International Conference on Nanotechnology for Renewable Materials

U.S. Endowment for Forestry and Communities

USDA Forest Service U.S. DEPARTMENT OF AGRICULTURE

Designing a Physical and Chemical Characterization Strategy for Cellulose Nanomaterials to Meet Regulatory Requirements in Multiple Markets

James D. Ede, Angel Precious-Egere, Yueyang Zhang, Jo Anne Shatkin

13 JUNE 2023

Vireo Advisors, LLC
Raising the Bar on Sustainability in Innovation

VTT

UBC

TAPPI NANO DIVISION

12-16 JUNE 2023 • VANCOUVER, B.C. CANADA

Intended Uses of Cellulose Nanomaterials

Food

Food Contact

Cosmetics

Chemical

Regulatory Requirements Vary by Use and Geography

Canada	Health Canada	NSP	

EU	EFSA	EFSA, BfR, etc.	EC	ECHA

US	FDA	EPA	

Alliance for Food Safety Acceptance of Fibrillated and Crystalline Celluloses (AFSAC)

- Collaborative public/private Alliance
  - P3Nano
  - CN Manufacturers
- Demonstrating the safety of CNs for food and food contact applications
- Pursuing regulatory authorization in markets worldwide

Data for submission - dossier

	Intended use	Chemistry	Toxicology
	• Technical function • Amount • Type of foods and conditions	• Identity • Migration	• Exposure • Toxicity
Testing	Proof of concept	Physical chemical analyses Migration and extraction testing	Exposure calculation Safety testing Risk assessment

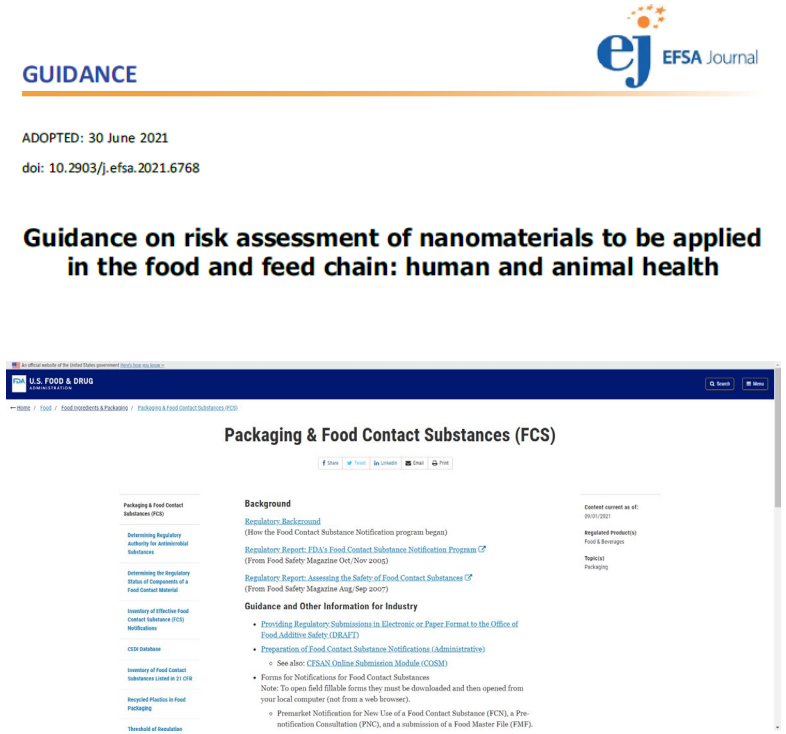
Food and Food Contact Packaging in Multiple Markets

GOAL: Physical and chemical testing plan for approval of MFCs in food and food-contact packaging applications

1. Meet regulatory requirements for the use of CNs in food and food-contact packaging
2. Key markets: European Union & United States
    - Endpoints
    - Methodologies
3. Form a panel of CN physical-chemical characterization experts to design testing plan
    - Academia
    - Industry
    - Government

FDA, EFSA, BfR Food and Food Contact Packaging Requirements

Develop list of physical-chemical characterization requirements for food and food contact applications in key markets


