Concentrations and Dilutions of Food Dyes

Learning Goals:

1. Develop an understanding of the use of volumetric glassware.
2. Prepare a series of dye solutions of known concentrations.
3. Explore the relationship between concentration of solutions and their absorbance using a UV-vis spectrometer

Abstract:

The dyes approved for use in Foods, Drugs and Cosmetics are called FD & C Dyes. FD&C Food Dyes are large, organic molecules developed to enhance the color of foods. Based on their chemical structure, these dyes produce intense colors in very small concentrations that are reportedly safe for human consumption.

Table I. Color Additives Permitted For Direct Addition To Human Food In The United States

<table>
<thead>
<tr>
<th>Certifiable Colors</th>
<th>Colors Exempt from Certification</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD&amp;C Blue No.1 (Dye and Lake), FD&amp;C Blue No.2 (Dye and Lake), FD&amp;C Green No.3 (Dye and Lake), FD&amp;C Red No.3 (Dye), FD&amp;C Red No.40 (Dye and Lake), FD&amp;C Yellow No.5 (Dye and Lake), FD&amp;C Yellow No.6 (Dye and Lake), Orange B*, Citrus Red No.2*</td>
<td>Anatto extract, B-Apo-8'-carotenal*, Beta-carotene, Beet powder, Canthaxanthin, Caramel color, Carrot oil, Cochineal extract (carmine); Cottonseed flour, toasted partially defatted, cooked; Ferrous gluconate <em>, Fruit juice, Grape color extract</em>, Grape skin extract* (enocianina), Paprika, Paprika oleoresin, Riboflavin, Saffron, Titanium dioxide*, Turmeric, Turmeric oleoresin, Vegetable juice</td>
</tr>
</tbody>
</table>

*These food color additives are restricted to specific uses.
### Table II. Color Additives Certifiable For Food Use

<table>
<thead>
<tr>
<th>Name/Common Name</th>
<th>Hue</th>
<th>Common Food Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD&amp;C Blue No.1</td>
<td>Bright blue</td>
<td>Beverages, dairy products powders, jellies, confections, condiments, icings, syrups, extracts</td>
</tr>
<tr>
<td>Brilliant Blue FCF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD&amp;C Blue No.2</td>
<td>Royal Blue</td>
<td>Baked goods, cereals, snack foods, ice cream, confections, cherries</td>
</tr>
<tr>
<td>Indigotine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD&amp;C Green No.3</td>
<td>Sea Green</td>
<td>Beverages, puddings, ice cream, sherbet, cherries, confections, baked goods, dairy products</td>
</tr>
<tr>
<td>Fast Green FCF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD&amp;C Red No.40</td>
<td>Orange-red</td>
<td>Gelatins, puddings, dairy products, confections, beverages, condiments</td>
</tr>
<tr>
<td>Allura Red AC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD&amp;C Red No.3</td>
<td>Cherry-red</td>
<td>Cherries in fruit cocktail and in canned fruits for salads, confections, baked goods, dairy products, snack foods</td>
</tr>
<tr>
<td>Erythrosine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD&amp;C Yellow No.5</td>
<td>Lemon Yellow</td>
<td>Custards, beverages, ice cream, confections, preserves, cereals</td>
</tr>
<tr>
<td>Tartrazine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD&amp;C Yellow No.6</td>
<td>Orange</td>
<td>Cereals, baked goods, snack foods, ice cream, beverages, dessert powders, confections</td>
</tr>
<tr>
<td>Sunset Yellow</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Food and Drug Administration HFI 140*

In this experiment the following four common dyes will be used:

<table>
<thead>
<tr>
<th>Food Dye</th>
<th>Chemical Name</th>
<th>Chemical Formula</th>
<th>Molecular Weight</th>
<th>Structural Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue#1</td>
<td>Brilliant Blue FCF, or Erioglaucine</td>
<td>C_{37}H_{34}N_{2}O_{9}S_{3}Na_{2}</td>
<td>792.84</td>
<td><img src="BLUE1.png" alt="Structural Formula" /></td>
</tr>
<tr>
<td>Red#3</td>
<td>Erythrosin B</td>
<td>C_{20}H_{6}O_{4}I_{4}Na_{2}</td>
<td>879.86</td>
<td><img src="RED3.png" alt="Structural Formula" /></td>
</tr>
</tbody>
</table>
Diluting dyes to different concentrations and obtaining UV-vis spectra of these dilutions can explore the relationship between absorbance and concentration in the UV-vis range.

