Lab 3: Making Solutions

Making solutions is a very common activity for lab workers in a biotechnology lab. Proper solution making requires basic math skills, accurate measurement, and the ability to follow instructions.

A **solution** is a homogeneous mixture of two or more substances. In a solution, the **solute** is the substance that is dissolved in the **solvent**. Most of the time, the solvent will be H_2O, so if it is not otherwise specified, assume that you should dissolve the necessary amount of solute calculated in H_2O.

Activity 3a
Making Solutions of Differing Mass/Volume Concentrations

Purpose and Background

In this activity, you will learn how to calculate and make copper sulfate ($CuSO_4$) solutions of differing mass/volume concentrations.

Solutions are prepared with a certain mass of solute in a certain volume of solvent, similar to the glucose solutions made in Activity 2c. Any metric mass in any metric volume is possible, but the most common units of mass/volume concentrations are as follows:

g/mL	grams per milliliter
g/L	grams per liter
mg/mL	milligrams per milliliter
µg/mL	micrograms per milliliter
µg/µL	micrograms per microliter
ng/mL	nanograms per milliliter
ng/µL	nanograms per microliter

Although concentrations can be reported in any mass/volume units, these 7 mass/volume units are the most common in biotechnology applications. The prefix "nano-" means one-billionth. A nanogram is equal to 0.001 µg, and there are 1000 ng in 1 µg.

To determine how to prepare a certain volume of a solution at a certain mass volume concentration, use the equation that follows.

Mass/Volume Concentration Equation:

(Volume desired) x (Concentration desired) = (mass of solute needed)
(for ex: mL) (for ex: g/mL) (for ex: g)

Note:
- the units in the numerator & denominator should cancel if the equation is set up properly.
- you must make sure that the units used in the calculation can be cancelled, and you may need to convert units within the metric system if necessary.

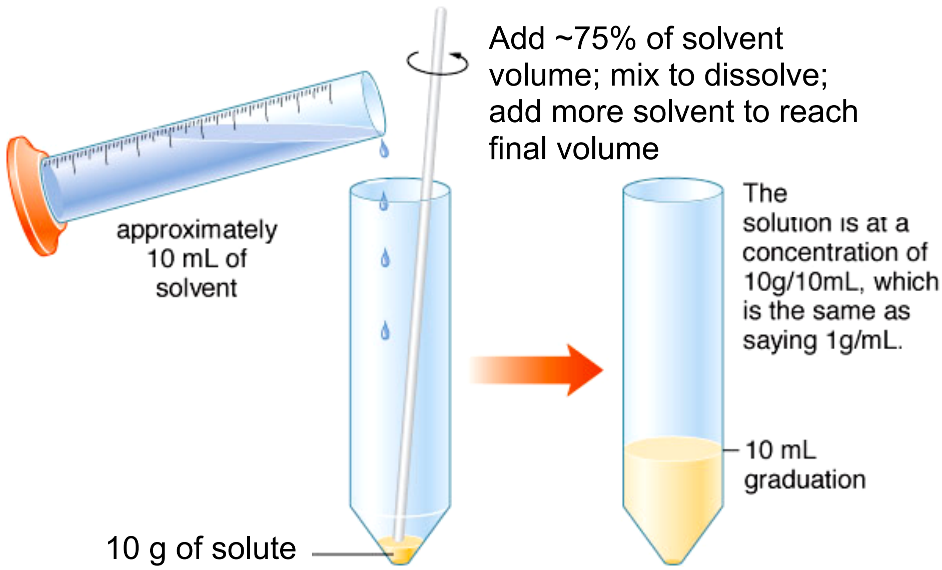
Example: You need to make 20 mL of a 40 mg/mL solution of glucose. How many grams of glucose will you need to measure to make this solution?

$$(20\ \cancel{mL})\ \left(\frac{40\ mg}{\cancel{mL}}\right) = 800\ mg$$

→ but you want to know how many g, not mg, so convert 800 mg to grams (= 0.8 g)

Notice how the mL units cancel out during multiplication so as to leave the answer in mg to be weighed out. Since the balances measure in grams, it was necessary to convert mg to g.

