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Physicochemical and functional characterization of microcrystalline cellulose from bamboo (Dendrocalamus longispathus)

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Abstract: The present study is an investigation of the physicochemical and functional properties of microcrystalline cellulose (MCC) prepared from bamboo (*Dendrocalamus longispathus*) fibers. *Dendrocalamus longispathus* bamboo is one of the 15 commercially important bamboo species in India that have been used in pulp and paper industries. The percentage cellulose and α-cellulose yield from the original material were 65.42 and 55.63 respectively. The properties of the prepared MCC were investigated and compared with standard Avicel PH101 MCC. Micromeritic properties such as average particle size, flow properties, porosity and density of the powder MCC samples were determined along with the total ash, moisture contents and swelling index. Fourier transformed infrared spectroscopy (FTIR), scanning electron microscopy (SEM), thermogravimetric analysis (TGA) and x-ray diffraction (XRD) spectroscopic studies were also performed on both the prepared and standard MCC samples. Results from these analyses indicate that *Dendrocalamus longispathus* bamboo has a great potential as a green source of MCC.
Keywords: microcrystalline cellulose; Dendrocalamus longispathus; physicochemical properties; functional properties.

1. Introduction

Polymers derived from natural resources especially those from non-food sources are attracting increased attention in recent years mainly due to their biodegradability, low cost and unique physical properties. Cellulose is highly abundant classical example of renewable and biodegradable structural plant polymer which can be processed into whisker like microfibrils[1]. It is widely employed as a raw material to prepare a number of excipients. Microcrystalline cellulose (MCC) represents a novel form of cellulose with unique physicochemical properties. It has an excellent compactibility and low chemical reactivity which makes it one of the most useful pharmaceutical excipients for direct compression tableting[2]. MCC also offered a significant opportunity for multiple uses in pharmaceutical industry, in food applications as a texturizing agent and fat replacer, and also, as

an additive in paper and composites applications[3]. MCC is listed as generally recognized as safe (GRAS) by the FDA and MCC obtained from natural sources are proven to be safe, stable and physiologically inert[4, 5]. Several approaches have been applied to prepare MCC from different sources[5-11], all of them leading to different types of microfibrillar materials. The chemical composition and physical structure of MCC depend significantly on the characteristics of the raw materials and the manufacturing conditions[12]. As a result, several grades of microcrystalline cellulose are available in the market with different physicochemical and thermal properties exhibiting different functional parameters and applications[13].

Bamboo, a naturally occurring composite material growing abundantly in tropical countries offers great potential as a green source for MCC preparation. It has been reported that the cellulose and α-cellulose content of bamboo is comparable to that of softwoods and hardwoods which are the commercial sources for MCC[14]. Moreover, compared to most wood species, bamboo is cheap and fast growing with superior physicochemical properties which makes it an ideal alternative to these woods[7].The present study reports the preparation and evaluation of MCC produced from *Dendrocalamus longispathus* bamboo, also commonly called as Rawnal bamboo, collected from Mizoram, India. *Dendrocalamus longispathus*, a large tufted bamboo, is one of the 15 commercially important species of bamboo growing in India that have been used in paper and pulp industries[15]. The present works has been aimed at exploring the potentials of making MCC from *Dendrocalamus longispathus* bamboo and systematically characterize its physicochemical and functional properties in view of its application in different industries.

2. Materials and Methods

2.1 Materials

The raw bamboo (*Dendrocalamus longispathus*) collected from Mizoram was identified and received from the Department of Forestry, Mizoram University. Microcrystalline cellulose (Avicel PH101) was procured from Sigma-Aldrich (India). All other chemical and reagents were of analytical grade and used as supplied without further purification.

2.2 Methods

2.2.1 Preparation of microcrystalline cellulose

The isolation of cellulose from the bamboo fiber was performed according to the method reported earlier by Abe and Yano[16]. The raw bamboo was cut into chips and passed through mesh 60. The sample was then dewaxed in a soxhlet apparatus with 2:1 (v/v) mixtures of toluene/ethanol for 6 hours. Lignins, hemicelluloses and residual starch and pectin were removed through sequential chemical treatment. Firstly, the dewaxed sample was treated with sodium chlorite at 70 °C for 1 hour followed by treatment at 90 °C with 2 % w/v potassium hydroxide (KOH) solution for 2 hours. It was the treated again with sodium chlorite at 70 °C for 1 hour and finally the sample was heated at 90 °C for 2 hours with 5 % w/v KOH solution. Sample was filtered and rinsed with distilled water until the residues were neutralized. Sample was the dried overnight at 50 °C in a tray drier.

