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In Vitro – In Vivo Evaluation of Floating Tablet Containing Anti Retroviral Agent Lamivudine

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ABSTRACT

This study aimed to develop hydrophilic matrix based controlled release gastroretentive drug delivery system of Lamivudine floating matrix tablets, which after oral administration are designed to prolong the gastric residence time, increase the drug bioavailability and diminish the side effects of irritating drugs. FTIR studies revealed that there is no interaction between the drug and polymers used for the formulation. Among all the formulations F21 containing HPMC K100M, Carbopol 934P, Polyox WSR 303 and sodium bicarbonate, as gas generating agent was selected as optimized formulation based on physico chemical properties, floating lag time (34 sec) and total floating time (>24 h). From *in vitro* dissolution studies, the optimized formulation F21 showed drug release of 99.36±5.36%, whereas 92.36±5.02% of the drug was released from the marketed product within 24h. From *in vivo* bioavailability studies, after oral administration of floating tablet containing 100 mg Lamivudine, the C_{max}, T_{max}, and $AUC_{0-\infty}$ of optimized gastroretentive formulation were found to be 32.11±3.16 µg/mL, 8.00±1.26 h and 225±28.14 µg*h/ml, respectively. C_{max} and AUC values of optimized formulation were found to be significantly higher than of marketed product, where longer gastric residence time is an important condition for prolonged or controlled drug release and also for improved bioavailability. Hence, gastro retention can be a promising approach to enhance bioavailability of Lamivudine with narrow absorption window in upper GIT.

Keywords: Lamivudine, AIDS, HPMC, Floating lag time, Pharmacokinetics.

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INTRODUCTION

Using current release technology, oral delivery for 24 h is possible for many drugs; however, the substance must be absorbed well throughout the whole gastrointestinal tract [1]. Based on the requirement, formulations can be changed from immediate release to extended release by using several polymers [2]. Gastroretentive floating dosage forms are continuously researched and developed as the stomach is a major absorption zone. The gastric emptying time which varies from 2-3 h is a disadvantage for gastroretentive dosage forms. Based on the formulation type and physiological condition of the patient, the gastric emptying process can vary from a few minutes to 12 h also. This variation may lead to unpredictable bioavailability and times to achieve peak plasma levels [3]. In addition, the relatively brief gastric emptying time in humans, through the stomach or upper part of the intestine (major absorption zone), can result in incomplete drug release from the drug delivery system, leading to reduced overall efficacy of the drug. Some drugs like Lamivudine exhibit region-specific absorption in different regions of the intestine because of different pH conditions, various enzymes and endogenous components like bile [1].

Some of the common approaches used to increase the gastric residence time of pharmaceutical dosage forms include floating systems, swelling and expanding systems, bio adhesive systems, unfolding and modified- shape systems, high density systems etc. Floating drug delivery systems have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period [4]. Whilst the system remains afloat, the drug is released at a desired rate from the system [5]. Following drug release, the residual system gets emptied from the stomach. This results in an increased gastric retention time and a better control of the fluctuations in plasma drug concentration. However, besides a minimal gastric content needed to allow the proper achievement of the buoyancy retention principle, a minimal level of floating force is also required to keep the dosage form reliably buoyant on the surface of the meal [6].

Lamivudine, a member from meglitinide class, is a post prandial glucose regulator used in treatment antiretroviral [7]. This study was conducted with an aim to develop floating gastroretentive tablet formulation incorporating 100mg Lamivudine into hydrophilic polymeric matrix which would release the drug in stomach and upper part of GIT in a controlled manner. Since Lamivudine has site-specific absorption from these regions, gastroretention of the dosage form will improve its oral bioavailability.

MATERIALS AND METHOD

Materials

Lamivudine was procured from Aurobindo Pharma Ltd., Hyderabad. HPMC K 4M, HPMC K 15M, HPMC K 100M were obtained from Ashland India Pvt. Ltd., Hyderabad. Carbopol 934p and POLYOX WSR 303 were obtained from Granules India Ltd, Hyderabad. Sodium bicarbonate, MCC, talc and magnesium stearate were procured from SD Fine Ltd., Mumbai and all other chemicals used were of analytical grade.

