# DEVELOPMENT AND IN-VITRO EVALUATION OF LAMIVUDINE HOLLOW MICROSPHERES

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***ABSTRACT:*** ***The objective of the present work was to formulate floating hollow microspheres of Lamivudine which is soluble and shows better absorption in gastric pH. Microspheres were prepared by emulsion solvent diffusion technique. Using various such as Ethyl cellulose and eudragit polymers. The formulations were evaluated for micrometrics properties, in vitro buoyancy, % yield, entrapment efficiency, and in vitro studies. They were characterized by FT-IR. FT-IR and studies indicated that there was no interaction between the drug and polymers. SEM photographs showed the outer surface of microspheres was smooth and dense whereas the internal surface was porous which helped to prolong floating to increase residence time in the stomach. The results showed that floating microspheres could be successfully prepared with better yield. Results showed larger the particle size, longer was the floating time. In vitro drug release studies showed controlled release of Lamivudine for over 8 h. From the results it can be concluded that gastric floating hollow microspheres can be successfully used for the delivery of Lamivudine to control blood glucose level.***

***Keywords: Lamivudine, Polymers, FTIR studies, emulsion solvent diffusion technique, floating time, in vitro drug release studies.***

## I. INTRODUCTION

Controlling the rate of drug release is microencapsulation where a drug material is coated with a various polymer substance. As a result, the process becomes invincible to safety hazards, and toxicity and decreases the cost of production making the techniques reproducible, economically and ecologically at an industrial scale.[1] Hollow Microspheres are gastro-retentive drug delivery systems based on a non-effervescent approach. Hollow Microspheres are in strict sense, spherical empty particles without the core. These Microspheres are characteristically free-flowing powders consisting of proteins or synthetic polymers, ideally having a size less than 200 micrometer.[2] Hollow Microspheres are prepared by solvent diffusion and evaporation methods to create the hollow inner core. Lamivudine is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of Hepatitis B. Lamivudine was originally developed as an antiretroviral drug. The drug is metabolized intracellularly to the active triphosphate moiety by both infected and uninfected cells.[3]Thus, the present study proceeds with the objective of formulation and evaluation of lamivudine hollow microspheres of varying polymers which corroborate the rate retarding properties of the lamivudine delivery.

## II. MATERIALS

Lamivudine was obtained from Hetero Labs, HYD. Eudragit and Ethyl cellulose were procured from Synpharma Research Labs, Hyderabad, and other chemicals the reagents used were of analytical grade.

### Methodology

**Drug excipient compatibility studies[4]**

FTIR is a useful technique to check and confirm any interaction that may occur between excipients and drug. The FTIR spectra of the drug, excipients, briefly, solid sample (1 mg) along with 100 mg dried potassium bromide were compressed into a disc. For liquid sample, few drops of the sample were dripped onto NaCl or KBr aperture plate and sandwiched it under another aperture plate, such that no gas bubbles were trapped. The sample allowed formation of a thin liquid membrane between the two aperture plates. Thereafter, sample was scanned for absorbance over the range from 4000 to 400 (cm-1) wave numbers.

**Preparation and evaluation of Lamivudine hollow microspheres**

**Table-1: Formulation development of Lamivudine hollow microspheres**

| F. no | Polymer | Drug and polymer ratio | Stirring speed |
|---|---|---|---|
| F1 | Eudragit | 1:1 | 1000 |

| | | | |
|---|---|---|---|
| F2 | Eudragit | 1:2 | 1000 |
| F3 | Ethyl cellulose | 1:1 | 1000 |
| F4 | Ethylcellulose | 1:2 | 1000 |

**Method:** [5]

Emulsion-solvent diffusion technique with some modifications was used to prepare Eudragit, ethyl cellulose microspheres containing Lamivudine. Briefly Lamivudine was dissolved in 5 ml distilled water. Polymers was dissolved in Dichloromethane at various drug - polymer ratios (1:1 and 1:2). Then these drug and polymer solutions were mixed and emulsified using a Remi Lab Magnetic stirrer at 500 rpm for about 10 min to form stable w/o emulsion. This stable w/o emulsion was slowly added to 200 ml aqueous solution containing 1 % PVA and stirred at 1000 rpm by a mechanical stirrer equipped with a three bladed propeller (Remi motors, India) at room temperature for 2 h to allow the solvent to evaporate completely. Microspheres were isolated by filtration and washed with distilled water several time to remove PVA. The produced microspheres were dried at ambient temperature for 24 h and dried in vacuum chamber at 25 $^0$C for 2 h to remove any residual solvent.

