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Nanocellulose: characterization and properties

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Learning objective

- Ability to address and recognize the main challenges in nanocellulose characterization

- Readiness to select a correct analytical technique for determining a certain feature in nanocellulose

- Awareness of the main properties of nanocellulose and what dictates them

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Outline

(1) Nanocellulose – recap and main practical difference
(2) Chief challenges in characterisation of nanocellulose
(3) Chemical and physical properties and their characterization

- Dimensions
- Chemical analysis
- Charge
- Degree of polymerisation
- Rheology (flow properties)
- Mechanical properties

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Nanocellulose - recap

Cellulose nanofibres (CNF)

Carboxylate content 1.5 mmol/g

100 nm
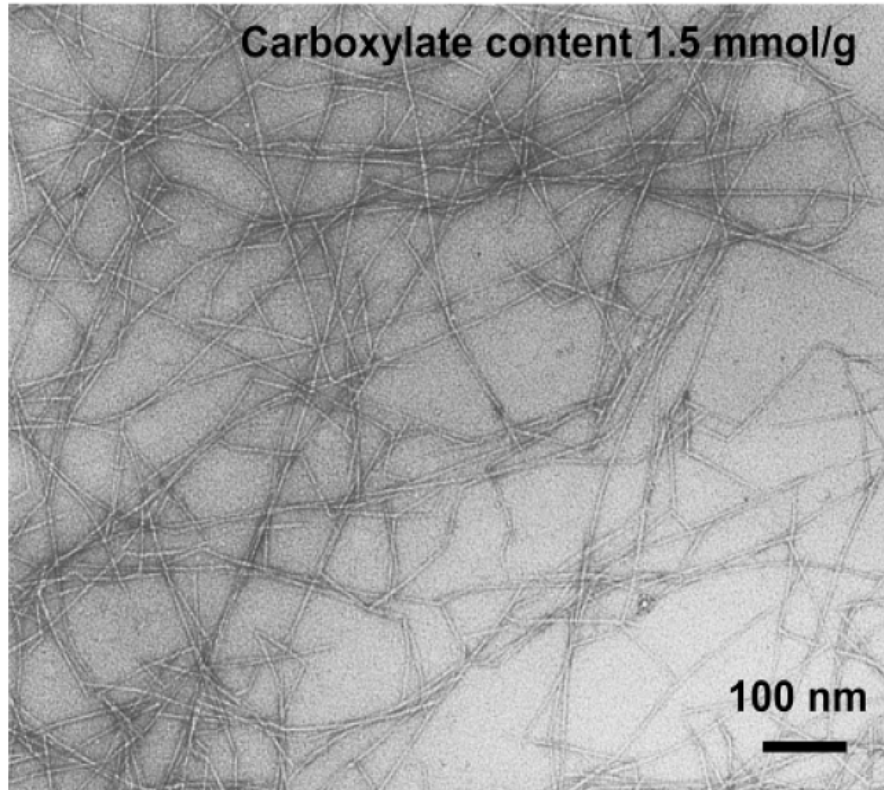
- Long threads of isolated cellulose microfibrils
- Very high aspect ratio
- Length: 0.5-5 µm
- Width: 3-50 nm
- Highly charged when prepared by chemical isolation with TEMPO-oxidation
- Low charge density when prepared with mechanical isolation

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Cellulose nanocrystals (CNCs)

1 µm
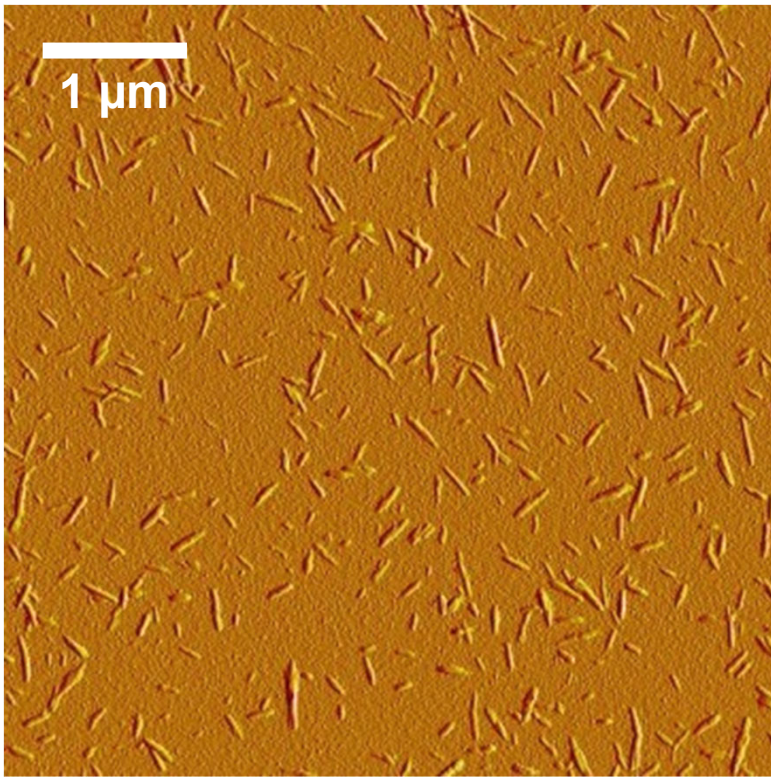
- Rigid rods of crystalline cellulose
- Length: 50-1000 nm
- Width: 3-20 nm
- Usually charged with sulphate groups on the crystal surface
- Suspensions form spontaneously chiral nematic liquid crystal phases

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Basic practical difference: CNF *vs.* CNC

Cellulose nanofibres
(CNF)
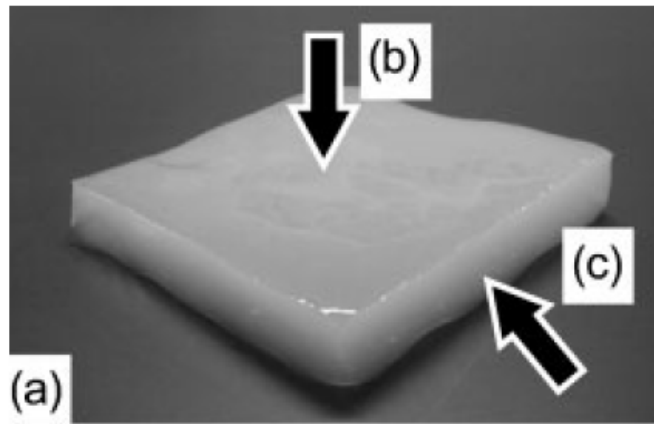
After preparation: gel

Cellulose nanocrystals
(CNC)

After preparation: suspension
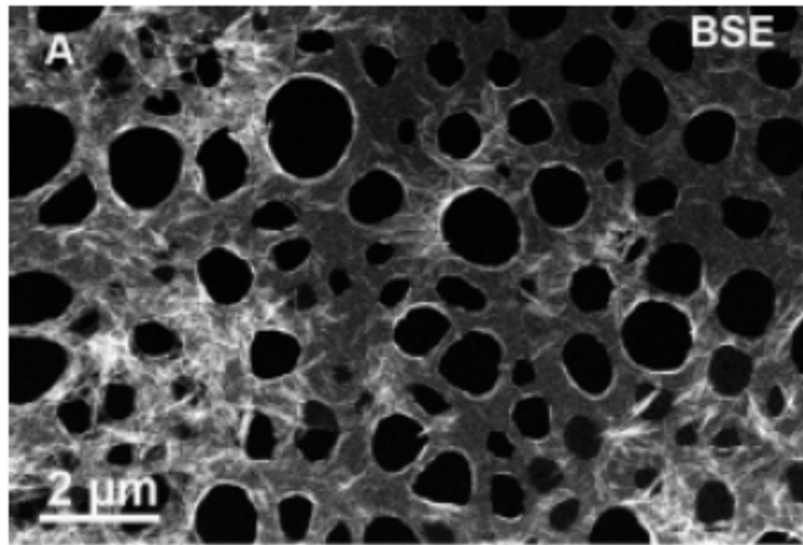
Chief practical challenges in characterisation of nanocellulose

