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FORMULATION, DEVOLOPMENT AND EVALUATION OF MICROSPHERES OF ANTI-VIRAL DRUG USING VARIOUS HYDROPHILIC NATURAL GUMS

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ABSTRACT

The main objective of this research work was to formulate and evaluate the microspheres of various hydrophilic natural sources occurring guar gum and xanthan gum in the view of bio-degradable, easy of availability, cheap, widely use and drug release rate controlling agent with antiviral drug. Lamivudine is an antiviral drug having biological half life of 4-6 hours and bioavailability is 86% for the treatment of HIV as well as Hepatitis-B. Similarity study was carried out by using FT-IR at the range of 400 $cm^{-1}$to 4000 $cm^{-1}$and show no significant change in the characteristic peak. Microspheres of Lamuvidine were prepared and analyzed by solvent evaporation technique using liquid paraffin. The prepwas are microspheres were subjected to various evaluation parameters such as flow and micromeritics properties, particle size analysis and stability study. In- Vitro drug release using USP dissolution rate apparatus type-II (paddle method)and data was subjected to various kinetic models. Microspheres thus obtained were found to free flowing, spherical shape and pale yellow in color. The Scanning Electron Microscopy (SEM) studies inferred the spherical shape and size range of 100micrometre to 200micrometer for the total of 9 formulations(F1.F2,F3,F4,F5,F6,F7,F8 &F9) and 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 formulations was stable. The research concluded that microspheres offer to prepare controlled release of Lamuvidine with xanthan gum as rate controlling agent to reduce in dose frequency and increase bioavailability.

KEYWORDS: Lamivudine, various natural gums, solvent evaporation, oral controlled drug delivery.

1. INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is a transmissible disease of the human immune system caused by the human immunodeficiency virus (HIV). AIDS first recognized in the United State in the year 1981. In this year, that causes it have wrought physical and social devastation around the world. People affected by the illness has increased markedly and the range of communities affected has expanded. As of 2010, AVERT (also known as the AIDS Virus Education and Research Trust) estimated that there are 34 million people worldwide living with HIV/AIDS, with 2.6 million new HIV infections per year and 1.8 million annual deaths due to AIDS. HIV infects a type of white blood cell in the body's immune system called a T-helper cell (also called a CD4 Cell).These vital cells keep us healthy by fighting off infections and diseases. HIV cannot reproduce on its own. Instead, the virus attaches itself to a T-helper cell and fuses with it (joins together). It then takes control of the cells. DNA makes copies of itself inside the cell and finally releases more HIV into the blood. HIV weakness the body's natural defenses and over time several damages the immune system and quickly the virus develops depends on a person's general health, how they are diagnosed and start antiviral drug(e.g. Lamivudine) for treatment. Lamivudine is a nucleoside reverse transcriptase inhibitors and work blocking the HIV reverse transcriptase and Hepatitis-B virus polymerase. It is also known as 3TC, molecular formula is $C_8H_{11}\ N_3\ O_3$S and molecular weight is 229.26g/mol for the treatment of AIDS, the dosage of conventional oral formulations of Lamivudine is 300mg per day (i.e. 150 mg twice daily) with an absolute bioavailability of 86% ± 16%, peak serum concentration of Lamivudine (C_{max}) of 1.5 ± 0.5 mcg/mL and mean elimination half-life (t½) of 5 to 7 hours, thus necessitating frequent administration for a prolonged period of time (lifelong in AIDS and for one year in hepatitis patients) to maintain constant therapeutic drug levels.

Microspheres can be defined as solid, small spherical particles with diameter from 1 to 1000µm. In some cases, microspheres are also known as microparticle. 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. 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.

The technique for the preparation of microspheres offers a variety of opportunities to control aspects drug administration and also enhances the therapeutic efficacy of the given drug. There are various approaches in delivering a therapeutic substance to target site in a sustained release controlled fashion. One of the major approaches is using microspheres as carriers for drugs also known as micro particles. A well designed controlled drug delivery system can overcome some of the problems of conventional and enhance the therapeutic efficacy of a given drug.

Natural gums are biodegradable in nature. They are naturally available biodegradable polymer are produced by all living organisms and capable of a massive amount of chemical modification so, they are safe enough for oral consumption in the food additives or drug carrier. Some of the examples of natural gums like agar, Guar gum, Gelatin, Carboxyl methyl cellulose, Xanthan gum, Sodium alginate etc. for potential pharmaceutical and biomedical applications. The advantages of natural gums over their synthetic counterparts are their biocompatibility, non-toxic, low cost, and environmental-friendly processing. An additional advantage of biodegradability confers the property of complete drug release from the dosage form due to the degradation of gums by colonic bacteria and enzymes present in the distal portion of the gastro-intestinal tract.

2. MATERIALS AND METHODS

2.1. Materials

Lamivudine was received as a gift from M/S Stadmed Private Ltd, Kolkata, Guar gum and Xanthan gum was received from Allied Agency, Silchar, Gluteraldehyde as a crosslinking agent and petroleum ether. All chemicals and solvents used were of pharmaceutical or analytical grade.

2.2. Pre-formulation studies

2.2.1. Solubility Analysis

Pre-formulation solubility analysis was done select a suitable solvent to dissolve the drug as well as various excipients used for formulation of microspheres.

2.2.2. Melting point determination

Melting point determination of the obtained drug sample i.e. Lamivudine was done as it is a first indication purity of the sample. The small quantity of purity can be detected by a lowering as well as widening in the melting point range. The melting point of Lamivudine was measured by Thiele's tube apparatus.

2.3. Formulation studies

Table No.1: Formulation of the natural gum based Lamivudine microspheres.

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

2.4. Spectophtometric method for Lamivudine

2.4.1. Determination of λ –max

Lamivudine was dissolved in 0.1N HCL, pH 1.2and phosphate buffer, pH 6.8 further diluted with the same and scanned for maximum absorbance in UV double beam spectrophotometer (Shimadzu 1800) in the range from200 to 400nm 0.1N HCL and phosphate buffer pH6.8 as blank. The $\lambda - max$ of was found to be 272nm.

