Ankamma Chowdary Y & Venkata Suresh Jilakara. / ***International Journal of Pharmacy & Therapeutics*****, 4(1), 2013, 25-33.**
e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

COMPARATIVE *IN VITRO* AND STABILITY STUDIES OF LAMIVUDINE FLOATING SUSTAINED RELEASE TABLETS

Y. Ankamma Chowdary*[1] and Venkata Suresh Jilakara[2]

*[1]Department of Pharmaceutics, NRI College of Pharmacy,
Pothavarappadu, Agiripalli, Krishna district, Andhra Pradesh, India.
[2]Department of Pharmacognosy and Phyto Pharmaceutical, KLR Pharmacy College,
Contractor's colony, Paloncha, Khammam, Andhra Pradesh, India.

ABSTRACT

The investigation was concerned with design, preparation and evaluation of oral floating sustained release tablets of Lamivudine using different natural polymers such as Chitosan, Guar gum and Xanthan gum. The formulated different ratio of oral floating sustained release tablets of Lamivudine were evaluated by different parameters for improve the drug effectiveness. The FT-IR studies were confirmed no chemical interaction between the Drug and Natural polymers. The Lamivudine oral sustained release matrix tablets were prepared by using wet granulation method. The prepared granules were evaluated for angle of repose, bulk density, compressibility index and hausner's ratio. The tablets were subjected to thickness, weight variation test, hardness, friability, drug content, *in vitro* release, kinetic release and stability studies. The results conclude that FM-7 (95.10%) can be considered as a optimized formula for sustained release of drug, when it is compared with Marketed product (Lamivudine 300mg tablets (Epivir) (91.67%)) for 24 hours. Kinetic treatment to the *in vitro* release data revealed that the drug release followed first order, non - fickian diffusion, It means the release of drug from tablet diffusion mechanisms are used.

Key Words:- Floating tablets, Lamivudine, Natural polymers, *In vitro* kinetics and Stability studies.

INTRODUCTION

The terms of "sustained or extended release", "prolonged release, and controlled release", as applied to drug formulations, have the meanings ascribed to them in Remington's pharmaceutical Sciences, Sustained or extended release (Remington's., 1996) drugs systems include any drug delivery system which achieves the slow release of drug over an extended period of time, and include both prolonged and controlled release system (Theodore JR *et al.,* 1983; Donald LW *et al.,* 1984). If such a sustained release system is effective in maintaining substantially constant drug level in the blood or target. It is considered a prolonged released system (Fig 1).

Lamivudine is a potent hydrophilic anti viral agent indicated for treatment of AIDS (Acquired Immunodeficiency Syndrome). It belongs to class III of the BCS Classification with High solubility and low permeability (Wells JI *et al.,* 1996). 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. It is phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. The lack of a 3'-OH group in the incorporated

Corresponding Author

Y. Ankamma chowdary
Email:- yarlagaddaac@yahoo.co.in

nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated.

Lamivudine is an active antiretroviral drug belongs to nucleosides reverse transcriptase cross inhibitor. Lamivudine treatment has gained immense popularity in the AIDS treatment in the present era conventional oral formulations of Lamivudine are administered multiple times a day because of its moderate half-life ($t_{1/2}$ = 5-7 hours) (www.wikipedia.com). Treatment of AIDS using conventional formulations of Lamivudine is found to have many draw backs, such as adverse side effects resulting from accumulation of drug, in multi dose therapy (Moyle G *et al.,* 2009), poor patient compliance and high cost.

The aim of designing sustained release delivery system is to increase the effectiveness of drug, reduce the frequency of dosing and side effect and maintain uniform drug level by localization at the site of action. Sustained release constitutes any dosage form that provides medication over an extended of time.

MATERIALS AND METHODS

Materials

Lamivudine was obtained as a gift sample from Strides Arcolab, Bangalore. All other chemicals and ingredients were used for study are of Analytical grade. Chitosan, Guar gum, Xanthum gum, Magnesium stearate, Talc were obtained from Yarrow Chem. Products, Mumbai. Sodium bicarbonate was a Gift sample from Molychem, Mumbai. Citric acid and Polyvinyl pyrrolidone K-30 was a Gift sample from Loba chemistry, Mumbai. All other chemicals and ingredients were used for study are of Analytical grade.