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Main challenge: small size

- Optical microscopy is diffraction limited (wavelength of light)
- Even most Scanning Electron Microscopes (SEM) cannot image nanocellulose
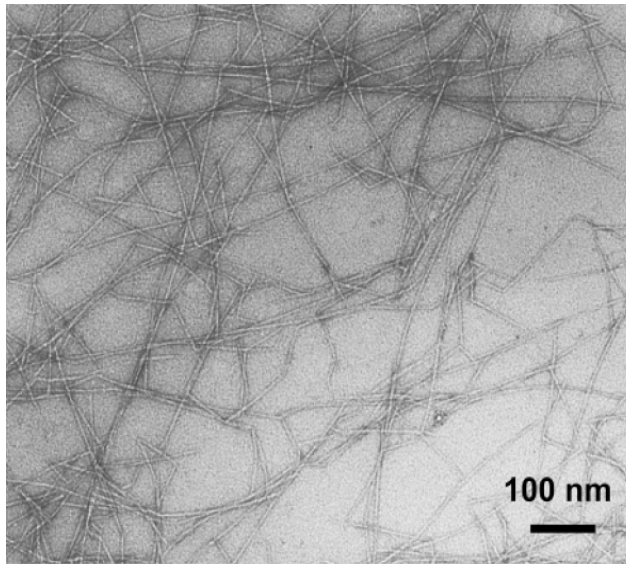

Note: Holes in the image are artefacts of the substrate

Image of cellulose nanocrystals by SEM – not proper resolution

Main challenge: small size

- Transmission Electron Microscopy (TEM) is generally required to yield informative and reliable images of nanocellulose
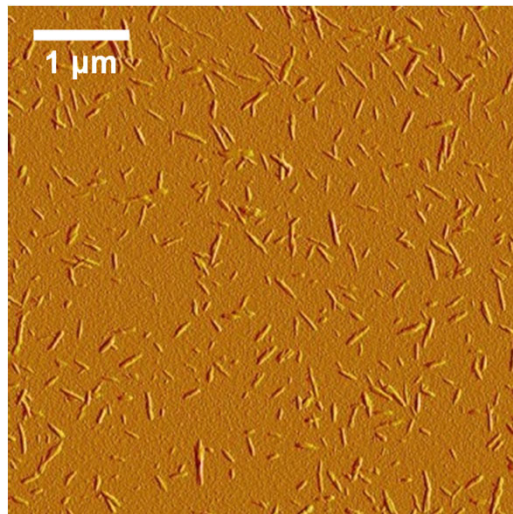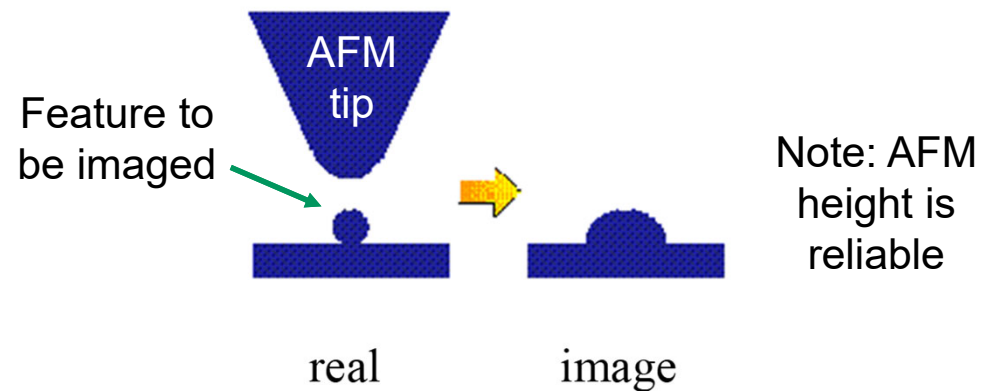

100 nm

CNF by TEM

CNCs by TEM

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Main challenge: small size

- Atomic Force Microscopy (AFM) is able to image nanocellulose
- However, the width of nanocellulose is so small that it gets exaggerated by the scanning tip which is roughly the same size
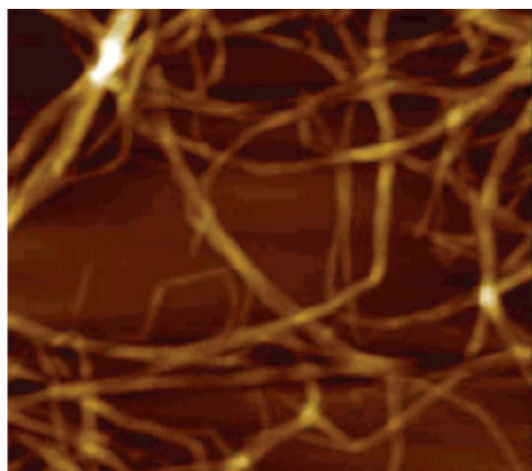
1 µm

AFM tip

Feature to be imaged

real

image

Note: AFM height is reliable

AFM image of CNCs

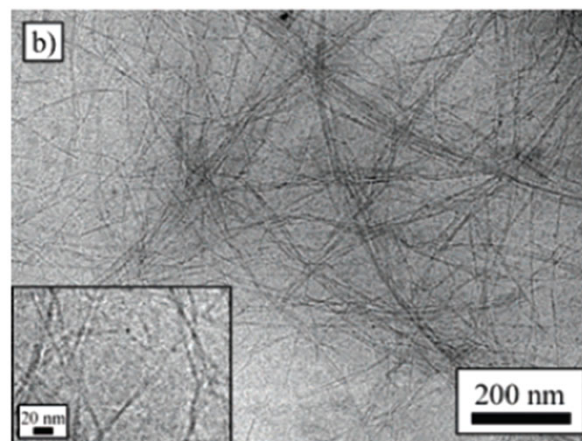Schematic representation of AFM tip exaggeration

AFM *vs.* TEM

AFM image of cellulose
nanofibres (CNF)

Fibril width: 10-30 nm

Cryo-TEM image of
cellulose nanofibres (CNF)

Fibril width: 5-10 nm

TIP EXAGGERATION IN AFM YIELDS UNRELIABLE WIDTHS FOR CNF

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Another challenge: high aspect ratio

- High aspect ratio of CNF means that whole nanofibres are difficult to image
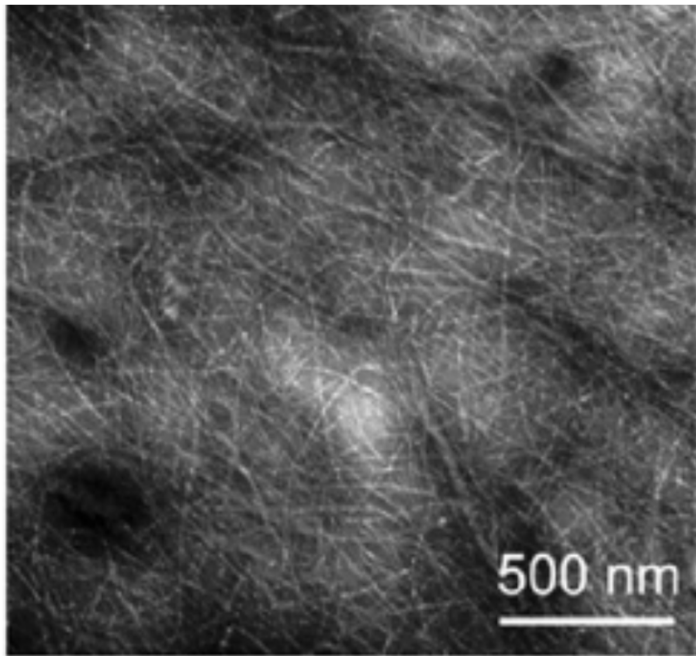
- High resolution is required to image the nanofibrils
- High resolution requires "zooming-in"
- → Width of CNF is easy to observe but entire nanofibrils do not fit into the image
- → Length cannot usually be determined

