Purpose

Apply dilution techniques and use a calibration curve to determine an unknown..

Learning Objectives

Develop good laboratory technique in data collection.

Use computer to generate and model a calibration curve.

Apply dilution concepts.

Develop and use a calibration curve.

Learn and apply Beer-Lambert law.

Discussion

Did you know that cranberries are used to treat many diseases such as urinary tract infections and kidney diseases? There have been numerous research studies performed analyzing the effects of cranberry in the human body. The results have shown that aside from cranberries treating urinary tract infections and kidney diseases, they also contain moderate levels of Vitamin C, dietary fiber, dietary minerals, manganese, and micronutrients that benefit the cardiovascular and immune systems. Cranberries also contain phytochemicals, such as anthocyanins (Figure CR.1), which have alternating single and double bonds that give cranberries their deep red color, and that result in the high absorbance of ultraviolet radiation.

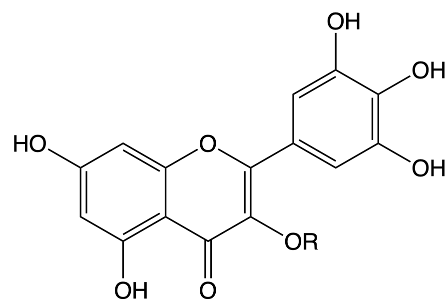

Figure CR.1: Anthocyanins: Molecular structure of myricetin, one of the anthocyanins that give cranberries their deep-red color. The "R" in the structure represents a sugar.

In this laboratory students will determine the percentage of pure, natural cranberry juice in cranberry-apple juice. Students will analyze a commercial juice found in the grocery store. An Agilent Cary 60 UV-Vis Spectrophotometer will be used to determine the percentage of natural cranberry juice in the cranberry-apple juice.

The use of a spectrophotometer for experimental analysis involves the interaction between electromagnetic radiation with matter. The degree of absorbed radiation, by matter, is measured via the spectrophotometer. The spectrophotometer will measure the transmitted light intensities with a photosensitive detector at specific visible wavelengths. The light regions used in a spectrophotometric analysis range from ultraviolet to infrared regions.

For this experiment, measurements will be taken in the visible light region since cranberry juice has a visible red color. Figure CR.2 shows a schematic of a spectrophotometer.

