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Research Article

Native and Modified *Oryza glaberrima* Steud Starch Nanocrystals: Solid-state characterization and Anti-tumour drug release studies

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ABSTRACT

In a bid to explore new biodegradable sourced materials for drug delivery, *Oryza glaberrima* starch was extracted, modified, and characterized. Nanocrystal starch (NS) and acetylated nanocrystal starch (ANS) were produced from the native starch (S). The physicochemical properties, scanning electron microscopy (SEM), X-ray diffraction (XRD), Differential scanning calorimetry (DSC) and Fourier Transform Infra-red spectroscopy (FTIR) were done. The loading and release studies of Doxorubicin-loaded starches were carried out. The density, solubility, swelling capacity, and hydration capacity of the starch increased after modification whereas the amylose content was reduced. SEM revealed no change in morphology with sizes of the starch granules to be 5-15µm and the shape to be polyhedral. Modification of the starch increased the percentage drug loading capacity (DLC) and loading efficiency (LE). The drug release kinetics of the Dox-loaded S and ANS was first-order release, while Dox-loaded NS was Korsemeyer-Peppas model. Improvement in the physicochemical properties of the NS and ANS made them useful carriers to sustain and control drug release.

INTRODUCTION

Starch is a naturally occurring polysaccharide found as reserves in roots, tubers, fruits, and seeds of some plants. Starch is insoluble, semi-crystalline in nature consisting of two polysaccharides, namely amylose and amylopectin (Olu-Owolabi et al., 2011; Wang and Copeland, 2015). In the food industry, starch has been used as thickening agents in processed food (Saha and Bhattacharya, 2010) and films for food packaging (Odeniyi *et al.*, 2018). Starch has also found useful applications in the pharmaceutical industry as a disintegrants (Odeniyi and Ayorinde, 2014), binders (Manek et al., 2012), microspheres (Okunlola and Ogunkoya, 2015), and films for drug delivery (Jaiyeoba et al., 2013).

However, the use of native starches have been restricted in pharmaceutical and food industries due to the production of tablets with low mechanical properties when used as binders, they have poor processing stability (Choi et al., 1999), low thermal and shear resistance and poor solubility in cold water (Mukerjea et al., 2007). Therefore, there is a need for modification of starches to improve their use in the pharmaceutical and food industries. Starch nanocrystals with varying morphology and sizes have been reported and include nanocrystals from platelet-like shaped waxy maize starch to those from potato starch granules with round and oval shape (Odeniyi et al., 2018). These nano-starches have been reported to have higher functionality compared with native starch with sizes ranging from 40 nm to 100

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nm observed under a transmission electron microscope.

Modifications of starch could be physical, chemical, or enzymatic (Yadav et al., 2013; Alcázar-Alay and Meireles, 2015). Physical modification includes pregelatinization (Wu et al., 2010; Majzoebi et al., 2011; Li *et al.*, 2014), Hydrothermal modification (Ovando-Martinez et al., 2013; Putri et al., 2014; Falade and Aiyetigbo, 2015). Chemical modifications involves acid hydrolysis (Ulbrich et al., 2014), oxidation (Ali and Hasnani, 2014) and acetylation (Kapelko et al., 2013; Zhang et al., 2014). Acetylation of starch has been observed to decrease swelling rate and increase functionality in sustained drug delivery (Xiao et al., 2016).

Modification some of the methods above have reduced the processing time in the production of starch nanocrystals. A combination of ball milling with sulphuric acid hydrolysis has been used for the production of waxy maize nanocrystal starch (Dai et al., 2018). Different studies have been performed on the use of starch nanocrystals (Xiao et al., 2016; Alwaan et al., 2019) and modified starch nanocrystals in drug delivery (Odeniyi et al., 2019).

Drugs are carried into cancer cells by nanoparticles generally by endocytosis and transport the drug to the cytoplasm or perinuclear region and release the drug inside the cells (Prasad et al., 2012). Some studies indicated that nanoparticles can accumulate in many solid tumors at much higher concentrations than in normal tissues or organs by a nonspecific targeting process known as the enhanced permeation and retention effect due to leaky vasculature and limited lymphatic drainage (Maeda et al., 2000; Maeda, 2001).

