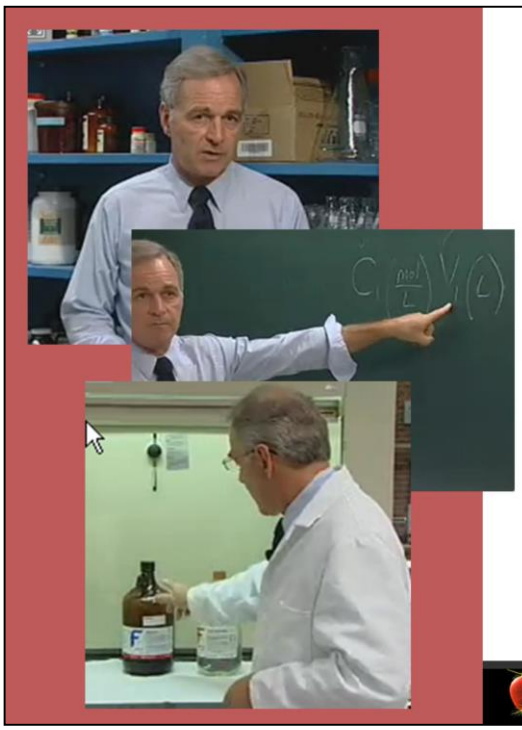

Quantitative Methods: Diluting Solutions Part II

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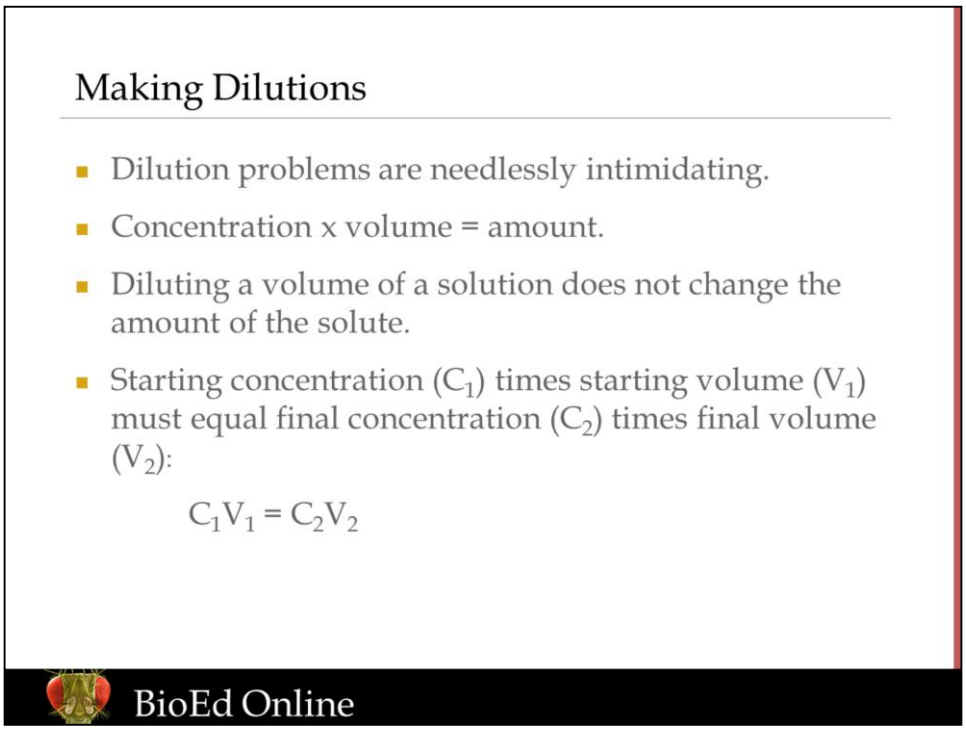

Making Dilutions

Many students panic when they must dilute something, yet the mathematics involve nothing worse than the simplest algebra. What is the objective? If the idea is to dilute a starting volume of known concentration to some final concentration, then the objective is to determine the volume of the final product. If the idea is to dilute stock solution to some final concentration AND final volume, then it is the starting volume of stock solution that must be determined.

It is easy to work out dilution problems once a solid frame of reference is established. First, consider that the total amount of solute in a solution is equal to its volume times its concentration (e.g., 1/2 liter of 0.2 M sucrose contains 0.1 mole sucrose). Next, consider that if you take a volume of solution of a certain concentration and dilute it, you are adding solvent without changing the total amount of solute. Thus, the starting concentration multiplied by starting volume must equal the final concentration multiplied by the final volume. Once students understand the principle behind the equation $c_1v_1=c_2v_2$, dilution problems should be easy for them.