Method

Preparation of Lamivudine floating tablets

Tablets were prepared by wet granulation method. Lamivudine Hydrochloride (300 mg) was mixed with required amount of polymers and other excipients (Table 1). All the excipients were passed through sieve no.40, mixed and granulated with 10% solution of PVP K-30 in isopropyl alcohol. The wet mass was passed through sieve no.16 and dried at 45^{o}C for 2 hrs. Dried granules were passed through sieve no.20 and mixed with magnesium stearate and talc (Raju DB *et al.,* 2010).

EVALUATION PARAMETERS

Precompression studies (Raju D.B *et al.,* 2010; Arunachalam A ***et al.,* 2012; Mohamed Raffick M *et al.,* 2012;** Lachman L *et al.,* 2009)

Bulk density

5gm of Lamivudine granules were weighed separately and transferred into 100ml measuring cylinder, initial volume was measured and calculated according to the formula

Bulk density = Mass / Volume

Tapped density

Tapped density is determined by placing a graduated cylinder containing a known mass of granules and mechanical tapper apparatus, which is operated for a fixed number of taps until the powder bed volume has reached a minimum volume. Using the weight of the granules in the cylinder and this minimum volume, the tapped density may be computed.

Tapped density = Weight of granules/ Tapped volume of granules

Angle of Repose

The manner in which stresses are transmitted through a bead and the beads response to applied stress are reflected in the various angles of friction and response. The most commonly used of this in angle of repose, which may be determined experimentally by number of methods. The method used to find the angle of repose is to pour the powder a conical on a level, flat surface and measure the included angle with the horizontal.

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

Where,

θ = Angle of repose,
h = Height of the powder cone,
r = Radius of the powder cone.

Compressibility Index or Carr's Index

Carr's Index is measured using the values of bulk density and tapped density.

The following equation is used to find the Carr's Index,

$$CI = \frac{(TD-BD)}{TD} \times 100$$

Where, TD = Tapped density
BD = Bulk density

Hausner's Ratio

It indicates the flow properties of the powder and ratio of Tapped density to the Bulk density of the powder or granules.

Postcompression studies of floating tablet

Hardness or Crushing strength Test

Hardness of the tablet was determined using the Monsanto hardness tester (The lower plunger was placed

in contact with the tablet and a zero reading was taken. The plunger was then forced against a spring by tuning a threaded bolt until the tablet fractured. As the spring was compressed a pointer rides along a gauge in the barrel to indicate the force.

Thickness Test

The thickness of the tablet is mostly related to the tablet hardness can be uses as initial control parameter. Ten tablets were randomly selected from each tablet thickness was determined using a Vernier calliper and the reading was recorded in millimeters.

Friability Test

The pre-weighed tablets were placed in the friabilator (EF-2, Electro lab, Mumbai) which was then operated for 100rpm, then dusted and reweighed. The Conventional compressed tablets that lose less than 0.5-1.0% of their weight are generally considered acceptable.

$$\text{Friability index} = \frac{I - F}{I} \times 100$$

Where,

I - Initial weight

F - Final weight

Weight variation test

Weights of 20 individual tablets were noted and their mean weight also calculated. The percentage deviation was calculated by using the following formula,

Percentage deviation = [X-X^*/ X] × 100

X - Average weight of the tablet

X^*- Actual weight of the tablet

Estimation of Drug Content

Ten tablets of each formulation were weighed and powdered. The quantity of powder equivalent to 100 mg of drug was transferred into 250 ml volumetric flask, it was shaken with 150 of distilled water and volume was adjusted to 250 ml with water. The solution was filtered, suitable dilutions were made and absorbance was recorded by using U.V. spectrophotometer (Labindia, Hyderabad) at 270nm. The experiment was repeated three times.

