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DEVELOPMENT AND EVALUATION OF LAMIVUDINE CONTROLLED RELEASE TABLET USING CROSS-LINKED STARCH

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ABSTRACT

The present investigation concerns with the development of controlled release tablets of lamivudine using cross-linked moth bean starch. The cross-linked moth bean starch was synthesized with phosphorous oxy chloride in basic pH medium. The cross-linked moth bean starch was tested for acute toxicity and drug-excipient compatibility study. The formulation were evaluated for physical characteristics like hardness, friability, % drug content and weight variations. The *in vitro* release study showed that the optimized formulation exhibited highest correlation (R) value in case of zero order kinetic model and the release mechanism study proved that the formulation showed a combination of diffusion and erosion process. There was a significant difference in the pharmacokinetic parameters (T_{max}, C_{max}, AUC, V_d, $T_{1/2}$ and MDT) of the optimized formulation as compared to the marketed conventional tablet Lamivir®. The *in vitro* and *in vivo* study of the optimized formulation revealed that the cross-linked moth bean starch in the CR tablets rendered the drug to be released in a controlled manner.

Key Words: Lamivudine, Controlled release tablet, Cross-linked, Moth bean starch.

INTRODUCTION

Recently, starch finds increasing applications in the pharmaceutical industry because of its easy availability, biocompatibility, and biodegradability. The applicability of starch in pharmaceutical formulations is often associated with its gel-forming capacity upon gelatinization. Among different technologies used in controlled drug delivery, hydrophilic

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matrix systems are the most popular because of their simplicity of formulation, ease of manufacturing, low cost and applicability to drugs with wide range of solubility (Aerde and Remon 1988). Cross-linking treatment is intended to add chemical bonds at random locations in a granule. The cross-linking stabilizes the granules and strengthens the relatively tender starch. Pastes from cross-linked starches are more viscous and are less likely to breakdown with extended cooking times, increased acid content or severe agitation. Epichlorohydrins, phosphoryl chloride ($POCl_3$), sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP) are the main cross-linking agents used to give cross-linking of food grade starches. Cross-linking alters not only the physical properties, but also the thermal transition characteristics of starch, and the effect of cross-linking depends on the botanical source of starch and the cross-linking agent (Solarek 1986). Decrease in retrogradation rate and increase in gelatinization temperature has also been observed with cross-linked starches, and these phenomena are related to the reduced mobility of amorphous chains in the starch granule as a result of the intermolecular bridges (Liu *et al* 1999). Drug release from these systems is the consequence of controlled matrix hydration, followed by gel formation, change of rheological behavior, matrix erosion, and/or drug dissolution and diffusion. The significance of such systems depends on drug solubility, concentrations and changes in matrix characteristics. Reservoir and matrix type tablets are the most commonly used orally administrated sustained release preparations. Especially matrix tablets, which are produced by direct compression using either hydrophilic polymer such as natural gums, HPMC, CMC, Carbopol or hydrophobic polymer, like ethyl cellulose and amylodextrin are relatively easy to manufacture (Narasimhan 2001). Lamivudine (LAM) is the first nucleoside analogue approved to treat chronic HBV infection and AIDS. Conventional oral formulations of LAM are administered multiple times a day 150 mg each time because of its moderate half-life ($t_{1/2}$ = 5 to 7 hours). Treatment of AIDS using conventional formulations of LAM is found to have many drawbacks, such as adverse side effects resulting from accumulation of drug in multi-dose therapy, poor patient compliance, and high cost (Perry and Faulds 1997). The aim of the present study was to evaluate the chemically modified (cross-linked) moth bean starch as hydrophilic inert matrix former in the formulation of controlled release tablets of LAM by direct compression technique. The formulated tablets were

evaluated for various physical characteristics, *in vitro* dissolution study and *in vivo* pharmacokinetic study in rabbit model.

