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Physicochemical Properties of Cassava Starch Retrograded in Alcohol

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ABSTRACT

Starch is an important excipient employed in the pharmaceutical industry but irrespective of its source, the native starch is undesirable for many applications because of its inability to withstand processing conditions and hence the need for it modification in order to improve its desired properties. The objective of this study was to extract, modify by retrogradation in alcohol and evaluate starch obtained from *Manihot esculentum* as a pharmaceutical excipient. The starch was extracted then modified by retrogradation using alcohol for a period of 0, 24, 48 and 96 hrs. The granular morphology, amylose and amylopectin fractions, ash value, true density, powder porosity, swelling capacity, hydration capacity, moisture sorption capacity, bulk density, tapped density, Carr's index, Hausner's ratio, angle of repose, X-ray diffractography, elemental and proximate analysis were used for the physicochemical characterizations and was subsequently compared with native *Manihot esculentum* starch. Starch obtained by retrogradation showed decrease in angle of repose, moisture sorption capacity, moisture content, water solubility index and showed increase swelling capacity, hydration capacity and crystallinity as compared to the native starch. In conclusion, retrograded starch showed better pharmaceutical potentials than the native starch as evident to its improved physicochemical properties.

INTRODUCTION

Cassava (*Manihot esculenta*) is a perennial tuber plant widely grown in many tropical countries including Nigeria as one of the most important commercial crops. Cassava is second only to sweet potato as the most important starchy root crop of the tropics (Grace, 1977). Native starches, irrespective of their source, are undesirable for many applications (Wang *et al.*, 1993) because of their inability to withstand processing conditions such as extreme temperature, diverse pH, high shear rate and freeze-thaw variation. In order to improve on the desirable functional properties and overcome its limitations, native starches are often modified. Physical modification involves the simultaneous action of several conditions such as temperature, pressure, moisture and shear. Temperature and moisture contents during processing of starch alter its functional properties. The molecular interactions (hydrogen bonding between starch chains) after cooling of the gelatinized starch paste have been called retrogradation (Hoover, 2001). During retrogradation, amylose forms double-helical associations of 40 – 70 glucose units (Jane and Robyt, 1984) whereas amylopectin crystallization occurs by association of the outermost short branches. When native starch is heated and dissolves in water, the crystalline structure of amylose and amylopectin molecules are lost and they hydrate to form a viscous solution. If the viscous solution is cooled or left at lower temperature for long enough period, the linear molecules, amylose, and linear parts of amylopectin molecules retrograde and rearrange themselves again to a more crystalline structure. The linear chains place themselves parallel and form hydrogen bridges. In viscous solutions the viscosity increases to form a gel. At temperatures between –8 °C and +8 °C the aging process is enhanced drastically. Cassava and sweet potato starches have low retrogradation tendency and therefore high paste stability. Retrogradation is induced by low temperature, high amylose content and the presence of polar substances, such as salts. Overall, starch susceptibility to retrogradation is also controlled by its molecular weight, concentration, temperature, and presence of non-starch components (salts, saccharides, lipids, acids, hydrocolloids, surfactants) (Sandhu and Singh, 2007). In consequence of Retrogradation, the intermolecular distances between starch molecules diminish. This leads to the removal of water from gel, and in consequence dehydration of the material.

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The phenomenon could be observed as occurrence of water on gel surface, known as syneresis (Karim *et al.*, 2000). Retrogradation occurs not only in amylose fraction but also amylopectin from gelatinized granules. Association of linear amylose molecules takes place quickly at the first stage of retrogradation, while slow increase in starch gel rigidity is attributed to amylopectin crystallization (Zobel, 1988). The objective of this study is to evaluate physico-chemical properties of cassava starch retrograded in alcohol for pharmaceutical applications.

MATERIALS AND METHODS

Materials

Roots of the plant *Manihot esculenta* were obtained from Bodinga local government central market, Sokoto State, Nigeria. All other chemicals and reagents used were of analytical grade.

