



Bio-chemical Preparation of Microcrystalline Cellulose Powder from Cotton Linters for Utilization as Tablet Excipients

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Authors' contributions

This work was carried out in collaboration among all authors. Author VM designed the study, reviewed and wrote the first draft of the manuscript. Author HS performed the experiments. Author VS analyzed the data. All authors read and approved the manuscript.

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ABSTRACT

Aims: The present work is aimed to prepare microcrystalline cellulose (MCC) powder from cotton linters by an eco-friendly bio-chemical process and to evaluate the prepared MCC as tablet excipients.

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Study Design: Randomized Complete Block Design.

Place and Duration of Study: Ginning Training Center, ICAR-Central Institute for Research on Cotton Technology, Nagpur, Maharashtra, India during June to December, 2019.

Methodology: The MCC was prepared from cotton linters by enzymatic and chemical methods and compared with commercial grade MCC (Avicel® PH101). The crude enzyme extract of *Aspergillus* sp. VM-1 was obtained by solid-state fermentation and used for hydrolysis of cotton linters at 60°C for 60 min. In the chemical process, 10% alkali was used for hydrolysis at boiling temperature for 60 min. In both the processes, the hydrolyzed cotton linters were bleached with hydrogen peroxide. The MCC powders were characterized for physico-chemical and tableting properties based on Indian Pharmacopeia (IP) and United States Pharmacopeia (USP) specifications.

Results: The α -cellulose content (%) in the synthesized MCC by enzymatic process was 98.1 while the commercial grade MCC, Avicel® PH101 had 98.5. The physico-chemical properties of synthesized MCC by enzymatic process were comparable with Avicel PH101 and meet the IP standards. The degree of polymerization (DP) of prepared MCC and Avicel PH101 were 215 and 157, respectively. The FT-IR spectrum of synthesized MCC had similarity to that of Avicel® PH101. The tableting properties of prepared MCC met USP standards. The MCC prepared from cotton linters by enzymatic was found to be superior to chemical process with respect to cellulose yield, degree of polymerization and tablet dissolution property.

Conclusion: The results showed MCC synthesized from cotton linters through enzymatic route is a promising candidate for direct compressible excipient of tablet. The present study highlights that the enzymatic process significantly reduces the alkali usage and heating temperature and thus saves the chemicals and energy in the process.

Keywords: *Aspergillus* sp.; bio-chemical; cotton linters; chemical process; crude enzyme; enzymatic process; microcrystalline cellulose; tablet excipient.

1. INTRODUCTION

During the past few decades, the bio-based materials are gaining importance in order to reduce the dependency on fossil fuels [1,2]. Cotton is considered as king of natural fibres as they are rich in cellulose (98%) and more comfortable. The major producers of cotton are India, China, United States of America, Brazil, and Pakistan [3]. "Microcrystalline cellulose (MCC) is partially purified de-polymerized crystalline cellulose having potential industrial applications. The quality MCC has high demands in the field of cosmetics, food and pharmaceutical industries because it can be used as good suspension stabilizer and a reinforcing agent for final products such as medical tablets and capsules" [4,5]. "Commercially available MCC is mostly derived from costly wood pulp and purified cotton by treating with acids like hydrochloric acid" [6]. "The need for cheaper sources of MCC has led to the investigation of other lignocellulosic materials based on different agricultural residues" [7]. The cotton linters are the short staple fibres attached to the cottonseed and it constitutes about 8 % of cottonseed by weight. About 6 to 10% of cotton generated during cultivation as well as processing is made up of short fibres which could not be woven and it is called non-spinnable cotton. The linters are short

fuzzy fibres (<10 mm staple length) obtained from ginned cottonseed after delinting process. The cotton linters contain about 90-98% of cellulose, hemi cellulose (0.4-1.5%), ash (0.3-0.6%), acid-insoluble materials (0.08-0.66%) [3].

Cellulose/microcrystalline cellulose were produced from various agro-residues through chemical (acid) or biological (enzymes) or combination of both. The crude enzyme prepared from lignocellulosic material was used in biological in biological process. The agro-residues reported for synthesis of MCC/Cellulose are bamboo [8], corn-cob [9-11], cotton gin waste [12], soybean hulls [13]. Cotton linters are rich in cellulose, available in plenty and therefore have potential in preparation of MCC. The crude enzyme of *Aspergillus* sp. VM-1 grown in banana pseudostem waste had the ability to increase the absorbency of cotton linters [14]. The present study is aimed to prepare the MCC from cotton linters by deploying the crude enzyme of *Aspergillus* sp. VM-1 and evaluate the prepared MCC in tablet excipients.

