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Preparation of cellulose nanocrystals in water–aprotic solvent mixtures

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Materials

To obtain CNCs, dried sulfate-bleached pulp of coniferous wood (State Standard 9571-89, Arkhangelsk Pulp and Paper Mill, Russia) (Table S1), sulfuric acid (98 %, chemically pure, State Standard 4204-77, Chimmed, Russia), deionized water, acetonitrile (MeCN), tetrahydrofuran (THF), and dioxane (Sigma–Aldrich, USA) were used. All solvents used were chemically pure or extra pure and were used without further purification.

Table S1 Chemical composition of sulfate-bleached pulp of coniferous wood (State Standard 9571-89).

Component	Content, %
Cellulose	93-96
Hemicellulose	3-6
Lignin	0.1-0.4
Oils, resins, waxes	0.1-0.2
Ash	0.1-0.15

Preparation of CNCs

The process of preparing CNCs was similar to the procedure described earlier.[S1] In brief, the pulp ground in a blender was subjected to treatment in sulfuric acid solutions of various concentrations (20–70 wt%) in an appropriate solvent at 50 °C for 2 h with vigorous stirring. The concentration of the pulp suspension was 0.025 g mL^{-1}. The reaction was carried out in a water bath using a flask equipped with a reflux condenser. After completing the treatment, the heating was stopped, and the reaction mixture was diluted 10-fold with ice-cold deionized water. The CNC suspension was left to settle overnight, followed by decantation of the supernatant. The suspension was then washed in successive cycles of centrifugation (3–5 times for 10 min at 8000 rpm) and removal of the supernatant, first with distilled water and then with the appropriate solvent. The final washing step involved treatment with water. Subsequently, the CNC aqueous suspension was treated with ultrasound (Sonorex DT100, Bandelin, Germany) for 15–30 min, followed by purification with ion exchange resin (TOKEM MB-50(R)) and a dialysis membrane (cut-off of 14 kDa, Roth, Germany) until a constant pH was reached.

Determination of CNC yield

The CNC yield was determined by the gravimetric method described elsewhere.[S1] The CNC aqueous suspension was left to stand at a temperature of 4 °C for one month, during which large particle aggregates with a small surface charge precipitated. The CNC suspension was separated from the sediment, and its volume was determined. Three parallel samples of a precisely measured volume were taken, poured into preweighed Petri dishes and air-dried until the weight was constant. Having thus determined the concentration of the suspension and knowing its volume, the total yield of the CNCs was calculated taking into account the initial pulp mass (under the assumption that all the water-soluble products had been removed at the stage of washing and dialysis). The relative error in determining the CNC yield in the three parallel samples did not exceed 3 %.

Characterization of CNCs

Transmission electron microscopy (TEM)
A JEOL JEM-1011 transmission electron microscope (Japan) with an 8.5 megapixel ORIUS SC1000 W digital camera, an acceleration voltage up to 100 kV, and an image resolution up to 0.3 nm was used to examine the shape and size of the CNC particles. A diluted CNC suspension (approximately 0.01 g dm^{-3}) was sonicated and then coated onto a copper TEM grid with a 200-mesh size. To improve the quality of the images, negative contrast was performed using uranyl acetate.

Fourier transform infrared spectroscopy (FTIR)
The FTIR spectra were obtained on a VERTEX 80v spectrophotometer (Bruker, Germany) in the frequency range of 4000-400 cm^{-1}. The samples were pressed into tablets containing 1 mg of the analyte and 100 mg of potassium bromide.

Dynamic light scattering (DLS)
The sizes of the CNC particles in the aqueous suspensions were measured by the dynamic light scattering method (DLS) (emission wavelength of 633 nm) on a Zetasizer Nano ZS device (Malvern Instruments Ltd., UK) operating in the range from 0.3 nm to 6 μm. The measurements were carried out in disposable polystyrene cuvettes at a 0.1 g dm^{-3} suspension concentration. During the measurements, the cuvette with the test sample was thermostated at a temperature of 20°C. The obtained particle size values are the results of averaging over five successive measurement cycles. The value obtained in each cycle is, in turn, the result of automatic processing of 10–15 measurements. The sizes of the CNC particles obtained by the DLS method are averaged values for the hydrodynamic diameters of equivalent spheres and do not reflect the real physical sizes of anisotropic rod-shaped CNC particles; rather, they are used for comparative analysis.
The surface charge of the CNC particles in an aqueous suspension was evaluated by the ζ-potential (Zetasizer Nano ZS). The obtained ζ-potential values were the results of averaging over five successive measurement cycles.

