MOHAWK

COLLEGE OF APPLIED ARTS AND TECHNOLOGY

CHEMICAL, ENVIRONMENTAL, AND BIOTECHNOLOGY DEPARTMENT

Spectrometry: Absorbance of Visible Light by a Food Colour Dye

by Professor David Cash

September, 2008

Contact information for Mohawk College will be found on the following page.

This Experiment is a 2 ½ hour General Chemistry laboratory exercise. It is designed for students in the common first term of a 2-year diploma program (Biotechnology, Environmental, or Health Technician).

For Information or Assistance
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Experiment 10

Spectrometry: Absorbance of Visible Light by a Food Colour Dye

OBJECTIVE

Calibration standard solutions of the red food colour dye Allura Red (Red 40) will be prepared from a supplied stock solution by serial and multiple dilutions. The visible light absorbance of each of the prepared calibration solutions will be measured. From the absorbance values of the prepared calibration solutions a calibration line of absorbance against concentration will be constructed. The absorbance of one or more supplied unknown solutions containing Allura Red will be measured. From the experimental absorbance values of the unknown solutions, the concentration of the dye in each unknown solution will be determined using the calibration line.

- A working solution of Allura Red will be prepared from the stock solution by simple dilution.
- A set of calibration standard solutions of Allura Red will be prepared from the working solution by multiple dilution.
- The concentration of Allura Red in each solution prepared will be calculated in ppm units.
- The percent transmission of light at 504 nm wavelength of the calibration standard solutions of Allura Red will be measured using a Spectronic 20 instrument.
- The absorbance of each calibration standard solutions of Allura Red will be calculated.
- A plot of absorbance against concentration for Allura Red will be constructed.
- A linear trendline will be added to the plot.
- The concentration of one or more solutions containing unknown amounts of Allura Red will be determined experimentally by the use of the trendline equation.
- The amount of Allura Red in specified portions of the unknowns will be determined.

REFERENCES

General Information Section of This Manual	Page
➢ Dilution	**Error! Bookmark not defined.**

Wikipedia	URL
➢ Food Colours	**http://en.wikipedia.org/wiki/Food_coloring**
➢ Brilliant Blue	**http://en.wikipedia.org/wiki/Brilliant_Blue_FCF**
➢ Allura Red	**http://en.wikipedia.org/wiki/Allura_Red_AC**
➢ Spectronic 20	**http://en.wikipedia.org/wiki/Spectronic_20**
➢ Beer-Lambert Law	**http://en.wikipedia.org/wiki/Beer-Lambert_law**

INTRODUCTION

Food Colour Dyes

According to **Pavia**, **Lampman**, and **Kriz**[1], there were more than 90 dyes regularly used in foods prior to 1906, many of them also used as textile dyes. As scientific knowledge of the hazards has become more precise and government safety regulation more stringent, the number of allowed food dyes has been gradually decreased. In 1938, the number of food dyes allowed in the U.S. was 15, and in 1950 it was 19. At the present time (2008), there are 7 FD&C (food, drug, and cosmetic) dyes allowed for food use in the U.S. The same 7 and one other dye are allowed for food use in Canada. Some other dyes are also allowed in some other countries around the world.

The eight food dyes allowed in Canada are distributed by Calico Food Ingredients[2] Ltd. of Kingston, Ontario in the form of solid powders or as 5 % solutions in water.

A food dye must absorb visible light. The visible range spectra of some food dyes, including Allura Red (Red 40) and Brilliant Blue (Blue 1) are shown on page 3. The chemical structures of the two dyes are shown in the Table on page 4.

The wavelength of maximum absorbance ($?_{max}$) for Red 40 is taken to be at **<u>504</u> nm**.
The substance absorbs **BLUE** light and appears **RED** to the human eye.

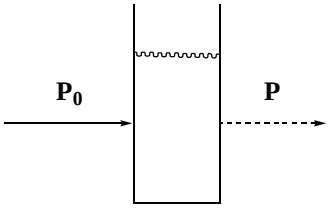
Quantitative Spectrometry

Lambert (˜ 1760) and **Beer** (˜ 1850) determined that the transmission of light energy through a solution of an absorbing substance was dependent on the **path length** and the **concentration** of the absorbing substance by a logarithmic relationship, as shown:

Beer - Lambert Law (Beer's Law):

$$A = abc$$

A = the Absorbance
a = the 'Constant'
b = the Path Length
c = the Concentration



P_0 = the Incident Intensity
P = the Emergent Intensity

Definitions and Terms:

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

$$\text{Percent T} = \%\,T = \frac{P}{P_0} \times 100\ \%$$

$$\text{Absorbance} = A = -\log T = \log \frac{P_0}{P} = \log\left(\frac{100}{\%\,T}\right)$$

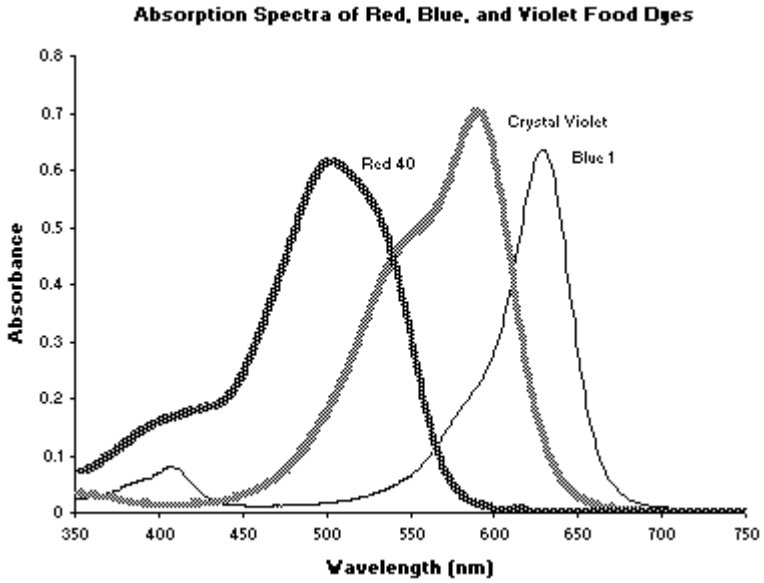
This relationship is now called the **Beer–Lambert Law** or **Beer's Law**.

Most quantitative methods using Beer's Law are based on the premise that a plot of absorbance (**A**) against concentration of the analyte is **linear** in the region of use. This must be proven for a new method, and should be verified for an analysis that is unfamiliar.

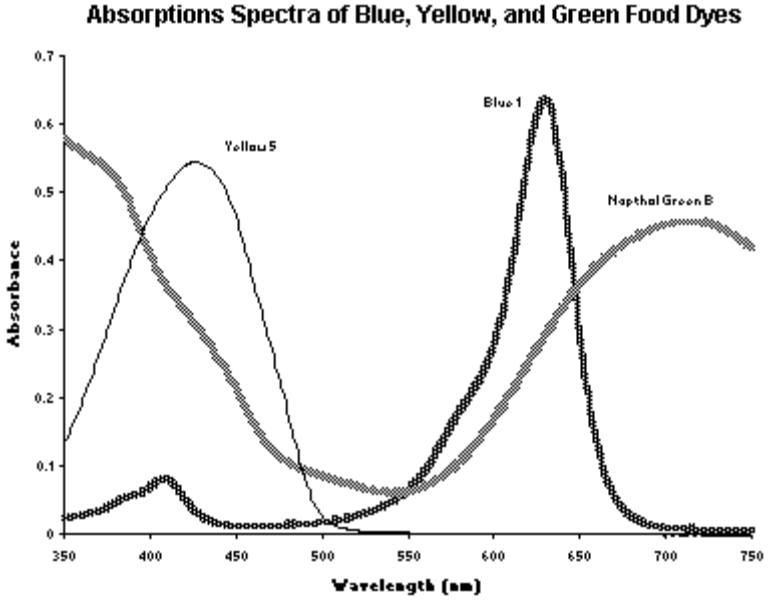
When a linear plot has been prepared (called a calibration line), it may be used to determine the concentration of the analyte in an unknown solution. This process will be demonstrated in this experiment.

[1] Pavia, Lampman, and Kriz, Introduction to Organic Laboratory Techniques, 3rd Edition, Saunders, pages 269-273.
[2] See http://www.calicofoods.com/

Visible Absorption Spectra of Some Food Dyes

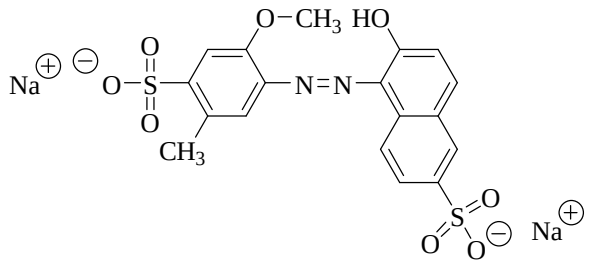
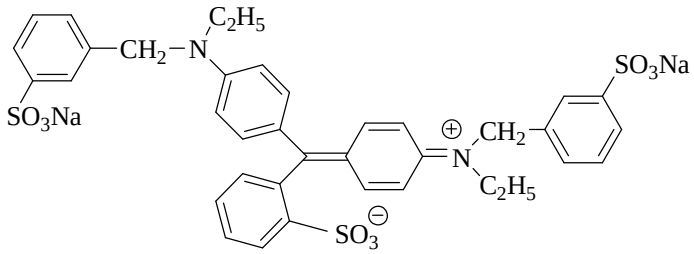


http://www.dartmouth.edu/~chemlab/chem6/dyes/full_text/chemistry.html **Retrieved 2008 02 15**



http://www.dartmouth.edu/~chemlab/chem6/dyes/full_text/chemistry.html **Retrieved 2008 02 15**

