CHAPTER 4

Solutions

Good solution making is an important skill for scientists because having properly made solutions is critically important to the success of any experiment. Solution making is also one of the least glamorous of laboratory tasks, and it is frequently passed to someone else. *However*, unless you are absolutely sure that your solution maker is skilled with mathematics, not making your own solutions can be very risky. An incorrectly made solution can ruin an experiment. Furthermore, when something does go wrong with an experiment, determining whether the solution is the source of the problem can be arduous.

The calculations involved in making (and unmaking) solutions have confused and frustrated legions of science students and have given rise to vast piles of calculation-covered paper scraps that radiate a desperate hope of stumbling across numerical revelation. Calculations lurk in almost every step of solution making—creating a recipe from a list of concentrations; calculating moles and molarity from masses, formula weights, and volumes; measuring accurately and determining the precision of the measurement; calibrating and using a pH meter; making aliquots; centrifuging; and diluting.

This chapter addresses the mathematical hurdles of solution making in two ways. First, there are short explanations and reminders of what you are doing when you are making a solution and why you are doing it. Second, there are "How to Quickly" shaded boxes. If you do not have time, at the moment of truth, to understand the rationale for the math that you are doing (you will come back later and learn, of course), you can learn "How to Quickly" do the necessary calculations to get on with your work.

TALKING ABOUT SOLUTIONS

Acid: The classical (Arrhenius) definition of an acid is a molecule that dissociates in water to yield H_3O^+. The Brønsted–Lowry definition of an acid is a molecule that can donate (release) a proton in the form of H^+.

Acid Dissociation Constant, K_a: K_a is the equilibrium constant of an acid:

$$K_a = \frac{[H]^+[OH^-]}{[H_2O]}.$$

If $K_a > 1$, the acid is strong; that is, it is likely to donate a proton readily. If $K_a < 1$, the acid is weak; that is, it is less likely to donate a proton.

Base: The classical (Arrhenius) definition of a base is a molecule that dissociates in water to yield OH^-. The Brønsted–Lowry definition of a base is a molecule that can accept a proton in the form of a hydrogen atom (H^+).

Buffer: A buffer is a weak acid that is added to a solution to prevent changes in the pH when a small amount of a strong acid or base is added. Buffers work by accommodating the added acid or base with changes in the relative concentrations of the weak acid and its conjugate base (i.e., the concentration of buffer molecules that can donate protons and the concentration that can accept protons).

cc: cc stands for "cubic centimeter." 1 cc = 1 mL. Use "mL," not "cc."

Diluent: The diluent is the medium or solvent added to a concentrated solution to dilute it.

Diluting 1: X: Recipes for solutions sometimes contain directions for diluting a stock solution according to a certain ratio. To read those directions, you need to know the following. A 1:X dilution means that your concentrated solution should be diluted to $1/X$th its current concentration. Add 1 volume of concentrate to $(X - 1)$ volumes of diluent to create a total volume equal to X. Looking at it yet another way, in the final solution what you diluted will be $1/X$-th of the total volume. Therefore,

1:100 means 1 part concentrate, 99 parts diluent;
1:14 means 1 part concentrate, 13 parts diluent;
1:2 means 1 part concentrate, 1 part diluent;
1:1 means straight concentrate.

Note: This is the original convention; however, it is not always followed. If you see "1:9," chances are that the author means 1:10 and the recipe should be checked. This is particularly true if you see "dilute 1:1" in a recipe; chances are that the author means 1:2. You must use your judgment. This confusion is one reason why it is always better to report starting and final concentrations explicitly.

λ: λ is an abbreviation for microliter (μL) and the symbol for wavelength. The context around the use of λ indicates which meaning is applicable to the situation.

Medium: A medium is a solution. The plural is media. In biology, the word "medium" is typically reserved for solutions made specifically to feed cells.

Meniscus: The surface of the liquid in a thin cylindrical container is concave, and the rounded surface is called the meniscus. Glassware is calibrated so that the correct volume is measured when the bottom of the concave meniscus lines up with the volume marking.

Molality: Molality is the number of molecules per *mass* of solvent. This is in contrast to molarity (see below), which is the number of molecules per *volume* of solvent. To measure molality, you only need to measure mass; thus, there is less uncertainty in the measurement than if you had to measure mass and volume. Still, this measure is not used much anymore.

Molar: Molar is an adjective describing molarity. For example, a bottle is labeled 1 M $MgCl_2$; the solution is "one molar magnesium chloride," which means that the molarity of $MgCl_2$ in the solution is 1 mol of $MgCl_2$ per liter of solution.

Molarity: The molarity of a solution is the moles of solute per liter of solvent. Molarity is an SI unit, and the symbol is M. If there is more than one component in a solution, the molarity of each is given separately. For example, a solution might be 1 M NaCl (58.43 g/mol; so the solution has 58.43 g of NaCl per liter of solution) and 0.5 M $CaCl_2$ (110.98 g/mol so the solution has 55.49 g of $CaCl_2$ per liter of solution).

Mole: Mole is an amount: 6.022×10^{23} of molecules of a substance. One mole of a substance has a mass equal to its molecular weight in grams. For example, HEPES has a molecular weight of 238.3; 1 mol of HEPES "weighs" (has a mass of) 238.3 g. Mole is the SI unit for amount of substance. It is abbreviated "mol."

Percent: Percent is a dimensionless parameter meaning per one hundred (as in per centum); 37% of $A = 37/100 \times A = 0.37A$.

Percent Volume per Volume (v/v) or Weight per Weight (w/w): The amounts of ingredients in a solution are sometimes described as a percentage of the total solution. To read those recipes, you must know the following. If the units are the same (e.g., w/w or v/v), the percentage is what you expect based on the definition of percent:

$$100\% = (1\,\text{g})/(1\,\text{g});$$
$$1\% = (10\,\text{mL})/\text{L}.$$

Percent Weight per Volume: This is one of those cases in which "weight" really means "mass." Percent mass per volume (abbreviated % w/v) is based on a convention that is the mass/volume of pure water: 1 mL of water has a mass of 1 g; hence, 1g/mL = 100%. It works as follows:

$$100\% = (1\,\text{g})/\text{mL};$$
$$10\% = (100\,\text{mg})/\text{mL};$$
$$1\% = (10\,\text{mg})/\text{mL}.$$

If you just see % without any units such as w/v, you can assume that it means w/v.

pH: pH is used to quantify the acidity of a solution. The "p" indicates that you take the negative log of what follows, which is H, signifying the concentration of protons. pH is therefore a measure of the concentration of protons, or H^+, in a solution. As the number of protons in solution increases, the magnitude of the pH decreases; thus, the lower the pH of a solution, the more acidic it is.

Q.S.: Q.S. stands for "quantity sufficient" and means "add enough solvent to bring the total volume to . . . ." For example, "Q.S. to 500 mL" means "Whatever volume you have now, add enough solvent to bring the total volume to 500 mL." To Q.S. a solution properly, it must be measured in an appropriately calibrated vessel (i.e., a graduated cylinder, not a beaker).

Reagent: A reagent is a compound or solution that will go into your reaction mixture.

Solute: The solute is the dissolved, or dispersed (as opposed to the liquid), phase of a solution; it is what you mixed in.

Solution: A solution is a homogeneous mixture (usually liquid) of two or more substances; one or more solutes dissolved in solvent. The solutes can be solid, liquid, or gas; the solvent can be liquid or gas.

Solvent: The solvent is the dispersing (i.e., liquid) phase of a solution, the substance into which a solute is dissolved—what you mixed stuff into.

NUMBERS FOUND ON CHEMICAL BOTTLES

The first item that you are likely to pick up when you begin to make a solution is a bottle of some chemical. Important numbers are found on the labels of chemical bottles and in chemical company catalog listings. Many of the

numbers included on chemical bottles have obvious meanings, and others do not; but each provides information that may be extremely useful. Below is a list of numbers typically reported on chemical containers and why you want to know them.

Activity and Unit Definition: Activity and unit in this context apply to enzymes and tell you how much activity to expect from an enzyme and what the units of that activity mean. For example, alcohol dehydrogenase can be purchased with the activity 300–500 units per milligram protein, where 1 unit will convert 1.0 µmol of ethanol to acetaldehyde per minute at pH 8.8 at 25°C. In this case, the activity is a rate.

Formula Weight (FW): This tells you the mass in grams per mole of the chemical as listed in the formula on the label; in this context, FW is being used interchangeably with molecular weight. If it is a hydrated compound, the FW includes the mass of the water. For more on this topic, including FW versus MW, see Chapter 2.

Lot Number: It is a good idea to record the lot number of a reagent in your laboratory notebook, especially when using reagents that may vary significantly from batch to batch, such as antibodies or enzymes. Having the lot number easily accessible can be very useful for troubleshooting your experiments. It may be a reagent, not you, that causes an experiment to go awry. When you call a company to ask if a batch of enzyme is known to have been contaminated or is of low activity, for example, technical support at the company will need to know the lot number.

Product Number: This is the company catalog number, and it is very useful for correct reordering, particularly when a chemical is available in several forms. (The compound may be available in a hydrated form or at a different concentration or purity.)

Purity: Sometimes trace amounts of by-products of the method used to isolate a chemical compound cannot be entirely eliminated. Purity is a measure of, well, the purity of a chemical; that is, it tells you how much is not contamination. Purity is typically represented as a percent: the percentage of what you bought that is actually what you want.

Risk and Safety Numbers: These numbers refer to lists found in the catalogs of companies that sell chemicals. They are like mini–Material Safety Data Sheets (MSDS); but the MSDS is the only reliable source for complete safety information. MSDSs are available free online at a number of sites, as listed in Resources at the end of this chapter.