Methods

Extraction

The procedure described by Alves *et al.* (2002) was adopted with little modification. The cassava tuber were peeled and cut into cubes which weighs 5.79 kg. These cassava cubes were then soaked in 10 litres 0.075 % of sodium metabisulphite solution overnight to avoid oxidation. This mixture was then milled, stirred and filtered using a double fold clean cheesecloth which was allowed to completely settle while the starch sediments. This was followed by decanting the water and centrifuging the suspension at 4000 rpm for 5 min using Lab centrifuge (Thermo electron co. IEC FL40R, France); after centrifugation, the starch was separated from water and non water soluble constituents. Finally the pure starch obtained from centrifugation was air dried in a hot air oven (Nurve FN055 Oven, Germany), grinded with a grinder (Super Master Co. Ltd. SMB-3377) and sifted through Sethi standard sieves. The flour was then packed into an airtight container and stored at room temperature for further analysis.

Starch Modification

This was achieved by adopting the method described by Bertolini (2009) with little modification. A 20 g quantity of native starch (NS) powder and 400 ml of water was used for the pasting process after which ethanol in a ratio 1:1 was added and stirred uniformly. This was transferred into an ice bath for 10mins with continues stirring (0 HR) after which it was kept in a refrigerator at 4 °C for 24, 48 and 96 hrs (to be known as 24HR, 48HR and 96HR respectively). At the end of the period, the sample was removed and the supernatant liquid was decanted and the sediment poured onto a clean tray and dried in an oven at 50 °C for 48 hrs. Afterwards, the completely dried flakes were removed and crushed into powder using a blender. This was followed by sieving using a 300 µm mesh.

Granular Morphology

Very small amount of the sample was mounted on a slide in dilute glycerol and viewed under light microscope (Optika B131 SN 303059, Italy) and the image captured using an electron eyepiece (YJEYE01-130).

Physicochemical properties

Iodine and pH test were carried out in accordance with British Pharmacopoeia (2010) specification

Moisture sorption capacity

2g of the starch material was accurately weighed and evenly distributed over the surface of a 70mm tarred Petri dish. The samples were placed in large desiccator containing saturated sodium chloride solution (RH=75%) in the reservoirs at room temperature and the weight gained by the exposed sample at the end of a five day period was recorded and the amount of water sobbed calculated from the weight differences .

True density

The true density (D_I) of the starch was determined by the liquid displacement method using xylene as the immersion fluid as described by Ohwoauvorhua *et al* (2004) and computed according to the following equation.

$$D_I = W/ [(a+w) – b] \times SG$$

Were w is the weight of the powder, SG is specific gravity of liquid, a, is Weight of bottle + liquid and b is weight of bottle + solvent + powder.

Bulk and tapped densities

A 10g quantity of the powder sample was Placed in 50ml clean, dry measuring cylinder and the volume V_o occupied by the sample without tapping determined. After 500 manual taps, occupied volume V500 was determined. The bulk and tapped densities was calculated as the ratio of the weight of weight of volume (V_o and V_{500} respectively). The Carr's index and Hausner's ratio were determined from the values of the bulk and tapped densities results obtained above.

Angle of repose

The static angle of repose, a was measured according to the fixed funnel and free standing cone method and the tangent of the angle of repose calculated using the equation

$$\text{Tan } a = 2h/D$$

Were h is the height of the heap of powder and D is the diameter of the base of the heap of powder.

Powder porosity

This was determined from the values of true and bulk densities when fitted into the equation according to the method of Ohwoauvorhua *et al. (*2004):

$$e = 1\text{-}B_b/D_t \times 10$$

Were BЬ, is the bulk density, D, is the true density and e is the porosity

Hydration capacity

The method of Kornblum and stoopak (1973) was used. A 1g sample was placed in each of four 15ml plastic centrifuge tubes and 10ml distilled water added from a 10ml measuring cylinder and then stopped. The contents were mixed on a vortex mixer for 2 min. The mixture then allowed to stand for 10min and immediately centrifuged. The supernatant was carefully decanted and the sediment weighed. The hydration capacity was taken as the ratio of the weight of the sediment to the dry sample weight.

Swelling capacity

This was determined at the same time as the hydration determination using the method of Okhamafe *et al* (1991) and computed according to the following equation;

$$S = (V_2 - V_1)/V_1 \times 100\%$$

Where S is the % swelling capacity, V_2 is the volume of the hydration or swollen material and V_1 is the tapped volume of the material prior to hydration.

