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ACETYLATION AND CHARACTERIZATION OF ENSET STARCH AND EVALUATION OF ITS DIRECT COMPRESSION AND DRUG RELEASE SUSTAINING PROPERTIES

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ABSTRACT: Enset (*Ensete ventricosum*, Musaceae), a plant widely cultivated in south and southwest of Ethiopia, has been shown to be a rich source of starch. In an effort to produce directly compressible matrix-forming excipient, native enset starch was acetylated. Starch acetates (SA) with degrees of substitution (DS) of 0.672 and 2.142 were evaluated. Fourier transform infrared (FTIR) spectra of the modified starches verified the acetylation of the starch molecules. Further investigations revealed that acetylation increased swelling power and solubility, improved flow property and compactability of the starch. The tensile strength of SA matrix tablets increased with an increase in DS. Plain tablets of SA with DS 0.672 disintegrated within 3 min while those of SA with DS 2.142 did not disintegrate over a period of 2 h. Dissolution studies of theophylline loaded SA tablets conducted in 0.1 N HCl for the first 1.5 h and in phosphate buffer pH 6.8 for the remaining study time revealed the change in drug release rate from rapid to sustained release (>12 h) as the DS increased from 0.672 to 2.142. The dissolution data obtained best fitted Higuchi model with R^2 > 0.99. The drug release diffusional exponent (n), obtained from Korsemeyer-Peppas model, varied between 0.4899 - 0.6369 for different theophylline/SA ratios and the goodness of the fit was > 0.99 in each case which indicated the deviation from Fickian diffusion. Accordingly, high degree of acetylation renders enset starch highly compressible and suitable for sustained release formulations that makes it amenable for use as an alternative pharmaceutical excipient.

INTRODUCTION: Native starches are less favored in direct compression of tablet dosage form and are inappropriate for controlling drug release. Hence, they are chemically modified to improve their direct compression and drug release sustaining properties [1-7].

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Most studies on the production of chemically modified starches have been limited to widely available starches such as maize, potato, wheat, tapioca and rice.

Enset (*Ensete ventricosum*, Musaceae), a plant cultivated in many parts of Ethiopia and used as staple food in south and southwest of the country, has been shown to be a rich source of starch with amylose content of 29% [8].

The potential applications of native enset starch have been extended by chemical modification technique [9, 10].

Acetylation, one of the chemical modification techniques of starch, can be effected by acetic anhydride or vinyl acetate in the presence of alkaline catalyst [11-15]. The hydroxyl moieties on C_2, C_3 and C_6 of the α-D gluco-pyranose unit are partially or almost completely substituted by acetate groups [16]. Depending on the reaction conditions, the DS varies and the theoretical maximum DS is 3 [17] Acetylation improves the bond-forming ability, drug sustaining and film forming properties of native starches [18-23].

In the present study, an attempt is made to acetylate starch obtained from *E. ventricosum* and to evaluate its potential application as directly compressible excipient for controlled release of active ingredients.

MATERIALS AND METHODS: *Boulla*, the wet starch of liquid exudates (obtained from the pseudostem and corms of enset plant) was purchased from the local Enset cultivation zone (Gurage zone, around Welene area, south-west Ethiopia). Sodium metabisulphite (Guangzhou Jinhaunda Chemical Reagent Co. Ltd, China), Acetic anhydride (May and Baker Ltd Dagenham, England), Avicel® PH 101, Ethylcellulose (EC) (BDH Chemicals Ltd, England), Magnesium stearate (BDH Chemicals Ltd, England), Paracetamol (China associate Co Ltd, China), Anhydrous theophylline (Shandong Xinhua Pharmaceutical Co. Ltd, China). All reagents and solvents used were of analytical grade.

Isolation of enset starch: Starch was isolated from *boulla* according to the method described elsewhere [8].

Acetylation of Enset starch: Starch acetates (SAs) were prepared by combination and modification of the methods described elsewhere.[24-26] The reactions were carried out in a 2 L oil bath jacketed glass reactor. Before acetylation, enset starch was dried in an oven (Kottermann® 2711, Germany) at 50 °C for 24 h to minimize the interference of moisture on the chemical reaction. The dried enset starch (150 g) was mixed with acetic anhydride (1:4 ratio) in the jacketed glass reactor at room temp (Table 1). After stirring for 5 min (at 100 rpm), 50% (w/w) aqueous NaOH solution was added as a catalyst drop by drop with constant stirring.

The temp of the oil bath was increased to 90 °C within 15 minutes and held at this temp for different reaction times. At the end of the reaction times, excess cold distilled water was added to the reactor with vigorous mixing to terminate the reaction and to thoroughly rinse the reaction products. The precipitate was then filtered in a Buchner funnel by vacuum filtration and washed with water. The white acetylated starch was dried in an oven at 50 °C. The dried products were milled before testing.

TABLE 1: REACTION CONDITIONS USED FOR THE SYNTHESIS OF ENSET SAS WITH TWO DIFFERENT DS

Reaction Condition	Starch/Acetic Anhydride Ratio	NaOH (50% w/w) per g of starch	Reaction Temperature (°C)	Reaction Time (hr)
I	1:4	0.35	90	5
II	1:4	0.2	90	1

Determination of degree of substitution (DS): DS was determined by saponification titration method, as described elsewhere.[27] Accurately weighed 1 g of pulverized Enset SA was added to 50 ml of aqueous solution of ethanol (75%). The slurry was kept in a water bath at 50°C for 30 min. After the slurry was cooled down to room temp,40 ml of 0.5 N aqueous solution of KOH was added to the mixture. The flask was fitted with a tight stopper and kept at room temp with occasional shaking for 72 hrs for complete saponification. Thereafter, the excess alkali was titrated with 0.5 N HCl with phenolphthalein as indicator until the endpoint. The solution was allowed to stand for 2 hrs and then any additional alkali, which might leach from the sample, was titrated. A blank test was performed with native enset starch following the same procedure.

