ORIGINAL ARTICLE

Pharmacopoeial and physicochemical properties of α-cellulose and microcrystalline cellulose powders derived from cornstalks

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Background: Suitable α-cellulose and microcrystalline cellulose powders for use in the pharmaceutical industry can be derived from agricultural wastes. **Aims:** The pharmacopoeial and physicochemical properties of cornstalk α-cellulose (CCC) and cornstalk microcrystalline cellulose powders (MCCC) were compared to a commercial brand of microcrystalline cellulose (Avicel PH101) to evaluate their usefulness as pharmaceutical excipients. **Settings and Design:** Physicochemical properties of an excipient play a very crucial role in the functions of the excipient; hence, these properties were evaluated and compared with a commercial brand. **Materials and Methods:** α-cellulose was extracted from cornstalks. Modification of this α-cellulose powder was carried out by its partial hydrolysis with hydrochloric acid (HCl) to obtain a microcrystalline cellulose powder. Their pharmacopoeial, physicochemical and microbiological properties were evaluated using standard methods. **Statistical Analysis:** OriginPro 8 SR2 v. 0891 (B891) software (OriginLab Corporation USA) was used for statistical evaluation. One-way analysis of variance was used to differentiate between samples and decide where significant differences were established. **Results:** The yield of α–cellulose from the cornstalks was 32.5%w/w and that of microcrystalline cellulose 26%w/w. All the cellulose samples met all the pharmacopoeial parameters that were carried out. The comparison of physicochemical properties of the CCC, MCCC and Avicel PH101 suggests that the microcrystalline celluloses might have better flow and compression properties than the CCC sample. The three cellulose powders were of high microbial excipient quality. For almost all parameters evaluated, it was generally observed that the MCCC has similar characteristics to Avicel PH101. **Conclusions:** MCCC can be a suitable alternative to the expensive Avicel PH101as pharmaceutical excipients.

Key words: Cornstalk, microcrystalline cellulose, pharmacopoeial properties, physicochemical properties, α-cellulose

INTRODUCTION

Cellulose is a poly-disperse polymer of high molecular weight and comprised of long chains of D-glucose units joined together by -1, 4-glucosidic bonds.[1] It is the chief constituent of plant cell wall and is widely distributed in plant foods.[2] Biomass, including agricultural residues represents a renewable and alternative source of cellulose and its derivatives available especially in agricultural producing countries. The conversion of biomass into cellulose and its derivatives for use as raw materials in different industries using different technologies is one of the most promising alternatives under study today. Given their renewable character, cellulose-containing materials have been proposed as sustainable alternatives to petroleum-based feedstock.[3] Among other uses, cellulose is widely employed as a raw material to prepare a number of excipients. Microcrystalline cellulose (MCC), a purified partially depolymerised non-fibrous form of cellulose is perhaps the best filler-binder currently available. It was first introduced in 1964 under the brand name Avicel and marketed by the FMC Corporation (Philadelphia, PA U.S.A.). Commercially available MCC is derived from high costly hard wood and also purified cotton. The need for cheaper sources of α-cellulose and MCC powders has led to investigation of other lignocellulosic materials.[4-7] The physical properties and chemical composition of α-cellulose and MCC depend significantly on the characteristics of the raw material employed and the manufacturing conditions.[8] As a result, several types of MCC are available in the market with different pharmacopoeial and physicochemical properties and therefore they will have different functional parameters and applications. These differences can affect their functional properties when employed in tableting.

