Spectroscopy

Objective: Beer’s Law or the relation between the concentration of a chromophore in solution and the optical absorbance of the solution will be used to determine the concentration of glucose in a solution of unknown glucose concentration. A series of solutions with known concentrations will be used to determine the molar extinction coefficient of the chromophore produced by the reaction of glucose with o-toluidine.

Methods and Data: A sample for the spectrophotometer was prepared by adding 3 ml of the o-toluidine reagent to 0.5 ml of a glucose (C₆H₁₂O₆) solution. The sample was heated in boiling water for 12 minutes. The color changed to a blue-green color. The sample was cooled down to room temperature. All samples used in this experiment were prepared by this procedure.

The absorbance spectrum of a sample prepared from the stock glucose solution (250 mg/L or 1.39x10⁻³ M) was measured on the Agilent 8453 Diode Array Spectrophotometer. The wavelength of greatest absorbance was found to be 634 nm. The Genesys20 spectrophotometer was then set to this wavelength.

The absorbance of samples prepared by diluting the stock solution 1:2, 1:5 and 1:10 with benzoic acid solution and then diluting with o-toluidine reagent was measured on the Genesys20 spectrophotometer. The absorbance results were graphed against the sample concentration in M. The slope of the line is the molar extinction coefficient with units of M⁻¹cm⁻¹.

The absorbance of a sample prepared from Unknown A was measured on the Genesys20. The concentration of the unknown sample was determined from the graph made using the absorbance measurements of the stock solution samples.

Calculations & Results: The concentration of the stock solution in Molar is:

\[ 250 \text{ mg/L} \times (1 \text{ g/1000 mg}) \times (1 \text{ mole/180 g glucose}) = 1.39 \times 10^{-3} \text{ M} \]

The concentration of the diluted samples is found using \( M_{\text{old}} V_{\text{old}} = M_{\text{new}} V_{\text{new}} \). As an example in order to make 5 ml of the 1:2 dilution solution from the stock solution 2.5 ml of stock was diluted with an equal volume of benzoic acid diluent solution to make 5 ml of the 1:2 diluted solution.

\[
M_{\text{stock}} V_{\text{stock}} = M_{1:2} V_{1:2} \\
1.39 \times 10^{-3} \text{ M} \times 2.5 \text{ ml} = M_{1:2} \times 5 \text{ ml} \\
M_{1:2} = 6.95 \times 10^{-4} \text{ M}
\]

The remaining solutions were prepared using a serial dilution procedure and the concentrations were calculated as above. 3.0 ml of o-toluidine reagent was added to 0.5 ml of each solution and heated to make a sample. The samples had a green-blue color and a wavelength of maximum absorbance at 634 nm. The concentration of each sample was
calculated using \( V_{\text{old}} = 0.5 \text{ ml} \), \( V_{\text{new}} = 3.5 \text{ ml} \) and \( M_{\text{old}} \) was the concentration of the appropriate diluted solution.

The sample absorbances are shown in the table below. The sample concentration was always a factor of 1/7 of the solution concentration from which it was made.

<table>
<thead>
<tr>
<th>Solution Conc. (M)</th>
<th>Sample Conc. (M)</th>
<th>Abs</th>
</tr>
</thead>
<tbody>
<tr>
<td>stock</td>
<td>1.39E-03</td>
<td>1.99E-04</td>
</tr>
<tr>
<td>1:02</td>
<td>6.95E-04</td>
<td>9.93E-05</td>
</tr>
<tr>
<td>1:05</td>
<td>2.78E-04</td>
<td>3.97E-05</td>
</tr>
<tr>
<td>1:10</td>
<td>1.39E-04</td>
<td>1.99E-05</td>
</tr>
<tr>
<td>blank</td>
<td>0</td>
<td>0.00E+00</td>
</tr>
</tbody>
</table>

Sample absorbance was graphed against concentration in the Beer’s Law plot.

A sample prepared from Unknown A had an absorbance of 0.354 AU. Using the equation for the trend line of the Beer’s Law plot the concentration of the sample is:

\[
0.354 = 2862.3x - 0.0072; \quad x = 1.26 \times 10^{-4} \text{ M}
\]

The concentration of Unknown A is:

\[
M_{\text{sample}} V_{\text{sample}} = M_{\text{UnkA}} V_{\text{UnkA}}
\]

\[
1.26 \times 10^{-4} \text{ M} * 3.5 \text{ ml} = M_{\text{UnkB}} * 0.5 \text{ ml}
\]

\[
M_{\text{UnkA}} = 8.83 \times 10^{-4} \text{ M}
\]

\[
8.83 \times 10^{-4} \text{ mole/L} * 180 \text{ g/mole} * 1000 \text{ mg/g} = 159 \text{ mg/L}
\]

**Discussion:** The extinction coefficient for the glucose/o-toluidine chromophore was found to be 2862 M\(^{-1}\)cm\(^{-1}\) from the slope of the trend line on the Beer’s Law plot. The trendline had a correlation coefficient of 0.9898, which is very, very close to a completely linear value of 1.0. Beer’s Law states that \( A = \varepsilon cl \), my data set was in good agreement with
this as the value of the intercept of the trendline was very small, -0.0072, indicating little deviation from the theoretical intercept value of 0.0.

The concentration of Unknown A was found to be $8.83 \times 10^{-4}$ M. This value was determined using a sample that had an absorbance within the range of values of the solutions of known concentration that were used to make the Beer’s Law plot. This allowed the concentration of the sample to be found by interpolation rather than the less accurate method of extrapolation, or going beyond the range of the collected data set.

**Conclusion:** The extinction coefficient of the glucose-o-toluidine chromophore was found to be $2862 \text{ M}^{-1}\text{cm}^{-1}$ at 634 nm using a Beer’s Law plot. This plot was used to find the concentration of glucose in an unknown sample. The concentration of glucose in Unknown A was found to be $8.83 \times 10^{-4}$ M or 159 mg/L.