Lab 2: Isolation of Nanocrystalline Cellulose

Objective

To understand the process of making nanocrystalline cellulose and how starting material can impact yield.

Background

The pulp and paper industry in Canada has suffered significant market reduction over the past decade, diminishing the need to harvest and process trees, impacting local economies with mill closings. However, a new product derived from pulp, nanocrystalline cellulose, is seen as a novel material for a variety of different markets, from composite materials to nanomedicine that can help the industry remain competitive. Nanocrystalline cellulose is a nanoparticle, meaning that one of its dimensions is less than 100nm. In fact most nanocyrstalline celluloses have a diameter that is less than 10nm, with a rod like shape that is about 150nm in length. The material is made by hydrolyzing the accessible portions of cellulose microfibrils away with mineral acid, like sulphuric acid, leaving the highly crystalline core of the fibril in place.

Q: Why is there an accessible portion of the cellulose microfibril?

Laboratory format

The lab will take place over three lab sessions along with lab activities outside of lab time. The first week the lab groups will hydrolyze various cellulose samples with strong acid and recover the cellulose nanoparticles. During the following week the class will purify the cellulose sample, through laboratory techniques of centrifugation and dialysis and measure yield of cellulose nanoparticles.

Data Analysis

The data from the class experiments (9 groups) will be aggregated into a single spreadsheet so each group can compare the yield of cellulose nanoparticles from the various starting materials. The bulk of the analysis will require an ANOVA comparison of the yield based on starting cellulose type.

Lab 2. Part 1: Cellulose Hydrolysis

Northern bleached hardwood Kraft (NBHK) pulp and high alpha cellulose content sulfite pulp are ground using a Wiley mill fitted with a 60 Mesh screen to isolate nanocrystalline cellulose. For this reaction a 3-neck round bottom flask containing a magnetic stir bar is lowered into an oil bath on a hotplate/stirrer. Slowly add 22 ml of 64% sulfuric acid to the flask using a glass funnel while mechanically stirred. When the temperature reaches 45°C, add 2.5 g cellulose powder [8.75 mL of a sulfuric acid per one gram of cellulose] to the flask and manually stir with a glass rod to ensure it is well mixed in the acid solution. The reaction flask is fitted with a condenser during the reaction. Set a timer for a reaction time of 45 minutes. Manually mix intermittently

using the glass rod. (Be careful where you place the glass rod as it has touched the 64% acid). Once the reaction time has been reached, transfer the yellowish cellulose suspension into a (labeled[1]) beaker containing 220 ml cold distilled water (10 times volume of the suspension) to stop the hydrolysis reaction. Allow suspension to sit overnight. Store in refrigerator for next week's lab.

Lab 2. Part 2: Hydrolyzed-Cellulose Purification

Carefully (without agitating solution) decant off the top clear liquid to a waste beaker. Transfer ~30ml of the sediments into three (labeled[2]) centrifuge tubes. Centrifuge the tubes for 10 minutes at 5000 rpm and carefully decant the clear supernatant to the waste beaker. Add ~30ml distilled water to each centrifuge tube and mix using a vortex machine. Centrifuge for 10 minutes at 5000 rpm. Repeat the 'washing' steps 2-3 times until a turbid supernatant is finally collected.

Collect the turbid supernatant from the three centrifuge tubes into a clean beaker, stir well and measure and record the pH. Transfer the collected supernatant to three wet dialysis tubes (molecular porous membrane tubing) using a glass funnel ensuring that one end of the tube is clipped first. Once filled, clamp the open end of the dialysis tube and place in a tank that is filled with distilled water (measure and record the pH of the water **BEFORE** placing the dialysis tubes in). Measure and record the pH of the water **AFTER** placing the dialysis tubes in the tank.

Each group must return **daily** to record the pH of the water and change the distilled until the pH of the outside water is stable (same pH reading prior to placing the dialysis tubes in the water). Once the pH is stable, collect the suspension from each dialysis tube into a clean labeled[3] beaker. Mix well and record the pH of the suspension of nanocrystalline cellulose. Place the beaker in a refrigerator for next week's lab.

Lab 2. Part 3: Nanocrystalline Cellulose yield

Label[4] and record the weight of 3 empty glass vials. Add 100 µl of the suspension to each vial. Record the weight of the vial + $suspension_{wet}$. Dry the suspension in a drying oven (105°C) for 15-20 minutes. Once the suspension is completely dried, allow to cool to room temperature in a desiccator. Record the weight of the vial + $suspension_{dry}$ and calculate the concentration. (see details in the data sheet provided)

Concentration = B/A x 100%

A = weight of $suspension_{wet}$ – weight of empty

B = weight of $suspension_{dry}$ – weight of empty

Laboratory report

Follow the guidelines for general lab reports included in the laboratory handout.

[1] Group 1,2 – Beaker A; Group 3,4,5 – Beaker B; Group 6,7 Beaker C; Group 8,9 – Beaker D

[2] Beaker A = tubes A1, A2, A3; Beaker B = tubes B1, B2, B3;
Beaker C = tubes C1, C2, C3; Beaker D = tubes D1, D2, D3

[3] Beaker A, Beaker B, Beaker C, Beaker D

[4] Vials A1, A2, A3; B1, B2, B3; C1, C2, C3; D1, D2, D3.