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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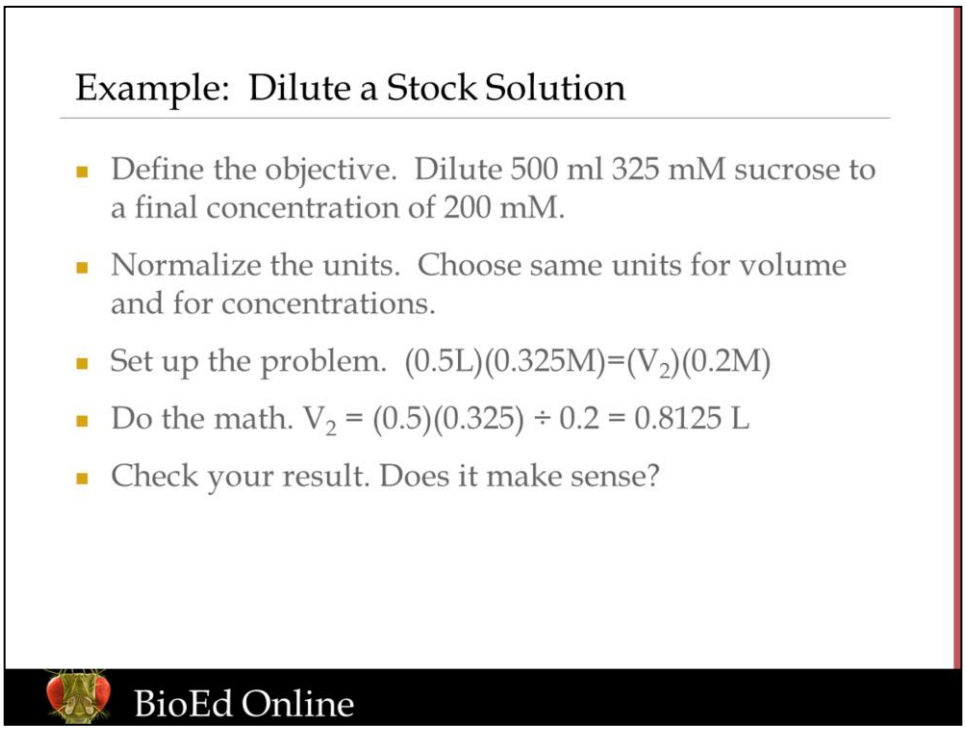

Example: Dilute a Stock Solution

To create a stock solution, first define clearly what you want to do. For example, suppose you have 500 ml of a stock solution of sucrose at 325 mM concentration. Then suppose you need to dilute it to 200 mM. First, make sure all the units agree. On other words, the units used for concentration must be the same on both sides of the equation, as must be the units used for volume.

Now you can set up the problem. The total amount of solute is given by the concentration of the solute times the volume of the solution, or C_1V_1. You have 0.5 liter times 0.325 moles/liter, which is 0.1625 moles. To dilute the solution means to increase its volume, obviously, by adding more solvent but no more solute. You know the final desired concentration, namely 200 mM, but you don't know the final volume yet. We'll call it V_2. You know that V_2 times the final concentration, 0.2 moles/liter, has to be 0.1626 moles. The final volume, then, is given by the equation

$V_2 = (0.5)(0.325)/0.2$

The equation yields 0.8125 L, which is 812.5 ml. You already have 500 ml, so you must add 312 ml to that solution, or simply bring the final volume to 812 ml in a graduated cylinder.

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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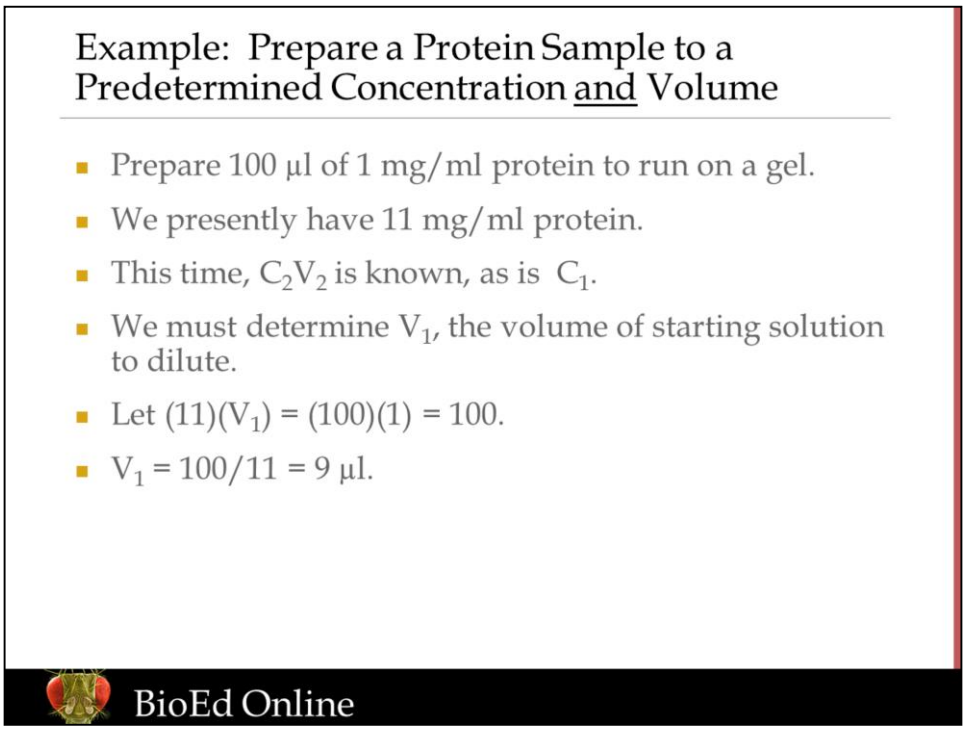

Example: Prepare a Protein Sample to a Predetermined Concentration and Volume

To work with macromolecules, we often must dilute a solution to some predetermined concentration, and we usually need some particular amount of solution. For example, it is common to conduct a protein assay to determine the concentration of an unknown protein mixture, and then to dilute the unknown to a predetermined amount and concentration in order to run it on a gel.

