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1. Solutions and Buffers

1.1 Introduction

Almost all the experimental techniques in biochemistry involve the use of solutions. These have to be used for extraction of biomolecules from tissues, their separation, quantitative estimation, purification and even for their physico-chemical characterization. Therefore, it is important that a student of biochemistry must be fully familiar with the proper preparation of solutions and buffers.

1.2 Solutions

A solution is homogeneous mixture of two or more non-reacting substances and has uniform properties such as chemical composition, density, refractive index etc. However, its composition can be varied within certain fixed limits.

A solution which is made up of two components is called binary solution. The dissolved substance in solution is called the solute and the medium in which it is dissolved is known as the solvent. The solute in a solution is always present in a smaller quantity than the solvent. The most commonly used solutions in biochemical work are of solid-liquid and liquid-liquid type. A few examples of such solutions are given below:

<i>Solute</i>	<i>Solvent</i>	<i>Example</i>
Solid	Liquid	Sugar in water, AMP in buffer, Tris in water
Liquid	Liquid	Glycerol in water, alcohol in water, 2-mercaptoethanol in buffer

Composition of a solution can be expressed in two ways: quantity and concentration. Quantity is the amount of any substance (solute) present in a solution/solvent irrespective of the amount/volume of the solvent/solution. In contrast, concentration refers to the quantity of the solute present in an exact or a specific amount of solvent or that of solution. For example, a solution containing 4 g NaOH in a volume of 100 ml or even 200 ml of a solution has a total quantity of 4 g NaOH. When this amount is expressed per unit volume such as 4 g/100 ml of the solution, then it is termed as 4% (concentration) solution. Thus in this example, per cent is a unit of concentration where as the amount is the unit of quantity.

The term mole, which is defined as the molecular weight of a compound in grams, is frequently used while indicating the quantity of a compound and for preparation of solutions. Thus,

1 mole = g molecular weight of the substance, and
 1 mole of glucose = 180 g (Mol. wt. = 180)
 1 mole of albumin = 68,000 g (Mol. wt. = 68,000)

1.2.1 Mode of expressing concentration of a solution

Following are some of the common ways of expressing the concentration of solutions:

1.2.1.1 Molarity (M)

This is the most common method for expressing the concentration of a solution in biochemical studies. The molarity of a solution is the number of moles of the solute dissolved per L of the solution. A solution which contains 1 mole of the solute in one L of the solution is called a molar solution. Molarity of a solution can be calculated as follows:

$$\text{Molarity} = \frac{\text{Weight of a solute in g/L of solution}}{\text{Mol. wt. of solute}}$$

It may be noted that in case of molar solutions, the combined total volume of the solute and solvent is one L. Thus for preparing 0.1 M NaOH, one may proceed as follows:

$$\text{Mol. wt. of NaOH} = 40$$

$$\text{Required molarity of solution} = 0.1 \text{ M}$$

$$\therefore \text{Amount (in g) of NaOH per L of solution} = \text{Mol. wt. of NaOH} \times \text{molarity} \\ = 40 \times 0.1 = 4 \text{ g}$$

Thus, weigh 4 g of NaOH, dissolve it in a small volume of solvent (water) and make the final volume to 1 L with the solvent.

Sometimes it is desirable to know number of moles of a substance in a reaction mixture. This can be calculated using a simple relationship:

$$\begin{aligned} 1 \text{ M solution} &= 1 \text{ mole of the substance/L of solution.} \\ &= 1 \text{ mmole/ml of solution} \\ &= 1 \text{ } \mu\text{mole}/\text{ml of solution} \\ 1 \text{ mM solution} &= 1 \text{ mmole/L of solution} \\ &= 1 \text{ } \mu\text{mole/ml of solution} \end{aligned}$$

1.2.1.2 Molality (m)

A solution which contains 1 mole of the solute dissolved in 1 kg of the solvent is called a molal solution. Hence,

$$\text{Molality} = \frac{\text{Weight of a solute in g/kg of solvent}}{\text{Mol. wt. of solute}}$$

It is important to remember that in a molal solution, the amount of solvent is 1000 g. Thus in case of aqueous solution, 1 molal solution is obtained by dissolving

1 mole of the solute in 1000 ml (since Sp. Gravity = 1) of water. For example, for preparing 1 m Na₂CO₃ solution, dissolve 106 g of Na₂CO₃ (Mol. wt. of Na₂CO₃ = 106) in one kg of water.

