1. **Problem Statement**

There are many situations in biomanufacturing where some substance must be diluted with water or an aqueous buffer. It is sometimes confusing to technicians to determine how much diluting substance to add to the substance to obtain a final solution with a particular volume and concentration.

2. **Scenario Description and Specific Example**

Many biomanufacturing companies have a media production division whose role is to prepare and provide buffers, culture media, and other reagents to the rest of the company. Frequently these aqueous solutions are initially prepared in a concentrated form and then must be diluted, either by the media production staff or at the point of use in the company. Technicians must be able to accurately determine how much diluting substance (usually water or buffer) and how much of the concentrated stock solution must be combined to obtain the correct final concentration and volume.

Box 1 shows a short portion of a standard operating procedure (SOP) that is followed when making an antibody pharmaceutical product (for example, the monoclonal antibodies that were sometimes used to treat COVID-19). Figure 1 shows two chemicals that have been supplied by the media prep department to the production staff who are following this SOP.

Questions:

**Question 1.** Do any of the supplied reagents require dilution by production staff?  
**Question 2.** If so, how should this be accomplished?

(Do not worry if you do not fully understand the SOP; the details are not important right now.)
6.5.1. Prepare a bottle with 150 mL of 1M NaHCO₃ in ultrapurified water as follows:

6.5.1.1 Weigh out 12.6 ± 0.1 g of NaHCO₃ and transfer to a 250 mL beaker.
6.5.1.2. Measure 145 mL ultrapurified water and add to the 12.6 ± 0.1 g of NaHCO₃ in the beaker. Add a magnetic stir bar and stir on a magnetic stirrer to dissolve.
6.5.1.3. Transfer the dissolved NaHCO₃ solution to a 250 mL graduated cylinder and bring to 150 mL volume with ultrapurified water.
6.5.1.4. Label the bottle as 1M NaHCO₃ with date, preparer initials, storage: room, temp, disposal: drain.

6.5.2. Add 100 mL of 1X PBS to the bioreactor vessel where cells will be cultured.

Figure 1. a. Sodium Bicarbonate Reagent Bottle. If you could look inside this bottle, you would see that this chemical has been supplied as a powder. b. PBS (Phosphate buffered Saline) Reagent Bottle. If you could look inside the bottle, you would see that it is a clear liquid.

3. Issues to be Addressed and 4. Mathematics (Calculations)

The first question asked in this example is whether, in this SOP, preparing either the sodium bicarbonate (NaHCO₃) or the PBS requires a dilution step. Beginners sometimes have difficulty identifying situations that require dilution. It is important to realize that dilutions (in our biomanufacturing context) begin with a concentrated aqueous solution, not with a chemical in powdered form. Thus, making the sodium
bicarbonate does not require dilution. In contrast, the PBS has been supplied as a concentrated stock solution. The PBS label, “10X,” means that the reagent has been supplied in a form that is ten times more concentrated than is required for normal use. Thus, the answer to the first question is that production staff preparing the sodium bicarbonate do not perform a dilution while they do so when preparing the PBS.

Answering the second question in this scenario requires determining how much of the PBS stock solution is required and how much diluent must be added to it to obtain 100 mL of 1X PBS. There is a very convenient formula, Equation 1, to do this calculation. It is:

\[
\text{Equation 1} \quad \text{THE } C_1V_1 = C_2V_2 \text{ EQUATION} \\
\text{HOW TO MAKE A LESS CONCENTRATED SOLUTION FROM A MORE CONCENTRATED SOLUTION} \\
(\text{Concentration}_{stock}) (\text{Volume}_{stock}) = (\text{Concentration}_{final}) (\text{Volume}_{final}) \\
\text{This equation can be abbreviated:} \\
C_1V_1 = C_2V_2
\]

In our scenario, the stock solution of PBS is labeled “10X.” In this expression, the “X” means “times.”

This SOP tells the technician to make 100 mL of the PBS at a concentration of “1X.” Applying Equation 1:

\[
C_1 \text{ is } 10X \\
C_2 \text{ is } 1X \\
V_1 \text{ is the unknown} \\
C_2 \text{ is } 100 \text{ mL} \\
\text{Thus:} \\
(10X) (?) = (1X) (100 \text{ mL}) \\
? = 10 \text{ mL}
\]

To make the PBS, take 10 mL of the concentrated stock and bring to a final volume of 100 mL.