Cornstalks are one of the largest sources of lignocellulosic biomass in the world; there has been limited use of

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cornstalks for pharmaceutical applications. Traditionally, cornstalks have been used as a source of fibres for manufacturing pulp for paper. Recently, fibres obtained from cornstalks were tried as reinforcements for starch foams for packing materials and also for composites used in the automotive and construction industries.[9-11] Cornstalk fibres used as reinforcement improved the tensile properties of the starch acetate foams in the laboratory.[9] Cornstalks have also been studied to obtain regenerated cellulose.[10]

To the best of our knowledge, there is limited literature available describing the pharmacopoeial, physicochemical and microbiological properties of α-cellulose and MCC powders obtained from cornstalks for possible application in the pharmaceutical industries. As one of the largest source of lignocellulosic biomass, cornstalks are a cheap and annually renewable resource suitable for producing α-cellulose and its derivatives. Producing α-cellulose and MCC powders of pharmaceutical quality from cornstalks will have several advantages. They will add value to the corn crops and provide an inexpensive source of raw materials to the industry and will also benefit the environment. In this paper, we report the process used to produce high quality α-cellulose and MCC powders. The pharmacopoeial, physicochemical and microbiological properties of the α-cellulose and MCC powders produced from cornstalks were also compared to commercial brand of microcrystalline cellulose (Avicel PH101). However, no attempt was made in this preliminary study to directly evaluate the tableting properties of the powders.

MATERIALS AND METHODS

Materials

Cornstalks were collected from ready-to-harvest corn fields in Enugu-Umuonyia, Orumba South Local Government Area, Anambra State, Nigeria. It was ground in a Fitz mill and screened with 40 mesh size for the experiment. Hydrogen peroxide (3%) used is a product of Jay-Kay Pharmaceutical Limited, Lagos, Nigeria and was used as received. All other reagents are of analytical grade unless otherwise stated.

Cellulose Production

The dried and ground cornstalks were used as the lignocellulosic raw material. α-cellulose was obtained from the lignocellulosic raw material using inorganic substances, following a modification of procedure based on that reported elsewhere.[12] The raw material was treated in a 15% w/v NaOH solution at 120°C for 2.5 hours using a liquor-to-stalk weight ratio of 20:1. The stalks and alkali solution were heated in a closed container on a hot plate with temperature control. After the treatment, the slurry was washed in warm water to remove the dissolved substances. The obtained material was neutralized using a solution of 10% (v/v) acetic acid. The product (unbleached cellulose) was bleached with 5g/L H_2O_2 at 70°C for 1 hour at pH 10. The bleached material was filtered, rinsed, neutralized, then dried in an oven at 60°C for 16 hour and weighed. The product was milled in a Kenwood blender, sifted through a 212 μ aperture sieve, dried further at 60°C for 1 hour, labelled CCC and stored in a tightly closed container, pending further analysis.

Microcrystalline Cellulose Production

Cellulose powder obtained from the cornstalks was hydrolyzed with 2N HCl acid under reflux for 15 minutes,[4] the solid-liquor ratio was 1:10. The hydrolyzed cellulose was thoroughly washed with cold distilled water until neutral to litmus paper and then air-dried. The microcrystalline cellulose obtained via this process was labelled MCCC, and their physicochemical properties were compared with that of Avicel PH 101 (commercial grade) (MCCA).

Characterization of Cellulose Powders

Yield of α-cellulose

The amount of α-cellulose extracted from the agricultural wastes was related to the quantity of starting material and the percentage yield calculated using the following mathematical relationship shown in equation 1.

$$\%\text{yield}=\frac{Wc}{Wm}\times 100 \quad (1)$$

where Wc is the weight of α-cellulose and Wm is the weight of the starting waste material

Estimation of purity of α-cellulose

The determination of the purity of α-cellulose of the products was carried out using Browning method.[13] 25 ml of 17.5% sodium hydroxide solution was added to about 3 g pulp (cellulose). The slurry was left to swell for 4 minutes at 20°C and was pressed for 3 minutes with a glass rod and another 25 ml of 17.5% sodium hydroxide solution was added. The suspension was thoroughly mixed for 1 minute and left covered at 20°C. After 35 minutes, 100 ml of distilled water was added, followed by filtration in a sintered glass crucible (G2 of 5 cm diameter and 4.5 cm length). The filtrate was poured twice on the paste before washing with distilled water until complete neutrality and then with 10% acetic acid followed by distilled water. The pulp was finally dried at 105°C for 5 hours and weighed. Percent α-cellulose was calculated as in equation 2.

$$\%\alpha\text{-Cellulose}=\frac{B}{A}\times 100 \quad (2)$$

Where A represents the weight of dry sample (before alkali treatment) and B is the weight of dry treated sample. The determination was made in triplicate.

