J Therm Anal Calorim (2012) 108:307–313
DOI 10.1007/s10973-011-1752-x

Synthesis, characterization, and compatibility study of acetylated starch with lamivudine

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Received: 24 May 2011 / Accepted: 15 June 2011 / Published online: 29 June 2011

Abstract In this study, highly substituted starch acetate was prepared by reaction with native moth bean starch and acetic anhydride. Physicochemical characterization of this modified starch was done using scanning electron microscopy, X-ray diffraction, and thermogravimetric analysis. Their formation was confirmed by titrimetric analysis and highest degree of substitution was observed with a value of 2.35. The synthesized modified starch was further studied for compatibility with model drug lamivudine using differential scanning calorimetry and isothermal stress testing for its controlled release tablet formulation.

Keywords Moth bean starch · Acetylation · Compatibility study · Lamivudine

Introduction

Native starches are utilized in different forms in various commercial purposes like food, pharmaceutical, textile, paper, and packaging industries. However, starch has disadvantages of being hydrophilic, having poor mechanical properties, and having poor dimensional stability, especially in aqueous environments. 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. Highly acetylated starch with a degree of substitution (DS) of 2–3 was of research interest because of their solubility into acetone and chloroform and for their thermo plasticity [1]. The demand for modified starches in developing countries like China, India is growing annually with a growth rate of 8–9%. So, there is a market demand to explore other tropical crops to fulfill the industrial demands [2]. The moth bean is an edible bean belonging to the Fabaceae family. Moth bean starch is obtained from *Phaseolus aconitifolius* Jacq. and grown well in Southeast Asian countries as a tropical crop [3]. Lamivudine is an analog of cytidine. It inhibits both types (I and II) 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 nucleoside analog prevents the formation of the 5′ to 3′ phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated [4].

This study reports synthesis and characterization of highly substituted moth bean starch acetate. This study also elucidates suitability of acetylated moth bean starch and common pharmaceutical excipients in lamivudine based tablets.

Materials and methods

Moth bean seeds were purchased from Indian Institute of Pulse Research, Kanpur India. Acetic anhydride and pyridine extra pure were purchased from Qualigens, India. Lamivudine was kindly donated by Ranbaxy Limited, Paonta Sahib, Himachal Pradesh, India. Spray dried lactose (SDL) was kindly gifted from DMV Fonterra excipients, The Netherland. Magnesium stearate (MST), talc, and

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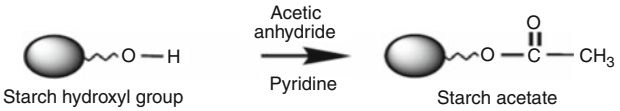
polyvinyl pyrrolidine K-30 (PVP) was purchased from Loba Chemie, Mumbai, India. All other chemicals used were of AR grade. Double distilled water is used in the study.

Synthesis of acetylated moth bean starch

Native moth bean starch (MBS) was firstly grinded and passed through a sieve (120 meshes). The uniform native MBS was pregelatinized by heating it with water at 70 °C. The pregelatinized starch was dried and sieved through mesh 60. The acetylation of 25 g pregelatinized starch was done with 100 g acetic anhydride in a medium of 200 g pyridine. The reaction was carried out at varying temperature (100 °C) and time (4 h). The product was precipitated with ethanol filtered, dried in vacuum oven and finally passed through a sieve (60 meshes) and stored till further study.



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Characterization of acetylated starch

Determination of degree of substitution (DS)

DS was determined using the method described elsewhere [5] 1.0 g of grounded sample accurately weighted was added to the aqueous solution of ethanol (75%). 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 N, 30 mL) was added and solution was stirred for 72 h. After this indicator (phenolphthalein) was added and the excess of alkali was titrated with 0.5 N hydrochloric acid.

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

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

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

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

Scanning electron microscopy of starches

The morphological features of the MBS and highly substituted starch acetate were observed with scanning electron microscopy (JEOL JSM-6100, JEOL Ltd., Tokyo, Japan). The dried samples were coated with gold to make the sample conductive. Micrograph's were recorded at different magnification.

X-ray diffraction (XRD) analysis of starches

X-Ray diffraction patterns of the MBS and starch acetate were analyzed using X-ray powder diffractometer (JADE, USA) at a voltage of 40 kV and a current of 200 mA. The scattered ration was detected in the angular range of 5–60° (2θ), with a scanning speed of 8° (2θ)/min and a step of 0.06° (2θ).