GUIDANCE

EFSA Journal

ADOPTED: 30 June 2021

doi: 10.2903/j.efsa.2021.6768

Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health

U.S. FOOD & DRUG ADMINISTRATION

Packaging & Food Contact Substances (FCS)

Background

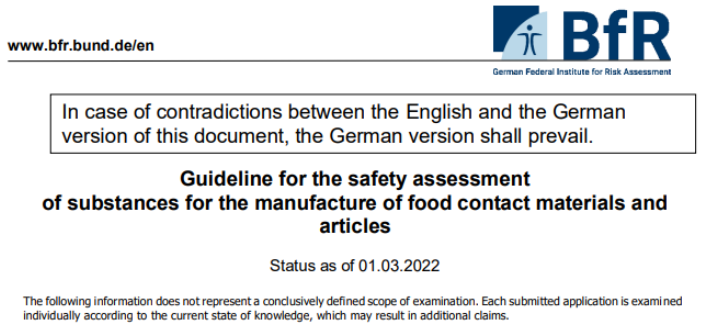
Regulatory Background
(How the Food Contact Substance Notification program began)

Regulatory Report: FDA's Food Contact Substance Notification Program
(From Food Safety Magazine Oct/Nov 2005)

Regulatory Report: Assessing the Safety of Food Contact Substances
(From Food Safety Magazine Aug/Sep 2007)

Guidance and Other Information for Industry

- Providing Regulatory Submissions in Electronic or Paper Format to the Office of Food Additive Safety (DRAFT)
- Preparation of Food Contact Substance Notifications (Administrative)
  - See also: CFSAN Online Submission Module (COSM)
- Forms for Notifications for Food Contact Substances
  Note: To open field fillable forms they must be downloaded and then opened from your local computer (not from a web browser).
  - Premarket Notification for New Use of a Food Contact Substance (FCN), a Pre-notification Consultation (PNC), and a submission of a Food Master File (FMF).


www.bfr.bund.de/en

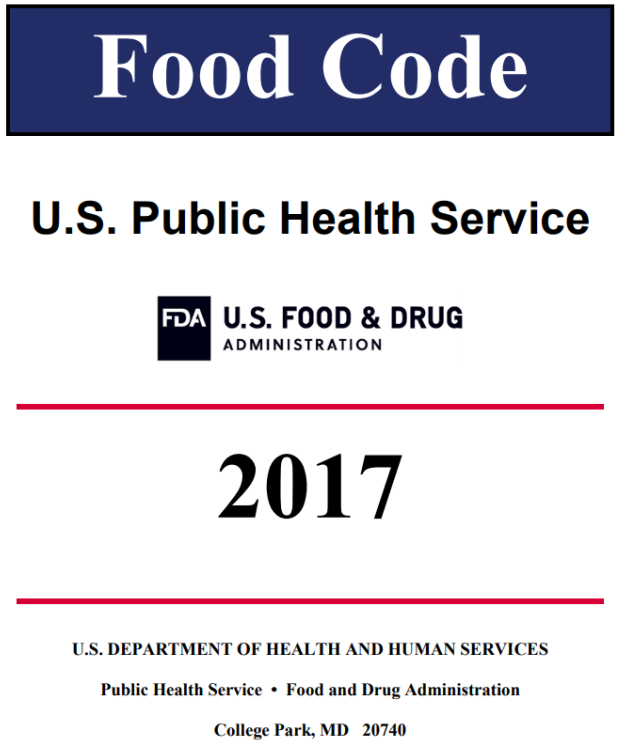
BfR German Federal Institute for Risk Assessment

In case of contradictions between the English and the German version of this document, the German version shall prevail.

Guideline for the safety assessment of substances for the manufacture of food contact materials and articles

Status as of 01.03.2022

The following information does not represent a conclusively defined scope of examination. Each submitted application is examined individually according to the current state of knowledge, which may result in additional claims.