**Evaluation of hollow microspheres**

**Particle size analysis**

Particle size analysis plays an important role in determining the release characteristics and floating properties. The sizes of hollow microspheres were measured by using a set of standard sieves ranging from 14, 16, 18, 22, 30, and pan. The sieves were arranged in increasing order from top to bottom. The hollow microspheres were passed through the set of sieves and the amount retained on each sieve was weighed and the % weight of hollow microspheres retained by each sieve was calculated.

Mean particle size for all formulation was determined by dividing the total weight size of formulation to % total weight of hollow microspheres. [6]

**Floating Property of Hollow microsphere**

100 mg of the hollow microsphere were placed in 0.1 N HCl (300 ml) containing 0.02% tween 20. The mixture was stirred with paddle at 100rpm. The layer of buoyant microspheres was pipetted and separated by filtration at 1, 2, 4 and 6 hours. The collected microspheres were dried in a desiccator overnight. The percentage of microspheres was calculated by the following equation:[7]

% Hollow microsphere = Weight of hollow microsphere / Initial weight of hollow microsphere x 100

**Drug Entrapment efficiency**

The various formulations of the hollow microspheres were subjected for drug content. 50 mg of hollow microspheres from all batches were accurately weighed and crushed. The powdered microspheres were dissolved with 10ml ethanol in 100ml volumetric flask and the volume was made up with 0.1 N HCl. This resulting solution is than filtered through Whatmann filter paper No. 44. After filtration, from this solution 10 ml was taken out and diluted up to 100 ml with 0.1 N HCl. Again from this solution 2 ml was taken out and diluted up to 10 m1 with 0.1 N HCI and the absorbance was measured at 239 nm against 0.1 N HCl as a blank. The percentage drug entrapment was calculated as follows. [8]

% Drug entrapment =- Calculated drug concentration / Theoretical drug concentration x 100

**Percentage Yield**

The percentage yield of different formulations was determined by weighing the hollow microspheres after drying. The percentage yield was calculated as follows.[9]

Total weight of % Yield = hollow microspheres/ Total weight of drug and polymer x 100

**Surface Characterization by SEM**

From the formulated batches of hollow microspheres, formulation which showed an appropriate balance between the buoyancy and the percentage release were examined for surface morphology and shape using scanning electron microscope. Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 20KV during scanning. Microphotographs were taken on different magnification and higher magnification (200X) was used for surface morphology. [10]

**In vitro drug release study**

In vitro drug release studies for all formulations were carried out using the USP type –II dissolution basket assembly. Microspheres equivalent to 100 mg of Lamivudine were taken. The dissolution media of 900 ml of stimulated gastric fluid (pH1.2) was maintained at 37±0.5 ºC and stirred at 100 rpm. 1 ml aliquots were withdrawn at a predetermined intervals and equal volume of dissolution medium was replaced to maintain sink conditions. The necessary dilutions were made with 1.2 pH buffer and the solution was analyzed for the drug content spectrophotometrically using UV-Visible spectrophotometer. [11]

**Stability Study**

From the prepared hollow microspheres which showed appropriate balance between the buoyancy and the percentage release was selected for stability studies. The prepared formulation were placed in borosilicate screw capped glass containers and stored at room temperature (27 ± 2° C), oven temperature (42±2° C) and in refrigerator (5-8° C) for a period of 30 days. The samples were assayed for drug content at regular intervals for 3 months.[12]

## III. RESULTS AND DISCUSSION

**FT-IR Spectrum of Lamivudine**

FT-IR Spectra of Lamivudine and F2 formulation were recorded. All these peaks have appeared in formulation and physical mixture, indicating no chemical interaction between Lamivudine and polymer. It also confirmed that the stability of drug during microencapsulation process.