Oryza glaberrima, also known as African rice, is grown in West Africa as a staple food, but at present, it is being replaced by Asian rice (*Oryzia sativa*) (Linares, 2002). *Oryza glaberrima* starch has been used in the formulation of microspheres and tablets to control or sustain drug delivery (Okunlola and Ogunkoya, 2015; Omoteso et al., 2019). Previous studies have focused on the native starch and minimal comparable work has been done on nanocrystal starch from *Oryza glaberrima*. Hence, in this study, the effect of modification on *Oryza glaberrima* starch nanocrystals on physicochemical characteristics, the drug loading capacity and loading efficiency were investigated. Doxorubicin, an anticancer drug, was incorporated and effect of modification on release profile and kinetics was also determined.

MATERIALS AND METHODS

Ofada rice (*Oryza glaberrima*) was purchased from Bodija Market, Ibadan, Nigeria and the starch extracted from the grains in the Pharmaceutics laboratory of University of Ibadan, Nigeria. Other materials used were Corn amylose and Doxorubicin (Sigma Chemical Co, St Louis, MO, USA), calcium oxalate monohydrate (European Pharmacopoeia Reference Standard), Lugol solution (Fisher Scientific, UK), Acetic anhydride (Fisher Chemical, USA), and Sodium hydroxide (BDH, England). All other reagents used were analytical grade.

Methods

Extraction of Oryza starch

Briefly, the grains were washed with distilled water to remove extraneous matter. The grains were re-washed separately with distilled water and milled until a fine pasty mass was achieved using a blender (Osterizer Dual range Pulse Matic Milling blender (Model 857, John Oster Manufacturing Co., Racine, Wisconsin, USA). The slurry was sieved using muslin cloth to remove the chaff and allowed to settle for 24 hours after which the supernatant was decanted leaving the starch sediment. The sediment was washed continuously for 4 days until the supernatant was observed to be colourless. The water was removed using a muslin cloth and the wet mass dried in a hot air oven (Model DHG-9053A, Ocean Medical England) at 60 ˚C for 48 h. The dried mass of Ofada rice was blended and the dry products were screened through a 120-mesh sieve (125 µm) to obtain a uniform powder which was weighed and stored in separate air-tight containers until required (Okunlola and Ogunkoya, 2015).

Preparation of nanocrystal starch

The method of Xiao et al. (2016) was used for the preparation of nanocrystals. 36.7 g of Ofada starch was mixed with 250 mL 3.16M sulphuric acid at 40^{o}C, stirred with a magnetic stirrer at 100 rpm for 6

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days. It was filtered with a Whatman filter paper size 1 and washed with 10 mL distilled water three times and centrifuged at 8000 rpm for 5 minutes with a Clinical 100 centrifuge (VWR, USA). The centrifuge tube containing the starch was immersed in ice until the temperature reached 10°C. Acetone was added at 10°C and centrifuged again for 15 minutes. The starch nanocrystal (NS) obtained was dried at 40°C for 6 hours (Model DHG-9053A, Ocean Medical, England).

Acetylated starch nanocrystal (ASN) was prepared by adding 100 mL distilled water to 15 g of starch nanocrystal (NS), dispersed by bath sonication (PCI Analytics, India) for 30 minutes at 25°C. 3% sodium hydroxide was added to the suspension to adjust the pH to 8.0 with continuous stirring on a magnetic stirrer (VWR mini stirrer model 12620-998, USA) at a speed of 700 rpm for 1 hour at 25°C. 9% acetic anhydride was added dropwise with stirring while maintaining the pH at 8.0-8.5 over a period of 30 minutes. The reaction was allowed to proceed for 1 hour after adding acetic anhydride. The slurry was adjusted to pH 4.5 with 0.5 M hydrochloric acid. The slurry was centrifuged at 8000 rpm for 5 minutes, and sediment was washed with 10 mL distilled water three times and then with 10 mL 95% ethanol. The ASN was dried at 40°C for 6 hours. The dried starch was passed through a 125 µm sieve, collected and stored in a tightly-closed container for further characterization

