COLORS WE CONSUME
A spectroscopic analysis of FD&C food colors in soda pop

LAB VIS 12
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PURPOSE
Utilizing Beer's Law, determine spectroscopically the concentration of two absorbing species in an unknown solution.

INTRODUCTION
As you know, most soft drinks contain artificial colors. The color of a beverage plays a big part in the impressions a consumer receives about a product. The general thinking is that if a product looks good, it probably is. The developers of Cherry 7UP and Mountain Dew have taken this fact into account. The color of Cherry 7UP is as expected, a bright red. Mountain Dew, a citrus color also has its own distinguishable yet appropriate color, yellow.

Each soft drink contains its own artificial color. Cherry 7UP contains red dye # 40 while Mountain Dew contains yellow dye # 5. The artificial colors in Mountain Dew and Cherry 7UP have a specific concentration. We will be calculating the concentrations of the dyes (Red # 40 and Yellow # 5) in each brand of pop.

The two dyes we will be analyzing are common in many soft drinks. Their structures are shown below:

Red # 40

![Red # 40 structure]

Empirical Formula: $C_{18}H_{14}N_2O_8S_2Na_2$
Molecular Weight: 496.42

Yellow # 5

![Yellow # 5 structure]

Empirical Formula: $C_{16}H_9N_4O_9S_2Na_3$
Molecular Weight: 534.36
As the electrons in molecules absorb light energy, the energy promotes electrons to higher energy levels. As photons of light interact with the absorbing particles the initial radiant power $P_0$ is reduced to $P$. The ratio of $P$ to $P_0$ is called the transmittance $T$. That is:

$$T = \frac{P}{P_0}$$

Transmittance can be expressed as a percent. The percent transmittance is defined as 100 times the ratio of $P/P_0$.

$$\%T = 100\left(\frac{P}{P_0}\right)$$

The logarithmic inverse of the transmittance is called absorbance $A$

$$A = \log_{10}T = \log(100 / %T)$$

The absorption of visible light by an absorbing molecule produces an electronically excited species. The amount of energy associated with the photon exactly corresponds to the energy required to promote an electron into a higher energy state. Quantum mechanics states that only certain electronic transitions are allowable. The energy of these transitions (and the energy of the photon) can be calculated using the equation:

$$E = \frac{hc}{\lambda}$$

where $E$ is the energy measured in joules. The symbol $(h)$ is Planck's constant and has a value of $6.626 \times 10^{-34}$ kg m$^2$/s, $c$ is the speed of light ($3.0 \times 10^8$ m/s), and $\lambda$ is the wavelength associated with the photon of light measured in nanometers ($10^{-9}$ m).

An absorption spectrum may be obtained by plotting absorbance vs wavelength. The peaks in the graph correspond to the wavelengths of light that produce transitions of highest probability.

![Absorbance Spectrum of Nickel (II) Nitrate](image)
The wavelength at which the most light is absorbed is called $\lambda_{\text{max}}$ in spectroscopic quantitative analysis. In the example above, $\lambda_{\text{max}}$ is 300 nm. The largest signal is obtained at this wavelength. In the experiment we will find the maximum absorbance for both dyes and use them to perform quantitative analysis.

At a fixed wavelength, the amount of light absorbed is proportional to two parameters. These two factors are summarized in **Beer's Law**:

$$A = a \ b \ c$$

where $A$ is the amount of light absorbed (absorbance), $a$ is the proportionality constant called absorptivity, $b$ is the pathlength of light through the solution and $c$ is the concentration of the solution. The magnitude of $a$ is dependent on the wavelength of light absorbed as well as the units used of $b$ and $c$. If the cell length $b$ is measured in centimeters and the concentration $c$ is measured in moles per liter, the absorptivity is called the molar absorptivity ($\varepsilon$). Under these conditions Beer's law can be expressed as:

$$A = \varepsilon \ b \ c$$

where $\varepsilon$ has the units L cm$^{-1}$ mol$^{-1}$.

Absorbance, like absorptivity, is dependent upon two factors: 1) the concentration of the absorbing species, and 2) the pathlength of light through the solution. The higher the concentration, the more compound is present to absorb light, and the more light will be absorbed. If the reading is made in cells of different pathlengths, different readings will be obtained. As the pathlength increases, the light has to pass through more solution and more compound, hence more light will be absorbed. In order to use Beer's Law to determine an unknown concentration, it is simplest to hold $\varepsilon$ and $b$ constant. By working at a fixed wavelength, $\varepsilon$ is constant and if all measurements are recorded in the same cell, then $b$ is constant. Using these conditions, the amount of light absorbed by the solution is directly proportional to the concentration of compound in the solution.

By preparing standard solutions of varying concentrations and analyzing them at a fixed wavelength we can construct a plot of absorbance vs concentration called a Beer's Law plot. A linear least squares calculation (done on a computer) produces a best-fit curve for the plot. As you can see from the example below, the relationship between absorbance and concentration is a linear one if $\varepsilon$ and $b$ are held constant.
The concentration of an unknown solution can be read directly from the graph if its absorbance is obtained. Another method of solving for the concentration of an unknown solution is to use the mathematical equation for a straight line.

The equation for a straight line is:

\[ y = mx + b \]

where \( m \) is the slope of the line and \( b \) is the y-intercept for the graph. The letters \( y \) and \( x \) correspond to a point on the graph located at coordinates \((x,y)\). Substituting an absorbance reading for \( y \), we can solve algebraically, for the concentration \((x)\) of an unknown solution.

