

SOLUTIONS & DILUTIONS

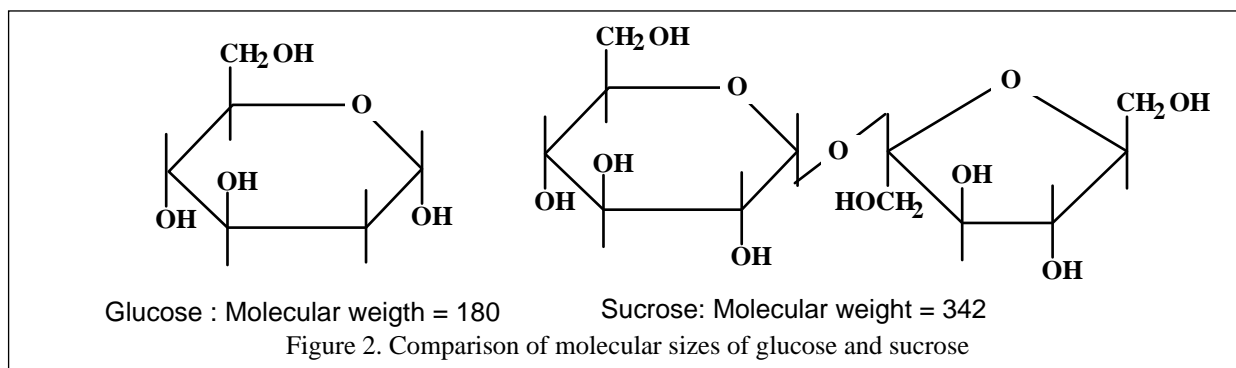
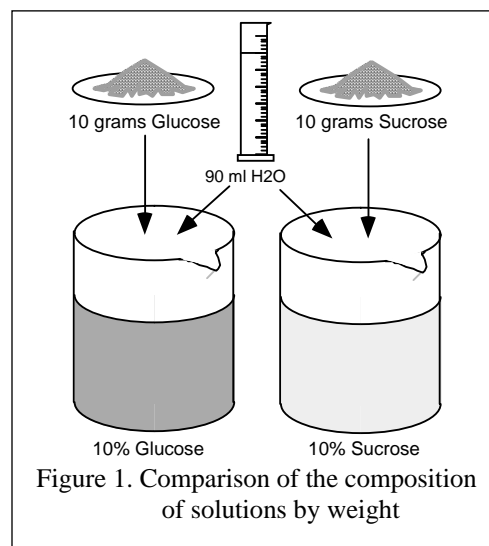
INTRODUCTION

The chemistry of life depends upon specific molecules interacting with each other. Successful interaction requires that the molecules be dissolved in water. One or more molecular species dissolved in water constitutes as solution. The molecules dissolved in water are called solutes, while the water is referred to as solvent. At this point you should review the discussion of solutions in your text book.

A key property of solutions is the concentration of solute. To a nonscientist, concentration is most often thought of in terms of percent. A “10% glucose solution” is something most of us can easily relate to and visualize (for every 100 parts of solution, 10 parts are glucose). The “parts” in a percent solutions usually refer to weight. Thus, the 10% glucose solution would consist of 10 grams of glucose for every 100 grams of solution (10 g glucose in 90 g H₂O).

Noting the concentration of solutions in percent by weight can lead to problems because it doesn't give any information about the concentration of molecules. For example, in comparing a 10% solution of glucose and a 10% solution of sucrose (Fig. 1), you see that each has the same weight of sugar (10g per 90g H₂O), but because a glucose molecule is about half the size of a sucrose molecule (Fig. 2), the glucose solution has about twice the concentration of dissolved solute molecules. The weight of sugar in each solution is the same, but there are twice as many sugar molecules in the glucose solution.