1.2.1.3 Normality (N)

The normality of a solution is the number of gram equivalents of the solute per L of the solution.

A solution having one g equivalent of the solute per L of solution is called 1N solution. Therefore,

$$\text{Normality} = \frac{\text{Amount of a substance in g/L of solution}}{\text{Eq. wt. of substance}}$$

For preparing 0.1 N Na₂CO₃ (Eq. wt. of Na₂CO₃=53) solution, dissolve 5.3 g Na₂CO₃ in a final volume of 1 L of solution.

1.2.1.4 Mass concentration

Substances like proteins, nucleic acids etc. which do not have a uniformly defined composition, their concentration is expressed in terms of weight per unit volume rather than moles per unit volume. The unit of volume is L, so all concentrations should be expressed with L (g/L, mg/L, μg/L etc.). The term per cent (%) is also quite commonly used. However, to avoid any ambiguity it is necessary to properly define the basis of % solution as illustrated by the following example. A 5% solution of acetic acid could mean:

$$\begin{aligned} &5 \text{ g of acetic acid per 100 g of solution (w/w)} \\ &5 \text{ g of acetic acid per 100 ml of solution (w/v)} \\ &5 \text{ ml of acetic acid per 100 ml of solution (v/v)} \end{aligned}$$

Thus, 1% (w/v) solution of casein would imply 1 g of casein dissolved in solvent to give a final volume of 100 ml of the solution.

1.2.1.5 Mass fraction

The mass fraction of a component in solution is the mass of that particular component per unit mass of the solution. If W_A and W_B represent the masses of components A and B in a solution, then

$$\text{Mass fraction of A} = \frac{W_A}{W_A + W_B}$$

$$\text{Mass fraction of B} = \frac{W_B}{W_A + W_B}$$

1.2.1.6 Mass percentage or % (w/w)

It is the weight of the component present in 100 parts by weight of the solution.

In a solution containing 10 g sugar in 40 g of water, then

$$\text{mass \% of sugar} = \frac{10 \times 100}{(10 + 40)} = 20\%$$

1.2.1.7 Percentage by volume or % (v/v)

It is the volume of the component in 100 parts by volume of the solution. In a solution containing 20 ml alcohol in 80 ml of water, the % volume of alcohol will be $\frac{20 \times 100}{(20 + 80)} = 20\%$

1.2.1.8 Parts per million (ppm)

This is generally employed for those solutions in which a substance is present in a very small quantity. It represents gram of a solute per million grams of solution or the gram of a solute per million ml of the solution.

$$\text{ppm} = \frac{\text{mass of the component}}{\text{total mass of the solution}} \times 10^6$$

$$\text{or} \quad \text{ppm} = \frac{\text{g or ml of solute or substance}}{\text{g or ml of solution}} \times 10^6$$

Thus, 1 ppm of solution of NaCl in water represents

$$1 \text{ ppm} = 1 \text{ mg NaCl/L of solution}$$

$$\text{or} \quad = 1 \text{ mg NaCl/1000 ml of solution}$$

$$\text{or} \quad = 1 \mu\text{g NaCl/ml of solution}$$

1.2.2 Types of Solutions**1.2.2.1 Stock solution**

Stock solution of a substance is the one having a concentration many folds higher than that actually required in the experiment. Stock solutions are prepared of the substances that are to be used frequently and are stable at higher concentration for several days and can be used after appropriate dilution just before use. It is sometimes convenient to weigh out a relatively large amount of the compound and prepare a stock solution of that compound from which small amounts can be withdrawn at convenience and added to solution of other components. The use of a stock solution thus cuts down on the amount of pipetting and at the same time reduces variability between a number of similar incubation mixtures, assay mixtures etc.

Frequently, a particular volume of a solution containing a desired concentration of a substance has to be prepared by using its stock solution. A simple mathematical equation can be used to calculate the volume of the stock solution needed to prepare a solution of required volume containing desired concentration of the compound:

$$N_1 V_1 = N_2 V_2$$

where N_1 = concentration of the solution to be prepared
 V_1 = volume of the solution to be prepared
 N_2 = concentration of the stock solution
 V_2 = volume of the stock solution

For example, for preparing 150 ml of a solution containing 10 mM Tris from a stock of 75 mM Tris, the volume of the stock solution which should be used can be calculated as follows:

$$V_2 = \frac{N_1 V_1}{N_2} = \frac{10 \times 150}{75} = 20 \text{ ml}$$

Thus by taking 20 ml of 75 mM stock solution of Tris and making its final volume to 150 ml (by adding water or other solutions as per the requirement of an experiment) a solution containing 10 mM Tris is obtained.