Acetyl content (% A) was calculated according to Eq. 1

$$\%A = [(V_o - V_n) \times N \times 0.043 \times 100] / M$$

……..Eq. 1

where V_o is the ml of HCl used to titrate blank, V_n is the ml of HCl used to titrate sample, N is the normality of HCl used, M is the sample amount as dry substance (g).

Acetyl content, % A, was used to calculate the DS, according to Eq. 2

$$DS = {162 \times \%A}\big/{[4300 - (42 \times \%A)]}$$

……Eq. 2

Determination of Fourier Transform Infrared (FTIR) spectra: About 10 mg of finely ground samples were mixed with mulling agent (Nujol) in a mortar and pestle. The sample mixture was then placed between potassium bromide (KBr) plates to form a thin film of the mull by compression. The sandwiched plates were placed in the IR spectrophotometer (FTIR-8400S, SHIMADZU, Japan) and the spectra were obtained. Each IR spectrum was collected with 20 scans and spectral resolution of 2 cm^{-1}. Scanning was performed between wave numbers of 4000 and 600 cm^{-1}. Background spectrum was collected before running each sample.

Powder properties: True density was determined by liquid displacement method using xylene as immersion fluid. Bulk density of the samples was determined by carefully pouring 30 g powder into a graduated glass cylinder and measuring the bulk volume. Tapped density was obtained after tapping 500 times using tapped densitometer (ERWEKA, Germany) to attain a constant volume reading. To assess the flowability of powders, Hausner ratio (HR) and the Carr's index (CI) were calculated from the bulk and tapped densities. The angle of repose and flow rate were also determined. Water content of the powders was measured by gravimetric method.

Kawakita analysis: Kawakita equation was applied to study the powder compression using the degree of volume reduction(C).Graduated measuring cylinder containing 30 g of powder was tapped with a tapping densitometer (ERWEKA, Germany) 5, 10, 20, 30, 40, 50, 75, 100, 200, 300, 400 and 500 times and the reduction in volume were measured. The volume reduction of a powder due to tapping was calculated by the Kawakita equation (Eq. 3).

$$N/C = N/a + 1/ab$$

.....Eq. 3

where N is the number of taps and both 'a' and 'b' are constants. The constants of the Kawakita equation were calculated from the slope and intercept of the line from the graphs of N/C versus N. The term 'C' describes volume reduction during the tapping treatment and is obtained from Eq. 4

$$C = {(V_o - V_N)}\big/{V_o}$$

……..Eq. 4

where V_o is the loose volume of the powder column before tapping and V_N is the volume of the powder column after a certain number of taps (N).

Determination of swelling power and solubility: Solubility and swelling power (SP) of starch samples were determined using the method outlined by Bello-Pérez *et al* with slight modification[11].Starch samples (0.5 g each) were dispersed in distilled water (10 ml) in pre-weighed centrifuge tubes. The tubes were then kept in a thermostatically controlled water bath at 20, 37, 50, 65, 75 and 85^oC for 30 min,with shaking every 5 min and then left to cool to room temp (18-20^oC).The suspensions were centrifuged for 15 min at 3000 rpm. The supernatants were decanted and dried in an oven for 2 h at 130^oC. The percent solubility (% S) was calculated from the residues obtained after drying (Eq. 5).The sediments obtained were weighed to obtain the swelling of the starches (Eq. 6).

$$S(\%) = \frac{W_1}{W_3} \times 100$$

…….....Eq. 5

$$SP = \frac{W_2 \times 100}{W_3 \times (100 - S)}$$

……...Eq. 6

where W_1 is the weight (g) of soluble material in the supernatant, W_2 is the weight (g) of precipitate and W_3 is the weight (g) of starch sample.

Determination of moisture sorption pattern: The method described by Odeku and Picker-Freyer was slightly modified and applied [28]. Starch samples were pre-dried in an oven for 4 hrs at 120^oC and spread evenly on a petri dish (dried and weighed) and transferred to particular RH chamber. Samples were equilibrated for four weeks at room temp.

The weights after four weeks were recorded and the moisture uptake of each sample was calculated.

Tablet formulation and tableting: Plain tablets of modified starches and reference excipients (EC and Avicel® PH 101) were prepared. In this, the modified starches or reference excipients were lubricated with 0.5% (w/w) magnesium stearate for 3 min in a Turbula mixer (Willy A. Bachofen AG, Turbula 2TF, Basel, Switzerland) at 45 rpm. The lubricated powders were then compressed with an instrumented eccentric tablet press (Korsch EK0, Korsch, Germany) using a 10 mm flat faced punches at a fixed compression pressure.

Paracetamol was mixed with an excipient (modified starches/Avicel PH 101) for 10 min and then magnesium stearate was added to the drug-excipient mixture and further mixed for 3 min in a Turbula mixer.

The resulting mixture was then compressed at a fixed compression pressure. In the drug release studies anhydrous theophylline was used as a model drug. The compositions (% w/w) of the formulations used for the drug release studies are given in **Table 2**. The weight of tablets was fixed to 400 mg.

TABLE 2: THE COMPOSITIONS OF TABLET FORMULATIONS USED IN DRUG RELEASE STUDIES

Formula	Theophylline (% w/w)	SA DS 2.142 (% w/w)	SA DS 0.672 (% w/w)	Avicel PH 101 (% w/w)	EC (%w/w)	Mg Stearate (% w/w)
I	25	74.5	-	-	-	0.5
II	30	69.5	-	-	-	0.5
III	40	59.5	-	-	-	0.5
IV	25	-	74.5	-	-	0.5
V	25	-	-	74.5	-	0.5
VI	25	-	-	-	74.5	0.5

Compactibility of the powder: The crushing strength of 10 tablets from each formulation was determined using hardness tester (Schleuniger, 2E/205, Switzerland) crushing strength (in Newton) that just caused each tablet to break was recorded. The radial breaking strength, volumetric dimensions and weight of the tablets were measured 24 h after tabletting. The radial tensile strength of the tablets, which allows the dimensions of the compact to be taken into account, was calculated from the breaking strength values, according to Fell and Newton (1970) by Eq. 7 [29].

$$\sigma = \frac{2 \cdot F}{\pi \cdot h \cdot d}$$

…… Eq. 7

Where F is the breaking force, h is the tablet height, and d is the tablet diameter.

