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Characterization and Evaluation of Carboxymethyl Starch of Cajanus Cajan Seeds as Tablet Binder

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ABSTRACT

This study was to characterize carboxymethylated cajanus cajan starch (CMCCS) and evaluate its capacity as tablet binder. Cajanus cajan starch (NCCS) was extracted and modified into CMCCS. The CMCCS was used in preparation of metronidazole tablet formulations and the effect of CMCCS concentration and wet massing time (WMT) on the physical properties of tablets was tested. The physicochemical and micromeritic properties of CMCCS were determined and shown acceptable characteristics as a tablet excipient. The prepared tablets showed increased hardness and decreased % friability with the increased % CMCCS, significantly, but no significant effect on disintegration time (DT) was observed unless the WMT is increased to its highest level, where DT was significantly increased. Also all the batches showed more than 90 % drug release within 15 minutes.

Keywords: Cajanus cajan, Amylose, Carboxymethylated, Metronidazole, Starch.

INTRODUCTION

Starch has been widely studied by many researchers as to its suitability as an excipient in pharmaceutical dosage forms particularly in tableting technology 1. Starch is primarily composed of two major molecular components, amylose and amylopectin and its physicochemical properties depend largely on its botanical origin and source 2. As a result of the competing demands for starch as food, pharmaceutical and industrial uses coupled with the need to attain self sufficiency in starch production, there is a need to find other high yield sources besides the existing sources e.g. cassava, maize and potato 3. Also the amylose/amylopectin ratio differs with the botanical source, which in turn leads to variable physicochemical and functional properties making variations in the pharmaceutical benefits of different starches, this means that search for new starch sources is a subject of scientific interest 4. Few research work have been reported on the isolation, physicochemical characterization and pharmaceutical application of starch from cajanus cajan 5. There are number of reports confirmed that Cajanus cajan contains high level of carbohydrates 6 and this makes it a potential alternative source for starch.

Modification of starch is performed to overcome the shortcomings of native starch, such as instability at extremes of pH and temperature, shear during processing and retrogradation 5.

Addition of bulky functional groups such as carboxymethyl groups in the starch molecule, leads to significant change in the physicochemical characteristics of the starch. Carboxymethyl starch (CMS) has high swelling capacity as well as high water permeability and these properties leads to fast disintegration and drug release from tablets contains CMS. CMS has also been reported to be able to overcome the negative effect of hydrophobic lubricants such as magnesium stearate on the disintegration time of tablets or capsules. The carboxymethylated products properties affected by the functionalization pattern of carboxymethyl groups and determined mainly by the overall degree of substitution (DS). CMS synthesized through the reaction of the starch with monochloroacetic acid or its sodium salt after activation of it with an aqueous solution of NaOH in a slurry of an aqueous organic solvent, mainly alcohol 7.

Factorial design is a technique introduced in 1926 by Fisher. The basis of the technique is to reveal the effects of different factors on experimental results simultaneously. All possible combinations of factors and their levels are studied, and thus all main effects and interactions can be evaluated. Thus it is a way of separating the factors that are important from that are not. The technique can be applied to many pharmaceutical problems, and is the basis for many tests that seek to find an optimal solution 8.

The objectives of this study were to modify the NCCS to its carboxymethylated form, investigate the physicochemical properties of the modified starch and determine its functionality as tablet binder.

Table 1 : Basic formula

Function	Ingredient	Quantity per 1 tablet (mg)
Active ingredient	Metronidazole	200 mg
Binder	CMCCS	(12.5 – 17.5)*
Disintegrant	SSG	4 %
Lubricant	Mg. St.	0.5 %
Granulating fluid	Water	22.5 %

* % of CMCCS as varied per experimental design

MATERIALS AND METHODS

The study was carried out through 2015-2016. Seeds of cajanus cajan were collected from Gezira scheme, Sudan and identified at the Agricultural Research Corporation, Shambat, Sudan, by Prof. Dawoud Hussein Dawoud. Gift samples of sodium starch glycolate (SSG), metronidazole powder and metronidazole working standard from (Bnp Co. Ltd., Sudan). Amylose standard powder was purchased from (Aladdin, China), magnesium stearate was purchased from (Techno pharmchem, India). Iodine and sodium hydroxide were purchased from (Central drug house, CDH, India). Ethanol, methanol, hydrochloric acid, acetone, glacial acetic acid and potassium iodide were purchased from (Scharlau, Spain). Sodium chloride was purchased from (Supertek, India). Chloroacetic acid was purchased from (Metlab, UK) and isopropyl alcohol and xylene were purchased from (LOBAChemie, India). All other chemicals and solvents were of analytical grade.

