Section I

# Introduction to Practical Biochemistry and pH Meter

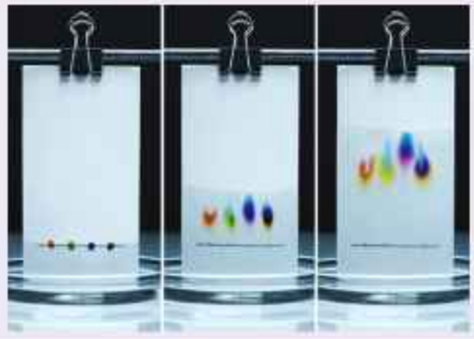
**Competency BC14.1:** Describe commonly used laboratory equipments, good/safe laboratory practice, biomedical hazards and waste management.

## BC14.1 COMMONLY USED EQUIPMENTS AND LABORATORY APPARATUS

| Sl. No. | Name of the instrument | Diagram | Description and uses |
|---|---|---|---|
| EQUIPMENTS | | | |
| 1 | Balances | Double pan chemical balance  | They are used for weighing out substances, and are useful in preparation of qualitative and quantitative reagents. Double pan chemical balance: They are mechanical balances in which chemicals can be weighed from 100 mg to a few grams. |
| | | Single pan electronic balance  | Single pan electric and electronic balances—they are very accurate and can easily measure milligram quantities. Sensitivity can range even in micrograms in highly sophisticated balances. Though easy to operate they are delicate and costly. |

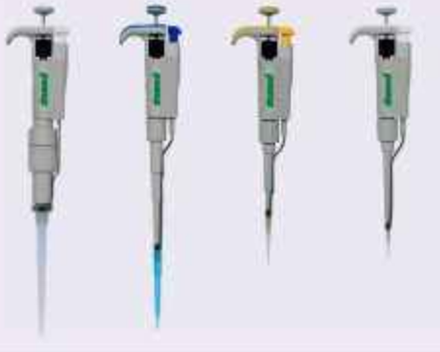
| Sl. No. | Name of the instrument | Diagram | Description and uses |
|---|---|---|---|
| 2 | **Water baths** |  | These are usually steel container fitted with heating device and thermostat to regulate the temperature. They have various kinds of sample containers fitted in them like test tube racks in which the sample can be easily heated or incubated.<br>Serological baths in which the temperature range is low (25–40°C) often used for incubation at 37°C.<br>Boiling water bath in which temperature is maintained at 100°C. |
| 3 | **Hot air oven** |  | Used for drying glassware, chemicals etc.<br>They have high temperature range up to 250°C with thermostat for regulation.<br>They are made up of stainless steel body with heating system and are available in different sizes. |
| 4 | **Centrifuges** | **Table top model**<br>  <br>**Cold/refrigerated centrifuge**<br>  <br>**Ultracentrifuge**<br>  | These are the devices used to separate particulate matter from a liquid suspension using centrifugal force. Centrifuges are designed to accelerate the sedimentation process by using centrifugal force, which acts on a substance in circular motion towards the periphery.<br>**Types of centrifuges**<br>Floor model for table top model: They are simple centrifuges to be kept on the floor or table for convenient use.<br>Cold or refrigerated centrifuge: They have in-built system to provide low temperature during centrifugation.<br>Ultracentrifuge: They have high speed centrifuge with r.p.m in lakhs commonly used to separate very fine particles like cell organelles.<br>**The centrifuge is used in a laboratory for various purposes such as**<br>• Separation of serum or plasma from red blood cells<br>• Separation of sediment in urine<br>• Separation of protein free filtrate<br>• Washing of red blood cells by normal saline<br>• Separation of antigen bound fraction or antibody bound fraction from the free fraction in immunoassays. |

| Sl. No. | Name of the instrument | Diagram | Description and uses |
|---|---|---|---|
| 5 | **pH meter** | **pH meter**<br>  | Measuring pH is a routine activity in a biochemistry laboratory. pH of a solution is measured using pH meter. |
| 6 | **Colorimeters and spectrophotometers** | **Colorimeter**<br>  | These are most commonly used photometric instruments in laboratory and they are indispensable for clinical biochemistry laboratory. Any substance which reacts with specific reagents to give coloured product can be measured by colorimeter and spectrophotometer. |
| | | **Spectrophotometer**<br>  | Semi-automated analyzers: They are automated versions of colorimeters and spectrophotometers. These instruments are typically used for kinetic assays and enzyme based colorimetric tests, which may involve multiple steps and require minimal sample volumes.<br>Fully automated biochemistry analyzers streamline diagnostic testing by performing a wide range of biochemical assays with minimal human intervention. These instruments handle sample processing, reagent management, and data analysis autonomously, ensuring high throughput and precision. They are designed for efficiency, capable of analyzing multiple parameters from a single sample in a short period.<br>With built-in quality control, they deliver reliable results, making them essential in clinical and laboratory settings. |
| 7 | **Electrophoresis apparatus** | **Electrophoresis apparatus**<br>  | It is a separation technique used for the separation of proteins based on the charge.<br>It is used in the diagnosis of multiple myeloma, liver cirrhosis, nephrotic syndrome, etc. |