Food Code

U.S. Public Health Service

FDA U.S. FOOD & DRUG ADMINISTRATION

2017

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service • Food and Drug Administration

College Park, MD 20740

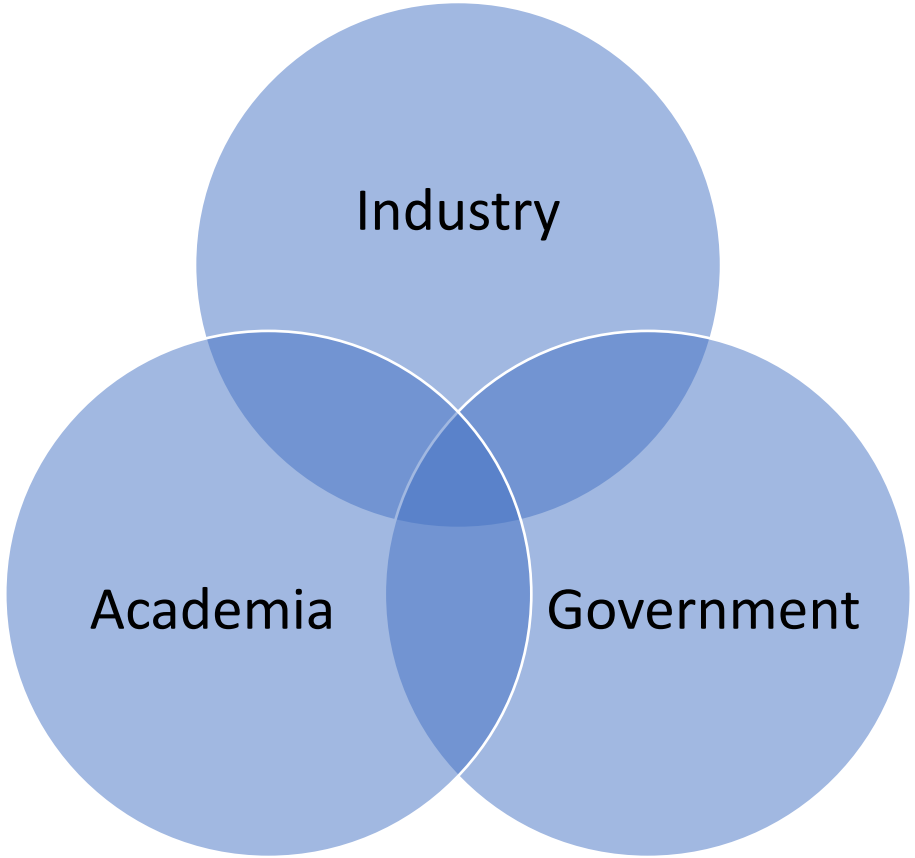
Regulatory Cross-walk

FDA, EFSA, BfR Food and Food Contact Packaging Requirements

Regulatory cross-walk to develop physical-chemical characterization requirements for food and food contact applications in key markets

- **Size/Morphology**
- **Nanoscale Fraction**
- **Elemental Analysis & Surface Chemistry**
- **Surface Area**
- **pH**
- **Crystallinity**
- **Impurities**
- **Reactivity**
- **Molecular Weight**
- **Degree of Polymerization**
- **Solubility and Partition Coefficients**
- **Density and Pour Density**
- **Melting Point, Boiling Point, Specific Gravity**
- **Dustiness**
- **Hydrogen Bonding**
- **Hydration**

Physical-chemical Subcommittee



- Relevant endpoints for MFCs
- Unique pchem considerations for MFCs
- Methods and standards for pchem endpoints
- Develop final pchem testing plan

Physical-chemical Characterization Strategy: Read Across

- **Raw materials:**

1. Five industrial MFC

2. A non-commercial MFC (RefMFC)
   - Produced by the University of Maine Process Development Centre

3. Two MFCs already found in food
   - Ketchup MFC: isolated from commercially available ketchup
   - Nata MFC: isolated from Nata de Coco

4. Reference microcrystalline cellulose (RefMCC)

5. Reference conventional cellulose (RefCC)

- **Methods:**

1. All materials were characterized side-by-side.

Example: Size and Morphology

Regulatory requirements

- Primary particle size, size range, and number size distribution (two methods, one being EM)
- Secondary particle size, size range and number size distribution (two methods, one being EM)
- Information on physical form
- Microfibril length distribution
- Particle/agglomeration size
- Microfibril diameter distribution