Determination of degree of substitution

The method of Ayucitra (2012) was used with slight modification. 0.5 g of ANS was suspended in 25 mL 75% ethanol solution. The slurry was kept in a water bath at 50°C for 30 minutes with constant stirring. The slurry was cooled at room temperature and 20 mL of 0.5M sodium hydroxide was added. The slurry was allowed to stand for 72 hours at room temperature with occasional swirling. The excess alkali was titrated with 0.5M hydrochloric acid using phenolphthalein as indicator. Starch was used as blank. The determination was done in duplicate. The native starch was used as blank. The degree of substitution was calculated using the equation:

$$\%\ Acetyl = \frac{(Blank - Sample)ml \times M_{HCl} \times 0.043}{Weight\ of\ sample} \times 100 \quad (1)$$

$$DS = \frac{162 - \%Acetyl}{4300 - (42 \times \%Acetyl)} \quad (2)$$

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

Determination of densities

The bulk density of the starches was determined by weighing 5 g of starch, poured into a 25 mL cylinder, and tapped once. The bulk volume was noted, and the bulk density was calculated using the equation

$$bulk\ density = \frac{Weight\ of\ starch\ (g)}{Volume\ occupied\ (mL)} \quad (3)$$

The determination was done in triplicate.

The tapped densities were determined by tapping the cylinder 100 times, and the volume occupied was noted.

Determination of Hausner's ratio and compressibility index

Hausner's ratio was determined as the ratio of tapped density to bulk density. Compressibility index was obtained from the bulk and tapped densities using the equation below:

$$compressibility = \frac{Tapped\ density - Bulk\ density}{Tapped\ density} \times 100 \quad (4)$$

Determination of pH

1% w/w starch slurry was prepared, and the pH was determined with a VWR pH meter (VWR Symphony H10P, USA) at room temperature.

Determination of Hydration capacity

Hydration capacity was determined according to the method of Kornblum and Stoopak (1973). A 1 g of starch was weighed into a centrifuged bottle and mixed with 10 mL distilled water with intermittent stirring for 10 mins. The slurry was centrifuged at 1000 rpm for 10 mins, and the supernatant was discarded. The starch remaining was weighed, and hydration capacity was calculated using the equation

$$\%\ hydration = \frac{weight\ of\ the\ dry\ starch}{weight\ of\ sedimented\ starch} \times 100 \quad (5)$$

Determination of swelling power and solubility

1% w/w slurry of each type of starch was heated on a water bath at 50°C for 30 mins. The slurry was

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centrifuged at 1500 rpm for 20 mins, and the supernatant was collected and dried in a hot air oven at 120°C for 24 hours. The residue was weighed to calculate the solubility. The weight of wet starch was calculated to determine the swelling power.

Determination of moisture content

A clean dry glass petri dish was weighed and 2 g of the sample was weighed and dried at 105˚C for three hours. The percentage moisture content was calculated from the expression:

$$\% \text{ moisture content} = \frac{W_i - W_f}{Wi} \times 100 \quad (6)$$

Where W_i= initial weight of the sample and W_f = final weight of the sample.

Determination of Amylose and Amylopectin content

Amylose determination was by the iodo-colorimetric method of Juliano (1971) and Williams *et al.* (1970). About 0.1 g of starch was weighed into 50 mL test tubes. 1mL of 95% ethanol was added to the samples. 9 mL of 1*N* NaOH was added and heated on a boiling water bath for 10 min. From this extract, 1 mL was taken and made up to 10 mL with distilled water. 0.5 mL aliquot was taken into test tubes from this solution and assayed by the addition of 0.1 mL 1*N* acetic acid and 0.2 mL of Lugol solution. The solution was diluted to 10 mL with 9.2 mL of distilled water and left for 20 min for color development. The absorbance was read at 620 nm (wavelength) on a spectrophotometer (Milton Roy Spectronic 601). The amylose content was calculated from a calibration curve obtained from solutions of different amylose/amylopectin concentrations. Corn amylose (Sigma Chemical Co, St Louis, MO, USA) was used as standard. Triplicate amylose content measurements were performed on each sample. Amylopectin content was calculated as follows:

$$\% \text{ Amylopectin} = 100 - \% \text{ Amylose} \quad (7)$$

Thermal analysis of starch

Thermogravimetric thermal analyses were performed in a Netzsch STA 449 F3 Jupiter Simultaneous Thermal Analysis (STA) instrument (Germany). Each sample was placed into a 25 µL Aluminum pan of an approximate weight of 38 mg. Pan was cold-welded with a lid, and a small pin-hole was opened on the top of the lid in order to have the released gases escape the system when the temperature is increased. All samples were measured under ultra-high purity Helium gas (flow of 50 mL/min) from room temperature up to 800 °C. The temperature was increased at a rate of 10°C/min. TGA and DSC signals were measured simultaneously. The buoyancy effect for Helium was corrected by measuring the empty Aluminum pan under the same measurement conditions used for the samples. The performance of the thermobalance in the STA was verified by using a certified sample of calcium oxalate monohydrate (European Pharmacopoeia Reference Standard) up to 1000 °C.

X-ray diffractometry

Powder X-ray diffraction of the native and modified starches was measured at room temperature on a STOE powder diffractometer (STADI MP, STOE & Cie GmbH, Germany) equipped with an asymmetric curved Germanium monochromator (CuKα1 radiation, λ = 1.54056 Å) and one-dimensional silicon strip detector (MYTHEN2 1K from DECTRIS). The line focused Cu X-ray tube was operated at 40 kV and 40 mA. The powder was sandwiched between two acetate foils (polymer substrate with neither Bragg reflections nor broad peaks above 10 degrees) and measured in transmission geometry in a rotating holder. Intensity data from 5 to 90 degrees two theta were collected over a period of 60 mins. Instrument was calibrated against a NIST Silicon standard (640d) prior to the measurement.

Scanning electron microscopy

Scanning Electron Microscopy was performed on a Hitachi S3400N-II scanning electron microscope. Samples were mounted on a metallic stub using carbon tape, and images were taken at room temperature and under vacuum.

Fourier transform infra-red

The FTIR analysis was done to identify the functional groups present in the starches. A small quantity of starch was placed on the diamond crystal of the FTIR machine (100 FTIR series, Perkin Elmer, MA, USA) The sample was examined for FTIR spectra in the range of 4000-400 cm^{-1} at 64 scans.

Preparation of calibration curve of Doxorubicin

Doxorubicin at different concentrations ranging between 0.01-0.12 mg/mL was prepared by

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dissolving 12 mg doxorubicin in 100 mL 0.01M phosphate buffer solution (PBS) pH 7.4. Serial dilution was done to obtain the different concentrations. The absorbance was determined with a UV-spectrophotometer at 480 nm wavelength. The standard plot was obtained by plotting the absorbance against concentration.

Drug loading studies

150mg of the different starch samples were suspended in 10 mL of 1.0mg/mL drug (doxorubicin) solution in distilled water and then incubated for 1 hour. The suspensions were centrifuged and washed with distilled water. The amount of drug in the supernatant was assayed using UV-visible Spectrophotometer according to the standard curves of the drug absorbance to concentration (Xiao et al., 2016). The Drug loading content (DLC) and the loading efficiency (LE) were calculated using the following equations:

$$\%\text{DLC} = \frac{\text{wt of drug in starch sample}}{\text{wt of starch sample}} \times 100 \quad (8)$$

$$\%LE = \frac{wt\ of\ drug\ in\ the\ starch\ sample}{Initial\ wt\ of\ drug\ in\ loading\ solution - wt\ of\ starch\ sample} x100 \quad (9)$$

In vitro release studies

Dox-loaded starches were air dried and encapsulated in gelatin capsules, and placed in 100 mL 0.01M PBS (pH 7.4) at a temperature of 37°C in a conical flask and placed on a shaker. 5 mL sample was withdrawn at intervals and replaced with fresh PBS. The absorbance was determined and amount of drug released determined using the standard calibration plot. The release rate was calculated using the equation:

$$R = \frac{M_1}{M_0} \quad (10)$$

Where M_1 is the cumulative of doxorubicin released at any given time, M_0 is the total amount of doxorubicin loaded in the starch. The release kinetics was determined using DD Solver, a dissolution analytical solver, an add-in programme for Microsoft Excel.

STATISTICAL ANALYSIS

Statistical analysis was carried out using analysis of variance with computer software GraphPad Prism® 4 (GraphPad Software Inc. San Diego, USA). Tukey–Kramers multiple comparison tests were used to compare the effects of modification on the starch properties. At 95% confidence interval, P values less than or equal to 0.05 were considered significant.

