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SERIAL DILUTION & IT'S USE IN HPLC

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ABSTRACT

Dilution is the process of decreasing the concentration of a solute in a solution, usually simply by mixing with more solvent like adding more water to the solution. To dilute a solution means to add more solvent without the addition of more solute. The resulting solution is thoroughly mixed so as to ensure that all parts of the solution are identical. The same direct relationship applies to gases and vapors diluted in air for example. Although, thorough mixing of gases and vapors may not be as easily accomplished. For example, if there are 10 grams of salt (the solute) dissolved in 1 litre of water (the solvent), this solution has a certain salt concentration (molarity). If one adds 1 litre of water to this solution, the salt concentration is reduced. The diluted solution still contains 10 grams of salt (0.171 moles of NaCl).

KEYWORDS: Dilution Liquid, HPLC, UV- Spectroscopy.

Serial dilution

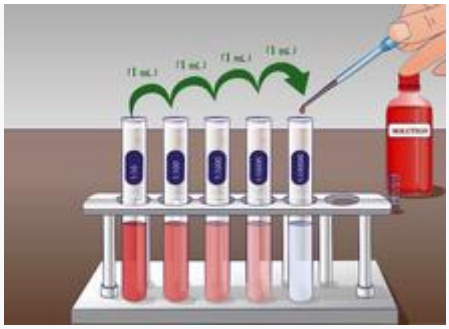


Figure-1: Logarithmic dilution.

A **serial dilution** is the stepwise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. A ten-fold serial dilution could be 1 M, 0.1 M, 0.01 M, 0.001 M ... Serial dilutions are used to accurately create highly diluted solutions as well as solutions for experiments resulting in concentration curves with a logarithmic scale. A tenfold dilution for each step is called a **logarithmic dilution** or **log-dilution**, a 3.16-fold ($10^{0.5}$-fold) dilution is called a **half-logarithmic dilution** or **half-log dilution**, and a 1.78-fold ($10^{0.25}$-fold) dilution is called a **quarter-logarithmic dilution** or **quarter-log dilution**. Serial dilutions are widely used in experimental sciences, including biochemistry, pharmacology, microbiology, and physics.[1]

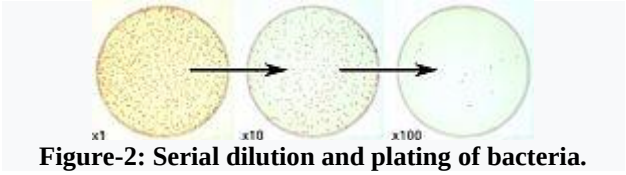


Figure-2: Serial dilution and plating of bacteria.

In homeopathy

Serial dilution is one of the core foundational practices of homeopathy, with "succussion", or shaking, occurring between each dilution. In homeopathy, serial dilutions (called potentisation) are often taken so far that by the time the last dilution is completed, no molecules of the original substance are likely to remain.

How to Do Serial Dilutions

A dilution in chemistry is a process that reduces the concentration of a substance in a solution. A serial dilution is the repeated dilution of a solution to amplify the dilution factor quickly. It's commonly performed in experiments requiring highly diluted solutions, such as those involving concentration curves on a logarithmic scale or when you are determining the density of bacteria. Serial dilutions are used extensively in experimental sciences like biochemistry, microbiology, pharmacology and physics.[2]

Performing a Basic Dilution

Figure-3: Basic Dilution Equipments.

1. Determine the proper dilution liquid. The liquid that you will be diluting your substance in is very important. Many solutions will be diluted in distilled water, but this is not always the case. If you are diluting bacteria or other cells, you will likely want to dilute in culture media. The liquid you choose will be used for every serial dilution.

If you're unsure what liquid to use, ask for help or check online to see if other people have performed a similar dilution.

