

Physiological Chemistry Laboratory CHEM1106

Solutions and Dilutions

Most “wet” experiments you do in chemistry involve at least one solution. A solution contains one chemical (“solute”) dissolved in another (“solvent”). Technically, in a solution, the chemical in the smaller quantity is referred to as the **solute**. The chemical in the larger quantity is generally referred to as the **solvent**. Thus, we have “1M NaCl” or “0.1 M NaOH” solutions. These solutes (NaCl, NaOH) are present in a lower concentration compared to the water solvent.

A concentration refers to the amount of a solute in a given amount of solvent. For example, a 1M solution of NaCl contains 1 mole of NaCl for every liter of solvent (water) that makes up the solution. If the solvent has a volume of 0.5 liter, that means there must be 0.5 moles of NaCl present (0.5 moles NaCl/0.5 liters = 1M). It is important to distinguish concentrations from amounts. An amount is a measure of mass or volume. If we put 1 gram of salt in a beaker, that is an amount. If we bring the volume in the beaker up to 1 liter with water, then we have a 1 g/L solution, which is described by its concentration (amount/volume of solution). 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: 1 mmol/1 liter) solution, which is described by its 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 between 1 and 2 g/L, depending on the volumes of the initial solutions.

% 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 99 ml of water to give 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 a volume of water that is much less than your desired, final volume (for example for example, begin with 500 ml for a one liter solution) and then bring the solution up to the final volume. This is the correct way to make any solution, be it a mixture of different liquids or of a solid compound dissolved in a solvent.

Molar Solutions

The most common types of solutions are those whose concentration is described in terms of *molarity*. For example, a 1 M solution describes a solution that contains 1 mole of solute for every liter of solution. A 1 mM solution has 1 mmol (10^{-3} mole) of solute in every liter of solution. A 1 micromolar (μM) solution has 1 micromole (10^{-6} mole) of solute for every liter of solution.

Remember that the number of moles present in any given amount of a pure substance is calculated by dividing the number of grams of the substance by its formula weight (in grams per mole). For example, if the formula weight of compound "X" is 39 g/mole then one mole of compound "X" weighs 39 g. If we have 13 g of "X", we can calculate the number of moles present by dividing by the formula weight:

$$13 \text{ g} \div 39 \text{ g/mole} = 0.33 \text{ mole (notice how units cancel)}$$

If we then take the 13 g of "X" and bring it up to a volume of 0.5 L with 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, μM , nM and so on, still refer to an amount (moles, millimoles, micromoles, etc) divided by **liters** of solution. Many students mistakenly think, for example, that a 1 mM solution refers to a solution of 1 mmol of a substance dissolved in every *milliliter* (ml) of solution. What it actually refers to is 1 mmol of a substance in every *liter* of solution.

Dilutions

Performing calculations for dilutions seems to be one of the harder concepts in the chemistry laboratory. When making a dilution, you always start with a particular volume of a more concentrated, or stock, solution and add more solvent to it, until you make a solution with the desired volume and (lower) concentration. There is a simple way of performing the calculation for determining how much of a stock (concentrated solution) will be needed to make a particular volume of a diluted solution. You should memorize it by heart:

$$C_1V_1 = C_2V_2$$

where:

C_1 = concentration of the stock solution

V_1 = volume of the stock solution to be used in making the dilution

C_2 = concentration of your diluted solution

V_2 = volume desired for your diluted solution

To solve for any of these variables, you will **need values for the 3 other variables**. A typical use of this problem will be to calculate the volume needed of a stock solution (say 1M) to prepare a particular volume (say 100 ml) of a diluted solution with a desired

concentration (say 0.1M). In such a cases, you know the volume and concentration of your desired solution (100ml, 0.1M) and you know the concentration of your stock solution (1M). Your task will be to calculate the *volume* (V_1) of stock solution required. This equation works for any units of concentration and volume, *provided the units are the same on both sides of the equal's sign*.

for example:

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

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

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

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

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

$$C_1 V_1 = C_2 V_2$$

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

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

$$V_1 = 10ml$$

This tells us we need 10 ml of the 1 M stock HCl solution, diluted to a total volume of 100 ml (i.e add 90 ml of water) to give the solution with the desired concentration and volume.

Another example:

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

$$C_1 \text{ (stock solution)} = 100 \text{ mg/ml}$$

$$C_2 \text{ (final solution)} = 1 \text{ mg/ml}$$

$$V_2 \text{ (final volume)} = 10 \text{ ml}$$

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

$$V_1 = \frac{(1mg / ml)(10ml)}{(100mg / ml)}$$

$$V_1 = 0.1ml$$

Therefore, take 0.1 ml of the 100 mg/ml glucose solution and add it to (10 – 0.1 = 9.9 ml) of water to make 10 ml (final volume) of a 1 mg/ml (final concentration) solution

Hint: If you need to make a dilution, 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 protein, prepare 1 ml of 0.02 mg/ml protein solution.

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

$$V_1 = \frac{(0.02 \text{ mg / ml})(1 \text{ ml})}{(100 \text{ mg / ml})}$$

$$V_1 = 0.0002 \text{ ml}$$

This is a unique situation, where a very small quantity (0.0002 ml) is called for. We cannot pipet 0.0002 ml (0.2 ul) accurately. To more accurately perform this dilution, we perform a stepwise dilution. That is, we would make an initial dilution (10-100 fold) from our concentrated stock solution and then make an additional dilution to produce a solution with our desired final volume and concentration. In this manner, both dilutions involve the transfer of reasonable volumes from the solution to be diluted. For instance, we might first dilute the stock (100 mg/ml) glucose solution 100-fold to produce 10 ml of an initial diluted solution with a concentration of 1 mg/ml:

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

0.1 ml is a small, but reasonable volume to transfer. We place 0.1 ml of the 100 mg/ml stock solution into a container and add (10 mL-0.1 ml) = 9.9 ml of solvent to produce 10 ml of 1 mg/ml protein. Now we perform an additional dilution, on this initially diluted solution, to prepare 1 ml of the solution with the concentration we desired in the first place:

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

Thus, we add (1.0 ml – 0.02 ml) = 0.98 ml of solvent to a tube and add 0.02 ml (20 ul) of the 1 mg/ml diluted protein solution to obtain the desired solution with a concentration of 0.02 mg/ml.

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

Sometimes we want to transfer a fixed amount of solute by moving **an amount of solution in which it is dissolved**. The amount of solution to transfer, which contains the amount of solute desired, say 1 mg, is simply calculated with the solution-dilution equation. Notice that the product “CV” in the solution-dilution equation is an amount: Concentration (equal to

an amount/volume) x Volume = amount. So, if we want to transfer an amount we make it equal to the product C_2V_2 .

An example:

How much of a 0.2 mg/ml stock insulin solution should we use to provide a patient with 8 micrograms (ug) of insulin?

Solution - Dilution equation: $C_1V_1 = C_2V_2$

C_1 is 0.2 mg/ml, C_2V_2 is the *amount* desired = 8 ug. The question becomes: what is V_1 ?

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

we want our final answer in micrograms (ug). Convert the 0.2 mg/ml term to "ug/ml", i.e. $0.2 \text{ mg/ml} \times 1000 \text{ ug/mg} = 200 \text{ ug/ml}$. The solution-dilution equation 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 the use of this equation until you can do it in your sleep.