To make this solution, 0.8 g of glucose is weighed and put into a graduated 50 mL tube. Approximately 15 mL (~75 % of final volume) of solvent (deionized water or buffer) is added to the tube, and the contents are mixed using the vortex. Then, additional solvent is added to reach a total volume of 20 mL. The reason that not all 20 mL of solvent is added initially is that the solute may take up some space when it dissolves in the solvent. Thus, if we had added all 20 mL to the solute at once, the final volume may have in fact been greater than 20 mL.



Procedure

1. Label all tubes with the sample name and concentration, and your group's initials.
2. Review the use of the balance and weigh boats before beginning.
3. Do the calculations necessary to prepare the solutions in Table 3.1 for tube numbers 1 through 5. Use the Mass/Volume equation to determine the mass of $CuSO_4$ to measure in order to give the correct concentration at the volume desired for each sample.

Table 3.1 $CuSO_4$ Mass/Volume Solution Preparation

Tube #	$CuSO_4$ solution	Total Volume	Concentrat'n (mg/mL)	Calculation of Mass Needed (g)
1	5.0 mL of 300 mg/mL	5.0 mL	300 mg/mL	5.0 mL x $\frac{300\ mg}{mL}$ =1500 mg = 1.5 g
2	4.5 mL of 150 mg/mL			
3	4.0 mL of 75 mg/mL			
4	3.5 mL of 37.5 mg/mL			
5	3.0 mL of 18.75 mg/mL			

4. Prepare the solutions in labeled 15 mL plastic tubes, using deionized water as the solvent. Use the vortex on your bench to help dissolve the $CuSO_4$. You may need to let the more concentrated samples sit in the warm water bath for several minutes in order for the $CuSO_4$ to dissolve fully.
5. Is the difference in concentration of the tubes obvious in one tube versus another? Compare your tubes' colors and volumes to the standard "key" solutions prepared by the instructor. If any of the volumes or colors are obviously wrong, try to identify where you may have made an error. Then dump them out, and remake them.
6. Keep tube #1 for use in Activity 3d.

Activity 3b
Making Solutions of Differing % Mass/Volume Concentrations

Purpose and Background

In this activity, you will learn how to prepare solutions of specific % mass/volume concentrations. You will prepare several % mass/volume solutions and use some of them as testing reagents. The underlying rule for % mass/volume solutions is that a 1% solution contains 1 g of solute in a total volume of 100 mL.

Solutions of Differing % Mass/Volume Concentrations

% Mass/Volume Concentration Equation steps:
Rule: a 1% solution means 1 g solute in 100 ml solvent

Step 1: Convert the % to a decimal by **dividing % by 100**.
This changes the units to g/mL

Step 2: (same as in previous equation for mass/volume concentrations)

(Volume desired) x (Concentration desired) = (mass of solute needed)
(for ex: mL) (for ex: g/mL) (for ex: g)

Procedure

You will do the calculations for and prepare the following solutions:

- 5 mL of 10% NaOH solution *
- 5 mL of 5% $CuSO_4$ solution
- 5 mL each of 5%, 2.5%, 1.25%, and 0.625% gelatin solutions

As an example, I will do the calculation for the preparation of 5 mL of 10% NaOH solution. I will also prepare this solution for you, because NaOH is extremely caustic.

Step 1: Convert 10% to a decimal by dividing by 100: (10%) / 100 = 0.1 g/mL

Step 2: $(5\ \cancel{mL})\left(\frac{0.1\ g}{\cancel{mL}}\right) = \mathbf{0.5\ g}$

Do all the necessary calculations in your lab notebook. Then, record the amounts of the solutes needed in a table similar to Table 3.2 to serve as a guide when preparing your solutions.