Figure 1: Sample UV-vis spectrum of green food color

The UV-vis spectrum shown in Figure 1 plots the wavelength on the X-axis and the absorbance on the Y-axis. In this spectrum, green food coloring shows two distinct absorbance maxima; one peak is at approximately 420 nm with an absorbance of 0.45 while the other is at 640 nm with an absorbance of 0.80. The shape of the spectrum and the wavelength of maximum absorbance for each peak are characteristic of the chemical compound.
As shown in Figure 2., the absorbance values are also directly related to the concentration of the sample.

Figure 2: Sample UV-vis spectra of four decreasing concentrations of green food color

Pre-Lab Assignment:

In your lab notebook, prepare the following information:

1. View the video clips on Using the UV-visible spectrometer, Using the Balance, and Making Solutions and Dilutions. You will need Quick Time video player to see them.
2. A brief (2-3 sentence) introduction to the lab.
3. A table of safety information including the chemicals used in the lab and any safety handling precautions. This information can be obtained from the MSDS safety sheets.
4. Calculate the weight of 0.00010 moles for all four food dyes.

Give the information to your TA at the beginning of the lab. You will not be allowed to work in the lab without this information.
### Procedure:

**A Flow Chart for Concentration and Dilution of Food Dyes**

1. Measuring volumes accurately with volumetric glassware.

   **A. Use of a volumetric flask**

   1. Weigh an empty, dry 100 mL volumetric flask, including the stopper, to the closest milligram (+/- 0.001 g).

   2. Fill the flask with distilled water to the mark (use an eye dropper when you get close to the mark) replace the stopper and dry the outside of the flask then reweigh the flask.

   Volumetric flasks can be used to accurately measure volumes of liquids. By measuring to the mark on a volumetric flask, less error will occur. When the meniscus of the solution just touches the line on the volumetric flask the volume is exactly the volume indicated on the flask label. The exact volume is reached by carefully adding liquid to the flask with a dropper as you approach the mark. The bottom of the meniscus of the liquid should reach but not exceed the mark. A meniscus is the curvature that occurs at the surface of a liquid when it is confined in a narrow glass tube such as a pipet or buret.

   3. Determine the weight of the water and its temperature. From a table of Water Densities at Different Temperatures, determine the density of the water at the measured temperature and calculate the volume of water in the volumetric flask. How many significant figures will you have in the final answer? How does this value compare to the accuracy given on the neck of the flask?
Volumetric glassware allows you to accurately measure a volume to at least three significant figures. Volumetric glassware includes volumetric flasks, pipettes, and burets. In contrast, graduated cylinders provide two significant figures of accuracy and beakers provide one. For instance, a pipet can be used to measure 5.00 mL of liquid, a graduated cylinder can be used to measure 5.0 mL of liquid and a beaker can measure 5 mL of liquid.

4. How can you improve on your technique for filling the flask so that you can be more exact? Use this improved technique whenever you use a volumetric flask to make solutions.

B. Use of a measuring pipet

1. Using a 10 mL graduated measuring pipet and a pipet filler, practice accurately transferring different amounts of water from one container to another.
2. Weigh a dry, 125 mL Erlenmeyer flask and then transfer exactly 5.00 mL of water using the measuring pipet.
3. Weigh the flask after transfer and determine the exact volume of liquid that was transferred.
4. Make a data table listing the number of times the water is measured, the attempted volume, the exact mass transferred and its calculated volume.
5. Repeat this exercise until you can accurately reproduce different volumes using the pipet. This skill is crucial to the next part of this lab and many of the subsequent labs in this course. The ability to accurately measure volume is as important in science as the ability to accurately measure mass on a balance.

2. Diluting the Assigned Dye:

A. Make a series of 1:10 dilutions

1. Using the analytical balance, a small spatula and a small plastic weighing boat measure 0.00010 moles (1.0 x 10^{-4}) (to two significant figures, +0.001 g) of your assigned dye into a small plastic weighing dish. (the mass needed does not have to be exactly the amount to give 0.00010 moles but you need to know the weight to the closest milligram so that the exact number of moles can be accurately calculated)
2. Transfer all of the dye to a 150 mL beaker. Use the wash bottle with distilled water to rinse any dye left in the weigh dish into the beaker. Add approximately 50 mL of distilled water to the beaker and stir carefully until the dye is completely dissolved. Transfer all of the solution to a 100 mL volumetric flask, rinsing the beaker several times. Add distilled water to the volumetric flask until the bottom of the
meniscus just reaches the mark on the flask neck. Replace the stopper. While holding the stopper, carefully invert the flask several times to insure complete mixing of the solution. You should now have 100 mL sample containing a known number of moles of the assigned dye. Pour the solution into a beaker. This solution is called the "stock" solution.