To extract the α-cellulose, the dried sample was treated with 17.5 % NaOH at 80 °C for 1 hour. The sample was filtered and repeatedly washed with water to neutralize and dried in tray drier at 50 °C overnight. Microcrystalline cellulose was prepared following the method of Ejikeme[5] by treating the α-cellulose obtained with 2.5 M HCl at 105 °C for 15 minutes to penetrate and hydrolyze the amorphous regions of the cellulose. The MCC prepared were collected by filtration, washed with distilled water to neutral pH and dried at 50 °C in a tray drier overnight. The percent yield of MCC was determined and identification test was performed with Iodine solution as described in Indian Pharmacopoeia[17].

2.2.2 Average particle size

The particle size of the MCC was determined by microscopic method. The ocular micrometer was calibrated using stage micrometer and each division of the ocular micrometer was measured in micrometer. About 100 particles were counted and done in triplicate.

2.2.3 Moisture content

Moisture content was expressed as percentage weight loss on drying (% LOD). About 2 g of MCC sample was weighed and oven dried at 105°C for 5 h to a constant weight. The experiment was done in three replications and an average of the three replicates was taken. The percent loss on drying was then calculated as follows[18]:

$$\%\ Loss\ on\ drying = \frac{Weight\ of\ water\ in\ the\ sample}{Total\ weight\ of\ wet\ sample}\ x\ 100 \qquad (1)$$

2.2.4 Ash content

The ash content was determined by following the method of Yebeyen et al[19]. About 2 g of the sample was first heated on a burner in air to remove its smoke. Then it was burned in a muffle furnace at 550 °C until it becomes totally white. The ash content was expressed as a percentage ratio of the weight of the ash to the oven dry weight of the powdered MCC.

2.2.5 Swelling index

The swelling index was determined on a graduated measuring cylinder of 25 ml capacity taking water as a medium. About 1 g of the sample was taken into the measuring cylinder and water was added making the volume up to 25 ml. The cylinder was shaken every 10 minutes for 1 hour to wet the sample thoroughly. After 24 hrs, readings were taken and the swelling index was calculated as follows:

$$Swelling\ index = \frac{H_f - H_0}{H_f}\ x\ 100 \qquad (2)$$

Where H_0 is the initial height of the MCC in the graduated measuring cylinder and H_f is the height of the swollen MCC in the measuring cylinder recorded after 24 hours.

2.2.6 Angle of repose (θ)

Angle of repose (θ) was measured using a fixed height funnel fitted at the height of 10 cm from the base (The funnel is 60 °, 10 cm in diameter, 0.7 cm internal stem diameter with 9.6 cm stem length). About 20g of the dried powder was allowed to flow through the funnel into the base and a pile was formed at the base. The angle of repose was then calculated as follows:

$$Angle\ of\ repose\ (\theta) = tan^{-1}\frac{h}{r} \qquad (3)$$

Where h and r are the height and radius of the pile respectively.

2.2.7 Compressibility index

The compressibility index of the powder was determined according to Carr's index and Hausner ratio after determining bulk (ρ_{bulk}) and tap densities (ρ_{tap}). About 20 g of the dried powder was taken into 50 ml graduated measuring cylinder and the initial volume (V_0) was recorded. The cylinder was then tapped 100 times using bulk density apparatus (ACM-157, Acmus Technocracy, New Delhi) to achieve a final volume (V_f). The bulk density was calculated from the initial volume and tap density from the final volume after hundred tappings. Carr's index and Hausner ratio were then determined by the following equations[20]:

$$Carr's\ Index = \frac{\rho_{tap} - \rho_{bulk}}{\rho_{tap}}\ x\ 100 \qquad (4)$$

$$Hausner\ ratio = \frac{\rho_{tap}}{\rho_{bulk}} \qquad (5)$$

2.2.8 True density

The true density (ρ_{true}) of the powder was determined by liquid displacement method taking xylene as the immersion liquid. The true density for both the prepared microcrystalline cellulose and Avicel PH101 were determined according to the following equation[21]:

$$\rho_{true} = \frac{w}{[(a+w)-b]} \; x \; SG \qquad (6)$$

Where 'w' is the weight of the powder, 'SG' is the specific gravity of the solvent, 'a' is the total weight of the bottle and the solvent, 'b' is the total weight of the bottle, solvent and the powder.

