Exp. 18: Spectrophotometric Analysis: Concentration of a Solution Using Beer’s Law

White light → violet (400nm) – red (800nm) → “visible spectrum”

Sample (light absorbed) → light transmitted
Basically, we can detect the amount of light absorbed by the sample by monitoring the amount of light before and after the sample.

\[
\text{sample (light absorbed)} \quad \frac{I_o}{I_T} \quad \text{light transmitted}
\]

Amount of light absorbed is proportional to the concentration of the sample (at proper \( \lambda \)):

\[
A \propto c \quad \text{c increases} \quad A \text{ increases}
\]

absorbance (unitless) \quad concentration
Basic instrument setup:

\[ A = \log \frac{I_m}{I_T} \]
Absorbance is dependent on the concentration of solution and also on the quantity of solution (meaning sample size); this relationship can also be written

\[ A = \varepsilon b c \]

\( \varepsilon \) – molar absorptivity of particular species
\( b \) – cell thickness; typically, 1 cm
\( c \) – conc. of species
\( A \) – absorbance, unitless

Referred to as **Beer’s Law**

Note: linear relationship between \( A \) and \( c \)

\[ A \propto c \]
Since linear relationship, we can make a calibration plot of $A$ vs. conc. for known standards.

Calibration Plot of Cobalt II Nitrate

$y = 0.015x + 0.0274$

$R^2 = 0.9387$

$A = m \cdot \text{conc} + y_{\text{inter}}$

Unknown has an absorbance of 0.400

$\frac{A - y_{\text{inter}}}{m} = \text{conc}$

$\frac{0.400 - 0.0274}{0.015} = 24.8 \text{ mg/mL Co(NO}_3\text{)}_2$
Standards: **50 mL** per group 0.200 M Co(NO$_3$)$_2$

Make standards:

<table>
<thead>
<tr>
<th>Vol.</th>
<th>Vol H$_2$O</th>
<th>M Co(NO$_3$)$_2$</th>
<th>mg/mL Co(NO$_3$)$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 mL (blank)</td>
<td>10.00 mL</td>
<td>0.00 M</td>
<td>0.00 mg/mL</td>
</tr>
<tr>
<td>1.00 mL</td>
<td>9.00 mL</td>
<td>0.0200 M</td>
<td>3.66 mg/mL</td>
</tr>
<tr>
<td>3.00 mL</td>
<td>7.00 mL</td>
<td>________</td>
<td>________</td>
</tr>
<tr>
<td>5.00 mL</td>
<td>5.00 mL</td>
<td>________</td>
<td>________</td>
</tr>
<tr>
<td>7.00 mL</td>
<td>3.00 mL</td>
<td>________</td>
<td>________</td>
</tr>
<tr>
<td>9.00 mL</td>
<td>1.00 mL</td>
<td>________</td>
<td>________</td>
</tr>
<tr>
<td>10.00 mL</td>
<td>0.00 mL</td>
<td>0.200M</td>
<td>________</td>
</tr>
</tbody>
</table>

\[ M: \quad C_b V_b = C_d V_d \quad \text{dilution with total volume 10 mL each time} \]

\[ \text{bulk} \quad \text{diluted} \]

\[ (0.200 \text{ M Co(NO}_3\text{)}_2 \text{) (1.00 mL)} = (C_d) (10.00 \text{ mL solution}) \]

\[ (0.200 \text{ M Co(NO}_3\text{)}_2 \text{) (1.00 mL) = 0.0200 M Co(NO}_3\text{)}_2 = C_d \]

10.00 mL solution
Standards: 50 mL per group 0.200 M Co(NO\(_3\))\(_2\)

Make standards:

<table>
<thead>
<tr>
<th>Vol.</th>
<th>Co(NO(_3))(_2)</th>
<th>H(_2)O</th>
<th>M Co(NO(_3))(_2)</th>
<th>mg/mL Co(NO(_3))(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.200 M</td>
<td>1.00 mL</td>
<td>9.00 mL</td>
<td>0.0200 M</td>
<td>3.66 mg/mL</td>
</tr>
</tbody>
</table>

