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Cross-linking with multifunctional excipients and its effect on the physicochemical properties and release profile of ibuprofen-loaded *Digitaria exilis* starch nanoparticles

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ABSTRACT: Nanoparticles have been used to overcome the limitations of oral drug delivery. This study was performed to examine the effects of modifying *Digitaria exilis* starch by cross-linking with multifunctional excipients: citric acid (CA) and sodium tripolyphosphate (STPP) to produce starch citrate and phosphate respectively at 20 % concentration. Thereafter, nanoparticles were synthesized via the nanoprecipitation method in the presence of Tween® 80, using ibuprofen as the model drug. The physicochemical properties of the modified starch were evaluated and the nanoparticles characterized by the encapsulation efficiciency, loading capacity, particle size, polydispersity index, scanning electron microscope (SEM), fourier transform infrared spectroscopy(FTIR), *in vitro* drug release and release kinetics. Results show that both cross-linkers improved the physicochemical properties of the starch, and produced particles in the nanometer range (616 nm and 933 nm) for citric acid and sodium tripolyphosphate nanoparticles respectively. Spherical and pitted particles corresponding to citric acid and sodium tripolyphosphate nanoparticles respectively were produced and they both showed a controlled release of ibuprofen from the formulation and both followed the Higuchi kinetic model with R^2 values that exhibited a non-fickian diffusion pattern. This study revealed that the two excipients had different effects on the size and morphology of the nanoparticles and controlled the release of drug from the nanoparticles.

KEYWORDS: Starch; citric acid; sodium tripolyphosphate; nanoparticles; ibuprofen; multifunctional excipient.

1. INTRODUCTION

Polymer nanoparticles, are solid colloidal, nanometric carriers with particle sizes of 1-1000 nm, utilized in the delivery of gene, vaccines, proteins, and drugs for controlled release at the desired site of action[1]. They are characterized by, high stability, protecting the biomolecule from degradation, readily biodegradable [2], high encapsulation efficiency, prolonged circulation within the biological system and improved kinetics of the biomolecule [3]. The application of nanotechnology to medicine, known as nano-medicine has increased significantly the diagnosis, imaging, and treatment of numerous diseases[4]. They have been exploited to overcome the limitations of oral drug delivery such as poor stability, water insolubility, low selectivity, high toxicity, and side effects[5]. Natural polymers are bio-renewable materials obtained from diverse sources that can be degraded into water, carbon dioxide and inorganic molecules. They are eco-friendly and possess functional groups that can be modified either by physical, chemical, biological or enzymatic means to yield products with high functionalities [6]. Some of these natural polymers include chitosan, starch, alginate, cellulose, hyaluronic acid, chondroitin sulfate, etc [7]. Starch is produced by many plants as a source of stored energy. It is the second most abundant biomass material in nature and found in plant roots, stalks, crop seeds, and staple crops such as rice, corn, wheat, tapioca, and potato [8]. They consist of a collection of amylose anhydrous glucose components connected by 1,4-d-glycoside links, and amylopectin that are extensively branched with (1–6) bonds[9]. They are hydrophilic in

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nature because of the numerous hydroxyl groups they possess, which makes them susceptible to brittleness, high moisture sensitivity, viscosity, retrogradation during storage, hence limiting their use in the development of starch-based products[10]. Modification of starch involves the alteration of the physical and chemical properties of native starch to improve its functionality. Some of these enhanced properties include an improvement in the mechanical and barrier properties of starch nanoparticles [11]. The most extensively utilized technique for the synthesis of nanoparticles from carbohydrate polymers is by chemical means. Cross-linking is one of such methods and it involves the use of multifunctional group reagents (such as hydrophobic ester groups) to react with the hydroxyl groups of starch (responsible for its hydrophilicity), to produce new chemical bonds within molecular starch chains[12]. This leads to improved swelling properties, biodegradability, biocompatibility, high temperature and high shear conditions in addition to the ease of processing, enhanced strength and stability of starch products, thus showing promise as effective carriers for drug delivery[13]. *Digitaria exilis* also known as acha or fonio is one of the earliest cereals found in Africa, grown for years as a major food source and has been incorporated as a key aspect of nutrition among the people, particularly for its flavor[14]. However, there is a lack of utilization of this crop compared to other cereals such as maize, millet, and sorghum[15]. The aim of this study, therefore, is to synthesize, characterize and evaluate citric acid and sodium tripolyphosphate cross-linked starch nanoparticles derived from *Digitaria exilis and* use it in the oral delivery of ibuprofen.