MATERIALS AND METHODS

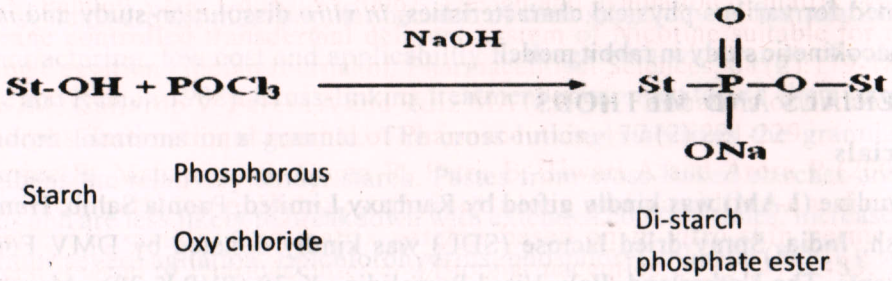
Materials

Lamivudine (LAM) was kindly gifted by Ranbaxy Limited, Paonta Sahib, Himachal Pradesh, India. Spray dried lactose (SDL) was kindly donated by DMV Fonterra Excipients, The Netherland. Poly Vinyl Pyrrolidine K-30 (PVP K-30) , Magnesium Stearate and Talc were purchased from Loba Chemie Limited, Mumbai, India. All other chemicals used were of analytical grade and purchased from SD Fine Chemical Limited, Mumbai, India. Digital Weighing Balance (Sartorius, Germany), Rotary Ten Station Tablet Punching machine (Shakti Engineering Limited, Ahmedabad, India), UV-Visible Spectrophotometer (UV-1700, Shimadzu, Japan), HPLC (Waters®, USA), Eight Basket Digital *in vitro* USP Dissolution Apparatus (Electrolab, Mumbai, India), Monsano Hardness Tester and Roche Friabilator (Campbell electronics, Mumbai, India), Laboratory scale stability chamber (Model TH-90 S/G, Thermolab, Mumbai, India) were used.

Methods

Cross-linking of moth bean starch

The native moth bean starch was pregelatinized and dried at 45°C and then sieved (120 mesh). Cross-linking of starch was done with phosphorous oxy chloride ($POCl_3$) in sodium hydroxide environment (Singh and Nath 2011a; Singh and Nath 2011b). The moth bean starch (50g, dry weight basis) was dispersed in distilled water (200 mL), and then the slurry was adjusted to pH 9.0 with 0.5 M NaOH solutions. The cross-linking reagent $POCl_3$ was added drop wise in different concentrations (0.5-2.5% w/w). The starch dispersion was stirred for 1 h and stored for 12 h at room temperature for completion of the reaction. The starch suspension was adjusted to pH 6.5 adding 1 M HCl which leads to termination of the reaction. Extensive washing was done to ensure the removal of un-reacted salt. After drying overnight at 40°C in a vacuum oven, the cross-linked starch was grounded and sieved (60 mesh).



Degree of Substitution (DS)

The DS value of starch derivatives is defined as the number of moles of substituted hydroxyl groups per mole of D-glucopyranosyl structural units. The phosphorus content of starch phosphate was colorimetrically determined by the reaction with ammonium molybdate according to the method described by Murphy and Riley 1962, and the DS value (phosphate group) was calculated as follows:-

$$DS = \frac{162\ P}{(3100 - 102P)} \qquad (2)$$

Where, P is the percentage of phosphorous on dry weight basis, 162 is the molecular weight of anhydroglucose unit and 3100 is the atomic weight of phosphorous, i.e. 31 attached to 100 unit of anhydroglucose.

Acute toxicity study of cross-linked starch

For the acute oral toxicity study, healthy male and female Swiss albino mice were used. The animals were housed in polypropylene cages and provided with bedding of clean paddy husk. The animals were acclimatized to laboratory conditions for one week prior to experiment. The temperature in the animal house was maintained at 25 ± 2°C with a relative humidity of 30-70% and illumination cycle set to 12 h light and 12 h dark. The mice were fed with standard laboratory pelleted feed (M/s Gold Mohur Foods and Feeds Ltd., Bangalore, India). All the mice of both sexes were fasted overnight before experiment and were allowed to take food one hour after the experiment. Modified starch was administered orally at a dose of 2 gm/kg body weight

in distilled water. The animals were observed for any mortality and morbidity (convulsions, tremors, grip strength and pupil dilatation) at an interval of 12 h for 14 days. This study was approved by the Animal Ethical Committee of Gayatri College of pharmacy (Regn. No.1339/ac/10/cpcsea).

Preformulation study

Drug-excipient compatibility study by DSC: A differential scanning calorimetry (JADE DSC, Perkin Elmer, USA) was used to study the thermal analysis of drug-excipient compatibility. Firstly, binary mixtures of lamivudine and excipients (in 1:1 mass/mass ratio) were prepared using physical mixture technique. The drug-excipient mixture was scanned in the temperature range of 50°C to 220°C under nitrogen atmosphere. The heating rate was 20°C/min and the obtained.

Drug-excipient compatibility study by FT-IR spectroscopy: FT-IR spectra was recorded on a Bruker Spectrophotometer (Model - 220, Germany) using KBr discs in the range of 4000 to 450 cm^{-1}. FT-IR analysis has been performed using sample of lamivudine with various excipients and chemically modified starches at 1:1 mass/mass ratio.

