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SYNTHESIS AND CHARACTERIZATIONS OF WATER SOLUBLE ACETAMINOPHEN STARCH CONJUGATE WITH METHYLENE CARBONYL BRIDGE AND IMPROVED SOLUBILITY

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ABSTRACT
Acetaminophen starch conjugate was synthesized by the reaction of acetaminophen and starch polymer via a spacer bridge. The solubility of acetaminophen starch conjugate was compared to the corresponding untreated acetaminophen in simulated stomach fluid (SSF) and simulated intestine fluid (SIF) at simulated body temperature. The structures of the acetaminophen starch conjugate and untreated acetaminophen were determined by FTIR, ^{1}H-NMR and ^{13}C-NMR. The thermal stability of the polymers was determined by TGA and the surface morphologies were characterized by SEM.The thermal stability of acetaminophen was retained even after conjugation.The esterification reaction between acetaminophen and starch causes the destruction of the matrix, with the modification of their size, shape and texture. The acetaminophen suffers destruction as it interacts with starch, resulting in the formation of different types of chemical bonds as illustrated by SEM analyses. The synthesized acetaminophen starch conjugate has improved solubility in SSF and SIF when compared to untreated acetaminophen. Conjugation of acetaminophen with starch can potentially be used to enhance the solubility and bioavailability of acetaminophen (Paracetamol) in the human system.
Key Words: Acetaminophen, starch, conjugate,methylene carbonyl bridge, drug delivery,

INTRODUCTION

Oral delivery is one of the popular routes for administration of drugs (Zamani *et al.*, 2017).The two critical stages for generating a therapeutic outcome are sufficient drug solubility in the gastro intestinal fluids and high permeability across the epithelial cells lining the gastro intestinal tract. Majority of the drugs coming through the drug discovery pipeline have limited aqueous solubility due to the deployment of non-aqueous solvents in the screening of drug libraries. Thus, low solubility limits absorption and results in low bioavailability. As a result, enhancement of solubility and dissolution for poorly water soluble drugs remain one of the most significant challenges facing the pharmaceutical industries (Gupta *et al.*, 2009; Khan *et al.*, 2012).

Paracetamol (acetaminophen) is a common analgesic and antipyretic drug with minimal side effects. It is freely soluble in ethanol (95%) and acetone; sparingly soluble in water, very slightly soluble in dichloromethane and ether. The drug suffers from poor bioavailability because of its poor aqueous solubility (Majid *et al.*, 2009).

Advances in polymer science have led to the development of novel drug delivery systems. These have explained the general properties and applications of different water soluble polymers, drug polymer conjugates, block co-polymers and hydrogels in the formulation of different dosage forms, novel delivery systems and biomedical applications (Gowda and Betagari, 2011).

Existing researches in polymer chemistry have explored various applications in drug delivery and related biomedical applications. Natural polymers, especially those derived from plant sources are currently receiving growing interest in this regard. These could be attributed to their relative abundance, low cost, biodegradable and eco-friendly profiles (Bhatia, 2016).

Orally administered drugs have been reported to face the problem of low water solubility, low permeability, and less retention in the bloodstream leading to unsatisfactory pharmacokinetic profile. Degraded and oxidized hydroxyethyl starch (HES), a highly biocompatible semi synthetic biopolymer, was reported to have been used as a drug carrier to overcome the solubility and permeability problems. The HES was coupled with synthesized N-arylsulfonylbenzimidazolones by creating an amide linkage between the two species. The coupled products were reported to possess a chemical bond between the biopolymer and the N-arylsulfonylbenzimidazolones. The coupled products show aninvivo antidiabetic potential on male albino rats (Abbas *et al.*, 2016). In the present work, we wish to report the synthesis of

acetaminophen starch conjugate through esterification of acetaminophen with cassava and yam starch. The products were characterized using various spectroscopic and analytical techniques. The solubility of the acetaminophen starch conjugate was tested in simulated stomach fluid and simulated intestine fluid.

EXPIREMENTAL

MATERIALS

Fresh native cassava and yamtubers were purchased from Rimi Market, Kano, Nigeria. Pure acetaminophen powder was supplied by Kano state Drugs Management Agency. Ethanol (99%, BDH), Hydrochloric acid (96%, Sigma-Aldrich), Potassium Hydroxide (99.5% , BDH), Monochloroacetic acid (99.5% , BDH), Sodium Hydroxide (98% , LovaChemie PVT LTD). All chemicals used are of analytical grade and used without further purification.

