Determination of Allura Red Concentration in Mouthwash

GOAL AND OVERVIEW

The spectral profile of Allura Red, or red dye #40, will be measured using a Spec 20 spectrometer. From the spectral profile, the wavelength of light of maximum absorbance, λ_{max}, will be determined. Using the absorbance values obtained for a series of volumetric dilutions made from a stock solution of known Allura Red concentration, a Beer's law plot of absorbance versus concentration will be constructed. The Beer's law plot will be used to determine the concentration of Allura Red in a sample of commercial mouthwash.

Objectives and Science Skills

- Explain dilution and how it relates to moles of solute in a solution and how it changes the molarity of a solution.
- Perform both standard and serial volumetric dilutions and calculate associated molarities.
- Understand and explain absorption spectroscopy and the mathematical relationships between percent transmittance, absorbance, concentration, path length, and extinction coefficient.
- Apply linear fitting methods to find relationships between dependent and independent variables, such as percent transmittance (absorbance) and concentration; explain and apply Beer's Law.
- Use absorption data to qualitatively and quantitatively analyze the concentration of Allura red in solution.

SUGGESTED REVIEW AND EXTERNAL READING

- reference material on spectroscopy and dilution; textbook information on spectroscopy

BACKGROUND

The study of the interaction of light with matter is called spectroscopy. Spectroscopy can be used for both qualitative and quantitative analysis of matter.

Recall that visible photons correspond to a very small part of the electromagnetic spectrum between 400 (violet) and 700 (red) nanometers (1 nm = 10^{-9} m). This lab uses ***absorption*** of visible light to measure the amount of a molecule present in solution.

Absorption and emission strictly follow the law of conservation of energy. The photon's energy is transferred directly to or from the molecule: $E_{photon} = \Delta E_{molecule}$. The absorption or emission of a photon of the appropriate energy can cause transitions between electronic and vibrational energy levels of a molecule. Molecular absorption and emission spectra are called band spectra because they appear as broad peaks ("bands").

