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MICROSPHERES PREPARATION AND EVALUATION BY NATURAL GUM FOR ANTIVIRAL DRUG

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ABSTRACT
The present study shows the preparation and evaluation of microspheres of natural gum containing antiviral drug. Lamivudine is an active anti-retroviral drug. Compatibility study was carried out by using FTIR at the range of 800cm^{-1} to 3800cm^{-1} and shows no significant change in the characteristic peaks of Lamuvidine and excipients in all the formulation. Lamuvidine Microspheres is formed by solvent evaporation technique by using xanthan gum and guar gum as rate controlling agent. The Scanning Electron Microscopy (SEM) studies inferred the spherical shape and size range of formulations. In-vitro drug release shows decreases as concentration of xanthan gum increases and release rate was zero order and Fickian diffusion controlled. Stability studies were carried out which indicate that selected formulation was stable. We conclude that microspheres offer a practical and suitable approach to prepare controlled release of Lamuvidine with natural occurring xanthan gum as rate controlling agent to enhance bioavailability and reduction in dose frequency.

KEYWORDS: - Microspheres, Anti-viral drugs, Pre-formulation studies

1. INTRODUCTION

New drug delivery technologies are revolutionizing the drug discovery, development and creating R&D focused pharmaceutical industries to increase the momentum of global advancements. In this view novel drug delivery systems (NDDS) have many benefits, which include improved therapy by increasing the efficacy and duration of drug activity, increased patient compliance through decreased dosing frequency and convenient routes of administration and improved site-specific delivery to reduce unwanted adverse effects.[1,2]

Oral route is the most commonly employed route of drug administration. Although differentroute of administration is used for the delivery of drugs, oral route remains the preferred mode. The popularity of the oral route is attributed patient acceptance, ease of administration, accurate dosing, cost effective manufacturing method and generally improved shelf-life of the product.[3,4]

Controlled Drug Delivery

Controlled drug delivery is one which delivers the drug at a predetermined rate, locally or systemically, for a specified period of time. Continuous oral delivery of drugs at predictable and reproducible kinetics for predetermined period throughout the course of GIT. Recently, a new generation of pharmaceutical products, called controlled release drug delivery systems, such as those developed from the osmotic pressure activated drug delivery system, have recently received regulatory approval for marketing, and their pharmaceutical superiority and clinical benefits over the sustained release and immediate release pharmaceutical products have been increased.[5,6]

MICROSPHERES

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000µm.They are made of polymeric, waxy or other protective materials that are biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes.[7,8] The solvents used to dissolve the polymeric materials are chosen according to the polymer and drug solubility, process safety and economic considerations. Microspheres are small and have large surface-to-volume ratio. At the lower end of their size they have colloidal properties. The interfacial properties of microspheres are extremely important, oftens including their activity.[9,10]

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Anti-viral drug

Antiviral drugs are a class of medication used specifically for the treating viral infections. Like antibiotics for bacteria, specific antivirals are used for specific viruses. Unlike most antibiotics, antiviral drugs do not destroy their target pathogen; instead they inhibit their development. Most of the antiviral drugs now available are designed to help deal with HIV, herpes viruses, the hepatitis B and C viruses, and influenza A and B viruses.[11,12]

The emergence of antivirals is the product of a greatly expanded knowledge of the genetic and molecular function of organisms, allowing biomedical researchers to understand the structure and function of viruses, major advances in the techniques for finding new drugs, and the intense pressure placed on the medical profession to deal with the human immunodeficiency virus (HIV), the cause of the deadly acquired immunodeficiency syndrome (AIDS) pandemic.[13,14]

The first experimental antivirals were developed in the 1960s, mostly to deal with herpes viruses, and were found using traditional trial-and-error drug discovery methods. Researchers grewcultures of cells and infected them with the target virus. They then introduced into the cultures chemicals which they thought might inhibit viral activity, and observed whether the level of virus in the cultures rose or fell. Chemicals that seemed to have an effect were selected for closerstudy.[15,16]

2. MATERIAL AND METHODS

2.1 Standard Calibration Curve of Lamivudine:

Accurately weighed 100 mg of Lamivudine was dissolved in 100 ml of 0.1 N HCl (pH 1.2) (Conc. 1000 µg/ml) to prepare first stock solution. 1 ml of above solution was pipetted out into 100 ml volumetric flask and volume was made up to with 0.1 N HCl (pH 1.2) (Conc. 10 µg/ml)to prepare stock II solution. The aliquot solution of stock II solution was further diluted with pH 1.2 to get 5µg, 10µg, 15µg, 20µg, 25µg and 30µg of drug in the final solution. Then the absorbance was measured in a double beam UV spectrophotometer at 270 nm against pH 1.2 as blank. The same procedure was repeated by using phosphate buffer pH 6.8.

