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EVALUATION OF THE TABLETING PROPERTIES OF ENZYME HYDROLYZED CASSAVA STARCH IN CHLOROQUINE TABLET FORMULATION

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ABSTRACT

Purpose: To investigate the tableting properties of Enzyme-Hydrolyzed Cassava Starch (EHS) used as a direct compression excipient in the formulation of Chloroquine Phosphate tablet and to compare it to the properties of tablets produced using Microcrystalline Cellulose (MCC)

Methods: Enzyme-hydrolyzed cassava starch (EHS) was produced by enzymatic hydrolysis of Native Cassava starch using α-amylase. The hydrolysis was allowed to proceed for 6h under optimum temperature and pH. The derived EHS was recovered by filtration after precipitation with ethanol (95 %v/v). The Powder properties were investigated and tablets of Chloroquine Phosphate were formulated using EHS and MCC as binary binders at different ratio of 0:100, 25:75, 50:50, 75:25 and 100:0.

Results: The tablets were evaluated and the crushing strength of the tablets was found to increase in binary mixture of MCC while Friability increased with increase in the proportion of EHS. The disintegration time was above 60 min, tablets continued to swell, absorbing more water without disintegrating for the period of the study.

Conclusion: Enzyme hydrolyzed starch may not be suitable for use as a direct compression excipient in the production of chloroquine tablet.

Keywords: Enzyme-hydrolyzed starch, Microcrystalline starch, Direct compression, α-amylase.

INTRODUCTION

Tablets are solid dosage form in which the drug component is combined with a number of excipients to aid formulation of the desired products. These excipients which include binders, disintegrants, fillers and lubricants all have some roles to play in the processing of drug substances into the end – product. The excipients and drug substance are processed through a series of operations such as blending, coating, mixing, granulation and tableting to form the final product [1].

Direct compression method has taken the centre stage in tablet manufacturing. This is due to the fact that the process avoids many of the problems associated with wet granulation methods. It is a simple, economical process which is suitable for unstable compounds. However, the success of the direct compression process is

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determined to a greater degree by the choice of excipients. This is because excipients impart flow and compression characteristics of the powder blend for direct compression. Microcrystalline cellulose (MCC) is a well-known excipient for direct compression with excellent compaction properties [2]. It possesses good disintegrants properties but tends to lose this action when high compression load is applied, it also has high porosity which promotes swelling and disintegration of formulated tablets and this is attributed to either penetration of water into the hydrophilic tablet matrix by means of capillary action of the pores or even by a disruption of hydrogen bonds. By increasing compaction pressure, water penetration into the tablet will decrease, therefore disintegration time will increase. [3,4] Due to this limitation, there is a need to increase the range of excipients available for direct compression.

Starches are polysaccharides which serve as energy storage for most green plants. It is the most common carbohydrate found in plants which are used by microbes and plants themselves. Starch is locally available in Nigeria and has been used as raw materials in the food and pharmaceutical industries. Native starch despite its usefulness has poor physico-chemical properties like poor compressibility, poor flowability, and ability to generate dust. Hence it has become very important therefore to modify starch to improve its properties. In order to improve native starch, enzymatic methods have been employed. Enzymatic modification improves the flowability and compressibility profile [5].

The objective of this study was to modify starch by partial enzymatic hydrolysis using α-amylase and to evaluate it tableting properties in the formulation of Chloroquine tablets.

MATERIALS AND METHODS

Materials

The materials used were 7595 α-amylase enzyme and Hydrochloric acid (Sigma Aldrich laborchemikalien GmbH, Germany), Chloroquine powder and Sodium Hydroxide (May and Baker Ltd., Dagenham, England), Ethanol (Park Scientific Ltd, Northampton, U.K), Magnesium Stearate and MCC (Hopkins and Williams, U.K), Iodine solution (BDH, Poole, England). Freshly harvested cassava tubers were obtained locally at Samaru market, Zaria and identified by the Institute of Agricultural Research (IAR), Ahmadu Bello University, Zaria.

Methods Extraction and Modification of Cassava Starch

Starch was extracted from freshly harvested tubers of cassava (*Manihot esculenta)* using a method described by Buwalda and Arends [6]. The cassava tubers were peeled, washed and cut into small pieces before been grated into fine pulps by a blender. The pulp was mixed with 10 L of distilled water and passed through a Calico cloth sieve. The starch was allowed to settle and excess water was decanted, residual starch was washed with 0.1N Sodium hydroxide to neutralize the slightly acidic nature of the starch. Excess alkali was removed by washing several times with distilled water. A suspension of the starch in distilled water was centrifuged and the supernatant fluid decanted. The brown protein layer was scraped off and the tightly packed starch was collected and spread on a tray to dry for 24 h,The starch obtained was further dried in an oven (Gallenkamp BS size

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3, England) at 40ºC for 6 h and then stored in an airtight container in preparation for further use.