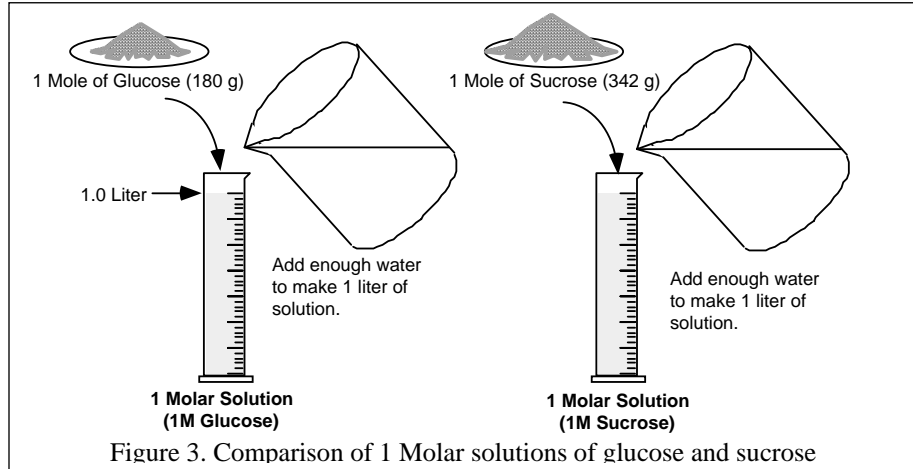
To get around this problem, solutions are often described by their solute concentration using the



Molar designation. A Mole of a substance is equal to its molecular weight in grams. Thus for any type of substance, the number of molecules in a Mole is always the same; namely 6.022×10^{23} (this is an extremely large number).

Molar solutions are simply the number of moles of solute in one liter of solution.

Figure 3 compares how 1 Molar glucose and sucrose solutions would be made. The concentration of solute molecules in both solutions is the same: 6.022×10^{23} molecules per liter of solution.

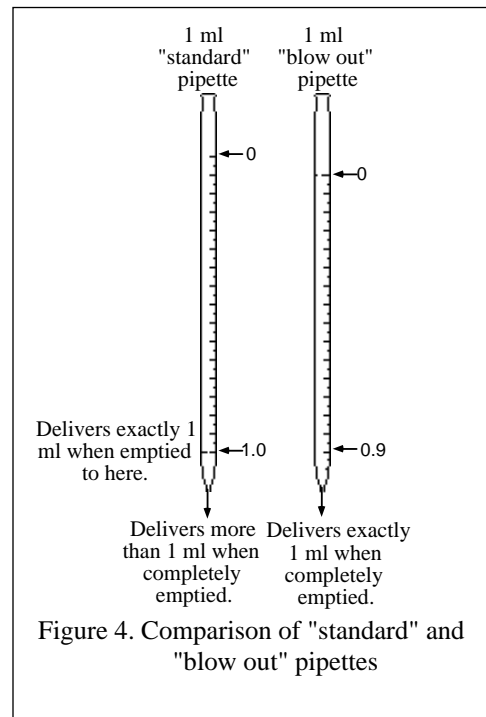


Since a great deal of experimental lab work in biology involves solutions, the ability to work with and manipulate them is very important. In this lab you will investigate several techniques for working with solutions.

In biology lab work, concentrations are often given in micro-moles or μM . A μM is one millionth of a mole per liter, or 1×10^{-6} M.

