# Biochemical Calculations

312 BCH

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## Reference:

Biochemical calculations by Irwin H. Segel


*Online copy:* http://site.iugaza.edu.ps/bzabut/wp-content/uploads/biochemical%20calculation.pdf

# Midterm dates

- 1st Midterm:
  - 5th week, 4 / 2 / 2019
  - Time 12-1 pm.
- 2nd Midterm:
  - 10th week, 11 / 3 /2019
  - Time 12-1 pm.

# Marks distribution

- Midterm 1: 15 marks (15%)
- Midterm 2: 20 marks (20%)
- Lab: 25 marks (25%)
- Final exam: 40 marks (40%)

# Glassware

## Bottles

Wide neck, amber, bottles; can be used for a wide range of light sensitive liquid or solid storage.


Reagent bottles

Narrow neck bottles; can be used for a wide range of liquid storage, media preparation and sampling applications.

# Glassware continued


Media-lab bottles


Wash bottles

# Glassware continued

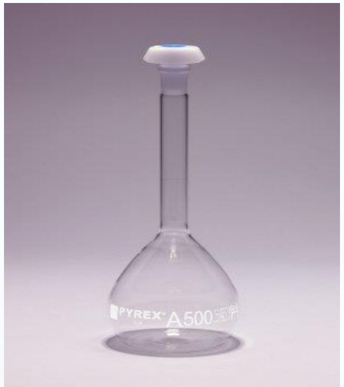
## Beakers


Conical shape beakers; ideally used for titrations and mixing application, these conical shape beakers are a cross between a standard beaker and conical flask.


Griffin beaker; is great for general laboratory use.

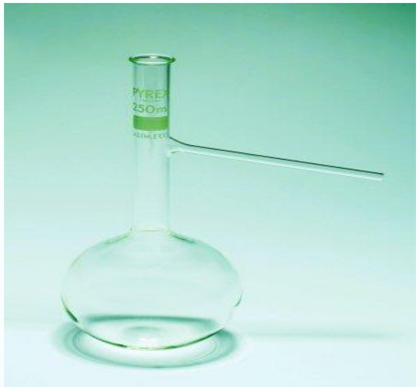
# Glassware continued

## Flasks

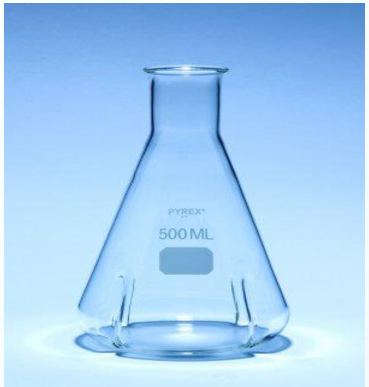



Volumetric flasks; are precision measuring instruments.


Boiling flasks



Distillation flasks




Cell culture flasks


Erlenmeyer
Conical flasks

# Glassware continued

## Volumetric ware

Burette

Burette clamp

Cylinder

Mixing cylinder

# Glassware continued

## Test tubes

Test tubes

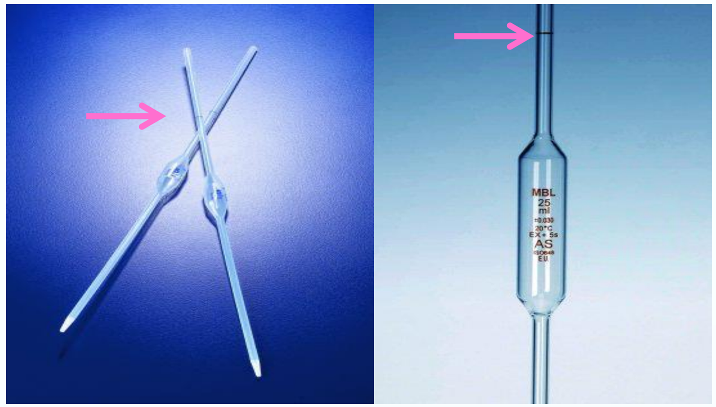
Test tubes with ground socket joint.