Chemical Structures of Red 40 and Blue 1

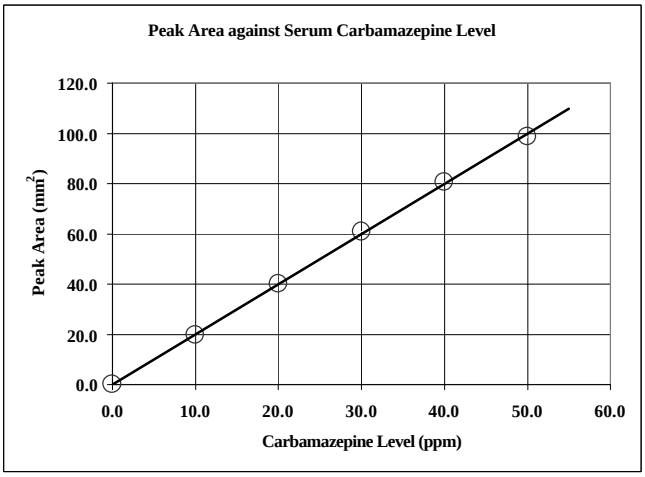
Name (Colour)	Chemical Structure
Allura Red AC or FD&C Red 40 (Red)	
Brilliant Blue FCF or FD&C Blue 1 (Blue)	

The Calibration Line Method

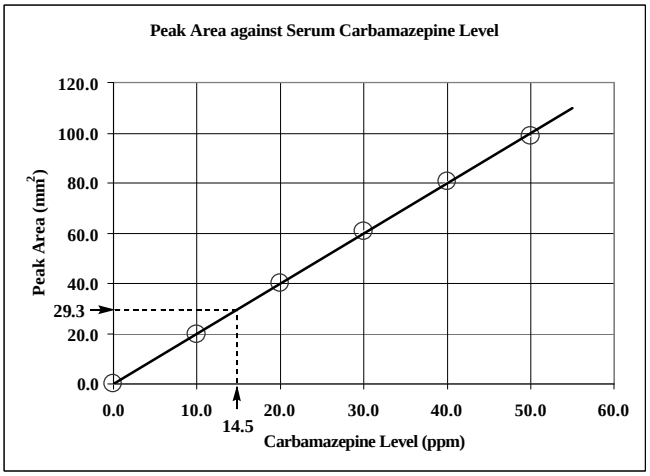
There are many chemical and physical systems used in analysis, where some measured property is directly proportional to the concentration of a certain substance. In such cases, a **Linear Trendline** or **Calibration Line** method may be utilized, as in this experiment.

The example below is taken from **Bender**[3]. This is an analytical calibration line (a linear trendline) for an analytical measurement due to the presence of **Carbamazepine**, an anticonvulsant prescription drug. The plot and the trendline were developed by using calibration standard solutions of **Carbamazepine** of known concentration, represented by the plotted data points. The analytical measurements were made using a High Performance Liquid Chromatograph (HPLC) instrument.

Concentration (ppm)	Peak Area (mm^2)
0.0	0.0
10.0	19.7
20.0	39.9
30.0	61.0
40.0	80.5
50.0	98.7



Using this calibration line plot and trendline, the unknown concentration of **Carbamazepine** in a serum sample may be estimated analytically. The method is shown graphically for a blood serum sample of peak area **29.3 mm^2**. The blood serum level of **Carbamazepine** is estimated as **14.5 ppm**.



Carbamazepine

http://upload.wikimedia.org/wikipedia/commons/b/b6/Carbamazepine.png

The construction and use of a calibration line for this experiment are described in detail in the **Sample Calculations** Section beginning on page 8.

[3] Bender, Gary T., Principles of Chemical Instrumentation, Saunders, 1987, pages 192-193.

Concentration in ppm Units

The unit **parts per million** (**ppm**) as an expression of concentration is an ambiguous term. There are three common different uses of the term **ppm**. It is possible to be mistaken about the meaning. A similar difficulty occurs with **percent** as a unit of concentration. It is not always easy to be sure which meaning is intended, but sometimes the context will make this clear to the reader.

Parts per Million

There are at least three common uses of this concentration unit:

- parts per million **mass** to **mass**;
- parts per million **mass** to **volume** for dilute aqueous solutions;
- parts per million **volume** to **volume.**

The three analogous uses of percent which may cause confusion are:

- percent mass / mass (**% w / w**);
- percent mass / volume (**% w / v**);
- percent volume / volume (**% v / v**).

Parts per Million Mass to Mass

This is the most general use of the term. This concentration unit refers to any mixture in which the amount of one component is conveniently expressed as a ratio of "x" parts by mass of a million total parts by mass. A typical example would be gold (solid) in an ore.

Examples: $\frac{\mu g}{g}$ $\frac{mg}{kg}$ $\frac{g}{tonne}$ **All are referred to as ppm**

Parts per Million Mass to Volume for Dilute Aqueous Solutions

This is a commonly used adaptation of the previous usage, **applicable only to dilute solutions in water**. The rationale for this usage is that a dilute solution in water has a density close to 1.0 g / mL or 1.0 kg / L or 1.0 tonne / m^3. A typical example would be gold, dissolved as a soluble salt in dilute aqueous solution.

Examples: $\frac{\mu g}{mL}$ $\frac{mg}{L}$ $\frac{g}{m^3}$ **All are referred to as ppm**

As the above two definitions and uses are very similar, no great harm can arise from any confusion.

Parts per Million Volume to Volume

This usage is reserved almost exclusively for gas phase mixtures or perhaps dusts and aerosols. It has most often been used in occupational hygiene and safety. This concentration unit refers to any mixture in which the amount of one component is conveniently expressed as a ratio of "x" parts by volume of a million total parts by volume. A typical example would be a contaminant in air.

Examples: $\frac{\mu L}{L}$ $\frac{mL}{m^3}$ **All are referred to as ppm**

This third definition is very different from the previous two. Any error or confusion will result in estimated values which are wrong by a factor of approximately three orders of magnitude (10^3). An error of this magnitude could be extremely serious in industrial health and safety applications.

Stock Solutions, Working Solutions, and Calibration Standard Solutions
There are a number of classification systems and terms for the solutions which are prepared for use in the laboratory. For example, refer to **Experiment 6**, page **Error! Bookmark not defined.**, for the definitions of a **primary standard** and a **secondary standard solution**. Another useful set of terms is a **stock solution**, a **working solution**, and a **calibration standard solution.**

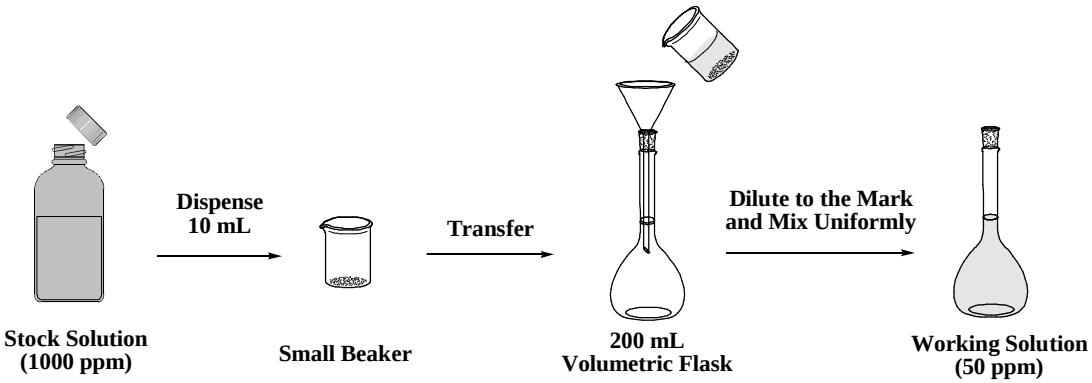
Stock Solution
A stock solution is usually a primary standard solution of relatively high concentration which is conveniently prepared and stable for storage. From this solution, diluted working solutions and calibration standard solutions may be prepared as needed.

Working Solution
A working solution is usually prepared by simple dilution of a stock solution. It is of a convenient concentration for short term use, but is too dilute for convenient storage.

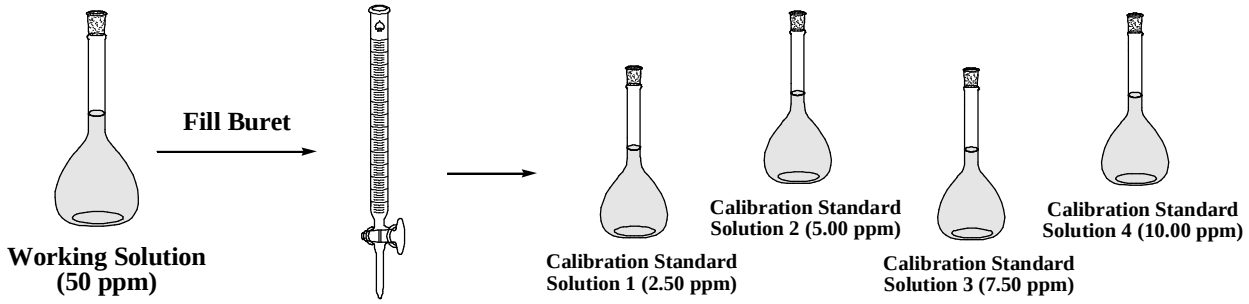
Calibration Standard Solutions
Calibration standard solutions are usually prepared by multiple dilution of either a stock solution or a working solution. They are required for short term use in the standardization of solutions or the calibration of instruments. They are inconvenient for storage.

Dilution Scheme of the Experiment

1. Preparation of a Working Solution



2. Preparation of Calibration Standard Solutions



Construction and Use of an Absorbance Calibration Line for Allura Red Food Dye

Sample Calculations

Example 1 (Preparation of a Working Solution by Simple Dilution)
A stock solution of **Allura Red** of concentration **870 ppm** was diluted **20-fold** (**1** to **20**) to prepare a working solution (**10.00 mL** to **200.0 mL**). Calculate the concentration **in ppm units** of the Allura Red dye in the working solution. State the answer to 2 place after the decimal point.