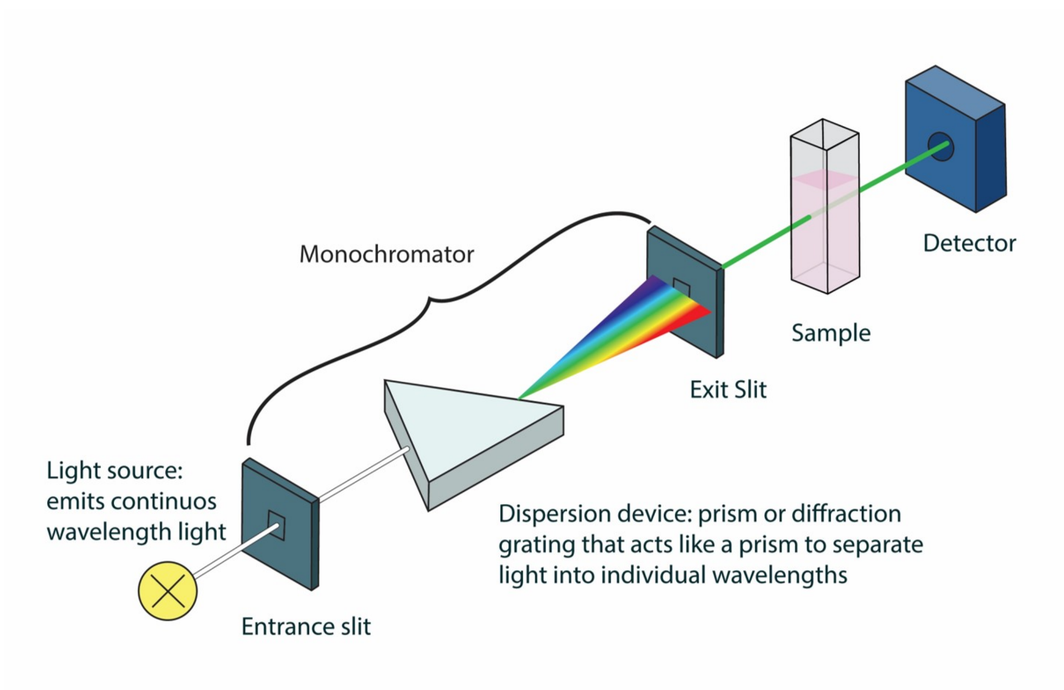

Figure CR.2: Schematic of a Spectrophotometer

The light source emits light with a range of wavelengths (λ_{total}), which then passes through a monochromator containing a diffraction grating to select a particular wavelength of light, λ_x. Comparing the intensity of the light before it goes through the sample (the incident light intensity, I_0) with the intensity of the light after it passes through the sample (the transmitted light intensity, I) gives a measure of the absorbance of the sample. This absorbance measure is detected and the measurement is given as output. Figure CR.4 displays the schematic of the Agilent Cary 60 UV-Vis Spectrophotometer (at the end of the Discussion section).

Absorbance values of a solution are directly proportional to its concentration. This relationship is known as the Beer-Lambert law, and takes the form $A = \epsilon bc$, where ϵ is a constant unique to each different substance, b is the path length of the sample through which light travels, and c is the concentration of the solution. This relationship generates a linear plot, an example of which is shown in Figure CR.3. The slope and intercept of this linear plot, also called a calibration curve, can be used to calculate the concentration of a similar solution of unknown concentration if the absorbance is known. Note: While the equation above, $A = \epsilon bc$, indicates an intercept equal to zero, the line may not go through zero because of random error effects being noticeable if just a few samples are used for the calibration curve; with a greater number of samples, the line should go through zero.

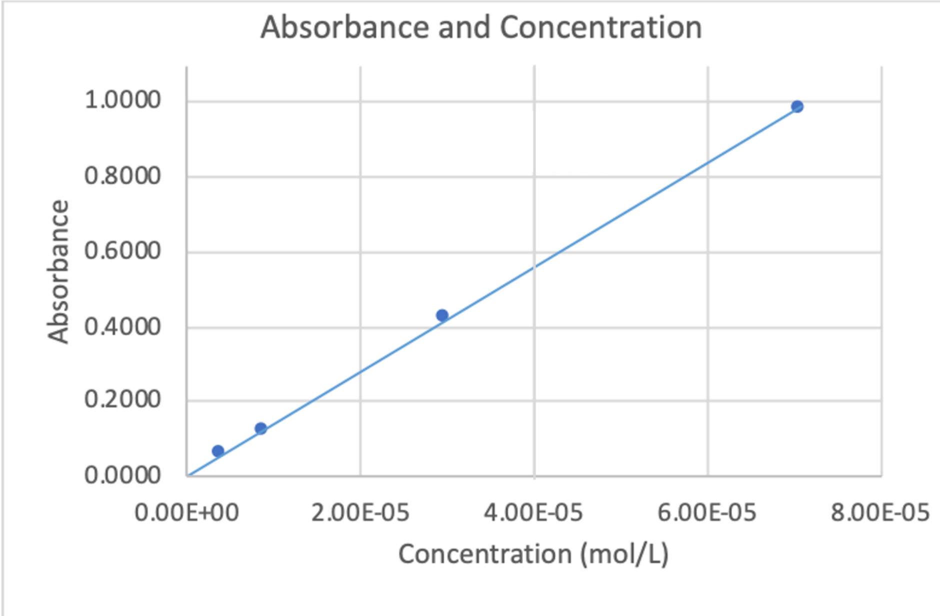

Figure CR.3: The Direct Proportional Relationship of Absorbance vs. Concentration

In Figure CR.3, the absorbance values of four solutions of various concentrations were measured. You will use five solutions for your calibration curve. To generate the calibration curve, a plot of absorbance versus concentration should be made. Once this plot is made, a linear trendline should be constructed to determine the equation of the straight line formed by the points. The standard equation of straight-line should be of the form $y = mx + b$, or, in the example above, $A = mc + b$. Given this equation, if the absorbance is measured of a solution of the same substance but of unknown concentration, the unknown concentration can be calculated by substituting the measured absorbance into the equation as the value of A and solving for c, the unknown concentration. Alternatively, the unknown concentration can be read directly off the graph by finding the value of the concentration that corresponds to the value of the measured absorbance.