Methods

Formulation method

Accurately weighed quantities of polymers and MCC were taken in a mortar and mixed geometrically, to this required quantity of Lamivudine was added and mixed slightly with pestle [8]. Accurately weighed quantity of sodium bicarbonate was taken separately in a mortar and powdered with pestle. The powder is passed through sieve no. 40 and mixed with the drug blend which is also passed through sieve no. 40. The whole mixture was collected in a plastic bag and mixed for 3 minutes .To this magnesium stearate was added and mixed for 5 minutes, later talc was added and mixed for 2 minutes [9]. The mixture equivalent to 400 mg was compressed into tablets with 10 mm round concave punches at a hardness of 6 kg/cm^2. The composition of floating matrix tablets of Lamivudine with different polymers is shown in Table 1, 2 and 3.

Table 1: Composition of floating matrix tablets of Lamivudine with HPMC K4M

Ingredients (weight in mg)	Formulations						
	F1	F2	F3	F4	F5	F6	F7
Lamivudine	100	100	100	100	100	100	100
HPMC K4M	50	55	60	65	70	75	80
Carbopol 934p	50	50	50	50	40	30	20
Polyox WSR 303	20	25	30	35	40	45	50
Sodium Bicarbonate	20	22	24	26	28	30	32
MCC	56	44	32	20	18	16	14
Talc	2	2	2	2	2	2	2
Mag. Stearate	2	2	2	2	2	2	2
Total Weight	**300**	**300**	**300**	**300**	**300**	**300**	**300**

Table 2: Composition of floating matrix tablets of Lamivudine with HPMC K15M

Ingredients (weight in mg)	Formulations						
	F8	F9	F10	F11	F12	F13	F14
Lamivudine	100	100	100	100	100	100	100
HPMC K15M	50	55	60	65	70	75	80
Carbopol 934p	50	50	50	50	40	30	20
Polyox WSR 303	20	25	30	35	40	45	50

Sodium Bicarbonate	20	22	24	26	28	30	32
MCC	56	44	32	20	18	16	14
Talc	2	2	2	2	2	2	2
Mag. Stearate	2	2	2	2	2	2	2
Total Weight	**300**	**300**	**300**	**300**	**300**	**300**	**300**

Table 3: Composition of floating matrix tablets of Lamivudine with HPMC K100M

Ingredients (weight in mg)	**Formulations**						
	F15	**F16**	**F17**	**F18**	**F19**	**F20**	**F21**
Lamivudine	100	100	100	100	100	100	100
HPMC K100M	50	55	60	65	70	75	80
Carbopol 934p	50	50	50	50	40	30	20
Polyox WSR 303	20	25	30	35	40	45	50
Sodium Bicarbonate	20	22	24	26	28	30	32
MCC	56	44	32	20	18	16	14
Talc	2	2	2	2	2	2	2
Mag. Stearate	2	2	2	2	2	2	2
Total Weight	**300**	**300**	**300**	**300**	**300**	**300**	**300**

Evaluation of floating matrix tablets of Lamivudine

Parameters like Weight Variation Thickness, Hardness [10] and Friability [11] were evaluated according to the reported method.

In vitro buoyancy studies

The buoyancy was determined by floating lag time. The tablets were placed in a 100ml beaker containing 0.1N hydrochloric acid. The time required for the tablet to rise to the surface and float was determined as floating lag time [12]. The duration of time for which the dosage form constantly remained on the surface of medium was determined as the total floating time.

Drug Content

Twenty tablets were taken and powdered. The powder equivalent to one dose each was transferred to a 100ml volumetric flask and 0.1N HCl was added. The volume was then made up to the mark with 0.1N HCl. The solution was filtered and diluted suitably and drug content in the samples was estimated using UV spectrophotometer at 270 nm [13].

***In vitro* drug release studies**

The *in vitro* drug release study was performed for the single and multiple-unit tablets using USP Type II dissolution apparatus using 900ml of 0.1N HCl at a temperature of 37±0.5ºC at 50 rpm. 5ml of sample was collected at 0, 2, 4, 6, 8, 12, 16, 20, 24 h and the same volume of fresh media was replenished [14]. The drug content in the samples was estimated using UV visible spectrophotometer at 270 nm.

