and Figure 2).

Drug Excipient Compatibility Study

FTIR spectroscopy was carried out to check the compatibility of Lamivudine with the polymer used in the formulation of floating microspheres. All significant functional group frequencies for Lamivudine showed no significant shifts in combination spectra indicating no interaction.

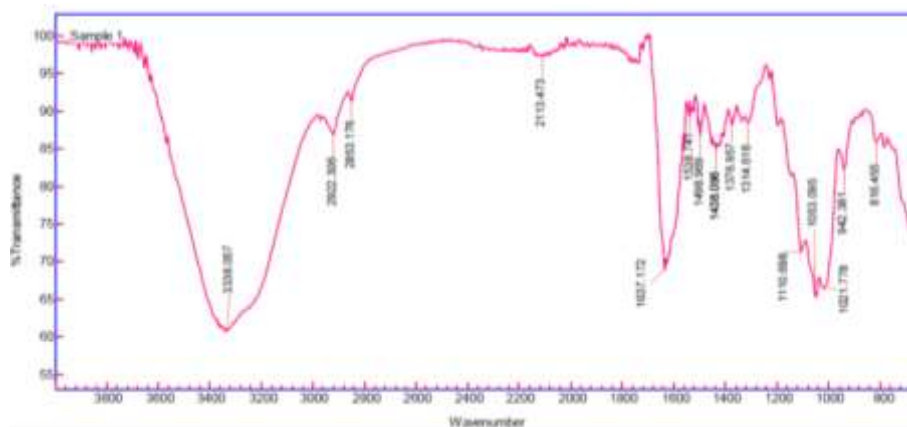


Figure 3: FTIR Spectra of Lamivudine and physical mixture

Micromeritic properties

The flowability of the microspheres was studied. The achieved data along with related parameters are presented in (Table 3). The values of θ in between ranged from 25.74 to 37.12 indicating that the obtained values were well within the limits. This result clearly shows that the prepared microspheres have

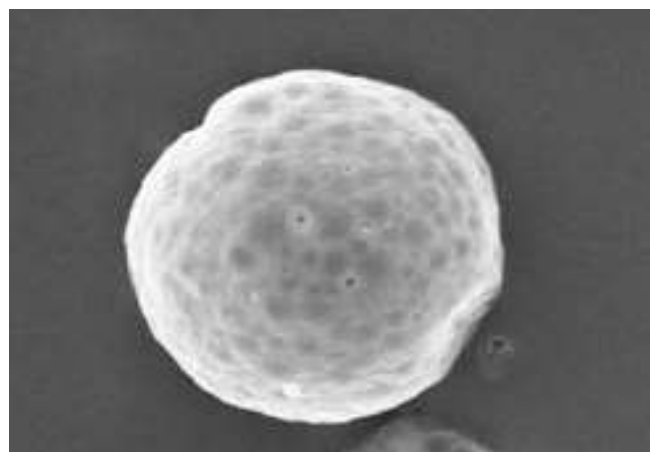
reasonably good flow potential. The value of the compressibility Index was found to be in the range of 12.36 to 16.16 %. The values of tapped density ranged between 0.51 to 0.64 g/cm³. The density difference between the formulations is negligible and the density values of formulations were well within the limits, indicating that the prepared microspheres were non-aggregated and spherical.

Table 3 Micrometric properties of Lamivudine Microspheres

F. code	Bulk Density(g/cm ³)	Tapped Density(g/cm ³)	Compressibility Index (%)	Hauser Ratio	Angle of repose (θ)
B1	0.5418±0.013	0.6183±0.001	12.36±1.04	1.141±0.02	28.67±0.36
B2	0.4986±0.008	0.5814±0.004	14.24±1.32	1.166±0.05	25.74±0.24
B3	0.5438±0.016	0.6432±0.014	15.45±0.84	1.183±0.026	37.12±1.51
B4	0.4426±0.005	0.5126±0.009	13.65±1.21	1.158±0.02	26.93±0.23
B5	0.0523±0.015	0.6243±0.008	16.16±1.27	1.193±0.011	32.94±0.17
B6	0.4813±0.009	0.5446±0.005	11.94±1.34	1.131±0.019	33.81±0.14

Determination of Particle size and Surface Morphology

The mean particle size for the formulations B1 to B3 containing guar gum was found to be in the range from 572 ± 12.51 to 991 ± 10.73 μ m for formulations B4 to B6 containing Xanthan gum the mean particle size was found to be in the range from 278 ± 7.14 to 913 ± 6.35 μ m respectively. The prepared microspheres were spherical. The microspheres of Lamivudine with Guar gum were smooth; spherical when compared with the microspheres of xanthan gum (**Figure 4**).