| Sl. No. | Name of the instrument | Diagram | Description and uses |
|---|---|---|---|
| 8 | **Paper chromatography** | **Paper chromatography**<br>  | It is a separation technique, used for the separation and identification of carbohydrates, proteins, lipids, hormones and growth factors based on the retention factor (Rf) value.<br>Rf = Distance travelled by solute/Distance travelled by solvent front.<br>This technique is used for the screening of aminoacidurias and inborn error of metabolism. |
| 9 | **Glucometer** | **Glucometer**<br>  | It is a point-of-care testing device known as a glucometer. It is used for testing blood glucose levels. This device uses capillary blood for the estimation of glucose.<br>It is mainly used for self-monitoring of blood glucose (SMBG). |
| **LABORATORY APPARATUS**<br>Commonly used apparatus in laboratory is largely glassware and plasticware like tubes, pipettes, syringes and vacutainers, etc. Glassware are made up of complex silicate containing boron dioxide. They are usually resistant to acid, alkali, corrosion, temperature, radiation, etc. They have low coefficient of expansion. | | | |
| **GLASSWARE** | | | |
| 10 | **Beakers** | **Beakers**<br>  | These have capacities from 5 mL to 5000 mL. They are cylindrical glassware usually with the flat bottom and has a spout for pouring and volume marking for approximate measurements. These are used mainly for preparation of solutions. |
| 11 | **Flasks** | **Flasks**<br>  | These have capacities of 25 mL to 5000 mL, different types of plastics used are:<br>• Conical/Erlenmeyer flask: It has flat bottom, a wide body and a narrow cylindrical neck. Used for mixing, heating and storing liquids/ chemicals without risk of spillage. Ideal for titration.<br>• Volumetric flasks: It is pear shaped with flat or round bottom for stability and along, narrow neck for precise volume measurement. |

| Sl. No. | Name of the instrument | Diagram | Description and uses |
|---|---|---|---|
| 12 | Measuring cylinders | Measuring cylinders  | They are available in 10 to 2000 mL capacities. They are used to measure quantity of the liquid. High degree of accuracy is not possible because of their wide bore. |
| 13 | Bottles | Bottles  | • Reagent bottles—used to store certain reagents.<br>• Screw capped bottles—used to store hygroscopic reagents.<br>• Drop bottles—used for delivery of drops of solutions. |
| 14 | Burettes | Burette  | These are used for measuring variable quantities of liquids, performing titrations and also to dispense corrosive reagents. |
| 15 | Condensers | Condenser  | Used in chemical reactions to cool hot gases and condense them into liquid form. They are used in distillation reflux, and other heating processes. |
| 16 | Desiccators | Desiccator  | These are available in various sizes. Chemicals like sulphuric acid, phosphorus pentoxide, calcium chloride and silica gel can be used as desiccants. They are used to desiccate chemicals used for preparation of accurate normal solutions and standards. |

| Sl. No. | Name of the instrument | Diagram | Description and uses |
|---|---|---|---|
| 17 | **Funnels** | **Funnels**<br>  | They are used in the separation of solids from liquids, liquids from liquids and for pouring liquid chemicals for solutions into a container. |
| 18 | **Tubes** | **Test tubes**<br>  <br>**Centrifuge tubes**<br>  <br>**Folin-Wu tube**<br>  | Based on the need of an experiment, different types of tubes are used in a clinical laboratory.<br>**Test tubes:** These are slender, U-shaped cylindrical container with an open top used for holding, mixing and heating small quantities of liquids in the laboratories.<br>**Centrifuge tubes:** These are graduated or plane available in conical shape and are used in centrifugation.<br>**Folin-Wu's tubes:** These tubes are mainly used for determination of blood sugar by Folin-Wu's method. |

