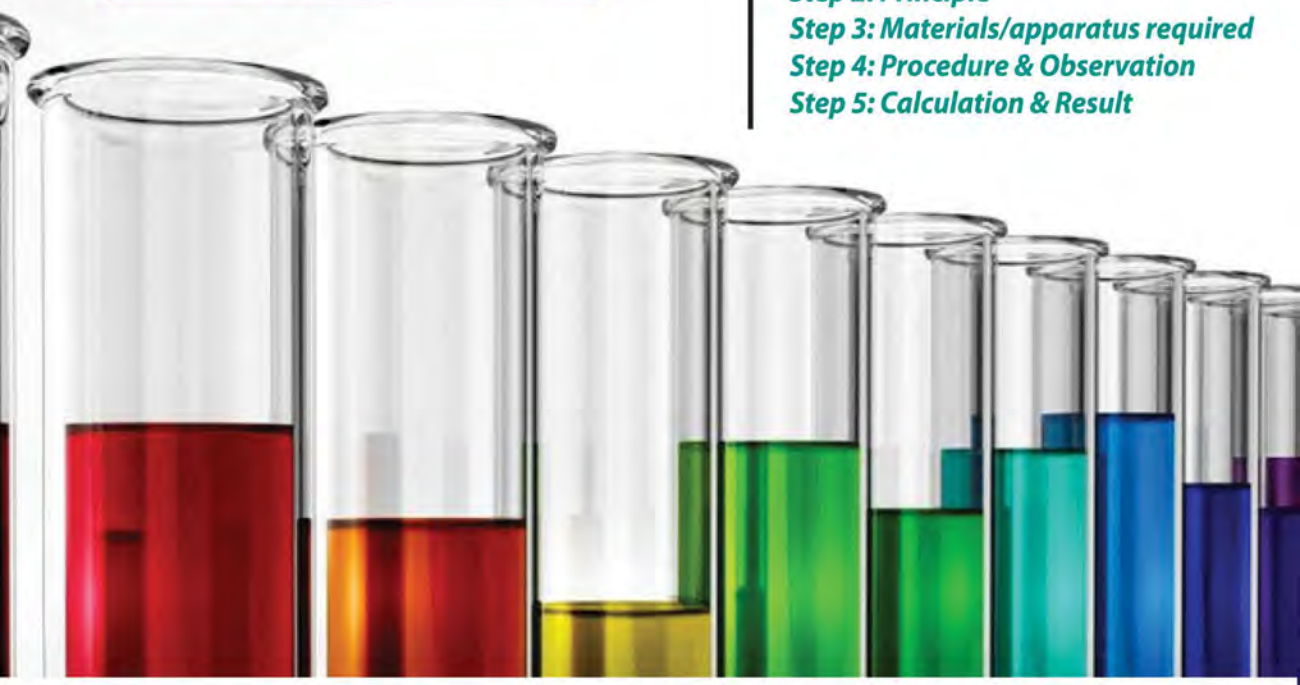
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Essentials of Practical Biochemistry

Exercises with interpretations
Model papers for revision
Methodological presentation of exercises

Easy to Perform and Easy to Learn
Just follow
Step 1: Introduction
Step 2: Principle
Step 3: Materials/apparatus required
Step 4: Procedure & Observation
Step 5: Calculation & Result



Prem Prakash Gupta
Neelu Gupta

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Commonly Used Glassware, Apparatus and Equipment

Chapter 2

In an undergraduate biochemistry laboratory, various glassware, apparatus and equipment are required to perform different practical exercises including both qualitative and quantitative exercises. The laboratory glassware like beakers, volumetric flasks, reagent bottles, test tubes, centrifuge tubes, measuring cylinders, conical flasks, glass pipettes, funnels, burettes, etc. are normally made of borosilicate glass [**ingredients:** silica (80.6%), boric acid (12.6%), sodium oxide (4.15%) and aluminum oxide (2.2%)]. You will find the uses of following glassware, apparatus and equipment in various exercises described in your practical manuals.



Fig. 2.1: Beaker

Beakers: A beaker is a container used for stirring, mixing, holding, transferring, and heating liquids/solutions in laboratories. Beakers are generally cylindrical in shape with a flat bottom and have a small beak that helps in pouring/transferring the liquids in other containers (*See* Fig. 2.1). Beakers are available in a wide range of volumes from 10 ml to several liters, e.g. 50, 100, 150, 200, 250, 500, 1000, 2000 ml, etc. Beakers may be graduated or nongraduated. Beakers are made of glass, plastic or related substances.