2. MATERIALS AND METHODS

2.1 Collection of Raw Material

The cotton linters were obtained from M/s Gimatex, Hinganghat, India. The staple length of cotton linter was 8mm.

2.2 Microorganisms

The fungal strain, *Aspergillus sp.* VM-1 was obtained from Microbiology Laboratory of Ginning Training Center, ICAR-CIRCOT, Nagpur. The fungal strain, *Aspergillus sp.* VM-1 were grown in malt extract broth (1x) (Himedia, India) for 48 h and maintained in malt extract agar slant at 4°C.

2.3 Substrates for Solid State Fermentation

“The agro-residues namely banana pseudostem wastes, cottonseed hulls and cottonseed meal, were purchased from local market. These substrates were dried in a hot air oven, powdered using pulverizer and passed through 1 mm sieve. The substrates thus prepared were used for solid-state fermentation” [11].

2.4 Solid State Fermentation

Solid state fermentation was carried out in one-liter conical flask containing 100 g of substrate (banana pseudostem, cottonseed hulls and cottonseed meal in the ratio of 60:30:10). Eighty milliliters of distilled water were added to maintain initial moisture content [up to 80% (v/w)]. The moistened substrate was autoclaved at 121°C for 15 lbs/in² for 20 min. After cooling, the substrate was inoculated with 5 ml of 48 h old fungal strain, *Aspergillus sp.* VM-1 (5% inoculums grown in malt extract broth) and incubated for one week at 30 ± 2°C.

2.5 Enzyme Extraction

“The enzyme was extracted by adding total one liter of distilled water in the one-week old fermented substrate, stirred for 30 min and filtered through muslin cloth. The enzyme extract was stored at 4 °C until use. The pectinase activity in crude enzyme was determined by estimation of polygalactouronase activity and analysis of reducing sugars released from citrus pectin during the reaction” [15]. One unit of enzymatic activity (U) was defined as the amount of enzyme which releases one μmol of galactouronic acid per minute.

2.6 Preparation of Microcrystalline Cellulose from Cotton Linters by Enzymatic Method

Ten g of cotton linters was taken in a beaker to which enzyme extract of *Aspergillus sp* VM-1

was added in the ratio 1:20 (cotton linters to enzyme solution). It was kept in a water bath and incubated for one hour at 60 °C. After incubation, it was subjected for open boiling with 0.5% (w/w) sodium hydroxide for 15min. The cotton linters was washed with water and neutralized with 0.2% (v/v) acetic acid. Subsequently, the bleaching was carried out using hydrogen peroxide 1% (w/v), along with sodium hydroxide 2% (w/v) at 90-95 °C for 30 min with material to liquor ratio 1: 20 and washed with water.

2.7 Preparation of Microcrystalline Cellulose from Cotton Linters by Chemical Method

Ten g of cotton linters was taken in a beaker and subjected for open boiling with 10% (w/w) sodium hydroxide for 60 min. The cotton linters were washed with water and neutralized with 0.2% (v/v) acetic acid. Subsequently, the bleaching was carried out by using hydrogen peroxide (1%w/v), along with sodium hydroxide 2% (w/v) at 90-95°C for 30 min with material to liquor ratio 1:20 and washed with water.

2.8 Physico-Chemical Characterization of Micro Crystalline Cellulose

The physico-chemical properties of MCC prepared from cotton linters by enzymatic and chemical process were determined according to Indian Pharmacopeia [11,16] and compared with commercial grade MCC (Avicel ® -PH 101). The physico-chemical properties tested were pH, moisture content, solubility characteristics, ash, cellulose, degree of polymerization, particle size, bulk density, tapped density, true density, porosity, angle of repose, Carr’s index, Hausner ratio, hydration capacity, swelling capacity and moisture sorption capacity.

2.9 Infrareds Spectroscopy

“To study the functional groups and chemical structure of MCC prepared from cornhusk fibres, Fourier transform infrared (FT-IR) spectroscopy was done. Shimadzu IR Prestige 21 analyzer was used to carry out the spectroscopy. MCC (0.5 mg) was mixed with 200 mg KBr (spectroscopy grade) and pelletized in a KBr press. A pressure of approximately 8 tons was used to form 13 mm diameter pellets. To eliminate air and moisture from the KBr powder degassing was done. The scanning range was 500 to 4500 cm⁻¹ [11].