Friability: Preweighed 10 tablets were placed in a friability tester (ERWEKA, TAR 20, Germany) and the plastic chamber was allowed to rotate at 25 rpm for 4 min. The tablets were then dusted and reweighed and the percent loss in weight was calculated.

Disintegration time: Disintegration time test was carried out according to USP/NF specification (USP XXX/NF XXV, 2009).

Tablet porosity: The apparent density (ρ_a) of the compact was calculated from the ratio of the tablet mass (m) to the volume of the compact (Eq. 8). The material densities of the modified starches, Avicel PH 101, magnesium stearate and anhydrous theophylline and their percentage in tablets were used to determine true density of the compact (ρ_t).

$$\rho_a = \frac{m}{\pi \cdot r^2 \cdot h}$$

……Eq. 8

The ratio of ρ_a/ρ_t is a measure of the relative density (D) (Eq. 9) or the solid fraction of the compact.

$$D = \rho_a / \rho_t$$

…… Eq. 9

Porosity (ε) of the tablet was calculated according to Eq. 10

$$\varepsilon = [1 - \frac{\rho_a}{\rho_t}] \times 100\%$$

……Eq. 10

***In vitro* drug release studies:** The *in vitro* drug release studies were performed in USP dissolution apparatus II (Paddle Method) (ERWEKA, DT600, Germany) adjusted to rotate at 100 rpm. Phosphate buffer solution (900 ml, pH 6.8), maintained at 37 ± 0.5°C, was used as the dissolution medium. Samples of dissolution medium (5 ml each) were withdrawn at predetermined time intervals (at 15 and 30 min, and then at 1, 2, 3, 4, 6, 8, 10 and 12 hr) and immediately replaced with an equal volume of the dissolution medium (at 37 ± 0.5 °C) in order to maintain the sink conditions. The samples withdrawn were filtered, properly diluted, and analyzed for theophylline content by UV spectrophotometer at 271 nm. The drug release from the matrix tablets was also studied in 0.1 N HCl for the first 1.5 hrs and then in phosphate buffer pH 6.8 for the next 10.5 hrs to verify the integrity of the matrix tablet in acidic pH.

Analysis of drug release kinetics: The dissolution data were fitted into different drug release models to evaluate the *in vitro* drug release kinetics from the hydrophobic SA matrix tablets.

I. Zero order release model:

$$Q = Q_o - Kt$$

……Eq.11

where Q is the amount of drug remaining at time t, Q_o is the quantity of drug present initially in the dosage form and K is the zero order release constant.

II. First order release model:

$$\ln Q = \ln Q_o - Kt$$

……Eq.12

where Q is the amount of drug remaining at time t, Q_o is the quantity of drug present initially in the dosage form and K is the first order release constant.

III. Higuchi square root model:

$$M_t / M_o = Kt^{1/2}$$

……Eq.13

Where M_t is the amount of drug released at time t, M_o the amount of total drug in the tablets, M_t/M_o is the fractional drug release at time t and K is a constant incorporating the matrix structure.

IV. Korsemeyer-Peppas release model:

$$M_t / M_o = Kt^n$$

……..Eq. 14

Where M_t is the amount of drug released at time t, M_o the amount of total drug in the tablets, M_t/M_o is the fractional drug release at time t, K is a constant incorporating the matrix structure and n is the diffusional exponent, which was used to characterize the transport mechanism.

V. **Hixson-Crowell cube root model:**

$$Q_o^{1/3} - Q_t^{1/3} = Kt$$

…….Eq. 15

Where Q_t is the amount of drug remaining in time t, Q_0 is the initial amount of the drug in the tablets and K is the rate constant for Hixson-Crowell rate equation.

Statistical analysis: Wherever appropriate, the data were subjected to further statistical analysis using Analysis of Variance (ANOVA) on Origin 7.0 statistical software. A confidence limit of $P < 0.05$ was fixed for interpretation of the results.

RESULTS AND DISCUSSION:

Degree of substitution: Among the various trials during preliminary studies, the highest DS of Enset SA obtained was 2.142 (with acetyl content of 36.55%) at reaction condition I. SA with DS of 0.672 (with acetyl content of 15.19%) obtained at reaction condition II was selected for comparative study (**Table 1**).

Studies conducted on starch acetylation indicated that the number of acetyl groups incorporated into the starch molecules depends upon different factors such as reactant concentration, reaction time, pH and the presence of catalyst [11, 17].

During the preliminary studies different reaction conditions were employed to synthesize enset SA with high DS as it is essential for controlled release properties of the polymer in tablet manufacturing by direct compression.

SA powders with two different DS (2.142 and 0.672), referred herein as SA DS 2.142 and SA DS 0.672, were obtained from reaction conditions I and II, respectively (Table 1). The theoretical maximum yields of SA from 150 g of native enset starch are 236 g and 177 g to produce SAs with DS of 2.142 and 0.672, respectively. The actual yield, however, were about 205 g (86.61%) and 169 g (95.51%). This difference could be due to losses during handling of the reaction products.

Fourier Transform Infrared (FTIR) spectra: The FTIR spectra of native enset starch and SA DS 2.142 are presented in **Fig. 1 and 2**, respectively. Amylose and amylopectin, the two structural units of starch, are composed of identical monomers, which have only one prominent functional group, OH group. There are two types of alcohol groups in these structural units. Primary alcohol moiety is present as CH_2OH and secondary alcohol moiety as the OH directly attached to ring carbons [30].

In the acetylation reaction, the OH moieties of monomers in starch are substituted with acetyl moieties. As depicted in Fig. 1 and Fig. 2, compared to native enset starch, SAs had strong absorption bands at 1,750 cm^{-1} which is attributed to the stretching of the ester carbonyl C=O indicating the acetylation of enset starch [31]. Increased peak intensity was observed as DS increased from 0.672 to 2.142.