Stability studies

Stability testing was conducted at 40˚C ± 2˚C/75% RH ± 5% RH for 3 months using stability chamber (Thermo Lab, Mumbai). Samples were withdrawn at predetermined intervals 0, 30, 60 and 90 days period according to ICH guidelines [15]. Various *in vitro* parameters like % yield, entrapment efficiency and *in vitro* release studies were evaluated.

***In vivo* bioavailability studies Lamivudine:**

Animal Preparation

Twelve New Zealand white rabbits of either sex rabbits were (weighing 2-3kg) selected for this study, all the animals were healthy during the period of the experiment. Animals were maintained at room temperature 25^0C, RH 45% and 12 hrs alternate light and dark cycle with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply and rabbits were fed with standard diet and water ad libitum. The protocol of animal study was approved by the institutional animal ethics committee with No. CPCSEA/1657/IAEC/CMRCP/PhD-15/37.

***In vivo* Study design**

Rabbits were randomly divided into two groups each group contains six animals. The group A, rabbits were fed with Lamivudine optimized formulation, group B fed with Innovator product with equivalent dose to animal body weight. Blood samples (approximately 0.5ml) were obtained with syringes by marginal ear vein at 0, 0.5, 1, 1.5,2, 4, 6, 8, 12, 16, 20 and 24 h post doses. During collection, blood sample has been mixed thoroughly with heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5minutes to 10 minutes and stored frozen at −20°C until analysis.

Determination of Lamivudine in rabbit plasma by HPLC method

For HPLC C8 column with 5µm particle size and the mobile phase containing water: tetrahydrofuran: acetonitrile (45.83: 20.83: 33.34 % v/v/v), Oyster BDS premium C18 (250 mm × 4.6 mm, 5 µm) analytical column in isocratic mode at room temperature and UV detection at 245 nm. The compounds were eluted at a flow rate of 1.15 ml min^{-1}. Internal standard didanosine was used. The retention times of Lamivudine and didanosine were found to be 2.01 ± 0.003 minutes and 3.01 ± 0.001minutes respectively [16].

Preparation of Plasma Samples for HPLC Analysis

Rabbit plasma (0.5ml) was prepared for chromatography by precipitating proteins with 2.5ml of ice-cold absolute ethanol for each 0.5ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was resuspended with 1ml of acetonitrile by vortexing for 1min. After centrifugation (5000 – 6000 rpm for 10min.), the acetonitrile was added to the ethanol and the

organic mixture was taken to near dryness by a steam of nitrogen at room temperature. Samples were reconstituted in 200µ1 of 50% of acetonitrile and 50% 0.1% orthophosphoric acid was injected for HPLC analysis.

Pharmacokinetic Analysis:

The pharmacokinetic parameters, peak plasma concentrations (C_{max}) and time to reach peak concentration (t_{max}) were directly obtained from concentration time data. In the present study, AUC_{0-t} refers to the AUC from 0 to 24 h, which was determined by linear trapezoidal rule and $AUC_{0-\alpha}$ refers to the AUC from time at zero hours to infinity.

Calculated using the formula $AUC_{0-t} + [C_{last}/K]$ where C_{last} is the concentration in µg/ml at the last time point and K is the elimination rate constant.

Various pharmacokinetic parameters like area under the curve [AUC], elimination half life ($t_{½}$). Volume of distribution (V_d), total clearance (Cl_T) and mean residence time for each subject using a non compartmental pharmacokinetic program. The pharmacokinetic parameters were performed by a non compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean ±SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Physico-chemical properties of Lamivudine floating tablets

The prepared tablets of Lamivudine were evaluated for different parameters like weight variation, thickness, hardness, friability and drug content were found to be within the limits and summarized in Table 4.