Synthesis of carboxymethyl cajanus cajan starch (CMCCS)

Native cajanus cajan starch was extracted using the method of Lawal (2008) 5. For the synthesis of CMCCS followed the method of Rachtanapun et al (2012) 9with some modifications. First, thirty grams of monochloroacetic acid was dissolved in 400 mL of isopropyl alcohol. Then 100 g of NCCS was dispersed in the solution with continuous stirring. Then 100 mL of aqueous NaOH solution 30% w/v was added into the mixture and heated up to 50 ºC for 20 minutes. At the end, the slurry was neutralized with glacial acetic acid and purified by filtration and washed four times with 95 % ethanol. The resulting modified CMCCS was air dried before being passed through BSS # 240 (63µm) mesh sieve.

DDetermination of the degree of substitution of carboxymethyl starch

A method of Li et al. (2010) 10 was used for the determination of the degree of substitution (DS) of carboxymethyl starch. 10 g of CMCCS was dispersed in 300 ml acetone followed by addition of 5 M HCl and then the dispersion was whipped for 30 minutes. This process leads to conversion of carboxymethyl cajanus cajan starch in sodium form to the hydrogen form of carboxymethyl cajanus cajan

starch (H- CMCCS). Methanol (80% v/v) was used to wash the obtained H- CMCCS till the pH test of the dispersion became neutral. The neutral dispersion was subjected to filtration and then suspended in acetone and filtered again and dried for 24 hours in a desiccator over silica gel. NaCl solution (1% w/v) was used dissolve 2 g from H- CMCCS and the obtained solution was titrated against 1M NaOH solution. The DS was calculated using the following equation:

$$DS = \frac{Q_{NaOH} X M_g}{W_{ds} - Q_{NaOH} X M_R}$$

Where Q NaOH; Amount of NaOH consumed per mol; Mg, Molar mass of anhydrous glucose units (162g/mol); Wds, Weight of starch per g (dry basis); MR , Molar mass of carboxymethyl residue (58g/mol).

Some physicochemical properties of starch

The moisture content of the starch samples was determined using a moisture analyzer (Kern, MLB 50-3N, Kern and Sohn GmbH, Balingen, Germany), Amylose/amylopectin ratio was determined according to Abdalla et al. (2009) 11, pH was evaluated using the method of Ashogbon and Akintayo (2012) 12. Method of Muazu et al. (2011) 13 was used to determine swelling, hydration and moisture sorption capacities. Also the cold water solubility (SC) was measured according to method of Zhang et al. (2013) 14.

Micromeritics properties of starch

Bulk, tapped and true densities and the angle of repose (θ) of the starch samples were determined according to method of Gbenga et al. (2014) 2. Also Carr's index and Hausner ratio were calculated from the bulk and tapped densities data using the following relations:

$$\text{Carr's index} = (\text{Tapped density} - \text{Bulk density})/(\text{Tapped density}) \ X100\%$$

$$\text{Hausner ratio} = (\text{Tapped density})/(\text{Bulk density})$$

Analysis of Fourier transforms infrared (FTIR) spectra

FTIR spectra of NCCS and CMCCS starches were acquired at room temperature using FTIR spectrometer (IRAffinity-1, Shimadzu, Japan) in transmittance mode according to method of Gbenga et al. (2014) 2.

Drug-excipient compatibility study

Compatibility Studies were carried out according to method of Khan et al. (2011) 15. Infrared spectra were matched for detection of any possible chemical interaction between drug (Metronidazole) and CMCCS.