Answer
This is a dilution calculation:

$$C_{dilute} = C_{concentrated} \times \left(\frac{V_{concentrated}}{V_{dilute}} \right)$$

$$C_{dilute} = 870 \text{ ppm} \times \left(\frac{10.00 \text{ mL}}{200.0 \text{ mL}} \right) = \underline{43.50} \text{ ppm}$$

Example 2 (Preparation of Calibration Standard Solutions by Multiple Dilution)
The working solution of **43.50 ppm** Allura Red dye was placed into a buret. The volumes listed in the table below were taken in turn from the buret and each was diluted to **100.0 mL** volume with distilled water in a volumetric flask. Calculate the concentration **in ppm units** of the Allura Red dye in each of the calibration standard solutions. State the answers to 2 places after the decimal point.

Answer
This is a set of dilution calculations. **For Standard Number 1**:

$$C_{dilute} = 43.50 \text{ ppm} \times \left(\frac{4.00 \text{ mL}}{100.0 \text{ mL}} \right) = \underline{1.74} \text{ ppm}$$

Standard Solution Concentrations

Standard Number	Volume of 43.50 ppm Solution	Diluted Volume	Standard Concentration
1	4.00 mL	100 mL	1.74 ppm
2	8.00 mL	100 mL	3.48 ppm
3	12.00 mL	100 mL	5.22 ppm
4	16.00 mL	100 mL	6.96 ppm
5	20.00 mL	100 mL	8.70 ppm

The Sample Calculations Section Continues on the Next Page →

A **Spectronic 20** spectrometer was warmed up and the wavelength selector was set to **504 nm** in the blue region of the visible spectrum. The **0 % Transmission** and **100 % Transmission** readings were properly adjusted, the latter using distilled water in the sample tube.

The **Percent Transmission** reading for each standard solution was determined in duplicate. Using the mean value of **% T** for each solution, an **Absorbance** value was calculated. These values are listed in the table below.

Standard Solution Absorbance Values

Standard Number	% T (Trial 1)	% T (Trial 2)	Mean % T	Calculated Absorbance
1	**79**	**80**	**79.5**	**0.100**
2	**63**	**63**	**63**	**0.201**
3	**49**	**50**	**49.5**	**0.305**
4	**38**	**38**	**38**	**0.420**
5	**31**	**33**	**32**	**0.495**

Example 3 (Calculation of the Absorbance Value from the Mean Percent Transmission Value)
Calculate the **Absorbance** of **Standard Solution Number 1** from the mean **% T** value.
State the answer to 3 places after the decimal point.

Answer
The required relationship is:

$$\textbf{Absorbance} = A = -\log T = \log \frac{P_0}{P} = \log\left(\frac{100}{\%\ T}\right)$$

$$\textbf{Absorbance} = A = \log\left(\frac{100}{79.5}\right)$$

Absorbance = <u>0.100</u>

The Sample Calculations Section Continues on the Next Page →

The values of absorbance for the standard solutions were plotted against concentration as a **Microsoft Excel XY Scatter Plot**. The following examples show how this may be done.

Example 4 (Construction of an XY Scatter Plot of Absorbance against Concentration)
Construct a scatter plot of the **Absorbance** values (**y-axis**) of the **Allura Red** Standard Solutions against **Concentration** values in **ppm units** (**x-axis**).

Answer

ppm	A
1.74	0.100
3.48	0.201
5.22	0.305
6.96	0.420
8.70	0.495

1. Open a new worksheet in **Microsoft Excel**; name and save the file.

2. The required plot must have the **Absorbance** values on the **y** (vertical) axis and the **Concentration** values on the **x** (horizontal) axis. Excel expects that you will enter the data as shown in the table, with the **x - axis** data in the left column, and the **y-axis** data in the right column.

3. Use the cursor to select the **Left** column cells. Go to the **Format** menu:
   - Choose **Cells**;
   - Choose the **Number** tab;
   - Choose the **Number** category;
   - Set the **Decimal places** value at **2**.
   -
4. Use the cursor to select the **Right** column cells. Go to the **Format** menu:
   - Choose **Cells**;
   - Choose the **Number** tab;
   - Choose the **Number** category;
   - Set the **Decimal places** value at **3**.

5. Use the cursor to select the 2 × 5 block of cells containing the data. Go to the **Insert** Menu:
   - Select **Chart**;
   - Select **XY Scatter Plot**; use the default option - a Scatter Plot with **NO LINES**;
   - Follow the steps to create the plot;
   - Give the plot a title and label the axes; you can edit these later.

Note

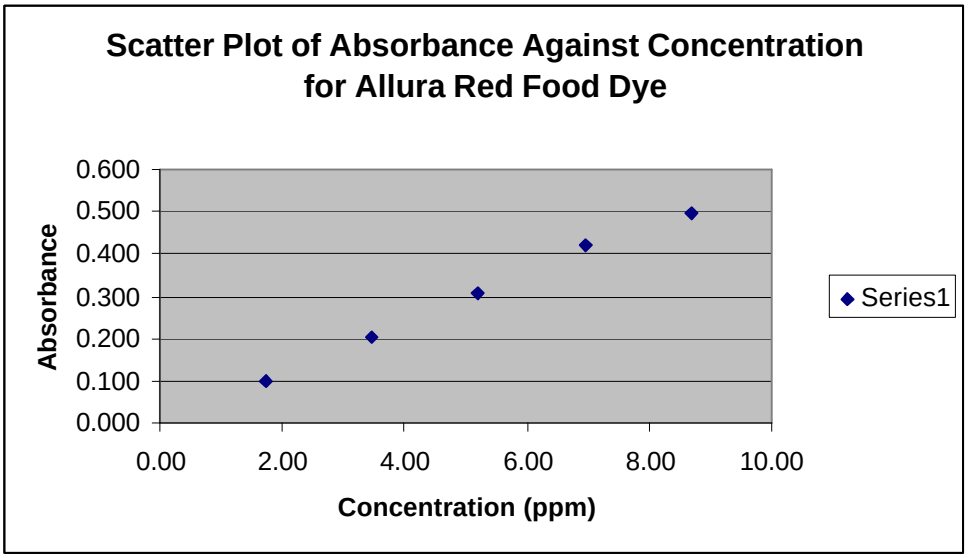
If your version of Excel has a **Chart Wizard**, you can use that method instead of the **Insert** Menu.

The resulting standard plot is shown on the next page.

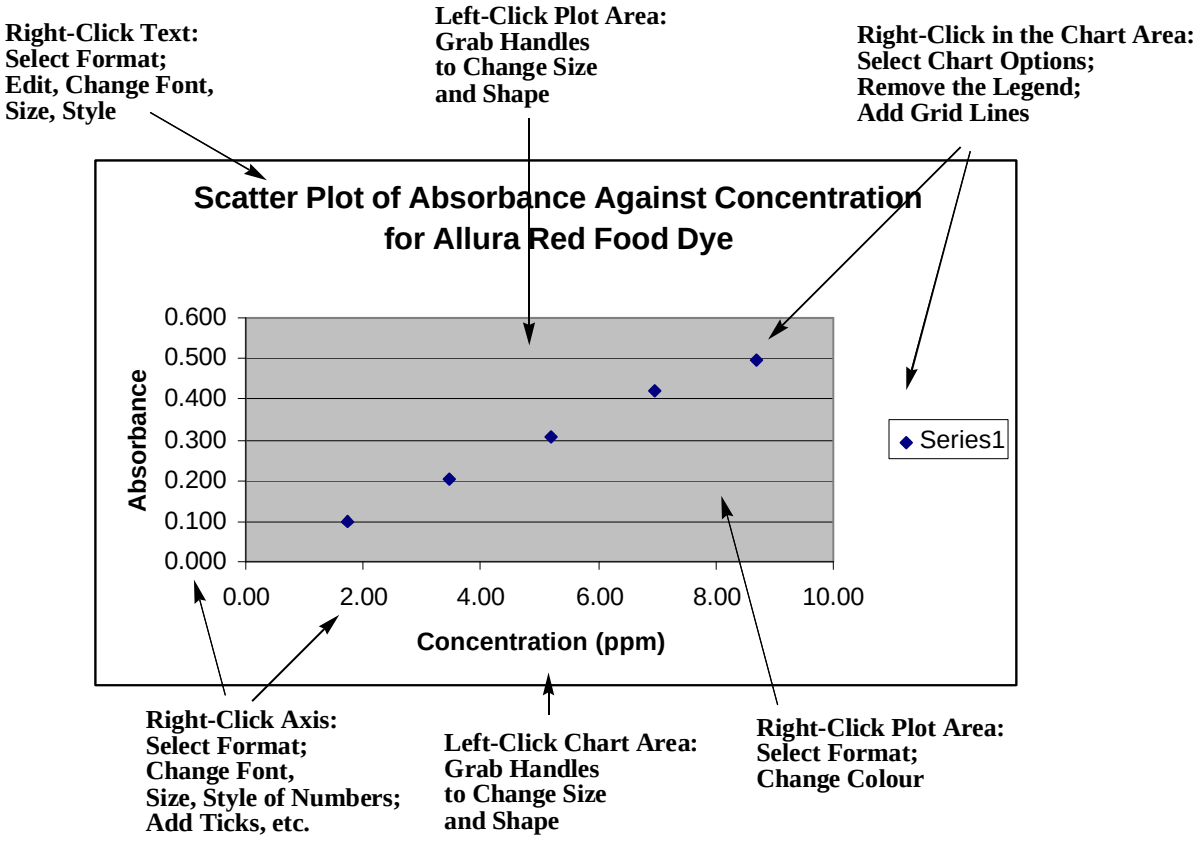
Example 4 Continues on the Next Page →

Example 4 (Cont.)