The method described by the World Intellectual Property Organization (WIPO) [7] was used for the production of Enzyme-hydrolyzed starch. Aqueous suspension of starch (40 % w/v) was brought into a double-walled reaction vessel under optimum pH and temperature for 6 h and dose with 0.1 mL of α-amylase with constant stirring. The action of the enzyme was terminated afterwards by lowering the pH to 2.5 with 0.1N HCl. The reaction medium was subsequently neutralized by raising the pH to 7 using NaOH. The resulting product was separated from the reaction medium after settling down. It was washed several times with distilled water and then dehydrated with 100 mL ethanol (95 % v/v). The dehydrated product was air dried and powdered after decanting the ethanol.

Determination of Powder Properties

Organoleptic Properties: The taste, odor and color of the starches (NCS, EHS and MS) were assessed by ten individuals and a matching assessment result given by at least eight of them.

Solubility: Two grams of each starch (NCS, EHS and MS) was dispersed in 10 mL of cold water, hot water, acetone, ethanol and chloroform. The resultant solution was left overnight. Five (5) mL of the clear supernatant solution was taken and heated to dryness over a water bath. The weight of the dried residue with reference to the volume of the solution was determined as the percentage solubility of the starch in the solvent.

Microscopy: A pinch of each starch was mounted on a slide in glycerol, the size of the starch grain was measured using the calibrated eyepiece micrometer.

Bulk density: 50 g quantity of each starch powder was poured gently into a 50 mL graduated measuring cylinder. The volume of the powder was read and the bulk density calculated.

$$\text{Bulk density} = \frac{weight\ of\ powder}{initial\ volume} \ldots\ldots\ldots [1]$$

Tapped density: The measure containing the 50 g of each starch powder was tapped 3 times on a flat surface at 2 s intervals for 5, 25 and 50 taps. The volume was noted and used in calculating the tapped density.

$$\text{Tapped density} = \frac{weight\ of\ powder}{final\ volume} \ldots\ldots [2]$$

Carr's index: The difference between the tapped and bulk density of each starch was divided by the tapped density. Carl's index was calculated and the ratio expressed as percentage.

$$\text{Carr's index (\%)} = \frac{(Tapped\ density - Bulk\ density)}{Tapped\ density}\ X\ 100 \ldots [3]$$

Hausner's ratio: The ratio of the tapped density to the bulk density of the starch was calculated as the Hausner's quotient.

$$\text{Hausner's ratio} = \frac{Tapped\ density}{Bulk\ density} \ldots\ldots\ldots [4]$$

Flow rate: Twenty gram (20 g) of each starch was placed in the flow rate apparatus (Erweka flow tester) and the time taken for the samples to flow through the orifice was noted and recorded. Flow rate was calculated using the equation below.

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Flow rate = $\frac{weight\ of\ powder\ in\ grams}{time\ taken\ in\ seconds}$ ….[5]

Angle of repose: Twenty gram (20 g) of each starch was poured into a clean funnel clamped to a retort stand with a 7cm height from the tip to the base. The starch was allowed to flow freely to the base. Height of the starch heap and radius were measured which were used to calculate angle of repose using the equation below.

Tan Ø = h/r ……[6]

Particle Density: A method described by Odeku *et al* [8] was adopted. 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 differences between W_1 and W was obtained as W_2. A 2 g quantity of the powder was weighed (W_3) and transferred into the pycnometer bottle. The excess solvent was wiped off and the bottle weighed again (W_4). The various weights recorded were used to calculate the particle density of the each of the starches using Equation 1.

$Pt = (W_2 \times W_3) / 50 (W_3 – W_4 + W_2 + W)$ ---- [7]

Swelling Capacity: A method described by Iwuagwu and Onyekweli [9,10] was adopted to measure the swelling capacity. The tapped volume occupied by 5 g of the each starch, Vx was noted. The starch was then dispersed in 85 mL of water and the volume made up to 100 mL with more water. After 24 h of standing, the volume of the sediment Vv was estimated. The swelling capacity was calculated using equation below.

Vv / Vx ---------- [8]

Hydration Capacity: The method described by Kornblum and Stoopak [11] was adapted.1 g weight of the starch was placed in each of four 15 mL plastic centrifuge tubes to which 10 mL distilled water was added and then stoppered. The contents were mixed on a vortex mixer for 2 min and allowed to stand for 10 min and then centrifuged at 1000 rpm for 10 min on a bench centrifuge. The supernatant was carefully poured out and the sediment weighed. The hydration capacity was determined as the ratio of sediment weight to the dry sample weight.