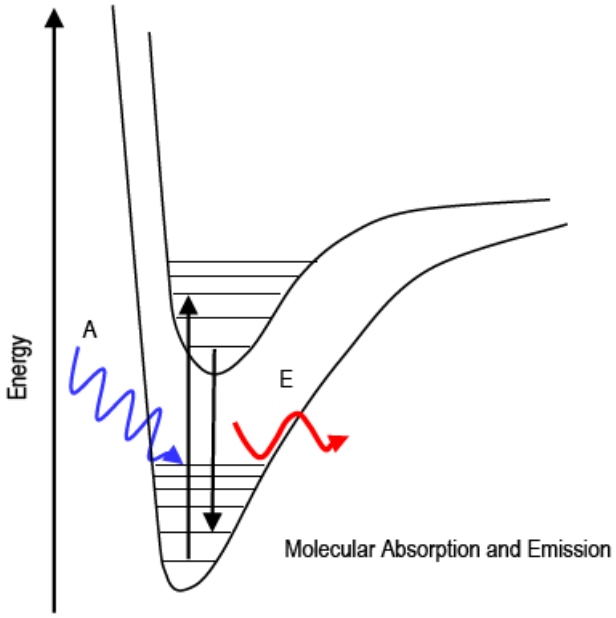

Figure 1

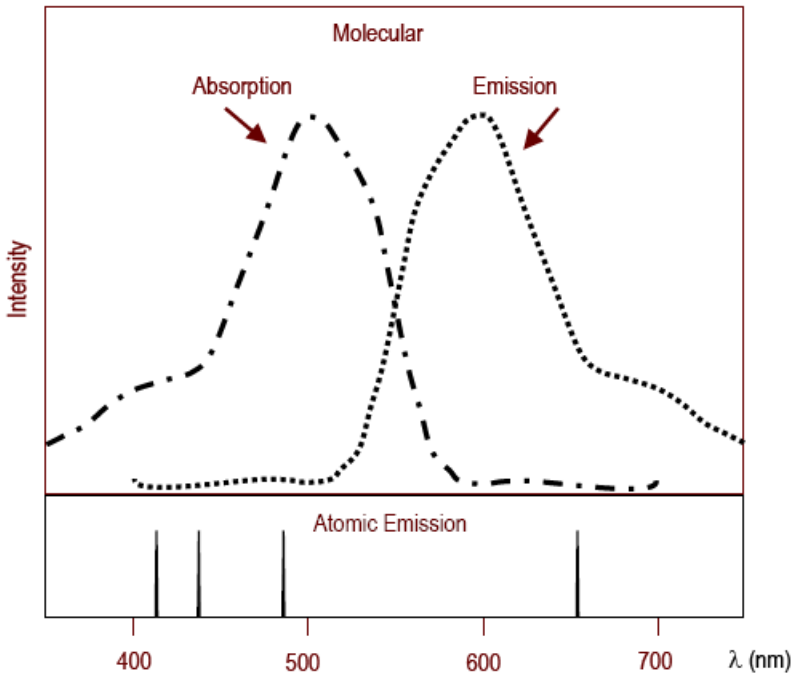

Figure 2

When our eyes see a colored substance, the observed color could be the result of two scenarios:

i the light comes from the emitting source directly; or,

ii the photons have been reflected or transmitted from some molecules.

Our brain interprets the following wavelengths as the indicated colors:

- Violet 400 - 420 nm
- Indigo 420 - 440 nm
- Blue 440 - 490 nm
- Green 490 - 570 nm
- Yellow 585 - 620 nm
- Red 620 - 700 nm

White light is a mixture of all visible wavelengths (400-700 nm). When white light shines on an object that appears colored, the object is absorbing some wavelengths and is transmitting (or reflecting) the rest; the "rest" is observed.

If one color of light is mixed with light of its "complementary color," the resulting light will look white. A color wheel for light is used to determine a color's complement, where: R = red, O = orange, Y = yellow, G = green, B = blue, V = violet. For an object to be colored under white light, it must absorb the light of its complementary color. For example, green leaves absorb red and violet, reflecting green to our eyes.

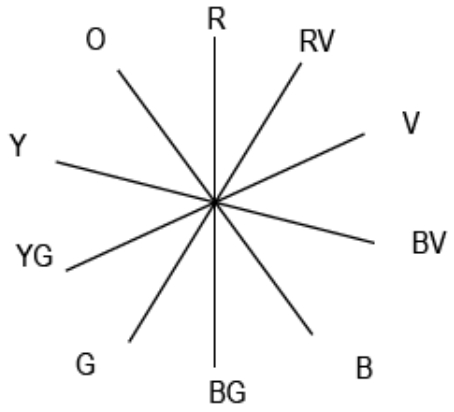

Figure 3

Few molecules undergo emission processes that can be measured spectroscopically, so absorption studies are much more common. Every molecule absorbs some wavelengths of light in some region of the electromagnetic spectrum. Quantitatively, Beer's law describes the relationship between the number of photons absorbed and the number of absorbing molecules in a sample. In a typical experiment, the spectrometer is used:

i to scan over a range of wavelengths to identify which wavelength is most strongly absorbed by the molecule of interest (λ_{max});

ii to quantify the concentration of absorbing molecules in the sample by measuring how many photons are absorbed at λ_{max} (Beer's Law).

The following section describes the mathematical basis for Beer's Law, with Allura Red acting as the absorbing molecule. A solution of Allura Red is placed into the sample holder ("cell"). The cell is then inserted in the sample holder in the spectrometer.