Formulation	Drug (mg)	Xanthan Gum (mg)	Guar Gum(mg)	Liquid Paraffin(ml)	Span 80(v/v)
F1	100	15	-	200	0.5
F2	100	20	-	200	0.5
F3	100	25	-	200	0.5
F4	100	30	-	200	0.5
F5	100	-	15	200	0.5
F6	100	-	20	200	0.5
F7	100	-	25	200	0.5
F8	100	-	30	200	0.5
F9	100	-	35	200	0.5

Table 1. Formulation of Microspheres

2.2 Preparation of Microspheres

Microspheres were prepared by using different ratios of drug: natural gum (1:1.15, 1:1.20, 1:1.25). Gums were allowed to hydrate in 20 ml water for 3 hrs. weighed quantity of drug (100mg) was dispersed in 10 ml of methylene chloride and add the aqueous solution of gum. The above drug-gum dispersion was acidulated with 0.5 ml of concentrated sulphuric acid to give a clear viscous solution. The resultant solution was emulsified into the oily phase by poured into 200 ml of paraffin liquid containing 0.5 % w/w span 80 as an emulsifying agent. Stirred mechanically at 1800 rpm for 210 min using a stirrer and heated by a hot plate at 50^0C. 1.2 % w/v dichloromethane was added as encapsulating agent and 0.15 % w/v of gluteraldehyde as cross-linking agent, stirring and heating were maintained for 2.5 hrs until the aqueous phase was completely removed by evaporation. The oil was decanted and collected microspheres were washed with water to remove surfactant residue and three times with 100 ml aliquots of n- hexane, filtered through whatman filter paper, dried in an oven at 80^0C for 2 hr to collect discrete, solid, free flowing microspheres and stored in a desiccator at room temperature.

2.3 EVALUATION OF LAMIVUDINE MICROSPHERES

2.3.1 Bulk Density

The bulk density is defined as the mass of powder divided by bulk volume.The bulk density was calculated by dividing the weight of the samples in grams by the finalvolume in cm.

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Bulk density = $\frac{\text{Mass of microspheres}}{\text{Volume of microspheres before tapping}}$

2.3.2 Tapped Density

Tapped density is the volume of powder determined by tapping by using a measuring cylinder containing weighed amount of sample. The cylinder containing Known amount of microspheres was tapped for about 1 minute on a tapped density apparatus until it gives constant volume.

Tapped density = $\frac{\text{Mass of microspheres}}{\text{Volume of microspheres before tapping}}$

2.2.3 Carr's Compressibility Index

This is an important property in maintaining uniform weight. It is calculated using following equation.

% Compressibility Index = $\frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}}$ X 100

Lower the compressibility values indicate better flow.

% Compressibility	Flowability
5-15	Excellent
12 – 16	Good
18 – 21	Fair to passable
23 – 35	Poor
33 – 38	Very poor
> 40	Extremely poor

Table 2. Relationship between % Compressibility and Flowability

2.2.4 Hausner's ratio

A similar index like percentage compressibility index has been defined by Hausner. Values less than 1.25 indicate good flow, whereas greater than 1.25 indicates poor flow. Added glident normally improves flow of the material under study. Hausner's ratio can be calculated by formula,

Hausner's ratio = $\frac{Tapped\ density}{Bulk\ density}$

2.2.5 Angle of Repose (θ)

Good flow properties are critical for the development of any pharmaceutical tablet, capsules or powder formulation. It is essential that an accurate assessment of flow properties be made as earlyin the development process as possible so that an optimum formulation can be quickly identified. Interparticle forces between particles as well as flow characteristics of powders are evaluated by angle of repose. Angle of repose is defined as the maximum angle possible between the surface and the horizontal plane.

Angle of Repose	Flowability
< 25	Excellent
25-30	Good
30-40	Passable
> 40	Very Poor

Table 3. Relationship between Angle of Repose and Flowability

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2.2.6 Particle Size Determination: The particle size of the microspheres was determined by using optical microscopy method. Approximately 100 microspheres were counted for particle size using a calibrated optical microscope.

2.2.7 Morphological Study using SEM: The morphological study was carried out by Scanning Electron Microscope (SEM). Microspheres were scanned and examined under Electron Microscope HITACHI SU 1500, Japan connected with Fine coat, JEOL JFC-1100E Ion sputter. The sample was loaded on copper sample holder and sputter coated with carbon followed by Gold.

