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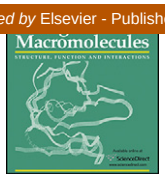

Evaluation of acetylated moth bean starch as a carrier for controlled drug delivery

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ABSTRACT

The present investigation concerns with the development of controlled release tablets of lamivudine using acetylated moth bean starch. The acetylated starch was synthesized with acetic anhydride in pyridine medium. The acetylated moth bean starch was tested for acute toxicity and drug–excipient compatibility study. The formulations 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 Higuchi 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®, which proved controlled release potential of acetylated moth bean starch.

1. Introduction

In recent years, a new generation of biomaterials has been introduced as tablet excipient for purpose of controlled drug release. Different modification techniques, such as chemical reaction, complexation reaction and grafting have been applied to alter native biomaterials by the process of substitution of functional groups with other compounds. Recent efforts in drug development resulted in a number of controlled drug delivery systems consisting of a drug encapsulated within a suitable polymer carrier or equally dispersed in a polymeric matrix [1]. Reservoir and matrix type tablets are the most commonly used orally administered 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, which are relatively easy to manufacture [2]. Acetylation of starches is an important modification that has been applied to the native starches to impart thickening and known for more than a century. In the acetylation, parts of the hydroxyl groups of the α-D-glucopyranose units have been converted by esterification to acetyl groups. High acetyl substituted starch with a degree of substitution (DS) of 2–3 was of research interest because of their solubility in acetone and chloroform and for their thermo plasticity [3,4]. Lamivudine (LAM) is the first nucleoside analogue approved to treat chronic HBV infection and AIDS. Conventional oral formulations of LAM are administered two times a day 150 mg each time because of its moderate half-life ($t_{1/2}$ = 5–7 h). 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 [5]. The aim of the present study was to evaluate the acetylated moth bean starch as hydrophobic inert matrix former in the formulation of controlled release tablets of lamivudine 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.

2. Materials and methods

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 Netherlands. Polyvinyl 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) and 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).

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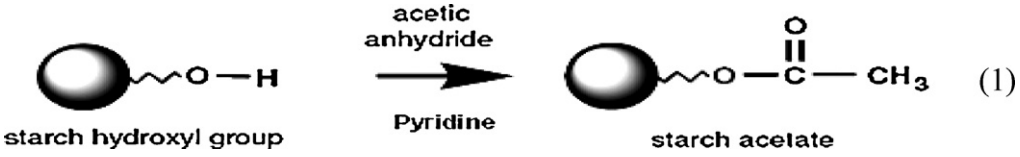
2.1. Synthesis of acetylated moth bean starch

Native moth bean starch was isolated from the wet grinding method. The dried starch flakes were crushed, grounded and finally passed through a sieve #120 (0.125 mm). The uniform moth bean starch was pregelatinized by heating it with water at 70 °C. The pregelatinized starch was dried and sieved through mesh #60 (0.251 mm). The acetylation of 25 g pregelatinized starch was done with 100 g acetic anhydride in a medium of 200 g pyridine [6,7]. The reaction was carried out at varying temperature of 50–150 °C and time 1–6 h. The final product was precipitated with ethanol filtered and dried in vacuum oven and passed through a sieve #60 and stored till further study.



(1)

2.2. Determination of degree of substitution (DS)

DS was determined using the method described elsewhere [8]. Accurately weighted 1.0 g of grounded sample was added to the aqueous solution of ethanol (75%). The slurry was kept in the water bath for 30 min, after the slurry was cooled down an exact amount of aqueous solution of potassium hydroxide (0.5 M, 30 mL) was added and solution was stirred for 72 h. Indicator (phenolphthalein) was added in excess to the alkali and was titrated with 0.5 M hydrochloric acid.

Acetyl content (*A*%) was calculated according to the following equation:

$$\text{Acetyl group (\%)} = \frac{(\text{Value for blank} - \text{Value for sample})\ (\text{mL})}{\text{Sampling weight (g)}} \times \text{molarity of HCl} \times 0.043 \times 100 \quad (2)$$

Acetyl content was used to calculate the degree of substitution, (DS), according to following equation:

$$\text{DS} = \frac{162 \times \%\ \text{Acetyl group}}{4300 - (42 \times \%\ \text{Acetyl group})} \quad (3)$$

where 162 is the molecular weight of the anhydroglucose unit, 42 is the molecular weight of replaceable acetyl group and 4300 is the molecular weight of the acetyl group attached with 100 anhydroglucose unit.

