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Synthesis and characterization of microcrystalline cellulose produced from bacterial cellulose

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Abstract In this study, microcrystalline cellulose (MCC) was prepared from the acid hydrolysis of bacterial cellulose (BC) produced in culture medium of static *Acetobacter xylinum*. The MCC-BC produced an average particle size between 70 and 90 μm and a degree of polymerization (*DP*) of 250. The characterization of samples was performed by thermogravimetric analysis, X-ray diffraction, and scanning electron microscopy (SEM). The MCC shows a lower thermal stability than the pristine cellulose, which was expected due to the decrease in the *DP* during the hydrolysis process. In addition, from X-ray diffractograms, we observed a change in the crystalline structure. The images of SEM for the BC and MCC show clear differences with modifications of BC fiber structure and production of particles with characteristics similar to commercial MCC.

Keywords Bacterial cellulose · Microcrystalline cellulose · *Acetobacter xylinum* · Acid hydrolysis

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Introduction

Cellulose is one of the most abundant organic substances on the planet, being the main constituent of vegetable fiber. Cellulose is a linear hydrophilic homopolysaccharide and this structure consists of units of β-D-glucopyranose (β-glucose) linked by glycosidic type β-(1 → 4) (Fig. 1). However, cellulose is not found only in plant organisms. In addition to plants, different microorganisms are capable of producing cellulose as *Vallonia*, *Disctystelium slime mold* (fungi), *Agrobacterium*, *Aerobacter*, *Pseudomonas*, and *Sarcina*. However, *Acetobacter xylinum* (*Glucanacetobacter xylinus*) is the only known species capable of producing cellulose in commercial quantities and is therefore extensively studied [1–5].

Cellulose, due to its high availability and outstanding chemical and physical properties, is a polymer widely used in a variety of commercial applications in the food, textile, and pharmaceutical industries. For these and other applications, cellulose is obtained from mechanical and chemical (pulping) processing of woods to remove lignin and hemicelluloses, in this case, a pulp is obtained commercially as a percentage of α-cellulose ranging between 96 and 98%. The bacterial cellulose (BC) obtained from *A. xylinum* is pure with about 99% cellulose without the need to perform steps of delignification and bleaching. The material in this condition can be employed in the production of cellulosic derivatives; since for production of these derivatives, cellulose must have the highest purity possible [6].

The production of cellulose derivatives significantly broadens the applications of cellulose by modifying the polymer chemically in terms of hydrophobicity, processability, and solubility. For application as excipient in pharmaceutical industry, an important aspect is the

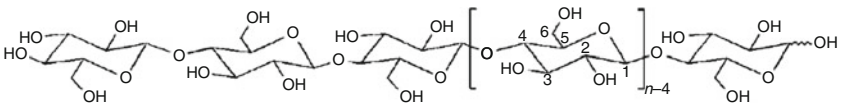

Fig. 1 Molecular structure of cellulose with degree of polymerization (*DP*) n

improvement in processability of cellulose; in this sense, the most important cellulose derivative is microcrystalline cellulose (MCC). MCC, introduced in the early 1960s, is regarded as the best excipient for direct compression tableting [7]. The MCC is typically produced from wood pulp and cotton linters; recently, new lignocellulosic sources have been used in the production of MCC. However, all these sources have in common the need for removal of lignin and hemicellulose for subsequent production of MCC.

In this study, we propose the use of BC produced by *G. xylinus* as a source for production of MCC due to its high purity, which excludes the need of carrying out the delignification and bleaching steps necessary for lignocellulosic materials. The MCC is obtained on an industrial scale by hydrolysis of cellulose using dilute mineral acids. Once the cellulose obtained from different sources differs in properties (crystallinity, moisture, surface and pore structure, and molecular mass), different properties of MCC are expected. Considering this aspect, in this study, MCC produced from BC was compared with vegetal MCC and commercial MCC. The samples were characterized by degree of polymerization (*DP*) obtained by technique of viscosity, via changes in the crystallinity degree and the pattern of X-ray diffraction (XRD) (Wide Angle X-ray Diffraction—WAXD) and evaluated in terms of change in the thermal stability of samples followed by thermogravimetric analysis (TG). The modification of fiber structure was accompanied by Scanning electron microscopy (SEM).