Culture Tubes

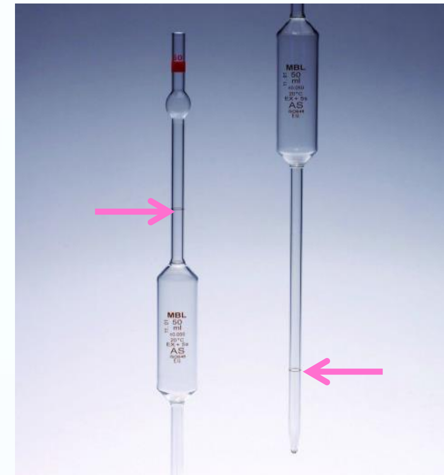

Centrifuge Tubes

# Glassware continued

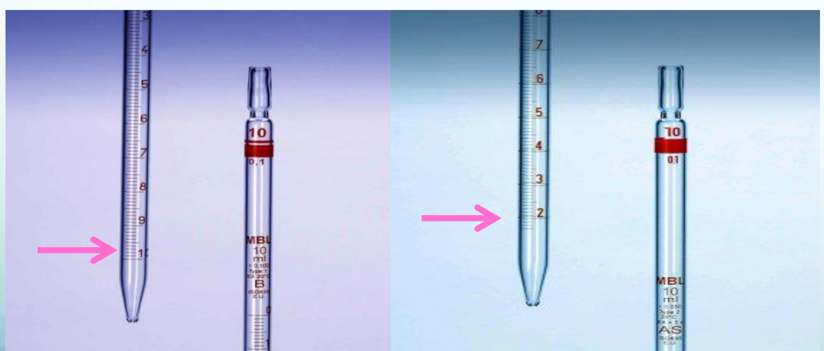
## Pipettes



One mark pipette



Two mark pipette
with safety bulb
at top.



Type 1 Type 2

Graduated pipettes

Pasteur Pipettes

# Glassware continued

## Funnels

Filter funnels

Dropping & Separating Funnels

Buchner Funnels

# Lab equipment

Petri dishes


Evaporating dishes


Vial Racks

pH meter

Balance

Ring Stand

Test Tube Clamp

Test Tube Rack

# Lab equipment continued

Plates

Micropipettes

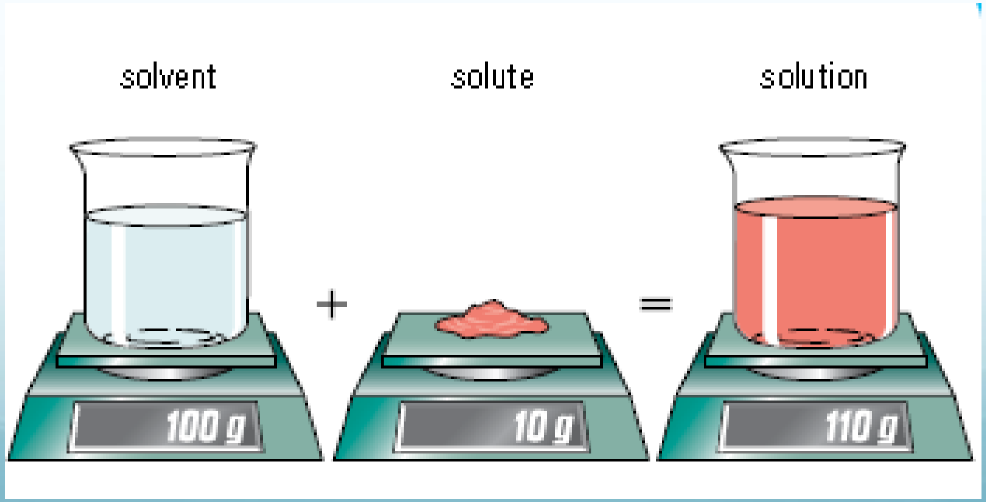
Multichannel micropipettes

Tips

Eppendorf tubes

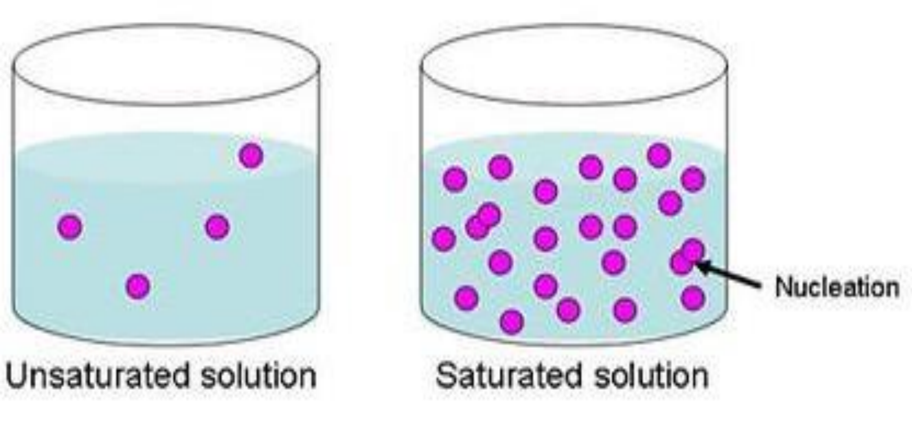
# Solution Composition

- A solute is the substance being dissolved.
- A solvent is the liquid in which the solute is dissolved.
- A solute is dissolved in a solvent.
- An aqueous solution has water as solvent.