Table 4: Physicochemical parameters of Lamivudine floating tablets

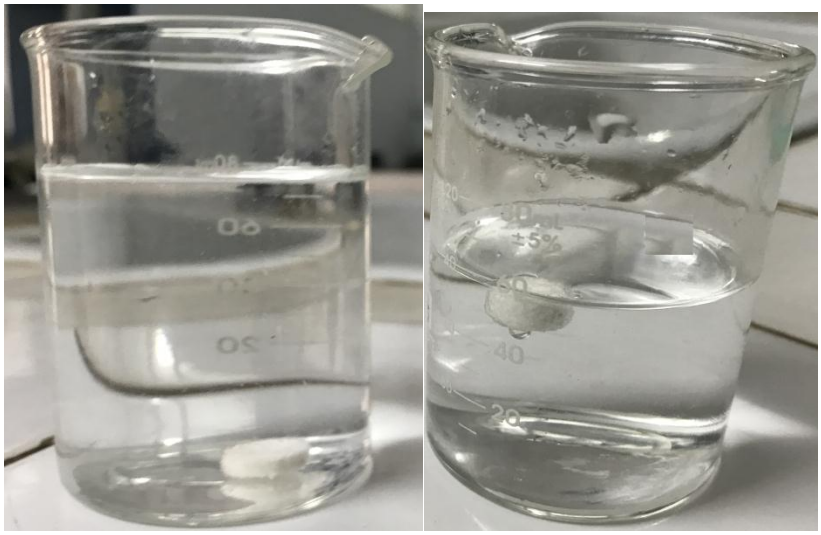
F. No	*Weight variation(mg)	#Thickness (mm)	#Hardness (Kg/Cm^2)	#Friability (%)	#Content uniformity(%)	Floating Lagtime(sec)	Total floatingtime(h)
F1	298±0.8	3.20±0.10	5.1±0.50	0.19	96.11±0.45	53	>24
F2	297±0.6	3.19±0.10	5.5±0.52	0.24	94.25±0.45	51	>24
F3	299±0.8	3.22±0.10	5.4±0.51	0.23	93.12±0.19	48	>24
F4	298±0.8	3.18±0.08	5.6±0.52	0.21	96.14±0.45	46	>24
F5	297±0.6	3.29±0.12	5.5±0.52	0.19	93.26±0.19	43	>24
F6	299±0.8	3.28±0.12	5.4±0.51	0.24	95.12±0.45	41	>24
F7	298±0.8	3.26±0.12	5.2±0.50	0.23	96.31±0.45	39	>24
F8	300±0.10	3.22±0.10	4.10±0.48	0.21	94.52±0.35	51	>24
F9	296±0.6	3.27±0.12	4.9±0.45	0.25	93.16±0.19	48	>24
F10	299±0.5	3.22±0.10	5.1±0.50	0.24	91.24±0.15	46	>24

F11	295±0.5	3.27±0.12	5.3±0.51	0.22	97.23±0.60	43	>24
F12	297±0.6	3.29±0.12	5.4±0.51	0.23	95.86±0.45	41	>24
F13	299±0.8	3.21±0.10	5.2±0.50	0.21	94.57±0.35	39	>24
F14	298±0.8	3.25±0.12	4.9±0.45	0.20	96.45±0.45	36	>24
F15	299±0.8	3.18±0.08	5.3±0.51	0.19	92.36±0.19	50	>24
F16	300±0.10	3.20±0.10	4.10±0.48	0.24	93.47±0.19	48	>24
F17	298±0.8	3.21±0.10	4.9±0.45	0.23	91.35±0.18	45	>24
F18	300±0.7	3.22±0.10	5.2±0.50	0.21	94.36±0.35	43	>24
F19	302±0.8	3.28±0.12	5.1±0.50	0.22	92.01±0.19	41	>24
F20	299±0.8	3.15±0.08	4.8±0.45	0.18	98.49±0.68	38	>24
F21	301±0.10	3.20±0.10	4.10±0.48	0.20	96.23±0.45	34	>24

*Values are expressed in mean± SD: (n=20) #Values are expressed in mean± SD: (n=3)

Tablets of all batches had floating lag time below 1 minute regardless of viscosity and content of HPMC because of evolution of CO_2 resulting from the interaction between sodium bicarbonate and dissolution medium; entrapment of gas inside the hydrated polymeric matrices enables the dosage form to float by lowering the density of the matrices. Total Floating time for all the formulations were more than 24 h.