The degree of polymerization (*DP*) of cellulose samples was determined by viscometry using an Ostwald viscometer according to Brazilian technical standard NBR7730 [8].

To achieve the synthesis of MCC, mineral acids (HCl, H_2SO_4, and HNO_3) are commonly used [9]. In this study, the cellulose fibers (BC and Vegetal Cellulose) were hydrolyzed in 2.5 mol L^{-1} of H_2SO_4 at a reflux system under constant stirring for 120 min according to Paralikar et al. [10]. The hydrolyzed pulp was thoroughly washed with distilled water until pH 7.0, and was wetted with ethanol and dried in an oven at 37 °C until constant mass.

X-ray powder diffraction studies

Diffraction patterns were obtained using a Kristalloflex Simens X-ray diffractometer. The diffraction patterns were recorded using Cu-Kα radiation with *Ni* filter from 4° to 70°. The samples were pressed into pellets (25 mm in diameter) by compression of 0.25 g. These diffraction patterns were intended to identify structural changes in crystalline and amorphous phases after processing the cellulose in the derivative studied (MCC). The separation of crystalline peaks and amorphous halo was performed by deconvolution using the function Pseudo—Voigt 1, whose equation is described below:

$$y = y_0 + A\left[m_u \frac{2}{\pi} \frac{w_L}{4(x - x_c)^2 + w_L^2} + (1 - m_u)\frac{\sqrt{4\,\mathrm{In}2}}{\sqrt{\pi} w_G}\left(-4\,\mathrm{In}\,2/w_G^2\right)(x - x_c)^2\right] \quad (1)$$

Experimental

MCC preparation

Bacterial cellulose membranes were supplied by Fibrocel Produtos Biotecnológicos LTDA, Ibiporã, Brazil. Vegetal cellulose (VC) sheets produced from bleached Kraft Eucalyptus pulp were supplied by Bahia Pulp, and commercial MCC was supplied by Merck (cellulose microcrystalline for thin-layer chromatography).

Since W_L and W_G are the widths at half height for the components of the Gaussian and Lorentz equation, A is the area and m_u is the form factor. The crystallinity index can be evaluated from the Eq. 2:

$$x_c(\%) = \frac{A_c}{A_c + A_A} \times 100 \quad (2)$$

where A_C and A_A are the areas of crystalline peaks and amorphous halo, respectively.

Scanning electron microscopy

Scanning electron microscopy was performed using a Top Com 600 system. The samples were coated with a carbon layer with a thickness of 15 nm.

Thermogravimetric analysis (TG)

A SDT (TA Instruments) Thermogravimetric analyzer was used to study the thermal stability of the different MCC samples. The heating rate was set at 10 °C min^{-1} over a temperature range of 10–900 °C. Measurements were carried out in nitrogen atmosphere, with a rate of flow of 50 cm^3 min^{-1}.

Results and discussion

Degree of polymerization

The hydrolysis of BC and the VC to prepare MCC was carried out using 2.5 mol L^{-1} of sulfuric acid. Viscometry experiments showed that these pulp samples reached a lower degree of polymerization after their reflux with acid during 120 min. It is known that lowering the degree of polymerization is due to degradation of the glycosidic chains of cellulose. Table 1 shows the *DP* of the MCC prepared from the BC and VC pulps. The results are compared to a commercial Avicell-MCC.