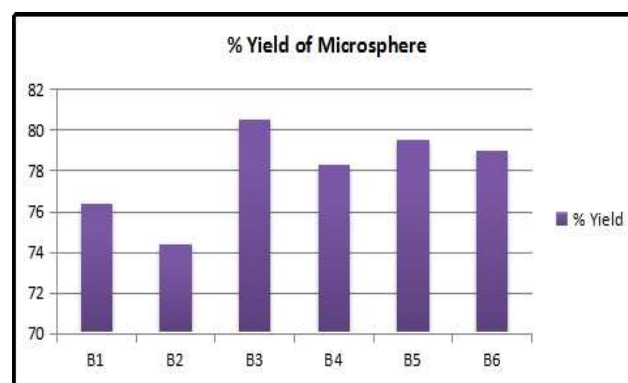
**Figure 4:** Surface morphology of microsphere containing lamivudine and guar gum

Percentage yield

The percentage yield of all the formulations was observed in the range of 74.4%-80.5%, the prepared microsphere batches B3 give a higher percentage yield (**Table 4** and **Figure 5**).

Table 4: Percentage yield of microsphere

Sr. No	Formulation Batch Code	% Yield
1	B1	76.4
2	B2	74.4
3	B3	80.5
4	B4	78.3
5	B5	79.5
6	B6	79.0

**Figure 5:** Comparison of % Yield of the Prepared Microspheres

Drug Loading and Drug Entrapment

The amount of drug-loaded and entrapped in the microspheres was calculated. The entrapment efficiency and drug loading of all the formulations ranged from 73.62-90.44 % and 27.05-38.56% respectively (**Table 5**).

Table 5: Data of % drug loaded and % Drug Entrapment

Sr. No.	Formulation Batch	% Drug loaded	%Drug Entrapment
1	B1	37.88	73.62
2	B2	32.86	78.44
3	B3	29.44	84.88
4	B4	38.56	78.12
5	B5	27.5	82.48
6	B6	22.60	90.40

Buoyancy percentage

The formulated floating microspheres show an average percentage of buoyancy of higher than 79 %. It shows that all preparations achieve a very ideal floating property that helps to good sustain drug release in the systemic circulation.

In vitro release study

The in vitro drug releases of all the preparations were found to be in the range of 79.86 % to 86.52 % at the end of 12 hours. The comparative in vitro release profile of the formulations was revealed in (Figure 6).

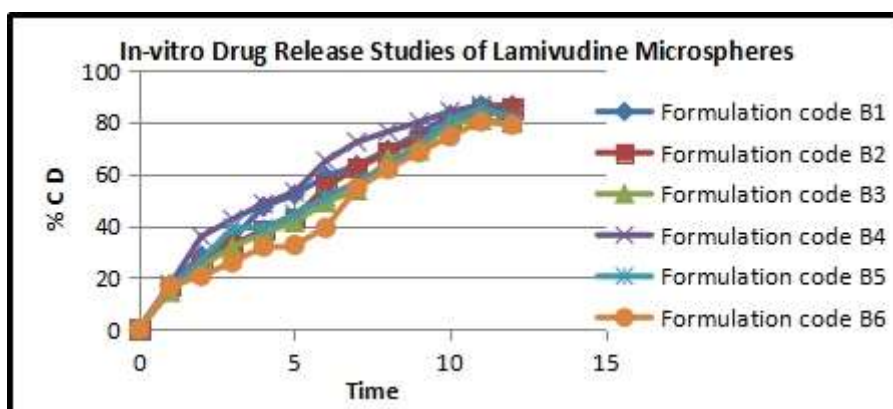


Figure 6: The *in-vitro* drug release data of different formulations

Release kinetics

The release data were fitted to different kinetic models to conclude the release constant and regression coefficient (R^2). The drug release profiles for formulations (B1 to B) were best

fitted with zero-order kinetics based on regression coefficients of 0.9868, 0.9834, 0.9896, 0.9552, and 0.9914 respectively (Figure 7). The diffusion exponent (n) values for all the formulations were greater than 0.5, representing the drug release by the non-fickian diffusion mechanism.