Analyses of Samples

^{1}H and ^{13}C NMR spectral data were obtained using 500 MHz NMR machine (Joel 1500 model). Chemical shifts were recorded in ppm using tetramethylsilane (TMS) as reference and DMSOas solvent. IR spectra were recorded in wavenumbers (cm^{-1}) using CARY 630 FTIR AGILENT spectrometer. Thermal stability of the products was investigated using thermogravimetric analysis (TGA) (Perkin-Elmer TGA 7 US), at a heating rate of 10^0C up to 700^0C under a nitrogen atmosphere. Sample morphologies were studied using a scanning electron microscope, JEOL JSM6610LV SEM.

Extraction of starch

Cassava andyam tubers were separately cleaned with fresh water, peeled, cut into smaller pieces, and crushed in a blender with addition of distilled water and NaCl. Each mixture was stirred using stirring rod and then filtered with nylon fabric. The starch was allowed to settle for -hours and the solution was separated by decantation.Each starch sample was air dried at room temperature for 48-hours.

Determination of the Intrinsic Viscosity and Molecular Weight (M_v) of Starch

The intrinsic viscosity was determined according to a published procedure (Markin, 2014). In this method, various concentrations (0.2, 0.4, 0.6, 0.8, and 1.0) % w/vof Cassava, and Yam starch in 10:90 v/v water and dimethyl sulfoxide (DMSO) solvent mixture contained in 250ml volumetric flask were prepared. The mixtures were separately placed in a rotatory shaker for 24 hours and filtered using filter paper. The pure solvent and polymer solution (filtrate) were separately poured into Ostwald Viscometer to the mark-point and filled with air to the upper mark-point. The flow time of pure solvent from upper marked-point to lower marked-point was recorded as t_o, the flow time of various concentrations of starch were also recorded as t_i i.e. ($t_{0.2}$, $t_{0.4}$, $t_{0.6}$, $t_{0.8}$ and $t_{1.0}$) respectively. The Relative Viscosity (η_r), Specific Viscosity (η_{sp}) and Reduced Viscosity (η_{red}) were calculated.

Synthesis of 2-Oxy-Acetanilido-Acetic Acid

The synthesis of 2-hydroxy acetanilido acetic acidwas conducted using an earlier published procedure (Leven *et al.*, 1989).Acetaminophen powder (30g, 0.987mol) was dissolved in 100ml ethanol and potassium hydroxide solution (prepared by dissolving 11.2g, 0.28mol in 50ml of distilled water). A trace amount of potassium iodide was added and the mixture was heated with stirring, while monochloroacetic acid (16g, 0.118mol) was added slowly. The reaction mixture was refluxed for eight hours and then the solution was concentrated in a water bath. The isolated solid product was washed twice with ether, filtered off and dried under vacuum at 25^0C.

Synthesis of Acetaminophen Starch Conjugate

Acetaminophen starch conjugate was prepared using an earlier published procedure Jing *et al.*, 2012. Dried starch (10g, 0.061mol) was added to 500ml distilled water contained in a 1L three necked round bottom flask equipped with a magnetic stirrer. The mixture was purged with nitrogen andheated at 100^oC with continuous stirring, until a clear viscous solution was obtained. 2-oxyacetanilidoacetic acid (12.83g, 0.061mol) was added to the mixture, the reaction was allowed to stand for 6 hours. It was then allowed to cool to 25^oC. The product was precipitated by the addition of absolute ethanol under vigorous shaking. The precipitate was washed further with ethanol. The product was concentrated, and oven dried in a vacuum at 40^0C and ground in to fine powder using motar and pistle followed by sieving.

Determination of Degree of Substitution (DS) of Acetaminophen Starch Conjugate

The degree of substitution was determined using a titration method (Varavini*et al.*, 2001). A sample of acetaminophen starch conjugate (1g/0.003mol) was accurately weighed and dispersed in 30ml of distilled water, 15ml of 0.5M NaOH solution was also added. The mixture was vigorously stirred at room temperature for 4-hours. Excess NaOH solution was then titrated to pH 7 with 0.1 M HCl solution.

The degree of substitution (DS) is the average number of hydroxyl groups replaced by 2-oxy-acetanilido acetic acid in an anhydrous glucose unit (AGU), as calculated using the following equation. Where; -

$$D_S = 162\,M\ \frac{(V_o - V)}{1000\ W}$$

- V_o is the volume in ml of 0.1M HCl solution used for titrating the blank
- V is the volume in ml of 0.1M HCl solution used for titrating the sample.
- M is the Molarity of HCl
- W is the weight of sample in grams
- 162 is the molecular weight of anhydrous glucose unit in starch molecule.

Anhydrous glucose unit is a unit of polymer consisting α-D-(1→4) glucosidic bonds and (1→6) forming branch-point.