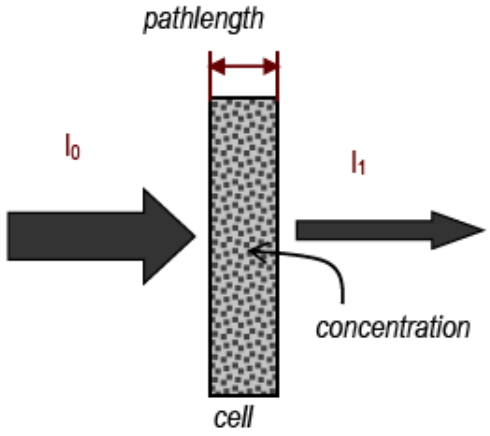

Figure 4

In the spectrometer, incident light of a known wavelength at an initial intensity I_0 is directed through the cell. Each Allura Red molecule has an equal probability of absorbing a photon as the light passes through the sample.

The light emerging from the far side of the cell has a reduced intensity, I_1, as shown in figure 4. The fraction of the incident light transmitted through the cell is I_1 / I_0. This ratio is called the transmittance and is given the symbol T. Regardless of the incident light intensity, a given cell transmits the same fraction of the incident light.

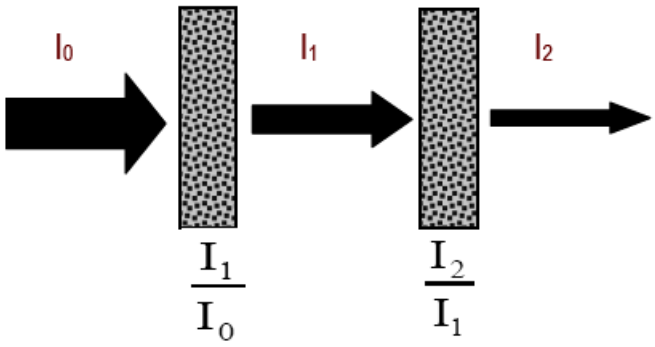

Figure 5

Suppose the light of intensity I_1 emerges from the first cell and enters another cell that is identical to the first one. The light that emerges from the second cell has intensity I_2. Beer's law asserts that the second cell transmits the same fraction of I_1 as the first cell did of I_0. The overall transmittance T_{total} is the product of the two individual transmittances. The same effect would be noted if a third (or fourth, fifth, ...) cell were inserted. The total transmittance is the product of the individual transmittances.

$$T_1 = \frac{I_1}{I_0} = \frac{I_2}{I_1} = T_2 \tag{1a}$$

$$T_{\text{total}} = \frac{I_2}{I_0} = \frac{I_1}{I_0} \cdot \frac{I_2}{I_1} = \left(\frac{I_1}{I_0}\right)^2 \tag{1b}$$

If a cell with pathlength b is used, its transmittance can be referenced to that of a 1 cm pathlength cell with the same concentration of absorbing molecules. The "cell number" is just the pathlength b in cm. In this more general case given below.

$$T_{\text{total}} = \frac{I_b}{I_0} = \frac{I_1}{I_0} \cdot \ldots \cdot \frac{I_b}{I_{b\,-\,1}} = \left(\frac{I_{1\text{ cm}}}{I_0}\right)^b = (T_{1\text{ cm}})^b \tag{2}$$

Note that the pathlength b appears as a power. Think of it this way: if one, 2-cm cell were used, it would be the same as using two, 1-cm cells.

This idea also explains the observed concentration dependence. For a concentration c, as referenced to a 1 M solution shown below.

$$T_{\text{total}} = \frac{I_{\text{conc}}}{I_0} = \left(\frac{I_{1\text{ M}}}{I_0}\right)^c = (T_{1\text{ M}})^c \tag{3}$$

If concentration were doubled, it would be equivalent to having two identical cells in the path of the incident light. Tripling the concentration would be equivalent to three identical cells, and T would be cubed. Note that the concentration c appears as a power.