2.10 Preparation of Paracetamol Tablet Using MCC

The MCC from chemical and enzymatic process was evaluated for excipient property in paracetamol tablet. The ingredients and its composition of prepared paracetamol tablets is given in Table 1. A starch paste (5.0 % w/v) was prepared with the help of soluble starch by heating till translucent paste formed. The paracetamol, lactose and microcrystalline cellulose were mixed using mortar and pestle and moisturized it with required quantity of starch paste to prepare a damp mass. The damp mass was passed through sieve no. 12 to form granules and dried them in a hot air oven at 60°C for 2 h. The starch powder, talc and magnesium stearate were added. The granules were compressed on tablet compression machine at 2.0 tons pressure to prepare the tablet.

Table 1. Formulation of paracetamol tablet using MCC

Name of ingredient	Activity	Quantity
Paracetamol	Analgesic, antipyretic and anti-inflammatory	500.0mg
Microcrystalline cellulose (MCC)	Diluent	150.0mg
Lactose	Diluent	100.0mg
Starch paste	Binding agent	q.s
Starch powder	Disintegrant	5.0% w/w
Talc	Glidant	0.5% w/w
Magnesium stearate	Lubricant	0.1% w/w

2.11 Evaluation of Tablet Properties

The tablet properties after addition of MCC prepared from cotton linters by enzymatic and chemical process were examined according to United states Pharmacopeia (1980) [17].

2.11.1 Shape and colour

Uncoated tablets were examined under a lens for the shape of the tablet, and colour was observed by under light.

2.11.2 Weight Variation test

Twenty tablets were selected randomly from each batch and weighed individually to check for weight variation. The results were expressed in mg.

2.11.3 Uniformity of thickness

Three tablets were picked from each formulation randomly and the thickness was measured using Vernier caliper and expressed in mm.

2.11.4 Hardness test

Three tablets were randomly picked and analyzed for hardness. Hardness indicates the ability of a tablet to withstand mechanical shocks while packaging, handling, and transportation. The hardness of the tablets was determined using Monsanto hardness tester and expressed in kg/cm².

2.11.5 Friability test

It is the phenomenon whereby tablet surfaces are damaged and show evidence of lamination or breakage when subjected to mechanical shock or attrition. The friability of tablets was determined using Roche Friabilator and expressed in %. Ten tablets were initially weighed (A) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes or run up to 100 revolutions. The tablets were weighed again (B). The % friability was then calculated by the formula, [(A-B)/A] × 100.

2.11.6 Disintegration of paracetamol tablets

For disintegration test, six tablets were placed in the tube of USP disintegration test apparatus. The assembly was suspended in the beaker with 900 ml distilled water as medium at 37 ± 2 °C at 50 rpm. The time (min) required to disintegrate each tablet was measured.

2.11.7 *In vitro* dissolution

"*In vitro* dissolution of paracetamol tablets was measured according to the USP30" [18]. "For this, six tablets were analyzed in USP dissolution type II apparatus at a speed of 50 rpm. The dissolution medium was 900 ml of 0.1 M phosphate buffer solution having pH 7.4 at 37 ± 0.5 °C. After 45 min, 5 ml solution from each basket of the apparatus was collected, filtered, diluted if necessary and measured the absorbance at 332 nm using UV-Vis spectrophotometer (Model: UV 1700, Shimadzu). The amount of drug dissolved after 45 min was calculated from the standard curve of paracetamol prepared by measuring the absorbance of paracetamol solution having different concentration" [18].

3. RESULTS AND DISCUSSION

The increasing awareness about the ill effects caused by the harsh chemical released from the industry and agriculture to the environment, forced us to look for alternatives. Enzymes are the biomolecules which catalyze the chemical reaction and found to be an eco-friendly alternative to chemicals. Several commercial enzymes are used for industrial applications. The crude enzyme extracted from fungi *Aspergillus* sp. VM-1 and *Pleurotus flabellatus* M-1 under solid state fermentation of banana pseudostem has reduced the alkali usage significantly while increasing the absorbency of cotton linters [14, 19, 20]. The pectinase activity of the enzyme extract was 551 U/ml (results not shown) The present study is aimed to prepare MCC from cotton linters by treatment with crude enzyme extracted from *Aspergillus* sp. VM-1 and compare them with MCC prepared from chemical process and commercial grade MCC (Avicel® PH101). The MCC prepared from the cotton linters is depicted in Fig. 1.