Table 3.2 Preparation of % Mass/Volume $CuSO_4$ and Gelatin Solutions

Tube label	Amt. of solute (g)	Volume (mL)	Calculations
5.0 % $CuSO_4$			
5.0 % gelatin			
2.5 % gelatin			
1.25 % gelatin			
0.625% gelatin			

You will prepare all the solutions in 15 mL plastic tubes (you can reuse your washed tubes from last week). When you are ready to prepare the gelatin solutions, there are some important tips that your instructor will demonstrate to help you. Gelatin, like a lot of other proteins, has a tendency to form a sticky clump when it is being dissolved. To avoid this, after you weigh out the needed mass of gelatin, pour it into your 15 mL tube with the tube at an angle, so that the gelatin powder forms a thin layer all along the side of the tube. Then, continue to hold the tube at an angle while adding the water with your squirt bottle. You may also need to let the tube sit in the warm water bath for a few minutes for the gelatin to fully dissolve. Also, do not vortex your gelatin solutions. Proteins don't like being vortexed, as the mechanical force shears the protein and causes lots of sticky bubbles to form.

Once you have made your solutions, you will use them to test for the presence of protein using a Biuret Test that produces a color change when protein is present. To do this, follow this procedure:

1. Get 5 microcentrifuge tubes and label them 5.0% gelatin, 2.5% gelatin, 1.25% gelatin, 0.625% gelatin, and 0% gelatin.
2. Add 250 μL of 10% NaOH to each tube (gloves!!!)
3. Add 125 μL of 5% $CuSO_4$ to each tube.
4. Add 1 mL of the appropriate gelatin solution to each tube (for the 0% gelatin tube, add 1 mL of water).
5. Close the microcentrifuge tube and vortex for 1 to 2 seconds.
6. Note the color changes that appear in your lab notebook. Do the colors indicate the expected difference in gelatin concentration? How so or how not?

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Activity 3c
Making Solutions of Differing Molarity Concentrations

Purpose and Background

In this activity you will be introduced to the concentration measurment of molarity, and you will learn how to do the necessary calculations to prepare molar solutions, or solutions of a specific molarity.

The concentration of many solutions is reported as moles/liter (mol/L or M; the M is spoken "molar") or some fraction of those units. This concentration measurement is called *molarity*. Molarity is sometimes a challenging concept to understand. However, with your recently acquired solution preparation skills, you will see that making molar solutions requires only one extra calculation.

To understand how to make a solution of a given molarity, you must know what a "mole" is. A mole of a compound is equal to 6.02×10^{23} molcules, but that is not really a very useful number. So, in biotech, it is easier to use this definition:

> *The unit "1 mole" is the mass, in grams, equal to the molecular weight (MW), also called "formula weight" (FW) of the substance.*

The FW can be determined by using a Periodic Table and adding the atomic weights of the atoms in the molecule. An easy way, though, is to just read the label of a chemical reagent bottle, which lists the "MW" or "FW." The molecular weight of NaCl is 58.5 atomic mass units (amu), since the Na atom weighs 23 amu, and a Cl atom weighs 35.5 amu.

Molarity concentrations are reported as the number of moles per liter (mol/L or M). The unit "M" is used as a base unit, just like meters, liters, or grams, so you can have "millimolar" (mmol/L, or mM) and "micromolar" (µmol/L, or µM) concentrations, both of which are used frequently in biotech labs.

If you wanted a 1 M NaCl solution, you would measure out 1 mole of NaCl (58.5 g) and dissolve it in water to a total volume of 1 L. This gives you 1 mole of NaCl per liter of solution, or 1 M NaCl. A liter of solution is a large volume for most purposes in biotechnology labs. Instead, mL or µL quantities are usually used. To determine how to mix up a smaller volume of solution, follow the equation below.

Solutions of Differing Molarity Concentrations

Molarity Concentration Equation:

(Volume desired) x (Molarity desired) x MW of solute = (mass of solute needed in g)
(L) (mol/L) (g/mol)

Convert smaller or larger units to these units as necessary.