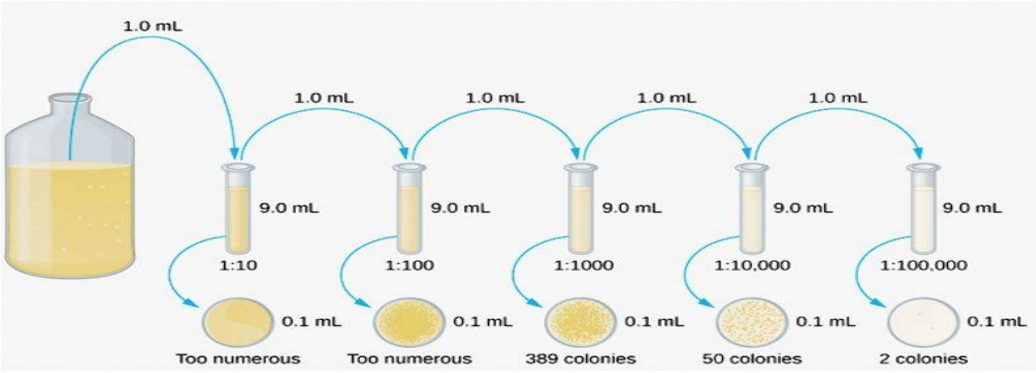
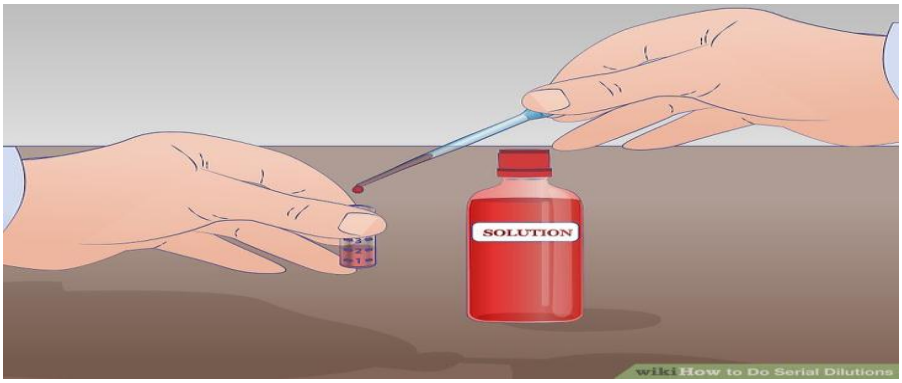


Figure-4: Determination of proper dilution fluid

2. Prepare several test tubes with 9 mL of dilution liquid. These tubes will serve as your dilution blanks.[3] You will be adding your undiluted sample to the first tube and then serially diluting into the following tubes.

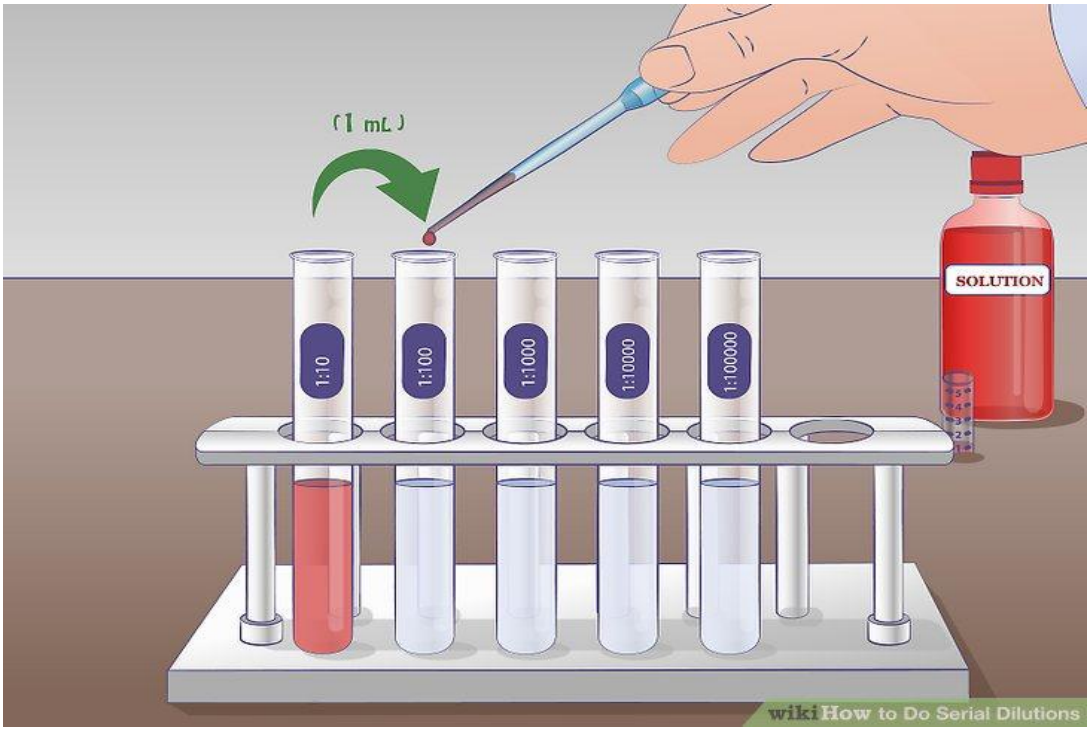
- It's helpful to label all of your tubes before you begin so you don't get confused once you begin with the dilutions.
- Each tube will be a 10-fold dilution starting from the undiluted tube. The first tube will be a 1:10 dilution, the second a 1:100, the third a 1:1000, etc. Determine the number of dilutions you need to do beforehand so you don't waste tubes or diluting liquid.