Storage Temperature: This tells you the temperature at which to store a reagent to avoid problems like loss of activity or decay. Pay attention to the

storage temperature (and expiration dates) of chemicals, because chemicals really can go bad.

−70°C	means keep it in the ultracold freezer (usually − 70°C to − 80°C);
−20°C	means keep it in the deep freeze;
−20°C−0°C	means keep it in the freezer compartment of the refrigerator;
0°C	means keep it in the freezer compartment of the refrigerator;
2°C−8°C	means keep it in the refrigerator;
RT	means keep it at room temperature.

In addition, watch labels for notes indicating whether a chemical should be stored desiccated or in the dark.

MOLES AND MOLARITY

Moles and molarity are both units (see Chapters 1 and 2). Mole is the SI unit for the dimension "amount of a substance" (symbol for dimension: N), and molarity is the SI unit for concentration, where concentration is the amount of a substance per liter of solution (dimension NL^{-3}). Therefore, the number of molecules of a chemical is counted in moles, and the concentration of molecules in a solution is quantified as the molarity.

The word *mole* is like the word *dozen* in that it is a special name for a particular number. *Dozen* is a name for the quantity 12; *mole* is a name for the quantity 6.022×10^{23}. The 6.022×10^{23} goes one better than 12 in that it actually has *two* special names. One of the names, at the risk of being repetitive, is "mole" and the other is "Avogadro's number" (in honor of Amedeo Avogadro, who in 1811 was the first to distinguish between atoms and molecules, and who stated that equal volumes of all gases at the same temperature and pressure contain the same number of molecules, now known as "Avogadro's principle"). When you talk about moles of a chemical, you are talking about the number of smallest indivisible units of that chemical, that is, the number of molecules of that chemical. A mole of a chemical is 6.022×10^{23} molecules, just like a dozen eggs is 12 "smallest indivisible units of eggness."

Why people talk about dozens of items goes way back and may have to do with the 12 cycles of the Moon per cycle of the Sun. Why people talk about moles of chemicals is a bit more subtle: One mole of a chemical has a mass equal to the molecular weight; put the opposite way, the molecular weight of a molecule is the mass of 1 mol of that molecule. To figure out how many moles of a chemical you have, you measure the mass of the chemical

(using a balance) and then divide that mass by the molecular weight of the chemical (which is listed as the FW on the label). The result is the number of moles you have. For example, HEPES has an FW of 238.3; 1 mol of HEPES weighs ("has a mass of") 238.3 g. If you weigh out 327.4 ± 0.8 g of HEPES on your balance and want to know how many moles that is, you simply divide the amount you have weighed out by the weight of 1 mol: $(327.4 \pm 0.8\,\text{g}) \div (238.3\,\text{g/mol}) = 1.374 \pm 0.003\,\text{mol}$ of HEPES.

The molarity of a solution is the moles of solute per liter of solution. Molarity is the SI unit of concentration, and saying "molarity" is the same as saying "moles of solute per liter of solution." The symbol for molarity is an uppercase M. Molar is an adjective describing molarity. For example, a bottle is labeled 1 M $MgCl_2$; the solution is "one molar magnesium chloride," which means that there is 1 mol of $MgCl_2$ per liter of solution (or 1 mmol of $MgCl_2$ per milliliter of solution, or 1 µmol per microliter, etc.). Another way to say it is "the molarity of the solution is 1." If there is more than one solute in a solution, the molarity of each is given separately.

MAKING SOLUTIONS

Making Solutions Using Dry Chemicals

GENERAL WARNING Sometimes recipes will say that upon completion a solution will be of a certain pH. But qualities such as the pH of the distilled water used to make solutions can vary from laboratory to laboratory or even day to day; therefore, you cannot blindly count on the final pH of your solution to be as predicted. It is important to check that the solution has actually achieved the desired pH. Many factors can alter your ultimate pH and, ultimately, ruin your experiment. It is so easy to check pH—you should always do it.

Solution making most typically involves dissolving a dry chemical into water or other specified solvent. The amount of chemical you add to a solvent depends on the final concentration (molarity) you want in the finished solution and the total amount (liters) of solution you have decided to make. The easiest way to measure chemicals, however, is by mass, because mass is what laboratory balances report (for more regarding balances, see Chapter 2). Therefore, to make a solution, typically you must determine the mass of chemical that you need, based on knowing the desired final concentration (usually molarity), the molecular weight of the chemical, and the final volume of solution. Here is how to do that.

When making any solution, you should start by putting ~80%–90% of the total volume of solvent into your mixing vessel and then add the chemicals one by one. Once all the chemicals have been added and your solution has been brought to the correct pH, transfer your almost finished solution to a graduated cylinder; then Q.S. (see page 106) to the final total volume. You do it that way because the chemical you are adding, and any pH adjusting, will contribute some volume to the solution and you do not want to overshoot the desired final total volume inadvertently.

METHOD Planning a Solution of a Particular Molarity

One simple way to plan the making of a solution from a dry chemical is as follows:

1. Figure out the mass that you would need if making a full liter.
2. Figure out what fraction of a liter you are making.
3. Use that same fraction of the mass of the chemical.

Example

This example shows how to figure out a recipe step by step. At the end of this example is the "How to Quickly" that you can use for reference to get on quickly with making your solution. Say you want 100 mL of a 5 M stock solution of calcium chloride, $CaCl_2$. The MW of $CaCl_2$ is 111.0 g/mol.

1. Determine the amount of grams of $CaCl_2$ that would go into 1 L by multiplying the number of moles in 1 L times the molecular weight of the compound:

   $5.00\,\text{M} = 5.00\,\text{mol/L};$

   $5.00\,\text{mol} \times 111.0\,\text{g/mol} = 555.0\,\text{g}.$

2. Figure out what fraction of 1 L you are making by dividing the desired volume by 1 L:

   $$\frac{(100\,\text{mL})}{(1000\,\text{mL})} = 0.1.$$

3. Multiply the fraction of a liter by the number of grams to make 1 L of solution of the same molarity (0.1 × 555.5 g):

   $0.1 \times 555.0\,\text{g} = 55.5\,\text{g}.$

To make 100 mL of a 5 M solution of $CaCl_2$, dissolve 55.5 g into ~80–90 mL of water and then Q.S. to 100 mL. Written as one expression, this looks like

$$5.00\,\text{mol} \times \frac{(110.0\,\text{g})}{\text{mol}} \times \frac{(10^{-1}\text{L})}{\text{L}} = 55.5\,\text{g}.$$

This expression can be simplified (which is always good). First, notice that the second term is the molecular weight of the chemical; therefore, substitute MW. Second, notice that the third term can be written as 10^{-1} L × L^{-1}. Because multiplication is commutative, the "× L^{-1}" can be moved over and combined with the first term; that makes the first term 5.00 mol/L, which, you may notice, is the desired molarity, 5.00 M. Thus, the above equation simplifies to

$$5.00\,\text{M} \times \text{MW} \times 10^{-1}\text{L} = 55.5\,\text{g},$$

or the desired molarity (mole/liter) times the molecular weight (gram/mole) times the desired volume (liter) equals the grams of chemical required.

DON'T FORGET

When you are actually making up the solution, put only ~80%–90% of the total volume of solvent into the beaker before adding the chemicals. (How much will become clear once you have made the solution a couple of times; you want the volume you must add at the end to have a minimal possible effect on pH.) Once the chemicals have been added and dissolved and the solution pH'd, only then should you transfer the contents to a graduated cylinder and Q.S. to the final total volume.

HOW TO QUICKLY CALCULATE THE GRAMS OF CHEMICAL NEEDED FOR A SOLUTION OF PARTICULAR MOLARITY

Plug your numbers into the following equation (calculate individually for each solute):

$\text{M} \times \text{MW} \times V = \text{g}$.

Add chemicals to 80% of volume then Q.S. to the final volume.

M = final molarity of compound (mole/liter);
MW = molecular weight of compound (listed on the bottle as FW)(g/mol);
V = desired final volume (L);
g = grams of compound to add to solvent (g);
Q.S. = Add sufficient solvent to bring the volume (to the final volume).

See also Plug and Chug in Chapter 8.

MAKING SOLUTIONS FROM HYDRATED COMPOUNDS

Some chemicals come with water molecules attached. First, remember that the MW (listed as FW on the bottle) of such a compound includes the mass of the water; whenever you would have used the MW of the unhydrated compound in the equations above, use instead the MW of the hydrated compound. If you have a recipe that tells you how many grams of the unhydrated compound to use, figure out the target concentration (see "Converting Recipes to Concentrations" below) and then calculate the amount of grams of hydrated compound to use.

Second, when using a hydrated compound, remember that the attached water molecules contribute water to the solution, potentially diluting the final concentrations (if the solvent is water). Therefore, you must account for the contribution of water from the hydrated compound when determining the volume of solvent (water) to add.

Example 1

1. You can buy sodium phosphate as $NaH_2PO_4 \cdot H_2O$ (sodium phosphate monobasic, FW = 137.99).
2. If you want the molarity of the NaH_2PO_4 to be 0.5 M in a liter, weigh out 68.99 g (i.e., 0.5 × 137.99) and add it to your solution.

   Here's the catch: For every 0.5 mol of $NaH_2PO_4 \cdot H_2O$ that you add to the solution, you are adding 0.5 mol of sodium phosphate *and* you are adding 0.5 mol of water. One mol of water takes up 18.015 mL. Just by adding 68.99 g of compound, you have added 9.008 mL of solvent; you need 991 mL (100 – 9.008) more to reach 1 L.