Thermogravimetric (TG) study of starches

Thermogravimetric analysis of native and highly substituted acetylated starch (AMBS) was performed using thermal analyzer (Model TGA-400, Perkin Elmer, USA). Samples of approximately 5 mg were heated in an aluminums cell from 30 to 500 °C temperature at a heating rate of 10 °C/min.

Drug-excipient compatibility study

Compatibility study by differential scanning calorimetry

A differential scanning calorimetry (JADE DSC, Perkin Elmer, USA) was used to study the thermal analysis of drug-excipient compatibility. First, binary mixtures of lamivudine and excipients (in 1:1 mass/mass ratio) were physically mixed and finally filled in aluminum pan. The drug-excipient mixtures were scanned in the temperature range of 50–220 °C under an atmosphere of nitrogen. The heating rate was 20 °C/min and the obtained curves were observed for any type of interaction.

Isothermal stress testing [6, 7]

In isothermal stress testing (IST), samples of drug and different excipients (Table 1) were weighed directly in 5 mL glass vials ($n = 3$). Mixing was done on a cyclomixer for 3 min, with 10% (w/w) water in each of the vial. The glass vials, after Teflon sealing, were stored at 50 °C in hot air oven. Drug-excipient blends without added water and stored in refrigerator served as controls. The drug-excipient blends were periodically examined for any change in physical appearance. Samples were quantitatively analyzed using

Table 1 Corresponding peak temperatures and enthalpy values of lamivudine in various Drug-excipient mixtures

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 + Mag. stearate	1:1	177.63	182.91	80.66
LAM + Talc	1:1	178.64	183.32	98.38

Abbreviations LAM Lamivudine, *AMBS* Acetylated moth bean starch, *SDL* Spray dried lactose, *Mag. stearate* Magnesium stearate

UV–visible spectrophotometer (Pharmaspec 1700, Shimadzu, Japan) after 4 weeks of storage at above conditions.

Analysis of samples in isothermal stress testing

The stored samples were quantitatively analyzed using UV–vis spectrophotometer. The drug-excipients samples were diluted in phosphate buffer solution (pH 6.8). The samples were centrifuged, filtered and analysed at 270 nm in UV–visible spectral analysis.

Results and discussions

Synthesis and characterization

The starch acetate with high degree of substitution (DS = 2.35) was synthesized using pyridine as organic solvent. The preliminary degree of substitution was done by titrimetric analysis and it was found to be 2.35. The MBS powder had semi-crystalline structure, as shown in Fig. 1 with a strong diffraction pattern. The degree of crystallinity decreased after achieving higher substitution in the starch granules. Intra and intermolecular hydrogen bonds present in amylopectin are responsible for the highly ordered crystalline structure. On achieving highest esterification, acetyl group replaced some of the hydrogen group on starch backbone, which reduced the formation of intermolecular hydrogen bonding and thereby reducing the orderly crystalline structure. As the degree of substitution of starch acetate is highly substituted the crystalline pattern is changed into amorphous form [8].

Native MBS granules were of oval in shape. The starch granules lost their smooth surface texture after esterification as a result of substitution of hydroxyl groups. After reaching the highest substitution (DS = 2.35), the native starch granules almost broken into small pieces. Once the reaction completed, the starch became fibrous in nature and break into small fragments (Fig. 2).

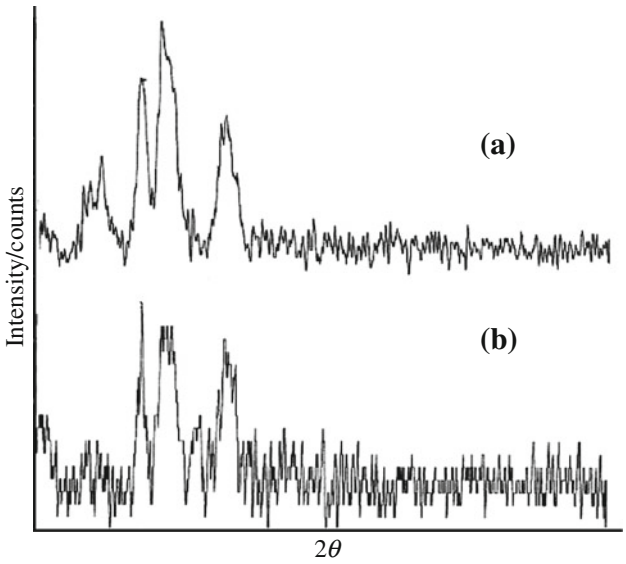

Fig. 1 XRD patterns of Moth bean starch (**a**), and starch acetate with DS 2.35 (**b**)