2.4.2. Standard Calibration Curve of Lamivudine

Accurately weighed 100 mg of Lamiv$udine$ was dissolved in 100ml of 0.1 HCl, pH 1.2 (conc. 1000μg/ml) to prepare stock-I solution. The adequate of stock-I solution was pipette out into 100ml volumetric flask and volume was made up to with 0.1 HCL of pH 1.2 (conc. 10μm/ml) to prepare stock –II solution. The adequate of stock-II solution was further diluted with pH 1.2 to get $5,10,15,20,25\ and\ 30\mu g$ 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. The absorbances obtained were tabulated as in Table No.-3 and Table No.-4. Calibration curve was plotted and shown in Figure No.-1 and 2 respectively. The maximum obtained in the graph was considered as λ -max for pure drug.

2.5. Preparation of microspheres by solvent evaporation technique

The Lamivudine microspheres were prepared by solvent evaporation method 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 adds the aqueous solution of gum. The above drug-gum dispersion was acidified 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 liquid paraffin containing 0.5 % w/w span 80 as an emulsifying agent. Stirred mechanically at 1800 rpm for 3hours30 minutes min using a stirrer and heated by a hot plate at 50^{o}C. 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 2hours 30 minutes 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^{o}C for 2 hours to collect solid, free flowing microspheres and stored in a well closed glass container at room temperature. The formulations are shown in Table No: 01.

2.6. Characterization and evaluation of Lamivudine microspheres

2.6.1. The microspheres prepared by the above technique were characterized for

I) Particle size

II) Drug-polymers interaction

I) Particle size

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.

Scanning Electron Microscopy (SEM) studies

Scanning Electron Microscopy (SEM) has been used to determine particle size distribution, surface morphology of the microspheres. SEM is probably the most commonly used method for characterization drug delivery systems, owing in large part to simplicity of sample preparation and ease of operation. Microspheres were scanned and examined under Electron Microscope HITACHI SU 1500, Japan connected with Fine coat, JEOL JFC-1100E Ion sputter. Dry microspheres were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microspheres were taken by random scanning of the stub.

II) Drug-polymers interaction

Fourier Transform- Infrared Spectroscopy (FT-IR) studies

There are two types of fundamental vibration in a molecule (Dyer, 1984).Stretching in which the distance between two atoms increased or decrease, but atoms remain to in same bond axis. Bending in which the position the atom changes relative to the original bond axis. The various stretching and bending vibration of bond occur at certain frequencies. When infrared light of same frequency is incident on the molecule, energy is absorbed and the amplitude of the vibration increased. Most groups such as C-H, C=O and C=N give rise to infrared absorption band which ultimately characterizes compound. For a non-linear molecule, which contain 'N' atoms have 3n-6 possible fundamental absorption bonds. Sometimes and additional (non-fundamental) absorption bond occurs because of the presence of overtones. The dried powder sample of 100mg was taken range of 4000 cm^{-1} to 400 cm^{-1}using FTIR (Perin Elmer, USA, Model: Spectrum one, Version A). The spectra obtained for Lamivudine and physical mixtures of Lamivudine with polymers were compared to check compatibility of drug with polymers.

2.6.2 The microsphere is prepared by the above technique were evaluated for

I) Percentage yield

II) Drug content

III) Entrapment efficiency

IV) In-vitro drug release

I) Percentage yield

The yield of the prepared formulation was calculated as the percentage of the weight of the dried product at room temperature compared to the theoretical amount. Product yield is calculated by using the following equation

$$\text{Product yield} = \frac{Weight\ of\ the\ product}{Weight\ of\ raw\ materials} \text{ X } 100$$

II) Drug content

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, pH1.2 repeatedly. The extract was transferred to a 100ml. volumetric flask and the volume was made up to with 100ml.with 0.1N HCl, pH1.2. The solution was filtered and

absorbance was measure using UV spectrometer at 212nm. The amount of drug content in the microspheres was calculated by the following formula:

$$\% \text{ Drug content} = \frac{\textit{Practical drug content in mg.}}{\textit{Total weight of microspheres in mg}} \text{ X } 100$$

III) Entrapment efficiency

The prepared formulations were examined for entrapment efficiency. 50 mg. of the prepared formulation was taken in equivalent quantity of 0.1NHCl, pH 1.2. The suspension is ultra-centrifuged at 17240 rmp for 40 minutes.

$$\% \text{ Entrapment efficiency} = \frac{\textit{Practical drug content in mg}}{\textit{Theoritical drug content in mg.}} \text{ X } 100$$

IV) In-vitro drug release Study

The in vitro drug release study for all the formulations was carried out by USP dissolution apparatus Type-II. The dissolution medium for the first 2 hr was 900 ml of 0.1 N HCl (pH 1.2) and continued in phosphate buffer pH 6.8 for the next 7 hrs The temperature of dissolution medium was maintained at 37 ±0.5 °C and the basket was rotated at 50 rpm. An aliquot of 5 ml was withdrawn at predetermined time intervals and replaced with an equal volume of the fresh dissolution medium to maintain sink conditions. The samples were analyzed at 272 nm, the percentage drug release using an UV and Visible spectrophotometer (Jasco double beam). The drug concentration was calculated using standard calibration curve. The results obtained are shown in Table No.-9.

Details of Dissolution test Apparatus

Apparatus: LABINDIA USP Type II

Speed: 100 rpm.

Stirrer: Paddle type

Volume of medium: 900 ml.

Volume with drawn: 5ml.

Media used: 0.1 N HCl (pH-1.2) and pH- 6.8 Phosphate buffer.

Temperature: 37±0.5°C.

λ-max: 272 nm.