2.2.8 Drug Loading and Drug Entrapment: Microspheres equivalent to 50 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl (pH-1.2) repeatedly. The extract was transferred to a 100 mL volumetric flask and the volume was made up using 0.1N HCl (pH-1.2). The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically (UV 1700, Shimadzu, Japan) at 212 nm against appropriate blank. The amount of drug loaded and entrapped in the microspheres was calculated by the following formulas:

$$\% \text{ Drug loading} = \frac{\text{Weight of the drug loaded in the microspheres(DC)}}{\text{Total weight of the microspheres}} \text{X } 100$$

$$\% \text{ Drug entrapment} = \frac{\text{Amount of drug actually prsent(DC)}}{\text{Theoretical drug loaded expected}} \text{ X } 100$$

2.2.9 Percentage yield

$$\% \text{ yield} = \frac{\text{Practical yield}}{\text{Theoritical yield}} \text{X}100$$

2.2.10 In vitro drug release Study

The matrix systems were reported to follow the Peppas release rate and the diffusion mechanism for the release of the drug. To analyze the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to, Zero order, First order, Higuchi matrix, Peppas and Hixson Crowell model. In this by comparing the r-values obtained, the best-fit modelwas selected.

3. RESULTS AND DISCUSSION

3.1 Standard calibration curve of Lamivudine

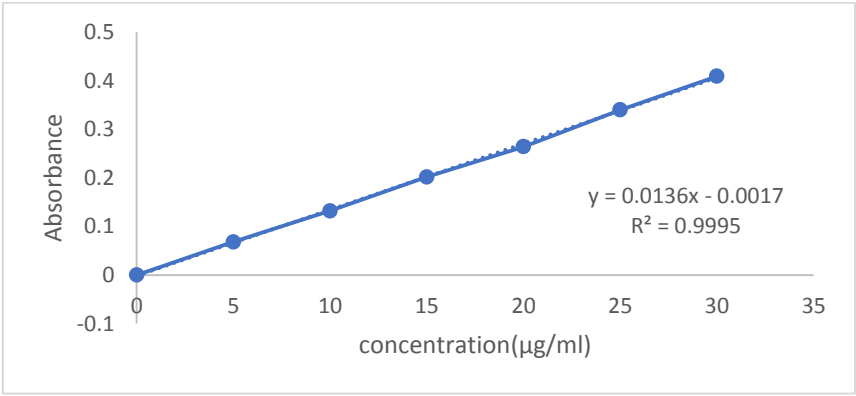

Figure 1. Standard Calibration Curve of Lamivudine in 0.1N HCl

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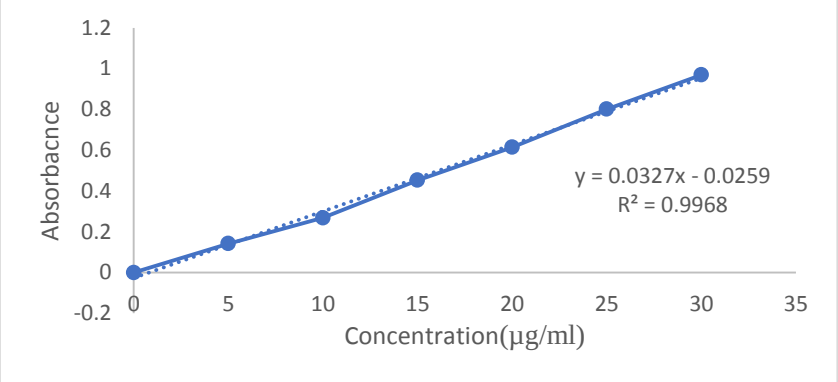

Figure 2. Standard Calibration Curve of Lamivudine in pH 6.8

3.2 Micromeritic Properties

Formulation Code	Bulk Density ($g/cm^{3)}$	Tapped Density (g/cm^3)	Compressibility Index (%)	Hausner's Ratio	Angle of Repose (θ)
F1	0.4426±0.005	0.5126±0.009	13.65±1.21	1.158±0.02	26.93±0.23
F2	0.4986±0.008	0.5814±0.004	14.24±1.32	1.166±0.05	25.74±0.24
F3	0.5234±0.015	0.6243±0.008	16.16±1.27	1.193±0.011	32.94±0.17
F4	0.4813±0.009	0.5446±0.005	11.94±1.34	1.131±0.019	33.81±0.14
F5	0.5418±0.013	0.6183±0.001	12.36±1.04	1.141±0.02	28.67±0.36
F6	0.6168±0.011	0.7136±0.012	13.56±1.02	1.156±0.08	27.08±0.16
F7	0.4576±0.014	0.5228±0.008	12.47±1.21	1.142±0.03	33.61±0.64
F8	0.4754±0.013	0.5845±0.011	15.24±1.03	1.229±0.023	34.54±1.07
F9	0.5438±0.016	0.6432±0.014	15.45±0.84	1.183±0.026	37.12±1.51