Fig. 2.2: Volumetric flask

Volumetric flasks: A volumetric flask (also known as **measuring flask** or **graduated flask**) is a kind of flask, **calibrated to contain accurate volume** of liquids/solutions (*See* Fig. 2.2). These flasks are usually pear-shaped, with a flat bottom and made up of borosilicate glass. A volumetric flask has a tall, narrow neck with a stopper closing the opening at top (*See* Fig. 2.2). There is a circular line on the neck to show how much to fill the flask for accurate measurement of volume. Volumetric flasks are used for doing precise dilutions and for preparation of standard solutions to be used in titration and quantitative estimation exercises. Volumetric flasks are generally colorless but may be amber-colored for containing solutions of photosensitive substances. The volumetric flasks are available in capacities of 25–5000 ml. Other flasks are **conical (Erlenmeyer) flask, flat bottom round flask and round bottom flask**.

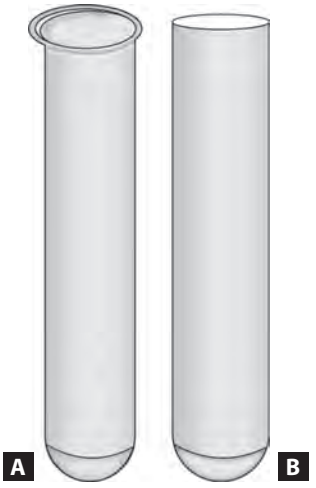
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Reagent bottles: Reagent bottles are used to store reagents, solutions and chemicals and body fluid like urine (24 hour collection). Reagent bottles of various volumes/capacities are available, e.g. 100, 250, 500, 1000, 2000 ml, etc. (*See* Fig. 2.3). They may be **graduated or non-graduated** and are generally colorless but may be amber-colored or red-colored for storing photosensitive reagents, solutions and chemicals (e.g. silver nitrate, chloroform, nifedipine, sodium nitroprusside, etc.). Reagent bottles are usually made up of borosilicate glass, but they may also be made up of plastic or related substances. They may be screw-capped or are fitted with glass stoppers (*See* Fig. 2.3). The caps may be made up of metal or plastic. **Drop bottles** are used for delivery of drops of solutions, such as stains. They have capacities of 50–100 ml and are mainly used in Microbiology and Pathology labs.



Fig. 2.3: Reagent bottles

Test tubes: Test tubes (with or without rim) are very common laboratory glassware that are cylindrical in shape with a rounded U-shaped bottom (*See* Figs 2.4). Test tubes of different sizes (lengths and widths) are available; typically from 10 to 18 mm wide and 75 to 150 mm long. In biochemistry laboratory, they are used to carry out simple chemical/biochemical reactions. A large test tube specifically used for the purpose of boiling of liquids/solutions is known as **boiling tube.** Other tubes are **centrifuge tubes, Folin-Wu's tubes and digestion tubes**. Folin-Wu's tubes are used for blood sugar estimation by Folin-Wu's method. All types of tubes of good quality are usually made up of borosilicate glass.



Figs 2.4 (A and B): Test tubes: A. Test tube with rim; B. Test tube without rim

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Fig. 2.5: Test tube stand

Test tube stand: The test tube stands are used to keep tubes at the work places. Both acrylic and metallic test tube stands are available in the market (*See* Fig. 2.5).



Fig. 2.6: Test tube/bottle brush

Test tube and Bottle brush: A test tube/bottle brush is a long handled device with rows of radial acrylic/plastic bristles or filaments (*See* Fig. 2.6). These brushes are used for cleaning of test tubes, centrifuge tubes, reagent bottles and other glassware.

Test tube holder: While performing a chemical test, a test tube holder is used to hold the test tube and to keep it away from us in a secure position without being held by hand (*See* Fig. 2.7).



Fig. 2.7: Test tube holder

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Centrifuge tubes: A test tube with a tapered conical bottom is called centrifuge tube (*See* Fig. 2.8). These tubes are available in various sizes/capacities and are made of glass/plastic. Microcentrifuge tubes are available with capacities from 250 μl to 2.0 ml, however, normal centrifuge tubes are 15 mm in width and 115 mm in length (*See* Fig. 2.8). Centrifuge tubes are used to separate substances of greater and lesser density with the help of centrifuge machine.