2.6 Determination of Percent Acetylation

This was determined according to a published method (Eloma*et al.*, 2004), using the relation as follows:-

$$\%\ \text{Acetylation} = \frac{(Vo - V)\text{ml} \times M\ \text{of Hcl} \times 0.209}{\text{Sample Weight (g)}}$$

0.209 = miliequivalent of the acetyl group.
Other parameters were explained in section 2.5

Solubility Test

The solubility of acetaminophen starch conjugate in contrast to the solubility of untreated acetaminophen in aqueous medium was determined at two different pH ranges (i.e 4.5 average pH of stimulated stomach fluid and 6.7 average pH of the stimulated intestine fluid) (Roger and Ake, 1999).

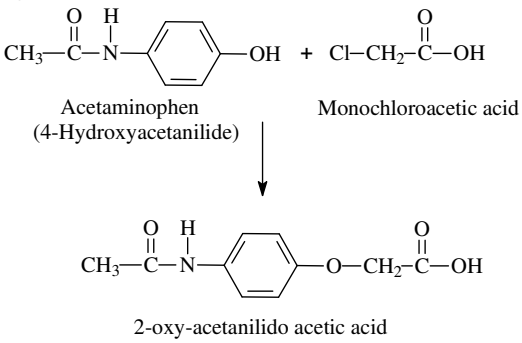
A 0.3g of sample of each acetaminophen starch conjugate (i.e. acetaminophen cassava starch conjugate and acetaminophen yam starch conjugate) were weighted accurately and transferred into 250ml conical flask. 100ml acidified water with pH 4.5 & 6.7 were separately added to each sample. The solutions were shaken at 32.5 ^{o}C in a closed shaker for 24 hours. The solutions were removed and allowed to settle for 4 hours with no agitation.A sample of the clear saturated solution (10ml each)were transferred with a clean syringe into a pre-weighed vials with the mass Mv. The mass of the vials with the saturated solution Mvs was also measured. The vials caps were removed and the solvent was allowed to evaporate in an air oven at 50^{0}Cfor 1- week. All vials caps and vials were marked concurrently to prevent replacement. With only solid remained in the vials, the temperature was raised to 100^{0}C.After 24-hours, the vials were removed and allowed to cool to room temperature, the mass of the residue together with the vials were measured as Mvdr. All measurements of vials were carried out together with vials caps.

The solubility was express in gram of solid per kilogram of solvent as follows: -

$$C_S = 10^3\ \frac{(M_{vdr} - M_v)}{(M_{vs} - M_{vdr})}$$

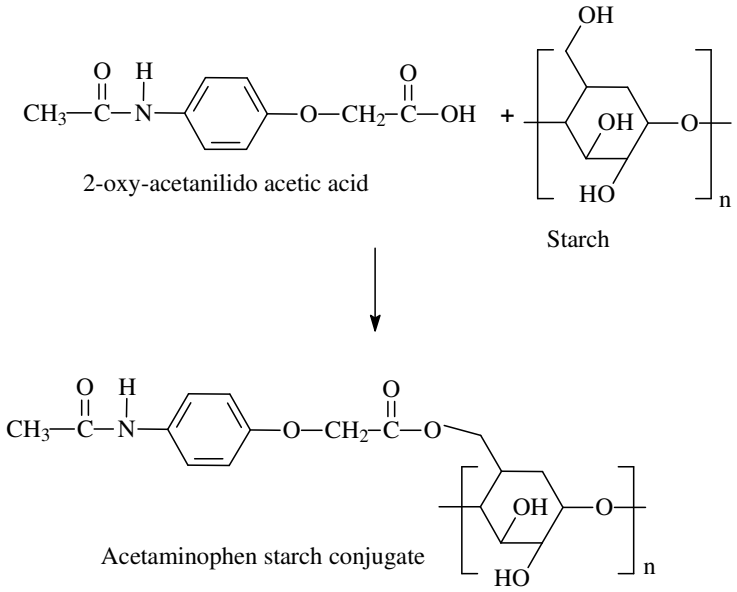
RESULTS AND DISCUSSION

Synthesisof Acetaminophen Conjugate of Cassava and Yam Starch



Scheme 1: Reaction of Acetaminophen with Monochloroacetic acid

The reaction between 2-oxy-acetanilido acetic acid (intermidiate) with starch was successfully carried out at 100^0C, as illustrated in scheme 2



Scheme 2: Reaction of 2-oxy-acetanilido acetic acid with Starch

Acetaminophen conjugate of cassava starch and yam starch were prepared in high yields from the acetylation of 2-oxyacetanilidoacetic acid with cassava starch and yam starch respectively (Scheme 2). Formation of the expected products was confirmed from the ^{1}H and ^{13}C NMR data discussed below.

