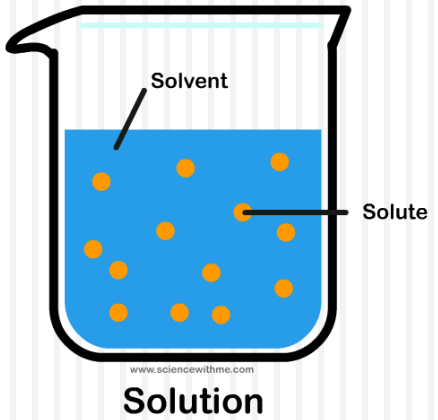
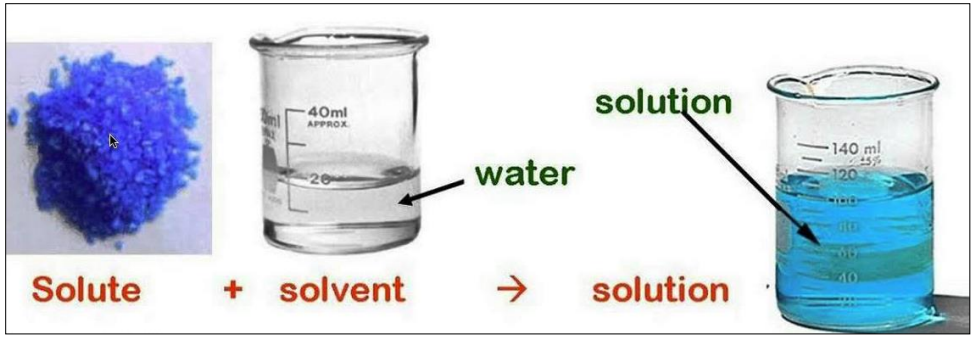
Preparation Of Biological Solutions And Serial Dilutions



Introduction :

- It is very important to understand how to prepare solutions and make dilutions and it is an essential skill for biochemists which is necessary knowledge needed for doing any experiment.

- A **simple solution** is basically two substances that are evenly mixed together.
- One of them is called the **solute** and the other is the **solvent**.
- A **solute** is the substance to be dissolved (sugar), The **solvent** is the one doing the dissolving (water). forming a homogenous mixture.

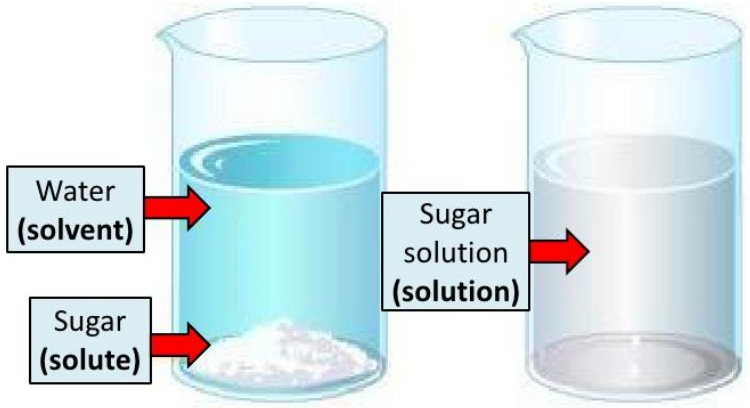


A. Preparation of biological solutions:

Solution concentration define as: quantity of a substance dissolved in per unit quantity of another substance (the relative amounts of solute and solvent in a solution)

There are different ways to express concentration:

1. Molarity.
2. W/V %.
3. W/W %.

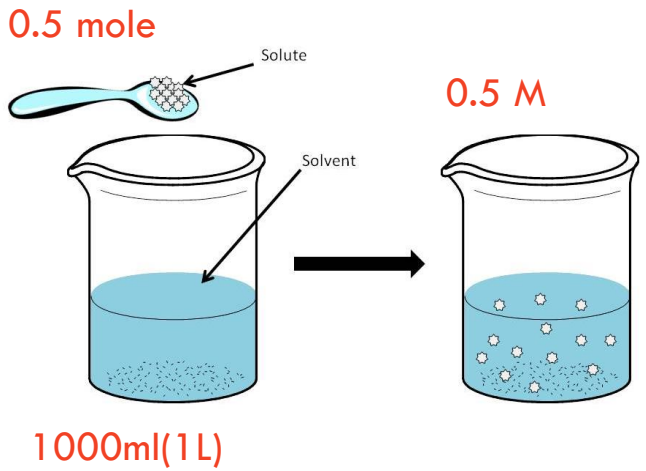


1.Molarity:

- is the number of **moles** of solute dissolved in **one liter** of solution.
- **Molar =** no. of mole/vol. in L.