The mathematics required to solve this equation is simple algebra – solving an equation with one unknown. The major difficulty for beginners is recognizing situations where this equation applies. Also, as will be discussed below, technicians must have a clear understanding of the meaning of “concentration” and the conventional ways of expressing the concentration of a solution. They also must understand the terminology used in the equation.
5. Extensions Beyond Biomanufacturing

The concept of “dilution” has a great many applications beyond biomanufacturing. For example, syrups used in food and beverage preparation are often prepared in a concentrated form and then diluted to make the final product.

Carbon fiber products are used in the manufacture of many items, such as aircraft, bicycle frames, and sporting goods. “Sizings” is a term used for coatings that are applied to the surface of carbon fibers during the manufacturing process. A manufacturer of carbon fiber products provided the following example of a scenario that is conceptually much like the biomanufacturing scenario, but might appear different to a beginner:

The technician is asked to prepare a 1.5% concentration sizing bath (sizing is the chemical treatment applied to the surface of the carbon fiber product) by diluting a 20% resin/80% water mixture.

In this case, the desired volume of 1.5% sizing bath is not specified and the technician would need to know what size is required for the application. The absence of this necessary piece of information means that contextual knowledge is required to perform the task. The term “20% resin/80% water mixture” is used by the supervisor in this manufacturing setting. In a biomanufacturing setting, the terminology would simply be “20%” and water would be assumed as the solvent. Here, slightly different terminology might obscure the fact that the underlying concept is the same in the biomanufacturing and carbon fiber manufacturing situations.

The supervisor provided calculations for his carbon fiber manufacturing scenario based on a 50-gallon sizing bath:

\[
(20\%) \ ? = (1.5\%) \ (50 \text{ gallons})
\]

\[
? = 3.75 \text{ gallons} \text{ (amount of the original resin required)}
\]

50 gallons – 3.75 gallons = \textbf{46.25 gallons} (amount of water required to get final 50-gallon volume)
Thus, the calculation tool used in the biomanufacturing and carbon fiber manufacturing settings is the same but the context, terminology, and assumed knowledge is different.

Further Notes

Why do beginners find dilution challenging? First, beginners sometimes confuse “concentrations” with “amounts.” Concentrations are ratios; amounts are not. Before solving calculation problems, such as shown above, it is essential that students can distinguish between an amount (such as a gram, liter, or mole) and a concentration (such as grams/liter or moles/liter). Observe that in biological/chemical terminology, the symbol “M” for molarity, indicates a concentration (moles/liter), although it does not appear to be a ratio at first glance.

A second issue that confuses beginners in biology (and chemistry), is there are multiple ways to express the concentration of molecular solutes in a solution. Concentration can be expressed as molarity, percent, molality, or as a simple ratio. The calculations involved with the different expressions of concentration are somewhat different and therefore confusing.

Terminology can be a source of confusion. In this scenario, the convention of using an “X” is shown. 10X means that a solution is ten times more concentrated than it is commonly used. For example, if a solution is routinely used at a concentration of 0.1 molar, then 10X means the solution is 1 molar. Technicians need to be familiar with this convention and understand its meaning.

At a more basic level, technicians need to have a clear understanding of ratios. They should see that 10 mg of substance in 100 mL of total solution is the same concentration as 1 mg of substance in 1 mL of total solution. 10X reagent is not the same concentration as 1X reagent.

Also note that, in the workplace, calculations often are required as technicians move through a procedure. In this scenario we show an SOP, that in and of itself requires knowledge to perform. In this example, the PBS was not supplied at the required concentration and the technician might not discover this until getting to that step in the procedure (though advance preparation is always
a good idea). Hence, the necessity to perform a calculation in the workplace often occurs when the technician is preoccupied with other task(s).

Another issue that might be confusing is that it is important to understand what each variable is in the \( C_1V_1 = C_2V_2 \) equation. Observe that \( V_2 \) is the final total volume. Biologists will typically bring a solution to the final volume by adding diluent to the amount calculated as \( V_1 \) using a graduated cylinder or volumetric flask. In the carbon fiber example, the volume of diluent required is calculated by subtraction, a different strategy. In yet other situations, the volume of stock required, \( V_1 \), is so small that the diluent is simply measured out and the stock added to it directly. The technician needs to know what strategy is appropriate in a given situation.

For a technician, the \( C_1V_1 = C_2V_2 \) is a handy calculation tool that applies in a great many situations. However, this also means that sometimes beginners try to apply the equation where it is not relevant. For example, in the scenario above, it is not relevant when asked to make 150 mL of 1M NaHCO\(_3\). This means that the technician needs to understand what a dilution is. Also, as will be described in the second part of this two-part scenario, there are other ways to think about dilutions that are useful in other situations.