2.7. EVALUATION OF LAMIVUDINE MICROSPHERES

2.7.1. Micromeritic Studies

The prepared microspheres are characterized by their micromeritic properties such as microspheres size, Bulk density, Tapped density, Carr's compressibility index, Hausner's ratio and angle of repose. The results obtained are shown in Table No-13.

I) Bulk Density

The bulk density is defined as the mass of powder or microspheres divided by bulk volume. The bulk density depends on particle size, distribution, shape and cohesiveness on particles. Accurately weighed quantity of powder or microspheres was carefully poured into graduated measuring cylinder through large funnel and volume was measured which is called initial bulk volume. Bulk density expressed in gm/cm^3 and given by -

$$\text{Bulk density} = \frac{Mass\ of\ microspheres}{Bulk\ volume\ of microspheres}$$

II) Tapped Density

The tapped density is defined as the mass of the powder or microspheres divided by tapped volume. The quantity of powder or microspheres was introduced into a clean, dry 100ml measuring cylinder. The cylinder was then tapped 100 times from constant height and tapped volume was read. It is expressed gm/cm^3 and given by-

$$\text{Tapped density} = \frac{Mass\ of\ microspheres}{Tapped\ volume\ of\ microspheres}$$

III) Carr's Compressibility Index (C.C.I.)

Use for compare the bulk density and tapped density. It is calculated using following equation.

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

Relationship between % Compressibility Index and flowability,

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

IV) Hausner's ratio

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

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

V) Angle of Repose (θ)

Angle of Repose is defined as the maximum angle possible between the surface and the horizontal plane. Angle of repose of granules was determined by the funnel method. The granules were allowed to flow through the funnel freely into the surface. The diameter of the powder or microspheres cone was measured and angle of repose was calculated using the following equation-Angle of Repose

$$(\theta) = Tan^{-1}\frac{h}{r}$$

Where, θ= Angle of Repose,

h = Height of the heap,

r = Radius of the heap.

Relationship between Angle of Repose and flowability,

Angle of Repose (θ)	Flowability
< 2	Excellent
25-30	Good
30-40	Passable
40>	Very poor

2.8. Release Kinetics

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 model was selected.

2.8.1. Zero Order Kinetics

Drug dissolution from Pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represent by following equation,

$$Qt = Qo+Kot$$

Where,

Qt = Amount of drug dissolved in time "t"

Qo=Initial amount of drug in the solution and

Ko= Zero Order release constant.

2.8.2. First Order Kinetic

To study the first order release kinetics the release rate data were fitted to the following equation,

$$\text{Log } Qt = \text{Log } Qo+\frac{K1t}{2.303}$$

Where,

Qt= Amount of drug dissolved in time "t"

Qo=Initial amount of drug in the solution and

K1= First Order release constant.

2.8.3. Higuchi Model

Higuchi development several theoretical models to study the release of water soluble and low soluble drugs incorporated in semi-solid or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The Higuchi equation is

$$Qt = KH \text{ X } t_{1/2}$$

Where,

Qt = Amount of drug dissolved in time "t" and

KH=Higuchi dissolution constant.

2.8.4. Korsmeyer-Peppas Model

To study this model, the release rate data is fitted to the following equation,

$$\frac{Mt}{M}=k.tn$$

Where,

$\frac{Mt}{M}$= Fraction of drug release

K= Release constant

t=Drug release time and

n=Diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form.

2.8.5. Hixson-Crowell Model

To study the Hixson-Crowell model, the release rate data are fitted to the following equation.

Wo 1/3-Wt1/3/Kst

Where,

Wo=Amount of drug in the Pharmaceutical dosage form

Wt=Remaining amount of drug in the Pharmaceutical dosage form

Ks=Constant incorporating the surface-volume relation.

2.9. Stability Studies

Stability of a drug has been defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light, and enables recommended storage conditions. Accelerated testing is 40^{o}C/75% RH for 6months (ICH guideline). The accelerated stability study of the formulation was carried out as per the ICH guideline.

In the present study, stability study was carried out for a period up to the two months for selected formulation. The selected formulations were analyzed for the physical appearance, drug entrapment efficiency and in-vitro release study.

3. RESULTS AND DISCUSSION

3.1. Pre-formulation studies

3.1.1. Solubility analysis

Lamivudine is freely soluble in water, sparingly soluble in methanol but practically insoluble in acetone. It is also soluble in 0.1N HCl (pH1.2) and Phosphate buffer (pH6.8).

3.1.2. Melting point determination

The melting point of the dry sample i.e. Lamivudine was found to be 161^{o}C It complies with the purity of the dry sample i.e. Lamivudine.

3.2. Standard graph of Lamivudine

3.2.1. Preparation of standard solution

Table No. 2: Preparation of standard solution.

Weight of Lamivudine taken	100mg
Volume made up to	100ml
Concentration of standard solution	100$\mu g/ml$
Aliquote of volume make up to	25ml

3.2.2. Data for standard graph of Lamivudine in 0.1HCl(pH1.2) at 272nm.

The absorbance was measured in a UV-spectrophotometer at 272nm against 0.1N HCl (pH 1.2) and phosphate buffer (ph6.8). The absorbencies of standard solution of Lamivudine ranging from 5 to 30$\lambda g/ml$ so obtained were tabulated as in Table No. - 3 calibration curves were plotted and shown Figure No.-1 respectively. The curves were found to be line in the ranging of 5 to 30 $\mu g/ml$ at 272nm. The regression values (R^2) were found to be 0.998 and 0.998 in pH 1.2 and pH 6.8 respectively.

Table No. 3: Spectrophotometric Data for the Estimation of Lamivudine in 0.1N HCl(pH1.2).

