EXERCISE 2 Name ___________________________

How do scientists prepare solutions with specific concentrations of solutes?

Objectives

After completing this exercise, you should be able to:

- define and correctly use the following terms: solute, solvent, solution, aqueous solution, concentration, mole, molar, formula weight, dilution series, parallel dilution, and serial dilution
- prepare a solution of specified volume and concentration from separate solute and solvent
- prepare a solution of specified volume and concentration by diluting a stock solution
- prepare a dilution series using both the parallel and serial dilution methods; and be able to determine which method is most appropriate for preparing a given dilution series
- define optical absorbance and transmittance; and explain how these measurements can be used to assay the concentration of a solution
- explain what a spectrophotometer is; and be able to use one to measure the optical absorbance and transmittance of a solution
- explain the difference between a dependent and an independent variable
- analyze the relationship between two experimental variables using tables, graphs (also called charts), and linear regression analysis
- carry out linear regression analysis for a set of paired numbers and be able to:
    a. determine the linear correlation coefficient for the set of paired numbers
    b. determine the slope and y-intercept of the "best fit" straight line
    c. determine the equation of the "best fit" straight line
    d. calculate the y-value if given the x-value for any point on the "best fit" straight line
    e. calculate the x-value if given the y-value for any point on the "best fit" straight line
    f. plot the "best fit" straight line

Prelab

Before you come to lab, read this entire exercise. You must also answer all questions and complete all assignments on the first 19 pages of this exercise. Your instructor will give you directions on when and where to turn in your work.

Solutions are liquid or gaseous mixtures. In a liquid solution, the liquid that makes up most of the volume of the solution is called the **solvent.** If the solvent is water, then the solution is called an **aqueous solution**. The minor components of the solution are called **solutes.** For example, if we dissolve one teaspoon of sugar and one teaspoon of salt in a glass of water, water is the solvent while sugar and salt are solutes. This would be an example of an aqueous solution. Since living organisms are composed mostly of water, studying the chemistry of life largely involves studying the chemistry of aqueous solutions. Therefore, preparing aqueous solutions is an essential part of molecular and cellular biology.

The **concentration** of the solution refers to the amount of solute per unit of solution. Concentration can be expressed as a ratio:

$$\text{Concentration of solution} = \frac{\text{amount of solute}}{\text{amount of solution}}$$

For example, if we have 10 g of sucrose in 2 L of solution, the concentration is 5 g/L, or "5 grams per liter."

Another way to describe the concentration of a solution is by **percent weight per unit volume**. This equals the number of grams of solute per 100 mL of solution. For example, if we have 10 g of sucrose in 2 L of solution, this equals 5 g in 1 L of solution, which equals 0.5g in 100 mL, or a 0.5% sucrose solution.

A third way to describe the concentration of a solution is by **molarity**. The molarity of a solution equals the number of moles of solute per liter of solution. Note that:

- a one **molar** (1M) solution contains one **mole** of solute per liter of solution.
- a one **millimolar** (1mM) solution contains one **millimole** of solute per liter of solution.
- a one **micromolar** (1µM) solution contains one **micromole** of solute per liter of solution.

One **mole** (1 mol) of any substance is equal to 6.02 x 10^{23} basic units. (Note: the basic units of an element are individual atoms, and the basic units of a compound are individual molecules.) The formula weight of a substance tells us how many grams one mole of that substance weighs. For example, the formula weight of potassium permanganate ($KMnO_4$) is 158.04 g/mole. Therefore, if you have 158.04 g of potassium permanganate in one liter of solution, the concentration would be 1 molar (1M).

⇒ **IMPORTANT: Please note that "gram", "mg", "liters", "mole", and "millimole" are all examples of units of amount. On the other hand, "g/L", "mg/mL", "millimoles per liter", "micromolar", and "molar" are all examples of units of concentration. Whenever you report the amount of a substance or the concentration of a solution, make sure you use appropriate units. Also note that the abbreviation for mole (a unit of amount) is "mol" and the abbreviation for molar (a unit of concentration) is "M".**

Electromagnetic radiation is a form of energy that travels through space with the characteristics of a wave. Electromagnetic radiation with wavelengths between about 400 nm and 740 nm is detected as visible light. Our eyes detect different wavelength of visible light as different colors. For example, light with a wavelength of 400 nm appears violet while light with a wavelength of 740 nm appears red. White light is actually a mixture of all the visible wavelengths. When white light passes through a solution, the color of the solution will depend on how much of each wavelength passes through the solution (called **optical transmittance**) and how much of each wavelength is absorbed (called **optical absorbance**).

A **spectrophotometer** is an instrument that can pass light of a single wavelength through a solution and measure the amount that passes through. First, the spectrophotometer must be zeroed by passing light of the chosen wavelength (500 nm for example) through a blank containing only the solvent. Then, the same wavelength of light is passed through the solution. The percentage of light that passed through the solution relative to the amount that passed through the solvent alone is called the **percent transmittance at 500 nm**. For example, if half as much light passes through a solution as passes through the solvent alone, we would record this as 50% T:

$$\textbf{Percent transmittance (\%T)} = \frac{\text{amount of light transmitted through the solution}}{\text{amount of light transmitted through the solvent}} \times 100$$

On the other hand, the amount of light at 500 nm that is absorbed by the solution is called the **absorbance at 500 nm** and is abbreviated A_{500}. Absorbance is the negative logarithm of the percent transmittance divided by 100. **Because logarithms have no units, absorbance has no units**:

$$\textbf{Absorbance (A)} = -\log(\%\ \text{transmittance} / 100)$$

Note that absorbance and transmittance are inversely related to each other. In other words, as the absorbance of a particular wavelength increases, the transmittance of that wavelength decreases. It turns out that, within limits, the optical absorbance of a solution at a specified wavelength is **linearly** related to its concentration. This means that if the concentration of a solution increases at a steady rate, then the absorbance will also increase at a steady rate. This is called a **linear relationship** because if we measure the absorbance of several solutions with different concentrations, and then plot a graph with concentration on the x-axis and absorbance on the y-axis, all of our data points should fall on a straight line.

In this lab, you will learn 3 methods for preparing solutions that contain a specific concentration of solutes. You will then use all 3 methods to prepare 14 solutions of $KMnO_4$, each with a different concentration of solute. Next, you will measure the absorbance of each solution with a spectrophotometer, and plot a graph of your data with the solution concentration on the x-axis and the absorbance on the y-axis**. If your solutions were prepared correctly, all of your data points should fall on a straight line.** Then you will use a statistical technique called **linear regression** to determine the equation of the straight line. Finally, you will use this equation to estimate the concentration of a solution of potassium permanganate of unknown concentration.

Why do scientists need to prepare solutions with specific concentrations of solutes?

When making solutions for use in the biology lab, controlling the concentration of solutes in the solution is often critical. For example, failure to carefully control the concentration of solutes in solutions used to culture cells can cause the cells die or develop abnormally. Likewise, inappropriate concentrations of solutes in solutions that contain biomolecules may cause the biomolecules to denature or aggregate, thus making them inactive. Concentrations of solutes can also affect the speed and types of chemical reactions that take place within a solution.

How do I prepare solutions with specific concentrations of solutes?

In this lab we will examine 3 methods for preparing solutions with specific concentrations of solutes. Make sure you understand WHEN to use each method as well as HOW to use each method:

A. Dissolving solid solutes in the solvent
B. Diluting an existing stock solution using the parallel dilution technique
C. Diluting an existing stock solution using the serial dilution technique

A. Dissolving solid solutes in the solvent

This method must be used when there is no existing stock solution. In this case, you dissolve the solid solute (usually granules or powder) in the solvent (usually dH_20.) To make a solution in this way, you must know how much of the solute to use and how much solvent to mix with it.

As mentioned previously, a 1 molar (1 M) solution contains 1 mole of solute dissolved in 1 liter of total solution. Obviously, we cannot measure moles directly because we have no way to count atoms or molecules. However, the **formula weight** of a substance tells us how much one mole of the substance weighs. Therefore, we can use the formula weight to measure moles indirectly by weight.

Your Turn

You want to prepare one liter of a 2 M potassium permanganate ($KMnO_4$) solution. How many moles of potassium permanganate should you dissolve in one liter of solution?

____

The formula weight of $KMnO_4$ is 158.04 g/mole. How much potassium permanganate should you weigh out in order to prepare one liter of the following $KMnO_4$ solutions?