1.2.2.2 Standard solution

A solution of known concentration is referred to as a standard solution. In many experiments, for quantitative determination of a particular substance in a sample preparation, a standard or reference solution is required. For example, for quantitative determination of total proteins in a sample preparation, a standard solution of a protein like bovine serum albumin is used for comparison. The standard solution is prepared accurately by weighing a fixed or known amount of bovine serum albumin and adding precisely measured volume of solvent. The standard solution serves the purpose of a reference. Very often the standard solution containing different concentrations of a compound is used for preparing a standard curve. This can be done by making a stock solution from which solutions of graded concentrations are made e.g. 1, 5, 10, 50 and 100 μg protein/ml and their experimental values such as O.D. are determined. Then a graph of O.D. *versus* amount of protein is plotted to obtain a standard or reference curve.

1.2.2.3 Saturated solution

Solubility of any substance in a particular medium, say water, varies with temperature. When a solution contains the solute in an amount in excess of that which can completely be dissolved at a given temperature and the solute in solution is in equilibrium with the excess of undissolved solute, the solution is said to be saturated.

1.2.2.4 Solutions of acids

Since most of the commonly used acids like hydrochloric acid and sulphuric acid are not pure, their effective strength is low which ought to be taken into account while making their solutions of desired concentrations. The following formula can be applied to compute the volume of the commercially available concentrated acid required for preparing a solution of required normality:

$$V_1 = \frac{\text{Eq. wt. of acid} \times V_2 \times \text{normality} \times 100}{1000 \times \text{specific gravity} \times \text{purity (\%)}},$$

where V_1 = required volume of the concentrated acid
 V_2 = total volume of the acid solution of desired normality to be prepared.

The use of above formula is illustrated by the following example.

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Exercise: Prepare 750 ml of 1N sulphuric acid from concentrated acid (purity, 96%; specific gravity, 1.84; Mol. wt., 98).

Eq. wt. of sulphuric acid = 49 (since sulphuric acid is diprotic)

Putting various values in the above equation, we get

$$V_1 = \frac{49 \times 750 \times 1 \times 100}{1000 \times 1.84 \times 98} = 20.8 \text{ ml}$$

thus, take 20.8 ml of concentrated sulphuric acid and dilute it to 750 ml with water to get 1 N solution of sulphuric acid.

1.2.3 Precautions

- (i) Aqueous solutions should always be made using pure water (deionized or double distilled).
- (ii) Solution should remain clear after preparation.
- (iii) Solution should always be properly stored, e.g. some solutions can be stored at room temperature while others require storage at low temperatures. Solutions which are sensitive to light should always be stored in brown bottles.
- (iv) Always note down the Mol. wt. printed on the bottle and take into account hydration state of the substance, purity, specific gravity etc.

1.2.4 Practical exercises

Use of solutions of specified compositions is one of the fundamental requisites for obtaining accurate, reliable and reproducible results in almost all biochemical investigations. Incomplete or improper knowledge of solution preparation is bound to give erroneous results. A few 'Exercises' are given below with a view to provide some practice to the students for preparing various types of solutions that are commonly used in biochemical experiments:

1. How many g of NaOH would be required to make 50 ml of 0.5 mole/L solution of NaOH? (Mol. wt. of NaOH = 40)
2. How many mmole/ml of the solute are contained in the following solutions:
 - (a) 0.20 M NaCl
 - (b) 10 mM Glucose
 - (c) 100 μM Glutamate
 - (d) 30 g of urea in 100 ml of solution (Mol. wt. of urea = 60)
3. How many g of alanine are present in 20 ml of a 50 mM solution of this amino acid (Mol. wt. of alanine = 89).
4. Conc HCl is 37.5% HCl by wt. and has a density of 1.19. Describe how would you prepare 500 ml of 0.2 N HCl solution (Mol. wt. of HCl = 36.5).
5. Describe preparation of 300 ml of 0.5 N CH₃COOH (Acetic acid is 100% pure, has a density of 1.05 and its Mol. wt. is 60).
6. Calculate the volume of 200 mM glucose solution required to make 25 ml of 50 mM glucose solution.
7. You are provided with 95% (v/v) ethanol. Explain how would you obtain 150 ml of 70% (v/v) ethanol?