This brings up a central ideal in Beer's Law: transmittance depends on the number of absorbing molecules in the path of the light. Transmittance will decrease (more light absorbed) as the number of absorbing molecules in the path of the light increases. It does not matter whether those molecules are there because the pathlength is longer or because the concentration is increased. If we use a 1-cm reference cell and a 1 M solution, total transmittance becomes the following equation.

$$T_{\text{total}} = (T_{1\text{ M, }1\text{ cm}})^{bc} = \left(\frac{I_{1\text{ M, }1\text{ cm}}}{I_0}\right)^{bc} \tag{4}$$

Whatever fraction of light is transmitted in a 1 cm path, that fraction squared will be transmitted in a 2 cm path, cubed in a 3 cm path, etc. Whatever fraction of light is transmitted by a 1 M solution, that fraction will be squared for a 2 M solution, cubed for a 3 M, etc.

The discussion this far has only spoken to the number of absorbing molecules, not their identity. To account for how strongly a molecule absorbs light of a given wavelength, the *molar extinction coefficient*, ϵ, has been defined. In terms of the fraction of light transmitted by a 1 cm path containing a 1 molar solution of the absorbing molecule, $T = 10^{-\epsilon}$. Note ϵ appears as a power.

ϵ is both molecule- and wavelength-specific. A larger value for ϵ means that the molecule absorbs "better" at that wavelength (lower transmittance). $\epsilon = 0$ means the molecule does not absorb that wavelength.

When the molar extinction coefficient is included in the general transmittance equation, the following relationship between transmittance, pathlength and (here) Allura Red concentration can be written as:

$$T = \left(\frac{I_{1\text{ cm, }1\text{ M}}}{I_0}\right)^{bc} = (10^{-\epsilon})^{bc} = 10^{-\epsilon bc} \tag{5}$$

where b is pathlength of the light through the cell in cm, c is the concentration in molarity, and ϵ is the molar extinction coefficient of Allura Red at that wavelength. Note the exponential dependence observed for ϵ, b, and c.

Many values of ϵ are listed in tables. These values are usually given at $\lambda_{\max}$, where the molecule absorbs most efficiently. Not only is the spectrophotometer most sensitive for quantitative analysis at $\lambda_{\max}$, $\lambda_{\max}$ represents the peak of the absorption curve, which is usually flat right around its maximum. This means that if the wavelength of the light generated by the spectrometer happens to fluctuate slightly during the experiment, the absorption should not be greatly affected (and precision is not greatly compromised).

Spectrometers can often be read in either percent transmittance, $\%\,T$ (which is $100\% \times T$) or absorbance, A. The relationship between A and T is Beer's Law.

$$A = -\log\left(\frac{\%T}{100\%}\right) = -\log(10^{-\epsilon bc}) = \epsilon bc \tag{6}$$

You should read the $\%\,T$ from the Spec 20, and then convert to absorbance, A. A is the preferred unit because it changes linearly with concentration. If concentration of a compound doubles, then its absorption doubles. By measuring absorption changes, you can qualitatively infer that concentration is changing. If you know ϵb, you can use absorption measurements to quantitatively calculate concentration.

Use of the Spec 20 Spectrometer

In a spectrometer, light from a source passes through a number of components before going through the sample and onto a detector. A bulb emits white light, which contains all wavelengths. The light passes through a monochromator, which is a device (usually a prism or a grating) that spreads out the different colors and lets only one get through. By adjusting the monochromator, the wavelength can be selected. This single wavelength is then passed through the sample. The light then strikes a detector, which is a photocell whose electrical output is proportional to the amount of light that strikes it. This output is amplified to drive a meter that shows how much light was absorbed or transmitted.

Source → lenses/slits/collimators → monochromator → sample → detector → computer/measuring device

We use a Bausch and Lomb Spectronic 20 (often called simply a "Spec 20"). The detectors used

in the Spec 20 are sensitive to only limited ranges of wavelengths. The blue-sensitive detector is used for the range 350-595 nm, and the red-sensitive detector is used for the range 600-950 nm. If the wrong detector is in place, you will not get data that makes sense.