Pharmacopoeial characterization

The following pharmacopoeial characteristics of the powders were carried out.[14]

(a) *Identification*

10 mg of the sample was placed on a watch glass and dispersed in 2 ml iodinated zinc chloride solution. The color of the powder was noted.

(b) *pH*

2 g of the sample was shaken with 100 ml of water for 5 minutes. The pH of the supernatant liquid was noted.

(c) *Starch*

0.1 g of cellulose was shaken with 5 ml of water, and 0.2 ml of 0.05 M iodine was added. The color change was noted.

(d) *Organic impurities*

10 mg of cellulose was placed on a watch glass and 0.5 ml of instantly prepared solution containing 0.1 g of phloroglucinol in 5 ml of hydrochloric acid was added. The color change was noted.

(e) *Heavy metals*

The test for heavy metals was carried out as detailed in British Pharmacopoeia.

(f) Solubility

The solubility test was carried out following a procedure based on that reported elsewhere.[15] The solvents tested for were water, 2.5 M HCl, 2.5 M H_2SO_4, ethanol, toluene, acetone and ammoniacal tetramine solution.

(g) Ash content

The ash was estimated by ignition in muffle furnace. 2 g of the sample was weighed and placed in a platinum crucible. The crucible with sample was then placed in the furnace and ignited for 30 minutes at 400°C and for further 45 minutes at 800°C. The percentage of ash was calculated from:

$$\%\text{ash} = \frac{\text{Weight of ash}}{\text{Weight of cellulose sample}} \times 100 \quad (3)$$

The determination was made in triplicate.

Physicochemical characterization

(a) Bulk and tapped density

For the determination of the bulk and tapped densities, the methods employed in an earlier study were adopted.[16] The bulk density, D_{bulk}, and tapped density, D_{tap}, were determined using Eqs. (4) and (5):

$$D_{bulk} = w / v_0 \quad (4)$$

$$D_{tap} = w / v_1 \quad (5)$$

where w is the weight of the powder, and v_0 and v_1 are the volumes of the bulk and tapped powders, respectively. The arithmetic mean of four replicate determinations was taken in each case.

(b) True density

The true density of the cellulose samples was determined using a model MPY-2 helium displacement pycnometer (Quantachrome Corporation, Syosset, NY, USA).[16] The true density, D_{true}, was calculated using Eq. (6):

$$D_{true} = w / v_p \quad (6)$$

where w and v_p are the weight of the sample and the true volume of the powder, respectively.

(c) Carr's index and Hausner ratio

Carr's index[17] and Hausner ratio[18] for cellulose were calculated from bulk and tapped densities using Eqs. (7) and (8), respectively:

$$Carr's\ Index = \frac{D_{tap} - D_{bulk}}{D_{tap}} \times 100 \quad (7)$$

$$\text{Hausner Ratio} = \frac{D_{tap}}{D_{bulk}} \quad (8)$$

(d) Porosity

The porosity (P_b) of the tested powders was evaluated from the true and tapped densities, by means of Eq. (9):

$$P_b = 1 - \frac{D_{tap}}{D_{true}} \quad (9)$$

(e) *Angle of repose*

The measurement of the angle of repose was carried out using a long cylindrical tube open at both ends as detailed in our earlier study.[16] The height, h, and radius, r, of the conical heap formed were measured, and then the angle of repose,θ, was calculated from Eq. (10). Determinations were done in triplicate, and the average was taken.

$$\theta = \tan^{-1}(h/r) \quad (10)$$

(f) Moisture content

The moisture content of the cellulose powders was calculated from the weight loss on heating from room temperature to 225°C on a Perkin Elmer series 7 thermal analyzer. Triplicate determinations were carried out.