Recommended Testing	Rationale	
Scanning Electron Microscopy	Recommended for nanoscale resolution	Average and distribution of fiber width
Laser Diffraction		Commonly used in food science
Fiber Analyzer	Recommended for microscale resolution	
Light Microscopy	Recommended for macroscale resolution	

Example: Size and Morphology

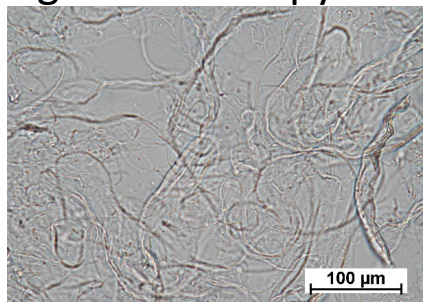
Regulatory requirements

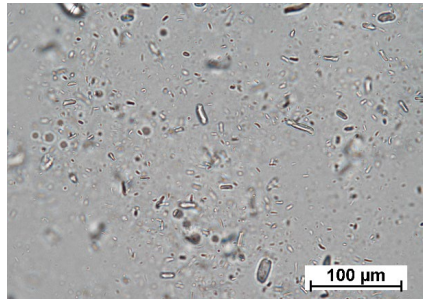
- Microfibril length distribution
- Particle/agglomeration size
- Microfibril diameter distribution
- Primary particle size, size range, and number size distribution (two methods, one being EM)
- Secondary particle size, size range and number size distribution (two methods, one being EM)
- Information on physical form

Recommended Testing	Rationale	
Scanning Electron Microscopy	Recommended for nanoscale resolution	Average and distribution of fiber width
Laser Diffraction		Commonly used in food science
Fiber Analyzer	Recommended for microscale resolution	
Light Microscopy	Recommended for macroscale resolution	
Transmission Electron Microscopy	High resolution SEM is preferable	
Dynamic Light Scattering	LD is a better alternative	
Confocal Microscopy; Atomic Force Microscopy	Redundant testing	

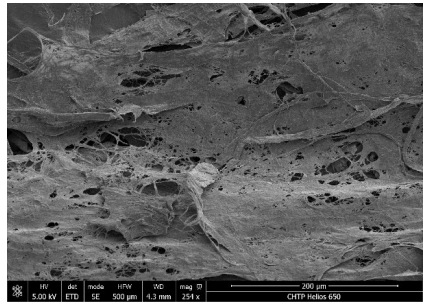
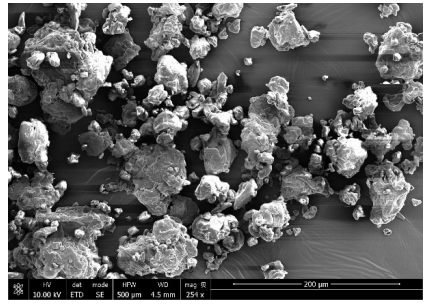
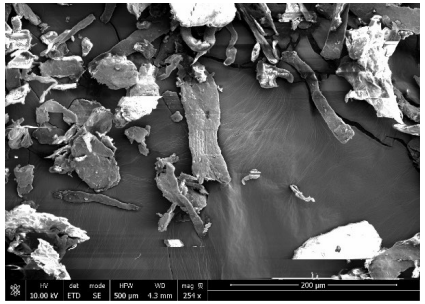
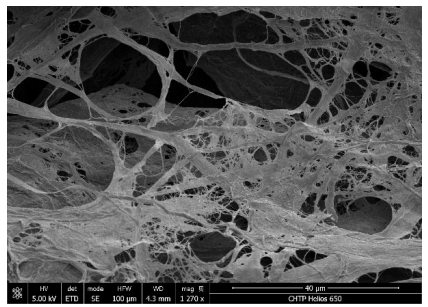
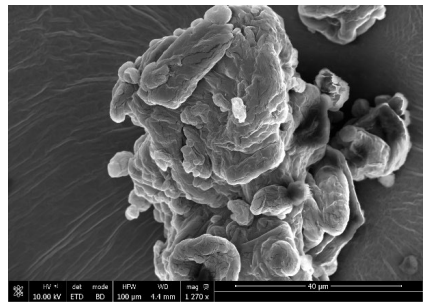
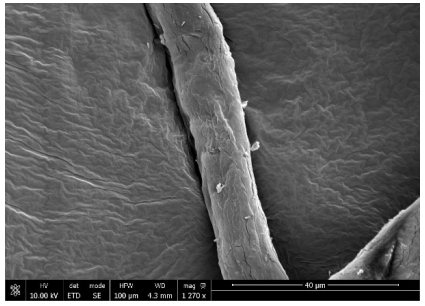
Example: Size and Morphology