Standard Excel Plot

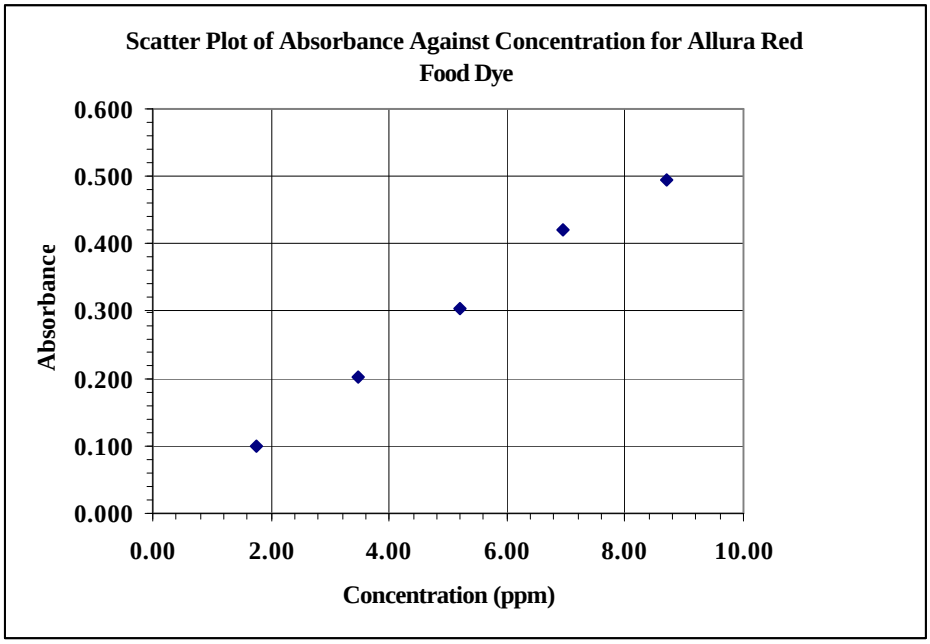


Plot Customization The plot may be **Customized** as shown below and on the next page.



The Sample Calculations Section Continues on the Next Page →

Customized Plot



Example 5 (Addition of a Linear Trendline to the XY Scatter Plot)
Add a linear trendline to the scatter plot.

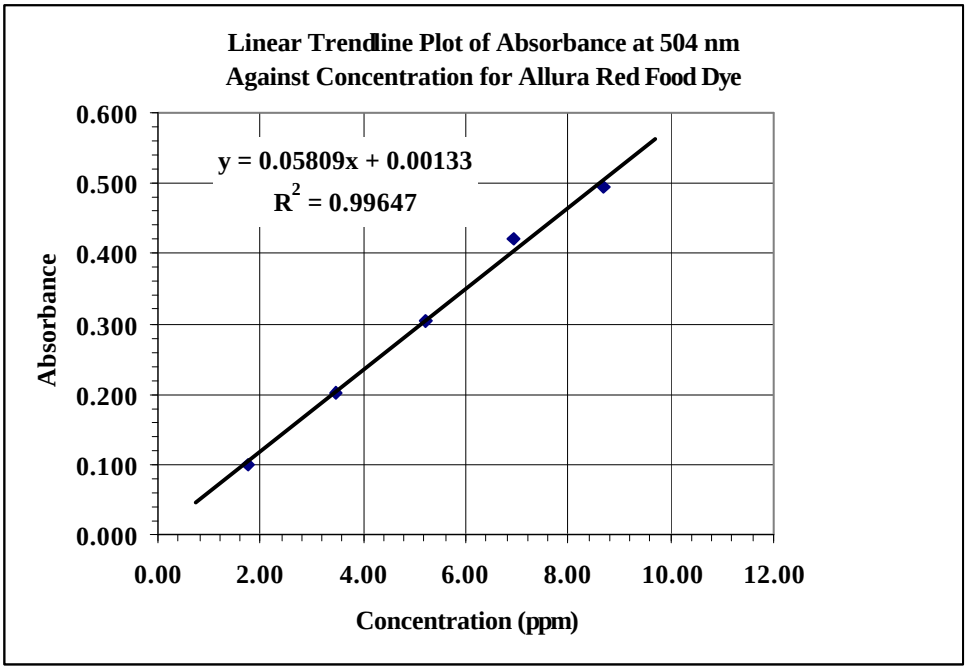
Answer

1. **Right-Click** the cursor on one of the data points on the scatter plot:
   - Select **Add Trendline** from the menu which appears;
   - Select **Linear** from the menu which appears;
   - Before clicking OK, select **Options**;
   - Click the boxes **Display Equation** and **Display R^2 value**;
   - Use **Forecast** to extend the trendline 1 unit backwards and forward;
   - Click **OK** to create the trendline.

2. **Right-Click** the cursor on the box containing the equation of the trendline:
   - Select **Format Data Labels**;
   - Select the **Number** tab; select the **Number** category; set the **Decimal places** to **5**;
   - Select the **Patterns** tab; select the **White** option for the **Area** – the equation background becomes opaque and covers the gridlines;
   - Use the cursor to grab and move the equation to a convenient spot.

The Sample Calculations Section Continues on the Next Page →

Customized Plot with the Added Trendline



Example 6 (Use of the Linear Trendline to Determine an Unknown Concentration)
A cherry-red solution in a **250 mL** bottle of a children's drink containing the food colour FD&C Red 40 (Allura Red) had a **0.263** absorbance value at **504 nm** wavelength.

Calculate the concentration **in ppm units** of the Allura Red in the drink solution using the calibration line equation of **Example 5**. State the answer to 2 places after the decimal point.

Answer
The equation of the trendline is: **y = 0.05809 x + 0.00133**
Translating:

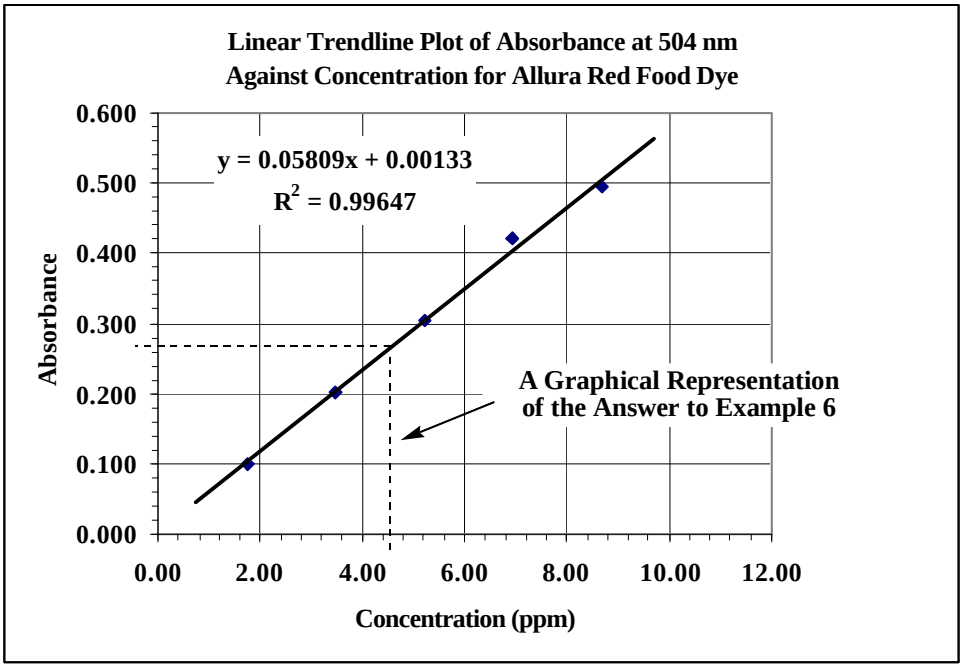
$$\textbf{Absorbance} = (0.05809)*(\textbf{Concentration in ppm units}) + 0.00133$$

$$\textbf{Concentration in ppm units} = \frac{\textbf{Absorbance} - 0.00133}{0.05809}$$

$$\textbf{Concentration in ppm units} = \frac{0.263 - 0.00133}{0.05809} = \underline{4.50}\ \textbf{ppm}$$

The Sample Calculations Section Continues on the Next Page →

A Graphical Solution to Example 6



Example 7 (Calculation of the Amount of the Analyte[4] in a Given Volume of Solution)
Calculate the total amount of Allura Red food dye **in mg units** in the **250 mL** (**0.250 L**) contents of the drink bottle of **Example 6**. The solution concentration was found to be **4.50 ppm** in **Example 6**.
State the answer to 2 places after the decimal point.

Answer
The unit **ppm** has the meaning **mg / L** in this application:

4.50 ppm = 4.50 mg / L

Total Content of Allura Red = 4.50 mg / L × 0.250 L = 1.13 mg

[4] Analyte: the substance whose concentration or amount is being determined by an analytical procedure.

Name		Day		Start Time	

PRE-LABORATORY PREPARATION for Experiment 10

To be completed before the laboratory session.
To be submitted before beginning the experiment.

Your Mohawk College ID Number is **nnnnnWXYZ**.

Calibration Line Exercise
The data in the **Table** below were obtained by the method of this experiment.
Use your Mohawk ID Number to determine and enter the value of each datum[5].

Allura Red Conc. (ppm)	Allura Red Conc. (ppm)	Absorbance	Absorbance
2.5W		**0.14Z**	
5.0X		**0.29Y**	
7.4Y		**0.45X**	
10.1Z		**0.59W**	

Instructions (See Examples 4 and 5 on pages 10 and 12)

1. Use a software package (e.g.: Microsoft Excel®) to display an **XY Scatter Plot** of the Absorbance against the Concentration in ppm units. **Choose the no-lines option**.

2. **Title** the plot, **including your name and the date**, and **label** both axes.

3. Choose a **linear trendline** to the data points.

4. **Display** the **trendline** on your plot and also display the **equation** and $\mathbf{R^2}$ value of the trendline.

5. Use the cursor to **select** the equation box.

6. Format as a **number** and choose the option **five** (**5**) places after the decimal point.

7. **Attach a Printed Copy of your Plot to the PRE-LABORATORY PREPARATION**.