Calculation

The amount of Metformin present in tablet can be calculated using the formula

A_t/As x S_w/100 x 100

Where, A_t = Absorbance of sample preparation

A_s = Absorbance of Standard preparation

S_w = weight at Metformin working standard (mg)

Floating or Buoyancy Test

The time taken for tablet to emerge on the surface of the medium is called the floating lag time (FLT) or buoyancy lag time (BLT) and duration of time the dosage form constantly remains on the surface of the medium is called the total floating time (TFT). The buoyancy of the tablets was studied in USP type II dissolution apparatus at 37±0.5^{0}C in 900ml of simulated gastric fluid at pH 1.2. The time of duration of floatation was observed visually.

***In vitro* Drug Release Study**

In vitro release studies were carried out by using USP paddle dissolution test apparatus. 900ml of 0.1NHCl (pH1.2) was taken in the dissolution vessel and the temperature of the medium were maintained at 37±0.5^{0}C. 100rpm was maintained, 1 ml of sample was withdrawn at predetermined time intervals for 24 hours and the same volume of the fresh medium was replaced. The samples were analysed at 270nm by using a UV spectrophotometer (Labindia, Hyderabad). The dissolution data obtained were plotted as percentage drug release versus time.

Bioequivalence studies

The bioequivalence study was carried out for 24 hours were using USP paddle type dissolution apparatus in 0.1NHCl (pH 1.2) at 100 rpm maintaining temperature at 37±0.5^{0}C. A 1ml samples were collected from each vessel at 0, 1, 2, 4, 8, 12, 16, 20 hours and analyzed by UV spectrophotometer at 270 nm. The withdrawn sample was immediately replaced by equal volume of fresh buffer. The dissolution data obtained were plotted as percentage drug release versus time.

KINETIC CHARACTERISTICS OF THE DRUG RELEASE

To know the mechanism of the drug release from the batches, the results obtained from the *In vitro* dissolution process (Korseyer R W *et al.,* 1983; Higuchi T *et al.,* 1963) were fitted into different kinetic equations as follows:

Zero order kinetics

Zero order release would be predicted by the following equation:-

$A_t = A_0 - K_0 t$

Where, A_t = Drug release at time't'.

A_0 = Initial drug concentration.

K_0 = Zero - order rate constant (hr^{-1}).

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys Zero – order equal to K_0.

First Order Kinetics

First – order release would be predicted by the following equation:-

$\text{Log } C = \log C_0 - Kt / 2.303$

Where,

C = Amount of drug remained at time't'.

C_0 = Initial amount of drug.

K = First – order rate constant (hr^{-1}).

When the data is plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follow first order kinetics. The constant 'K' can be obtained by multiplying 2.303 with the slope values.

Higuchi's model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation: -

$Q = [D\varepsilon / \tau (2A - \varepsilon Cs) Cst]^{½}$

Where,

Q = Amount of drug released at time't'.

D = Diffusion coefficient of the drug in the matrix.

A = Total amount of drug in unit volume of matrix.

Cs = the solubility of the drug in the matrix.

ε = Porosity of the matrix.

τ = Tortuosity.

t = Time (hrs) at which 'q' amount of drug is released.

Above equation may be simplified if one assumes that 'D', 'Cs' and 'A' are constant. Then equation becomes:-

$Q = Kt^{1/2}$

When the data is plotted according to equation i.e. cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K'.

Korsemeyer equation / Peppa's model

To study the mechanism of drug release from the transdermal patches, the release data were also fitted to the well-known exponential equation (Korsemeyer equation/ Peppa's law equation), which is often used to describe the drug release behavior from polymeric systems.

$M_t / M_\alpha = Kt^n$

Where,

M_t / M_α = the fraction of drug released at time't'.

K = Constant incorporating the structural and geometrical characteristics of the drug / polymer system.

n = Diffusion exponent related to the mechanism of the release.

Above equation can be simplified by applying log on both sides,

$\text{Log } M_t / M_\alpha = \text{Log } K + n \text{ Log } t$

When the data is plotted as log of drug released versus log time, yields a straight line with a slope equal to 'n' and the 'K' can be obtained from y – intercept. For Fickian release 'n' <0.5 while for anomalous (non - Fickian) transport 'n' ranges between 0.5 and 1.0.