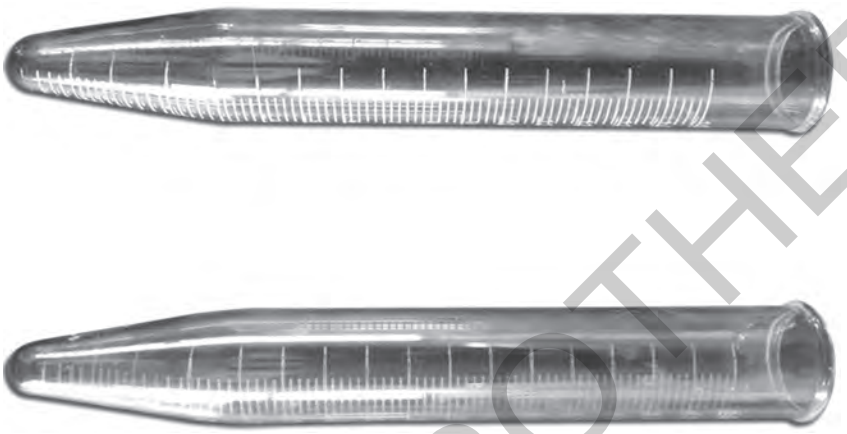


Fig. 2.8: Centrifuge tubes

Centrifuge machine: A centrifuge machine is electric equipment, driven by an electric motor to put the object in rotation around a fixed axis in order to generate centrifugal force (*See* Fig. 2.9). Application of centrifugal force causes sedimentation of denser substances from a liquid suspension. Under the influence centrifugal force, the lighter substances tend to move to the top of the centrifuge tube and thus the substances of greater and lesser density are separated.



Fig. 2.9: Centrifuge machine

Centrifuge machines are used for separation of cell organelles, blood plasma, lipoproteins, etc. Centrifuge machines are also used to sediment precipitates, urine sediments or any undissolved substance from a liquid suspension, e.g. for complete sedimentation of protein precipitate during deproteinization of blood. Commonly used tabletop laboratory centrifuge machines have speed ranging from few hundred to ten thousand r.p.m., whereas the ultracentrifuge machine has a speed of about 1,00,000 r.p.m.

Measuring cylinders: A **graduated cylinder**, also known as measuring cylinder, is used for accurate measurement of the volume of a liquid and for transferring it into another

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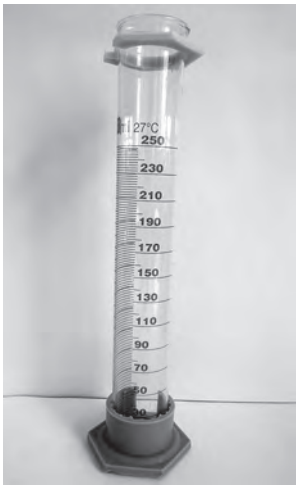


Fig. 2.10: Measuring cylinder

container (*See* Fig. 2.10). Graduated cylinders are generally more precise for this purpose than graduated beakers or conical flasks. Measuring cylinders of various volumes/capacities are available varying from 10 to 2000 ml. They are usually made of glass, but they may also be made up of plastic.

Conical flasks: A conical flask, also known as **Erlenmeyer flask** (named after the German chemist **E Erlenmeyer**), is a glassware of conical shape with narrow cylindrical neck and flat bottom (*See* Fig. 2.11). Conical flasks are used for stirring of solutions/liquids. The narrow neck of conical flasks keeps the contents from spilling out; because of this reason the conical flasks are also used for titration (*See* Fig. 2.18). Conical flasks of various volumes/capacities are available varying from 25 to 2000 ml. They may be **graduated or non-graduated** and with or without glass-stopper (*See* Fig. 2.11). Conical flasks are usually made up of borosilicate glass.