Concentration ($\mu g/ml$)	Absorbance (nm)
0	0
5	0.066
10	0.134
15	0.205
20	0.267
25	0.342
30	0.407

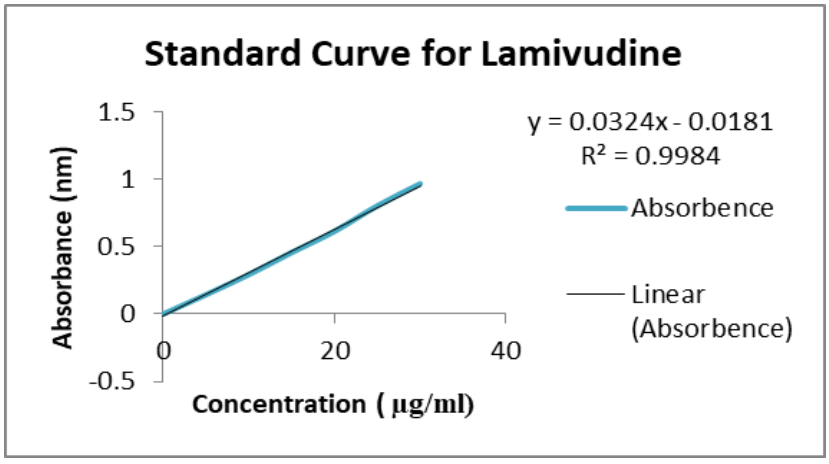
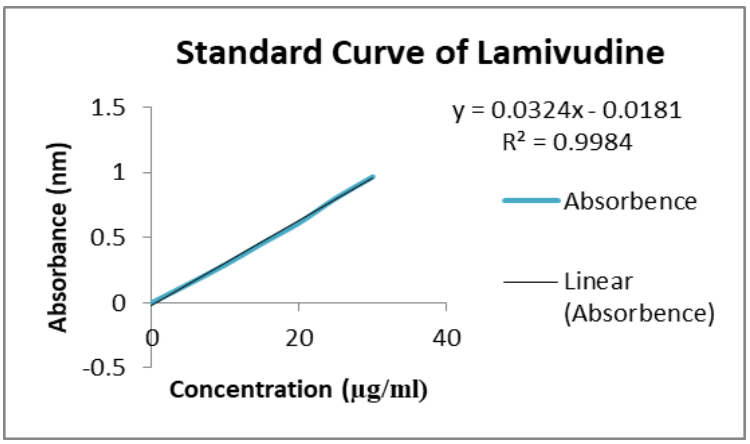


Figure No. 1: Standard graph of Lamivudine using 0.N HCl (pH 1.2).

3.2.2. Data for standard graph of Lamivudine in 0.1Phosphate buffer(Ph6.8) at 272nm.

Table No.-4, Spectrophotometric Data for the Estimation of Lamivudine in 0.1N Phosphate buffer(pH6.8).

Concentration($\mu g/ml$)	Absorbance(nm)
0	0
5	0.144
10	0.293
15	0.457
20	0.614
25	0.803
30	0.967



Fighure No.-2: Standard graph of Lamivudine using 0.N Phosphate buffer (pH 6.8).

3.3. Particle Size Analysis

Table No.-5: Average particle size of Lamivudine Microsperes.

Formulation Code	Average particle size (µm)
F1	916
F2	939
F3	457
F4	274
F5	992
F6	741
F7	652
F8	588
F9	573

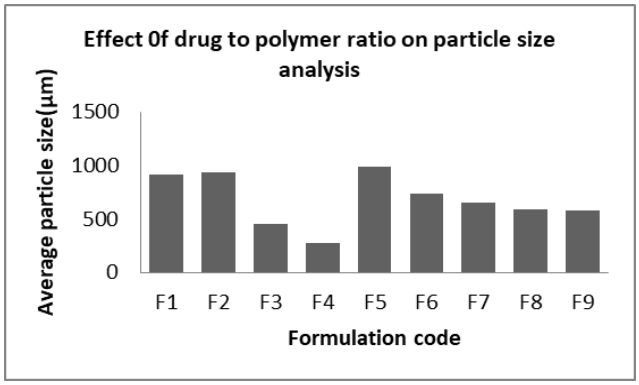


Figure No.3: Comparison of average size of the prepared Lamivudine microspheres.

Average particle size of Lamivudine microspheres can be determined by optical microscopy are shown in Table No.-5 and Figure No.-3. The mean particle size for the formulation F1 to F4 and F4 to F9 containing Xanthan gum and Guar gum was found to be in range from 274µm to 916µm and 573µm to 992µm respectively with increase in polymer concentration in the microspheres from F1 to F9. The particle size of microspheres increase respectively because the viscosity of polymer solution increasing polymer concentration, which in turn decrease the stirring efficiency.

3.3.1 Scanning Electron Microscope (SEM) Studies

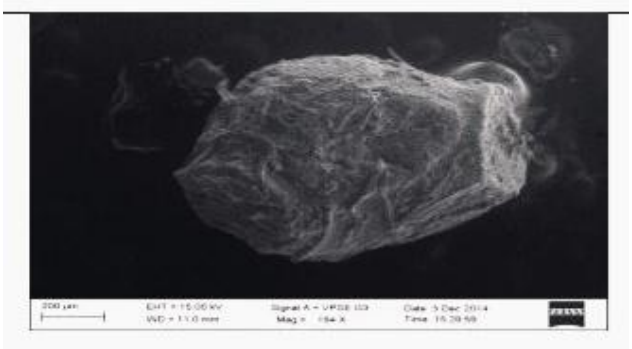


Figure No.-4: F1(A).

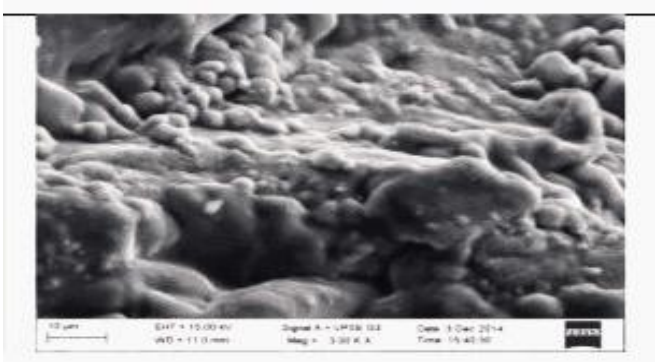


Figure No.-4: F1(B).