Figures 1 and 2 represent the ^{1}H NMR spectra of acetaminophen conjugate of cassava starch and yam starch respectively. In both spectra, the singlet peak at 1.99 ppm is assigned to C*H*$_3$resonance. The peaks between 3.0 ppm to 6.0 ppm are assigned to the various methoxy protons (OC*H* and OC*H*$_2$) present in the acetaminophen starch conjugate. The aromatic protons are observed between 6 -8 ppm, while the singlet peak at 9.7 ppm is due to the amide proton (CON*H*).

In the ^{13}C NMR spectra of acetaminophen conjugate of cassava starch (Fig. 3) and yam starch (Fig. 4) the peak at 25 ppm is assigned to the methyl (*C*H$_3$) carbon. The methoxy carbon (O*C*H) peaks are observed between 60 - 100 ppm. The peaks between 115 – 131 ppm are due to the C=C in the benzene ring. The peaks between 165 – 175 ppm are assigned to the C=O resonace of ester and amide groups.

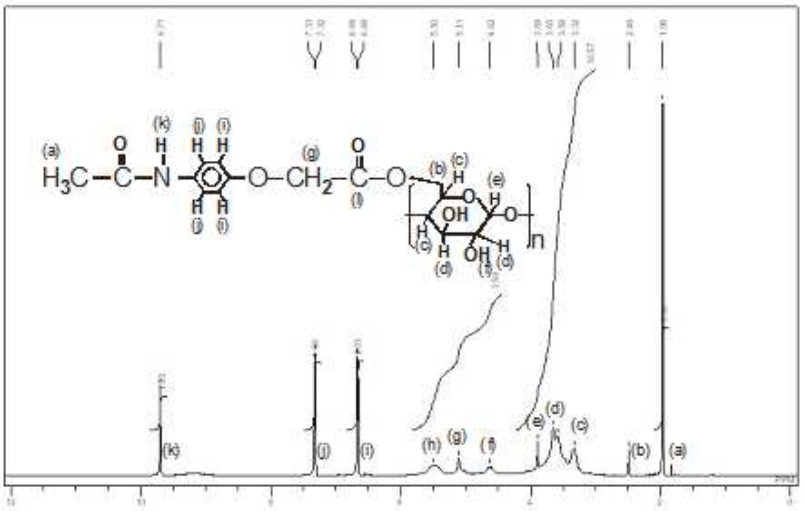


Fig. 1: ^{1}H NMR spectrum of Acetaminophen Conjugate Cassava Starch

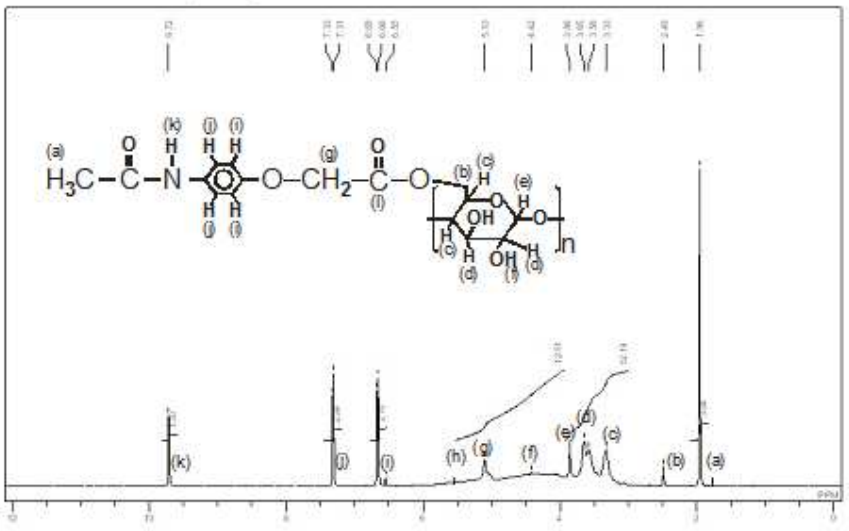


Fig. 2: ^{1}H NMR spectrum of Acetaminophen Conjugate Yam Starch

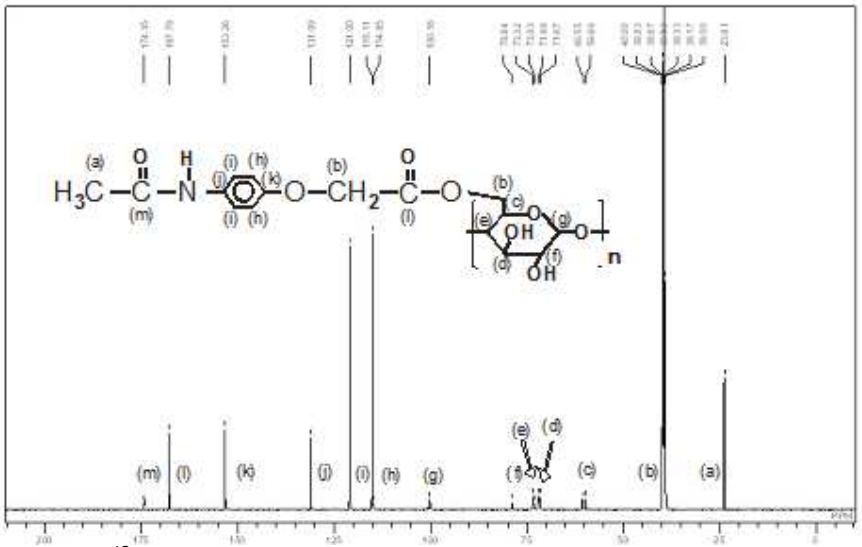


Fig. 3: ^{13}C NMR spectrum of Acetaminophen Conjugate Cassava Starch

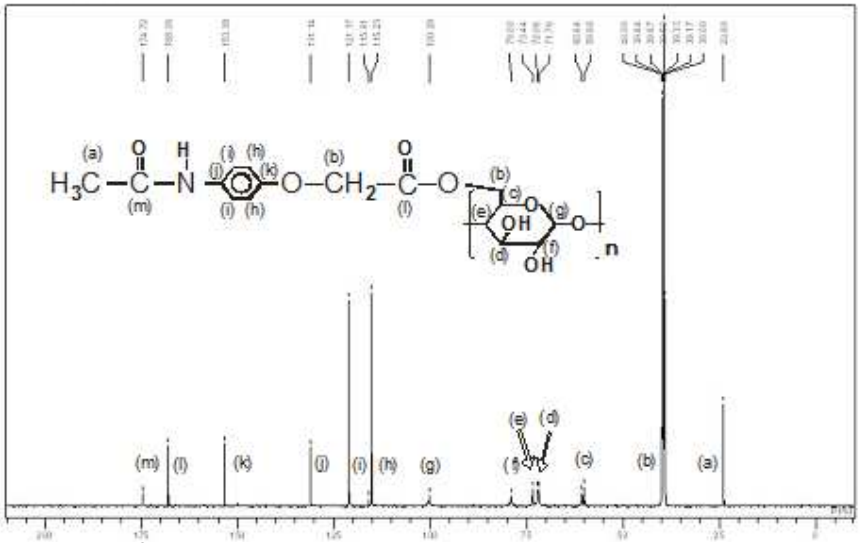


Fig. 4: ^{13}C NMR spectrum of Acetaminophen Conjugate Yam Starch

FTIR RESULTS

Table 1 Represent the Infrared Absorption Bands of Acetaminophen, Chloroacetic Acid, 2-Oxy-Acetanilido Acetic Acid, Native Starch and Acetaminophen Starch Conjugate.