Another challenge: high aspect ratio

- Length of CNF can be deduced by imaging a string of TEM images but this is laborious and it is difficult to gain proper statistical data
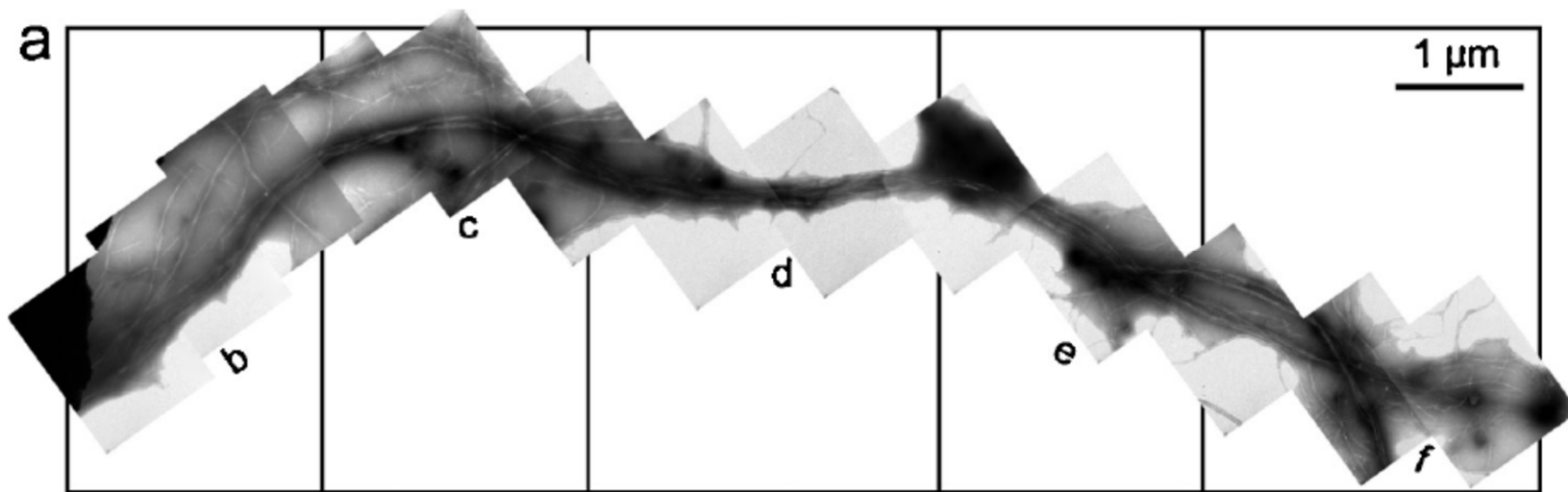
String of TEM images of a 12.7 µm long cellulose nanofibril

Main challenge: small size

- Mechanical properties can be measured from nanocellulose networks (e.g., nanopaper), but it is very difficult to measure them for individual CNF or CNC
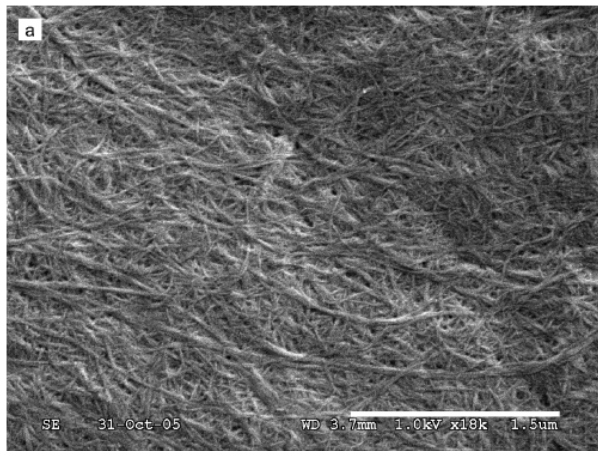

CNF network (nanopaper)

Individual nanofibrils

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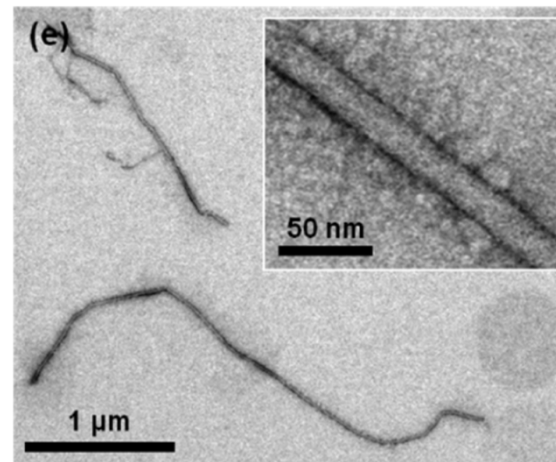
Main challenge: small size

Elastic modulus of individual cellulose nanofibril/nanocrystal by Atomic Force Microscopy (AFM)
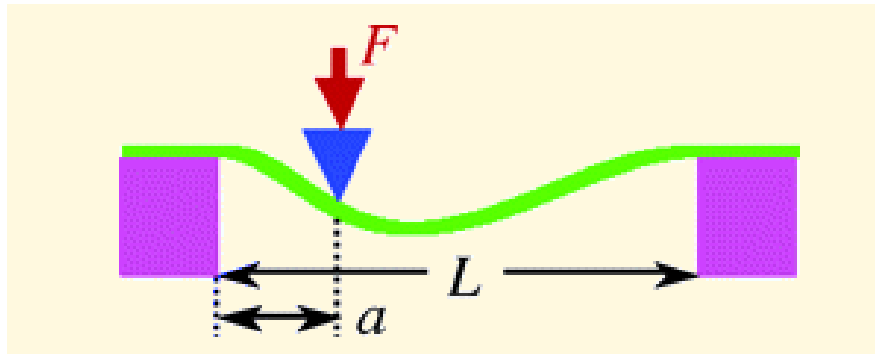
- the force (F) exerted at location a by the cantilever (through the AFM tip) causes deformation in the sample
- the deformation can be monitored through cantilever deflection
- the deformation at a known force is proportional to the elastic modulus

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Main challenge: small size

$\bar{\sigma}$: 2–6 GPa
Solvent flow
Imploding cavitation bubble
Tensile stress
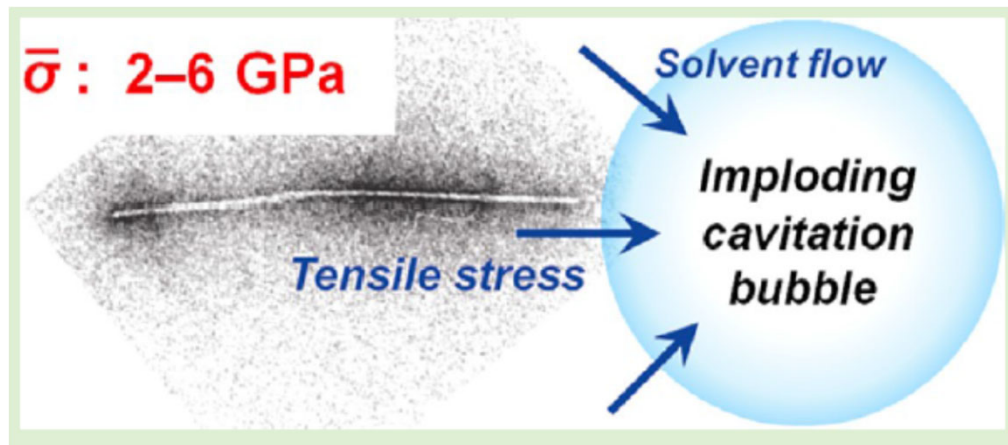
- Individual nanofibres is so small that it cannot be applied for tensile testing
- Breaking the nanofibres to smaller fragments by cavitation (ultrasonics) can be used to implicitly yield the tensile strength of CNF with mathematical models
- The acquired tensile strength (2-6 GPa) is close to that of steel

Another challenge: gel-like nature of CNF

- CNF gels at very low concentrations (often at 0.5-1.0 w-%)
→ Solution based techniques (e.g., titration) require substantial dilution
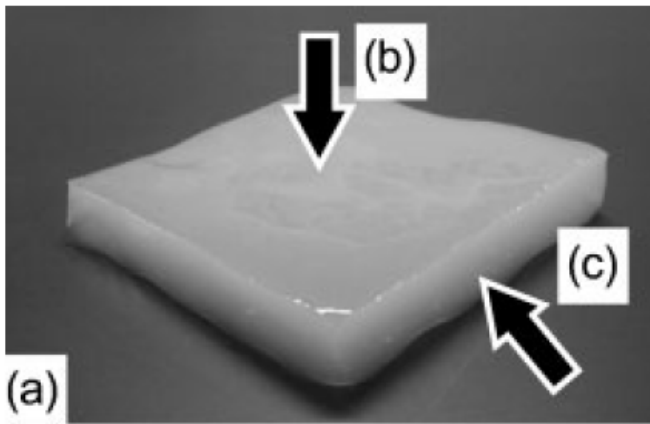
Properties: Dimensions