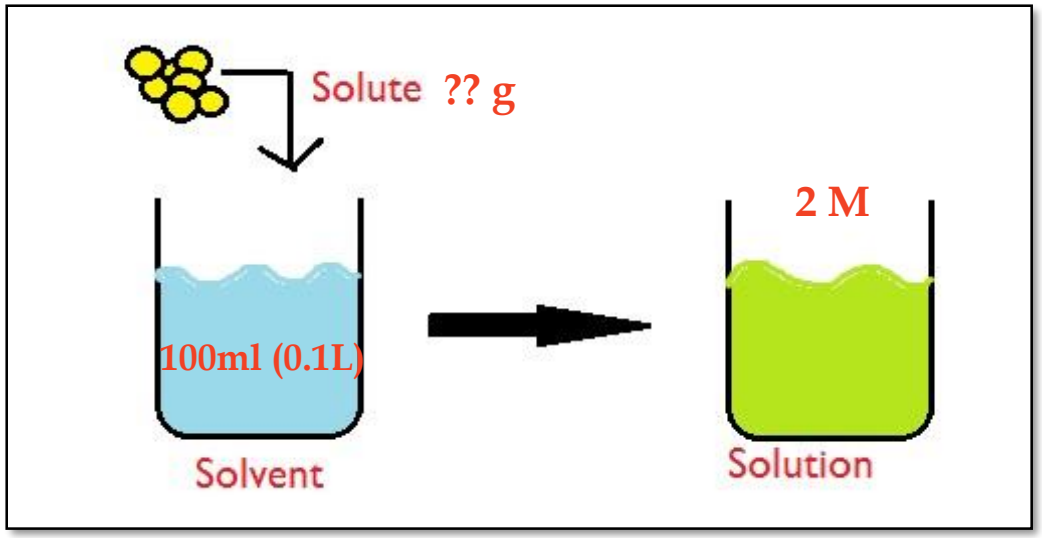
Molarity= moles of solute (mole) / volume of solution in (L)

- **Mole =** weight (g) / molecular weight (g/mole),
  Mole= Wt/ M.W
- **Example: 0.5 Molar (M) solution:** that mean there are 0.5 mole dissolved in 1000ml (1L).
- Units of molarity are : M, molar or mole/L



Example:

- **Prepare 2 M of NaCl in 100 ml, starting from solid NaCl. (MW of NaCl is 58.44 =(35.5+23)**
- To prepare a solution you need to know the amount of solvent in ml (known) and the amount of solute in grams (unknown)



So you must find "how many grams of NaCl you need to prepare 2 Molar solution"?

Con't.

Tow ways to solve it

1 2

(**1) 2 mole** of NaCl present in 1000 ml [or 1Liter] of solvent.

***But,** this mole needed if 1000 ml is required to be prepared. Since we need to prepare only **100 ml,** So:

(2) 2 mole → 1000 ml.

? mole →100 ml.

[(2 x 100)/1000] = 0.2 mole.

And we know that:

Mole = weight (g) / molecular weight (g/mole)

Mole= Wt / M.W

(3) So, [0.2 mole= weight (g) / 58.5 (M.w of NaCl)]

→ **weight (g) =** 0.2 x 58.5 = 11.7 g.

* **11.7 g** of NaCl dissolved in small volume of dis.H_2O, then complete the volume up to 100 ml.

$$\text{Molarity} = \frac{\text{moles of solute (mole)}}{\text{volume of solution in (L)}} \rightarrow \text{Molarity} = \frac{\text{weight (g)}}{\text{volume (L) x M.W}}$$

Molarity= 2M

Solution volume= 100 ml → convert to L = 100/1000 = 0.1L

Molecular weight (M.W) = 58.5 g/mole

Weight = ?

So:

Weight = Molarity x volume (L) x M.W

Weight = 2 x 0.1 x 58.5 = 11.7g

* **11.7 g** of NaCl dissolved in small volume of dis.H2O, then complete the volume up to 100 ml.

Practically how to prepare 2M NaCl:

- Place a beaker in a balance and zero the balance.
- Weight 11.7 grams of NaCl , in the beaker and dissolve in little water (less than 100 ml).
- Once the solid is dissolved the volume is transferred to 100 ml volumetric flask.
- Brought up to a final volume 100 ml.

