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Mixtures and Solutions

The word mixture can be defined as a heterogeneous association of substances that cannot be represented by a single chemical formula. This definition does not limit mixtures to solids mixed with liquids, nor is every mixture considered to be a solution. Two or more gases, solids, or liquids can be mixed, and two or more different phases of matter can be combined in a mixture. A biologist must be able to work with a variety of mixtures, many, but not all, of which are made by mixing a solid with water. Not all such mixtures are true solutions, either. Here are some examples of mixtures that a biologist might encounter in a laboratory.

- physiological saline solutions
- buffers
- cell suspensions
- soil suspensions
- staining solutions
- microbiological media
- chromatography slurries
- dishwater
- milk
- protein solutions
- DNA solutions
- density gradients

Wilhelm Ostwald, Nobel Laureate in Chemistry in 1909, was one of the founders of modern physical chemistry. He is reported to have said, "There are no sharp differences between mechanical suspensions, colloidal solutions, and molecular [true] solutions. There is a gradual and continuous transition from the first through the second to the third." A mixture, regardless of type, is described as "uniformly dispersed." This means that one or more minor components are evenly distributed throughout a major component. The major component is the substance that is present in the greatest proportion. In the biology laboratory the major component is often a liquid, and minor components can be solids, other liquids, or even gases.

The "mechanical suspension" to which Ostwald referred is the easiest to describe. The minor component in a suspension is typically visible in an optical microscope and is often visible to the naked eye. A colloidal mixture is sometimes called a colloidal system, a colloidal suspension, or simply a "colloid." The smallest dimension of the minor component of a colloidal mixture can range from approximately one nanometer (1 billionth of a meter) to one micrometer (1 millionth of a meter). Examples of liquid colloidal mixtures are milk, paints, and muddy water. The medium can be a gas, in the cases of smog, smoke, or aerosol sprays. Some solids are considered to be colloidal mixtures, as in steel or foam rubber.

In a true solution, one or minor components interact at the molecular level or ionic level with the major component. The minor components are atoms or molecules, and are not distinguishable in any optical microscope. Learn to apply the correct term when describing a mixture. For example, mixing cells in buffer does not usually produce a solution. Solutions are completely homogeneous mixtures, a property that is often attributed to suspensions and colloids as well. The minor components of a true solution, however, remain dispersed due to interactions at the molecular level. A substance is considered to be soluble in a particular solvent if it is capable of interaction with the solvent so as to form a solution.

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Water and Glassware for Solution Making

Water is by far the most commonly used solvent in biology because it is the major component of all living organisms. Most known biochemical reactions take place in an aqueous environment and water is frequently a reactant in or is a product of biochemical reactions. Biologically important macromolecules, organelles, cells, and organs are all designed to function in an aqueous environment.

Water quality is highly variable, and for any task an appropriate grade of water must be chosen. For example, tap water is fine for washing dishes. It is not recommended for making solutions because the quality of such water is unknown. Tap water typically contains sediments (suspended particles), metal and other ions, deliberately added chemicals such as chlorine or fluoride, and/or traces of organic solvents. Although tap water is generally safe for drinking and other personal uses, materials in tap water can be toxic to some cells or may interfere with assays or biochemical reactions. It is recommended that glassware that has been washed and rinsed in tap water be thoroughly rinsed with a higher quality water.

Distilled water, obtained from the condensation of steam, is of better quality because distillation eliminates all of the sediment and most of the inorganic solutes. Organic contaminants and some of the inorganic contaminants remain.

Deionized water is produced by running tap water through a resin cartridge or series of them. A home deionizing system might simply replace divalent cations with sodium ions, producing what is commonly known as "soft" water. Laboratory deionized water is usually treated so as to remove both cations and anions, which are exchanged for hydrogen and hydroxyl ions respectively. Deionized water is often of better quality than distilled water although on the downside, the resins used in the cartridges may release organic contaminants into the water.