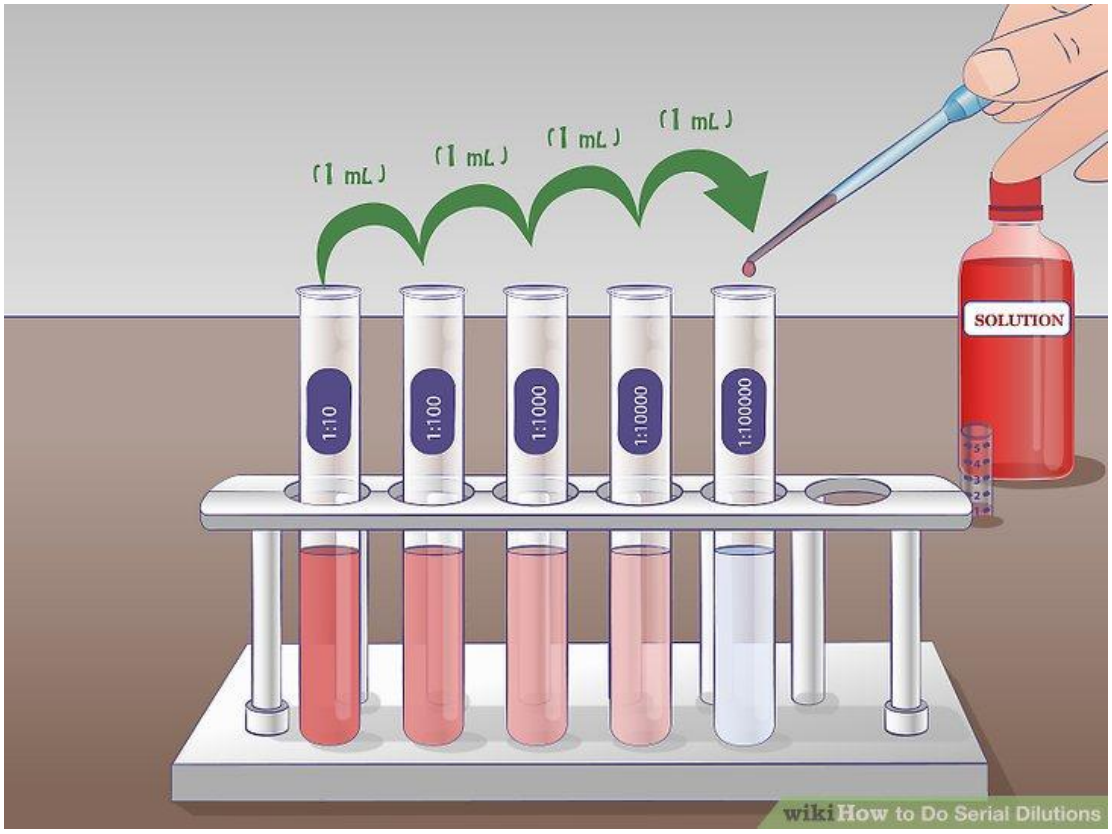
3. Prepare a test tube with at least 2 mL of your undiluted solution. The minimum amount needed to perform this serial dilution is 1 mL of undiluted solution. If you only have 1 mL you will not have any remaining undiluted solution. Label this tube *US* for undiluted solution.