2. W/V %:

- **W/V%** ➡ Weight/Volume Percentage Concentration.
- **W/V% define as** : The number of **grams** of solute dissolved in **100 mL** of solution (100%).
- **W/V % = $\frac{\text{weight of solute in (g)}}{\text{volume of solution in (ml)}}$ X100**
- **For example: 3% of NaOH** ➡ Mean 3 grams of NaOH is dissolved in 100 ml of the solution.
- Unit of molarity is : %

Example:

how many grams of NaOH we need to prepare 50ml of 4%NaOH solution?

- **Prepare 50 ml of 4% NaOH**

- 4% NaOH ➔ Mean 4 grams of NaOH is dissolved in 100 ml of the solution.
- we need to prepare **50 ml** not 100 ml.

- 4g------> 100 ml
- ?-------->50 ml

- **Weight** in grams of NaOH needed to prepare 4% NaOH is = (4 x 50)/100 = **2 g**.

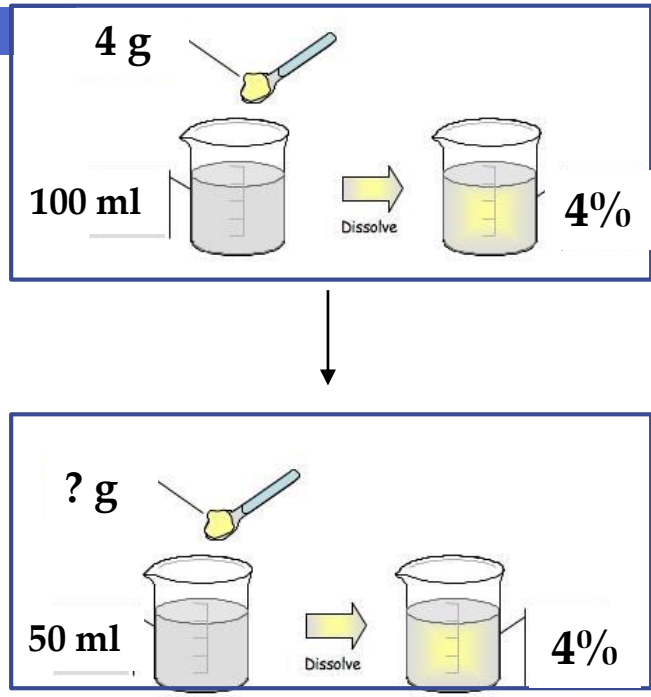
- * **2 grams** of NaOH is dissolved in little water and the volume made up to 50 ml

$$\text{W/V \%} = \frac{\text{weight of solute in (g)}}{\text{volume of solution in (ml)}} \times 100$$

- $4 = \frac{\text{weight of solute in (g)}}{50} \times 100$

- $g = (4 \times 50) / 100 = 2g$

- * **2 grams** of NaOH is dissolved in little water and the volume made up to 50 ml



3. W/W % :

- **W/W%** - Weight/Weight Percentage Concentration.
- W/W% is The number of **grams** of solute dissolved in **100 gram** of solution.
- **W/W % = $\frac{\text{weight of solute in (g)}}{\text{weight of solution in (g)}}$ X100**
- The concentrations of many commercial **acids** are giving in terms of **w/w%**.
- In order to calculate the volume of the stock solution required for a given preparation the **density (specific gravity)** of stock solution should be provided.
- **Weight (wt) = volume (ml) x SG x w/w% (as decimal)**.
- To calculate w/w% as decimal = (w/w)/100. For example: w/w%= 13% ➔ 13 / 100 = 0.13

Example

how many ml of concentrated HCl we need to make 0.4M HCl solution?

- **Prepare 100ml with 0.4 M HCl solutions starting with the concentrated HCl solution you are provided with. (w/w% = 36% , S.Gr = 1.15).**

