	YILDIZ TECHNICAL UNIVERSITY **BIOMEDICAL ENGINEERING DEPARTMENT** **BME2901- BIOCHEMISTRY COURSE** **2020-2021 FALL SEMESTER**

EXPERIMENT 1
BASIC BIOCHEMICAL TECHNIQUES

1.1.PURPOSE OF THE EXPERIMENT:

To learn solution preparation methods, concentration units, to understand concepts of pH and buffer solution and to learn how buffer solutions are prepared.

1.2.THEORETICAL KNOWLEDGE

1.2.1. SOLUTION & UNIT

Homogeneous mixtures of two or more substances are called "**Solution**".The disintegration medium in solutions is referred to as the solvent, and the disintegrating agent is called the solute. The solubility of a substance is determined by the maximum amount of dissolved in a solvent at a given temperature and pressure. Solubility of a solid or liquid material is expressed in grams of the material dissolved in 100 g of solvent. If the amount of dissolved substance is small, the solution is called dilute solution, and too, it is called concentrated solution.

Solutions containing less substances than the solvent can dissolve are called unsaturated solutions. If the substance is dissolved to the limit of solubility, such solutions are called saturated solutions.

The most commonly used solutions in chemistry according to solvent and soluble are:

- **Liquid in liquid:** Water-alcohol solution
- **Solid in liquid:** Salt water solution
- **Gas in liquid:** Aqueous ammonia solution

The amount of dissolved substance in a given amount of solvent for any solution is called the concentration and is indicated by "C".

$$c = \frac{m_{solute}}{V_{solution}}$$

Here;
C = Concentration of solution
m_{solute}= Amount of solute
$V_{solution}$ = Amount of solute and amount of solvent.
The types of concentration are grouped as volume, mass and moles.

Volume Based Concentrations:
- Molarity (M),
- Normality (N),
- Mass/Volume Percent

Mass Based Concentrations:
- Mass percent,
- Molality (M),
- Millions (ppm),
- Billion (ppb)

Mole Based Concentrations:
- Mole percent
- Mole fraction (mostly used for physicochemical quantities)

Molarity (M): Solutions containing 1 mole of substance per liter are called "**Molar solutions**". Molarity is the number of moles of material dissolved in 1 liter (1000 cm^3= 1000 ml) of solution. Molarity is defines as

$$M = \frac{The\ mole\ number\ of\ solute}{Volume\ of\ the\ solution} = \frac{n}{V(L)}$$

Molarity M; the number of moles of the solute n and the volume of the solution V.
For example; 1 M (or 1 molar) sodium chloride solution means 1 mole, ie 58.44 g NaCl, per liter of solution. The concentration of said NaCl solution is expressed in any of the terms 1 M, mol / L or 1 molar.

Molality (m): In 1000 grams of solvent, it is called the number of moles of dissolved substance and is indicated by m. The most important difference from molarity is that the amount of solvent and solute is known but the solution volume is not known. For example, 3 molal NaOH solution is a solution prepared by dissolving 3 moles (3x40 = 120 g) of NaOH in 1000 grams of water.

$$m = \frac{Amount\ of\ solute\ (mol)}{Amount\ of\ solvent\ (kg)}$$

Example: 12 g of NaOH was dissolved in 120 g of water (120 ml of water). What is the molality of this solution?

12 g NaOH = (12/40) = 0,3 mol NaOH

$$m = \frac{Amount\ of\ solute(mol)}{Amount\ of\ solvent(kg)}$$

120 ml water = 120 g water = 0,120 kg water

$$m = \frac{0,3}{0,120} = 2,5 molal$$

<u>Normality (N):</u> It is referred to as the number of equivalents of the solute in 1 liter of solution and is indicated by N. It is an important part of calculating the equivalent mass in the preparation of such solutions.