The chain length of cellulose, expressed in numbers of constituents of anhydrousglucose, with *DP*, varies with the source and type of treatment of raw material. In the case of cellulose from wood pulp, the values are generally 300–1700. In the case of cotton and other fibrous plants the values of *DP* are in the range 800–1500. For the BC, the *DP* obtained from viscometry was 2800, which equates to an average molecular mass of 453,600 g mol^{-1}. The MCC obtained commercially (Avicell) presents values of *DP* between 150 and 300 [11]. For the MCC produced in this study, it was obtained a value of degree of polymerization of 250 for the pulp produced with BC, 170 for the MCC produced with plant cellulose, and for the commercial MCC, it was obtained a value of degree of polymerization of 225. These results are within the range reported in the literature.

Table 1 Degree of polymerization obtained from viscosity measurements

Samples	Degree of polymerization
BC	2800
VC	1300
MCC-BC	230
MCC-VC	170
MCC-Avicell	225

X-ray diffraction studies

X-ray diffraction is a useful technique to follow the change in cellulose crystallinity as the sequence of reactions progressed [12]. The XRD pattern of BC, VC, and MCC samples is shown in Fig. 2. The calculated crystallinity indexes of the different samples are given in Table 2. As shown in Fig. 2, all samples of MCC have a typical crystal lattice for cellulose I [13]. Also, all samples had similar *CrI* values with slightly higher values for MCC derived from vegetal cellulose. One possible explanation for this may be related to the morphological differences between VC and BC. Bacterial cellulose has a higher purity and degree of polymerization than vegetal cellulose, moreover, the fibers are arranged to form a hollow space and network structure like a membrane. This allows the BC to absorb large amounts of water when ground and exposed to acidic or basic aqueous solutions. In plants, cellulose is organized in a cellular hierarchical structure linked to other components such as hemicellulose, lignin, and pectin. After delignification and bleaching, cellulose pulp absorbs less water than the BC. These structural differences significantly influence the accessibility and reactivity of cellulose, as well as the final properties of MCC produced. This fact can be observed in the patterns of XRD and degree of crystallinity of the samples of bacterial MCC and BC. The reduction of crystallinity of MCC-BC after the hydrolysis can be explained due to the extensive swelling during hydrolysis possibly leading to disruption of crystalline regions, with the increase of the amorphous sample in comparison with BC and the samples produced from plant cellulose.

The XRD of BC shows three diffraction peaks at $2\theta = 15°$, 17°, and 22.5°, which are attributed to the interplanar distance characteristic of the phases $I\alpha$ and $I\beta$ of the elementary cellulose crystalline structure ($100_{I\alpha}$, $110_{I\beta}$, and $010_{I\beta}$ refer to the angle 15° and $110_{I\alpha}$ and $200_{I\beta}$ to 22.5°) [14]. The hydrolysis modifies the pattern of X-rays with change of relative intensity of the peaks and the appearance of a shoulder around 2θ equals to 21.50. The etching occurs preferentially in the amorphous regions of cellulose. However, changes in the profile of MCC diffractograms indicate change in crystalline structure with a possible contribution of the polymorphic form of cellulose II.

Scanning electron microscopy (SEM)

Figure 3 shows SEM micrographs of the different samples. Through the images we observed that BC (Fig. 3a) presents a three-dimensional structure formed by nanometric fibers.