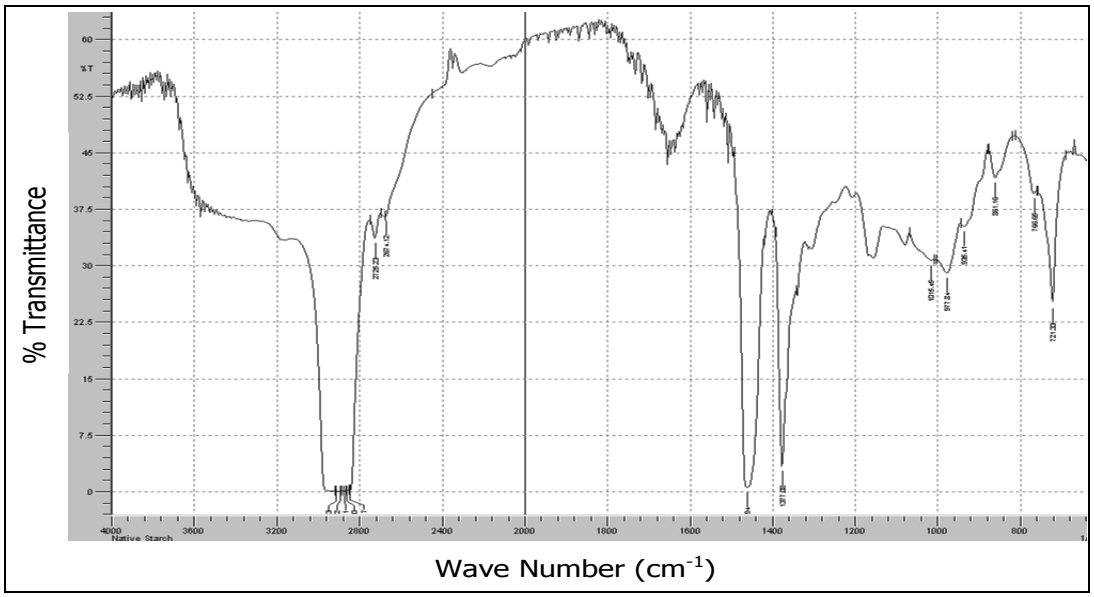

FIGURE 1: FTIR SPECTRUM OF NATIVE ENSET STARCH

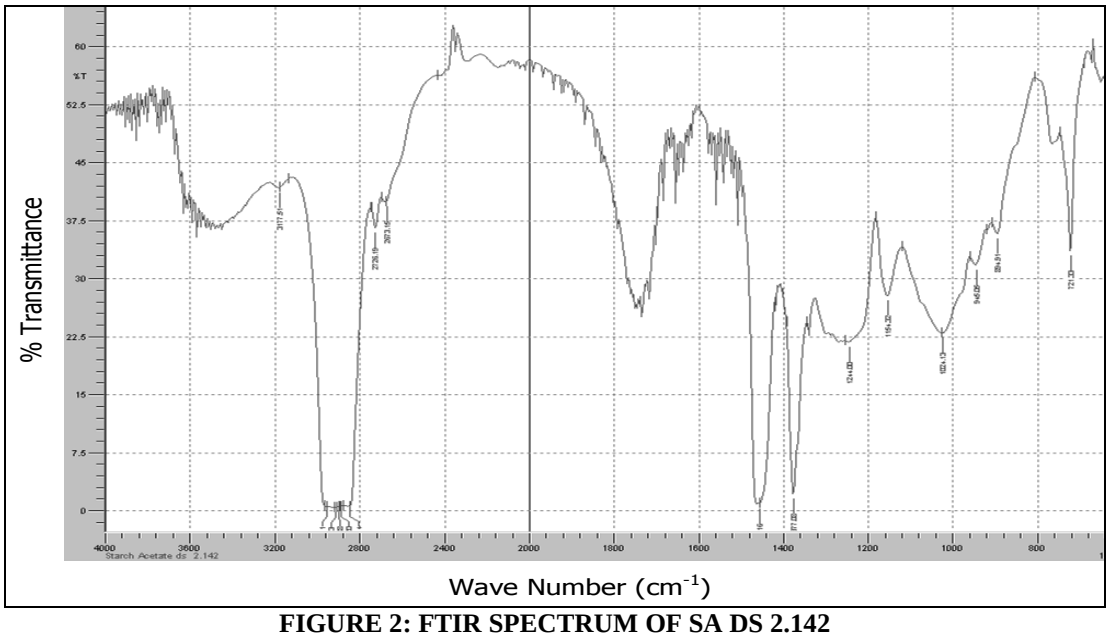

FIGURE 2: FTIR SPECTRUM OF SA DS 2.142

Powder properties: Some powder properties of the starches and reference excipients are shown in **Table 3 and 4**. Acetylation of enset starch significantly improved its flow property, i.e., the CI and HR of native enset starch reduced significantly ($P < 0.05$) upon chemical modification. Moreover, the extent of acetylation also impacted the flow property as SA DS 2.142 powder showed significantly ($P < 0.05$) better flowability than SA DS 0.672 powder. The results of the angle of repose were in line with CIs and HRs (Table 3). The improved flow property could be attributed to variations in particle size and particle shape of the modified starches compared with the native one.

TABLE 3: POWDER PROPERTIES OF NATIVE ENSET STARCH, MODIFIED STARCHES AND REFERENCE EXCIPIENTS

Property	Native Enset starch	Enset SA DS 2.142	Enset SA DS 0.672	EC	Avicel PH 101
Bulk density (g/cm^3)	0.536 (0.010)	0.472 (0.002)	0.367 (0.004)	0.391 (0.011)	0.336 (0.003)
Tapped density (g/cm^3)	0.711 (0.002)	0.562 (0.003)	0.471 (0.004)	0.449 (0.015)	0.439 (0.001)
True density (g/cm^3)	1.506 (0.091)	1.368 (0.095)	1.319 (0.139)	*	1.731 (0.086)
Carr's Index (%)	24.869 (1.126)	16.037 (0.439)	22.345 (1.591)	12.833 (1.090)	23.424 (0.344)
Hausner Ratio	1.328 (0.020)	1.191 (0.006)	1.288 (0.026)	1.147 (0.014)	1.306 (0.016)
Angle of Repose (^{o})	X	28.96 (1.34)	32.84 (0.85)	31.09 (1.30)	X
Flow Rate (g/sec)	X	1.769 (0.104)	1.806 (0.120)	2.909 (0.157)	X
Moisture Content (%)	10.966 (0.431)	8.933 (2.996)	8.383 (0.732)	2.850 (0.879)	8.333 (2.250)

The values in parenthesis are the standard deviation, X indicates that the powders did not flow through the funnel, * The true density of EC was not determined.

