Electronic supplementary information (ESI)

Esterification and hydrolysis of cellulose using oxalic acid dihydrate in a solvent-free reaction suitable for preparation of surface-functionalised cellulose nanocrystals with high yield

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Experimental

Esterification and hydrolysis of cellulose by oxalic acid dihydrate

Commercially available softwood dissolving pulp (Domsjö Fabriker AB, Sweden) was mixed with oxalic acid dihydrate (OAD, melting point 104-106°C) according to the molar ratio of the anhydrous glucose units (AGU)/OAD=1/5. Each mixture was heated to 110°C for 15 min, 30 min, 60 min, and 120 min, respectively, under constant mixing and reflux, to obtain cellulose oxalates (COX), denoted as COX15, COX30, COX60, COX120, respectively. Excess oxalic acid dihydrate was removed by dissolution in THF followed by filtration and Soxhlet extraction using THF for 24 h. All samples were dried in a fume hood at ambient conditions.

Preparation of nanocellulose suspension

475 mg of the cellulose oxalate was mixed with 100 ml of deionized water. The pH of the suspension was adjusted to 9-10 with 0.01 M NaOH. The suspension was then sonicated 6 times for 3 min (3 min × 6 = 18 min) with 40% amplitude (Vibra-Cell VC 750, Sonics, USA) and centrifuged at 7448 g for 30 min (ROTINA 420, Hettich Zentrifugen, Germany). The supernatant was collected to obtain the nanocellulose suspensions.

Characterisation

Fourier transform infrared (FTIR) spectra of the cellulose oxalates were recorded on a PerkinElmer Spectrum 2000 FT-IR (PerkinElmer, USA) equipped with a heat-controlled single reflection attenuated total reflection (ATR) accessory (Golden Gate heat-controlled). 32 scans were recorded for each spectrum.

Nuclear magnetic resonance (NMR) was performed on COX15 by first dissolving 25 mg of the sample in a solution of 0.33 g of tetrabutylammonium fluoride trihydrate in 1.65 g of DMSO-d6. The 13C NMR spectrum was collected on a Bruker UltraShield 400 MHz (Bruker, USA) spectrometer at 50 °C. The number of accumulations was 11 000.

The gravimetric yields of CNCs were calculated based on the dry weights of the raw materials.

Total carboxyl content (TCC) of each cellulose oxalate was determined by alkaline hydrolysis and back titration as described elsewhere. 0.5 g of each sample was mixed with 40 ml of a 70% ethanol aqueous solution and the suspension was stirred for 30 min. The hydrolysis was carried out at 60°C for 48 h after adding 20 ml of a 0.5 M NaOH solution into the suspension. A 0.5 M HCl solution was used to titrate the un consumed NaOH after the hydrolysis and phenolphthalein was used as the indicator. The equations for calculating TCC are shown below:

\[
\text{Total carboxyl content} = \frac{C_{\text{HCl}} \times (V_B - V_S)}{m}
\]

where \(C_{\text{HCl}}\) is the exact concentration (mol/l) of the HCl solution, \(V_B\) and \(V_S\) are the exact volumes (l) of the HCl solution used for titrations of the blank and the sample, respectively. \(m\) is the dry weight (g) of cellulose oxalate.

Free carboxyl content (FCC) of each cellulose oxalate was determined by conductometric titration as previously described. 100 mg of each cellulose oxalate was mixed with 100 ml of water and 10 ml of a 0.01 M NaCl solution. The suspension was then stirred for 1 h and titrated with 0.01 M NaOH. All titrations were carried out under constant nitrogen flow. The calculation of the content of free carboxyl groups is based on the equation below:

\[
\text{Free carboxyl content} = \frac{C_{\text{NaOH}} \times V_{\text{NaOH}}}{m}
\]

where \(C_{\text{NaOH}}\) is the exact concentration (mol/l) of the NaOH solution, \(V_{\text{NaOH}}\) is the exact volume (l) of the NaOH solution used for titration before the conductivity increased from the plateau of the titration curve. \(m\) is the dry weight (g) of the oxalate sample.
The degree of substitution (DS) of each cellulose oxalate was calculated from the two above titrations using the following formula:

\[
\text{Degree of substitution} = \frac{162.14 \times C_{\text{HCl}} \times (V_B - V_S) \times (1 - FC)}{m - C_{\text{HCl}} \times (V_B - V_S) \times (1 - FC) \times (90.03 - 18.02)}
\]

where FC is the ratio of free carboxyl content to total carboxyl content (FCC/TCC). 90.03 g/mol is the molecular weight of oxalic acid.