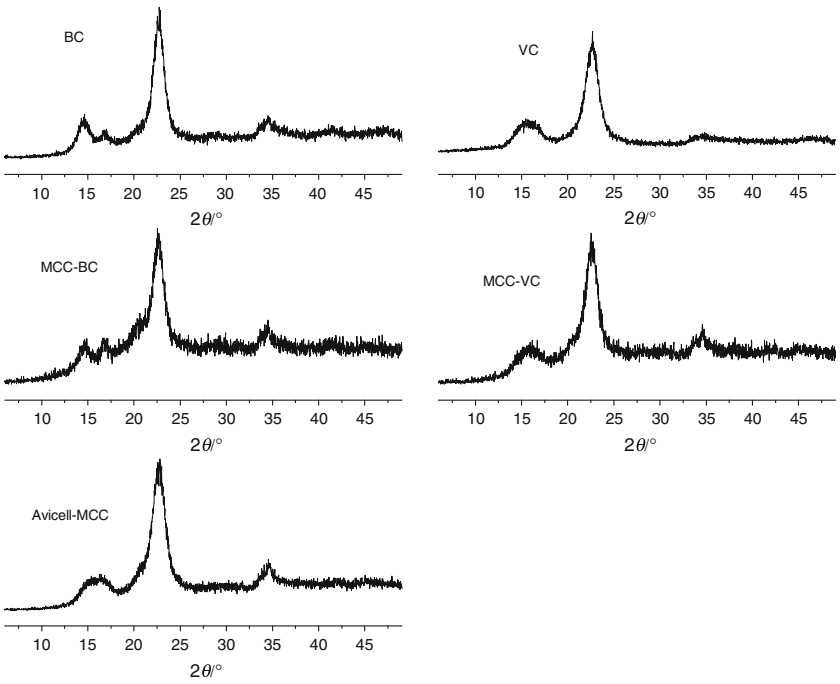

Fig. 2 X-ray diffraction patterns of BC, VC, MCC-BC, MCC-VC, and Avicell-MCC

Table 2 Crystallinity index (*CrI*) of all samples

Samples	*CrI*/%
BC	76
VC	79
MCC-BC	69
MCC-VC	88
MCC-Avicell	72

Figure 3b shows the structure of the vegetal cellulose, with thicker fibers than those seen in BC.

According to the images of the samples of MCC, it can be inferred that, by acid hydrolysis, cellulose particles were obtained, similar in appearance and size to those observed for commercial MCC (Fig. 3c) and produced from other sources such as lignocellulosic materials [15]. The images also show that the particles formed are smaller than 30 μm, and also that for the MCC obtained from BC showed more uniformity compared with other samples.

Thermogravimetric analysis

The thermal behaviors of BC and plant cellulose have been reported [16, 17].Thermal stability of the different MCC samples was studied using TG. Figure 4 shows the TG curves of these samples and the Table 3 gives the data obtained from these curves.

The TG curves for all the samples show two stages of mass loss within the temperature range 25–600 °C. The first degradation stage for the samples occurs at a temperature range 25–100 approximately. This process is assigned to water evolution. For BC and VC (Fig. 4a, b), between 270 and 398 °C, we observe a mass loss due to degradation processes of cellulose, such as depolymerization, dehydration, and decomposition of glycosyl units followed by the formation of a charred residue [18]. The same event is observed for the MCC commercial and MCC prepared from BC (MCC-BC) and cellulose plants MCC (VC). For MCC (Fig. 4c (MCC-BC), d (MCC-VC)), we observed a decrease in decomposition temperature compared to original celluloses and commercial MCC. This difference can be attributed to several aspects like the difference between the sources, considering the use of bacterial and plant cellulose and processing differences, since the process for obtaining the commercial MCC is not known in all its stages [19].

The difference between thermal stability of some cellulosic materials showed in Fig. 4 and Table 3 can be attributed to crystallinity, moisture content, the porous structure, and the polymerization degree of materials before and after chemical and/or mechanical treatment. Roman and Winter [20] showed that for samples hydrolyzed with sulfuric acid, the increase of sulfate ions concentration leads to a decrease in degradation temperature of cellulose prepared. The presence of these ions can influence the thermal stability of MCC in different ways according to the porosity of the structure and the ability to absorb water and promote the swelling of bacterial and plant cellulose. In this case, the decrease in thermal stability of MCC-BC in relation to BC can be explained in terms of reduced structural order during the hydrolysis due to swelling of the cellulose, caused by