Example 2

1. You can buy sodium phosphate hydrated with 12 waters: $Na_3PO_4 \cdot 12H_2O$ (sodium phosphate tribasic, FW = 380.12).
2. If you want the molarity of Na_3PO_4 to be 0.5 M in a liter, weigh out 190.06 g of $Na_3PO_4 \cdot 12H_2O$ (0.5 × 380.12) and add it to your solution.

   If you are using this compound, for every 0.5 mol of sodium phosphate that you add, you are adding 6 mol of water, which has a volume of 108.09 mL. To Q.S. to 1 L only requires 891.91 mL (1000 – 108.09).

The moral of these stories is that you must know in advance what volume of water will be contributed by the hydrated compound, and you must accommodate that volume. One way to accommodate the volume of water contributed by a hydrated compound is to calculate exactly what volume of water you will be adding when you add the hydrated compound and then subtract that volume from the volume of water you planned to add. Using the second of the above examples, if you wanted 100 mL of 0.5 M Na_3PO_4, you could calculate that if you use the $Na_3PO_4 \cdot 12H_2O$, you will be adding 10.81 mL of water (1/10th the volume of 6 mol of H_2O calculated above) along with the compound; so you will only need to add 89.19 mL (100 – 10.81) more water. The steps of that calculation are as follows:

1. Multiply the desired molarity times the desired volume. This tells you the number of moles of the compound that you will be adding.
2. Multiply the number of moles of the compound times the number of moles of H_2O in the compound. The result is the number of moles of H_2O that you will be adding.

3. The number of moles that you are adding, times 18.015, tells you the number of milliliters of water that you will be adding.
4. Subtract the number of milliliters that you are adding from the total volume.

HOW TO QUICKLY CALCULATE WATER CONTRIBUTED BY A HYDRATED COMPOUND

Plug your numbers into the following equation:

$\text{M} \times V \times \#H_2O \times 18.015 = \text{mL}\ H_2O$ contributed by hydrated compound.

Calculate individually for each hydrated solute.

M = desired molarity of compound (mol/L);
V = desired final volume of solution (L);
$\#H_2O$ = number of H_2O molecules in the hydrated compound;
18.015 = milliliters per mole of H_2O (mL/mol).

See also Plug and Chug in Chapter 8.

The calculation to figure out how much water you are adding is useful for making sure that your measuring container does not overflow. The easier way to deal with hydrated compounds is to follow the directions for making any solution: Start with less than the total volume of solvent, mix in the chemicals, transfer it all to a graduated cylinder, and Q.S. to the final volume. When working with hydrated compounds, however, you must be particularly careful regarding how much you start with. It is a good idea to do the above calculation, subtract the volume that will be added by the hydrated chemical from the total volume, and then start with 80%–90% of the difference that you just calculated. This will ensure that you leave enough room. Again, with experience, you will know how much to start with.

HOW TO QUICKLY MAKE A SOLUTION USING A HYDRATED COMPOUND

1. Calculate about how much water will be needed after the hydrated compounds are added using How to Quickly Calculate Water Contributed by a Hydrated Compound: $\text{M} \times V \times \#H_2O \times 18.015 = \text{mL}\ H_2O$ contributed. Subtract that many milliliters from the desired final volume (V).
2. Put 80% of the resulting volume of H_2O into a mixing vessel.
3. Add your compounds. Mix well.
4. Transfer the solution so far to a graduated cylinder.
5. Q.S. to the desired final volume in a graduated cylinder.

MAKING STOCK SOLUTIONS

In many laboratories, or for particular experiments, certain solutions are used frequently and are therefore made up in large quantities. To minimize the volume actually occupied by these solutions, they are often made at a higher concentration than that which will be used. These concentrated solutions are referred to as stock solutions. Stock solutions save time, in addition to space, because when you need a solution stored as a concentrated stock, you need only to dilute the stock; you do not have to start from scratch. Some commonly encountered stock solutions are: 10× TBE (in laboratories that run a lot of gels), 10× SSC (in laboratories that do lots of hybridizations), and 10× PBS (in laboratories that do just about anything with cells).

Making a stock solution is just like making any solution; you simply scale up the concentration of all of the ingredients. Typical stock solutions are 2×, 5×, and 10×, meaning double the normal concentration, five times the normal concentration, and 10 times the normal concentration. The upper limit to the concentration of a stock solution is set by the solubility of those chemicals in that solvent.

It is important to be very careful not to contaminate a stock solution. If a stock solution gets contaminated, you *must* dispose of it *immediately*. If you do not, you *will* mess up experiments for what will feel like an eternity. To avoid contaminating a stock solution, *never* put *anything* into its container—no pipettes, nothing. Always pour some stock out into a second, clean container, and then take what you need from there. This is just being considerate to others and preventing headaches for yourself.

Diluting a Stock Solution to a Particular Concentration

Having concentrated stock solutions available in the laboratory is a great time-saver if everyone who will be using them is comfortable diluting. The following instructions make diluting easy. The section begins with an explanation, and ends with a How to Quickly.

If you know what concentration of a solution you want and need to determine how much of a concentrated stock solution to dilute, use the following equation:

$$\frac{(\text{Concentration you want})}{(\text{Concentration you have})} \times \text{final volume} = \begin{array}{c}\text{volume of concentrated stock to} \\ \text{add to mixture.}\end{array}$$

You may recognize this as a rearrangement of $M_1V_1 = M_2V_2$, where M = molarity and V = volume. You can use the above equation to calculate everything but the solvent volume, add the stock to 80%–90% of the final volume of solvent, pH, and then Q.S. with solvent to the final volume.

Example

You want 25 mL of the following solution:

0.5 M $CaCl_2$, 1.0 M $MgSO_4$.

You have the following stock solutions:

5.0 M $CaCl_2$;
2.5 M $MgSO_4$.

Step 1. Figure out how much of the $CaCl_2$ stock to add:

$$\frac{(0.5\,\text{M})}{(5.0\,\text{M})} \times 25\,\text{mL} = 2.5\,\text{mL of } CaCl_2 \text{ stock.}$$

Step 2. Figure out how much of the $MgSO_4$ stock to add:

$$\frac{(1.0\,\text{M})}{(2.5\,\text{M})} \times 25\,\text{mL} = 10\,\text{mL of } MgSO_4 \text{ stock.}$$

Step 3. Add 80% of the final volume of water:

$$80\% \times [25\,\text{mL} - (2.5\,\text{mL} + 10\,\text{mL})] = 80\% \times 12.5\,\text{mL of water}$$
$$= 10\,\text{mL of water.}$$

Step 4. Q.S to 25 mL.

It is always a good idea to check your work. Use the method for calculating concentrations from recipes given on the following pages.

If you are interested in understanding this better, you can think of it in terms of proportions. The proportion of the final volume that is contributed by the concentrated solution:

$$\frac{\text{Volume to add}}{\text{Final volume}}$$

should be the same as the proportion of the final concentration of chemical that is contributed by the concentrated solution:

$$\frac{\text{What you want}}{\text{What you have}}.$$

That is

$$\frac{\text{Volume to add}}{\text{Final volume}} = \frac{\text{what you want}}{\text{what you have}}.$$

Now, rearrange and you have the How to Quickly.

HOW TO QUICKLY DILUTE TO A PARTICULAR CONCENTRATION

Plug your numbers into the following word equation:

$$\frac{\text{What you want}}{\text{What you have}} \times \text{final volume} = \text{volume to add to mixture.}$$

Q.S. to the final volume.

What you want = desired final concentration (M);

What you have = concentration of your stock solution (M);

Final volume = total volume of solution with final concentration (L);

Volume to add to mixture = volume of stock solution to be diluted (L).

"What you want" and "what you have" must have the same units.

See also Plug and Chug in Chapter 8.

Using Dilution Ratios

Another common method for figuring out how to dilute a solution to a particular concentration is to determine the ratio of diluted to undiluted (stock) concentrations and then convert that ratio to a fraction and simplify. That fraction of the final volume should be made of concentrated solution; the rest should be solvent.

Revisiting the above example, you want to make 25 mL of a solution that is 0.5 M $CaCl_2$, 1.0 M $MgSO_4$. The stock solutions available are 5.0 M $CaCl_2$ and 2.5 M $MgSO_4$. For $CaCl_2$, the ratio of diluted to undiluted is 0.5:5.0, which simplifies to 1:10; therefore, the 5.0 M $CaCl_2$ should make up 1/10th of the final volume. The final volume is 25 mL, 1/10th × 25 mL = 2.5 mL; therefore, you should add 2.5 mL of 5.0 M $CaCl_2$. For $MgSO_4$, the ratio is 1:2.5; therefore, the 1.0 M $MgSO_4$ should make up 1/2.5th times the final volume, which equals 10 mL. As before, 10 + 2.5 = 12.5, and 25 – 12.5 = 12.5, thus it will take 12.5 mL of solvent to Q.S. to the final volume of 25 mL. Written as a word equation, this looks like

$$\frac{[\text{Diluted}]}{[\text{Undiluted}]} \times \text{final volume} = \text{volume to add},$$

where

[Diluted] = concentration of chemical in the final diluted solution (M);
[Undiluted] = concentration of chemical in the starting stock solution (M);
Final volume = volume of diluted solution needed;
Volume to add = volume of undiluted stock solution to use.