- Thoroughly mix your solution before starting any dilutions.

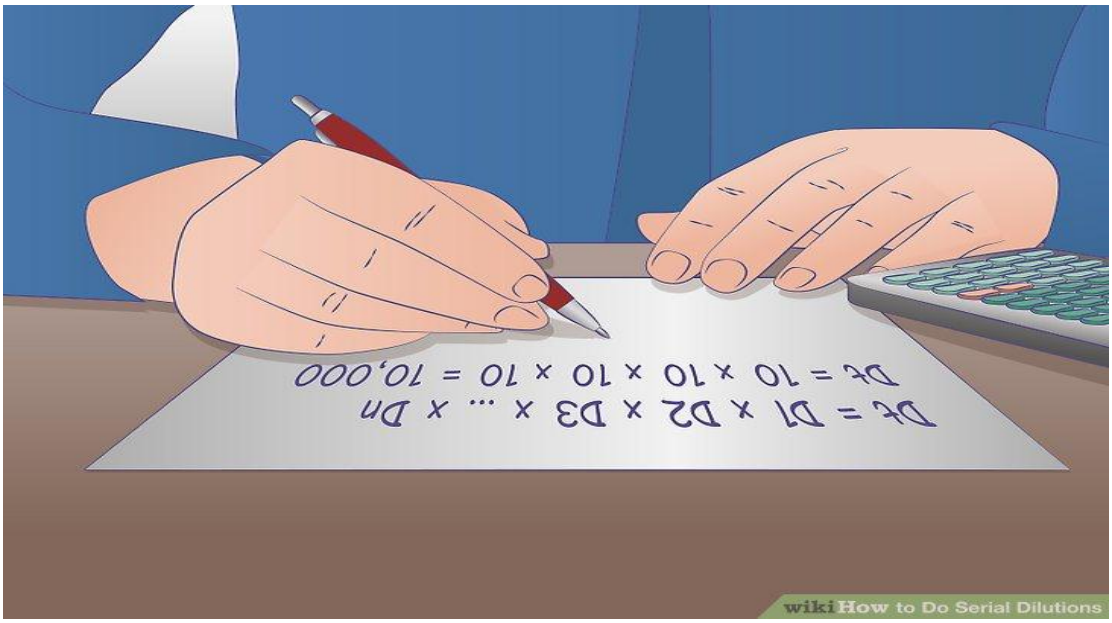
4. Perform the first dilution. Draw 1 mL of undiluted solution from test tube *US* with a pipette and transfer it to the test tube labeled *1:10* containing 9 mL of the dilution liquid and mix thoroughly. There is now 1mL of the undiluted solution in 9 mL of the dilution liquid. The solution, therefore, has been diluted by a factor of 10.



5. Perform the second dilution. For the second serial dilution, you will take 1 mL of solution from tube *1:10* and add it to the 9 mL of dilution liquid in the tube *1:100*. Thoroughly mix tube *1:10* before adding to the next tube. Again, mix the tube *1:100* following dilution. The solution from test tube *1:10* has been diluted 10-fold into test tube *1:100*.