Water Solubility Index

The water solubility index of starches was carried out as described by Anderson and Sefa-dedeh (2001) with little modification. 1 g each of the starches and 10 ml of water were mixed in a 15 ml plastic centrifuge tube and were immersed in water bath for 30 min at 37 ^{0}C. This was then centrifuged at 4000 rpm for 10 min after which the supernatant was collected in a pre-weighed beaker and the residue was weighed after the water was evaporated at 105 ^{0}C; the percentage of residue with respect to the amount of starch used was taken as the water solubility index.

Proximate and Elemental analysis

The proximate analysis for moisture, crude protein, crude lipid, fiber and ash content of the native and retrograded starches were carried out according to the method of the AOAC (1990). The conversion factor of total nitrogen to crude protein was 6.25. Percentage total carbohydrate was determined by subtracting the sum total of ash, crude protein, lipid and fiber from 100. Apparent amylose content was determined by the method as described by William *et al*., (1958) and the elemental analysis was carried out using an atomic absorption spectrophotometer.

X- Ray Diffraction

The X-ray diffraction pattern of native and retrograded Manihot esculentus starch were recorded with a diffractometer (schimadzu) set to work at 2θ for 1200 sec while the diffractogram was observed on the monitor.

RESULTS AND DISCUSION

The native starch (NS) and 0HR gave a blue black colouration; the remaining samples gave a slight reddish blue coloration with iodine solution. This could be due to a variation in the amylose content of all the samples. The photomicrographs (Fig.1) indicates the shapes of *Manihot esculentum* starches varied considerably with modification; NS granules were shown to be ovoid, oval and closely packed together though not aggregate, while the 0HR has a polygonal shape granules with smooth walls and are closely packed together. Finally the retrograded sample also has predominant polygonal shape granules with smooth walls.

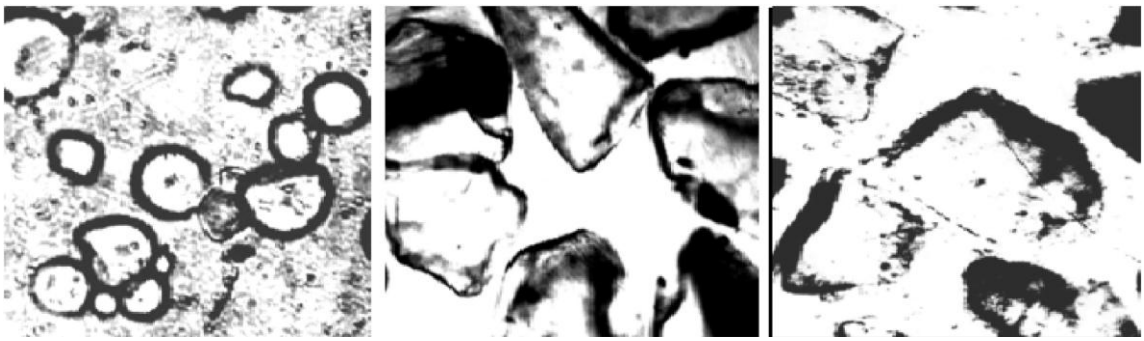
The flow properties of powders are of significance in determining whether a material is suitable as a direct compression excipient. The angle of repose, Hausner index and Carr's percent compressibility are considered as indirect measurements of powder flow property, The Hausner index is indicative of inter-particle friction, while the Carr's index shows the aptitude of a material to diminish in volume (Staniforth, 1996). As the values of these indices increase, the flow of the powder decreases. In general, Hausner ratio greater than 2.5 indicates poor flow; Carr's index below 16 % indicates good flowability while values above 35 % indicate cohesiveness (Staniforth, 1996). When the angle of repose exceeds 50^0 the flow is referred to as poor flow (Shimelis, 2006). The Hausner's ratio previews the degree of densification which could occur during tableting. The higher the ratio, the greater the propensity of powder densification and this phenomenon may cause tablets which lack uniformity of weight and content to be produced. The result obtained (Table 1) for Hausner's ratio range signifies a good to fair property and was observed to be decreasing with increase period of retrogradation. From the results, it could be observed that the angle of repose is far lower than 50^0 compared to the native starch while the Carr's index is less than 35 % for all the samples. Therefore, a low angle of repose and Carr's index would imply a good initial packing arrangement, with less volume of voids (Achor *et al*., 2010).