The highest grade of water is called 18 megohm water. Eighteen megohms is 18 million ohms, which are units representing resistance to the flow of electricity. Eighteen meghoms is more than a million times the electrical resistance of a typical household electric circuit. Very pure water does not conduct electricity well compared with contaminated water because it contains no inorganic ions with which to carry electric current. Eighteen megohm water is usually produced in multiple steps, including reverse osmosis and the passage of product through ion exchange resins, activated carbon beds and filters.

Pure water is somewhat acidic, with pH close to 5. It is also what we call an aggressive reagent, meaning that it will leech ions from plastic or glass containers. It does so because of the polar nature of water molecules. Ions dissolve most readily in 18 megohm water because the system (water plus dissolved ions) is more stable than when pure water is separated from soluble materials. Because very pure water accumulates contaminants during storage, it should be freshly prepared. The use of plastic tubing, funnels, and especially metal containers, should be avoided.

Beakers are suitable for mixing solutions because they have large open tops for pouring in solvent or large amounts of dry chemicals. Flasks are a bit easier to handle and solution is less likely to splash out of a flask. The narrow opening discourages evaporative loss and contamination from the outside. A powder funnel can be used to add dry chemicals to a flask and a glass funnel can be used to add liquid. For measurement of liquid volumes from 10 milliters on up, graduated cylinders are usually the practical choice. Cylinders are accurate to perhaps 1% of total volume, which is more than sufficient for most solutions. We seldom have a need for volumetric flasks in biology, since we don't need such a high level of precision.

It is good practice to choose graduated cylinders and containers that are as close as possible to the intended

volume of the contents. For example, it is not very accurate to use a 2 liter cylinder to measure out 100 ml of water. The same principle holds for weighing materials. It does not make sense to weigh out one hundredth of a gram of substance in a container that weighs 100 grams.

A magnetic stirring rod is useful when it takes some time for a solute to go into solution, although it is possible to introduce additional contamination into the solution. Use heat only if a formula calls for it.

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Volumes, Amounts, and Concentrations

The common language of scientists uses units of measurement that are recognized in labs all over the world, appropriately called the International System of Units. The seven SI (for Systeme Internationale) base units are all defined in terms of well established physical quantities or standards. Other units of measure were derived from the SI base units and are called SI-derived units. In solution making we work with SI or SI-derived units for volumes, amounts, and concentrations.

Volume describes the space taken up by something. Since space is three-dimensional, a unit of volume is the cube of a unit of length, such as centimeters, feet, inches, or meters. An SI-derived unit for volume is the liter, defined as 1000 cubic centimeters. One cubic centimeter, or one thousandth liter, is a milliliter. We use prefixes in order to simplify quantitative expressions of volume, amount, and concentration. For example, we might find it convenient to describe a volume in microliters (one millionth liter) or deciliters (1 tenth liter).

The SI base unit for an amount of substance is the mole. The mole and its use in defining solutions will be discussed later. A less specific unit with which to describe an amount of substance is a unit of mass. The SI base unit for mass is the kilogram, which is 1000 grams. We may describe quantities using milligrams (one thousandth of a gram), micrograms (one millionth of a gram), or perhaps even nanograms (one billionth of a gram).

Concentration refers to the amount of substance within a specified volume. Solutions are defined by the type of solute or solutes, the type of solvent, the concentration of each solute, and often (for aqueous solutions), pH. Suspensions can also be defined as the amount of a minor component per unit total volume (volume of minor components plus volume of solvent). The concentration of a suspension can be described as the number of particles per unit volume, as is often the case for cell suspensions.

When you settle on units with which to describe quantities, you should select an appropriate prefix so that the expressions are simplified as much as possible. For example, if most of the concentrations you report are less than a thousandth of a mole per liter, it is much preferable to report them using millimoles per liter. The quantity 0.00045M is the same as the quantity 0.45 mM. You should reduce large numbers to smaller ones if you can. For example, 48,000 milligrams (mg) is the same as 48 grams.

Prefixes (International System of Units)

A student of the natural sciences really should be comfortable using prefixes to modify units. You should be familiar with at least some of the prefixes, from prior course experience.