Figure No. 5: F5(A).

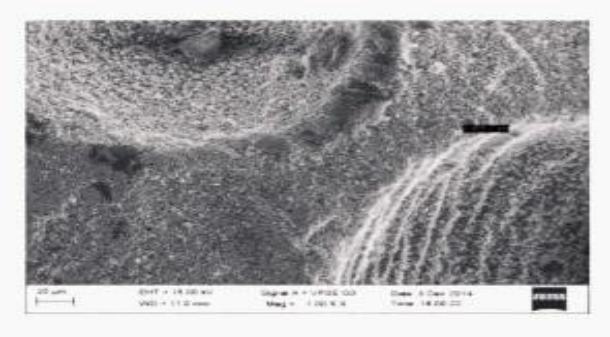


Figure No.-5: F5(B).

The Shape and surface morphology can be determined by scanning electron microscope. Scanning electron microscope analysis of the samples revealed that all microspheres prepared were spherical shape. The microspheres of Lamivudine with guar gum were smooth, spherical and slightly aggregated particles. When compared with the microspheres of Lamivudine with xanthan gum which were porous, rough, grossly and discrete spherical. Scanning electron photomicrographs of the formulations F1(A,B) and F5(A,B) are shown in Figure No.-4,5.

3.3.2. Fourier Transform- Infrared Spectroscopy (FTIR) Studies

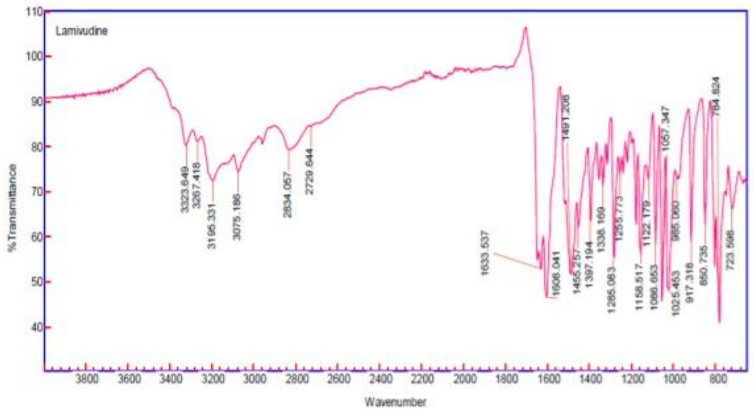


Figure No.6(A): FTIR Spectra of Lamivudine.

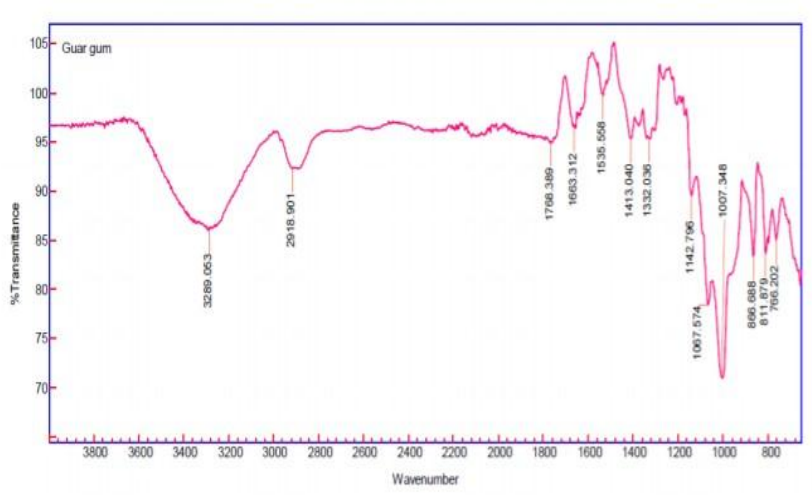


Figure No.-6(B): FTIR Spectra of Lamivudine with Guar gum.

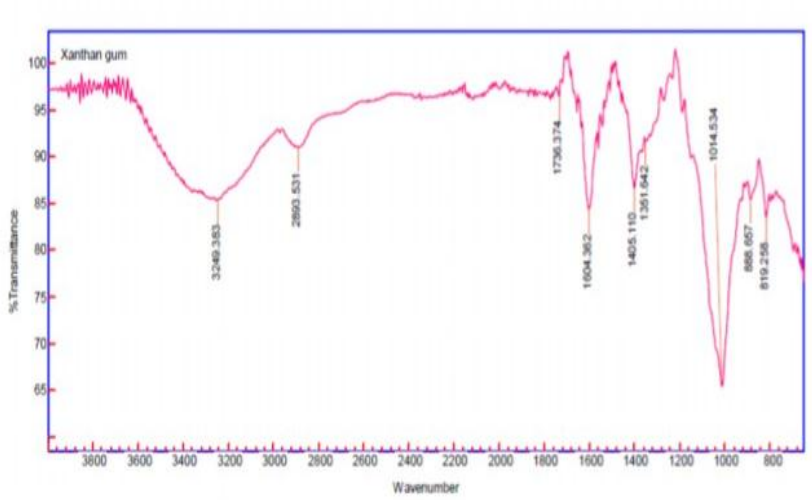


Figure No.-6(C): FTIR Spectra of Lamivudine with Xanthan gum.