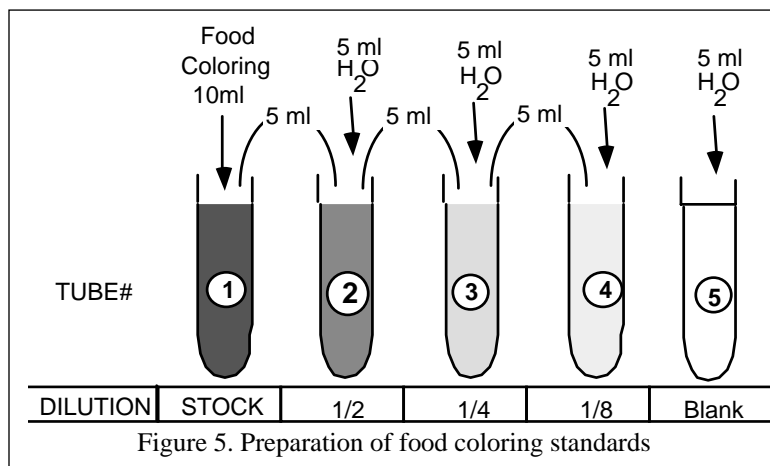
USING PIPETTES TO MEASURE SMALL VOLUMES

1. For most work in the biology laboratory, it is necessary to prepare smaller volumes of dilutions. In next series you are going to make a dilution series using and ending up with relatively small volumes.
2. There are two kinds of pipettes commonly used in laboratories (Fig. 4). The "blow out" type delivers the desired amount when completely emptied. The other type delivers the desired amount when the liquid level reaches the last calibration mark. This type is more accurate but the "blow out" type is more convenient because once it is filled to a known level, it can then be emptied completely for an accurate measurement. You will use both types at one time or another in later exercises, so always check the type you are using.



USING STANDARDS TO ESTIMATE CONCENTRATION OF UNKNOWN

1. Obtain five test tubes and a 10 ml pipette. Label the tubes #1-#5 and place them in order in a rack over white paper towels.
2. Obtain a container of the stock food coloring.
3. Prepare the dilution series stock solution of food coloring as outlined in Figure 5. Note that this is a 1/2 dilution series rather than the 1/10 that you did before. Also note that you end up with 5 ml of solution in each tube.



4. Unknowns #1 and #2 are food coloring solutions of unknown concentration. You are going to use your series of known food coloring concentrations to estimate the concentration of the unknowns. A series of known concentrations used for such comparisons is called a standard series.
5. Place 5 ml each of Unknowns #1 and #2 in test tubes, and one at a time, compare them to your standard series.
6. You should be able to see which of the standards most closely matches the color of the unknown. If the color exactly matches one of the standards, then the concentration of the unknown is that of the standard. If the color falls between two standards, then you can describe the unknown's concentration as greater than one but less than the other. You can even note that it is between them, but closer to one than the other.
7. The important point to realize here, is that you must interpolate if your unknown does not exactly match one of the standards. This is done with your eye judging color differences.
8. Use your standards to estimate the concentrations of your unknowns and record in Table 1.

Solution	"Eyeballed" Concentration
Unknown #1	
Unknown #2	

USING THE SPECTROPHOTOMETER TO ESTIMATE CONCENTRATION

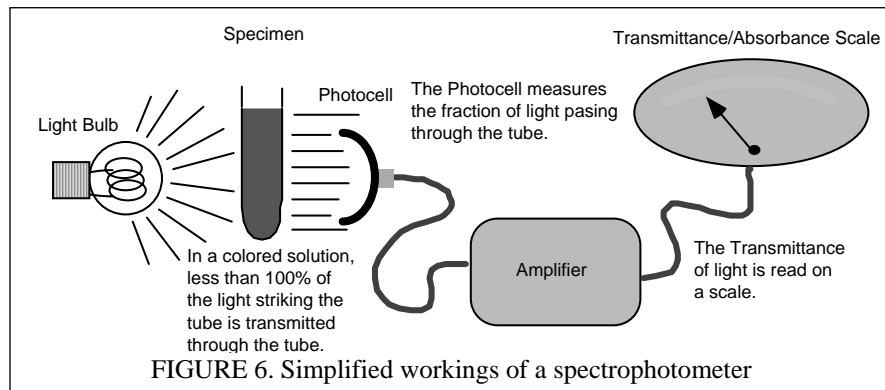
The Spectrophotometer is an instrument that can accurately measure what our eyes were judging in step 7 above. It measures how much color there is in a solution by analyzing how much light passes through the solution.

Examine Figure 6. If the specimen in the test tube is perfectly clear, then none of the light striking it is absorbed and 100% is transmitted through it, and 100% of that light strikes the

photocell. Test tubes used in Spectrophotometers are made of high quality glass or plastic and are called cuvettes.

On the other hand, if the solution is colored, some of the light will be absorbed, and only a certain percentage will be transmitted to strike the photocell. The amount of light transmitted depends on the concentration of colored solute. The spectrophotometer will accurately measure the percent transmittance of light through a specimen.