Dimensions of cellulose nanocrystals

Table 1. Examples of the Length (L) and Width (w) of CNs from Various Sources Obtained by Different Techniques

source	L (nm)	w (nm)	technique	ref
bacterial	100−1000	10−50	TEM	105
	100−1000	5−10 × 30−50	TEM	80, 104
cotton	100−150	5−10	TEM	127
	70−170	~7	TEM	128
	200−300	8	TEM	129
	255	15	DDL	119
	150−210	5−11	AFM	117
cotton linter	100−200	10−20	SEM-FEG	97
	25−320	6−70	TEM	79
	300−500	15−30	AFM	130
MCC	35−265	3−48	TEM	79
	250−270	23	TEM	101
	~500	10	AFM	100

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Dimensions of cellulose nanocrystals

Table 1. Examples of the Length (L) and Width (w) of CNs from Various Sources Obtained by Different Techniques

source	L (nm)	w (nm)	technique	ref
ramie	150−250	6−8	TEM	81
	50−150	5−10	TEM	131
sisal	100−500	3−5	TEM	82
	150−280	3.5−6.5	TEM	92
tunicate		8.8 × 18.2	SANS	118
	1160	16	DDL	119
	500−1000	10	TEM	107
	1000−3000	15−30	TEM	132
	100−1000	15	TEM	129
	1073	28	TEM	79
Valonia	>1000	10−20	TEM	125
soft wood	100−200	3−4	TEM	95, 113
	100−150	4−5	AFM	96
hard wood	140−150	4−5	AFM	96

According to recent, established knowledge the most common CNC dimensions:
- Cotton-based: w 6-7 nm; L 50-300 nm
- Wood-based: w 3-4 nm; L 50-150 nm

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Chem. Rev. **2010**, *110*, 3479.

Dimensions of cellulose nanocrystals
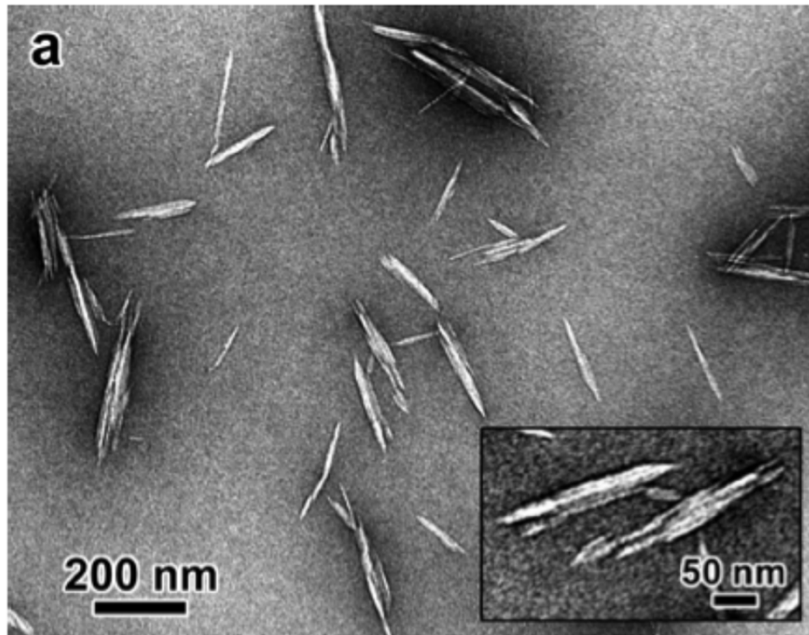
TEM image of cotton-based CNCs

Some nanocrystals do not bear the width of individual microfibrils but they consist of aggregates of several crystallites.

This is not an artefact of drying (the figures have been taken by cryo-TEM directly from a vitrified dispersion).

→ CNCs are not necessarily single crystals of cellulose

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Dimensions of cellulose nanofibres

- Depends heavily on the preparation method
- Mechanically isolated CNF is wider and more heterogeneous in width as compared with CNF prepared by TEMPO-mediated oxidation
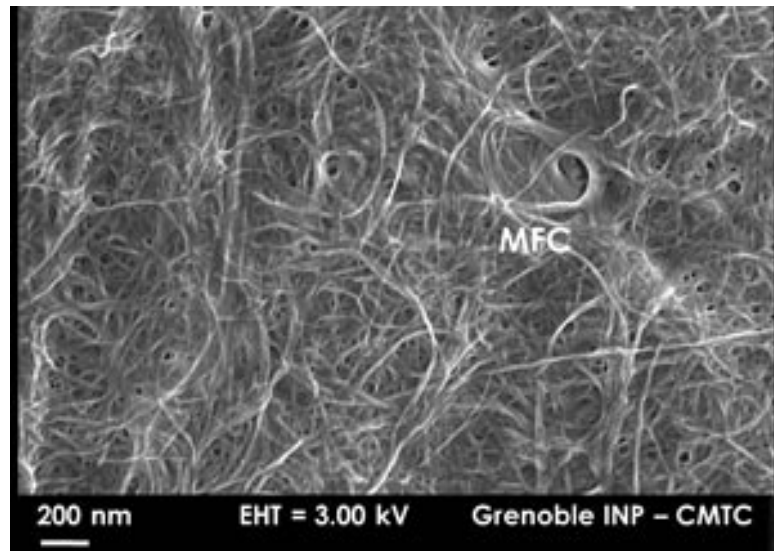
Mechanically ground CNF
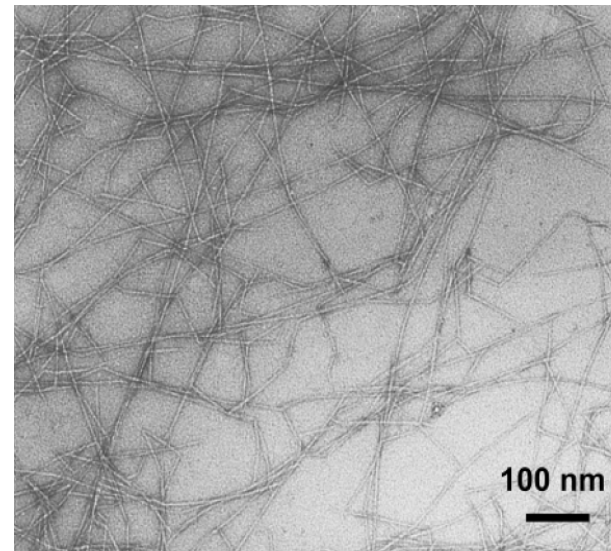
TEMPO-oxidized CNF

Dimensions of cellulose nanofibres

The starting material affects the CNF width in the same manner as it does with CNCs but is nearly always made from wood-based materials (mainly pulp)

wood
pulp

cotton

tunicate

bacterial
cellulose

Saito et al. *Biomacromolecules* **2006**, 7, 1687.
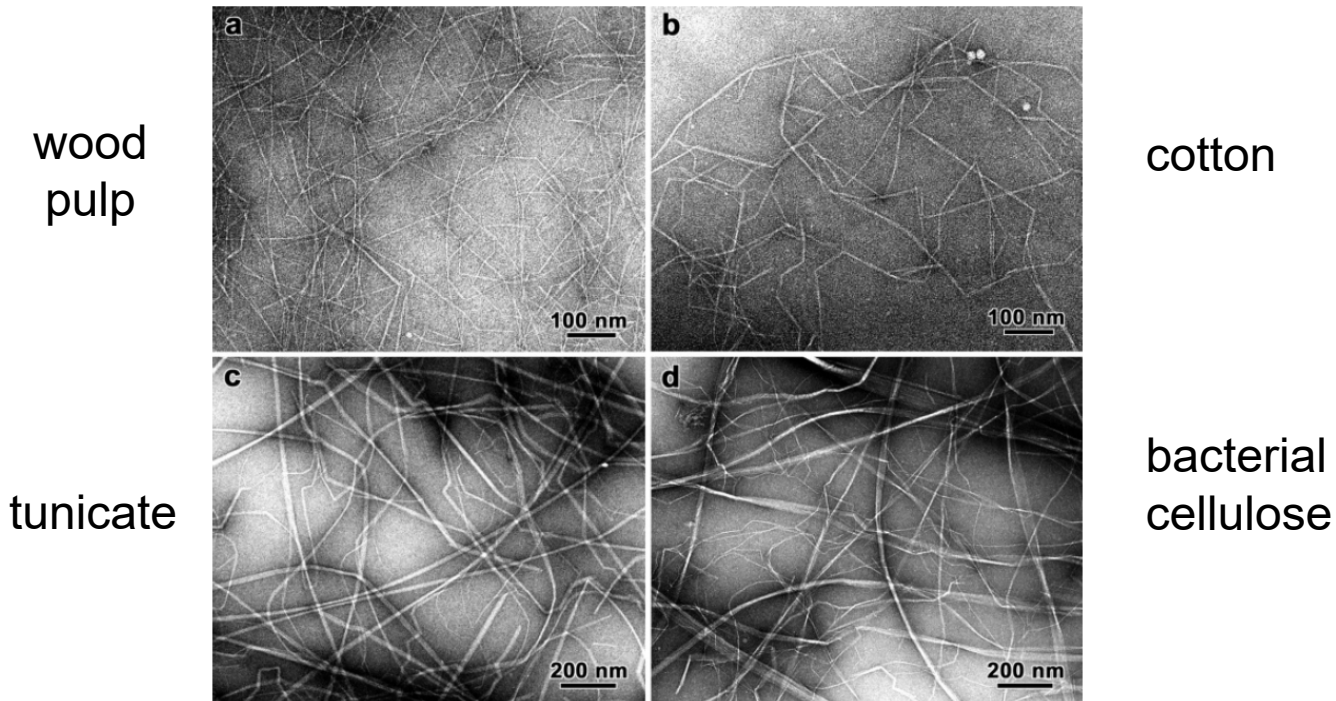
Dimensions of cellulose nanofibres