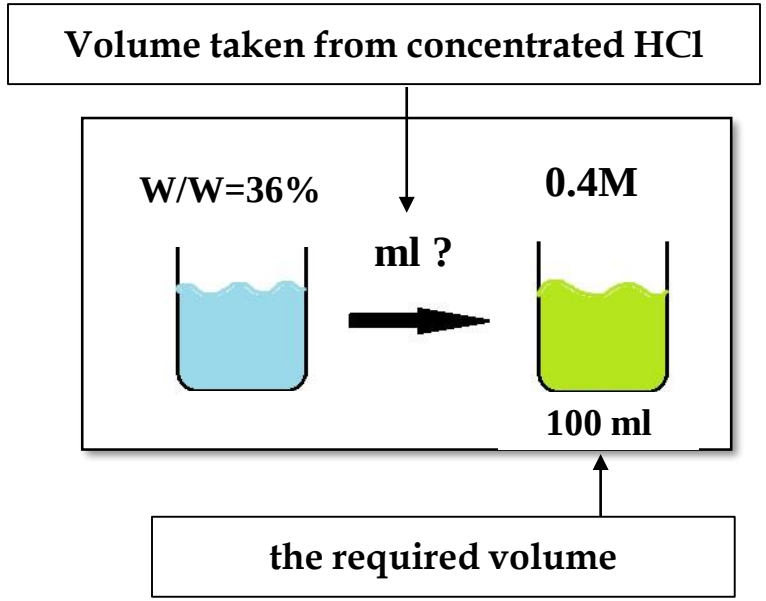
- **Weight= volume(ml) x SG x w/w% (as decimal)**

Note : the volume in this formula is not the required volume in the question, it is the volume of the concentrated HCl that you must add.

- **(1)** we must calculate the **weight** by the following:
- **from molarity formula:** Mole=Molarity x volume in liter (the required volume)
  = 0.4 x 0.1=0.04 mole
- Weight= mole x MW
  =0.04 x 36.5= 1.46 g

- **(2)** Volume (of stock must added)= weight/ (SG x w/w%)
- 1.46=volume x 1.15 x 0.36
- Volume= **3.53 ml**

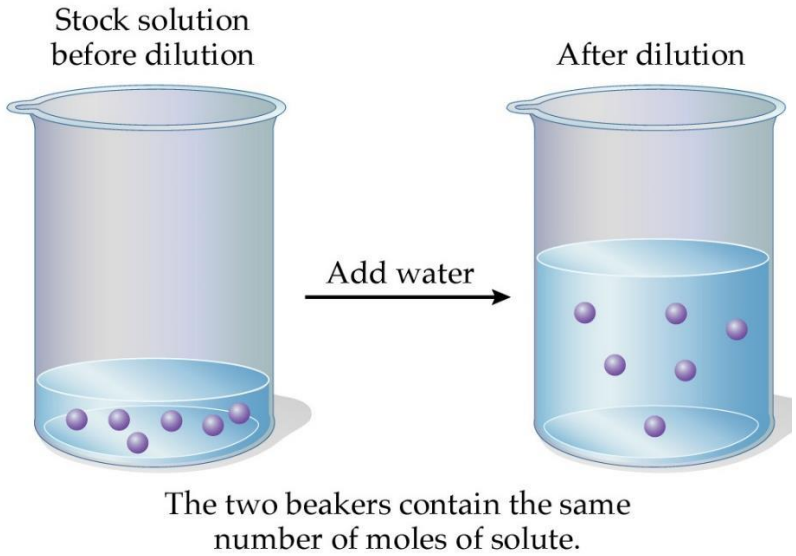
- So, **3.53 ml** of stock (i.e. concentrated HCl) solution is needed and the volume made up to 100 ml by the addition of water.



B .Dilution of Solution :

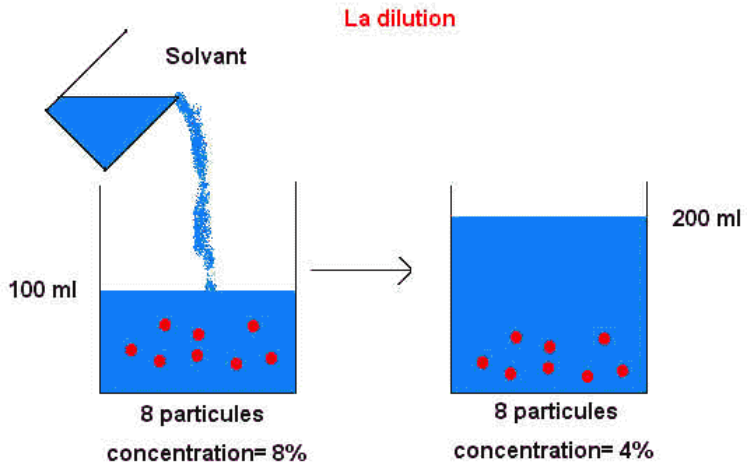
- **Dilution of solution:** means to add more solvent without the addition of more solute

  ⟶ To make it less concentrated.