Fig. 2.11: Conical flasks

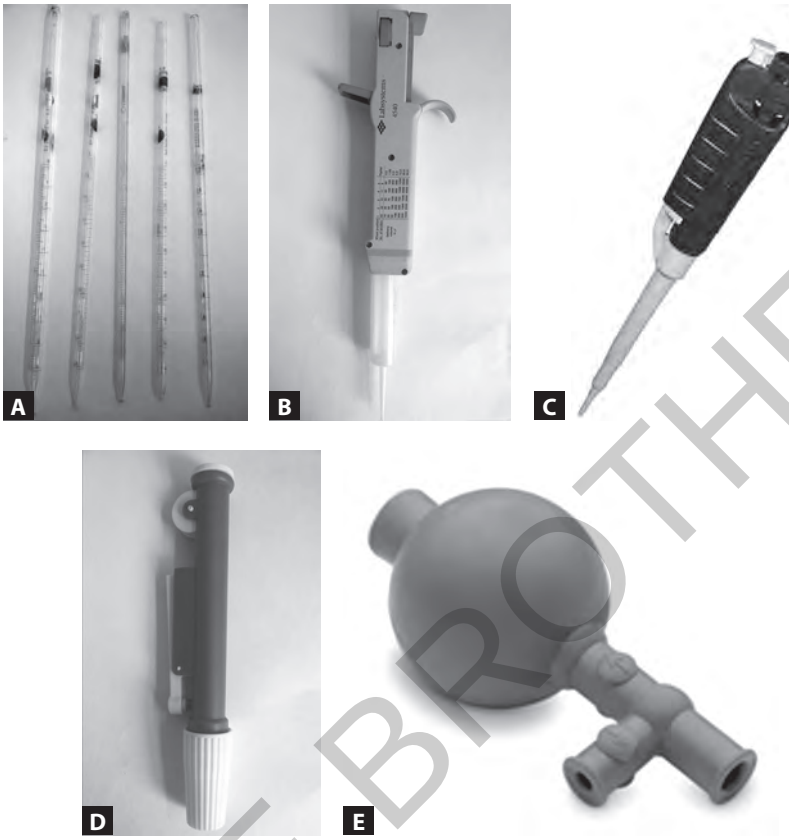


Fig. 2.12: Thermometer

Laboratory thermometers: A thermometer is a device that measures the temperature (*See* Fig. 2.12). The name thermometer has been derived from two words: 'thermo' means heat and 'meter' means to measure. The **thermometer measures temperature in Fahrenheit, Celsius and another scale called Kelvin.** In biochemistry lab, we normally use the Celsius scale, which is also called the 'centigrade' scale. The Celsius scale is named after **Anders Celsius. Clinical thermometers** are used for measuring body temperature.

Pipettes: A **pipette** (also called **pipet**) is a laboratory device which is used to aspirate and transfer an accurate and desired volume of liquid (solution/reagent/chemical) from one container to other (*See* Figs 2.13A, B and C). Pipettes exist in several forms (designs) as **glass pipettes,**

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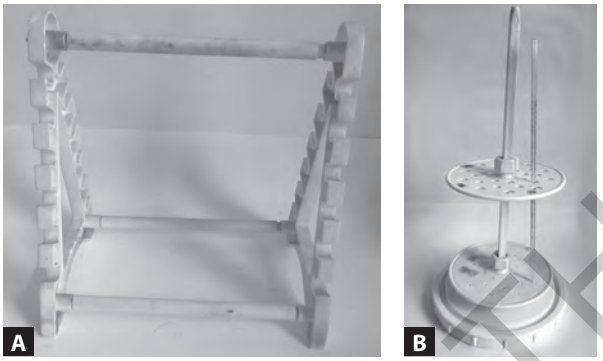


Figs 2.13 (A to E): A. Glass pipettes; B and C. Auto-pipette; D. Pipette pump; E. Rubber-bulb

auto-pipettes/push-button pipettes (adjustable or fixed volume pipettes), pipetting-syringes, etc. Auto-pipettes/push-button pipettes operate by piston-driven air displacement, whereas glass pipettes are operated by mouth-pipetting. Glass pipettes are broadly of two types—**graduated glass pipettes** and **volumetric glass pipettes**. Graduated glass pipettes (*See* Fig. 2.13A) are graduated and are available from 0.1 to 10 ml capacities and are of two types—**serological glass pipettes** and **Mohar glass pipettes**. The serological glass pipettes are graduated up to tip, whereas the Mohar glass pipettes are graduated above the tip so that even if the pipette-tip is broken, the pipettes can be used. The volumetric glass pipettes are not graduated, but are **fixed-volume pipettes**. They are designed specifically with a central bulb to deliver a fixed volume (e.g. 10 ml and 25 ml). These pipettes are also known as **bulb-pipette** and are used for titration. Since mouth pipetting with graduated glass pipettes is considered unsafe due to the possibility of accidentally ingesting or inhaling toxic chemicals, **rubber-bulbs** and **pipette-pumps** (*See* Figs 2.13D and E) are attached to the graduated glass pipettes for easy and safe aspiration and dispensing. The mouth pipetting with volumetric glass pipettes is considered safe. **Auto-pipettes/push-button pipettes (adjustable or fixed volume pipettes)** operate by piston-driven air displacement (*See* Figs 2.13B and C). These pipettes (also known as micropipettes) dispense between 5 and 1000 µl and are used in quantitative estimation exercises.