Important points of notice

- If a CNF or CNC is truly individualized, they bear the width of the original (non-aggregated) microfibril in the plant cell wall

- In essence, CNCs are like shorter, rigid fragments of CNFs

Chemical composition

Points on chemical analysis of nanocellulose

- Ideally (or in a simplified presentation), nanocellulose consists of pure cellulose
- The only exception of pure cellulose is usually charge on the surface (usually sulphate on CNC, and carboxylate on CNF)
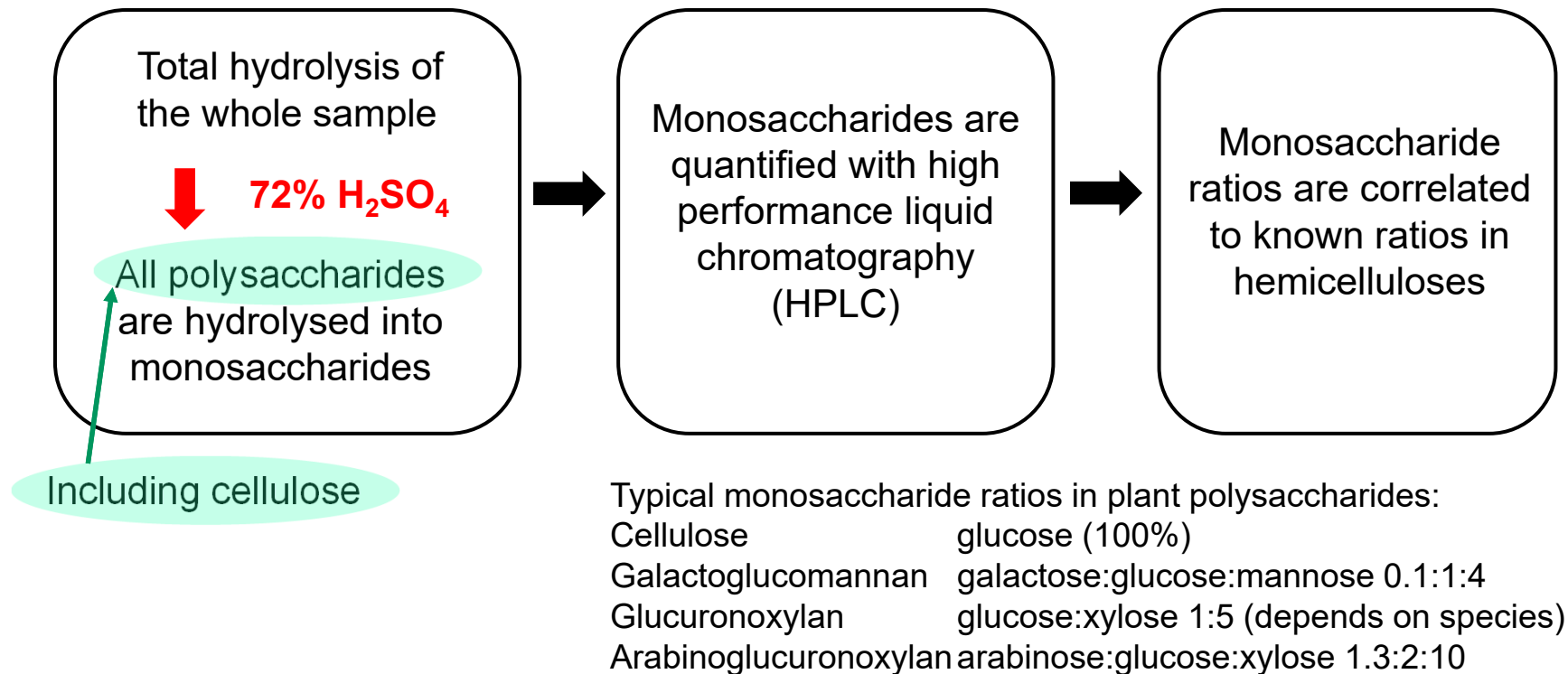- In reality, however, CNF usually contains significant portions of hemicellulose (often from 5% to even 20%) because of wood-based origin
- The hemicellulose content is not often characterized or even addressed in literature
- Trace amounts of lignin may also remain in CNF or even CNC; this is addressed very rarely in literature

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Hemicellulose analysis for CNF

Total hydrolysis of the whole sample

⬇ **72% H_2SO_4**

All polysaccharides are hydrolysed into monosaccharides

Including cellulose

→

Monosaccharides are quantified with high performance liquid chromatography (HPLC)

→

Monosaccharide ratios are correlated to known ratios in hemicelluloses

Typical monosaccharide ratios in plant polysaccharides:

Cellulose	glucose (100%)
Galactoglucomannan	galactose:glucose:mannose 0.1:1:4
Glucuronoxylan	glucose:xylose 1:5 (depends on species)
Arabinoglucuronoxylan	arabinose:glucose:xylose 1.3:2:10

Hemicellulose content of CNF

Example of monosaccharide composition in CNF prepared from Birch kraft pulp
Glucose – 73%
Xylose – 25%
Mannose – 1%
Methyl glucuronic acid – 0.6%

- Glucose is mainly from cellulose
- Hemicelluloses in birch are glucuronoxylan and glucomannan
- Xylose and methyl glucuronic acid are exclusively from glucuronoxylan
- Mannose is exclusively from glucomannan

NOTE: This CNF sample contains an exceptionally high amount of hemicellulose.

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Hemicellulose in NFC

- Hemicellulose resides always on the surface of the nanofibrils (it cannot be inside the crystallite)
- Hemicellulose has a genuine effect on the surface properties of the nanofibrils (dispersion properties, bonding between nanofibrils in a nanopaper network etc.)

	Ultimate tensile strength (MPa)	Young's modulus (GPa)	Strain-to-failure (%)	Yield strength (MPa)	
NFC	199 ± 35	10.0 ± 3.1	5.24 ± 0.6	113 ± 22	← Containing xylan
NFC–Xyl[a]	243 ± 38	13.1 ± 1.9	4.81 ± 0.3	126 ± 24	← Not containing xylan

a) NFC treated with xylanase enzyme (xylan has been removed)

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Charge

Points on nanocellulose charge determination

- Charge on CNCs is very often from sulphate groups (sometimes, if the CNCs have been TEMPO-oxidized, they bear a high number of carboxylic groups)