Isothermal stress testing (IST) analysis

In isothermal stress testing (Singh and Nath 2011c; Singh and Nath 2011d) samples of drug and different excipients (Table-3) were weighed directly in 5 mL glass vials (n=3). After mixing on a cyclomixer for 3 min, 10% (w/w) water was added in each of the vial. The glass vials, after teflon sealing, were stored at 50°C in a hot air oven. Drug-excipient blends without adding water and stored in refrigerator served as controls. The drug-excipient blends were periodically examined for any change in physical appearance. Samples were quantitively analyzed using UV-Vis Spectrophotometer (Pharmaspec 1700, Shimadzu, Japan) after 4 weeks of storage at above conditions.

Table-1: Composition of lamivudine controlled release tablets using CLMBS

Ingredients (mg)	Formulation code					
	F1	F2	F3	F4	F5	F6
Lamivudine	100	100	100	100	100	100
Cross-linked moth bean starch	50	100	150	200	250	300
PVP K-30	20	20	20	20	20	20
Spray dried Lactose	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Magnesium Stearate	4	4	4	4	4	4
Talc	4	4	4	4	4	4
Total weight	500	500	500	500	500	500

qs = quantity sufficient

Formulation of tablets

The lamivudine controlled release tablets were formulated by incorporating selected excipients using model drug lamivudine. Lamivudine, cross-linked moth bean starch (CLMBS), spray dried lactose and PVP K-30 were dispensed accurately. Each ingredient was shifted through # 80 sieves, transferred in to a polyethylene bag and mixed for 20 min. The dry mixture was subjected to direct compression on a ten station rotary tablet machine. The slugs were broken into granular form and were shifted through #20 sieves, weighed and subjected to evaluation of flow properties. The quantity of remaining ingredients of the Table-1 i.e. magnesium stearate and talc required were calculated as per the formula of the Table-1 to compensate any loss during the direct compression process. The lubricated dry mixture was compressed into tablets at 6 kg/cm^2 pressure using 10 mm standard concave and flat punch set in 10 station rotary tablet punching machine. The tablets were double wrapped in polyethylene bag till further study.

Evaluation of tablets

The tablets from each batch was picked randomly in order to evaluate the weight variation, the hardness, % drug content and the friability (Banker and Andsi 1982). The hardness and friability of the tablet were measured using Monsanto hardness tester and Roche friabilator, respectively.

***In vitro* dissolution rate study**

In vitro dissolution rate study of the formulations (n=6) was carried out in USP Type-II dissolution rate test apparatus. Dissolution rate study was performed in simulated gastric fluid (0.1M HCl) and in simulated intestinal fluid (PBS pH 6.8) for first 2 h and for successive 10 h, respectively.The each dissolution medium (900 mL) was maintained at 37±0.5°C throughout the study. The samples (5 mL) were withdrawn at predetermined time (1, 2, 3, 4, 6, 10 and 12 h) and replaced with an equivalent volume of fresh medium. The samples were filtered through membrane filter (0.45 µm) and analysed by UV-Visible Spectrophotometer at 270 nm. The cumulative percent drug release was plotted against time to determined the release profile.

Kinetics of drug release

The kinetics of drug release (Costa and Loba 2001; Hadjiioannou, Christian and Koupparis 1993; Higuchi 1963; Korsemeyer *et al* 1985) is important because it is an useful tool to correlate the *in vitro* drug release and *in vivo* drug responses or to compare the results of pharmacokinetics with dissolution profiles of the formulations. Different mathematical models i.e. zero order, first order, higuchi and korsemeyer-peppas eqution were applied for describing the kinetics of the drug release process from controlled released tablets of LAM, the most suited being the one which fitted best in the experimental results.

Pharmacokinetic study

The pharmacokinetic study of optimized controlled release tablet (F5) of lamivudine was carried out in two groups of six male white albino rabbits each weighing 1.5 to 2.5 kg. All animals were fasted overnight (12 h) before dosing and continued till 4 h after administration of tablets, thereafter rabbit chew diet was provided *ad libitum*. Drinking water was deprived of before dosing and continued till two hours of post dose, thereafter it was provided *ad libitum*. The tablets (n=3) of batch F5 was administered orally to three rabbits of each group along with 10 mL of water using feeding tube.

The blood samples (50 µL) were collected from orbital sinus into the microcentrifuge tubes containing 50 µL of 10% w/w of disodium EDTA as anticoagulant according to the sampling schedule (pre dose, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 9.0, 12.0 and 24.0 h) . The collected blood samples were centrifuged immediately at 1000×g for 10 min. The supernatent plasma layer was seperated and stored at -20°C till analysis.