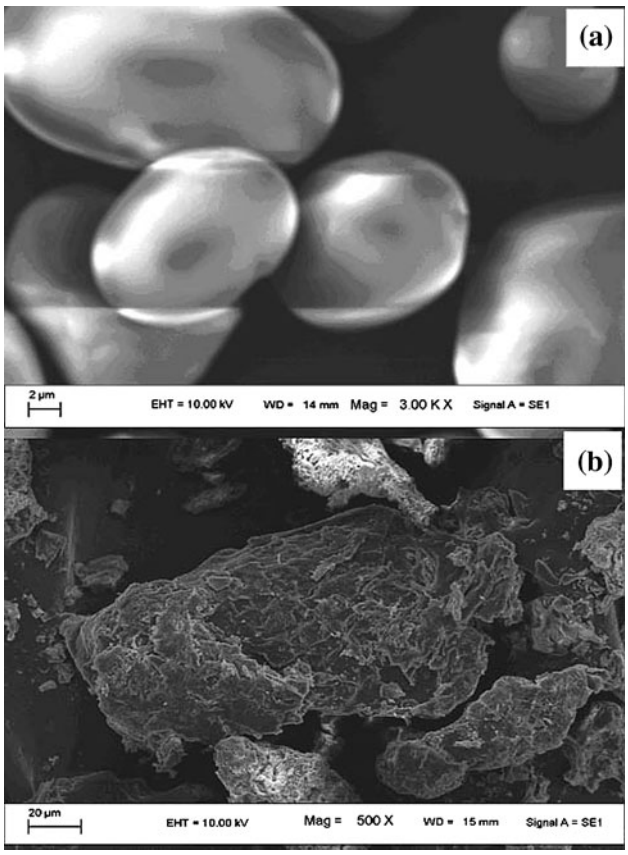

Fig. 2 Scanning electron micrograph of moth bean starch (**a**), highly substituted starch acetate (**b**)

The thermogravimetric analysis was used to examine the thermal stability of MBS and starch acetate. The native MBS showed thermal decomposition at 236.54 °C and highest

decomposition at around 398.56 °C. The modified starch acetate showed shifting of peaks, i.e., onset at 246.21 °C and highest decomposition at 403.20 °C (shown in Fig. 3). Shifting of peak was observed after acetylation confirmed its higher thermal stability; this might be due to replacement of hydroxyl groups in starch molecules after acetylation [9, 10].

Compatibility study

DSC curves of drug and drug-excipient mixtures are shown in Figs. 4, 5, 6, 7, 8, 9, and corresponding peak temperatures and enthalpy values (ΔH) of LAM with various excipient mixtures are summarized in Table 1. DSC curve of LAM showed a sharp endothermic peak at 182.73 °C corresponding to its melting point (Fig. 4). The endothermic peak of the drug was well-retained in majority of cases. However, in some combinations there was slight change in peak temperature and peak shape, which might be due to mixing of excipients with the drug as this reduces the purity of component in mixtures [11].

In the DSC curve of LAM + AMBS, the endothermic peak of LAM was well-retained in the mixture (Fig. 5), with a slight change in the enthalpy value. The thermogram of spray dried lactose and drug combination showed an early peak at 148.18 °C that is observed due to the bound water present in lactose. The endothermic peak of drug was well-retained in the DSC curve of LAM + SDL mixture (Fig. 7), from this it can be concluded that SDL is compatible with the drug lamivudine. The DSC curve of PVP K-30 shows that the melting endotherm of LAM was well-preserved in the mixture and heat of enthalpy is nearly same to the parent drug, concluding its suitability with LAM. One extra peak was observed at 82 °C (Fig. 6), which is of the adsorbed water present on PVP K-30. In the DSC curve of drug and magnesium stearate mixture (Fig. 8), one peak at 116 °C was observed that might be due to bound water present in magnesium stearate, while the peak of parent drug was well-retained in the mixture, which confirming its suitability with the drug. In the DSC curve of Lam + Talc mixture (Fig. 9), no shifting was observed in the endothermic peak of the drug, while there is slight increase in the enthalpy value of the mixture that might be a solid–solid interaction rather than incompatibility [12]. To check its suitability the combination was further checked in isothermal stress testing.