2.3. Acute toxicity study of acetylated moth bean starch

Healthy male and female Swiss albino mice (8 weeks) used for the acute oral toxicity study were breed and reared at the Animal House of Gayatri College of Pharmacy, Orissa. 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 the sexes were fasted overnight before experimentation and were allowed to take food one hour after the experiment. Modified starch was administered orally at a dose of 2000 mg/kg b.w. 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).

2.4. Preformulation study

2.4.1. 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 by using physical mixture technique. The drug–excipient mixture was scanned in the temperature range of 50–220 °C under an atmosphere of nitrogen. The heating rate was 20 °C/min and the obtained thermograms were observed for any type of interaction.

2.4.2. 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 4000–450 cm^{-1}. FT-IR analysis has been performed using sample of lamivudine with various excipients and acetylated moth bean starch at 1:1 mass/mass ratio.

2.4.3. Isothermal stress testing (IST) analysis

In isothermal stress testing [9,10] samples of drug and different excipients 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 hot air oven. Drug–excipient blends without adding water were stored in refrigerator served as controls. The drug–excipient blends were periodically examined for any change in physical appearance. Samples were quantitively analysed using UV-Vis spectrophotometer (Pharmaspec 1700, Shimadzu, Japan) after 4 weeks of storage at above conditions.

2.5. Formulation of tablets

The lamivudine controlled release tablets were formulated by incorporating selected excipients using model drug lamivudine. Lamivudine, acetylated moth bean starch (AMBS), 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 slugs prepared were crushed into granules and the mass was shifted through #20 sieves and weighed. The quantity of remaining ingredients *i.e.* magnesium stearate and talc required for tablets was calculated as per the formula to compensate any loss during the direct compression process. The lubricated dry powder was compressed into tablets at 8 kg/cm^2 pressure by 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.

2.6. Evaluation of tablets

The tablets from each batch were picked randomly in order to evaluate the weight variation, the hardness, % of drug content and the friability [11]. The hardness and friability of the tablet were

measured using Monsanto hardness tester and Roche friabilator respectively.

2.7. 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 (0.1 M HCl) and in simulated intestinal fluid (PBS pH 6.8) for first two hours and for successive 10 h respectively. The each dissolution medium (900 mL) was maintained at $37 \pm 0.5\,^{\circ}C$ 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–Vis spectrophotometer at 270 nm (λ_{max}). The cumulative percent drug release was plotted against time to determine the release profile.

2.8. Kinetics of drug release

The kinetics of drug release [12–15] is important because it is a 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 equations were applied for describing the kinetics of the drug release process from controlled released tablets of AMBS, the most suited being the one which fitted best in the experimental results.

2.9. Pharmacokinetic study

The pharmacokinetic study of optimized controlled release tablet (F7) of lamivudine was carried out in two groups of three male white albino rabbits each weighing 1.5–2.5 kg. All animals were fasted overnight (12 h) before dosing and continued till 4.0 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, F7 were administered orally to three rabbits of each group along with 10 mL of water by using feeding tube. This study was approved by the Animal Ethical Committee of Gayatri College of Pharmacy (Regn. No.1339/ac/10/cpcsea).

The blood samples each of about 50 μL from each animal 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 $1000 \times g$ for 10 min. The supernatent plasma layer was separated and stored at $-20\,^{\circ}C$ till analysis.

2.9.1. 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 s. Acetonitrile (1.5 mL) was added into the mixture, vortexed for 3 min and centrifuged at 10,000 rpm for 10 min. The supernatent was transferred into a glass centrifuge tube and evaporated to dryness at $45\,^{\circ}C$ under a stream of nitrogen. The residue was reconstituted with 200 μL of reconstitution solvent (mobile phase). 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 developed in the laboratory [16]. The area under the curve of peaks of LAM and IS was determined and the concentration of drug present in sample was estimated by using the linear regression 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 [16–18].

2.9.2. Determination of pharmacokinetic parameters

The pharmacokinetic parameters were determined from the data of plasma drug concentration at different time points by using MS-Excel 2003 Software according to the procedure described elsewhere [16–18].