Table 4. Micromeritic properties of Lamivudine microspheres

3.3 Particle Size Analysis

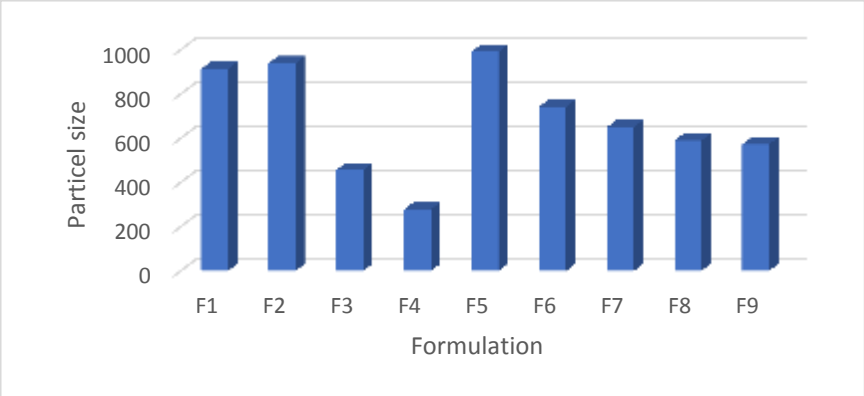

Figure 3. Comparison of Avg. Particle Size of the Prepared Microspheres

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3.4 Scanning Electron Microscopy