8. How would you prepare 85 ml of 0.02% (w/v) solution of ninhydrin in acetone?
9. You need 10 ml of 0.5 M maltose for an experiment. Explain how would you make this solution (Mol. wt. of maltose = 342).

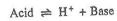
10. According to an experimental procedure you are required to prepare 5 ml of a reaction mixture containing 20 mM MgCl₂. This amount of MgCl₂ has to be supplied by adding 0.1 ml of MgCl₂ solution to 4.9 ml of the reaction mixture. Calculate how much MgCl₂ would you take for preparing 1 ml of its stock solution to fulfill the above requirement (Mol. wt. of MgCl₂ = 95.3).

1.3 Buffers

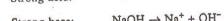
Buffers are extensively used in biochemical studies since these aid in maintaining a near constant pH of the media while performing various laboratory operations such as during extraction, isolation and purification of various biomolecules. Selection of an appropriate buffer with optimal pH is important as it may have a profound influence on extractability, stability and even biological functioning of cell constituents. For example, all the biochemical reactions in a cell are catalysed by enzymes whose stability as well as activity is highly dependent on the pH of the system. In several reactions protons may be consumed or released during an enzyme reaction. In such cases it, therefore, becomes increasingly important to provide a system i.e. buffer which could stabilize the hydrogen ion concentration and obviate adverse effects due to change in pH on the enzyme under investigation. Before considering how buffers function, it is necessary to know some of the important terms associated with the concept of pH and buffers.

1.3.1 Acids and bases

The modern concept of acids and bases defines acid as proton donor and a base as proton acceptor. On ionization, an acid donates a proton and at the same time a corresponding base (which is capable of accepting a proton) is formed. Such a base is known as conjugate base:



Acids and bases are classified into two groups: strong and weak acids and bases. Strong acids or bases are those which get completely ionized in solution so that the concentration of free H⁺ or OH⁻ is the same as the concentration of the acid or base. Example:



On the other hand, weak acids or bases dissociate only to a limited extent and the concentration of free H⁺ and OH⁻ depends on the dissociation constant.



1.3.2 Dissociation of water, its ionic product K_w and concept of pH
Water is weak electrolyte which dissociates only slightly to form H^+ and OH^- ions.



The equilibrium constant of this dissociation reaction is 1.8×10^{-16} mole/L at 25°C

Thus $K_{eq} = \frac{[H^+][OH^-]}{[H_2O]} = 1.8 \times 10^{-16}$

1000 ml of pure water contains 1000 g of water. Since the molecular weight of water is 18 its molar concentration can be calculated and is equal to 1000/18 or 55.5 moles/L. Because the concentration of H_2O in dilute aqueous solution is essentially unchanged from that in pure H_2O , this figure may be taken as constant. Thus,

$$\frac{[H^+][OH^-]}{55.5} = 1.8 \times 10^{-16}$$

$$[H^+][OH^-] = 1.8 \times 10^{-16} \times 55.5$$

$$= 1.01 \times 10^{-14}$$

or $K_w = 1.01 \times 10^{-14}$

where K_w is ion product of water which expresses the relationship between the concentration of H^+ and OH^- ions in aqueous solutions. This equation may be used to calculate the concentration of H^+ in pure water. Let x be the concentration of H^+ on dissociation of water. Since production of one H^+ is associated with generation of one OH^- , the concentration of OH^- will also be x .

Hence, $[H^+][OH^-] = 1.01 \times 10^{-14}$

or $x^2 = 1.01 \times 10^{-14}$
 $x = 1.01 \times 10^{-7}$ moles/L

A more convenient method of expressing concentration of H^+ ions is by means of pH which is defined as negative logarithm of H^+ ion activity.

Thus, $pH = \log \frac{1}{aH^+} = -\log aH^+$

where aH^+ is defined as the activity of H^+ . In this context no distinction is made between activities and concentration. Hence,

$$pH = \log \frac{1}{H^+} = -\log [H^+]$$

It is important to note that the pH is a logarithmic function, thus when the pH of a solution decreases by one unit from 5 to 4, the H^+ ion concentration increases by ten fold, i.e. from 10^{-5} to 10^{-4} M. If we now apply the term pH to the ion product of water

$$[H^+][OH^-] = 1.0 \times 10^{-14}$$

$$\log [H^+] + \log [OH^-] = \log (1.0 \times 10^{-14})$$

$$-\log [H^+] - \log [OH^-] = 14$$

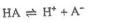
$$pH + pOH = 14$$

or $pH = 7$

Hence pH of pure water is 7.0

1.3.3 Henderson-Hasselbalch equation

The Henderson-Hasselbalch equation is simply another way of expressing dissociation constant of an acid



