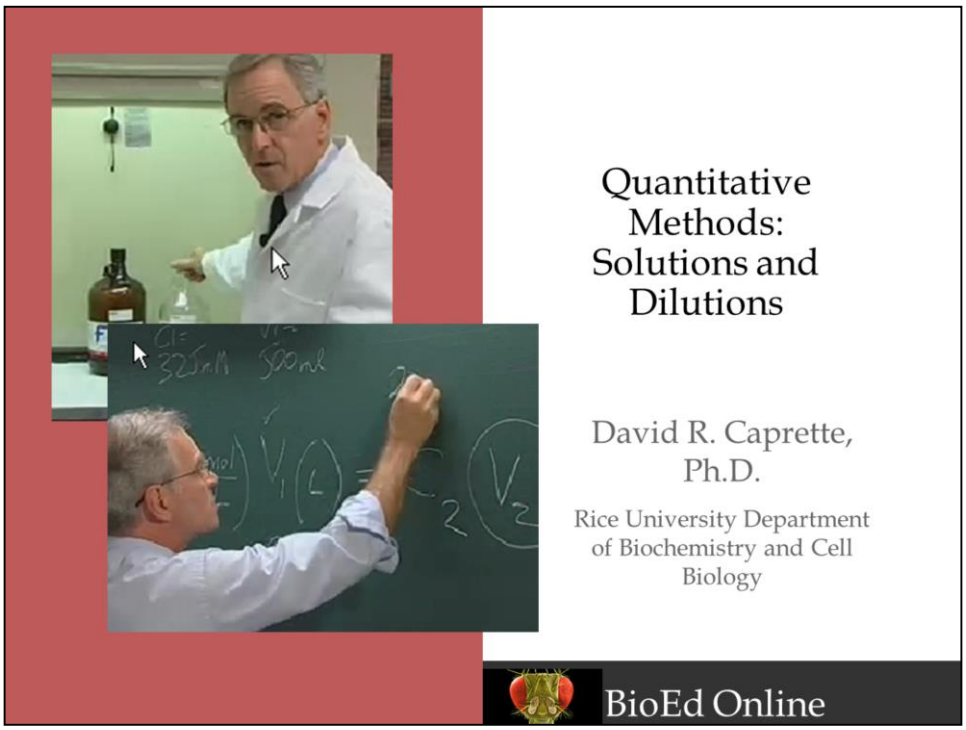

Quantitative Methods: Solutions and Dilutions

Part I of this series presented types of mixtures that might be prepared and/or encountered in a biological laboratory, and discussed their properties. It discussed the properties of water, the most important biological solvent, and described grades of water with respect to quality. It also introduced basic equipment for solution making, terms and concepts related to formulas, and the concept of the mole. Part II will show you how to prepare solutions from common types of formulas and will show you how to carry out dilutions with confidence.

References:

Farone, M.B. & Farone, A.L. (1999). *Dilution Solutions*. Kendall-Hunt. [This work is a practical student guidebook and workbook that includes descriptions of types of formulas and how to conduct dilutions, problem sets, and chapters on working with cells, viruses, nucleic acids, and proteins].

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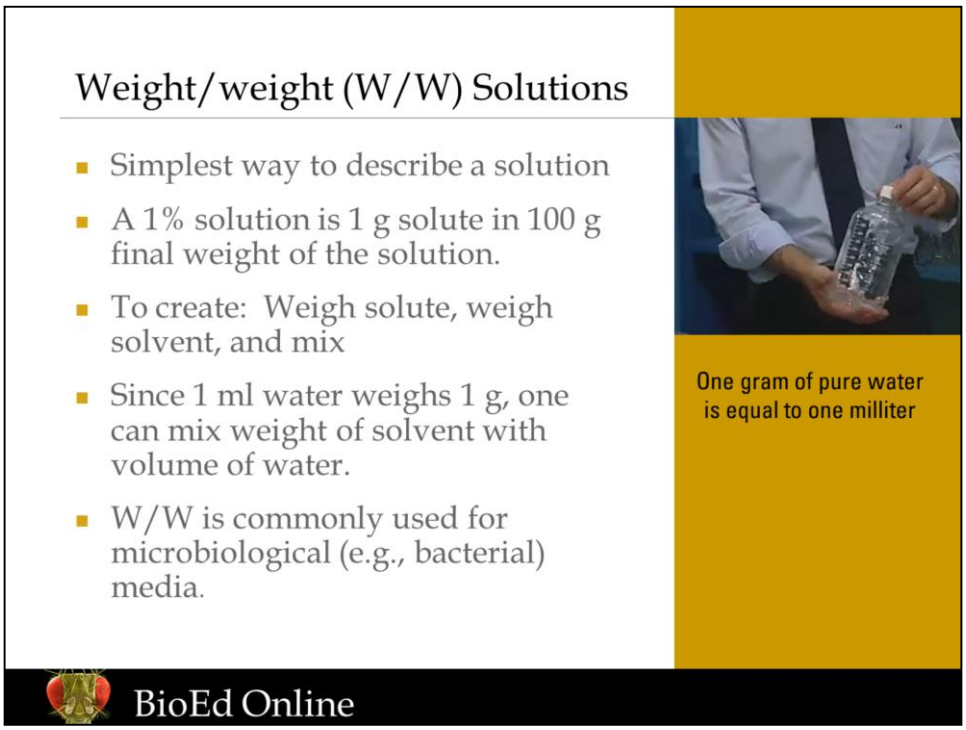

Weight/Weight (W/W) Solutions

Perhaps the easiest way to describe a solution is in terms of weight-in-weight (w/w). The weight of the solvent, relative to the weight of the final solution, is described as a percentage. For example, suppose you are working with a dye that is soluble in alcohol. Rather than write the instructions, "take 3 grams dye and mix with 97 grams absolute alcohol," you can describe the solution simply as 3% dye in absolute alcohol. The formula applies to any volume of solution that might be required. Three grams dye plus 97 grams alcohol will have final weight of 100 grams, so the dye winds up being 3% of the final weight. Note that the final weight is not necessarily equal to the final volume.