2.2.9 Porosity

The porosity (ε) of the test powders was derived from the values of the true and bulk densities fitted into the following equation[22]:

$$\varepsilon = \left[1 - \left(\rho_{tap} / \rho_{true}\right)\right] x \; 100 \qquad (7)$$

2.2.10 FTIR spectroscopy

FTIR spectrum was recorded on Alpha FT-IR spectrophotometer (Bruker, USA). Powder samples were taken and the absorbance was recorded directly between 400 and 4000 cm^{-1} for both the prepared MCC sample and the standard Avicel PH101. The spectra thus obtained were analyzed for functional groups and compared to characterize the MCC samples.

2.2.11 Thermogravimetric analysis

TG analysis was performed on TGA (Pyris TGA, Perkin Elmer) between 40˚C - 855˚C at a heating rate of 10˚C/min while nitrogen purging was maintained at 20 ml/min. For each analysis, about 6 mg of the sample was taken into the aluminium sample pan and sealed. Empty aluminium pan was used as a reference and the thermogram was then recorded for the prepared R-MCC as well as the Avicel PH101.

2.2.12 XRD studies

XRD patterns were recorded on Philips analytical X-ray diffractometer (Philips Co., Netherland). The data were collected at 2θ between 5° and 45°. The degree of crystallinity was calculated and expressed as percentage crystallinity index using the equation[8]:

$$Crystallinity\ Index\ (\%) = \frac{I_{002} - I_{am}}{I_{002}} \; x \; 100 \qquad (8)$$

Where I_{002} is the counter reading at peak intensity at 2θ angle close to 22° representing the crystalline part and I_{am} is the counter reading at peak intensity at 2θ close to 18° representing the amorphous part of the cellulose.

2.2.13 Scanning electron microscopy

The shape and surface characteristics of the MCC was analyzed by scanning electron microscopy (JEOL JSM-6300, Japan). Samples were mounted on the aluminium stub and photomicrographs of the powders were taken after sputter coating with a thin layer of gold. The quality of the MCC with respect to surface properties was studied.

2.3 Statistical analysis

Statistical analysis was performed using computer software SigmaStat 2.03 (SPSS, USA). One-way analysis of variance followed by Tukey Test was performed to compare the properties of the two MCC samples.

Table I Physicochemical properties of Avicel PH101 and R-MCC

Powder property	Avicel PH101	R-MCC
Moisture content (%LOD)	4.22 ±0.44	4.87±0.54
Swelling index	24.00	25.00
Ash (%)	0.12	0.15
Average particle size (µm ± SD)	69.11±24.29	108.45±45.05
Bulk density (g/ml)	0.3941	0.3788
Tap density (g/ml)	0.5167	0.5271
True density (g/ml)	1.474	1.57
Porosity (%)	66.9	68.22
Carr's Index	23.73	28.14
Hausner ratio	1.311	1.39
Angle of repose	31.61	48.45
Crystallinity index	72	72.5

Table II Cellulose fractions yield of R-MCC in comparison to other sources

Source	Cellulose	α-Cellulose	MCC
Rawnal bamboo (*Dendrocalamus longispathus*)	65.42	55.63	51.18
Cotton (*Cochlospermum planchonii*)[21]	-	32	21
Orange mesocarp[5]	-	62.5	25.3
Moso bamboo[23]	-	41.8	-
Wood[23]	-	46.4	-
Wheat straw[23]	-	39.8	-
Jute[9]	59.8	97.8% of cellulose	48-52.8