Formulation of tablets

Batches of 200 tablets containing 200 mg metronidazole per tablet were prepared using the basic formula in Table (1)

Experimental design

A two factor two level (22) factorial design was employed to study and evaluate the effect of two independent variables (X1 = % CMCCS as binder and X2 = WMT) on the disintegration time, % friability and hardness of metronidazole tablets prepared by wet granulation process. Formulations B1 to B4 were prepared by varying the levels of the independent variables as required by the experimental design and factor levels were suitably coded in Table (2). A first-order model was established for the responses and the linear equation was generated and the validation of the mathematical model was performed through analysis of variance.

Preparation of granules

Preparation of granules

The specified amount of metronidazole, CMCCS and half the amount of SSG mixed in poly ethylene bag for 10 min. The granulating fluid was added to the powder mix till the amount of water reached 22.5 % w/w of specified formula. A planetary mixer was used to perform the granulation process at 34 rpm to the designed time. Then the resulted wet mass was passed through BSS (1.4 mm) and dried till loss on drying (LOD) came in range 2.50 and 4 % w/w, then rescreened through BSS (850μm).

Preparation of tablets

The remaining amount of the SSG was used as extra granular disintegrant by mixing it with granules for 3 min, then the mixture was lubricated with 0.5 % w/w Mg. St., then compressed using a single punch tableting machine (Erweka Type EP-1, Heusenstamm, Germany) into tablets of 9 mm in diameter.

Evaluation of prepared tablets

A 24 hours period was allowed for stress relaxation and then, all the batches of tablets were subjected to weight uniformity, friability, hardness, disintegration time and dissolution tests.

RESULTS AND DISCUSSION

The properties of the carboxymethylated starch mainly determined by the total degree of substitution (DS), the DS of obtained CMCCS was found to be 0.252. The amylose contents of starches have been determined and found to be 32.9% and 20.92% for NCCS and CMCCS, respectively. It is obvious that, carboxymethylation led to a significant decrease in the amylose content ($P < 0.05$).

Table (3) shows the results of physicochemical properties of the starches. The moisture contents of NCCS and CMCCS were 9.6% and 8.86%, respectively, which are within the acceptable limits of less than 15%. NCCS showed the highest moisture content and this may be attributing to its larger grain size, which means that there are larger pore sizes leading to high ability for trapping water. The aqueous dispersions of starch usually have a pH in the range of 4 to 8 16, both of the starch samples showed pH values within that range.

Table 2: Factor combinations as chosen by 2^2 factorial design

Formulation code	Factor levels	
	X_1	X_2
B1	-1 (12.5%)	-1 (6 min)
B2	+1 (17.5%)	-1 (6 min)
B3	-1 (12.5%)	+1 (12min)
B4	+1 (17.5%)	+1 (12min)

The results of cold water solubility, swelling capacity and hydration capacity which found to be 0.9, 1.46 and 1.99, respectively for NCCS, while the CMCCS was found to be ultimately soluble in water. The cold water solubility values could be attributed to the amylose level in the starch and to differences in crystalline structure of the starch 17. 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 18. Generally, water penetration into the starch granules of CMCCS may be increased due to the incorporation of hydrophilic carboxymethyl group and to amylose/amylopectin ratio.

The moisture sorption capacity is a measure of moisture sensitivity of a material and it reflects the relative physical stability of the tablets formulated with the material when stored under humid conditions 19. The results show that CMCCS absorbed the moisture (48.5%) and form transparent viscous solution more than the NCCS (17.34%). This could indicate that NCCS based tablets have ability to absorb less moisture and so more physically stable tablets than those tablets formulated with CMCCS.

Table 3: Some physicochemical characters and micromeritics properties of starch

Parameter	Starch	
	NCCS	CMCCS
Amylose % w/w	32.9	20.92
Moisture content	9.6%	8.86%
pH	6.12	6.9
Cold water solubility % w/v	0.9	**
Swelling capacity	1.46	**
Hydration capacity	1.99	**
Moisture uptake %	17.34	48.5
Angle of repose	35.05 ± 0.922	24.14 ± 4.051
Bulk density	0.667	0.571
Tapped density	0.95	0.833
Carr´s index	29.8%	31.45%
Hausner ratio	1.42	1.46
True density	1.51 ± 0.154	1.35 ± 0.025

** CMCCS was ultimately soluble in water

Micromeritics

The results of micromeritics properties were presented in Table (3). The bulk, tapped and true densities for NCCS were higher than those for CMCCS. The differences observed in the bulk density values could be due to the different particle sizes and shapes which affected the packing arrangement of the powder particles.