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Glass pipette stands: These stands are used to keep glass pipettes in upright or horizontal position. While working, glass pipettes are kept horizontally in pipette stands (*See* Fig. 2.14A), whereas the glass pipettes are air dried by placing them in upright position in vertical pipette stands (*See* Fig. 2.14B). A variety of acrylic pipette stands are available in the market.



Figs 2.14 (A and B): Glass pipette stands: A. Glass pipette stand (horizontal); B. Glass pipette stand (vertical)



Fig. 2.15: Funnel

Funnels: Funnels, made up of glass, stainless steel, aluminum or plastic, are used in transferring liquids from one container to other (*See* Fig. 2.15). Otherwise, without a funnel, spillage would occur. Commonly used funnels have diameter of 50–100 mm. There are different kinds of funnels like filter funnel, thistle funnel and dropping funnel.

Stopwatch: A stopwatch is a mechanical device used to measure the amount of time elapsed from a particular time. It is used when a biochemical reaction is to be run for a specific time period (*See* Fig. 2.16).



Fig. 2.16: Stopwatch

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Wash bottles: A wash bottle, mostly made up of polyethylene, is a squeeze bottle with a nozzle (*See* Fig. 2.17) and is used to keep glass distilled or deionized water for the purpose of rinsing laboratory glassware like test tubes, beakers, funnel, etc. For its use, the wash bottle is pressed by hands so that the water present inside the wash bottle is forced to come out of the nozzle into a narrow stream of liquid. Wash bottles of various volumes/capacities are available varying from 100 to 1000 ml.



Fig. 2.17: Wash bottle

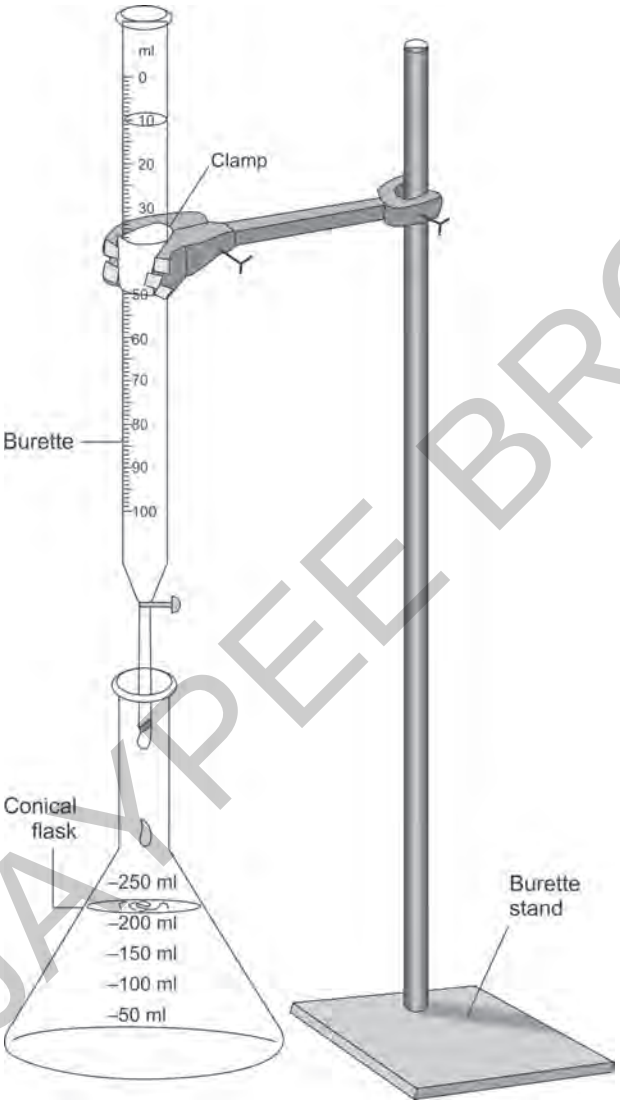


Fig. 2.18: Burette and burette stand

Burette: Burette, laboratory glass-ware with a volumetric graduation, is used to dispense known volume of a solution or liquid with accuracy in volumetric analysis. Burettes are most often used for titration, to deliver one reactant in titrating mixture until the precise end point of the reaction is obtained. Burettes of various volumes/capacities are available varying from 10 to 100 ml (*See* Fig. 2.18). Burettes are also used to dispense corrosive reagents.