There are situations where Beer's law does not apply. Beer's law is successful in describing the absorption behavior only if the solution is dilute. In a solution that is of high concentration, the average distance between the absorbing species is reduced until each molecule affects the charge distribution of its neighbors. This in turn can alter their ability to absorb a given wavelength of light.

A similar effect is sometimes observed in solutions with low absorber concentrations but high concentration of other species. The close proximity of ions affects the molar absorptivity of the absorbing species by electrostatic interactions. Neither of these problems will be encountered in this experiment because the concentrations selected for analysis are dilute enough to eliminate these problems.

A third deviation from Beer's law occurs when changes in concentration affect the refractive index of the solution. This is a problem because \( \varepsilon \) is dependent on the refractive index of the solution. This deviation will not be encountered in this lab.
EQUIPMENT/MATERIALS

- Spect 20
- 2 cuvets
- 2 beakers
- 1 package of kimwipes
- 1 squirt bottle with distilled water
- 3 50 mL beakers
- 1 250 mL beaker

- 8.05 x 10^-5 M Red # 40 stock solution
- 4 additional Red # 40 standard solutions
- 7.48 x 10^-5 M Yellow # 5 stock solution
- 4 additional Yellow # 5 stock solutions
- Soda pop containing Red # 40 (Cherry 7UP)
- Soda pop containing Yellow # 5 (Mountain Dew)

PROCEDURE

λ_{max} search

1. In a 50 mL beaker, obtain about 15 mL of the Yellow # 5 stock solution. In another 50 mL beaker, obtain 15 mL of the Red # 40 stock solution. In a third 50 mL beaker, obtain about 15 mL of the solvent solution. Using the solvent as the blank, identify λ_{max} for Yellow # 5 by recording the absorption readings of the 7.48 x 10^-5 M stock solution every 5 nm beginning with 400 nm and ending with 450 nm. Discard all washings into the 250 mL beaker as waste. Identify λ_{max} by creating a wavelength vs absorption plot.

2. Use the solvent as the blank and put all waste and cuvet washings in the 250 mL beaker. Identify λ_{max} for Red # 40 by recording the absorption readings of the 8.05 x 10^-5 M stock solution every 5 nm beginning with 475 nm and ending with 525 nm. Identify λ_{max} by creating a wavelength vs absorption plot. Clean and dry the 50 beakers.

SPECTOSCOPIC ANALYSIS

3. Obtain about 35 mL of Mountain Dew in a clean and dry 50 mL beaker.
4. Obtain about 35 mL of the Cherry 7UP in a clean and dry 50 mL beaker.
5. At the λ_{max} for yellow # 5, measure the absorbance of the yellow # 5 stock solution and four standard solutions in order of increasing concentration. Measure the absorbance of the absorbance of the Mountain Dew as well. Use the 250 mL beaker to collect waste.
6. At the λ_{max} for red # 40, measure the absorbance of the stock solution and the four standard solutions in order of increasing concentration. Measure the absorbance of the Cherry 7UP at the λ_{max} as well. Use the 250 mL beaker to collect waste.

CLEAN UP

All solutions can be poured down the sink with copious amounts of water. Be sure to wash all glassware with soap and water.
**COLORS WE CONSUME**

<table>
<thead>
<tr>
<th>Color</th>
<th>$\lambda_{\text{max}}$ between</th>
<th>Color</th>
<th>$\lambda_{\text{max}}$ between</th>
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</thead>
<tbody>
<tr>
<td>Yellow #5</td>
<td>400nm and 405nm</td>
<td>Red #40</td>
<td>475nm and 480nm</td>
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<tr>
<td></td>
<td>410nm $\star$</td>
<td></td>
<td>485nm $\star$</td>
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<tr>
<td></td>
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<td></td>
<td>450nm</td>
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<td>525nm</td>
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**Standardization of Yellow #5**

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<thead>
<tr>
<th>Solution</th>
<th>Concentration</th>
<th>Absorbance</th>
<th>Solution</th>
<th>Concentration</th>
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<tbody>
<tr>
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<td>_____</td>
<td>_____</td>
<td>Red Stan 1</td>
<td>_____</td>
<td>_____</td>
</tr>
<tr>
<td>Yellow Stan 2</td>
<td>_____</td>
<td>_____</td>
<td>Red Stan 2</td>
<td>_____</td>
<td>_____</td>
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<tr>
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<td>_____</td>
<td>Red Stan 3</td>
<td>_____</td>
<td>_____</td>
</tr>
<tr>
<td>Yellow Stan 4</td>
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<td>_____</td>
<td>Red Stan 4</td>
<td>_____</td>
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</tr>
<tr>
<td>Yellow Stock</td>
<td>_____</td>
<td>_____</td>
<td>Red Stock</td>
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Spectroscopic Analysis of Unknown Soda Pops:

<table>
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<th>Absorbance</th>
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<th>Absorbance</th>
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</thead>
<tbody>
<tr>
<td>Yellow # 5</td>
<td>Mountain Dew</td>
<td>Red # 40</td>
<td>Cherry 7UP</td>
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**Graphs and Calculations:**
1. Construct a graph of the peak absorbance for both of the dyes. Be sure to identify $\lambda_{\text{max}}$ of each.
2. Construct a Beer's Law plot for both dyes. Perform a best fit curve (linear). Be sure to identify the data points with no connecting lines, only the best fit curve.
3. Using the Beer’s Law plots to determine the concentrations of the dye(s) in the Cherry 7UP and the Mountain Dew.

**Question:**
(to be included in conclusion)
1. Why could we use water as a solvent for the pop samples when we used the butanol-ethanol-ammonia solution for the standard solvent?