In this section you will use the SPECTRONIC 20 GENESYS spectrophotometer (Spec 20) to measure the % transmittance of each standard solution and the unknowns.



1. Turn on the spectrophotometer at your station. Allow a 10 minute warm-up.
2. Set the wavelength to 630 nm.
3. Select "Absorbance" mode using A/T/C key (A is for absorbance and T is for transmittance).
4. Pour the contents of tube #5 into a cuvette and insert the cuvette into the specimen well. Press the "0 ABS/100%T" key. This sets the Spec 20 to 100% Transmittance (0 Absorbance) for the blank.
5. Pour the contents of tube #4 into a different cuvette and insert it into the Spectrophotometer. This cuvette will be referred to as the "experimental cuvette" from now on. Observe the Absorbance and record the reading in Table 2.

6. Empty the contents of the experimental cuvette back into tube #4. Shake out any remaining liquid and pour the contents of tube #3 into the experimental cuvette. Rinsing here is not necessary since you are going from a weaker to stronger concentration, but be sure to empty the tube completely before adding the new solution.

Tube #	Absorbance	Concentration
5		0 (blank)
4		
3		
2		
1 (stock)		
Unknown #1		
Unknown #2		

7. Measure the Absorbance of the experimental cuvette (tube #3 contents) and record in Table 2.
8. Continue the process until you have measured the Absorbance of tubes #1 through #4, and recorded the data in Table 2.
9. Measure the Absorbance of the two unknowns and record them in Table 2. You will need to rinse the cuvettes in between measurements here.

ANALYSIS OF SPECTROPHOTOMETER DATA

Once you have measured the Absorbance of a group of standards, you can make a "Standard Curve" to more accurately estimate the concentration of the unknown solutions. A standard curve is a plot of the concentration of each standard against its spectrophotometer reading.

As you work through this section, you will learn how to use Excel to make a basic spreadsheet and graph of your standard curve data.

ENTER YOUR DATA IN A SPREADSHEET

1. Open the *Excel* program to bring up a blank spreadsheet.
2. A spreadsheet consists of data cells arranged in rows and columns. A single spreadsheet file in *Excel* is called a "Workbook." Each Workbook can have one or more "Sheets." In a Sheet, rows are identified with numbers and columns with letters. This means each data cell can be identified by its row and column, e.g. A1, B4, D2, etc.
3. When you open a new Workbook in *Excel*, it opens with one Sheet. Sheets can easily be added or deleted from the workbook. More on this later.
4. When you click on a data cell, it is selected and is outlined. Note how the cell number is shown in the upper left part of the window and the cell's contents are shown in the "Formula Bar" to the right.
5. Click on cell A1 and type in "Tube #." Note how the letters appear in both cell A1 and in the formula bar above as you type. You can edit what you've typed by clicking the cursor on the area of text in the formula bar you want to change.
6. Click on cell B1 and type in "Abs." Continue entering the other column headings.
7. Enter the tube # in descending order in column A.
8. Enter the absorbance data from Table 1 in each tube in column B.
9. Enter your known concentration of food coloring into column C of the spreadsheet.
10. Leave out your unknown data for now.

MAKE THE GRAPH SHOWING THE RELATIONSHIP BETWEEN CONCENTRATION AND ABSORBANCE

1. The next step is to have Excel draw a graph that expresses the relationship between concentration and optical transmittance in your data. Unfortunately there is a small conflict in terminology between Excel and most of the scientific community. A graphic representation of data is referred to as a "graph" by most scientists, whereas Excel refers to the same as a "chart." So keep in mind when this manual instructs you to make a graph of a data set, you will be using the "Chart Tools" in Excel.
2. Click on cell B2 and drag to cell C6 to "select" the range of data cells you want to graph. The selected cells will appear blue.
3. With the data selected, click on the "Chart Wizard" button on the toolbar at the top of the *Excel* window (Fig. 9).
4. This brings up the "Chart Wizard Window. It is presently showing the first of 4 screens that you will work through to select the type and appearance of your graph.