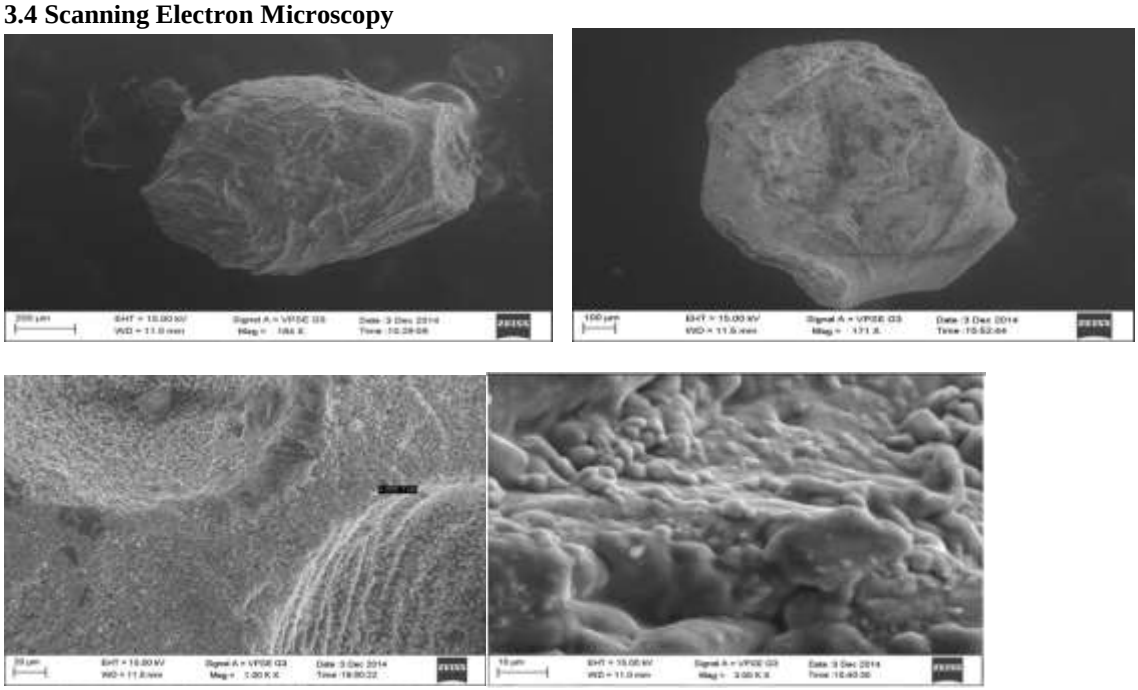

Figure 4. SEM images of F1 and F5 formulation

3.5 COMPATIBILITY STUDY:

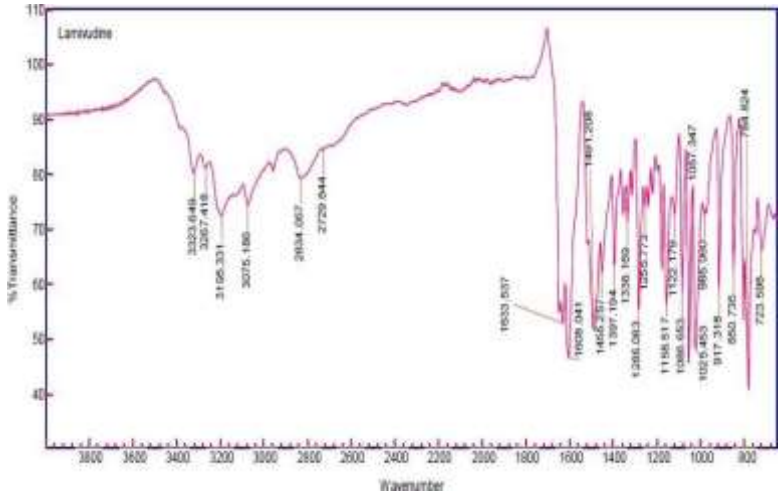

Figure 5. IR Spectrum of pure drug Lamivudine

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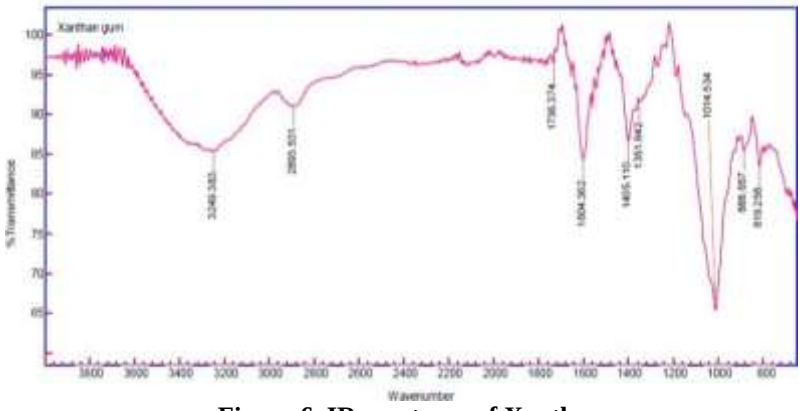

Figure 6. IR spectrum of Xanthan gum

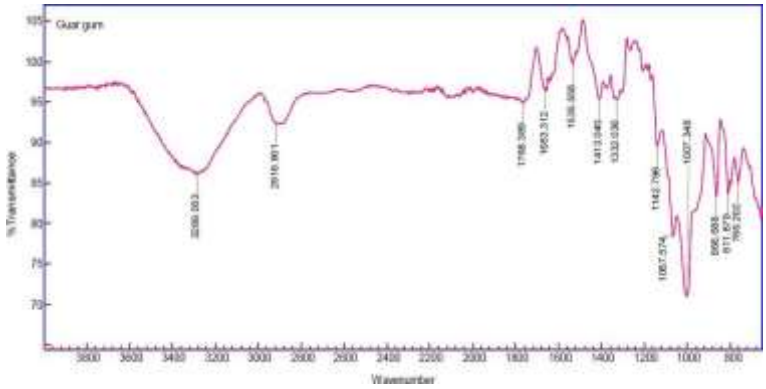

Figure 7. IR spectrum of Guar gum

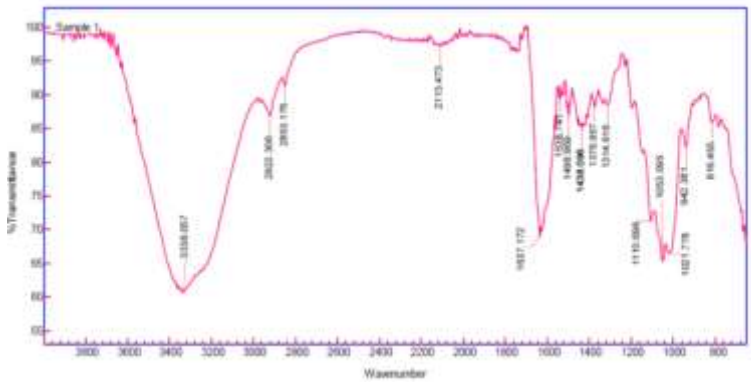

Figure 8. IR Spectrum of Lamivudine with xanthan gum + guar gum

3.6 Solubility analysis

The Lamivudine is freely soluble in water; sparingly soluble in methanol; practically insoluble inacetone. It was soluble in 0.1N HCL (pH 1.2) and phosphate buffer (pH 6.8). Solubility analysis is important because the drug has to dissolve in the solvents and also in thedissolution medium used.

