

Ex. No. 4: Separation of Photosynthetic Pigments by Paper Chromatography.

Aim: To separate photosynthetic pigments by paper chromatography.

Principle: The separation of photosynthetic pigments is based on partition takes place between the static phase adsorbed to the cellulose matter of the paper and mobile phase.

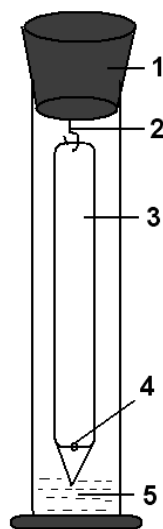
Materials Required: Mulberry leaves, pestle and mortar, 80% acetone, calcium carbonate, Buchner filter, beaker, measuring cylinder, glass jar/test tube with a tight cork, Whatmann No.1 filter paper, petroleum ether, acetone, hook, micropipette.

Procedure:

1. Take 50 g of fresh leaves in a pestle and mortar. Crush them with 20 ml of 80% acetone. Add a pinch of calcium carbonate and again crush.
2. Filter the extract on a Buchner filter or with double layered muslin cloth. The deep green coloured filtrate containing chlorophylls and carotenoids is obtained. Evaporate the extract to concentrate.
3. Take a glass jar (about 45cm high) with a tight cork fitted in it. The cork should have a hole in the centre to fix the hook.
4. Now prepare the solvent by mixing 25 ml petroleum ether and 3 ml acetone. Pour the solvent into the jar and allow the jar to become saturated.
5. Cut a strip of filter paper of the size which can easily be hung on the hook. Apply a circular spot of pigment extract about 3cm from the base of strip with the help of a micropipette. Now hang the strip inside the jar to the hook of cork and close the cork. Care should be taken that the spot is not dipped in the solvent. Make the apparatus air tight and observe.

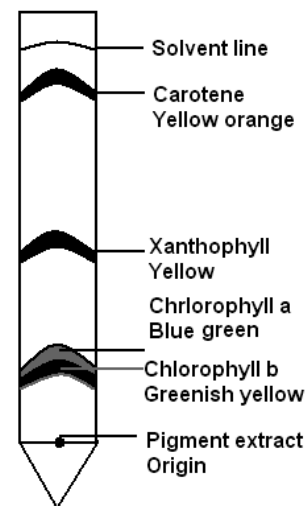
Observation:

The solvent will run on the filter paper. After few hours, the chloroplast pigments will be separated in the form of different spots on the paper. Take out the paper when the solvent reaches up to the upper level. After drying the paper, identify the different pigments with the help of their specific colours. Carotene is yellow, Xanthophyll is yellow-brown, Chlorophyll-a is blue green and Chlorophyll-b is olive green in colour.



Experimental set up during running

1. Cork to minimize evaporation of solvent
2. Paper clip hook to hold the paper
3. Filter paper, 4. Spot of pigment, 5. Solvent



Chromatogram