Functional Group	Acetaminophen (cm^{-1})	Chloroacetic acid(cm^{-1})	2-oxy- acetanilido-acetic acid (cm^{-1})	Native Starch (cm^{-1})	Acetaminophen Conjugate Starch(cm^{-1})
O—H	3322 str	2568	3322	3293	3324
C—O	1175	1289 1175	1225	1078	1227
C-O-C	-	-	1110	1153	1110
C—H	2880 str 3110 aro	2959	2810 str 3108 aro	2929 str 1423 ben	2928 str
C=O	1706	1709	1709	-	1737
C=C	1652 aro	-	1652		1611
N—H	3445 str	-	3445	-	3448
C—N	1220	-	1017		1017
C—C	1110	1199	1110	996	1080
C—Cl	-	786	-	-	-

Intrinsic Viscosity and Molecular Weight

A plot of reduced viscosity against concentration (Figure 5)was made which was extrapolated to Y-axis and the point of the intercept was the intrinsic viscosity [η] obtained as a major parameter used to calculate molecular weight of the starch polymer.The molecular weight of thepolymer solution was calculated using the Mark-Houwink equation which relates the intrinsic viscosity and molecular weight of polymer as follows;-

$$[\eta] = KM^{\alpha}$$

$$\text{Log}[\eta] = \text{Log } K + \alpha \text{LogMw}$$

$$\alpha \text{LogMw} = \text{Log}[\eta] - \text{Log } K$$

$$\text{LogMw} = \frac{\text{Log}[\eta] - \text{Log } K}{\alpha}$$

$$\text{Mw} = \text{Anti Log } \left(\frac{\text{Log}[\eta] - \text{Log } K}{\alpha}\right)$$

The intrinsic viscosity of yam and cassava starch were 0.39, 0.44 and 0.54 repectivelyas presented in Figure 6.The molecular weight of Yam and Cassava starch were calculated using Mark-Hauwink equationand the valueswere found to be 2.61 x 10^6, 3.79 x 10^6 and 7.37 x 10^6g/mol respectively.

