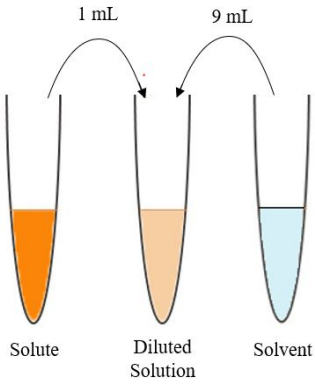
Dilution



Dilution is the process of making a solution less concentrated. This is accomplished by adding a solvent (such as water) to dissolve a solute (such as a dye) into a solution, reducing the solute's concentration.

Terms to express dilution

Dilution ratio (1:x dilution) - The ratio of initial volume to final total volume.

Dilution factor (DF) - The ratio of final volume to initial volume.

To the right is an example of a 1:10 dilution.

Dilution ratio = 1:10 (1 mL solute in 9 ml solvent - 10 mL total solution)

Dilution factor = 10 (10 mL total solution / 1 mL solute)

Calculating the dilution

When diluting, we add solvent to a set amount of solute - because of this, while the total volume (V) of the solution changes, the actual amount of solute in the solution remains constant. The amount of solute in a solution is equal to the concentration of the solute (C) multiplied by the total volume of the solution (V). For example, a solution containing 1g of salt per 100 mL of water (C=1g/100mL) should have how many grams of salt in 500 mL of solution (V=500 mL)?

$$C * V = \textit{Solute amount}$$
$$1g/100mL * 500mL = 5g\ salt$$

Since the amount of solute is always equal to concentration times volume, we can compare diluted solutions to each other and calculate dilutions using this fundamental equation:

$$C_{initial}V_{initial} = \textit{Solute amount} = C_{final}V_{final}$$

or

$$C_1V_1=C_2V_2$$

We also know that, when diluting, the total volume of the solution will change according to how much solvent is added. This gives us the following equation:

$$V_{final} = V_{initial} + V_{solvent}$$

Using the equation

Usually, $C_{initial}$ is known to us. C_{final} is the target concentration we're trying to obtain.

To calculate C_{final}, we can rearrange the equation as follows:

$$C_{initial}V_{initial} = C_{final}V_{final}$$
$$C_{final} = \frac{C_{initial}V_{initial}}{V_{final}}$$
$$= C_{initial} \times \left(1 \div \frac{V_{final}}{V_{initial}}\right)$$
$$= C_{initial} \times (1 \div DF)$$

$= \frac{C_{initial}}{DF}$

Since these equations are all equivalent rearrangements of each other, they can all be used to solve for C_{final}.

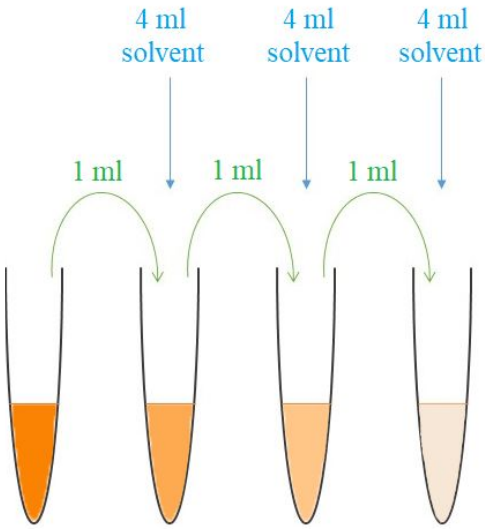
Although we are often attempting to solve for C_{final}, we can solve for any single missing variable in a similar manner. For example, if we want to make a certain amount of diluted solution, V_{final} is the target volume we want the diluted solution to have. We can thus calculate the initial volume by rearranging the above equations to obtain $V_{initial} = \frac{C_{final} V_{final}}{C_{initial}}$. We can also calculate the added solvent volume by $V_{solvent} = V_{final} - V_{initial}$

Serial dilution

Serial dilution is a technique where multiple simple (1-step) dilutions are performed consecutively, each using the diluted solution obtained in the previous step.

To the right is an example of a serial dilution with 1:5 dilution for each step, meaning that there is a total 1:125 dilution from the first solution to the final solution.

Serial dilutions are used when the dilution factor is large, or when the initial volume to be used is too small to be accurately measured.



