Electronic Supplementary Information

Separation and characterization of cellulose nanocrystals by multi-detector asymmetric flow field-flow fractionation

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Figure S1: Transmission electron microscopy image of uranyl acetate stained CNCD-1, showing the characteristic needle-like shape. Image provided and reproduced with permission of the National Research Council - Canada.

Table S1. AF4 conditions and parameters evaluated in the optimization of analytical and semipreparatory fractionation of CNCD-1.

		Analytical fractionation	Semi-preparatory fractionation
	Membrane	PES and RC	RC
Channel	Membrane cut-off	10 kDa	10 kDa
parameters	Spacer	250 and 350 µm	490 μm
Mobile phase	NaCl	0.1, 1.0, and 5 mmol/L	1.0 mmol/L
	Elution time	2 min	0.5 min
Time	Focus time	2 min	0.5 min
parameters	Focus + Injection time	2, 3, and 4 min	1-3 min
	Focusing time	2, 3, and 4 min	1-3 min
	Injection volume	100 - 275 μL	100-400 μL
	Sample concentration	1 mg/mL	10 mg/mL
Sample quantities	Injection flow	0.2 mL/min	0.5 mL/min
and flow rates	Focus flow	1, 2, and 3 mL/min	1-3 mL/min
	Channel flow (V_c)	0.3, 0.4, and 0.5 mL/min	0.8-1.2 mL/min
	Cross flow (V_x)	0.1-1.0 mL/min	0.1-0.3 mL/min



Figure S2: Power calibration of sonicator. Calorimetric data, linear fits and corresponding calculated delivered (output) power for different operational settings (S), obtained using a Branson 450* analog disruptor with ¹/₄" solid titanium probe.

Off-line DLS and zeta potential measurements: additional details and discussion

^{*} The identification of any commercial product or trade name does not suggest endorsement or recommendation by the National Institute of Standards and Technology.

A 3-measurement mean value and standard deviation under repeatability conditions for D_h and PDI of each prepared sample were obtained at a scattering angle of 173° (backscatter optics). Folded capillary (FC; Malvern model# DTS1070) cuvettes, and a palladium dip cell (DC; Malvern model# Zen1002) in combination with polystyrene disposable cuvettes (PDC) were used to measure zeta potential, while size measurements were conducted in FCs, PDCs, and glass cuvettes (GC; PCS8501).

The comparative measurements of D_h , PDI, and zeta potential using different cuvettes and cells, as applicable, exhibited low variation and were very consistent with the values assigned for CNCD-1, thereby independently validating the NRC dispersion protocol. Results are summarized below in Figures S3-S5. The relative lack of variability in results obtained using different types of cells and cuvettes is germane for the practical use of CNCD-1 by different laboratories.

For context, the reported mean length and height/width for CNCD-1 CNCs (derived from the stock suspension) are, respectively, (76 ± 5) nm and (3.4 ± 0.8) nm by AFM, and 87 nm and 7.3 nm by TEM.¹ The 2-fold difference between the AFM height and TEM width of the imaged CNCs was attributed to lateral aggregation of individual CNCs.

Influence of rotational diffusion on DLS measurements

Because DLS data may be influenced by the rotational diffusion of rod-like CNCs, as noted by Guan et al. (2012), ² the issue is worth addressing. De Souza Lima et al. (2003)³ examined the diffusive dynamics of rod-like cellulose "whiskers" prepared by sulfuric acid hydrolysis of cotton and tunicate fibers, using vertically-polarized and depolarized angle-dependent DLS and transient electric birefringence. In this study, two diffusive modes were observed in all three measurements: fast and slow. This is demonstrated by linearity over a wide angular range in plots of the decay constant, Γ , versus q^2 (where q is the scattering vector magnitude proportional to $sin(\theta)$ and θ is the scattering angle).

The slow mode, associated with diffusion of the whiskers, was dominant (representing ≥ 90 % of the scattering intensity and upwards of 95 %) and concentration-independent (below about 0.27 mass % for cotton-derived). The faster mode was attributed to sample polydispersity (diffusion of shorter whisker fragments). The effective translational diffusion coefficient obtained from the slow mode in *depolarized* DLS was nearly identical to the slow mode-derived *vertically-polarized* DLS diffusion coefficient, demonstrating that the latter is principally representative of translational diffusion and hydrodynamic size over the angular range used; DLS measurements in the present study were vertically polarized (at a fixed angle of 173° or 99°, for off-line or on-line analysis, respectively) and mass concentrations were within the linear region observed by De Souza Lima et al. We conclude from this prior work that the DLS results obtained in the present study are largely reflective of the effective hydrodynamic size of CNCs.



Figure S3: Hydrodynamic diameter (D_h) obtained by off-line (batch mode) dynamic light scattering with cumulants analysis for 0.05 % mass fraction CNCD-1 in 5 mmol/L NaCl, prepared per the NRC Canada protocol as described in the main text. A) polystyrene disposable cells (PDC) and folded capillary cuvettes (FC), and B) glass cells (GC) and FC cuvettes. Error bars represent one standard deviation of the mean. Each sample represents an independently prepared dispersion of the dry CNC powder.



Figure S4: Polydispersity index (PDI) obtained by off-line (batch mode) dynamic light scattering with cumulants analysis for 0.05 % mass fraction CNCD-1 in 5 mmol/L NaCl, prepared per the NRC Canada protocol as described in the main text. Measured in A) polystyrene disposable cells (PDC) and folded capillary (FC) cuvettes, and B) glass cells (GC) and FC cuvettes. Error bars represent one standard deviation of the mean. Each sample represents an independently prepared dispersion of the dry stock CNC powder.



Figure S5: Zeta potential measurements of 0.05% CNC in 5 mmol/L NaCl using the dip cell (DC) and folded capillary (FC) cuvettes. Error bars represent one standard deviation of the mean of replicate measurements. Each sample represents an independently prepared dispersion of the dry stock CNC powder. The pH of suspensions was 5.0 ± 0.1 .



Figure S6: Plot of rms radius (R_g) as a function of hydrodynamic radius (R_h) for 0.1 % mass fraction CNCD-1 dispersion in 1 mmol/L NaCl, measured on-line post-fractionation by AF4. The mean value of the shape factor ($\rho = R_g/R_h$) was determined from the slope of a linear fit to the data. The slope, in this case, is equivalent to the arithmetic mean of all data points.



Figure S7: Comparison of rod lengths calculated using the cylindrical form factor model in Astra to those calculated using the simple relationship $R_g^2 \approx L^2/12$, as a function of retention time for 0.1 % mass fraction CNCD-1 in 1 mmol/L NaCl, Lengths are overlaid on the light scattering trace measured at 90°.



Figure S8. Variation of M_w (Da) and R_h with retention time during AF4 fractionation. F1-12 represent fractions collected at 2 min intervals for comparative purposes (see Table 1 in main text). The solid black line represents the dRI trace.



Figure S9: Semi-preparative method: Plot of R_g as a function of R_h for 1% CNC dispersion in 1 mmol/L NaCl. The average value of the shape factor ($\rho = R_g/R_h$) was calculated from the slope of the graph. The slope, in this case, is equal to the arithmetic mean of all data points.

References

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