X-ray diffraction (XRD) analysis
X-ray diffraction analysis was carried out on a Bruker D8 Advance diffractometer according to the Bragg-Brentano scheme using Cu-*Kα* radiation (λ = 0.1542 nm). The angular scanning range was 2–45° with a 0.01° step. A Vantec-1 high-speed meter was used. The pulse acquisition time at each scanning point was 0.5 s. The CNC crystallinity index according to Segal[S2] was determined as

$$IC = (I_{200} - I_a)/I_{200},$$

where I_{200} is the intensity of the reflection corresponding to the crystallographic plane (200) and I_a is the intensity of the amorphous halo (the minimum between the peaks corresponding to crystallographic planes (200) and (110)).

Degree of cellulose polymerization (DP)
The degree of polymerization of the CNC samples was determined by the viscosity of the solutions in cadoxene, as described earlier.[S3]

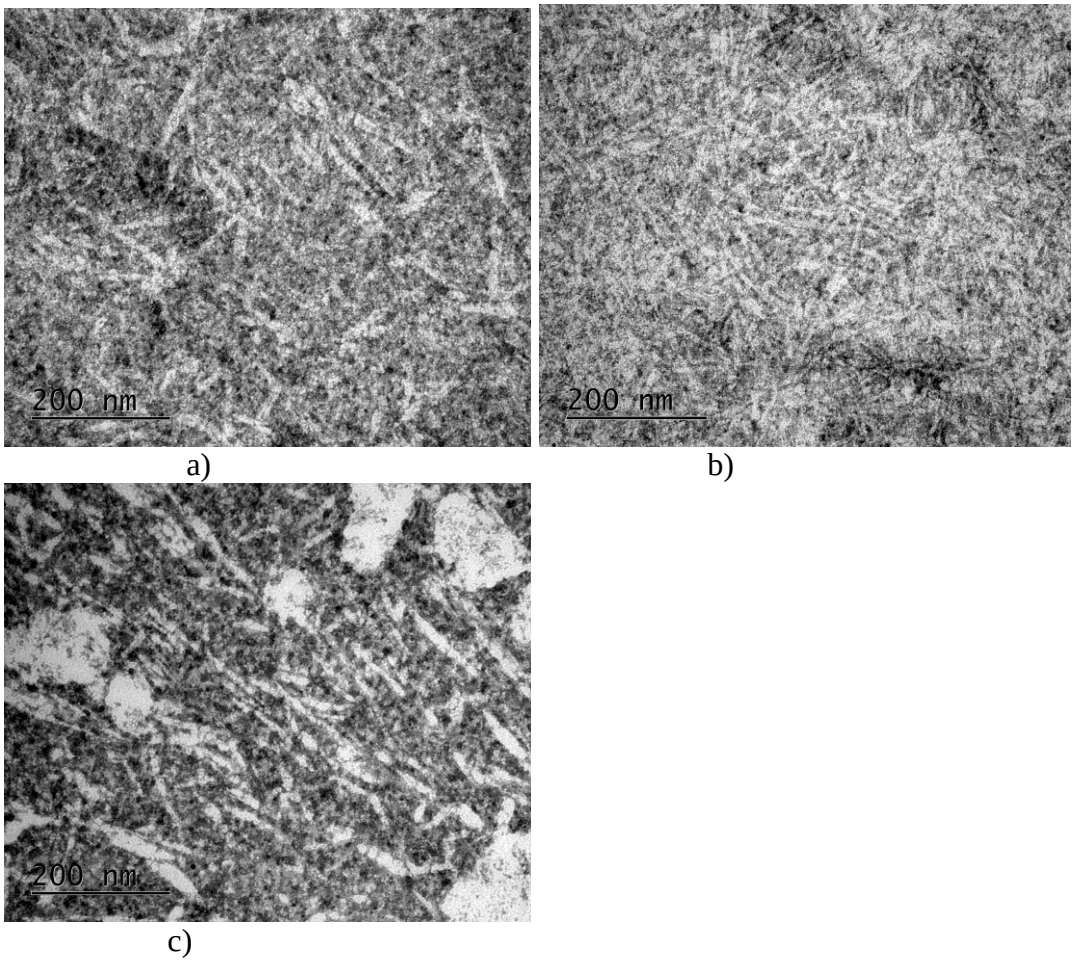

Figure S1 TEM-images of the CNC particles obtained through hydrolysis in water/aprotic solvent mixtures: (a) 1:3 water–MeCN, (b) 1:5 water–dioxane, and (c) 1:3 water–THF. The scale is 200 nm.