In the isothermal stress testing, drug-excipient binary mixtures showed no change in physical appearance at

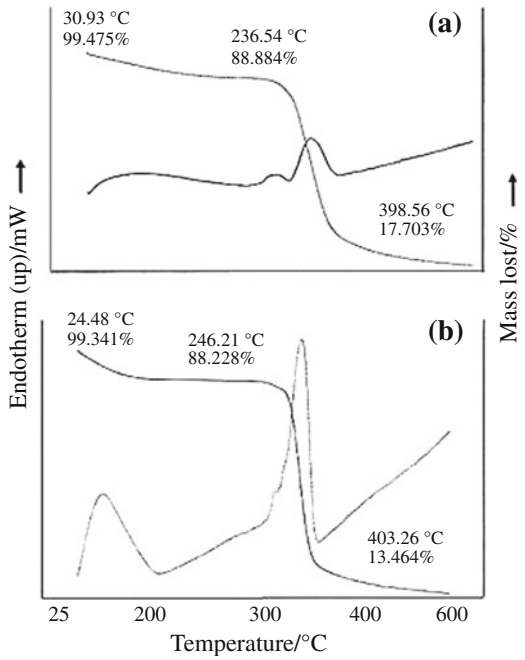

Fig. 3 TG curve of moth bean starch (**a**), and starch acetate with DS 2.35 (**b**)