2.10. 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 guideline. The optimized controlled release tablets were kept in polypropylene bottle and stored in a stability chambers maintained at $40 \pm 2\,^{\circ}C$ and $75 \pm 5\%$ RH for six months. Samples were checked initially, after three months and further after six months.

3. Results and discussion

3.1. Synthesis of highly substituted acetylated moth bean starch

The starch acetate with high degree of substitution (DS = 2.35) was synthesized using pyridine as organic solvent. The optimized temperature for synthesizing highly substituted starch was found to be $100\,^{\circ}C$. The reaction duration for synthesizing highest substitution was found to be 4 h, increasing the duration caused the starch granule destruction which restricts acetylation of moth bean starch. The preliminary degree of substitution was done by trimetric analysis and it was found to be 2.35.

3.2. Acute toxicity study

The purpose of this study was to evaluate toxicity profile of the modified starch. A 14-day acute oral toxicity study was performed in swiss albino mice. The LD_{50} of the modified starch (acetylated) was not further studied as it was found to be safe up to 2000 mg/kg on 24 h study basis. It was observed that the animal fed with the modified starch was 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 was found in growth behavior between the control and treatment groups in 14-day study. The body weights 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.

3.3. Preformulation study

3.3.1. Drug–excipient compatibility study by DSC

DSC thermograms of drug and drug–excipient mixtures with their corresponding peak temperatures and enthalpy values (ΔH) of lamivudine (LAM) with various excipient mixtures are summarized in Table 1. DSC thermogram of LAM showed a sharp endothermic peak at $182.73\,^{\circ}C$ 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 changes 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.

Table 1
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)	ΔH (J g^{-1})
LAM	–	177 40	182 73	74 54
LAM + AMBS	1:1	177.58	183.79	101.76
LAM + PVP K-30	1:1	174.32	180.09	72.43
LAM + SDL	1:1	171.56	179.44	48.78
LAM + Magnesium stearate	1:1	177.63	182.91	80.66
LAM + Talc	1:1	178.64	183.32	98.38

3.3.2. 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 and 3198.77 cm^{-1} represents the amino and hydroxy groups present in lamivudine. Peaks present at 1287.37 and 1160.32 cm^{-1} represents the asymmetrical and symmetrical stretching of C—O—C group present in oxathiolane ring of lamivudine. All the binary mixtures of drug and excipient showed none type of physical interaction except with magnesium stearate. In FT-IR spectral diagram of drug-magnesium stearate there is introduction of absorption bands at 2955.18 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.

3.3.3. 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 2 shows percent of drug remaining at the end of the study at 50 °C.

3.4. Formulation of controlled release tablets

The formulae of the lamivudine (LAM) controlled release tablets have been depicted in Table 3. Spray dried lactose was selected as direct compression diluent 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 were used as lubricant and glidant respectively due to their widespread applicability in industry and relative moisture insensitivity. Highly substituted acetylated moth bean starch (AMBS) was used as hydrophobic polymeric carrier material for the production of LAM oral controlled release tablet. The batches (F1–F8) of tablets were prepared to select the suitable grades of AMBS. The tablets were formulated to attain 8 kg/cm^2 hardness with varying concentration of AMBS (25–200 mg/tablet). The tablets were formulated without any processing difficulties (stickiness, lamination, capping and picking).

3.5. Evaluation of physical parameters of tablets

3.5.1. General appearance and thickness

The tablets of all the batches were white in color, flat, round shaped and plane from both sides. The thickness (4.61 ± 1.1 mm to 4.62 ± 1.4 mm) of all the batches of tablets is shown in Table 4.

3.5.2. Weight variation

The average weight of 20 tablets along with standard deviation of entire formulations has been presented in Table 4. The percentage of weight variation of individual tablets from the average weight was found to be within ±5% (w/w) which proved that the entire tablets have passed the USP weight variation test.

3.5.3. Drug content

The drug content of all the tablets in each batch was found to be in the range of 99.98 ± 1.4% to 103 ± 1.6% which is shown in Table 4. The results indicated that tablets of entire batches have passed the USP criteria for the drug content of tablets.