$$K_{ion} = k' = \frac{[H^+][A^-]}{[HA]}$$

on rearranging,

$$[H^+] = k' \frac{[HA]}{[A^-]}$$

Taking negative logarithm on both sides

$$-\log [H^+] = -\log k' - \log \frac{[HA]}{[A^-]}$$

Substituting pH for $-\log [H^+]$ and pK' for $-\log k'$, where pK' is $-\log$ of dissociation constant

$$pH = pK' - \log \frac{[HA]}{[A^-]}$$

or $pH = pK' + \log \frac{[A^-]}{[HA]}$

This is known as Henderson-Hasselbalch equation which can be written in a more general form as

$$pH = pK' + \log \frac{\text{conjugate base}}{\text{conjugate acid}}$$

It is an extremely useful equation from which either pH of the solutions of various concentration ratios of a conjugate acid-base pair of known pK' can be calculated or the ratio of conjugate acid-base of known pK' to obtain a buffer of desired pH can be found out.

1.3.4 Buffer system

A buffer system is one that resists a change in pH on the addition of acid or alkali and constitutes of conjugate acid-base pair. Most commonly, the buffer solution

consists of a mixture of a weak acid and its conjugate base, e.g. a mixture of acetic acid and sodium acetate is a buffer solution. A well known physiological buffer system is the carbonate-bicarbonate system of blood.

The basis for functioning of a buffer with regards to its ability to resist change in pH can be illustrated by the following example. Suppose we have a buffer containing 5 ml of 0.1 M sodium acetate and 4 ml of 0.1 M acetic acid. Let us examine the effect on pH on addition of 1 ml of 0.1 N HCl to this buffer:

(a) Before adding acid

$$\begin{aligned} \text{Total volume of buffer is } (5 \text{ ml} + 4 \text{ ml}) &= 9 \text{ ml} \\ \text{Conc of acetate} &= 5/9 \times 0.1 \text{ M} = 0.5/9 \text{ M} \\ \text{Conc of acetic acid} &= 4/9 \times 0.1 \text{ M} = 0.4/9 \text{ M} \\ \text{pK}' \text{ of acetic acid} &= 4.76 \\ \text{Thus, pH} &= 4.76 + \log \frac{0.5/9}{0.4/9} \\ &= 4.76 + (0.097) = 4.86 \end{aligned}$$

(b) After adding acid

HCl provides H^+ which combine with the acetate ion to give acetic acid. Thus, it reduces the amount of acetate ions present and increases the amount of undissociated acetic acid leading to an alteration in the salt/acid ratio and hence to a change in pH.

The final volume of buffer after acid addition = 10 ml

$$\begin{aligned} \text{Conc of acetate after acid addition} &= 5/10 \times 0.1 \text{ M} - 1/10 \times 0.1 \text{ M} \\ &= 0.04 \text{ M} \\ \text{Conc of acetic acid after acid addition} &= 4/10 \times 0.1 \text{ M} + 1/10 \times 0.1 \text{ M} \\ &= 0.05 \text{ M} \\ \text{Thus, pH} &= 4.76 + \log 0.04/0.05 \\ &= 4.76 + (-0.097) \\ &= 4.66 \end{aligned}$$

Thus, the pH changes marginally from 4.86 to 4.66 on addition of 1 ml of 0.1 N HCl. However, if the same amount of the acid is added to pure water its pH will change from 7.0 to 2.0.

1.3.5 Preparation of buffers

Information regarding preparation of most of the commonly used buffers is available in tabulated form in several books (see Methods in Enzymology Vol. 1 and Vol. 182). These tables have been computed using Henderson-Hasselbalch equation. It is, however, quite simple to do calculations for preparing a buffer of required pH as shown in an example as follows:

Suppose one wishes to prepare 1 L of 0.1 M acetate buffer of pH 5.22 (pK' of acetic acid is 4.74). First determine the required ratio of conjugate base (acetate ion) to weak acid (acetic acid) in this buffer solution using Henderson-Hasselbalch equation.

$$\text{pH} = \text{pK}' + \log \frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$$

$$\text{Putting the values} \quad 5.22 = 4.76 + \log \frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$$

$$\text{or} \quad \log \frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]} = 5.22 - 4.76 = 0.48$$

$$\text{or} \quad \frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]} = \text{antilog } 0.48 = 3$$