The angle of repose, θ, could be used as a qualitative measure of the cohesiveness or the tendency of powdered or granulated materials to flow, for instance, from hoppers through the feed frame into tableting machines. Such uniformity of flow will minimize weight variations in tablets produced 2. Also Carr's index and Hausner's ratio have been reported to preview the degree of densification that would occur during tableting. As the values of these indices increase, the flow of the powder decreases and gives more likelihood of producing tablets with more weight variation 19. The results obtained showed that the CMCCS had good flow characteristic, while the NCCS had passable flow characteristic. Also the NCCS had lower value of the Hausner's ratio and Carr's index, suggesting lesser densification than the CMCCS.

FTIR Analysis

The FTIR spectra of NCCS, CMCCS and Metronidazole are shown in the Figure (1). The FTIR study of physical mixture of metronidazole-CMCCS (Figure 1d) showed no appearance or disappearance of FTIR peaks in CMCCS-drug mixture, which confirms absence of any chemical interaction between drug and starch.

Tablets

The effect of CMCCS concentration and WMT on prepared tablets was evaluated in wet granulation process using a 22 factorial design study. The responses were analyzed by Design-Expert® 8 software and the best fitting model was

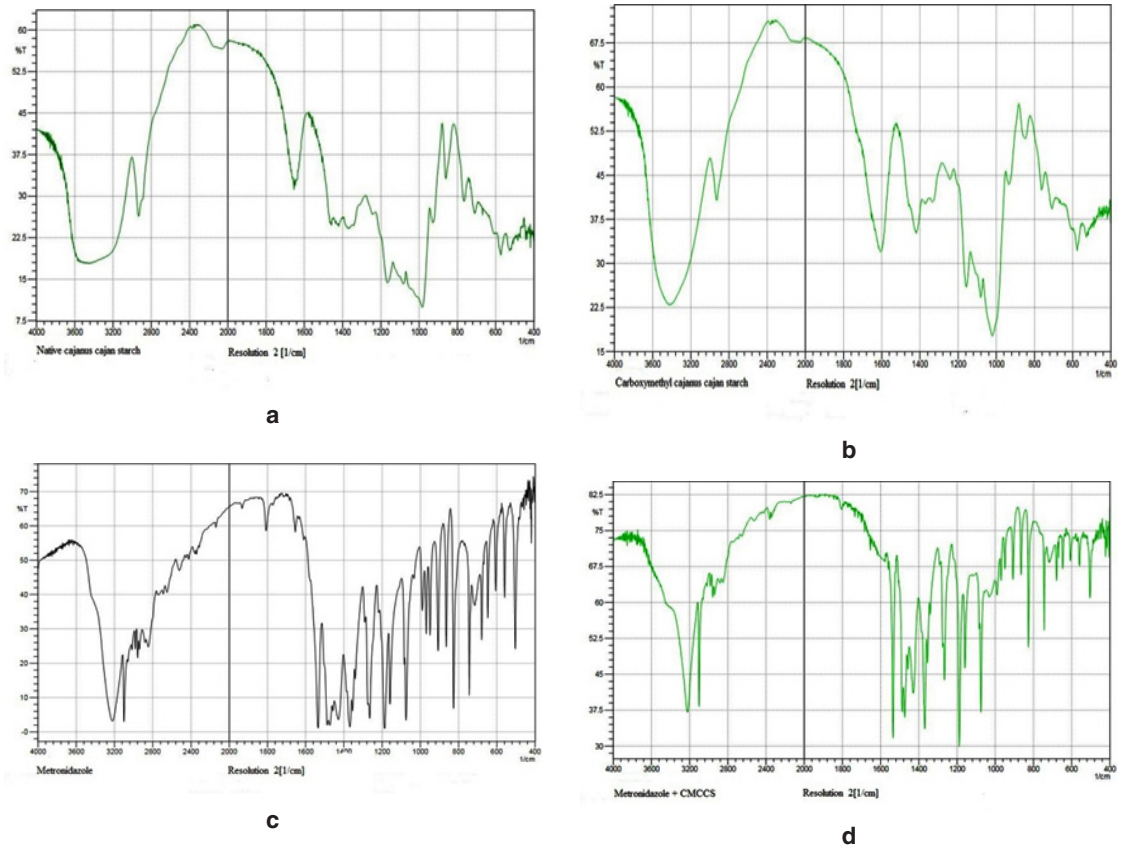

Fig. 1 : FTIR Spectra of NCCS (a); CMCCS (b) Metronidazole (c) and Metronidazole-CMCCS physical mixture (d)

