Original Article

Thai Journal of Pharmaceutical Sciences

# Evaluation of modified *Pennisetum glaucum* starch as a suspending agent in metronidazole benzoate suspension

**Nkemakolam Nwachukwu, Kenneth Chinedu Ugoeze, Dorathy Rokalke Igoni**

*Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Port Harcourt, Nigeria*

**Corresponding Author:**
Kenneth Chinedu Ugoeze, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Port Harcourt, Nigeria.
E-mail: kenneth.ugoeze@uniport.edu.ng

**Received:** Oct 30, 2020
**Accepted:** Jan 13, 2021
**Published:** Dec 13, 2021

## ABSTRACT

The properties of modified starch derived from Pennisetum glaucum (PT) seed as a suspending agent in metronidazole benzoate suspension was evaluated. Matured PT seeds were steeped for 72h, wet milled and the slurry washed with water through a muslin cloth to obtain native PT starch (NPS) which was dried at 60°C. NPS slurry was heated (70°C), cooled (40°C), and submerged in 3.5%w/v sodium hypochlorite for 10 min, washed with 95%v/v alcohol till neutral to litmus. The modified starch (MPS) was dried (50°C), classified (180$\mu$m). NPS and MPS were characterized using standard methods. Metronidazole benzoate (MTBZ) suspensions were formulated using 2.5%w/v, 5.0%w/v and 7.5%w/v of MPS as suspending agent. Similar concentrations of acacia and methylcellulose were used as standard. An MTBZ suspension without polymer served as control. The suspensions were evaluated for pH, sedimentation, flow rate, viscosity and re-dispersibility within 28 days. Results show that modification enhanced the densities, compressibility, hydration capacity and swelling index of MPS. The suspensions had stable pH and viscosities. Sedimentation volume increased with increase in concentration of polymer (MPS>Methylcellulose>acacia>control) while flow rate decreased with increased polymer concentrations. The MTBZ suspensions were re-dispersed on agitation. MPS formed more stable suspensions than acacia and methylcellulose at similar concentrations.

**Keywords:** *Pennisetum glaucum*, starch, suspending agent, metronidazole benzoate.

## INTRODUCTION

A pharmaceutical suspension is a two-phase system with uniform dispersion of finely divided solid drug particles in a continuous phase of solid, liquid, or gas in which the drug has minimum solubility.[1] The continuous or external phase is usually liquid or semisolid. Stabilizers or suspending agents are added to stabilize the suspension and reduce the rate of sedimentation of the suspended indiffusible particles as well as permitting the ease with which suspended particles are redispersed on agitation. Suspending agents also increase the viscosity of the suspending medium as well as provide a protective colloidal sheath around the particles which reduces inter particulate interaction thereby increasing the stability of the suspension.[2,3] Suspensions are often used for drugs that are poorly or completely insoluble in water especially when ingestion of tablets or capsules is not possible such as in geriatric and pediatric patients. Common routes of administration of suspensions include the oral, topical, and parenteral route where they are available as ready to use preparations or as dry powders/granules that would be re-constituted with an appropriate solvent before use.

Natural or green materials such as plant gums and mucilages (acacia [AC], tragacanth, xanthan, guar, *Brachystegia eurycoma*, *Chrysophyllum albidium*, *Abelmuscus esculenta*, *Aloe babadensis*, etc.) are often employed as suspending agents in the formulation of suspensions because of their safety level in terms of toxicity as well as their ability to increase the viscosity of the continuous phase of a suspension which would decrease the rate of sedimentation of the in-diffusible active pharmaceutical ingredient powder.[4,5] Besides plant gums and mucilages, semi synthetic agents such as sodium carboxymethylcellulose, microcrystalline cellulose, methylcellulose (MC), hydroxyl ethyl cellulose, or synthetic agents are also used as suspending agents. Natural gums have

several advantages over semi synthetic and synthetic gums as they are biodegradable, cheap, readily available, effective, and eco-friendly.[5]

Carbohydrates are a major component of food consumed by humans and likewise other animals which do not have the capacity to generate carbohydrates. Starch is the most important source of carbohydrates and accounts for more than 50% of the carbohydrate intake by humans. It exists in large quantities in photosynthetic plant parts such as seeds and tubers where they are stored in the form of carbohydrates.[6,7] The quantity of starch contained varies depending on the botanical origin and type of plant. Cereal seeds such as wheat, corn and rice are rich in starch. Starch is a mixture of two polymers: amylose and amylopectin. Natural starches consist of about 10–30% amylase and 70–90% amylopectin. Amylose is a linear polysaccharide composed entirely of D-glucose units joined by the α-1,4-glycosidic linkages. Commercial starch is presented as a dry white powder.[8,9]

Pearl millet (*Pennisetum glaucum*) is the most widely grown type of millet. It has been grown in Africa and the South Asia since prehistoric times.[10] Millet is important because of its uniquely high content of nutrients, including impressive starch levels, vitamin B, calcium, iron, potassium, zinc, magnesium, and fats. It also has significant levels of protein and dietary fiber.[10]

Metronidazole benzoate (MTBZ) is the benzoate ester of metronidazole, a synthetic nitroimidazole derivative with antiprotozoal and antibacterial activities against amoebiasis, trichomoniasis, giardiasis, and many other parasitic diseases.[11,12] Metronidazole is available as yellow crystals that are slightly soluble in water, methylene chloride and alcohol.[13] It inhibits nucleic acid synthesis by disrupting the DNA of microbial cells. It is often used in the formulation of suspensions when metronidazole is indicated as a drug of choice for pediatric populations because it is more palatable than the metronidazole base.

Starch in its native form has poor suspending properties but has found more application in pharmaceutical drug delivery as either a binder, disintegrant, diluent or glidant in granules, tablet and capsule manufacture. In this study, modified *P. glaucum* starch (MPS) is used as a suspending agent in the formulation of MTBZ suspension. MTBZ was chosen for this study because of its poor aqueous solubility.

## MATERIALS AND METHODS

### Materials

The following reagents were used as procured: Matured *P. glaucum* (millet) seeds (purchased from Rumuokwuta market, Port Harcourt), 3.5% w/v Sodium hypochlorite solution (Hypo®, Multipro Enterprises Limited, Nigeria), Ethanol (JHD Guangdong Guanggua Sci. Tech Co., Limited, China), n-hexane (Sigma, USA).

### Methods

Dry matured *P. glaucum* (millet) seeds were purchased from Rumuokwuta market, Rumuokwuta, Port Harcourt, Rivers State. Identification and authentication was done by Dr. A. T. Oladele of the Department of Forestry and Wildlife management, University of Port Harcourt. The sample was deposited in the herbarium and assigned voucher number FHUPH-101.