Spectrometers read the transmittance, which is the fraction of the entering light that is transmitted:

$$\begin{aligned} \%\text{ Transmittance} &= \%T = \%\text{incident light transmitted} = \frac{\text{intensity of light leaving cell}}{\text{intensity of light entering cell}} \times 100\% \\ &= \frac{I}{I_0} \times 100\%. \end{aligned} \tag{7}$$

They also read the absorbance, A, which is related to T as described in Eq. 6. Calculate absorbance from Eq. 6.

Steps for Using Spec 20 to Measure Absorbance

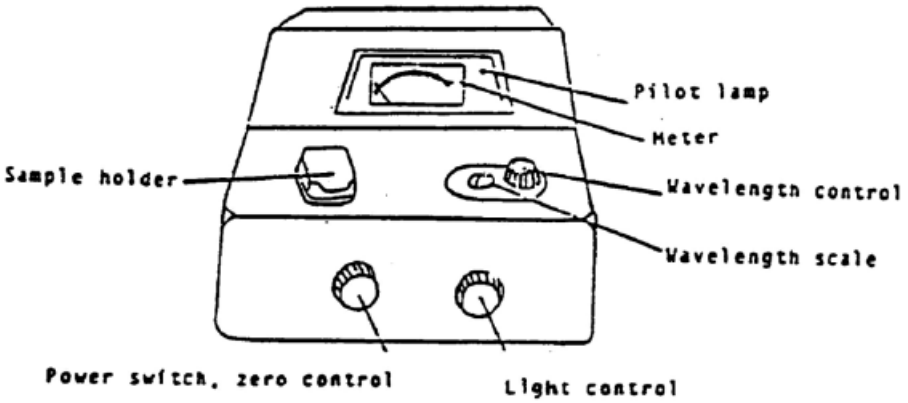

Figure 6

Do not fill the cuvet (or cell) more than 2/3 full.

Be sure to **dry** the cuvet with Kimwipe before you put it into the sample holder.

Do not leave a cuvet in the sample holder when you have finished a reading. Take it out and close the sample holder cover.

1 If the spec is off, make sure sample holder is empty, close the cover, and turn the spec on. It should warm up 30 minutes. Your instrument should already be warmed up.

2 Be sure that the correct detector is in place (blue sensitive, 350-595 nm, or red-sensitive, 600-950 nm).

3 Set the wavelength that you want.

4 You must blank the spec every time you change the wavelength setting. Adjust to 0% transmittance with sample holder empty and cover closed (turn zero control knob to give 0% T reading

on meter). Adjust to 100% transmittance with the sample holder containing a cuvet 2/3 full of solvent (the "blank"). *Line up the mark on the cuvet with the mark on cell holder* to insure a constant pathlength for all readings. Close the cover and turn the light control knob to the 100%T reading. If needle flickers or wanders after about 5 sec, report the malfunction. Remove the cuvet and close the cover.

5 Re-check 0%T and 100%T until no further changes are needed.

6 Measure %T of the sample. Fill the other cuvet 2/3 full of the sample. Insert the cuvet and close the cover. Read the %T. Remove the cuvet and close the cover.

You will determine ϵ for Allura Red by preparing a Beer's Law plot, which graphs absorbance versus concentration (y vs. x) at $\lambda_{\max}$. The slope should be straight and equal to ϵb; the y-intercept should be 0. You must:

i collect the spectral profile (absorbance over a range of wavelengths) and plot the data to find $\lambda_{\max}$; and,

ii take absorbance measurements at $\lambda_{\max}$ using solutions of known concentration to create a Beer's Law plot. The slope divided by the cell pathlength (measured in cm) is the experimentally-determined value for ϵ.