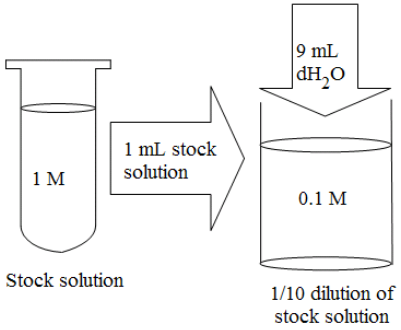
- **3 ways of dilution:**

1. Volume to volume dilutions (ratio).
2. Preparing dilutions by using the V1XC1=V2XC2 formula.
3. Serial Dilutions.



1) Volume to volume dilution (ratio):

- This type of dilutions describes the ratio of the **solute** to the **final volume** of the dilute solution.
- **For example,** to make 1:10 dilution of 1M NaCl solution, one part of the 1M NaCl solution, should be mixed with nine parts of water, for a total of ten parts.



- Therefore **1:10** dilution means 1 part + 9 parts of water .
- **Thus**

➡ If 10 ml of the **1:10** dilution was needed, then 1ml of 1M NaCl should be mixed with 9 ml of water.

➡ If 100 ml of **1:10** dilution was needed, then 10 ml of the 1M NaCl should be mixed with 90 ml of water.

The final concentration of NaCl in both cases will be 0.1 M (1/10) = 0.1

Example:

1:4 dilution

vol. Of solute **Total vol.**

1 ml from solute+ 3 ml from solvent= Total volume 4

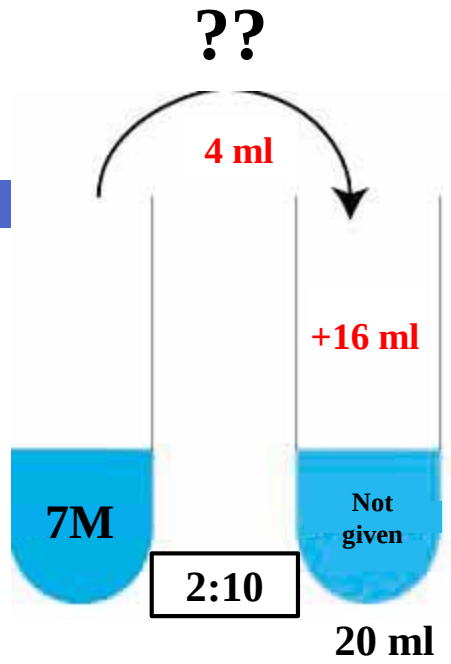
Example:

- Prepare 2:10 dilution of solution (A)with 7 M , but the total volume is 20ml not 10 ml?

- 2 ml → 10 ml

  ?? → 20 ml = (2 X 20) / 10 = **4 ml**

how many ml of 7M solution A we need to make 20 ml of 2:10 A solution?



- **So, 4 ml** from solution(A) of 7 M is needed and complete volume up to 20 ml (adding 16 ml water). [16 ml water= 20 ml - 4 ml].

How to Know the concentration of solution A after dilution?

First we will find the **DILUTION FACTOR** by the following :

Dilution factor (D.F) = final volume / aliquot volume =10/2 = 5

Then we will **divide** the stock concentration (before dilution) by the D.F: 7/5 = 1.4M

Note :To find out the stock concentration you will **multiply** the diluted concentration by the D.F

(2)Preparing dilutions by using the $C_{1X}V_1=C_{2X}V_2$ formula

- Sometimes it is necessary to use one solution to make a specific amount of a more dilute solution . To do this the following formula can be used:

$$C_1 \, X \, V_1 = C_2 \, X \, V_2$$

- Where:

- V_1= Volume of starting solution needed to make the new solution.
- C_1= Concentration of starting solution.
- V_2= Final volume of new solution.
- C_2= Final concentration of new solution.

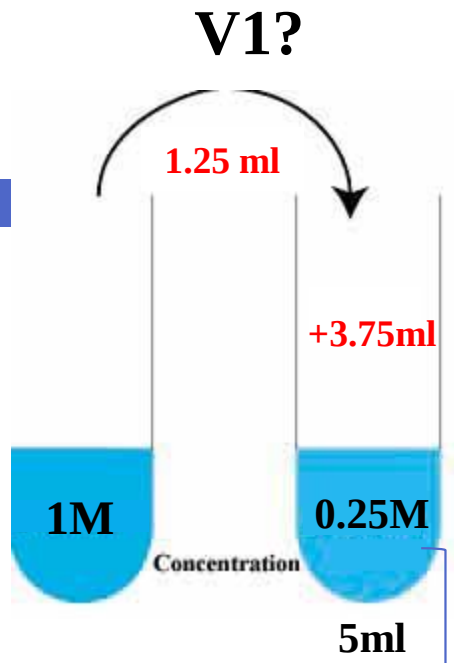
Example:

- **Make 5ml of 0.25M solution from a 1M solution:**

- Since: $\mathbf{V_1 \times C_1 = V_2 \times C_2}$.