In vitro buoyancy lag time of the optimized formulation F21 is showed minimum floating lag time of 34 sec, when compared with other formulations and shown in Figure 1.



At time 0 **After 34 sec**

Figure 1: *In vitro* buoyancy lag time of the optimized formulation F21

***In vitro* dissolution studies**

From dissolution studies it can be observed that the polymer HPMC K100 M has controlling effect on the release of drug from the floating matrix tablet of Lamivudine compared to HPMC K4M and HPMC K15M. The difference in the drug release profiles of various formulations was due to the presence of different concentrations of polymer HPMC K100M. The concentration of polymer was

added in increasing order to check its drug release retarding ability and F21 was considered as best one among the all the formulations, and the drug release was 99.36±5.36, when compared with marketed product of 92.39±5.02 within 24h (Figures 2, 3 and 4).

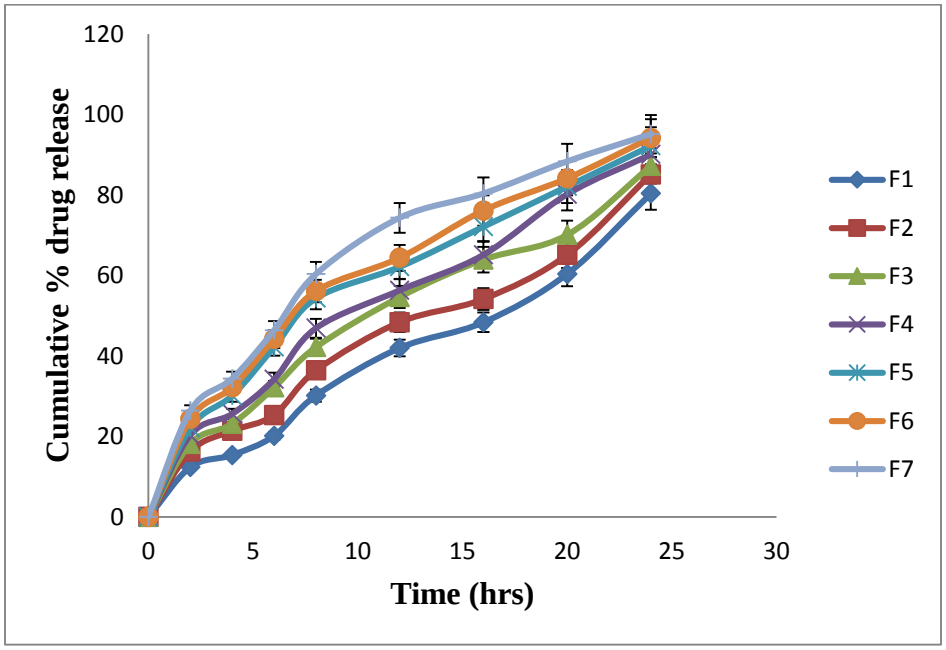


Figure 2: *In vitro* drug release profile of Lamivudine floating tablets F1-F7

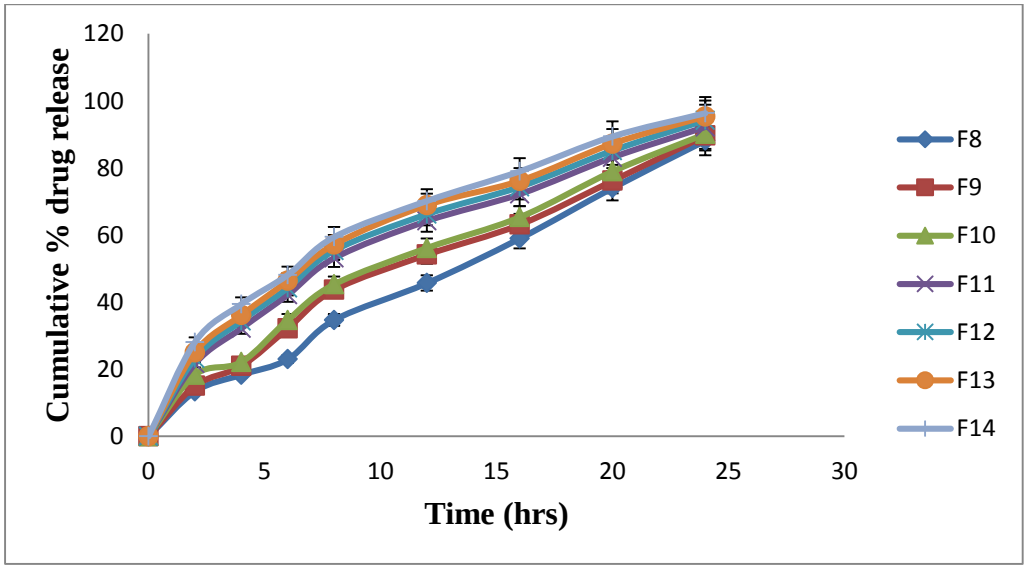


Figure 3: *In vitro* drug release profile of Lamivudine floating tablets F8-F14

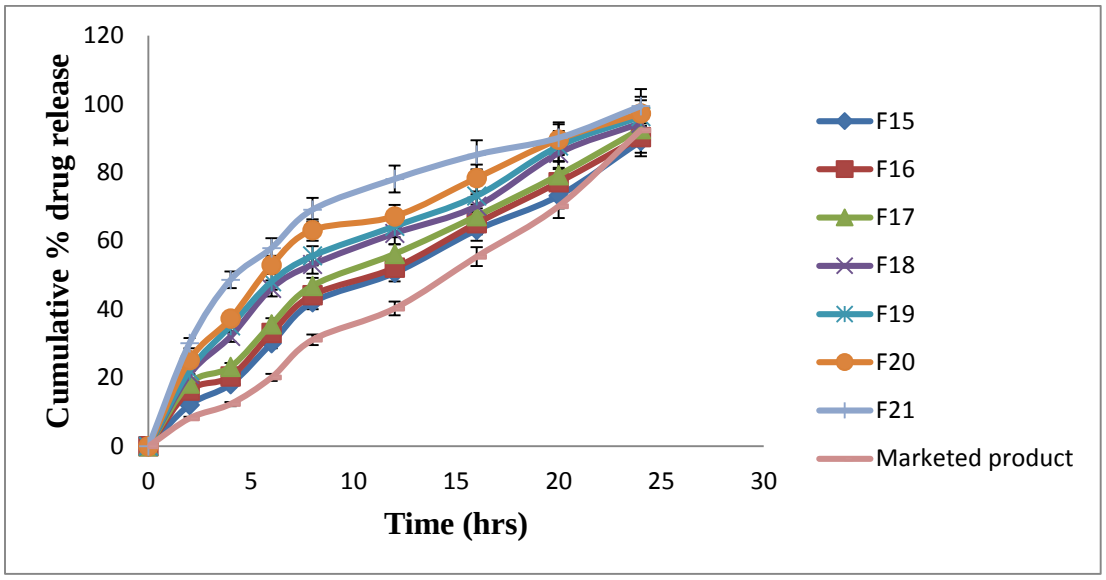


Figure 4: *In vitro* drug release profile of Lamivudine floating tablets F15-F21 and Marketed product

Stability studies

There were no changes observed in % drug content, *In vitro* drug release studies and floating lag time during storage of the optimized formulation. Hence the optimized formulation was found to be stable.

Bioavailability parameters

Mean plasma concentration profiles of prepared Lamivudine optimized formulation and marketed product are presented in Figure 5**.**

