

Biochemistry I Laboratory
CHEM 4401
Units, Concentrations, Solutions & Dilutions

Let's face it. It's been over a year or more since you've had general chemistry and you've forgotten what all those terms like "molarity" and "molality" mean. It's time to do some review on these terms and brush up on our conversion factors, as we will be using them extensively in biochemistry laboratory.

"Molarity" is a concentration term. It refers to the number of moles of a substance per liter of solution. If you recall, a "mole" is the quantity of substance that contains 6.02×10^{23} (Avogadro's number) items. You may also recall that the atomic or formula weight of a substance, in grams, contains 1 mole (6.02×10^{23} units) of that substance. For example, the atomic weight of oxygen gas (O_2) is 32. Therefore, 1 mole of O_2 weighs 32 g and contains $6.02 \times 10^{23} O_2$ molecules. NaCl provides another example. 1 mole of NaCl weighs approximately 58.4 g (Na atomic wt. = 23, Cl atomic weight = 35.4) and, you guessed it, contains 6.02×10^{23} atoms of both Na and Cl.

The atomic or formula (for substances which don't exist as molecules) weight of a substance can also be easily used to determine how many moles of a substance are contained in a given quantity. For example, if we have 25 g of NaCl on hand, we can calculate the number of moles by dividing this amount by the formula weight of NaCl:

$$\text{Number of moles} = \frac{\text{given weight in grams}}{\text{weight of one mole}} = \frac{25 \text{ g NaCl}}{58.4 \text{ g per mole}} = 0.43 \text{ moles NaCl} \quad (1)$$

It is relatively easy to extrapolate calculations for the number of moles of substance into the concentration term "molarity". To calculate the molarity of a solution, simply divide the number of moles of substance it contains (solute) by the volume of the solution, in liters. Using our NaCl example, a 1 molar (M) solution of NaCl contains:

$$\frac{1 \text{ mole NaCl}}{\text{liter solution}} \quad (2)$$

a 0.5 molar solution would contain 0.5 moles, and so forth.

A variation on this theme refers to the use of terms such as millimolar (mM), micromolar (uM), etc. This is where the beauty of the metric system becomes apparent. Just as in other units of measurement (length, for example), the "milli-" prefix means "one thousandth" (10^{-3}), micro-means "one millionth" (10^{-6}) and nano- means "one billionth" (10^{-9}). Thus, a millimolar solution contains 1 millimole (10^{-3} moles) of substance (solute) per liter of solution.

$$1 \text{ millimolar (mM)} = \frac{1 \text{ millimole solute}}{1 \text{ liter of solution}} \quad (3)$$

These types of calculations can also be used in reverse, which is most useful when you need to make up solutions of a particular molarity. For example, if a particular experiment requires you to make 1 liter of a 0.25 M solution of NaCl, you know that all you have to do is obtain 0.25 moles of NaCl and dissolve it in enough solvent (usually water) to make 1 liter of solution. To determine how much NaCl is contained in 0.25 moles, set up your equation as in (1) above and solve for the unknown:

$$0.25 \text{ moles NaCl} = \frac{\text{? grams NaCl}}{58.4 \text{ g NaCl per mole}} \quad (4)$$

Usually, it will not be this straightforward. Often, you will be asked to make up volumes other than 1 liter. In these cases, the problem will be to determine how much solute will be required. To do this, you will have to factor in a conversion to determine the actual number of moles, and hence grams, required. For example, if you are asked to make up 200 ml (0.2 liter) of a 0.25 M solution of NaCl, you will need to calculate the number of moles required in 0.2 liter by multiplying the number of moles in a one liter solution by 0.2. This value will then be used to determine the amount of NaCl, in grams, required:

$$\frac{0.25 \text{ moles NaCl}}{\text{liter of solution}} \times 0.2 \text{ liter required} = 0.05 \text{ moles NaCl} \times \frac{58.4 \text{ g NaCl}}{\text{mole NaCl}} = 2.92 \text{ g NaCl} \quad (5)$$

Notice how all the units canceled until we ended with the units required (grams). This is a good way to double check your results. If after canceling units, you come up with something other than the units desired, you have made a mistake.