2 M _______________ 0.75 M _______________

Suppose you do not need an entire liter of solution. How much potassium permanganate should you weigh out in order to prepare the following amounts of a 0.75 M $KMnO_4$ solution?

0.5 L _______________ 350 mL _______________

HINT: Multiply the amount of potassium permanganate needed to make one liter of solution by the number of liters you actually need (remember, 350 mL = 0.35 L).

When preparing a solution by dissolving a solid solute in the solvent, use the following formula to calculate the weight of solute needed:

Weight of solute (g) = formula weight of solute (g/mole) X molarity (mole/L) X final volume (L)

For example, to make 600 mL of 0.35 M $KMnO_4$, use the following values:

Formula weight of $KMnO_4$ = 158.04 g/mole
Molarity desired = 0.35 mole/L
Final volume = 0.6 liters

Weight of $KMnO_4$ needed = 158.04 g/mole X 0.35 moles/L X 0.6 L = 33.19 g
(Notice how all the units except for grams cancelled out of this equation!)

Your Turn

Calculate the weight of potassium permanganate ($KMnO_4$) needed to prepare 80 mL of a 0.01 M solution. Write your answer in the space below **and in the space provided on page 19 of the Lab Procedures.**

Weight of potassium permanganate ($KMnO_4$) needed = ______________

Once you have weighed out the required amount of solute, place it in an appropriate volumetric measuring device (such as a graduated cylinder) and add enough solvent until the total volume of the solution reaches the desired amount. Notice that if you want to make 80 mL of solution, you must add less than 80 mL of solvent to the solute because the solute occupies some volume. If you added a full 80 mL of solvent to the solute, the total volume of the solution would be greater than 80 mL, and your solution would be too dilute.

General Procedure for Preparing a Solution by Dissolving a Solid Solute in the Solvent:

1. **Determine the amount of solute you need, and weigh it out with an electronic balance.**
2. **Place the solute in an appropriate volumetric measuring device. For example, if you need 80 mL of solution, a 100 mL graduated cylinder would be an appropriate choice.**
3. **Carefully add water to your measuring device until the bottom of the meniscus reaches the level of the final volume of your solution.**
4. **Seal the top of the measuring device so no solution can leak out. In the case of a graduated cylinder, you can seal it by stretching and tightly wrapping a piece of Parafilm around the top and holding it securely in place with your fingers or the palm of your hand (make sure you are wearing protective gloves.)**
5. **Gently invert the measuring device several times until all of the solute is dissolved.**

Your Turn

A student wants to make 80 mL of a 1.0 M solution of sucrose (formula weight 342.3 g/mole). How much sucrose should he weigh out to prepare this solution?

____

The student weighs out the correct amount of sucrose, places it in a beaker, measure 80 mL of dH2O with a graduated cylinder, adds the dH2O to the beaker, and then mixes the solution with a stir bar until all of the sucrose is dissolved. What did he do wrong?

____

____

If the final volume of the solution he prepared was 95 mL, what is the actual molarity of his solution?

____

B. Diluting an existing stock solution using the parallel dilution technique

When preparing solutions, it is often easier to dilute an existing stock solution than it is to weigh out a solid solute and then dissolve it in the solvent. This is especially true if you want to prepare several solutions that each have a different concentration of the same solute.

One way to dilute an existing stock solution is by using the **parallel dilution** technique. When using this technique, first calculate the amount of stock solution needed to make your dilution.

When making parallel dilutions, use the following formula to calculate the amount of stock solution needed for each dilution:

$$\mathbf{C_1 \ X \ V_1 = C_2 \ X \ V_2} \qquad \text{or} \qquad \mathbf{C_1V_1 = C_2V_2}$$

C_1 is the concentration of the starting (stock) solution
V_1 is the volume of starting (stock) solution needed to make the dilution (this is your unknown)
C_2 is the desired concentration of you final (dilute) solution
V_2 is the desired volume of you final (dilute) solution

For example, if you want to make 50 mL of 0.15 M calcium chloride ($CaCl_2$) from a 2.0 M stock solution:

$$C_1 = 2.0 \text{ M} \quad C_2 = 0.15 \text{ M} \quad \text{and} \quad V_2 = 50 \text{ mL}$$

To determine the volume of stock solution required (V_1):

$$\mathbf{C_1V_1 = C_2V_2}$$

$$(2.0 \text{ M})\, V_1 = (0.15 \text{ M})\,(50 \text{ mL})$$

$$(2.0 \text{ M})\, V_1 = 7.5 \text{ M mL}$$

$$V_1 = 3.75 \text{ mL}$$

After you have calculated the required amount of stock solution (V_1), pour the stock solution into a graduated cylinder and then add enough dH_2O to bring the final volume up to 50 mL. Alternatively, you can pour the required amount of stock solution into a container, and then calculate and add the amount of dH_2O needed to give you a final volume of 50 mL. (In this example, 50 mL minus 3.75 mL of stock solution equals 46.25 mL, so 46.25 mL of dH_2O must be added to the 3.73 mL of stock solution.)

Your Turn

Calculate the amount of 0.01 M potassium permanganate ($KMnO_4$) stock solution needed to prepare 25 mL of a 2 mM solution. (Note that 2 mM = 0.002 M.) Write your answer in the space below **and in the space provided on page 20 of the Lab Procedures.**

Volume of stock solution needed = ______________

Sometimes you must prepare several solutions that each have a different concentration of the same solute. This is referred to as a dilution series. When preparing a **parallel dilution series**, use the formula $\mathbf{C_1V_1 = C_2V_2}$ to calculate how much stock solution is needed to make each dilution.

Your Turn

Calculate the amount of 0.01M potassium permanganate (KMnO4) stock solution and the amount of dH_2O needed to make 10 mL each of the following dilutions. Write your answers in the spaces below **and in the table on page 20 of the Lab Procedures**:

Diluted Concentration	Volume of 0.01 M KMnO4 Stock Solution Needed	Volume of dH_2O needed
1.0 mM KMnO4	_______________	_______________
0.6 mM KMnO4	_______________	_______________
0.4 mM KMnO4	_______________	_______________
0.2 mM KMnO4	_______________	_______________
100 µM KMnO4	_______________	_______________
50 µM KMnO4	_______________	_______________
20 µM KMnO4	_______________	_______________

C. Diluting an existing stock solution using the serial dilution technique

When to make a serial dilution

Another way to make dilutions is to use some of your existing stock solution to make a dilute solution, then use some of the dilute solution to make an even more dilute solution, then use some of that solution to make an even more dilute solution, and so on. This procedure is called the **serial dilution technique**. There are two situations where serial dilutions should be used rather than parallel dilutions:

1. Use a serial dilution when you need several solutions of the same solute and there is a constant **dilution factor**. For example, suppose you have a 2 M stock solution of $KMnO_4$ and you want to make 15 mL of each of the following concentrations of $KMnO_4$: 0.2 M, 20 mM, 2 mM, and 0.2 mM. Notice that the concentration of each solution is $1/10^{th}$ the concentration of the previous solution in the series. The factor by which each solution is diluted compared to the previous one is called the dilution factor.

   To calculate the dilution factor for each dilution, divide the concentration of the starting solution by the concentration of the diluted solution. For example, for the first dilution 2 M divided by 0.2 M equals 10. For the second dilution, 0.2 M divided by 20 mM equals 10. For the third dilution, 20 mM divided by 2 mM equals 10. And for the fourth dilution 2 mM divided by 0.2 mM equals 10. Therefore, this series has a constant dilution factor of 10.

2. Also use a serial dilution when the dilution factor is so large that the amount of stock solution needed to make the dilution in one step (using the formula $C_1V_1 = C_2V_2$) is too small to measure accurately. Remember that the smallest volume you can measure with the micropipettors is 2 µL.

Your turn

1. You have a stock solution of 1.6 M sucrose and you need to prepare solutions with the following concentrations of sucrose: 0.4 M, 0.1 M, 25mM, and 6.25 mM. What is the dilution factor for this series?

   ____

2. You plan to prepare 4 solutions by serial dilution. The concentration of your stock solution is 2.7 M and the dilution factor is 3. What will be the molarity of your four solutions?

______________ ______________ ______________ ______________

3. You have a 1.0 M stock solution of glycine (an amino acid), and you need 5 mL of a 0.1 mM solution. By what factor do you need to dilute your stock solution?

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   Using the formula $\mathbf{C_1V_1 = C_2V_2}$, calculate the amount of stock solution you need to make 5 mL of a 0.1 mM solution from a 1.0 M stock solution?