RESULTS AND DISCUSSION

Degree of substitution

Polysaccharides such as starches are excellent materials for synthesizing drug carriers as they possess remarkable physicochemical and biological properties. They are also naturally biocompatible, biodegradable and have a low immunogenicity. The presence of the hydroxyl functional group renders them suitable to modifications thereby increasing their functionality. The degree of substitution of the ANS starch was 0.06±0.02. Ayucitra (2012) obtained 0.08-0.12 degree of substitution for corn starch when this method was used. Colussi et al., (2015) obtained DS of 0.05-0.10 for rice starch. The degree of substitution depends on the individual characteristics of the starch and the acetylation condition used (Ayucitra, 2012). Acetylated starch with low degree of substitution (0.01-0.2) has several applications in forming films, thickeners, adherents, stabilisers, texturisers and encapsulation agents (Alcarzar-Alay, 2015).

Density and flow properties

The density measurement of starches is of importance in manufacturing as this has been shown to have a direct effect on the powder flow and compaction of the powder. The result presented in Table 1 showed that the densities of the starch increased with modification: ANS > NS > S. Compressibility index and Hausner ratio are indirect indicators for determining the flow properties of powders, which is essential for direct compression excipients (Odeniyi *et al*., 2017). Literature has shown that the compressibility index, also known as Carr's index, describes the flow properties of powders as excellent (5–15%), good (12–16%), fair (18–21%), and poor (23–28%). Similarly, a Hausner ratio value of less than 1.20 is indicative of good flow, while a value greater than 1.50 indicates poor flow properties (Manek et al., 2012). According to Table 1, the compressibility index of the native and modified starches was above 28%, and the Hausner ratio was above 1.5 for native starch, this indicates the compressibility and flow of all starches were poor.

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however there was slight improvement but it was not significant (p >0.05).

Table 1. *Material properties of native (N), nanocrystal starch (NS) and acetylated nanocrystal (ANS) Ofada starches*

Properties	N	NS	ANS
Bulk density (g/mL)	0.385±0.011	0.412±0.023	0.466±0.019
Tapped density (g/mL)	0.589±0.013	0.590±0.016	0.648±0.004
Compressibility index (%)	34.60±0.42	30.09±3.82	28.06-3.19
Hausner's ratio	1.53±0.01	1.43±0.08	1.39±0.06
pH	4.85±0.05	4.00±0.10	5.30±0.10
Solubility (%)	1.95±0.64	2.63±0.13	3.03±0.44
Swelling Capacity (%)	3.72±0.22	3.93±0.11	3.75±0.18
Water hydration capacity (%)	39.16±3.05	41.24±1.46	47.01±2.02
Moisture content (%)	1.67±0.00	0.89±0.01	0.92±0.08
Amylose (%)	29.31±1.02	17.20±1.22	22.85±1.26
Amylopectin (%).	70.69±2.06	82.80±1.05	77.15±1.42

Solubility, Swelling capacity, and Hydration capacity

The pH of the starches was between 4.0 and 5.3, indicating the starches were slightly acidic. There was no significant ($p>0.05$) increase in apparent solubility of starch with modification in the order of ANS > NS > S, while swelling capacity is in the order NS > ANS > S. However, there was significant increase ($p<0.05$) in the water hydration capacity of ANS. Increase in solubility and swelling capacity after modification of starch has also been reported by other researchers (Olu-Owolabi et al., 2011; Ayucitra, 2012). Acetylation of starch has been shown to improve starch solubility (Berski et al., 2011), and this was observed in our result with an increase in apparent solubility of ANS. It has been suggested that the swelling power of starch is directly related to the amylose/amylopectin content (Tester and Karkalas, 1996; Singh et al., 2003). It was also reported that high amylose content in starch granules reinforced and made them more rigid, hence reduction in swelling (Li et al., 2016).

Moisture content

Moisture content is of importance in powder as it has been known to affect powder flow (Nokhodchi, 2005). The ranking of moisture content was S>ANS>NS. There was significant difference ($p<0.0001$) in the moisture content of the starches. Increase in moisture content caused a decrease in bulk density and increase in compressibility index of the starches.