A few notes about calculations for molar solutions:

- As before, the units in the numerator & denominator should cancel if the equation is set up properly, and you should be left with the units desired.
- You must make sure that the units used in the calculation can be cancelled, and you may need to convert units within the metric system if necessary. Often, you are given a volume that is not in L (might be in mL or μL) or a molarity that is in millimolar (mM) or micromolar (μM). If this is the case, you will need to convert the volume to L and the molarity to mol/L, and then plug these numbers into the molarity concentration equation above.

Procedure

You will do the calculations for and prepare the solutions in Table 3.3 below. The molecular weight (MW) for $CuSO_4$ (actually copper sulfate pentahydrate, or $CuSO_4 \cdot H_2O$) is 250 g/mol. The first calculation has been done for you as an example.

Table 3.3 $CuSO_4$ Molarity Solution Preparation

Tube #	$CuSO_4$ molarity	Total Volume	Mass of $CuSO_4$ to use (g)	Calculation of Mass Needed (g)
1	1.0 M	5 mL	1.25 g	$(0.005\ L)\left(\frac{1\ mol}{L}\right)\left(\frac{250\ g}{mol}\right) = 1.25\ g$
2	0.5 M	5 mL		
3	0.1 M	5 mL		
4	0.05 M	5 mL		
5	0.01 M	5 mL		

1. Once you have done the calculations, label 5 tubes 1 through 5, and weigh out the needed amounts of CuSO4 to make your solutions. Feel free to check your group's calculations with the instructor before you begin.
2. Prepare the solutions in labeled 15 mL plastic tubes, using deionized water as the solvent. Add the solute first, then add some water to dissolve, using the vortex to mix. Then add more water to bring the final volume up to 5 mL.
3. Are the differences in concentration of your five tubes obvious? Compare the colors and volumes of your samples to others in the class and to the "key" tubes made by the instructor. If any of the volumes or colors are obviously wrong, try to identify where you may have made an error. Then dump them out, and remake them.

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Activity 3d
Making Dilutions of Concentrated Solutions

Purpose and Background

In this activity you will learn how to make diluted solutions from a more concentrated stock solution. You will learn how to use the dilutions equation, and prepare several different dilutions from a concentrated $CuSO_4$ stock solution.

Very often in biotech labs, you will dilute a concentrated "stock solution" to use as a "working solution." This is done to save time, as well as space in the lab. Also, it is often more accurate to prepare a concentrated stock solution because it involves weighing larger masses of chemicals (therefore less error in measurements).

The working solution concentration is represented as 1X, and the concentrated stock solution is some multiple of this (5X, 10X, etc.). For example, a buffer solution may be used at a concentration of 0.01 M Tris. This is the working concentration of the Tris solution (1X). But because of shipping costs, a small amount of the buffer is shipped as a 10X solution with a concentration of 0.1 M Tris. When the technician is ready to use the buffer, it is diluted with deionized water down to 1X (0.01 M Tris).

To figure out how to dilute something from a concentrated solution, we use a simple ratio equation shown here:

Diluting Concentrated Solutions

Diluting Concentrated Solutions Equation:

$$C_1V_1 = C_2V_2$$

Where C_1 = concentration of starting (stock) solution
V_1 = volume to use of the stock solution to make diluted solution
C_2 = desired concentration of diluted solution
V_2 = desired volume of the diluted sample

The $C_1V_1 = C_2V_2$ equation may be used with any concentration units (i.e. mass/volume, %, or molar) as long as the units are the same on each side of the equation, for canceling purposes. Thus, you can't have mL for V_1 and L for V_2—you must change one to match the other. Once you have the equation set up and the numbers plugged in, then solve for the variable you want to find. Here is an example. Let's say you want to make 200 mL of 0.15 M NaCl from a stock that is 3 M.