The PRE-LABORATORY PREPARATION Continues on the Next Page →

[5] **Datum** (Merriam-Webster Dictionary) - something used as a basis for calculating or measuring. Plural: data or datums.

PRE-LABORATORY PREPARATION for Experiment 10 (Cont.)

Questions: Answer in the space provided. **Show work**.
Your Mohawk College ID Number is **nnnnnWXYZ**.

A cherry cough tablet containing the food colour Allura Red was completely dissolved in distilled water and diluted in a volumetric flask to **100 mL** (**0.100 L**) volume.
The solution had a **0.30Z** absorbance value at **504 nm** wavelength.

Q-1. Use the equation of the trendline of your Pre-Laboratory Preparation plot.
Calculate the concentration of the Allura Red dye in the solution in **ppm units**.
State the answer to 2 places after the decimal point. Show your work.
See **Example 6** on page 13.

Your Trendline Equation:

0.30Z = _____

Concentration of Allura Red in the Cough Tablet Solution (ppm) = _____ ppm

Q-2. Calculate the total amount of Allura Red present in the **100 mL** (**0.100 L**) solution containing the dissolved cough tablet **in mg units**. State the answer to 2 places after the decimal point. Show your work. See **Example 7** on page 14.

Total Amount of Allura Red in the Cough Tablet Solution (mg) = _____ mg

Bring a Calculator with you for Use in the Laboratory Period

PROCEDURE

All of the solids and solutions used in this experiment are non-hazardous wastes. They may be discarded into the garbage and the sink. The food dye may cause non-hazardous stains on exposed skin or on clothing.

A. Preparation of Glassware and Apparatus

The following **clean** glassware and laboratory apparatus is required for the experiment:

- ☐ a **50 mL** buret and a buret stand
- ☐ a glass funnel
- ☐ a small plastic buret funnel
- ☐ a dropper (pasteur) pipet
- ☐ a **200 mL** volumetric flask with a stopper
- ☐ a **100 mL** volumetric flask with a stopper (extra equipment)
- ☐ a **small** beaker
- ☐ a small or medium watch glass
- ☐ a rubber squeeze bulb
- ☐ one shared spectrometer tube

A-1. Clean the glassware and apparatus if necessary with a 1 % solution of detergent in warm water. See **Box: Cleaning and Drying of Glassware** on page **Error! Bookmark not defined.**. Rinse the cleaned glassware and apparatus with tap water and then with distilled water. Drain dry.

To avoid breakage, do not leave any glassware standing in an unstable position.

A-2. Set up your buret stand and your **50 mL** buret at your bench station.

A-3. After cleaning, assemble the buret **securely**, and check that the buret tap is working.

A-4. Drain the buret, upside-down (tap open), and the pipet, upside-down, in the buret stand. Check that the inner walls of the buret and the transfer pipet are clean and that the capillary tips are not broken or plugged.

A-5. Check that the volumetric flasks can be capped securely without leaking.

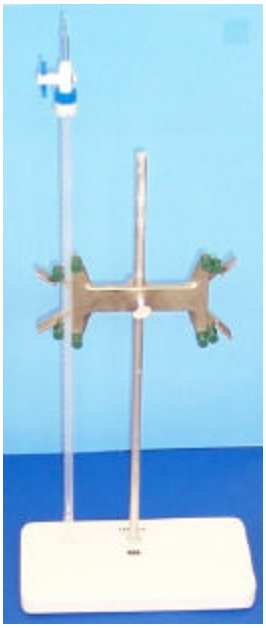


Photo: Emily Girard

B. Preparation of a Working Solution of Allura Red by Simple Dilution

A stock solution of the food colour dye **Allura Red AC (FD&C Red # 40)** will be supplied. The concentration of the Allura Red dye in the solution will be stated **in ppm units**.

The instructor will explain how the stock solution is to be dispensed: either from a buret or by the use of a pump dispenser from a stock bottle.

B-1. Record the concentration of the stock solution of the Allura Red dye **in ppm units** in the **Solution Concentrations Table** in the **REPORT PAGES** Section on page 29.

B-2. You require a clean **small** beaker, a clean glass funnel, and a clean **200 mL** volumetric flask.

B-3. Obtain a **10 mL** sample of the stock solution of Allura Red **in your small beaker**.
- If the solution is dispensed by a pump, record the volume as **10 mL** (**exact**).
- If the solution is dispensed from a buret, read the start and final levels to 2 places after the decimal point. Record the volume measurements in the **Stock Solution Volume Area** in the **REPORT PAGES** Section on page 29.

B-4. Transfer the **10 mL** sample of the Allura Red stock solution **totally** into your **200 mL** volumetric flask using your glass funnel. Rinse the inside of the the beaker and the inside of the funnel several times with distilled water, into the flask. Remove the funnel.

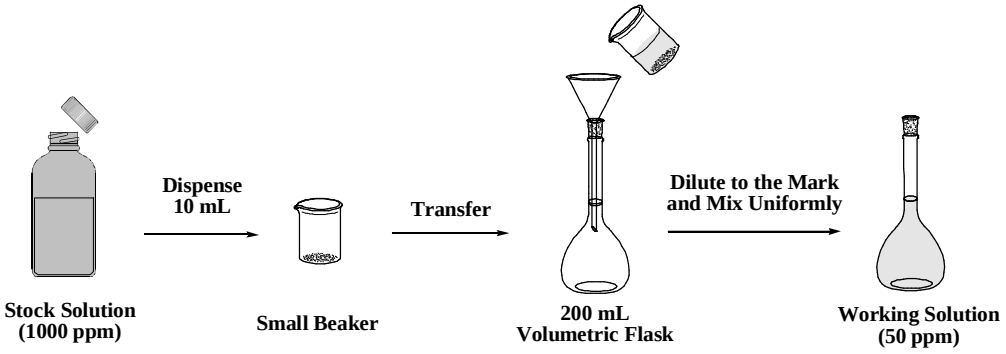
B-5. Add distilled water to the flask, mixing from time to time. Dilute to the mark line using distilled water from your dropper pipet.

B-6. Cap the flask securely. Invert slowly at least 17 times to ensure that the working solution is completely uniform before proceding.

B-7. Complete the calculations necessary to determine the concentration of the Working Solution and record the values as required on page 29.

B-8. Show your calculations to the instructor for initialing.

Preparation of a Working Solution



C. Buret Preparation

C-1. Rinse your small beaker with distilled water if necessary.

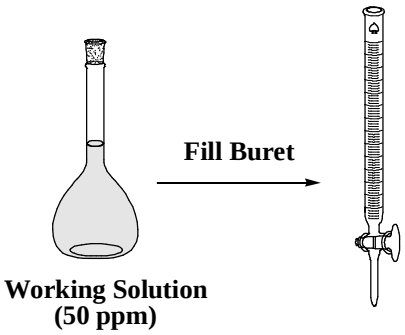
C-2. Place about **20 mL** of the **Working Solution** from your **200 mL** flask into the beaker. Rinse the beaker and your buret and its buret funnel with **Working Solution**. Collect the rinse portion for disposal into the sink.

C-3. Repeat the rinse procedure of the beaker and the buret and funnel with a second **20 mL** portion of the dye working solution.

C-4. Take a third, larger portion of the working solution into the beaker.

Fill the buret with the dye working solution.

C-5. Clear the tip of the buret of air bubbles.



D. Preparation of the Calibration Standard Solutions

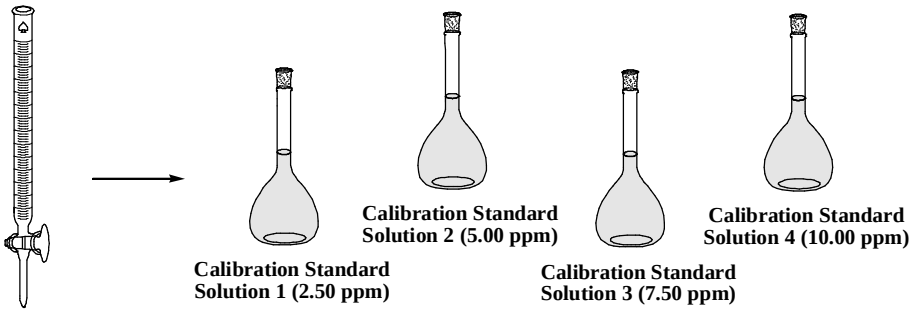
D-1. Your **100 mL** volumetric flask and its stopper should be clean and ready for use.

D-2. Read and record the initial volume level in the buret to 2 places after the decimal point in the **Standard 1** column of the **Calibration Standards Preparation Table** in the **REPORT PAGES** Section on page 30.

If the solution is too intensely coloured to allow you to read the bottom of the meniscus, read the top of the solution level in each case.

D-3. For **Standard 1** add **5.0 ± 0.5 mL** of the solution from the buret directly into your **100 mL** volumetric flask. The amount taken does not have to be exactly 5.00 mL, but it must be known precisely. If you lose some solution, discard, rinse the flask and begin again.

Volume of Working Solution Required	Standard 1 5.0 ± 0.5 mL	Standard 2 10.0 ± 0.5 mL	Standard 3 15.0 ± 0.5 mL	Standard 4 20.0 ± 0.5 mL



D-4. Read and record the final volume level in the buret to 2 places after the decimal point in the **Standard 1** column of the **Calibration Standards Preparation Table** in the **REPORT PAGES** Section on page 30.

D-5. Add distilled water to the flask, mixing from time to time. Dilute to the mark line using distilled water from your dropper pipet.

D-6. Cap the flask securely. Invert slowly at least 17 times to ensure that the working solution is completely uniform before proceding.

D-7. Complete the calculations necessary to determine the concentration of **Calibration Standard 1** and record the value as required in the **Calibration Standards Preparation Table** on page 30.