Amylose and amylopectin content

The amylopectin of the native and modified starches is in the order NS >ANS > S. The result of amylose to amylopectin ratio corroborates the swelling power result. The amylose content for native starch (S) was found to be 29.31%; however, this was significantly higher than that reported by Ali *et al.* (2016) for Kohsar rice starch. This difference could have been due to a botanical source of starch, climatic, and soil conditions during grain development (Sandhu *et al.*, 2008: Ali *et al.*, 2016). for Kohsar rice starch. This difference could have been due to a botanical source of starch, climatic, and soil conditions during grain development (Sandhu *et al.*, 2008: Ali *et al.*, 2016).

Moore et al., (1983) classified starch into three groups according to the amount of amylose content: normal starch (18-30%), waxy starch (about 1%), and high amylose starch (above 70%). According to this classification, both the native and modified starches are normal starch since their amylose content was 17.20-29.31%.

The reduction of amylose content in NS after acid treatment could be attributed to preferential hydrolysis of the amorphous regions of the starch in which the amylose content is located (Jekins and Donald, 1995; Wang and Copeland, 2015).

Scanning electron microscopy

The morphology of the starches was analyzed using scanning electron microscopy, as shown in figure 1. The image showed that the majority of the particles were in the 5-15 µm range and polyhedral in shape, appearing isotropic (no elongated faces). Okunlola and Ogunkoya (2015) also found the shape of native ofada starch to be polyhedral with a mean granule size of 2.20µm. There was no change in the particle size and shape of the particles after modification, which was also observed by different researchers in other starches such as corn starch after acetylation (Ayucitra, 2012), sword bean starch after hydrothermal treatment (Olu-Owolabi, 2011). Although, some other researchers found that there

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was a reduction in particle size after modification of starch (Kong et al., 2012; Ulbrich et al., 2014).

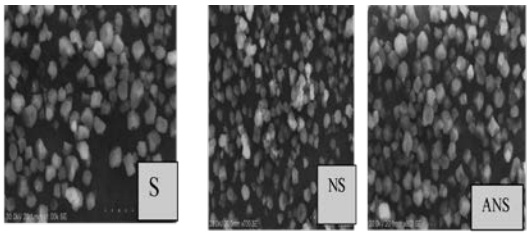

Fig. 1. Scanning electron micrographs of native (S), nanocrystal starch (NS) and acetylated Ofada starch nanocrystals (ANS)

X-ray diffraction analysis

X-ray powder diffraction patterns of the starches are very similar, showing broad diffraction peaks between 10 and 30 degrees two theta (Cu-radiation), Figure 2. The broad nature of the peaks is indicative of the lack of long-range crystallinity in the structure. Crystalline particle analysis using the Scherrer equation gave an approximate crystalline particle size of 70 nm using the first intense peak at ~ 15.2 two thetas. Full Width at Half Maximum of this peak is around 0.17 degrees, and the instrumental broadening is around 0.05 degrees. Width of the diffraction peaks is not uniform, i.e., reflection at around 27 degrees two theta appears much sharper than all other peaks.

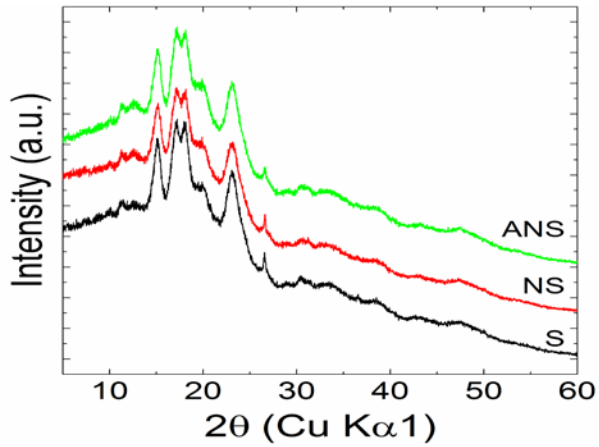

Fig. 2. X-ray diffraction of native (S), nanocrystal starch (NS) and acetylated Ofada starch nanocrystals (ANS)

Thermal Analysis

Weight loss as a function temperature was found to be similar for all samples. Under the Helium environment, weight loss (%) for samples 'S', 'NS,' and 'ANS' was 9.7%, 9.1%, and 9.2%, respectively, from room temperature to 200 °C, Figures 3. The weight loss started at around 50 °C with a broad decrease, which completes at around 180 °C. Differential TG curves (dTG/dt) were very similar to the observed DSC curves; therefore, the curves were not included. DSC thermal event appeared in the same region with the weight loss, and presumably, this endothermic DSC peak was associated with weight loss. The integrated area of the DSC peak was similar for samples 'NS' and 'ANS' with 640 µVs/mg where sample 'S' has a slightly higher area of 805 µVs/mg.