'n' values can be used to characterize diffusion release mechanism as:

n < 0.5	Fickian diffusion
n >0.5 < 1	Non-fickian diffusion
n > 1	Class – II transport

STABILITY STUDIES

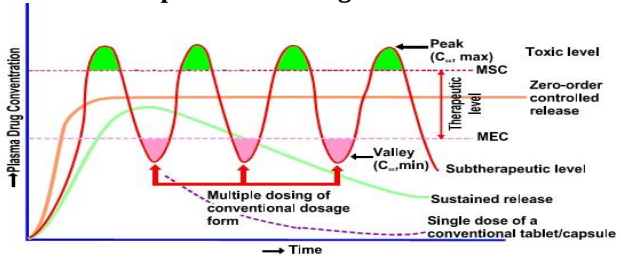
Stability conditions

Once the final formula is arrived at, and then the stability batches are prepared where three batches of the same formula are prepared and packed in Alu strip, Alu Blister, amber PVDC 60, and HDPE. These packed samples are stored in three conditions.

- 25^0C and 60%RH
- 30^0C and 65% RH
- 40^0C and 75% RH

For periods of 1, 2, 3, 6, 9, 12, 18, 24 and 36 months as may be the case applicable. These are in line with the ICH guidelines the optimized formulations of FM-7 were chosen for stability studies, Tablets were packed in Alu-Alu Blister and kept for 90 days at 40^0C / 75% RH and 30^0C / 65% RH in a stability chamber (New tronic walk in humidity chamber, India). The tablets were evaluated interval of 1, 2, 3 months analysis as per in-house specification.

Fig 1. Hypothetical plasma concentration-time profile from single doses of sustained or extended delivery formulations



RESULTS

Preformulation studies

Compatability studies (Fourier Transform Infrared Spectroscopic studies)

Fig 2. FTIR spectrum of Pure Drug (Lamivudine)