Prefix	Factor base 10	Symbol	Prefix	Factor base 10	Symbol
deci	-1	d	deca	1	da
centi	-2	c	hecto	2	h
milli	-3	m	kilo	3	k
micro	-6	µ	mega	6	M
nano	-9	n	giga	9	G
pico	-12	p	tera	12	T
femto	-15	f	peta	15	P
atto	-18	a	exa	18	E

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Formulas for Solutions

Suppose that someone has already worked out the details, so all that you have to do is read a formula and make a solution. We can usually assume that a solution is to be aqueous unless stated otherwise. What about the concentration of the substance to be added? Common ways of describing the concentrations of solutions are weight-in-weight, weight-in-volume, volume-in-volume, and molarity. Less commonly used descriptions include normality and molality. These formulas all have one thing in common. A quantity of solute is measured out, mixed with solvent, and the volume is brought to some final quantity after the solute is completely dissolved. That is, solutions are typically prepared volumetrically. Because solutes add volume to a quantity of solvent, this method of preparation of solutions is necessary to ensure that an exact desired concentration is obtained.

There are exceptions, of course. For example, culture media for bacteria are typically made up by adding a measured amount of powdered medium to a measured volume of water. In such cases it isn't critical that a precise concentration be obtained, thus a weight-to-volume method is appropriate, instead of weight-in-volume.

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 solute relative to the weight of the final solution is described as a percentage. For example, suppose you have 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 solutions 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.

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 is often the case with complex dyes, enzymes or other proteins. Solutions that require materials from natural sources are often 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 that is needed in order 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.

Volume/volume (v/v) solutions

Volume-in-volume is another rather simple 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 is often used to describe alcohol solutions that are 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 in a bit, when we discuss how to make dilutions.

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

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 will likely 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, so that the same amount in grams of one form of the chemical contains a different amount of it than another form. For example, you may work with a chemical that can be in one of several forms of hydration. Calcium chloride 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 calcium chloride. The same mass of this chemical will contain fewer molecules of calcium chloride.

When we are interested in the actual concentration of molecules of a chemical in solution, it 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).

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). In order to calculate the desired mass of solute you will need to know the formula weight. Formula weights are usually 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, and 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. For $CaCl_2 \cdot 6H_2O$ (hexahydrate) the formula weight is 219.1 g/mol.

A hydrated compound is a compound that is surrounded by water molecules that are 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 give a 1 mole per liter solution, abbreviated 1M.

Suppose that you need one liter of a solution of 10 mM calcium chloride (10 millimolar, or 0.01 moles per liter),

and suppose that you have only CaCl2 dihydrate. To make your 10 mM solution you weigh out 1/100 of the formula weight for dihydrated CaCl2, which is 0.01 x 147.0 = 1.47 grams and bring it to one liter.

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 that when you purchase the chemical the formula weight may not be identical to the molecular weight. Suppose that 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 that you need 10 grams of pure CaCl2 (m.w. 111.0 g/mol), then discovered that all you have is the hexahydrated form (CaCl2•6H2O, f.w. 219.1 g/mol). Take 219.1 divided by 111.0 and multiply by 10. You need 19.7 grams of CaCl2•6H2O.

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 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 hydroscopic. This problem should not be confused with the state of hydration of a substances, which refers to the direct association of water molecules with molecules of the substance through hydorgen bonding. Magnesium chloride is commonly used in biological buffers, and is notoriously hydroscopic. 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 is usually 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.

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Examples: Making Solutions

Two simple examples are presented here. A third example is of a complex solution for which the description lists the concentrations of components using different expressions.

Weight in volume: 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 which is 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 that we tap out 16.97 grams of NaCl. Should we go to the trouble to get that last 0.03 gram? Nope! Consider that 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 precise then 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.

Molarity: Prepare 200 ml of 70 mM sucrose

Suppose that 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 C12H22O11 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. Take 0.07 moles/liter times 342.3 grams per mole and you have 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 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.