3.5.4. Hardness

The hardness of tablets of entire batches was found to be in the range of 8.1 ± 1.4 kg/cm^2 to 8.3 ± 2.1 kg/cm^2 and the results are depicted in Table 4.

3.5.5. Friability

The results of the friability test of entire formulations are depicted in Table 4. It was observed that the tablets of entire batches had passed USP criteria of friability testing (<1.00%, w/w). The results revealed that tablets possessed good mechanical strength.

3.6. In vitro drug release study

The comparative results (n = 6) of *in vitro* drug release study of all the batches of tablets are shown in Fig. 1. From the drug release profile of the batches from F1 to F4 it is seen that the total amount of drug was released within 6 h, while from the batches F5 and F6, the total amount of drug was released within 8 h. Release profile of F7 showed a superior fit to the desired controlled drug release profile among all the batches. The tablets of batch F8 (with highest concentration of AMBS) also showed a controlled release pattern but significantly a less amount of drug was released from them so, batch F8 was not considered for further study. The result of drug release profile of the batch F7 showed the release of 28% (w/w) of drug during initial 2 h, while within the first 6 h 68% of drug was released and the remaining 32% (w/w) of the drug was released within the remaining 6 h. Hence, a controlled release pattern of drug was observed from the batch F7 throughout the 12 h of dissolution study.

3.7. Kinetics and mechanism of drug release

The release rate constant was calculated from the slope of the appropriate equations and the correlation coefficient (R) was

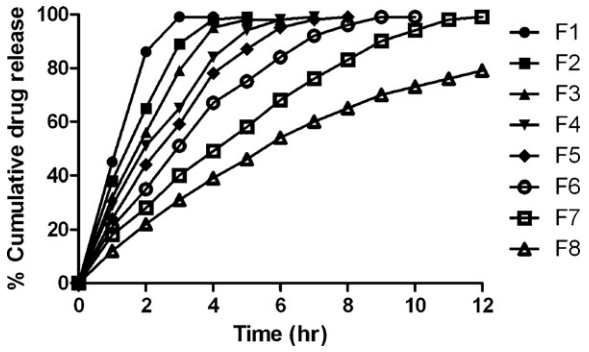


Fig. 1. *In vitro* dissolution rate profile of all the batches of tablets.

Table 2
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 + AMBS	1:2	103.36 ± 2.5	101.12 ± 1.6	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 + Magnesium Steanate	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.

Table 3
Composition of lamivudine controlled release tablet using AMBS.

Ingredients (mg)	Formulation code							
	F1	F2	F3	F4	F5	F6	F7	F8
Lamivudine	100	100	100	100	100	100	100	100
Acetylated moth bean starch	25	50	75	100	125	150	175	200
PVP K-30	20	20	20	20	20	20	20	20
Spray dried lactose	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Magnesium stearate	4	4	4	4	4	4	4	4
Talc	4	4	4	4	4	4	4	4
Total weight	400	400	400	400	400	400	400	400

q.s., quantity sufficient.

Table 4
Physical properties of lamivudine CR tablets using AMBS as release retardant.

Batches	Drug content (%)	Weight deviation (%)	Hardness (kg/cm^2)	Friability (%)	Thickness (mm)
F1	101.2 ± 2.1	402 ± 1.4	8.1 ± 1.4	0.65 ± 1.3	4.66 ± 0.5
F2	99.98 ± 1.4	398 ± 0.8	8.3 ± 1.7	0.45 ± 1.7	4.65 ± 1.2
F3	100.56 ± 1.3	403 ± 1.2	8.2 ± 2.1	0.43 ± 1.1	4.67 ± 1.4
F4	102.12 ± 0.9	405 ± 1.7	8.3 ± 2.0	0.34 ± 1.7	4.66 ± 2.1
F5	101.6 ± 1.2	401 ± 1.4	8.2 ± 1.3	0.36 ± 1.5	4.63 ± 2.2
F6	99.98 ± 1.4	397 ± 1.1	8.2 ± 1.1	0.29 ± 1.4	4.61 ± 1.1
F7	103.1 ± 1.6	409 ± 2.1	8.3 ± 2.1	0.27 ± 1.2	4.62 ± 1.8
F8	101.2 ± 1.5	401 ± 1.5	8.2 ± 1.9	0.26 ± 0.21	4.64 ± 1.4