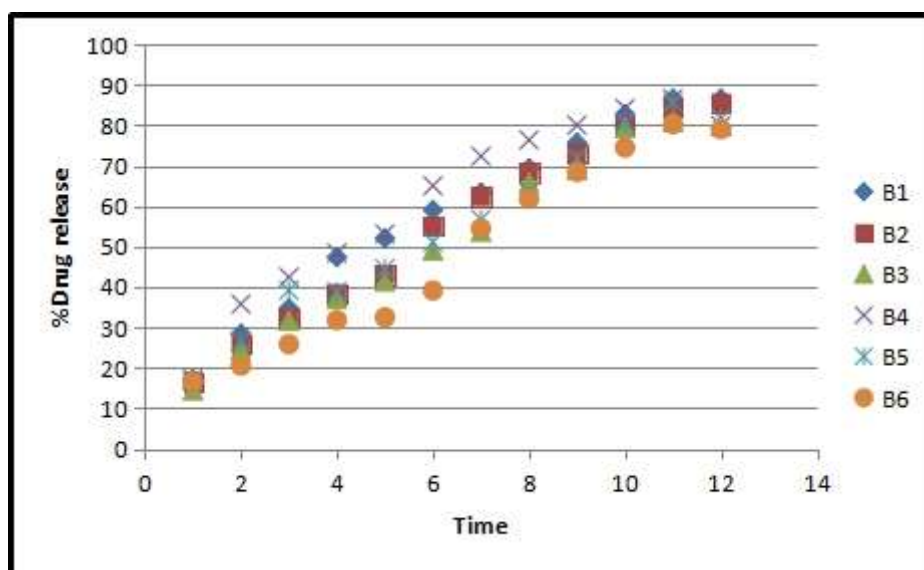


Figure 7: Zero order release kinetics for prepared Microspheres

Stability study

There was no significant change observed in the entrapment efficiency and in vitro drug release as conducted at an interval of 15,30,45 days after 2 months at $40 \pm 2^\circ\text{C}$.

DISCUSSION

Lamivudine floating microspheres were successfully prepared by using guar gum and xanthan gum as polymers, along with liquid paraffin and Span 80. FTIR spectra of the physical mixture revealed that the drug and the polymers used were compatible. The Flow properties of all formulations were within the acceptable range. The particle size of floating

microspheres was found to increase with the increase in polymer concentration i.e. The particle size of guar gum-containing batches was found to be in the range from 572 ± 12.51 to 991 ± 10.73 μm and for xanthan gum, the mean particle size was found to be in a range from 278 ± 7.14 to 913 ± 6.35 μm respectively. The surface topography study of floating microspheres revealed that the prepared microspheres were spherical. The microspheres of Lamivudine with Guar gum were smooth and spherical when compared with the microspheres of xanthan gum. The in vitro drug releases of all the formulations were found to be in the range of 79.86 % to 86.52 % at the end of 12 hours. The percentage yield obtained in all the batches was good and in

the range of 74.4%-80.5%. The drug release decreased with the increase in polymer concentrations in floating microspheres. All formulations B1-B6 followed zero-order kinetics with a non-fickian drug release mechanism.

CONCLUSION

The Lamivudine Floating Microspheres were successfully formulated and estimated for various parameters. In the present study of lamivudine floating microspheres, a satisfactory attempt was made to develop a formulation with improved bioavailability, efficient targeting capacity, and dose decrease capacity. From the experimental results, it can be concluded that the guar gum was a suitable polymer for the preparation of floating microspheres of Lamivudine with improved patient compliance.

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Conflict of interest

Authors do not have any conflict of interest

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