HOW TO QUICKLY USE RATIOS TO DILUTE TO A PARTICULAR CONCENTRATION

1. Write the ratio of diluted concentration to undiluted concentration as a fraction.
2. That fraction times the total volume is the volume of undiluted stock solution to add.
3. Repeat for each stock solution to be diluted and used.
4. Q.S. to the final volume.

Diluted concentration = concentration you are working to achieve (M);
Undiluted concentration = concentration of the solution you will be diluting (M);
Final volume = volume of diluted solution that you want to make.

MAKING SERIAL DILUTIONS

If you start with a concentrated solution and dilute it, take some of that diluted stock and dilute it more; then, take some of that diluted diluted stock and dilute it some more; then take some of that diluted diluted diluted stock and dilute it some more, and so on, and you are doing a serial dilution. A series of dilutions like this is a quick and easy way to produce a solution in a variety of concentrations and is also sometimes the only way to effectively and accurately dilute an extremely concentrated solution or, often, a cell suspension.

Often, the goal of a serial dilution is to make a variety of concentrations so that you can then determine which concentration is most appropriate for the job at hand. You could make each of your dilutions separately using the following:

$$\frac{\text{Concentration you want}}{\text{Concentration you have}} \times \text{final volume} = \text{volume to add.}$$

But if you want a series of different dilutions that vary in a constant way, if the dilution you want is very dilute but you want to conserve solvent, or if you cannot measure out a small enough volume of what you have to dilute it accurately, it is easier to start with the high concentration and do serial dilutions.

The difference between each of the various concentrations that result from a serial dilution is constant. For example, each is one-half the concentration of the next highest, or each is 1/10th the concentration of the next highest.

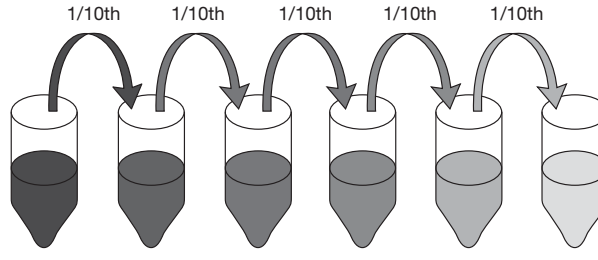
Serial dilutions are often used to dilute cell suspensions. In this case, the units of concentration are cells per milliliter (cells/mL) it works the same way. Serial dilutions are particularly well suited for cell suspensions because these suspensions are often too concentrated to dilute easily in one step.

METHOD Serial Dilution the Simple, but Imprecise, Way

If you do not have to worry about saving reagents or minimizing waste, you can do certain serial dilutions very easily. Specifically, 1:10 and 1:2 dilutions are a snap.

1:10 Serial dilutions

1. Determine how many different dilutions you want and approximately how much volume of each you need. Make ~20% more volume than you need; use containers large enough to hold ~50% more than the volume that you are making. Place the containers in a row on the bench and label them 1:10, 1:100, 1:1000, and so on; or, even better, label them with the concentrations of the solutions that they will hold.
2. Place 9/10th of the volume that you are making (i.e., 9/10ths of the volume that you need plus 20%) of solvent in each container.
3. Take 1/10th of the volume of concentrated solution or suspension that you are making and dispense it into the 1:10 container. Mix.



Dilution	1:1	1:10	1:100	1:1000	1:10,000	1:100,000
Example of concentration	5 M	0.5 M	50 mM	5 mM	0.5 mM	50 μM
Fraction of volume you are mixing that you will have at the end		9/10th	9/10th	9/10th	9/10th	10/10th

4. Take 1/10th of the volume of 1:10 solution or suspension you that are making, and dispense it into the 1:100 container. Mix.
5. Take 1/10th of the volume of 1:100 solution or suspension that you are making and dispense it into the 1:1000 container. Mix.
6. And so on.

1:2 Serial dilutions

1. Determine how many different dilutions you want and approximately how much volume of each that you need. Make about double the volume that you need; use containers large enough to hold more than double the volume that you are making. Place the containers in a row on the bench and label them 1:2, 1:4, 1:8, and so on; or, even better, label them with the concentrations of the solutions that they will hold.
2. Place the volume of solvent that you are making (i.e., double the volume that you need) in each container.
3. Take the volume of concentrated solution or suspension that you are making and dispense it into the 1:2 container. Mix.
4. Take the volume of 1:2 solution or suspension that you are making and dispense it into the 1:4 container. Mix.
5. Take the volume of 1:4 solution or suspension that you are making and dispense it into the 1:8 container. Mix.
6. And so on.

METHOD Serial Dilution the Slightly Less Simple, but More Precise, Way

If you need to be very precise with your reagents because they are either expensive or hazardous, the following steps will tell you how to make exactly the amount that you need:

1. Decide on the final volume (V_f) of each dilution that you need. (If you need to dilute to a particular concentration, see page 115.)
2. Decide on how many different concentrations (N) that you need and line up that many containers.

3. Decide on the dilution factor (X). If you are doing a first experiment to determine the appropriate concentration, a good rule of thumb is to cover a few orders of magnitude, meaning that you will be doing 1:10 dilutions. If you are doing an experiment to narrow down the concentration, do 1:2 dilutions.

4. Calculate the concentration in each container when you are finished:
   - The first will have a concentration of $\left(\frac{1}{X}\right)$ × the initial concentration (C_i), where X is the dilution factor.
   - The second will have a concentration of $\left(\frac{1}{X^2}\right)$ × the initial concentration (C_i).
   - The third will have a concentration of $\left(\frac{1}{X^3}\right)$ × the initial concentration (C_i).
   - And so on.

5. Label the containers with the final concentration that they will hold.

6. Place V_f solvent into each container.

7. Set your pipette to $\frac{V_f}{(X-1)}$.

8. Go:

   Put a new pipette tip on your pipetter or get a new pipette.

   Pick up $\frac{V_f}{(X-1)}$ of the most concentrated solution.

   Dispense it into the first vessel, the one whose label indicates that the concentration will be

   $\frac{1}{X}$ × the initial concentration.

   - Mix.
   - Change the pipette tip or pipette.

9. Pick up $\frac{V_f}{(X-1)}$ of the solution you just made. (You will be leaving behind exactly the volume you need of that dilution.)

   Dispense it into the next vessel, the one whose label indicates that the concentration will be

   $\frac{1}{X^2}$ × the initial concentration.

Mix.

Change the pipette tip or pipette.

10. Pick up $\frac{V_f}{(X-1)}$ of the solution you just made.

    Dispense it into the next vessel, the one whose label indicates that the concentration will be

    $\frac{1}{X^3} \times$ the initial concentration.

    Mix.

    Change the pipette tip or pipette.

11. And so on.

Example 1

For a 1:10 serial dilution of a 10 M (C_i) solution, you need

10 mL of each dilution ($V_f = 10$ mL);
Six different concentrations ($N = 6$);
1:10 dilutions ($X = 10$).

Note: 1:10 dilutions are the simplest to calculate because each dilution is 1/10th the concentration of the last, which means, whatever the concentration was, just move the decimal point one digit to the left to get the concentration of the next most dilute solution.

Container 1: $\frac{10\text{ M}}{10} = 1\text{ M}$;

Container 2: $\frac{10\text{ M}}{10^2} = 0.1\text{ M}\left(\text{or } \frac{1\text{ M}}{10} = 0.1\text{ M}\right)$;

Container 3: $\frac{10\text{ M}}{10^3} = 0.01\text{ M}\left(\text{or } \frac{0.1\text{ M}}{10} = 0.01\text{ M}\right)$;

Container 4: $\frac{10\text{ M}}{10^4} = 0.001\text{ M}\left(\text{or } \frac{0.01\text{ M}}{10} = 0.001\text{ M}\right) = 10^{-3}\text{ M} = 1\text{ mM}$;

Container 5: $\frac{10\text{ M}}{10^5} = 0.0001\text{ M}\left(\text{or } \frac{0.001\text{ M}}{10} = 0.0001\text{ M}\right) = 10^{-4}\text{ M}$;

Container 6: $\frac{10\text{ M}}{10^6} = 0.00001\text{ M}\left(\text{or } \frac{0.0001\text{ M}}{10} = 0.00001\text{ M}\right) = 10^{-5}\text{ M} = 10\,\mu\text{M}$.

1. Label the containers.

   #1: 1 M;

   #2: 0.1 M ($= 1 \times 10^{-1}$ M);

   #3: 0.01 M ($= 1 \times 10^{-2}$ M);

   #4: 0.001 M ($= 1 \times 10^{-3}$ M = 1 mM);

   #5: 1×10^{-4} M;

   #6: 1×10^{-5} M (= 10 μM).

2. Place 10 mL (V_f) of solvent into each container.

3. Set your pipette to

$$\frac{V_f}{(X-1)} = \frac{10\,\text{mL}}{(10-1)} = \frac{(10\,\text{mL})}{9} = 1.111\,\text{mL}.$$

4. Put a new pipette tip on your pipetter.

   Pick up 1.111 mL of the 10 M solution.

   Dispense it into the container labeled #1: 1 M.

   Mix.

   Change the pipette tip.

5. Pick up 1.111 mL of the #1: 1 M solution.

   Dispense it into the container labeled #2: 1×10^{-1} M.

   Mix.

   Change the pipette tip.

6. Pick up 1.111 mL of the #2: 1×10^{-1} M solution.

   Dispense it into the container labeled #3: 1×10^{-2} M.

   Mix.

   Change the pipette tip.

7. Pick up 1.111 mL of the #3: 1×10^{-2} M solution.

   Dispense it into the container labeled #4: 1×10^{-3} M (= 1 mM).

   Mix.

   Change the pipette tip.