Alternative concentration terms such as “molality” are not nearly as useful. Molality refers to the number of moles per *kilogram* of solvent. We will not be concerned with this definition in biochemistry.

Unit reviews

milligram	10^{-3} g	0.001 g
microgram	10^{-6} g	0.000001 g
nanogram	10^{-9} g	0.000000001 g
picogram	10^{-12} g	0.000000000001 g
milliliter	10^{-3} liter	0.001 L
microliter	10^{-6} liter	0.000001 L

Solutions and Dilutions

Most experiments you do will involve at least one solution. A solution contains one chemical dissolved in another liquid. When chemicals are in the form of liquids, they can be either pure liquids or solutions. A pure liquid would be something like absolute ethanol; it contains nothing but molecules of ethanol. When dealing with solutions, the chemical in the *smaller quantity* is the **solute**. The liquid it is dissolved in is the **solvent**. When you calculate the amount of solute dissolved in the solvent, you have determined the concentration.

Concentrations are used to describe the relative amount of solute in a solution. It is important to be able to distinguish values that are concentrations from those that are not, which we will call amounts. If we put 1 gram of salt in a beaker that it is an amount. If we bring the volume up to 1 liter with water then we have a 1 g/L solution, which is described using values for concentration. If we have 1 millimole (mmol) of salt in a beaker that is an amount. If we bring the volume up to 1L with water then we have a 1mM (millimolar) solution, which is also described using values for concentration.

Amounts are always additive, concentrations are not. Adding 1 g of salt from one beaker to 1 g of salt in another beaker gives 2 g of salt. If we add a solution that is 1 g/L to another solution of 1 g/L, we *will not* produce a solution that is 2 g/L. In fact, we will produce a solution that is 1 g/L.

% Solutions

Solutions based on percent are the easiest to calculate, because they do not depend on knowledge of the molecular weight. Your instructor can give you a tube with an unknown white powder, and tell you to make up a 1% w/v solution, and you can do it without knowing anything about the white powder.

%w/v means percent weight to volume and has units of grams/100 ml. Therefore a 1% w/v solution has 1 g of solute in a total of 100 ml of solution.

%v/v means percent volume to volume and has units of mL/100 ml. Therefore a 1% v/v solution of ethanol has 1 ml of pure ethanol in 100 ml of total solution.

Remember that when you make solutions, the important thing is the *final* volume of the solution. If you are to make one liter of a 10% w/v solution, you would add 100 g of solid chemical to water (begin with ~ 700 ml, for example) and then bring the solution up to a final volume of 1 liter.

Molar Solutions

The most common types of solutions are molar solutions. A 1 M solution means 1 mole of solute in a total volume of 1 liter. A 1 mM solution has 1 mmol (10^{-3} mole) in a total of 1 L of solution. A 1 micromolar (uM) solution is 1 micromole (10^{-6} mole) of solute in a total of 1 L of solution.

Remember that the number of moles of a substance are calculated by dividing the amount in grams by the formula weight (grams per mole). For example, if the formula weight of compound “X” is 39 g/mole, this means that one mole of compound “X” weighs 39 g. If we have 13 g of “X”, we calculate the number of moles present as:

$$13 \text{ g} \div 39 \text{ g/mole} = 0.33 \text{ mole}$$

If we take 13 g of “X” and dissolve it in 0.5 L of water, our molar concentration will be

$$0.333 \text{ mole} \div 0.50 \text{ L} = 0.666 \text{ mole/L} = 0.67 \text{ M}$$

Remember that derivatives of molar solutions, such as mM, uM, nM and so on, still refer to an **amount** (millimoles, micromoles, etc.) divided by **liters** of solution. Many students mistakenly think that a 1 mM solution means 1 mmol of solute dissolved in 1 ml. What it actually means is 1 mmol in 1 L!