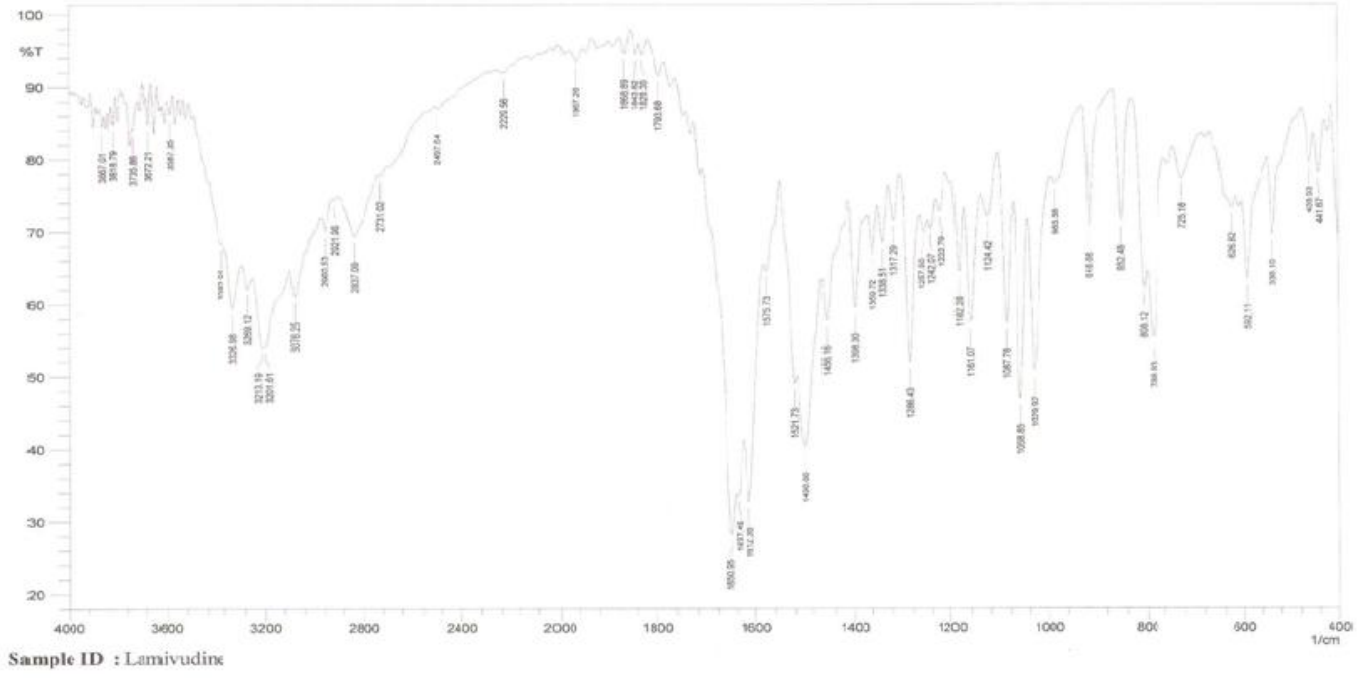
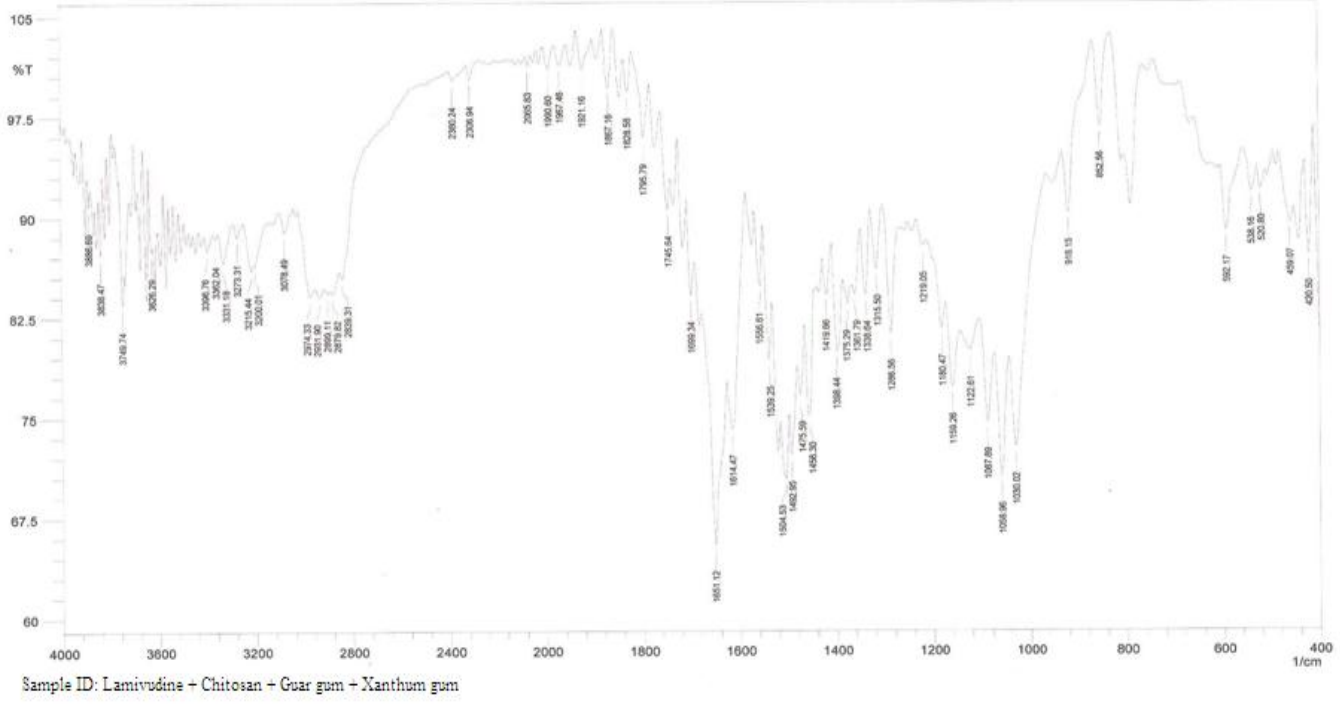


Fig 3. FTIR spectrum of Lamivudine + Chitosan + Guar gum + Xanthum gum

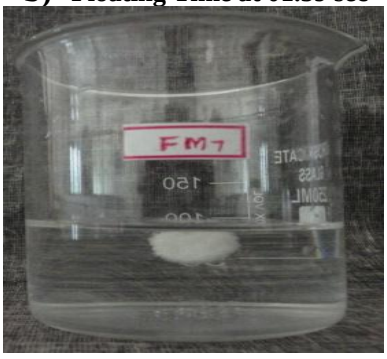


Ankamma Chowdary Y & Venkata Suresh Jilakara. / *International Journal of Pharmacy & Therapeutics*, 4(1), 2013, 25-33.