The Kawakita plots of N/C versus N for the starches and the reference excipients gave linear plots. Kawakita constants (Table 4) indicate the behaviour of the powder from the bulk density state to the tapped density state. In general, small values of constants 'a' (compressibility, or the amount of densification due to tapping) and '1/b' (cohesiveness, or how fast or easily the final packing state is achieved) indicate good flowability and small cohesiveness [19].

The result of powder densification study showed that SA DS 2.142 and EC densified the least (i.e., small compressibility values) and attained the final packing state slowly (i.e., the greater cohesive values). On the other hand, SA DS 0.672, native enset starch and Avicel PH 101 densified considerably and achieved the final packing state rather quickly, of which native enset starch is the fastest to attain the final packing state. The smaller 'a' values of SA DS 2.142 and EC indicate good flowability.

TABLE 4: KAWAKITA CONSTANTS OF NATIVE ENSET STARCH, SA DS 2.142, SA DS 0.672 AND REFERENCE EXCIPIENTS

	Kawakita Compressibility (a)	Kawakita Cohesiveness (1/b)	Correlation Coefficient (R^2)
SA DS 2.142	0.167	26.561	0.999
SA DS 0.672	0.225	18.446	0.996
Native Enset starch	0.247	7.286	0.998
Avicel PH 101	0.232	10.313	0.995
EC	0.131	23.359	0.995

The SP and solubility profiles of the starches are depicted in Fig. 3a and b, respectively. The SP of the native enset starch was low at low temp which may be attributed to extensive and strongly bonded structures making the starches relatively resistant to swelling (**Fig. 3a**).

The SP of the starch, however, increased with temperature probably due to macromolecular disorganization and degradation of starch during thermal treatment. The SP of native enset starch increased dramatically as the temperature raised from 50^{o}C to 85^{o}C.

The SP of SA DS 0.672 powder was higher than those of the corresponding enset starch powder at the temperature lower than 75°C. Acetylation appreciably increased starch SP at the lower DS than the higher DS. Chen *et al* studied the effect of acetylation to different extent (DS) on swelling and enzymatic degradation of maize starch, and reported that the swelling of SA particles were higher than those of the corresponding native maize starch granules [32]. According to the finding, acetylation appreciably accelerated starch swelling at lower DS, but inhibited at higher substitution levels.

The present study also was in accordance to it. The introduction of the acetyl group (at lower levels of substitution) reduces the interaction between starch molecules and, thereby, increase the SP. Bello-Pérez *et al* acetylated banana starch (DS about 0.04) and the acetylated sample showed higher swelling value [11]. This was explained by the introduction of acetyl groups, allowing the retention of water molecules because of their ability to form hydrogen bonds. As can be seen from the solubility profiles (**Fig. 3b**), acetylation significantly increased solubility which may be attributed to the obstruction of chain association by the acetyl groups.

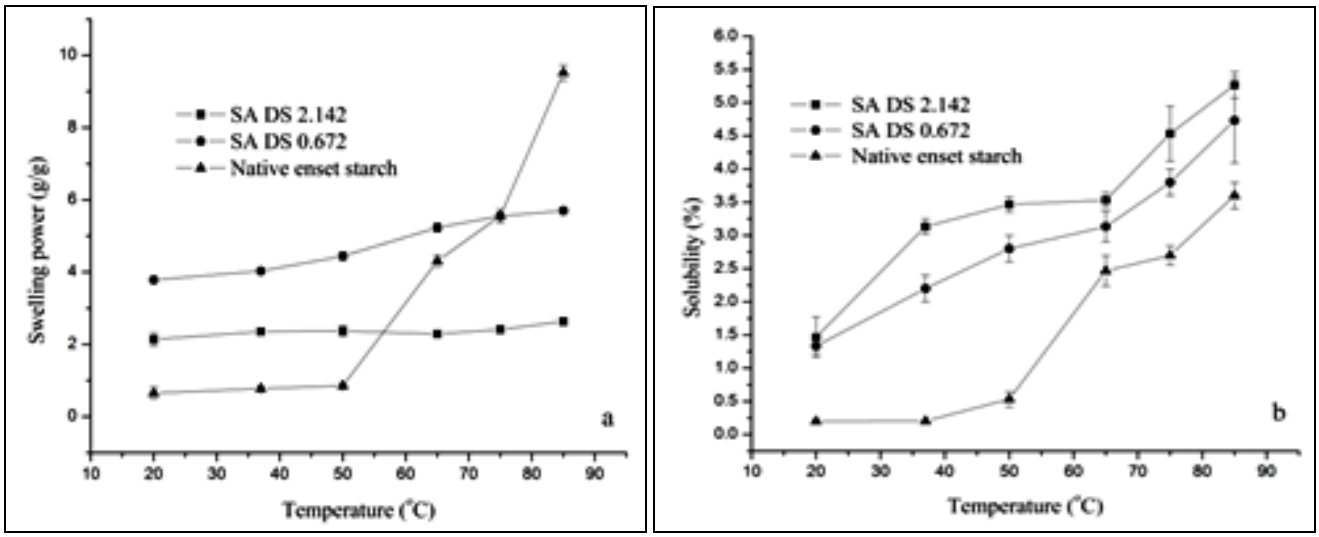

FIGURE 3: SWELLING POWER (A) AND SOLUBILITY (B) OF SA DS 2.142 (—■—), SA DS 0.672 (—●—) AND NATIVE ENSET STARCH (—▲—) AT DIFFERENT TEMPERATURE

The moisture sorption profiles of starches are given in Fig.4. Hygroscopic materials can experience significant increase in moisture content when exposed to humid air.[33] Knowledge of moisture sorption profiles of starches is necessary where controlled powder flow and/or compaction are critical. Moisture modifies the flow and mechanical properties of many powders including starch.