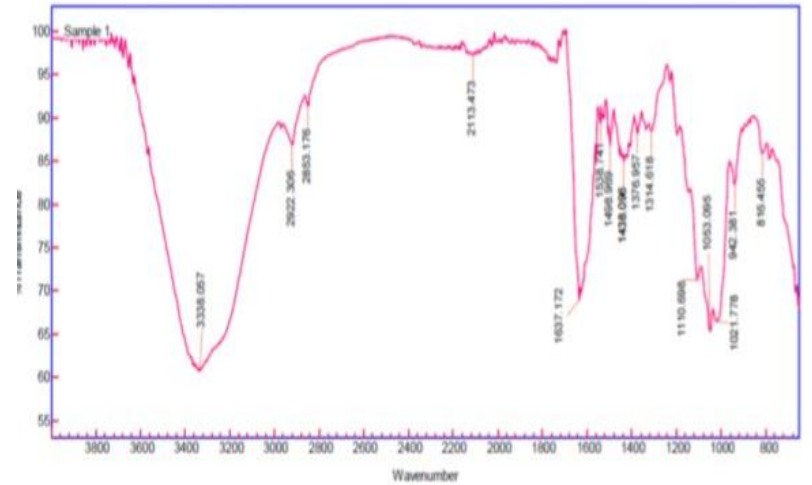


Figure No.-6(D): FTIR Spectra of Lamivudine with guar gum and xanthan gum.

3.3.3. Percentage yield

Table No.6: Percentage yield of Lamivudine microspheres.

Formulation Code	Theoretical yield in mg	Actual yield in mg	Percentage yield
F1	1000	754	74.40
F2	1500	1091	72.73
F3	2000	1286	64.30
F4	1000	769	76.90
F5	1500	1096	73.06
F6	2000	1282	64.00
F7	1000	741	74.10
F8	1500	1082	72.13
F9	2000	1308	65.40

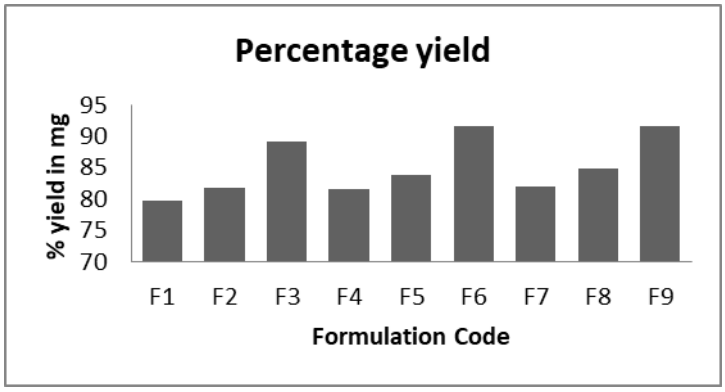


Figure No.7: Comparison of percentage yield of the prepared of Lamivudine microspheres.

The percentage yield for Lamivudine loaded microspheres were 75.40%, 72.73%, 64.30; 76.90%,73.06%, 64.00%; 74.10%,72.13%, 65.40% for formulation F1, F2, F3; F4, F5, F6; F7, F8, F9 respectively are given above Table No.-6 and Figure No.-7.

3.3.4. Drug Content

Table No.7, Drug loading of Lamivudine microspheres.

Formulation Code	Practical drug content in mg	Theoretical drug content in mg	Total weight of microspheres in mg	Percentage of drug content
F1	10.12	24	50	38.24
F2	13.55	16.57	50	27.10
F3	11.20	12.55	50	22.40
F4	19.57	24	50	39.14
F5	13.97	16.67	50	27.94
F6	12.46	12.52	50	24.92
F7	19.69	24	50	39.38
F8	14.11	16.62	50	28.22
F9	11.48	12.54	50	22.96

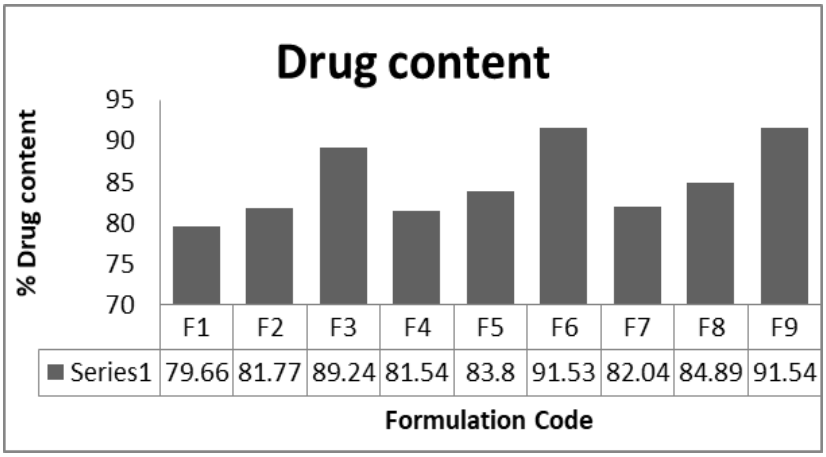


Figure No.8: Comparison of percentage of drug loading of Lamivudine microspheres.

3.3.5. Drug entrapment efficiency

Table No.8: Comparsion of percentage of drug entrapment efficiency of Lamivudine microspheres.

Formulation Code	Practical drug content in mg	Theoretical drug content in mg	Total weight of microspheres in mg	Percentage of drug entrapment efficiency
F1	10.12	24	50	79.66
F2	13.55	16.57	50	81.77
F3	11.20	12.55	50	89.24
F4	19.57	24	50	81.54
F5	13.97	16.67	50	83.80
F6	12.46	12.52	50	91.53
F7	19.69	24	50	82.04
F8	14.11	16.62	50	84.89
F9	11.48	12.54	50	91.54

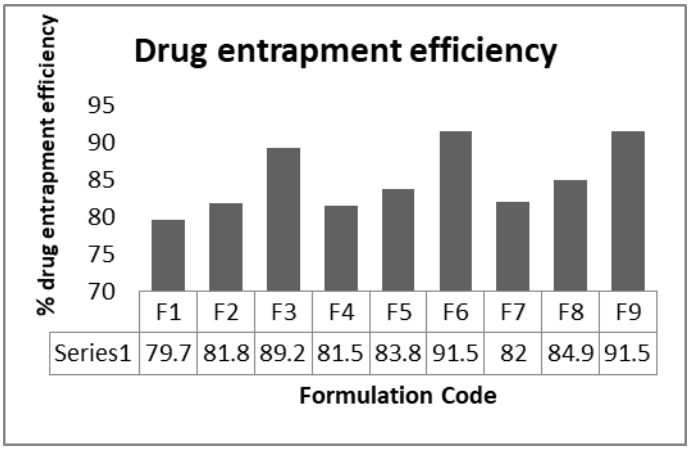


Figure No.9, Comparison of percentage of drug entrapment efficiency of Lamivudine microspheres.