Dilutions

Dilutions seem to be one of the hardest concepts for most beginning biochemistry students. However, doing problems is the only way to really understand dilutions. When doing a dilution, you always start with a sample of concentrated, or **stock**, solution and add additional solvent to it, thus lowering the concentration. There is a simple way of doing dilutions problems. This formula forms the foundation of all dilutions, and you should memorize it by heart:

$$C_1 V_1 = C_2 V_2$$

The terms of the equation describe the concentrations (C) and volumes (V) of two solutions: the stock solution (1) and the diluted solution (2) that you wish to make. To use the formula, you need to know 3 of the 4 variables. A typical use of this problem will be to calculate the volume needed of a stock solution to prepare a particular volume of a diluted solution. In such cases, you will know the volume and concentration of your final concentration (through written instructions, directions from your instructor, etc.) *and* the concentration of your stock solution. Your task will then be to calculate the volume of stock solution required to make a dilution. The equation works for any units of concentration and volume, *provided the units are the same on both sides of the equal's sign.*

Some examples:

- (1) Given a stock of 1 molar (M) HCl, prepare 100 ml of 0.1 M HCl.

$$C_1 (\text{stock solution}) = 1 \text{ M}$$

$$C_2 (\text{final solution}) = 0.1 \text{ M}$$

$$V_2 (\text{final volume}) = 100 \text{ ml}$$

$$V_1 (\text{volume of stock solution required}) = ?$$

$$C_1 V_1 = C_2 V_2$$

$$(1\text{M}) \times V_1 = (0.1\text{M})(100 \text{ ml})$$

$$V_1 = \frac{(0.1\text{M})(100\text{ml})}{(1\text{M})}$$

$$V_1 = 10\text{ml}$$

This tells us we need 10 ml of the 1 M stock HCl solution, diluted to a total volume of 100 ml (final volume) to give the desired solution.

(2) Given a stock solution of 100 mg/ml glucose, prepare a 10 ml solution of 1 mg/ml glucose.

$$\begin{aligned}
 C_1 (\text{stock solution}) &= 100 \text{ mg/ml} \\
 C_2 (\text{final solution}) &= 1 \text{ mg/ml} \\
 V_2 (\text{final volume}) &= 10 \text{ ml} \\
 V_1 (\text{stock solution}) &= ? \\
 V_1 &= \frac{(1\text{mg / ml})(10\text{ml})}{(100\text{mg / ml})} \\
 V_1 &= 0.1\text{ml}
 \end{aligned}$$

Therefore, take 0.1 ml of the 100 mg/ml glucose solution and add it to $(10 - 0.1 = 9.9 \text{ ml})$ of water to make 10 ml (final volume) of a 1 mg/ml (final concentration) solution. Hint: If the volume of a dilution (V_2) is not specified, the first thing to decide is **how much (what volume)** of the dilution you want to make. And make it convenient (i.e. 1,10,100 ml)

(3) Given a stock of 100 mg/ml BSA, prepare 1 ml of 0.02 mg/ml BSA solution.

$$\begin{aligned}
 C_1 (\text{stock solution}) &= 100 \text{ mg/ml} \\
 C_2 (\text{final solution}) &= 0.02 \text{ mg/ml} \\
 V_2 (\text{final volume}) &= 1 \text{ ml} \\
 V_1 (\text{stock solution}) &= ? \\
 V_1 &= \frac{(0.02\text{mg / ml})(1\text{ml})}{(100\text{mg / ml})} \\
 V_1 &= 0.0002\text{ml}
 \end{aligned}$$

