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Hydrated fractions of cellulosics probed by infrared spectroscopy coupled with dynamics of deuterium exchange

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ABSTRACT

This article presents a novel method to selectively probe the non-crystalline, hydrated fractions of cellulosic biomass. The method is based on time-resolved infrared spectra analyzed to provide information on spectral and dynamical features of deuterium exchange (OH → OD) in D_2O atmosphere. We assign deuterium exchange spectral regions (700–3800 cm^{-1}) and explore changes due to relative humidity, different cellulosic samples, and infrared polarization. Here, two results are highlighted. First, a wide range of celluloses isolated from plants show remarkable spectral similarities whatever the relative amounts of cellulose and xylan. This result supports an inherent type of hydrated disorder which is mostly insensitive to the molecular identities of the associated polysaccharides. Second, polarized infrared analysis of cotton reveals hydrated cellulose having chains preferentially aligned with those of crystals, while the hydroxyls of hydrated cellulose present much more randomized orientation. Our results provide new insights on molecular and group orientation and on hydrogen bonding in hydrated fractions of cellulosic biomass.

1. Introduction

Cellulose is the main component of cell walls from higher plants, which makes cellulose the most abundant organic polymer on Earth (Klemm, Heublein, Fink, & Bohn, 2005). Cellulosic biomass forms supramolecular structures comprising cellulose crystals as well as disordered, water-accessible fractions. Crystalline cellulose is understood in appreciable detail, with knowledge of crystal polymorphism (O'Sullivan, 1997), native crystal structures (Nishiyama, Johnson, French, Forsyth, & Langan, 2008; Nishiyama, Langan, & Chanzy, 2002), and some recent insights on crystal imperfections (Ciesielski et al., 2013; Driemeier & Francisco, 2014). For hydrated disordered fractions, on the other hand, comparatively less is known. Most in-depth structural studies have investigated model systems of amorphous cellulose, either experimental models (e.g., ball-milled cellulose) (Fink, Philipp, Paul, Serimaa, & Paakkari, 1987; Mori et al., 2012) or computational molecular models (Kulasinski, Keten, Churakov, Derome, & Carmeliet, 2014; Kulasinski, Keten, Churakov, Guyer, et al., 2014; Mazeau & Heux, 2003). Although valuable, such model studies miss the possible diversity of structures across relevant cellulosic biomasses, in which hydrated fractions often include naturally associated polymers such as hemicelluloses.

Isotopic exchange in deuterated water (D_2O) has a long history in experimental characterization of water-accessible fractions of cellulosics. At room temperature, D_2O molecules cannot penetrate into cellulose crystals, but do penetrate into disordered regions, promoting OH → OD exchange in accessible hydroxyl groups (Frilette, Hanle, & Mark, 1948; Mann & Marrinan, 1956; Nelson & O'Connor, 1964). Disordered regions can therefore be probed *selectively* by employing room temperature deuteration coupled to measurement techniques that provide isotopic contrast. Such strategy has been widely exploited to quantify (Hishikawa, Inoue, Magoshi, & Kondo, 2005; Hofstetter, Hinterstoisser, & Salmén, 2006; Mann & Marrinan, 1956; Nelson & O'Connor, 1964; Suchy, Virtanen, Kontturi, & Vuorinen, 2010) and to localize (Fernandes et al., 2011; Nishiyama et al., 2003; Thomas, Altaner, & Jarvis, 2013; Thomas, Forsyth, et al., 2013) disordered fractions, but not yet to derive spectroscopy signatures of disorder across wide set of specimens. A notable exception is the study by Müller, Czihak, Schober, Nishiyama, and Vogl (2000), which observed common lattice vibration signals from disordered regions of several native celluloses; however that investigation was performed with inelastic neutron scattering, a tool inaccessible to most studies.

In the present study, we develop the principles and demonstrate the applicability of a novel spectroscopic method to probe hydrated

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fractions of cellulosics. The method has basis on isotopic contrast provided by Fourier-transform infrared spectroscopy (FTIR), with monitoring of deuterium exchange dynamics promoted by controlled exposure to D_2O vapor. Although the idea of monitoring the dynamics of deuterium exchange is not new (Hishikawa et al., 2005; Hofstetter et al., 2006), we go beyond the usual analysis of O—H and O—D stretching modes (at ~3300 cm^{-1} and ~2500 cm^{-1}, respectively) and focus on the more informative 700–1900 cm^{-1} spectral region (Maréchal & Chanzy, 2000). Furthermore, we employ principal component analysis (PCA) (De Juan & Tauler, 2003; Johnson & Wichern, 2007) to reduce hundreds of time resolved spectra into coefficients containing the essential spectral features from hydrated disorder. By analysis of representative cellulosic samples, new insights on molecular and group orientation, water transport, and hydrogen bonding are obtained with the novel technique.

