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STUDIES ON THE PHYSICOCHEMICAL PROPERTIES OF MICROCRYSTALLINE STARCH OBTAINED BY ENZYMATIC HYDROLYSIS USING α-AMYLASE ENZYME

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ABSTRACT

The aim of this study was to produce directly compressible microcrystalline starch by enzymatic hydrolysis of cassava starch obtained from the tubers of *Manihot esculenta.* The hydrolysis was carried out using α-amylase (Ban 240L) under a suitable temperature and pH (55°c, 6) for 5h. The microcrystalline starch was recovered from the reaction mixture by precipitation using ethanol. The bulk, tapped and true densities were determined. Hausner's ratio (HR) and Carr's Index (CI) were also determined. The yield of microcrystalline starch was 83.5% and the bulk, tapped and true densities were found to be 0.66, 0.86 and 1.38g/ml respectively. Hausner's ratio and Carr's index gave 1.3 and 23% respectively. The dilution potential of this modified starch was determined using ascorbic acid and metronidazole and was found to be 40:60 (40% drug: 60% filler-binder). These physical properties were compared with those of microcrystalline cellulose which was used as a basis for comparison. The physicochemical properties of this modified starch form a basis for the development of model tablet formula for directly compressed ascorbic acid and metronidazole tablets.

Keywords: Cassava starch, Microcrystalline starch, Enzymatic hydrolysis, α-amylase, Direct compression, Dilution potential.

INTRODUCTION

Drugs are rarely administered as pure chemical substances alone and are mostly given as formulated preparations or medicines (Manjanna *et al,* 2010). Drug dosage forms contain many other components in addition to the active pharmaceutical ingredient(s) otherwise known as excipients. The International Pharmaceutical Excipients Council (IPEC) defines excipients as "substances, other than the active drug substance of finished dosage form, which have been evaluated for safety and are included in a drug delivery system during its manufacture; protect; support; enhance stability, bioavailability, or patient acceptability; assist in product identification; or enhance any other attributes of

the overall safety and effectiveness of the drug delivery system during storage or use."

Due to advances in drug delivery technology, excipients are currently included in novel dosage forms to fulfill specific functions and in some cases; they directly or indirectly influence the extent and/or rate of drug release, hence the need to study their physicochemical properties. New and modified excipients continue to emerge with better drug delivery performance.

Starches play a prominent role as pharmaceutical excipients in tablet formulation. They are used as fillers, binders, disintegrants and lubricants in tablet formulation due to their inherent physicochemical properties and relative inertness (Odeku and Itiola, 2007).

Obtained from renewable sources, starch is a natural polymer composed of amylose and amylopectin homopolymers of anhydroglucose units (Joshi and Neves, 2005). Considered a traditional excipient and trusted through many years of use, starch is also the source of a variety of complex derivatives and starch manufacturers have utilized various physical, chemical and biotechnological means to convert native starches into specialized derivatives thereby widening the application of starches in the pharmaceutical industry. These modified starches provide benefits such as improved binding (pregelatinized starch), direct compressibility (mannitol, sorbitol), encapsulation (cyclodextrins), rapid disintegration (sodium starch glycollate), sugar-free applications (maltitol, xylitol) and parenteral applications (hydroxyethyl starch, pyrogen free dextrose and polyols) (Joshi and Neves, 2005).

Physical and chemical modifications have been used to improve the compaction properties of some native starches (Odeku *et al*, 2008). Acid modified starches have been used as filler-binder in the production of pharmaceutical tablets (Puchongkavarin *et al*, 2003).

Microcrystalline starch, a modified starch product was derived by the action of acid and/or enzymes on starch granules below the gelatinization temperature. Partial hydrolysis and dissolution of the amorphous portions of the starch granules takes place leading to an increase in the content and accessibility of the crystallites (Buwalda and Arends-Scholte, 1997).

Modified starches with improved functional properties have been derived using conventional physical and chemical methods but the use of enzymatic hydrolysis is more specific in its action and so fewer byproducts are formed with a greater percentage yield (Aiyer, 2005).

The shift in tableting toward direct compression and high speed manufacturing has forced the excipient industry to search for new excipients hence the need for this study (Nachaegari and Bansal, 2004).

This study aims to modify starch using enzymatic hydrolysis and evaluate its potential as a direct compression filler-binder in tablet formulation in comparison with microcrystalline cellulose, a standard direct compressible excipient.