3.7 Melting point determination

The melting point of the obtained drug sample was found to be 161^{0}C which is within thereported range of 160-162^{0}C. It complies with the purity of the drug sample.

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3.8 Drug Loading and Drug Entrapment

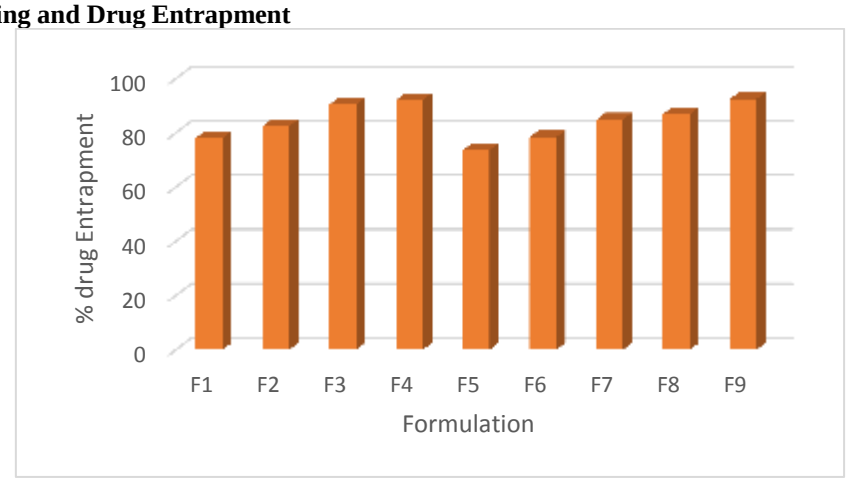

Figure 9. Comparison of % Drug Entrapment of the Prepared Microspheres

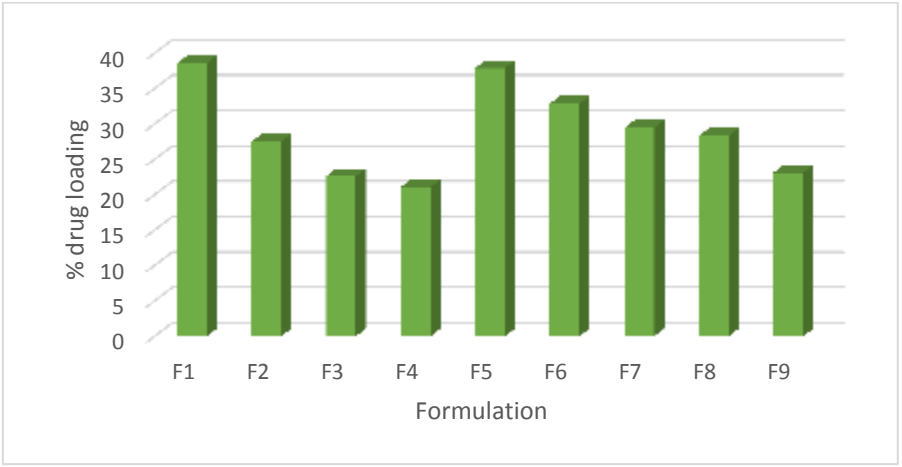

Figure 10. Comparison of % Drug Loading of the Prepared Microspheres

3.9 Percentage yield

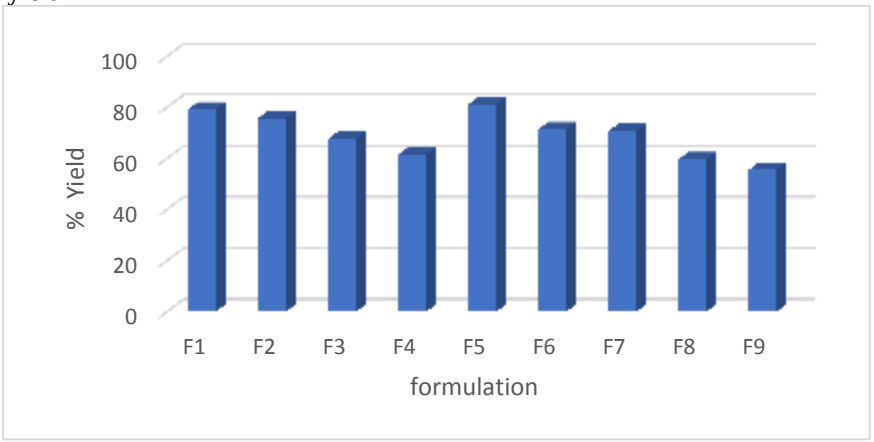

Figure 11. Comparison of % Yield of the Prepared Microspheres

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3.10 *In-vitro* drug release studies