Convert mol/L to mg/mL:
Convert through molar mass of Co(NO\(_3\))\(_2\) (182.95 g/mol) and units

\[
\left( \frac{0.0200 \text{ mol Co(NO}_3\text{)}_2}{L} \right) \left( \frac{182.95 \text{ g Co(NO}_3\text{)}_2}{\text{ mol Co(NO}_3\text{)}_2} \right) \left( \frac{1000 \text{ mg}}{1 \text{ g}} \right) \left( \frac{1 \text{ L}}{1000 \text{ mL}} \right) = 3.66 \frac{\text{mg}}{\text{mL}} \text{Co(NO}_3\text{)}_2
\]

\[
\left( \frac{0.0200 \text{ mmol Co(NO}_3\text{)}_2}{\text{mL}} \right) \left( \frac{182.95 \text{ mg Co(NO}_3\text{)}_2}{\text{ mmol Co(NO}_3\text{)}_2} \right) = 3.66 \frac{\text{mg}}{\text{mL}} \text{Co(NO}_3\text{)}_2
\]
Unknown: **10 mL** per group of **unknown**

**Changing unknown instructions from manual (pg 124 last paragraph)**

Unknown will be made by diluting 5 mL of unknown with 5 mL deionized water. This should give an absorbance reading less than the 0.2 M Co(NO$_3$)$_2$ sample

Determine diluted conc of unknown directly off handplot and calculate with equation of line on computer plot (**we will do both**).
The calibration plot of Cobalt II Nitrate has the equation:

\[ y = 0.0175x - 0.0086 \]

where \( R^2 = 0.9935 \).

The concentrations are given in milligrams of Cobalt (NO3)2 per milliliter (mg Co(NO3)2 / mL), and the absorbance values are shown on the y-axis.

The equation for the calibration plot is:

\[ C_{\text{bulk}} V_{\text{bulk}} = C_{\text{diluted}} V_{\text{diluted}} \]

For a specific unknown sample, the calculation is:

\[ (C_{\text{bulk}}) (5.00 \text{ mL bulk}) = (C_{\text{diluted from plot or eq}}) (10.00 \text{ mL solution}) \]
A 15.00 mL solution of unknown conc is diluted to 250.00 mL. A 10.00 mL aliquot (portion) of the 250.00 mL solution is analyzed with a spectrophotometer and the conc. of this soln is found to be $3.50 \times 10^{-2}$ mg/mL. What is the conc. of the original soln.?

\[
C_{\text{bulk}} V_{\text{bulk}} = C_{\text{diluted}} V_{\text{diluted}}
\]

\[
(C_{\text{bulk}}) (15.00 \text{ mL bulk}) = (3.50 \times 10^{-2} \text{ mg/mL}) (250.00 \text{ mL solution})
\]

\[
C_{\text{bulk}} = \frac{(3.50 \times 10^{-2} \text{ mg/mL}) (250.00 \text{ mL solution})}{15.00 \text{ mL bulk}} = 0.583 \text{ mg/mL}
\]
Why do we have to dilute the unknown and make sure its absorbance is below highest absorbance reading of standards?
Other changes in experiment:

Section – Plotting the Spectrum of Co(NO$_3$)$_2$

We will not do this section. We will use $\lambda_{\text{max}}$ of 626 nm for the Beer’s Law Plot section – standards and unknown.

Important points:
Be sure to use the same spec 20 for entire experiment (record serial #)

Do hand plot in notebook and also computer plot using excel.

Report conc. of bulk unknown in mg / mL Co(NO$_3$)$_2$ by hand plot data and computer (eq of line)
**Spec 20 operation**

Instrument should be warmed up for 15 minutes before used.

Select filter by switching lever on left side to 600-950nm

Select wavelength (626 nm) with knob on top and put on transmittance mode

With no sample and sample door closed, turn left knob (0%T) until you get a reading of zero

Fill cuvette with deionized water to middle of white mark on tube. Wipe the cuvette dry with Kimwipe. Place cuvette in sample holder with mark facing out towards you. Closed door. Put spec 20 in absorbance mode and turn right knob until read 0 absorbance.

Fill cuvette with either standard or unknown into place into spec 20. Record absorbance reading.

Check zero with blank; adjust as needed; new sample and read.