

Making Dilutions

Katherine Dorfman, UMass Biology Department, 2019

It is often very important to know the precise concentration of some chemical you are using in your experiment. Various units of concentration are used in biology and chemistry:

Molarity (M) = moles/Liter

mg/mL = g/L

% w/vol = g/100 mL (because 1 mL of water weighs 1 g)

% vol/vol = mL/100 mL

Sometimes you can start from scratch, that is, weigh out the substance and dissolve it in the appropriate amount of solvent (usually water for biological applications).

What if I need 10 mL of 5M NaCl (MW = 58)? You can calculate how much NaCl to dissolve in 10 mL water this way (notice how all the units but g cancel out):

$$\frac{5\text{mol}}{L} \times \frac{58\text{g}}{\text{mol}} \times 10\text{mL} \times \frac{1L}{1000\text{mL}} = 2.9\text{g}$$

What if I need 1 mL of 5mM NaCl?

$$\frac{5\text{mmol}}{L} \times \frac{1\text{mol}}{1000\text{mmol}} \times \frac{58\text{g}}{\text{mol}} \times 1\text{mL} \times \frac{1L}{1000\text{mL}} = 0.00029\text{g}$$

Unfortunately, 0.00029 g is an almost imaginary amount of sodium chloride. Even if you made 10 times what you needed, you'd have to weigh out 0.0029 g, and most of our prep room balances only report three decimal places. You could make a liter of 10mM NaCl with 0.29 g, because sodium chloride is cheap to buy and legal to pour down the drain, but many reagents are much harder to come by and dispose of, so we need other ways of making solutions.

If you already had your 10 mL of 5 M NaCl, you could make your 1 mL of 5 mM NaCl by dilution, that is, by taking a small volume of your 5 M NaCl and diluting it in water. To calculate how, we use the lab preparer's best friend:

$$c_1v_1 = c_2v_2$$

c_1 = the concentration of the initial solution used to make the more dilute solution

v_1 = the initial small volume of the first solution used to make the dilute solution

c_2 = the concentration of the second, more dilute solution

v_2 = the final volume of the second solution after the dilution is carried out

So we need to figure out what volume (v_1) of the 5 M (c_1) solution to use to make 1 mL (v_2) of a 10 mM NaCl (c_2) solution.

$$v_1 = \frac{c_2v_2}{c_1} = \frac{5\text{mM} \times 1\text{mL}}{5\text{M}} \times \frac{1\text{M}}{1000\text{mM}} = 0.001\text{mL}$$

2 Making Dilutions

Another way to look at this is to calculate the dilution factor, that is, the ratio between the initial and final concentrations. Diluting a 5M solution to a 5mM solution is a 1000-fold dilution:

$$\frac{c_2}{c_1} = \frac{5mM}{5M} \times \frac{1M}{1000mM} = \frac{1}{1000}$$

Therefore, you need 1 part stock solution to make 1000 parts of your final solution.

$$v_1 = \frac{c_2}{c_1} \times v_2 = \frac{1}{1000} \times 1mL = 0.001mL$$

Fortunately, I have a micropipettor that can deliver 0.001mL (=1 μ L). So I can make the dilute solution by mixing 0.001mL of my concentrated solution with 0.999 mL of water.

What if I don't really trust¹ my pipettor down in the single μ L range?

Worse, what if my concentrated sodium chloride stock solution is 1M, rather than 5M? The initial volume would be 0.0002 mL (=0.2 μ L), and I *really* don't have a pipettor I trust to deliver that small a volume.

I can solve the problem of a v_i too small to deliver by using a *series* of dilutions, each transferring a volume I *can* accurately deliver, to achieve my intended volume and concentration. For this procedure, I use a modification of $c_1v_1=c_2v_2$:

$$c_1v_i = \left(\frac{c_1}{d}\right)(v_f + v_i)$$

c_1 = the concentration of the initial solution used to make the next dilution in the series

v_i = the transfer volume

d = the dilution factor (*e.g.*, 2 for a 2-fold dilution series, where the concentration of each solution in the series is half that of the previous one)

$c_2 = \frac{c_1}{d}$ = the concentration of the second solution in the series

v_f = the desired final volume of each solution

$v_f + v_i$ = the initial volume of a diluted solution, until the transfer volume is removed to make the next dilution in the series

This procedure is illustrated in Figure 1.