   ______________ mL or ______________ µL

   Do you have a measuring device available that will accurately measure this amount? **Explain.**

   ____

   ____

Your turn

For each of the following situations, state whether the parallel or serial dilution technique would be more appropriate, **and explain your reasoning**.

1. You need 20 µL of a 5 mM glycerol solution. You have a 1.0 M glycerol stock solution on hand.

Which dilution technique is appropriate? ______________________

Why? ____

2. You have a 2.5 M stock solution of EDTA. You need 50 mL each of 2 M, 0.5 M, 0.2 M and 0.1 M solutions of EDTA

Which dilution technique is appropriate? ______________________

Why? ____

3. You have a 2.0 M stock solution of sucrose. You need 100 mL each of 1 M, 0.5 M, 0.25 M, 0.125 M, and 62.5 mM sucrose solutions.

Which dilution technique is appropriate? ______________________

Why? ____

How to make a serial dilution

Let's return to our example where you have a 2 M stock solution of $KMnO_4$ and you want to make 15 mL of each of the following concentrations of $KMnO_4$: 0.2 M, 20 mM, 2 mM, and 0.2 mM. In this case you must make a total of 4 dilutions. First, the 2 M stock solution is diluted to make the 0.2 M solution. Next, the 0.2 M solution is diluted to make the 20 mM solution. Next, the 20 mM solution is diluted to make the 2 mM solution. Finally, the 2 mM solution is diluted to make the 0.2 mM solution. How would you make these 4 dilutions using the serial dilution technique? Before you begin, you must determine the values of 3 variables: **df,** $\mathbf{v_2}$, and $\mathbf{v_1}$.

df For each dilution, **df** is the dilution factor. We have already seen that this dilution series has a constant dilution factor of 10.

$\mathbf{v_2}$ For each dilution, $\mathbf{v_2}$ is the volume of diluted solution that you want to make. Since we want to make 15 mL of each diluted solution, $\mathbf{v_2}$ = 15 mL

$\mathbf{v_1}$ For each dilution, $\mathbf{v_1}$ is the volume of more concentrated solution that you mix with solvent to make the more dilute solution. If you know **df** and $\mathbf{v_2}$, you can calculate the value of $\mathbf{v_1}$ using the following formula:

When making serial dilutions, use the following formula to calculate the value of v_1:

$$\frac{V_1 + V_2}{V_1} = df$$

In our example: $\frac{V_1 + 15\text{ mL}}{V_1} = 10$

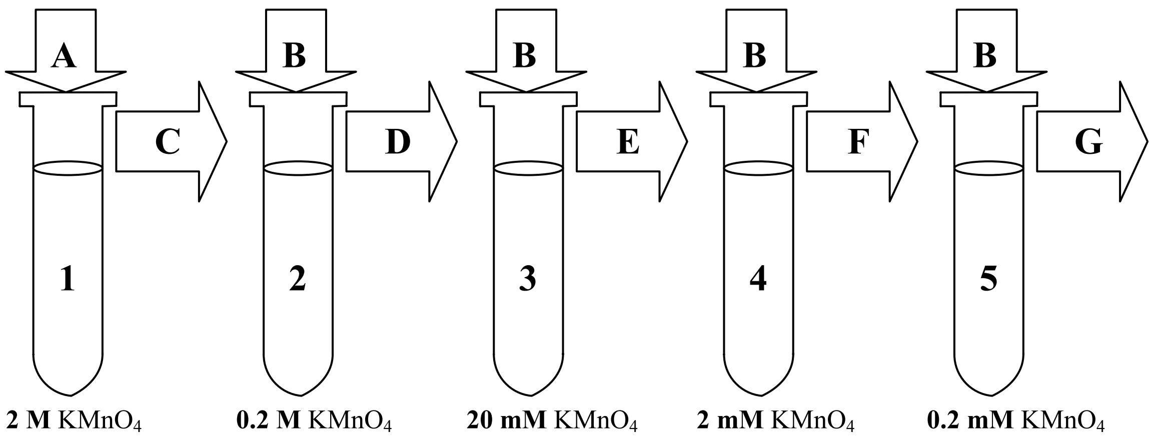
$$V_1 + 15\text{ mL} = 10\,V_1$$

$$15\text{ mL} = 10\,V_1 - V_1$$

$$15\text{ mL} = 9\,V_1$$

$$V_1 = 15/9\text{ mL} \quad\text{or}\quad 1.67\text{ mL}$$

Once you know the values of **df, v_2**, and **v_1**, set up 5 tubes (one for the stock solution and one for each of the dilute solutions) as shown in the following diagram and then follow the procedure described below the diagram:



⇒ **NOTE: The letters below correspond to the letters on the arrows in the diagram above.**

A. Place the correct amount of stock solution ($V_1 + V_2$) into tube 1. In the case of our example, place 16.67 mL of 2 M $KMnO_4$ into tube 1.

B. Place the correct amount of solvent (V_2) into the remaining tubes. In the case of our example, place 15 mL of dH_20 into tubes 2 through 5.

C. Transfer the correct amount of solution (V_1) from tube 1 to tube 2 and then thoroughly mix the contents of tube 2. In the case of our example, transfer 1.67 mL of solution from tube 1 to tube 2 and thoroughly mix. This will leave 15 mL of 2 M $KMnO_4$ in tube 1.

D. Transfer the correct amount of solution (V_1) from tube 2 to tube 3 and then thoroughly mix the contents of tube 3. In the case of our example, transfer 1.67 mL of solution from tube 2 to tube 3 and thoroughly mix. This will leave 15 mL of 0.2 M $KMnO_4$ in tube 2.

E. Transfer the correct amount of solution (V_1) from tube 3 to tube 4 and then thoroughly mix the contents of tube 4. In the case of our example, transfer 1.67 mL of solution from tube 3 to tube 4 and thoroughly mix. This will leave 15 mL of 20 mM $KMnO_4$ in tube 3.

F. Transfer the correct amount of solution (V_1) from tube 4 to tube 5 and then thoroughly mix the contents of tube 5. In the case of our example, transfer 1.67 mL of solution from tube 4 to tube 5 and thoroughly mix. This will leave 15 mL of 2 mM $KMnO_4$ in tube 4.

G. Remove the correct amount of solution (V_1) from tube 5 and discard it. In the case of our example, remove 1.67 mL of solution from tube 5 and discard it. This will leave 15 mL of 0.2 mM $KMnO_4$ in tube 5.

Your turn

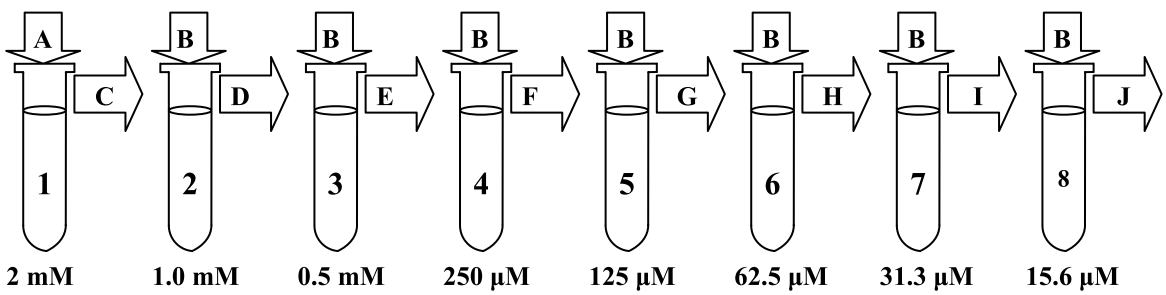
Starting with a stock solution of 2 mM $KMnO_4$, you plan to make 6 mL of each of the following concentrations of $KMnO_4$: 1.0 mM, 0.5 mM, 250 µM, 125 µM, 62.5 µM , 31.3 µM , and 15.6 µM

.What is df for this dilution series? _______________

What is V_2 for this dilution series? _______________

What is V_1 for this dilution series? _______________

Label the arrows in the diagram below **and in the diagram on page 21 of the Lab Procedures** to show how you would make the required solutions. Above each vertical arrow, write down the volume and type of liquid you will place in the tube (e.g. 13 mL of stock solution or 9 mL of dH_2O.) Below each horizontal arrow, write down how much solution you will transfer from one tube to the next (e.g. 3 mL):



How do scientists analyze the relationship between 2 experimental variables?