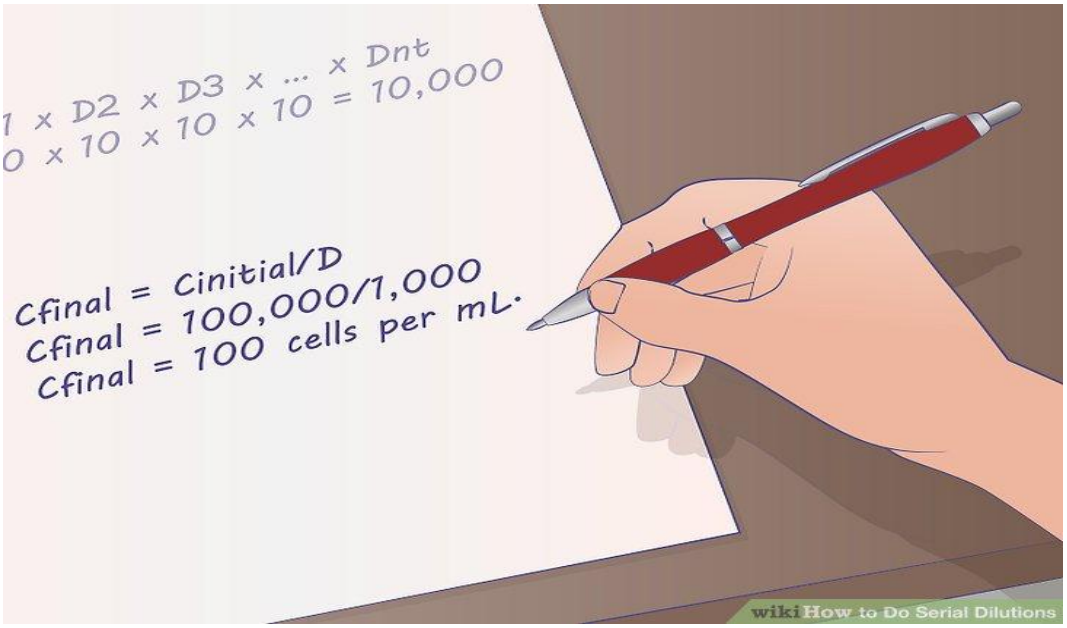


6. Extend this procedure to perform longer serial dilutions. This process may be repeated as many times as necessary to achieve the desired solution. In an experiment involving concentration curves, you can use a serial dilution to create a series of solutions with dilutions of 1, 1:10, 1:100, 1:1,000.



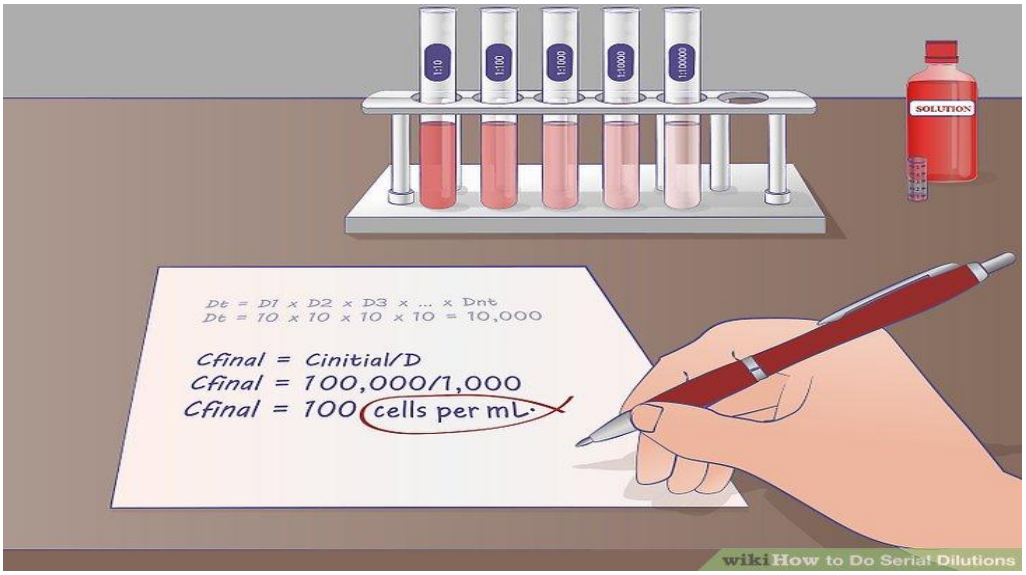
Calculate the final dilution ratio in a serial dilution. The total dilution ratio can be determined by multiplying the dilution factor of each step leading up to the final step. This can be mathematically illustrated with the equation $D_t = D_1 \times D_2 \times D_3 \times \ldots \times D_n$ where D_t is the total dilution factor and D_n is the dilution ratio.