Aqueous weight-in-weight solutions are the easiest to prepare. Since 1 milliliter of water weighs one gram, we can measure a volume instead of weighing the solvent. A very common use of w/w formulas is with media for the culture of bacteria. Such media come in granular or powdered form, often contain agar, and often require heat in order to dissolve the components. Microbiological media, especially when they contain agar, are difficult to transfer from one vessel to another without leaving material behind. They coat the surfaces of glassware, making quite a mess. Using a w/w formula the

media and water can be mixed, heated, then sterilized, all in a single container. For example, tryptic soy agar, a very rich medium used for growing a variety of bacterial species, comes with instructions to simply mix 40 grams agar with one liter (equivalent to 1 kilogram) of deionized water, without adjusting the final volume. Very little material is wasted and there is less of a mess.

References:

Farone, M.B. & Farone, A.L. (1999). *Dilution Solutions*. Kendall-Hunt. [This work is a practical student guidebook and workbook that includes descriptions of types of formulas and how to conduct dilutions, problem sets, and chapters on working with cells, viruses, nucleic acids, and proteins].

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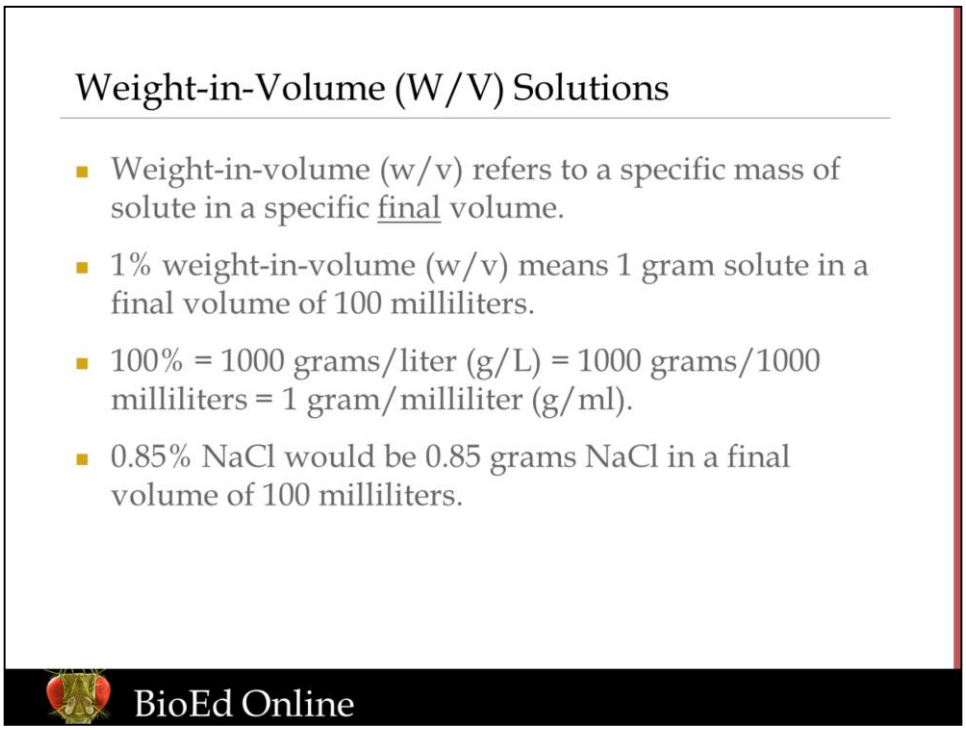

Weight-in-Volume (W/V) Solutions

When we describe a concentration as a percentage without specifying the type of formula, we imply that the solution is to be made using the weight-in-volume (w/v) method. As with w/w, weight-in-volume is a simple type of formula for describing the preparation of a solution of solid material in a liquid solvent. This method can be used to describe any solution, but is commonly used for simple saline solutions and when the formula weight of the solute is unknown, variable, or irrelevant, which often is the case with complex dyes, enzymes or other proteins. Solutions that require materials from natural sources often are prepared w/v because the molecular formula of the substance is unknown and/or because the substance cannot be described by a single formula.

A one percent solution is defined as 1 gram of solute per 100 milliliters final volume. For example, 1 gram of sodium chloride, brought to a final volume of 100 ml with distilled water, is a 1% NaCl solution. To help recall the definition of a 1% solution, remember that one gram is the mass of one milliliter of water. The mass of a solute needed to make a 1% solution is 1% of the mass of pure water of the desired final volume. Examples of 100% solutions are 1000 grams in 1000 milliliters or 1 gram in 1 milliliter.