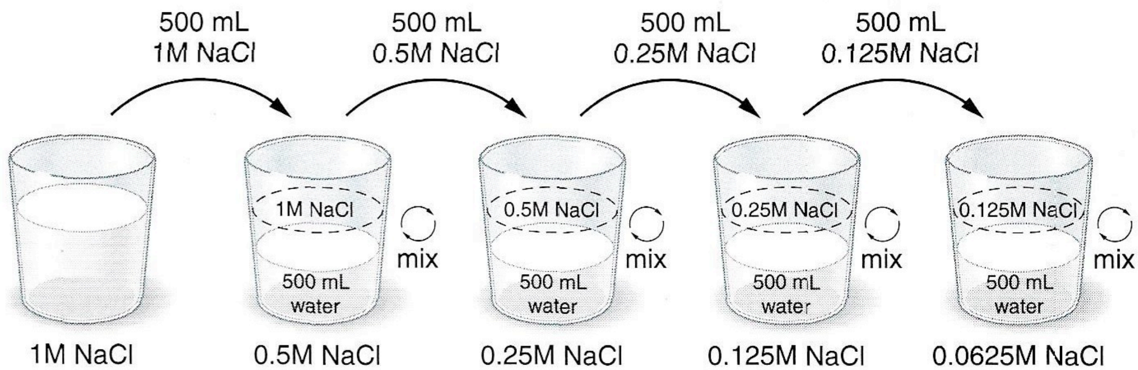
C_1 = 3 M
V_1 = the amount of stock to use (this is what you're solving for)
C_2 = 0.15 M
V_2 = 200 mL

$$(3\text{ M})(V_1) = (0.15\text{ M})(200\text{ mL}) \qquad V_1 = \frac{(0.15\text{ M})(200\text{ mL})}{3\text{ M}} = 10\text{ mL}$$

Therefore, to make the solution, you would measure out 10 mL of the 3 M NaCl stock solution and add 190 mL of deionized water to dilute to the final concentration of 0.15 M.

When a number of dilutions must be made, and each is proportionally the same dilution as the one before, it is called a serial dilution (see figure below). Doing a serial dilution makes sense for many experiments when many samples of varying concentrations are needed. A serial dilution is also useful for preparing very dilute solutions that are hard to make from scratch, because the solute masses can be too small to measure on a balance.

Each succeeding sample is made with the same ratio of sample and diluent as the one before.



Serial dilution. **Each of these dilutions is one part (previous) sample and one part solvent. This is called a 1:2 dilution, or one part sample in two total parts. This could be read "1 to 2", which could erroneously result in a preparation with three parts. In practice, by convention, a 1:2 dilution is actually "1 in 2" total parts.**

Procedure

1. You should still have a tube of 5 mL of 300 mg/mL CuSO4 solution (tube #1 from Activity 3a). If not, make this solution. This solution will now serve as your **300X stock solution**.
2. Label 5 tubes with the concentrations listed in Table 3.4 (150X, 30X, 15X, 3X, and 1X) and your group's initials. Then, do the calculations in your lab notebook and prepare the solutions in Table 3.4 below. The first calculation has been done for you as an example.

Table 3.4 Dilutions of the 300X Stock $CuSO_4$

Concen-tration	Volume	Calculation of volume of stock needed	Vol. stock to use	Vol. H_2O to add
150X	**5 mL**	$(300X)(V_1) = (150X)(5mL)$ $V_1 = \frac{(150X)(5mL)}{300X} = 2.5$ mL	2.5 mL	2.5 mL
30X	**7 mL**			
15X	**5 mL**			
3X	**5 mL**			
1X	**6 mL**			

3. Prepare a 1:10 serial dilution of the concentrated (300 mg/mL) stock according to Table 3.5 below. Do the calculations in your lab notebook and prepare the solutions in 15 mL plastic tubes. Feel free to check your calculations with the instructor before beginning to prepare the dilutions.

Table 3.5 Serial Dilution (1:10) of the 300X Stock $CuSO_4$

Concen-tration	Volume	Calculation of volume of stock needed	Vol. of V_1 to use	Vol. H_2O to add
30 mg/mL	3 mL			
3 mg/mL	3 mL			
0.3 mg/mL	3 mL			

4. When you are done, review your calculations and consider why this is a 1:10 serial dilution. Does this make sense? Compare the colors and volumes of your samples to others in the class and to the "key" tubes made by the instructor. If any of the volumes or colors are obviously wrong, try to identify where you may have made an error. Then dump them out, and remake them.