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

determined for all formulations (Table 5). The *in vitro* drug release of optimized batch (F7) was best fitted and explained by Higuchi kinetics with the highest linearity ($R_H = 0.981$), followed by zero order ($R_o = 0.9619$) and first order ($R_F = 0.896$). This explains that batch F7 was found to be best fitted in Higuchi kinetics as compared to other batches. All other batches showed neither good (high) correlation value (R) nor acceptable K value (low). This explains that the drug release from monolithic matrix tablet prepared with AMBS with highest DS value followed primarily diffusion controlled mechanism. The model drug LAM was assumed to be diffused only through the pores that were formed by dissolution of dispersed drug particles *i.e.* the drug release mechanism was leaching of dissolved drugs through the pores. Inter-particulate porosity is essential for solvent penetration into the monolithic matrix and consequently for drug release. The drug release mechanism from controlled release devices is very complex to explain and still not yet completely understood. Although some controlled release processes may be classified as either purely diffusional 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 becomes equal to the square root of

Table 5
Comparative release kinetics parameter of all batches of tablets.

Formulation code	Release kinetic parameters							
	Zero order		First order		Higuchi		Korsemeyer & Peppas	
	R_O	K_O	R_F	K_F	R_H	K_H	R_p	n_p
F1	0.8611	25.2	0.9238	−0.57	0.9616	53.86	0.8896	0.591
F2	0.8978	19.9	0.9601	−0.42	0.9764	8.03	0.9566	0.624
F3	0.8909	16.6	0.9531	−0.32	0.9674	4.47	0.9536	0.657
F4	0.9136	14.11	0.9549	−0.29	0.9784	1.14	0.9748	0.653
F5	0.9159	12.46	0.9561	−0.25	0.9723	9.16	0.972	0.702
F6	0.9197	9.9	0.9115	−0.20	0.9753	5.43	0.9771	0.701
F7	0.9619	8.08	0.896	−0.15	0.9813	1.98	0.995	0.719
F8	0.9589	6.42	0.9991	−0.05	0.9782	5.29	0.992	0.762

Table 6
Pharmacokinetic parameters of marketed and optimized lamivudine controlled release tablet (F7) after a single oral dose of 100 mg lamivudine to rabbits ($n = 3$).

Pharmacokinetic parameters	Observed value (Lamivir®)	Observed value (F7)
Maximum plasma concentration, C_{max} (ng/mL)	12,784.91 ± 2.1	8342.56 ± 3.4
Time required to reach maximum plasma concentration, T_{max} (h)	1.00 ± 1.6	4.00 ± 2.2
Area under curve at 24 h, $AUC_{(0\rightarrow\infty)}$ (ng h/mL)	54,206.284 ± 3.7	57,075.323 ± 4.5
Area under momentum curve at 24 h, $AUMC_{(0\rightarrow\infty)}$ (ng h^2/mL)	227,067.968 ± 0.6	306,847.633 ± 1.2
Volume of distribution, V_d (L)	9.574 ± 2.8	16.910 ± 3.1
Plasma half life, $T_{1/2}$ (h)	3.594 ± 0.2	6.690 ± 4.1
Absorption rate constant, K_a (h^{-1})	2.142 ± 1.8	0.796 ± 1.0
Elimination rate constant, K_e (h^{-1})	0.193 ± 1.5	0.105 ± 2.4
Mean residence time, MRT (h)	4.189 ± 2.1	5.376 ± 2.1
Clearance, Cl (L/h)	1.846 ± 3.0	1.753 ± 1.7

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 *i.e.* combination of both diffusion or swelling and erosion mechanism takes place. For $n_p > 1$, non-Fickian case-II, erosion controlled or zero order release kinetics is followed. The R_p and n_p values of various batches of tablets are presented in Table 5. The R_p values of 0.8896, 0.9566, 0.9536, 0.9748, 0.972, 0.9771, 0.995 and 0.992 for the tablets F1, F2, F3, F4, F5, F6, F7 and F8 respectively indicated good linearity between log cumulative amount of drug release and log time. The R_p value of all the tablets was found to be linear and highest linearity was observed with the batch F7, where the concentration of AMBS as release retardant polymer was quite high. The value of n_p was 0.591, 0.624, 0.657, 0.653, 0.702, 0.701, 0.719 and 0.762 for the batches of tablets F1, F2, F3, F4, F5, F6, F7 and F8 respectively. Hence, the mechanism of drug release from the tablets was predicted from Korsemayer–Peppas equations and from the obtained n_p values of all the batches of tablets ($n_p > 0.5$) it is revealed that the mechanism of drug release was a coupling of both the process of diffusion and erosion.