# Aqueous Solution

- The majority of reactions occur in solutions.
- There are several ways to express the concentration of a substance in a solution based on:
  - The volume
  - The weight
  - Degree of saturation



# Concentrations Based on Volume

# Concentration based on volume

- Here the concentrations are based on the amount of dissolved solute per unit volume
- The calculations depending on volume include:
  - Molarity (M)
  - Normality (N)
  - Activity (a)
  - Weight/Volume percent (w/v %)
  - Volume/volume percent (v/v%)
  - Milligram percent (mg %)
  - Osmolarity (osm)

# Avogadro’s Number

- It is the number of molecules per mole of substance
- <u>OR</u> it is the number of atoms per gram-atom of substance
- <u>OR</u> it is the number of ions per g-ion of substance
- Avogadro’s number = $6.023 \times 10^{23}$
- For example: 1 mole of water contains Avogadro’s no. of $6.023 \times 10^{23}$ molecules

# 1- Molarity

- Is the number of moles of solute per liter of solution

$$M = \frac{\text{No. of moles}}{\text{Volume of solution in L}}$$

- No. of moles = $Wt_g$ /MWT (molecular weight)
- 1 mole contains Avogadro's number of molecules per liter ($6.023 \times 10^{23}$).
- Molar concentrations are usually given in square brackets.
  - Example: $[H^+]$ = molarity of hydrogen ion
    [NaOH] = molarity of Sodium Hydroxide

# Examples

- A solution of NaCL had 0.8 moles of solute in 2 liters of solution. What is its molarity?

$$M = \frac{\text{no. of moles}}{\text{volume of solution in L}}$$

$$M = \frac{0.8}{2}$$

M = 0.4 molar

**Given values:**

No. of moles= 0.8 mol

V= 2 L

**M=??**

# Examples

- How many grams of solid NaOH are required to prepared 500 ml of 0.04 M solution?

$$M = \frac{\text{no. of moles}}{\text{volume of solution in L}}$$

no. of moles = 0.04 × 0.5

no. of moles = 0.02 mole

$$\text{no. of moles} = \frac{\text{weight in gram}}{\text{molecular weight (MWT)}}$$

MWT of NaOH = 23+16 + 1 = 40

Wt in grams = no. of moles × MWT

wt in grams = 0.02 × 40

wt in grams = 0.8 grams

**Given values:**

M= 0.04 M

V= 500 ml = 500 ÷ 1000 = 0.5 L

Wt=??

# 2- Normality

- Is the number of equivalents of solute per liter of solution

$$N = \frac{\text{no. of equivalents}}{\text{volume of solution in L}}$$

$$\text{no. of equivalents} = \frac{\text{weight in gram}}{\text{Equivalent weight (EW)}}$$

$$\text{equivalent weight} = \frac{\text{MWT}}{n}$$

*n = is the number of replaceable hydrogen (H+ in **acids**) or hydroxyl ions (OH- in **bases**) per molecule*

*OR*

*n = is the number of electrons gained or lost per molecule (**in oxidizing or reducing agents**)*

# Relationship between molarity and normality

$$N = \frac{\text{no. of equivalents}}{\text{volume of solution in L}}$$

$$N = \frac{\text{weight in gram}}{\text{Equivalent weight}} / \text{volume of solution in L}$$

$$N = \frac{\text{weight in gram}}{\text{MWT / n}} / \text{volume of solution in L}$$

$$N = \frac{\text{weight in gram} \times n}{\text{MWT}} / \text{volume of solution in L}$$

no of moles

$$N = n \times M$$

- For example: A 0.01 M solution of $H_2SO_4$ is 0.02 N

# Example

What is the normality of $H_2SO_4$ solution that contains 24.5 g of solute in a total volume of 100 ml?

**Given values:**

Wt= 24.5g

V= 100 ml = 100 ÷ 1000 = 0.1 L

n= 2

N=??