Due to the procedure used to measure the concentration of cranberry in apple-cranberry juice, students must make sure that apple juice does not contribute any color to the deep red color of cranberry juice. In this laboratory, you will be generating an absorption spectrum for cranberry juice. Students will compare this absorption curve to the absorption spectrum of apple juice. This comparison will determine whether apple juice does or does not contribute to the deep red color of cranberry juice.

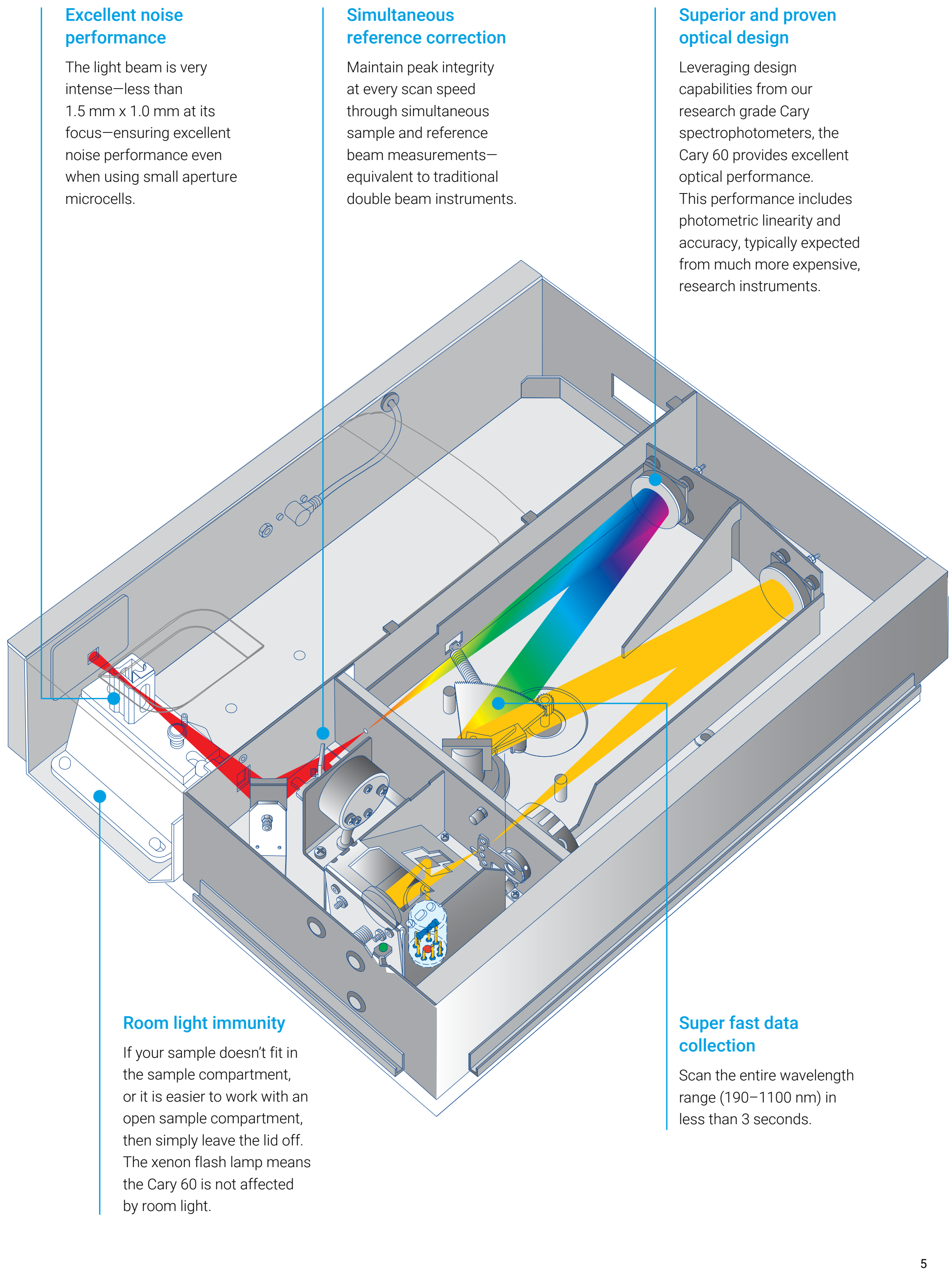

Figure CR.4: Schematic of the Agilent Cary 60 UV-Vis Spectrophotometer

Procedure

Special Procedure

Instructions for operation of the instrument will be posted near the instrument. In order to get accurate absorbance readings, use a Kimwipe to clean the outside surface of the cuvet. ONLY handle the rim of the cuvet. Follow this procedure whenever you fill and handle a cuvet.

Safety Precautions

No hazardous materials.

Before beginning this experimental procedure, obtain approximately 100 mL of the cranberry juice, 5 mL of cranberry-apple juice, and 25 mL of apple juice in separate beakers. Label each beaker appropriately; these amounts of juice samples will be used throughout the experiment.

Apple and Cranberry Juice Absorbance Spectrum

1. 1. Prepare 20% Cranberry Juice Diluted Solution using commercial cranberry juice cocktail (27% cranberry).
    a. Obtain a pipet, a 100 mL volumetric flask, and a clean cuvet.
    b. Measure the required amount of purchased cranberry juice cocktail with a pipet and pipet pump into a 100 mL volumetric flask.
    c. Fill up the volumetric flask with deionized water to the 100 mL mark. Cap and invert the flask several times to ensure that the cranberry juice and deionized water have mixed thoroughly.

2. Prepare 20% Apple Juice Diluted Solution using commercial apple juice (100% apple) using a smaller volumetric flask.