Thus, in this buffer solution the molar ratio of CH_3COO^- to CH_3COOH should be 3. In other words, 75% of the buffer components should be present as the conjugate base CH_3COO^- and 25% of the components as acetic acid. Since 1 L of 0.1 M acetate buffer should contain 0.1 mole of acetate plus acetic acid, the solution should have 0.075 moles of acetate ions and 0.025 moles of acetic acid. Finally calculate the amount in g of sodium acetate required to obtain 0.075 moles of acetate ions. Similarly, to obtain 0.025 moles of acetic acid 1.5 g of acetic acid would be required. Prepare these solutions, mix them and make the final volume to 1 L with water. This will give 1 L of buffer of the desired pH and concentration.

1.3.6 Criteria for selection of buffers

While choosing a buffer the following factors need to be taken into account. It should:

- (i) possess adequate buffering capacity in the required pH range. Generally buffers are most effective over a range of one pH unit on either side of their pK' value, e.g. Tris which has pK' value of 8.3 has an effective pH range of 7–9. The pK' values of some important buffers are given in Appendix (Table 6)
- (ii) be chemically inert and not react or bind with biomolecules or other components, particularly, for assaying activities of enzymes which require a metal ion for their functioning
- (iii) be available in high degree of purity and should not contain impurities which may interfere with estimations
- (iv) be enzymically and hydrolytically stable
- (v) maintain pH that is minimally influenced by temperature, ionic composition and concentration or salt effect of the medium
- (vi) not be toxic
- (vii) not absorb light in the visible or ultraviolet regions.

1.3.7 Measurement of pH

By using pH indicators (natural or chemically synthesized organic compounds) an approximate value of pH of a solution can be obtained. The indicators dissociate like a weak acid on coming in contact with solution and give range of colours depending on pH. Dissociated and undissociated form of the indicator have different colours. pH papers coated with indicators of different pH range are commercially available. However, accurate pH can be measured using pH meter which measures e.m.f. of a concentration cell developed from a reference electrode, test solution and a glass electrode sensitive to H⁺ ions. The combined electrode consisting of glass and reference electrode is dipped into the test solution for accurate measurement of pH.

1.3.8 Composition of some commonly used buffers

Buffers can be made in stock solutions and these are diluted before use. Preparation of some of buffers frequently used in biochemical studies is given below. Unless otherwise stated, by following these procedures 0.1 M buffer will be obtained.

(a) Acetate buffer**Stock solutions**

- A. 0.2 M acetic acid (11.55 mL/L)
- B. 0.2 M sodium acetate (16.4 g of sodium acetate or 27.2 g of sodium acetate 3H₂O per L)

x ml of A + y ml of B, diluted to a total volume of 100 ml

x	y	pH	x	y	pH
46.3	3.7	3.6	25.5	24.5	4.6
44.0	6.0	3.8	14.8	35.2	5.0
41.0	9.0	4.0	10.5	39.5	5.2
36.8	13.2	4.2	8.8	41.2	5.4
30.5	19.5	4.4	4.8	45.2	5.6

(b) Phosphate buffer**Stock solutions**

- A. 0.2 M monobasic sodium phosphate (27.8 g in 1 L)
- B. 0.2 M dibasic sodium phosphate (53.65 g of Na₂HPO₄ · 7H₂O or 71.7 g Na₂HPO₄ · 12 H₂O in 1 L)

x ml of A + y ml of B, diluted to a total volume of 200 ml

x	y	pH	x	y	pH
93.5	6.5	5.7	56.5	43.5	6.7
90.0	10.0	5.9	39.0	61.0	7.0
85.0	15.0	6.1	16.0	84.0	7.5
77.5	22.5	6.3	5.3	94.7	8.0
68.5	31.5	6.5			

(c) Tris (hydroxymethyl) aminomethane buffer or Tris buffer:**Stock solutions**

- A. 0.2 M Tris (hydroxymethyl) aminomethane (24.2 g per L)
- B. 0.2 M HCl

50 ml of A + y ml of B diluted to a total volume of 200 ml. 0.05 M Tris-HCl buffer will be obtained.

y	pH	y	pH
5.0	9.0	26.8	8.0
8.1	8.8	32.8	7.8
12.2	8.6	38.4	7.6
16.5	8.4	41.4	7.4
21.9	8.2	44.2	7.2

(d) Preparation of imidazole, MOPS and HEPES buffers

For preparing these buffers, the molar ratio of the protonated to non-protonated species for obtaining buffer of required pH can be accomplished by mixing the computed amount of free buffer and one of its salts (from Henderson-Hasselbach equation). In the Table given below, buffer as free acid and its salt can be mixed in the ratio ranging from 90:10 to 10:90 to obtain the desired pH. For example, for preparing 100 ml 0.1 M HEPES buffer of pH 7.22, mix 70 ml of 0.1 M HEPES and 30 ml of 0.1 M sodium salt of HEPES.