Complex solution: Prepare a sample buffer for SDS-PAGE

The following formula describes the composition of the 2x concentrated buffer that we use to denature proteins for electrophoresis. The formula descriptions v/v or w/w would not be listed in a methods section since it is obvious which components are liquids or solids.

50% (v/v) concentrated SDS-PAGE stacking gel buffer, pH 6.8
4.6% (w/v) sodium dodecyl sulfate
20% (v/v) glycerol
160 mM dithiothreitol (Cleland's reagent)
0.01% bromphenol blue dye

For 100 ml of sample buffer a 125 ml erlenmeyer flask is an appropriate mixing vessel. The liquid components will take up 70% of the total volume so we start by placing 50 ml stacking gel buffer and 20 ml glycerol in the flask. Glycerol is very viscous, so to be accurate you might use a syringe to deliver the stuff. We need 4.6 grams (4.6%) of sodium dodecyl sulfate (also called lauryl sulfate). To mix it evenly it should be added while stirring the solution. The concentration of dye is also given as weight-in-volume. One hundredth of 1% is only 0.1 mg/ml. You'll need just 10 mg of it. Because the bromphenol blue serves as a tracking dye and its concentration is not critical, you can weigh out something close to 10 mg or just use a "pinch" by adding the amount on the narrow end of a spatula.

The formula weight for Cleland's reagent is 154 grams/mole. The amount to weigh is given by (0.16 mole/L)(0.1 L)(154 gms/mole) = 2.46 gms (rounds off to 2.5 gms).

Most of the time it is not necessary to heat a solution to mix it, but in this case the detergent does not go into solution completely until it is heated. After popping the flask in a microwave oven for a minute or so on a low setting and stirring a bit the solution should be ready to pour into a 100 ml graduated cylinder for topping off with distilled water.

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Making Dilutions

Many of you appear to panic when you must dilute something, yet the mathematics involve nothing worse than the simplest algebra. One reason is simply that when you are busy with a laboratory procedure you are distracted and it is difficult to think in the abstract. That problem can be overcome by practicing in advance of the need. Even with practice, though, you may find dilution problems confusing unless you very clearly define your objectives. We will give you a useful formula for making dilutions, one that you may have seen before. The formula is worse than useless, though, if you don't use it properly.

Notes on using micropipettors

- To obtain the best accuracy with variable volume pipettors pre-rinse each new disposable tip
- To avoid error due to hysteresis when setting volume on a variable volume pipettor be consistent in the direction in which you change volume (either always increase to the desired volume or always decrease to the desired volume)
- When conducting a dilution using a micropipettor make sure that the tip can reach the bottom of the test tube; for example, our 1000 µl pipettors with blue tips cannot reach the bottom of a 13 x 100 mm culture tube; use an Eppendorf sample tube instead
- It is very awkward to have one person hold a tube while the other pipets from it; when students work in pairs it is better simply to take turns pipetting

Establish a frame of reference

For the sake of simplicity, lets say we are talking about sucrose solutions. Suppose you have a starting solution of sucrose (in water) with volume V1 and concentration C1. What is the total amount of sucrose in your solution? Answer: C1•V1.

Example. Volume = 0.2 liter; concentration is 50 grams/liter. C1•V1 = 50 grams/liter • 0.2 liter= 10 grams sucrose.

Now suppose that you dilute that solution with water—the whole thing—to some larger, predetermined volume (V2). What amount of sucrose is present in the new, diluted solution? If you said 10 grams, you get the gold star. But wait a minute—C1•V1 = 10 grams, and the new solution has a different volume, V2. The same amount of sucrose is present in the new solution as was in the original solution, so the following relationship must hold:

C1*V1 = C2*V2, where C2 = concentration of the new solution.

Example. Dilute the previous sucrose solution to 2 liters. What is the concentration of the new solution? We must solve for C2, of course. C1•V1 = C2•V2 = 10 grams. We know that V2 = 2 liters, so now we have

C2•(2 liters) = 10 grams

Solve for C2 to obtain 5 grams/liter.