3.1 Physico-Chemical Characterization

The physico-chemical properties of MCC prepared from cotton linters and Avicel® PH 101 are presented in Table 2. The pH of MCC prepared by enzymatic (6.4) and chemical (5.9) process was found to be slightly less than commercial grade MCC (6.8). The moisture content of MCC prepared by enzymatic and chemical from cotton linters was 5.1 and 5.6 %, respectively while it was 5.0 % in Avicel® PH 101. The results agree with the previous studies where the moisture content of MCC was between 5-7% [13, 21]. The moisture content of MCC has an effect on compaction properties, tensile strength and viscoelastic properties [22,23]. As relative humidity increases, the tablet strength decreases [24].

The ash content of MCC prepared by enzymatic and chemical process was found to be 0.04 and 0.09 %, respectively while 0.06% in Avicel® PH101. According to U.S.P.1980, ash content should not be more than 0.1%. In a similar study, the ash content of MCC prepared from cornhusk fibre was 0.05% [14]. "The cellulose content in MCC prepared from cotton linters was 98.05% and 91.95 % in enzymatic and chemical, respectively while 98.5% in Avicel® PH101. According to U.S.P, the cellulose content should not be less than 97%. In a similar study, cellulose content of MCC prepared from soybean hulls,

banana fibres and corn husk fibres were 97.5, 99.0 and 98.2 %, respectively" [11,13,21]. Thus, the MCC prepared by enzymatic process in this study meet the U.S.P specifications. The starch content was absent in MCC prepared from cotton linters (results not shown) which is comparable to the specification of U.S.P. for MCC. The MCC prepared from cotton linters were partially soluble in distilled water and 1% NaOH, insoluble in 1% HCl and completely soluble in petroleum ether, acetone and groundnut oil (results not shown). The solubility properties meet the standards of Pharmacopoeia of India, 1985.

The degree of polymerization (DP) is an important parameter and helps us to understand the number of repeating units of glucose in a polymer. The DP of MCC prepared from cotton linters was found to be 215 and 280, in enzymatic and chemical process, respectively. The DP of Avicel® PH101 was found to be 157. In a similar study, the DP of MCC derived from hemp stalks and corn husks as 125 and 245, respectively [25]. The pharmacopoeia MCC has a DP below 350 glucose units while compared to DPs in the order of 10,000 units for the original native cellulose [26, 27]. The particle size (μm) was analyzed by particle size analyzer and the results showed, the range of particle size in enzymatic, chemical and Avicel® PH101 were 30-40, 35-45 and 30-50, respectively. In a similar study, the particle size (μm) of MCC prepared from corn husk fibres by anaerobic fermentation method was 35-45 [11].

The bulk and tapped densities of the MCC from cotton waste fibres were slightly lesser than the Avicel® PH101. The bulk and tapped densities of MCC prepared by enzymatic process were 0.26 and 0.4 g/ml, respectively. "The bulk and tap densities represent the property of a material to flow and rearrange under compression. Similar observations were made in MCC powder obtained from Indian bamboo and corn husk fibres" [8,14]. The true density of MCC powder prepared from cotton linters was comparable to that of Avicel® PH101. According to Stamm (1964) [28] "there is a direct correlation between the degree of crystallinity of cellulose and its true density when determined in a non-polar liquid". "Similar observations were made in our study. The voids and pores within the particles make up the total porosity of a porous powder. The results showed that the porosity of MCC prepared from enzymatic and chemical process is slightly higher than Avicel® PH 101. The angle of repose of MCC powder gives a quantitative assessment of