3. Results and discussion

3.1 Physicochemical properties

The physicochemical properties of the MCC prepared from *Dendrocalamus longispathus* (R-MCC) and the standard Avicel PH101 are summarized in Table 1. A white or slightly yellowish white MCC powder was obtained which give a blue-purple coloration on identification test performed confirming the identity of the MCC. The yield of cellulose and α-cellulose was about 65.42 % and 55.63 % respectively from the original raw material as given in Table 2. The yield of R-MCC from the obtained α-cellulose was about 92 % which is about 51.18 % from the original material. The α-cellulose content of 55.63 % from the original Rawnal (*Dendrocalamus longispathus*) bamboo fiber is higher than 46.4, 41.8 and 39.8 % reported for wood, Moso bamboo and wheat straw fibers respectively[23]. The final MCC yield of 51.18 % for R-MCC was also higher than the reported MCC yield of 25.3 % for orange mesocarp[5], 21 % for raw cotton[21] and similar to 48-52.8 % reported for Jute[9].

An excess of water in plant materials encourage microbial growth and deterioration by hydrolysis. When the moisture content of MCC exceeds 5 %, water molecules act as plasticizer affecting the mechanical properties of the MCC which results in lower tensile strength of MCC tablets[24]. Pharmacopoeial limit of water content for MCC has been set at 6 %[17]. The percent LOD determined on the R-MCC and Avicel PH101 was found to be 4.87±0.54 % and 4.22 ±0.44 % respectively. These findings indicate that the moisture content of both the samples is within the suggested pharmacopoeial limit. Both the standard Avicel PH101 and R-MCC swells on contact with media when the swelling index was determined on water. R-MCC was found to show slightly higher swelling index than the standard Avicel. Determination of swelling property is important particularly for MCC in view of its application in pharmaceutical industries as its rapid disintegration property

is mainly attributed to the swelling of MCC particles in aqueous media resulting in the destruction of bonding forces holding them together.

The total ash content determination measured the total amount of materials remaining after ignition which includes both the physiological ash from the plant tissue and non-physiological ash which is a residue of extraneous matter such as sand and soil adhered to the sample. The low percentage of total ash in both the samples may be due to the low inorganic material contents of cellulosic derivatives and the level of care taken during the preparation of the MCC[5]. Both the samples were found to be within the pharmacopoeial limit of 0.2 % for MCC.

Micromeritic properties of MCC such as particles density, angle of repose, average particle size and powder flow characteristics are important indicator for functionalities of the MCC products. The flow property of the MCC powder is essential in determining the suitability of its use in pharmaceutical products especially as a direct compression excipient. The bulk density of the R-MCC was found to be less than the standard Avicel PH101 and there was statistically significant difference in bulk density of the two samples ($p=0.0396$). However, bulk density determined for both the standard and R-MCC was found to be comparable with the values reported for MCC obtained from other sources[5, 21]. A higher bulk density would result in a lower loading volume during tablet manufacturing. The average particle size as determined by microscopic method was found to be 69.11±24.29 µm and 108.26±30.42 µm for Avicel and R-MCC respectively. There was statistically significant differences in the average particle size ($p<0.01$) between the R-MCC and Avicel PH101. Small particle size and low moisture content results in higher bulk density[25]. Therefore, the smaller average particle size and lower moisture content of the Avicel may be the reason for its higher bulk density. There was no significant difference in the true density of R-MCC with the standard Avicel PH101 ($p=0.4932$). It was reported earlier that there is a direct correlation between the true density of the particles and the degree of crystallinity[5]. Results from true density determination suggest that the degree of crystallinity for both the samples would be very close.

Compressibility index, Hausner ratio and angle of repose provides information on the powder flowability and compactibility for which, a small values indicate better flowability[24]. The compressibility and Hausner indices were determined from the tap and bulk densities. Compressibility index of less than 15 % and angle of repose less than 35 ° are indicative of very freely flowing powders[5]. Avicel was found to exhibit smaller compressibility index and angle of repose indicating and confirming its better flow property. During tableting, enhancement of flowability of the powders or granules would decrease the weight variation of tablets resulting in the production of uniform tablets.