The seeds were sorted of leaves, husk, stones, and steeped in distilled water for 72 h at ambient conditions. The millet seeds were washed, wet milled and the starch was separated from the fiber by washing with water through a muslin cloth. The filtrate was allowed to settle, the supernatant was decanted and the wet starch squeezed through a muslin cloth. Drying was done at 60°C in an oven (Memmet, England), the starch was pulverized, passed through a 180 μm stainless steel sieve (Retsch, Germany) and labeled as natural *Pennisetum* starch (NPS).

A 200 g quantity of NPS was dispersed in 50 ml of distilled water at room temperature to obtain a slurry. 250 ml of hot water (80°C) was added to the cold starch slurry in a 1 L beaker and heated over a hot water bath to 70°C. The beaker was removed from the hot water bath and stirred till the temperature of the mucilage reduced to 40°C. A 500 ml volume of sodium hypochlorite (3.5% w/v) was added to the mucilage and stirred at room temperature (30 ± 2°C). Alcohol (95% v/v) was added to precipitate the starch. It was washed with more alcohol until it was neutral to litmus. The alcohol was squeezed out through a muslin cloth and the starch dried at 50°C in the oven (Memmet, England). It was screened through a 180 μm sieve (Retsch, Germany) and was labeled as MPS.

### Physicochemical Properties

*Organoleptic properties*

The organoleptic properties such as the color, smell, taste and texture of NPS and MPS were investigated.

*Identification of starch*

A 0.2 g quantity of both NPS and MPS were placed separately in a white porcelain mortar and a few drops of iodine were placed on each sample until sufficiently damp. Observations were made with the naked eye for change in color.[7]

*pH and viscosity*

A 100 ml volume of a 2% w/v dispersion of both NPS and MPS was prepared and the pH determined using a pH meter (Hannah, USA). The viscosity of NPS and MPS dispersion was determined using a viscometer (Brookfield, UK).

*Ash contents and other extractives*

The total ash, acid insoluble ash, water soluble ash, ethanol, and water extractive content each of NPS and MPS were determined using the methods stated in the United States Pharmacopoiea[14] and the World Health Organization quality control methods for herbal materials (1998).[15]

Total ash

A quantity of 4.0 g each of NPS and MPS was weighed into separate tarred nickel crucibles and heated gradually up to 675 ± 25°C in a Haraeus model D-2800 muffle furnace (Bremen, Germany) until they were free from carbon, The crucibles were allowed to cool in a dessicator and reweighed. The percentage of the total ash for each was calculated.

Acid insoluble ash

A quantity of 0.19 g of total ash was boiled for about 5 min with 25 ml of 2 M hydrochloric acid. The insoluble material was collected in a sintered glass crucible (funnel) and the residue was washed with hot water and ignited. The percent acid insoluble ash was calculated.

Water soluble ash

A quantity of 0.19 g total ash was boiled for about 5 min with 25 ml of deionized water. The insoluble sample was collected in a sintered glass funnel and ignited at 500°C for 20 min in the furnace. The weight of the residue was subtracted from the weight of the ash, and the difference was taken to be the water soluble ash. The percent of water soluble ash was calculated.

Ethanol extractive yield

A quantity of 2 g of dried NPS was soaked in 100 ml of 90% v/v ethanol in a closed flask for 24 h, with frequent shaking for 6 h. The mixture was allowed to stand for 18 h. This was filtered rapidly and 20 ml of the filtrate was evaporated to dryness in a 25 ml beaker over a water bath. The residue was dried at 105°C to constant weight. The percentage of ethanol extractive yield was calculated. The same process was carried out using MPS.

*Hydration capacity*

The hydration (water retention) capacity each of NPS and MPS was determined by the method of Ring.[16] A 1 g quantity of the starch was placed in a 15 ml plastic centrifuge tube and 10 ml of water was added. The tube was shaken vigorously, ensuring that the sample is well mixed and was allowed to stand for 10 min. This was then centrifuged for 10 min at 3000 revolutions per minute (rpm). The supernatant was decanted and the weight of the powder after water uptake and centrifugation was determined. This was carried out in triplicate and mean values were determined. The hydration capacity was determined using equation 1:

$$\text{Hydration Capacity} = y/x \quad (1)$$

Where y is the weight of moist powder after centrifugation and x is the weight of the dry powder.

*Swelling index*

The dry powder each of NPS and MPS was poured into a 100 ml measuring cylinder and tapped until a tapped volume of 5 ml was obtained. Distilled water (85 ml) was poured into the cylinder and the powder dispersed in it. Sufficient water was used to make up to the 100 ml mark of the measuring cylinder. The dispersion was allowed to swell without agitation for 24 h. The volume occupied by the swollen wet powder was recorded.[17,18] This was done in triplicate and an average obtained.

$$\%\ \text{swelling index} = V_F/V_I \times 100 \quad (2)$$

Where $V_F$ is the final volume and $V_I$ is the initial volume.

*Moisture absorption/hysteresis*

The moisture sorption studies was carried out by storing 0.5 g of NPS and MPS in respective air-tight desiccators containing saturated aqueous solutions of potassium sulfate, potassium chloride, sodium chloride, and magnesium nitrate at 30 ± 2°C to maintain relative humidity environments of 96, 84,75, and 52%, respectively.[19] Observations were made for 5 days. The increase in weight of the sample was calculated as percentage moisture gain using equation 3:

$$\%\ \text{Moisture gain} = (\text{moisture gain})/(\text{original weight}) \times 100 \quad (3)$$

*Moisture content (loss on drying)*

An empty clean and dry watch glass was weighed ($M_1$) on an Acculab Sartorius Balance, Germany, and the weight recorded. 1 g of the sample (M) was transferred into the watch glass and the new weight ($M_2$) obtained. The watch glass and its content were placed in the oven at 105°C for 1 h, 1.5 h, and 2 h. The watch glass and its content (starch) were weighed intermittently over the period of heating in the oven until a constant (final) weight ($M_3$) was obtained. The procedure was carried out in triplicate on NPS and MPS, respectively. Moisture content was calculated from the data obtained using equation 4:

$$\text{Moisture content} = (M_3 - M_2)/M \times 100 \quad (4)$$

Where $M_3$ is the final weight of watch glass and sample after drying, $M_2$ is the weight of watch glass and sample before drying and M is the weight of sample.

*Scanning electron microscopy (SEM)*

The SEM of NPS and MPS were done to determine the morphology or shape of the samples using a scanning electron microscope (Phenom Prox, Phenom-World, Netherlands).

*Fourier transform infrared spectroscopy (FTIR)*

The NPS, MPS, MTBZ and a combination of MTBZ and MPS in a ratio 1:1 proportion/mix were evaluated for preformulation compatibility using an FTIR equipment (FTIR- 8001, Shimadzu, Japan) by the potassium bromide pellet method.