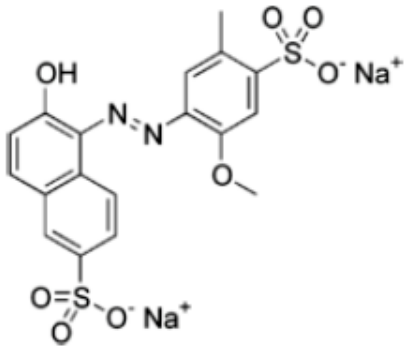

Figure 7

Allura Red, red dye #40, has a molar mass of 496.42 g/mol. It usually comes as a sodium salt, but it is soluble in water. In aqueous solutions, its $\lambda_{\max}$is around 504 nm. Allura Red was approved for use in food in 1971 after Allied Chemical Corporation subjected it to the most expensive and thorough testing program that had ever been given to a food dye.

You will use your Beer's Law plot to analyze a commercial mouthwash for its Allura Red content.

PROCEDURE

Be sure to use the same Spec 20 for the entire experiment.

Part 1: Spectral Profile for Allura Red to Find the Maximum Wavelength

1 Take about 40 mL of the stock solution of Allura Red.

2 Record the concentration of the stock solution.

3 Fill the spectrometer cell about 2/3 full with the stock solution.

4 Record percent transmittance (%T) to 0.1% over the entire visible range (400 - 700 nm).

- **i** Near the absorbance maximum (transmittance minimum), use 5 - 10 nm intervals.
- **ii** Away from the minimum T range, use 20 nm intervals.

5 Calculate absorbance at each wavelength to three significant figures using Eq. 6.

6 Create the spectral profile by graphing A vs. λ. This must be included in your results.

7 Determine the value of λ_{max}. Does your value of λ_{max} make sense, given that you are looking at a red dye?

Part 2: Transmittance (and Absorbance) for Known Concentrations of Allura Red

The Beer's law plot requires absorbance data for a range of concentrations. The concentration range needs to include the unknown concentrations you plan to measure (the mouthwash). Four dilutions of a stock solution of Allura Red are needed. During dilution, the number of moles of solute does not change, so the concentration of the dilute solution is given by the dilution equation, $M_1V_1 = M_2V_2 = n_{\text{solute}}$. Here, each of the four dilutions will be 1/2 the concentration of the one before it; the process is called "diluting by a factor of two". The volume goes up by a factor of 2, so the concentration goes down to half of what it was.

To make a volumetric dilution:

- **i** Take a known volume (aliquot), V_1, of the stock solution of concentration c_1. Use a volumetric pipet to deliver the solution into a volumetric flask. Do not use graduated glassware like a graduated cylinder.
- **ii** Add pure solvent until the final total volume is V_2. Use a disposable pipet for the last few drops in order to get the bottom of the meniscus to be at the mark on the flask.
- **iii** The dilution equation works for any consistent concentrations units (here, mol/L), and any consistent volume units (here, mL).

Starting with the Allura Red stock solution:

1 Record the concentration of the stock solution of Allura Red. It is approximately 0.002% by mass: $g_{\text{Allura Red}}/g_{\text{solution}} \times 100\%$. Calculate the molarity to two significant figures. Assume the density of the solution is roughly that of water.

2 Use a 25 mL volumetric pipet and a 50 mL volumetric flask.

3 Make a set of four serial dilutions using the procedure outlined above. Dilution 1: take 25 mL of the stock solution; dilute to 50 mL. Dilution 2: take 25 mL of dilution 1 and dilute to 50 mL. Repeat for dilutions 3 and 4. The four dilutions and the stock solution are five solutions of known concentration of Allura Red; the sixth should be the blank (deionized water), where you should have zero absorbance for zero concentration.

4 For each solution, measure the $\%T$ at $\lambda_{\max}$ to 0.01% (6 points).

5 Calculate the absorbance for each to three significant figures. Use Eq. 6.

6 Plot absorbance against concentration (include in results).

7 Draw the best straight line fit to the data points.

8 Determine the slope to the ones place: A = slope $\times$ concentration. Recall $A = (\epsilon b)c$. You should now be able to:

- **i** find absorbance, given concentration; or,
- **ii** find concentration, given absorbance.