3.2 Scanning electron micrographs (SEM)

The morphology of R-MCC powder after acid hydrolysis was studied through scanning electron micrograph and compared to the Avicel PH101. As shown in Fig. 1, the particles size distribution of R-MCC fibers is uneven and rod shaped compared to the more spherical Avicel PH101 particles. Narrow channels were formed on the surface of the R-MCC particles and both the R-MCC and Avicel PH101 were shown to exhibit rough surface. The Avicel PH101 was found to have much more uniform particle sizes than the prepared R-MCC. The obvious morphological difference of R-MCC and Avicel PH101 was probably due to difference in the source of raw materials and difference in the method of MCC preparation. Previous studies also reported that cellulose obtained from different sources and hydrolysis conditions differ in overall characteristics of MCC such as particle size and aggregation[1].

3.3 FTIR spectroscopy

FTIR spectroscopy is a powerful tool for studying the physicochemical and conformational properties of polysaccharides. FTIR spectra for both the samples were recorded for analysis of functional groups present in the structure. Absorption at 1430, 1158, 1109, 1025, 1000 and 970 cm^{-1} is a typical absorption peak of cellulose[7]. These peaks are observed in the spectra of both the MCC samples as depicted in Fig. 2. Peaks at 3200 – 3450 cm^{-1} are assigned to stretching of –H bond of –OH group. This –OH stretching peaks are observed at 3275 cm^{-1} for both the Avicel and R-MCC samples. Peak at 2887 cm^{-1} for Avicel and 2884 cm^{-1} for R-MCC is attributed to the C–H stretching. Peak due –C–O– stretching of the carboxyl and acetyl groups in

hemicelluloses lignocelluloses fibres was not observed in both the Avicel and the R-MCC spectrum indicating the purity of the samples. A small peak appeared at 1638 cm^{-1} and 1636 cm^{-1}in Avicel and R-MCC respectively due to the adsorbed water. The bands at 1424 and 1363 cm^{-1} in Avicel spectrum and 1420 and 1366 cm^{-1} in R-MCC spectrum were attributed to the asymmetric $–CH_2$ bending and wagging. Peak associated to the –C–O–C– stretch of the β-1,4- glycosidic linkage in cellulose was observed at 1154 and 1157 cm^{-1} for Avicel and R-MCC respectively. This band has a lower intensity in R-MCC spectrum which could be due to the presence of trace amounts of non-cellulosic constituents in R-MCC[8]. The absorption peak at 892 cm^{-1} is assigned to –C–O asymmetric stretching. The weak shoulder at around 750 cm^{-1} was assigned to the I_β (monocyclic) cellulose and the absorption peak at 699 cm^{-1} was also assigned to the presence of I_α (tricyclic) cellulose.

Fig. 1 Scanning electron micrographs of (A1& A2) Avicel PH101 and (B1 & B2) R-MCC