This time, suppose you already have determined the final desired concentration and volume (C_2 and V_2). You know the starting concentration, C_1, from the protein assay. However, you must do the algebra to find out the volume, V_1, of the starting solution you will need to dilute to the final desired volume.

In this example, the protein assay gave a concentration of 11 milligrams/milliliter unknown protein sample. You need 100 µl at a concentration of 1 mg/ml. Since three of the four variables are known, you only need to solve for the fourth variable, namely the volume of 11 mg/ml protein with which to start. To avoid confusion I have converted the concentrations to micrograms per microliter. One milligram per milliliter is the same as one microgram per microliter or 1 gram per liter.

$V_1 = (100\ \mu l)(1\ \mu g/\mu l)/11\ \mu g/\mu l = 9\ \mu l$

References:

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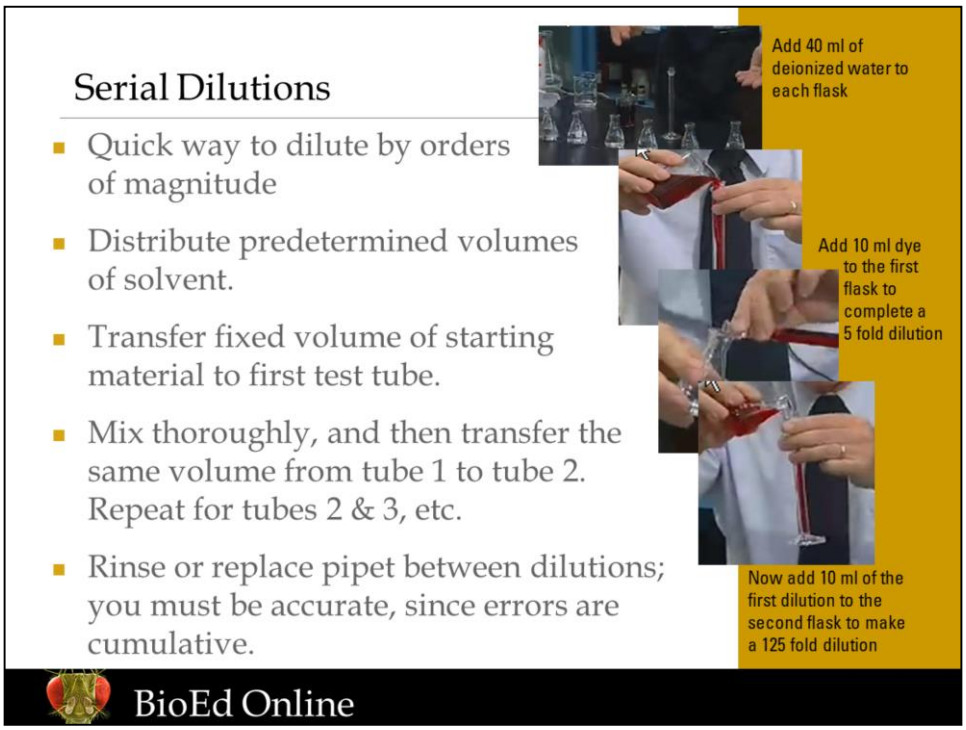

Serial Dilutions

It often is convenient to make dilutions of a stock solution. For example, to conduct an assay on a substance when nothing is known about the starting concentration, one can make several dilutions and determine which dilution gives a concentration that falls within the sensitive range of the assay. Serial dilutions, in which one systematically prepares dilutions sequentially by the same factor, accomplish this purpose very well. Other uses for serial dilutions include determining the effective concentration of an antibody solution, the best dilution for conducting bacterial counts, or the best dilution for an enzyme assay.

Start your serial dilutions by deciding how much to dilute each successive sample. It is common to make ten-fold dilutions. Then, decide on a container. Larger volumes tend to reduce error introduced by pipetting inaccuracies.

Place a predetermined volume of solvent in each container. To make 5 serial dilutions of a bacterial sample, you might start with five 125 ml Erlenmeyer flasks and aseptically put 90 ml of culture medium into each flask. Next, transfer 10 ml of liquid bacterial culture to the first flask and mix completely to make a 10 fold dilution. Transfer 10 ml from that flask to the next one and mix again, repeating for the remaining five flasks. The fifth flask should contain a 10^5 dilution of the starting material.

The pipette used for transfer must be rinsed each time, or even replaced, since any remaining liquid is ten times concentrated, compared to the solution you will transfer next. Errors will be compounded, so it is crucial to use an accurate transfer technique.

References:

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