Scientists are often interested in studying the relationship between 2 experimental variables. For example:

- What relationship, if any, exists between the amount of fiber in a person's diet and the risk of developing colon cancer?
- What relationship, if any, exists between the amount of exercise a person gets each week and the probability of developing heart disease?
- What relationship, if any, exists between the wavelength of light shined through a solution of $KMnO_4$, and the amount of light that is absorbed?
- When light of a particular wavelength is shined through a solution of $KMnO_4$, what relationship, if any, exists between the concentration of the solution and the amount of light that is absorbed?

To examine some methods scientists use to analyze the relationship between experimental variables, let's look at an example where a scientist is studying the relationship between the protein concentration of a solution and the amount of UV light at 280 nm that the solution will absorb. To study this relationship, the scientist prepares 10 solutions with the following protein concentrations in µg/mL: 0, 10, 20, 40, 80, 160, 320, 640, 1280, and 2560. He then places each solution in a spectrophotometer and measures the absorbance of UV light at 280 nm.

Notice that the data this scientist collects will consist of **pairs** of values. For each observation, he records both the **concentration** of the solution, and the $\mathbf{A_{280}}$ **value** of the solution. Since both concentration and the A_{280} values can change as he makes his observations, they are called **variables**.

In the example described above, before the experiment even starts, the scientist must decide which protein concentrations he wishes to test. Because the value of the concentration variable at each reading was determined beforehand by the experimenter and did not depend, in any way, on the A_{280} values of the solutions or on any other variables in the experiment, it is called an **independent variable**. On the other hand, the A_{280} values are not determined by the experimenter. These values depend on the experimental conditions that have been set up. Therefore, the A_{280} variable is called a **dependent variable**. In general, the number you record at the end of each experimental trial is the value of your dependent variable.

Your turn

For each example below, identify the independent and dependent variables:

Example #1 - A botanist measures the height of four groups of pea plants that are grown using different concentrations of a nutrient solution. She takes her measurements once per week over the course of 3 months.

Which is/are independent variable(s)? ________________________________

Which is/are dependent variable(s)? __________________________________

Example #2 - A scientist studies the survival rate of 4 groups of AIDS patients. One group receives no drug treatment, the second group receives the drug AZT only, the third group receives the drug saquinavir (a protease inhibitor) only, and the fourth group receives both AZT and saquinavir. The survival rate is recorded once per month over the course of 2 years.

Which is/are independent variable(s)? ________________________________

Which is/are dependent variable(s)? __________________________________

Now, let's take a look at several methods scientists use to help determine whether 2 experiment variables are related, and if the variables are related, exactly what the relationship between them is.

1. Tables or Spreadsheets

Often, the first step in analyzing the relationship between two experimental variables is to organize your data in a **table** or **spreadsheet**. A table or spreadsheet is an orderly presentation of data aligned in rows and columns. Data for paired variables are conveniently shown in a table with 2 columns. Values for the independent variable are usually shown in the left column while the corresponding values for the dependent variable are shown in the right column. The following table presents hypothetical results for the scientist who is studying the possible relationship between protein concentration and A_{280} values:

Table 2.1 Absorbance of UV light by protein solutions

Protein Concentration (µg/mL)	**A_{280}**
0	0.000
10	0.045
20	0.098
40	0.195
80	0.373
160	0.711
320	1.398
640	1.833
1280	1.999

IMPORTANT: When displaying data in a table:

1. The table should be self explanatory. In other words, the table should have a descriptive title and all parts of the table should be clearly labeled so that the reader knows exactly what every number or entry in the table represents.

2. All measurements listed in the table should include appropriate units. (Note, however, that absorbance values are unusual in that they are one of the few measurements that lack units.)

Tables help us visualize relationships between the variables we are studying. In Table 2.1, for example, we can immediately see that as protein concentration increases, the A_{280} values also increase. This means that there is a **direct** relationship between these 2 variables. This is in contrast to an **inverse** relationship where the values of the 2 variables go in opposite directions. For example, absorbance and transmittance are inversely related because as the amount of light absorbed by a solution increases, the amount of light transmitted decreases.

2. Graphs

Graphs often make it easier to see relationships between the variables you are studying. There are many types of graphs, but the one you will use most often in this class is a simple **scatter diagram**. To make a scatter diagram, begin by drawing the axes for the graph on a sheet of graph paper. The horizontal axis of the graph is called the **x-axis** or abscissa, and the vertical axis is called the **y-axis** or ordinate. Each axis must be labeled with a description of the variable you are plotting (e.g. "Protein concentration" or "A_{280} values.") **You should always plot the independent variable on the x-axis and the dependent variable on the y-axis.** Each axis must also be labeled with appropriate numerical values AND units of measurement.

Values on the x axis should increase as you move from left to right, and values on the y axis should increase as you move upwards. The simplest scatter diagrams uses a **linear scale** for both the x and y axes. With a linear scale, a given change in distance along the axis always represents the same change in value for the variable, no matter where on the axis the change occurs. For example, if you are plotting protein concentration on the x-axis and one square represents a change of 100 µg/mL, then every other square must also represents a change of 100 µg/mL. Similarly, if you are plotting A_{280} values on the y-axis and one square represents an increase of 10.000, then every other square must also represents a change of 10.000. **In other words, as you move along each axis the value of the variable must increase at a constant rate.** Note that the scale on one axis does not have to match the scale on the other axis. However, both scales should be linear and should be adjusted so that your completed graph will nearly fill the entire page. After both axes are completely labeled, simply plot the paired values on your graph paper. Finally, include a clear, descriptive title above your graph.

Checklist for Preparing a Graph or Chart

1. **Each axis should be clearly labeled with a description of the variable you are plotting. Make sure you plot the independent variable on the x-axis and the dependent variable on the y-axis. Independent variables are variables that the experimenter controls during the experiment. The dependant variable is the one the experimenter measures at the end of each trial.**

2. **Each axis should also be clearly labeled with appropriate numerical values and appropriate units of measurement.**

3. **Values on the x axis should increase as you move from left to right, and values on the y axis should increase as you move upwards.**

4. **Both axes should have a linear scale, meaning that the same increments are consistently the same distance apart. The size of the increments on one axis does not have to match the size of the increments on the other axis, but both scales must be linear.**

5. **Mark off the numerical values on each axis so that you completed graph will nearly fill the entire page.**

6. **Give your graph a brief title that explains the relationship you are graphing.**

⇒ **For more detailed information on how to draw graphs, please see Appendix E at the back of this manual.**

Your turn

Use the grid below to make a scatter diagram of the data in Table 2.1 (Absorbance of UV light by protein solutions). **Plot each point using a single dot or small circle. Make sure you follow ALL of the directions listed in the checklist on the previous page.**

3. Trendlines and Linear Regression

After a scatter diagram has been prepared, the relationship between the 2 variables can sometimes be seen more clearly if we draw a simple **line graph** by connecting the points on the scatter diagram. However, a line graph includes apparent irregularities in the relationship that may actually be due to error and/or chance variations that occur when you collected your data. For this reason, rather then connect the points, it is often more useful to determine the **straight line** or **smooth curve** that can best be drawn as close as possible to the points on the scatter diagram. This is referred to as the **best fit line** or **trendline**. If the trendline is straight, rather than curved, then the relationship between the 2 variables is described as **linear**.

In this course you will often be asked to determine the “best fit” straight line for a set of data points. Many calculators have statistics programs that can determine how well a set of data points fit a straight line. These programs can also determine the equation of the “best fit” straight line. This type of analysis is called **linear regression analysis**. Linear regression is an extremely powerful technique because the equation of the best fit straight line (also called the **linear regression line**) indicates the exact mathematical relationship between the 2 variables. This means that if you know the value of one variable, you can use the linear regression equation to calculate the value of the other variable.

In the following discussion we will describe some general principles of linear regression. Detailed instructions on how to use the TI-36X calculator to carry out linear regression can be found in **Appendix B** at the back of this manual. If you are using a different calculator, refer to the instructions that came with your calculator or check for instructions on the Web.

Examine the scatter diagram that you plotted on the previous page, and try to visualize the smooth line that would most closely match the 9 data points. Notice that this line would have a steeper slope at low protein concentrations (up to about 320 µg/mL), but would gradually “flatten out” as you move towards higher protein concentrations. This is because once all or most of the UV light at 280 nm has been absorbed, further increasing the protein concentration of the solution will not produce any additional increase in absorbance.