Fig 4. Floating or Buoyancy Test

a) Initial Time at 0 sec

b) Floating Time at 01.59 sec



c) Total floating time at >24 hr

Fig 5. Comparative dissolution study of different formulations with various ratios of polymers

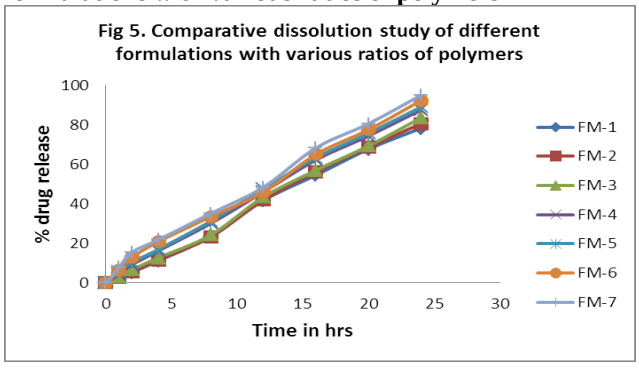


Fig.6 Comparative dissolution study of formulation-7(FM-7) and Marketed sample (Epivir)

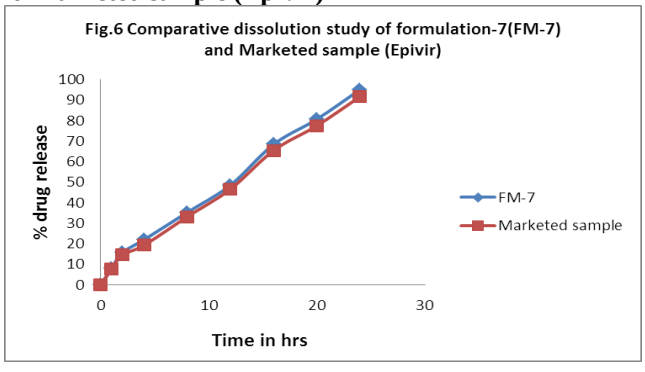


Table 1. Formulation of different batches of Lamivudine Floating tablets (mg/tab)

S.No	Formulation	Drug (Lamivudine)	Polymers			Citric acid	NaH CO_3	PVP K_{30}	Magnesium stearate	Talc
			Chitosan	Guar Gum	Xanthum Gum					
1	FM-1	300	120	-	-	10	40	Q.S	20	10
2	FM-2	300	-	120	-	10	40	Q.S	20	10
3	FM-3	300	-	-	120	10	40	Q.S	20	10
4	FM-4	300	60	60	-	10	40	Q.S	20	10
5	FM-5	300	-	60	60	10	40	Q.S	20	10
6	FM-6	300	60	-	60	10	40	Q.S	20	10
7	FM-7	300	40	40	40	10	40	Q.S	20	10

Pre and post compression studies Table 2. Precompression studies of granules

S.No	Formulations	Bulk Density (gm/cm^3)	Tapped Density (gm/cm^3)	Angle of Repose (θ)	Carr's Index (%)	Hausner's Ratio
1	FM-1	0.523	0.612	33.85	14.54	1.193
2	FM-2	0.547	0.639	34.34	14.39	1.168
3	FM-3	0.568	0.654	35.86	13.14	1.151
4	FM-4	0.547	0.639	34.23	14.39	1.168
5	FM-5	0.552	0.646	35.67	14.55	1.170
6	FM-6	0.572	0.667	36.07	14.24	1.166
7	FM-7	0.523	0.612	33.85	14.54	1.170