This is a very small quantity; we cannot pipet 0.0002 ml (0.2 μl) accurately. We have to do a stepwise dilution. First, dilute the stock solution 100-fold to make a 1 mg/ml solution. How can we do this? First, let's make 10 ml of the 1/100 dilution:

$$\begin{aligned}
 C_1 V_1 &= C_2 V_2 \\
 (100\text{mg / ml})V_1 &= (1\text{mg / ml})(10\text{ml}) \\
 V_1 &= \frac{(1\text{mg / ml})(10\text{ml})}{(100\text{mg / ml})} \\
 V_1 &= 0.1\text{ml}
 \end{aligned}$$

So, we place 0.1 ml of the 100 mg/ml stock solution of BSA into a container and add (10 mL-0.1 ml = 9.9 ml) of solvent to get 10 ml of 1 mg/ml solution of BSA. Now to prepare the solution we wanted in the first place we use the 1/100 dilution as our new “stock” solution:

$$\begin{aligned}
 C_1 V_1 &= C_2 V_2 \\
 (1\text{mg / ml})V_1 &= (0.02\text{mg / ml})(1\text{ml}) \\
 V_1 &= \frac{(0.02\text{mg / ml})(1\text{ml})}{(1\text{mg / ml})} \\
 V_1 &= 0.02\text{ml}
 \end{aligned}$$

Thus, we add $(1.0 \text{ ml} - 0.02 \text{ ml}) = 0.98 \text{ ml}$ of solvent to a tube and add 0.02 ml ($20 \mu\text{l}$) of the 1 mg/ml BSA to obtain the desired solution.

When you need to transfer an amount of substance that is in solution.

Sometimes we want to transfer a fixed amount of solute by transferring **a portion of solution in which it is dissolved**. The amount of solution to transfer, that is the amount of solution that contains, say, 1 mg of solute is simply calculated with the solution-dilution equation. The product CV in the equation is an amount; $(\text{concentration} = \text{amount}/\text{volume}) \times \text{volume} = \text{amount}$. So, if we want to transfer an amount we make it equal to the product C_2V_2 .

Example:

How much of a 0.2 mg/ml stock solution of insulin (a protein) solution is required to provide a patient with $8 \text{ micrograms (ug)}$ of insulin?

Dilution equation: $C_1V_1 = C_2V_2$

C_1 is 0.2 mg/ml , C_2V_2 is 8 ug . Question becomes: what is V_1 ?

remember, make the units on both sides of the equation the same

i.e. $0.2 \text{ mg/ml} \times 1000 \text{ ug/mg} = 200 \text{ ug/ml}$. The solution then becomes:

$(200 \text{ ug/ml}) V_1 = C_2V_2 = 8 \text{ ug}$

$$V_1 = \frac{8 \text{ ug}}{200 \text{ ug/ml}}$$

$$V_1 = 0.04 \text{ ml}$$

This procedure always works and is the same for any concentration scale. You should practice this until you can do it in your sleep. In the experimental write-ups, you will be told to prepare solutions of specified concentrations given stocks of specified concentration.

Mixing solutions of the same substance to a new concentration.

You will often be faced with the problem of reaching a specified final concentration of a solute when there are two or more sources. Suppose, for example, that a final concentration of 5 mM MgCl_2 was required in an enzyme assay. You have a stock solution of 50 mM MgCl_2 that you can use to adjust the concentration. However, the matter is complicated because the enzyme to be used in the assay is stored in a buffer solution that contains 1 mM MgCl_2 . In this case there are two sources of MgCl_2 , the stock solution and the buffer solution that contains the enzyme.

Problems of this type can be solved by a straightforward extension of the C_1V_1 process explained above. Since there are two sources of the required component write the material balance equation as:

$$C_1V_1 + C_2V_2 = C_fV_f$$

The expression C_fV_f represents the **final** amount of added substance present in the solution. It is the product of the final volume required, V_f , and the final concentration achieved, C_f . The products on the left of the equal's sign represent the sources of the component.

