Aim 35

To Separate the Anthocyanin Pigment by Paper Chromatography

Introduction

Angiospermic flowers are characterized by varied colours of different kinds. The pigments responsible for the colours of angiospermic flowers are present in petals particularly. The most important pigments in floral colouration are known as flavonoids pigments belonging to one major group of flavonoids are called anthocyanins which are major determination of flower colour. Most red, purple and blue plant pigments found in angiospermic flowers are due to angiospermic.

Angiospermic are water soluble and colour of anthocyanins pigments depends upon the acidity of the cell rap. There are several different type of anthocyanins. All anthocyanins have a basic structure of anthocyanidin but they differ from one another in the attachment of sugar, primarily at 3-OH position. Anthocyanin absorbs strongly between 475 and 560 nm. However, different anthocyanin have different absorption spectra and can be separated from one another with chromatographic methods.

Requirements

Red rose petals, Pestle mortar, Whatman's paper no.- 4, funnel, centrifuge, water bath. *Chemicals*:- H_2SO_4, methanol, lead acetate, ammonia solution. *Glassware*:-Beakers, measuring cylinder, micropipette, dryer.

Preparation of Running solvent:-

Running solvent was prepared by mixing n-butanol : Acetic acid : H_2O in the ratio of 4:1:5, in a separating funnel. After

one hour, the upper layer was taken and lower layer was discarded. The upper layer was used as running solvent.

Reagents

Acidic methanol:- Mix 99.5 ml of mixing 99.5 ml methyl alcohol with 0.5 ml Conc. H_2SO_4 acid. *Ammonium Solution:-* Make this solution 50% by mixing it with equal volume of distilled water.

Lead acetate solution:- A pinch of lead acetate was dissolved in 10 ml of distilled water.

Procedure

1. 2 g of rose petals were weighted and grinded in 25 ml of acidic methanol made by
2. After grinding extract was filtered using muslin cloth and filtrate was retained.
3. Take the filtrate and add diluted ammonia solution drop wise until its pH becomes 9.
4. Then, add saturated lead acetate solution drop by drop which leads to the formation of green precipitates (add lead acetate solution till complete precipitates takes place).
5. After complete precipitation, precipitates were filtered out using filter paper.
6. The precipitate was scrapped out and then dissolved in 25 ml of acidic methanol which gives red colour solution.
7. This solution was again filtered and filtrate was taken discarding the residence.
8. The filtrate was then concentrated to 1/3 of total volume on water bath.

Chromatogram

1. A chromatogram of Whatman's no. 4 was cut of size 28 cm and 5-6 cm width (dimensions as per running chamber).

2. At a distance of 2.5 cm, a line was marked and at this line the pigment was loaded 4-6 time using a micropipette.
3. Dry the loading in cool current of air.
4. After loading, the chromatogram was kept in running solvent.
5. After few hours of running, chromatogram was dried out and Rf value was calculated.

Results

The Rf values of the bands were calculated by using formula:

$$Rf = \frac{\text{Distance travelled by solute from the loading point}}{\text{Distance travelled by solvent from the loading point}}$$

Discussion

Chromatographic technique is an important application in separating mixture of solutes. The pigments loaded on chromatographic paper on interaction between mobile and stationary phase as well as difference in solubility of sample leads to development of characteristic bands. Moreover, the solubility faster the migration of compound.

In the given experiment conducted 3-4 bands are seen on chromatogram which were labeled an A,B,C for ray reference.

Precautions

1. During running of solvent, the strip should not touch the ride of base of running chamber.
2. Over running of solvent should be avoided.
3. Loading should be done on minimal area.