Let's make a 0.85% solution of sodium chloride—a solution often called physiological saline—by combining 0.85 gram of NaCl in a final volume of 100 ml of high quality water. We may have a use for this solution because sodium and chloride ions are the principal inorganic ions in vertebrate blood. This means they are the principal inorganic ions in the fluid in tissues that surrounds our cells. The concentration of NaCl in blood is about 0.85%, so if we are working with organs, tissues, or cells, we use such solutions (often containing other components in lower concentrations) to keep cell membranes from deteriorating and to maintain an osmotically neutral environment.

Solutions that we use for organs, cells, and tissues generally will be high in sodium, since sodium is the principal cation among the electrolytes in extracellular fluid, the liquid that is outside of cells. Solutions that we use for organelles are often high in potassium, since potassium is the principal cationic electrolyte in cytoplasm.

References:

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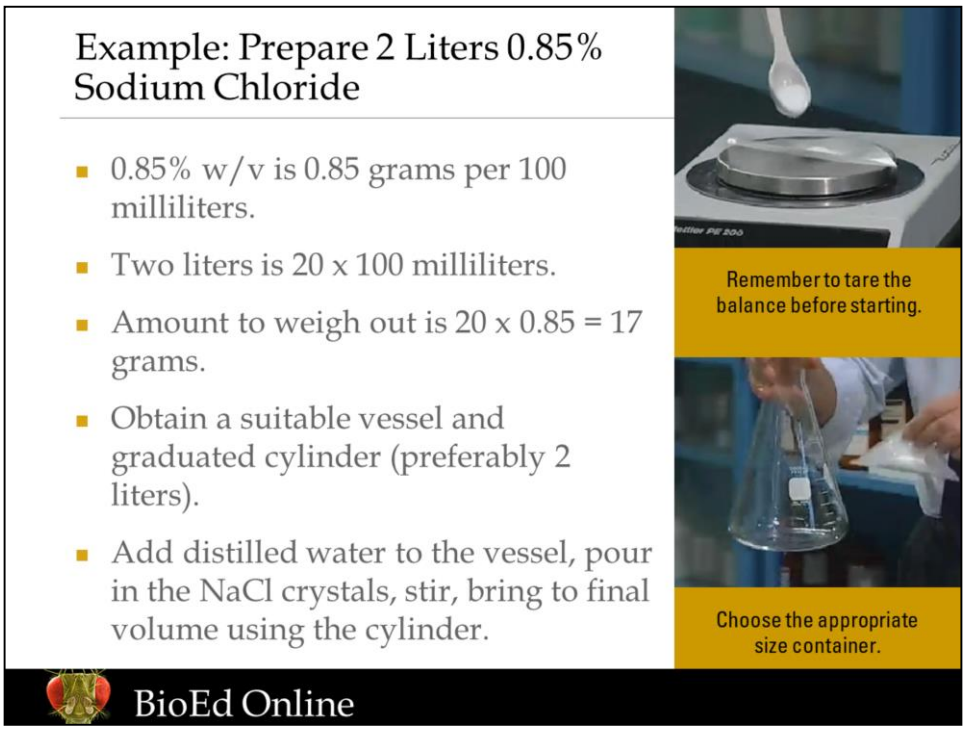

Example: Prepare 2 Liters 0.85% Sodium Chloride

With 1% defined as 1 gram per 100 ml, 0.85% is 0.85 grams per 100 ml. Since two liters is 20x the volume of 100 ml, we need 20 x 0.85 grams, or 17 grams NaCl. For this quantity, we can use a top loading balance or even a trip balance.

A typical electronic balance is accurate to one hundredth of a gram, which is sufficiently accurate for weighing out 17 grams. First we "tare" the instrument by placing a weigh boat onto the pan and setting it to "zero." We don't want to contaminate our chemical stocks, so we either clean the spatula or spoon before dipping it into the container, or we simply shake the chemical out onto the boat.

Suppose we tap out 16.97 grams of NaCl. Should we go to the trouble to get that last 0.03 gram? No, we are close enough. If it was necessary to be more accurate, we would describe the formula as something like 0.846% NaCl, or maybe 0.8495%. If there is some advantage to being that precise, we should exercise precision. Otherwise, trying to be too precise just wastes time.

Remember how to use significant digits? Seventeen grams means greater than 16.5 grams and less than 17.5 grams. If we wanted to be more accurate we would write "17.0" grams, meaning greater than or equal to 16.95 grams and less than or equal to 17.05 grams.

References:

Farone, M.B. & Farone, A.L. (1999). *Dilution Solutions*. Kendall-Hunt. [This work is a practical student guidebook and workbook that includes descriptions of types of formulas and how to conduct dilutions, problem sets, and chapters on working with cells, viruses, nucleic acids, and proteins].

