Exercise 3

MEASURING pH, CALIBRATION OF pH METER AND PREPARATION OF BUFFERS

Structure

3.1 INTRODUCTION

In the previous exercise you have studied about how to prepare solutions with different concentrations. The major aim of this exercise is to learn about how to measure pH, in addition you'll know about calibration of pH meter along with preparation of various buffers. However in the second Unit (water) of course BBCCT-101 you have learnt about what is pH and its biological significance. For better understanding of the current exercise it would be better if you recollect the concepts studied in the unit water.

In your lower classes you might have studied that various parts of our body have different ranges of pH according to their functions (Table: 3.1). Do you know that pH plays a significant role in many biochemical reactions especially in enzyme catalysed reactions (Refer BBCCT-107 enzyme catalysis). It is essential to measure the pH for conducting biochemical experiments. Buffers play an important role in maintaining pH of the body and regulate biochemical reactions. Hence, in this exercise we'll be studying about preparation of buffers and their compositions. There are three major ways to measure pH like using pH paper strips, voltage gated pH meter and digital pH meter. In this exercise we'll be discussing about digital pH meter that is widely used.

Expected Learning Outcomes

After going through this exercise, you should be able to:

- define pH;
- operate pH meter;
- determine the pH of a solution; and
- perform the preparation of different types of buffers.

3.2 MEASURING pH

pH is defined as negative logarithm of H^+ ion concentration. pH meter is a electric device and routinely used in biochemistry laboratory. We all know that pH scale is used to measure concentration of hydrogen (H^+) ions and tells us whether the given solution is acidic, alkaline or neutral in its nature.

Principle: pH meter designed in such a way that, it can measure the effective concentration of H^+ ions in solution. A typical pH meter can measure the potential difference i.e., Electro Motive Force (EMF) which is developed between selective glass electrode and test solution containing H^+ ions. The magnitude of this EMF varies with the varying temperature of the solution. The output potential is in millivolts [mv] and it is recorded glavanometrically or digitally on a scale graduated in pH units. This relationship is defined by the following equation.

$$V = \frac{E_o + 2.303RT}{F} = pH$$

Where, V= Voltage of the completed circuit [observed EMF]

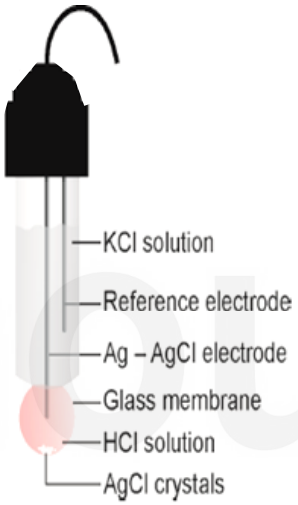
E_0= Potential of reference Electrode

R= the gas constant [8.314 J/mole/^{o}K]

T= the absolute temperature in ^{o}K [25^{o}C= 298 ^{o}K]

F=the Faraday's constant (964846 Coulombs/ equivalent weight or 9.64846 x 10^4 Coulombs mol^{-1}).

(Source: reference 1: Experimental Biochemistry)



A glass electode

The pH meter having combined electrode that is reference electrode and pH sensitive glass electrode connected by potassium chloride bridge present in single glass tube.

Source: Course BCHCL-134 of B.Sc. Gen.

3.2.1 Calibration of pH Meter

So for we have studied the principle behind pH meter. Let us explore how to calibrate pH meter.

Materials:

pH meter, Thermometer, Beakers, Wash bottle, Distilled water, Commercial buffer capsules and Tissue Paper.

Test Sample:

We can use any of the following liquids as test sample: Normal tap water, bottle water, diluted acid or base and Urine sample.

Procedure:

pH meter standardization: Prior to start using the pH meter,immerse the electrode in electrode storage solution provided by the manufacturer or dip in 100 mL of pH 7.0 buffer with 0.5 grams of potassium chloride (KCl)

added. Later connect the electrode to the pH meter. Prepare three different buffer solutions of pH 7.0, pH 4.0 and pH 9.0 that represent neutral, acidic and alkaline pH respectively. For this dissolve respective commercial buffer capsule into100 mL of deionized water separately. Then wash the electrode with deionized water using a wash bottle, later gently wipe the electrode with tissue paper. Now, immerse the electrode in pH 7.0 buffer solution and use a glass rod to stir the buffer with moderate speed. Wait for 1-2 minutes, if pH meter does not display the value of buffer automatically, set the value of buffer manually to pH 7.0 (Fig. 3.1). Remove the electrode from pH 7 buffer solution and wash with deionized water, later wipe the electrode with tissue paper without touching the bottom membrane. Repeat the standardization procedure with already prepared pH 4.0 followed by pH 9.0 buffer solutions. Now the pH meter has been standardized, and is ready for measuring the pH of given/test sample. Do not change any settings before measuring the unknown samples.