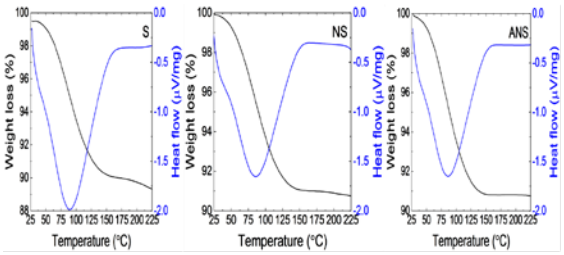

Fig. 3. Weight loss and heat flow (DSC) as a function of temperature for native (S), nanocrystal starch (NS) and acetylated Ofada starch nanocrystals (ANS).

FTIR Analysis

The FTIR of native and modified starches are shown in Figure 4. The spectra of the starches were found to be similar. This was also observed by Kalita and co-workers (2014) in the acetylation of waxy rice starch. This similarity in the spectra could have been due to the low degree of substitution (Kalita et al., 2014).

Although, there was a slight change in the O-H stretching band in the native starch from 3419 cm^{-1} to 3367 cm^{-1} when the nanocrystal starch was formed. The peaks at 2929 cm^{-1}, 1647-1649 cm^{-1}, 1363-1368 cm^{-1} indicate C-H stretching, C-O bending associated with OH group and C-H symmetric bending respectively (Abdullah et al., 2018).

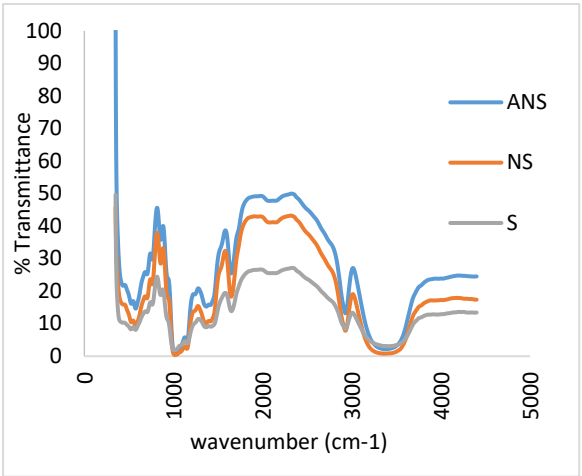

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Fig. 4. FTIR of native (S), nanocrystal starch(NS) and acetylated Ofada starch nanocrystals (ANS)

Drug loading

The results (Table 2) showed that ANS has highest drug loading and loading efficiency of 7.05% and 91.18%, respectively. This could be due to the acetylation of the starch, which made it more hydrophilic thus causing doxorubicin to be more attracted to it (Xiao et al., 2016). There was no significant difference in the %DLC and %LE of the different starches (Table 2). The ranking of %DLC and %LE was ANS > NS > S. This result agrees with that obtained by Xiao et al., 2016, when starch from broken rice was used as drug carrier, whereas, Odeniyi and co-workers (2019) observed that native Acha starch had highest %DLC and %LE when naproxen was used as the drug.

Table 2. *The percentage drug loading capacity and loading efficiency of native (S), nanocrystal starch (NS) and acetylated Ofada starch nanocrystals (ANS)*

	S	NS	ANS
%DLC	6.94±0.03	7.01±0.08	7.05±0.07
%LE	89.80±0.41	90.66±0.97	91.18±0.91

Drug release studies

The release of doxorubicin from Dox-loaded S, NS and ANS is shown in figure 5. Dox-loaded S with the lowest DLC and LE had initial drug release of 14% after 1 hour. This could be due to the low solubility of the native starch. Dox-loaded ANS released more than 50% of the drug within 1 hour and the remaining drug was released steadily over a 24-hour period. This could be due to the relatively higher solubility of the ANS compared to the two other starches and also Dox also has high solubility. Several factors such as drug type, polymer, drug-excipient interaction, solubility of drug have been shown to affect the release of drug from a carrier (Son et al., 2017). Hence, the initial higher rate of drug release from the acetylated nanocrystalline starch could provide a loading dose of the drug with subsequent sustained release. This could be of advantage in the treatment of solid tumors where nanoparticles can accumulate in many solid tumors at much higher concentrations than in normal tissues (Maeda, 2001)