selected. Figure (2) show the physical properties of the prepared formulations.

The percentage coefficients of weight variation (% CV) were calculated and shown in Figure (2a). From the results, all the formulated tablets passed the weight uniformity test as the standard deviation from the mean was within the specifications given in the B. Pharmacopoeia 20.

The response surface plot (Figure 2c) and contour plot (Figure 2b) elucidate the effect of variables on hardness and friability, respectively. All batches exhibited % friability less than 1% and showed acceptable hardness profile, ranging from 4.5 to 6.29. At both 12 and 6 min WMT, increase of CMCCS percentage from 12.5% up to 17.5% led to significant decrease of % friability and significant increase of hardness (P-value < 0.05, One-Way ANOVA, Tuke test). On other hand increase of WMT from 6 to 12 min has no significant effect on hardness value of tablets and decreases the % friability only at the high CMCCS concentration. The obtained equations for the friability percent and hardness were:

Friability = 0.84 – 0.092 (CMCCS concentration) - 0.048 (Wet massing time)

Hardness = 5.41 + 0.84 (CMCCS concentration) + 0.055 (Wet massing time)

As observed from equations, the CMCCS concentration has positive effect on hardness and negative effect on friability.

The response surface plot (Figure 2d) elucidates the effect of variables on DT. All formulated batches showed good and fast DT. At 6 min WMT increase of CMCCS concentration from 12.5 %