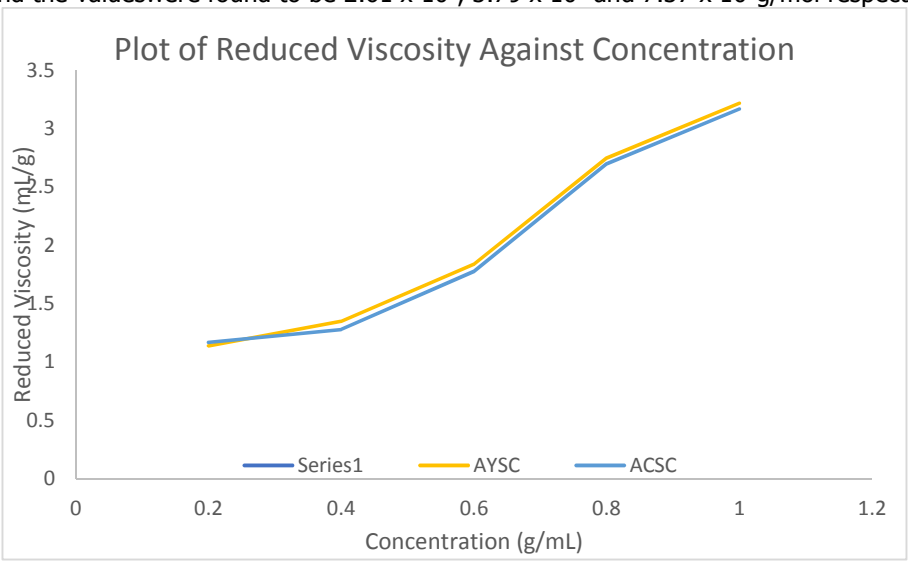


Fig. 5: Intrinsic Viscosity of Acetaminophen starch conjugate

Percent Acetylation And Degree of Substitution (D.S)

The reaction between untreated Acetaminophen powder and monochloroacetic acid was done at 100^0C in the presence of potassium hydroxide and trace amount of potassium iodide using ethanol and water as solvent.The product obtained was 2-oxy-acetanilido-acetic acid (intermediate) as illustrated in scheme 1

The % acetylation with respect to acetaminophen cassava starch conjugate and acetaminophen yam starch conjugate were found to be: 28.0 and 24.9 respectively.Concurrently, with respect to degree of substitution it was found to be 0.217 and0.193 respectively. This may be due to the differences in their molecular weights.

Results of Thermogravimetric Analysis (TGA)

The thermogravimetric curves of the untreated acetaminophen and acetaminophen conjugates of yam starch and cassava starch are shown as figures 6and 7 respectively. The decomposition temperature of acetaminophen starch conjugates of cassava and yam were found to be 235^oC and 245^oC respectively. This implies that the differences in their thermal stability is very negligible and concluded that the thermal stability of unmodified acetaminophen which is 250^oC is still retained even after the conjugation.

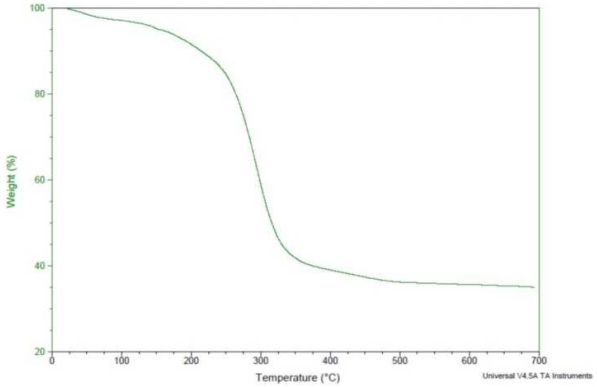


Fig. 6: TGA Curve ofUntreated Acetaminophen.