| Sl. No. | Name of the instrument | Diagram | Description and uses |
|---|---|---|---|
| 19 | Pipettes | Graduated pipette  | These are used for dispensing controlled quantities of liquids. **Graduated pipettes:** These are available from 0.1 mL to 10 mL capacities. These pipettes are used for quantitative determinations. |
| | | Serological pipette  | **Serological pipettes:** These are graduated pipettes with graduations present till the tip. These are used for separating solutions mainly used to perform serological tests. |
| | | Mohr pipette  | **Mohr pipettes:** These are graduated pipettes but graduations are not present till the tip. They are mainly used to accurately measure and transfer variable volumes of liquids in analytical procedures (titrations). |
| | | Volumetric pipette  | **Volumetric pipettes:** These pipettes are not graduated but designed specifically with the central bulb to deliver specific quantity of the specimen. |
| | | Pasteur pipette  | **Pasteur pipettes:** The drops of a specimen can be conveniently added in a reagent using a Pasteur pipettes. |
| 20 | Plasticware |  | Nowadays, most glassware is being replaced by plasticware. They are usually made of high density polyethylene and Teflon. Advantage is that they are unbreakable and can be used for highly acidic and alkaline solutions. The major disadvantage of plasticware is its tendency to bind various solutes and release them later into solutions. |
| 21 | Micropipettes and auto pipettes |  | Automated pipetting devices are now replacing the conventional mouth pipettes. The advantage with these is their precision and easy use. They are time saving especially where large numbers of samples are to be handled. |

## BC14.1 GOOD LABORATORY SAFETY PRACTICES (GLSP)

Good Laboratory Safety Practices (GLSP) are essential guidelines designed to protect laboratory personnel, equipment, and the environment from potential hazards. They promote a culture of responsibility, awareness, and careful handling of chemicals, biological materials, and equipment. Following GLSP minimizes the risk of accidents, contamination, and occupational illnesses. These practices include proper use of personal protective equipment, correct disposal of waste, and adherence to standard operating procedures. Overall, GLSP ensures a safe, efficient, and productive laboratory environment.

**TABLE 14.1.1:** General safety rules and precautions to be taken in a laboratory

| Category | Precaution | Explanation |
|---|---|---|
| **Personal protective equipment (PPE)** | Wear lab coat, gloves, safety goggles, and closed-toe shoes | Protects against chemical spills, biological hazards, and physical injuries |
| **Laboratory awareness** | Know location of fire extinguisher, safety shower, eyewash station, first aid kit, and emergency exits | Being prepared helps respond quickly in emergencies |
| **Chemical handling** | Read labels and SDS before use; avoid inhaling fumes; never taste chemicals | Ensures safe handling of corrosive, toxic, or flammable substances |
| **Cleanliness and organization** | Keep workspace tidy; clean spills immediately; store materials properly | Reduces contamination and accident risk |
| **Equipment use** | Operate only trained equipment; check for damage; ensure proper grounding | Prevents equipment malfunction, electrical hazards, and injuries |
| **Eating and drinking** | Do not consume food or beverages in the lab | Prevents accidental ingestion of hazardous substances |
| **Waste disposal** | Segregate and dispose of chemical, biological, and sharp waste properly | Protects the environment and prevents contamination |
| **Behaviour and conduct** | Avoid horseplay; stay attentive; follow lab rules | Reduces likelihood of accidents due to carelessness |
| **Labelling and documentation** | Label all samples, reagents, and solutions clearly; maintain accurate records | Prevents confusion, errors, and ensures reproducibility |
| **Accident reporting** | Report all accidents, injuries, or near-misses immediately | Helps manage hazards and prevent recurrence |

**TABLE 14.1.2:** Laboratory safety: Sterilization, disinfection, and first aid procedures

| Category | Procedure | Explanation |
|---|---|---|
| **Sterilization and disinfection of instruments and glassware** | **Boiling:** Immerse items in water and boil for 10 minutes | Kills most bacteria, viruses, and fungi, reducing risk of contamination |
| | **Soaking in disinfectant solution (20 min):** | Ensures thorough disinfection of instruments and surfaces |
| | • Sodium hypochlorite 1% | Effective against bacteria, viruses, and fungi |
| | • Glutaraldehyde 2% | High-level disinfectant; useful for heat-sensitive instruments |
| | • Lysol 2.5% | Suitable for surface and equipment disinfection |
| **First aid—skin burns** | Wash affected area under running water or iced water | Removes heat and prevents further tissue damage |
| | Apply petroleum jelly or appropriate ointment and cover with sterile gauze | Protects burn from infection and promotes healing |
| **First aid—chemical injury to eyes** | Immediately rinse eyes with running water | Dilutes and removes chemical contaminants |
| | Rinse with sterile saline solution | Ensures thorough cleaning and reduces irritation |

(Contd...)