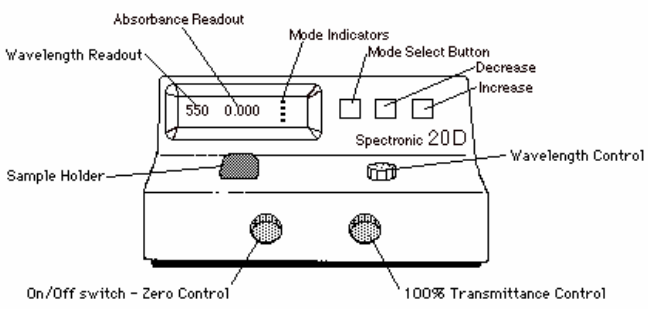
D-8. Now measure the absorbance of light by **Calibration Standard 1** as directed in **Part E** and **Part F** of the **PROCEDURE** Section beginning on page 22.

Box: The Spectronic 20 Spectrometer

Quoting from Wikipedia:
"The Spectronic 20 is a spectrometer developed by Bausch & Lomb in 1954. While of simple design, requiring manual setting of the wavelength and making readings from a moving-needle analog display, the unit is rugged, accurate, and easy to use. This venerable instrument is still in production use in analytic chemistry labs in both commercial and educational settings around the world."

A web search on the term **Spectronic 20** will turn up many useful sites.
Some recommended sites are:
http://www.thermo.com/com/cda/product/detail/0,1055,12100,00.html
http://www.chemistry.nmsu.edu/Instrumentation/Spectronic_20.html
http://www.wellesley.edu/Biology/Concepts/Html/analogspec20instructions.html

Analog Spectronic 20



Digital Spectronic 20D

Preparation of the Spectrometer
The instructor will demonstrate the preparation and use of the spectrophotometer. Each type of instrument used by the department has printed instructions for its use available in the laboratory. In general, the following steps are required:

- Plug in, turn on and allow sufficient warm-up time (about 10 minutes).
- Set the required wavelength (**504 nm**).
- With the sample compartment empty (light path blocked) and the cover closed:
  - Adjust **no transmission** = **0 %** transmission of light = **infinite** Absorbance value.
- Using a clean sample cell with a dry surface:
  - Set **full transmission** with either **distilled water** or a **blank solution** in the cell.
  - Adjust **full transmission** = **100 %** transmission of light = **zero** Absorbance value.
- Cycle between **0 % and 100 %** three times or until no further adjustment is required.

The **0 % and 100 %** adjustments are performed manually on the Analog Spectronic 20.
They are automatic on some microprocessor equipped instruments.

E. Preparing to Use the Spectronic 20

The instructor will assign you to a specific Spectronic 20 or other spectrometer. There should be a large disposal beaker near the spectrometer to collect rinse solutions and other solutions.

E-1 You will be given a special sample test tube, or be asked to share a sample test tube with other students. Clean the sample test tube. Rinse well with distilled water after cleaning. Be careful not to scratch the surface of the glass.

You must use the same tube for all of your measurements.

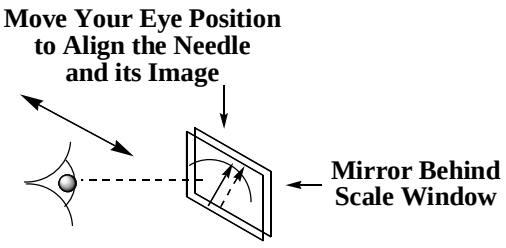
Notice that the tube has a vertical white marker line at the top. This marker line is used to ensure that the tube is always placed into the instrument in the same position.

E-2. When using the spectrometer, check that the wavelength is set to **504 nm**, but **DO NOT TOUCH** the wavelength setting dial if there is one.

All readings are made with the sample compartment lid closed to exclude stray light.

E-4. Check that with no tube in place (light path blocked) and the sample compartment lid closed the instrument reads **0 % Transmission**.
Adjust if necessary to zero.

Read the position of the needle marker with one eye, by lining up the needle with its mirror image.



E-3. Fill your sample test tube **three times** with distilled water. Empty the tube twice into the discard beaker. After the third refill, dry off the outside of the sample test tube carefully with the special **low-lint wipes** provided.

The tube needs to be only about two-thirds full; the light beam passes through the lower part of the tube.

E-5. Place the sample test tube in the sample compartment of the instrument with the white marker line in line with the marker notch on the cell compartment.

E-6. Set **Full 100 % Transmission** (**Zero Absorbance**) with the **distilled water** in the sample test tube.

E-7. Remove the test tube. Cycle between **0 % with the sample compartment empty** and **100 % with distilled water in the sample compartment** three times, or until no further adjustment of either value is required.

F. Percent Transmission / Absorbance Measurements

F-1. Empty the Spectronic 20 sample test tube into the discard beaker. Rinse the test tube with your prepared solution **twice** and then fill the sample test tube a **third time** to be about two-thirds full. Dry the outside. Insert the test tube, correctly positioned, into the instrument.

When the reading is stable, read and record the value for the solution in the **Percent Transmission Values Table** (**Trial 1**) in the **REPORT PAGES** Section on page 31.

Spectronic 20	Other Direct Reading Instrument
Read % Transmission Scale Value to Nearest 1 %	**Read Absorbance Value Displayed (All Digits)**

Notes:

1. **DO NOT** attempt to read the Absorbance scale of the **Spectronic 20** directly. The scale is not linear and is very difficult to interpret correctly.

2. If you are using an instrument which displays Absorbance values directly, record the values in the **Absorbance Values Table** in the **REPORT PAGES** Section on page 32.

F-2. Repeat the process of filling the test tube with a fresh sample of your solution (**Trial 2**). Remeasure the reading of the instrument. Do not worry if the value differs on the second reading by up to **± 2 %** Transmission (or **± 0.01** Absorbance).

If the **Trial 2** reading differs by more than this amount from the **Trial 1** value, consult the instructor.

F-3. When you are satisfied that you have made the required measurements, discard the solution in the **100 mL** volumetric flask and rinse the flask with distilled water.

F-4. Return to **Part D**.

F-5. Prepare **Standard Solution 2** and measure its light absorbance. Record the measured values in the **Percent Transmission Values Table**.

F-6. Repeat the process for **Standard Solution 3** and **Standard Solution 4**.

G. Calculations and Approximate XY Scatter Plot

G-1. If you are using a **Spectronic 20**, you must **CALCULATE** the Absorbance of each solution as follows:

a. Determine the mean value of % Transmission (**= % T**) for each solution. State each mean value to the nearest 1 %. Record the values in the **Calculated Absorbance Values Table** in the **REPORT PAGES** Section on page 31.

b. Calculate the value of the Absorbance of each solution using this equation:

$$\textbf{Absorbance} = A = -\log T = \log \frac{P_0}{P} = \log\left(\frac{100}{\%\ T}\right)$$

Fill in the calculated values of Absorbance for all of your Calibration Standard Solutions in the **Calculated Absorbance Values Table** on page 31.

G-2. Copy the values of Standard Solution Absorbance from the **Calculated Absorbance Values Table** into the **Calibration Line Data Table** in the **REPORT PAGES** Section on page 33.

G-3. Copy the values of Standard Solution Concentration from the **Calibration Standards Preparation Table** on page 30 into the **Calibration Line Data Table** on page 33.

G-4. Construct an **Approximate XY Scatter Plot** of the **Absorbance** against **Concentration** data in the **Calibration Line Data Table** on page 33. Use the plot area below the Table.

G-5. Show your approximate plot to the instructor for initialling. The instructor will decide whether the data is acceptable. If not, one or more of the solutions must be re-prepared and re-measured on the spectrometer.

G-6. **When you have completed all of the required tasks, show all of your data and calculation tables to the instructor for initialling**.

The PROCEDURE Section Continues on the Next Page →

One or more unknowns will be analyzed for Allura Red content.
The instructor will determine which of the following analyses you will perform.

H. Unknown Sample 1 - Analysis of a Children's Drink Containing Allura Red

The procedure assumes that the sample supplied is a solution whose percent transmission at 504 nm may be measured directly on the spectrometer without dilution.

A sample of **Simply Kids™ Vitamin Enriched Water (Cherry Colour)** is a suitable unknown.

H-1. Check that the Spectronic 20 wavelength selector is set to **504 nm**.

H-2. Adjust the **0 %** and **100 % Transmission** settings if necessary as in **Part E**.

H-3. Read the percent transmission of the unknown sample **in duplicate** as in **Part F**.

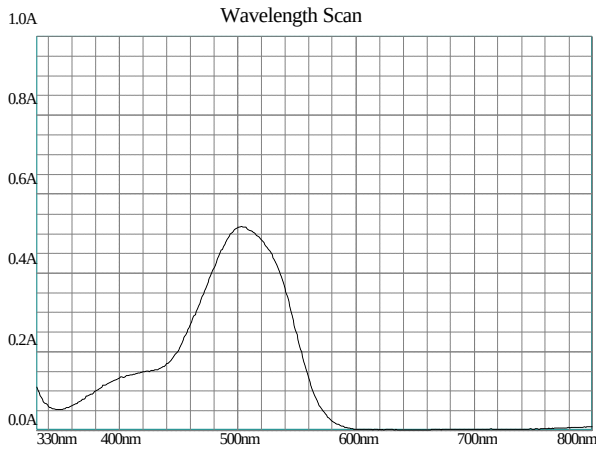
H-4. Record the observed values in the **Unknown Samples Table** in the **REPORT PAGES** Section on page 34.

H-5. Calculate the mean percent transmission and the absorbance of the solution at **504 nm**. Record these values in the **Unknown Samples Table** on page 34.

