Characterization of the bacterial cellulose dissolved on dimethylacetamide/lithium chloride

The main barrier to the use of cellulose is its insolubility on water or organic solvents, but derivatives can be obtained with the use of ionic solvents. Bacterial cellulose, is mainly produced by the bacterium *Acetobacter xylinum*, and is identical to the plant, but free of lignin and hemicellulose, and with several unique physical-chemical properties. Cellulose produced in a 4% glucose medium with static condition was dissolved on heated DMAc/LiCl (120 °C, 150 °C or 170 °C). The product of dissolved cellulose was observed with $^{13}$C-nmr and the effect on crystalline state was seen with x-ray crystallography. The crystalline structure was lost in the dissolution, becoming an amorphous structure, as well as Avicel. The process of dissolution of the bacterial cellulose is basics for the analysis of these water insoluble polymer, facilitating the analysis of these composites, by $^{13}$C-NMR spectroscopy, size exclusion chromatography and light scattering techniques.

Keywords: Bacterial Cellulose, Acetobacter xylinum, Dimethylacetamide, lithium chloride, Nuclear Magnetic Resonance, X-ray diffraction.

Introduction

Bacterial cellulose is a homo-polysaccharide formed for glucose molecules with β-(1-4) linkages that can be produced with different substrates and acquire the format of the recipient that contains it. The hydroxyl groups of the bacterial cellulose, as well as its similar plant, tends to form hydrogen bonds with the neighbor chain stabilizing its structure on a crystalline pattern. The crystallinity depends on the variability of the possible associations, intra and intermolecular. The crystalline states affect directly the solubility of the cellulose, which is insoluble in water or in usual organic solvents. Therefore this affect the degree of substitution and the distribution of the substituents concerning the hydroxyls disponibility when the cellulose is submitted to chemical derivatization as esterification.

The potential of cellulose as substrate can be increased with strategies to make it soluble. Thus, the polymer is dissolved in water and in ionic compounds as cupramonium hydroxid (Cuam), NaSCN/KSCN/LiSCN/H₂O, LiClO₄·3H₂O; H₂SO₄/H₃PO₄) and non-aquous as dimethylacetamide/litium cloride (DMAc/LiCl), n- methylmorpholine-n-oxide (NMNO) and dimethylsulfoxide (DMSO).
The solvent system DMAc/LiCl consists of an excellent solvent system for the homogeneous derivatization\(^6\). Still, the solution of LiCl in DMAc is one of the most important systems to dissolved cellulose for organic synthesis, as well as for analytical field\(^7\) allowing a structural modification of the polysaccharide. The question that remains is if the dissolution in adjusted solvent systems prevent or induce the degradability and the loss of the physical properties\(^5\). Bacterial cellulose membrane produced by Acetobacter xylinum, in 4 % glucose, was dissolved in order to get a soluble cellulose in the solvent system selected. For this, some conditions were tested as temperature of dissolution and the result was evaluated with nuclear magnetic resonance. Therefore, the effect of the solvent DMAC/LiCl on crystalline arrangements was seen by X-ray diffraction.

**Experimental**

**Bacterial strain**

The Acetobacter xylinum ATCC 23769 used in this study was gotten in the Foundation André Tosello-Brasil.

**Production of Bacterial Cellulose**

The Bacterial cellulose was produced on 10 mL cell culture flasks on pH 5.4 buffered Hestring-Schram medium. After inoculum, membrane was grown for 10 days on static condition, than cleaned with NaOH 1 %, at 60 °C for 60 min, and washed with water. The membrane was lyophilized to dry.

**Dissolution and acetylation of the bacterial cellulose**

A mixture of dimethylacetamide and bacterial cellulose, 50:1 (v/w), was agitated, warmed at 120-170 °C, in oil bath-heated, during one hour in a condensing system. Lithium chloride (0.4 %) was added, heated to 110 °C, 20 minutes. Than at the ambient temperature the mixture was shaken for 12 hours. The DMAc/LiCl was removed by dialysis in a membrane of 16 kDa for 72 hours. The product was separated through centrifugation.

**Regeneration of the Dissolved Bacterial Cellulose**

Bacterial and Avicel\(^\circledR\) cellulose, previously dissolved in the DMAc/LiCl system was regenerated as a pellicle with distilled water placed on a petri dish, getting a translucent film in both samples.

**Characterization of the dissolved cellulose**

*Nuclear Magnetic Resonance*: The soluble cellulose an the derivatives were analyzed on a spectrometer AC 300-P, Bruker, at 300.16 MHz to \(^1\)H-NMR and 75.13 MHz to \(^{13}\)C-NMR. The sample of the soluble cellulose was prepared dissolving 1 % (w/v) of the lyophilized material on 0.5 mL of DMAc/LiCl and 3 drops of dimethylsulfoxide-d₆ was added to the lock. The cellulose derivatives was solubilized on 0.5 mL acetone-d₆.
X-ray diffraction: measurements were performed on a ERD 7000, Schimadzu system. The intensity of Cu, Ni filter, 40 kv X 20 mA, was measured in a 20 range between 5° and 60°.