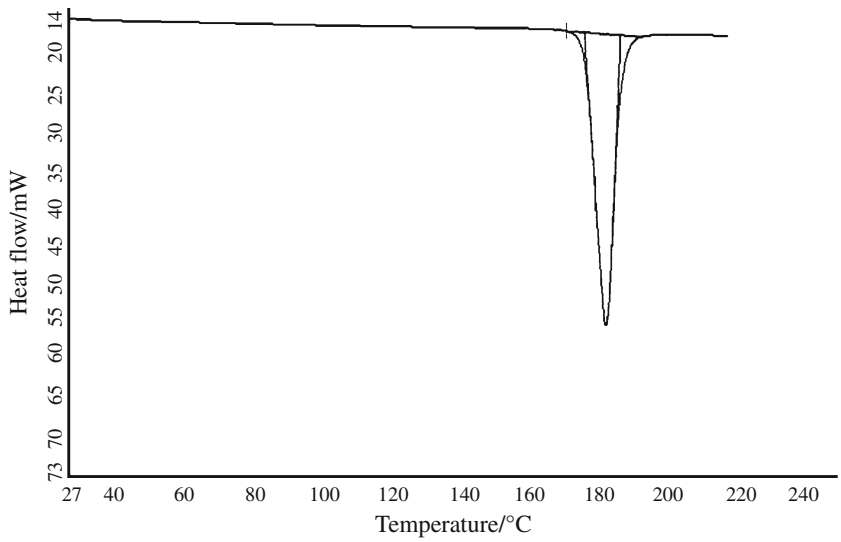

Fig. 4 DSC curve of pure Lamivudine drug

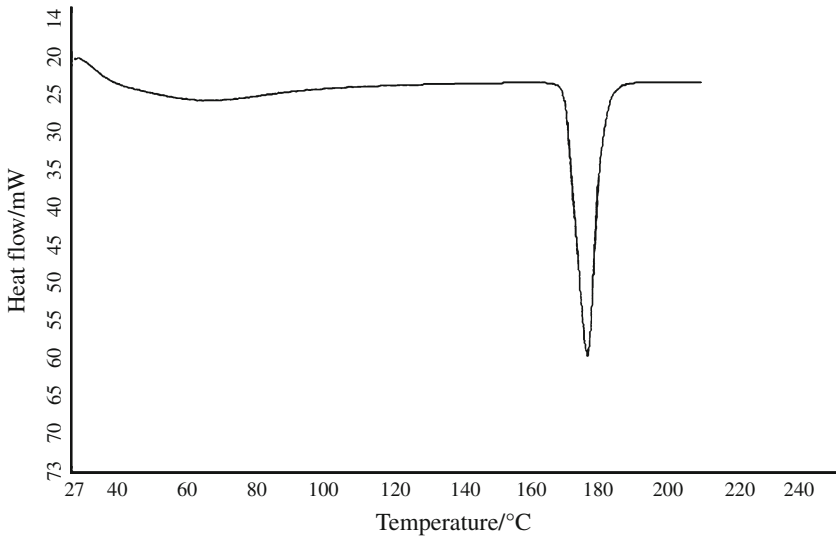

Fig. 5 DSC curve of Lamivudine and acetylated moth bean starch (1:1)

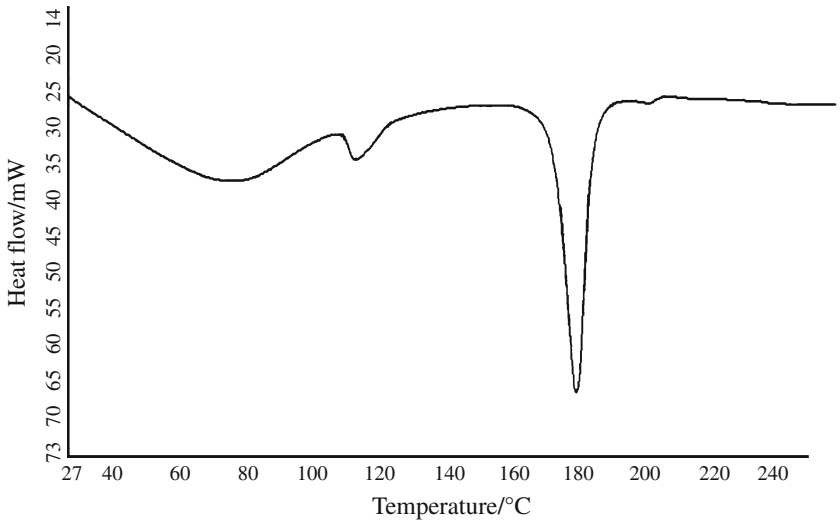

Fig. 6 DSC curve of Lamivudine and PVP K-30 (1:1)

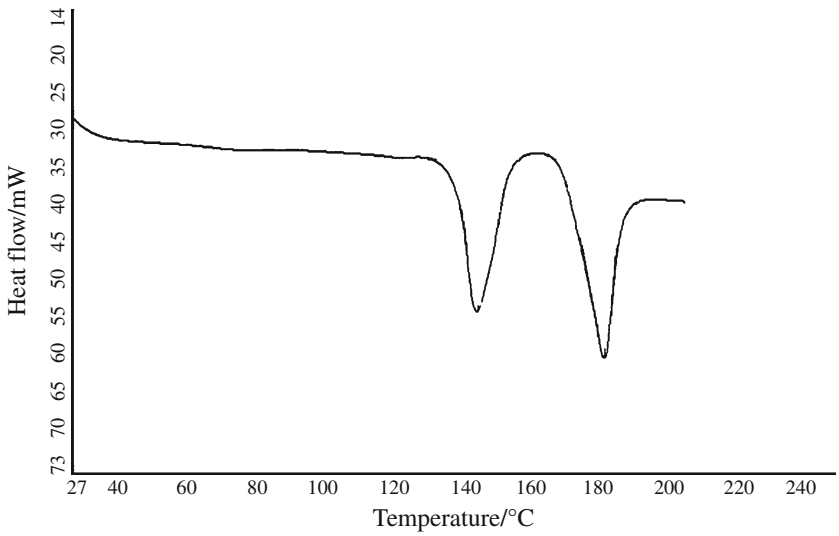

Fig. 7 DSC curve of Lamivudine and Spray dried lactose (1:1)

