Supplementary information

The content of acidic functionalities of the starting oxidized cellulose thin films as well as the degree of esterification after thermal treatment, carbodiimide-activation and methylation were studied in parallel by x-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy in the attenuated total reflectance mode (ATR-FTIR) and group selective fluorescence labelling in combination with GPC (FDAM)[1].

Materials and Methods

Cellulose thin films (for XPS) and cellulose volume samples (for ATR/FTIR, FDAM) were regenerated after dissolution in $NMMO{\cdot}H_2O$ at 110 °C for 2.5 hours. The cellulose volume samples were obtained through direct precipitation of the cellulose solution into MilliQ water. Cellulose thin films were produced by spin coating as described in the Materials & Methods section. After washing with MilliQ water to remove solvent residues the samples were freeze dried prior to use.

XPS. The chemical composition of the cellulose films was determined using an Axis Ultra spectrometer (Kratos Analytical, Manchester, United Kingdom) equipped with a monochromatized Al Kr X-ray source of 300 W at 20 mA. For a take off angle (angle between sample surface normal and the electron optical axis of the spectrometer) of 0°, the maximum information depth of the measurements is approximately 8 nm.[2] All spectra were charge-corrected by setting the energy of the C_x-H_y bond to 285.0 eV. Quantitative elemental compositions were determined from the peak areas of the survey spectra using experimentally determined sensitivity factors and the spectrometric transmission function. The high-resolution spectra were deconvoluted using software routines supplied by Kratos Analytical. Fitting parameters were the binding energy, peak height, full width at half-maximum, and the Gaussian-Lorentzian ratio of the component peaks.

ATR/FTIR. FTIR spectra were collected on a Bruker Tensor 27 in the ATR modus using a diamond crystal. The spectra were evaluated in a range of 1800-1680 cm^{-1} to analyze the C=O stretching bond of the cellulose carboxylic acid groups (1730-1700 cm^{-1}).

FDAM. Heterogeneous fluorescence labelling of cellulose volume samples was carried out using 9H-fluoren-2-yl-diazomethane (FDAM) as the fluorescence label.[1]
GPC measurements were performed with a setup containing the following components: online degasser Dionex DG-2410, pump Kontron 420; puls damper; autosampler HP series 1100; column oven Gynkotek STH 585; fluorescence detector Agilent FLD G1321A; MALLS detector Wyatt Dawn DSP with argon laser (488 nm); RI detector Shodex RI-71. Chromeleon, Astra and GRAMS/32AI software were used for data evaluation. The samples were analyzed by four serial GPC columns (PL gel, mixedA, ALS, 20 µm, 7.5 x 300 mm), and monitored by fluorescence (excitation, 286 nm, emission 330 nm emission (carbonyl groups); excitation, 252 nm; emission, 323 nm (carboxyl groups), injection volume, 100 µl; flow, 1.00 ml/min; run time 45s), MALLS, and RI detection. DMAc/LiCl (0.9%, m/V) was used as eluant with a refractive index increment of 0.140 ml/g at 25 °C and 488 nm. The calibration of the system was carried out using reference pulps.[3]

Results

XPS.

Figure 1 gives the high-resolution carbon 1s peak (C 1s) of the NMMO-regenerated oxidized cellulose film. The obtained signal can be divided into four different component peaks according to the chemical environment of the carbon atom. Peak A (Binding Energy (BE) = 285.0 eV) represents hydrocarbon contaminants that commonly occur on cellulose surfaces.[4] Increasing the number of carbon-oxygen bonds on a given carbon atom also increases their binding energies, therefore the sub-peaks could be characterized with respect to the number of their C-O bonds. Thus, Peak B (BE = 286.7 eV) represents carbon atoms being bound to one oxygen atom (C-OR bonds; R=H, C), whereas Peak C (BE= 288,1 eV, two carbon oxygen bonds) can be attributed to carbonyl- and/or acetal groups (C=O, O-C-O). Finally, subpeak D at a BE of 289,3 eV is related to carboxylic acid groups or ester groups. The evaluation of regenerated cellulose films revealed a content of carboxylic acid groups of approximately 2.2±0.2 atom-%. However, as the BE of ester functionalities is in the same range as the BE of the carboxylic acids[5], XPS does unfortunately not allow for an identification of the relative contribution of either one of these species. Thus, XPS does not permit to determine the degree of esterification (cross-linking) of the differently treated cellulose films.