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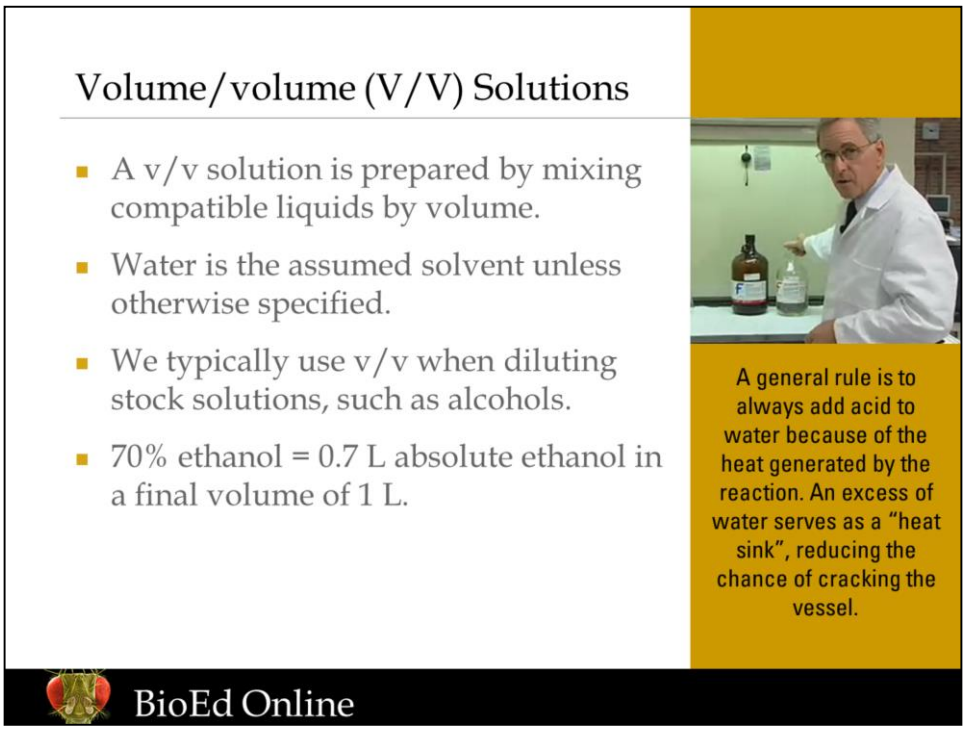

Volume/volume (V/V) Solutions

Volume-in-volume is another common way of describing a solution. We simply describe the percent total volume contributed by the liquid solute. As with the other types of formulas used in biology, we assume that the solvent is water unless some other solvent is specified.

V/v often is used to describe alcohol solutions used for histology or for working with proteins and nucleic acids. For example, 70% ethanol is simply 70 parts pure ethanol mixed with water to make 100 parts total. To make a liter of such a solution we would start with 0.7 L absolute ethanol and bring the final volume to 1 liter with water. More often, we might find ourselves with 95% alcohol. To make a 70% solution from a 95% stock solution requires a little more calculation. We will talk about that later, when we discuss how to make dilutions.

Destaining of protein gels refers to the soaking of a stained gel in acidified alcohol to remove all dye that is not bound to proteins, thus revealing the bands. A useful destaining solution consists of 7% methanol and 10% acetic acid. This means using 100 ml pure (or "glacial") acetic acid and 70 ml

methanol per liter of final solution.

References:

Farone, M.B. & Farone, A.L. (1999). *Dilution Solutions*. Kendall-Hunt. [This work is a practical student guidebook and workbook that includes descriptions of types of formulas and how to conduct dilutions, problem sets, and chapters on working with cells, viruses, nucleic acids, and proteins].

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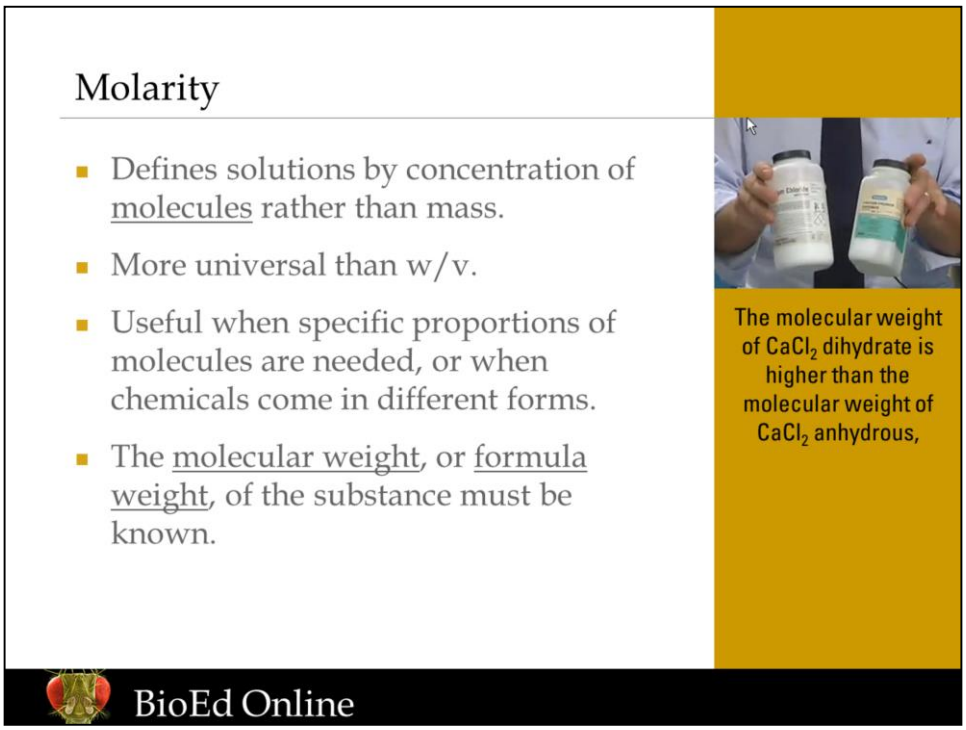

Molarity

A disadvantage of describing formulas as w/v (%) is that the description says nothing about the actual concentration of molecules in solution. What if we want equal amounts of two chemicals to be mixed together, so that for each molecule of substance #1 there is a single molecule of substance #2? The same amount in grams probably will not contain the same number of molecules of each substance.