Table 3. Precompression studies of Lamivudine floating tablets

S.No	Formulations	Hardness Test (kg/cm)	Thickness Test (cm)	Friability Test (%)	% of Weight variation test	Estimation of Drug Content
1	FM-1	12.45	0.34	0.234	99.25	97.28
2	FM-2	12.36	0.34	0.228	99.34	95.46
3	FM-3	12.65	0.34	0.236	99.42	96.62
4	FM-4	12.14	0.34	0.252	99.57	96.43
5	FM-5	12.75	0.34	0.240	99.62	95.72
6	FM-6	12.28	0.34	0.212	99.68	97.38
7	FM-7	12.86	0.34	0.204	99.75	98.24

Floating or Buoyancy Test

Table 4. Floating or Buoyancy Test

S.No	Formulations	Buoyancy lag time *(minutes)*	Total floating time (hrs)
1	FM-1	02.13	>24
2	FM-2	02.24	>24
3	FM-3	02.18	>24
4	FM-4	01.75	>24
5	FM-5	01.83	>24
6	FM-6	02.08	>24
7	FM-7	01.59	>24

Table 5. Comparative dissolution study of different formulations with various ratios of polymers

S.No	Time (hrs)	% of drug release (FM-1)	% of drug release (FM-2)	% of drug release (FM-3)	% of drug release (FM-4)	% of drug release (FM-5)	% of drug release (FM-6)	% of drug release (FM-7)
1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	1	2.85	2.91	3.14	4.37	5.54	6.39	7.95
3	2	5.42	5.53	6.72	9.32	10.51	13.068	15.65
4	4	11.25	11.73	12.86	16.27	17.06	20.90	21.96
5	8	23.49	23.46	24.34	30.23	31.32	33.66	35.26
6	12	41.82	42.18	43.57	46.13	47.53	46.12	48.53
7	16	54.63	56.23	57.10	62.33	63.46	65.16	68.42
8	20	67.80	68.76	69.46	74.46	75.87	77.65	80.67
9	24	78.40	80.73	83.66	87.60	89.12	92.43	95.10

Bioequivalence studies

Table 6. Comparative dissolution study of formulation-7(FM-7) and Marketed sample (Epivir)

S.No	Time (hrs)	% of drug release (FM-7)	% of drug release Marketed sample
1	0	0.000	0.000
2	1	7.95	7.66
3	2	15.65	14.52
4	4	21.96	19.24
5	8	35.26	33.14
6	12	48.53	46.60
7	16	68.42	65.45
8	20	80.67	77.48
9	24	95.10	91.67

KINETIC CHARACTERISTICS OF THE DRUG RELEASE

Table 7. Kinetic models of optimized batch (FM-7)

S.No	Release kinetics	Correlation coefficient(R^2)
1	Zero order equation	0.865
2	First order equation	0.987
3	Higuchi(diffusion)co-efficient	0.953
4	Korsmeyer Peppas equation	0.990

Table 8. Stability studies of Lamivudine floating tablets for 3 months (FM-7)

S.No	Storage condition	Description	Dissolution	Drug content
			Lamivudine	
			300mg/tab	300mg /tab
1	Appearance	No change	-	-
2	Average weight	No change	-	-
3	Hardness of tablets	No change	-	-
4	Initial	Complies	95.10	98.24
5	1st month	Complies	94.42	97.58
6	2nd month	Complies	93.68	96.47
7	3rd month	Complies	92.49	95.74

DISCUSSION

Floating tablets of Lamivudine were prepared by wet granulation method. The prepared floating tablets are round in shape. FTIR spectrum of the formulated floating tablets, pure drug and polymers was recorded. The Fourier Transform Infrared Spectroscopy study reveals that there is no interaction between the polymers and drug. The precompression studies values are within the limits, indicating that the powder blends have the required flow property for wet compression and the post compression studies values have within the acceptable range such as hardness, friability, thickness, weight variation etc. The *in vitro* drug release profile of tablets from each batch (FM-1 to FM-7) was carried in 0.1N HCl for 24 hours by using paddle type device. From the *in vitro* dissolution data, FM -7 formulation was found that the drug release is best (formulation containing Chitosan, Guar gum and Xanthan gum) and the cumulative % of drug release was 95.10 % respectively. The promising formulation FM-7 was found by evaluation studies were compared with Marketed product (Lamivudine 300mg tablets (Epivir)), the FM-7 formulation gave 95.10% of the drug release and the Marketed product gave 91.67 % of drug release in 24 hours of dissolution study. The formula FM-7 with 95.10 % of drug release has better control over release of drug is compared to marketed product.

