

Making dilutions is one of the most common practices in chemistry. Concentrated solutions take less storage space, are less expensive to ship, and last longer than dilute solutions. Analytical samples are often too concentrated for direct analysis. For these and other reasons, chemists are often diluting solutions and thus require a working knowledge of the techniques and calculations associated with this procedure.

Descriptions of diluted solutions are as diverse, requiring careful reading and understanding of the directions associated with their preparations.

Adding “n” volumes of solvent:

Chemists are often directed to add a specific number of volumes of additional solvent to a mixture. For example, “Add 2 volumes of water to the sample.” Or, “Add 1 volume of alcohol to the solution.” These directions are direct and easily understood. If the sample contains 50mL of sample, then adding “two volumes” of water means to add 100mL of water to the original 50mL of sample. If the original sample contains 2mL, then adding “one volume” of alcohol means that 4mL of alcohol should be added.

Diluting “s:n”

Another way of expressing this dilution is to state the ratio of sample-to-solvent (*sample volume : added volume*). For example, if 1 volume of sample is to be diluted by adding 2 volumes solvent, then we say that the sample should be “diluted 1:2” with solvent. When dilutions ratios are written this way, it refers to the individual volumes of sample and solvent that should be mixed. The sum of both parts equals the total volume of the final mixture. For example, if a sample of salt water were diluted 1:3 with water, one volume of salt water would be mixed with three volumes of water, yielding a total final volume of 4 volumes.

Dilution by Adding Solvent to an End Volume:

Another method for describing a dilution is stating that a starting volume be “diluted with enough solvent” to reach a final volume. For example, “20mL of 6N HCl is mixed with enough water to make 500mL of final solution.” Or, “50mL of ethanol was added to a 100mL volumetric flask and water was added to the mark.” In both cases, the initial volume of sample was diluted to a final volume, rather than stating directly what volume of diluent was added.

Another way of describing this type of dilution is to state to what extent the sample is diluted. Consider the case of the alcohol above. Since 50mL of the alcohol was diluted to a final volume of 100mL, we say the alcohol was diluted “two times,” “twice,” or “2x.” For the acid, 20mL was diluted to 500mL, so it would be described as being diluted 25 times ($500/20 = 25$). As seen, this calculation is simply the final solution volume divided by the original (sample) volume (final volume/original volume). The individual volume of solvent added is not stated directly, but must be calculated separately.

Consider the case of 10mL of soda pop that is being prepared for analysis. Exactly 10mL of the beverage is mixed with 40mL of water resulting in a final volume is 50mL. We say the sample has been diluted 1:4. Another way of describing the same process is to state that it has been diluted 5 times (5x).

Dilution by Mixing Two Solutions Together:

All of the above procedures describe the dilution of a concentrated sample by adding additional pure solvent. What if one solution is diluted by mixing it with a different solution? For example, when 100mL of 1M KCl solution is mixed with 100mL of 1M NaBr solution, the KCl is diluted to 0.5M and the NaBr is also diluted to 0.5M. So, both solutions are being diluted into each other.

Increasing Concentration by Removal of Solvent:

Dilution decreases solute concentration by adding solvent. By removing solvent, the concentration of solute increases. This type of “reverse dilution” is a common practice for juices that are sold on the commodities market. For example, frozen orange juice is often sold at retail as a 4x concentrate. This means that normal orange juice was processed by heating to remove enough water to reduce the juice to ¼ of its original volume. Before drinking this concentrate, it is diluted four times (4x) with water, or by adding three volumes (1:3) of water.

Sometimes it is necessary to increase the concentration of a solute prior to analysis. Consider a sample of water that is to be tested for arsenic. The original concentration of arsenic is only 10ppb, but the analytical method has a quantitative limit of detection of only 100ppb. To overcome this problem, a 1000mL sample is heated to evaporate enough water to reduce the solution volume to only 10mL, thus increasing the arsenic concentration by 100x (to 1000ppb or 1ppm).