*Bulk density*

A 20 g quantity each of NPS and MPS was gently transferred into a clean and dry 50 ml measuring cylinder placed on a smooth flat surface. The volume occupied by the powder was noted. The determination was done in triplicate. Their respective densities were calculated using equation 5:

$$\text{Bulk density} = \text{Weight of powder}/\text{Bulk volume} \quad (5)$$

*Tapped density*

A 20 g quantity of both starches was transferred into a clean and dry 50 ml measuring cylinder. The powder was tapped on a flat surface from a height of 3–5 cm until no further change in volume was observed. The determination was carried out in triplicate and their respective densities calculated using equation 6:

$$\text{Tapped Density} = \text{Weight of Powder}/\text{Tapped Volume} \quad (6)$$

*Particle density*

The particle densities each of NPS and MPS was determined by solvent displacement method using a 25 ml volume pycnometer and n-hexane as a non-solvent. The empty clean and dry pycnometer (Mettler, Germany) was weighed (W), and thereafter filled with n-hexane the excess wiped off, and the filled pycnometer was weighed (W1), The weight of n-hexane

(W2) was calculated as the difference between W1 and W. 0.5 g of either NPS or MPS was weighed (W3) and carefully transferred into the filled pycnometer and the excess fluid on the body of pycnometer wiped off. The pycnometer and its content was weighed (W4). The procedure was carried out in triplicate and the particle density, Pt calculated using equation 7:

$$Pt = (W2 \times W3)/V\ (W3 - W4 + W2 + W) \quad (7)$$

*Flow rate*

The funnel method was used for the determination of flow rate of the starch powder. A funnel was clamped to a retort stand at a distance of 4 cm from the orifice to the flat platform. The orifice of the funnel was stoppered, and then 20 g of NPS was transferred into the funnel. A stop watch was simultaneously started as the stoppered orifice was opened. The time taken for the powder to freely flow through the orifice was noted. The same procedure was carried out using MPS. Triplicate determinations were done. The flow rate was calculated from equation 8:

$$\text{Flow rate} = \text{Mass of powder/time of powder flow} \quad (8)$$

*Angle of repose*

The angle of repose of NPS and MPS was determined using the static method with some modification. A 13.5 cm long plastic pipe open at both ends with internal diameter of 4 cm was placed on a paper on a flat surface, and 50 g of the powder was poured from the upper end. The pipe was lifted up to discharge the powder to form a heap, and the edge of the powder heap was gently marked without distortion to estimate the powder heap diameter. The procedure was repeated thrice and the angle of repose calculated using equation 9:

$$\theta = Tan^{-1}(2h/d) \quad (9)$$

Where h is the height of powder heap and d is the diameter of heap.

*Hausner's ratio (HR) and Carr's compressibility index (CI)*

The HR of NPS and MPS was calculated from the data obtained from the determination of bulk density and tapped density, respectively, using equation 10:

$$HR = \text{Tapped density/bulk density} \quad (10)$$

While the CI, was calculated using equation 11:

$$\text{Carr Index} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}} \times 100 \quad (11)$$

*Porosity*

The porosity (P) of NPS and MPS powder bed was determined from the expression:

$$P = (1 - [\text{bulk density/particle density}] \times 100) \quad (12)$$

## Formulation of MTBZ Suspension

The MTBZ suspensions were formulated using the ingredients and amounts described as follows: Formulations I, II, and III consists of suspensions prepared with 2.0 g (4.0% w/v) MTBZ, 0.05 g (0.1% w/v) benzoic acid, 1.25 g (2.5% w/v) MPS, AC and MC, respectively. Formulations IV, V and VI consist of 2.0 g (4.0% w/v) MTBZ, 0.05 g (0.1% w/v) benzoic acid, 2.5 g (5.0% w/v) of MPS, AC and MC, respectively. Formulations VII, VIII, and IX consists of 2.0 g (4.0% w/v) MTBZ, 0.05 g (0.1% w/v) benzoic acid, 3.75 g (7.5% w/v) of MPS, AC and MC, respectively. Formulation X did not contain any suspending agent and served as control. The polymer was triturated with MTBZ and benzoic acid with distilled water in a mortar to obtain a pourable paste, which was transferred to a 50 mL measuring cylinder. The mortar was rinsed with distilled water into the measuring cylinder up to the 50 ml mark (100%). All the formulations were made in triplicates, plugged with cotton wool and stored.

*Evaluation of MTBZ suspension*

Determination of pH

The pH of the various MTBZ suspensions were determined using a pH meter (Corning, model 10, England) on days 1 to 7, 14, 21 and 28.

Flow rate

The time required for 10 ml of each batch of MTBZ suspension to flow through a 10 ml glass pipette was determined, and the apparent viscosity ($n\alpha$ in $mls^{-1}$) was calculated using equation 13.[20]

$$\text{Flow rate } (n\alpha) = \text{volume of pipette (ml)/Flow time (s)} \quad (13)$$

Freeze-thaw test

Each batch of the preparations was then subjected to a freeze-thaw test. The samples were frozen for 24 h, allowed to thaw at room temperature (30 ± 1°C) for 24 h, stored at 40°C for 24 h and allowed to equilibrate at room temperature for 24 h. The samples were analyzed for significant particle size growth using a microscope (Model XSZ-107 BN, Zenithlabo, USA) fitted with a camera and phoenix micro image analysis software (PHMIAS 2006 Ver. 2.0).

Sedimentation height

The MTBZ suspensions were stored in 50 ml graduated glass measuring cylinders that were kept on a flat surface undisturbed. The sedimentation behavior of the suspension was monitored on days 1 to 7, 14, 21 and 28. Both the initial ($V_o$) and final ($V_t$) sedimentation volumes this period was recorded. The respective percentage sedimentation volume (F) was calculated using equation 14.[21,22]

$$F = (V_t/V_o) \times 100 \quad (14)$$

Viscosity measurement

The viscosity of a 50 ml volume of each batch of MTBZ suspension was determined with a Brookfields viscometer (Dv2, Brookfield Engineering Laboratories, Massachusetts, USA) using Lv-02 (number 62) spindle at a speed of 12 rotations per minute (rpm) speed, and at a temperature of 29.5 ± 0.5°C. Determinations were carried out in triplicates.

Redispersibility tests

On the 28th day of storage, the MTBZ suspensions were shaken by covering the mouth of the measuring cylinder, turning it through an angle of 180° in an inverted position clockwise and returning the cylinder back to its former position again. This process was noted as a cycle.[23] The number of cycles for the sediments to completely re-disperse to produce a homogenously mixed suspension was noted for each formulation.[24]

## Statistical Analysis

All statistical analysis of data was carried out using the one-way analysis of variance with IBM Statistical Package for the

Social Sciences statistics 21 software. Differences in value of data were considered significant where $P < 0.05$.

## RESULTS AND DISCUSSION

### Physicochemical Properties

The yield of the native starch, NPS extracted from matured dry millet seed is 87% while the yield of the modified starch, MPS is 60 % of NPS. Both starches had an off white color, bland taste and granular texture although MPS was more granular. Both starches had an odor that is characteristic of starch.