Another disadvantage of the w/v method is that the same chemical can come in many forms, and one gram of a chemical in one form may contain a different amount of the chemical than one gram in another form. Imagine working with a chemical that can be used in several forms of hydration. For example, calcium chloride ($CaCl_2$), can be purchased as a dry chemical in anhydrous form, so that what you weigh out is nearly all pure calcium chloride. On the other hand, you may have a stock of dry chemical that is hydrated with seven water molecules per molecule of $CaCl_2$. The same mass of this chemical in this form will contain fewer molecules of calcium chloride than in anhydrous form.

Since we frequently want to know the actual concentration of molecules of a chemical in solution, it often is better to have a universal measurement that works regardless of how the chemical is supplied. As long as the molecular weight (sometimes called formula weight) is known, we can describe a solution in the form of moles per liter, or simply molar (M).

References:

Farone, M.B. & Farone, A.L. (1999). *Dilution Solutions*. Kendall-Hunt. [This work is a practical student guidebook and workbook that includes descriptions of types of formulas and how to conduct dilutions, problem sets, and chapters on working with cells, viruses, nucleic acids, and proteins].

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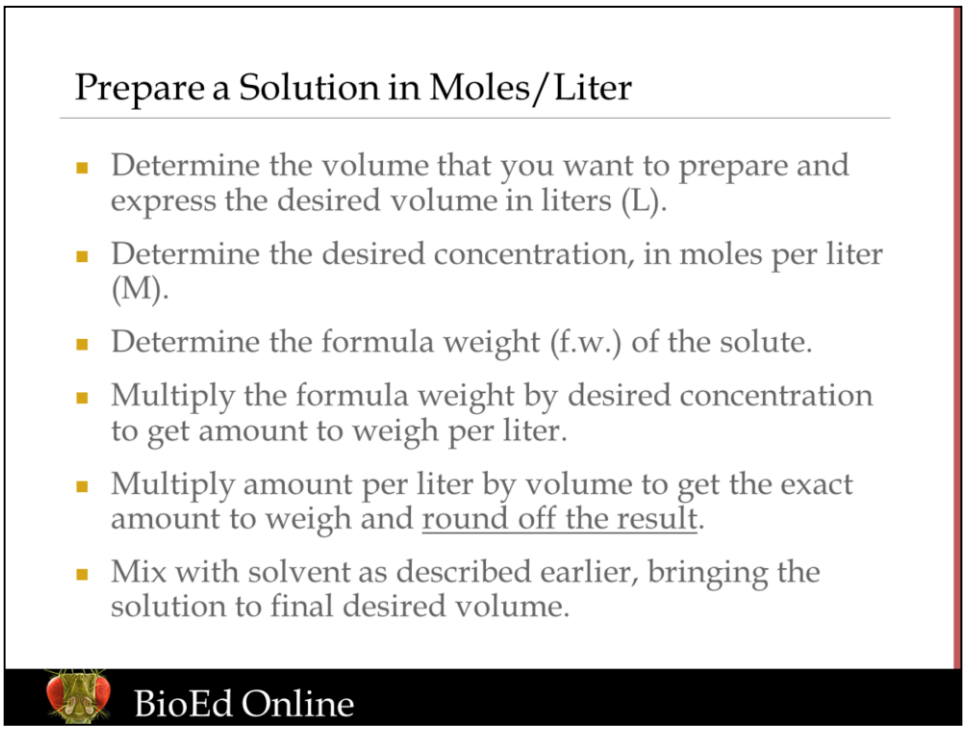

Prepare a Solution in Moles/Liter

The first step in the preparation of any solution is to decide how much to make. To simplify the calculations, express the desired volume in liters. For example, if you want to prepare 200 milliliters of a solution, express the volume as 0.2 liter.

Your formula may describe the concentration in moles per liter. For example, 0.25M means 0.25 moles/liter. It may call for a millimolar (mM) solution, a micromolar (µM) solution,a nanomolar (nM) solution, or even a picomolar (pM) solution. One mM, one µm, one nM, and one pM are 1/1000, 1/1,000,000, 1/1,000,000,000, and 1/1,000,000,000,000 mole/liter, respectively. Whatever prefix is used to describe the desired molar concentration, it is easier to do the calculation by converting the concentration to moles/liter. For example, 25 µM can be expressed as 0.000025M, or 2.5 x 10^{-5}M.

If you know the desired concentration in moles/liter and the formula weight of the substance, you can multiply these numbers and obtain mass per liter (moles/liter X grams/mole = grams/liter). And if you know the volume you need in liters, you then can multiply mass per liter times the desired volume to figure the amount to weigh (grams/liter X volume in liters = grams to weigh).

Remember that extreme precision is seldom either necessary or practical. It usually is good enough to get the actual concentration to within 1% of the desired concentration.

References:

Farone, M.B. & Farone, A.L. (1999). *Dilution Solutions*. Kendall-Hunt. [This work is a practical student guidebook and workbook that includes descriptions of types of formulas and how to conduct dilutions, problem sets, and chapters on working with cells, viruses, nucleic acids, and proteins].