Determining what you already knows and putting the informatin into the equation C1•V1 = C2•V2 establishes the relationship that you need in order to solve dilution problems.

Determine the objective

What do you want to do? Or, more realistically, what does the instructor want you to do? Two types of diution problems are quite common in biology and biochemistry labs.

- Dilute a known volume of known concentration to a desired final concentration
- Dilute a known concentration to a desired final concentration AND volume

The second type of problem really throws people off! Let's start with the first one, though. You know V1, C1, and C2 is predetermined. It remains, then, to solve for V2, namely the final volume to which to dilute the solution. This one is easy, since you keep the amount of solute the same and only have to change one factor.

Now for the second type problem. You know C1, namely the concentration of the starting solution. You have predetermined both V2 and C2, namely the final volume and concentration that you desire. There is one undetermined variable left, namely V1. V1 is the volume of original solution that you will dilute to the desired final volume and concentration.

C1•V1 = C2•V2

V1 = (C2•V2)/C1 or,

V1 = (final amount of solute)/C1

Example. You have a sucrose solution of 47 grams/liter. You want to prepare 100 milliliters (0.1 liter) of sucrose solution of concentration 25 grams/liter. Since you know the starting concentration of sucrose and you know both the final concentration and volume of solution that you want, all you need to find out is what volume of starting solution (V1) to use.

V1 = (C2•V2)/C1, that is, V1 = (25 grams/liter•0.1 liter) ÷ 47 grams/liter = 0.053 liter = 53 ml

Notice that the calculation comes out to 0.05319149L using full precision, but I rounded off the required volume to the nearest ml. There is a limit to the precision with which we can prepare a solution, and also a limit to the precision that we really need. You would use a 100 ml graduated cylinder to determine final volume. You would be able to read the markings to the nearest 1 ml, as a rule.

Units

It is critical that you report units for concentrations, volumes, and amounts, and when you make calculations for dilutions you must not mix up the units. For example, it doesn't work to write,

V1 (160 milliters)•C1(160 milligrams/liter = V2 (unknown)•C2(desired-3 grams/liter)

However, because concentration represents a proportional relationship, you can select from a variety of units. For example, 1 milligram/milliliter is the same as 1 gram/liter, 1 microgram/microliter, or 1 nanogram/nanoliter. It is also the same as 1000 milligrams/liter, but why would we write it that way?

Select units that simplify your expressions.

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Working with Stock Solutions

We define a *stock solution* as a concentrate, that is, a solution to be diluted to some lower concentration for actual use. We may use just the stock solution or use it as a component in a more complex solution. We refer to the solution that we end up using as a *working solution.* If you are comfortable making dilutions then you can appreciate the many advantages of working with stock solutions. Although it is never absolutely necessary to use a stock solution, it is often impractical *not* to use them. Stock solutions can save a lot of time, conserve materials, reduce needed storage space, and improve the accuracy with which we prepare solutions and reagents. Here are several illustrated types of applications using stock solutions.

Storing a solution as a concentrate

We frequently run protein gels using the Laemmli method of SDS-PAGE. The driving force that separates proteins in a gel is an electric field. To produce the field both ends of a gel and respective positive and negative electrodes are immersed in a solution that we call electrode buffer, and a typical class goes through 30 liters or so of the stuff. It would be nice to make up the buffer in advance, but 30 liters is a lot to store, and it has to be sterilized to be stored more than a few days. Sterilizing 30 liters of buffer takes a long time.

Electrode buffer can be made up in advance as a concentrate, sterilized, and stored until needed. With all of the components dissolved in a stock solution, it is only necessary to dilute the stock to make the working electrode buffer. The components of normal strength electrode buffer are 25 mM trizma base (known as tris buffer or simply tris), 192 mM glycine, and 1% sodium dodecyl sulfate (known simply as SDS). We can multiply all of the concentrations by a factor of five and still have it all go into solution. When we do that we have made a 5 fold concentrate, or what we call a 5x stock. When it is time to use the buffer we dilute each liter to a final volume of 5 liters and the buffer is ready to use.