Equivalent weight is calculated by dividing the molecular mass by the valence.

$$Gram\ Equivalent = \frac{(Solute)\ Mass\ of\ substance(m)}{Equivalent\ weight}$$

$$Equivalent\ Weight = \frac{Molecular\ mass(M_A)}{Valence}$$

Valence : The number of H^+ ions that the acids give to the environment and the number of OH^- ions that the bases give to the environment are called valence.

For example, for acids containing single H^+ ions such as HCl, HNO_3, CH_3COOH and single OH- ions such as NaOH, KOH, the equivalent weight is equal to the formula weight (effect value 1). Since H_2SO_4 contains two H^+ ions, the equivalent weight is equal to half of the formula weight (effect value 2).

In salt, the effect value is equal to the number of electrons given to or taken from the medium. For example, in salts such as NaCl, $AgNO_3$, the equivalent weight is equal to the formula weight (effect value 1). In the case of salts such as $BaCl_2$, $MgSO_4$, the equivalent weight is equal to half of the formula weight (effect value 2).

Example: 100 ml of concentrated H_2SO_4 (98%, d = 1.85) were diluted to 500 ml. What is the normality of this solution?

First, calculate how many grams of pure H_2SO_4 are present in 100 ml of concentrated sulfuric acid and find 108.32 g.

$$d = \frac{M}{V} \rightarrow M = d.V$$

$$M = 1,84 \text{ x } 100 = 184 \text{ g}$$

$$\frac{100}{98} = \frac{184}{X} \rightarrow X = 180,32\ g\ pure\ H_2SO_4$$

Sulfuric acid gives water 2 hydrogen. Therefore, the effective value is 2. Its molecular mass is 98. The equivalent mass of sulfuric acid is 98/2 = 49 g. In this case, the number of equivalents of dissolved substances (180.32 / 49) = 3.68, the normality (3.68 / 0.5) = 7.36 N would be.

Percent Concentration: It is called the amount of substance dissolved in 100 units of solution and is indicated by the % sign. The percent can be expressed in three ways, mass percent, volume percent and mass/volume percent.

Mass Percent: 100 mass units (g, kg, mg, ton, etc.) indicate how many mass units are dissolved in the solution. With the following equation,

$$Mass\ Percent\left(\%\frac{m}{m}\right) = \frac{Mass\ of\ Solute}{Mass\ of\ Solution} x100$$

mass percent describes.

For example, 20% by mass sodium chloride solution means that there is 20 g of solid sodium chloride in 100 grams of sodium chloride solution or 20 kg of solid NaCl in 100 kg of NaCl solution.

Volume Percent: 100 Volume units (mL, L, m^3, etc.) indicate how many volume units are dissolved in the solution. With the following equation,

$$Volume\ Percent\left(\%\frac{v}{v}\right) = \frac{Volume\ of\ Solute}{Volume\ of\ Solution} x100$$

volume percent describes.

For example, 40% by volume alcohol solution means that there is 40 ml of pure alcohol in 100 ml of alcohol solution or 40 L of pure alcohol in 100 L of alcohol solution.

Mass/Volume Percent: 100 Volume units indicate how many weight units are dissolved in the solution. With the following equation,

$$\frac{Mass}{Volume} Percent \left(\% \frac{m}{v}\right) = \frac{Mass\ of\ Solute}{Volume\ of\ Solution} x100$$

mass/volume percent describes. This concentration is used for solutions of solids in water.

For example, 20% by volume-mass sodium chloride solution means that there is 20 g NaCl in 100 ml NaCl solution or 20 kg NaCl in 100 liters NaCl solution. Here the amount of the solution should be expressed in volume units and the amount of solute should be expressed in mass units.

Mole Fraction: The ratio of the number of moles of a component in the solution to the total number of moles is defined as the molar fraction of that component and is denoted by X.