- $N = n \times M$
- $M$ = No. of moles / $V_{(L)}$
- No. of moles = $Wt_g$ /MWT
- MWT of $H_2SO_4 = 2 + 32 + (16 \times 4) = 98g$
- No. of moles = 24.5/ 98
- No. of moles = 0.25 mole
- $M$ = No. of moles / $V_{(L)}$
- $M = 0.25/0.1 = 2.5$ molar
- $N = n \times M$
- $N = 2 \times 2.5 = 5$ normal

# Another way to solve it

- Normality (N) = No. of equivalents / $V_{(L)}$
- No. of equivalents = $Wt_g$ of solute / equivalents weight (EW)
- EW= MWT of solute / n
- MWT of $H_2SO_4$ = 2 + 32 + (16 × 4) = 98 g
- EW= 98 / 2 = 49
- No. of equivalents = $Wt_g$ of solute / equivalents weight (EW)
- = 24.5 g / 49 = 0.5 eq
- Normality (N) = No. of equivalents / $V_{(L)}$

=0.5 / 0.1 = 5 Normal

# 3- Osmolarity

- It is the molarity of particles in a solution.

$$\text{Osmolarity} = n \times M$$

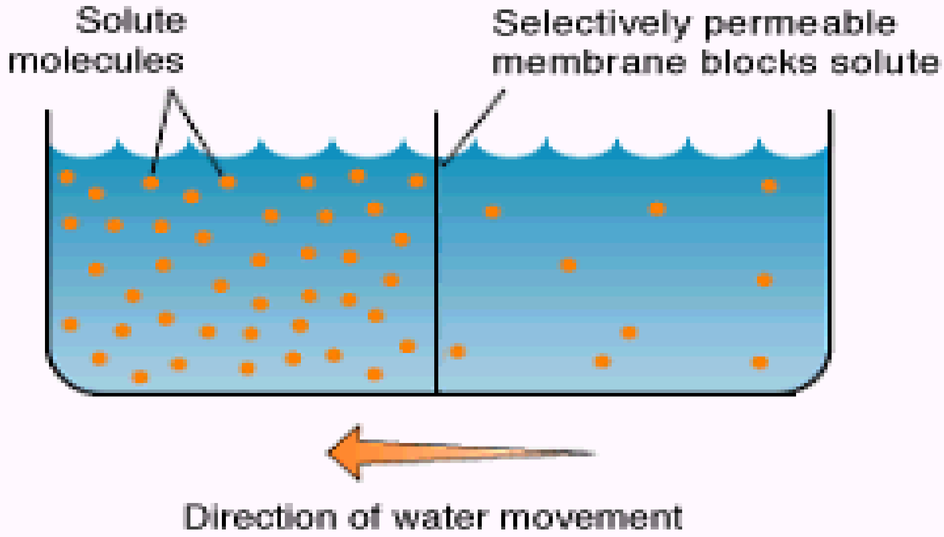
- *$n$ is the number of ions (or dissociable particles) produced per molecule.*
- *A 1M solution of a non-dissociable solute is also 1 osmolar.*