8. Pick up 1.111 mL of the #4: 1×10^{-3} M solution.

   Dispense it into the container labeled #5: 1×10^{-4} M.

   Mix.

   Change the pipette tip.

9. Pick up 1.111 mL of the #5: 1×10^{-4} M solution.

   Dispense it into the container labeled #6: 1×10^{-5} M (= 10 μM).

   Mix.

   Throw away the last pipette tip.

Example 2

For 1:2 serial dilution of a 12.6 mg/mL solution, you need

150 mL of each dilution;
Three different concentrations;
$X = 2$.

Container 1: $\dfrac{(12.6\,\text{mg/mL})}{2} = 6.30\,\text{mg/mL}$;

Container 2: $\dfrac{(12.6\,\text{mg/mL})}{2^2} = 3.15\,\text{mg/mL}$ $\left[\text{or } \dfrac{(6.30\,\text{mg/mL})}{2} = 3.15\,\text{mg/mL}\right]$;

Container 3: $\dfrac{(12.6\,\text{mg/mL})}{2^3} = 1.57\,\text{mg/mL}$ $\left[\text{or } \dfrac{(3.15\,\text{mg/mL})}{2} = 1.57\,\text{mg/mL}\right]$.

1. Label the containers.

   #1: 6.30 mg/mL;
   #2: 3.15 mg/mL;
   #3: 1.57 mg/mL.

2. Put 150 mL of solvent into each container.
3. Pick a graduated cylinder that holds $\frac{150\,\text{mL}}{(2-1)} = 150\,\text{mL}$.
4. Go:

   Measure out 150 mL of 12.6 mg/mL solution.

   Dispense it into the container labeled #1: 6.30 mg/mL.

Mix.

Clean the cylinder or get an unused one.

5. Measure out 150 mL of #1: 6.30 mg/mL solution.

   Dispense it into the container labeled #2: 3.15 mg/mL.

   Mix.

   Clean the cylinder or get an unused one.

6. Measure out 150 mL of #2: 3.15 mg/mL solution.

   Dispense it into the container labeled #3: 1.57 mg/mL.

   Mix.

   Wash the glassware.

This example illustrates that when picking out your containers, you need to account for what the volume will be after the more concentrated solution has been pipetted in and before the less concentrated solution has been pipetted out. In other words, your container must hold $V_f + \frac{V_f}{(X-1)}$. If you are doing 1:2 dilutions, you must pick out a container that can hold twice the final volume. You will always end up with $V_f + \frac{V_f}{(X-1)}$ of the final dilution—another reason to use a container that is large enough.

In brief, do the calculations for your dilutions *first*, and then set up a series of *carefully labeled* containers, in order. Add the appropriate amount of solvent to the containers, and then pipette concentrate into the first container, mix, change pipette tips, pipette the newly diluted solution into the next container, mix, change pipette tips, repeat. The mixing and changing of pipette tips is not just cosmetic: If you do not do it, your concentrations will not be correct. The following is not exactly a How to Quickly, but it does summarize the process.

HOW TO DO A SERIAL DILUTION

Set up N containers with capacities to hold $V_f + \frac{V_f}{(X-1)}$.

Label them $\frac{C_i}{X}, \frac{C_i}{X^2}, \frac{C_i}{X^3}, \ldots, \frac{C_i}{X^N}$.

Put V_f solvent into each container:

Put $\frac{V_f}{(X-1)}$ of solution C_i into container $\frac{C_i}{X}$. Mix. Change the pipette.

(Continued)

(*Continued*)

Put $\frac{V_f}{(X-1)}$ of solution $\frac{C_i}{X}$ into container $\frac{C_i}{X^2}$. Mix. Change the pipette.

Put $\frac{V_f}{(X-1)}$ of solution $\frac{C_i}{X^2}$ into container $\frac{C_i}{X^3}$. Mix. Change the pipette.

Put $\frac{V_f}{(X-1)}$ of solution $\frac{C_i}{X^{N-1}}$ into container $\frac{C_i}{X^N}$. Mix. Toss the pipette.

N = number of different concentrations;
V_f = final volume of each diluted solution;
X = dilution factor;
C_i = initial concentration (M)

See also Plug and Chug in Chapter 8.

Serial Dilutions to Achieve a Particular Concentration

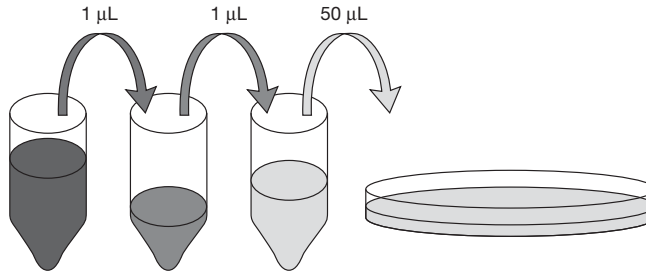
Sometimes you need to serially dilute a solution or a cell suspension to a particular concentration; in this case, you are not interested in the concentrations along the way, only the final concentration. A common scenario is diluting a cell suspension so that you can replate the cells at an optimal density.

First, you must figure out the final dilution. If that dilution is obviously a power of an integer, for example, 1:10,000 and 10,000 is 10^4, you can do four 1:10 dilutions (or two 1:100 dilutions, or 1:10 once and 1:1000 once; the sum of the exponents on the 10s must equal 4 because 4 was the exponent on the 10 of the originally calculated final dilution). More likely, each dilution will be different.

An easy way to plan each dilution is to calculate the final dilution and then write that number in scientific notation. The first dilution should be 1:(the mantissa); the rest will be 1:10 (or 1:100, etc.) dilutions. Therefore, if you calculated that the final dilution would be 1:36,850, that is 3.6850×10^4. The first dilution would therefore be 1:3.685; for example, 1 mL of suspension plus 2.685 mL of medium. Then dilute 1:10 four times (or 1:100 two times, as above).

Example

You have *Escherichia coli* suspended at 3×10^6 cells/mL, which is 3×10^3cells/μL. You want to be able to replate so that you will end up with about 10 colonies, and you need those 10 cells suspended in ~50 μL for delivery. Thus, the final concentration you need is 10 cells/50 μL, which



Dilution	Concentrate	1:15	1:1000	
Concentration	3×10^3 cell/µL	2×10^2 cell/µL	2×10^1 cell/µL	10 cell/50 µL
Volume of medium		14 µL	999 µL	
		+	+	
Volume added		1 µL	1 µL	

equals 0.2 cells/µL. You want to make six new plates, so you will need a total of 300 µL of this final suspension. Your best pipetter can pick up 1 µL accurately.

The ratio of diluted to undiluted is $0.2{:}3 \times 10^3$, or $1{:}15 \times 10^3$. Therefore, the first dilution can be 1:15 (1 µL of concentrated suspension + 14 µL of medium), and the second, 1:1000 (1 µL of the first dilution + 999 µL of medium).

You could also have done 1:150 for the first dilution (1 µL of concentrate + 149 µL of medium) and 1:100 for the second.

CAREFUL: Because you need to end up with 300 µL, the second dilution must be 3 µL of the first dilution + 297 µL of medium.

There is no difference in what you end up with, but there is a difference in the amount of waste generated: 714 µL in the first case, 147 µL in the second. The amount of waste can be very important, for example, if the reagents are in short supply, expensive, or hazardous. If you have a dilution that you do frequently, you can fiddle with the numbers and determine the optimal (least wasteful or easiest) series. If you do that, write it down for everyone.

CONVERTING RECIPES TO CONCENTRATIONS

Sometimes, when you are reading scientific literature or someone's protocol for making a solution or performing an experiment, you will encounter

a recipe for exactly how to make a particular volume of a particular solution (e.g., "dissolve 2.7 mg of EDTA in 40 mL of water"). You, however, need to know the final concentration of the EDTA in solution, not simply how to make up the same amount of the same solution. If this happens, you must know how to convert a recipe into a concentration.

Essentially, this is a word problem, and the trick to solving a word problem is to convert the words into math. The key to this process is knowing the translation. The translations you need to make solutions are

- "Of" means multiply.
- "Put amount *A* into volume *B*" (or "add *B* volume to *A* amount") means *A* divided by *B*.

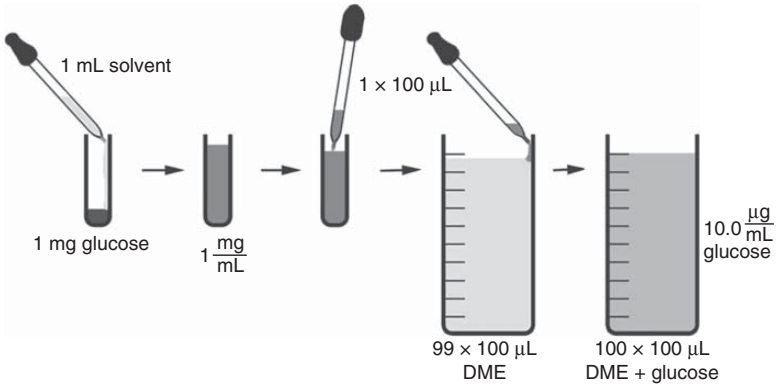
It can help to start by drawing a picture of what was done. This may seem like a time waster, but before you dismiss the idea, think about the amount of time you would otherwise spend scribbling random equations on scrap paper.