### Identification

Both NPS and MPS showed a blue black color on the addition of iodine, and this test is a typical identification test for starch.[25]

### Ash Contents and Other Extractives

The ash profile results are shown in Table 1. The ash content of a material shows how well the material is free of inorganic substances.[26] The higher values of total ash and acid insoluble ash obtained with MPS shows a level of impurity which could have resulted from the chemical substances used during the modification steps.

**Table 1:** Ash profile of NPS and MPS

| S/N | Parameters (%) | NPS | MPS |
|---|---|---|---|
| 1 | Total Ash (%) | 1.3 | 3.6 |
| 2 | Acid insoluble (%) | 0.5 | 2.1 |
| 3 | Water soluble ash (%) | 0.7 | 0.2 |
| 4 | Ethanol extractive yield (%) | 23.4 | 4.2 |

NPS: Natural *Pennisetum* starch, MPS: Modified *Pennisetum glaucum* starch

### pH and Viscosity

The pH of NPS and MPS were 5.40 ± 0.01 and 6.88 ± 0.01 respectively [Table 2]. The almost neutral pH of MPS makes it a good excipient for the formulation of both basic and acidic drugs. The viscosity of the cold aqueous dispersion of NPS and MPS had viscosity values of 3.07 ± 0.06 and 4.23 ± 0.06 cP respectively [Table 3]. This suggests that modification enhanced the viscosity of MPS which makes it more suitable as a suspending agent than the native starch.

### Hydration Capacity

The hydration (water retention) capacity of both NPS and MPS are shown in Table 4. MPS had a greater hydration capacity compared to NPS ($P < 0.05$). Modification enhanced the hydration capacity of MPS by more than twice that of NPS. Hydration capacity is an index that could help in the determination of the behavior of a material when in contact with an aqueous solvent. The hydration capacity indicates the amount of water a material is able to absorb on hydration.[27] Thus MPS is expected to have better suspending properties in suspension formulations as well as better disintegrant properties in tablet formulations.

### Swelling Index

Results of the swelling index of the starches are shown in Table 2. The swelling index of MPS was significantly higher (391.67 ± 7.64%) than that of NPS (123.33 ± 2.89%) ($P < 0.05$). The swelling index of a material shows the ability of a material to take up water as well as retain such water after absorption. Thus modification enhanced the swelling index of MPS.

### Moisture Absorption/Hysteresis

The moisture absorption results are shown in Table 2. In general, there was an increase in the amount of water adsorbed

**Table 2:** Physicochemical properties of NPS and MPS powders

| S/N | Parameter | NPS | MPS |
|---|---|---|---|
| 1 | pH | 5.40±0.01 | 6.88±0.01 |
| 2 | Viscosity (cP) | 3.07±0.06 | 4.23±0.06 |
| 3 | Hydration capacity (%) | 186.03±1.51 | 497.46±0.82 |
| 4 | Swelling index (%) | 123.33±2.89 | 391.67±7.64 |
| 5 | Bulk density (g/ml) | 0.31±0.01 | 0.48±0.00 |
| 6 | Tapped density (g/ml) | 0.53±0.01 | 0.72±0.08 |
| 7 | Particle density (g/ml) | 2.15±0.00 | 1.69±0.08 |
| 8 | Hausner's ratio | 1.71±0.48 | 1.50±0.23 |
| 9 | Angle of Repose ( °) | 33.46±0.88 | 29.21±0.46 |
| 10 | Porosity (%) | 85.49±0.24 | 71.28±0.20 |
| 11 | Carr's index (%) | 41.50±1.69 | 33.33±0.66 |
| 12 | Moisture content (%) | 14.73±0.69 | 11.12±0.69 |
| 13 | Moisture absorption (96% RH) (%) | 28.74±1.29 | 38.79±0.74 |
| 14 | Moisture absorption (84% RH) (%) | 11.58±0.75 | 20.78±1.87 |
| 15 | Moisture absorption (75% RH) (%) | 10.67±0.49 | 16.57±0.91 |
| 16 | Moisture absorption (52% RH) (%) | 10.17±0.88 | 16.51±1.32 |

Key: RH represents relative humidity, NPS: Natural *Pennisetum* starch, MPS: Modified *Pennisetum glaucum* starch

**Table 3:** Particle size of suspensions before and after freeze thaw

| S/no. | Materials/Batch | Mean particle size of suspension | Particle size after freeze thaw test | *P*-values |
|---|---|---|---|---|
| 1 | MPS | 2.88±2.26 | 2.88±2.26 | >0.05 |
| 2 | MTBZ | 6.95±1.44 | 6.95±1.44 | >0.05 |
| 3 | I | 4.72±1.33 | 4.92±1.73 | >0.05 |
| 4 | II | 6.86±1.92 | 5.28±1.03 | >0.05 |
| 5 | III | 7.52±1.56 | 6.34±1.79 | <0.05 |
| 6 | IV | 6.53±1.79 | 4.81±1.73 | <0.05 |
| 7 | V | 7.21±1.64 | 6.64±1.80 | <0.05 |
| 8 | VI | 7.18±1.07 | 4.31±1.32 | <0.05 |
| 9 | VII | 5.38±1.64 | 4.31±1.53 | <0.05 |
| 10 | VIII | 5.03±2.19 | 4.07±1.15 | <0.05 |
| 11 | IX | 4.88±1.63 | 4.88±1.63 | >0.05 |
| 12 | X | 4.15±1.86 | 6.38±1.90 | <0.05 |