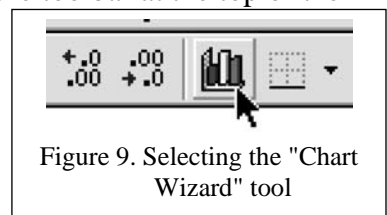
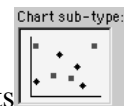


Figure 9. Selecting the "Chart Wizard" tool

5. Click on the "XY (Scatter)" from the "Chart type" menu on the left.



6. Now click on the top "Chart sub-type" that has no lines between points

7. Click on the "Next" button at the bottom of the window to move on to the next screen (Step 2).

8. Step 2 gives you a miniature view of your graph and options of the data range you selected. Make sure that "Columns" is selected in the "Series in:" option.

9. Click on the "Next" button at the bottom of the window to move on to Step 3.

10. This screen also gives you a miniature view of the graph, and has lots of options on it. You can see the different options ("cards") by clicking on the tabs at the top of the screen.

11. Enter your chart title and the labels for the X axis is the horizontal one. *Excel* calls the X axis the "Category axis." Enter "Food Coloring Standard Curve" for the title, "Absorbance (Abs. Units)" for the X axis, and "Concentration (% or M)" for the Y axis.

12. Since you have only one data set, your graph does not need a legend. Click on the "Legend" tab to bring up the card and click in the "Show legend" box so that the check mark disappears.


13. Click on the "Next" button at the bottom of the window to move on to Step 4.

14. Step 4 give you the option of having your graph appear as a new sheet in the workbook, or as an object on your existing sheet. For now, choose "As new sheet" option. Now click on the "Finish" button and your graph should appear as a new sheet in your workbook.

REMOVE THE GRAY BACKGROUND FROM YOUR GRAPH

You will notice that *Excel* draws the graph with a dark gray background. This looks good, especially on the computer screen, but uses a tremendous amount of laser printer toner when the graph is printed, which in turn makes a big difference in the life span of our toner cartridges here in the lab. For this reason, you should always remember to remove the gray background from your graphs before printing them.

1. Click anywhere on the graph to select the "plot area." Make sure you don't click on the gridlines, axes, or data series, since you can't change the background from these. When selected, "Plot Area" should appear in the name box in upper left corner of the *Excel* screen.

2. With the Plot Area selected, you can change the background color in two ways. The shortcut way is to click on the "Fill Color" button () on the toolbar and select the "No Fill" option from the palette.

3. The second way is (with the Plot Area selected) to select the "Selected Plot Area..." from the "Format" pull down menu, and select "None" from the fill options.


4. Make sure you remove the gray background from any graph you print in our lab.

PLOT THE REGRESSION LINE (TRENDLINE) FOR YOUR DATA POINTS

1. Since you are going to use this graph to estimate the concentration of unknown solutions, there are several changes you need to make to make it more accurate.

2. First, chances are your data points don't form a perfect straight line. *Excel* can plot the best straight line for your data
3. Click anywhere on the chart to select it.
4. Select "Add Trendline" from the "Chart" pull down menu. Click on "Linear" to choose a linear trendline. Choose "options" and click on "display equation on chart." You will use this equation to calculate the concentration of your unknowns.
5. Click on the "Finish" button. Now your graph should have the points plotted with the best straight line shown.
6. The present graph only shows "major" gridlines for the Y-axis. Since you are going to use this graph to determine the concentration of unknown solutions, you should add major and minor gridlines to both axes.
7. Gridlines may be added in step 3 of the Chart Wizard steps.
8. Click anywhere on the chart and click on the "Chart Wizard" tool. Go to step 3. Select major and minor gridlines in this graph
9. Because of the linear relationship, the relationship between Concentration and Absorbance may easily be calculated. This means the Absorbance of an unknown solution may be applied to the graph to give an accurate estimate of its concentration. A graph used in the manner is called a "Standard Curve."
10. Take the absorbance readings of your unknowns from Table 1 and calculate corresponding concentration using the equation. You should be able to solve for "y" by plugging in "x". Verify that the numbers you have generated are approximately right. You can do this by locating your absorbance readings on your standard curve. Find the absorbance data on the X axis and using a straight edge, visually look vertically up until you hit your trendline. Now turn your straight edge so that it tracks across to your Y axis and the number there should be fairly close to what you calculated.