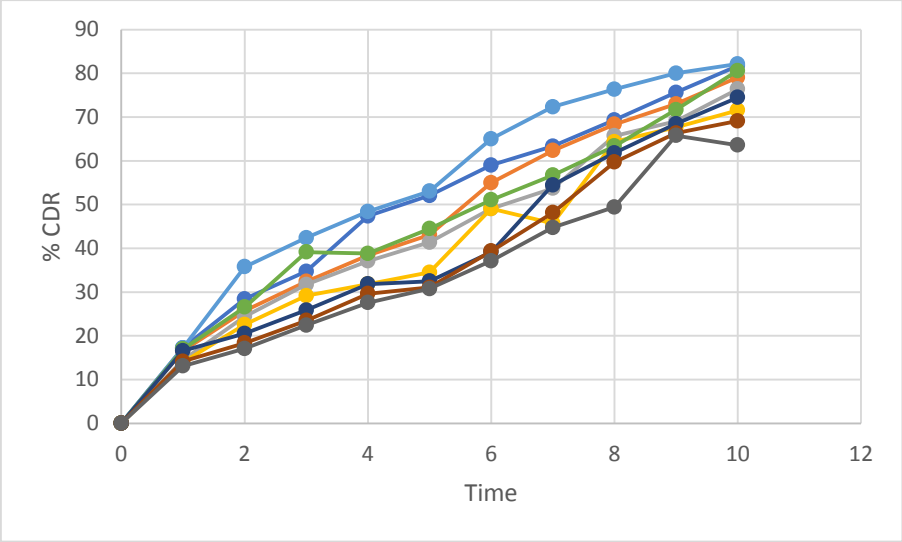

Figure 12. Comparative In-vitro Dissolution Profile of Lamivudine Microspheres

3.11 Release Kinetics

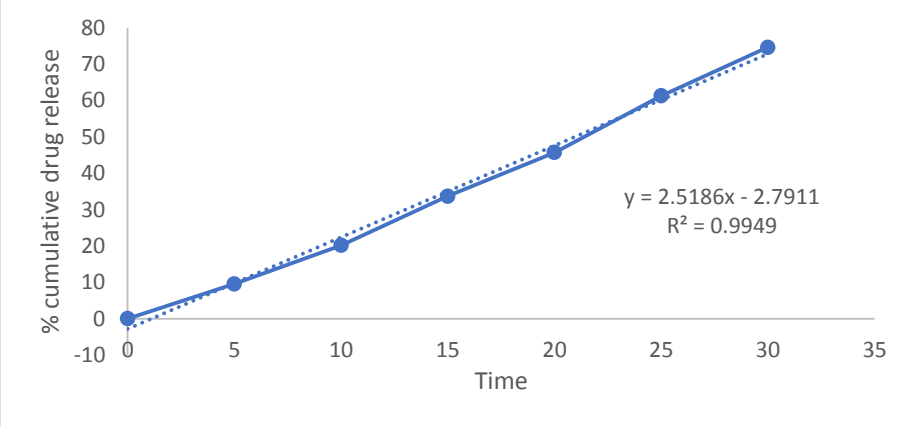

Figure 13. Zero order kinetics

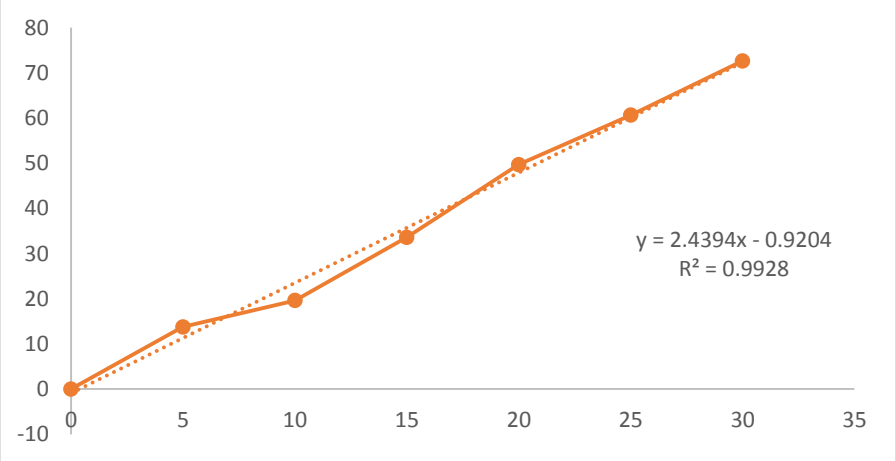

Figure 14. First Order Kinetics

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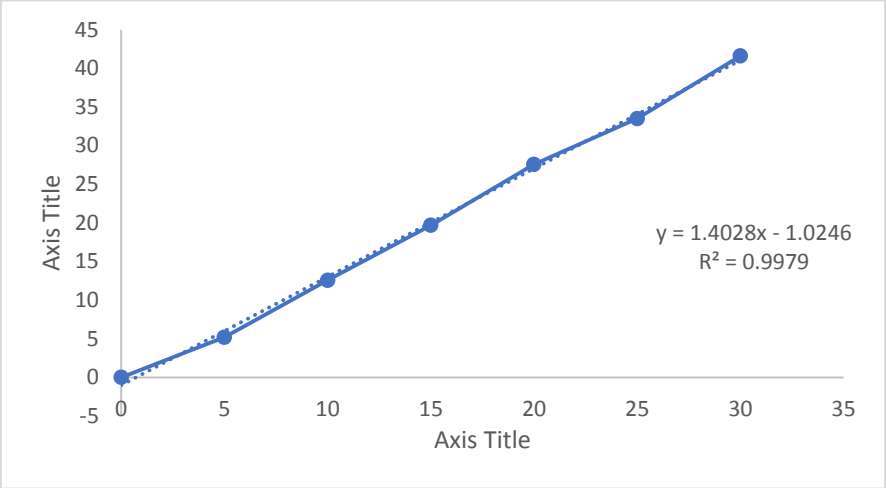

Figure 15. Higuchi plot

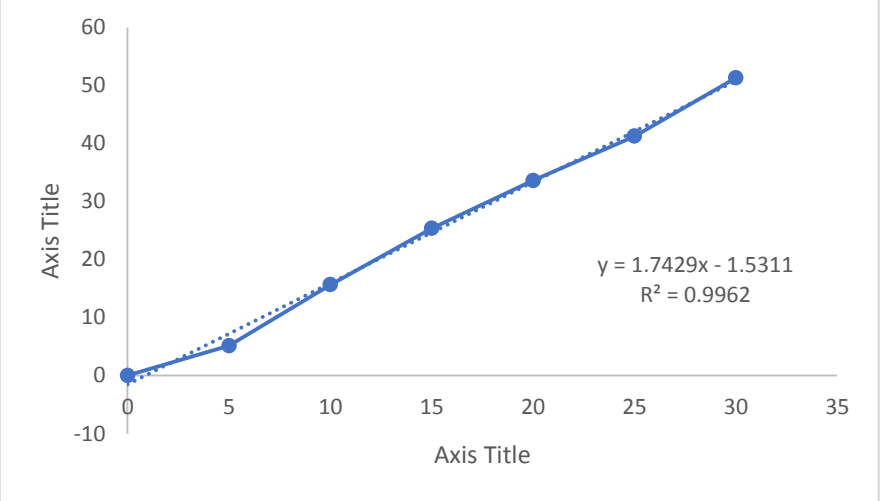

Figure 16. Peppa's plot

3.12 Stability study

Stability study was conducted for the prepared Lamivudine microspheres of formulation F1 and F5 at 40°C/75% RH respectively for a period of 60 days. Then, the sample was analyzed for physical appearance, entrapment efficiency, and drug release studies of the microsphere at the end of 15, 30, 45 and 60 days. There was no significant change in the physical appearance, drug entrapment, and *in-vitro* release study of the microspheres.

Tested after days	% Drug entrapment		% CDR	
	F1	F5	F1	F5
15	78.12	73.26	81.723	82.175
30	77.37	73.29	80.234	82.147
45	77.41	72.23	81.173	81.765
60	78.26	72.32	81.69	82.251

Table 5. Stability Studies for Formulations Stored at 40ºC/75% RH

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4. SUMMARY & CONCLUSION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body and also achieve and maintain the desired plasma concentration of the drug for a particular period of time. However, incomplete release of the dug, shorter residence times of dosage forms in the upper GIT leads to lower oral bioavailability. Such limitations of the conventional dosage forms have paved way to an era of controlled and novel drug delivery systems.

The present study reports a novel attempt to formulate microspheres of the Lamivudine by using natural gums like xanthan gum and guar gum as carrier for better treatment of HIV and chronic hepatitis B. Microspheres of Lamivudine were prepared by solvent evaporation method. Various evaluation parameters were assessed, with a view to obtain controlled release of Lamivudine.

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