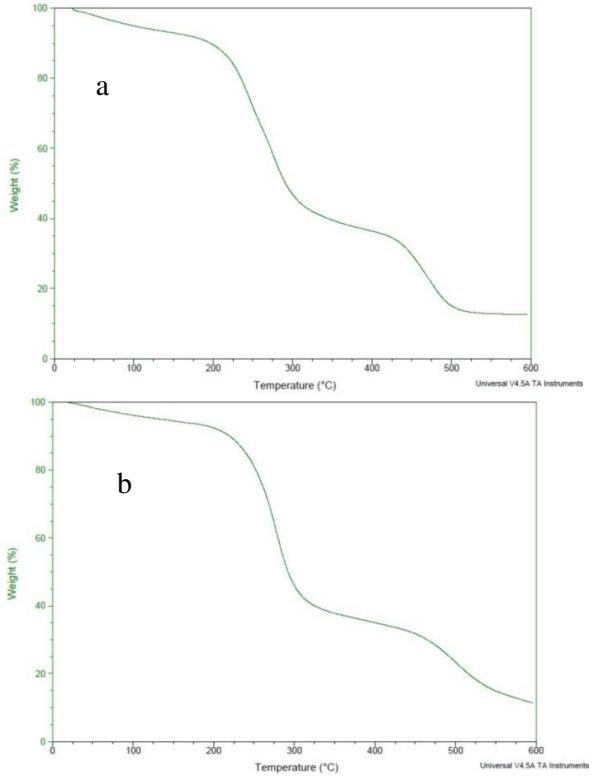


Fig. 7: TGA Curve of (a) Acetaminophen Yam Starch Conjugate. (b) Acetaminophen Cassava Starch Conjugate.

Scanning Electron Microscope (SEM)

Figure 8(a) shows the scanning electron micrograph of untreated acetaminophen, it shows particles in forms of granules which are long, spherical and rectangular but also shows some deformed pyramids and other truncated forms. Figure 8 (b and c) shows the micrographs of acetaminophen starch conjugates.

The reaction between acetaminophen and starch causes the destruction of the matrix, which is evidence of the modification of their size, shape and texture. The acetaminophen suffers destruction as it interacts with starch. The size of the agglomerates in the conjugate reveals different types of intergranular cohesion which may be due to the changes in functional group and this result in the increase in hydrogen bonding, and results in the increase in the solubility of the conjugate in aqueous medium.