MAKE PRINTOUTS OF YOUR GRAPH

1. The size and shape of your graph on the computer screen does not control how the graph will look when printed. To see how the graph will look in the printout, select "Print Preview" from the "File" pull down menu, or click on the  button in the toolbar.
2. This will bring up the preview window and show you exactly how your printout will look on the printed page.
3. You can change the orientation of the graph by clicking the "Setup" button at the top of the preview window.
4. This brings up the "Page Setup" dialog box. You can also access the "Page Setup" dialog box from the "File" pull down menu.
5. From this window you can change the page orientation, margins, header & footers, and whether your graph uses the whole page, or remains the same scale as shown on your screen.
6. Try several different options, e.g. "Portrait" orientation, and see how they look when you click on the "OK" button.
7. When your graph appears the way you want it to in the "Preview" window, click on the "Print" button at the top of the preview window.

8. This will bring up the “Printer” dialog box. You shouldn’t have to make any changes in this box. The number of “Copies” is preset to 1. Click on the “Print” button.
9. This will send the image to the printer and provide you with a printout of the graph. Make printouts of both graphs and your data spreadsheet. Be sure to save this file before moving on.

MAKING DILUTIONS THAT ARE NOT PART OF A SERIES

It is often necessary to make a single dilution that is not part of a series. These dilutions are easily calculated using the relationship: $C_1 \times V_1 = C_2 \times V_2$

For example, suppose you want to make 15 ml of 1.0 M sucrose solution from a stock solution on 1.5 M sucrose (you will be doing this in next week’s lab):

1. Call the stock solution “Solution 1” and the dilution you are going to make “Solution 2.”
2. To make Solution 2, you have to use some of Solution 1 and dilute it with water.
3. You don't know how much Solution 1 or how much water to use.
4. You do know that the concentration of Solution 1 is 1.5 M and that Solution 2 is to be 15 ml at 1.0 M concentration.
5. The relationship between Solution 2 and the amount of Solution 1 used to make it is:
 $C_1 \times V_1 = C_2 \times V_2$, where **C** is concentration and **V** is volume.
6. Plugging in the known values, you get: **$1.5M \times V_1 = 1.0M \times 15ml$**
7. Note that V_1 is the only unknown. Solving for it you get: **$V_1 = 10ml$**
8. Now you know that 10ml of Solution 1 are used to make 15ml of Solution 2. This means that **5ml (15ml-10ml)** of water are used in the dilution.

QUESTIONS FOR YOUR REPORT

1. Present an abstract of your work. Your abstract should include a brief description (1/2 page) of 1) what you set out to learn, 2) what procedures you performed, and 3) a summary of your data or discoveries.
2. Present your spreadsheet and standard curves of the spectrophotometer data. Include tables 1 and 2.
3. What is a spectrophotometer? Explain how it is used in estimating concentration. Explain how you calculated the concentration of the unknowns and how the values compare to your “eyeballed” values.

You may neatly write out the solutions to the questions below:

4. If current population growth continues, the world population will reach 10 billion (10,000,000,000) in about 40 years (it is presently 6 billion). Roughly, how many times larger or smaller is the number of glucose molecules in 1 liter of a 1 molar solution, compared to the world population in 40 years? Show how you arrived at your answer.
5. The molecular weight of lactic acid ($C_3H_4O_3$) is 88. How many grams would be in 2 liters of a 2 M solution? How many molecules of lactic acid?
6. In terms of weight, compare the amount of solute in a 1 M glucose solution and a 1 M sucrose solution. Now compare them in terms of the number of molecules.
7. How much of each, stock and water, would be necessary to directly make 10 ml of a 1/100 dilution?
8. If you were to directly make 20 ml of a 0.02% solution using a 2% stock, how much of each, stock and water would you use?
9. Given a stock of sugar that is 10%, how would you make 100 ml of 3% sugar solution?
10. Suppose you have a 3.5M glucose solution. What is the concentration in $\mu\text{Mol/ml}$? Show how you arrive at this answer.