Example

Suppose that the Materials and Methods section of an article said "100 µL of D-(+)-glucose (ChemCo, 1 mg/mL) was diluted 1:100 in DME." That means that the following actually happened:

Start by translating what was written into a description of what was done; use the words "of" and "put into." 1 mg *OF* glucose was *PUT INTO* 1 mL of solvent (ChemCo did that), or

$$(1\text{ mg}) \div (1\text{ mL}) = 1\text{ mg/mL}.$$



100 µL *OF* 1 mg/mL glucose solution was *PUT INTO* a total of 10 mL of solution, or

$$\frac{100\,\mu L \times 1\,mg/mL}{10\,mL}.$$

Calculate.

$$\frac{100\,\mu L \times 1\,mg/mL \times 10^{-3}\,mL/\mu L}{10\,mL} = 10^{-2}\,mg/mL = 10\,\mu g/mL.$$

Therefore, the concentration that they used was

10 µg/mL glucose in DME.

pH

pH is the way that the acidity of a solution is quantified. The pH of a solution is usually important and often critical, because many reactions will only take place as expected if the pH is correct. Sometimes, pH is essential to the solution-making process itself. For example, EDTA will not dissolve until the pH is brought to 7.

In the term pH, the p means "the negative of the log of" and the H stands for "the concentration of protons." If pH = 6, that means $[H^+] = 10^{-6}$; if pH = 8, that means $[H^+] = 10^{-8}$. So, pH is a logarithmic measure of the concentration of protons in a solution. Because pH is the negative of log$[H^+]$, the lower the pH, the higher the concentration of H^+, and the higher the pH, the lower the concentration of H^+. The pH scale runs from 1 to 14, with pH 7 (the pH of pure water) considered neutral. A pH below 7 means that a solution is acidic; a pH above 7 means that a solution is basic (or alkaline).

Because pH is logarithmic, a change in pH of 1 means a $10^1\times$ or a 10× change in $[H^+]$. A solution of pH 6, therefore, has 10 times as many protons as a solution of pH 7. A change in pH of 2 means a $10^2\times$ or 100× change in $[H^+]$; a change in pH of 3 means a $10^3\times$ or 1000× change in $[H^+]$; and so on. pH is not typically calculated; it is usually measured directly using a pH meter or estimated using pH paper.

Measuring pH

To measure pH, you can use indicator dyes, litmus paper, or a pH meter. A solution being "pH'd" must be well stirred.

Indicator dyes

Indicator dyes change color when pH changes. These dyes are calibrated so that you can compare, by eye, the color of a solution with dye in it to a chart with a key of colors and their corresponding pHs. Indicator dyes are the pH-measuring method most pet stores sell for measuring the pH of fish tanks. Many biological media contain indicator dyes, like phenol red, so that users can tell at a glance whether the pH of their solution is correct.

Litmus paper

Litmus paper is coated with an indicator dye that changes color with pH. It is calibrated so that you can easily observe the color that results when you drop some of your solution onto the paper (never dip the paper into the solution—you will contaminate it). By comparing the color to the chart provided, you can determine the pH. Litmus paper comes in a variety of pH ranges to accommodate different precisions of measurement.

pH meters

pH meters work by measuring voltage changes that occur when an electrode is placed in a solution. The tip of the electrode is permeable to hydrogen ions. When the electrode is placed in a solution, a certain number of ions, proportional to the pH, get inside the electrode and change the electrical properties of a sensor. That change is monitored and converted into a measure of pH.

The conversion made by a pH meter is dependent on calibration, which is why it is critical to calibrate any pH meter properly before each use. For more information about pH meters, see Chapter 3. pH meters should be calibrated using at least two buffers that bracket the pH being measured. If at any time you are uncertain in any way about your pH meter, use caution and recalibrate it.

Note: There are two very important considerations when using a pH meter. One is that the electrode of many pH meters must not dry out. The electrode should always be kept submerged in liquid, even when the meter is not in use. You can buy storage buffers or just store the electrode in one of the standard buffers (pH 7 or pH 4). The second consideration is never to transfer the electrode from one liquid to another without first rinsing it well, for example, with distilled water (catch the runoff in a waste container).

METHOD **Measuring pH with a pH Meter**

Below are some general guidelines. Follow the precise instructions that came with your pH meter to calibrate and use your particular meter properly.

1. Calibrate the pH meter.
   a. To calibrate a pH meter, choose buffers to which you will standardize that bracket the final pH you are measuring. For most biological applications, using standards of pH 4.0 and of pH 10 will bracket the pHs you are likely to measure. Another common standard to use is pH 7. If you will only be measuring pHs below 7, calibrate using standards of pH 4 and pH 7; if you will only be measuring pHs above 7, use standards of pH 7 and pH 10. Some pH meters allow you to use all three standards to calibrate. Whichever standards you use, the meter is taking the calibration points and graphing a line that will tell it the relationship of voltage to pH at that moment in time. You must use at least two standards, because it takes two points to define a line. The instructions that come with your meter and the electrode will have more details regarding calibrating and topics such as the uncertainty associated with measuring extreme pHs.
   b. Submerge the *well-rinsed* electrode into the pH 4.0 standard. Use the appropriate button or knob to set the pH to 4.0.
   c. Submerge the *well-rinsed* electrode into the pH 10 standard. Use the appropriate button or knob to set the pH to 10.
2. Put the *well-rinsed* electrode tip into the well-mixed (usually contemporaneously mixing) solution to be measured.
3. Wait for the measurement of pH to stop fluctuating.
4. Read the pH.

From Acid–Base to Buffers: Conjugate Acid–Base Pairs

Every acid has a conjugate base, and every base has a conjugate acid. An acid's conjugate base has one fewer H and one more negative charge than the acid; a base's conjugate acid has one more H and one less negative charge than the base. In other words, if an acid gives up a proton, what is

left is its conjugate base; if a base picks up a proton, the result is its conjugate acid.

Example

$H_2PO_4^- \leftrightarrow H^+ + HPO_4^{2-}$

Acid Conjugate base

A buffer is a weak acid that is chosen such that it prevents changes in pH by substituting changes in the relative concentrations of the weak acid and its conjugate base. Thus, a buffer works by replacing a change in $[H^+]$ with a change in relative amounts of the weak acid and its conjugate base in the solution. When you add acid to a buffered solution, the pH stays the same, the amount of buffer goes up, and the amount of the buffer's conjugate base goes down. When you add base to a buffered solution, the pH stays the same, the amount of buffer goes down, and the amount of its conjugate base goes up.

Examples

Compare the following scenarios:

1. You add NaOH to a solution.

   Result: The NaOH dissociates into Na^+ and OH^-. The pH of the solution consequently rises. OH^- provided by the dissociation of the NaOH combines with free H^+, bringing down the $[H^+]$ in the solution. In addition, the number of molecules of Na^+ goes up, as does the number of molecules of H_2O.

2. You add NaOH to a buffered solution.

   Result: The NaOH dissociates into Na^+ and OH^-. The acid component *of the buffer* donates H^+ to combine with the free OH^-, and the pH does not change. What *does* change is the relative amount of acid (decreases) and conjugate base (increases) in the solution.

pK_a: Judging a buffer by its number

An important number used to characterize buffers is pK_a. A full explanation of pK_a is given below, but the simple, critical factor to remember regarding pK_a and buffers is that you want to choose a buffer with a pK_a as close as possible to the pH you ultimately want to maintain. For biological solutions, a good rule of thumb is to get as close as you can or a little lower. Although it is not covered in this book (because readily available buffers work quite well

for most purposes), you might want to know that there is a way to design and make your own buffers with a particular pK_a. If you do need a buffer that does not seem to exist, ask a friendly biochemist for help using the Henderson–Hasselbalch equation (see below) to create a recipe for the buffer you need.

Acid–base reactions and pK_a

To understand pK_a, the number that describes buffers, you can think about the classical definitions of acids and bases. Every acid–base reaction resembles this generic formula:

$$HA + H_2O \Leftrightarrow H_3O^+ + A^-,$$

which, because the H_2O to H_3O^+ is assumed, is sometimes written:

$$HA \Leftrightarrow H^+ + A^-,$$

where HA is an acid and H_2O is the base it reacts with to form H_3O^+ (or H^+, the conjugate acid of H_2O), and A^- (the conjugate base of HA). This generic reaction is quantified by the dissociation constant, which is the acid–base equivalent of an equilibrium constant (K) (for more on equilibrium constants, see page 104):

$$K = \frac{[H_3O][A^-]}{[HA][H_2O]}.$$

K is (in this case) a dimensionless quantity that measures the proton (i.e., H^+) affinity of the HA/A^- pair relative to the proton affinity of the H_3O^+/H_2O pair; that is, it tells you whether you are more likely to have HA or H_3O^+. In other words, K tells you whether the generic reaction ($HA \Leftrightarrow H^+ + A^-$) is more likely to go to the right (the acid is strong and gives up a proton easily; the acid will dissociate even if there are already lots of protons around) or to the left (the acid is weak and does not give up a proton easily; the acid will not dissociate so easily). If K is above 1.0, the reaction goes to the right and HA is a stronger acid (one that readily dissociates). If K is below 1.0, the reaction goes to the left and HA is a weaker acid (one that does not dissociate easily). To get from K to K_a, just rearrange the definition of K:

$$K[H_2O] = \frac{[H_3O^+][A^-]}{[HA]} = K_a,$$

and that is the definition of K_a:

$$K_a = \frac{[H_3O^+][A^-]}{[HA]} = \frac{[H^+][A^-]}{[HA]}.$$

CAREFUL: Sometimes the subscript is left off.