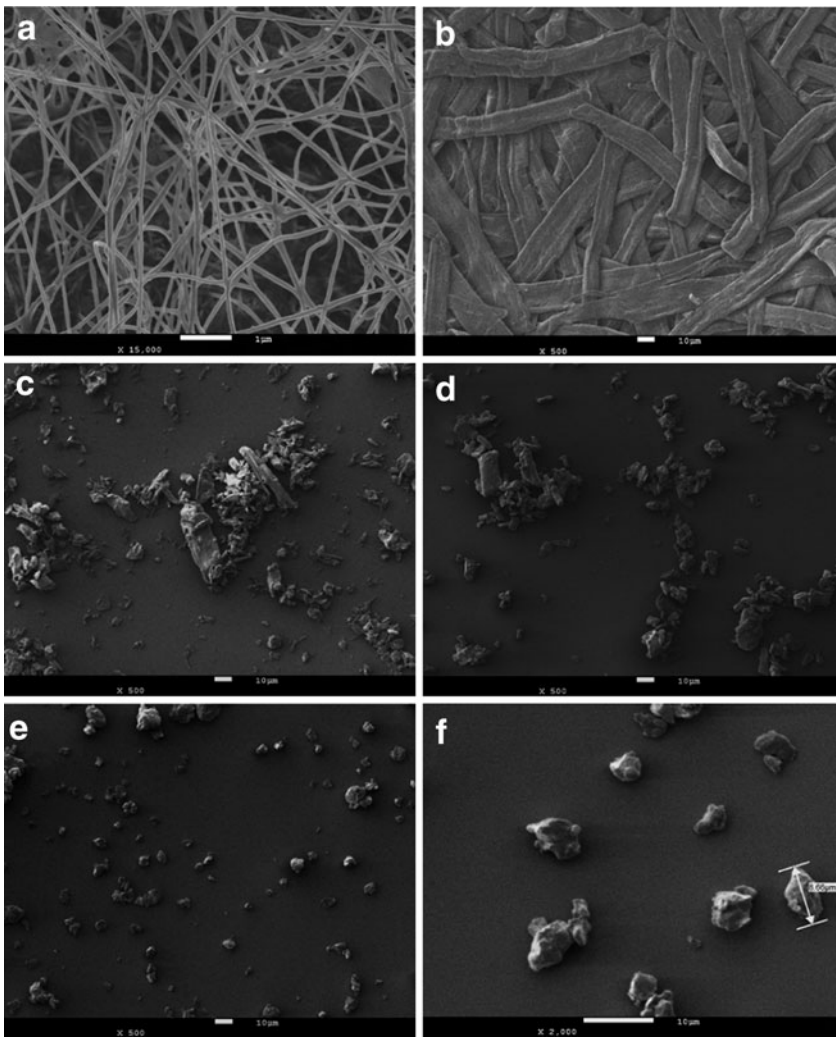

Fig. 3 SEM graphs of BC (**a**), VC (**b**), MCC-Avicell (**c**), MCC-VC (**d**), and MCC-BC (**e** and **f**)

sulfuric acid solution, a good swelling agent for cellulose. This swelling can change crystalline regions on cellulose and increase amorphous fraction. Because of increased accessibility in this case, the degradation temperature of MCC-BC was decreased. The reduction of crystallinity can be confirmed by the results obtained from analysis of X-ray patterns of the samples. For the sample of vegetal MCC (MCC-VC), a decrease in the polymerization degree is one of the aspects that reduce its thermal stability catalyzed by the presence of sulfate ions leading to degradation temperatures of MCC-VC to a value lower than that found for MCC-BC and commercial MCC.

A more detailed observation of the samples TGS MCC-BC (Fig. 4c) and MCC-VC (Fig. 4d) shows a decrease in the slope of the event which occurs between 200 and 450 °C, moreover, the peak in the curves DTG has a shoulder. These aspects are indicative of the presence of a heterogeneous structure [21], which could lead to production of fractions of material with different crystallinities and polymerization degree. In this case, the thermal stability of MCC also depends on the heterogeneity of the sample produced. This fact is not unusual, since the acid hydrolysis proceeds in heterogeneous phase. The existence of heterogeneity can generate fractions of material with greater or lesser resistance to hydrolysis, in this case, the fraction of material less resistant to hydrolysis is responsible for the decrease in thermal stability. The hydrolysis time is one of the essential parameters in reducing the heterogeneity of the samples produced. This is one of the key parameters that have been optimized and will be presented in future study.

