

# Exercise 21

**Aim:** To detect the presence of proteins.

**Principle:** Proteins respond to some colour reactions due to the presence of one or more radicals or groups of the complex protein molecule. All proteins do not contain the same amino acids, and hence they do not respond to all colour reactions. Nitrogen atoms in the peptide chain form a complex (violet colour) with copper ions in the Biuret test. (Biuret test is for peptide bond in the molecule of a protein.) Xanthoproteic test is specific for protein containing aromatic amino acids. The benzene ring in the amino acids is nitrated by heating with nitric acid and forms yellow nitro-compounds which turns to orange colour with alkali.

**Requirement:** Glasswares: test tube, spirit lamp; Chemicals: 40% NaOH, 1% CuSO<sub>4</sub> solution, Concentrated HNO<sub>3</sub>, 20% NaOH solution; Miscellaneous: test tube holder, test tube stand.

## Procedure

### (a) Biuret test

- Take 2 mL of protein solution (milk, albumin of egg or gram seed extract) in a test tube.
- Add 1 mL of 40% NaOH solution and 1 or 2 drops of 1% CuSO<sub>4</sub> solution.
- A violet colour indicates the presence of proteins. Care must be taken that excess of copper sulphate is not added otherwise there will be blue colour instead of violet colour.

### (b) Xanthoproteic test

- Add carefully 1 mL of concentrated HNO<sub>3</sub> to 2 mL of protein solution (albumin of egg, milk or gram seed extract).
- A white precipitate is formed.
- Boil the solution and the colour changes to yellow.
- Cool the test tube and add 2 mL of 20% NaOH (or ammonia solution) to make it alkaline.
- The colour changes to orange indicating the presence of proteins.

## Discussion

A yellow stain is often observed on skin when it comes in contact with nitric acid. The reason of yellow stain is xanthoproteic reaction.

## Questions

1. Why does the skin turn yellow when it inadvertently comes in contact with  $\text{HNO}_3$ ?
2. Why are only few drops of  $\text{CuSO}_4$  solution added during the biuret test?

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# Exercise 22

**Aim:** To detect the presence of fats (lipid) in different plants and animal materials.

**Principle:** Lipids (fats) are usually insoluble in water but soluble in organic solvent. Sudan III reagent gives a specific red colour with fats.

**Requirement:** Glassware; test tubes; Chemicals: Sudan III, ethyl alcohol, chloroform, ether, benzene; Miscellaneous: test tube holder, test tube stand, oil/ghee/butter.

## Procedure

### (a) Solubility test

- Arrange five dry test tubes in a test tube stand.
- Add 2 mL of water, ether, chloroform, benzene and ethyl alcohol to each test tube followed by one drop of mustard oil, ghee or butter.
- Shake thoroughly.
- Oil, ghee or butter is broken into small droplets in water which float at the surface indicating that fat is insoluble in water. But in acetone, benzene and ethyl alcohol no such droplets are formed as the oil, ghee or butter dissolves in these organic solvents.

### (b) Sudan III test

- Take 2mL of mustard oil in a test tube.
- Add equal volume of water to it and shake.
- Add a pinch of sudan III and shake again.
- As the layers separate out, the lipid layer is seen to be stained red, whereas water layer remains uncoloured. This indicates the presence of lipids.

## Questions

1. What is the simplest form of fat?
2. Will fat dissolved in organic solvent give a positive result with sudan III?

# Exercise 23

**Aim:** Separation of plant pigments (chloroplast pigments) by paper chromatography.

**Principle:** The chloroplasts contain photosynthetic pigments - Chlorophyll a, Chlorophyll b, Carotenes and Xanthophylls. Pigments absorb solar radiation at different wavelengths of the visible spectrum for photosynthesis. These pigments differ in their chemistry, and hence in their physicochemical properties, such as molecular weight, solubility in the solvent etc.

Paper chromatography is a popular technique widely used for separating various chlorophyll pigments from a mixture. In chromatography, pigments move to different distances, depending on their solubility in the solvent system, on a fine quality cellulose paper (Whatmann No.1 chromatography paper). Movement of pigments on the chromatography paper is governed by the principle of adsorption and capillary action. The solvent system components vary in density and thus move at different rates due to wick action through the chromatography paper. Lighter components move faster than the heavier components. Differential solubility of pigments in the solvent system and the differential rates of mobility of solvent system components is used for separation of pigments.

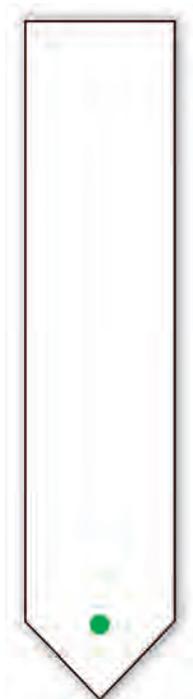
**Requirement:** Mucilage-free leaves of locally available herbaceous plants, test-tubes (6"x1"), boiling tube with split rubber cork, chromatography chamber or wide mouth test tube, 100 ml and 10 ml measuring cylinders, 50 mL beaker, capillary tube, mortar and pestle, acetone, petroleum ether, muslin cloth, sand, scissors, pins, Whatmann No. 1 filter paper, ordinary blotting sheet.