Microbiological evaluation

The microbiological evaluation of the cellulose samples were carried out following a procedure based on that reported elsewhere.[19] Briefly, for each of the cellulose sample (CCC, MCCC and MCCA), 0.1 g of the sample was

suspended in 10 ml of nutrient broth from which 10^{-1}, 10^{-2}, and 10^{-3} dilutions in sterile distilled water were made. 0.1 ml each of the dilutions was inoculated on plates containing nutrient agar in triplicates. The plates were incubated at 30°C for 72 hours. In a similar manner, 0.1 ml each of the cellulose suspension was inoculated on plates of 7% salt agar, McConkey agar, and Saboraud agar in triplicates.

RESULTS

Cornstalk α-cellulose (CCC) and cornstalk microcrystalline cellulose (MCCC) powders were obtained from the cornstalks and their pharmacopoeial, physicochemical and microbiological properties were compared with that of Avicel PH 101 (commercial grade) (MCCA). The yield of the α-cellulose from the cornstalks was 32.5%w/w. The percentage purity of CCC was 97.8%±1.4.

The data for pharmacopoeial characterization for the three samples (CCC, MCCC and MCCA) is shown in Table 1. The CCC and MCCC were almost white in color, tasteless and odourless.

The physicochemical properties of the α-cellulose directly isolated from the cornstalks (CCC), the cornstalk microcrystalline cellulose (MCCC) and the commercial brand of microcrystalline cellulose (MCCA) are reported in Table 2.

The results of the microbial counts are shown in Table 3. It is important to note that only *Bacillus subtilis* grew on nutrient agar plates. There was no growth on the other media (McConkey agar, 7% salt agar, and Saboraud agar) for all the cellulose samples.

DISCUSSION

The 32.5%w/w yield of the α-cellulose reported in this work for CCC differs with 30 and 23%w/w, respectively reported for maize cob[12] and Sorghum caudatum stalk.[20] The yield of the microcrystalline MCCC, obtained from α-cellulose was approximately 80% w/w. Thus the yield of MCCC was approximately 26% w/w of the starting dry plant material. It has been reported that cornstalk has a cellulose content of 42.4%.[21] The MCCC content is expected to be lower than that reported in the literature since during the hydrolysis and washing steps, the abundant amorphous regions are solubilized and eliminated.

The percentage purity of CCC (97.8%±1.4) met the British Pharmacopoeia[14] purity specification for α-cellulose (i.e., powdered cellulose) of 97.0 to 102.0%. This implies that the extraction procedure employed yielded α-cellulose of pharmacopoeial quality in terms of purity. They percentage purities of MCCC and MCCA samples were also within the British Pharmacopoeia acceptable range [Table 1].

Table 1: Pharmacopoeial properties of the cellulose samples, with standard deviations in parentheses

Property	Cellulose powder samples		
	CCC	MCCC	MCCA
Identification	Violet-blue	Violet-blue	Violet-blue
pH	6.5 (0.4)	6.0 (0.5)	6.1 (0.6)
Starch	Nil	Nil	Nil
Organic impurities	Nil	Nil	Nil
Heavy metals	<10 ppm	<10 ppm	<10 ppm
% Purity (Cellulose)	97.8 (1.3)	98.8 (1.4)	99.8 (1.1)
% Ash	0.152 (0.005)	0.045 (0.023)	0.035 (0.005)

CCC – Cornstalk α-cellulose; CCCC – Cornstalk microcrystalline cellulose; MCCA – Avicel® PH 101

Table 2: Physicochemical properties of the cellulose samples, with standard deviations (corresponding to the last significant figure) in parentheses

Property	Cellulose powder samples		
	CCC	MCCC	MCCA
Bulk density (g cm^{-3})	0.23 (2)	0.33 (5)	0.34 (5)
Tap density (g cm^{-3})	0.32 (3)	0.43 (4)	0.42 (2)
True density (g cm^{-3})	1.48 (4)	1.59 (4)	1.60 (3)
Carr's index	28.13	23.26	19.05
Hausner ratio	1.40	1.30	1.26
Powder porosity	0.78	0.73	0.74
Angle of repose (°)	44 (2)	41 (4)	39 (5)
Moisture content (%)	5.7 (6)	5.6 (4)	5.4 (5)