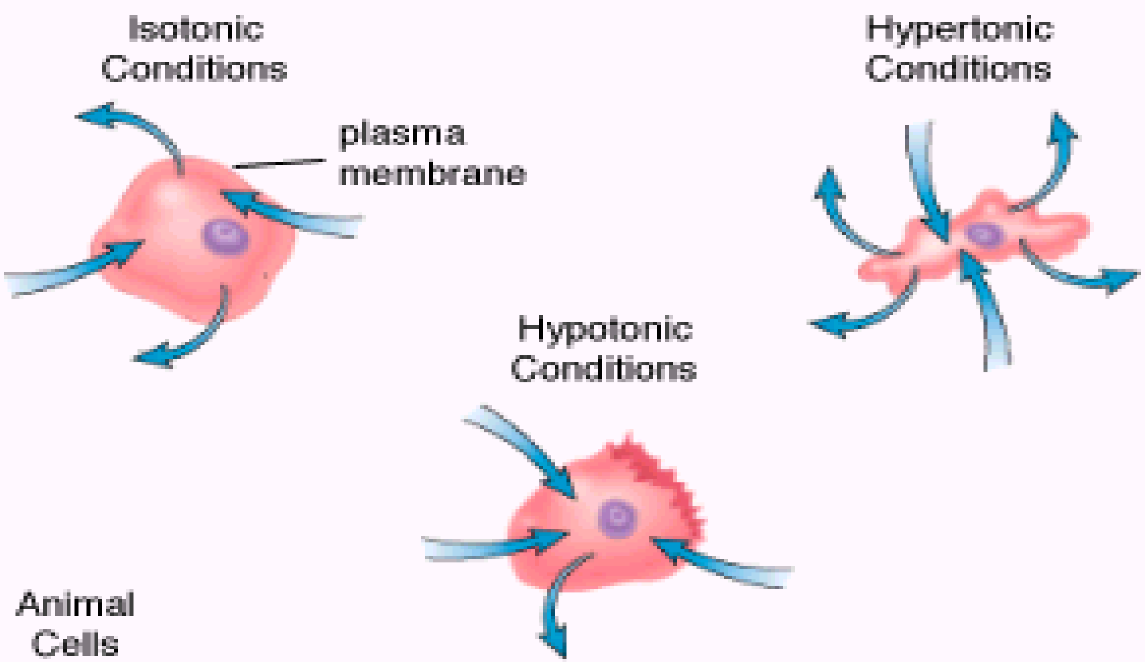
- It is often considered in physiological studies where tissues or cells must be bathed in a solution of the same osmolarity as the cytoplasm in order to prevent the uptake or release of water.

# Isotonic solution

- ISO - means alike.
- TONICITY - refers to osmotic activity of body fluids; tells the extent that fluid will allow movement of **water** in & out of the cell.
  - *Meaning* .. that the solutions on both sides of selectively permeable membrane have established equilibrium.

# Osmosis




Osmosis is the diffusion of water across a membrane. Like other molecules, water will move from an area of high concentration to an area of low concentration. The more solute there is in a solution, the lower the concentration of water in that solution. There is terminology to describe concentration differences between two solutions. A solution with higher solute concentration is hypertonic relative to one with lower solute concentration. Conversely, a solution with lower solute concentration is hypotonic relative to one with higher solute concentration. If two solutions have the same concentration they are isotonic. Water will move from a hypotonic to a hypertonic solution.

# Example (1)

- A solution of KCl that has a molarity of 0.03 M what is the osmolarity ?

Given values:

Molarity= 0.03 M

KCl is dissociable ($K^+$, $Cl^-$), → $n$ = 2

**Osmolarity =??**

Osmolarity = M x n

= 2 x 0.03 = 0.06 Osmolar

# Example (2)

- When you want to study RBC and its osmolarity in the cytoplasm is 0.308 osmolar.
    - What do you think the osmolarity of the *in vitro* solution should be?

**Memorize** → 0.308 osmolar.

Red blood cells suspended in a 0.308 osmolar NaCl solution would neither shrink nor swell.

# Example (3)

- Classify these solution in regards to the RBC osmolarity

1) 0.56 osmolar → Hypertonic

2) 0.21 osmolar → Hypotonic

3) 0.154 M NaCl → Isotonic

$osmolarity = n \times M = 2 \times 0.154 = 0.308$ *osmolar*

# 4- Weight/Volume Percent (wt/v%)

- It is the weight in gram of a solute per 100 ml of solution.

$$\text{wt/v }\% = \frac{\text{Wt in gram of solute}}{\text{100 ml of solution}}$$

- It is often used for routine laboratory solutions where exact concentration *are not too important*.

# 5- Milligram Percent (mg%)

- It is the weight in *mg* of a solution per 100 ml of solution.

$$\text{mg \%} = \frac{\text{Wt in mg of solute}}{\text{100 ml of solution}}$$

- It is often used in clinical laboratories.
  - For example, a clinical blood sugar value of 255; means 255mg of glucose per 100 mg of blood serum.

# 6- volume / volume percent (v / v %)

- It is the volume in *ml* of a solute per 100 ml of solution.

# Example

- A 0.04M solution of NaOH was prepared by the use of 0.8 g of NaOH and the total volume of the solution was 500 ml.
  a. Express the concentration g/l.
  b. W/V %.
  c. mg%.
  d. Osmolarity.
  e. Normality.