Light Microscopy



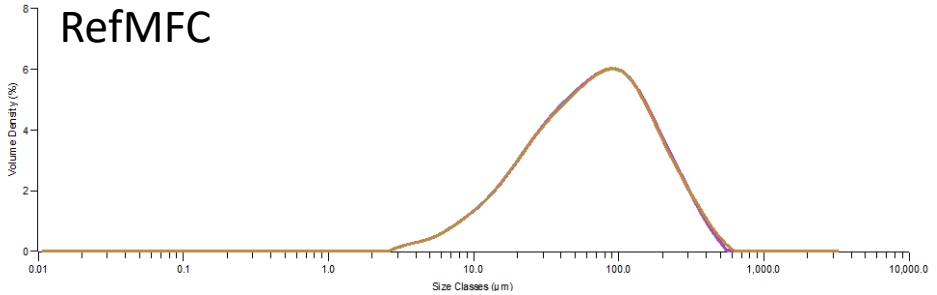


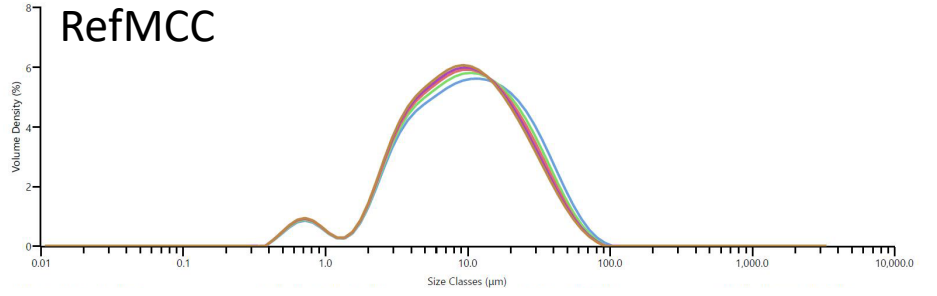
Scanning Electron Microscopy

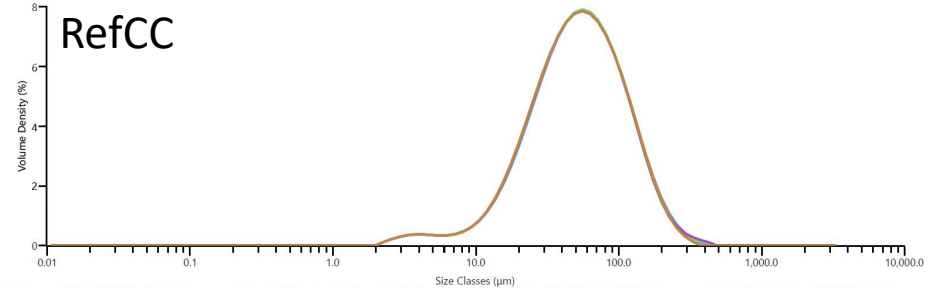







RefMFC RefMCC RefCC

Laser Diffraction







Example: Size and Morphology - Finest Fraction

Regulatory requirements

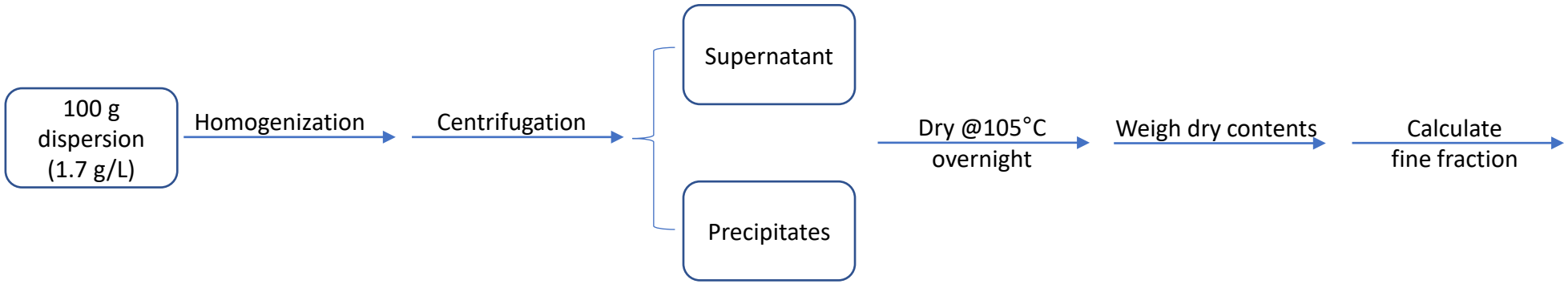
- Nanoscale 'fraction' of samples, If individual nanoscale fibrils present

Recommended Testing	Rationale
Fractionation by centrifugation and then studied by atomic force microscopy (AFM)	Aim to show (i) no/limited free nanoscale fibrils; (ii) similar 'nanoscale fraction' present in conventional celluloses already approved for food/contact

- **Method:**