2. RESULTS

2.1.Physicochemical Properties of Cross-Linked Starch

The physicochemical properties of native and cross-linked *Digitaria exilis* starch are presented in Table 1. The bulk and tapped densities of native starch (NS) was decreased with cross-linking with CA and STPP. Cross-linking with STPP improved the Hausner's ratio of NS from poor to passable flow, but was unaffected by CA; in addition, the Carr's index of NS was improved to fair flow by cross-linking with STPP, but had poor flow with CA, and angle of repose of NS was slightly improved to passable flow with CA and STPP. The pH of NS was grossly affected by the pH of the CA and STPP and the moisture content was also influenced by the excipients.

Table 1: Physicochemical properties of native and cross-linked *digitaria exilis* starch

	Formulation		
Material	**CA-20**	**STPP-20**	**NS**
Bulk Density (g/ml)	0.45 ± 0.01	0.44 ± 0.01	0.56 ± 0.03
Tapped Density (g/ml)	0.57 ± 0.01	0.57 ± 0.00	0.76 ± 0.02
Hausner's Ratio	1.40 ± 0.02	1.26 ± 0.03	1.36 ± 0.08
Carr's Index (%)	28.78 ± 0.82	20.43 ± 1.81	26.30 ± 1.97
Angle of Repose (°)	45.10	43.80	46.30
pH	2.79 ± 0.02	8.15 ± 0.06	5.11 ± 0.19
Moisture Content (%)	11.35 ± 0.03	15.00 ± 0.01	11.00 ± 0.02

Values are presented as mean of triplicate readings ± standard deviation
STPP - Sodium tripolyphosphate
CA – Citric acid
NS - Native Starch

2.2.Swelling Capacity

The swelling capacities of native and cross-linked starch are shown in Figure 1. Cross-linking reduced the swelling capacity of NS, the highest degree of swelling was observed at 80 °C and lowest at 35 °C with all samples. The degree of swelling was comparable between the cross-linked samples and very slightly lower with STPP than CANP.

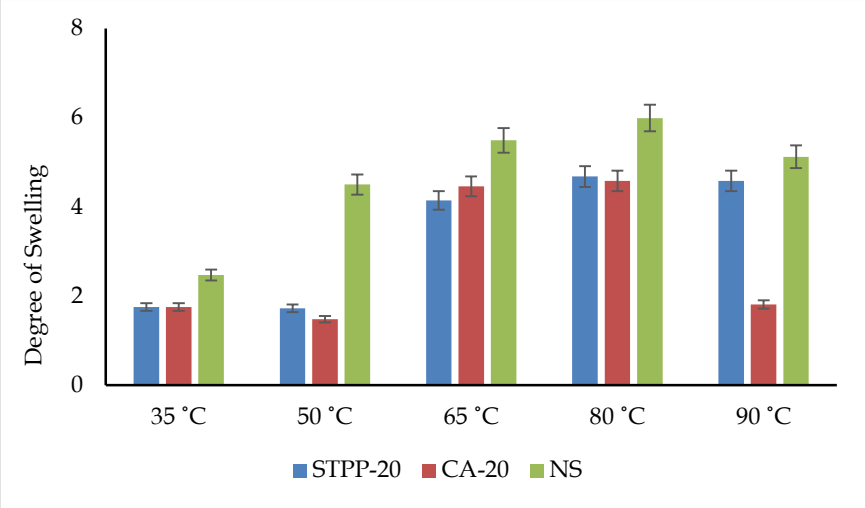


Figure 1: Swelling capacity of native and cross-linked *digitaria exilis* starch
Values are presented as mean of triplicate readings ± standard deviation
CA – Citric acid naoparticles
STPP – Sodium tripolyphosphate nanoparticles
NSNP – Native starch nanoparticles

2.3. Physicochemical properties of nanoparticles

The yield of native and cross-linked starch nanoparticles is shown in Table 2. The native starch nanoparticles (NSNP) has a higher yield compared to CANP and STPP NPs.

The results of encapsulation efficiency (EE) and loading capacity (LC) are shown in Table 2. Cross-linking increased both the EE and LC of the formulations, with CANP having the highest EE and LC compared to STPP nanoparticles.

Table 2: Parameters for ibuprofen-loaded cross-linked starch nanoparticles

	Formulation		
Parameters	**CANP-20**	**STPP-20**	**NSNP**
Yield (%)	60.70 ± 0.01	63.40 ± 0.02	66.76 ± 0.02
Encapsulation Efficiency (%)	98.24 ± 0.07	98.08 ± 0.07	97.40 ± 0.04
Drug Loading (%)	11.56 ± 0.01	11.05 ± 0.01	10.42 ± 0.00
Particle Size (nm)	933	616	1940
Polydispersity Index	0.489	0.351	0.531

Values are presented as mean of triplicate readings ± standard deviation
CA – Citric acid naoparticles
STPP – Sodium tripolyphosphate nanoparticles
NSNP – Native starch nanoparticles

Cross-linking also produced particles in the nanometer range, however, CANP had a lower particle size (616 nm) compared to STPP (933 nm) as shown in Table 2. Similarly, polydispersity index of the NPs are 0.351, 0.489 and 0.531, corresponding to STPP, CANP and NSNP respectively. The particle size distribution of the nanoparticles are shown in figure 2.