Scanning electronic microscopy (SEM): the SEM images was done at a JEOL JSM-6360LV of metalized samples and HITACHI TM-100.

Results and discussions

Analysis for $^{13}$C-NMR of the Bacterial Cellulose dissolved on the DMAc/LiCl

The $^{13}$C-NMR spectra of 1 % of bacterial cellulose dissolved (Figure 1. A) under the conditions of dissolution of cellulose/DMAc/LiCl (1:50:4) (w/v/w), the signals of the cellulose, between 55 - 105 ppm, are coherent with literature$^3$ for the CP-MAS $^{13}$C-nmr of solid samples. Moreover, the solvent signals at 170 ppm, and at 20 ppm are evidently but not affect the quality of the cellulose resonances.

The same can be observed for the Avicel® (Figure 1. B) dissolved in solvent system. The chemical displacements of the dissolved plant and bacterial cellulose can be seen on the Table 1, in confrontation with literature to CP-MAS $^{13}$C-nmr.
Table 1. Chemical shifts of $^{13}$C-NMR (solution) of the dissolved Avicel® and bacterial cellulose, comparing with literature (Kono, 2004).

<table>
<thead>
<tr>
<th></th>
<th>Avicel®</th>
<th>Bacterial Cellulose</th>
<th>Avicel® – Solid NMR Kono et al. (2004).</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>102.7</td>
<td>103.2</td>
<td>102.4</td>
</tr>
<tr>
<td>C2</td>
<td>74.79</td>
<td>75.87</td>
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<td>C3</td>
<td>74.79</td>
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<td>C4</td>
<td>78.18</td>
<td>79.16</td>
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<td>C5</td>
<td>73.13</td>
<td>74.39</td>
<td>72.9</td>
</tr>
<tr>
<td>C6</td>
<td>59.29</td>
<td>59.89</td>
<td>62.5</td>
</tr>
</tbody>
</table>

**Characterization by X-ray diffraction of the bacterial cellulose**

In the X-ray diffraction to the plant cellulose the indicative of the presence of crystalline cellulose of type I is through the occurrence of peaks in $14^\circ$, $16^\circ$ and $22,6^\circ$ $2\theta$ $^{5,6}$, as can be observed the Figure 3. For cellulose type II the peaks if present in $12^\circ$ and $20^\circ$ $2\theta$ $^8$, that doesn’t appear on diffractograms. Bacterial cellulose native, without treatment of dissolution, represented in the Figure 3. A $^9$, possess the peaks in angles of characteristic Bragg $2\theta$ of cellulose type I, with a crystalline structure of approximately 53 %. However, when is compared the native BC with the dissolved, the crystalline structure became amorphous, Figure 3. C.

Native plant cellulose (Avicel®) (Figure 3. C) has high crystallinity (80 %) and crystal structure typical of cellulose type I. After the dissolution in DMAc/LiCl, the crystallinity is broken. Because after reacting with the solvent, the structure becomes amorphous (Figure 3. C). The high crystallinity not necessarily result in low solubility. Scanning electronic microscopy (SEM) of the regenerated cellulose show a homogeneous material without distinction of fibers (Figure 2.

![Figure 2: SEM of bacterial cellulose native, 100x (A), (B) photography of the film of bacterial cellulose regenerated on water after dissolution with DMAc/LiCl, (C) SEM of the film of bacterial cellulose regenerated 300x of magnification.](image)

Despite the excellent mechanical properties of the crystalline structure of cellulose, the amorphous structure is more reactive and has higher amount of hydroxyl available with the solvent, while the crystal structure due to the connections of hydrogen are more strongly connected, is not available to react with the solvent.
3 Conclusions

The system solvent dimethylacetamide/lithium chloride was effective to dissolve bacterial cellulose as well the plant cellulose. Bacterial cellulose produced by the bacterium Acetobacter xylinum was completely dissolved at a concentration of 1 % of cellulose in the system solvent DMAc/LiCl, presenting a clear solution.

The bacterial cellulose dissolved in DMAc/LiCl lost the crystallinity, becoming an amorphous structure, and the native cellulose (Avicel®) dissolved under the same conditions when regenerated on water. The dissolution process occur by the cision of the linkage between hydroxyls and hydrogens of pared chains. These hydrogen linkages were substituted by the solvent/ions bridge. The flexible and translucent pellicle obtained as seen on Figure 2. B, consist on a non pared amount of cellulose fibrils.

Acknowledgment

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References