Therefore, the moisture sorption profiles of starches were determined to extrapolate the extent of the influence of a humid environment on the powder properties. The moisture sorption profiles (**Fig. 4**) of the starch samples indicate that the water uptake of the modified starches changed slightly at RH levels below 75%. Hence, lower moisture levels are recommended for storage to obtain SA powders with optimum flow and compaction properties and also to prevent the deterioration of tablets.

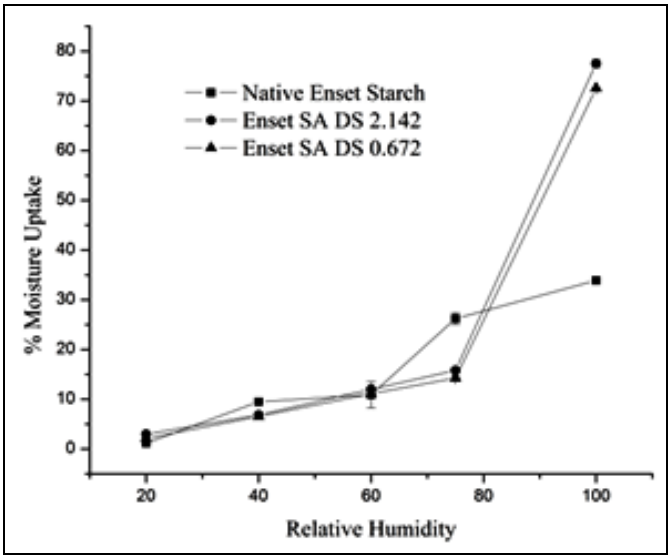

FIGURE 4: MOISTURE SORPTION PATTERNS OF NATIVE ENSET STARCH (—■—), SA DS 2.142 (—♦—) AND SA DS 0.672 (—▲—) AT ROOM TEMPERATURE

Tablet properties: Some properties of plain and paracetamol loaded tablets are shown in **Table 5 and 6**. As can be seen from Table 5, plain tablets prepared from SA DS 2.142 showed about threefold higher tensile strength than the tablets prepared from SA DS 0.672 which reflected the better compactibility of the former modified starch. Incorporation of paracetamol resulted in a decrease in the compactibility of paracetamol/SA powder blends when compared to plain SA powders (Table 6). This may be attributed to weaker interaction forces between SA and paracetamol particles than the cohesive forces within SA powders [2].

The tensile strength of SA DS 2.142 tablets was significantly higher than SA DS 0.672 tablets which in turn are harder than the native enset starch tablets indicating better compactibility of SA DS 2.142. Paracetamol was used as a model drug in compactibility studies of the modified starch. Raatikainen *et al* in their study focusing on the deformation properties of substituted SAs, indicated that the acetate moiety of SA, perhaps in combination with existing OH groups, is a very effective bond-forming substituent [1].

Strong molecular bonds are formed leading to a very firm and intact tablet structure. They also reported the induction of some fragmentation due to slightly harder and more irregular shape of high-substituted SA particles and slight enhancement of plastic flow under compression.

TABLE 5: HARDNESS, RADIAL TENSILE STRENGTH, FRIABILITY, DISINTEGRATION TIME AND POROSITY OF PLAIN TABLETS OF SA DS 2.142, SA DS 0.672, AVICEL PH 101 AND EC

	SA DS 2.142	SA DS 0.672	Avicel PH 101	EC
Hardness (N)	258.2 (8.311)	82.8 (4.894)	74.7 (3.860)	135.7 (5.889)
Tensile Strength ($Kgcm^{-2}$)	39.9 (1.258)	12.5 (0.739)	12.2 (0.627)	22.0 (0.956)
Friability (%)	0.050	0.438	0.434	0.157
Disintegration Time (min)	> 120**	2.9 (0.467)	1.6 (0.083)	> 120**
Porosity (%)	10.8 (1.45)	22. 7 (1.26)	44.6 (0.90)	*

The values in the parenthesis are the standard deviations, * The true density of EC was not determined, ** Tablets did not disintegrate during the measurement time of 2 h

Friability results (Table 5 and 6) were in agreement with the mechanical strength of the tablets Except for those prepared with native enset starch, all tablets met the limits of the test for loss of weight. In disintegration tests, all plain tablets made of SA DS 0.672 disintegrated completely within a few min while those made of SA DS 2.142 and EC did not disintegrate at all during the measurement time of 2 h (Table 5). As the degree of acetylation increases, SA becomes more hydrophobic and the mechanical strength increases.

As a result, disintegration does not occur anymore and the drug release from these SA matrix tablets is extended for longer period of time. On the other hand, paracetamol loaded SA DS 2.142 tablets disintegrated within 30 min (Table 6), which could be attributed to the poor compressibility and elastic recovery of paracetamol.

Moreover, native starch, SA DS 0.672 and Avicel PH 101 tablets disintegrated within 3 min. The release rate of a drug from hydrophobic matrix can be modified by changing in the porosity and tortuosity of the matrix, i.e., its pore structure. Interparticulate porosity is essential for solvent penetration into the matrix and, consequently, for drug release. Compression force controls the porosity of the matrix, which in turn controls drug release [34].

The interparticulate porosity of tablets also increases by void formation during stress relaxation and tablet expansion after the densification process [2]. Generally, a more rigid and less porous matrix will release drug more slowly than a less consolidated matrix.

The plain matrix tablets of SA DS 2.142 which showed some threefold increase in their tensile strength were about half as porous as the corresponding plain SA DS 0.672 tablets (Table 5). This is in line with the findings of Pohja *et al* who reported inverse exponential relationship between the porosity and tensile strength of tablets compressed from plain SA powders [2].