Burette stand: A burette stand with clamp is used in chemical laboratory to hold burette while it is being used (*See* Fig. 2.18).

Glass droppers and Glass/ Plastic/Metal spatulas: Glass-droppers are used to transfer and add small volume of liquids/solutions/ reagents drop by drop. They are glass tubes tapered to a narrow point and fitted with a soft rubber bulb at the top. Plastic droppers are also available. Glass/plastic/metal spatulas are used to transfer solid chemicals/substances. Spatulas are used in biochemistry labs like spoons in kitchen.

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Tripod stand and Wire gauge: A tripod stand is a metallic stand with three legs and is often used in chemistry lab, as a platform to hold various glassware like conical flasks, beakers, etc. during experiment especially during heating. A wire gauge is also kept on tripod stand before heating the glassware. The wire gauge is kept on tripod stand to ensure the even distribution heat in the bottom of glassware (*See* Fig. 2.19).

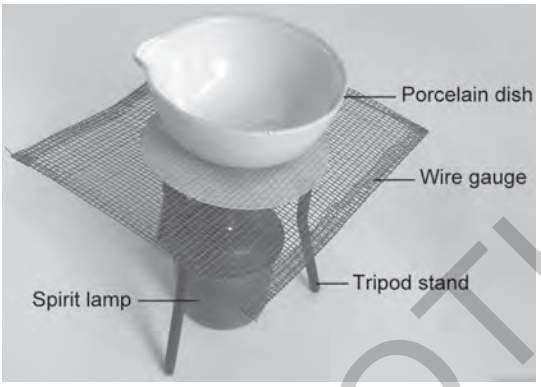


Fig. 2.19: Tripod stand and wire gauge



Fig. 2.20: Spirit lamp

Bunsen burner and Spirit lamp: Gas burner or **oil burner** is a device that burns a gas or liquid fuel into a flame in a controlled manner. **Spirit lamp** (*See* Fig. 2.20) is a device that burns alcohol/methylated spirit as a fuel into a small flame. Spirit lamp is generally used in biochemistry laboratory for mild heating, whereas Bunsen burners are used for intensive heating.

Stirring glass-rods: A stirring glass-rod or **stir glass-rod** is a piece of solid glass-rod (about 15–30 cm long and 5 mm in diameter) and is used to mix or stirrer reagents/solutions/liquids in a lab. Using a stir glass-rod, the stirring or mixing is done by hands.

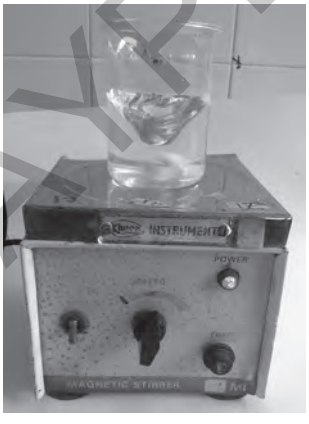


Fig. 2.21: Magnetic stirrer

Magnetic stirrer: A magnetic stirrer or **magnetic mixer** is an electrical device that rotates a magnet to cause a stir bar (also known as 'flea') immersed in a liquid to spin, in a controlled manner, for the purpose of stirring or mixing (*See* Fig. 2.21). The stir bars are typically iron pieces coated in teflon, or less often in glass. A magnetic stirrer is used for quick and prolong stirring or mixing. By using **hot-plate magnetic-stirrer device**, heating can also be done to speed up mixing/dissolution process; because at high temperature, solutes are easily dissolved in solvents. Heating elements may range from 120 W or lower to 500 W or more. A **cyclo-mixture**, an electrically driven device, is used for mixing/stirring of samples in test tubes, without shaking or inverting them.

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Porcelain dishes: Porcelain dishes have fully glazed interior surfaces and can withstand temperatures up to about 1000 °C. Porcelain dishes are used in chemical laboratory for the purpose of heating, evaporation of solutions and drying at high temperature (up to about 1000 °C) (*See* Figs 2.22 and 2.19).



Fig. 2.22: Porcelain dish

Desiccators: Hygroscopic chemicals need to be kept under moist-free environment. Such chemicals are kept in desiccators. Calcium chloride, silica gel, phosphorus pentoxide, copper sulfate, etc. can be used as desiccants. Desiccators of variable sizes are available in market (*See* Fig. 2.23).