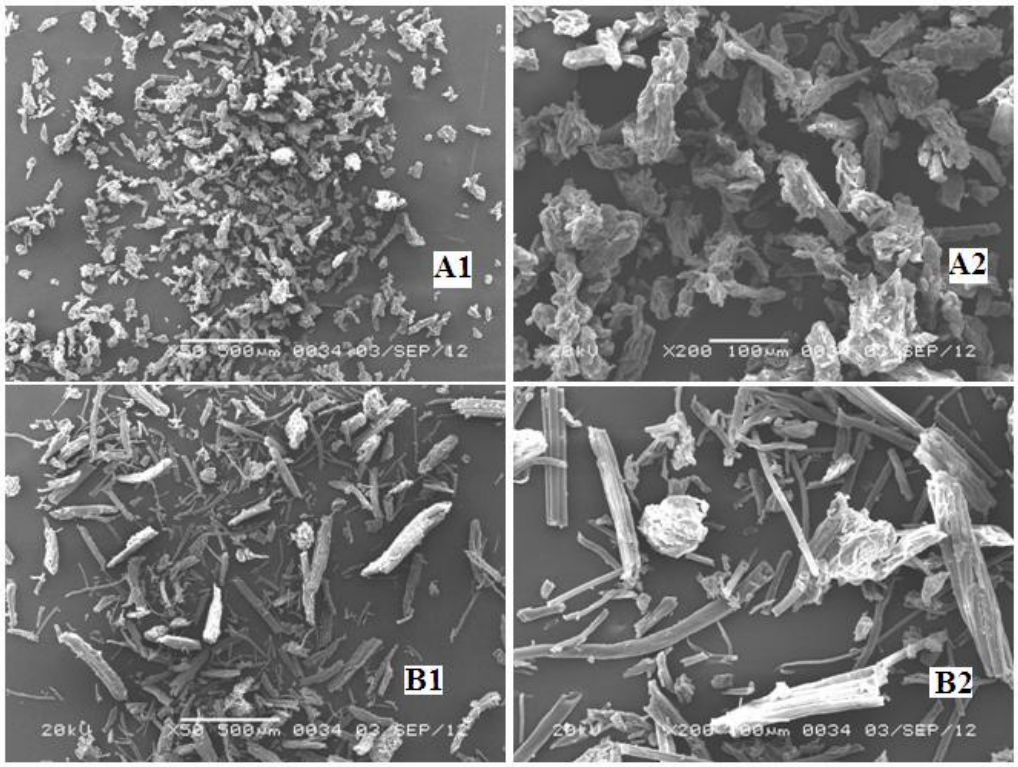
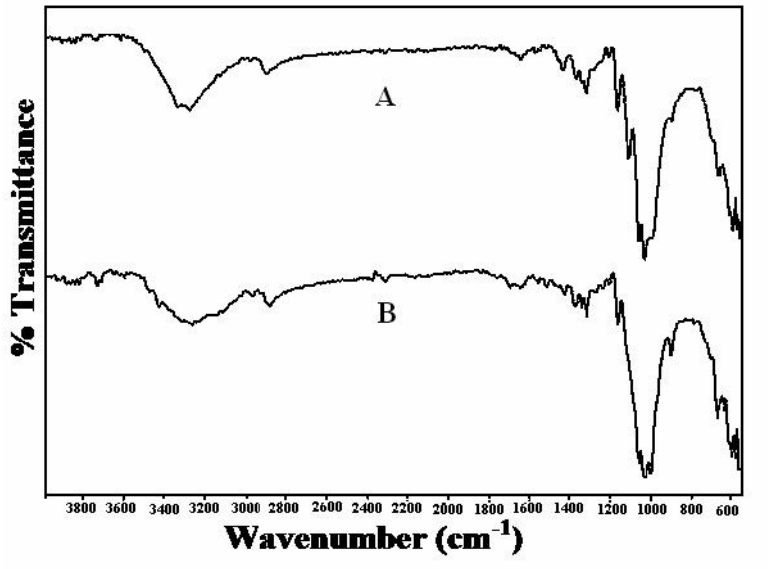


Fig. 2 FTIR spectrum of (A) Avicel PH101 and (B) R-MCC



3.4 Thermogravimetric analysis (TGA)

Thermal stability of cellulose is critical for many applications including the use of MCC for the production of bio-composites where the processing temperature may rise above 200 °C[9]. Thermogravimetry was performed to investigate the thermal stability of the prepared R-MCC and compared it to the Avicel PH101. Analysis of TG curve (Fig. 3) for R-MCC fiber showed a first thermal event occurring between 0-96.89 °C during which about 4.411 % weight loss was observed. This event can be attributed to the evaporation of water and results from moisture content determination also supported this observation. The first thermal event for evaporation of water was also observed for Avicel PH101sample between 0-90.83 °C where there was about 3.479 % weight loss. Thermal weight loss occurring in the range of 200-400 °C was attributed to depolymerization of cellulose[26]. This thermal depolymerization of cellulose event for R-MCC was observed between 260.11 – 380.43 °C during which about 70 % weight loss took place. The second thermal event for Avicel occurred at 284.67 – 386.00 °C resulting in 83.450 % weight loss. At 400 °C almost all the cellulose was pyrolyzed and the solid residuals were about 15 % and 8 % respectively for R-MCC and Avicel. According to earlier reports, natural bamboo fiber has maximum thermal degradation at about 365.1 °C and regenerated bamboo fiber showed maximum at about 339.9 °C[27]. This higher thermal stability of the natural bamboo fiber than reinforced or mercerized bamboo fiber is probably due to the removal of lignin during material preparation such as in preparation of MCC. The maximum thermal degradation for the sample was observed at 320.27 °C and 335.34 °C for R-MCC and Avicel respectively indicating the slightly higher thermal stability of the Avicel PH101.