We typically refer to the strength of a stock solution by a number followed by the times symbol x. For example, a stock solution that is concentrated by a factor of 10 is called a 10 times concentrated stock, a 10x concentrate, a solution of 10x strength, or simply a 10x solution. A normal working solution is a 1x, or normal strength solution.

A Stock solution as a component of a complex working solution

A stock solution can be mixed along with other components, including other stock solutions, to make a working solution. For example, to study respiration by isolated mitochondria we need to suspend them in a complex medium consisting of 70 mM sucrose, 220 mM mannitol, 2 mM HEPES buffer, 5 mM magnesium chloride, 5 mM potassium phosphate, 1 mM EDTA, and 0.1% fatty acid free bovine serum albumin, pH 7.4. It is practical to weigh out and dissolve the sucrose, mannitol, HEPES, and albumin, however we run into complications with the magnesium chloride, EDTA, and potassium phosphate.

Magnesium chloride is extremely hydroscopic, that is, the dry chemical accumulates moisture from the atmosphere when stored on a shelf. Sometimes one will open even a new bottle, and depending on where it has been and for how long you might find your crystals immersed in a semi-liquid slurry. Because we don't know how much water has accumulated and because water adds weight, it is impossible to obtain an accurate concentration of magnesium chloride by weighing out the chemical unless it has been stored in a very effective dessicator. A trick is to purchase a known quantity of the material, for example 500 gms, and without even

weighing it to prepare the entire lot as a stock solution. You can prepare, for example, a liter of 0.5M (500 mM) magnesium chloride. To use the stock to prepare respiration medium, simply include 10 ml of stock solution per liter of working solution. For this application, then, 0.5M magnesium chloride is a 100x stock.

Ethylene diamine tetraacetic acid (EDTA) as a free acid will not go into solution without bringing the pH to near neutrality. Unfortunately, because the material continues to reduce the pH as it dissolves, dissolving a quantity can be a tedious process. EDTA salts tend to dissolve more readily, but you would have to keep two different salts for sodium based and potassium based solutions respectively. In addition, we might use EDTA in a concentration as low as 0.1 mM in a small volume. It is usually easier to deliver a small volume of liquid accurately than to accurately weigh out and deliver a small amount of dry chemical. A concentrated stock of EDTA can be prepared using either NaOH or KOH to adjust pH, to be available whenever a solution requires EDTA as a component. For example, our respiration medium is potassium based so we might prepare 100 mM EDTA and use 1 ml of the stock per liter of working solution.

The issue with phosphate buffers calls for a write-up of its own.

Working with phosphate buffer stocks

When we include a phosphate buffer in a solution we choose a salt that is compatible with the intended use of the solution. For example, the principal positive electrolyte inside cells is potassium, while the principal extracellular cation is sodium. Because mitochondria are intracellular organelles we suspend them in solutions buffered with potassium salts. The name potassium phosphate refers to a family of inorganic salts, including dibasic and monobasic potassium phosphate. The formula for the dibasic form is K2HOP4 and for monobasic it is KH2PO4. The monobasic form is actually quite acidic. We can make a stock solution of potassium phosphate at a desired pH by mixing monobasic and dibasic stocks. A practical concentration for each stock is 100 mM.

For a pH near neutrality we start by stirring dibasic solution and slowly adding monobasic stock while monitoring pH, stopping when we reach the desired value. The concentration of phosphate ion remains 100 mM because that was its concentration in both of the "parent" stock solutions. To use the buffer as a component of respiration medium we simply add an appropriate volume of 100 mM stock solution before bringing the medium to final volume. What volume will you need to add to obtain a final concentration of 5 mM phosphate? What is the strength of the concentrate?

Saline solutions

Biologists often work with Ringers solutions, relatively simple buffers that contain a limited mixture of inorganic and perhaps some organic salts. A marine biologist or developmental biologist might work with artificial seawater (ASW), also a relatively simple solution of common salts. The composition of Ringers or ASW may be varied for experimental purposes. For example, the concentrations of potassium and sodium ions both have profound individual effects on the level of the membrane potential in muscle cells immersed in Ringers. It would be inconvenient to weigh out different amounts of dry potassium and/or sodium over and over as different solutions are required. On the other hand, it is a quick and simple matter to pipet different volumes of stock solutions before bringing a Ringers solution to final volume.