We see that the polymer concentration was increased the percentage of drug loading decreased and percentage of drug entrapment efficiency was increased due to increase in the viscosity of the solution. The value of percentage of drug loading and percentage of drug entrapment efficiency were given in Table No.-8 and their histograms shown in Figure No.-9 This can be attributed to the permeation characteristic of each polymer used that could facilitate the diffusion of part of entrapped drug to the surrounding medium during preparation of microspheres.

3.3.6. In-vitro drug release studies

Table No.9: In-vitro drug release of Lamivudine microspheres against time.

Time in hours	<--CUMULATIVE % DRUG RELEASE FORMULATION→								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	16.245	17.521	14.212	13.982	17.115	17.212	16.593	14.129	13.091
2	27.980	25.621	24.425	22.642	35.782	26.512	20.515	18.294	17.185
3	35.102	32.445	31.694	29.129	41.997	39.137	25.819	23.442	22.392
4	47.339	38.827	37.075	31.792	47.927	37.992	31.752	28.792	26.729
5	51.943	43.152	41.359	34.479	53.057	44.482	32.329	31.151	30.279
6	58.992	55.104	49.072	49.059	64.914	51.069	39.142	38.726	36.124
7	64.314	63.215	53.725	45.792	72.321	56.692	54.369	48.297	45.129
8	69.420	68.224	65.715	64.370	76.325	63.282	62.102	59.661	49.369
9	74.926	73.105	69.115	67.664	79.892	71.669	67.927	66.215	65.627
10	82.105	79.138	76.412	71.625	82.147	80.212	74.521	69.112	64.623

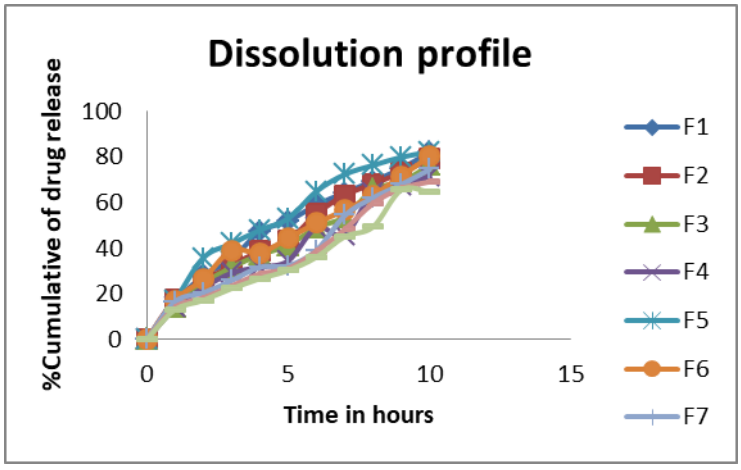


Figure No.10, Comparative In-vitro drug release of Lamivudine microspheres against time.

Table No.10: In-vitro release kinetic parameters for Lamivudine microspheres.

Formulation code	$< -R^2$value of different rate of kinetics-→						Best fit model
	First order	Zero order	Higuchi	Hixson Crowell	Korsmeyer-Peppas		
	R^2	R^2	R^2	R^2	R^2	n	
F1	0.8882	0.9895	0.9635	0.9662	0.9417	0.4532	Zero order
F2	0.8979	0.9842	0.9682	0.9632	0.9342	0.4480	Zero order
F3	0.8674	0.9562	0.9497	0.9309	0.9201	0.4408	Zero order
F4	0.9085	0.9915	0.9741	0.9729	0.9472	0.4369	Zero order
F5	0.9106	0.9872	0.9746	0.9687	0.9427	0.4576	Zero order
F6	0.8875	0.9638	0.9616	0.9424	0.9339	0.4234	Zero order
F7	0.9306	0.9962	0.9907	0.9899	0.9671	0.4458	Zero order
F8	0.9482	0.9944	0.9882	0.9867	0.9712	0.4190	Zero order
F9	0.9469	0.9898	0.9854	0.9810	0.9672	0.4356	Zero order

The In-vitro release study for all nine formulations was carried out by USP dissolution test apparatus type-II. The temperature of dissolution medium 0.1N HCl,pH1.2 and pH6.8 was maintained at 37°C with stirring rate of 50 rmp. In-vitro drug release data of different formulations are shown in Table No.-9 and Figure No.-10. The percentage of cumulative drug release after twelve hours was found to be in the range of 82.105%, 79.138%,76.412% and 71.625% for the formulations F1 to F4 respectively whereas percentage of cumulative drug release after twelve hours was 82.147%, 80.212%, 74.521%, 69.112% and 64.623% for the formulation F5 to F9 respectively. The cumulative drug release significantly decrease with increase in polymer concentration. The increase density of the polymer matrix at higher concentrations results in an increased diffusional path of length. This may decrease the overall drug release from the polymer matrix. Further small microspheres are formed at a lower polymer concentration and have a larger surface are exposed to dissolution medium giving rise to faster drug release.

The regression coefficient obtained for formulation F1 to F9 Zero order kinetics were found to be higher(R^2 :0.9562 to 0.9962) when compared with other kinetic model (First order, Higuchi, Hixson Crowell and Korsmeyer-Peppas). The results were shown in Table No.-10.

3.3.7. Stability studies

Table No.11, Stability Studies for formulations stored at 40℃ /75%RH.