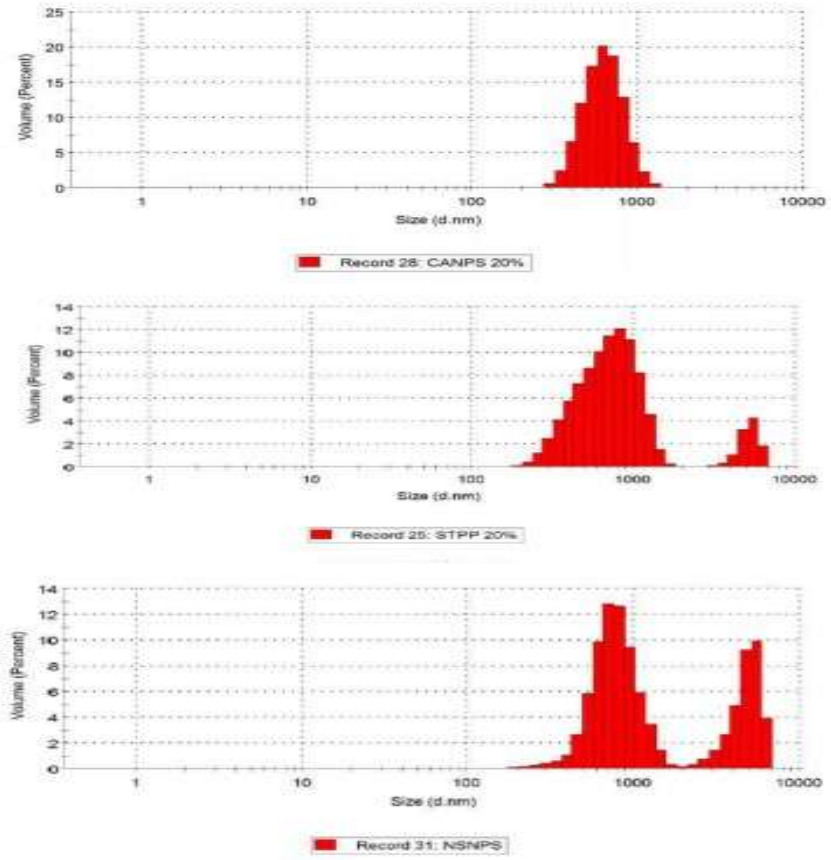


Figure 2: Particle size distribution of native and cross-linked starch nanoparticles
CANP – Citric acid nanoparticles
STPP – Sodium tripolyphosphate nanoparticles
NSNP – Native starch nanoparticles

2.4. FTIR of Native and Cross-linked Starch Nanoparticles

The IR spectra of synthesized nanoparticles presented in Table 3 shows the absorption spectra of NS, NSNP and the cross-linked starch nanoparticles (CANP and STPP). There is an introduction of vibration bands at 1871 cm^{-1} on the CANP-20 spectra due to C=O vibration confirming the formation of a citrate bond and the introduction of 1207.7cm^{-1} peak on the STPP-20 spectra due to the C=C vibrations resulting from the formation of phosphate linkage.

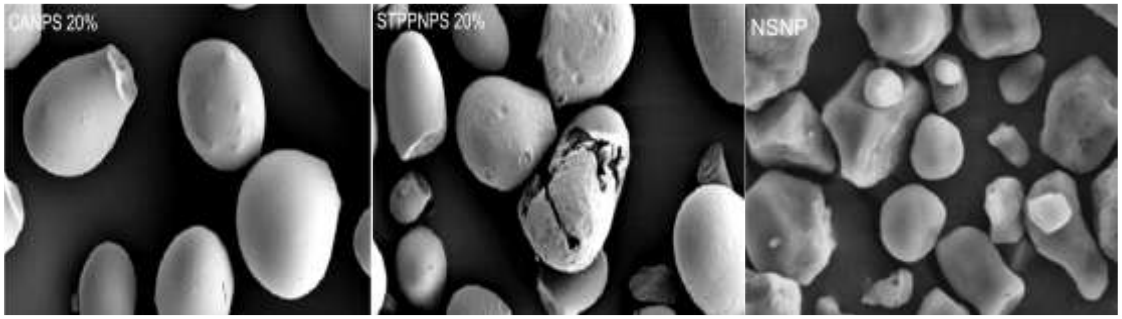
Table 3: FTIR absorption bands for native and cross-linked *Digitaria exilis* starch