¹ When I say I don't trust the pipettor, I mean that I worry about tiny errors in delivery representing a large percentage of the volume I'm transferring.

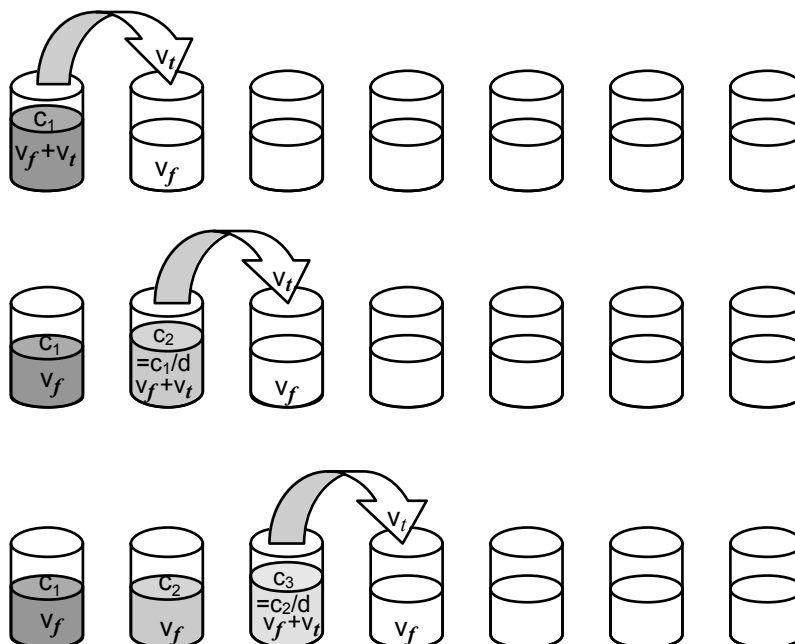


Figure 1. A serial dilution. A small volume (v_t) of solution is transferred to a vessel containing a volume (v_f) of solvent. After these are mixed, v_t of the second solution is transferred to the third vessel, also containing v_f of solvent. The concentration of each solution is $1/d$ the concentration of the previous one in the series.

So if I didn't think I could trust a $1\ \mu\text{L}$ transfer volume, I might try a 10-fold serial dilution ($d = 10$) (both because calculations with 10 are easy, and because I want the last dilution in my series to be one one-thousandth of the initial concentration). Suppose that the $1\ \text{mL}$ volume in the example above was more than enough for my experiment. An easy 10-fold dilution series could be done with a $0.1\ \text{mL}$ transfer volume, as follows:

Starting with the general equation:

$$c_1 v_t = \left(\frac{c_1}{d}\right)(v_f + v_t)$$

Substituting the initial concentration, the dilution factor, and the transfer volume:

$$1\text{M} \times 0.1\text{mL} = \left(\frac{1\text{M}}{10}\right)(v_f + 0.1\text{mL})$$

Solving for v_f :

$$\left(1\text{M} \times 0.1\text{mL} \times \frac{10}{1\text{M}}\right) - 0.1\text{mL} = v_f = 0.9\text{mL}$$

So by starting with $1\ \text{mL}$ ($v_f + v_t$) of my 1M (c_1) solution, and transferring $0.1\ \text{mL}$ (v_t) of it to $0.9\ \text{mL}$ (v_f) of solvent gives me $1\ \text{mL}$ ($v_f + v_t$) of 0.1M ($c_2 = \frac{c_1}{d} = \frac{c_1}{10}$) solution.

Repeating this process will give me a series of solutions, each one-tenth the concentration of the one before it.

What if I don't know what concentration range of a particular substance is suitable for use in my experiment or in my measuring device? You need to test a wide range of concentrations. Serial dilution is the best way to achieve such a range. With a 2-fold dilution series of 10, you can take a solution down to less than a 1000th of its original value:

$$\frac{1}{2^{10}} = \frac{1}{1024} = 0.000977$$

With a 10-step 10-fold dilution series, you can get down to 10⁻¹⁰ (less than a billionth!) of the original concentration.

What if the manufacturer gives me exactly 1 mg of the chemical? You can take advantage of the manufacturer's precision by dissolving the chemical, still in its original package to prevent loss of material, in a small, known volume of suitable solvent (which the package insert should identify for you), thereby making a stock solution of known concentration (*e.g.*, 1 mg/mL). From that, you can make whatever dilute concentrations you need.