Objective: Determine concentration of iron in a water solution by spectrophotometry.

Concepts:
- Complexation
- Polydentate Ligands
- Volumetric Dilution
- Oxidation-Reduction
- pH Control with Buffers
- Beer’s Law

Techniques:
- Preparing precise dilutions & related calculations
- Initializing and Using a Visible-UV Spectrophotometer

Apparatus:
- Spectronic 20
- Volumetric Flask
- Buret

Organization of the Lecture
- Beer’s Law - Review
- Spectronic 20 - Review
- Our Complexing Agent - Phenanthroline
- Complexes - Nomenclature
- Stoichiometry of the Complex
- Our Reducing Agent - Hydroquinone
- Our Buffer - Citric Acid
- The Procedure
- Calculating Dilutions for Beer’s Law
- The Beer’s Law Spreadsheet
- The Beer’s Law Constant
- The Unknown - Dilution
- Why Two Measurements of same solution?
- Datasheet

Beer’s Law

Absorbance is related to concentration by:

\[ A = \varepsilon b c \]

Where:
- \( A \) = Absorbance at a particular wavelength
- \( \varepsilon \) = Absorptivity of the target substance
- \( b \) = Path length of light through the sample
- \( c \) = Concentration of target substance

Blank = All reagents except target substance

For Blank, Absorbance, \( A = 0 \) \( \%T = 100 \)

\[ A = 2.000 - \log \%T \]

(NO ABSORBING SAMPLE = NO ABSORBANCE)
Stoichiometry of complex is 3 phen : 1 Iron
i.e., 1 mol of **Ferrous Ion** (Fe$^{2+}$)
reacts with 3 mol of 1,10-Phenanthroline (phen)
The complex is red-orange
Analytical Wavelength (Absorbance maximum) is at 508 nm

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1,10-Phenanthroline complex in this exercise requires that IRON be in the **+2 state** **NOT +3**
BUFFERS

Use Sodium Hydrogen Citrate Buffer.

**CITRIC ACID** is a weak triprotic acid with acid ionization constants ($H_3Cit$):

- $K_1 = 7.41 \times 10^{-4}$, $pK_a1 = 3.13$
- $K_2 = 1.74 \times 10^{-5}$, $pK_a2 = 4.76$
- $K_3 = 3.98 \times 10^{-7}$, $pK_a3 = 6.40$

Its partially neutralized salts (NaH$_2$Cit) are natural buffers over a range of acidic pH's.

PROCEDURE

**Use in Pairs** for BEER’S LAW Determination

ALONE for Unknown

1. Obtain ~50 mL of iron stock solution in a clean, DRY beaker

2. **BEER’S LAW**

   Rinse buret with iron stock solution before preparing dilutions

   Prepare a BLANK solution

   BLANK solution must contain EVERYTHING other than the IRON

   Keep BLANK in cuvette for entire exercise!

   **Note Concentration**

   **Why do?**

   Concentration of Stock Solution = 40.0 mg/L

   \[
   \text{Vol of stock solution (mL)} \times \text{Conc of Fe}^{2+} (\text{mg/L}) = \text{Absorbance}
   \]

   \[
   V_1 \times C_1 = V_2 \times C_2
   \]

   \[
   V_1 = \frac{M_2}{M_1} V_2
   \]

   Concentration of Stock Solution = 40.0 mg/L

   Vol of stock solution (mL) | Conc of Fe$^{2+}$ (mg/L) | Absorbance
   --------------------------|------------------------|---------
   BLANK                      | 0                      | 0       
   1.12                      | 0.448                  | 0.093   
   3.24                      | 0.448                  | 0.274   
   4.86                      | 0.448                  | 0.418   
   7.0                       | 0.448                  | 0.83    
   10.22                     | 0.448                  | 0.848   
   \[
   V_1 = \frac{V_2}{V_4} \times 100 \text{ mL}
   \]

   Concentration of Stock Solution = 40.0 mg/L

   **Measure ABSORBANCE of solutions in order of INCREASING CONCENTRATION** - (most dilute first)

   Save dilutions in case you need to repeat absorption measurements after you plot the Beer’s Law data.

   Tabulate your data, calculate concentrations and determine the slope and R$^2$ of your Beer’s Law plot.

   Mix each solution thoroughly - then add water to bring to 100 mL mark and mix again

   **Wait for complex to form!** At least 10 minutes

   Then, measure absorbance of each solution at 508 nm

   **Remember:** Beer’s Law Determination is critical to everything that follows. Make sure it is done correctly!
Concentration of Stock Solution = 40.0 mg/L

<table>
<thead>
<tr>
<th>Vol of stock solution (mL)</th>
<th>Conc of Fe²⁺ (mg / L)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.12</td>
<td>0.448</td>
<td>0.093</td>
</tr>
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<tr>
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<td>2.82</td>
<td>0.593</td>
</tr>
<tr>
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<td>4.09</td>
<td>0.848</td>
</tr>
</tbody>
</table>

Spreadsheet requires 5 data points, (+ blank) in order of increasing concentration.

If you use the same or identical cuvettes for all Absorbance measurements:

\[
A = k_{\text{Beer}} c
\]

\[
R^2 > 0.98
\]

Beer’s Law Plot - Fe(phen)₃⁺²⁺

508 nm

SLOPE = 0.737 / 3.50 = 0.211 L / mg

CONVERGE IRON TO COMPLEX

To the 10 mL sample in the 100 mL volumetric flask, we add:

1. 2 mL of 25 g/L Sodium Citrate Buffer solution

2. 2 mL of 10 g/L HYDROQUINE solution

3. 4 mL of 2.5 g/L PHENANTHROLINE solution

4. Mix thoroughly

5. Bring to 100 mL by adding distilled water - carefully

6. Mix thoroughly again

The order of addition of the reagents is important!
7. Wait for complex to form
8. Measure the absorbance of diluted solution
9. Calculate concentration of diluted solution
10. Calculate concentration of original solution (X 10.00)
11. Repeat absorbance measurement a second time with a fresh sample of the **SAME** diluted unknown solution

**Why do we measure two separate samples of the same solution?**

1. The second sample had longer to form the colored complex.
   - If there is no change in absorbance, the reaction that forms the complex was probably complete.
   - If the second absorbance is greater than the first, the reaction may not have been complete.

2. Was the solution homogeneous?
   - If the second absorbance is different from the first, the solution may not have been homogeneous.
   - OR
   - If the two absorbance measurements differ by more than 3%, you should consider preparing a second dilution of your unknown.

**DATASHEET FOR UNKNOWN**

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance of diluted solution</td>
<td>0.471</td>
</tr>
<tr>
<td>Conc Fe²⁺ in diluted solution</td>
<td>2.23 mg/L</td>
</tr>
<tr>
<td>Conc Fe²⁺ in original solution</td>
<td>22.3 mg/L</td>
</tr>
</tbody>
</table>

**Slope of Beer's Law plot** = 0.211 L/mg

**From Part 1**

% Diff = \[ \frac{0.477 - 0.471}{0.471} \times 100 \% = 1.3 \% \]

Don't throw away any solutions until you are sure the data you collect are valid.

Be sure to rinse out volumetric flasks thoroughly before putting each new solution into them.