9 Use your ruler to measure b, the inner diameter (pathlength) of your cuvette to 0.01 cm.

10 Calculate your experimental value of ϵ, the extinction coefficient, for Allura Red.

An example plot is shown in figure 8. Where $\epsilon b = 24943$.

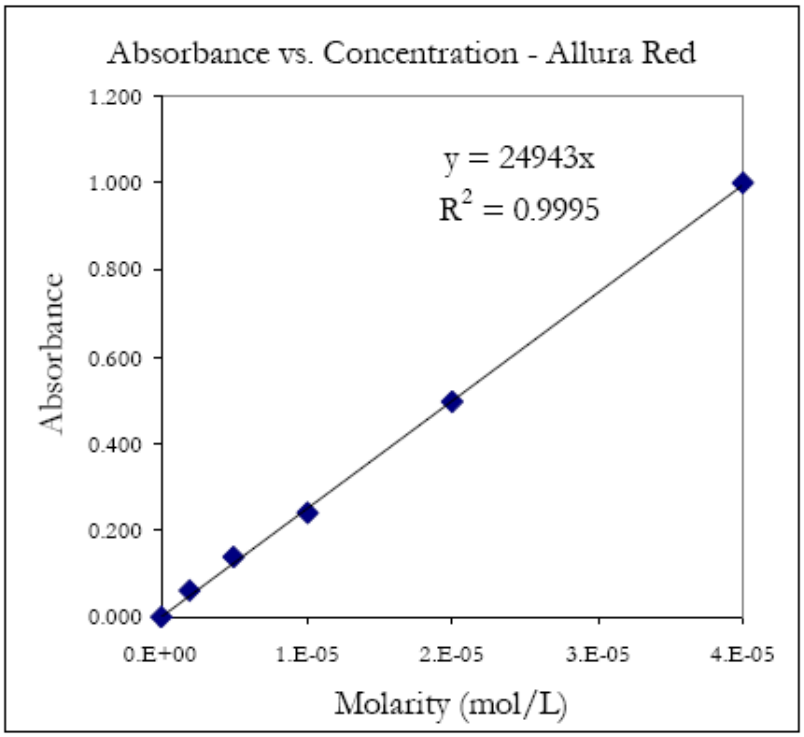

Figure 8

Part 3: Determination of Allura Red (Red Dye #40) Concentration in Mouthwash

The concentration of Allura Red in the commercial mouth wash is too high to read directly, so you will have to dilute it.

1 Obtain about 7 mL of the mouthwash. This is enough for three trials.

2 Make a 1 to 25 dilution using a 2 mL volumetric pipet and 50 mL volumetric flask. Repeat this two more times.

3 Measure $\%T$ for each diluted sample, and convert to A.

4 Calculate the concentration of dye in each diluted solution to two significant figures using your Beer's Law plot results.

5 Take into account the dilution factor and report the concentrations of Allura Red in the original mouthwash (undiluted) to two significant figures.

6 Determine the average concentration and the standard deviation of the three values obtained from your three trials.

7 Also calculate the average number of Allura Red molecules in one milliliter of mouthwash.

Waste Disposal: Follow your TA's instructions for waste disposal.

REPORTING RESULTS

Complete your lab summary or write a report (as instructed).

Abstract

Results

λ_{max} and ϵ of Allura Red (include spectral profile plot)

$\%T$ measurements for known concentrations (include Beer's Law plot)

$\%T$ of mouthwash (dilutions and undiluted)

Average concentration and standard deviation of Allura Red in undiluted mouthwash

Sample Calculations

Absorbance from transmittance

Dilution

Slope and extinction coefficient

$[\text{Allura Red}]_{\text{cuvette}}$ and $[\text{Allura Red}]_{\text{mouthwash}}$

No. of Allura Red molecules in one mL of mouthwash

Discussion/Conclusion

What you did, how you did it, and what you determined.

Does λ_{max} agree with observed color?

How was Beer's law used?

What were the final results?

Review