**Given values:**

- Molarity= 0.04 M
- Wt = 0.8 g
- V= 500 ml → 0.5 L

## a. Concentration as g/L.

Since 0.8g → 500 ml

? → 1000 ml (1L)

The weight of NaOH in 1L = (1000 x 0.8)/500 = 1.6gm

## b. W/V%

= (Weight in gram solute)/(100ml of solution)

0.8 g → 500 ml

? → 100 ml

The weight of NaOH in 100 ml = (100x0.8)/500 = 0.16%

## c. mg%

= (weight in mg of solute)/(100 ml of solution)

Since 0.16% x 1000 = 160 mg%

## d. Osmolarity

*NaOH yeilds two particals (Na+ and OH-) per molecule*

→O= n *x* M = 2 x 0.04 = 0.08 Osmolar

## e. Normality

*NaOH contains one OH per molecule*

→ N = n × M = 1 × 0.04 = 0.04 N

# Concentrations Based on Weight

# Concentrations based on weight

- They include:
  - weight/weight percent (w/w%)
  - Molality (m)

# Weight/weight percent (w/w%)

- The weight in *g* of a solute per 100g of solution.

$$w/w\% = \frac{\text{no. of gram of solute}}{\text{100g of solution}}$$

- The concentration of many commercial acids are giver in terms of w/w% → so, in order to calculate the volume of stock solution required for a given preparation you must know its *density* or *specific gravity*

*P*=density = weight per unit volume

SG= specific gravity = density relative to water. Density of water is 1 g/ml,

SG is numerically equal to density.

# Weight/weight % cont'ed

- The relationships between the weight, density, and % w/w can be combined into a single expression.

$$wt_g = Vol_{ml} \times P_{g/ml} \times w/w\ \%$$

Where:

$wt_g$ = weight of pure substance required in g

$Vol_{ml}$ = volume of stock solution needed in ml (attention!!!)

w/w% = fraction of total weight that is pure substance *as a decimal*

# Example

Describe the preparation of 2 liters of a 0.4M HCl solution starting with a concentrated (stock) solution of HCl with 28% w/w%, the specific gravity is 1.1

$$wt_g = Vol_{ml} \times P_{g/ml} \times w/w\ \%$$

Given values:

Total volume = 2L

M = 0.4 M

w/w%= 28% → as decimal ( ÷100) = 0.28

*P = 1.15*

MW of HCl = 1+35.5 = 36.5 g/mole

*First we must calculate the wt:*

No. of moles of pure HCl needed = MxV = 0.4x 2 = 0.8 moles

The weight in grams of pure HCl needed = no. of moles* MW = 0.8* 36.5 = 29.2g

*Apply the formula above:*

29.2 = ? X 1.15 x 0.28 → V = 90.7 ml

So the volume of the stock HCl needed is 90.7ml and make up the volume to 2 liters with distilled water

# Molality (m)

- It is the no. of moles of solute per **1000 g (1kg) of solvent**

$$m = \frac{\text{no. of moles of solute}}{\text{1000 g of solvent}}$$

- Used in physical calculations (e.g., calculations of boiling point elevation)

# Example (1)

- Calculate the molality of a concentrated HCl stock solution which has a 28% w/w%, S.G= 1.15.

Since the weight of solution = weight of solvent + weight of solute.

Thus, the weight of solvent = weight of solution - weight of solute.

= 100g – 28 g = 72g

MW of HCl = 1+35.5 = 36.5 g/mole

No. of moles of solute = 28 / 36.5 = 0.77 mole

0.77 mole of solute → in 72 g of solvent

? mole of solute→ in 1000 g of solvent *( according to molality's definition)*

No. of moles of solute 1000 g of solvent = ( 0.77 * 1000) / 72

= 10.69 moles → **The molality is 10.69m**

# Example (2)

- What is the molality if you had 2 moles of solute dissolved into 1 L of solvent

1 liter of solvent is equal to 1000 g (1Kg) of solvent

*{when the density (specific gravity)is not given}*

$\rightarrow m = 2 / 1 = 2\ m$

# Mole Fraction

- also called *molar fraction,* is the number of moles of solute as a proportion of the total number of moles in a solution

- Example: in a sol. containing $n_1$ moles of compound 1, $n_2$ moles of compound 2, $n_3$ moles of compound3 , the mole fraction of compound 2 equals:

$$MF_2 = n_2 / (n_1 + n_2 + n_3)$$

# Example

- **Calculate the mole fraction of concentrated HCl solution 28 w/w %**

No. of moles = wt / Mwt

In 100 g of solution :

28 g HCl / 36.5 = 0.767 moles of HCl

And

72 g $H_2O$ / 18 = 4 moles of $H_2O$

$MF_{HCl}$ =0.767 / (0.767 + 4) = 0.161