Summary: Solutions & Dilutions

Solutions of Differing Mass/Volume Concentrations

Mass/Volume Concentration Equation:

(Volume desired) x (Concentration desired) = (mass of solute needed)
(for ex: mL) (for ex: g/mL) (for ex: g)

Solutions of Differing % Mass/Volume Concentrations

% Mass/Volume Concentration Equation steps:
Rule: a 1% solution means 1 g solute in 100 ml solvent

Step 1: Convert the % to a decimal by **dividing % by 100**.
This changes the units to g/mL

Step 2: (same as in previous equation for mass/volume concentrations)
(Volume desired) x (Concentration desired) = (mass of solute needed)
(for ex: mL) (for ex: g/mL) (for ex: g)

Solutions of Differing Molarity Concentrations

Molarity Concentration Equation:

(Volume desired) x (Molarity desired) x MW of solute = (mass of solute needed in g)
(L) (mol/L) (g/mol)

Convert smaller or larger units to these units as necessary.

Dilutions

Diluting Concentrated Solutions Equation:

$$C_1V_1 = C_2V_2$$

Where C_1 = concentration of starting (stock) solution
V_1 = volume to use of the stock solution to make diluted solution
C_2 = desired concentration of diluted solution
V_2 = desired volume of the diluted sample

Lab 3 Homework

Name: ______________________

1. For each of the following mass/volume solutions, show the calculation (equation with all units) for the preparation of each solution. Then, describe how you would make the solution in an appropriate container.

   a) 25 mL of 2.5 g/mL NaCl solution

   b) 10 mL of 50 mg/mL $CuSO_4$ solution

   c) 2 L of 0.5 g/mL dextrose solution

   d) 100 mL of 0.005 g/mL NaOH solution

2. Express the following % mass/volume concentrations in g/mL units:
   a) 10% NaOH: _________ g/mL NaOH
   b) 5% $CuSO_4$: _________ g/mL $CuSO_4$
   c) 1.25% gelatin: _________ g/mL gelatin

3. For each of the following % mass/volume solutions, show the calculation (equation with all units) for the preparation of each solution. Then, describe how you would make the solution in an appropriate container.

   a) 40 mL of 3% $CuSO_4$ solution

   b) 200 mL of 8% NaCl solution

   c) 0.75 L of 5% dextrose solution

d) 10 mL of 1.25% NaOH solution

4. For each of the following molar solutions, show the calculation (equation with all units) for the preparation of each solution. Then, describe how you would make the solution in an appropriate container.

   a) 3 L of 0.5 M $CuSO_4$ solution (The MW of $CuSO_4$ is 250 g/mol)

   b) 80 mL of 0.1 M NaCl solution (The MW of NaCl is 58.5 g/mol)

   c) 125 mL of 10 M NaOH solution (The MW of NaCl is 40 g/mol)

   d) 400 mL of 50 mM glucose solution (The MW of glucose is 180 g/mol)

5. For each of the following dilutions, show the calculation (equation with all units) for the preparation of each solution. Then, describe how you would make the solution in an appropriate container.

   a) 50 mL of 15 mg/mL NaOH solution from 100 mg/mL NaOH

   b) 10 mL of 0.5 M $CuSO_4$•$5H_2O$ solution from 10 M $CuSO_4$•$5H_2O$ solution

c) 2 L of 5 mg/mL gelatin solution from 1 g/mL gelatin solution

d) 950 mL of 1X $CuSO_4 \cdot 5H_2O$ solution from 25X $CuSO_4 \cdot 5H_2O$ solution

e) 5 L of 0.2 M dextrose solution from 5 M dextrose solution

f) 100 mL of 2.5 X NaOH solution from 50X NaOH solution

g) 50 mL of 5 mM NaCl solution from 1 M NaCl solution