Table 1: Flow Properties of Native and Retrograded Starches obtained from *Manihot esculentum*.

Parameters	NS	0HR	24HR	48HR	96HR
Bulk density (g/ml)	0.55	0.66	0.66	0.66	0.62
Tapped density (g/ml)	0.66	0.80	0.83	0.76	0.71
Hausner ratio	1.20	1.20	1.25	1.15	1.14
Compressibility index (%)	16.7	17.5	20.5	13.2	12.7
Angle of repose (°)	49.1	20.3	25.7	26.2	26.8

NS (Native starch), 0HR (Zero hour retrogradation), 24HR (24 hours retrogradation), 48HR (48 hours retrogradation) and 96HR (96 hours retrogradation)

Depending on the molecular arrangement of the material, the true density can equal the theoretical density of the material and therefore be indicative of how close the material is to a crystalline state or the proportions of a binary mixture. In the result (Table 2), the true densities of the retrograded starches were virtually found to be higher than that of the native starch. Because powders normally flow under the influence of gravity and that dense substances are generally less cohesive than lighter ones; hence it would be expected that retrograded starches will flow better than the native form. It is known that porosity determines the swelling capacity of starch, i.e. the higher the porosity the more the inter-particulate spaces where water could be absorbed. Generally the results (Table 2) shows lower porosity values for retrograded starch as compared to the native starch which could be



NS OHR 48HR

Fig. 1: Photomicrograph of starches obtained from Manihot esculentum at 100 resolutio
[NS (Native starch), OHR (Zero hour retro gradation), 24 HR and 48 hours retrogradation)].

Table 2: Physicochemical Properties of Native and Retrograded Starches obtained from *Manihot esculentum*

	WSI (%)	Hydration capacity (%)	Swelling capacity (%)	Water sorption capacity (%)	True density	Porosity	PH
NS	3.80	1.77	17.69	16.59	1.83	2.43	7.11
0HR	1.95	7.45	484.38	17.02	1.87	1.62	7.31
24HR	2.10	7.45	344.44	14.02	1.91	1.82	7.25
48HR	1.40	8.55	431.25	17.97	1.89	1.68	7.22
96HR	1.80	7.35	384.38	15.25	1.89	1.98	7.22

WSI (Water solubility index), NS (Native starch), 0HR (Zero hour retrogradation), 24HR (24 hours retrogradation), 48HR (48 hours retrogradation) and 96HR (96 hours retrogradation)

attributed to low inter-particulate spaces resulting from particle size and shape or the increased true densities of the starch. Swelling which is generally accepted as an indication of tablet disintegration ability can be assessed by the determination of hydration capacity, swelling capacity and moisture sorption profile (Caramella, 1991). The hydration capacity value obtained (Table 2) indicates that the retrograded starch is capable of absorbing and retaining more water than its native form. The swelling capacity which reflects the increase in volume of samples following water absorption followed the same trend as the hydration capacity. From the result (Table 2), it will be observed that retrograded starch has higher water sorption capacity compared to the native starch which could be due to its high amylopectin content, also water sorption capacity decreases with decreased amorphous region. pH account for the acidic and basic nature of the starch and is attributed to the concentration of Hydrogen cyanide in the starch which was established to depreciate due to fermentation. As such, the result (Table 2) of both modified and unmodfied starch generally shows a neutral pH which is an indication of low HCN content.

Mineral matter influence a number of starch characteristics such as paste viscosity and swelling (Moorthy, 2001) of which at a low concentration, their effect is minimal. The values obtained for minerals (Table 3) are significantly low except for phosphorus which range from 3.80 to 4.03 and potassium ranging from 2.0 to 4.3 mg/kg. These might be from the cassava origin. The moisture content of starch is the amount of moisture present in it. The higher the moisture content the lower the amount of dry solids in the flour. The maximum allowable limit for moisture in most starch type should not be more than 15 % as stated by the British Pharmacopoeia (2010). Going through the result (Table 4), all the samples passes this specified value. Low Moisture content defines the stability of a drug when starch is used as an excipient by decreasing the rate of deterioration of the drug.

Table 3: Elemental Analysis of Native and Retrograded Starches obtained from *Manihot esculentum*.