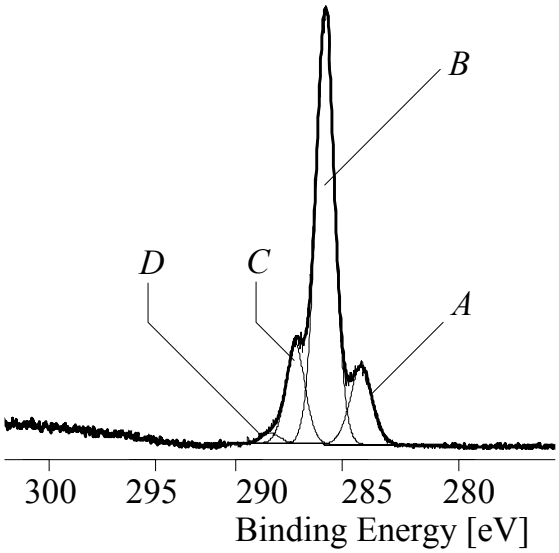

Figure 1. C 1s peak of the oxidized cellulose film. Peak A: 16.9±1.1 atom-%, Peak B: 65.3±0.1 atom-%; Peak C: 15.6±0.1 atom-%, Peak D: 2.2±0.2 atom-%.

ATR/FTIR.

The ATR/FTIR spectrum of the NMMO-regenerated cellulose volume sample does not contain any C=O stretching bond of the carboxylic acid groups, typically obtained in a range of 1700-1730 cm^{-1} indicating that the content of oxidized functionalities of our starting cellulose remains below the detection limit of this method. The cellulose raw material Avicel demonstrates a similar spectrum without an absorption band within this wavelength range (see Figure 2).

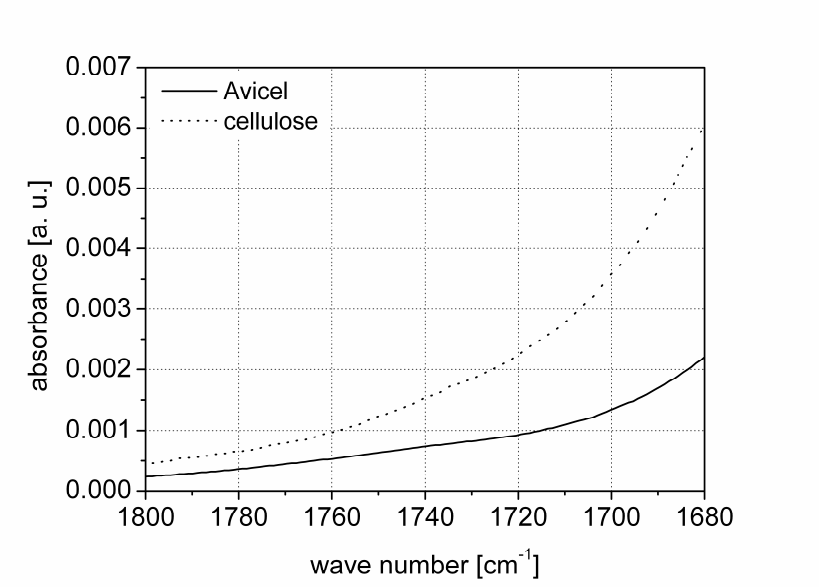

Figure 2. ATR/FTIR spectra of the oxidized cellulose and the corresponding cellulose raw material Avicel in the range of the C=O stretching bond (1700-1730 cm^{-1}) of the carboxylic acid group.

FDAM.