How many ml of 1M solution we need to make 5 ml of 0.25M solution (V1) ?

- ($V_{1??}$) x (1M) = (5ml) x (0.25M).
- V_1 = [(5 x 0.25)/1] = **1.25 ml**



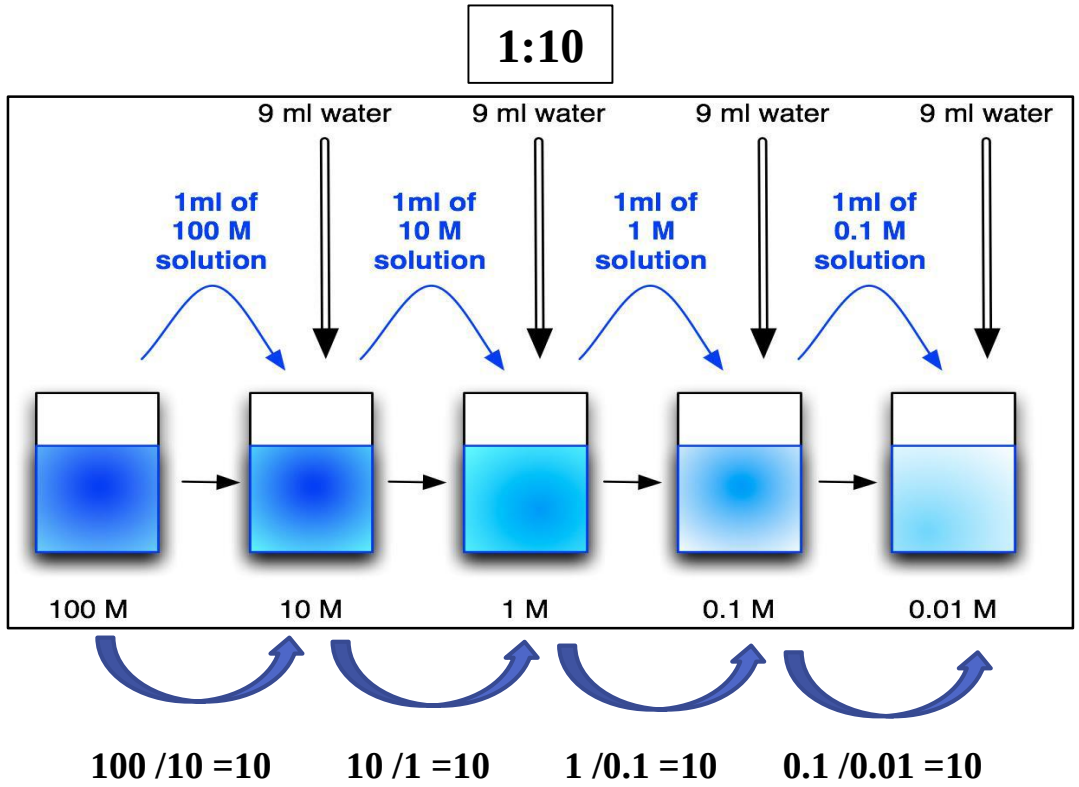
- So **1.25ml** of the 1M solution is needed (starting solution) then complete the volume up to 5 ml by water.

- (V_1-V_2) = 5ml – 1.25ml = 3.75ml).
- 3.75ml of diluent (generally water) should be added to the 1.25ml of starting solution.

The concentration after dilution is given

3) Serial Dilutions:

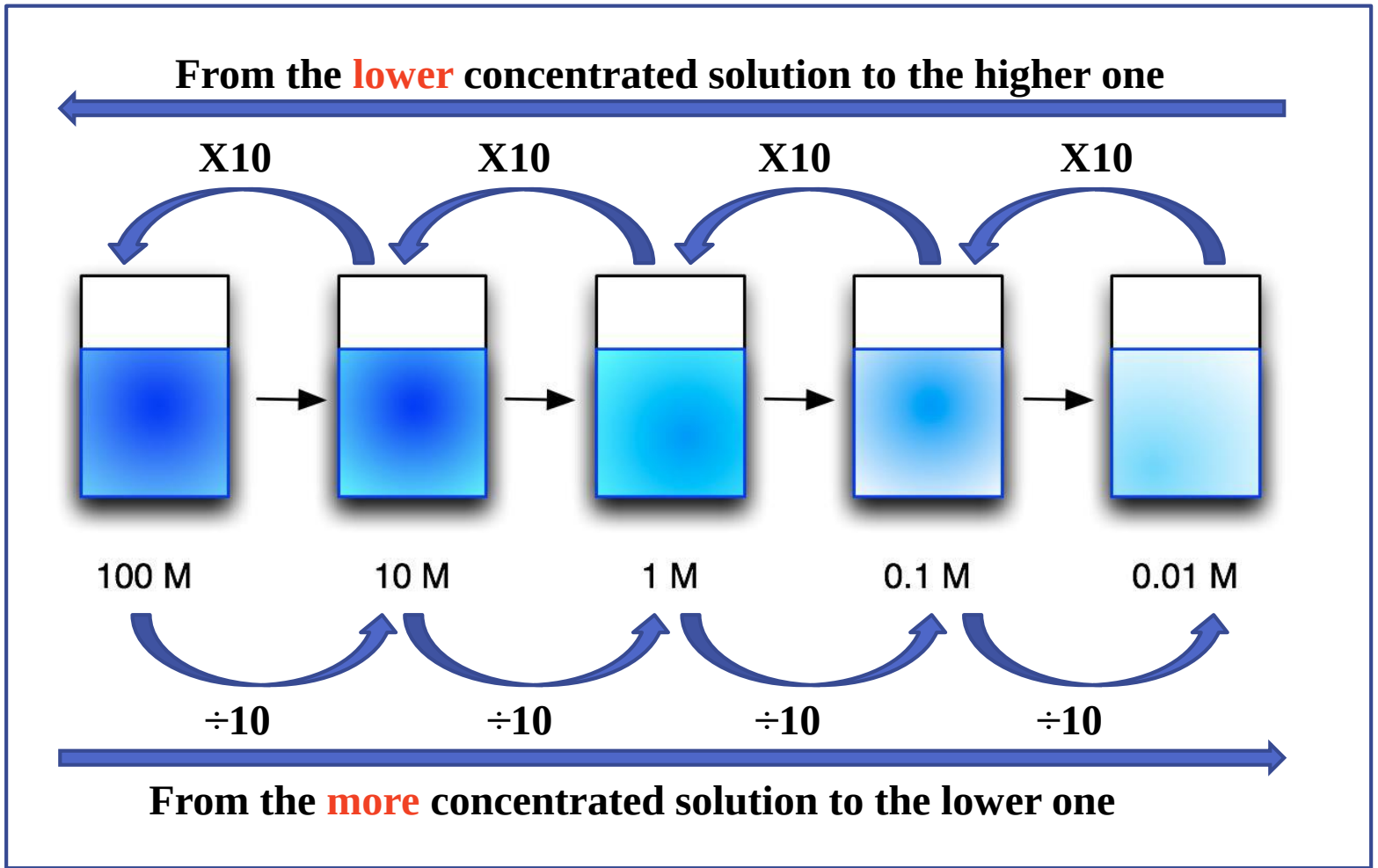
- It is a stepwise dilution of a solution, where the dilution factor is constant at each step.
- The source of dilution material for each step comes from the diluted material of the previous step.



Dilution factor (D.F) = final volume / aliquot volume
= 10 /1 = 10 (for each step)

Find out the concentration of the diluted solutions

Dilution factor (D.F) = final volume / aliquot volume = 10 /1 = 10 (for each step)



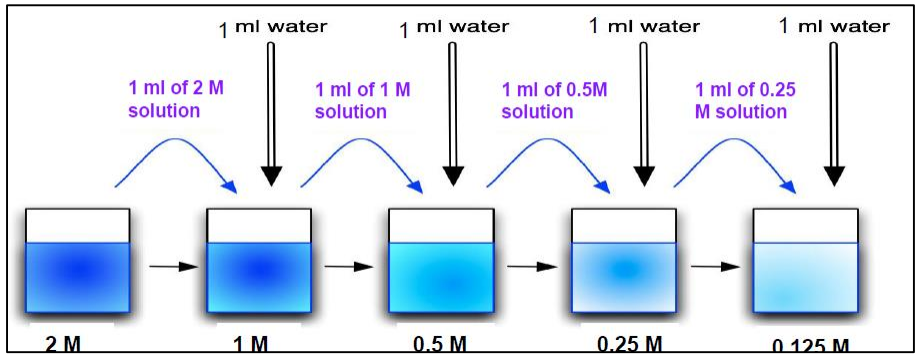
Example:

Starting with a 2.0 *M* stock solution of hydrochloric acid, prepare 1ml of four standard solutions by serial dilution of the following Molarity respectively 1 M, 0.5 M, 0.25 M, 0.125 M. [with 1:2 dilution]

- **Dilution factor (D.F) = final volume / aliquot volume**
  = 2/1 = 2 → 1:2

- To prepare standard solution 1:
  1 ml of the stock 2.0M solution is needed and volume made up to 2 ml with distilled water (1ml of water), mix properly.