MPS: Modified *Pennisetum glaucum* starch, MTBZ: Metronidazole benzoate

**Table 4:** pH of metronidazole suspensions containing MPS, acacia and methyl cellulose

| Day/ Batch | I | II | III | IV | V | VI | VII | VIII | IX | X |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 4.31±0.06 | 4.31±0.06 | 5.75±0.02 | 3.98±0.00 | 4.15±0.07 | 4.21±0.07 | 3.33±0.02 | 3.25±0.02 | 3.28±0.02 | 3.36±0.07 |
| 1 | 4.29±0.04 | 4.29±0.04 | 5.87±0.07 | 4.00±0.07 | 4.12±0.07 | 4.18±0.04 | 3.45±0.02 | 3.42±0.02 | 3.36±0.03 | 3.27±0.07 |
| 2 | 4.36±0.07 | 4.35±0.07 | 5.91±0.07 | 4.01±0.07 | 4.16±0.05 | 4.17±0.01 | 3.48±0.04 | 3.50±0.02 | 3.43±0.03 | 3.26±0.06 |
| 3 | 4.32±0.03 | 4.32±0.07 | 6.01±0.07 | 4.02±0.07 | 4.15±0.06 | 4.22±0.07 | 3.56±0.01 | 3.49±0.02 | 3.48±0.02 | 3.44±0.07 |
| 4 | 4.32±0.07 | 4.32±0.07 | 6.03±0.07 | 4.04±0.02 | 4.18±0.06 | 4.23±0.07 | 3.57±0.03 | 3.55±0.22 | 3.52±0.03 | 3.69±0.07 |
| 5 | 4.33±0.28 | 4.33±0.28 | 6.12±0.07 | 4.08±0.04 | 4.18±0.07 | 4.26±0.07 | 3.57±0.01 | 3.51±0.02 | 3.54±0.02 | 3.47±0.07 |
| 6 | 4.38±0.28 | 4.38±0.28 | 6.17±0.02 | 4.02±0.02 | 4.17±0.04 | 4.22±0.02 | 3.57±0.01 | 3.53±0.06 | 3.55±0.01 | 3.49±0.21 |
| 7 | 4.28±0.04 | 4.28±0.04 | 6.15±0.07 | 4.01±0.02 | 4.16±0.04 | 4.23±0.03 | 3.51±0.11 | 3.54±0.02 | 3.57±0.05 | 3.38±0.06 |
| 14 | 4.27±0.02 | 4.29±0.01 | 4.30±0.03 | 4.01±0.01 | 4.15±0.01 | 4.21±0.01 | 3.51±0.01 | 3.53±0.02 | 3.54±0.03 | 3.39±0.01 |
| 21 | 5.06±0.07 | 5.06±0.08 | 5.23±0.02 | 4.01±0.01 | 4.15±0.02 | 4.20±0.01 | 3.48±0.02 | 3.54±0.04 | 3.53±0.03 | 3.42±0.05 |
| 28 | 4.31±0.06 | 4.31±0.06 | 5.75±0.02 | 3.98±0.00 | 4.15±0.07 | 4.21±0.07 | 3.33±0.02 | 3.25±0.02 | 3.28±0.02 | 3.36±0.07 |

MPS: Modified *Pennisetum glaucum* starch

by both starches as the relative humidity conditions increased. Modified millet starch adsorbed more moisture than the natural starch which implies that modification improved its moisture adsorption ability. This is important not only in the choice of excipient to be used in a formulation but also helps in the choice of packaging materials and conditions of storage.

## Bulk, Tapped and Particle Densities

Table 2 contains results of the density determinations of the starches. The bulk and tapped densities of MPS was higher than NPS. These suggest a greater compressibility and inter particulate arrangement within the powder bed of MPS. A lesser particle density for MPS would translate to a more organized lattice structure for MPS. Thus modification improved the bulk and tapped density properties of the starch.

## Flow Properties

The flow properties of the starch grains are shown in Table 2. Generally, starch grains are known to have a poor flow.[6,28]

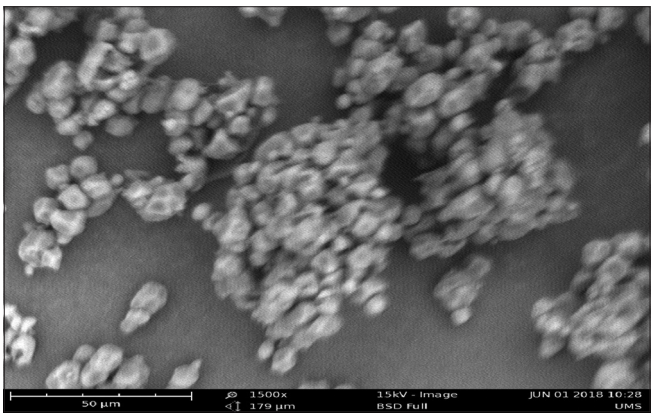



**Figure 1:** Scanning electron microscopy of Natural Pennisetum Starch 1500× (magnification)

Modification improved the flow properties of MPS ($P < 0.05$). This is shown by the Hausner's quotient, Carr's index, and angle of repose values of NPS and MPS.

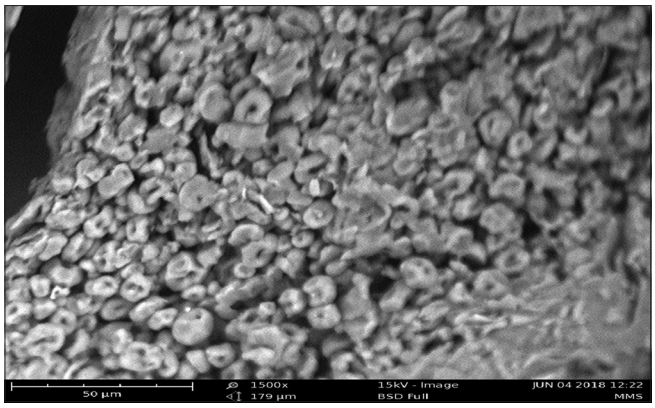


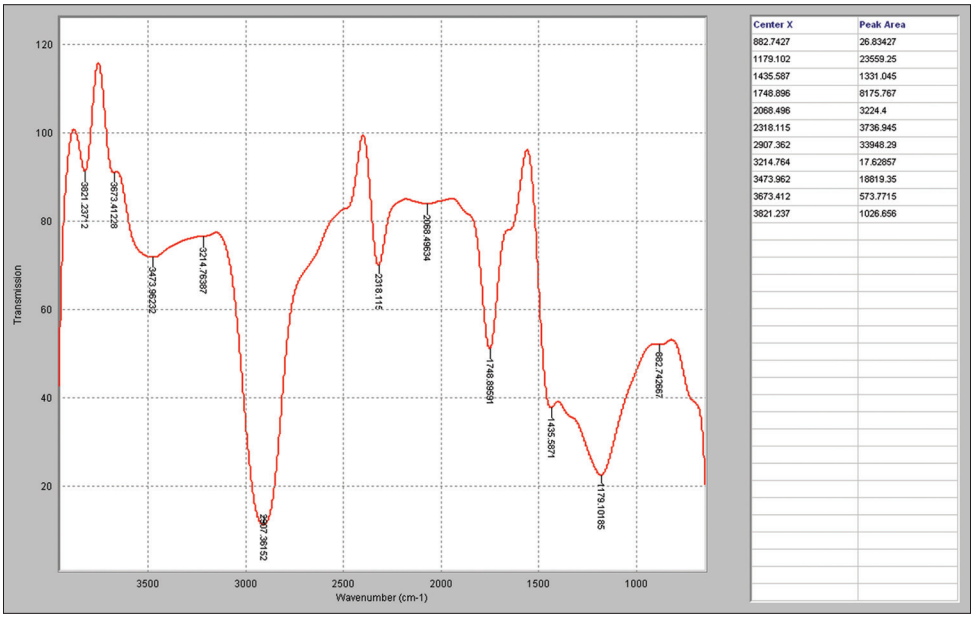

**Figure 2:** Scanning electron microscopy of modified *Pennisetum* starch 1500× (magnification)

## SEM

The scanning electron micrographs of NPS and MPS are shown in Figures 1 and 2 respectively. Their morphologies show clustered rectangular to polygonally shaped granular materials with a hilum in the middle of each grain. The SEM of the various samples was visually different from each other, which shows that the modifications done had effect on the surface morphology of the starches.