TABLE 6: HARDNESS, RADIAL TENSILE STRENGTH, FRIABILITY AND DISINTEGRATION TIME OF PARACETAMOL (25%) LOADED TABLETS OF SA DS 2.142, SA DS 0.672, AVICEL PH 101 AND EC

	Native starch	SA DS 2.142	SA DS 0.672	Avicel PH 101	EC
Hardness (N)	49.8 (2.974)	132.0 (5.598)	73.3 (3.164)	95.4 (4.648)	161.2 (8.149)
Tensile Strength ($Kgcm^{-2}$)	9.2 (0.552)	22.0 (0.932)	13.2 (0.570)	20.0 (0.973)	25.5 (1.290)
Friability (%)	3.2 (0.021)	0.320 (0.031)	0.65 (0.047)	0.37 (0.050)	0.08 (0.001)
Disintegration Time (min)	0.26 (0.035)	29.3 (1.527)	0.76 (0.067)	2.9 (0.254)	> 120*

The values in the parenthesis are the standard deviations, *Tablets did not disintegrate during the measurement time of 2 h

***In vitro* drug release and drug release kinetics:** The dissolution profiles of tablets in different media are depicted in **Fig. 5 and 6**. As can be seen from Fig. 5, the dissolution rate of theophylline changed from rapid release to sustained release as the DS of SA increased from 0.672 to 2.142. The rapid drug release from SA DS 0.672 tablets can be attributed to short disintegration time of the tablets.

The SA tablets with DS 2.142 demonstrated sustained-release profiles with higher initial drug release. The tested reference direct compression excipient, i.e., MCC (Avicel PH 101) had no sustained release property and released the drug within few minutes, as expected. The drug release from the hydrophobic SA DS 2.142 matrix containing 25% (w/w) of theophylline showed comparable profile to the corresponding EC matrix tablet.

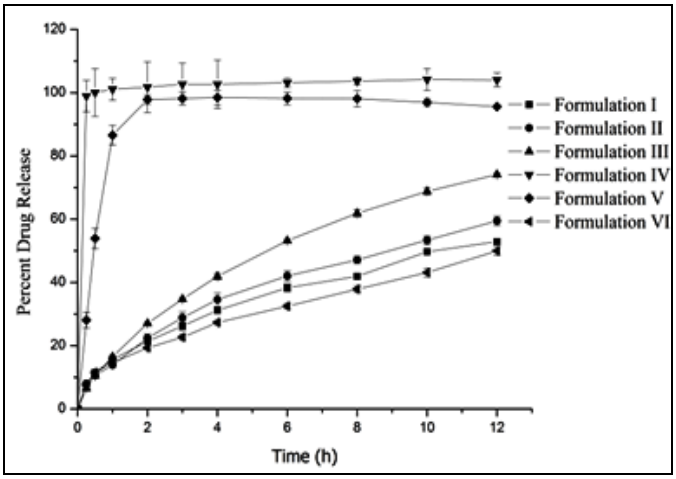

FIGURE 5: THEOPHYLLINE RELEASE PROFILES FROM SA DS 2.142, SA DS 0.672, EC AND AVICEL PH 101 TABLETS IN PHOSPHATE BUFFER pH 6.8

In the present, study the effect of theophylline to SA ratio on theophylline release from matrix tablets was also investigated. Incorporation of theophylline clearly decreases the compactibility of powders, because adhesive forces between SA and theophylline particles are weaker than the cohesive forces within the SA particles.

The drug release from SA DS 2.142 matrices with 25% (w/w) theophylline ($t_{50\%}$ = 618 min) was slightly more sustained than that of SA DS 2.142 matrices with 30% (w/w) theophylline ($t_{50\%}$ = 540 min) which in turn sustained the drug release more than SA DS 2.142 matrices with 40% (w/w) theophylline ($t_{50\%}$ = 338 min). The study done by Pohja *et al* demonstrated that an increase in SA concentration in tablets decreases the drug release rate. Hence, both DS and SA concentration can be varied to control drug release from SA matrices [2].

To verify the integrity of the matrix tablets in acidic environment, the drug release from SA DS 2.142 and EC tablets, each containing 25% theophylline, was studied in 0.1 N HCl for the first 1.5 h and then in phosphate buffer pH 6.8 for the next 10.5 h. As can be seen from Fig. 6, the drug release was more sustained in phosphate buffer pH 6.8 alone than in 0.1 N HCl/phosphate buffer pH 6.8. The higher initial drug release in the acidic medium might be attributed to the erosion and dissolution of surface drug particles without any control by the matrix as a result of decreased integrity of the matrix in the medium.

On the contrary, EC matrix tablets exhibited similar drug release profiles in both phosphate buffer pH 6.8 alone and 0.1 N HCl/phosphate buffer pH 6.8 media, as depicted in Fig. 6. However, drug release studies in 0.1 N HCl alone for 12 h showed similar drug release profile as in 0.1 N HCl/phosphate buffer pH 6.8. This result confirmed that, although there is high initial surface erosion in 0.1 N HCl, the matrix tablets were able to withstand the acidic environment with no sign of disintegration and sustained the drug release for more than 12 h. This is an interesting finding as large unit dosage forms, including modified release tablets and capsules, may exhibit delayed gastric emptying for several hours in the presence of food in the stomach.

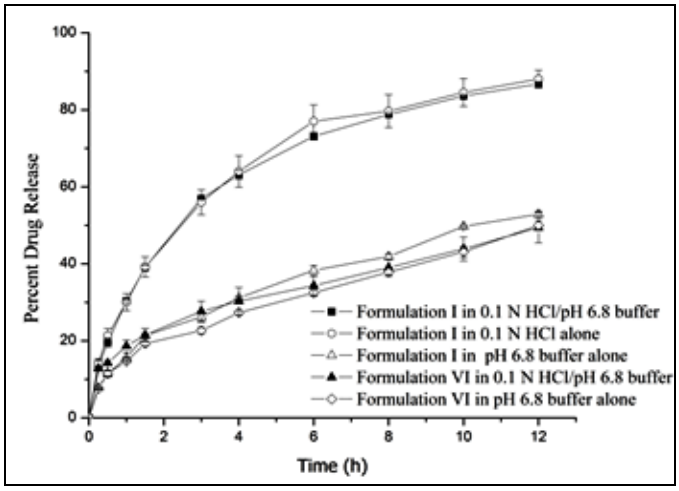

FIGURE 6: THEOPHYLLINE RELEASE PROFILES FROM SA DS 2.142 AND EC TABLETS IN PHOSPHATE BUFFER, 0.1 N HCl/ PHOSPHATE BUFFER PH 6.8 AND 0.1 N HCl

Theophylline/SA DS 2.142 tablets showed considerable tablet structure changes, i.e., macroscopic cracks, on the horizontal plane in the middle of the tablets during drug dissolution. The upper part of the tablet was almost separated from the lower part and this effect may be due to incorporation of theophylline into the SA matrix, which could weaken the SA matrix laterally. This phenomenon has also been reported by Pohja *et al* [2].