1. Centrifugation protocol to isolate smallest fibers and fibrils (wt. %).

2. AFM to characterize average fiber length and width in finest fraction.



Example: Size and Morphology - Finest Fraction

Regulatory requirements

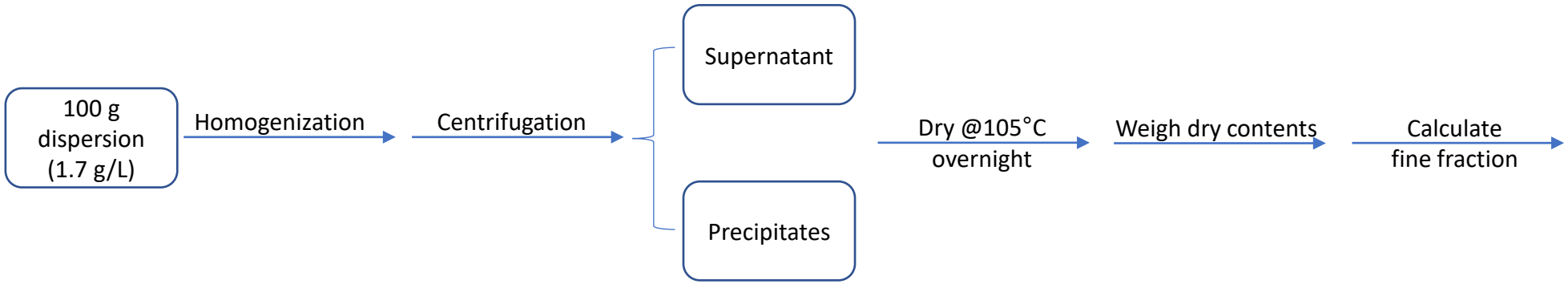
- Nanoscale 'fraction' of samples, If individual nanoscale fibrils present

Recommended Testing	Rationale
Fractionation by centrifugation and then studied by atomic force microscopy (AFM)	Aim to show (i) no/limited free nanoscale fibrils; (ii) similar 'nanoscale fraction' present in conventional celluloses already GRAS
Mechanical fractionation by sieves and membranes	Limited access to equipment; No longer commonly used