Parameters (mg/kg)	NS	0HR	24HR	48HR	96HR
Nitrogen	0.06	0.11	0.12	0.10	0.08
Calcium	0.25	0.30	0.25	0.25	0.25
Magnesium	0.60	0.50	0.50	0.55	0.60
Phosphorus	3.80	3.91	3.96	4.03	3.85
Sodium	1.10	1.80	0.80	1.00	1.10
Potassium	4.30	2.40	2.40	2.00	2.20

NS (Native starch), 0HR (Zero hour retrogradation), 24HR (24 hours retrogradation), 48HR (48 hours retrogradation) and 96HR (96 hours retrogradation)

The ash content of a sample is the non-volatile inorganic matter of a compound which remains after subjecting it to a high decomposition temperature. Hence the ash content can be considered as an indication of clean processing where the British Pharmacopoeia (2010) specify that starch samples should have ash value of less than 0.6 %. It could be observed from the result (Table 4) that all the samples have passed this specification. Fats; the saturated form of lipid could be the reason for the result obtained for lipid content as it was established to be the measure for starch's water solubility and swelling power. That is: the higher the fat content of a starch, the less its solubility and swelling power. The results (Table 4) indicates lower lipid content of retrograded starch as compared to native starch, hence this could attributed to the high swelling power and increase sorption capacity of the starch seen above. But the expected low value of water solubility index (which is an indicative of starch ability to dissolve in water) may be as result of the modification process or as confirmed by Moorthy and Ramanujam (1986) that wherever

the granules have low swelling volume they have high solubility hence the low values of solubility index obtained from the result could be attributed to the high values of swelling capacity of the starch.

Table 4: Physicochemical Properties of Native and Retrograded Starches obtained from *Manihot esculentum*.

Parameters (%)	NS	0HR	24HR	48HR	96HR
Crude protein	0.35	0.70	0.78	0.61	0.52
Moisture content	12.4	11.6	12.0	11.9	12.1
Ash value	0.15	0.10	0.15	0.15	0.05
Lipid content	0.75	0.65	0.65	0.50	0.60
Fibre content	0.05	0.05	0.05	0.05	0.05
Carbohydrate content	98.7	98.5	98.4	98.7	98.7
Amylose content	40.1	35.7	32.1	28.8	52.7
Amylopectin content	59.8	64.2	67.8	71.1	47.2

NS (Native starch), 0HR (Zero hour retrogradation), 24HR (24 hours retrogradation), 48HR (48 hours retrogradation) and 96HR (96 hours retrogradation)

Considering the result obtained in Table 4, it will be observed that at the beginning amylose content starts to depreciate until amylopectin reached its peak at 48 hrs of modification before amylose content begin to rise; this shows the period of re-association of these polymers as modification time progresses. The amylose content of starch affects starch solution properties such as starch solubility and swelling power, which depend on the leaching of amylose out of the crystalline network of amylopectin into solution (Moore and Amante, 2005). From the result, it could be observed that water solubility and swelling power of the modified starch follow the same trend by decreasing with decrease in amylose and increase in amylopectin contents. The diffractography is an important technique in establishing batch to batch reproducibility of a crystalline form. Random orientation of a crystal lattice in a powder sample causes the X-ray to scatter in a reproducible pattern of peak intensities at distinct angles (θ) relative to the incident beam (Leon *et al.,* 2009). As seen in Fig. 2 -6, more peak intensities were observed with increase in modification period which indicates an increase in crystallinity of the starch.