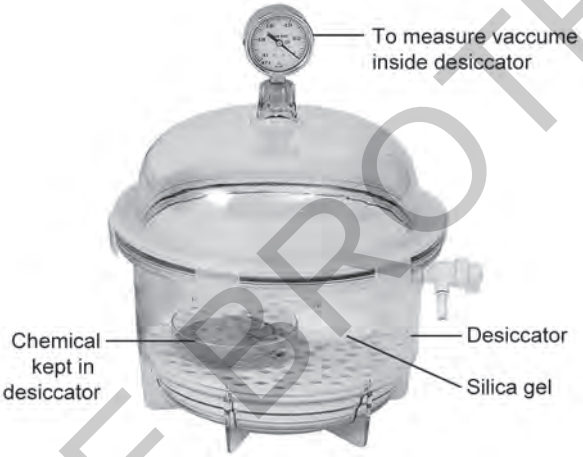


Fig. 2.23: Desiccator

Water-bath: A hot water-bath (*See* Fig. 2.24) is an apparatus, which is used to carry out a chemical/biochemical reaction at a constant desired temperature, e.g. enzyme assays are normally carried out at 37 °C. In a water-bath, the vessels containing the assay mixture are heated by heating the water that surrounds the vessels. A thermostat immersed in the water precisely controls the heating of water in water-bath. Temperature between 37 to 100 °C can be maintained in water-bath. Water-baths provided with test tube racks and shaking system are also available in the market.

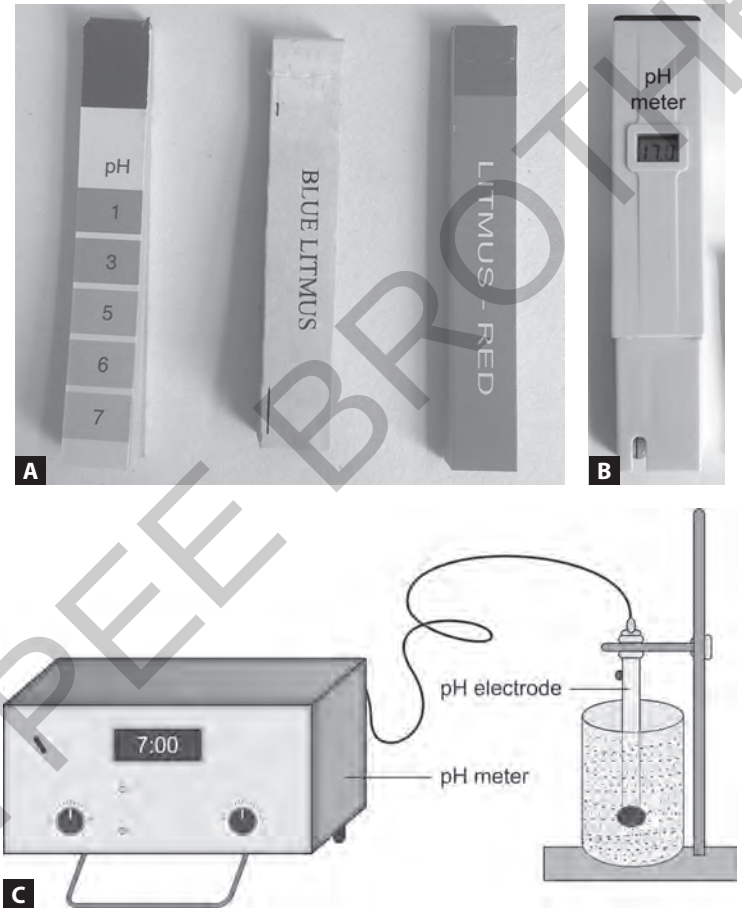


Fig. 2.24: Water-bath

Litmus paper, pH paper strip and pH meter: Litmus paper (*See* Fig. 2.25A) is used to find out whether a test material is acidic or basic/alkaline. Blue litmus paper turns red in acidic condition, whereas the red litmus paper turns blue in basic/alkaline condition. Litmus is a water-soluble mixture of different dyes extracted from lichens, especially ***Roccella tinctoria***. Litmus can also be prepared as an aqueous solution that functions similarly; the test solution

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turns red in acidic condition and the test solution turns blue in basic/alkaline condition. The approximate pH (from 1 to 14) of a test solution or body fluid like saliva, urine, blood, etc. can be determined by placing a drop of the test solution/body fluid on a piece of pH paper strip (*See* Fig. 2.25A). Depending on pH of the test material, the pH paper strip provides excellent color separation in both the acidic range (< pH 7) and alkaline range (> pH 7), e.g. a pH paper strip gives light orange, light green and violet color at pH 5, 8 and 13, respectively. For accurate measurement of pH of a test solution, electronic **pH-meters** are used (*See* Figs 2.25B and C). **Various indicators** like methyl orange (red at pH 3.0 and yellow at pH 4.4), phenolphthalein (colorless at pH 7.0, pink at pH > 8.3 and red at pH 10), methyl red (red at pH 4.4 and yellow at pH 6.0), etc. are also used to find out approximate pH.