- For example, let's say you did a 1:10 dilution of your liquid 4 times. Plug your dilution factor into the equation: $D_t = 10 \times 10 \times 10 \times 10 = 10{,}000$
- The final dilution factor of the fourth tube in your serial dilution is 1:10,000. The concentration of your substance is now 10,000 times less than the original undiluted solution.



Determine the concentration of the solution following dilution. To determine the final concentration of your solution following serial dilution you will need to know your starting concentration. The equation is $C_{final} = C_{initial}/D$ where C_{final} is the ending concentration of the diluted solution, $C_{initial}$ is the starting concentration of the original solution and D is the dilution ratio previously determined.[3]

- For example: If you started with a solution of cells with a concentration of 1,000,000 cells per mL and your dilution ratio is 1,000, what is the final concentration of your diluted sample?
- Using the equation:
- $C_{final} = C_{initial}/D$
- C_{final} = 1,000,000/1,000
- C_{final} = 1,000 cells per mL.



Confirm that all units match. When performing any calculation, you want to make sure that your units always match at the end. If you started with cells per mL make sure you are ending with cells per mL. If your starting concentration is parts per million (ppm), then your final concentration must be ppm.

Relation of Serial Dilution With HPLC

When working with high performance liquid chromatography (HPLC), good calibration is absolutely essential to ensure reliable, quality results. Proper calibration of an HPLC instrument begins with the making of a suitable calibration standard. In most instances, calibration in fact requires a series of standards of increasing concentration in order to produce what is known as a calibration curve. This is a plotted line and associated equation which describes the relationship between the concentration of the chemical being tested for and the response of the HPLC detector.

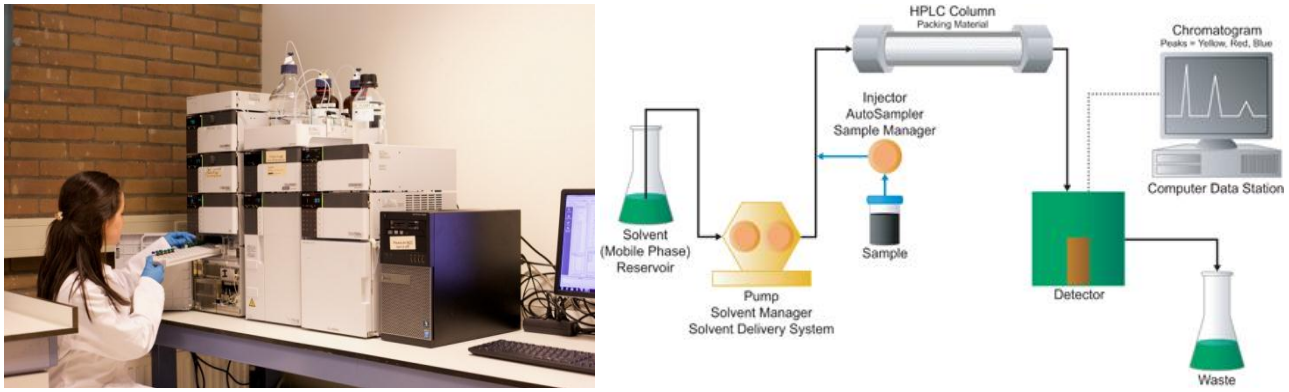


Figure-5: Instrumentation of HPLC.

Determine the chemical which you wish to test for using the HPLC (the "analyte"). For example, you might wish to test a series of soft drinks for fructose content, in which case fructose would be the analyte.

Obtain a quantity of the analyte chemical of suitable purity. Normally purity should be above 99% and the analyte should be purchased from a reputable chemical supply company. In the case of fructose, for example, you would purchase pure fructose from a chemical vendor and not from a grocery store.

