

Chemistry of Our Environment – Lab

PAPER CHROMATOGRAPHY

Introduction:

At the turn of the 20th century a Russian botanist named Tswett developed a process which separated the components of a green plant extract by color. He named this process chromatography, which, in Greek, means color writing. (Kromos = color; graphin = writing) Tswett's discovery has led to a multi-billion dollar per year industry and has provided a method for the isolation and identification of components in mixtures. Perhaps the most important application has been in the study of complex biological processes.

Procedure:

1. Fill a chromatography tank to a depth of 3 cm with the 0.1% NaCl solution provided.
2. Using a square of chromatography paper 24 x 24 cm in size, mark a **pencil** line 1 cm from the bottom of the paper across the sheet. Mark a similar line 2 cm from the bottom of the paper. Leaving 2 cm on each edge of the paper place pencil dots at 2 cm intervals across the second line.
3. Choose one of the standard dyes provided and place a small spot on the first dot made in the previous step. Try to keep this spot as small as possible. Label the name of this dye in **pencil** at the top of the sheet.
4. Repeat this step for each of the remaining standard dyes. In addition, spot any unknown sample or samples provided by your instructor. Your instructor will demonstrate the method for your unknown samples.
5. Hang the chromatographic paper from a rod using whatever clip is provided by your instructor, so that the 0.1% NaCl solvent level reaches **no higher** than the first pencil line. **Make sure, however, that the paper is in the solvent.**
6. After 30 minutes or until the solvent rises about 2-thirds up the paper, remove the paper from the tank and place it someplace to dry (on a paper towel or hanging in from the desk top). Immediately mark the solvent front and circle the darkest region of the dye spots with a pencil. For the unknown, there will probably be more than one colored area. Circle **each** one. As the paper dries the spots and solvent front will continue to move a little.
7. Measure the distance in, mm, the solvent front has traveled for each dye from the second pencil line and the distance from the second pencil line to the center of the circle you have made for each dye. List this data in a table. Also, make note of the final color of each dye,

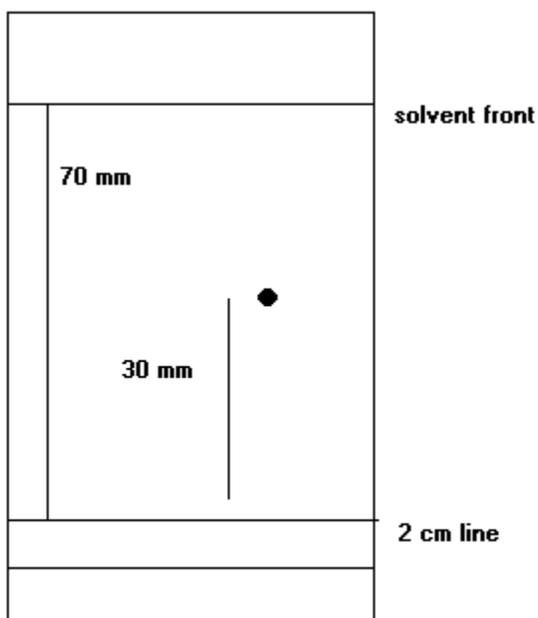
including the multiple colors of the unknown.

8. Compute the retention factor, R_f , for each standard and for **each** color in the unknowns, using the formula in the next section.
9. In your lab report, hand in the actual chromatogram and using the final colors and the R_f values, identify which standard dyes are in your unknowns.

Calculations:

$$R_f = \frac{\text{Dye distance travelled (mm)}}{\text{Solvent (NaCl solution) distance travelled (mm)}}$$

Example:



Dye traveled 30 mm

Solvent traveled 70 mm

$$R_f = \frac{30 \text{ mm}}{70 \text{ mm}} = 0.428 = 0.43$$

Questions:

1. Compute R_f

Sample spot traveled 55 mm; Solvent front traveled 132 mm.

2. What are the **final units** for R_f ?

TYPICAL CHROMATOGRAM