Actually, this is a fairly typical result when comparing two variables in a biological experiment. Often there is a linear (i.e. straight line) relationship between the variables when the independent variable has low and/or moderate values. But this relationship may “break down” as we approach extremely high (or in some cases extremely low) values of the independent variable, causing the “best fit” line to either “flatten out” or “steepen”. Therefore, although we could try to fit a straight line to all of the data points on our scatter diagram, we should look for signs that the linear relationship is “breaking down” at extreme ends of the data range. When using linear regression analysis, you should also keep in mind that a minimum of 5 data points are needed to get reliable results. In our example, because the curve seems to “flatten out” with the last two data points, most scientists would try to fit a straight line to the first 7 data points only. Most likely, it is only in this region where a true linear relationship exists.

You can use your calculator to determine how well a set of points actually fit a straight line by calculating the **linear correlation coefficient**, usually designated by the letter “r”. The value of “r” ranges from +1 to -1. A positive correlation coefficient means that as one variable increases, the other also increases. A negative correlation coefficient means that as one variable increases, the other decreases. If the linear correlation coefficient is close to +1 or -1 this indicates that the data fit a straight line very well. If the correlation coefficient is close to zero (either positive or negative) then the data do not fit a straight line well. In general, most scientists will conclude that 2 variables are linearly related if the absolute value of the linear correlation coefficient is greater than 0.95.

You can also use your calculator to determine the equation of the straight line that best fits your data. The general equation for a straight line can be written as:

$$y = mx + b$$

where m is the **slope** of the line (how steep or flat the line is),

and b is the **y-intercept** (the value of y when the line crosses the y axis).

A **positive slope** means the value of y increases as the value of x increases (positive correlation), and a **negative slope** means the value of y decreases as the value of x increases (negative correlation.) To determine the equation of the straight line that best fits your data, use your calculator to determine the slope and y-intercept of the “best fit” straight line, and then substitute these values into the equation above. The resulting equation indicates the precise mathematical relationship between the 2 variables you are studying, within the region where they are linearly related.

Your turn

Examine the scatter diagram that you plotted on page 13. Enter the paired values for **all 9** data points into your calculator's memory, and calculate the linear correlation coefficient for your data. Follow the instructions that came with you calculator; or use the instructions in Appendix B if you have the TI-36X; or get help from someone who knows how to use your calculator. Write down the linear correlation coefficient ("r") in the space below:

____

Should you conclude that protein concentration and A280 values are linearly related when the protein concentration of a solution is between 0 and 1280 μg/mL? Explain your answer.

____

____

Now, **clear your calculator's memory**, and circle the **first 7** data points on your scatter diagram. Enter the paired values for these 7 data points **only** into your calculator's memory, and calculate the linear correlation coefficient for your data. This time, **do not** erase the memory until after you have finished ALL of the remaining calculations in the Prelab. Based on the first 7 points of your scatter diagram, what is the linear correlation coefficient ("r") for protein concentration and A280 values?

____

Should you conclude that protein concentration and A280 values are linearly related when the protein concentration of a solution is between 0 and 320 μg/mL? Explain your answer.

____

____

Do you expect the slope of the best fit straight line for these 7 data points to be positive or negative? Explain your answer.

____

____

With the values for the first 7 data points still in your calculator's memory, calculate the slope and y-intercept of the "best fit" straight line. Follow the instructions that came with you calculator; or use the instructions in Appendix B if you have the TI-36X; or get help from someone who knows how to use your calculator. Write the equation for the best fit straight line in the space below:

____

The equation of the best fit straight line indicates the exact mathematical relationship between the 2 variables you are studying. In our example, the variables are protein concentration (x) and A280 values (y). This means that if you know the value of one variable (either x or y), you can substitute that value into the linear regression equation and calculate the value of the other variable. [Note: With the TI-36X, you can simply enter the value of one variable into the calculator, press two buttons, and the calculator will display the value of the other variable. See Appendix B for detailed instructions.] This allows you to determine the coordinates (x and y values) of any point that lies on the linear regression line. In addition, if you wish to plot the linear regression line on your scatter diagram, simply determine the coordinates of any two points on the line, plot the points on the scatter diagram, and then draw the straight line that runs through the two points. When doing this, it is best to plot two points that are near the two extremes of the region where your data suggest a linear relationship exists.

⇒ **IMPORTANT: If you know the value of one variable, you can use the linear regression equation to determine the value of the other variable ONLY when the value of the variables lies within the data range that was used to calculate the linear regression equation. In our example, the linear regression equation was calculated using data points where protein concentration varied from 0 to 320 µg/mL, and A280 values varied from 0 to 1.398. Therefore, you should NOT use this equation to determine the A280 value of a solution that has a protein concentration greater than 320 µg/mL. Likewise, you should NOT use this equation to determine the protein concentration of a solution that has an A280 value greater than 1.398.**

Your turn

Using your linear regression equation, calculate the A280 of a solution that has a protein concentration of 5 µg/mL. A280 = ________

Using your linear regression equation, calculate the protein concentration of a solution that has an A280 of 1.300. protein concentration = ________

Plot these 2 data points on your scatter diagram using a small "x" to plot each point so that you can visually distinguish these points from the data points that you plotted earlier. Now, plot the linear regression line by drawing a straight line through the 2 points that you just plotted. Make sure the line does not extend beyond the data range that was used to calculate the linear regression equation.

THOUGHT QUESTION: You are given an unknown solution and you are told to determine the protein concentration of the solution using a spectrophotometer and the linear regression equation that you calculated using the data from Table 2.1 on page 11. You place a sample of the solution in a spectrophotometer and get an A280 value of 1.891. Explain how you would determine the protein concentration of this solution:

____

____

____

____

____

4. Graphs and Linear Regression with Excel

As previously mentioned in Lab Exercise #1, three *Excel* quizzes have been posted on the Internet to help you learn the features of *Excel* that are required for this class. These Excel quizzes are located at the following address:

http://www.austincc.edu/biology/labmanuals/manualsindex.html

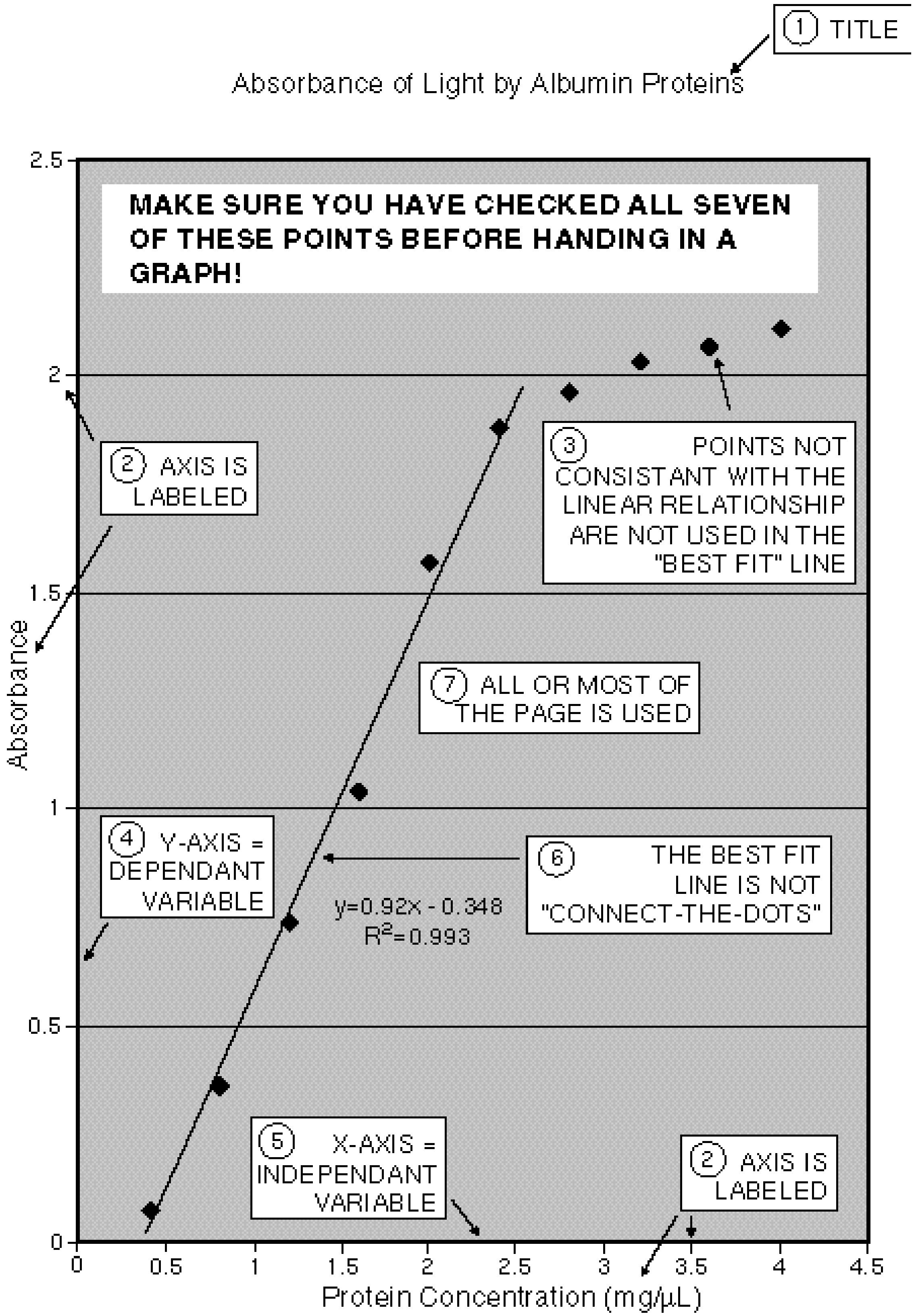
Introduction to Excel 3 explains how to plot graphs and carry out linear regression with *Excel.*