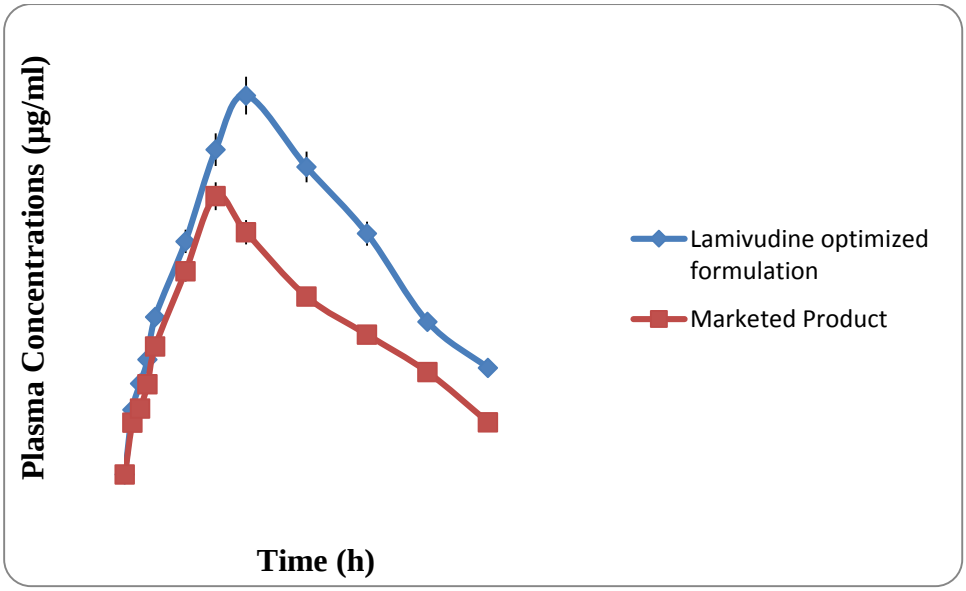


Figure 5: Plasma concentrations at different time intervals for Lamivudine optimized formulation and Marketed Product

Lamivudine optimized formulation exhibited as sustained release *in vivo* when compared with marketed tablet. All the pharmacokinetics parameters are summarized in Table 5**.**

Table 5: Comparison of pharmacokinetic parameters of Lamivudine optimized formulation and Marketed Product

Parameters	Lamivudine Optimized formulation	Marketed Product
C_{max}(µg/ml)	32.11±3.16	25.11±3.13
AUC_{0-t}(µg*h/ml)	196±56.44	157±44.26
$AUC_{0-\infty}$ (µg*h/ml)	225±28.14	186.25±38.12
T_{max} (h)	8.00±1.26	6.00±0.14
$t_{1/2}$ (h)	12.053 ± 0.5	9.164 ± 0.01
K_{el} (h-1)	0.057 ± 0.016	0.075 ± 0.018

From *in vivo* bioavailability studies, after oral administration of floating tablet containing 100mg Lamivudine, the C_{max}, T_{max}, and

$AUC_{0-\infty}$ of optimized gastroretentive formulation were found to be 32.11±3.16 µg/mL, 8.00±1.26 h and 225±28.14 µg*h/ml, respectively. C_{max} and AUC values of optimized formulation were found to be significantly higher than of marketed product, where longer gastric residence time is an important condition for prolonged or controlled drug release and also for improved bioavailability. The results indicated that the optimized formulation could increase the bioavailability of Lamivudine in Rabbits effectively. In this study, the Lamivudine floating tablet produce higher bioavailability than that of a marketed product, this overall increase in bioavailability and increased gastric residence time, caused by flotation of dosage form in the stomach.

CONCLUSION

In the present work, it can be concluded that the Lamivudine floating tablets can be an innovative and promising approach for the delivery in the treatment of AIDS. The optimized formulation F21 containing HPMC K100M, Carbopol 934p, Polyox WSR 303 and gas-generating agent. *In vitro* release profile of Lamivudine and marketed product when compared, the optimized formulation F21 showed drug release of 99.36±5.36% whereas 92.36±5.02% of the drug was released from the marketed product within 24 hrs. From *in vivo* bioavailability studies, after oral administration of floating tablet containing 100mg Lamivudine, the C_{max}, T_{max}, and $AUC_{0-\infty}$ of optimized gastroretentive formulation were found to be 32.11±3.16 µg/mL, 8.00±1.26 h and 225±28.14 µg*h/ml, respectively. C_{max} and AUC values of optimized formulation were found to be significantly higher than of marketed product, where longer gastric residence time is an important condition for prolonged or controlled drug release and also for improved bioavailability. Hence, gastro retention

can be a promising approach to enhance bioavailability of Lamivudine with narrow absorption window in upper GIT.

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