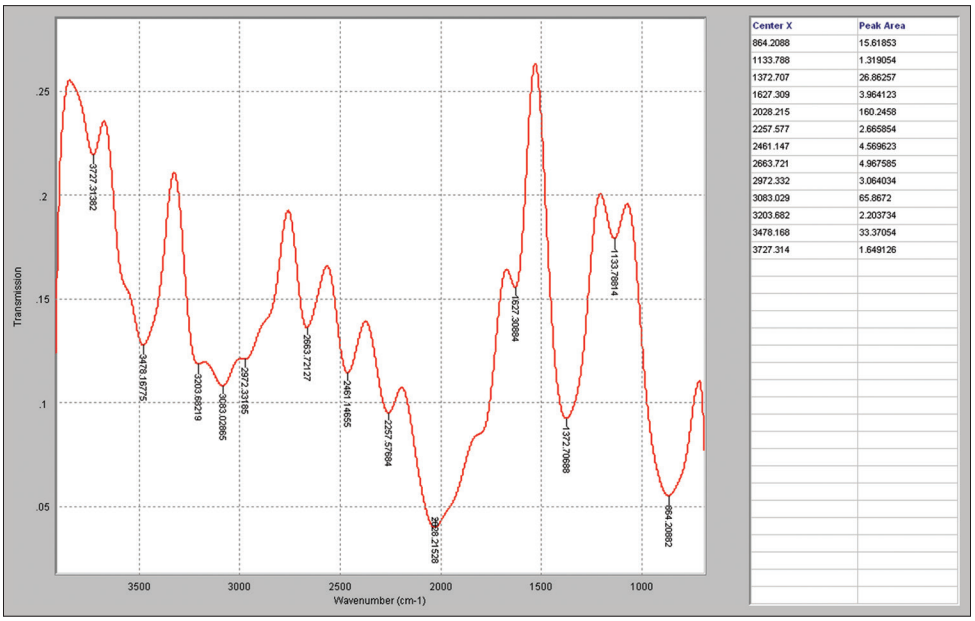
## FTIR

The FTIR spectra of NPS, MPS, MTBZ, and a combination of MPS and MTBZ in the ratio 1:1 are shown in Figures 3-6. More peaks were observed in MPS at the wavelength range of 2500-3000 $cm^{-1}$ than in NPS while no major shift in the spectral




| Center X | Peak Area |
|---|---|
| 882.7427 | 26.83427 |
| 1179.102 | 23559.25 |
| 1435.587 | 1331.045 |
| 1748.896 | 8175.767 |
| 2068.496 | 3224.4 |
| 2318.115 | 3736.945 |
| 2907.362 | 33948.29 |
| 3214.764 | 17.62857 |
| 3473.962 | 18819.35 |
| 3673.412 | 573.7715 |
| 3821.237 | 1026.656 |

**Figure 3:** Fourier transform infrared spectroscopy of natural *Pennisetum* starch




| Center X | Peak Area |
|---|---|
| 864.2088 | 15.61853 |
| 1133.788 | 1.319054 |
| 1372.707 | 26.86257 |
| 1627.309 | 3.964123 |
| 2028.215 | 160.2458 |
| 2257.577 | 2.665854 |
| 2461.147 | 4.569623 |
| 2663.721 | 4.967585 |
| 2972.332 | 3.064034 |
| 3083.029 | 65.8672 |
| 3203.682 | 2.203734 |
| 3478.168 | 33.37054 |
| 3727.314 | 1.649126 |

**Figure 4:** Fourier transform infrared spectroscopy of modified *Pennisetum* starch

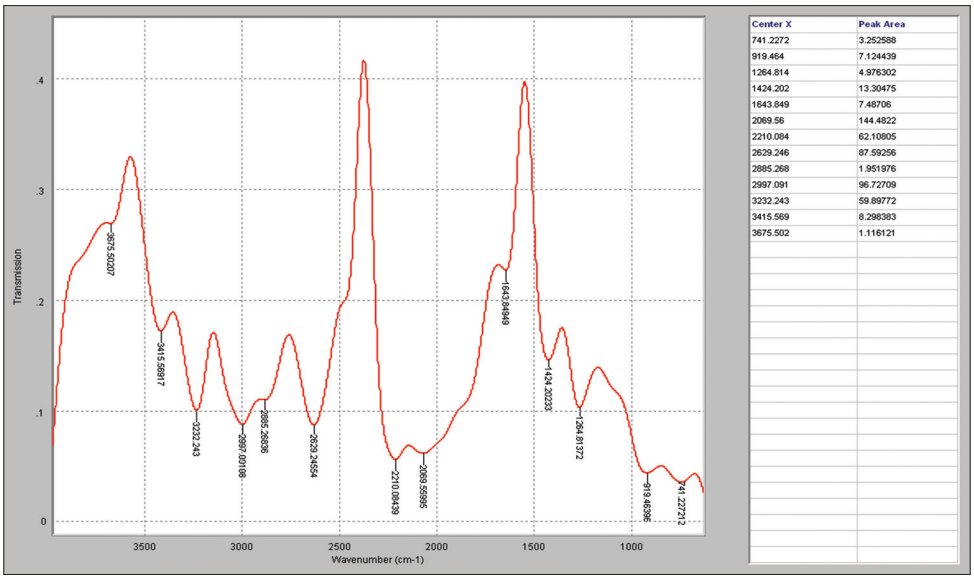



**Figure 5:** Fourier transform infrared spectroscopy of metronidazole benzoate

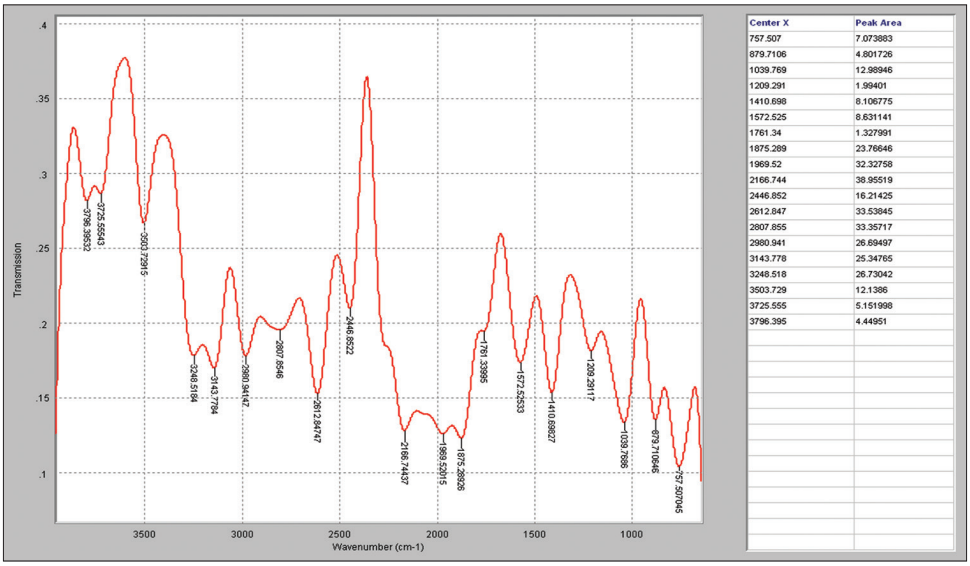



**Figure 6:** Fourier transform infrared spectroscopy of metronidazole benzoate+modified *Pennisetum* starch

peaks were observed between equal mixtures of MPS and MTBZ implying compatibility between the two compounds.