Plasma sample analysis

The 200 µL of each sample was taken into 2 mL centrifuge tube and 50 µL of nelfinavir solution (50 µg/mL) was added as an internal standard (IS) .The mixtures were vortexed for 10 seconds. Acetonitrile (1.5 mL) was added into the mixture, vortexed for 3 min and centrifuged at 10000 rpm for 10 min. The supernatent was transferred into a glass centrifuge tube and evaporated to dryness at 45°C under a stream of nitrogen .The residue was reconstituted with 200 µL of reconstitution solvent. The samples were filtered through 0.45 µm membrane filter using syringe filter. An aliquot of 20 µL of the sample was injected into the injector of the HPLC system. The samples were analysed by using the chromatographic condition described elsewhere (Singh, Nath and Pani 2011e). The area under the curve of peaks of LAM and IS was determined and the concentration of drug present in sample was estimated using the linear regrassion equation of standard calibration curve (concentration of LAM vs. ratio of LAM to IS. The amount of drug present in 200 µL of plasma [(quantity obtained from linear regression equation/20) × 1000)] was calculated (Jambhekar and Breen 2009).

Determination of pharmacokinetic parameters

The pharmacokinetic parameters were determined from the data of plasma drug concentration at different time points using MS-Excel 2003 software according to the procedure desribed elsewhere (Singh, Nath and Pani 2011e; Jambhekar and Breen 2009).

Stability study

Stability study is used to assess expiration dating and storage conditions for pharmaceutical products. Stability study of lamivudine controlled release tablets were performed as per ICH guidelines. The optimized controlled release tablets were kept in polypropylene bottle and stored in a stability chambers maintained at 40 ± 2°C and 75 ± 5% RH for six month. Samples were checked initially, after three month and further after six month.

RESULTS AND DISCUSSION

Synthesis of highly cross-linked starch

The cross-linking of starch granules depends on various experimental factors. Duration of reaction is one of the major parameters that influence the extent of modification in case of biomaterial modification. The highly substituted (DS=0.55) cross-linked strach was successfully synthesized.

Acute toxicity study

The purpose of this study was to evaluate toxicity profile of the modified starch. A 14 days acute oral toxicity study was performed in swiss albino mice. The LD_{50} of all the modified starches were not further studied as they were found to be safe up to 2 gm/kg on 24 h study basis. It was observed that the animal fed with the modified starches were found healthy. No unusual changes in behavior, or in locomotor activity, ataxia, and signs of toxicity were observed during the 14 days period. No difference were found in growth behavior between the cotrol and treatment group in 14 days study. The body weight of male and female swiss albino mice were found to be normal after treatment. There was no change observed in body weight of control and modified starch treated mice.

Preformulation study

Drug-excipient compatibility study by DSC: DSC thermograms of drug and drug-excipient mixtures with their corresponding peak temperatures and enthapy values (ÄH) of Lamivudine (LAM) with various excipient mixtures are summarized in Table-2. DSC thermogram of LAM showed a sharp endothermic peak at 182.73°C corresponding to its melting point. The endothermic peak of the drug was well retained in majority of cases. However, in some combinations there were slight change in peak temperature and peak shape, which might be due to reduction of the purity level of component in mixtures on mixing of excipients with the drug.

FT-IR analysis of drug-excipients mixture: Pure lamivudine showed the characteristic band peaks at 1651.12 cm^{-1}, which corresponds to cystedine nucleus. A characteristic bands peak at 3407.58 cm^{-1} and 3198.77 cm^{-1} represents the amino and hydroxy group present in lamivudine, respectively. Peaks present at 1287.37 cm^{-1} and 1160.32 cm^{-1}

represents the asymmetrical and symmetrical stretching of C-O-C group present in oxathiolane ring of lamivudine, respectively. All the binary mixture of drug and excipient showed none type of physical interaction except with magnesium stearate (Figure 1). In FT-IR spectral diagram of drug-magnesium stearate there is introduction of absorption bands at 2955.18 cm^{-1} and 2850.32 cm^{-1}, which might be a type of physical interaction, but in thermal analysis (DSC and IST) there was no confirmation for the same.

Isothermal stress testing study

In the isothermal stress testing, drug-excipient binary mixtures showed no change in physical appearance at ambient temperature. The blends remain physically stable and no discoloration, liquefaction or gas formation was observed during storage. There was no significant drug degradation observed in the excipients. Table-3 showed percent drug remaining at the end of the study at 50°C.