3. Measure the absorption spectra of both the 20% diluted cranberry juice, and 20% diluted apple juice.
    a. Fill a clean cuvet at least three-fourths full with this diluted cranberry juice using a plastic pipet. Wipe the outside of the cuvet with a Kimwipe. Handle the cuvet only by the rim when inserting it into the UV spectrophotometer.

4. Compare the spectra of the apple juice to the spectra of the cranberry juice, to determine if apple juice contributes any color to the deep red color of cranberry juice.

5. Identify the lambda maximum (λ_{max}), which is the wavelength corresponding to the maximum absorbance in the visible region (between 400 nm–700 nm) of the cranberry juice.

Cranberry Calibration Curve

1. Prepare a series of standard solutions of cranberry juice as given below, starting with your 20% cranberry juice sample.
   a. Use the 20% cranberry solution to make the following dilutions in the appropriate volumetric flasks: 100 mL of 4% cranberry 50 mL of 8% cranberry 25 mL of 12% cranberry 10 mL of 16% cranberry

2. Fill four cuvets with each of the diluted cranberry juice standards.

3. Measure the absorbance of 20% dilution sample and the four samples above at the λmax determined in the absorption spectrum of the cranberry juice. There will be five control standards of diluted cranberry juice.

4. Prepare a calibration curve of absorbance versus % concentration, and verify that a linear relationship exists. Redo any points that deviate significantly from the line.

5. Determine the equation of the straight line and R^2 value.

Absorbance of Cranberry-Apple Juice

1. Fill a clean cuvet with undiluted cranberry-apple juice.

2. Measure the absorbance of the cranberry-apple juice at the λ_{max} determined in the absorption spectrum of the cranberry juice.

3. Calculate both graphically and using the linear regression model the percentage of cranberry juice in the cranberry-apple juice.

> **Waste Disposal**: All juice waste can be disposed of down the drain.