CCC – Cornstalk α-cellulose; CCCC – Cornstalk microcrystalline cellulose; MCCA – Avicel® PH 101

Table 3: Microbiological evaluation of the cellulose samples in various media

Medium	Cellulose powder samples / No. of Colonies*		
	CCC	MCCC	MCCA
Nutrient agar[a]	100 cfu/gm	50 cfu/gm	50 cfu/gm
7% Salt agar[b]	No growth	No growth	No growth
McConkey agar[c]	No growth	No growth	No growth
Saboraud agar (Yeast)	No growth	No growth	No growth
Saboraud agar (Fungi)	No growth	No growth	No growth

*Results of triplicate experiments expressed in colony forming units (cfu). [a]bacterial mesophilic count [b]bacterial pseudomonads counts [c]bacterial coliform counts; CCC – Cornstalk α-cellulose; CCCC – Cornstalk microcrystalline cellulose; MCCA – Avicel® PH 101

Pharmacopoeial Characterization

The identification test gave a violet-blue color for the three samples, indicating a cellulose sample. The test for starch and dextrin did not give any color change for all the samples; hence, they were thus adjudged absent in the samples. Furthermore, the three samples were free from organic impurities. The solubility data the celluloses indicated that they are insoluble in most solvents tested except ammoniacal solution of copper tetramine in which they dissolved completely. The pH of the samples, which ranged from 6.0 – 6.5, fell within the acceptable limits of between 5 and 7.[14,22] This indicates that the celluloses were adequately washed with water following extraction.

The test for heavy metals carried out indicates the three cellulose samples complied with the limit test for heavy metals of 10 ppm.[14] Total ash for the microcrystalline cellulose samples, (MCCC, 0.045%; MCCA, 0.035%) fall within the acceptable limits of < 0.05%.[22] However, the ash value of 0.152% obtained for the CCC sample was above the limit. Total ash value is of importance in determining the safety of pharmaceutical excipients used for oral and parental products and indicates, to some extent, the level of care taken in the preparation of the substance.[23] The low percentage of ash in the MCC samples may be as a result of very low inorganic materials usually present in the MCC materials and care taken in the preparations of the MCC samples.

Physicochemical Characterization

The bulk and tap densities of the microcrystalline celluloses (MCCC, 0.33 g cm^{-3}; MCCA, 0.34 g cm^{-3}) are significantly higher than that of the α-cellulose (CCC, 0.23 g cm^{-3}). This suggests that the microcrystalline celluloses might have better flow properties than α-cellulose, although their higher bulk densities would imply the need for larger amounts for compression in tablet manufacturing.[24] Small particle size and low moisture content has been suggested to lead to increased bulk density.[25] The bulk density of MCCC, 0.33 g cm^{-3} is lower than 0.42 g cm^{-3} reported for orange mesocarp[15] and higher than 0.27 g cm^{-3} reported for sorghum caudatum,[20] as well as 0.238 g cm^{-3} reported for sisal fibre MCC.[26]

The higher values of the true density for the microcrystalline celluloses, compared to the α-cellulose reported in Table 2, suggest that the microcrystalline celluloses might exhibit better compressibility. It could also be inferred that the MCCC and MCCA are more crystalline in nature compared to the CCC, as the degree of crystallinity of cellulose has been reported[20] to increase directly with true density determined in a non-polar solvent.

The suggestion of better flow properties for the MCC celluloses than for the α-cellulose is also supported by the results of the Carr's index and Hausner ratio. In Table 2, a decrease of both magnitudes is observed for the MCC samples, compared to the cornstalk α-cellulose. Carr's index, also called "per cent compressibility," measures the potential powder arch or bridge strength and stability, and has been widely used to estimate the flow properties of powders.[17] Values of this index in the ranges 5–10, 12–16, 18–21, and 23–28 indicate excellent, good, fair, and poor flow properties of the material, respectively. In this study, CCC has a Carr's index of 28 (poor flowability), the indices for the microcrystalline celluloses (MCCC and MCCA) are in the range of 15–20 (fair-good flowability).