What is the concentration in molarity of your stock solution?

3. Prepare 5 volumetric dilutions of the stock solution. Each should be 1/10 as concentrated as the previous one. (By pipeting exactly 10.00 mL of stock solution into a 100 mL volumetric flask and filling to the mark the first 1:10 dilution can be preformed.)

4. Calculate the concentration for each of the dilutions.

**B. Obtain spectra of 1:10 dilutions**

1. Fill a small plastic UV-vis cuvet 1/2 full of the stock solution. Repeat with each of the dilutions. Scan their spectra using a UV-vis spectrometer; water will be used for the blank. Be sure to record their file names. Obtain a print out of your spectra.

2. Some of the spectra may be of solutions that are too concentrated to be usable. Since Absorbance values are logarithmic any value above 2.0 begins to become too large to use. Determine the first spectra that has a maximum peak that is smooth and with a lmax value of 2 or less. This will be the spectrum of the solution, which has the maximum concentration of the dye solution, which will give a useful smooth UV-vis spectrum.

3. Determine which spectrum has the minimum detectable amount of dye at λmax (minimum concentration of the dye solution detectable by the UV-vis spectrometer.)

4. Good clear spectra are smooth (except for the interference from the plastic cuvets in the 200-300 nm range) and have distinctive peaks. Figure 3 is an example of an acceptable spectrum.
5. Poor spectra have jagged, unclear peaks, a high baseline and can be off scale. This sample should be diluted in order to obtain a usable spectrum.

6. You will need to determine the best wavelength for recording absorbance values for your assigned dye.
The maximum absorbance is the absorbance reading (y-axis) for the top of a peak. The wavelength for the maximum absorbance peak is read from the x-axis. The shape and the location of the peaks in UV-visible spectra are defined by the wavelength and maximum absorbance.

C. Make additional solutions and obtain their spectra

1. Starting with the most concentrated solution, which gives a smooth spectrum, make several more diluted solutions with smaller and smaller concentrations until you reach the concentration with the minimum detectable amount of your dye. You will need at least four more diluted solutions whose concentrations fall between the maximum concentrated with a smooth curve and the minimum detectable concentration.

2. Obtain spectra of these dilutions. The spectra of the additional dilutions should look somewhat like Figure 2, although the absorbance values, shape and number of peaks will probably be different.

3. Record the dilution factors and calculate the concentration in molarity of each dilution. (See the sections on Concentrations and dilutions in your text.)

4. From the spectral data taken prepare a table of concentration vs. absorbance at a wavelength of strong absorbance for your dye solutions (remember to include the point (0,0) as the UV-VIS was zeroed with distilled water which has a 0 absorbance and a 0 concentration of food coloring).

5. Plot this data [concentration (the independent variable) on the x axis, absorbance the (dependent variable) on the y axis] using the ICN Spectra View program at the top of your Progress Page or a Spread Sheet Program such as Excel.
6. Determine the resulting equation for the above plotted line and the $R^2$ value (a measure of how well the points fit the straight line).

7. Obtain a spectrum of an unknown concentration your assigned dye sample. Use the above plot to determine the concentration of this sample.

Post Lab Assignment:

Include the following in your lab report. Remember to show examples of all calculations.

1. The temperature and mass of water contained in your 100 mL volumetric flask with it's corresponding calculated volume.
2. A copy of the data table listing the number of times the water is measured, the attempted volume, the exact mass transferred and its calculated volume.
3. The concentration in molarity of your dye stock solution.
4. The dilution factors and the concentrations in molarity for the diluted (1:10) solutions.
5. A printout of the spectra from the above dilutions. The solution with the maximum concentration of the dye solution, which will give a useful smooth UV-vis spectrum and the solution with the minimum concentration of detectable dye, should be labeled. The peak with the clearest maximum should be designated the wavelength, $\lambda_{\text{max}}$, for collecting absorbance values and should be labeled.
6. A table containing the concentration and absorbance for each of the next set of solutions, which were made to fall between the maximum concentration and the minimum concentration dilutions. Print the spectra of these dilutions plus the maximum concentration and the minimum concentration spectra and again label $\lambda_{\text{max}}$.
7. A plot of concentration on the x axis and absorbance at $\lambda_{\text{max}}$ on the y-axis for these solutions.

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