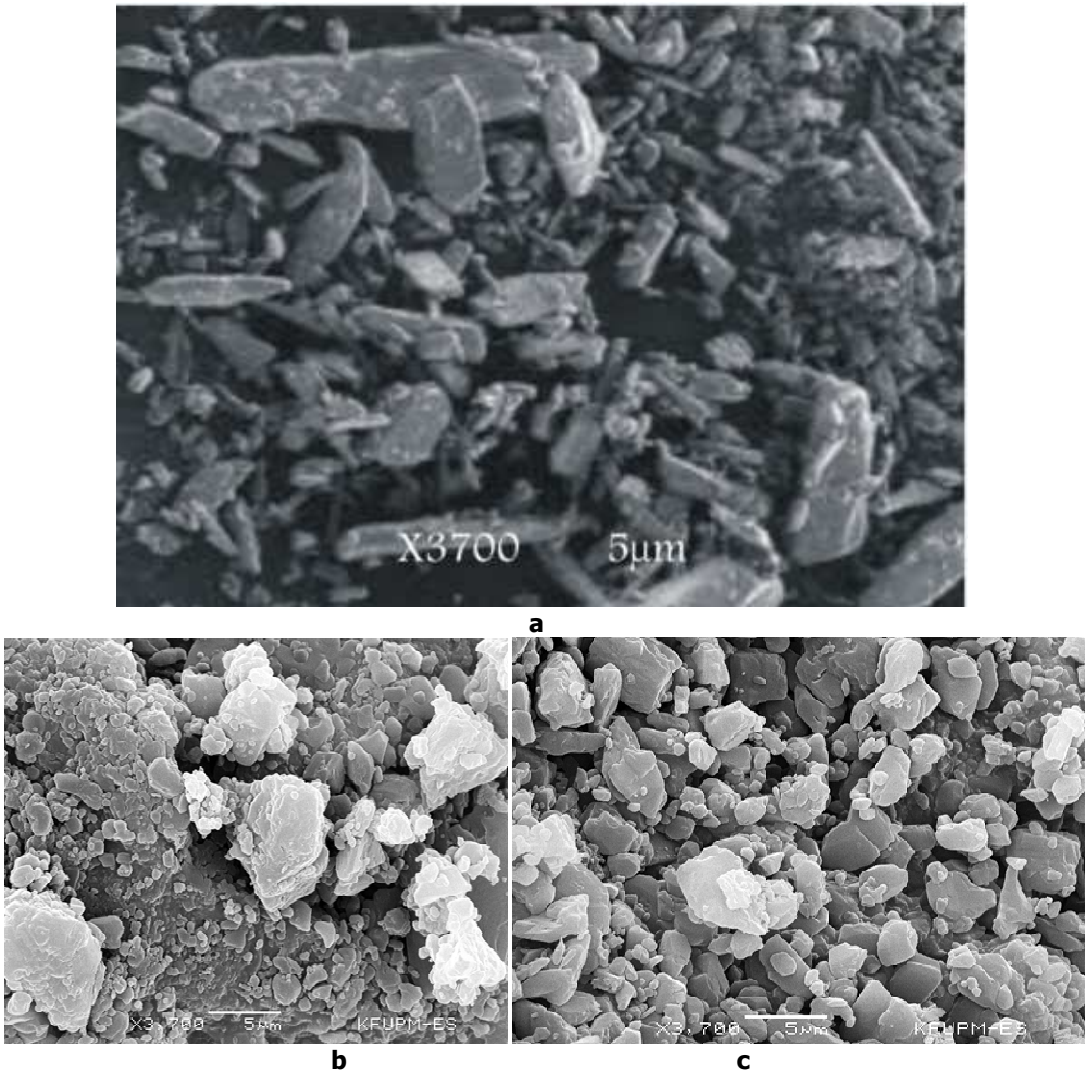


Fig. 8: Morphology of (a) untreated acetaminophen (b) acetaminophenyam starch conjugate (c) acetaminophen cassava starch conjugate

Solubility Test

The following chart indicate the solubility of untreated acetaminophen, acetaminophen cassava (ACSC)and yam (AYSC) starch conjugates respectively, expressed as g solid / Kg solvent.

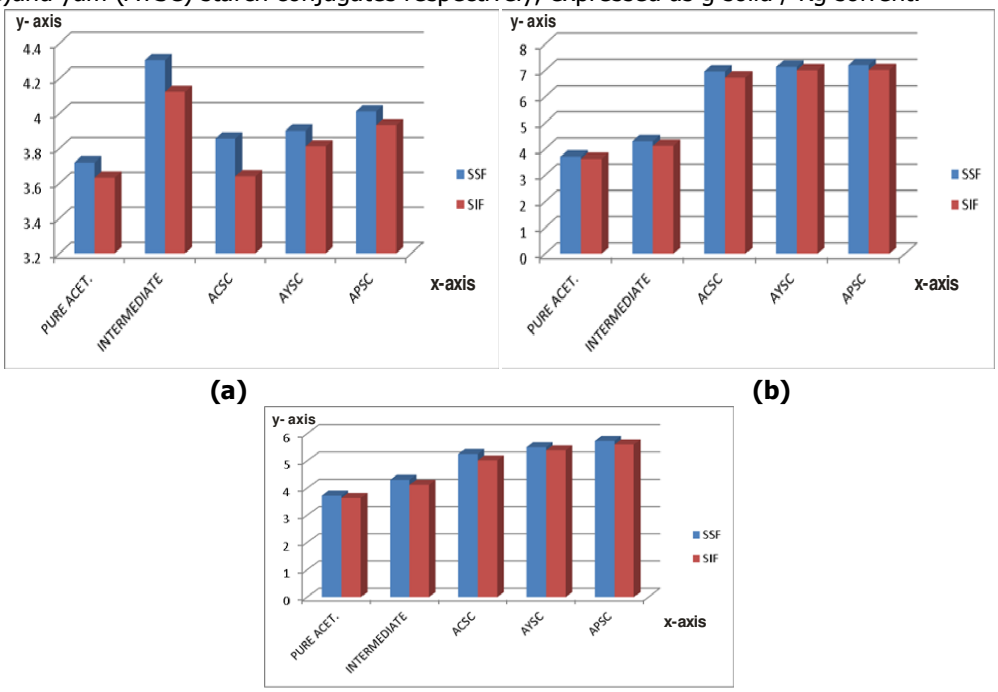


Figure 9: Solubility of acetaminophen: starch (a) 3:1 reaction ratio (b) 2:1 reaction ratio (c) 1:1 reaction ratio

SSF =Average pH of simulated stomach fluid is 4.0
SIF = average pH of simulated intestine fluid is 6.7

The above results shows that 3:1 reaction ratio has the highest solubility followed by 2:1, then 1:1reaction ratio. This may be due to the presence of highly electron rich centers in 3:1 acetaminophen: starch reaction ratio that can participate in formation of hydrogen bonding as the key parameter for solubility in aqueous medium.

CONCLUSION

The synthesis of acetaminophen starch conjugate was achieved by the reaction of Cassava and Yam starch with derivatized acetaminophen. The thermal stability of acetaminophen was retained after conjugation. The esterification reaction between acetaminophen and starch causes the destruction of the matrix, with the modification of their size, shape and texture. The synthesized acetaminophen starch conjugate has improved Solubility in SSF and SIF when compared to untreated acetaminophen.

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