3.8. 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 comparison of the different parameters was done by using a 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 1.0 h for the marketed conventional tablet (Lamivir®), which indicates the slow absorption rate from the CR tablet due to extended release effect of hydrophobic polymer present in controlled release tablets. The average C_{max} value of the controlled release tablets was decreased as compared to conventional tablets (from 12,784.91 ± 2.1 ng/mL to 8342.56 ± 3.4 ng/mL). The $AUC_{0-\infty}$ of controlled release tablets of lamivudine exhibited high value (57,075.323 ± 4.5 ng h/mL) as compared to conventional tablets (54,206.284 ± 3.7 ng h/mL). The $AUMC_{0-\infty}$ of controlled release tablets was found to be higher 306847.63 ± 1.2 ng h^2/mL as compared to the low value of conventional tablets (227067.96 ± 0.6 ng h^2/mL). The mean Vd and clearance values for controlled release tablets were found to be 16.91 ± 3.1 and 1.753 ± 1.7 L/h respectively. The K_a and K_e value for controlled release tablets was found to be 0.796 ± 1.0 and 0.105 ± 2.4 h^{-1} which is lower than the values of the conventional tablets. The mean residence time (MRT) of controlled release tablets was found to be higher (5.376 ± 2.1 h) than that of the conventional tablets (4.189 ± 2.1 h) confirming the controlled release property of the modified starch AMBS. The results revealed that the drug

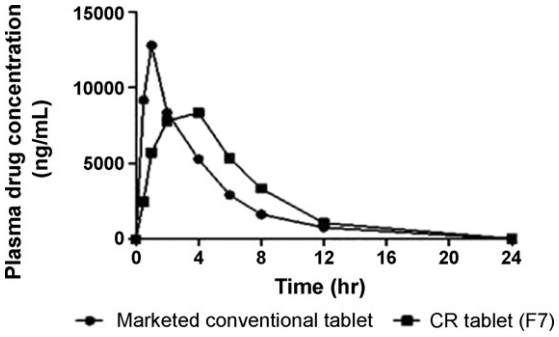


Fig. 2. Comparative *in vivo* pharmacokinetic study of marketed conventional tablet of Lamivir® and optimized formulation (F7).

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 AMBS in the tablets as evident from Fig. 2.

3.9. Stability study

The selected optimized formulation (F7) was evaluated for various parameters (drug content, dissolution study) after 3 and 6 months 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 months of storage at accelerated stability conditions. The dissolution profile of formulation at initial stage was considered as the reference for dissolution study. The results obtained revealed that the dissolution profile of the formulation after 6 months of storage at accelerated condition was found to be similar to that of reference one. Based on the results it was opinioned that the tablets of batch F7 was stable after 6 months of storage at accelerated stability conditions.

4. Conclusion

The present study focused on the formulation of 12 h controlled release tablets of lamivudine using AMBS as matrix forming hydrophobic polymer. It was summarized from the dissolution study of the entire batches that the tablets contain less concentration of AMBS were disintegrated with in 1.0 h and unable to control the release of drug. At higher concentration of AMBS the tablets released the drug in controlled release manner over 12 h study. The study of release mechanism exhibited anomalous non-Fickian diffusion which involved combination of both diffusion and erosion mechanism. The pharmacokinetic study of the optimized batch of formulation (F7) in rabbits was carried out in replicate. The plasma drug concentration *versus* time interval was estimated by using in house developed RP-HPLC method. The optimized controlled release tablets exhibited first order rate kinetics in release of drug from the tablets. The increase in T_{max} value and decrease in C_{max} value in case of the optimized formulation F7 from the values

of the conventional tablet of lamivudine (Lamivir®) revealed that the AMBS in the CR tablets rendered the drug to be released in a controlled manner.

Declaration

The authors declared no conflict of interest.

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