Tablet Production: The formula for Chloroquine tablets is given below on table 1. Tablets were prepared by the process of direct compression. The formula in table 1 was used to prepare 100 tablets per formulation. The batches contain different proportions of Enzyme-hydrolyzed starch and MCC in the following proportions; 0:100, 25:75, 50:50, 75:25 and 100:0. Binary mixture of Enzyme-hydrolyzed starch and MCC were prepared by weighing Enzyme-hydrolyzed Starch and MCC using a weighing balance (Mettler balance, Switzerland). They were transferred into a mortar and mixed using a pestle. Chloroquine phosphate powder 20 g was weighed and transferred to the mortar, the mixture was well mixed after which it was lubricated with 125 mg magnesium stearate and compressed directly using single punch tableting machine. The tablets were kept for 24 h to allow elastic recovery before the evaluation process.

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Table 1: **Tablet formula for Chloroquine Tablet Formulation using Direct Compression Formulations**

Materials	I	II	III	IV	V
Chloroquine (mg)	250	250	250	250	250
Enzyme-hydrolyzed Starch (EHS)	0.0	93.44	186.88	280.30	373.76
MCC. (mg)	373.76	280.30	186.88	93.44	0.0
Magnesium Stearate (0.2% w/w)	1.25	1.25	1.25	1.25	1.25
Tablet weight (mg)	625	625	625	625	625

MCC = *Microcrystalline cellulose,* Batch I-EHS: MCC. 0: 100, Batch II- EHS : MCC. 25 : 75, Batch III-EHS : MCC. 50 : 50, Batch IV-EHS : MCC. 75 : 25, Batch V- EHS : MCC. 100 : 0

Evaluation of Tablet Properties

Uniformity of weight: The weights of twenty tablets for each formulation were randomly selected and weighed individually and collectively. The mean and the percentage deviations determined according to the British Pharmacopoeia method [12].

Measurement of tablet diameter: Ten (10) tablets were randomly selected from each formulation and the diameters were taken using a micrometre screw gauge 24 h after the tablets were compressed.

Measurement of tablet thickness: The thicknesses of the tablets were measured using a sliding vernier calliper (Moore and Weight Sheffield, England).

Crushing and tensile strength: The crushing strength of 5 tablets for each formulation was determined using a hardness tester (Pharma tester PTB 301). Mean crushing strength was calculated and the tensile strength values were determined using the equation below:

$$Ts = 2Cs / 3.14dt \text{ ---------- [7]}$$

Where Ts is the tensile strength, Cs is the crushing strength, d and t is the diameter and thickness of the tablet respectively.

Friability test: Friability was determined using an Erweka Friabilator. Ten tablets per formulation were weighed and allowed to tumble in the drum of the friabilator which was operated at 25 rpm for 4 min. The tablets were dusted properly and reweighed. The difference in weight was determined and expressed as percentage friability value using this formula.

$$\text{Friability (\%)} = \left[\frac{(W1-W2)}{W1}\right] X\ 100 \text{.....[8]}$$

Where W_1 is original weight and W_2 the final weight

Disintegration test: Disintegration time of six tablets per formulation was individually determined in distilled water at 37±0.5ºC using the Erweka disintegration test apparatus. The time taken for each tablet to break into small particles and pass through the mesh was recorded as described in the

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British pharmacopoeia [12]. The mean disintegration time was calculated for each batch.

RESULTS AND DISCUSSION

Physicochemical Properties

The organoleptic properties result obtained for the three starches are similar. The details are presented on Table 2.

The results for flow rate and angle of repose are presented on Table 3. The largest angle of repose was obtained with NCS (33.3°) and that of MS was the smallest (26.0º). Native Cassava starch has Carr's index of 16.67 % and Hausner's ratio of 1.2, while EHS has Carr's index of 9.62 % and Hausner's ratio of 1.08. Materials having Carr's index above 16 % and a Hausner's ratio greater than 1.2 possess poor flow properties hence, EHS showed improved flow properties. NCS has the highest true density of 1.63 g/mL compared to that of EHS of 1.49 g/mL. Powder porosity ranged from 27 % for NCS and 35 % EHS. The EHS contained 9.55 % of amylose and 90.45 % of amylopectin.

The average diameter of particle size for NCS is 79.77 μm and that of MCS is 197.2 μm. Modification of the NCS improved its physicochemical properties.