In this first study, we are concerned to show the feasibility of producing MCC from a cellulose source was little explored in the production of cellulose derivatives. The results show that the material produced has the characteristics of MCC. The system shows an ivory-white aspect when a viscous suspension is produced; this aspect has been reported in the literature [22].

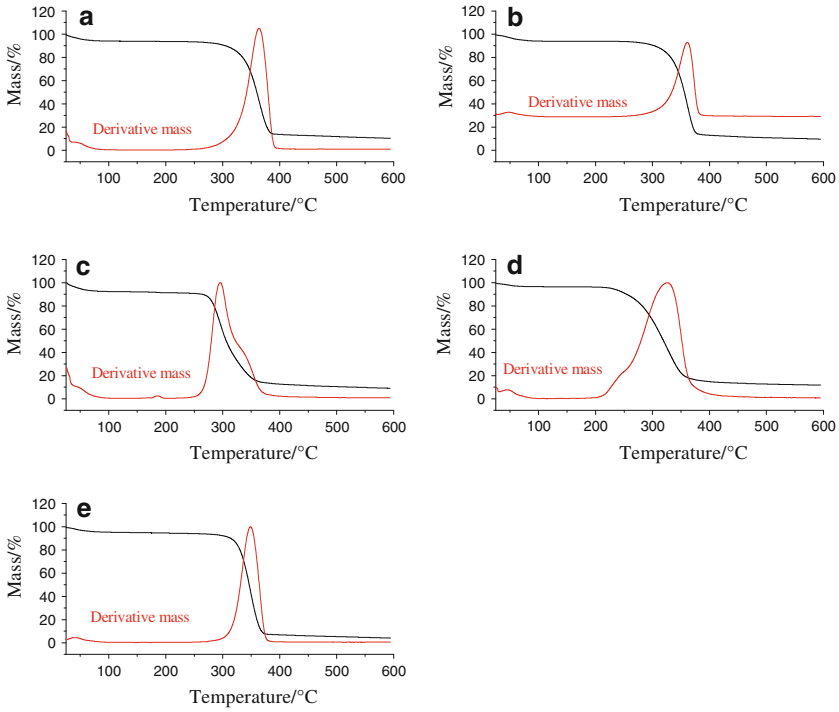

Fig. 4 TG curves and differential TG curves of BC (**a**), VC (**b**), MCC-BC (**c**), MCC-VC (**d**), and MCC-Avicell (**e**)

Table 3 TG data obtained for cellulose and MCC samples

Samples	Onset stage degradation temperature/°C	Maximum stage mass-loss temperature/°C
BC	278	363
VC	255	360
MCC-BC	250	295
MCC-VC	206	326
MCC-Avicell	280	350

Conclusions

In this study, we report the viability of producing MCC from the BC from the acid hydrolysis using sulfuric acid. The material was produced as a powder with a *DP* 250 within the expected range for a commercial MCC. The standard X-ray indicates preferential attack of the amorphous regions with small changes in crystal structure that can be attributed to a contribution of cellulose II.