## Procedure

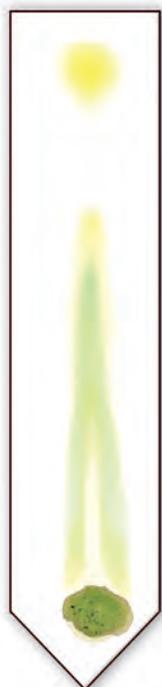
### Preparation of pigment extract

- Select tender, herbaceous, mucilage free leaves for the experiment. Remove the petiole and mid rib from 20-30 mature leaves.
- Cut the leaves into smaller bits and transfer them into a mortar. Add a pinch of sand, a few mL of acetone and grind thoroughly until the leaf extract becomes dark green in colour.
- Filter extract through a muslin cloth and collect the filtrate in 50 ml beaker.
- Prepare 9:1 solution of petroleum ether and acetone. Transfer 3-5 ml of this mixture into the chromatography chamber or wide mouthed test tube. Close the chamber or the test-tube with the split cork tightly as the solvents are volatile and keep it in standing position.

## Exercise 23



**Fig. 23.1** Loading of pigment extract



**Fig. 23.3** A chromatogram of chlorophyll

- Cut a strip of Whatmann No. 1 chromatography paper (6" X 1/2"), which can easily fit into the chamber or the test-tube.
- Using scissors make one end of the chromatography paper into a triangular shape with a pointed end.
- Using the capillary tube, load the pigment extract on the lower side of the chromatography paper in the spot (labelled S in Fig. 23.1) till the spot is dark green in colour. Make sure that the diameter of the loaded spot is not more than 3-5mm. Dry the spot by blowing warm air on it. Never use ink/ ball pen for marking on chromatography paper.
- Suspend the loaded chromatography paper into the boiling tube with the help of a split rubber cork or in chromatography chamber (Fig. 23.2) or wide mouth test tube.
- Make sure that the loaded spot is clearly above the solvent.
- Keep the apparatus straight and leave the set up in a test tube stand undisturbed for 40 minutes. Note the rise of solvent and pigments.
- Remove the chromatography paper after the four different coloured pigments appear.
- Cover the chromatogram with black paper to prevent photo oxidation.



**Fig. 23.2** Experimental setup of the chromatography

### Observation

Identify the pigments on the basis of colours. Sequentially from the bottom, the four colours visible are: greenish yellow (chlorophyll b); bluish green - (chlorophyll a), yellow (carotene) and orange (xanthophyll) (Fig. 23.3).

### Discussion

The four major photosynthetic pigments have been separated by chromatography and identified. This process of separation is called monodimensional ascending chromatography since the solution and pigments move upwards in one direction only.

## Questions

1. Which photosynthetic pigment moves farthest and why?
2. Leaves in general appear green although they possess yellow and orange pigments. Comment.
3. Why do we add sand while preparing the pigment extract?
4. Among the two solvent system components in chromatography, which moves farthest and why?

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# Exercise 24

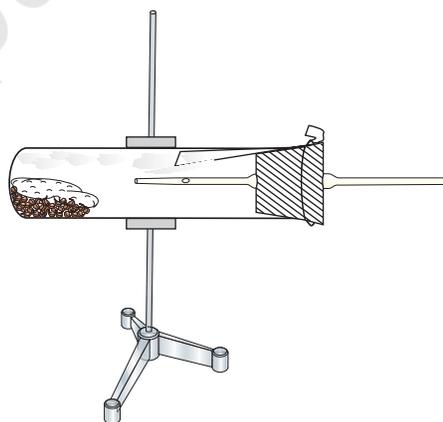
**Aim:** To study the rate of respiration in flower buds/germinating seeds.

**Principle:** Respiration is a vital process in living organisms and generates energy through break down of food materials in presence/absence of  $O_2$ . The released energy is used for all life processes. Rate of respiration depends on internal and external factors (age, physiological status and type of cell, temperature, and availability of oxygen).

**Requirement:** Flower buds/germinating seeds, boiling tube, single bore rubber cork fitted with a pipette, cotton, stand with burette clamp, KOH solution, black paper and filter paper.

## Procedure

- Take about 10-15 buds or 10-15g germinating seeds in a boiling tube or wide mouth test-tube. Introduce a wad of cotton. Dip a 2 × 1cm strip of filter paper in KOH solution and place it in the tube ensuring that it does not touch the cotton swab or seeds.
- Dip the tip of the pipette in water and slowly suck-in water in such a manner that a small air bubble is trapped in it. Now insert the attached rubber cork (along with pipette) into the tube as shown in Fig. 24.1. The test tube should be fixed in horizontal position with burette clamp.
- Note the position of air bubble in the pipette.
- Record the distance travelled by the bubble at 2 minute intervals for a period of time.
- Now shift the set up to bright sunlight (outside the laboratory). After a few minutes, note the distance travelled by the bubble at 2 minute intervals for the same period of time.



**Fig. 24.1** Experimental setup for the study of rate of respiration

## Observation

Compare the two sets of values obtained in the experiments. It is likely that in the experiment conducted in bright sunlight the bubble moves much faster indicating higher rate of respiration. One of the factors that is responsible for increase in rate of respiration is temperature. Can you think of a reason?

## Discussion

Notice that the rates of respiration are not same in different materials and under different conditions. Respiration is an enzymatic process where food materials are broken down to release energy. Light and temperature affect the process. Young meristematic cells show high rate of respiration.

## Questions

1. What is the role of KOH strip used in the experiment?
2. Why does the air bubble move in the pipette towards the boiling tube?
3. What will happen if the setup is not air tight?