Fig. 3.1: pH meter

3.2.2 Measuring pH of a Biological Test Sample

Once you calibrate the pH meter remove the electrode from buffer solution and wash with deionized water and wipe the electrode with tissue paper. Then take the given test solution into a beaker and note the temperature value using a thermomete. Now immerse the electrode into the test solution. Note the values displayed on LED window of the pH meter.

Results: The pH of the given test sample is ..... .

Discussion:

The pH value of a solution indicates the concentration of hydrogen ions in a solution, indicates the relative abundance of H^+ ions in it. Living cells require either acidic or alkaline pH. Enzymes being significant biocatalysts require optimum conditions for their activity. Alterations in the levels of pH and temperature will affect the activity of cells, in gastric acidity the pH value of gastric juice raises above pH 6 (alkaline) many gastric enzymes will work in the range of (pH 1-3). Here it is important to remember that, the pH values are not same in all parts of our body (Table 3.1). Note: pH value depends on the role and functional status of the cell.

Table 3.1: pH values of different biological fluids

Biological Fluid	pH
Urine	6 to 7.6
Serum	7.8
Bile	7.8 to 8.6
Pancreatic Juice	8.0
Saliva	6.3 to 6.5
Blood	7.4
Gastric Juice	1.6 to 3.0

3.3 PREPARATION OF BUFFERS

So far we have discussed about calibration of *p*H meter and measuring *p*H. Before we start discussing on how to prepare a buffer solution, it is better to go through the concept of buffers, *Henderson-Hasselbalch* equation and the biological importance explained in the second Unit (water) of course BBCCT-101. A buffer is defined as a solution (chemical system) that can resist change in *p*H upon the addition of small amounts of acid or alkali. Buffer solution has a significant role in maintaining constant *p*H while performing a biochemical reaction. In general, buffers are mixtures of a conjugate acid and a conjugate base (refer Unit-2 of BBCCT-101). A buffer has its maximum buffering capacity at its pK_a value (refer table 3.2).

Table 3.2: Routinely used buffers and their buffering range

Buffer	pK_a value	Buffering *p*H range
Phosphate	6.86	6.5-7.5
Carboxylic acid		
(a) Acetate	4.76	3.0 - 6.0
(b) Citrate	4.74	
Borate	9.24	8.5 - 10.0
Amino acid & peptide		
(a) Glycine	9.6 (pK_{a2})	2.0 - 3.0 & 9.5 - 10.0
(b) Histidine	6.0 (pK_{a2})	5.5 - 6.0
(c) Glycylglycine	8.4 (pK_{a2})	8.0 – 9.0
*Zwitterionic** *(Good's Buffer)*		
(a) Tris	8.10	7.5 – 9.0
(b) MOPS	7.20	6.5 -7.9
(c) HEPES	7.55	7.0 – 8.0

Tris – Tri (hydroxymethyl) aminomethane; MOPS- 3-(N-morpholino)-propane sulphonic acid; HEPES-N-2-hydroxyethyl piperazine-N'-2-ethane sulphonic acid.(Source: Experimental Biochemistry: A student Companion.BeeduSashidhar Rao and Vijay Deshpande. ISBN 81-88237-41-8, I.K. International Pvt. Ltd.*)

3.3.1 How to Prepare a Buffer Solution

While studying Unit-2 of BBCCT-101 we came to know that, buffer is combination of conjugate acid and its conjugate base. Hence to start preparation of specific buffer solution i1t is advised to prepare stock solutions of respective acid and base salt. Table 3.3 and 3.4 will show how to prepare acetate and carbonate-bicarbonate buffers with diverse range of pH.

Example 1. Preparation of Acetate Buffer

To begin with prepare the stock solution of acid (A) and stock solution of base (B) as following

Acetic acid stock solution A: Prepare 0.2 mol/litre solution of acetic acid (to obtain this take 11.55 mL of acetic acid and makeup to the one litre using distilled water).

Sodium acetate stock solution B: Prepare 0.2 mol/litre solution of sodium acetate (to prepare this dissolve 16.4 grams of sodium acetate in one litre of distilled water).