**TABLE 14.1.2:** Laboratory safety: Sterilization, disinfection, and first aid procedures (*Contd...*)

| Category | Procedure | Explanation |
|---|---|---|
| **First aid—accidental swallowing of poisonous reagents** | Spit out substance immediately | Minimizes absorption of toxic material |
| | Rinse mouth with tap water | Helps remove residual chemical |
| | Induce vomiting by drinking warm salt water (if advised) | Expels remaining chemical from the stomach |
| **First aid—accidental swallowing of corrosive acid** | Rinse mouth and throat immediately with plenty of tap water | Dilutes the acid and reduces damage |
| | Take antidotes orally (e.g. 5% soap solution, 8% magnesium hydroxide, egg white mixed with ~500 mL water) | Neutralizes the acid and minimizes tissue injury |
| **First aid—accidental swallowing of alkalis** | Take antidotes orally (e.g. lemon juice, 5% acetic acid) | Neutralizes the alkali and prevents chemical burns |
| **First aid—inhalation of toxic fumes** | Move person to uncontaminated, well-ventilated area | Reduces further exposure and allows breathing of fresh air |
| **General action** | Seek immediate medical help for any chemical exposure, burns, or poisoning | Professional medical attention is critical for proper treatment and recovery |

## BC14.1 HAZARDS IN BIOCHEMISTRY LABORATORY

Laboratory safety necessitates the effective control of all hazards that exists in the laboratory at any given time. The laboratory environment can be a hazardous place to work. The laboratory personnel are exposed to various potential hazards. The hazards could be physical, chemical or infective in nature.

**TABLE 14.1.3:** Laboratory hazards: Physical, chemical, and infection risks

| Type of hazard | Examples | Potential risks |
|---|---|---|
| **Physical hazards** | Electric shock, burns, radiation exposure, cuts from broken glassware or needles | Can cause serious injury, long-term health effects, or fatalities if precautions are not taken |
| **Chemical hazards** | **Acetic acid**—reacts dangerously with chromic acid, nitric acid, hydroxyl compounds, ethylene glycol, peroxides, permanganates | Can produce violent reactions, toxic fumes, or fire |
| | **Acetone**—reacts with sulfuric acid and nitric acid mixtures | Risk of explosion or fire |
| | **Alkali metals** (e.g. sodium, potassium, calcium)—react with water or chlorinated hydrocarbons | Violent reaction, fire, or explosion |
| | **Chlorine**—reacts with ammonia, hydrogen, benzene, and some metals | Produces toxic gases or explosions |
| | **Flammable liquids**—react with chromic acid, hydrogen peroxide, nitric acid, ammonium nitrate, halogens | Highly flammable, fire hazard |
| | **Hydrogen peroxide**—reacts with copper, iron, chromium, and most metals | Can cause violent decomposition or explosion |
| | **Oxalic acid**—reacts with silver and mercury | Toxic fumes and corrosive reaction |
| | **Sodium azide**—reacts with lead, copper, and other metals | Can generate explosive compounds |
| | **Sulfuric acid**—reacts with chlorates, perchlorates, permanganates, and water | Risk of violent reaction, heat release, or explosion |
| **Infection hazards** | Blood, tissue, or other human/animal specimens | Potential exposure to infectious agents such as viral hepatitis, HIV/AIDS, or bacterial infections |

## BC14.1 BIOMEDICAL WASTE DISPOSAL

### Definition

Biomedical waste (BMW) is the waste, which is generated during the diagnosis, treatment or immunization of human beings or animals or in research activities. It includes all the waste generated from health care facility which can have any adverse effect to the health of a person or to the environment in general if not disposed properly. The international symbol for biological hazard is given in figure.

To avoid the risk of biological hazard, safe handling and disposal of these infected materials is required. It is recommended that waste should be segregated at the point of generation and disposed in bags with correct colour coding with biohazard symbol.