References

1. Klemm D, Heublein B, Fink HP, Bohn A. Cellulose: fascinating biopolymer and sustainable raw material. Angew Chem Int Ed. 2005;44:3358.
2. Barud HS, Ribeiro CA, Crespi MS, Martines MAU, Dexpert GHYS, Marques J, Rodrigo FC, Messaddeq Y, Ribeiro SJL. Thermal characterization of bacterial cellulose—phosphate membranes. J Therm Anal Calorim. 2007;87:815–8.
3. Sjöström E. Wood chemistry—fundamental and applications. San Diego: Academic Press; 1981. p. 52–5.
4. Iguchi M, Yamanaka S, Budhiono A. Bacterial cellulose—a masterpiece of nature's arts. J Mater Sci. 2000;35(2):261–70.
5. Jonas R, Farah LF. Production and application of microbial cellulose. Polym Degrad Stab. 1998;59:101.
6. Bielecki S, Krystynowicz A, Turkiewicz M, Kalinowska H. Bacterial cellulose. In: Steinbuchel A, editor. Biotechnology of biopolymers, from synthesis to patents, vol. 14. Heidelberg: Wiley; 2005. p. 381.
7. El-Sakhawy M, Hassan ML. Physical and mechanical properties of microcrystalline cellulose prepared from agricultural residues. Carbohydr Polym. 2007;67:1–10.
8. NBR 7730.Pulp—determination of viscosity in cupriethylenediamine solution (CUEN) using capillary viscometer. 1998.
9. Bolhuis GH, Chawhan ZT. Materials for direct compaction. In: Alderbon G, Nyström C, editors. Pharmaceutical powder compaction technology. New York: Marcel Dekker; 1996. p. 419–501.
10. Paralikar KM, Aravindanath S. Crystallization of cellulose. J Appl Polym Sci. 1988;35(8):2085–9.
11. Muñoz-Ruiz A, Antequera VV, Parales CM, Ballesteros RJC. Tabletting properties of new granular microcrystalline celluloses. Eur J Pharm Biopharm. 1994;40(1):36–40.
12. Filho ECS, Santana SAA, Melo JCP, Oliveira FJVE, Airoldi C. X-ray diffraction and thermogravimetry data of cellulose, chlorodeoxycellulose and aminodeoxycellulose. J Therm Anal Calorim. 2009;100:315–21.
13. Nelson ML, O'Connor RT. Relation of certain Infrared bands to cellulose crystallinity and crystal lattice type. Part II. A new infrared ratio for estimation of crystallinity in cellulose I and II. J Appl Polym Sci. 1964;8:1325–41.
14. Ott E. High polymers e cellulose and cellulose derivatives. New York: Interscience Publishers Inc.; 1943.
15. Nitin A, Tayade T. Evaluation of microcrystalline cellulose prepared from sisal fibers as a table excipient: a technical note. AAPS PharmSciTech. 2007; 8(1): Article 8.

16. Barud HS, Ribeiro CA, Capela JMV, Crespi MS, Ribeiro SJL, Messadeq Y. Kinetic parameters for thermal decomposition of microcrystalline, vegetal, and bacterial cellulose. J Therm Anal Calorim. 2010. ESTAC2010 Special Issue: 1–6.
17. Cabrales L, Abidi N. On the thermal degradation of cellulose in cotton fibers. J Therm Anal Calorim. 2010;102:485–91.
18. Zohuriaan MJ, Shokrolahi F. Thermal studies on natural and modified gums. Polym Testing. 2004;23:575–9.
19. Uesu NY, Pineda AG, Hechenleitner AAW. Microcrystalline cellulose from soybean husk: effects of solvent treatments on its properties as acetylsalicylic acid carrier. Int J Pharm. 2000;206: 85–96.
20. Roman M, Winter WT. Effect of sulfate groups from sulfuric acid hydrolysis on the thermal degradation behavior of bacterial cellulose. Biomacromolecules. 2004;5:1671–7.
21. Rodrigues Filho G, Assunção RMN, Vieira JG, Meireles CS, Cerqueira DA, Barud HS, Ribeiro SJL, Messaddeq Y. Characterization of methylcellulose produced form sugar cane bagasse cellulose: crystallinity and thermal properties. Polym Degrad Stab. 2007;92:205–10.
22. Dong XM, Revol Jf, Gray DG. Effect of Microcrystalline preparation condition on the formation of colloid crystal of cellulose. Cellulose. 1998;5:19–32.