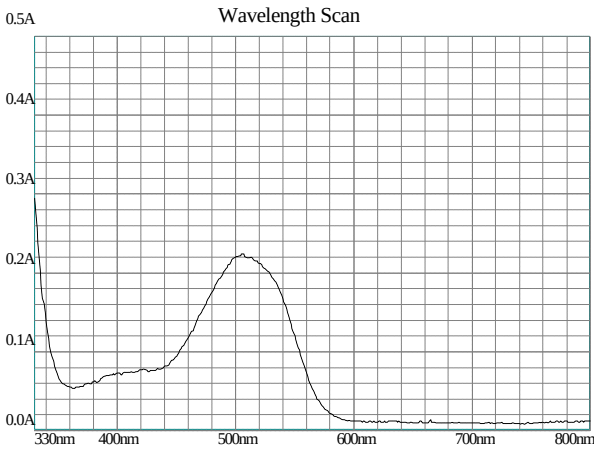
H-6. Use your calibration line equation to calculate the concentration of the Allura Red dye in the drink solution **in ppm units**. State the answer to 2 places after the decimal point. Show your work. See **Example 6** on page 13. Enter your concentration value in the **Unknown Samples Table** on page 34.

H-7. Calculate the total amount of Allura Red present in a **250 mL** portion of the drink solution **in mg units**. State the answer to 2 places after the decimal point. Show your work.
See **Example 7** on page 14.

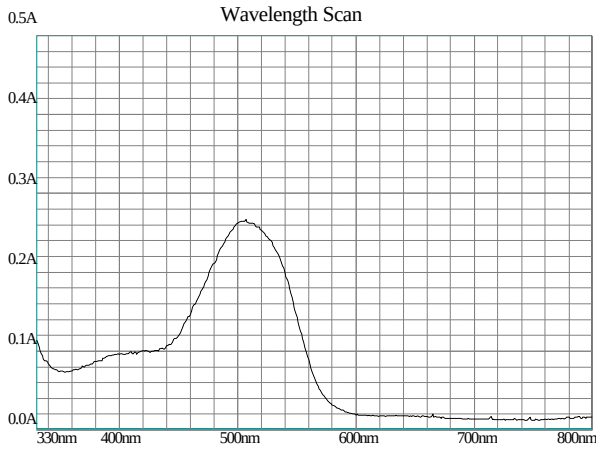
NovaSpec Plus Diode Array Spectrophotometer
Scans of Absorbance against Visible Wavelengths
1.0 cm Pathlength Cell



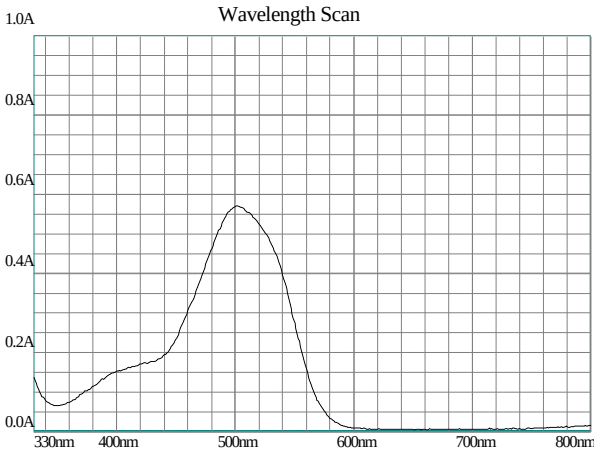
Allura Red (Red 40) Food Dye
(9.94 ppm Standard Solution)



Simply Kids Vitamin Enriched Drink[6]
(Undiluted From Plastic Bottle)



Halls Cherry Cough Tablet[7]
(One Tablet Dissolved in 100 mL Solution)



Cherry Kool-Aid Singles Powder[8]
(0.944 g Dissolved in 100 mL Solution)

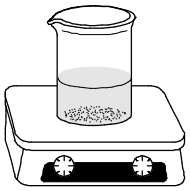
[6] Colour Ingredients: No Label Information; No Website Information; No Response to E-mail Query. **http://www.simplykids.ca/**
[7] Colour Ingredients: Label and Website: Red 40 and Blue 2. **http://www.gethalls.com/prod_halls_drugfacts.asp**
[8] Colour Ingredients: Website: Red 40. **http://www.kraftfoods.com/kf/Products/ProductInfoDisplay.htm?SiteId=1&Product=4300002347**

I. Unknown Sample 2 - Analysis of a Cherry Cough Tablet Containing Allura Red

The procedure assumes that the sample supplied is a water soluble tablet.
A **Halls Menthol-Lyptus Cherry Cough Tablet** is a suitable unknown.

Use one (1) tablet for a group of up to four (4) students. A clean small beaker and watch glass, a clean funnel, and a clean **100 mL** volumetric flask will be required.

I-1. Place the tablet in a clean small beaker. Add **30** to **40 mL** of distilled water to the beaker. A hot-plate will be available in a fume hood. Place the beaker on the hot-plate. Cover the beaker with a small or medium size watch glass. Set the control to a **LOW** heat setting.



I-2. When the tablet is fully dissolved, remove the beaker from the hot-plate onto a heat-proof pad (**CAUTION: HOT**). When the beaker is cool enough to handle, transfer the solution completely to a clean **100 mL** volumetric flask, using a funnel. Rinse the beaker and funnel with distilled water, collecting the rinse solution into the flask.

I-3. Fill the flask to the mark-line with distilled water. Cap the flask securely, and invert slowly many times to mix the solution completely. **This solution will now be used by all members of the group**. Each student will measure the percent transmission of the solution individually.

I-4. Check that the Spectronic 20 wavelength selector is set to **504 nm**.

I-5. Adjust the **0 %** and **100 % Transmission** settings if necessary as in **Part E**.

I-6. Read the percent transmission of the unknown sample **in duplicate** as in **Part F**.

I-7. Record the observed values in the **Unknown Samples Table** in the **REPORT PAGES** Section on page 34.

I-8. Calculate the mean percent transmission and the absorbance of the solution at **504 nm**. Record these values in the **Unknown Samples Table** on page 34.

I-9. Use your calibration line equation to calculate the concentration of the Allura Red dye in the cough tablet solution in **ppm units**. State the answer to 2 places after the decimal point. Show your work. See **Example 6** on page 13. Enter your concentration value in the **Unknown Samples Table** on page 34.

I-10. Calculate the total amount **in mg units** of Allura Red present in the **100 mL** (**0.100 L**) cough tablet solution. State the answer to 2 places after the decimal point. Show your work.
See **Example 7** on page 14.

Optional Bonus Analysis (10 Points Bonus)

J. Unknown Sample 3 - Analysis of a Cherry Powdered Drink Mix Containing Allura Red

The procedure assumes that the sample supplied is a powdered (solid) cherry drink mix.
A **Cherry Kool-Aid Singles** is a suitable unknown. A clean small beaker, a clean funnel, and a clean **100 mL** volumetric flask will be required.

J-1. **The sample is provided as a solid powder**. Tare a clean weighing boat.
Place **0.60 ± 0.05 g** of the solid powder into the boat.

J-2. Zero the balance. Weigh the boat and solid powder. Record the mass measurement to 3 places after the decimal point in the **Kool-Aid Mass Data Table** in the **REPORT PAGES** Section on page 36.

J-3. Transfer the solid into the clean small beaker. Zero the balance. Re-weigh the boat and solid residue. Record the mass measurement to 3 places after the decimal point in the **Table**.

J-4. Add **20** to **30 mL** of distilled water to the beaker. Dissolve the solid and transfer the solution through the funnel into the clean **100 mL** volumetric flask. Add more distilled water to the beaker if necessary to dissolve the solid. Rinse the beaker and funnel into the flask.

J-5. Fill the flask to the mark-line with distilled water. Cap the flask securely, and invert slowly many times to mix the solution completely.

J-6. Check that the Spectronic 20 wavelength selector is set to **504 nm**.

J-7. Adjust the **0 %** and **100 % Transmission** settings if necessary as in **Part E**.

J-8. Read the percent transmission of the unknown sample **in duplicate** as in **Part F**.

J-9. Record the observed values in the **Unknown Samples Table** in the **REPORT PAGES** Section on page 34.

J-10. Calculate the mean percent transmission and the absorbance of the solution at **504 nm**. Record these values in the **Unknown Samples Table** on page 34.

J-11. Use your calibration line equation to calculate the concentration of the Allura Red dye in the **100 mL** solution of drink powder **in ppm units**. State the answer to 2 places after the decimal point. Show your work. See **Example 6** on page 13. Enter your concentration value in the **Unknown Samples Table** on page 34.

J-12. Calculate the total amount of Allura Red present in the **100 mL** solution of drink powder **in mg units**. State the answer to 2 places after the decimal point. Show your work.
See **Example 7** on page 14.

J-13. The portion size of the drink is **one 8.3 g package**. Calculate the total amount of Allura Red present in an **8.3 g** package of drink powder **in mg units**. State the answer to 1 place after the decimal point. Show your work.

Name		Day		Start Time	

REPORT PAGES for Experiment 10
This section will be handed in as your report for this experiment.

Solution Concentrations

Solution	Nominal[9] Concentration (ppm)	Actual Concentration (ppm)
Supplied Stock Solution	**1000**	
Prepared Working Solution	**50.00**	
Calibration Standard 1	**2.50**	
Calibration Standard 2	**5.00**	
Calibration Standard 3	**7.50**	
Calibration Standard 4	**10.00**	

Stock Solution Volume

If Dispensed by Pump:

Volume = 10.00 mL (Exact)

If Dispensed by Buret (2 Places After Decimal):

Final Volume = _____ mL

Initial Volume = _____ mL

Volume Delivered = _____ mL

Transfer the Volume of the Stock Solution Dispensed into the Table below as $\mathbf{V_{conc}}$.

Working Solution Preparation Data

C_{conc}	V_{conc}	V_{dil}	C_{dil}
_____ ppm	**_____ mL**	**200.0 mL**	**_____ ppm**

Instructor's Initials on Completion: _____

Calculate the concentration of the Allura Red dye in the working solution **in ppm units**.
State the answer to 2 places after the decimal point. Show your work. See **Example 1** on page 8.

Concentration of Allura Red Dye in the Working Solution (ppm) = _____ ppm

Also enter this value in the **Calibration Standards Preparation Table** on page 30.