Biohazard symbol

### Steps of BMW Management

The various steps involved in the management of biomedical waste management are as follows:

**TABLE 14.1.4:** Steps of biomedical waste management

| Step | Description |
|---|---|
| 1. Collection | Waste should be gathered immediately at the point where it is generated by trained healthcare staff to maintain safety and accountability. |
| 2. Segregation | At the source, waste must be separated into **colour-coded, non-chlorinated containers or bags** according to guidelines, which helps reduce infection risk and aids in safe disposal. |
| 3. Pre-treatment | Laboratory waste and materials with high infection potential should be disinfected using **1% sodium hypochlorite (or equivalent)** with a minimum contact time of **30 minutes** before further handling. |
| 4. Transportation and storage | Properly segregated waste should be moved using **covered trolleys or containers** to a central storage area, which serves as a **temporary holding zone** before disposal. |
| 5. Treatment and disposal | Waste is finally processed in an authorized facility through methods such as **autoclaving, microwaving, shredding, or other approved technologies,** ensuring it becomes non-infectious and safe for the environment. |

**Segregation of BMW and its disposal** should be done as per BMW management and handling rules 2016 with amendments in 2018 as shown in Table 14.1.5.

**TABLE 14.1.5:** Biomedical waste categories, types, and treatment options

| Category | Colour code and container | Type of waste | Treatment and disposal option |
|---|---|---|---|
| Yellow  | Yellow non-chlorinated bag or container | **Human anatomical waste:** Human tissues, organs, body parts. | Incineration or plasma pyrolysis or deep burial |
| | | **Animal anatomical waste:** Experimental animals' tissues, organs. | Incineration or plasma pyrolysis |
| | | **Soiled waste:** Items contaminated with blood or body fluids (e.g. cotton swabs, dressings). | Incineration or plasma pyrolysis or deep burial |
| | | **Expired/discarded medicines:** Cytotoxic drugs, items contaminated by them, and antibiotics. | **Cytotoxic:** Return to manufacturer for incineration/encapsulation/plasma pyrolysis. **Other drugs:** Incineration. |
| | | **Chemical waste** (solid) | Incineration or encapsulation or plasma pyrolysis |
| | Separate collection system leading to effluent treatment | **Chemical liquid waste:** Liquid from labs, floor washings, etc. | Pre-treat before mixing with other wastewater. Sludge from treatment is sent for incineration. |
| | Yellow non-chlorinated bag or suitable packing | **Contaminated linen:** Mattresses, beddings, etc. | Non-chlorinated chemical disinfection followed by incineration or plasma pyrolysis |
| | Autoclave-safe plastic bags or containers | **Microbiology/biotechnology waste:** Lab cultures, stocks, specimens of microorganisms, blood bags. | Pre-treat to sterilize with non-chlorinated chemicals on-site, then send for incineration. |
| Red  | Red non-chlorinated plastic bags or containers | **Contaminated recyclable waste:** Tubings, gloves, syringes (without needles), IV sets, etc. | Autoclaving or microwaving/ hydroclaving followed by shredding or mutilation. Not for landfill. |
| White  | Puncture-proof, leak-proof, tamper-proof containers | **Waste sharps including metals:** Needles, scalpels, blades, etc. | Autoclaving or dry heat sterilization followed by shredding, mutilation, or encapsulation. |
| Blue  | Cardboard boxes with blue coloured marking | **Glassware:** Broken/discarded contaminated glass (e.g. medicine vials, ampoules). Excludes cytotoxic waste and Metallic body implants. | Disinfection (detergent wash followed by sodium hypochlorite soak) or autoclaving/microwaving/ hydroclaving, then sent for recycling. |

## Reader's Notes

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**Competency BC14.2 and BC14.8:** Describe the estimation of pH by pH meter or ABG analyser and interpretation of results with paper case scenarios.

## BC14.2A PREPARATION OF BUFFERS

### Introduction to Buffers

Buffers are special solutions that resist changes in their pH when small amounts of acid or alkali are added to them. This property is essential for maintaining a stable chemical environment in many biological and chemical processes.

### Types and Composition of Buffers

Buffers are typically mixtures of:

a. **Acidic buffers:** A weak acid and its salt with a strong base (e.g. acetic acid + sodium acetate).

b. **Alkaline (basic) buffers:** A weak base and its salt with a strong acid (e.g. ammonium hydroxide + ammonium chloride).

#### Common Examples:

| Acidic Buffers | Alkaline buffers |
|---|---|
| Succinate buffer | Phosphate buffer |
| Acetate buffer | Bicarbonate buffer |
| Citrate buffer | |

### Principle of Buffer Action

The pH of a buffer solution is governed by the **Henderson-Hasselbalch equation**: **For a weak acid (HA) and its salt ($A^-$):**

$$pH = pKa + \log_{10}([Salt] / [Acid])$$

Where:

**pKa** is the acid dissociation constant (a property of the weak acid).

**[Salt]** is the concentration of the salt.

**[Acid]** is the concentration of the weak acid.

This equation shows that the pH of a buffer depends on the pKa of the weak acid and the **ratio of the concentration of the salt to the acid**. By varying this ratio, buffers of different pH values can be prepared from the same pair of chemicals.