The new macroscopic cavity formed by cracking, slightly decreased the diffusion path length due to a decrease in tortuosity of pores. The high increase in area available for Fickian diffusion (porosity increase) increases drug release rate from SA matrices.

Van Veen *et al* classified the characteristic changes in physical appearance during dissolution into three different groups: tablets that maintained more or less their appearance, but cracks appeared in the tablets, tablets that exhibit surface erosion and tablets that disintegrate rapidly during dissolution [35].

In the present study, tablets containing 25% (w/w) and 30% (w/w) theophylline maintained more or less their appearance but cracked with some surface erosion. However, surface erosion was observed for tablets containing 40% (w/w) theophylline with some cracks appearing in the tablets. Tablets containing 25% (w/w) theophylline in SA DS 0.672 and MCC (Avicel PH 101) disintegrated rapidly during the dissolution studies and the release profiles coincided with the observed tablet behaviors in the dissolution medium.

Disintegrating SA DS 0.672 tablets released the total drug content rapidly, whereas those of SA DS 2.142 that showed only cracking and/or erosion displayed sustained release of the drug.

After the dissolution tests, the structure of the hydrophobic matrix tablets showed a distinct solvent front. The outer layers of the tablets consisted of various channel sizes, where the drug was completely dissolved, compared to the core of the tablets where the drug was not dissolved.

Similar results were reported by Pohja *et al* [2]. They pointed out that water penetration into tablets would increase the effective dissolution area and the cavities left by dissolution of dispersed drug particles together with the initial voids in the matrix allow diffusion of drug from the matrix tablets.

The drug release parameters and the statistical estimates are indicated in **Table 7**. The statistical estimates of the drug release data (Table 7) indicates that the best linearity was obtained for Higuchi model (R^2 > 0.99). Different studies reported the attenuation of the release rate from matrix tablets that follow the Higuchi drug release pattern.

As indicated previously, the primary reason for the continuously decreasing rate of drug release is the increasing distance that must be traversed by water and drug molecules into, and out of, the tablet, respectively.

When a matrix tablet is placed in the dissolution medium, the initial drug release occurs from the tablet's outer surface and, consequently, the release rate is relatively fast.

As time passes, the external layers of the tablet become depleted of the drug and water molecules must travel through long, tortuous channels to reach the drug remaining in the deeper layers of the tablet.

Similarly, the drug solution that is formed within the tablet must diffuse through long capillaries to reach the external dissolution medium [36].

TABLE 7: DRUG RELEASE PARAMETERS AND STATISTICAL ESTIMATES OF THE ZERO ORDER, FIRST ORDER, HIGUCHI, KORSMEYER-PEPPAS AND HIXSON-CROWELL MODELS FITTED TO DISSOLUTION DATA

Models		Formulation I	Formulation II	Formulation III	Formulation VI
Zero order	R^2	0.9589	0.9569	0.9514	0.9828
	K (h^{-1})	3.724	5.162	9.127	3.394
First order	R^2	0.9854	0.9889	0.9969	0.9943
	K (h^{-1})	0.0560	0.0681	0.1089	0.0489
Higuchi	R^2	0.9978	0.9973	0.9984	0.9918
	K ($h^{-1/2}$)	15.250	17.631	23.588	13.688
Korsemeyer-Peppas	R^2	0.9979	0.9947	0.9973	0.9937
	n value	0.4899	0.5364	0.6369	0.5112
	K (h^{-n})	15.6350	15.6566	16.9746	13.2495
Hixson-Crowell	R^2	0.8745	0.8704	0.8439	0.9156
	K ($h^{-1/3}$)	0.136	0.161	0.211	0.131

The drug release diffusional exponent (n) obtained from Korsemeyer-Peppas model varied between 0.4899 - 0.6369 for different theophylline/SA DS 2.142 ratios and the goodness of the fit was > 0.99 in each case. The drug release kinetics of the formulations deviated considerably from Fickian diffusion (n = 0.45). The hydrophobic SA DS 2.142 matrix tablets were cracked laterally during dissolution tests, which increased porosity and the available surface area for dissolution thereby decreasing the tortuosity, and consequently, caused the deviation from Fickian diffusion release. Similar findings were reported from other studies [2, 35].

The dissolution profiles plotted in different scales (i.e., normal time and the square root of time) resulted in a non-zero y-intercept due to immediate release of drug present at the surface of the tablets. The burst effect existed for the formulations at the beginning of the dissolution process (0 - 1 hr). The compacts were matrix tablets compressed from a binary mixture of drug and SA or EC and the drug particles from the surface of the tablet were able to dissolve without any control by the polymers. This is also believed to contribute for the deviation from Fickian diffusion. These results agreed with those of Pohja *et al* and Korhonen who reported that both diffusion and erosion mechanisms exist when SA is used as a release controlling excipient [2, 21].

CONCLUSION: The physicochemical properties of the modified enset starches differed from those of the native starch and were related to the degree of acetyl substitution. As the degree of acetyl moiety increased, SA became more hydrophobic and formed mechanically stronger tablets than the unsubstituted starch. The drug release also changed gradually from rapid release in tablets with low DS to slow release in tablets with high DS. The drug to SA ratio and the type of dissolution medium were found to influence the rate of drug release. The acetylated enset starches possessed powder properties appropriate for direct compression tableting process and for sustained drug release. Thus, SA is potentially useful as multifunctional excipient, and it may be useful for novel formulation design as directly compressible matrix-forming agent.

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