% buffer (as free acid)	% buffer (as free base)	Imidazole	pH	MOPS	HEPES
90	10	6.09	6.19	6.60	
80	20	6.44	6.54	6.95	
70	30	6.68	6.78	7.18	
60	40	6.87	6.97	7.37	
50	50	7.05	7.15	7.55	
40	60	7.18	7.32	7.73	
30	70	7.22	7.52	7.92	
20	80	7.35	7.75	8.15	
10	90	8.00	8.05	8.50	

In laboratory, these buffers are normally prepared by titrating a solution of the

free buffer with base (NaOH or KOH) and the pH is continuously monitored on a pH meter. For example, if 1 L of 0.1 M MOPS buffer of pH 6.6 is needed, first calculate the amount of MOPS (free acid) required to make 0.1 M solution. Dissolve this amount in approximately 800 ml water. Titrate this solution with concentrated solution of NaOH (e.g. 1 M) till the desired pH is obtained and then make the volume to 1 L with water.

1.3.9 Laboratory Exercises

Using the information in the above Tables, students may gain practice and experience in preparation of the buffers. Some such experiments are suggested below:

- EXPERIMENT 1:** Prepare a 0.1 M phosphate buffer of pH 7.5
EXPERIMENT 2: Examine the effect on pH of using double strength solutions of the components A and B of Tris buffer and mixing them in a selected fixed ratio.
EXPERIMENT 3: Determination of effect of addition of a fixed volume of an acid to a buffer of different molarities. For this prepare 0.025, 0.05, and 0.1 M buffer having identical pH. Take 50 ml of each of these buffers and add 5 ml of 0.1 M HCl. Note the change in pH. Also observe whether there exists any relationship between molarity of the buffer and its capacity to resist the change in its pH.

Suggested further reading

- Blanchard J.S. (1984). Buffers for enzymes. In *Methods in Enzymology*, Vol. 104 Part C, (Jackoby WB, ed.), pp 404–417, Academic Press Inc., New York.
Cone E.B. and Stumpf P.K. (1987). Outline of Biochemistry, 4th ed., pp 3–24, Wiley Eastern Ltd., New Delhi.
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Gordon D. (1959). Preparation of buffers for use in enzyme studies. In *Methods in Enzymology*, Vol. 1 (Clewellick PS and Kaplan NO, eds.), pp 138–146, Academic Press Inc., New York.
Sagel I.H. (1976). Biochemical Calculations, 2nd ed., Wiley, New York.
Stoll V.S. and Blanchard J.S. (1990). Buffers: Principles and Practice. In *Methods in Enzymology*, Vol. 182, pp 24–38 (Deutscher MP, ed.), Academic Press Inc., New York.

Molarity (M) = moles of solute/litres of solution

Molality (m) = moles of solute/Kgs of solvent

Normality (N) = n X M ; n= integer

For an acid, n = no of H⁺ ion provided by a formula units of acid

3 M HCl = 3N HCl

3M H₂SO₄ = 6N H₂SO₄

Q. What is the pH of 0.1 N HCl?

$$\text{pH} = - \log [\text{H}^+] = - \log [10^{-1}] = - (-1) = 1$$

It is important to note that pH is a logarithmic function, thus when the pH of a solution decreases by one unit from 5 to 4, the H⁺ ion concentration increases by 10 fold (from 10⁻⁵ M to 10⁻⁴M).

Q. Conc. HCl is 37.5 % HCl by wt. and has a density of 1.19. Describe how could you prepare 500 ml of 0.2 N HCl solution? (Mol. Wt of HCl = 36.5).

Density of HCl = 1.19

∴ the wt. of 1 ml of HCl = 1.19 gm

Specific gravity = the numerical value of the mass in grams of 1 ml of any substance.

Specific gravity = (density of solid or liquid) / (density of water at 4 °C)

The mass of 1 ml of pure water at 4 °C.

The no. of grams of HCl per ml of solution = 1.19 X 0.375 = 0.44625 gms of HCl per mL of solution.