Interesting aside: Why pH 7, the pH of pure water, is neutral

Because H_2O is an acid (albeit a weak one), it has a dissociation constant.

$$K_a = \frac{[H^+][OH^-]}{[H^2O]}.$$

Rearranging gives

$$K_a[H_2O] = [H^+][OH^-],$$

and that is the definition of K_w, the dissociation constant for water,

$$K_w = K_a[H_2O] = [H^+][OH^-].$$

At 25°C, $K_w = 10^{-14}$ M^2. Moreover, because in pure water the concentration of H^+ must equal the concentration of OH^- (because they are present in equimolar amounts in H_2O), $[H^+]$ must equal the square root of K_w, that is, 10^{-7} M. If $[H^+] = 10^{-7}$ M, then pH = 7. Hence, the pH of pure water is 7, and that is defined as neutral.

The Relationship of pK_a to pH

We can manipulate the definition of K_a to show the relationship between pH and the concentration of an acid and its conjugate base. First, taking the log of both sides of the equation defining K_a gives

$$\log(K_a) = \log[H^+] + \log[A^-] - \log[HA].$$

Rearranging gives

$$-\log[H^+] = -\log(K_a) + \log[A^-] - \log[HA],$$

which is the same as

$$-\log[H^+] = -\log(K_a) + \log\frac{[A^-]}{[HA]}.$$

Now, we substitute the shorthand "p" for "–log" and get

$$\mathrm{pH} = \mathrm{p}K_a + \log\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}.$$

This is the Henderson–Hasselbalch equation, which tells you the relationship between the concentration of an acid [HA], the concentration of its conjugate base [A^-], and pH. The Henderson–Hasselbalch equation is useful because it allows you to talk about the strength of an acid (the readiness with which it dissociates) with reference to the pH scale. To say an acid is strong is to say that it dissociates (donates protons), even if there is already a high concentration of protons around (i.e., it has a high K_a, which means that it has a low pK_a; rule of thumb: $K_a > 10^{-2}$ is a large K_a, so a pK_a of 2 is low). If the pK_a of an acid is 2, then in a solution of pH = 2, [A^-] must equal [HA] (because the second term on the right-hand side must equal zero; thus, the fraction must equal 1), which means that half of the acid has dissociated. Any chemical that can be 50% dissociated into protons and conjugate base in a solution that already has a pH of 2 is certainly a strong acid. If the pK_a of another acid is 5, then in a solution of pH 2, the second term must equal –3; thus, the fraction must equal 10^{-3}, which means that [A^-] is a thousand times lower than [HA], which means that less of this second acid dissociated, that is, it is a weaker acid.

Let us go back to the Henderson–Hasselbalch equation, which says that if [A^-] = [HA], then pH = pK_a. This relationship between pH and pK_a is important to understand: When choosing an acid to use as a buffer, you want [HA] to be about equal to [A^-] because that will be an acid that is about equally good at buffering against increases or decreases in pH. In other words, you want a buffer with a pK_a close to the pH you wish to maintain. Because buffers only work over a certain range of pHs (rule of thumb: buffers buffer in a range of pK_a ± 1), you want to get as close as possible.

HOW TO QUICKLY CHOOSE A BUFFER

1. Decide what pH you would like to maintain in your solution.
2. Look up buffers in the table in Chapter 8 or in a catalog.
3. Choose a buffer with a pK_a close to the desired pH.
4. Keep in mind that the buffer will only work over a certain range of pHs (pK_a ± 1).

WARNING: pH is sensitive to changes in temperature and to what is in the solution being buffered. In addition, different buffers work differently. Unless you are absolutely positive, owing to long experience with a particular buffered solution, it is a very good idea to confirm the pH of important solutions close to the time when you will be using them.

Biological solutions frequently become acidic over time. When selecting a buffer to use in a biological application, choose one with a pK_a that is slightly lower than the desired pH. That way, as the pH of the solution tries to change, the buffer is still working over the range at which it is good at buffering.

Some buffers can donate more than one proton and thus have more than one pK_a. The range over which these buffers work will therefore be larger than the range of buffering possible from an acid that donates only one proton.

How much buffer to include in a solution

Most recipes will tell you how much buffer to add. If you need to figure it out for yourself, you should know that there are no hard-and-fast rules for deciding how much buffer to add, but there are some considerations. The concentrations of various buffers in humans are as follows:

$$\left[HCO_3^-\right] = 24\ \text{mM};$$
$$\left[HPO_4^{2-}\right] = 1\ \text{mM};$$
$$\left[SO_4^{2-}\right] = 0.5\ \text{mM}.$$

For solutions to be used in biological applications, buffer concentrations are usually between 10 mM and 100 mM. Some catalogs include a good working concentration for the buffers they sell. The higher the concentration of buffer, the more you must worry about the effect that the buffer may have on the ionic strength and/or osmolarity of your solution.

METHOD Adjusting pH Using a pH Meter

1. Calibrate the meter.
   a. Choose buffers you will standardize to that bracket the pH for which you are aiming. For most biological applications, using a standard of

pH 4.0 and a standard of pH 10 will bracket the pHs you are likely to measure.

b. Submerge the *well-rinsed* electrode into the pH 4.0 standard. Use the appropriate button or knob to set the pH to 4.0.

c. Submerge the *well-rinsed* electrode into the pH 10 standard. Use the appropriate button or knob to set the pH to 10.

2. Put the *well-rinsed* electrode tip into the actively mixing solution.

3. Wait for the measure of pH to stop fluctuating.

4. Read the pH.

5. Change the pH.

a. If the pH of a solution is too low, checking the pH between the addition of each drop, add *one drop at a time* of high-concentration (usually 10 N) NaOH (or KOH) until the pH is within 1.0 of the desired value. (Remember, the pH is a log scale, whereas the number of H^+ or OH^- molecules in the drops you are adding remains constant. Therefore, one drop will affect a solution with a pH of 5 ($[H^+] = 10^{-5}$ M) much less than it will affect a solution with a pH of 10 ($[H^+] = 10^{-10}$ M). Then, *one drop at a time*, add low-concentration (1.0 N) NaOH (or KOH).

b. If the pH of a solution is too high, checking the pH between the addition of each drop, add one drop at a time of high-concentration (usually 12.1 N) HCl until the pH is within 1.0 of the desired value. Then, one drop at a time, add low-concentration (usually 1.2 N) HCl.

UNMAKING SOLUTIONS

Sometimes you want to remove certain objects from solution, or separate the components of a mixture. Here are some techniques for doing that.

Separating Sample Components Using Centrifuges

The centrifuge is a frequently used piece of equipment in a laboratory. It is used to pellet cells out of suspension, for subcellular fractionation, and as a part of many routine protocols such as those for protein or DNA isolation.

The idea of centrifugation is to spin a sample very fast, so that it experiences the effects of inertia and centripetal forces (see explanations below). Components of the sample that differ in certain physical properties, such as mass, will move differently in response to the forces and thus will end up in distinct places within their container; that is, they will be physically separated. For a centrifuge to work properly, weight in the centrifuge rotor *must* be distributed evenly. Tubes and volumes of liquid, for example, must be balanced. This is an important safety issue, because the forces generated by a centrifuge can be dangerous to you, not to mention your expensive centrifuge.

As mentioned above, two forces are acting on the components of a sample inside a tube in a spinning centrifuge. Centrifugal force, however, is not one of them. Centrifugal force does not actually exist: There is no force that pushes outward on the contents of a spinning tube. There are two real forces acting on objects in a centrifuge. The first is inertia. Each component in a centrifuge is essentially "trying" to travel in a straight line because of its inertia. The second force is the inward, or centripetal, force on the sample that is attributable to the tube being attached to the center of the machine (centripetal means "center-seeking"). This inward force bends the path of the sample into a circle.

Centrifugation depends on different components of a sample having different masses and, therefore, different amounts of inertia. Items with more mass and hence more inertia will be able to take a path that is straighter than the path of the items with less inertia. Within a revolving tube, the straighter path is found further away from the axis of the revolution, because the greater the radius of the circle traced, the straighter is the path of the particle tracing it. Thus, the denser components of a sample move further into the tube, whereas the less dense items end up closer to the top.

There are many different types of centrifuges, such as fixed angle and swinging bucket, and many methods of centrifugation, such as differential centrifugation and density gradient centrifugation. Each type exploits various physical phenomena to separate samples based on different physical properties.

How to Use the Centrifuge That You Have to Get the Separation That You Need

If you wish to use a protocol that includes a centrifugation step, you need to figure out how to get the right acceleration. Acceleration in a protocol may be referred to in terms of *g*-force or *g*s, and this is written as some number

followed by a *g*, for example, "centrifuge for 15 min at 1500*g*." This nomenclature relates the acceleration within the centrifuge to gravitational acceleration, which is a constant symbolized by the letter *g*; you can think of *g* as a unit for the property acceleration. Confusingly, however, *g*-force is not a force, and the accelerations indicated in this way are not multiples of the gravitational constant. Nevertheless, it is how the accelerations in a centrifuge are reported, so that is what you are attempting to accomplish with your centrifuge. Some centrifuges will set themselves to deliver the appropriate number of *g*s if you input the acceleration you want. Others, however, require you to determine how to accomplish the necessary *g*s. You may therefore have to calibrate your centrifuge.

On many centrifuges, you cannot directly change the *g*s, also known as the relative centrifugal field (RCF). What you can change on a centrifuge is the rotations per minute (rpm). The relationship between the rotations per minute and the relative centrifugal field (i.e., the number of *g*s) is given by the following equation:

$$\text{RCF} = 1.1 \times 10^{-6} \times (\text{rpm})^2 \times \text{radius},$$

where

RCF = relative centrifugal field dimensions LT^{-2}; reported using *g* as a unit);
rpm = rotations per minute (min^{-1});
Radius = distance from the center of the rotor to a relevant position in the tube (mm).