The cumulative drug released were subjected to the different kinetic models Zero order, first order, Higuchi, Hixson Crowell and Korsemeyer-Peppas. The parameters obtained i.e. correlation coefficient (r^2), diffusional release exponents (n) and the kinetic constants of the formulations are presented in Table

3. The model with the highest correlation coefficient values is the best fit. It was observed that Dox-loaded S and ANS had highest correlation of 0.975 and 0.969, respectively, with the first order model indicating release of drug was dependent on the amount of drug remaining in the carrier, while Dox-loaded NS had highest correlation coefficient of 0.944 with Korsemeyer-Peppas model.

The release exponent (n) of the Dox-loaded NS and ANS were 0.371 and 0.310, respectively, indicating Quasi-Fickian diffusion mechanism. The n-value for Dox-loaded S was 0.710 which is <0.890 but >0.45, indicating non-Fickian transport drug release mechanism.

The Akaike information criterion (AIC) shows how well a model fits a data set. The model with the least AIC value was chosen as the best fit (Odeniyi et al., 2019). From our experiment, the models with the least AIC values were first order for S and ANS, while Korsemeyer-Peppas was least for NS. This also confirmed the best models obtained when the formulations were subjected to the different kinetic models

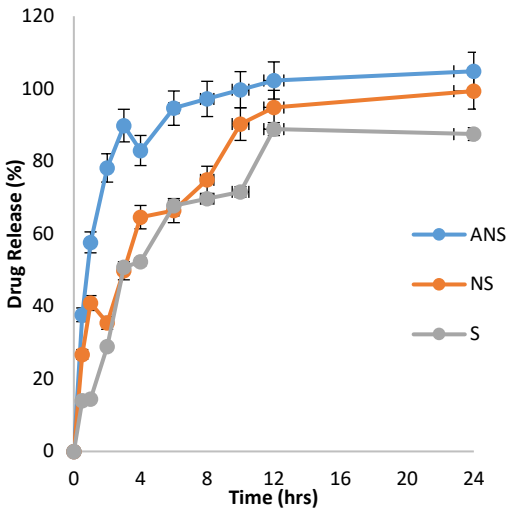

Fig. 5. Release profiles of doxorubicin from Dox-loaded S (native starch), NS (nanocrystal starch) and ANS (acetylated nanocrystal starch) in PBS (pH 7.4)

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Table 3: *In vitro release kinetics for Dox-loaded native (S), nanocrystal starch (NS) and acetylated Ofada starch nanocrystals (ANS)*

		S	NS	ANS
Zero order	K_o	12.79	14.06	21.16
	r^2	0.917	0.887	0.83
First order	K_1	0.195	0.249	0.789
	r^2	0.975	0.771	0.969
Higuchi	K_H	25.67	29.58	45.35
	r^2	0.929	0.915	0.88
Hixon	K_{HC}	0.057	0.07	0.021
	r^2	0.969	0.707	0.929
Korsemeyer	n	0.71	0.371	0.31
	k	19.62	34.65	57.18
	r^2	0.97	0.944	0.962
Akaike information criterion (AIC)	Zero	35.66	50.17	41.77
	First	33.65	48.17	39.76
	Higuchi	40.99	41.23	49.11
	Hixon	35.23	49.89	45.42
	Korsemeyer	36.9	40.25	43.01

CONCLUSIONS

Extracted starch from *Oryza glaberrima* rice was successfully modified to produce starch nanocrystals and acetylated starch nanocrystals with better flow properties, increased solubility, swelling power, and hydration capacity. All the starches had loading efficiency greater than 80%, and modification of the starch through nano-crystallization and subsequent acetylation was observed to improve the loading capacity and efficiency of doxorubicin with potentials for use in cancer therapy.

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