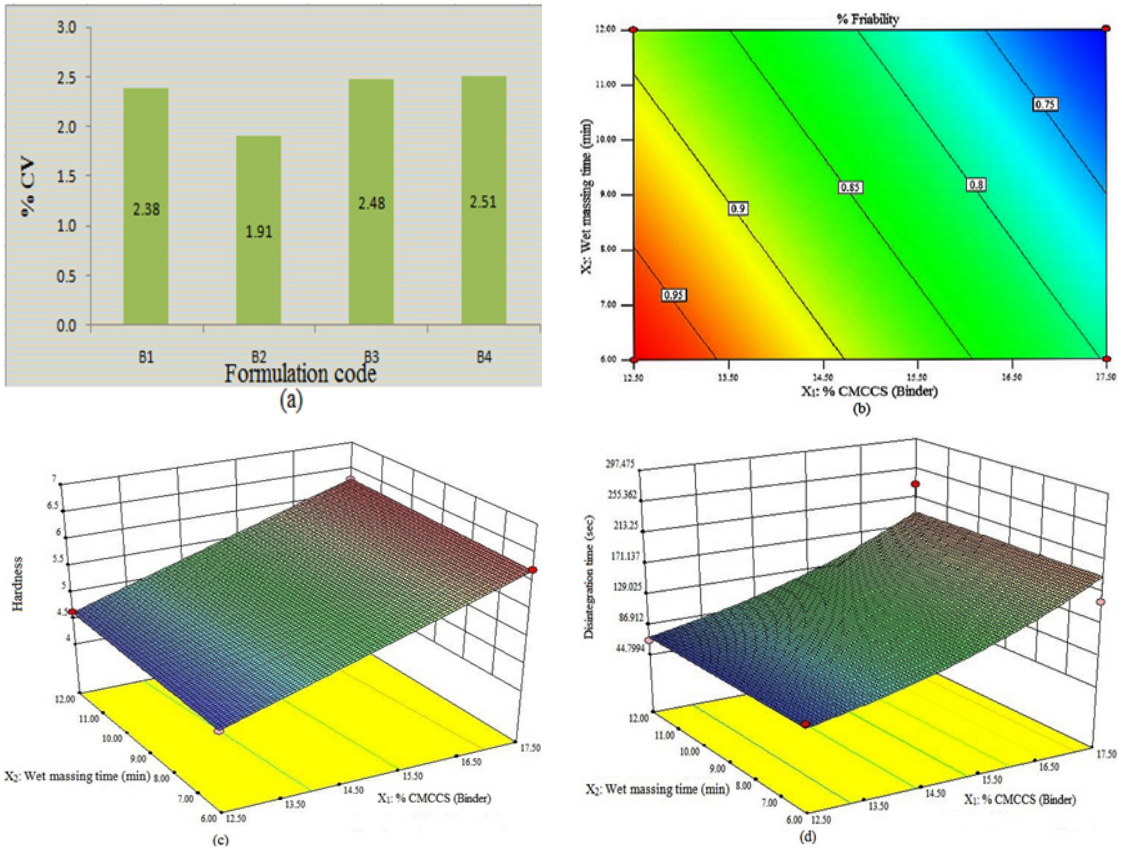

Fig. 2: (a) % CV; Effect of wet massing time and % CMCCS on (b) % friability (c) hardness and (d) DT of metronidazole tablets

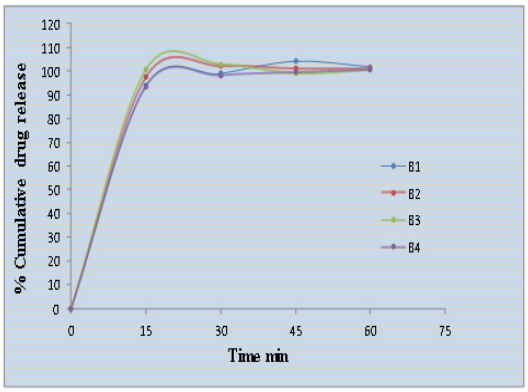

Fig. 3: Drug release profile of tablets formulated with CMCCS

to 17.5 % have slight increase of DT from 1.25 to 2.65 min. Similarly, at 12.5 % CMCCS increasing of WMT from 6 to 12 min have no significant effect on DT, while at 12 min WMT, increasing of CMCCS percentage caused significant increase in DT from 1.08 to 3.87 min. The obtained equation for DT was

Log10 DT = 2.06 + 0.22 (CMCCS concentration)

From equation, it is obvious that the main effect term (% CMCCS) has dominant positive effect on the value of log10 DT, while the effect of WMT on DT was not noticed.

The drug release profile of the B1, B2, B3 and B4 was shown in Figure (3). All batches show fast release profiles i.e. more than 90% of the drug was released in 15 min from all formulations. Faster tablet disintegration resulted in a faster drug release, confirming the fact that disintegration usually plays a vital role in the dissolution process since it determines to a large extent the areas of contact between the solid and the liquid media 21.

CONCLUSIONS

In this study, CMCCS was evaluated as tablet excipient in metronidazole tablet formulations. CMCCS demonstrated favorable physicochemical characteristics as a pharmaceutical excipient. Superior bulk properties such as higher true density, bulk and tapped densities qualify CMCCS as a robust and effective diluent. Modification of NCCS starch by carboxymethylation lead to CMCCS improving several physicochemical properties such as water solubility, water sorption capacity, increased flowability, enhanced drug release properties and also decreased amylose content of the starch. This alteration of the physicochemical properties resulted in different and broader applications of the modified starch, especially in pharmaceutical industry. According to the results of this study, Cajanus cajan starch, especially its carboxymethylated form is a possible alternative to replace the expensive imported starches. Furthermore, introduction of Cajanus cajan starch into pharmaceutical industry paradigm will add an extra value to this local crop.

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