- **Method:**

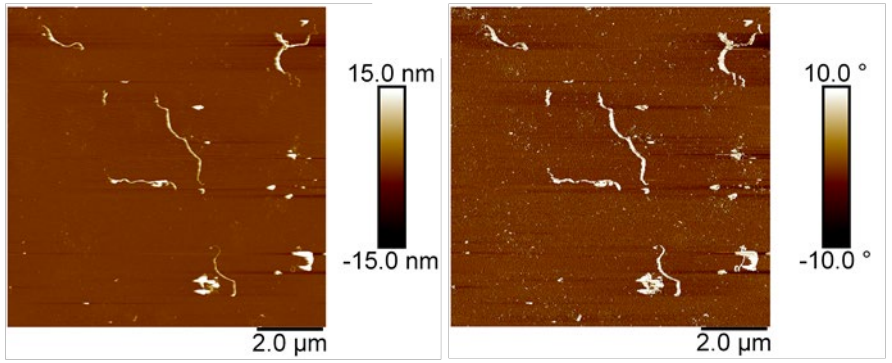
1. Centrifugation protocol to isolate smallest fibers and fibrils (wt. %).

2. AFM to characterize average fiber length and width in finest fraction.

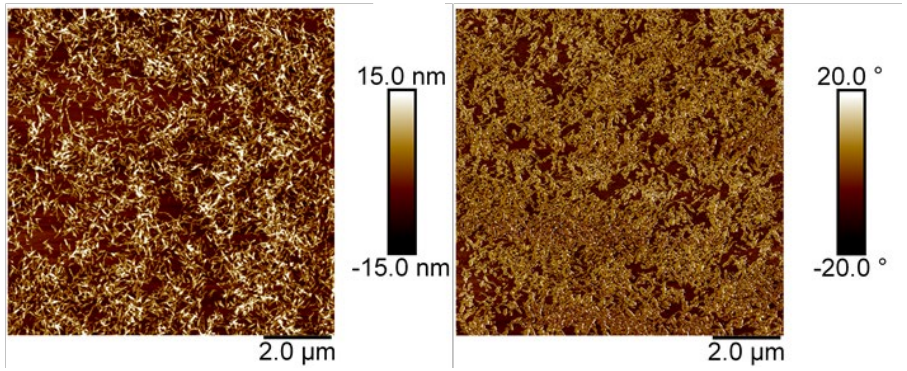


Example: Size and Morphology - Finest Fraction

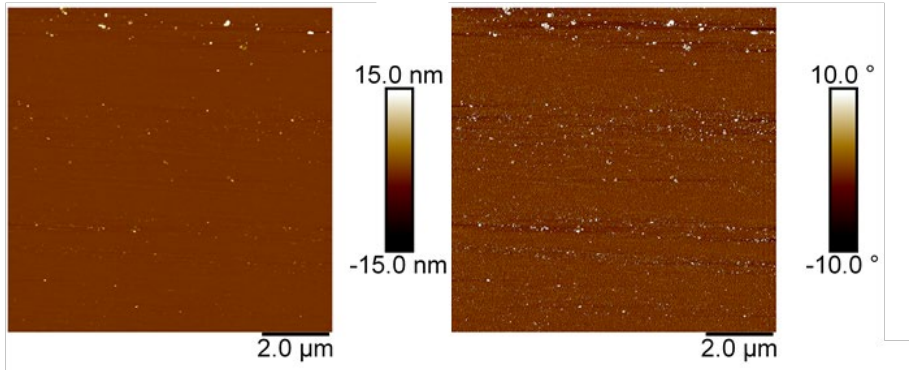
RefMFC



RefMCC



RefCC



Sample	Finest Fraction (%)	Fibril length (µm)	Fibril width (nm)	Notes
RefMFC	3 ± 0.6	1.76 ± 1.32	52.8 ± 19.1	
RefMCC	74.1 ± 6.1	0.26 ± 0.09	46.6 ± 12.1	Separation challenging
RefCC	7 ± 1.5	N.A.	N.A.	The upper phase contained visible fibers. No fibrils in AFM image