A description of how to plot graphs and carry out linear regression with *Excel* may also be found in Appendix E at the end of this manual.

Your turn

As part of this Prelab, you must complete **Introduction to Excel 3.** To complete the quiz**,** go to the Web address listed above, click on **Introduction to Excel 3**, and then follow the directions.

To complete the quiz you will need a computer that has a copy of *Excel* and an Internet connection. If you do not have a computer with *Excel* and an Internet connection, go to one of the ACC computer labs, or check with your instructor. After you have completed the quiz, save your spreadsheet and email a copy to your instructor. Check with your instructor about the deadline for submitting your quiz.


1 TITLE
Absorbance of Light by Albumin Proteins
MAKE SURE YOU HAVE CHECKED ALL SEVEN OF THESE POINTS BEFORE HANDING IN A GRAPH!
2 AXIS IS LABELED
3 POINTS NOT CONSISTANT WITH THE LINEAR RELATIONSHIP ARE NOT USED IN THE "BEST FIT" LINE
7 ALL OR MOST OF THE PAGE IS USED
4 Y-AXIS = DEPENDANT VARIABLE
6 THE BEST FIT LINE IS NOT "CONNECT-THE-DOTS"
y=0.92x - 0.348
R2=0.993
5 X-AXIS = INDEPENDANT VARIABLE
2 AXIS IS LABELED
Absorbance
Protein Concentration (mg/µL)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5

Summary of Data Analysis in BIOL 1406

Below is a list of procedures that you will frequently use to analyze data in BIOL 1406. In addition to being able to carry out each procedure, you should be able to explain what it is used for and what it tells you.

1. The following procedures can be carried out using EITHER a hand-held calculator with pen and graph paper OR a computer with spreadsheet program and printer. **You should know how to use both methods.** For each assignment during the semester, check with your instructor to see if he/she requires one particular method or the other.

    - Organize data in a **clearly labeled** table
    - Calculate the mean for a single list of numbers
    - Calculate the percent error of the mean for a single list of numbers
    - Calculate the standard deviation for a single list of numbers
    - Plot a **clearly labeled** scatter diagram for a paired list of numbers
    - Carry out linear regression for a paired list of numbers (You should be able to determine the linear correlation coefficient and the equation of the best-fit straight line for a paired list of numbers. You should also be able to use the equation to calculate the y-value if given any x-value within the linear region of the curve, and to calculate the x-value if given any y-value within the linear region of the curve.)
    - Plot the best-fit straight line (trend line) for a paired list of numbers on your scatter diagram

2. You should be able to perform the Student's T test using a computer with spreadsheet program and printer**.**

Lab Procedures

⇒ **CAUTION: Potassium permanganate has a health hazard rating of 2 and a reactivity rating of 3. You must wear approved safety eyewear and gloves during this entire lab, avoid breathing dust, and notify your instructor immediately if any spills occur.**

⇒ **CAUTION: Potassium permanganate is a hazardous waste. You must discard any potassium permanganate solutions in the special waste containers provided.**

⇒ **If your Spec-20 spectrophotometer has not already been turned on, now is a good time to turn it on so that it will be warmed up by the time you are ready to use it.**

I. Prepare a solution of a specific molarity using a solid solute

1. The formula weight of potassium permanganate ($KMnO_4$) is 158.04 g/mole. Calculate the weight of $KMnO_4$ needed to prepare 80 mL of a 0.01 M solution.

   Weight of $KMnO_4$ = _____________ (**Have your instructor check this calculation before proceeding.**)

2. Weigh out the calculated weight of $KMnO_4$ using a top-loading balance and a small weigh boat.

   NOTE: Free-flowing solids are removed from stock bottles by slowly pouring from the bottle or its lid into the weigh boat. Small quantities are best transferred by first pouring some of the solid into the lid of the stock bottle, then pouring from the lid into the weigh boat. Any excess can be poured from the lid back into the stock bottle. To avoid contamination, **never** place any objects into a stock bottle and **never** pour chemicals from the weigh boat or another container back into a stock bottle.

3. Pour approximately 40 mL of deionized water (dH_2O) into a clean 100 mL beaker, estimating the volume from the calibration marks on the side of the beaker. Place a stir bar into the beaker and set the beaker on a stir plate. Switch on the stir-plate and adjust the stirring rate so mixing is moderately fast but the solution does not splash.

4. Slowly pour the weighed $KMnO_4$ into the beaker and continue stirring until the $KMnO_4$ is completely dissolved. To transfer any $KMnO_4$left behind on the weigh boat into the beaker, use a plastic squeeze bottle of dH_2O.

5. Once the solute is completely dissolved, transfer the solution to a 100 mL graduated cylinder without transferring the magnetic stir bar: hold a large stir-bar against the outside of the beaker so that the stir-bar inside is held against one side. Slowly pour the solution into a 100 mL graduated cylinder while making sure the stir-bar does not fall out of the beaker.

6. Transfer 3 or 4 mL of dH_2O from a plastic squeeze bottle into the beaker, forcing the water all around the inside of the beaker to rinse any remaining solution to the bottom. Swirl the liquid for a few seconds, and then pour it into the graduated cylinder containing the $KMnO_4$ solution while preventing the stir bar from falling out of the beaker.

7. Rinse the beaker again with a few milliliters of dH_2O and pour the liquid into the graduated cylinder. Repeat, if necessary, in order to transfer all of the solute to the graduated cylinder, but be careful that the total volume of your solution does not exceed 80 mL.

8. Use the squeeze bottle or a Pasteur pipet to slowly add enough dH_2O to the graduated cylinder in order to bring the total volume of solution to 80 mL.

9. Stretch a piece of Parafilm® securely over the top of the graduated cylinder and mix the solution by tipping the graduated cylinder ***slowly*** upside down several times while holding the Parafilm® firmly in place.

10. Attach a piece of marking tape to the graduated cylinder. With a permanent marking pen, write the concentration and composition of the solution on the tape (i.e. 0.01 M $KMnO_4$).

II. Prepare a solution of a specific molarity using a previously prepared stock solution

1. Now, calculate the volume of 0.01 M $KMnO_4$ solution that should be diluted in order to make 25 mL of a 2 mM $KMnO_4$ solution.

   Volume of 0.01 M $KMnO_4$ solution needed = ________________ (**Have your instructor check this calculation before proceeding.**)

2. Using an appropriate measuring device, transfer the required solution into a 25 mL graduated cylinder.

3. Add enough dH_2O to the graduated cylinder to bring the total volume to 25 mL. Stretch a piece of Parafilm® over the top of the graduated cylinder to make a tight seal and then mix the contents of the cylinder.

4. Attach a piece of marking tape to the graduated cylinder. With a permanent marking pen, write the concentration and composition of the solution on the tape (i.e. 2 mM $KMnO_4$).