Determine the maximum and minimum anticipated concentrations of the analyte in the samples you intend to test on the HPLC. In the case of soft drinks, you would examine the labels of the drinks and determine the lowest and highest fructose content amongst the drinks you will test. Keep in mind that the initial sample (the soft drink) may be diluted or otherwise manipulated during preparation for analysis (depending on the HPLC method being used) and so the analyte concentration in the samples actually injected onto the HPLC may be modified. It is the analyte concentration in the samples as run on the HPLC that must be considered.[4]

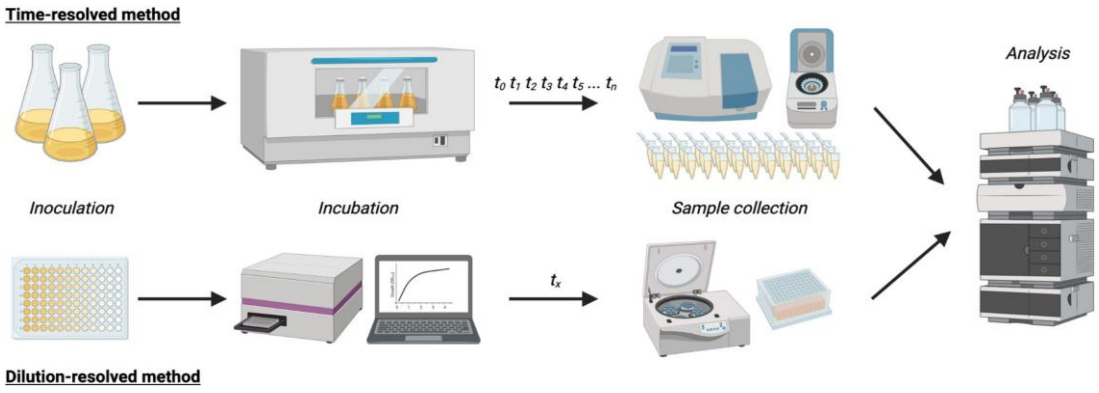
Determine the solvent in which you will dissolve your analyte to make up calibration standards. This solvent must be able to properly dissolve the analyte over a relatively wide range of concentration (at least as high as in the samples you intend to test). As well, this solvent should ideally be quite similar to the "mobile phase:" the solvent used to carry samples through the HPLC instrument.

Calculate the amount of analyte required to make a "stock standard" solution of the analyte. This is found by multiplying the required concentration of the stock standard by the desired volume. The concentration of the analyte in this solution should be at least 10% higher than the highest anticipated sample concentration. If the highest anticipated fructose concentration in a soft drink sample is 8 grams/100 milliliters, then the stock standard could be made to a concentration of 10 grams fructose/100 milliliters. A reasonable volume is 500 milliliters, thus 8/100 mL x 500 mL = 40 grams fructose would be required.

Weigh out the required amount of analyte to a suitable level of precision. Often a weight value in grams precise to one or two decimal places is suitable, but more precision may be necessary for some methods.

Transfer the weighed analyte to a volumetric flask of the necessary volume and add the desired solvent to the fill mark on the flask. Use of a volumetric flask (rather than a graduated beaker, for example) increases the precision of the stock standard concentration value. Ensure that all the analyte is transferred to the flask; use some of the solvent to wash it in, if necessary.

Stopper the volumetric flask and gently shake or invert until the analyte is completely dissolved.[5]



Make a series of varying dilutions of the stock standard by transferring known volumes of the stock standard into volumetric flasks, using pipettes for accurate transfer, then adding solvent. The lowest standard concentration should be below the lowest anticipated sample to be analyzed. In the soft drink example, if the lowest expected fructose concentration in a sample is 2 grams/100 mL, then a 1 gram/100 mL standard could be made. This would be made by a tenfold dilution of the stock standard. The standard series should include a total of 5 or 6 concentrations, so additional dilutions to produce standards of perhaps 3, 5 and 8 grams fructose/mL would be required. You now have a series of standard solutions with which to calibrate the HPLC.

CONCLUSION

In biology and medicine, besides the more conventional uses described above, serial dilution may also be used to reduce the concentration of microscopic organisms or cells in a sample. As, for instance, the number and size of bacterial colonies that grow on an agar plate in a given time is concentration-dependent, and since many other diagnostic techniques involve physically counting the number of micro-organisms or cells on specials printed with grids (for comparing concentrations of two organisms or cell types in the sample) or wells of a given volume (for absolute concentrations), dilution can be useful for getting more manageable results. Serial dilution is also a cheaper and simpler method for preparing cultures from a single cell than optical tweezers and micromanipulators.

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