Figs 2.25 (A to C): A. Litmus paper and pH paper strip; B and C. pH meter

Weighing balances: A weighing balance (also known as **weighing machine**) is an instrument used for measuring the weight or mass of an object/material/chemical substance (*See* Fig. 2.26). Double-pan **chemical balance** and single-pan (mono-pan) **electronic analytical balance** are used for accurate/precise measurement of weight from few milligrams to few grams. Since single-pan electronic analytical balance is more easy to use, electronic balance is preferred over

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chemical balance for accurate measurement of weight of chemical substance in a biochemistry laboratory. Single-pan electronic analytical balances up to a least measurement of 0.0001 g are available in the market. Electronic analytical balances are more costly than chemical balances. For gross measurement of weight, **single-pan spring balances** or traditional **double-pan mechanical balances** (provided with standard weights) are used (*See* Fig. 2.26).

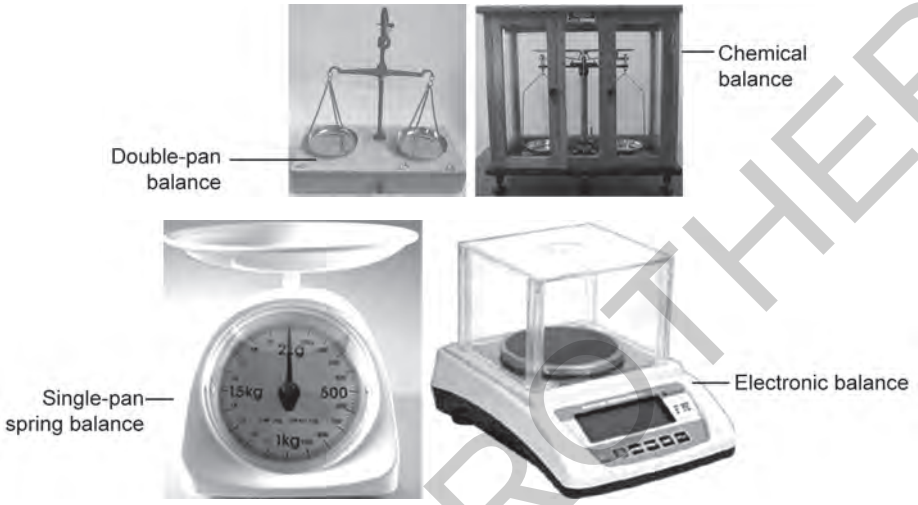


Fig. 2.26: Weighing balances

Laboratory ovens: Electric oven (hot-air oven) is a thermally insulated steel chamber used for the heating and drying of glassware, syringes, needles, etc. In an electric oven (*See* Fig. 2.27), electricity is needed as fuel to generate heat. In electric ovens, hot plates and thermostat are used to produce heat (temperature ranging from 40 to 340 degree Celsius) and to control temperature, respectively. Electric ovens are used in chemical laboratories. In wood-burning ovens, gas ovens and coal-burning ovens, wood, gas and coal are respectively used as fuel to generate heat.



Fig. 2.27: Laboratory oven

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Essentials of
Practical Biochemistry

The present textbook on Essentials of Practical Biochemistry has been prepared to provide systematic information on various practical exercise a prescribed at undergraduate level. This textbook is also useful for postgraduate students and includes practical exercises on qualitative tests for carbohydrates, proteins, amino acids, lipids and non-protein nitrogenous substances, qualitative and quantitative analysis of urine, and of various body fluids, quantitative estimation of various serum/plasma metabolites like glucose, cholesterol, urea, creatinine, triglycerides, electrolytes, uric acid, albumin, etc. estimation of various serum diagnostic enzymes, organ function tests and biochemical techniques. For quick and easy understanding, each practical exercise starts with introduction and finishes in several headings like principle, materials/apparatus required, procedure, observations, calculation and result. In each practical exercise, blank space has been provided for students to write down their own observations, readings and calculations then and there in the laboratory itself. Therefore, present textbook can also be used as a practical manual as well while working in laboratory.



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