III. Prepare several solutions using the parallel dilution technique

1. Calculate the amount of 0.01M $KMnO_4$ solution and the amount of dH_2O needed to make 10 mL each of the following solutions:

	Solution	**Volume of 0.01 M $KMnO_4$ Solution Needed**	**Volume of dH_2O needed**
1	1.0 mM $KMnO_4$		
2	0.6 mM $KMnO_4$		
3	0.4 mM $KMnO_4$		
4	0.2 mM $KMnO_4$		
5	100 µM $KMnO_4$		
6	50 µM $KMnO_4$		
7	20 µM $KMnO_4$		

➔ **Have your instructor check your calculations before proceeding.**

2. Use tape to label seven 20 mL test tubes with the final concentrations of the solutions being prepared, and then place the tubes in a test tube rack.

3. To make your first solution, use an appropriate measuring device to transfer the required amount of 0.01M $KMnO_4$ solution into a 10 mL graduated cylinder. Make sure you use the 0.01M $KMnO_4$ solution in the 100 mL graduated cylinder not the 2 mM $KMnO_4$ solution in the 25 mL graduated cylinder.

4. Add enough dH_2O to the graduated cylinder to bring the total volume to 10 mL.

5. Stretch a piece of Parafilm® over the top of the graduated cylinder to make a tight seal and then mix the contents of the cylinder.

6. Pour the contents of the cylinder into the tube labeled with the $KMnO_4$ concentration that you just prepared.

7. Clean your 10 mL graduated cylinder with dH_2O and prepare the rest of the dilutions in the same way.

8. Place the tubes with the 7 parallel dilutions into a test tube rack in order of decreasing concentration and label the rack "parallel dilutions."

9. Look at the 7 test tubes. Can you tell if your dilutions were made correctly? Well, there should be the same volume of liquid in each tube, and the color should get lighter as the solutions become more dilute, but other than that there is no way to tell if your dilutions were made correctly simply by looking at them. Therefore, it is extremely important that all calculations and procedures for preparing your dilutions are done carefully and accurately. **Any mistakes will be difficult to detect, and incorrectly prepared dilutions can invalidate the results of many long hours of lab work!**

IV. Prepare several solutions using the serial dilution technique

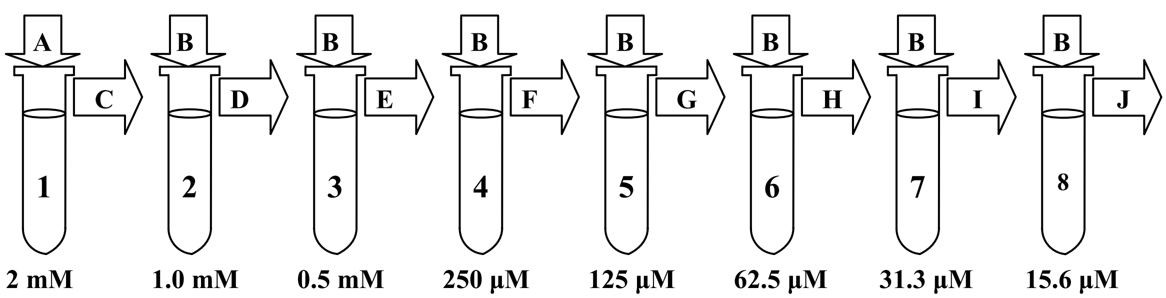
Starting with your solution of 2 mM $KMnO_4$, you will make 6 mL of each of the following concentrations of $KMnO_4$: 1.0 mM, 0.5 mM, 250 µM, 125 µM, 62.5 µM , 31.3 µM , and 15.6 µM.

What is df for this dilution series? ______________

What is V_2 for this dilution series? ______________

What is V_1 for this dilution series? ______________

Label the arrows in the diagram below to show how you would make the required solutions. Above each vertical arrow, write down the volume and type of liquid you will place in the tube (e.g. 13 mL of stock solution or 9 mL of dH_2O.) Below each horizontal arrow, write down how much solution you will transfer from one tube to the next (e.g. 3 mL):



➔ **Have your instructor check your calculations and diagram before you proceed.**

Now, use tape to label eight 20 mL test tubes–the first tube with the molarity of your stock solution and the remaining tubes with the final concentrations of the dilutions being prepared. Place the tubes in a test tube rack.

Performing your serial dilutions:

⇒ **NOTE: The letters below correspond to the letters on the arrows in the diagram above.**

A. Using an appropriate measuring device, place the correct amount of 2 mM $KMnO_4$ ($V_1 + V_2$) into tube 1.

IMPORTANT: Make sure you use the 2 mM $KMnO_4$ from your 25 mL graduated cylinder and not the 0.01M $KMnO_4$ from your 100 mL graduated cylinder.

B. Using an appropriate measuring device, place the correct amount of solvent (V_2) into the remaining tubes.

IMPORTANT: When making a dilution series, make sure you use a clean measuring device each time you transfer a solution with a different solute concentration.

C. Transfer the correct amount of solution (V_1) from tube 1 to tube 2 and then thoroughly mix the contents of tube 2 with a Vortex mixer.

D. Transfer the correct amount of solution (V_1) from tube 2 to tube 3 and then thoroughly mix the contents of tube 3 with a Vortex mixer.

E. Transfer the correct amount of solution (V_1) from tube 3 to tube 4 and then thoroughly mix the contents of tube 4 with a Vortex mixer.

F. Transfer the correct amount of solution (V_1) from tube 4 to tube 5 and then thoroughly mix the contents of tube 5 with a Vortex mixer.

G. Transfer the correct amount of solution (V_1) from tube 5 to tube 6 and then thoroughly mix the contents of tube 6 with a Vortex mixer.

H. Transfer the correct amount of solution (V_1) from tube 6 to tube 7 and then thoroughly mix the contents of tube 7 with a Vortex mixer.

I. Transfer the correct amount of solution (V_1) from tube 7 to tube 8 and then thoroughly mix the contents of tube 8 with a Vortex mixer.

J. Remove the correct amount of solution (V_1) from tube 8 and discard it.

Finally, place the 8 tubes with your serial dilutions into a separate test tube rack in order of decreasing concentration and label the rack "serial dilutions."

V. Spectrophotometer: Absorption spectrum for $KMnO_4$

Make sure that your Spec-20 spectrophotometer has been turned on for at least 5 minutes to warm up.

In this section, you will be determining the **absorption spectrum** for a $KMnO_4$ solution. An absorption spectrum shows you how much light is absorbed by the solution at various wavelengths. In this experiment, you will measure the absorbance of light by the $KMnO_4$ solution at wavelengths ranging from 480nm to 580nm. The wavelength which gives you the highest absorbance is called the **wavelength maximum** for the solution.

1. Label a Spec-20 cuvette "B" with a small piece of label tape placed near the top of the cuvette. (Never write on Spec-20 cuvettes!) Fill the cuvette with dH_2O. This will be your zero standard or **blank**.

2. **Appendix D** at the back of this manual contains instructions on how to use the Spec-20 spectrophotometers. Remove Appendix D from your manual and keep it handy for reference while you are using the machine.

3. Determine whether you will be using an **ANALOG** Spec-20 (has a meter with a needle that moves across a printed scale) or a **DIGITAL** Spec-20 (has an LED readout). Read the section of Appendix D that corresponds to the type of the Spec-20 you will be using. Regardless of the type of Spec-20 used, you must perform 3 steps every time you change the wavelength of light used, in this order:

   a) adjust to the correct wavelength (using the knob on top of the Spec-20)

   b) BEFORE placing any cuvette in the instrument, adjust the **% transmittance** to zero (using the **left** knob on the front of the Spec-20)

   c) AFTER placing the blank cuvette (labeled "B") in the instrument, adjust the **absorbance** to zero (using the **right** knob on the front of the Spec-20)

4. Set the wavelength of your Spec-20 to 480 nm and adjust the filter if necessary. Now, calibrate the Spec-20 using the instructions in Appendix D or the instructions that are printed on the instrument. Remember, the Spec-20 must be calibrated at 2 points every time you set a new wavelength: the **% transmittance** must be set to zero when the holder is empty, and the **absorbance** must be set to zero when the blank is in the holder. **Ask your instructor to check your calibration of the Spec-20 before you take any measurements.**

5. Select one of your parallel dilutions to use for determining the absorption spectrum of $KMnO_4$. Choose a sample that has a distinct color, but that is light enough that you can see through it. **Ask your instructor to check your selection.**

6. Draw a data table in your lab notebook to record the absorbance, by the selected solution, of light at the following wavelengths: 480 nm, 500 nm, 520 nm, 540 nm, 560 nm, and 580 nm.