Fig. 3 TGA curves of (A) Avicel PH101 and (B) R-MCC

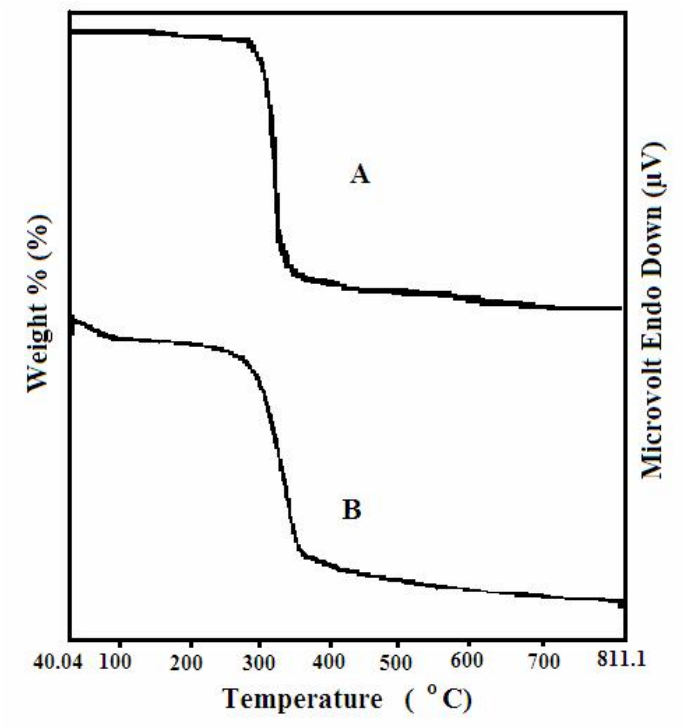
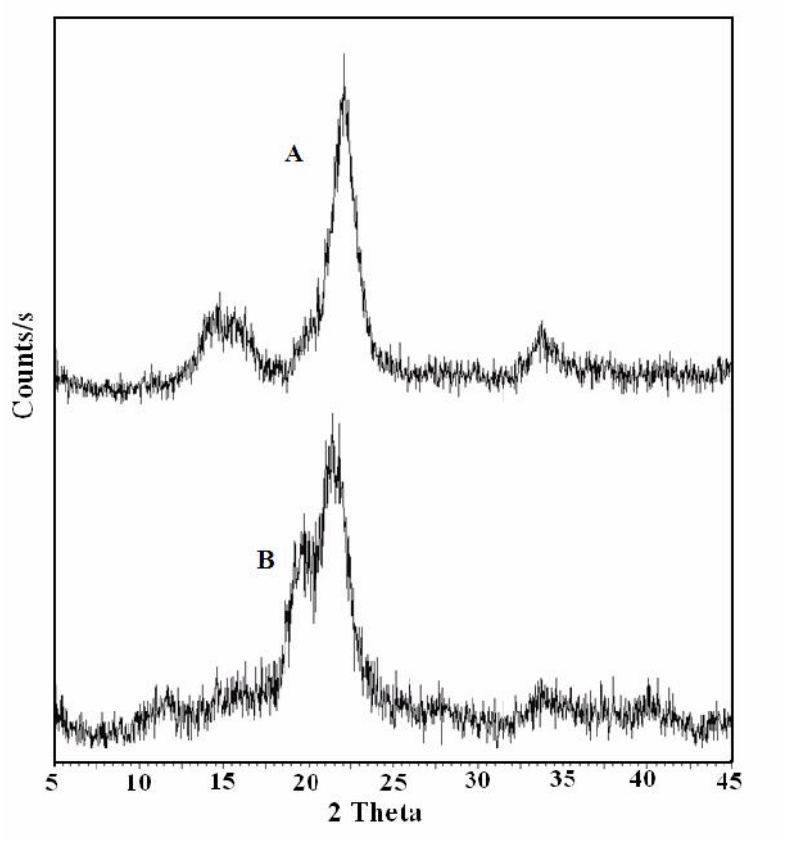
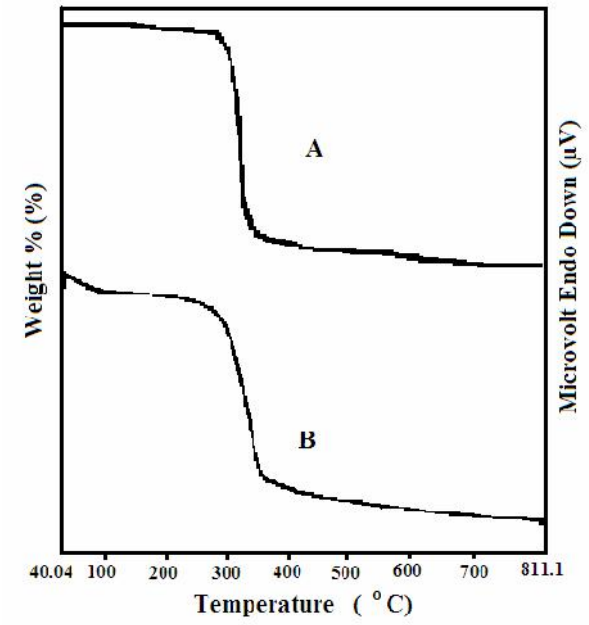