For example, in a solution of components A, B, C ...

$$Mole\ fraction\ for\ A\ component,\ X_A = \frac{n_A}{n_A + n_B + n_C \ldots}$$

$$Mole\ fraction\ for\ B\ component,\ X_B = \frac{n_B}{n_A + n_B + n_C \ldots}$$

The sum of molar fractions of the components in the solution is one and can be expressed as X_A + X_B + X_C + ... = 1.

PPM and PPB Solutions: Sometimes in very sensitive analyzes the concentrations are so small that “ppm” or ppb ”are used as units.

Million (ppm): It is a concentration unit in terms of parts per million (ppm, abbreviated form of English part per million)

$$ppm = \frac{mg\ amount\ of\ solute}{kg\ amount\ of\ solution}$$

For example, when 2 ppm Hg^{+2} (mercury) solution is mentioned; It is understood that 1 kg of water contains 2 mg of mercury.

$$\frac{2mg}{1kg} = \frac{2mg}{10^6 mg} = 2ppm$$

In very dilute solutions; the volume of the 1 kg solution is one liter (since the density of the water is 1 g / ml = 1 kg / liter). Accordingly, this unit in solutions,

$$ppm = \frac{amount\ of\ solute}{amount\ of\ solution}$$

describes in formula.

For example, 20 ppm Fe means 20 mg Fe^{+2} in 1 liter of solution.

Billion (ppb): Another concentration unit is used for very small concentrations. **ppb,** which means parts per billion (abbreviated parts per billion in English); liters is the amount of solvent expressed in micrograms.

$$ppb = \frac{mg\ amount\ of\ solute}{to\ namountofsolution} \qquad \text{or} \qquad ppb = \frac{ml\ amount\ of\ solute}{m^3\ amount\ of\ solution}$$

<u>Dilution of Solutions:</u> Solutions are generally prepared from **stock** solutions of known concentration. For this purpose, the solution taken from the stock solution is taken into the flask selected according to the desired volume and the solvent is added to the line indicating the volume. In this way a more dilute solution is prepared than the initial concentration.
The dilution calculations are based on the fact that the number of moles of solute taken from the stock solution is the same as the number of moles of the solute in the dilute solution and is expressed as (stock concentration) x (stock volume) = (desired concentration) x (desired volume). It should be careful that the concentration and volume units are the same on both sides of the equation.
Since most concentrations are expressed in molarity and normality,
In short, it can be written as $\mathbf{M_1V_1 = M_2V_2}$ **or** $\mathbf{N_1V_1 = N_2V_2}$.

$\mathbf{N_1}$**,** $\mathbf{M_1}$**,** $\mathbf{V_1}$ are the **initial** values of normality, molarity and volume, and $\mathbf{N_2}$**,** $\mathbf{M_2}$**,** $\mathbf{V_2}$ are the normality, molarity and **final** values of volume.

1.2.2. pH AND BUFFER SOLUTION

pH was defined as negative logarithm of molar concentration of hydrogen ion for the first time by Sorensen at 1909.

$$pH = \log[H^+]$$

or

$$[H^- = 10^{-pH}]$$

For pure water at 25^{o} C, value of pH is 7.

pH= -log [H^{+}]= -log(10^{-7}) = - (-7)=7

As the pH value decreases, [H^{+}] increases, and as the pH increases, [H^{+}] decreases. Acids are proton donors, bases are proton acceptors. Strong acids (HCl, H_2SO_4) are completely ionized in the solution. Weak acids are partially ionized in their solutions. The same situation is valid for the bases. Most body fluids are weak acids.

pH Meter: The strength of acids or bases can be defined by pH values. The scale that shows the acidity or alkalinity (basicity) of substances is called a pH meter. The pH meter ranges from 0 to 14.

pH is often depicted on a graphical colour scale as shown below:

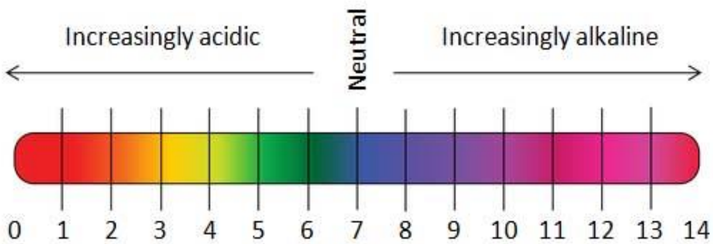

Figure 1.1- Graphical colour scale of pH

Accordingly, the table below shows the pH values of some substances:

Table 1.1 - pH values of some substances

SUBSTANCE	pH
1M HNO_3	0
Gastric juice	1.0-3.0
Lemon juice	2.4
Vinegar	3.0
Orange juice	3.5
Tomato	4.0- 4.4
Urine	5.0-7.0
Saliva	7.0-7.5
Pure water not in contact with air	7
Blood	7.35 – 7.45
Tear	7.4
Sea Water	8.5- 10.0
Ammonia used for household cleaning	11.5
1 M NaOH solution	14

Strength of Acids and Bases: In the nineteenth century, Arrhenius was the first to describe acids as substances that produce hydrogen ions (H^+) when they dissolve in water. On the other hand, bases are ionic compounds that dissociate into a metal ion and hydroxide ions (OH^-) when they are dissolved in water.

Acids are typically classified according to their ability to donate protons and the strength of the acids depends on the percentage of ionization. As the percentage of ionization increases, the strength of acidity increases. The percentage of ionization of an acid is related to the binding strength of the acidic hydrogen to the molecule. The weaker the bonding strength of the acid hydrogen is, the more easily it can be separated (ionizable) from the molecule. When dissolved in water, acids that are 100% or nearly 100% ionizable are called strong acids. Acids that can ionize to a small extent when dissolved in water are called weak acids. Since HCl, a strong acid, is completely ionized in its solution, the H^+ molar concentration is equal to the HCl molar concentration.

Bases are classified in terms of their ability to accept protons. Strong bases have a strong attraction for protons, whereas weak bases have less attraction for protons. For example, the Arrhenius bases such as LiOH, KOH, NaOH, and $Ca(OH)_2$ are strong bases that dissociate completely (100%). In addition, the KOH molar concentration is equal to the OH^- molar concentration.

Indicators are substances that used to understand whether the medium is neutral, acidic or basic. Indicators can change color depending on the acidity or alkalinity of the medium. Also, they give different colors to the solution at different pH values. For example; if phenol red is added to a solution of pH range between 6.6 to 8.2, the solution color turns to orange. If the pH of the solution is less than 6.6, the solution turns to yellow.

The following table lists some of the acid base markers and the effective pH ranges.

Table 1.2 – Indicator list with the effective pH ranges

	Color		
Indicator	**Acid**	**Base**	**pH Ranges**
Thymol Blue	Red	Yellow	1.2 - 2.8
Methyl Orange	Orange	Yellow	3.1 - 4.4
Methyl Red	Red	Yellow	4.2 - 6.3
Bromothymol Blue	Yellow	Blue	6.9 - 7.6
Cresol Red	Yellow	Red	7.2 - 8.8
Phenolphthalein	Colorless	Pink Red	8.3 - 10.0

Neutralization: It is a reaction between an acid and a base to produce a salt and water.

In the neutralization reaction, H^+ from the acid reacts with OH^- from the base to form water, leaving the spectator ions from the salt in the solution.

$$H^+ \quad + \quad OH^- \quad \leftrightarrow \quad H_2O$$

Acidity Alkalinity Neutrality

As we understand from the above equation, 1 mole of H^+ ion is completely combined with 1 mole of OH^- ion to form 1 mole of water. In this case, numbers of H^+ ion and OH^- ion coming from the mixed solutions specify the acidity or alkalinity of the medium.