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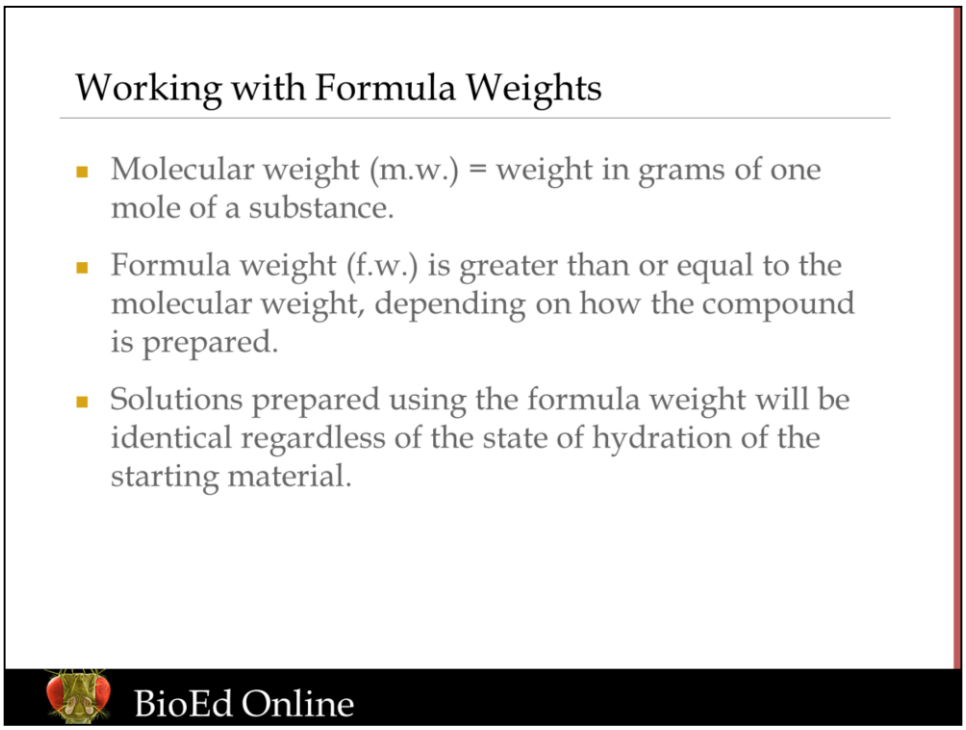

Working with Formula Weights

As with w/v solutions, we weigh out a specific amount of chemical when making a molar solution. Unlike w/v solutions, the amount to weigh depends on the molecular weight (m.w.) of the substance in grams per mole (g/mol). To calculate the desired mass of solute, you will need to know the formula weight. Formula weights usually are printed on the label and identified by the abbreviation f.w. Formula weight is the mass of material in grams that contains one mole of substance. It may include inert materials and/or the mass of water molecules, in the case of hydrated compounds. For pure compounds, the formula weight is the molecular weight of the substance and may be identified as such.

For example, the molecular weight of calcium chloride is 111.0 grams per mole (g/mol), which is the same as the formula weight if the material is anhydrous. Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) is 147.0 g/mol. The formula weight for $CaCl_2 \cdot 6H_2O$ (hexahydrate) is 219.1 g/mol.

A hydrated compound is a compound that is surrounded by water molecules held in place by hydrogen bonds. The water molecules in a hydrated compound become part of the solution when the material is dissolved. Thus, 111.0 grams of anhydrous $CaCl_2$, 147.0 grams of dihydrated $CaCl_2$, or 219.1 grams of $CaCl_2$ hexahydrate in one liter final volume all produce a 1 mole per liter solution, abbreviated 1M.

Suppose you need one liter of a solution of 10 mM calcium chloride (10 millimolar, or 0.01 moles per liter), and you have only $CaCl_2$ dihydrate. To make your 10 mM solution, you would weigh out 1/100 of the formula weight for dihydrated $CaCl_2$, which is 0.01 x 147.0 = 1.47 grams and bring it to one liter.

References:

Farone, M.B. & Farone, A.L. (1999). *Dilution Solutions*. Kendall-Hunt. [This work is a practical student guidebook and workbook that includes descriptions of types of formulas and how to conduct dilutions, problem sets, and chapters on working with cells, viruses, nucleic acids, and proteins].

