PROTEIN ASSAY BY MICROBIURET: STANDARDIZATION

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From DBF's Hopkins Notebooks, III, p. 102 & VI, p. 75.
http://biology.clc.uc.edu/fankhauser/Labs/Cell_Biology/Microbiuret/Microbiuret.htm

CAUTION: Wear safety goggles and handle the microbiuret reagent with care since it is a caustic solution of ~30% NaOH (lye). Any hint of slipperiness on your fingers should be rinsed off with a solution of 5% boric acid (in squirt bottles) followed by thorough hand washing.

Microbiuret reagent is an alkaline solution of copper ions which complex with the peptide bonds in protein to produce a blue-purple color (absorption max = 310 nm). Following Beer's Law, the color intensity should be proportional to protein concentration, allowing the amount of protein in a sample to be assayed in unknown solutions. The first stage is to construct a “standard curve” by assaying known amounts of protein and deriving a conversion factor to convert from A325 to mg protein. Then we will assay the concentration of protein in a variety of unknowns.

EQUIPMENT AND SUPPLIES, FOR A TABLE OF TWO (perform experiment in pairs):
- Microbiuret reagent
- Eppendorf Repipetor with 10 mL syringe (front of room)
- Squirt bottles: 5% boric acid to neutralize microbiuret spills
- test tube racks: 2 for 13x100 mm, 1 for 16x150 tubes
- 12 13 x 100 mm test tubes
- 2 16x150 test tubes
- 1 mL displacement pipet (for dH₂O and protein solution)
- 1 6 mL of mg/mL protein standard in 13x100 test tube
- 30-40 mL dH₂O in 125 mL flask
- vortex
- spectrophotometer, warmed up
- two cuvettes in test tube rack
- Kimwipes, paper towel

STANDARDIZATION OF MICROBIURET REAGENT:
1) Write out an experiment table in your book (or use the table on page 24), then set up 13x100 mm tubes for the standardization, then add in sequence 1) water (use 1 mL pipet twice), 2) protein and 3) microbiuret reagent (from repipet):

<table>
<thead>
<tr>
<th>tube</th>
<th>mL water</th>
<th>mL 1 mg/mL</th>
<th>mL microbiuret</th>
<th>mg protein</th>
<th>A₃₂₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>2.0</td>
<td>0.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.9</td>
<td>0.1</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.8</td>
<td>0.2</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.6</td>
<td>0.4</td>
<td>1.0</td>
<td>1.0</td>
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</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>0.8</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

2) Calculate the mg protein in each tube and enter in your table. (1 g = 10⁶ mg = 10⁹ μg).
3) Vortex to mix well, let sit 15 min, then
4) Read A₃₂₅ of each tube in a spectrophotometer, using tube B as the blank. (Read at 310 nm if you have a UV capabilities.) (Read at the lowest wavelength that can be zeroed on the machine.)
5) Plot standardization curve (protein vs A₃₂₅), determine the inverse of the slope for the conversion factor to convert optical density (OD) units to mg protein. (If the slope of the line were linear, A₃₂₅ of 1 mg/tube = 1.25 mg/OD unit).

Wash work all areas to clean up any spilled caustic materials. Pour assay tubes into waste beaker.

1 Our Spectronic 20s cannot measure in the UV range, but only measure absorbency down to 325 nm.

2 MICROBIURET REAGENT: (Safety glasses should be worn during this experiment since this solution is close to a 30% solution of NaOH. Handle with extreme caution.)
Solution A: 40 g NaOH (caution, caustic) 100 mL dH₂O to dissolve NaOH with caution 400 mg CuSO₄ water, agitate to dissolve
Solution B: 40 mL dH₂O to dissolve NaOH with caution 1) When cooled to around 30-40 C, q.s. solution A to 150 mL with dH₂O 2) Add solution B slowly to solution A with stirring.
Store in labeled bottle marked CAUTION: caustic.

3 STANDARD PROTEIN, mg/mL: Prepare 1 mg/mL solution of standard protein (bovine serum albumin [BSA]) by adding 100.0 mg of powder to 80 mL dH₂O, thoroughly dissolve with stirring, avoiding foaming which denatures protein. If possible, let sit overnight at 4°C to completely dissolve. Q.s. to 100.0 mL. Store at 4°C. (Need ~5 mL/student).