7. Pour the selected solution into a clean, dry cuvette and place the cuvette into the Spec-20 holder.

   IMPORTANT: Before you place a cuvette into the Spec-20 holder, always wipe the outside of the cuvette with a Kimwipe to remove any fingerprints or dust. Also, make sure the line on the cuvette is lined up with the line on the holder.

8. Read the absorbance at 480nm and enter the result into the data table in your lab notebook.

   IMPORTANT: Make sure you measure absorbance and not % transmittance.

9. Remove the cuvette from the Spec-20. Change the wavelength to 500nm and re-calibrate the Spec-20. Remember, you must re-calibrated the Spec-20 at 2 points every time you set a new wavelength: the **% transmittance** must be set to zero when the holder is empty, and the **absorbance** must be set to zero when the blank is in the holder.

10. Place the same solution that you used to measure absorbance at 480 nm back into the Spec-20. Read the absorbance at 500 nm and enter the result into the data table in your lab notebook.

11. Continue in this way until you have measured the absorbance of this same solution at all of the wavelengths listed in the data table of your lab notebook.

12. When you have completed your table for all measurements between 480 nm and 580 nm, examine your data. What is the wavelength maximum for your $KMnO_4$ solution? __________________

13. Set your Spec-20 to the wavelength maximum for $KMnO_4$. In the next section, you will use this wavelength to measure the absorbance of **all** your diluted $KMnO_4$ solutions.

VI. Spectrophotometer: Effects of concentration on absorbance

In this part of the lab, you will use the wavelength maximum determined in part V to measure the absorbance of all of the parallel and serial dilutions of $KMnO_4$ that you prepared in parts III and IV. Because you will be using a single wavelength, you do not have to re-calibrate the Spec-20 between each measurement. However, you should check the calibration every 15-20 minutes to make sure your Spec-20 hasn't "drifted."

1. Re-calibrate the Spec-20 with the wavelength set at the wavelength maximum that you determined for $KMnO_4$. (If you did not do part V of this lab, set the spectrophotometer at 540 nm to measure absorbance in this section.)

2. Measure the absorbance at the wavelength maximum for each solution that you made using the parallel dilution method. Record the absorbance of each sample in your lab notebook using a clearly labeled data table.

3. Measure the absorbance at the wavelength maximum for each solution that you made using the serial dilution method. Record the absorbance of each sample in your lab notebook using a separate data table.

4. Before you discard of your solutions, ask your instructor to check your absorbance measurements for plausibility.

VII. Determine the concentration of an unknown $KMnO_4$ solution

1. You will be given a solution with a concentration of $KMnO_4$ that is unknown to you. Measure the absorbance of this solution using the wavelength maximum for $KMnO_4$ that you determined in part V. Record the results in your lab notebook.

Clean up

Discard the $KMnO_4$ solutions: Make sure you discard all $KMnO_4$ solutions in the waste containers provided; do not dump them into the sink.

Clean the Spec-20 cuvettes: Spec-20 cuvettes are not ordinary test tubes. They are expensive, and great care must be taken to avoid scratching them. Scratches interfere with the passage of light through the tube and that can lead to inaccurate results. Rinse them only---do not use test tube brushes on cuvettes. Rinse the cuvettes thoroughly with tap water and then with dH_2O. Ask your instructor where they should be left to dry.

➔ ***Never use brushes or abrasives on Spec-20 cuvettes!***

Wash all regular glassware:

1. Pour contents into an approved container (Ask your instructor if you are unsure of how to discard a particular chemical.)
2. Rinse with tap water.
3. Scrub with soapy water and a test tube brush.
4. Rinse 3 times with tap water.
5. Rinse 3 times with distilled water.
6. Store inverted over absorbent towels until dry.

Wipe off your work space with a damp paper towel. You have been working with a purple dye. Don't be responsible for dyeing students purple in the next class! Or their belongings!

All instruments should be turned off and unplugged. Make sure everything that you have used is clean, put away, or discarded. Ask your instructor to check your work area before you leave.

Postlab

1. Use your data from Part V of the Lab Procedures to plot a scatter diagram showing an absorption spectrum for potassium permanganate. Do not attempt to draw a best-fit straight line on your graph because the relationship between wavelength and absorbance is not linear. Instead, draw a smooth curve between your data points to reveal a peak in absorbance at the wavelength of light that the potassium permanganate absorbs best. **Use the "Graphing Check List" on p. 18 of the Prelab to make sure you have included all necessary information on your graph**. Also, write the wavelength maximum for $KMnO_4$ on your graph.

2. Explain how you could have obtained a more accurate estimate of the wavelength maximum for $KMnO_4$.

3. Plot a scatter diagram of concentration vs. absorbance using the data from Part VI of the Lab Procedures. Plot the data from your parallel and serial dilutions on the same graph, but use different symbols (e.g. dots, small circles, or x's) to represent each series. **Use the "Graphing Check List" on p. 18 of the Prelab to make sure you have included all necessary information on your graph.**

4. Concentration and absorbance are linearly related, so all of your data points should appear to fall on a straight line, at least for low and moderate concentrations of $KMnO_4$.

   a) Do the data points from both dilution series that you prepared (parallel and serial) seem to fit a straight line?

   b) Do they seem to fit the same straight line?

   c) Do the data points from one series (either parallel or serial) seem to fit a straight line better than the other? If so, what does that tell you about the relative precision of the two dilution techniques?

   d) If a group of students obtained data points that did not fall close to a straight line, what can you conclude about the solutions they prepared?

5. Plot a second scatter diagram of concentration vs. absorbance using the data from both parallel and serial dilutions, but this time do not include points from the high end of the concentration range if the linear relationship does not seem to hold in this region (i.e. the slope of the line seems to significantly increase or decrease at relatively high concentrations of $KMnO_4$.) Use the "Graphing Check List" on p.18 of the Prelab to make sure you have included all necessary information on your graph. Calculate the correlation coefficient and linear regression equation for your data, and write these on your graph. Also, plot the best fit straight line for your data points.

6. Use the linear regression equation for your data to determine the $KMnO_4$ concentration of your unknown solution

7. The F.W. of NaCl is 58.4g. Write a short paragraph describing ***exactly*** how you would prepare 900 mL of a 0.5 M solution of this salt. Describe your actions–exactly what you would ***do*** –step-by-step–when preparing this solution. Also show all calculations. Be sure all amounts include units of measurement.

8. Describe how you would prepare 30 mL of a 0.5M NaCl solution from a 2M NaCl stock solution. Describe your actions–exactly what you would ***do*** –step-by-step–when preparing this solution. Also show all calculations. Be sure all amounts include units of measurement.

9. Describe how you would prepare 90 mL of a 5 mM NaCl solution from a 2 M NaCl stock solution. Describe your actions–exactly what you would ***do*** –step-by-step–when preparing this solution. Also show all calculations. Be sure all amounts include units of measurement.

10. Describe how you would prepare 10 mL each of 2 M, 1.5 M, and 1 M NaCl solutions from a 2 M stock NaCl solution. Describe your actions–exactly what you would ***do*** –step-by-step–when preparing this solution. Also show all calculations. Be sure all amounts include units of measurement.

11. Scientists sometimes measure concentration using percent rather than molarity. A 1% solution of NaCl means that 1% of the solution is NaCl. Therefore, if we want 500 mL of a 1% NaCl solution, 1% of the total weight or 5 g should be NaCl. (Remember that 1 mL of water weighs 1 g. Therefore, 500 mL of an NaCl solution–which is mostly water–weighs about 500 g.) To prepare the solution, we would weigh out 5 g of NaCl and add enough water to produce a final volume of 500 mL.

    a) Explain how you would prepare 900 mL of a solution containing 0.02% thimerasol. Show your calculations.

12. A student wishes to prepare 300 mL of 0.25 M sucrose. He puts 25.7 g of sucrose in a beaker and then adds 300 mL of dH2O. The total volume of the solution after the sucrose has been dissolved is 330 mL.

    a) What did he do wrong when preparing his solution?

    b) What is the actual concentration of the sucrose solution he prepared?

    c) What is the percentage error in the concentration of sucrose from the desired 0.25 M?

References:

ASM Style Manual for Journals and Books, American Society for Microbiology, Washington DC, 1991

Seidman, L.A. & C. Moore, *Basic Laboratory Methods for Biotechnology*, Prentice Hall, 2000