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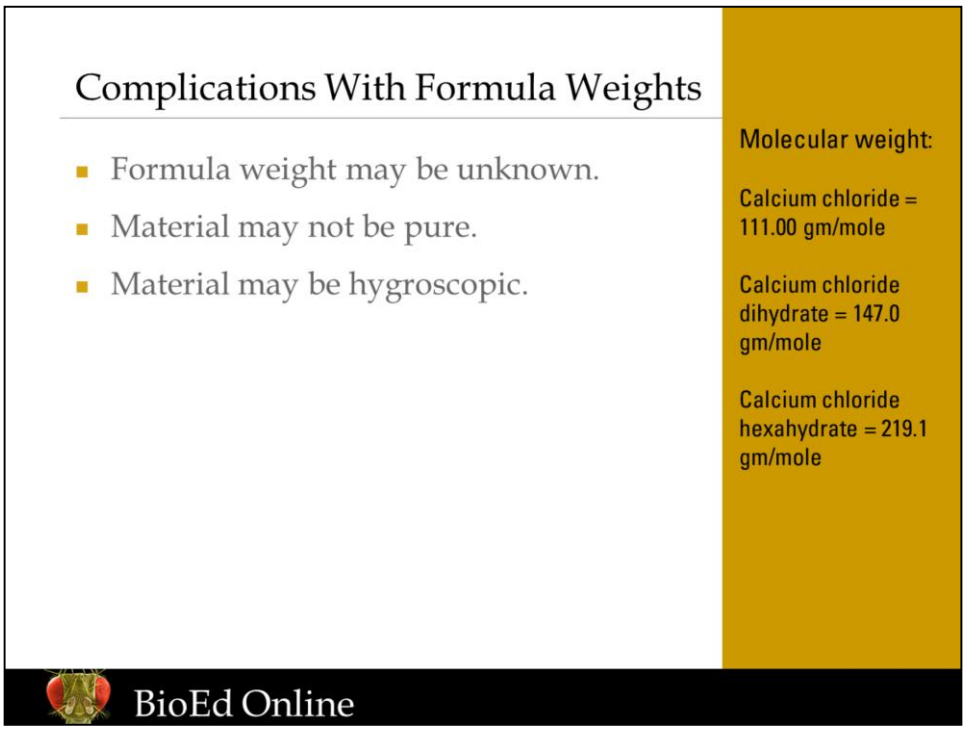

Complications With Formula Weights

Perhaps you cannot find a formula weight on a label, or perhaps you are planning a protocol and do not have the actual chemicals on hand. You can calculate molecular weight from the chemical formula with the aid of a periodic table. You must keep in mind when you purchase the chemical that the formula weight may not be identical to the molecular weight. Suppose you have already determined how much to weigh out, based on the molecular weight, but the formula weight is greater due to hydration or the presence of inert material. Your remedy is simply to multiply your calculated mass by the ratio of formula weight to molecular weight (or simply recalculate the weight needed).

For example, suppose you need 10 grams of pure $CaCl_2$ (m.w. 111.0 g/mol), but discovered that all you have is the hexahydrated form ($CaCl_2 \cdot 6H_2O$, f.w. 219.1 g/mol). Take 219.1 divided by 111.0 and multiply by 10. You need 19.7 grams of $CaCl_2 \cdot 6H_2O$.

Materials are not always available in 100% pure form. The description on the label might indicate that the chemical is >99% pure. Such is often the case with enzymes or other proteins that must be purified from natural sources. Most of us do not worry about purity if it is above 99%. Greater precision might be important to analytical chemist, for example, but is seldom needed in biological applications. If there are significant impurities or if you insist on being as precise as you can, then calculate the amount of material you need and divide by the fraction representing the purity of the substance. For example, if you need 10 grams of pure substance A but what you have is 95% pure, then divide 10 grams by 0.95 to get 10.5 gram (note that the result has been rounded to a reasonable level of precision).

Most chemicals tend to absorb water unless they are kept desiccated, that is, to some extent they are hygroscopic. This problem should not be confused with the state of hydration, which refers to the direct association of water molecules with molecules of the substance through hydrogen bonding. Magnesium chloride is commonly used in biological buffers, and is notoriously hygroscopic. The formula weight does not include the added mass of water that is absorbed from the atmosphere. In fact, the amount of contamination depends on how long and under what conditions the chemical has been shelved, especially with respect to humidity.

It usually is not practical to worry about water content, since it is so difficult to control. If precision is critical, then chemicals should be maintained under desiccating conditions or used immediately before they can absorb a significant amount of water.

References:

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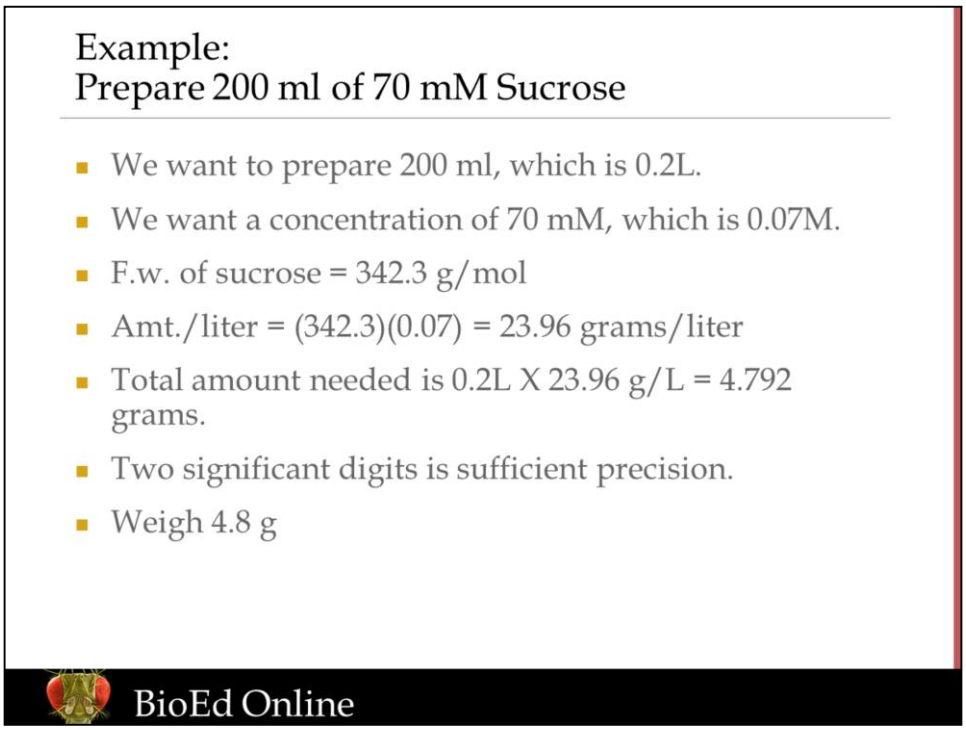

