

Separate the Coloured Components Present In the Given Grass/Flower By Ascending Paper Chromatography & Determine Their R_f Values

Apparatus

Gas jar, glass rod, filter paper strip (What man No. 1 filter paper), jar cover, fine capillary tube.

Requirement

A mixture of red and blue inks, alcohol and distilled water.

Procedure

1. Take a What man filter paper strip (20 x 2 cm) and draw a line with pencil above 3 cm from one end. Draw another line lengthwise from the centre of the paper as shown in Fig.

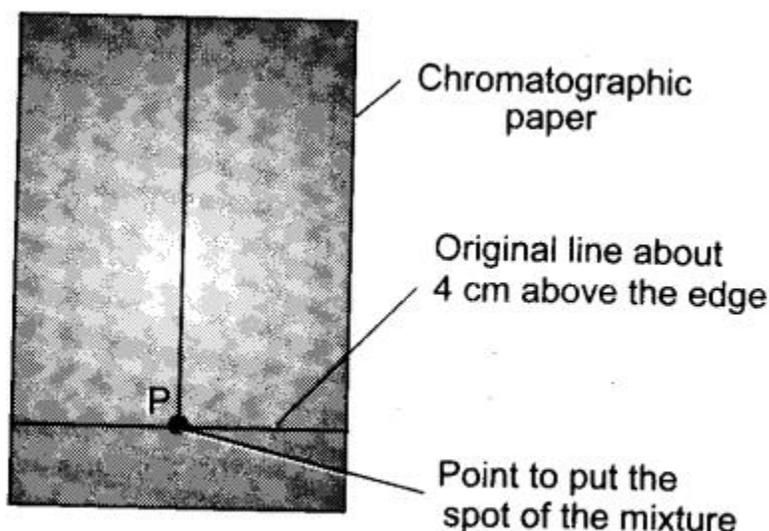


Fig. Spotting of the mixture.

2. With the help of fine capillary tube, put a drop of the mixture of red and blue inks at the point P. Let it dry in air. Put another drop on the same spot and dry again. Repeat 2-3 times, so that the spot is rich in mixture.
3. Suspend the filter paper vertically in a gas jar containing the solvent (eluent) with the help of a glass rod in such a way that the pencil line (and the spot) remains about 2 cm above the solvent level (50% alcohol + distilled water).

- Cover the jar and keep it undisturbed. Notice the rising solvent along with the mixture of red and blue inks. After the solvent has risen about 15 cm you will notice two different spots of blue and red colours on the filter paper.
- Take the filter paper out of the jar and mark the distance that the solvent has risen on the paper with a pencil. This is called the solvent front.
- Dry the paper. Put pencil marks in the centre of the blue and red spots.
- Measure the distance of the two spots from the original line and the distance of the solvent from the original line.
- Calculate the R_f values of the blue and red inks by using the formula :

$$R_f = \frac{\text{Distance travelled by the blue or red ink from the point of application}}{\text{Distance travelled by the solvent from the original line}}$$

Observations and Calculations

<i>Colour of the spot</i>	<i>Distance travelled by the spot from the original line</i>	<i>Distance travelled by the solvent from the original line</i>	<i>R_f Values</i>
Green (Chlorophyll)	A cm	X cm	A/X
Yellow Xanthophyll)	B cm	X cm	B/X
Red (Carotene)	C cm	X cm	C/X

Note: In the above experiment, crush fresh flowers or grass in a mortar and extract the juice with acetone. Use this juice for making the spot.

Precautions

- Use good quality pencil for drawing the reference line so that the mark does not dissolve in the solvent in which the chromatography is carried out.
- Always make use of a fine capillary tube.
- Keep the jar undisturbed and covered during the experiment.
- A spot should be small and rich in mixture.
- Allow the spot to dry before putting the strip in the jar.
- Keep the strip erect. Do not let it to be curled.
- Do not allow the spot to dip in the solvent.