	Infrared Signal Assignment(cm^{-1})			
Functional Groups	**Native Starch**	**NSNP**	**CANP-20**	**STPP-20**
O-H Stretch	3257.7	3257.7	3268.9	3254
C-H Stretch	2929.7	2926	2922.2	2822.2
C=O vibration	-	-	1871	-
H_2O absorption band	1640	1651.2	1640	1654
CH_2-OH side chain	1338.1	1364.2	1364.2	1207.7
C=C vibration	-	-	-	1207.7
C-O vibration	1148	1148	1148	1151.7
C-O-H bend	1077	1080	1017.6	1017.6
C-O-C vibration	928.1	935.6	931.8	924.4

CANP – Citric acid naoparticles
STPP – Sodium tripolyphosphate nanoparticles
NSNP – Native starch nanoparticles

2.5. Scanning Electron Microscopy (SEM)

Scanning electron microscope (SEM) image of native and cross-linked starch NPs is shown in Figure 3(i, ii and iii). The NPs are shown to be spherical, pitted and squamous corresponding to CANP, STPP and NSNP respectively.



(i) (ii) (iii)

Figure 3: Scanning electron micrograph of: (i) CANP, (ii) STPP and (iii) NSNP particles at 8,000 ×
CANP – Citric acid nanoparticles
STPP – Sodium tripolyphosphate nanoparticles
NSNP – Native starch nanoparticles

2.6. In-Vitro Release Studies

The *in-vitro* drug release study was carried out in phosphate buffer (pH 6.8) solution, using tablets (containing ibuprofen-loaded starch nanoparticles) compressed by direct compression with prosolv as the directly compressible excipient. The i*n-vitro* release of ibuprofen from NPs ranged from 22 % to 40.44 %, 18 % to 47.22 % and 6.35 % to 27.72 % for CANP, STPP and NSNP respectively within the period of 2 to 24 h (Figure 3). However, over 100 % of the plain ibuprofen was released from the formulation within 30 min. The *in-vitro* release profile shows a distinction between a type of conventional formulation and the nanoparticle formulation.

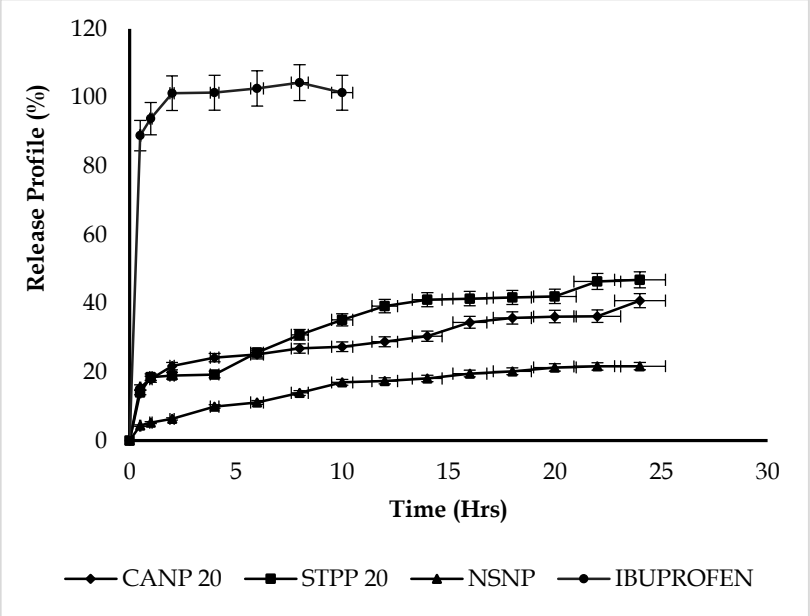


Figure 4: *In-vitro* drug release profile of pure ibuprofen, and ibuprofen-loaded native and cross-linked starch nanoparticles
CA – Citric acid nanoparticles
STPP – Sodium tripolyphosphate nanoparticles
NSNP – Native starch nanoparticles

2.7. Release Kinetics of ibuprofen from Optimized Formulation

Table 4 shows the data for the release kinetic models of CANP-20, STPP-20, NSNP and ibuprofen from nanoparticles. The linear factors (R^2) of 0.994, 0.995, 0.998, 0.999 represent CANP-20, STPP-20, NSNP and ibuprofen respectively. The release exponent (n) values also varied from 0.721 to 2.219.