Formulation of tablets

The Lamivudine (LAM) controlled release tablets were formulated using direct compression technique. Spray dried lactose was selected as direct compression diluents by considering its advantages in terms of good compressibility, easy availability, cost effectiveness and low moisture sensitivity. PVP K-30 was used as dry binder considering its widespread applicability in industry and relatively low moisture sensitivity. Magnesium stearate and talc was used as lubricant and glidant, respectively, due to their widespread applicability in industry and relatively low moisture sensitivity. Highly substituted cross-linked moth bean starch (CLMBS) was used as hydrophilic polymeric carrier material for the production of oral controlled release tablet. The batches of tablets from F1 to F6 were prepared to select the suitable grades of CLMBS. The tablets were formulated to attain 6 kg/cm^2 hardness with varying concentration of CLMBS (50-300 mg/ tablet).The tablets were formulated without any processing difficulties (stickiness, lamination, capping and picking).

Table-2: Corresponding peak temperatures and enthalpy values of lamivudine in various Drug-excipient mixtures in DSC study

Sample	Ratio (Drug: excipient)	T_{onset}(°C)	T_{peak}(°C)	$\Delta H(Jg^{-1})$
LAM	----------	177.40	182.73	74.54
LAM+ CLMBS	(1:1)	174.14	179.39	50.56
LAM+ PVP K-30	(1:1)	174.32	180.09	72.43
LAM+ SDL	(1:1)	171.56	179.44	48.78
LAM+ Mag. Stearate	(1:1)	177.63	182.91	80.66
LAM+ Talc	(1:1)	178.64	183.32	98.38

Table-3: Results of UV Analysis of the drug-excipient mixtures, under Isothermal stress testing after 4 weeks of storage

Sample	Ratio (Drug: excipient)	% Drug remaining[a]		Change in physical appearance
		Control[b] sample	Stressed[c] sample	
LAM	--------	101.12±3.2	99.97.±3.1	No
LAM+ CLMBS	1:2	101.12±1.3	99.10±2.2	No
LAM+ SDL	1:2	102.51±2.1	102.34±0.7	No
LAM+PVP	1:1	103.67±2.2	101.87±1.1	No
LAM+ Mag. Stearate	1:1	101.45±1.5	100.12±2.2	No
LAM+ Talc	1:1	101.22±4.1	100.02±1.1	No

[a]Values expressed as average ± standard deviation (n=3).

[b]Drug excipient blends without added water and stored in refrigerator.

[c] Drug excipient blends with 10% added water and stored at 50°C for 4 weeks.

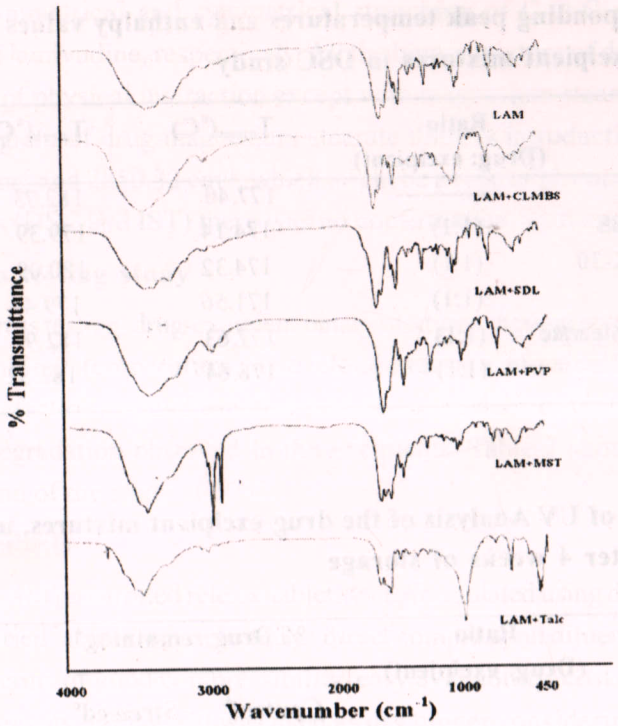


Figure 1: FT-IR spectrum of drug-excipient binary mixtures

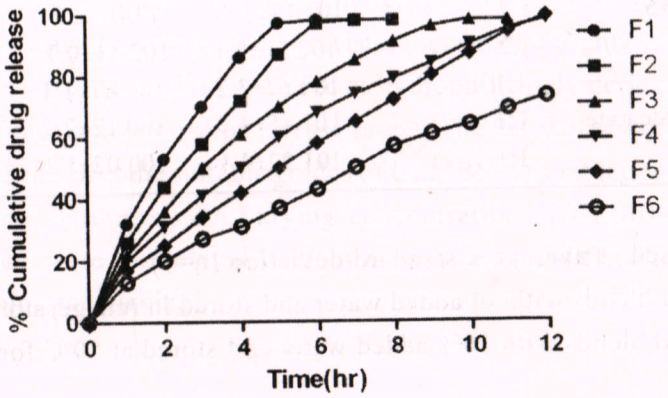


Figure 2: *In vitro* dissolution rate profile of all the batches of controlled release tablets

Evaluation of physical parameters of tablets

General appearance and thickness: The tablets of all the batches were white in color, flat, round shaped and plane from both sides. The thickness (4.91±2.2 mm to 4.96±3.5 mm) of all the batches of tablets are shown in Table 4.