Dilution Calculations:

Since dilutions are simply changes in the ratio of solute to solvent, a simple ratio of concentration to volume can be used for almost all calculations involving dilutions:

$$C_i V_i = C_f V_f$$

Where C is the concentration of the solute and V is the volume of the solution at initial (i) and final (f) conditions. An easy way to see how this equation works is to use units of molarity and liters:

$$M_i V_i = M_f V_f$$

$$(\text{moles/liter})_i \times (\text{liters})_i = (\text{moles/liter})_f \times (\text{liters})_f$$

Therefore,

$$\text{moles}_i = \text{moles}_f$$

Thus we see that regardless of the changes in volume, the total quantity of solute remains unchanged. If three of the quantities are known, the fourth is easily calculated.

Example 1: Describe how to prepare 500mL of 0.10N HNO₃ by diluting 12N HNO₃.

Solution: Solving for V_f, $V_i = C_f V_f / C_i = (0.10\text{N}) \times (500\text{mL}) / (12\text{N}) = 4.2\text{mL}$. Mix 4.2mL of 12N HNO₃ with enough water to make 500mL of final solution.

Example 2: What was the original concentration of uranium if evaporating water reduces 10.0 L solution to only 200 mL results in a concentration of 20ppm U?

Solution: Solving for C_i, $C_i = C_f V_f / V_i = (20\text{ppm}) \times (0.200\text{L}) / (10.0\text{L}) = 0.40\text{ppm} = 400\text{ppb}$.

Dilution Factors:

To simplify calculations involving dilutions prior to analysis, *dilution factors* are often used. A dilution factor is simply a multiplier that changes the final analyzed concentration back to the original concentration of the undiluted solution. Dilution factors are simply the ratio of the final volume divided by the initial volume:

$$C_i = C_f [V_f/V_i] \quad ,$$

where $[V_f/V_i]$ is the dilution factor.

Note that during dilution, the final volume is larger than the initial volume, resulting in dilution factors that are always greater than 1. However, in some cases, the solvent is evaporated to concentrate the solute. Since the final volume is less than the initial volume, dilution factors in this case are less than 1.

Example 3: Calculate the dilution factor for a sample if 4.0 mL were diluted with water to final volume of 1,000 mL.

Solution: Dilution factor = $[V_f/V_i] = 1,000/4.0 = 250$.

In Example 2 above, in which the solvent is evaporated to reduce volume from 10.0 L to 200 mL, the dilution factor is

$$[V_f/V_i] = 0.200/10.0 = 0.020.$$

Serial Dilutions:

To save solvent and utilize smaller volumetric glassware, dilutions are often performed as serial dilutions. That is, a sample is diluted stepwise. For example, if 10mL of sample is transferred to a 100mL volumetric flask and water is added to the mark, the sample has been diluted 10x. Then, if a 5 mL portion of this resulting solution is transferred to another 100 mL volumetric flask and enough water is added to fill the flask to the mark, the result is a second dilution of 20x. The total dilution is a product of both dilutions, resulting in a $10 \times 20 = 200x$ dilution. In other words, the combined dilution factor would be

$$\text{Combined Dilution Factor} = \frac{100}{10} \times \frac{100}{5} = \frac{10,000}{50} = 200$$

Dilution is common technique in quantitative microbiology, where the number of bacteria per milliliter must be determined. If the concentration is too high, the organisms are too numerous to count on a single agar plate, so serial dilutions are performed. A series of tubes is prepared, each containing 9 mL of buffer solution. One mL of original solution is added to the first tube and mixed. Then, a 1 mL aliquot of this tube is transferred to the next tube and mixed. One mL from this second tube is then transferred to the next tube in the series and so on. Each transfer represents a 10x dilution. A highly concentrated suspension of bacteria is easily determined after a series of 5-7 tubes is performed. If 60 organisms (CFU or colony-forming units) are counted per mL in the 6th tube in the series, then the original concentration is determined by multiplying this quantity by the dilution factor (10^6):

$$\text{Original Bacteria Concentration} = 60 \text{ cfu} \times 10^6 = 6 \times 10^7 \text{ cfu}.$$