Table 4: Release kinetics of pure ibuprofen, and ibuprofen-loaded native and cross-linked starch nanoparticles

	Release Kinetic Models										
	Zero Order Model		First Order Model		Higuchi Model		Hixson-Crowell Model		Korsmeyer–Peppas Model		
Formulation	K_0	R^2	K_1	R^2	K_H	R^2	K_{HC}	R^2	K_{KP}	R^2	n
CANP 20	0.809	0.971	-0.028	0.984	-1.088	0.994	-0.028	0.976	0.319	0.936	1.136
STPP 20	1.330	0.963	-0.021	0.990	-1.120	0.995	-0.043	0.861	0.468	0.945	1.048
NSNP	0.739	0.987	-0.048	0.961	-0.541	0.998	-0.038	0.852	0.466	0.967	0.721
Ibuprofen	0.367	0.999	-0.005	0.948	-13.81	0.994	-0.007	0.895	-0.298	0.862	2.219

CANP – Citric acid nanoparticles
STPP – Sodium tripolyphosphate nanoparticles
NSNP – Native starch nanoparticles

3. DISCUSSION

The results show that cross-linking reduced the bulk and tapped densities of the native starch. All the powders had fair to poor flow (USP 35). These results are similar to those obtained by Musa *et al.*[16] who reported 35.67 °, 32.7 %, and 1.49 % for angle of repose, Carr's index and Hausner quotient respectively, but much lower than the values of 39.2 %, 1.65 % and 64.2 ° for CI, HQ and angle of repose respectively reported by Odeniyi *et al.*[17].

The moisture content obtained in this study for native starch is very similar to the 11.6% reported by Musa *et al.*[16] but lower than the 14.8% reported by Odeniyi *et al*. [17]. The slightly higher moisture content in the STPP compared to the CA modified starch may be due the hygroscopic nature of STPP which was conferred on the starch [18].

The CA and STPP cross-linkers had the same effect by reducing the swelling of the starch granules, and both achieved their peak swelling below 80 ˚C compared to native starch that peaked at 80 ˚C. Cross-linking has the ability to restrict the movement of the granule structures due to the formation of covalent bonds between the starch and the cross-linking agents[19].The reduced swelling capacity as a result of cross-linking, therefore, can be utilized as an excipient in controlled-release formulations [20].

A decrease in yield observed with cross-linked NPs can be explained in terms of the steric hindrance cross-linking confers on starch granules, which leads to a reduction in water absorption by the starch granules during synthesis[21].

Encapsulation efficiency signifies the ability of the drug to be entrapped within the nanoparticle while drug loading capacity is the amount of the active ingredient loaded per weight of the nanoparticle. These values for EE in this study are higher than the 91.31 % reported by El-Feky *et al.*[22]. Some factors that affect the EE are temperature, pH of the media, method, and type of solvent used in the preparation of the nanoparticles, the concentration of surfactant and size distribution[23].

Particle size analysis explains the dispersion and aggregation of prepared nanoparticles and demonstrates how stable the formulation is. On the other hand, polydispersity index (PDI) measures the consistency and the homogeneity of the dispersed system and has a range between 0 – 1 [24]. The ability of nanoparticles to continue in circulation is correlated to their surface properties, and these are carefully designed in order to achieve targeted delivery with high efficacy[25].

The FTIR spectra for CANP and STPP reveals changes in vibrational frequencies that signifies the formation of starch nanoparticles[26]. The new peak observed at 1207.7 cm^{-1} for STPP cross-linked starch is attributed to the anti-symmetric stretching vibration of the phosphate group signifying the cross-linking of the –OH group of starch and the polyphosphate ions of the cross-linker which agrees with that observed by El-Naggar *et al*. [23].

The structural characteristics of native and cross-linked starch nanoparticles reveals a spherical, rough and porous morphology for CANP and STPP nanoparticles that agrees with the study conducted by Shen *et*

al.[27]. Zhang *et al*.[28] also observed that the porous starch granules obtained in their study had rough surfaces and internal hollow spaces that make the starch useful as an adsorbent for different purposes.

The peak plasma concentration of ibuprofen from an immediate release formulation is usually attained within 60 - 120 min with a bioavailability of 80 - 90 % after oral administration[29]. This correlates with the release of ibuprofen from the formulation in the present study. However, for the onset of analgesia, the mean blood plasma concentration of ibuprofen is between 6.8 - 10.1 µg/mL [30]. At 2 hours, the concentration of ibuprofen released from nanoparticles were 21.8 µg/mL, 18.9 µg/mL and 6.5 µg/mL and was sustained to 40.8 µg/mL., 46.9 µg/mLand 21.7 µg/mL in 24 hours for CANP, STPP and NSNP respectively.The results therefore show that drug release from cross-linked starch nanoparticles gave a better result compared to native starch nanoparticles. The release of ibuprofen in this study is found to be higher than those of El-Feky *et al*. [22], where they utilized different concentrations of STPP in generating starch nanoparticles and showed 15, 10, 8 and 6 % release of indomethacin from 4 different formulations in 2 hours. Another study conducted by El-Naggar *et al*. [23], revealed about 20 % of drug was released from the nano-formulations in 4 hours.