- Charge on CNF is very often from carboxylic groups
    - Mechanically ground CNF has methyl glucuronic acid from xylan
    - TEMPO-oxidized CNF has a high amount of carboxylic groups from the oxidation of primary alcohols on the CNF surface
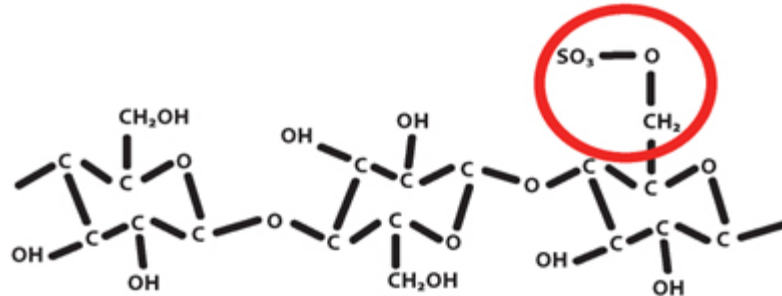
Cellulose nanocrystals: charge
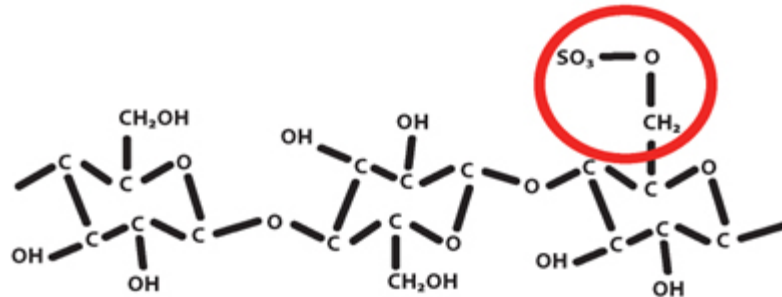
- Charge consists of sulphate groups on the CNC surface, originating from the preparation method with sulphuric acid
- Two predominant ways to determine the charge:
  - Elemental analysis of sulphur
  - Conductometric titration

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Cellulose nanocrystals: charge

- Charge content of cotton based nanocrystals is usually around 200-400 μmol/g, amounting to around 0.3-0.7 e/nm^2 (e – elementary charge)
- Sulphate groups plays a big role in CNCs
  - Stability of the CNC dispersion is brought in by charge repulsion
  - Many functionalities of CNCs are dictated by their charge
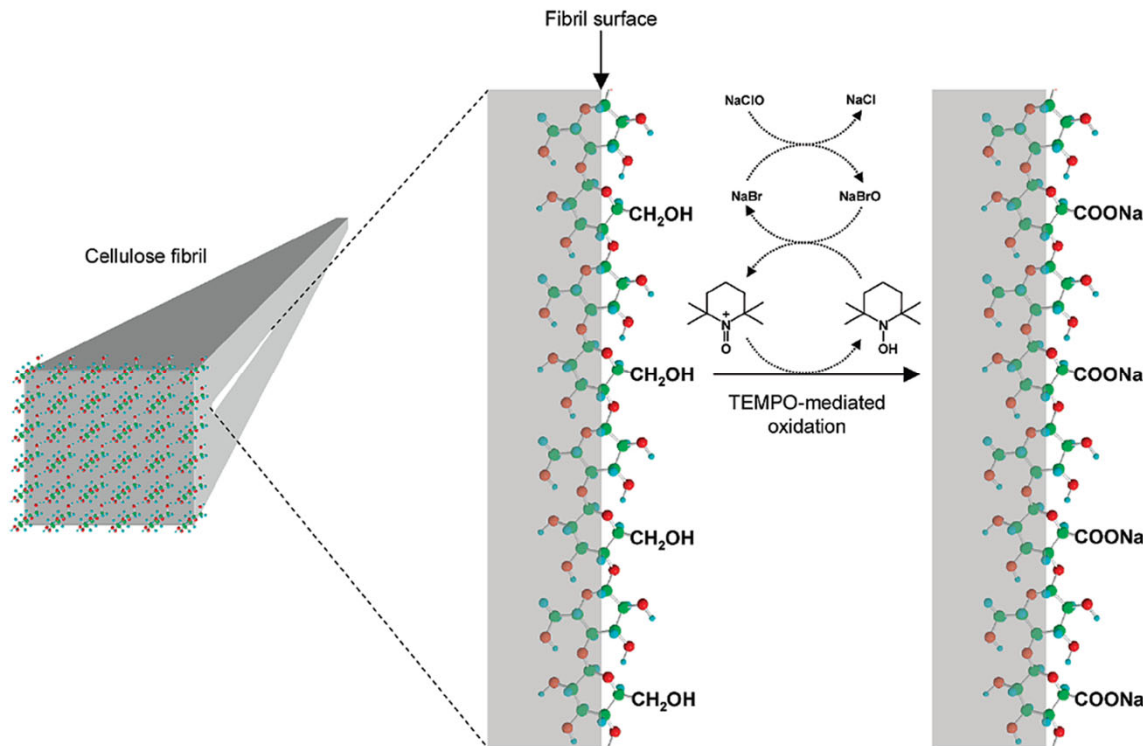
Cellulose nanofibres: charge

- With CNF prepared from untreated pulp, the charge stems predominantly from xylans on the surface

- Sometimes pulp is pre-treated with, e.g., carboxymethylation, resulting in increased charge in the eventual CNF

- With TEMPO-oxidized CNF, the charge originates predominantly from primary alcohols that have been oxidized to carboxylic acid groups

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Mechanically prepared CNF: charge

- Mechanically prepared CNF is usually prepared from hardwood pulp and the charge originates from xylan on the CNF surface (methylglucuronic acid or hexenuronic acid groups)

- Charge content of mechanically prepared CNF from hardwood is usually in the order of 50-100 µmol/g

- Although the charge is fairly small, it is important for CNF preparation and its dispersion / rheological properties

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TEMPO-oxidized CNF: charge

Fibril surface

Cellulose fibril

NaClO → NaCl
NaBr ⇄ NaBrO

TEMPO-mediated oxidation

CH$_2$OH → COONa

- Originates from carboxylic groups on the CNF surface, brought in by oxidation of primary alcohols of cellulose
- Charge content usually 1.0-1.5 mmol/g for wood-based CNF

TEMPO-oxidized CNF: charge
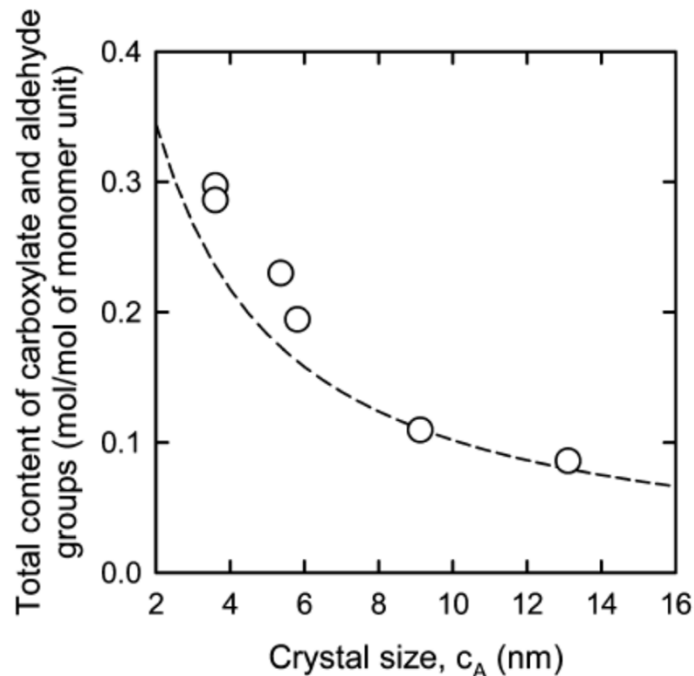
- When the primary hydroxyls are totally oxidized, the charge content depends on the size of the cellulose microfibril (source of cellulose)
- Larger microfibrils have less surface
- → CNF from larger microfibrils possess less charge

Degree of polymerization

Important points on degree of polymerization (DP) in nanocellulose

- DP of cellulose in plants is usually in the order of 6000-10,000

- After pulping and bleaching, cellulose DP is often reduced to 1000-1500

- Mechanical CNF preparation from pulp is not generally considered to reduce the DP of cellulose further but new data shows otherwise

- TEMPO-oxidation is known to reduce the cellulose DP further

- Gel permeation chromatography is the proper way to assess cellulose DP in TEMPO-oxidized CNF; standard viscosity is misleading: cuen dissolution breaks up cellulose