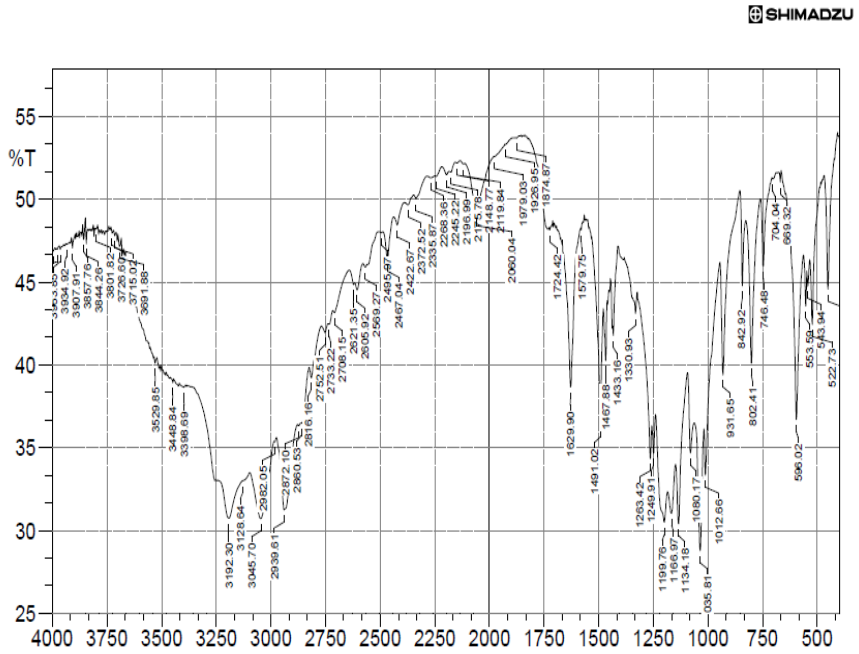


Fig.1.FTIR Studies of Lamivudine

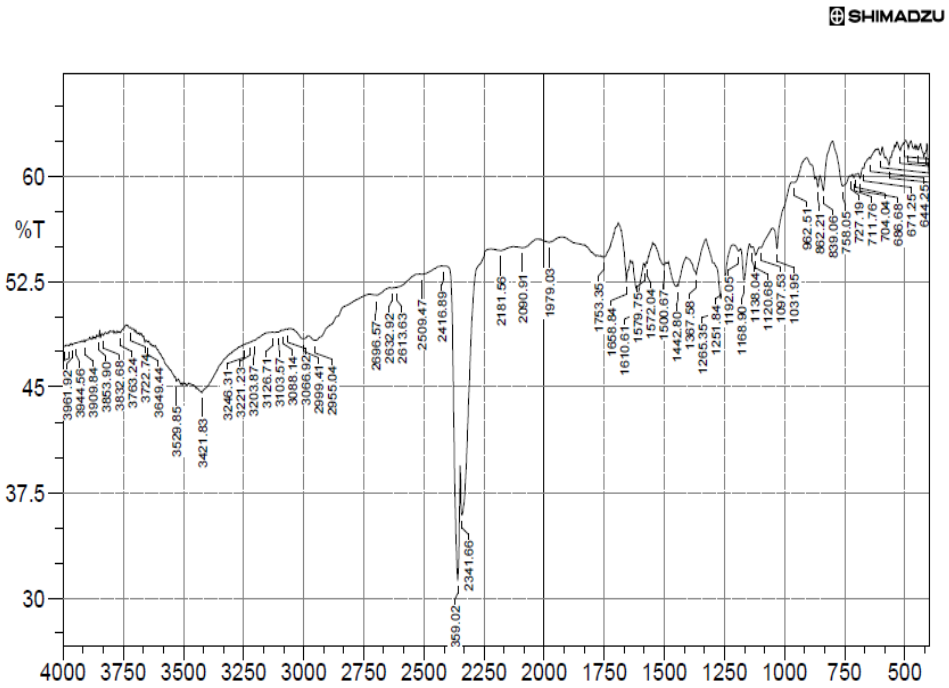


Fig. 2. FTIR Studies of optimized formulation

**Evaluations of hollow microspheres**

**Particle size analysis**

Particle size was determined by sieving method it plays important role in floating ability and release corrected of drug from hollow microspheres. If size of Hollow microspheres less than 500 mm so release rate of drug will be high and floating ability will reduce, while Hollow microspheres range between 500mm - 1000mm, floating ability will be more and release rate will be in sustained manner. The mean particle size of hollow microsphere was in range 500-520 nm.

**Table-2: Particle size of Different formulations of Hollow microsphere**

| Formulation code | Mean particle size* (nm) |
|---|---|
| F1 | 509 |
| F2 | 500 |
| F3 | 515 |
| F4 | 520 |

**Floating Property of hollow microsphere**

Hollow Microsphere were dispersed in 0.1 N HCl to simulate gastric fluid. Floating ability of different formulation were found to be differed according to polymer ratio.