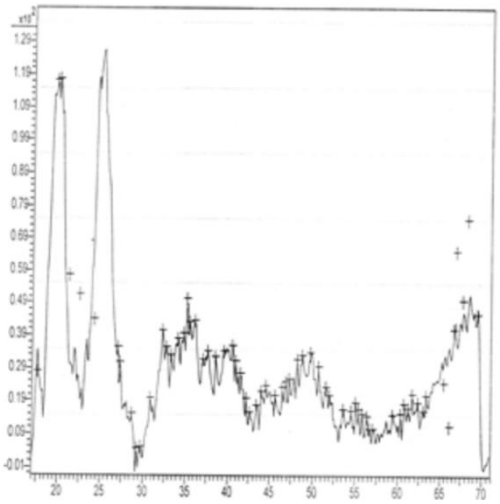


Fig. 2: x-ray diffractogram of native starch obtained from. Manihot esculentum

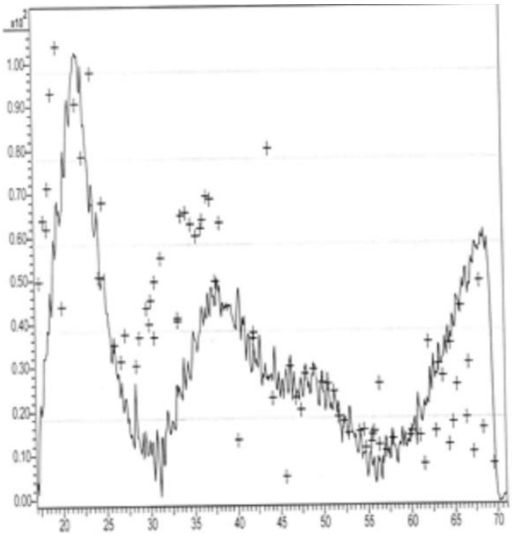


Fig. 2: x-ray diffractogram of retrograded starch (0Hrs) obtained from. Manihot esculentum.

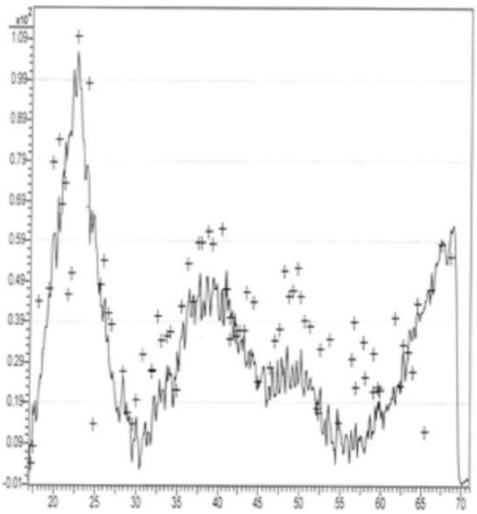


Fig. 2: x-ray diffractogram of retrograded starch (24Hrs) obtained from. Manihot esculentum.

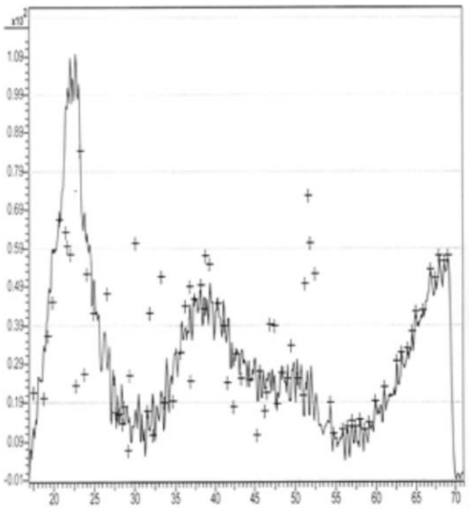


Fig. 2: x-ray diffractogram of retrograded starch (48Hrs) obtained from. Manihot esculentum.

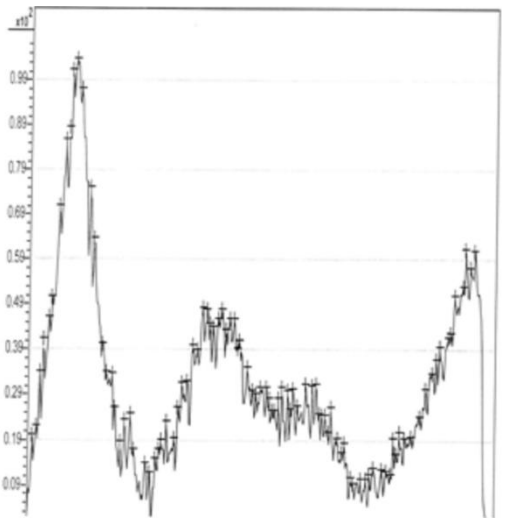


Fig. 2: x-ray diffractogram of retrograded starch (96Hrs) obtained from. Manihot esculentum.

CONCLUSION

Conclusively, on comparing the results obtained for both retrograded and native starches, retrograded starch exhibits better flow, compatibility and swelling properties. Hence in view of this, retrograded cassava starch could be used in direct compression or as disintegrant in tablet production.

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