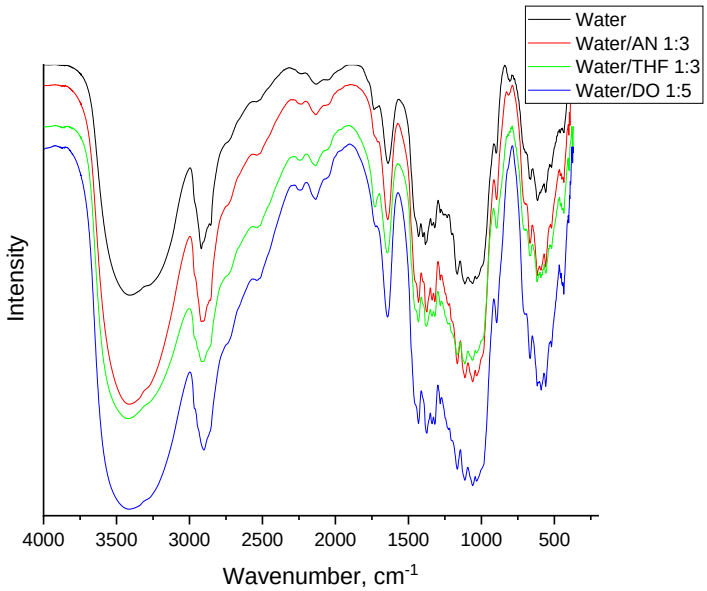

Figure S2 FTIR spectra of the CNC samples obtained in 1:3 water–MeCN, 1:3 water–THF, and 1:5 water–dioxane mixtures. For comparison, the FTIR spectrum of the CNC sample obtained by hydrolysis in water is shown.[S1]

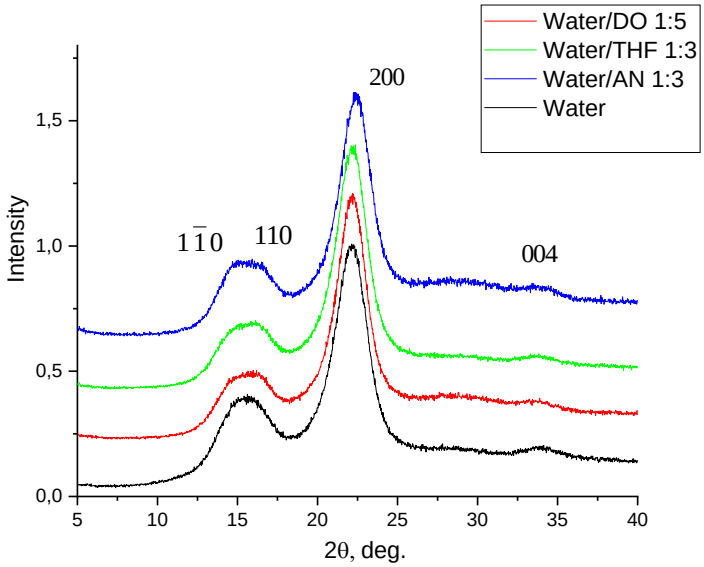

Figure S3 X-ray diffraction patterns of the CNC samples obtained in 1:5 water–dioxane, 1:3 water–THF, and 1:3 water–MeCN mixtures. For comparison, the X-ray diffraction pattern of the CNC sample obtained by hydrolysis in water is shown.[S1]

Table S2 Properties of CNCs obtained by hydrolysis in water and in water–aprotic solvent mixtures.

Medium	Properties of CNCs			
	Crystallinity index, %	Degree of polymerization	ζ-potential value, mV	CNC particle size, nm
1:5 water–dioxane	82±4	100±10	-40±2	150 ± 20
1:3 water–MeCN	81±4	110±10	-42±2	130 ± 20
1:3 water–THF	83±4	90±10	-41±2	140 ± 20
Water	80±4	80±8	-38±2	120 ± 20

References

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S2 L. Segal, J. J. Creely, A. E. Martin and C. M. Conrad, *Text. Res. J.*, 1959, **29**(10), 786; https://doi.org/10.1177/004051755902901003.

S3 N. V. Rubleva, E. O. Lebedeva, A. V. Afineevskii, M. I. Voronova, O. V. Surov and A. G. Zakharov, *ChemChemTech*, 2019, **62**(12), 85; https://doi.org/10.6060/ivkkt.20196212.5984.