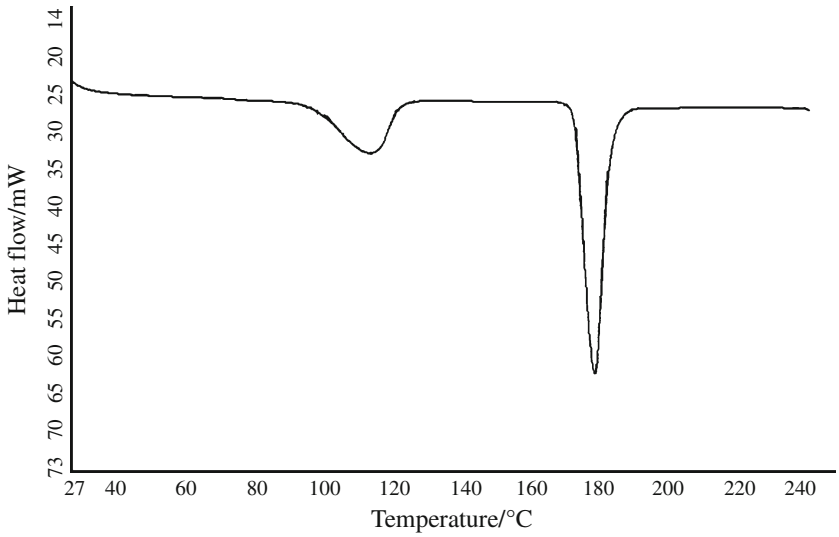

Fig. 8 DSC curve of Lamivudine and Magnesium stearate (1:1)

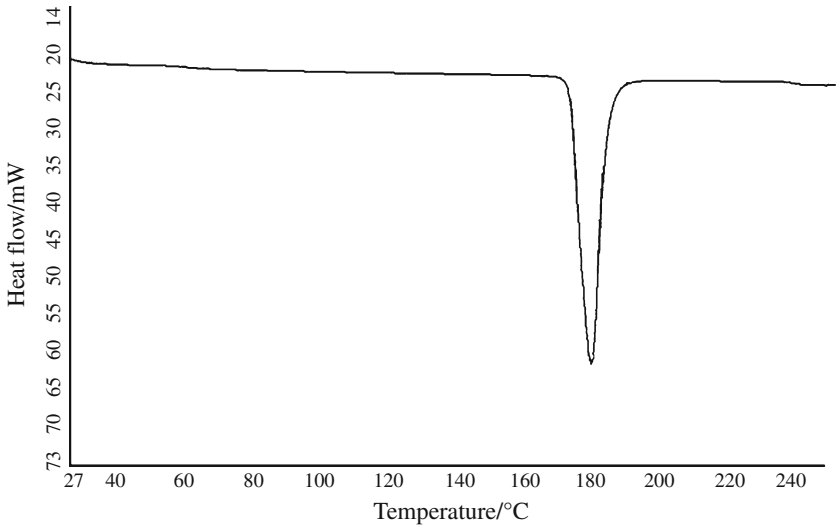

Fig. 9 DSC curve of Lamivudine and Talc (1:1)

Table 2 Results of UV analysis of the samples, under Isothermal stress testing after 4 weeks of storage

Sample	Ratio/drug-excipient	% Drug remaining[a]		Change in physical appearance
		Control sample[b]	Stressed sample[c]	
Lamivudine/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 + 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

ambient temperature. The blends remain physically stable and no discoloration, liquefaction, or gas formation was observed during storage. There is also no significant drug degradation was observed with any type of excipients. Table 2 showed % drug remaining at the end of the study at 50 °C.

Conclusions

Moth bean starch acetate with high degree of substitution (DS = 2.35) was synthesized by the reaction with acetic anhydride in pyridine medium. The XRD pattern showed conversion of crystalline MBS into amorphous after high substitution. It has also been shown that acetyl substitution increased the thermal stability, which is confirmed by TG. Compatibility study in Preformulation stage of formulation development is now become an essential step. The thermal analysis provides information about the thermal stability and decomposition of drug and used excipients. The results confirmed the suitability of drug lamivudine with various common tablet excipients like spray dried lactose, PVP K-30, magnesium stearate, talc, and a novel synthesized acetylated moth bean starch. The DSC and IST study showed none type of interaction in all drug-excipient combinations.

Acknowledgements The authors are thankful to Prof. Harkesh B Singh, Department of Chemistry, IIT Mumbai for carrying out XRD, FT–IR, and TG analysis. Authors are also thankful to AICTE, New Delhi for funding this project under RPS scheme.

Conflict of interest None.

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