VTT

Endpoints Not Relevant for MFC

Regulatory requirements

- Require hydrogen bonding
- Solubility in relevant solvents and partitioning between aqueous and organic phases, and influence of pH on solubility
- Information on density/porosity and pour density (for granular materials)
- Require melting point, boiling point and specific gravity
- Dustiness for dry powders

Endpoint	Rationale
Solubility and Partition Coefficients	Cellulose anticipated to be insoluble at pHs relevant for food applications
Density and Pour Density	Literature values are available for cellulose
Melting point, boiling point, specific gravity	• Several endpoints not applicable (e.g. boiling point) • Literature values are available for cellulose
Dustiness	Not applicable since MFC is aqueous gel/cakes
Hydrogen bonding	Difficult endpoint to characterize but can be informed by hydration and other planned endpoints (*e.g.* surface area, surface charge density)

Challenges

- Existing regulatory guidelines and testing strategies may not be suitable for MFC
  - Requires method development
  - Regulators require methodological validation for modified approaches; time- and cost-implications
  - No directly applicable reference materials for method validation
- Complex morphology of MFC makes characterization difficult
  - Large entangled network
  - High aspect ratio
  - Especially difficult to characterize size and size distribution
- MFC undergoes significant physical changes depending on dispersion conditions and physical state (e.g. hornification during drying)
  - Different methodological approaches require dry versus aqueous suspensions; influences results (e.g. surface area measurements)

Acknowledgement

P³Nano, the U.S. Endowment for Forestry and Communities and the USDA US Forest Service
Forest Product Laboratory (FPL).

The partners of the Alliance for the Food Safety Acceptance of Fibrillated and Crystalline Celluloses

TAPPI committee

The Vireo Team

The Vireo Team

Dr. Jo Anne Shatkin is an expert in novel product safety and environmental and health policy issues, with over 20 years experience leading projects in risk analysis, safety and regulatory policy work including numerous publications.

She is founder and president of Vireo Advisors in Boston, Massachusetts.

Dr. James D. Ede is a toxicologist experienced in testing strategies for novel materials, including molecular, biochemical and cellular techniques, and is experienced in life cycle risk assessment.

Dr. Kimberly J. Ong is a biologist and environmental scientist. Dr. Ong is an expert in developing protocols specific for novel material testing to improve reliability for risk and exposure assessment and is experienced in regulatory analysis for novel products.

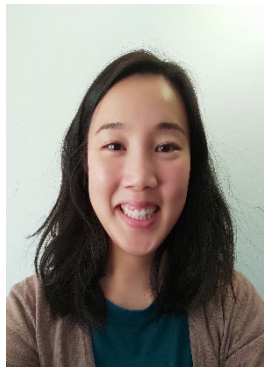
Dr. Shaun Clancy is a chemist with over 30 years experience in the chemicals industry, directing programs in health, safety, and regulatory affairs in major corporations. He is ANSI Co-Chair and participates in ISO TC229 and other international safety committees.

Fiona Case is a content writer with more than 20 years experience covering scientific innovations in foods, personal and home care products, sustainability, and computer-aided materials design.

Patricia Hodgkinson is Vireo's research consultant with a background in Food Policy law.

Leslie Hockman has industry experience working for a commercial manufacturer of cellulose nanomaterials and is Vireo's administrator.

Yueyang (Brian) Zhang is a toxicologist and a post doctoral fellow at University of Alberta and a MITACS Fellow with Vireo.



Wei Ng is an intern and is currently pursuing her PhD in Biological and Biomedical Science from Yale University.

Angel Precious-Egere is an intern and is pursuing a master's degree with a focus on antimicrobial nanotechnology.

Lauren Payne is a MPH student at Boston University and Vireo Intern with a business background.

Kora Kukk is a fellow and a UMaine graduate with an M.S. in Biomedical Engineering.

Padmapriya Srinivasan is an intern and is pursuing a Bachelors of Science degree from Mount Allison University.

Tatiana von Rheinbaben is a fellow and a M.S. Environmental Engineering and Science graduate of Stanford University.

Thank you

James Ede, Ph.D.

jede@vireoadvisors.com

CHECK OUT OUR BLOG AT:
WWW.VIREOADVISORS.COM/BLOG