BACKGROUND

- The reducing end of a carbohydrate is a carbon atom that can be in equilibrium with the open-chain aldehyde or ketone form.

![Diagram of reducing end of cellulose]

*Reducing End of Cellulose

- This protocol determines the reducing end concentration of carbohydrate or cellulose samples.
- A temperature dependent reaction occurs where the reducing ends in the carbohydrate sample reduce Cu^{2+} ions from the copper(II) sulfate to Cu^{+}. The amount of Cu^{2+} reduced is proportional to the amount of reducing ends present in the solution. Next, two molecules of bicinchoninic acid chelate with each Cu+ ion, forming a lavender –colored product that strongly absorbs light at a wavelength of 560 nm.

A. MATERIALS

- BCA disodium salt hydrate
- Copper(II) sulfate pentahydrate (CuSO₄·5H₂O)
- Glass Dish (pyrex)
- Graduated cylinder (pyrex or quartz)
- Greiner 96 well micro plate
- Hot plate with electronic stirring
REDUCING END DETERMINATION THROUGH BICINCHONINIC ACID (BCA) ASSAY

- L-serine
- Magnetic, Teflon coated stir bar
- Measuring balance
- Metal Stand
- Milli-Q
- Sodium bicarbonate (NaHCO₃)
- Sodium carbonate (Na₂CO₃)
- Sorvall Micro21R Centrifuge (Thermo Scientific)
- Styrofoam micro centrifuge stand
- Thermometer that goes up to 100°C
- Vortex Mixer (Fischer Scientific)
- 1.5mL Micro centrifuge tubes

B. PROTOCOL
1. Prepare Solution A with a pH of 9.7 by dissolving 27.14g of Na₂CO₃, 12.1g of NaHCO₃ and 0.971g of BCA disodium salt hydrate in 500 mL distilled water.
2. Prepare Solution B with a pH of 3.4 by dissolving 0.624g of CuSO₄·5H₂O and 0.631g of L-serine in 500mL of distilled water.
3. Freshly prepare the BCA reagent by mixing equal volumes of solution A and solution B for each assay.
4. Mix equal amounts of the BCA reagent and sample. The amounts and concentrations of samples used are as follows:
   - BMCC- 0.5mL of 6 mg/mL (approx.)
   - Avicel- 0.5mL of 5 mg/mL
   - CF-11- 0.5mL of 5 mg/mL
   - CNC- 0.5mL of 2 mg/mL
5. After agitation using a vortex mixer, incubate the tubes at 75°C for 30 min in a water bath (prepared using the glass dish, heating plate and magnetic stirrer).
6. Cool the tubes for about an hour at room temperature.
7. Agitate the samples using a vortex mixer.
8. For insoluble samples, separate by centrifugation for 2 min at a speed of 10,000g.
9. Measure the absorbance at 560nm using a 96 well micro plate.

Note:
1. Solutions A and B are stable for at least a month at 4 °C.
2. 1µM to 70µM glucose or cellobiose can be used as standards.

C. DISPOSAL
1. Pour the solution in the appropriate chemical waste container.
D. SAMPLE RESULTS

Examples of Reducing End Concentration Measured for Cellulosic Substrates:

<table>
<thead>
<tr>
<th>Name</th>
<th>Reducing end (umol/g)</th>
<th>Error</th>
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</thead>
<tbody>
<tr>
<td>Avicel PH-101</td>
<td>9.304377247</td>
<td>0.181488</td>
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<td></td>
<td>9.101460602</td>
<td>0.270536</td>
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<tr>
<td>BMCC (p-NDC)</td>
<td>9.408505169</td>
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<td>3.165469349</td>
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<td>3.429898844</td>
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<td>CNC (dil. 25%)</td>
<td>3.286862141</td>
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<td>21.18618158</td>
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<td>22.27209933</td>
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<td>CF-11</td>
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<td>0.193498</td>
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