**Buffer capacity** is the magnitude of a buffer's resistance to pH change. It is greatest when the [Salt]/[Acid] ratio is 1 (i.e. they are present in equal concentrations) and is directly proportional to the total concentration of the buffering agents.

### Preparation of Common Buffers

#### a. Acidic buffers

| | |
|---|---|
| **Succinate buffer (pH ~4.0)** | • Dissolve 11.8 g of succinic acid and 100 mg of sodium azide (preservative) in 800 mL distilled water.<br>• Adjust the pH to 4.0 by adding approximately 40–0 mL of 1N NaOH solution.<br>• Make the final volume up to 1000 mL with distilled water. |
| **Acetate buffer (0.2M)** | • **Solution A (0.2M acetic acid):** Dilute 1.15 mL of glacial acetic acid to 100 mL with distilled water.<br>• **Solution B (0.2M sodium acetate):** Dissolve 0.64 g of anhydrous sodium acetate (or 2.72 g of sodium acetate trihydrate) in 100 mL distilled water.<br>• To prepare 100 mL of acetate buffer, mix 36.2 mL of solution B and 14.8 mL of glacial acetic acid. Make up to 100 mL with distilled water. Measure the final pH with a pH meter. |
| **Citrate buffer** | • Dissolve 42 g of citric acid in approximately 376 mL of 0.1N NaOH.<br>• Dilute the mixture to 1000 mL with distilled water. |

### b. Alkaline buffers

| | |
|---|---|
| **Phosphate buffer (pH 7.4)** | • **Solution A (1/15 M $KH_2PO_4$):** Dissolve 9.078 g of $KH_2PO_4$ in 1000 mL distilled water.<br>• **Solution B (1/15 M $Na_2HPO_4$):** Dissolve 11.879 g of $Na_2HPO_4$ in 1000 mL distilled water.<br>• Mix **840 mL of solution B** with **160 mL of solution A**. |
| **Bicarbonate buffer (pH 9.9)** | • Dissolve 636 mg of anhydrous $Na_2CO_3$ and 336 mg of anhydrous $NaHCO_3$ in distilled water.<br>• Make the final volume up to 100 mL. |

### Preparation of Phosphate Buffer Solutions of pH 5.8 and pH 7.2.

*Materials and Reagents Required*

- 1/15 M disodium hydrogen phosphate ($Na_2HPO_4$)
- 1/15 M potassium dihydrogen phosphate ($KH_2PO_4$)
- 0.1 M acetic acid
- 0.1% sodium carbonate
- pH papers/pH meter
- Universal indicator
- Methyl red indicator
- Phenol red indicator

### Procedure for Preparing Phosphate Buffers

1. Take two clean, dry test tubes and label them **A** and **B**.
2. Using separate graduated pipettes, add the reagents as specified in the table below:

| Reagent | Tube A (for pH 5.8) | Tube B (for pH 7.2) |
|---|---|---|
| **1/15 M $Na_2HPO_4$** | 2.0 mL | 14.4 mL |
| **1/15 M $KH_2PO_4$** | 23.0 mL | 5.6 mL |

3. Mix the contents of each tube thoroughly by stirring or gentle swirling. The buffer solutions are now ready.
4. Verify the pH of each solution using a pH meter or precise pH paper.

### Applications of Buffers

Buffers are critically important in:

1. Various **qualitative and quantitative analytical estimations** in biochemistry.
2. Maintaining the correct pH for **enzymatic reactions** and **microbial cell culture**.
3. **Electrophoretic techniques** for the separation of biological molecules like proteins, lipoproteins, and hemoglobin.
4. Calibration of pH meters.
5. Functioning of biological systems (e.g. bicarbonate buffer in blood).

## BC14.2B ESTIMATION OF pH USING pH METER

### Aim

To understand the principle and procedure of measuring the pH of a solution using a pH meter.

### Introduction

The pH scale is a numerical representation of the acidity or alkalinity of a solution, defined as the negative logarithm of the hydrogen ion concentration: $\mathbf{pH = -\log[H^+]}$. This scale typically ranges from 0 (very acidic) to 14 (very alkaline), with 7 being neutral. While indicators and pH papers provide an approximate value, a pH meter is the most accurate and reliable instrument for determining pH.