Weight variation: The average weight of 20 tablets alongwith standard deviation value of entire formulations has been presented in Table-4. The percentage of weight variation of individual tablet from the average weight was within ±5% w/w, which indicate that the entire tablets have passed the USP weight variation test.

Drug content: The drug content of the tablets in each batch was found to be in the range of (100.98 ± 2.4% to 103.6 ± 1.2%) and shown in Table-4. The results indicate that tablets of entire batches have passed the USP criteria for the drug content of tablets.

Hardness: The hardness of tablets of entire batches was found to be 6.2 ± 2.0 kg/ cm^2 to 6.4 ± 2.1 kg/cm^2 and the results are depicted in Table-4.

Friability: The results of the friability test of entire batches are depicted in Table-4. It is observed that the tablets of entire batches have passed USP criteria of friability testing (<1.00 %w/w). The results revealed that tablets have possesed good mechanical strength.

***In vitro* drug release study**

The comparative *in vitro* drug release results of all the batches of tablets are shown in Figure 2. From the drug release profile of the batches F1 to F4, it is seen that the total amount of drug was released within 6 h. Release profile of batch F5 showed a superior fit to the required drug release profile among all the batches. The F6 batch (with highest concentration of CLMBS) also showed a controlled release pattern but significantly a less amount of drug was released so it was not considered for further study.

Table-4: Physical properties of lamivudine controlled release tablets using cross-linked moth bean starch as release retardant

Batches	Drug content (%)	Weight deviation (%)	Hardness (kg/cm^2)	Friability (%)	Thickness (mm)
F1	101.2±3.1	507±3.4	6.3±1.4	0.43±2.3	4.96±3.5
F2	102.98±3.1	505±2.2	6.4±1.7	0.41±2.7	4.95±3.2
F3	101.56±2.2	507±0.5	6.4±2.1	0.36±1.1	4.93±1.4
F4	102.12±1.7	503±2.5	6.2±2.0	0.31±2.7	4.94±3.1
F5	103.6±1.2	499±2.6	6.2±2.3	0.25±2.5	4.91±2.2
F6	100.98±2.4	497±2.7	6.3±3.1	0.21±2.4	4.93±1.1

All values represent mean±standard deviation (n=3).

Table-5: Comparative release kinetics parameter of all the batches of controlled release tablets

Formulation Code	Release Kinetic Parameters							
	Zero order		First order		Higuchi		Korsemeyer and Peppas	
	R_O	K_O	R_F	K_F	R_H	K_H	R_p	n_p
F1	0.8861	13.85	0.9467	-0.2794	0.9754	40.94	0.9665	0.604
F2	0.923	14.38	0.9351	-0.2425	0.9746	38.78	0.9816	0.6719
F3	0.9199	8.65	0.9532	-0.1629	0.985	32.91	0.9829	0.653
F4	0.9503	8.12	0.9345	-0.1482	0.9802	31.86	0.9935	0.678
F5	0.999	7.87	0.8477	-0.177	0.9658	30.07	0.9962	0.731
F6	0.9841	5.82	0.9922	-0.046	0.9693	22.35	0.9956	0.712

The result of drug release profile of the batch F5 showed that 25% w/w of drug was released during the first 2 h, while 58% w/w of drug was released within 6 h and remaining 42% w/w of the drug was released in the next 6 h. Hence, a controlled release pattern of drug was observed from the batch F5 throughout the 12 h of dissolution study.