MATERIALS AND METHODS

Materials

The materials used for the study includes the following: ascorbic acid, metronidazole, xylene, ethanol (95%v/v) (BDH Chemicals Ltd, Poole England), Microcrystalline cellulose 101 (India), A7595 α-amylase, Hydrochloric acid (Sigma-Aldrich laborchemikalien GmbH), Sodium Hydroxide pellets GRG (Avondale laboratories Ltd, Banbury, Oxen, England). The experimental starch, cassava starch was prepared in the process laboratory of the department of pharmaceutics & pharmaceutical microbiology, Ahmadu Bello University, Zaria.

Methods

Extraction of Cassava Starch

Freshly harvested tubers of cassava (*Manihot esculenta* (Fam. Euphorbiaceae)) was peeled,

washed, cut into smaller pieces and then grated. The grates were reduced to a fine pulp using a grinding machine. The pulp was then sieved using a calico cloth with sufficient distilled water. 0.1N NaOH was added to neutralize the acidity and allowed to settle. The excess water was decanted. A suspension of starch in distilled water was then centrifuged to separate the non-starch layer from the starch layer. The tightly packed starch was then collected and spread to dry in an oven at 40°C .The dried starch was then size reduced to fine powder using a blender.

Preparation of Microcrystalline Starch

Microcrystalline starch was prepared as described by Buwalda and Arends-Scholte, 1997.

A starch slurry was prepared containing cassava starch (40%w/w) in an aqueous medium and placed in a water bath (Digital Thermostatic water bath).The temperature was brought to 55°c and the pH adjusted to 6. 0.2ml per 100g of the enzyme was introduced into the reaction medium and hydrolysis was allowed to proceed for 5h. The reaction was then terminated by lowering the pH to 2.5 with 0.1N HCL and subsequently neutralized with 0.1N NaOH. The microcrystalline starch formed was recovered by precipitation using ethanol (95%v/v). It was then allowed to dry and powdered.

Particle Density

The particle density was determined using a method described by Odeku *et al.* (2005).

The particle density was determined with a pycnometer bottle using xylene as the displacement fluid. An empty 50ml pycnometer bottle was weighed (W), filled with xylene and the excess wiped off. The filled bottle was weighed a second time (W_1) and the difference between W_1 and W obtained as W_2. A 2g quantity of the sample was weighed (W_3) and transferred into the pycnometer bottle. The excess solvent was wiped off and the bottle weighed again (W_4). The particle density, ρ_t (g/cm^3), was then calculated from the following equation:

$$\rho_t = (W_2 \times W_3) \div 50(W_3 - W_4 + W_2 + W)$$

Bulk and Tapped Densities

Exactly 50g of starch was weighed on an electronic balance and transferred into a 100ml measuring cylinder. The cylinder was dropped on a wooden platform from a height of 2.5cm three times at 2 s intervals. The volume occupied by the starch was recorded as bulk volume. The cylinder was then tapped on the wooden platform until the volume occupied by the starch became constant. The bulk and tapped densities were calculated as the ratio of weight to volume.

Carr's Index (CI)

Compressibility Index was determined using the following equation (Schwartz *et al*., 1975).

$$CI = (TD\text{-}BD/TD) \times 100$$

Where CI = Compressibility Index

TD = Tapped density

BD = Bulk density

Hausner's Ratio

This was calculated from the ratio of the tapped bulk density to poured bulk density.

Powder porosity

This was derived from the values of true and bulk densities when fitted into the equation,

$$\varepsilon = 1 - D_b/D_t \times 100$$

Where ε is the porosity, D_b is the bulk density and D_t is the true density.

Hydration Capacity

The method of Kornblum and Stoopak (1973) was adopted. A 1g sample was placed in each of four 15ml plastic centrifuge tubes to which 10ml distilled water was added and then stoppered. The contents were mixed on a vortex mixer for 2min. The mixture was allowed to stand for 10min and then centrifuged at 1000rpm for

10min on a bench centrifuge. The resulting supernatant was carefully decanted and the sediment weighed. The hydration capacity was taken as the ratio of sediment weight to the dry sample weight.

Swelling Capacity

The method described by Iwuagwu and Onyekweli, 2002 was used.