A formula for frog Ringers is 0.65% NaCl, 0.014% KCl, 0.012% CaCl2, 0.1% NaHCO3. Notice that to prepare a liter of working solution would require weighing as little as 0.1 gm of dry chemical, creating a high potential for mistakes. On the other hand you could prepare a liter or more of 10x NaCl stock by weighing, dissolving, and bringing to 1 L volume 65 gms NaCl. You could prepare a liter each of 100x KCl, CaCl2, and NaHCO3 by weighing, dissolving, and bringing to volume 14, 12, and 10 gms per liter of each respective chemical. Not only is it more accurate to weigh a larger amount of material, but now you can change the Ringers formula at will. For example, for normal Ringers you would include 10 ml KCl stock per liter working solution. Suppose you want to raise the concentration of potassium five-fold. Then include 50 ml KCl stock per liter.

Working with samples

Suppose that you want to test the effectiveness of a substance in preserving enzyme activity. You plan to mix enzyme with preservative in aliquots and store them frozen, conducting an assay periodically to see how long the enzymes remain functional. To give your new preservative a good test you conducted a dozen enzyme preparations and have each prep in solution. You have measured the protein concentration of each enzyme solution.

Suppose also that you have stored your preservative as a 5x concentrate. To facilitate the assays and keep the

storage conditions uniform you want to prepare each enzyme solution to the same protein concentration (say, 1 mg/ml) in 1x preservative. How do you go about deciding what volumes to mix together?

First, decide what approach to take to diluting the enzyme preparation itself. Do you want to prepare the whole batch to a final concentration of 1 mg/ml or do you want to prepare a specific volume to 1 mg/ml and save the rest? Recall how to use the C1V1 = C2V2 relationship to set up each type of problem. Next, apply the relationship to determine your unknown quantity, which is either the final volume of your solution (V2) or the volume of enzyme solution to start with (V1). Separately, decide how much 5x preservative solution to include in your diluted enzyme solution to end up with 1x preservative.

For example, let the protein concentration of one enzyme batch be 12 mg/ml. Suppose that you have 3 ml and want to bring it all to 1 mg/ml. Using the "C,V" equation you can calculate the desired final volume to be 36 ml. After all, 3 ml of 12 mg/ml contains 36 mg protein, and 36 mg protein in 36 ml volume give you 1 mg/ml. You can arrive at the same conclusion by treating the enzyme solution as a stock solution. A concentration of 12 mg/ml is a 12x stock when your working concentration is 1 mg/ml.

Before bringing your enzyme solution to volume, factor in the preservative stock. You will prepare a final volume of 36 ml of enzyme-preservative. The preservative concentration is 5x. Then 1/5 of the final volume must be preservative stock, and 1/5 of 36 ml is 7.2 ml.

So, the answer to your problem is to combine 7.2 ml preservative solution with 3 ml enzyme solution and bring the final volume to 36 ml with water or buffer.

What do the ratios mean?

Suppose someone asks you to "prepare a one to ten dilution of solution X." Does it mean take one part solution X and add ten parts water, or does it mean take one part solution X and bring the volume to a total of ten parts? A biologist would likely apply the second definition to a buffer or reagent solution. In another discipline, though, the former definition might be more relevant. Even in biology, we often prepare complex media as weight-to-volume (w:v) instead of weight-in-volume (w/v), that is, we add a prescribed mass of material to a prescribed volume of water instead of mixing the materials and bringing the mixture to a prescribed final volume.

To avoid confusion you might say "please prepare a one to ten dilution of solution X, weight-in-volume," or if you want to bring materials together in a precise proportion, say "please prepare a one to ten dilution of solution X, weight-to-volume." In the latter case it might be less confusing just to say "please combine one part solution X with ten parts water."

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