On the other hand, the Hausner ratio is the quotient of tap and bulk densities, providing a measure of interparticle friction, and is also used to predict the flowability of a material.[27] A value of less than 1.20 indicates good flowability, whereas a value of 1.50 or higher suggests that the material will have poor flow properties. In our study, the Hausner ratio for the α-cellulose (CCC) is 1.40 (poor flowability), whereas those ratios for the microcrystalline celluloses (MCCC, 1.30; MCCA, 1.26) lie around the threshold of 1.20 (good flowability). Therefore, the values obtained for the Hausner ratio are fully consistent with those of the Carr's index.

The angle of repose of a powder gives a qualitative assessment of its internal and cohesive frictions. Angles of up to 40° indicate reasonable flow potential of the solid powders, whereas those samples with angles greater than 50° exhibit poor or absent flow.[28] In this study, the angles of repose for the celluloses (CCC- 44°; MCCC- 41°; MCCA- 39°) reported lie around the threshold of fair flow potential; however, the microcrystalline celluloses show superior flow properties than the α-cellulose. The observed values for the angles of repose are fairly consistent with those of the Carr's index and the Hausner ratio.

The moisture contents measured for all the cellulose samples in this work are less than the maximum allowable limit of 8%.[29] There is no significant difference among the values of moisture content of the cellulose samples used obtained in this study.

The total porosity of a porous powder is made up of voids between the particles as well as pores within the particles. The high value of total porosities reported in Table 2 for the three cellulose samples correlates with the high tapped densities obtained in this work.

Microbiological evaluation

Apart from *Bacillus subtilis that* grew on nutrient agar plates; there was no growth on the other media (McConkey agar, 7% salt agar, and Saboraud agar) for all the cellulose samples. This is indicative of the absence of *Enterobactereacea,* such as *E. coli*, *Klebsiella species*, *Staphylococci*, and fungi, respectively for all the three cellulose samples. The absence of *Enterobactereacea,* such as *E. coli*, *Klebsiella species*, Staphylococci, and *fungi* is suggestive of high microbial excipient quality. The only microorganism that grew on the nutrient agar, *Bacillus subtilis* is a ubiquitous bacterium commonly recovered from water, soil, air, and decomposing plant residue. It is considered a benign organism as it does not possess traits that cause disease. It is not considered pathogenic or toxigenic to humans, animals, or plants. Although the British Pharmacopoeia does not prescribe microbial standards which powdered cellulose must meet,

it does however, specify that *Escherichia coli* must be absent from pharmaceutical grade starch. If this requirement could be extended to a non-sterile excipient such as cellulose, we can conclude that the cellulose powders obtained from corn cobs and the commercial brand met the British Pharmacopoeia requirement in terms of microbial quality. Moreover, powdered excipients are normally stored under dry conditions, in these conditions; it is unlikely to result in spoilage due to growth of microorganisms in the final products. Contrarily, when they are used in the formulations of solid dosage medicaments, e.g. tablets and capsules, they are stored under tropical conditions (high temperature and relative humidity). These conditions can precipitate a dramatic loss of microbial integrity within a relatively short period of time.[30] Hence, it is worthy to note that the findings of this study do not preclude the possibility that microbial integrity of medicaments containing these cellulose powders will be sustained when stored in a humid environment.

CONCLUSION

Alpa cellulose and MCC powders obtained from cornstalks have some desired pharmacopoeial, physicochemical and microbiological properties required for pharmaceutical applications. Such materials are renewable, vastly available in many regions of the world and are generally burned or disposed for ambient degradation. Cornstalks could be used for the preparation of low-cost MCC using HCl hydrolysis. MCC prepared in this work from cornstalks has comparable physicochemical properties to that of commercial brand MCCA.

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