In this equation, the radius of interest is the distance from the center of the axis of rotation to the relevant position in the spinning tube—often either (1) the point in the sample that is furthest away from the axis, which will tell you the maximum acceleration, experienced at the tip of the tube; or (2) the point halfway between the furthest and the nearest points within the sample, which will tell you the mean acceleration experienced by the whole tube. The information may be provided by the manufacturer of the centrifuge, or you may have to measure it yourself. Once you know the radii of the standard rotors for your centrifuge, it is very useful to write the numbers down and tape them up in an obvious place (the lid is the usual place) so that no one else has to go through the measuring.

How the numbers on the speed-setting dial on a centrifuge translate to rpm is described in the literature that comes with the centrifuge. In many laboratories, this information is photocopied and taped to the wall near the centrifuge. Even better, the RCF for each number on the dial is often

calculated and then taped to the wall. You can also have your centrifuge calibrated professionally.

To match the rpm to a particular RCF (number of *g*s), you can either look at a chart that someone has kindly posted near your centrifuge or you can divide the value of the RCF you are trying to accomplish by the radius (in centimeters), divide that number by 1.118^{-6}, take the square root of that number, and choose the setting that corresponds to that rpm. In other words, solve this equation:

$$rpm = \left(\frac{RCF}{(r \times 1.118 \times 10^{-5})}\right)^{\frac{1}{2}}.$$

Then, run the centrifuge at the setting that delivers that rpm. Another way to figure out what setting to use (i.e., what rpm to use) is to use a chart called a nomogram; this is a graphical representation of the relationship between rpm, RCF, and rotor diameter.

To use the nomogram to determine the rpm, draw a straight line from the radius of the rotor, through the RCF you want, to the rpm line. This will tell you the rpm needed to achieve the *gs* you want.

Removing the Water from a Solution

A common way to remove solvent, and therefore concentrate and dry the solutes, is lyophilization, more commonly known as freeze-drying and less commonly known as cryodesiccation. The equipment works by lowering the temperature to freeze (solidify) the solvent, then lowering the pressure so that the frozen solvent will sublime. Sublimation is the process of going directly from solid to gas, without going through a liquid stage. As the water vapor leaves, the solution becomes more and more concentrated. Actual lyophilization is performed in three steps—(1) freezing (by one of a few different methods, although the "shell" method is the standard), (2) primary drying, which removes the unbound solvent, and (3) secondary drying, which removes the bound solvent.

The numbers you must think about are the following.

The Eutectic Point: This is the temperature at which a solution transitions between being meltable and being not-meltable; that is, entirely solid. (Compare this with the triple point, which is the temperature/pressure conditions under which all three phases—gas, liquid, solid—coexist.)

The Temperature of the Collector: This should be 15°C–20°C lower than the eutectic temperature of the solvent.

The Ice Holding Capacity: The volume in liters of the collector should be twice the volume of the solution.

Liters per Hour Removed: Most companies report this rate for solutions that are frozen using the "shell" method, in which special freezing containers full of solution are rotated in a heat-removing bath so that the solution freezes in cylindrical sheets, or shells, from the outside in.

Some eutectic points are as follows:

Solvent	Eutectic temperature (°C)
Acetone (dilute to 15%)	−94
Acetonitrile	−42
Acetic acid	16
Chloroform	−63
DMSO	18
Ethanol (dilute to 15%)	−117
Hexane (dilute to 15%)	−95
Methanol (dilute to 15%)	−98
Methylene chloride (dilute to 15%)	−97
Salt water	Varies with salt concentration
Sugar water	Varies with sugar concentration
Water	0

Removing Solutes Using Dialysis Tubing

Dialysis is the process of removing solutes from a solution by exploiting the fact that items move down their concentration gradients. For example, some procedures require adding salts that must be removed subsequently. If you put such a solution on one side of a semipermeable membrane and on the other, a buffer in which those salts are at a lower concentration (e.g., zero), the salts, which are small enough to fit through the pores in the membrane (the holes that make it "semi"-permeable, i.e., permeable to anything smaller than the holes), will diffuse through the holes, until the concentration of salts is equal (at equilibrium) on the two sides of the membrane. At the end of that process, the concentration of salt will be lower in the solution, which is what you wanted. If you make a semipermeable container, fill it with solution, then keep moving it to fresh buffer every time the

concentrations of whatever you want to remove reach equilibrium, you can get a lot of whatever it is out of the solution.

Dialysis tubing is thin-walled, flexible tubing, usually made of cellulose, with different-size pores; you cut off a length of it, tie a knot in one end, pour in your solution, tie a knot at the other end, and voilà, you have a container. You can remove anything smaller than the pore size—such as salts and other unwanted small molecules such as contaminants.

The numbers you must think about are the following:

Tubing Size (width/diameter/volume): Dialysis tubing is sold flattened on rolls, and then it opens up to a tube when it is filled. Thus, the size of the tube can be characterized either by its width when flat or its diameter when filled. You determine the length by where you cut it, but too long is possible. Therefore, a range of reasonable volumes that you might put in the tubing is sometimes given as a measure of tubing size. This is the information you need for determining what to buy based on the volume of solution that you want to work with.

Volume of Buffer Relative to Volume of Solution: The greater the ratio of buffer to solution, the more solute will diffuse out. Commonly used volumes are 200–500 times the volume of the solution.

Concentration of Solutes in the Buffer Relative to Concentration of Solutes in the Solution: If this ratio is very large, you might think it would speed things up. Yes, but. Because semipermeable membranes go both ways, there is nothing to stop solutes—or solvent—from moving from the buffer into the solution, contaminating your precious solution with new solutes or diluting it with solvent. The best way to make sure that you only do what you want is to design your buffer carefully and use many small steps (i.e., small differences in buffer solute concentrations) to accomplish your removing. That prevents large gradients in the wrong direction from messing up your solution.

The Amount of Time You Leave the Solution in Each Buffer: The steepness of the gradient will determine the speed at which solutes leave the solution; thus, as time passes and the gradient of interest gets shallower, the process slows down. But you do not want to waste buffer, and time, by changing every couple of minutes. A common regimen is to change the buffer every 1–2 h.

Temperature at Which You Perform the Dialysis: The warmer it is, the faster the molecules will be moving and diffusing and the faster equilibrium will be reached. But too warm a temperature can hurt your solution. Dialysis is commonly performed at room temperature or overnight at 4°C when there will not be any buffer changes.

Pore Size: This is **important**—get this wrong, and it will not work no matter how perfect everything else is. Pore size determines what will diffuse out of the solution and what will be retained. Anything smaller goes, and anything larger stays. You choose pore size based on what you want removed and what you want kept, keeping in mind that the larger the pore size relative to the solute size, the faster the solute will diffuse through it. Pore sizes are usually given in terms of minimum molecular weight of whatever will be retained. That is because pore size is determined by putting globular proteins of known molecular weights into the tubing and seeing which ones are small enough to come out. This is OK because molecular weight correlates pretty well with protein size for globular proteins. It is not so OK for determining whether objects that are not globular proteins will go through or not. Some items may be the right shape for their size, but they might clump up and therefore, in reality, be too big. Stringy objects, like DNA, can thread their way through pores that are *much* smaller than you would choose if you just went by molecular weight. If you are dialyzing stuff out of a solution of linear molecules, choose a pore size that is at most one-third of what you would choose based on molecular weight.

SPECIAL NOTE: CHELATORS AND OTHER DIFFUSION GAME CHANGERS

Chelators are molecules that bind ions. EDTA, for example, is a commonly used Ca^{2+} chelator. Chelators are used to keep the concentration of the free ion low. The existence of chelators means that you can lower the concentration of an ion by *adding* something, rather than by removing the ion itself, and adding is much easier than subtracting. Other molecules that can affect diffusion by changing the concentration gradient of something include enzymes that modify molecules enough to prevent the concentration gradient from getting shallower. A good example of exploiting this is by linking something hydrophobic onto a hydrophilic molecule using an ester linkage; that allows the molecule to cross a membrane, but once inside a cell, esterases will cleave off the hydrophobic moiety, and the molecule is now trapped inside the cell. There is also zero of the original linked molecule inside; thus, its concentration gradient still favors it moving into the cell. It is a clever way to get hydrophilic objects into cells. There are not really any numbers involved, but these are important ways to affect concentrations of items in solutions, so there it is.

RESOURCES

Acid–base and buffers

www.chemguide.co.uk/physical/acidbaseeqia/buffers.html
www.chemtutor.com/acid.htm

www.khanacademy.org/science/chemistry/acids-and-bases/v/acid-base-introduction

***Converting RPM to* g**

www.endmemo.com/bio/grpm.php

Dialysis

www.piercenet.com/files/TR0020-Dialysis-overview.pdf

Henderson–Hasselbalch equation

www.sbu.ac.uk/biology/biolchem/acids.html

Laboratory techniques

Barker K. 1998. *At the bench: A laboratory navigator*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
www.dartmouth.edu/~chemlab

Lyophilizing

www.labconco.com/news/need-a-lyophilizer-read-this-before-you-buy

Safety

www.ehs.mit.edu/site/topic
www.hazard.com/msds
www.cdc.gov/niosh/ipcs/icstart.html
www.ilpi.com/msds/index.html

Vocabulary

www.chem.purdue.edu/gchelp/gloss/terms.html
www.lhup.edu/~dsimanek/glossary.htm
www.nashua.edu/walshly/Chemistry/chemistryvocabulary.htm