TEMPO-oxidation and DP of cellulose

- TEMPO-mediated oxidation of cellulose reduces DP, especially with regenerated cellulose grades
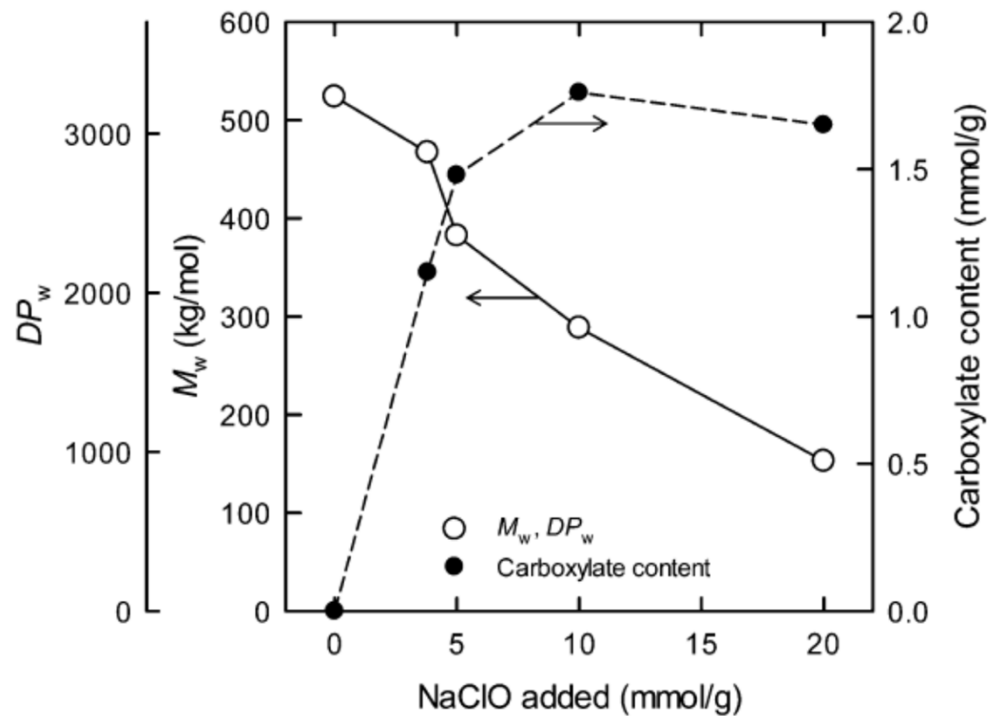- At neutral conditions in a TEMPO/NaClO/NaClO$_2$ system, the DP reduction is minimized
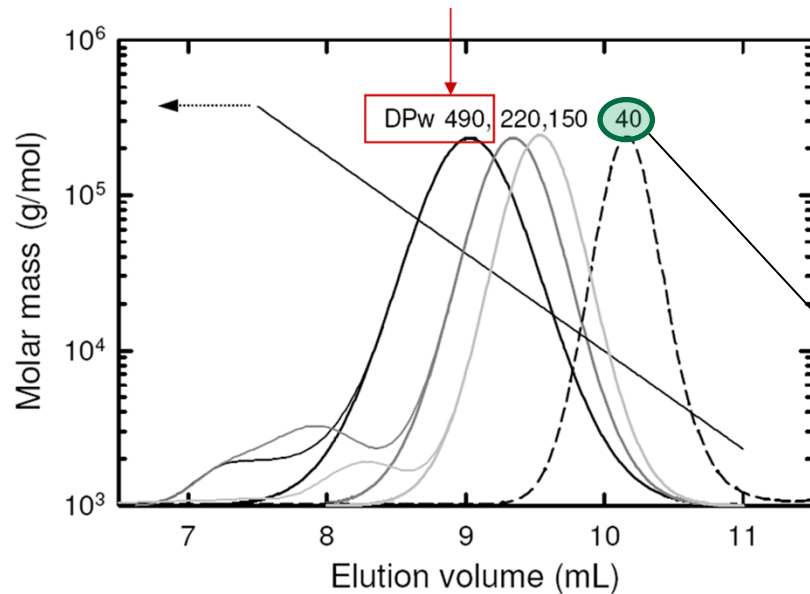
Original DP: **680**

DP after TEMPO/NaClO/NaClO$_2$ (oxidation for 3 days, pH 5.8): **490**
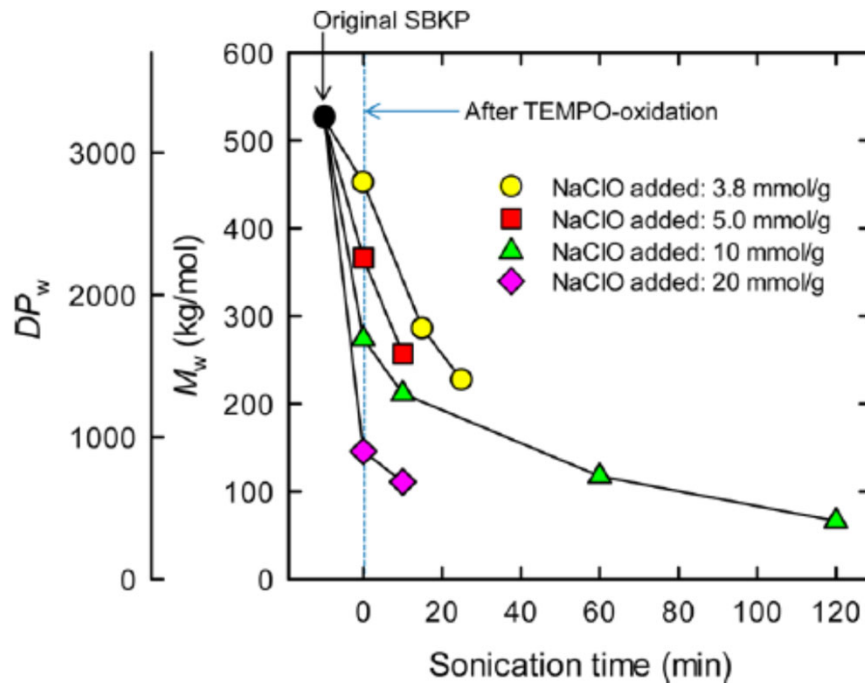
DP after TEMPO/NaBr/NaClO (2 hours, pH 10): **40**

Note: these results are not for CNFs but for regenerated cellulose to emphasize the loss in DP

Hirota et al. *Carbohydr. Polym.* **2009**, *78*, 330.

TEMPO-oxidation of cellulose: DP

- NaClO (hypochlorite) and alkaline conditions are detrimental to cellulose in terms of DP

TEMPO-oxidation coupled with mechanical treatment: DP

- TEMPO-oxidation with varying hypochlorite doses coupled with mechanical (ultrasonic) treatment shows a dramatic reduction in cellulose DP
- The results report only ultrasonication but the DP reduction may well occur with other mechanical treatments as well

Rheology

# Important points on rheological analysis of nanocellulose dispersions

- Hundreds of studies exist

- CNF is usually a gel

- CNCs usually form a fluid (running) suspension

- Rheological properties are very important for many nanocellulose applications, particularly those of CNF