The tapped volume occupied by 5g of the powder, V_x, was noted. The powder was then dispersed in 85ml of water and the volume made up to 100ml with distilled water. After 24hrs of standing, the volume of the sediment, V_v, was read off. The swelling capacity was obtained as follows:

Swelling capacity = V_v/V_x

Moisture Sorption Capacity

2g of the starch material was weighed and evenly distributed over the surface of a 70mm tarred petri-dish. The sample was then placed in a large dessicator containing distilled water in its reservoir (RH = 100%) at room temperature and the weight gained by the exposed samples at the end of the five day period was recorded and the amount sorbed was calculated from the weight difference

Loss on Drying

5g of the powder sample was dried in an oven at 105°C to constant weight. The % loss in weight was calculated as the moisture content.

Dilution Potential

Graded portions of the drug and modified starch were mixed in the following ratios: 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20 and 90:10. The crushing strength for each binary mix was determined.

RESULTS

Extraction of cassava starch and preparation of microcrystalline starch

Freshly harvested cassava tubers contained 16.94% cassava starch. The properties of obtained cassava starch met the requirements specified in the United States Pharmacoepia (USP27/NF 22). Enzymatic hydrolysis of cassava starch in aqueous medium at 55°c for 5h yielded 83.49% MCS.

Properties of cassava starch and microcrystalline starch

Organoleptic properties of CS and MCS were similar with a particle size of 5-20µm and 2.5-12.5µm for CS and MCS respectively. Loss on drying was 4% and 4.64% for CS and MCS respectively. The details of the physicochemical properties are available on Table 1.

DISCUSSION

Physicochemical properties

The bulk and tapped densities of CS and MCS were within the same range while that of MCC was much lower as seen in table 1. This could be due to the particle size, shape and distribution of the material.

Carr's index and Hausner's ratio are a measure of the flowability and compressibility of a powder and the values obtained for all the materials were high giving an indication of poor flow of the material. This could be attributed to the small particle size of the material. However, the lower the Carr's index of a material, the better the flowability, but the poorer the compressibility (Carr, 1965).

Hausner's ratio greater than 1.25 indicate poor flow while Carr's index below 16% indicate good flow and values above 35% indicate cohesiveness (Staniforth, 1996).

The swelling power is presented in table 1. The decreasing order of the swelling power was MCS>MCC>CS. The swelling power of starch

has been attributed to amylopection and has a negative correlation with amylose (Tester et al, 2004). For MCS, the swelling power was higher than that of native cassava starch. This is because the amylose content reduces with hydrolysis (Nattalpulwat *et al.*, 2008). The

The dilution potential for both materials being investigated as shown in table 3 reveals that MCC has a higher drug loading capacity. It is defined as the ability of a given quantity of an excipient to bind a specified amount of an active ingredient to form an acceptable tablet and still The results of the physicochemical properties of microcrystalline starch derived from cassava starch by enzyme hydrolysis shows an improvement in the compressibility and dilution

moisture sorption capacity is a measure of the moisture sensitivity of the material (Ohwoavworhua and Adelakun, 2005). MCC is more likely to give more stable tablets compared with MCS because of its

low sensitivity to moisture.

retain its compressibility. This high dilution potential of MCC is related to its low bulk density and its superior binding properties (Chowhan, 1998).

CONCLUSION

potential of native starch and will likely produce tablets with desired properties when used as a direct compression filler-binder.

Table 1: Physicochemical properties of native starch, derived starch and microcrystalline cellulose

Properties	**CS**	**MCS**	**MCC**
Bulk density (g/cm3)	0.63	0.66	0.36
Tapped density (g/cm3)	0.89	0.86	0.53
True density (g/cm3)	1.43	1.38	1.48
Hausner's ratio	1.40	1.30	1.50
Carr's index (%)	29	23	32
Powder porosity (%)	56	52	76
Swelling power	1.25	1.50	1.31
Hydration capacity	1.29	0.82	0.84
Moisture sorption	19	20	10
Capacity (%)			

CS: Cassava starch
MCS: Microcrystalline starch
MCC: Microcrystalline cellulose

Table 2: Some properties of native and derived starches

Properties	CS	MCS
Color	White	White
Taste	Tasteless	Tasteless
Texture	Fine	Fine
Iodine test	Blue-black	Blue-black
Yield (%)	16.94	83.49
Loss on drying (%)	4.00	4.64
Particle size range (µm)	5-20	2.5-12.5

Table 3: Dilution potential

	Drug-Excipient ratio	
	AA	**MET**
MCS	40:60	40:60
MCC	70:30	60:40

AA: Ascorbic acid
MET: Metronidazole

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