### Principle

The pH meter operates on the principle of **potentiometry**. It measures the electromotive force (e.m.f.) generated by an electrochemical cell, which consists of two electrodes dipped into the test solution:

1. **A reference electrode** (e.g. calomel electrode): Maintains a constant, stable potential.
2. **A measuring electrode** (glass electrode): Develops a potential that varies with the $H^+$ ion concentration of the test solution.

The thin glass bulb at the tip of the glass electrode is permeable only to $H^+$ ions. When immersed in a solution, a potential difference develops across this bulb due to the difference in $H^+$ ion concentration between the internal solution (usually 0.1 M HCl) and the external test solution. This potential difference is measured by the pH meter and converted into a direct pH reading.

## Requirements

- pH meter with glass and reference electrodes (often a combined electrode)
- Standard buffer solutions (pH 4.0, 7.0, and 9.2)
- Beakers (100 mL)
- Distilled water wash bottle
- Soft tissue paper
- Test solution (e.g. phosphate buffer)
- 0.1N HCl and 0.1N NaOH (for adjustment)

## Procedure

1. **Initialization:** Turn on the pH meter and allow it to warm up for 15 minutes. Ensure all solutions are at room temperature and set the temperature compensation knob accordingly.
2. **Zero check:** Set the function switch to 'STANDBY'. Adjust the meter needle to zero if using an analog model (digital meters often auto-calibrate).
3. **Calibration with first buffer:**
   - Rinse the electrodes with distilled water and gently blot dry.
   - Immerse them in a beaker containing standard pH 4.0 buffer.
   - Switch the function from 'STANDBY' to 'READ'.
   - Adjust the 'CALIBRATION' control knob until the display reads exactly pH 4.0.
   - Switch back to 'STANDBY' and remove the electrodes.
4. **Calibration with second buffer (verification):**
   - Rinse and dry the electrodes as before.
   - Immerse them in a beaker of standard pH 7.0 buffer.
   - Switch to 'READ'. The reading should be close to pH 7.0. If not, repeat the calibration process.
5. **Measurement of test solution:**
   - Rinse and dry the electrodes thoroughly.
   - Immerse them in the beaker containing the test solution (e.g. prepared phosphate buffer).
   - Switch to 'READ', wait for the reading to stabilize, and note the pH value.
6. **Adjustment (if required):** If the measured pH is not the desired value, adjust it dropwise using 0.1N NaOH (to increase pH) or 0.1N HCl (to decrease pH), stirring continuously. Re-measure until the target pH is achieved.
7. **Shutdown:** After use, rinse the electrodes with distilled water, switch to 'STANDBY', turn off the meter, and store the electrodes as per manufacturer's instructions (often in a storage solution or pH 4.0 buffer).

## Observations

- pH of the standard buffer solution (pH 4.0): _____
- pH of the standard buffer solution (pH 7.0): _____
- pH of the test solution (phosphate buffer): _____

## Result

The pH of the given solution is _____

**Note:** ABG analyzer is given in BC14.18

## QUESTIONS

**1. Define buffers and acids.**

- **Acids:** Acids are substances that can donate a proton (a hydrogen ion, $H^+$) when dissolved in a solution. The strength of an acid is determined by how readily it donates this proton. Strong acids (like hydrochloric acid, HCl) dissociate completely, while weak acids (like carbonic acid, $H_2CO_3$) dissociate only partially.
- **Buffers:** A buffer is a chemical system that resists significant changes in the pH of a solution when small amounts of acid or base (alkali) are added to it. It acts as a protective mechanism against pH fluctuation. A buffer typically consists of a weak acid and its conjugate base (e.g. $H_2CO_3/HCO_3^-$) or a weak base and its conjugate acid. They work by "soaking up" excess $H^+$ ions or donating $H^+$ ions to neutralize added base.

