Unit 3: Review
Spectrophotometry Lab

I. Parts of the spectrophotometer

Be familiar with the parts of the spectrophotometer.

- Digital display: displays the output of the detector (wavelength and % transmittance or absorbance).
- Sample holder: holds sample for reading.
- 0% T knob: to calibrate machine.
- 100% T knob: adjusted to negate any absorbance by the solution that is not due to the absorbing molecule.
- Mode button: toggle between % T and absorbance readings.
- Wavelength control knob: set to a desired wavelength.

II. Relationship between wavelength & color

With a strip of white paper in a Spectronic tube, you recorded the range of wavelengths for red, orange, yellow, green, blue, and violet colors.

Below is the wavelength of visible light.

III. Using the Spectrophotometer to determine % transmittance (% T) and absorbance (A)

Making the blank and sample tubes:

- A blank tube: water + indicator
- A sample tube: water + indicator + protein

Water, protein solution, and biuret reagent (indicator) were needed to prepare the test tubes.

Using the Spectrophotometer to determine % T and A continued:
Using the Spectrophotometer to determine %T and A continued

*To calibrate the spectrophotometer

• Adjust to desired wavelength using the wavelength control knob (on right side of the Spec 20)
• Set to 0%T (with empty sample holder and closed lid) using the 0%T knob (on bottom, left side of machine)
• Place blank tube in sample holder and close lid
• Set to 100% T (on bottom, right side of machine)

*Note: Whenever wavelength is changed, 100% transmittance must be reset. Also, if the instrument is operated for an extended period of time, periodic checks must be made to ensure proper calibration of machine.

Measuring %T and A

• Place sample tube in sample holder and close lid
• Press mode button to select %T and record
• Press mode button to select Absorbance and record

Using the Spectrophotometer to determine %T and A continued

A series of dilutions was prepared then results plotted on a graph. Independent variable (protein concentration) on the X-axis and the dependent variable (A) on the Y-axis.

<table>
<thead>
<tr>
<th>X axis</th>
<th>Y axis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube 1</td>
<td>Tube 2</td>
</tr>
<tr>
<td>Tube 3</td>
<td>Tube 4</td>
</tr>
<tr>
<td>Tube 5</td>
<td>unknown</td>
</tr>
</tbody>
</table>

An example of a standard curve is found in your lab manual.

Development of a standard curve continued

Serial dilution

Tube 1 has 1.00 ml of a known protein concentration (10.0 mg/ml).
Tubes 2-5 have 1.00 ml of distilled water.
1.00 ml of the protein solution (10.0 mg/ml) was added to tube 2 and mixed. 1.00 ml from tube 2 was removed and placed into tube 3 and mixed. Then 1.00 ml from tube 3 was removed and placed into tube 4 and mixed. 1.00 ml from tube 4 was removed and placed into tube 5 and mixed. 1.00 ml from tube 5 was removed and discarded.

Each tube then received 5.00 ml of the indicator solution.
The absorbance (A) of the each sample tube was recorded.

Development of a standard curve continued

The protein concentration (mg/ml) column was calculated. Absorbance vs. protein concentration was plotted on the graph and the best fit line was drawn.

Best fit line

Plot your points on the graph. Draw a straight line starting at 0,0 point through the middle of your points (some points will fall above the line and others below the line).

An test tube with an unknown concentration protein solution was prepared and its absorbance measured. You determine the concentration of the unknown protein solution by using your standard curve.

Development of a standard curve continued

The concentration of an unknown solution can be calculated by comparing the absorbance reading of the unknown to your standard curve. Draw a straight line from the recorded A on the Y-axis to the curve. Drop your line from the curve to the concentration on the X-axis.

For example, if the A of your sample measured 0.35 then your protein concentration is approximately 7.0 mg/ml