REDUCING END DETERMINATION THROUGH BICINCHONINIC ACID (BCA) ASSAY

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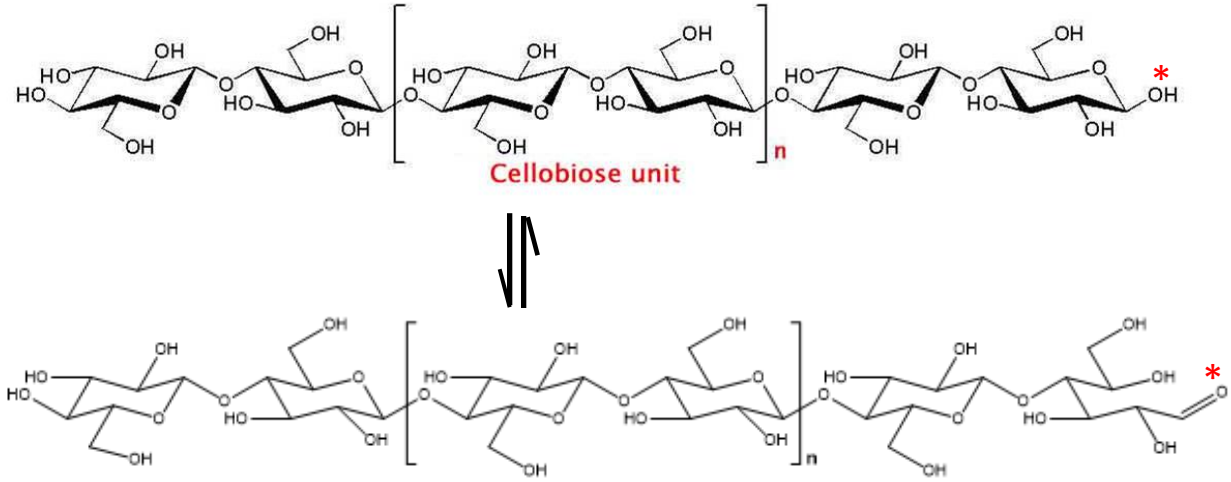
BACKGROUND

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



*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 ($CuSO_4 \cdot 5H_2O$)
- Glass Dish (pyrex)
- Graduated cylinder (pyrex or quartz)
- Greiner 96 well micro plate
- Hot plate with electronic stirring

- L-serine
- Magnetic, Teflon coated stir bar
- Measuring balance
- Metal Stand
- Milli-Q
- Sodium bicarbonate ($NaHCO_3$)
- Sodium carbonate (Na_2CO_3)
- 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 Na_2CO_3, 12.1g of $NaHCO_3$ 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 $CuSO_4 \cdot 5H_2O$ 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.

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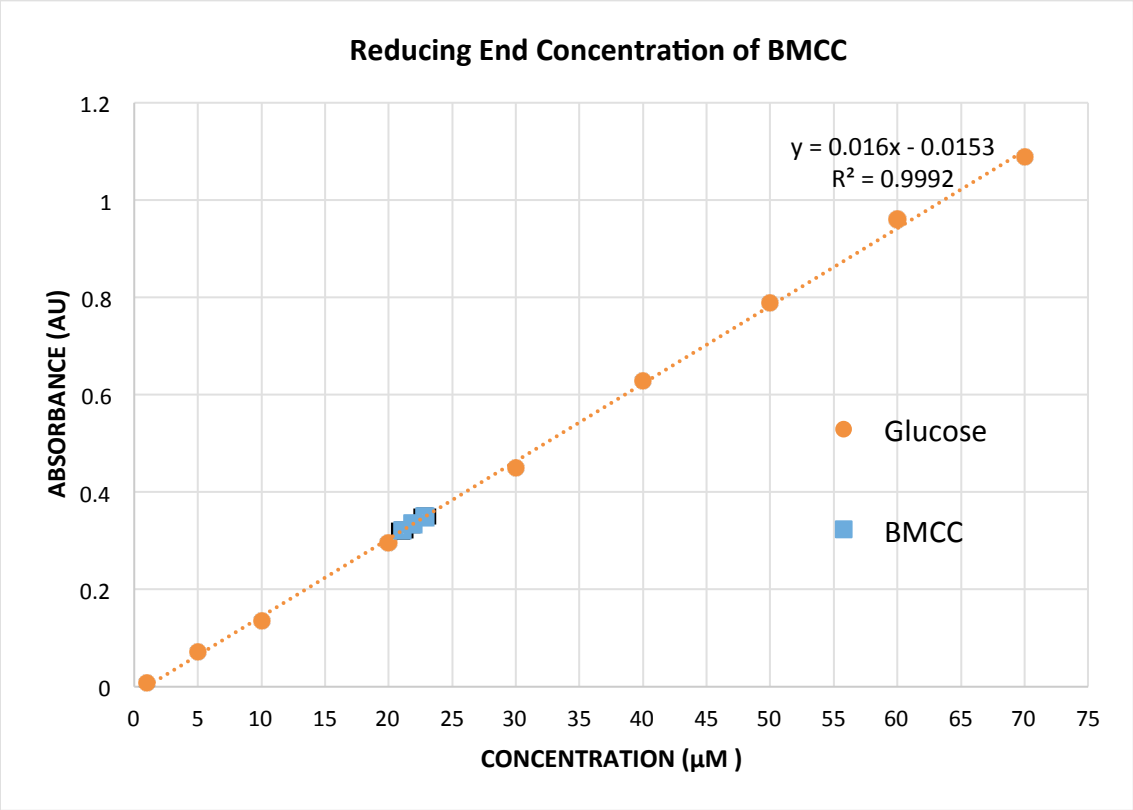
D. SAMPLE RESULTS

Sample Calibration Curve with Reducing End Concentration of BMCC:



Examples of Reducing End Concentration Measured for Cellulosic Substrates:

Name	Reducing end (umol/g)	Error
Avicel PH-101	9.304377247	0.181488
	9.101460602	0.270536
	9.408505169	0.241984
BMCC (p-NDC)	3.165469349	0.111751
	3.429898844	0.115432
	3.286862141	0.081015
CNC (dil. 25%)	21.18618158	0.382342
	21.12062851	0.401058
	22.27209933	0.527542
CF-11	4.786769839	0.212963
	5.632591075	0.14403
	6.034831387	0.193498