The REPORT PAGES Section Continues on the Next Page →

[9] **Nominal**: of, being, or relating to a designated or theoretical size that may vary from the actual: **APPROXIMATE**

REPORT PAGES for Experiment 10 (Cont)

Calibration Standards Preparation (Measured Values in Ink)

	Calibration Standard 1	Calibration Standard 2	Calibration Standard 3	Calibration Standard 4
Working Solution Final Volume (mL) (2 Places After Decimal Point)				
Working Solution Initial Volume (mL) (2 Places After Decimal Point)				
Working Solution Volume Delivered (mL) (2 Places After Decimal Point)				
C_{conc} (ppm) of Working Solution (From Previous Page)	**Working Solution (ppm) = _____ ppm**			
V_{dil}	**V_{dil} of All Calibration Standard Solutions = 100.0 mL**			
C_{dil} (ppm) (Actual Concentration of the Standard)				

Instructor's Initials on Completion: _____

Calculate the concentration of the Allura Red dye in each of the calibration standard solutions **in ppm units**. State the answers to 2 places after the decimal point.
Show one example calculation here. See **Example 2** on page 8.

Calibration Standard No. ____ Concentration of Allura Red Dye (ppm)

= _____ ppm

Enter the calculated values in the **Table** above. Copy the concentration values also to the **Solution Concentrations Table** on page 29 and the **Calibration Line Data Table** on page 33.

The REPORT PAGES Section Continues on the Next Page →

Name		Day		Start Time	

REPORT PAGES for Experiment 10 (Cont.)

If Using the Spectronic 20

Percent Transmission Values to the Nearest 1 % (Measured Values in Ink) and Calculated Absorbance Values to 3 Places

% Transmission Values of Calibration Standards	Trial 1	Trial 2	Mean % T	Calculated Absorbance
Standard 1				
Standard 2				
Standard 3				
Standard 4				

Instructor's Initials on Completion: _____

Calculate the absorbance of each of the calibration standard solutions.
State the answers to 3 places after the decimal point. Show your work for one calculation.
See **Example 3** on page 9.

$$\textbf{Absorbance} = A = -\log T = \log \frac{P_0}{P} = \log\left(\frac{100}{\%\ T}\right)$$

Example: Mean % T = 35 %

$$\textbf{Absorbance} = \log\left(\frac{100}{35}\right) = \underline{0.456}$$

Absorbance for Standard _____ = _____

Enter the calculated values of absorbance in the **Table** above.
Copy the absorbance values to the **Calibration Line Data Table** on page 33.

The REPORT PAGES Section Continues on the Next Page →

REPORT PAGES for Experiment 10 (Cont)

If Using a Direct Reading Instrument

Absorbance Values to 3 Figures (Measured Values in Ink)

	Trial 1	Trial 2	Mean A
Standard 1			
Standard 2			
Standard 3			
Standard 4			

Instructor's Initials on Completion: _____

Question 1: Why must you always use the same spectrometer tube? Why must you not scratch the tube glass surface? Why must the tube be clean and dry on the outside surface? Why must the tube always be put in in the same position using the marker line?

The answers to these questions are all related.

Question 2: Why must you not change the wavelength selector position during the experiment?

Hint: See the absorbance spectrum of Allura Red on page 26.

Question 3: Why is it necessary to close the sample compartment cover when reading the percent transmission value?

The REPORT PAGES Section Continues on the Next Page →

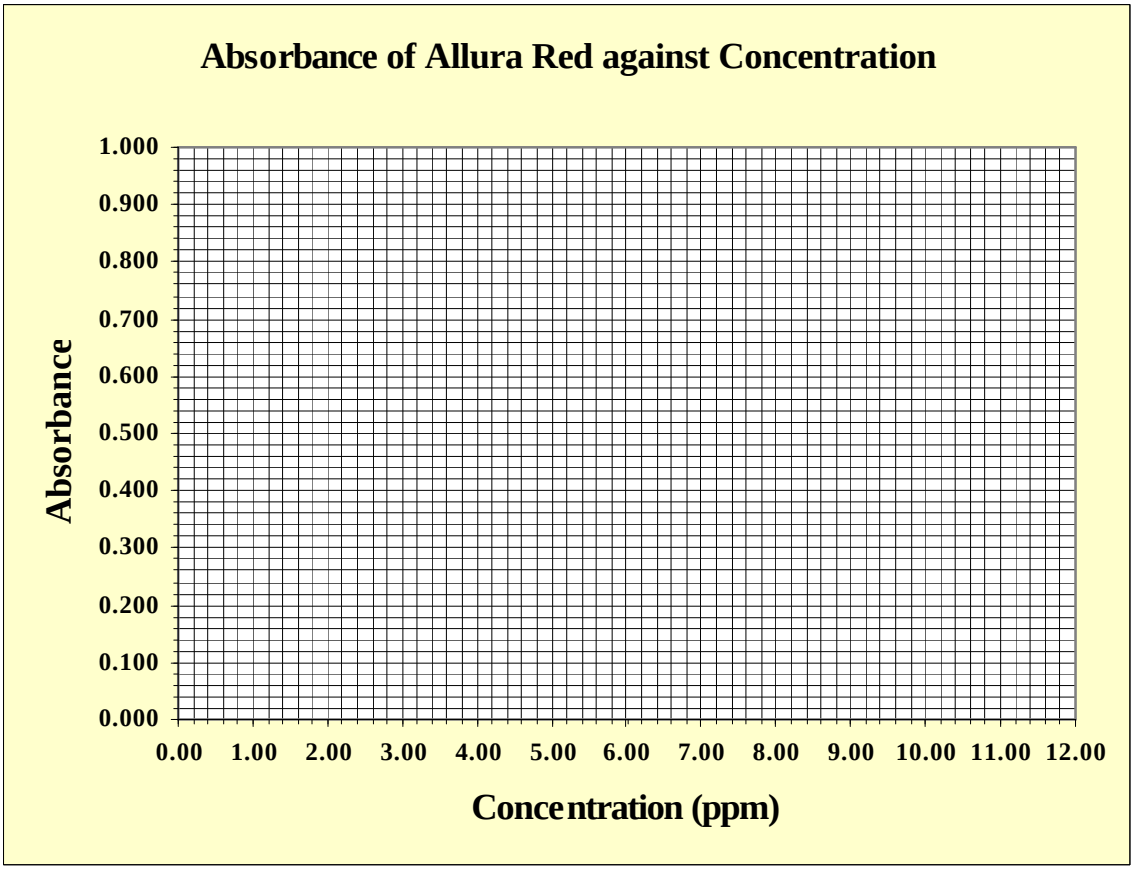
Name		Day		Start Time	

REPORT PAGES for Experiment 10 (Cont.)

Calibration Line Data

Solution	Calculated Concentration (ppm) 2 Places after the Decimal From Page 30	Calculated Absorbance 3 Places after the Decimal From Page 31
Standard 1		
Standard 2		
Standard 3		
Standard 4		

Approximate XY Scatter Plot Use a pencil. Circle the points.



Instructor's Initials on Completion: _____

The REPORT PAGES Section Continues on the Next Page →

REPORT PAGES for Experiment 10 (Cont)

Produce an Excel XY Scatter Plot and Linear Trendline for the Data in the Calibration Line Data Table on page 33 (See Examples 4 and 5 on pages 10 and 12).

1. Use a software package (e.g.: Microsoft Excel®) to display an **XY Scatter Plot** of the Absorbance against the Concentration in ppm units. **Choose the no-lines option**.

2. **Title** the plot, **including your name and the date**, and **label** both axes.

3. Choose a **linear trendline** to the data points.

4. **Display** the **trendline** on your plot and also display the **equation** and $\mathbf{R^2}$ value of the trendline.

5. Use the cursor to **select** the equation box.

6. Format as a **number** and choose the option **five** (**5**) places after the decimal point.

7. **Attach a Copy of your Experimental Plot to the REPORT PAGES**.

Unknown Samples (Measured Values in Ink)

Sample	Unknown 1	Unknown 2	Unknown 3
Sample Description			
Sample Treatment			
Percent Transmission Trial 1 (± 1 %)			
Percent Transmission Trial 2 (± 1 %)			
Mean % T (± 1 %)			
Calculated Absorbance (3 decimal places)			
Solution Concentration (ppm) (2 decimal places)			

Instructor's Initials on Completion of Data Collection: _____

The REPORT PAGES Section Continues on the Next Page →

Name		Day		Start Time	

REPORT PAGES for Experiment 10 (Cont.)

Equation of Your Experimental Linear Trendline:

Calculations for Unknown Sample 1 (See Example 6 and Example 7 on Pages 13 and 14)

Concentration of Allura Red in the Solution (ppm) = _____ ppm

Amount of Allura Red in 250 mL of the Solution (mg) = _____ mg

Calculations for Unknown Sample 2 (See Example 6 and Example 7 on Pages 13 and 14)

Concentration of Allura Red in the Solution (ppm) = _____ ppm

Amount of Allura Red in 100 mL of the Solution (mg) = _____ mg

The REPORT PAGES Section Continues on the Next Page →

REPORT PAGES for Experiment 10 (Cont.)

Optional Bonus Analysis (10 Points Bonus)

Kool-Aid Mass Data (Measurements Recorded in INK to 3 Places After the Decimal Point)

	Mass (g)
Weighing Boat + Solid **Kool-Aid** Sample (Initial Mass) (3 decimal places)	
Weighing Boat + Solid **Kool-Aid** Residue (Final Mass) (3 decimal places)	
Mass of **Kool-Aid** Sample (Initial Mass – Final Mass) (3 decimal places)	

Instructor's Initials on Completion: _____

Equation of Your Experimental Linear Trendline:

Calculations for Unknown Sample 3 (See Example 6 and Example 7 on Pages 13 and 14)

Concentration of Allura Red in the Solution (ppm) = _____ ppm

Amount of Allura Red in 100 mL of the Solution (mg) = _____ mg

Amount of Allura Red per 8.3 g Package of Drink Powder (mg) = _____ mg

Attach a Copy of your Experimental Plot to the REPORT PAGES