Example: Prepare 200 ml of 70 mM Sucrose

Suppose you need 200 milliters of a 70 mM solution of sucrose. Two hundred milliliters is 0.2L and 70 mM is 0.07M. The molecular weight of sucrose can be determined from its chemical formula, namely $C_{12}H_{22}O_{11}$, and the atomic weights of carbon, hydrogen, and oxygen. The formula weight for sucrose is identical to its molecular weight, namely 342.3 grams per mole. A 1M solution would consist of 342.3 grams sucrose in one liter final volume.

A concentration of 70 mM is the same as 0.07 moles per liter. Multiply 0.07 moles/liter by 342.3 grams per mole and you get 23.96 grams needed per liter. To make 200 milliliters of your solution, multiply grams/liter by liters needed. Since 200 milliliters is 0.2L, multiply 23.96 grams by 0.2L to get the 4.792 grams needed. Since a typical top loading electronic balance displays mass to the nearest 0.01 gram, the amount to be weighed should be rounded to 4.79 grams, although it is perfectly acceptable and perhaps even preferable to round to 4.8 grams.

References:

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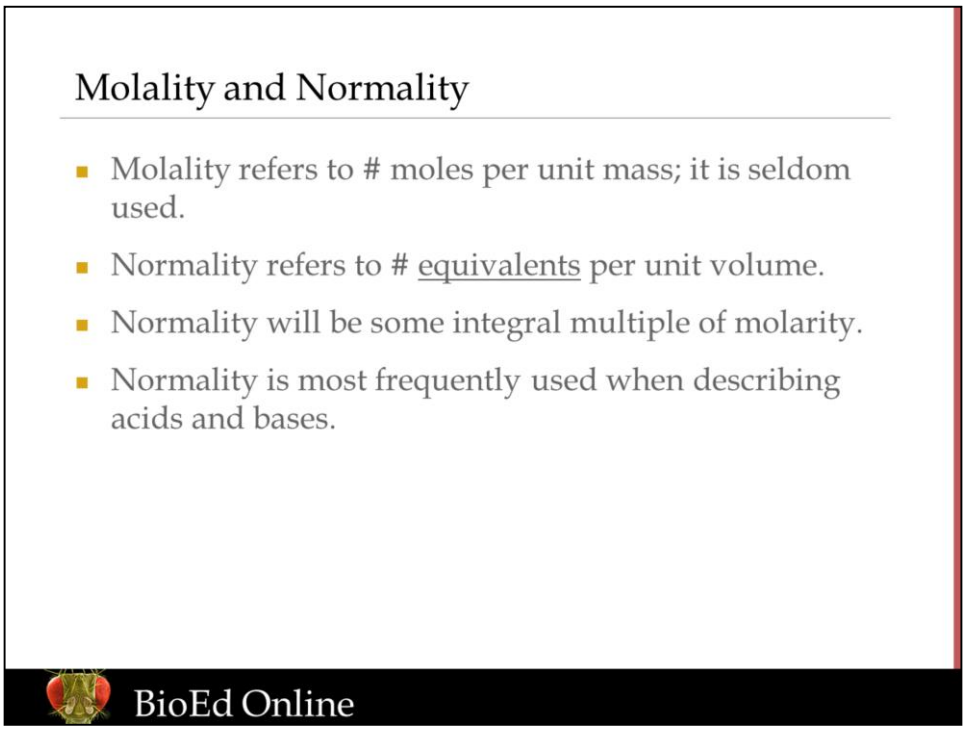

Molality and Normality

I have never prepared a solution from a molal formula. Molality refers to a number of moles per kilogram of solvent (pure solvent, that is, not total kilograms of the solution). Molality is useful when the concern is the effect of a solute on the physical properties of a solution, particularly on the boiling or freezing point. We have few applications for molal formulas in biology. If you do encounter a molal formula, the rules for preparing a molar solution apply. However, you must substitute moles per gram of the completed solution for moles per final volume.

Normality refers to the number of moles of a solute (number of equivalents), relative to some product. These days, normality is most often used when describing the concentrations of acids and bases. Sodium hydroxide (NaOH) is commonly used to make biological solutions more alkaline. One mole of NaOH dissociates to produce one mole of hydroxyl ions. Therefore there is one equivalent of NaOH per mole of NaOH. One mole of HCl dissociates to produce one mole of hydrogen ions, so there is one equivalent of HCl per mole.

On the other hand, sulfuric acid (H_2SO_4) releases two moles of hydrogen ions per mole upon dissociation. Thus, there are 2 equivalents of sulfuric acid per mole.

The number of equivalents depends on the chemical reaction of concern. If sulfuric acid reacted with another chemical to produce water, for example, one mole of sulfuric acid would be one equivalent, since one water molecule requires two hydrogen ions. In a similar reaction, a mole of HCl would be 1/2 equivalent. Normal solutions are prepared just as molar solutions are prepared, except that the molar concentration refers to the concentration of reactive groups, not of the parent compound.

References:

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