Fig. 4 X-ray diffraction profiles of (A) Avicel PH101 and (B) R-MCC





3.5 XRD study

The powder X-ray diffraction patterns for both the R-MCC and the standard Avicel PH101 are shown in **Fig. 4.** The two materials were found to exhibit slightly different X-ray diffraction patterns. Cellulose I, represented by native cellulose showed typical peaks at 15, 16 and 23°2θ due to $1\bar{1}0$, 110 and 002 reflections respectively, while Cellulose II types represented by mercerized and regenerated cellulose exhibited typical

peaks at about 12, 20 and 22°2θ due to $1\bar{1}0$, 110 and 002 reflections respectively[22]. The diffraction spectrum of Avicel PH101 showed typical cellulose I diffractogram peaks at 15, 16 and 23°2θ assigned to $1\bar{1}0$, 110 and 002 reflections respectively which is usually a characteristic of wood cellulose. However, the diffraction spectrum of R-MCC exhibited diffraction patterns typical of both cellulose I and cellulose II. The diffraction peaks for R-MCC were observed at 12, 15, 16, 20 and 23° 2θ which indicates that the MCC sample obtained from *Dendrocalamus longispathus* bamboo fibers is made up of both cellulose I and cellulose II polymorphs. This diffraction pattern obtained for the R-MCC sample can be attributed to the mercerization process carried out on the α-cellulose fibers with a strong sodium hydroxide solution. It was reported earlier that the lattice transition from cellulose I to cellulose II sets in at above 10 % sodium hydroxide, but not completed below 15 % of the alkali solution[13]. In contrast to cellulose I, which has a parallel up arrangement, the chains in cellulose II are in an antiparallel arrangement yielding a more stable structure preferable for various textiles and paper materials[3]. The diffraction pattern obtained with the R-MCC sample was found to be consistent with earlier reports on the effects of mercerization on the cellulose fibers as mercerization was carried out at 17.5 % sodium hydroxide in the present study which led to the presence of both cellulose I and cellulose II in the final product.

The degree of crystallinity calculated for both the R-MCC and Avicel PH101 shoed almost similar value of 72.5 % and 72 % respectively. The degree of crystallinity obtained for the Avicel PH101 was also found to be in agreement with the values reported in earlier studies[10, 22]. The high degree of crystallinity obtained for both the samples indicate an ordered, compact molecular structure which translates into a dense properties, while a low crystallinity would implies a more disordered structure representative of a more amorphous form[13]. As indicated earlier, there is a direct correlation between the true density and the degree of crystallinity of the sample. Therefore, result obtained from X-ray diffraction study and the degree of crystallinity calculated supported the true density values obtained forthe samples.

4. Conclusion

MCC was produced successfully from the Rawnal (*Dendrocalamus longispathus*) bamboo fibers. The R-MCC product conformed to the official specifications in the Indian Pharmacopoeia[17]. Compared to the standard Avicel PH101 there was statistically significant differences in micromeritic properties of the powder which could be attributed to the differences in the source of material and method of preparation. However, considering the high yield of α-cellulose and the availability of the bamboo, the Rawnal bamboo (*Dendrocalamus longispathus*) could be a potential green source of MCC.

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