**Size exclusion chromatography (SEC)** was used to analyse the molecular weight (Mw) properties of the cellulose oxalates and the softwood dissolving pulp. The SEC system consisted of a DGU-20A3 degasser (Shimadzu, Japan), a LC-20AD liquid chromatograph (Shimadzu), a Rheodyne 7725i fixed loop (100 µl) and a RID-10A refractive index detector (Shimadzu). The injection volume was 100 µl. The separation system consisted of a mixed-A 20 µm guard column (7.5×50 mm, Polymer Laboratories) and 4 mixed-A 20 µm columns (7.5×300 mm, Polymer Laboratories). The flow rate was set at 0.5 ml/min. The columns were kept at a constant temperature of 80°C and the eluent was 0.5% LiCl/DMAc. Pullulan standards (Fluka) with nominal molar weights of 1.3 MDa, 805 KDa, 366 KDa, 210 KDa, 113 KDa, 48.8 KDa, 21.7 KDa, 10 KDa, 6 KDa, 1.32 KDa, and 342 Da were used for composing the calibration curve (Mw vs. retention time). The linear coefficient of the determination (r²) is 0.996. LC Solution software (Shimadzu) was applied to control the system as well as record the data.

Prior to the injection, 5 mg of each cellulose oxalate was dispersed in 1 ml of DMSO. After shaking for 15 min at room temperature, the suspension was centrifuged at 6700 g for 10 min and the sediment was collected. The same procedure was performed 4 additional times with DMSO followed by 5 times with DMAc. Thereafter, the sample was dissolved in 0.32 ml of 8% LiCl/DMAc (w/v) and diluted with 4.68 ml DMAc so that the concentration of LiCl/DMAc was 0.5% (w/v). Finally, each cellulose oxalate solution was filtrated through a 0.45 µm polytetrafluoroethylene syringe filter before injection.

**Dynamic light scattering (DLS)** was performed on a Zetasizer ZEN3600 instrument (Malvern Instruments Ltd., UK) to determine the average particle size of the nanocellulose. The measurement was performed at pH 9 and 25°C on a nanocellulose suspension with a concentration of approximately 0.1 g/l. The calculation of average particle size was made based on the assumption that the particles were spherical⁶.

**Transmittance Electron Microscopy (TEM) graphs** were recorded on an HT7700 transmission electron microscope (Hitachi, Japan) with a voltage of 80 kV. A drop of a diluted nanocellulose suspension (pH=3.5, concentration approximately 0.002 g/l) was placed on a carbon-coated grid. After 1 min, the liquid drop was removed by dabbing with a piece of filter paper. The size of the nanocellulose was manually measured with ImageJ⁵ (version 1.48) as the tool.

**X-ray diffraction (XRD)** was performed on a PANalytical X’Pert PRO MRD X-ray diffractometer (The Netherlands) equipped with a PANalytical X’Celerator detector (The Netherlands). Diffractograms were collected in a range of 2θ = 10 to 50°. Cu Kα radiation was monochromatized with a Nickel filter. The crystallinity index was estimated as described elsewhere⁶.

![Graph showing wave number and absorption intensity]
**Figure S1.** FTIR spectra of cellulose oxalate samples after esterification. COX: cellulose oxalate. Reaction times of 15, 30, 60, and 120 min. SDP: softwood dissolving pulp as the reference.

![FTIR spectra](image)

**Figure S2.** $^{13}$C NMR spectrum of COX15.

![NMR spectrum](image)

**Figure S3.** Tentative structures of cellulose oxalates (a). Esterification primarily occurs at C6-OH (b).

![Chemical structures](image)

**References**