Biuret Protein Assay

**Introduction**
Substances containing more than two peptide bonds form a purple complex with copper salts in alkaline solution. The Biuret reagent is prepared by adding sodium hydroxide to a copper sulphate/sodium potassium tartrate solution. This can be used to quantify proteins in the concentration range from 0.5 to approximately 10mg/ml. This assay therefore requires more protein than other common methods such as the BCA, Lowry and Bradford assays. There are very few interfering substances to the Biuret assay, however it cannot be used in the presence of ammonium salts and the reducing agent dithiothreitol does enhance the colour. It is always advisable to prepare the standard in the same buffer as the sample to minimise any interference effects. Proteins with an abnormally high or low percentage of amino acids with aromatic side groups will give high or low readings, respectively.

**Materials required**
Copper (II) sulphate pentahydrate (Sigma C7631)
Potassium sodium tartrate tetrahydrate (Sigma S2377)
Potassium iodide (Sigma 207969)
Sodium hydroxide (Sigma S8045)
1 litre volumetric flask
1 litre beaker
Suitable tubes to hold and mix 3.0ml samples
Plastic disposable cuvettes (Jenway 060 084)
Standard protein solution of known concentration (up to 10mg/ml)

**Method**

**Preparation of the Biuret reagent**
1. Dissolve 1.5g copper (II) sulphate pentahydrate and 6g sodium potassium tartrate in 500ml water.
2. Add 300ml 10% (w/v) NaOH and make the volume to 1 litre with water.
3. Add 1g potassium iodide to inhibit the reduction of copper.
4. Store in a plastic container in the dark. Discard if any black or reddish precipitate is observed.

**Preparation of samples and standards**
1. Turn on the Genova and allow it to warm up. See below for set up instructions.
2. Prepare a series of protein standards ranging in concentration from 0.5 to 10mg/ml such that the final volume for the assay is 0.5ml. Examples of dilutions are given in Table 1 below. Note that the Genova can accept up to 6 standards, not including the blank.
3. Prepare the unknown samples in a similar way such that the final volume is 0.5ml.
4. Add 2.5ml of Biuret reagent to each sample and standard, vortex and allow to react for 30min.
5. Transfer the samples to cuvettes and measure the absorbance at 540nm using the Biuret protein mode on the Genova as detailed below.
Table 1: Examples of standard dilutions for preparation of a calibration curve.

<table>
<thead>
<tr>
<th>Final concentration (mg/ml)</th>
<th>Volume of a 10mg/ml standard (µl)</th>
<th>Volume of water or dilution buffer (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (blank)</td>
<td>0</td>
<td>500</td>
</tr>
<tr>
<td>0.5</td>
<td>25</td>
<td>475</td>
</tr>
<tr>
<td>1.0</td>
<td>50</td>
<td>450</td>
</tr>
<tr>
<td>1.5</td>
<td>75</td>
<td>425</td>
</tr>
<tr>
<td>2.0</td>
<td>100</td>
<td>400</td>
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<td>350</td>
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<tr>
<td>4.0</td>
<td>200</td>
<td>300</td>
</tr>
<tr>
<td>5.0</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>6.0</td>
<td>300</td>
<td>200</td>
</tr>
<tr>
<td>8.0</td>
<td>400</td>
<td>100</td>
</tr>
<tr>
<td>10.0</td>
<td>500</td>
<td>0</td>
</tr>
</tbody>
</table>

Setting up the Genova for the Biuret assay

1. From the main screen of the Genova select PROTEIN MODE and then BIURET.
2. Select SETUP. The wavelength should already be set to 540nm. Adjust the units to mg/ml or other units as appropriate.
3. Select the required resolution. Note that at high resolution i.e. 0.001, the concentrations will appear to fluctuate more.
4. Select EXIT and then CURVE. Select a METHOD number (0-9); the standard curve data generated will be stored with the method number displayed.
5. Enter the number of standards (maximum of 6).
6. In the table under the unit heading (mg/ml), enter the concentration values of each of the standards.
7. Place the sample blank into the Genova and press CAL to begin the calibration procedure. Press CAL again to zero the instrument on the blank.
8. Following the instructions on the screen, insert each standard as requested pressing CAL each time. Once all standards have been measured, the ABS column of the table will be updated with the actual absorbance.
9. To view the standard curve, select VIEW. To obtain the equation of the curve, select STATISTICS. The curve fit is quadratic of the form $y = ax^2 + bx + c$.
10. Press any key to clear the statistics then exit back to the main measurement screen.
11. Place the sample blank in the Genova and press CAL to zero.
12. Remove the blank and insert each sample. Read the concentration from the screen in the chosen units.

An example calibration curve is shown in Figure 1.
Biuret assay standard curve

\[y = -0.0006x^2 + 0.0462x - 0.0006\]

\[R^2 = 0.9997\]

Figure 1: A typical Biuret standard curve with samples ranging from 0.5 to 3mg/ml protein.

References


The protocols described here are for guidance only. Be aware of any hazardous compounds, take precautions where necessary and dispose of any waste in the appropriate manner.