## pH of Metronidazole Suspension

The pH of the different MTBZ suspensions is shown in Table 4. It was observed that increase in concentration of the polymer did not alter the pH of the suspension on storage within the 28 day storage period of the study ($P > 0.05$). This could be attributed to the absence of microbial degradation as well as chemical stability of the active pharmaceutical ingredient with the excipients used in the formulation.

## Freeze Thaw

The mean particle sizes of the suspensions before and after freeze thaw is shown in Table 3. No significant difference ($P > 0.05$) was observed in the particle sizes of MPS, MTBZ, MTBZ suspension formulations I, II and IX (suspensions containing MPS at 2.5%, 5.0% and MC respectively at 7.5% w/v) which signifies stability of the suspension. Formulations III (contains MPS at 7.5% w/v), IV, V and VI (contains AC at 2.5, 5.0 and 7.5% w/v), VII, VIII (contains MC at 2.5 and 5.0% w/v) and X (control) showed significant differences ($P < 0.05$) in their particle sizes after freeze thaw which suggests particle size growth (coalescence) that would result in the instability of the suspensions. Thus, the suspensions containing MPS showed more resilience in terms of stability over stress conditions that they were exposed to.

## Flow Rate and Viscosity of Metronidazole Suspensions

The flow rates of the MTBZ suspensions are shown in Table 5. It was observed that increase in the concentration of the

**Table 5:** Viscosity, flow rate and redispersibility of metronidazole suspensions containing MPS, acacia and methylcellulose

| Batch/Parameter | I | II | III | IV | V | VI | VII | VIII | IX | X |
|---|---|---|---|---|---|---|---|---|---|---|
| Viscosity (cP) | 4.00±0.03 | 5.00±0.10 | 12.50±0.13 | 5.00±0.25 | 7.50±0.02 | 10.00±0.14 | 3.00±0.11 | 4.00±0.05 | 12.50±0.13 | 2.00±0.01 |
| Flow-rate (ml/s) | 1.77±0.10 | 1.68±0.04 | 1.52±0.05 | 1.83±0.10 | 1.69±0.10 | 1.56±0.08 | 1.90±0.05 | 1.83±0.12 | 1.52±0.05 | 1.89±0.01 |
| Redispersibility | 2.00±0.02 | 2.00±0.00 | 3.00±0.01 | 12.00±1.01 | 13.00±0.73 | 18.00±0.47 | 7.00±0.51 | 9.00±0.25 | 3.00±0.01 | 13.00±0.01 |

MPS: Modified *Pennisetum glaucum* starch

polymer led to a decrease in the flow rate and an increase in the viscosity of the suspension. At 7.5% w/v polymer concentrations, MPS had the highest viscosity of 12.5 cP and lowest flow rate of 1.52 ml/s, while AC and MC at similar concentrations had viscosities of 10.0 cP and 5.0 cP, and flow rates of 1.56 ml/s and 1.75 ml/s, respectively. In general, the relationship between increase in polymer strength, decrease in flowability and increase in viscosity of suspensions has been reported as a characteristic that is common in suspensions formulated with hydrophilic polymers as suspending agents.[29-31]

## Redispersibility of Metronidazole Suspension

The redispersibility behavior of the MTBZ suspensions is shown in Table 5. All the suspensions were re-dispersed on shaking. The suspensions containing MPS were more easily re-dispersed than those containing AC and MC. Suspensions containing AC were the most difficult to re-disperse. The number of shakes for the control which had no suspending agent was greater than that of the suspensions containing the polymers. This can be attributed to a closely packed bed (cake) devoid of inter particulate pores on settling which did not allow an easy inflow of the vehicle into the bed upon agitation in order to aid dispersibility. The order of performance was MPS > AC > MC. A good suspension usually possesses a low redispersibility number and is easily redispersed so as to ensure uniformity of administered doses of medicaments upon shaking or agitation.[22]

## Sedimentation Height/Volume

The sedimentation heights/volumes of the MTBZ suspensions during a 28 day storage period are shown in Figure 7. A sharp decline of the sedimentation volume was observed for all the suspensions on the first day after formulation and storage. Thereafter, the volume of the sediments either gradually decreased or was almost stable for the rest of the period especially from day 7 ($P > 0.05$). Since sedimentation volumes toward 100% depicts stability,[32] the suspensions containing MPS at all concentrations had higher volumes of sediments than the suspensions formulated with other polymers ($P <$

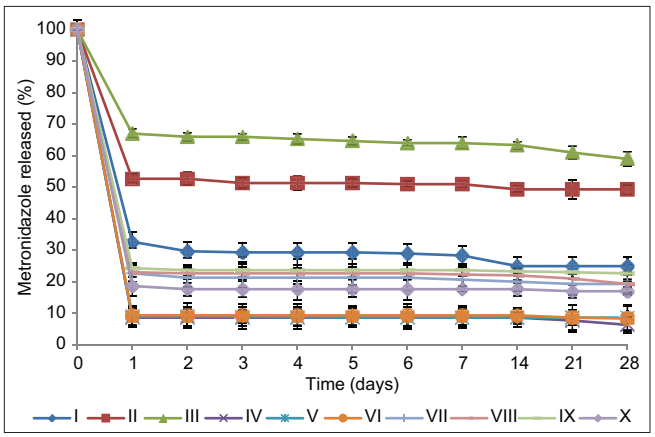



**Figure 7:** Sedimentation volume of metronidazole benzoate suspensions

0.05), and therefore, could be regarded as the most stable suspensions (7.5% w/v >5.0% w/v >2.5% w/v). In general, the order of stability of the suspensions based on the polymers that were used as suspending agents are MPS > MC > AC. Suspending agents could reduce sedimentation volume as well as the rate of sedimentation because they increase the viscosity of the suspensions and serve as a protective coating to the suspended solids. Thus, a reduction in the rate of sedimentation and collision between the insoluble components of the suspension is achieved. The higher the concentration of the polymer that was used, the more stable is the suspension produced. However, differences in the individual polymer characteristics such as its viscosity when hydrated, and its ability to maintain its physical and chemical composition over a given period of time play a major role to its functionality as a suspending agent when it is used in formulation of suspensions.