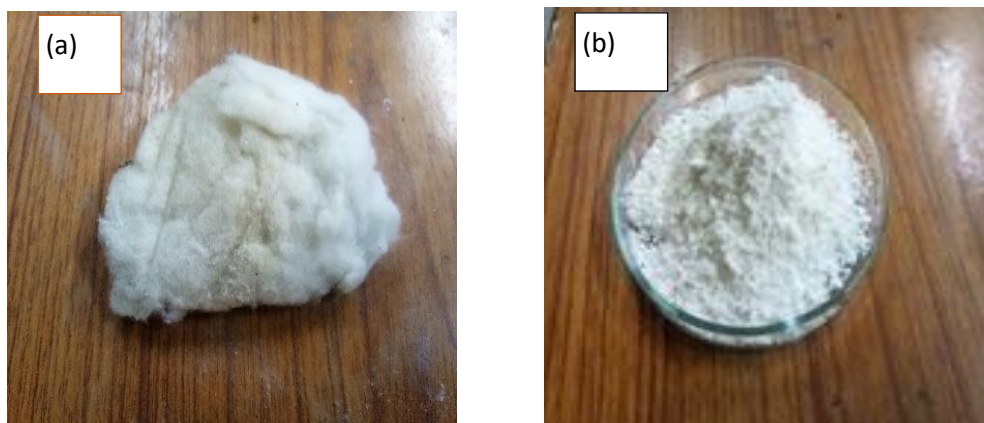


Fig. 1. (a) Cotton linters (b) MCC from cotton linters

its internal and cohesive frictions. The angle of repose around 35° for the MCC samples indicates good flow” [29]. In this study, the angles of repose of both the MCC powders obtained from cotton linters were comparable to Avicel® PH 101.

“In order to determine the suitability of a material to be used as a direct compression excipient, its flow properties are important. The flow properties of a powder can be indirectly determined by measuring its Hausner’s index and Carr’s percent compressibility” [30]. “The Hausner’s index indicate interparticle friction and measures cohesion between particles. The compressibility of a powder can be measured by Carr’s index. The values for Hausner’s index and Carr’s index vary inversely with particle flow. As the values of these indices increases, the flow of powder decreases. It is accepted that Hausner index ratio greater than 1.25 indicates poor flow and Carr’s compressibility index less than 16% indicates good flowability of the powders. The values more than 35% indicate cohesiveness” [30]. “The flow indices of MCC from cotton linters and Avicel® PH101 powders have showed poor flow as indicated by their Carr’s compressibility index. Similar observations were made in MCC powder obtained from corn husk fibres” [11]. “The hydration capacity value shows that MCC obtained from cotton linters was able to absorb more water compared to that of Avicel® PH 101. The swelling capacity for MCC from cotton linters by enzymatic and chemical method was 27 and 25 %, respectively which reflects the increase in the volume of cellulose due to water absorption. The measurement of moisture sensitivity of a material gives its moisture sorption capacity” [11]. The moisture sorption capacity value for MCC prepared from cotton linters was slightly

lesser than that of Avicel PH 101 which indicates the relative physical stability of tablets prepared from cotton linters MCC when stored under humid conditions.

3.2 IR Spectroscopy

“FT-IR spectroscopy has the capability to calculate structural differences not seen by other analytical methods. To understand the chemical groups, present in commercial MCC and MCC prepared from cotton linters, FTIR spectra of the samples were studied. No major differences were observed between the FT-IR spectra of the MCC samples prepared from cotton linters and Avicel-PH 101. Fig. 2, shows the general characteristic spectrum of typical cellulosic material. For example, absorption bands are clearly observed at 900, 2900 and 3300–3500 cm^{-1} which are corresponding to -glycosidic linkages, C–H asymmetric and symmetric tensile vibration and –OH stretching respectively” [31]. “Samples showed a strong broad absorption band in the range of 3170–3360 cm^{-1} corresponding to O–H stretching of hydroxyl groups of cellulose. A sharp medium band appeared in both the samples at its normal position of 2900 cm^{-1} is due to C–H stretching in cellulose molecule. The sharp peak at 1640 cm^{-1} may be attributed to the stretching vibrations of C=C. A strong and broad band observed at around 1400 cm^{-1} in both the samples corresponds to C-H bending vibrations. Whereas, peak at 1050 cm^{-1} is attributed to C–O stretching. This study shows that the samples are same in terms of functional groups presents and crystallinity as the peaks for crystallinity observed at 924 cm^{-1} are of same strength” [11].