Directly compressible excipient is expected to possess high dilution capacity, the dilution potential of an excipient is its ability to retain its compressibility and form a coherent compact when mixed with poorly compressible drug [17]

The results of the tableting properties of enzyme hydrolyzed cassava starch in chloroquine tablet formulation using direct compression are shown on Table 2. The weights of tablets were between 625.2 mg and 632.5 mg which is within the ±5 % weight variation specified by British Pharmacopoeia [13] for all tablets of theoretical weight greater than 250 mg. This implies that all the formulations of the tablets produced passed the weight variation test. The values of the crushing strength obtained for all the formulations fell within the acceptable limit of 4- 8 kgF for uncoated tablets [13]. However, the Crushing strength of the tablets decreased as the percentage of enzyme hydrolyzed starch in the binary mixture increased. Tablets produced with 100% and 75% EHS have the lowest crushing strength and the tablets produced by microcrystalline cellulose were well compressed and the crushing strength increases as the percentage of microcrystalline cellulose increases. The strength of a tablet depends on the magnitude of plastic deformation occurring during deformation. This implies that microcrystalline cellulose undergoes higher plastic deformation than enzyme hydrolyzed starch [14]. The values obtained for the friability were within the acceptable limit of 1% for all the batches except for the batches containing 75 and 100 % of enzyme hydrolyzed starches which have higher values than the acceptable limit and this could be as a result of high percentage of the test starch. The Friability increases as the

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proportion of microcrystalline cellulose reduces and it increases as the proportion of enzyme hydrolyzed starch increases. This is because microcrystalline cellulose form stronger bonds which confer resistance to fracture and abrasion compared to enzyme hydrolyze starch [15]. The disintegration time obtained for all the formulations are above 60 min. The tablets continued to swell without dissolving completely on time for the duration of the study. This could be because of the high swelling capacity of microcrystalline cellulose and forms very strong interparticulate bonds even at low pressure [16]. This agrees with the findings of Apeji that all the tablets formulated with MCC in direct compression did not disintegrate before 60 min. [5]

The Crushing Strength-Friability index reduced with increase in the proportion of enzyme hydrolyzed starch and increase with increase in the proportion of microcrystalline starch. This index is a better tool for measuring the quality of tablet, it measures the mechanical strength of tablet better than crushing strength and friability alone. The higher the index, the stronger the tablet [16]. This result shows that microcrystalline cellulose form stronger tablets than enzyme hydrolyzed starch.

Table 2: **Preliminary investigations on Native cassava starch (NCS), Enzyme hydrolyzed starch (EHS) and Maize starch (MS)**

Properties	NCS	EHS	MS
Odour	odourless	odourless	odourless
Colour	white	white	white
Taste	tasteless	tasteless	tasteless
Texture	smooth	smooth	smooth
Percentage yield (% w/w)	20	85	
Iodine test	blue-black	blue-black	blue-black
pH	6.25	6.96	6.50
Loss on drying (% w/w)	0.5	1	4
Particle size range (μm)	2-10	2-11.8	2-12.70
Particle shape	spherical	spherical	spherical

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Table 3: Physicochemical Properties of NCS, EHS and MS

	NCS	MCS	MS
Angle of repose (^{o})	33.3	26.6	26.0
Ash value %	1.0	1.0	1.0
Flow rate (g/s)	1.92	1.62	1.50
Bulk density (g/cm^3)	0.61±1.24	0.47± 0.08	0.45± 0.08
Tapped density (g/cm^3)	0.72	0.52	0.62
Hausner's ratio	1.20	1.08	1.17
Carr's index (%)	16.67	9.62	10.9
Particle density (g/cm^3)	1.63	1.49	1.47
Powder porosity (%)	27	35	63.5
Swelling power	1.45	1.23	1.20
Moisture sorption capacity (%)	11	8	10.5
Moisture loss (%)	0.5	1	5.6
Hydration capacity	1.29	0.9	1.35
Solubility in cold Water (% w/v)	0.02	0.02	0.02
Solubility in Hot Water (% w/v)	0.20	0.20	0.02
Solubility in Ethanol (% w/v)	0.30	0.30	
Solubility in Acetone (% w/v)	0.20	0.20	
Solubility in Chloroform (% w/v)	0.20	0.80	

Table 4: Evaluation of Tablets produced by Direct Compression

Binary mixture of MCC:EHS	Mean weight (mg)	Thickness (mm)	Diameter (mm)	TS (MN/m^2)	CS (Kgf)	FR (%)	DT (min)	CS-FR
0:100	630.1	3.32	11.0	0.09	5.0	1.20	70	3.8
25:75	625.2	3.28	10.9	0.08	5.5	1.14	75	4.36
50:50	625.0	3.28	11.0	0.11	6.0	0.60	90	5.4
75:25	630.3	3.24	10.0	0.13	6.5	0.55	100	5.95
100:0	632.5	3.25	10.0	0.14	6.9	0.50	120	6.4

Key: EHS: Enzyme hydrolyzed starch, MCC: Microcrystalline Cellulose (Avicel®), TS: Tensile Strength, CS: Crushing strength, FR: Friability, DT: Disintegration time

CONCLUSION

The findings from this study have shown that modification of native cassava starch by enzymatic hydrolysis has been able to impart some desirable features required for direct compression. However, it failed disintegration test for the duration of this studies hence, it is

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not suitable to be employed as a directly compressible excipient in the formulation of chloroquine tablets.

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