Dilution Practice

What you need:

- Micropipette
- water
- dye
- beaker
- microplate
- microplate reader

1) Simple dilution

    1) Create a table on your notebook like the one shown below:

Dilution	Starting dye concentration	Starting dye volume	Final dye concentration	Final total volume	Volume of water to be added	Absorbance at 460nm
1:2	20 %			2 ml		
1:5	20 %			2 ml		
1:8	20 %			2 ml		

2) Based on the $C_1V_1=C_2V_2$ equation, calculate and fill in the column "Starting dye volume," "Final dye concentration" and "Volume of water to be added."
3) Label 3 test tubes - one for each dilution. Add water to your beaker.
4) Pipette dye stock and water into the labelled test tubes based on your calculation results
   Note: Don't let your pipette barrel go below the rim of the test tube - only the pipette tip should go into the test tube. Tilt the test tube if necessary.
5) You will be assigned 3 wells on a 96 well plate to test your dilution results. Write down the wells assigned to you and which diluted solution will be used for each well.
6) Vortex your test tubes. Add 200 µl of each diluted solution to separate wells according to your labeling.
7) The plate will be processed by the accuSkan FC microplate reader once everyone finishes this step.
   Note: The microplate reader will test the absorbance of blue light (λ = 460 nm) for the solution in each well.
8) Write down the readings for each of your dilutions.

2) Serial dilution

1) Create a table on your notebook as below:

Dilution	Step starting dye concentration	Starting dye volume	Final dye concentration	Final total volume	Volume of water to be added	Absorbance at 460nm
1:10	20 %			3 ml		
1:25				3 ml		
1:50				3 ml		
1:100				3 ml		
1:200				3 ml		
1:500				3 ml		

Note: The dilution ratio refers to the overall dilution of the original dye stock - we are creating a 1:200 dilution from a 1:100 diluted solution, not diluting the 1:100 dilution another 200 times!

2) **This time, instead of using the stock food dye solution for all dilutions, each dilution should use the diluted solution from the previous dilution.** Calculate and fill in the column "Starting dye volume," "Final dye concentration" and "Volume of water to be added."
3) Label 6 test tubes according to each dilution.

4) Add solutions and water to the labelled test tubes based on your calculation results. After each dilution step, vortex the mixed solution before moving on to the next dilution.
5) You will be assigned 6 wells on a 96 well plate to test your dilution results. write down the wells assigned to you and which diluted solution will be used for each well.
6) Vortex your test tubes. Add 200 µl of your diluted solutions to the wells assigned to you based on your notebook. The plate will be processed by the accuSkan FC microplate reader once everyone finishes this step.
7) Write down the readings for each of your dilutions.

3. Data analysis
   1) Copy your tables into an Excel file.
   2) Select both the "Final concentration of food dye" and the "Absorbance at 460nm" columns.
   3) Create a scatter plot correlating both variables(click on "Insert" on the menu bar, then "Insert Scatter (X,Y) or Bubble Chart" and then "Scatter.")
   4) Make sure the x-axis is for the final concentration and the y-axis is for the absorbance reading.
   5) On the menu bar, click on "Design," then "Add Chart Element," and then add a trendline. Which trendline type should you choose?
   6) Double click on your trendline, "Format Trendline" should pop up on the right. Check "Display R-squared value on chart." It shows the coefficient of determination, and its value indicates how well your trendline represents the pattern of your data. A value close to 0 means the trendline does not explain the variation between data points well, while a value close to 1 means the trendline represents the meaning behind your data very well.
   7) Click on "Add Chart Element" again and add "Axis Titles" for both of the axes. Use them to label your axes properly.
   8) Create a Word file and copy your graph into the file.
   9) Below the graph, write a legend for your graph as instructed as follows:
      a) Start with figure number and title. Type "Figure 1." and write a title that describes what this figure is about. Bold this line.

      Example: **Figure 1. Title Here.**

      b) Following the title, write down information that a reader needs to fully understand your graph. For example: What is your raw data? How is your data plotted to create this figure? How many data points do you have?

      Note: A legend is not to be confused with Materials and Methods or Results. It should only contain just enough information to assist figure interpretation. If you're not sure what to put here, search for scientific papers and check what they say in their figure legends.

      Based on your data, how accurate were your dilutions? Does this data look like you'd expect? If not, can you identify possible sources of error?