The results obtained for the kinetic models showed that the cross-linked starch nanoparticles (CANP and STPP) and native starch nanoparticle (NSNP) followed the Higuchi kinetic models, which agrees with Jain *et al*.[31] for drug release from nanoparticles while the pure ibuprofen formulation followed the Zero-order kinetics model which suggests that a higher amount of the polymer will be needed to achieve a time-independent drug release.The "n" values from Korsmeyer-Peppas model shows the mechanism of drug release: for Fickian diffusion (n = 0.5), non-Fickian diffusion (0.5 < n < 1.0) and case II transport (n > 1.0). hence, it can be observed that only the native starch nanoparticles (NSNP) exhibited a non-Fickian diffusion *because* the value of "n" was 0.721. The release pattern from semi and solid dosage forms are governed by either dissolution or diffusion or both[32].

4. CONCLUSION

Ibuprofen-loaded starch nanoparticles were prepared by cross-linking *Digitaria exilis* starch with multifunctional reagents (citric acid and sodium tripolyphosphate) and the results showed that cross-linking influenced the physicochemical properties of starch, producing spherical and pitted particles in the nanometer range (616 nm and 933 nm) for citric acid and sodium tripolyphosphate respectively. A sustained delivery of ibuprofen up to 24 h with the highest cumulative drug release of 40.44 % and 47.22 % for CANP and STPP respectively was also observed, and their *in vitro* release kinetics followed the Higuchi model with only the uncross-linked nanoparticles exhibiting the non-Fickian diffusion.

5. MATERIALS AND METHODS

5.1. Materials

Digitaria exilis was purchased from Karmo market, Abuja, ibuprofen powder, potassium dihydrogen phosphate, sodium tripolyphosphate, Tween®80 and citric acid were purchased from Bristol Scientific Co. Ltd. Ethanol and sodium hydroxide were purchased from Finlab, Nigeria Ltd. All other chemicals were of analytical grade.

5.2. Methods

5.2.1. Sample Collection and Preparation

The method of Kunle *et al*. [33] was adopted with slight modifications. *Digitaria exilis* grains were bought from Karmo market, Abuja. Appropriate quantities of the grains was weighed and washed with a sufficient amount of water to remove dirt and sand. It was soaked in water containing 0.075 % w/v sodium metabisulphite for 24 h and then steeped, it was further rinsed with copious amount of water containing sodium metabisulphite and milled to a fine paste. Thereafter, it was transferred to a large bowl containing 10 L of freshly prepared water. The slurry was sieved using a muslin cloth and allowed to stand for 12 h. The sediment obtained was further centrifuged at 4,500 rpm for 30 min to obtain the starch which was air-dried for 6 h and dried in an oven at 50 °C for 16 h. The resulting powder was pulverized, passed through a 250 µm mesh sieve, dried again at 50 °C and packed in an airtight container for further analysis.

5.2.2. Modification of Digitaria exilis starch

The method of Moses *et al*. [34] was adopted with slight modification. Briefly; starch citrate/phosphate (30 % w/w; of starch) was prepared by dissolving CA/STPP (15 g) in 15 ml of distilled water, which was then made up to 50 ml in a beaker (Table 5). *Digitaria exilis* starch (50 g) was added to the resulting solution and mixed thoroughly, after which, it was transferred to a tray and allowed to stand for 48 h at room temperature (25^{o}C) to effect cross-linking. The cross-linked starch obtained was further dried at 60 ^{o}C for 6 h in the oven, pulverized, passed through a 250 µm mesh sieve and packed in an air-tight container for further analysis.