2. Materials and methods

2.1. Cellulosic specimens

In the present work we analyzed five celluloses isolated from plants. These celluloses are a subset from a previous study, in which samples were characterized by X-ray diffraction, vapor sorption, and chemical composition (Driemeier & Bragatto, 2013). All samples have cellulose I phase, negligible amounts of lignin, but variable amounts of hemicellulose, mainly xylan, which complemented the amounts of cellulose (Driemeier & Bragatto, 2013). The samples of the present study are microcrystalline cellulose Avicel PH-101 (Avicel) and α-cellulose (Alpha) acquired from Sigma–Aldrich (catalog codes 11365 and C8002, respectively); Whatman #1 filter paper (FP); a bleached Eucalyptus kraft pulp from a Brazilian mill (Ekp); and a peracetic pulp of sugarcane bagasse (Bpa) produced in our laboratory in a 1:1 mixture of 8.74 M glacial acetic acid and 21.6 M hydrogen peroxide at 60 °C for 24 h. For the FTIR characterization of the present work, Avicel and Alpha were characterized as received, while FP, Ekp, and Bpa were first ground to mean particle size of 130 μm by employing an impact and shear mill (Pulverizette 14, Fritsch, Germany). In addition to the aforementioned celluloses isolated from plants, we investigated cotton fibers kindly provided by Dr. Alfred French. Cotton belongs to genetic line MD90, grown under standard field conditions in New Orleans, LA, and harvested 40 days post-anthesis, which would be considered mature cotton fibers.

2.2. Instrumentation

FTIR analysis was performed in a benchtop mid-infrared spectrometer (Frontier, PerkinElmer, United States) having a single bounce Universal Attenuated Total Reflectance (UATR) sample module with diamond/ZnSe crystal and polarization accessory. The spectrometer was controlled by Spectrum Timebase Software, which allowed time-resolved spectra acquisition. Water vapor was provided by two humidity generators (GenRH-A, Surface Measurement Systems, United Kingdom). Both generators operated under identical conditions of relative humidity (RH), flow, and nitrogen carrier gas. However, one generator operated with deionized water (H_2O), while the other one with deuterated water (D_2O, 99.9 at% D, acquired from Sigma Aldrich). Spectrometer and humidity generators operated at laboratory room temperature (22–27 °C). The outlet of each humidity generator was connected to a 3-way valve so that the flow from one generator was directed to a sink while the other was directed to the specimen. The vapor environment in contact with the specimen was isolated from the surroundings by a custom-built stainless steel chamber placed on top of the UATR plate. Deuterium exchange was started by switching the vapor flow ($H_2O \rightarrow D_2O$) to the specimen. Fig. S1 (Supporting information) shows photographs of the experimental setup.

2.3. Spectra acquisition

For analysis of cellulosic powders the FTIR spectrometer was operated without IR polarization. Powders were carefully positioned on top of the UATR crystal by employing a perforated disk to contain the powder while pressing it against the UATR crystal. On the other hand, analysis of cotton fibers employed the polarization accessory to have IR linearly polarized on the plane of the UATR crystal surface. Cotton fibers were analyzed oriented either parallel (0°) or perpendicular (90°) to the IR polarization. For all analyses vapor was generated with a flow of 100 sccm (standard cubic centimeter per minute) and 40% RH, with the exception of selected Avicel analyses run at variable RH.

Specimens were initially air dried (i.e., in equilibrium with atmospheric RH). Before starting spectra acquisition, each specimen was conditioned during 2 h to equilibrate with the H_2O environment created by the humidity generator. This conditioning was performed with the specimen on top of the UATR plate, isolated from external environment by the steel chamber. At the end of this conditioning period, background spectrum was acquired. Then, specimen was positioned on top of the ATR crystal and pressed against it. This specimen manipulation was performed through a small hole at the top of the steel chamber. With specimen properly positioned and pressed, spectra acquisition was started.

Infrared spectra were acquired in the 450–4000 cm^{-1} range, with 4 cm^{-1} resolution, intensity recorded in 1 cm^{-1} intervals, and averaging 16 acquisitions for each recorded spectrum. Spectra were recorded in intervals of 1.5 min during a total period of 6 h. Thus, 240 spectra were recorded in each analysis. Vapor was switched ($H_2O \rightarrow D_2O$) after recording the spectra #10, which was considered the spectrum at time zero. The following 230 spectra (345 min) monitored the OH → OD exchange process. Analysis of each sample/condition was run in triplicate and presented results are means of triplicates.

2.4. Data analysis

Data analysis discarded the 450–700 cm^{-1} spectral region because of excessive noise. Spectra #1–9 were also discarded because this initial phase presented a stabilization period unrelated to deuterium exchange, which, as mentioned before, started after spectrum #10. The remaining data was organized in matrix form, having 231 spectra as matrix rows, and spectral wavenumbers as matrix columns. Outliers in data matrix were recognized and corrected based on the expected smoothness of spectral and time dependencies. Typically less than 0.25% of matrix entries was treated as outliers and then corrected.

Two spectral regions (700–1900 cm^{-1} and 2100–3800 cm^{-1}) were analyzed separately by PCA (De Juan & Tauler, 2003; Johnson & Wichern, 2007). Prior to PCA, flat spectral baselines were subtracted to minimize detrimental effect of baseline fluctuation during OH → OD exchange. Mean absorbance between 1850–1900 cm^{-1} and 3900–4000 cm^{-1} were used as baselines for the 700–1900 cm^{-1} and the 2100–3800 cm^{-1} spectral regions, respectively. Outlier correction, baseline correction, and PCA were performed through scripts in MATLAB computing language.

3. Results and discussion

3.1. Spectra, difference spectra, and principal components

Fig. 1 exemplifies spectra acquired during the course of deuterium exchange, showing data from Avicel conditioned at 40% RH.