Now you can mix the respective volumes of these two stock solutions and makeup to 100mL with distilled water as mentioned in the table 3.3.

Table 3.3: Shows the volume of stock solutions and distilled water to be taken to prepare acetate buffer with specific pH range.

pH	Volume of Solution A	Volume of Solution B	Volume of distilled water (mL)	Final volume (mL)
3.6	46.3	3.7	50	100
3.8	44.0	6.0	50	100
4.0	41.0	9.0	50	100
4.2	36.8	13.2	50	100
4.4	30.5	19.5	50	100
4.6	25.5	24.5	50	100
4.8	20.0	30.0	50	100
5.0	14.8	35.2	50	100
5.2	10.5	39.5	50	100
5.4	8.8	41.2	50	100
5.6	4.8	45.2	50	100

Example 2. Preparation of Carbonate-bicarbonate buffer

Sodium carbonate stock solution A: Prepare 0.2 M solution of sodium carbonate (anhydrous) (dissolve 21.2 grams of sodium carbonate in 1000 mL of distilled water).

Sodium bicarbonate stock solution B: Prepare 0.2 M solution of sodium bicarbonate (dissolve 16.8 grams of sodium carbonate in 1000 mL of distilled water).

Now you can mix the respective volumes of these two stock solutions and makeup to 100mL with distilled water as mentioned in the table 3.4.

Table 3.4: Shows the volume of stock solutions and distilled water to be taken to prepare Carbonate-bicarbonate buffer with specific pH range.

pH	Volume of Solution A	Volume of Solution B	Volume of distilled water (ml)	Final volume(ml)
9.2	4.0	46.0	50	100
9.3	7.5	42.5	50	100
9.4	9.5	40.5	50	100
9.5	13.0	37.0	50	100
9.6	16.0	34.0	50	100
9.7	19.5	30.5	50	100
9.8	22.0	28.0	50	100
10.9	25.0	25.0	50	100
10.0	27.5	22.5	50	100
10.1	30.0	20.0	50	100
10.2	33.0	17.0	50	100
10.3	35.5	14.5	50	100
10.4	38.5	11.5	50	100
10.5	40.5	9.5	50	100
10.6	42.5	7.5	50	100
10.7	45.0	5.0	50	100

3.4 PRECAUTION

- Do not touch the lower sensitive bulb of the electrode by naked hand or using any other rough material always use cotton or tissue paper for wiping the electrode.

- Always cover the tip of electrode with storage solution (if not in use for long time).
- Allow all of the buffers to reach the same temperature, since pH readings are temperature dependent.
- Use separate beaker to wash/rinse the electrode.
- Note that, the electrode tip and junction are fully immersed in the buffer, and stir the buffer at a moderate, uniform rate.

Self-Assessment Questions

1. Define pH?
2. Enlist the pH of saliva, urine and blood?
3. What is the importance of buffer solution?
4. Calculate the volume of sodium carbonate solution and sodium bicarbonate solution required to prepare 200 mL of carbonate-bicarbonate buffer with pH9.6. (Refer table 3.4)?

3.5 SUMMARY

- pH is negative logarithm of hydrogen ion concentration.
- Electro Motive Force(EMF) plays a crucial role in measuring the pH.
- Calibration of pH meter is essential before we start using it.
- Buffer is the solution that resists change in pH of a solution upon addition of small amounts of acid or base.
- Buffer solution is a combination of conjugate acid and its conjugate base.
- Cleaning, maintenance and handling of electrode is essential to obtain error free values.

3.6 FURTHER READINGS

1. Experimental Biochemistry: A student Companion. Beedu Sashidhar Rao and Vijay Deshpande. ISBN 81-88237-41-8, I.K. International Pvt. Ltd.
2. Practical Biochemistry: for medical, dental and allied courses. 2nd edition, Dr. G. Rajagopal and Dr.B.D. Toora.ISBN 81-901769-5-1, Ahuja publishing house.
3. Preparative Organic Chemistry CHE-08 (L), Chemistry Lab-III. ISBN 81-7263-333-5, Published by Indira Gandhi National Open University, 1993 (Reprint December-2006).
4. Segel, I.H. Biochemical Calculations. 2nd ed. John Wiley & Sons. Inc. NewYork (1976).
5. Laboratory manual of Microbiology and Biotechnology (second edition), K.R. Aneja. ISBN 978-93-87025-49-3.MEDTECH a division of Scientific international (Pvt. Ltd).