3.3 Tableting Properties of MCC

The MCC prepared from cotton linters were evaluated for direct compressible tablet materials (Table 3). The different physical parameters of the tablets such as thickness, hardness, friability, disintegration and dissolution were evaluated. The shape and colour of the prepared paracetamol tablets were round and white, respectively. The weight variation and uniformity of thickness of the prepared tablets comply with the USP specifications. The hardness and friability test were performed to confirm whether the prepared tablets can withstand the physical force during transportation. The hardness of tablet prepared using Avicel® PH101, enzymatic and chemically prepared MCC were 6.25, 5.25 and 4.78, kg/cm², respectively. According to USP (1980) specification, oral tablets should have a hardness of 4 to 10 kg/cm². This indicates that the cotton linters MCC based tablet formulation comply with the USP specification for hardness test. The weight loss of the tablets prepared from Avicel® PH101, enzymatic and chemically prepared MCC were 0.063, 0.085 and 0.099 %, respectively after 100 revolutions (25 rpm for 4 min) in the friabilator. This result complies with the USP specification for friability test where the weight loss should not be more than 0.8%. The *in vitro* disintegration was performed for the tablets using USP apparatus. The tablets formulated using cotton linters MCC disintegrated within 10 min indicating a good disintegration property [26]. The result follows USP specification for disintegration test. The formulated tablets were subjected to *in vitro* dissolution study using USP dissolution

apparatus. All the formulations showed more than 90 % drug release in 900 ml of 0.1 M phosphate buffer (pH 7.4) at 50 rpm within 45 min. The dissolution of tablet prepared using MCC of enzymatic process was higher (96.6) compared to chemical process (90.2). However, all the tablets prepared in this study confirms the USP specification for dissolution test where 80% or more must be released within 45min in specified conditions. The results are in accordance with the tablet properties of MCC prepared from waste paper [32], corn husk [33]. Thus the results showed, the MCC prepared from cotton linters by enzymatic and chemical process meet the physico-chemical standards of USP and IP. Also, the prepared MCC from cotton linters was found to be a good candidate as a direct compressible excipient of tablets.

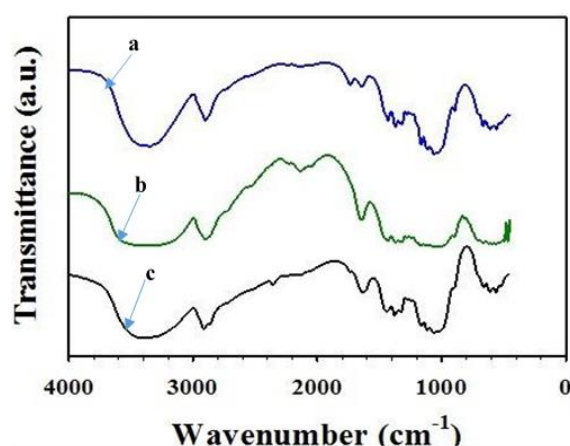


Fig. 2. FT-IR spectra of MCC a) commercial b) Enzymatic c) Chemical

Table 2. Physico-chemical properties of MCC

Property	Enzymatic process	Chemical process	Avicel® PH101
pH	6.4	5.9	6.8
Cellulose content (%)	98.05	91.95	98.5
Ash content (%)	0.04	0.09	0.06
Moisture content (%)	5.1	5.6	5.0
Degree of Polymerization	215	250	157
Particle size in μm	30-40	35-45	30-50
Bulk density(g/ml)	0.26	0.21	0.31
Tapped density(g/ml)	0.40	0.35	0.42
True density(g/ml)	1.52	1.60	1.54
Porosity (%)	32.2	36.0	30.19
Angle of repose	38°	42°	39°
Hausner index ratio	1.39	1.43	1.35
Carr's Compressibility Index (%)	30.5	32.8	26.19
Hydration capacity	3.56	3.78	3.49
Swelling index (%)	27	25	24
Moisture sorption Capacity (%)	5.86	5.09	6.05

Table 3. Tablet properties of MCC

Parameters	Enzymatic process	Chemical process	Avicel® PH101
Shape and colour	Round, white	Round, white	Round, white
Weight variation (mg)	0.565	0.612	0.627
Uniformity of thickness (mm)	4.9	5.2	6.15
Hardness (kg/cm ²)	5.25	4.78	6.25
Friability (%)	0.085	0.099	0.063
Disintegration (min)	9.21	8.34	4.50
Dissolution (%)	96.6	90.2	98.3

4. CONCLUSION

Cotton linters are rich in cellulose and annually available agro-residue and thus can be a potential raw material for MCC production.

The MCC prepared by chemical and enzymatic process contained cellulose of 92 and 98.2 %, respectively.

The physico-chemical characterization of MCC and the tableting properties of MCC showed that MCC prepared from cotton linters is at par with commercial grade MCC and also meets the pharmacopeia standards.

Considering the reduction in use of chemicals and energy, the enzymatic process reported in the study is an environment-friendly alternative to chemical process in the preparation of MCC from cotton linters.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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