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CNF and CNC comparison

[η]: intrinsic viscosity of aqueous nanocellulose dispersion

ρ: density of nanocellulose

p: aspect ratio of nanocellulose

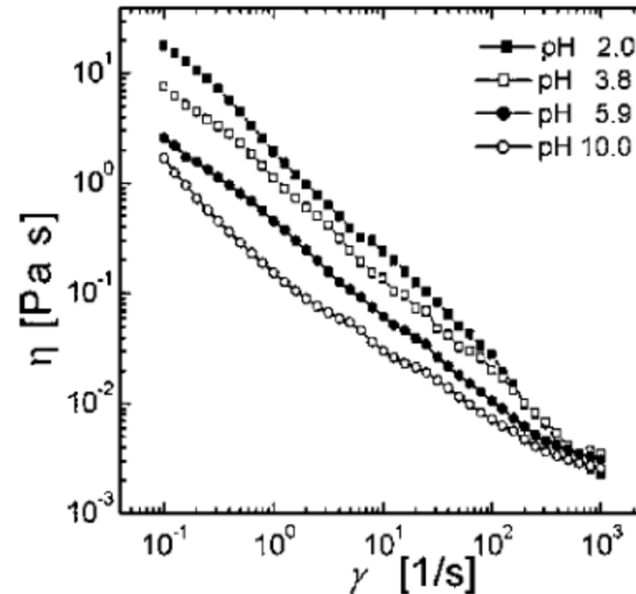$$\rho\,[\eta] = 0.15 \times p^{1.9}$$

CNF

CNCs
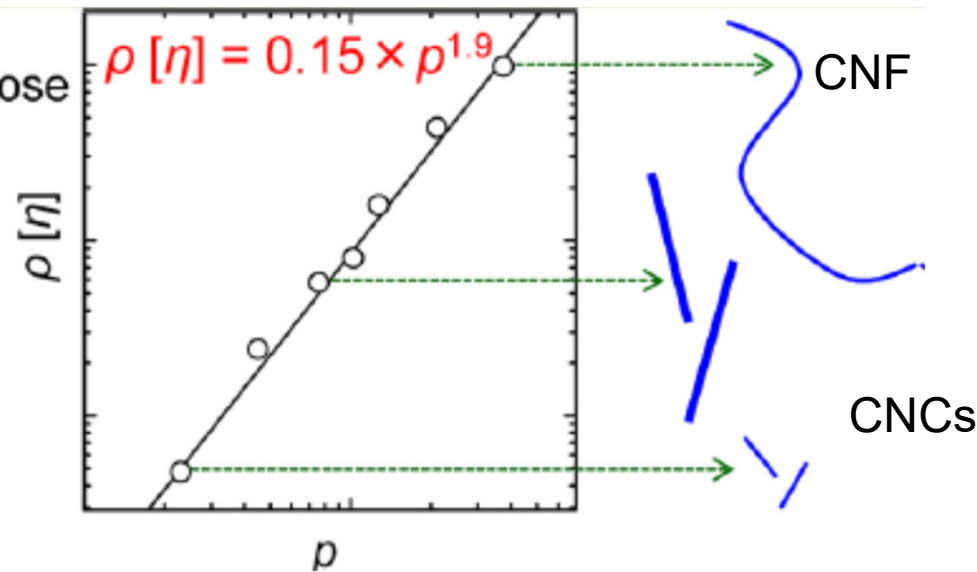
- Viscosity of aqueous nanocellulose dispersions can be expressed as a function of aspect ratio of nanocellulose

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Viscosity of CNF gels
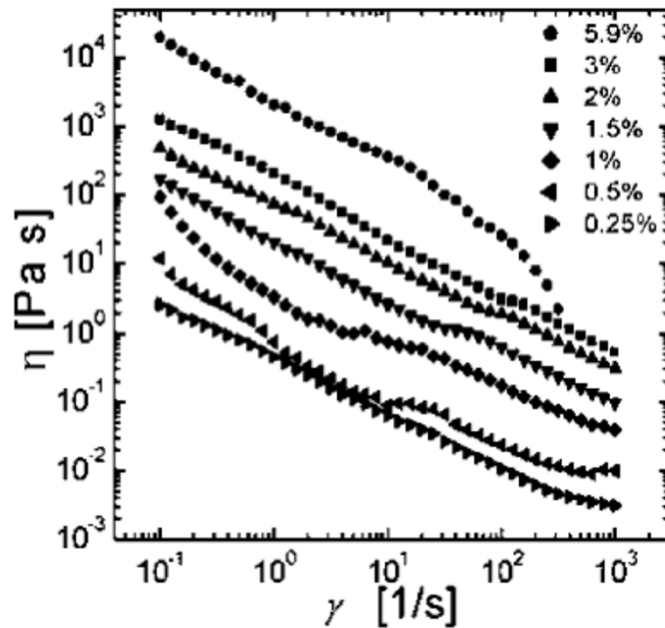
Effect of increased shear rate on viscosity: shear thinning

Effect of pH on viscosity: increased pH → more charge → more repulsion between fibrils → lower viscosity

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Mechanical properties

Important points on mechanical properties of nanocellulose

- Cellulose fibril and especially cellulose crystal is very strong with very high modulus

- However, the strength and modulus of a macroscopic nanocellulose network is not on par with the qualities of individual fibrils of crystals

- Mechanical properties of CNF are particularly surveyed: very high aspect ratios with high entanglement are optimal for strong networks (nanopaper)
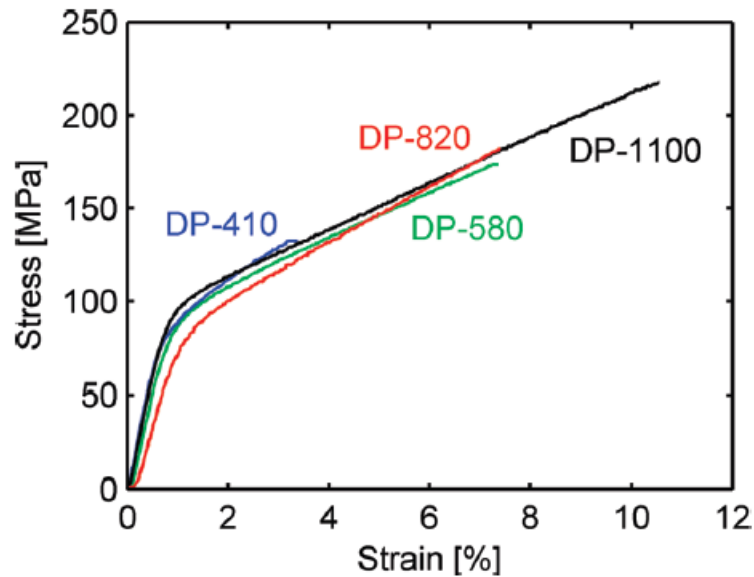
CNF nanopaper

Figure 4. Typical stress–strain curves for MFC films prepared of MFC with different DP.

- Nanopapers prepared from mechanically ground CNF

- Ultimate strength depends on the DP of cellulose

Henriksson et al. *Biomacromolecules* **2008**, 9, 1579.

CNF nanopaper

Work of fracture (toughness) with nanopapers is very high

Table 2. Physical and Average Mechanical Properties for MFC Films Prepared of MFC with Different DP[a]

material	porosity [%]	modulus, E [GPa]	slope in the plastic region, n [GPa]	yield stress, $\sigma_{0.2}$ [MPa]	tensile strength, σ_c [MPa]	strain-to-failure, ϵ_c [%]	work to fracture, W_A [MJ/m^3]
DP-410	20	13.7 (0.3)	-[b]	81.5 (4.7)	129 (8.7)	3.3 (0.4)	3.0 (0.5)
DP-580	24	10.7 (1.2)	1.27 (0.13)	83.6 (2.1)	159 (16.4)	6.4 (1.7)	7.1 (2.5)
DP-820		10.4 (0.5)	1.50 (0.07)	83.6 (2.8)	181 (12.7)	7.4 (1.5)	9.1 (2.3)
DP-1100	28	13.2 (0.6)	1.28 (0.16)	92.2 (5.2)	214 (6.8)	10.1 (1.4)	15.1 (1.9)

[a] The values in parentheses are the sample standard deviations. [b] Due to low ϵ_c, the plastic region is limited and this value can not be calculated.

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Individual CNFs vs. NFC network

Individual NFC
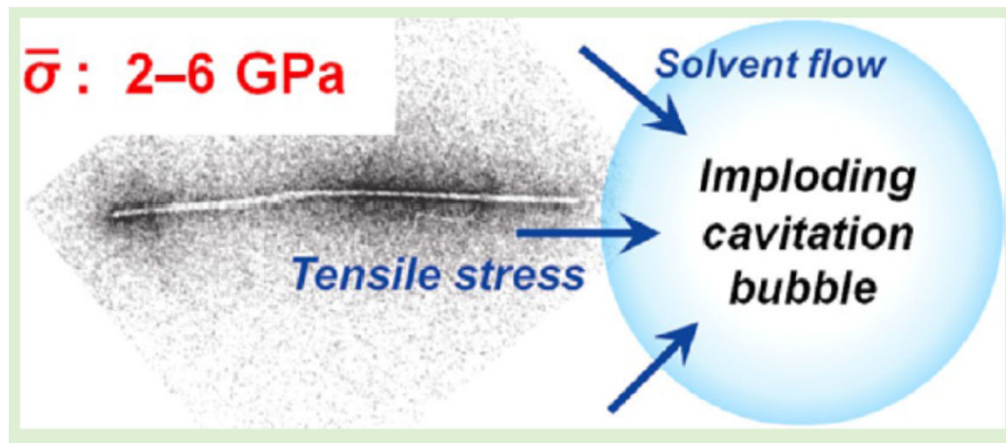
CNF network

tensile strength, σ_c [MPa]
129 (8.7)
159 (16.4)
181 (12.7)
214 (6.8)

Summary

- Predominantly: CNF – gel; CNCs – running suspension

- Small size (width) of nanocellulose provides the biggest challenges to the characterisation of its dimensions and individual tensile properties

- Charge of nanocellulose is important for the dispersion properties

- Rheological properties are mainly dictated by size (length) and charge

- Strength of an individual nanofibril is much stronger than that of their network

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