**2. Define blood buffers.**

The blood contains several efficient buffer systems that work simultaneously to maintain a stable pH. The most important are:

- **Bicarbonate buffer system:** This is the most significant **extracellular** buffer. It consists of carbonic acid ($H_2CO_3$) and bicarbonate ions ($HCO_3^-$). It is crucial because both components are regulated by the lungs (which exhale $CO_2$) and the kidneys (which excrete or reabsorb $HCO_3^-$).
  - Reaction: $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$
- **Phosphate buffer system:** ($NaH_2PO_4/Na_2HPO_4$) is an effective buffer in the blood, but its low concentration makes it less important than the bicarbonate system in extracellular fluid. It is, however, a major buffer in urine and intracellular fluid.
- **Protein buffer systems:** Proteins, particularly hemoglobin in red blood cells and plasma proteins, are excellent buffers.
  - **Hemoglobin:** The most important **intracellular** buffer. It binds to $H^+$ ions generated from the conversion of $CO_2$ to bicarbonate in the blood, playing a direct role in the chloride shift. Deoxygenated hemoglobin is a better buffer than oxygenated hemoglobin.
  - **Plasma proteins:** Albumin and other plasma proteins can act as buffers by accepting or releasing $H^+$ ions.

**3. Define urinary buffers.**

The kidneys regulate pH by excreting excess acid or base in the urine. The urine's pH can vary widely (from 4.5 to 8.0), so powerful buffers are needed to prevent damage to the urinary tract and to allow for the excretion of large amounts of acid.

- **Phosphate buffer system:** This is the primary urinary buffer. The monohydrogen phosphate ion ($HPO_4^{2-}$) acts as a base to "trap" $H^+$ ions, forming dihydrogen phosphate ($H_2PO_4^-$), which is then excreted in the urine.
- **Ammonia buffer system:** This is the most important adaptive mechanism for excreting large acid loads. Renal tubule cells generate ammonia ($NH_3$) by metabolizing the amino acid glutamine. $NH_3$ diffuses into the urine, where it binds with $H^+$ to form ammonium ion ($NH_4^+$). $NH_4^+$ is unable to diffuse back into the cells and is excreted, effectively removing $H^+$ from the body.

**4. What is the normal pH of blood?**

The normal pH of arterial blood is tightly maintained within a narrow range of **7.35 to 7.45**. A pH below 7.35 is called acidosis, and a pH above 7.45 is called alkalosis. Even slight deviations outside this range can have severe consequences for cellular function and can be life-threatening.

**5. Define renal regulation of pH of blood.**

The kidneys are the body's long-term regulators of blood pH, acting over hours to days. They perform three key functions:

1. **Reabsorption of bicarbonate ($HCO_3^-$):** The kidneys filter huge amounts of $HCO_3^-$ into the urine. To prevent its loss, the renal tubules must reabsorb almost all of it. They do this by secreting $H^+$ ions into the tubular fluid. These $H^+$ ions combine with filtered $HCO_3^-$ to form $CO_2$ and water, which are then reabsorbed, effectively reclaiming the bicarbonate.
2. **Excretion of hydrogen ions ($H^+$):** The kidneys excrete the non-volatile acids produced by metabolism (e.g. sulfuric acid, phosphoric acid). This is achieved by secreting $H^+$ ions into the urine, where they are buffered by phosphate and ammonia systems and excreted.
3. **Generation of new bicarbonate:** When the body needs to generate new $HCO_3^-$ to replenish its buffering capacity, the kidneys excrete $H^+$ ions buffered by ammonia ($NH_3$). For every $H^+$ ion excreted as $NH_4^+$, a new $HCO_3^-$ ion is generated and added back to the blood.

**6. Define chloride shift.**

The chloride shift (also known as the Hamburger phenomenon) is an ionic exchange process that occurs in red blood cells (RBCs) in tissues and lungs to maintain electrical neutrality.

- **In tissues (peripheral capillaries):**
  1. $CO_2$ diffuses from tissue cells into the blood and then into RBCs.
  2. Inside RBCs, the enzyme **carbonic anhydrase** rapidly converts $CO_2$ and $H_2O$ into carbonic acid ($H_2CO_3$), which dissociates into $H^+$ and $HCO_2^-$.
  3. The $HCO_3^-$ ions diffuse **out** of the RBC into the plasma.
  4. To balance the loss of this negative ion (anion), **chloride ions ($Cl^-$)** from the plasma move **into** the RBC. This inward movement of $Cl^-$ is the "chloride shift."
  5. The $H^+$ ions are buffered by hemoglobin.
- **In lungs (pulmonary capillaries):**
  1. The process reverses. $CO_2$ diffuses out of the blood into the alveoli to be exhaled.
  2. $HCO_3^-$ moves **back into** the RBCs.
  3. $Cl^-$ moves **out of** the RBCs into the plasma (the reverse chloride shift).
  4. Inside the RBC, $HCO_3^-$ and $H^+$ recombine to form $CO_2$ and $H_2O$, and $CO_2$ is exhaled.

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