- To prepare standard solutions 2-4 :
  1 ml of the previously diluted solution is taken and volume is made up to a final volume of 2 ml by the addition of distilled water.



How to calculate the concentration of the diluted solutions if they unknown ?

➔First: find the D.F:
Dilution factor (D.F) = final volume / aliquot volume
= 2/1 = 2

➔Second: divide the previous solution concentration by the D.F:

-concentration of solution 1= 2.0 M stock solution /2 =**1 M**
-concentration of solution 2= 1M/2 =0.5 M
-concentration of solution 3= 0.5M/2 =**0.25 M**
-concentration of solution 4= 0.25/2 =**0.125 M**

* or from **C1XV1 = C2XV2**

Practical Part

Objectives:

- To learn how to prepare solutions with different concentration expression.
- To get familiar with solution dilutions by different methods.

Method

A. preparation of solutions

- (1)……
- **You are provided with solid NaOH, Prepare 50ml with 0.08M NaOH solution.**

- Calculation:

- ……

  ……

- To prepare the 0.08M NaOH solution ……..g of solid NaOH should be dissolved in a little volume of water then the volume made up to ……ml ,by the addition of water.

- (2)……
- **You are provided with solid NaCl, Prepare 50ml with 1.5 w/v% solution of NaCl.**

- Calculation:

- ……

  ……

- To prepare the 1.5 w/v% solution ……g of NaClshould be dissolved in little water and the volume made up to …….ml by the addition of water.

- (3)……
- **Prepare 100ml with 0.4 M HCl solution starting with the concentrated HCl solution you are provided with: (w/w%= 36 , S.Gr=1.15).**

- Calculation:

- ……

  ……

- To prepare the 100ml of 0.4M HClsolution …….ml of stock (i.e. concentrated HCl) solution is needed and the volume made up to ……..ml by the addition of water.
- Measure and record the pH value of the acid you prepared……
- Calculate the pH of the acid (pH= -log [H+]) ……
- Determine your accuracy? ……..

B. solution Dilution

- (1)……
- **Prepare 50ml with 1:20 dilution using the 0.08M NaOH solution you previously prepared.**

- Calculation:

- ……

  ……

- To prepare the 1:20 dilution ……..ml of the starting solution (0.08M NaOH) is needed and volume made up to a final volume of ……..ml.

- (2)……
- **Prepare 100ml of 0.2M HCl from the previously 0.4M HCl solution you previously prepared.**

- Calculation:

- ……

  ……

- To prepare the 0.2M HCl……..ml of the starting solution (0.4M HCl) is needed and volume made up to a total volume of ……..ml by adding water.

- (3)……
- **Starting with a 2.0 M stock solution of hydrochloric acid, prepare 10 ml of four standard solutions (1 to 4) of the following Molarity respectively (dilution 1:5) :**

  (1) ……. M (2) ……. M (3) ……. M (4) ……. M .

- Calculation:
- ……

  ……

- To prepare standard solution 1: …… ml of the stock 2.0M solution is needed and volume made up to …….. ml with distilled water.
- To prepare standard solution 2-4: …… ml of the previously diluted solution (8.00×10-2 M) is taken and volume is made up to a final volume of …… ml by the addition of distilled water.

H.W

- **A student needed to prepare 1L of a 1M NaCl solution, which of the following methods is more accurate in preparing the solution? Why?**
- **a)** Weighing 58.5g of solid NaCl carefully ,dissolving it in 300ml of water, then adding 700ml of water.
- **b)** Weighing 58.5g of solid NaCl carefully ,dissolving it in a small volume of water then making the final volume up to 1L by adding water.

- 3. **A solution was prepared by taking 6ml of a 0.22M solution and then the volume was made up to a final volume of 30ml .What is the concentration of the final solution?**
- **4. How would you prepare 80ml of a 1:25 dilution of a 2.1M KCl solution?**
- **5. How would you prepare 50ml of a 6% NaCl solution?**