Table 5: Reaction conditions for the preparation of native and cross-linked starch

Parameters	Formulation		
	CA 20	**STPP 20**	**NS**
Weight of starch (g)	50	50	50
Weight of CA (g)	10	-	-
Weight of STPP (g)	-	10	-
Total Weight (g)	60	60	50

CA – Citric acid
STPP – Sodium tripolyphosphate
NS – Native starch

5.2.3. Preparation of Ibuprofen–Loaded Cross-Linked Starch Nanoparticles

The method of El-Feky *et al*.[22] was adopted with some modifications. Briefly, ibuprofen-loaded cross-linked starch nanoparticles was prepared by the nano-precipitation method. The native and cross-linked starch (5 %$^{w}/_{v}$) was solubilized in 30 % ($^{w}/_{w}$) of sodium hydroxide solution with the aid of a magnetic stirrer for a period of 1 h at room temperature (25 ^{o}C). A 0.5 % aliquot of Tween®80 was added to the resulting solution accompanied with stirring for a further 5-minute period after which 10 % (w/w) of model drug was introduced. Absolute ethanol was added dropwise to the starch solution under high magnetic stirring to produce nano-precipitates. This was centrifuged at 4,500 rpm for 30 min to collect nanoparticles, lyophilized and then packed in air-tight jars for further analysis. The composition of nanoparticle preparation is given in Table 6.

Table 6: Composition of ibuprofen-loaded starch nanoparticles

Ingredients	Formulation		
	CANP 20	**STPP 20**	**NSNP**
Ibuprofen (g)	0.5	0.5	0.5
Qty of Excipient (g)	5	5	5
Qty of NaOH (g)	1.5	1.5	1.5
Ethanol (mL)	100	100	100
Tween 80 (mL)	0.5	0.5	0.5
Water (mL)	100	100	100

CANP – Citric acid cross-linked nanoparticles
STPP – Sodium tripolyphosphate
NSNP – Native starch nanoparticles

5.3. Characterization of Native and Cross-linked Starch

5.3.1. Percentage Yield

The percentage yield of extracted starch and starch nanoparticles was calculated as the weight of the starch/starch nanoparticles obtained with respect to the original weight of the starting material. It is calculated using the formula below:

$$\text{Yield (\%)} = \frac{\text{Total weight of the prepared particles (mg)}}{\text{Total weight of the drug and polymers used (mg)}} X\ 100 \qquad \text{(Eq. 1)}$$

5.3.2. pH Measurements

The pH of the cross-linked starch solutions was measured using pH (Mettler Toledo) meter with a microprocessor. A 1 % dispersion of the native and cross-linked starch was prepared, and the pH was taken at room temperature (25 ºC). This was performed in triplicates.

5.3.3. Moisture Content

The moisture content of native and cross-linked starch was carried out with the aid of a moisture balance (OHAUS MB 45). A 1.0 g of the native and cross-linked starch was weighed with the aid of a weighing balance (Mettler Toledo- ME 303E) and placed in the moisture content analyser that was set at 100 °C for 10 min. The moisture content was performed using the formular below and computed in triplicates.

$$Moisture\ content\ (\%) = \frac{weight\ of\ wet\ mass - weight\ of\ dry\ mass}{weight\ of\ dry\ mass} \times 100 \quad \text{(Eq.2)}$$

5.3.4. Swelling Capacity

The swelling capacity was determined according to the method of Babu and Parimalavalli[35],by making a dispersion of 1 g of native and cross-linked starch in 10 mL of distilled water in pre-weighed centrifuge tubes. This was placed in a water bath (Karl Kolb Sci. Co, Germany) equilibrated at 35, 50, 65, 80 and 90 °C and the samples were agitated for 30 min. The swollen starch gel was cooled to 25 °C, centrifuged at 1,500 rpm for 10 min and the supernatant was discarded. The weight of the tubes with the swollen starch gels were further weighed and the swelling capacity calculated as follows:

$$Swelling\ Capacity = \frac{weight\ of\ swollen\ granules}{weight\ of\ dry\ sample} \quad \text{(Eq. 3)}$$

5.3.5. Encapsulation Efficiency (EE)

The encapsulation efficiency of drug-loaded NPs was determined according to Nagarajan *et al.*[36] by utilizing the supernatant obtained when the NPs were prepared. After centrifuging at 4,500 rpm for 30 min, aliquot of the supernatant was assayed for non-bound drug concentration using the UV spectrophotometer (Cary-60, UV-Vis, Agilent technologies, UK) which was read at 215 nm. The encapsulation efficiency (EE) was calculated in triplicates using the formula below:

$$\text{EE}\ (\%) = \frac{\text{Amount of drug in a definite mass of prepared particles (mg)}}{\text{Theoretical amount of the drug in the same mass (mg)}}\ X\ 100 \quad \text{(Eq. 4)}$$

5.3.6. Drug Loading Capacity (LC)

The drug loading capacity of each batch of ibuprofen-loaded nanoparticles was determined by evaluating the actual content of the drug in nanoparticles with respect to the weight of the nanoparticles. These values were obtained after EE was evaluated and it was calculated using the formula:

$$Drug\ Loading\ Capacity\ (\%) = \frac{weight\ of\ drug\ in\ nanoparticles}{weight\ of\ nanoparticles} \times 100 \quad \text{(Eq. 5)}$$

5.3.7. FT-IR Analysis

Fourier-transform infrared spectroscopy (Cary 630, Agilent technologies, USA) was performed on the cross-linked starch and drug-loaded nanoparticles obtained to determine and identify the functional groups present. The analysis was conducted at room temperature within a range of 4000–400 cm^{-1}, at a resolution of 2 cm^{-1} using the potassium bromide (KBr) pellets technique. About 5 mg of finely ground solid sample was ground with 100 mg of dry potassium bromide, and a 7 mm pellet was formed under high pressure. The background and sample spectra were obtained from 64 scans.