Kinetics and mechanism of drug release

The release rate constant was calculated from the the slope of the appropriate equations and the correlation coefficient (R) was determined for all the batches (Table-5). The *in vitro* drug release of optimized batch (F5) was best fitted and explained by zero order kinetics with highest linearity (R_O= 0.999), followed by higuchi model (R_H=0.9658) and finally first order kinetics (R_F=0.8477). All other batches showed neither good correlation value (R) nor acceptable K value.This explains that the batch F5 was found to be best fitted in zero order kinetics as compared to other kinetic study. The result signifies that the drug release from the optimized controlled release tablets was primarily by diffusion followed by dissolution. The model drug LAM was diffused only through the pores that are formed by dissolution of dispersed drug particles i.e. the drug release mechanism was leaching phenomenon. Interparticulate porosity is essential for solvent penetration in to the monolithic matrix and consequently for drug release. The drug release mechanism from controlled release devices is very complex and still not yet completely understood. Although some controlled release processes may be classified as either purely diffusion or purely erosion controlled and many others can only be interpreted as being governed by both the mechanism. To evaluate the *in vitro* drug release profile the data at various time points were fitted into the Korsemayer-Peppas equation, where K_p is the release rate constant and n_p is characteristics for the mechanism of the drug release. With an n_p value of 0.5, the equation became equal to the square root model described by higuchi, which signifies that drug release from the matrix is governed by fickian diffusion, for $n_p > 0.5$, anomalous non-fickian drug diffusion occurs which is the combination of both diffusion or swelling and erosion mechanism. For $n_p > 1$, non-fickian case-II, erosion controlled or zero order release kinetics is followed. The Rp and n_p values of various batches of tablets are depicted in Table- 4. The Rp values of 0.9665, 0.9816, 0.9829, 0.9935,

0.9962, 0.9956 for the tablets F1, F2, F3, F4, F5 and F6, respectively, showed good linearity between log (cumulative amount of drug release) versus log (time) and highest linearity was observed with F5, where the concentration of CLMBS as release retardant polymer was quite high in concentration. The values of n_p was 0.604, 0.671, 0.653, 0.678, 0.731 and 0.712 for the tablets F1, F2, F3, F4, F5 and F6, respectively. From the obtained n_p values of all the batches of tablets ($n_p > 0.5$) it is revealed that mechanism of drug release was a coupling of both the process of diffusion and erosion.

***In vivo* pharmacokinetics**

The results of the plasma drug concentration at different time intervals, after administration of controlled release tablets containing 100 mg of lamivudine to three rabbits, are presented in Table-6. The drug was remained in the body of the animals up to 24 h of administration of the tablet. The data of plasma drug concentration at various time intervals was analysed by one way Analysis of Variance (ANOVA). A value of $p < 0.05$ was considered statistically significant. The pharmacokinetic parameters were derived from plasma drug concentrations versus time profile of all the subjects and the results are shown in Table-6. The T_{max} of controlled release tablets was found to be 4 h, as compared to 1 h for the marketed conventional tablets (Lamivir®), which indicate the slow absorption rate from the controlled release tablets due to extended release effect of hydrophilic polymer present in controlled release tablets. The average C_{max} value of the controlled release tablets was decreased as compared to conventional tablets (from 12.78±2.1 µg/mL to 7.58 ±3.5 µg/mL). The $AUC_{0-\infty}$ of controlled release tablets exhibited high value (58.35±2.9 µg.hr/mL) as compared to conventional tablets (54.21±3.7 µg.hr/mL). The mean $AUMC_{0-\infty}$ of CR tablets was found to be 372.24 µg.hr/mL±3.1, which is higher than the conventional tablet (227.06±0.6 µg.hr/mL). The plasma half-life has been increased from 3.594±0.2 h (Lamivir®) to 9.96±1.7 h (F5), which confirms longer duration of action of the drug in systemic circulation from controlled release tablets F5. The mean Vd and clearance values for CR tablets were 24.56 ± 3.3 L and 1.718 ± 0.9 L/h, respectively. The Ka and Ke value for controlled release formulation was found to be 0.466 ± 2.1 h^{-1} and 0.070 ± 1.1 h^{-1}, respectively, which are lower than Lamivir® (2.142 ± 1.8 h^{-1} and 0.193 ± 1.5 h^{-1}, respectively). The mean residence time (MRT) of controlled release

tablets was found to be higher (5.170 ± 2.5 h) than that of conventional tablets (4.189 ± 2.1 h) confirming the controlled release property of the CLMBS. The results revealed that the drug was made available to the body in a controlled release manner and the controlled release effect was due to the presence of higher proportion of CLMBS in the tablets. The comparative pharmacokinetic study (Figure 3) exhibit controlled release behavior of the optimized formulation.

