**Development of thermosensitive hybrid hydrogels based on xylan-type hemicellulose from agave bagasse: Characterization and antibacterial activity**

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**Supplementary material**

1. **Isolation of hemicellulose and characterization**

The agave bagasse powder (40-60 mesh) was dewaxed with 2:1 (v/v) toluene-ethanol in a Soxhlet apparatus for 12 h. The dewaxed powder was delignified with 1 g/g dry matter sodium chlorite and 0.6 mL/g of dry matter of acetic acid with a solid to liquid ratio of 1:32 (g/mL) at 70 °C for 8 h. The residue, holocellulose, was subsequently washed with distilled water and then dried at room temperature to a constant weight. The holocellulose was extracted by 10% KOH solution with a solid to liquid ratio of 1:20 (g/mL) at room temperature for 12 h and then filtrated to separate HC and cellulose fractions. The filtrated was acidified to pH 5.5-6.0, then precipitated in 3 volumes of ethanol to yield the HC and dried in a vacuum. The obtained HC was characterized by nuclear magnetic resonance (NMR) and High-Performance Liquid Chromatography (HPLC).



Figure SM1. A )13C and B) 1H liquid NMR of hemicellulose extracted from agave bagasse.

The 13C-NMR spectrum contains five intense signals at 102, 72.2, 73.2, 75.9 and 63.6 ppm are characteristics of C-1, C-2, C-3, C-4 and C-5 of 𝛽-D-xylose residues, respectively. The characteristic peaks of L-arabinofuranose for C-2 and C-3 linked to the D-xylose unit can be observed at 82.2 and 76.9 ppm. The signals observed at 98, 70.2, 72.2, and 59.7 ppm originate from 4-O-methylglucoronic acid residues; likewise, the signal at 172.3 ppm which corresponds to the carbonyl group (-C=O). Figure SM1b (1H-NMR) shows signals at 5.08, 3.48, 3.02 and 4.25 ppm attributed to H-1, H-2, H-4 and H-5, respectively of 4-*O*-methyl-𝑎-D-glucuronic acid residues. Also, it is possible to observe the signal corresponding to 𝛽-Dxylose at 4.48 (H-1), 3.17 (H-2), 3.35 (H-3), 3.87 (H-4), 3.98 (H-5eq) and 3.23 ppm (H-5ax).



Figure SM2. HPLC Chromatogram of: (A) standards mixture and (B) sugars generated from hydrolysis agave bagasse hemicellulose.

Figure SM2 (A) shows a standard chromatogram mixture (1 mg/mL) of four monomeric carbohydrates separated on an Aminex HPX-87P column in approximate 20 min at 80 °C, using a HPLC (waters 2414, refractive index detector). The standards were eluted with water as a solvent at a flow rate of 0.6 mL/min. Baseline separations are achieved for the glucose (Glu), xylose (Xyl), mannose (Man) and arabinose (Ara) at 12.46, 13.68, 17.53 and 16.34 min, respectively, elution time. In Figure SM3(B), the constituent neutral sugars after hydrolysis with sulfuric acid in the extracted hemicellulose are observed [[1](#_ENREF_1)]. The HPLC chromatogram for the hemicellulose shows that the sugar present in hemicellulose are mainly xylose, arabinose and traces of glucose.

According to both 1H and 13CNMR spectrum and HPL chromatogram analysis, it is possible to conclude that the hemicellulose from agave bagasse is constituted by xylose, arabinose and 4-O-methyl glucuronic acid.



Figure SM3. A) Scheme for Preparation of Xylan-Type Hemicellulose-Based hybrid hydrogel; B) Solid state 13C-NMR in D2O of the thermo-responsive HC-*g*-TMSPMA/PVCL hydrogel.

Figure SM3 (B) shows the solid 13C NMR of hydrogel HC-*g*-TMSPMA/PVCL synthetized according to the scheme shown in Figure SM3(A). Briefly, hybrid hydrogels were prepared with sylanized hemicellulose and NVCL by free radical polymerization and crosslinking. In the NMR spectra, it can be seen the peaks at 23.6 and 30.4 ppm, which are assigned to carbon atoms in the methylene groups (–CH2). The signals at 38.3 and 44 ppm correspond to the signal of C atoms in -CH2-groups near to –C=O- and –NCH–. The peaks at 102, 75, 67 and 63.3 ppm were assigned to the C1, C4, C3, 2 and C5 of the xylopyranose units from HC. Carbonyl groups (-C=O) from HC as well as from PNVCL can been observed at 178 ppm. The small peak at 100 ppm corresponds to carbon atom of the silyl ether (-C – O – Si-).



Figure SM4(A): M-H agar plates with cylindric perforation for antimicrobial hydrogels analysis and antibiotic time-release. Figure SM4(B): Representative plate of antimicrobial assay using hybrid hydrogels.

Figure SM4(A) shows a representative M-H agar plate used in the bacterial growth inhibition analysis and ciprofloxacin time-release. Figure SM4(B) shows a representative M-H agar plate containing the hybrid hydrogel G1/5 (marked as 17-23 and 17-22, duplicated), a negative control as G1/5 composites without antibiotic (marked as 17-C) discarding a potential self-antibacterial effect of the hybrid hydrogel composition, no halo was detected. As positive control, ciprofloxacin 0.005 x10-3 g was used (not marked) as recommended by CLSI standard M07[[2](#_ENREF_2), [3](#_ENREF_3)] .

**References**

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Supplementary material

Supplementary material contents three figures of characterization by TGA and DSC (Fig.

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