5.3.8. Particle Size Analysis (PSA) / Polydispersity Index (PDI)

The particle size and polydispersity index of drug-loaded NPs were performed with the aid of a Malvern Zeta Sizer (Zen 1600, UK). A colloidal suspension of the NPs was prepared and poured into the nano-sizer cell and placed in the analysing chamber and the analysis was performed at 25 °C, with a

detection angle of 90 °. Prior to measurements of each sample, 1-3 mg of the sample were suspended in distilled water, then vortexed and/or sonicated for a few minutes to produce a suitable scattering intensity and each sample measured in triplicate.

5.3.9 Scanning Electron Microscope (SEM)

The scanning electron microscope (JOEL-JSM 7600F, Germany) was used to determine the morphology, shape, and surface characteristics of ibuprofen-loaded nanoparticles. The sample was prepared by sprinkling the dispersed nanoparticles onto a double-sided adhesive carbon conductive tape which was mounted on a microscopic stub of copper. Then the sample was sputter-coated with gold using ion sputtering device of the equipment.

5.3.10. In-Vitro Release Studies

Ibuprofen belongs to class II of the Biopharmaceutical Classification of drugs, having low solubility at pH 1.2 and 4.5 and high solubility at pH 6.8 but high permeability [37]. Therefore, this study is intended to demonstrate the ability of the nanoparticles to sustain the release of ibuprofen at pH 6.8.

The *in-vitro* drug release study was done in phosphate buffer (pH 6.8) solution. Tablets containing ibuprofen-loaded starch nanoparticles were compressed by direct compression with prosolv as the directly compressible excipient (Table 7), using Erweka (Germany) single punch tablet machine with a 12 mm punch and compression pressure of 6 KN. Likewise, 50 mg of pure ibuprofen was compressed using an 8 mm punch at a pressure of 8 KN.A tablet was placed in a basket and immersed into a vessel containing 900 mL of the dissolution medium and maintained at 37 ˚C under mild agitation (50 rpm). Samples were withdrawn within a 24-hour period at regular time intervals of 0, 1, 2, 3, 4, 5, 6 hours, and then replaced with the same volume of fresh phosphate buffer solution to maintain sink conditions. The samples were analysed using the UV spectrophotometer (Cary-60, UV-Vis) at 215 nm.

Table 7: Composition of tablets containing ibuprofen-loaded nanoparticles

Materials	CANP 20	STPP 20	NSNP	IBUPROFEN
Equivalent weight of nanoparticles containing 50 mg of Ibuprofen (mg)	494	524	589	50
Prosolv (mg)	156	126	61	156
Total Quantity (mg)	650	650	650	206

CANP – Citric acid nanoparticles
STPP – Sodium tripolyphosphate nanoparticles
NSNP – Native starch nanoparticles

5.3.11. In-vitro Release Kinetics

To evaluate the release kinetics of ibuprofen from native and cross-linked starch nanoparticles, the data obtained from *in vitro* dissolution studies were fitted into five (5) kinetic models, which include the zero-order, first-order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas kinetic models. The equations representing these kinetic models are given as follows:

Zero Order Kinetics	$Q_t = K_o t$	(Eq. 6)
First Order Kinetics	$\log Q_0 - \log Q_t = K_1 t / 2.303$	(Eq. 7)
Higuchi Kinetic Model	$Qt = K_H \sqrt{t}$	(Eq. 8)
Hixson-Crowell Model	$Q_0^{1/3} - Q_t^{1/3} = K_{HC} t$	(Eq. 9)
Korsmeyer–Peppas Model	$Q_t = K_{KP} t^n$	(Eq. 10)

where t is the time, Q_t is the amount of drug released at time t, Q_0 is the initial amount of drug in the nanoparticles, K_0 is the zero-order rate constant, K_1 is the first-order rate constant, K_H is the Higuchi constant showing the design variables, K_{HC} is the Hixson-Crowell rate constant, K_{KP} is the Korsmeyer–Peppas rate constant and n is the release exponent[38].

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