Now, let's solve the problem presented as an example above. We need two more critical pieces of information: the final volume of the solution to be made and the amount of the enzyme solution to be used. Usually the amount of one required component ($MgCl_2$ in this case) will be specified, in this case by the amount of enzyme to be added to the assay. Let's say the final volume of the assay will be 1 ml, and that it will require 50 μl of the enzyme solution. Since we have two sources of $MgCl_2$ we will use the equation above.

First, let's restate the entire problem: We want to use 50 μl of an enzyme solution in an assay with a total volume of 1 ml. The enzyme is dissolved in a buffer containing 1 mM $MgCl_2$. We want the final solution to contain 5 mM $MgCl_2$. We can use a stock solution of 50 mM $MgCl_2$ to adjust the $MgCl_2$ concentration.

The following steps are a useful way to approach problems like this:

1. Identify the final concentration and final volume required.

In our case it is 5 mM $MgCl_2$ in a final volume of 1 ml.

2. Count the number of sources of the required component, if two sources exist, then use the C_fV_f summation formula given above.

We have two sources of $MgCl_2$, the enzyme solution and the stock solution.

$$C_1V_1 + C_2V_2 = C_fV_f$$

3. Fill in all the given information

Let's let the first solution be the enzyme so $C_1 = 1$ mM

The second solution is the stock $MgCl_2$ $C_2 = 50$ mM

$V_2 = ???$

So our equation looks like this:

$$(1mM)(50\mu l) + (50mM)V_2 = (5mM)(1ml)$$

4. Look at the equation and decide if it is solvable. In more complicated examples you may be left with two unknowns. If you are, go back at this point and find a relation between the remaining unknowns. Our equation is solvable.

5. Adjust the units. This is the next to the last thing to worry about, not the first. The units on both sides of the equal's sign must be the same for both volumes and concentration.

In our example the concentration units are all the same, however the volume units are not. We can either change microliters to milliliters or vice-versa before we solve for the unknown.

6. Now solve for the unknown. Remember if we are looking for a certain final volume, the unknown may only represent how much of a stock solution is needed to supply the amount of the substance in question needed for the final concentration. **The final volume will be reached by adding water or any other solution that contains none of the component whose concentration is being adjusted.**

Our answer is 0.099 ml of stock 50 mM MgCl₂ (check this)

To reach the final volume we still need to add (1-0.05-0.099) = 0.851 ml of something else. Usually this volume is comprised of water or other components of the mixture, for example, the buffer being used.

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Show your work in the spaces for each question. Use the periodic table for atomic weights.

1. How many moles are there in 100 g of glucose ($C_6H_{12}O_6$)? (1 pt)
 2. How many grams of NaCl are required to make 2.5 liters of 0.25 M solution? (1 pt)
 3. How many moles of estradiol (MW = 272) are there in 2 ml of a 100 μM solution? (1 pt)
 4. How much Tris-Acetate-EDTA (TAE) stock buffer (3.25 M) is required to make 400 ml of solution that has a concentrations of 250 mM? How much water? (2pt)
 5. How many milliliters (ml) of 5 M $MgCl_2$ must be added to 25 ml of a 0.5 M $MgCl_2$ solution to produce 1500 ml with a final $MgCl_2$ concentration = 1.75 M? How much water? (2 pt)
 6. How much sodium phosphate buffer (1.5M, pH 7.2) is required to make 125 ml of a 50 mM solution? How much water? (2 pt)

7. You wish to make 3 reactions (1 ml each) with the specified amounts of protein. The remainder of each reaction consists entirely of buffer. The source of your protein is a stock solution that has a concentration of 0.5 mg/ml. What is the volume of stock solution required for each reaction (column 3). What is the volume of buffer required (column 4)? Show your work. Be sure to pay attention to units. (6 pt)

Reaction number	Amount of protein (ug)	Volume of Protein solution (ul) required	Volume of Buffer (ul) required	Final Volume (ml)
1	2			1
2	5			1
3	10			1