Tested after days	% Drug entrapment		% Cumulative drug release	
	F1	F5	F1	F5
15	78.14	73.24	81.73	82.18
30	77.28	73.27	80.24	82.14
45	77.32	72.22	81.17	81.77
60	78.21	72.34	81.71	82.25

Stability study was conducted for the prepared Lamivudine microspheres of formulations F1 and F5 at 40℃ /75% RH respectively for a period of 60 days. Then the sample was analyzed for physical appearance, drug entrapment efficiency and drug release studies of the microspheres at the end of 15, 30, 45 and 60 days. The results of stability studies are given above Table No.-11. There was no significant change in the physical appearance, drug entrapment and in-vitro release study of the microspheres.

4. EXPERIMENTAL EVALUATION

4.1. Evaluation of Micromeritic properties of Lamivudine microspheres

Table No. 12: Micormeritic properties of Lamivudine microspheres

Formulation Code	Angle of Repose (θ)	Bulk Density (g/Cm^3)	Tapped Density (g/Cm^3)	Compressibility Index (%)	Hausner's Ratio
F1	27.01	0.4427	0.5134	13.62	1.156
F2	25.69	0.4967	0.5816	13.34	1.165
F3	32.95	0.5240	0.6240	16.15	1.194
F4	33.89	0.4811	0.5449	12.12	1.135
F5	27.97	0.5423	0.6180	12.54	1.139
F6	27.12	0.6149	0.7134	13.82	1.153
F7	33.72	0.4567	0.5226	12.48	1.137
F8	34.56	0.4745	0.5851	15.22	1.130
F9	37.34	0.5483	0.6430	15.45	1.182

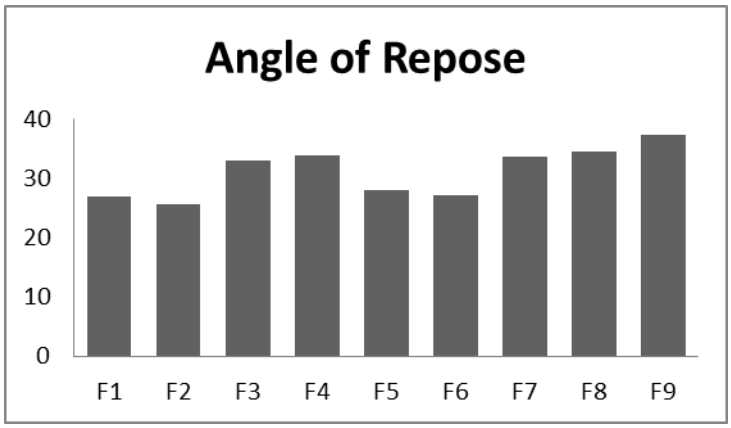


Figure No.11: Micromeritic properties (Angle of Repose) of Lamivudine microspheres.

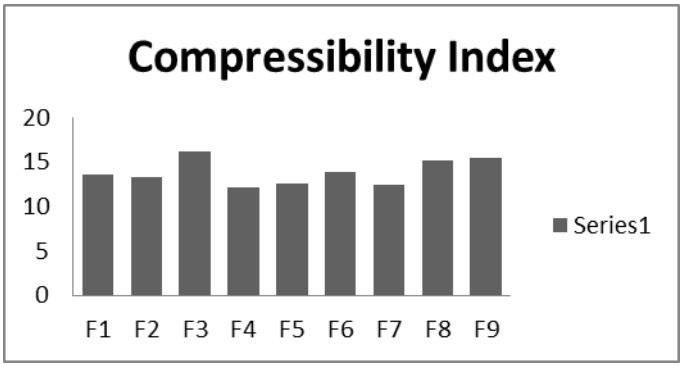


Figure No.12: Micromeritic properties (Carr's compressibility index) of Lamivudine microspheres.

The results of all formulation F1 to F9 are shown in Table No.-12 which evaluated for variable parameter as Angle of Repose, Bulk Density, Tapped Density, Carr's compressibility Index, Hausner's Ratio.

The Angle of Repose for the formulation F1, F2, F3 and F6 to F9 was found to be in the range 25 to 30 shows good flow property.

The % of Carr's compressibility index for the formulation F1 to F9 found between 11% to 18% indicating the good flow property.

5. CONCLUTION

Lamivudine is an anti-viral agent belonging to nucleoside reverse transcriptase inhibitor (NRTI). The formulate microspheres of the Lamivudine by using natural gums like xanthan gum and guar gum as carried for better treatment of HIV and chronic Hepatitis –B. It has a half life of 5 to 7 hours.

The aim of formulating microspheres containing Lamivudine is to offers a suitable practical approach to prolong therapeutic effect by continuously releasing the medication over extended period of time.

In this research work microspheres of Lamivudine were prepared by solvent evaporation method using natural gums like xanthan gum and guar gum. Pre-formulation studies likes solubility analysis, melting point determination and FTIR were studied. The prepared microspheres were subjected angle of repose, bulk density, tapped density, Hausner's ratio, Carr's compressibility index, drug loading, drug entrapment efficiency, in-vitro release studies and stability studies.

The formed micro particles were good flowing properties and exhibited good micrometric properties. The mean particle size of the prepared microspheres was within the range of 274µm to 992µm.

SEM analysis of the microspheres revealed that guar gum containing microspheres were smooth, spherical and slightly aggregrated particles when compare with microspheres of xanthan gum which are porous, rough, grossly, discrete spherical. FTIR studies should no interaction between drug and polymer. The percentage of cumulative drug release significantly decreases with increase in polymer concentration. The data obtained are fitting kinetic models indicated that the drug release followed zero order kinetics. The "n" value obtained from the Korsmeyer- Peppas model showed that the microspheres followed non-Fickian drug release mechanism.

The formulations F1 and F5 were selected for stability studies on the basis of their better and satisfactory evaluation. There was no variation in physical parameter even after the period of 60 days. So that the formulations F1 and F5 are found to be stable and retain their original properties.

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