- When water has an equal concentration of H^+ ions and OH^- ions, it is said to be neutral (pH=7)

  $nH^+ = nOH^- \Rightarrow$Solution is neutral. pH = pOH = 7

- When water has a greater concentration of H^+ ions, it is said to be acidic (pH<7)

  $nH^+ > nOH^- \Rightarrow$ Solution is acidic. pH < 7 < pOH

- When a solution has a greater concentration of OH^-, it is said to be alkaline (pH>7)

  $nH^+ < nOH^- \Rightarrow$ Solution is basic. pH > 7 > pOH

Buffer Solutions: Buffer solutions are those solutions pH of which does not change with a certain dilution or with the addition of a small amount of strong acid or base. Therefore, they are vital in biochemical reactions. Even a slight change in pH of organisms is life-threatening. Thus, all organisms are naturally buffered to provide an appropriate metabolism.

Buffer solutions are those solution which contain a weak acid and its conjugate base or a weak base and its conjugate acid.

$$CH_3COOH_{(aq)} \leftrightarrow H^+_{(aq)} + CH_3COO^-$$

Weak Acid Conjugated Base

$$NH_{3_{(aq)}} \leftrightarrow NH^+_{4_{(aq)}} + OH^-_{(aq)}$$

Weak Base Conjugated Acid

$$pH = pKa + Log\frac{[A^-]}{[HA]} \qquad (\text{Handerson} - \text{Hasselbach Equation})$$

Buffer Capacity: Buffer capacity is defined as the concentration of H^+ or OH^- ions which the buffer solution can neutralize without much change in its pH. The buffer capacity is indicated by β. The capacity of a buffer does not only depend on the total concentration of the conjugated acid-base pair but also the ration between their concentrations. As the concentration ratio increases to the values greater than or less than 1, the decrease in buffer capacity increases. A good buffer;

- should not be toxic,

- should not give no absorption in the UV region,

- should be biologically and chemically inactive,

- pKa value should change to a minimum level with temperature and ion strength.

The pH range at which the prepared buffer solution can resist pH change by buffering against the added acid or base is calculated as follows:

pH capacity = pKa ± 1

1.3.MATERIALS AND METHODS:

1.3.1. MATERIALS: Precision balance, Spatula, Distilled water,
Weighing bottle, Wash bottle, Graduated cylinder, 100 ml erlenmeyer flask, 100 ml solution bottle, Magnetic stirrer, Fume hood, NaH_2PO_4, Na_2HPO_4, NaCl, HCl, KCl, NaOH

1.3.2. METHOD:

Preparation of 50 ml of KCl (3 M) solution:

1. Determine the amount of solute using the appropriate equations.
2. Weigh the calculated amount of KCl on a precision balance.
3. Transfer the KCl to the erlenmeyer flask.
4. Add 50 ml of water.
5. Mix until the magnetic stirrer becomes homogeneous.
6. Transfer your solution to a clean and dry solution bottle.

Preparation of 200 ml of 3M NaOH solution:

1. Determine the amount of solute using the appropriate equations.
2. Weigh the calculated amount of NaOH on a precision balance
3. Transfer the NaOH to the erlenmeyer flask.
4. Add 50 ml of water.

5. Mix until the magnetic stirrer becomes homogeneous.

6. Transfer your solution to a clean and dry solution bottle.

<u>Preparation of 200 ml of 3 M HCl (37%) solution:</u>

1. The calculated amount of acid solution is added to distilled water.

2. Mix until homogeneous.

3. The solution is transferred to a suitable solution bottle which is clean and dry.

<u>Preparation of 250 ml 0.01M pH 7.4 Phosphate Buffered Saline (PBS)</u>

1. NaCl, KCl, Na_2HPO_4 and KH_2PO_4 amounts are calculated so that their solution will be 0.01 M and weighed on a precision balance.

2. The weighed substances are transferred to the flask, 200 ml of distilled water is added and the mixture is stirred on the magnetic stirrer.

3. After the substances are completely dissolved and the homogeneous solution is obtained, the pH is adjusted to 7.4.

4. After pH adjustment of buffer, volume of solution is completed to a total volume.