**Table-3: Percentage buoyancy for different formulation**

| Formulation | 1 hour | 2 hours | 4 hours | 6 hours |
|---|---|---|---|---|
| **F1** | 96.15 | 96.46 | 95.23 | 93.25 |
| **F2** | 96.62 | 92.42 | 92.68 | 93.16 |
| **F3** | 94.50 | 91.65 | 89.90 | 93.18 |
| **F4** | 90.18 | 90.18 | 93.15 | 93.20 |

**Drug Entrapment**

The drug entrapment efficacy of different formulations were in range of 74.27% - 78.92 % w/w. Drug entrapment efficacy slightly increases eudragit content ratio in Hollow microspheres. This is due to the permeation characteristics of Eudragit that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of hollow microspheres.

**Table-4: Drug entrapment for different formulation**

| Formulation | Drug Entrapment (% w/w) |
|---|---|
| **F1** | 76.50 |
| **F2** | 78.92 |
| **F3** | 75.81 |
| **F4** | 74.27 |

The entrapment efficiency values ranged from 76.50 to 74.27 for all the formulations. For selected formulation (Eudragit) entrapment was found to be more in F2. So it is indicated only optimum concentration is suggestible. From the above result F2 (drug and polymer) was selected as a optimized formulation.

**Percentage Yield**

Percentage yield of different formulation was determined by weighing the Hollow microspheres after drying. The percentage yield of different formulation were in range of 83.52- 85.46 % as shown in Table.

**Table –5: Percentage yield for different formulation**

| Formulation | Percent Yield*(%) |
|---|---|
| **F1** | 83.69 |
| **F2** | 85.46 |
| **F3** | 84.90 |
| **F4** | 83.52 |

**Scanning Electronic Microscopy**

Shape and surface characteristic of hollow microspheres examine by Scanning Electronic Microscopy analysis as shown in Fig. Surface morphology of F2 formulation examine at different magnification 40X and 200X, which illustrate the smooth surface of floating Hollow microspheres and small hollow cavity present in microsphere which is responsible for floating property.

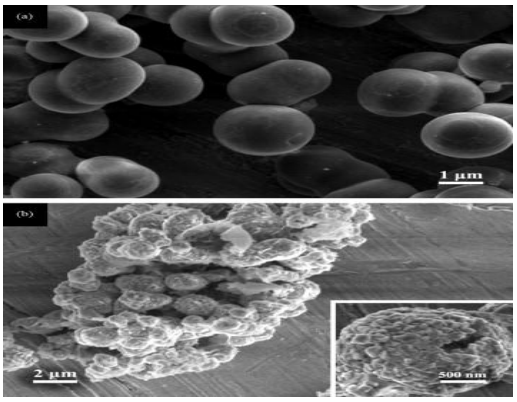


Fig.3. SEM Analysis of Optimized formulation

The optimized formulation F2 was evaluated for its surface morphology by using SEM analysis. The particle size was found to be 500nm. The hollow microspheres were found to be smooth and spherical in shape.

***In*-vitro Drug release study**

*In-vitro* drug release study of Hollow microspheres was evaluated in pH 1.2 buffer. Eudragit RS100 which is present in all formulation, have low permeability in acid medium. Since Eudragit is less soluble in acidic pH, release of drug in 0.1 N HCl was generally low.

**Table-6: Comparative *In-Vitro* Drug Release Profile for Formulation in 6.8 phosphate**

| Time (hrs) | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 1 | 15.98 | 16.34 | 16.82 | 16.02 |
| 2 | 26.75 | 27.53 | 28.15 | 21.88 |
| 3 | 40.14 | 36.82 | 32.88 | 34.25 |
| 4 | 56.03 | 45.15 | 44.15 | 50.14 |
| 5 | 67.25 | 58.05 | 59.18 | 69.29 |
| 6 | 72.65 | 69.89 | 66.63 | 75.18 |
| 7 | 84.92 | 86.48 | 74.16 | 83.85 |
| 8 | 91.53 | 94.85 | 90.90 | 92.12 |