FM-7 shows the higher R^2 value for first order plots (R^2- 0.987). This indicates that the drug releases is concentration independent and following 'first' order kinetics. It is also expressed by Higuchi equation and showed high linearity. To confirm the diffusion mechanism the data were fitted in korsemayer equation with slope (n) and R^2 value is 0.990. This indicates the release of drug follows non-fickian transport. It means the release of drug from tablet is diffusion mechanism (Table 7).

The tablets from trials FM-7 was charged for stability at 30 ^oC/ 65% RH and 40^oC/75% for three months and the three months results was found to be satisfactory (Table 8).

CONCLUSION

From the above results the aim of study concluded that the floating tablets of Lamivudine were successfully formulated by wet granulation technique. The Floating tablets of Lamivudine containing Formulation-7 (containing combination of Chitosan, Guar gum and Xanthan gum (FM-7)) showed satisfactory results with short buoyancy lag time, total buoyancy time more than 24 hrs and the drug release Formulation-7(FM-7) has better control over release of drug (95.10%) when compared to marketed product (91.67). The data obtained from *in vitro* release study were fitted to various mathematical model like Zero order, First order, Higuchi model and Peppas model. The results of mathematical model fitting of data obtained indicated that, the best fit model in all the cases the release was found to be by diffusion for optimized formulation (FM-7). Thus the release of the drug from the dosage form was found to be diffusion and non-fickian release. The formula optimized and it was selected for stability studies as per ICH guidelines.

REFERENCES

Anonymous 1. www.wikipedia.com- Lamivudine.

Arunachalam A, Sudhakar Babu AMS, Varatharajan P. Preparation and *in vitro* evaluation of sustained release tablets of Aceclofenac, *International Journal of Research in Pharmaceutical and Nano Sciences*, 1(1), 2012, 1-10.

Donald LW. Bioploymeric controlled release system, *USA: CRS Press Inc.* 1, 1984, 253- 65.

Higuchi T. Mechanism of sustained action medication: Theoretical analysis of rate of release of solid drug dispersed in solid matrix. *J Pharm Sci*, 52, 1963, 1145-1149.

Korseyer RW, Gurny R. Peppas. Mechanism of solute release from porous hydrophilic polymers. *In.J Pharm*, 15(1), 1983, 25-35.

Lachman L, Lieberman H, Kanig J. The theory and practice of industrial pharmacy, *Varghese publication house.* 3rd edition, 2009, 297-303.

Mohamed Raffick M, Muthu Mohamed J, Vetriselvan S. Preparation and Evaluation of *in-vitro* release kinetics of novel bilayer metoprolol succinate as sustained release and amlodipine besylate as immediate release tablets. *International Journal of Biological & Pharmaceutical Research*, 3(2), 2012, 285-297.

Moyle G. Clinical manifestations and management of antiretroviral nucleoside analog-Related mitochondrial toxicity, *Clin Ther*, 22, 2009, 911-936.

Raju DB, Sreenivas R and Varma MM. Formulation and evaluation of floating drug delivery system of Metformin Hydrochloride. *J. Chem. Res.* 2(2), 2010, 274-278.

Remington's. Pharmaceutical Sciences and practice of pharmacy, *Mack Publication.* 18th edition, 2, 1996, 1677.

Theodore JR, Mansdorf SY. Controlled release drug delivery system, *New York: Marcel Dekker.* 1, 1983, 1-5.

Wells JI, Swarbick J, Boylan JC. Encyclopedia of pharmaceutical technology, *New York; Marcel Dekker*, 2nd edition, Vol X, 1996, 411.