## CONCLUSION

The modification of native starch (NPS) obtained from *P. glaucum* resulted in a starch (MPS) that had improved physicochemical properties such as pH, viscosity, hydration capacity and swelling index. The SEM showed that the morphology of NPS differed from the morphology of MPS while FTIR showed compatibility between MTBZ and MPS. The MTBZ suspensions formulated with MPS compared favorably with those formulated with AC and MC which were used as comparing/standard suspending agents in terms of parameters such as pH, flow rate, viscosity, sedimentation volume and redispersibility. MTBZ suspensions formulated with MPS were more stable and redispersible than the suspensions containing AC and MC.

## REFERENCES


1. Martin A, Swarbrick J, Cammarata A, editors. Course dispersions. In: Physical Pharmacy. 3rd ed. Philadelphia, PA: Lea and Febiger; 1983. p. 544.
2. Femi-Oyewo MN, Adedokun MO, Olusoga T. Evaluation of the suspending properties of *Albizia zygia* gum on sulphadimidine suspension. Trop J Pharm Res 2004;3:279-84.
3. Kumar R, Patil BM, Patil SR, Paschapur SM. Evaluation of *Albelmoschus esculentus* mucilage as suspending agent in paracetamol suspension. Int J Pharm Res 2009;1:658-65.
4. Uhumwangho MU, Ileje IL. Preliminary evaluation of the suspending properties of *Brachystegia eurycoma* gum on metronidazole suspension. Int Curr Pharm J 2014;3:328-30.
5. Bakre GL, Ajakore O. Suspending properties of natural gums extracted from *Abelmuscus esculentus* pod and *Chrysophyllum albidum* fruit. Afr J Pharm Pharmacol 2015;9:321-6.
6. Kunle OO. Review: Pharmaceutical grade starch and some of its potential sources in Nigeria. J Phytomed Ther 2002;7:1-17.
7. Builders PF, Arhewoh MI. Pharmaceutical applications of native starch in conventional drug delivery. Starch Starke 2016;68:864-73.
8. Emeje MO, Rodrigues A. In: Valdez B, editor. Starch: From Food to Medicine, Scientific, Health and Social Aspects of the Food Industry. London: InTech; 2012.
9. Manek RV, Kunle OO, Emeje MO, Builders P, Rao GV, Lopez GP, *et al*. Physical, thermal and sorption profile of starch obtained from tacca leonpetaloides. Starch Starke 2005;57:55-61.
10. Gulia SK, Wilson JP, Carter J, Singh BP. Progress in grain pearl millet research and market development. In: Issues in New Crops and New Uses. Alexandria, VA: ASHS Press; 2007. p. 197-203.
11. Snydman DR, Jacobus NV, McDermott LA. *In vitro* evaluation of the activity of imipenem-relebactam against 451 recent clinical isolates of *Bacteroides* group and related species. Antimicrob Agents Chemother 2016;60:6393-7.
12. Dinsdag SA, Hunter N. Metronidazole: An update on metabolism, structure cytotoxicity and resistance mechanisms. J Antimicrob Chemother 2018;73:265-79.
13. The British Pharmacopoeia. Vol. 2. Cambridge: Her Majesty Stationary Office, University Press; 2012.
14. USP 28-NF 23 United States Pharmacopeia and National Formulary 2005. Rockville: The United States Pharmacopoeia, U.S.P, NF. The United States Pharmacopeial Convention; 2005.
15. World Health Organization. Quality Control Methods for Herbal Materials. Geneva: World Health Organization; 1998. Available from: http://apps.who.int/medicinedocs/documents/h1791e/h1791e.pd [Last accessed on 2019 Jun 20].
16. Ring SG. Some studies on gelatin. Starch 1985;37:80-7.
17. Bowen FE, Vadino WA. A simple method for differentiating sources. Drug Dev Ind Pharm 1984;10:505-11.
18. Iwuagwu MA, Okoli PC. The disintegrant properties of pregelatinized cassava and white yam starch. Pharm World J 1992;9:49-53.
19. Pharmaceutical Codex. In: Walter L, editor. Principles and Practice of Pharmaceutics. 12th ed. London: The Pharmaceutical Press; 1994. p. 696.
20. Mahmud HS, Oyi AR, Allagh TS, Gwarzo MS. Evaluation of the suspending property of *khaya senegalensis* gum in cotrimoxazole suspension. Res J Appl Sci Eng Technol 2010;2:50-5.
21. Ugoeze KC, Nwachukwu N. Evaluation of the suspending properties of a novel hydrophilic biopolymer derived from the tubers of *Ipomoea batatas* on sulphamethoxazole suspension. J Adv Med Pharm Sci 2017;15:1-8.
22. Zhu Z, Wang H, Peng D. Dependence of sediment suspension viscosity on solid concentration: A simple general equation. Water 2017;9:474.
23. Brhane Y. Evaluation of carboxymethylated *Plectranthus edulis* starch as a suspending agent in metronidazole benzoate suspension formulations. PLoS One 2020;15:e0228547.
24. Okoye E, Edochie C, Adegbemi JO. Preliminary evaluation of *Delonix regia* seed gum as a suspending agent in a liquid oral dosage form. Int J Pharm Sci Drug Res 2014;6:114-9.
25. Fleischer H. The iodine test for reducing sugars: A safe, quick and easy alternative to copper (II) and silver (I) based reagents. World J Chem Educ 2019;7:45-52.
26. Swamy P, Mulla SK. Preliminary pharmacognostical and phytochemical evaluation of *Portulaca quadrifida* Linn. Int J PharmTech Res 2010;2:1699-702.
27. Amidon GE, Houghton ME. The effect of moisture on the mechanical and powder flow properties of microcrystalline cellulose. Pharm Res 1995;12:923-9.
28. Odeku AO. Potentials of tropical starches as pharmaceutical excipients: A review. Starch Starke 2013;65:89-106.
29. Anyebe SN, Apeji YE, Olayemi OJ. The suspending properties of *Cissus rubiginosa* fruit mucilage in paracetamol suspension formulation. Nigr J Pharm Res 2017;13:19-25.
30. Aremu OI, Oduleya OO. Evaluation of metronidazole benzoate suspensions. Afr J Pharm Pharmacol 2015;9:439-50.
31. Bamiro OA, Ajala TO, Uwaezoke OJ, Akinwumi AG. The suspending properties of *Terminalia randii* gum in magnesium carbonate suspension. Afr J Pharm Pharmacol 2014;8:87-92.
32. Alok KK, Onkar NS, Michael GW. Pharmaceutical suspensions. In: From Formulation Development to Manufacturing. New York: Springer Science + Business Media; 2010. p. 52-3.