Table-6 : Pharmacokinetic parameters of marketed lamivudine tablet (Lamivir®) and optimized lamivudine controlled release tablets (F5) after a single oral dose to rabbits (n=3)

Pharmacokinetic parameters	Observed value (Lamivir®)	Observed value (F5)
Maximum plasma concentration, C_{max} (µg/mL)	12.79±2.1	7.58±3.5
Time required to reach maximum plasma concentration, T_{max} (h)	1.00±1.6	4.00±2.2
Area under curve at 24 h, AUC ($_0 \longrightarrow \alpha$) (µg.h/mL)	54.21±3.7	58.35±2.9
Area under momentum curve At 24 h, AUMC ($_0 \longrightarrow \alpha$) (µg.$h^2$/mL)	227.06±0.6	372.24±3.1
Volume of distribution, V_d (L)	9.574±2.8	24.567±3.3
Plasma half life ($T_{1/2}$) (h)	3.594±0.2	9.960±1.7
Absorption rate constant, Ka (h^{-1})	2.142±1.8	0.466±2.1
Elimination rate constant, Ke (h^{-1})	0.193±1.5	0.070±1.1
Mean residence time, MRT (h)	4.189±2.1	6.379±1.7
Clearance, Cl (L/h)	1.846±3.0	1.718±0.9

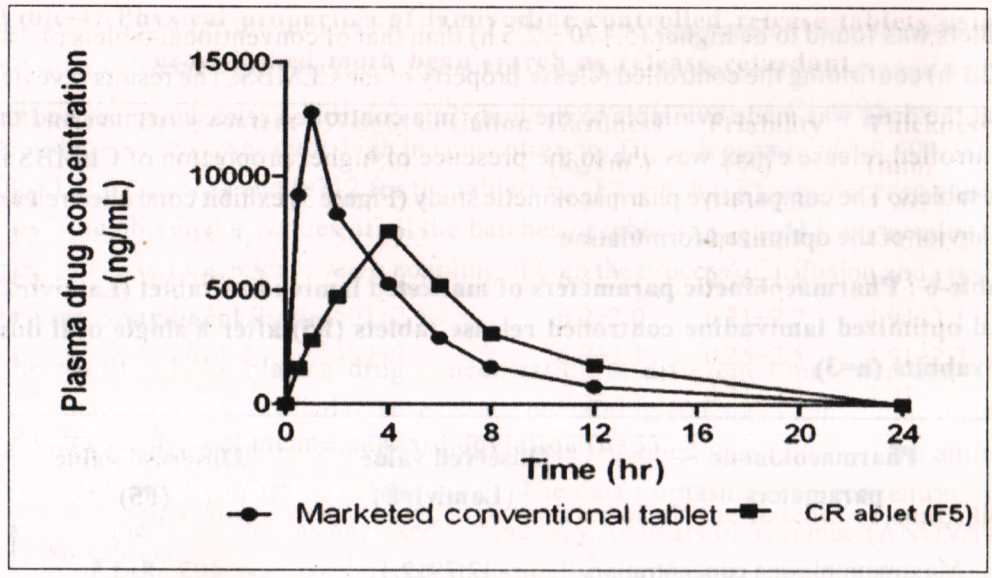


Figure 3: Comparative *In vivo* pharmacokinetic study of marketed conventional tablet (Lamivir®) and optimized formulation (F5)

Stability study

The selected optimized formulation (F5) was evaluated for various parameters (drug content, dissolution study) after 3 and 6 month of storage at accelerated stability conditions (40 ± 2°C and 75% ± 5% RH). There was no significant amount of change observed in the drug content of tablets after 6 month of storage at accelerated stability conditions. The dissolution profile of formulation at initial stage was considered as reference for dissolution study. The results obtained revealed that the dissolution profile of the formulation after 6 month of storage at accelerated condition was found to be similar to that of the reference one. Based on the results it is opined that the tablets of batch F5 are stable after 6 month of storage at accelearate stability conditions.

CONCLUSION

The present study focussed on the formulation of 12 h controlled release tablets of lamivudine using CLMBS as matrix forming hydrophilic polymer. It is concluded from the dissolution study of the entire batches that the tablets contain less concentration

of CLMBS were disintegrated within 1 h and unable to control the release of drug. At higher concentration the tablets released the drug in nearly controlled manner over 12 h study. The study of release mechanism exhibited anomalous non-fickian diffusion mechanism, which involved both diffusion and erosion mechanism. The pharmacokinetic study of the optimized batch (F5) in rabbits was carried out in replicate (n=3). The plasma drug concentration versus time interval was estimated using in house developed RP-HPLC method. The optimized batch (F5) exhibited first order rate kinetics in absorption of drug from the tablets. The increase in Tmax value and decrease in C_{max} value in case of the optimized formulation F5 than that of the conventional tablet of lamivudine (Lamivir®) revealed that the cross-linked moth bean starch in the CR tablets rendered the drug to be released in a controlled manner.

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