Now, 1000 ml 36.5 gm HCl's strength = 1N

1N = 0.0365 gm/ml

∴ 0.0365 gm/ml = 1N

$$0.44625 \text{ gm/ml} = (0.44625/0.0365) = 12.226 \text{ N}$$

$$V_1S_1 = V_2S_2$$

$$500 \times 0.2 = V_2 \times 12.226$$

$$V_2 = 8.2 \text{ ml.}$$

$$\text{Water} = (500 - 8.2) = 491.8 \text{ ml}$$

Mathematics for Practice

1. 1gm = mg = ug

2. 1 litre= ml= ul

3. Define Molarity, Molality and Normality

4. How many gm of NaOH would be required to make 50 ml of 0.5 mole/L solution of NaOH? (Mol. Wt. of NaOH= 40)

5. How many mmole/ml of the solute are contained in the following solutions:

- (a) 0.20 M NaCl
- (b) 10 mM Glucose
- (c) 100 uM Glutamate
- (d) 30g of urea in 100 ml of solution (Mol.wt. of urea = 60)

6. How many gm of alanine are present in 20 ml of a 50 mM solution of this amino acid? (Mol. Wt of Alanine = 89)

7. Conc. HCl is 37.5 % HCl by wt. and has a density of 1.19. Describe how would you prepare 500 ml of 0.2N HCl solution? (Mol. Wt. of HCl = 36.5)

8. Describe the preparation of 300 ml of 0.5N acetic acid (acetic acid 100% pure, has a density of 1.05 and its Mol.wt is 60).

9. Calculate the volume of 200 mM glucose solution required to make 25 ml of 50 mM glucose solution?

10. You are provided with 95% (v/v) ethanol. Explain how would you obtain 150 ml of 70 % (v/v) ethanol?

11. What is the pH of 0.1N HCl?

Calculate the pH of 10-8 M HCl.

If we use the relation, $\text{pH} = -\log [\text{H}_3\text{O}^+]$, we get pH equal to 8. But this is not correct because an acidic solution cannot have pH greater than 7. It may be noted that in very dilute acidic solution, when H^+ concentrations from acid and water are comparable, the concentration of H^+ from water cannot be neglected.

Therefore,

$$[\text{H}^+]_{\text{total}} = [\text{H}^+]_{\text{acid}} + [\text{H}^+]_{\text{water}}$$

Since HCl is a strong acid and is completely ionized

$$[\text{H}^+]_{\text{HCl}} = 1.0 \times 10^{-8}$$

The concentration of H^+ from ionization is equal to the $[\text{OH}^-]$ from water,

$$[\text{H}^+]_{\text{H}_2\text{O}} = [\text{OH}^-]_{\text{H}_2\text{O}}$$

$$= x \text{ (say)}$$

$$[\text{H}^+]_{\text{total}} = 1.0 \times 10^{-8} + x$$

But

$$[\text{H}^+] [\text{OH}^-] = 1.0 \times 10^{-14}$$

$$(1.0 \times 10^{-8} + x) (x) = 1.0 \times 10^{-14}$$

$$x^2 + 10^{-8}x - 10^{-14} = 0$$

Solving for x, we get $x = 9.5 \times 10^{-8}$

Therefore,

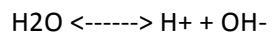
$$[\text{H}^+] = 1.0 \times 10^{-8} + 9.5 \times 10^{-8}$$

$$= 10.5 \times 10^{-8}$$

$$= 1.05 \times 10^{-7}$$

$$\text{pH} = -\log [\text{H}^+] = -\log (1.05 \times 10^{-7}) = 6.98$$

Calculate the pH of a 1×10^{-8} M NaOH(aq) solution? Show work and explain please?



$$[\text{H}^+] = [\text{OH}^-]$$

$$K_w = [\text{H}^+][\text{OH}^-] = 1.0 \times 10^{-14}$$

$$[\text{OH}^-] = 1.0 \times 10^{-7}$$

NaOH is a strong base $\text{NaOH} \Rightarrow \text{Na}^+ + \text{OH}^-$

$$[\text{OH}^-] = 1 \times 10^{-8} \text{ M}$$

$$\text{total concentration OH}^- = 1 \times 10^{-7} + 1 \times 10^{-8} = 1.1 \times 10^{-7} \text{ M}$$

$$\text{pOH} = 6.96$$

$$\text{pH} = 14 - 6.96 = 7.04$$