The cellulose carboxylic acid groups were labelled with 9H-Fluoren-2-yl-diazomethane (FDAM) (exemplarily shown in Figure 3 for the labelling of the standard (Methyl-β-D-glucopyranosid)uronic acid used for calibration).[1] NMMO-dissolution is known to primarily result in the oxidation of reducing end groups of cellulose into carboxylic acid groups (onic acids, C_1-COOH, main heterolytic pathway of NMMO degradation). Also, oxidation of the C_6-atom of cellulose can lead to the formation of uronic acid groups (C_6-COOH) while formation of COOH-groups at the C_2- and C_3 atom of the cellulose repeating unit after splitting the ring structure plays a minor role within the degradation process in presence of NMMO and is therefore neglected.[6] The fluorene-2-yl methyl onates primarily formed after labelling of the onic acids (C_1-COOH) with FDAM (see first reaction scheme in Figure 3) act like activated esters promoting the formation of onolactones with concomitant release of fluorene-2-yl methanol from the fluorescence label. Consequently only the uronic acid moieties (C_6-COOH) will be labelled quantitatively leading to the determination of only one type of acid groups present within the cellulose structure (see second reaction scheme in Figure 3).[1] - The total amount of the cellulose carboxylic acid groups is therefore not accessible via fluorescence labelling. The degree of substitution of the NMMO-regenerated cellulose determined by group selective fluorescence labelling of the cellulose uronic acid moieties (C_6-COOH) with FDAM was determined to be in a range of 20-22 μmol/g cellulose.

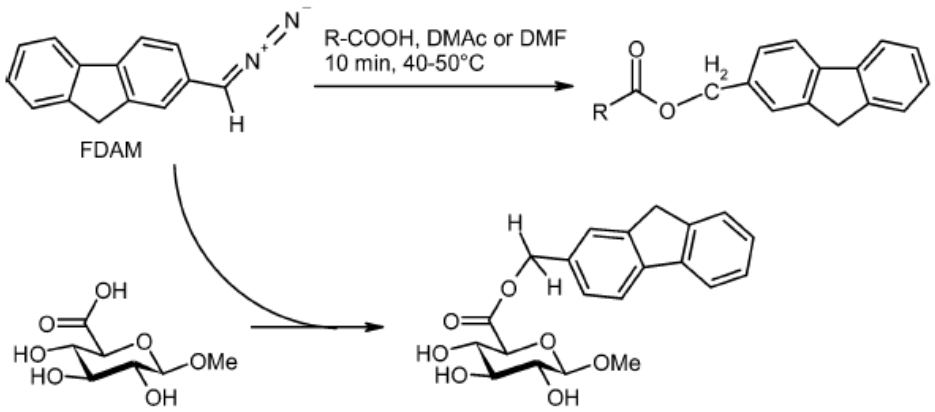

Figure 3. Fluorescence labelling of the standard (Methyl-β-D-glucopyranosid)uronic acid using 9H-Fluoren-2-yl-diazomethane (FDAM).[1]

In sum, the above-mentioned methods do unfortunately not permit to quantitatively evaluate the carboxylic acid content of partly oxidized cellulose nor the degree of esterification of the differently modified samples.

[1] B. Bohrn, A. Potthast, S. Schiehser, T. Rosenau, H. Sixta and P. Kosma, *Biomacromolecules* 2006, **7**, 1743-1750.
[2] M. P. Seah and W. A. Dench, *Surf. Interface Anal.* 1979, **1**, 2-11.
[3] J. Röhrling, A. Potthast, T. Rosenau, T. Lange, A. Borgards, H. Sixta and P. Kosma, *Biomacromolecules* 2002, **3**, 969-975.
[4] E. Kontturi, P. C. Thüne and J. W. Niemantsverdriet, *Polymer*. 2003, **44**, 3621.
[5] U. Freudenberg, S. Zschoche, F. Simon, A. Janke, K. Schmidt, S. H. Behrens, H. Auweter and C. Werner, *Biomacromolecules* 2005, **6**, 1628-1634.
[6] T. Rosenau, A. Potthast, H. Sixta and P. Kosma, *Prog. Polym. Sci.* 2001, **26**, 1763-1837.