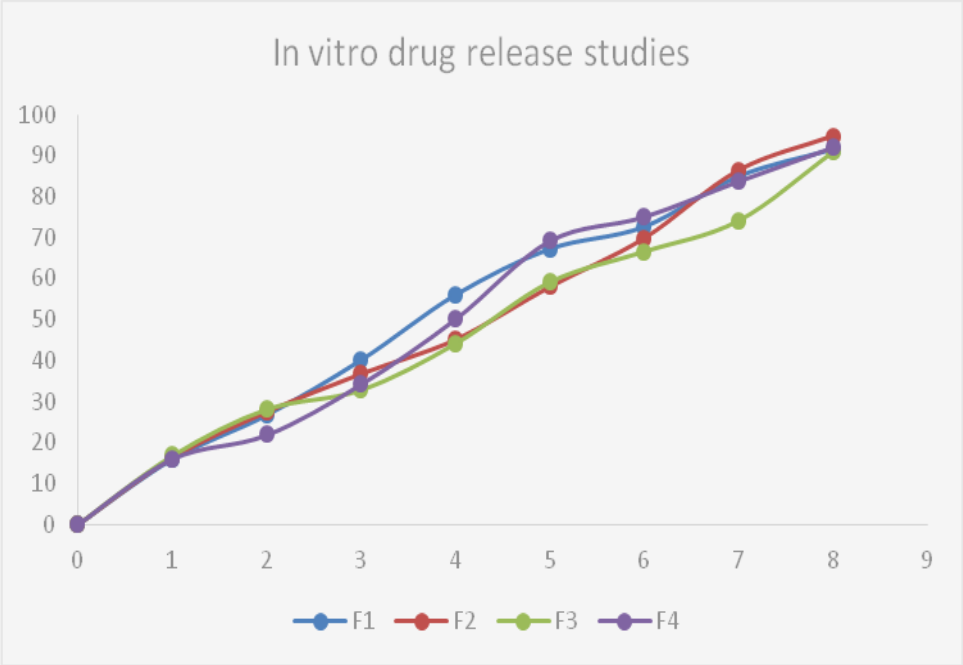


Fig.4. Comparative In-Vitro Drug Release Profile Of ( F1-F4 ) formulation

All the 4 formulations of hollow microspheres were subjected to dissolution studies. Dissolution was carried out in franz diffusion cell apparatus at 100 rpm in the volume of 10 ml dissolution media of 0.1N HCL for 8 hours. F2 showed a release rate of 94.85 by end of 8$^{th}$ hour of dissolution study.

**Stability Study**

Stability study was carried out for the F2 formulation by exposing it to different temperature 25°C, 30°C and 40°C for 90 days. The sample was analysed for drug content at the regular intervals. It was found that no remarkable change in the drug release of F2 formulation. This indicates that F2 was stable for following temperature.

**Table-7: Results of stability studies of optimized formulation F-5**

| S.NO | Time in days | Physical changes | Mean % drug release | | |
|---|---|---|---|---|---|
| | | | Lamivudine | | |
| | | | 25$^0$C/60% | 30$^0$C/75% | 40$^0$C/75% |
| 1. | 01 | No Change | 94.85 | 94.85 | 94.85 |
| 2. | 30 | No Change | 92.45 | 92.14 | 92.05 |
| 3. | 60 | No Change | 91.25 | 91.57 | 91.69 |
| 4. | 90 | No Change | 90.98 | 90.68 | 90.54 |

The optimized formulation was stored in different conditions to check the stability. Drug release of the optimized formulation F2 initially was 94.85%. From the above result it can be concluded that there was no significant change in physical and chemical properties of the hollow microspheres of formulation F-2 after 3 Months.

## IV. CONCLUSION

The view of microspheres showed a hollow spherical structure with rough surface morphology. It was also evident that the hollow microspheres exhibited porous surfaces. Drug content determination from the hollow microsphere inferred that there was a proper and uniform distribution of drug. The percentage encapsulation efficiency of microspheres also showed that the drug loading was optimum and increased with increasing amounts of polymers. The prepared microspheres exhibited good micrometric properties. From the results of particle size analysis, all the process variables were within the limits and the process was reproducible. The study of micromeritic properties indicated fair to good flow of microspheres. All the formulations floated for more than 8 hours. In vitro tests showed that larger the particle size, the longer the floating time. The microspheres of all the formulations were spherical and free-flowing. In vitro, floating behaviour studies showed that all formulations had a maximum percentage floating ability, the best being F2. The stability studies showed that there were no significant changes in the drug content and physical appearance.

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