تفريغ مختبر عضوية

Exp 6 : اسم الموضوع:

Chromatography

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EXPERIMENT 6: CHROMATOGRAPHY

A Separation and Purification Technique

INTRODUCTION

Chromatography is a technique that may be used to separate the components of a mixture as well as to identify organic substances and examine their purity. Chromatography encompasses several techniques such as column, thin-layer, paper, gas liquid, etc. chromatography. Two principles are basically involved in chromatography: adsorption (as in thin- layer chromatography) and partition (as in paper chromatography), andcertain terms are common to both types of chromatography.

In adsorption chromatography, separation depends on the selective desorption of the components of a mixture by the eluent (mobile phase) from the surface of a solid adsorbent (stationary phase). The adsorbent maybe packed in a column (column chromatography) or spread as a thin layer on a glass plate as in thin-layer chromatography.

In partition chromatography, separation depends on partition of the components of a mixture between the stationary and mobile phases. The mobile phase may be a liquid (liquid-liquid partition chromatography) or a gas (gas-liquid partition chromatography).

ANALYSIS OF CHROMATOGRAMS

In thin layer and paper chromatography, substances are characterized by their R_f-values (retardation factor). The R_f-value is a number (less than one) which is characteristic of a compound for a given adsorbent and developing solvent. It is defined as:

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent}}$$

In gas-liquid chromatography, compounds are characterized by their retention times.

THIN-LAYER CHROMATOGRAPHY (TLC)

This is one application of adsorption chromatography in which an adsorbent, usually silica gel or alumina, is spread out as a thin layer on an inert surface, such as a glass plate or microscope slide. The mixture is applied at one end of the coated plate and, as the mobile phase (a liquid) moves up the solid adsorbent by capillary action, the adsorbed components of the mixture get desorbed and carried along at different rates by the moving solvent.

Adsorption of the components of the mixture, on the surface of the adsorbent, occurs to differing extents depending on their structural features and polarity. The more strongly adsorbed a given compound is, the slower it is transported by the mobile phase, and conversely, the more weakly adsorbed the compound is, the faster it is transported up thestationary phase. The result is that the components of the mixture are separated into different zones or spots (Figure 20).

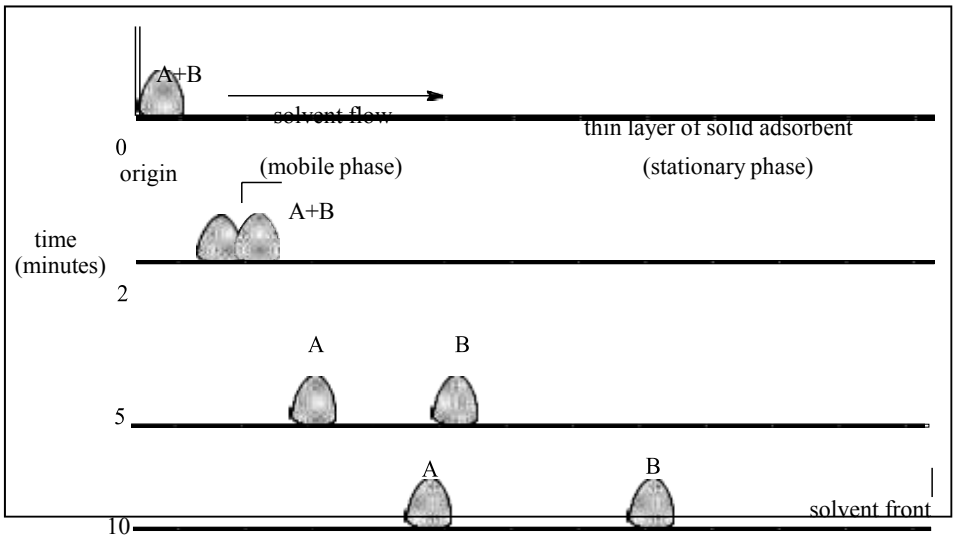


Figure 20. Separation by thin-layer chromatography

Separation by thin-layer chromatography depends on the kind and activity of the adsorbent (stationary phase), the polarity of the eluent (mobile phase) and on the chemical nature of the components of the mixture. The most common adsorbents employed in *TLC* are silica (SiO_2).

xH_2O) and alumina (Al_2O_3. xH_2O), and the activity of these adsorbents is largely determinedby their water content. For a given adsorbent and compound, the greater the polarity of the eluent, the greater is its ability to dislodge a compound from the surface of the adsorbent, and therefore the higher the R_f-value.

Eluting power of solvents:

Acetic acid > Ethyl alcohol > Acetone > Diethyl ether > Dichloromethane >Hexane.

GENERALIZED EXPERIMENTAL PROCEDURE

PREPARATION OF TLC PLATES

Large glass plates (20x20 cm) are commonly used for quantitative separations, while microscope slides are usually used for qualitative purposes. A homogeneous slurry of the adsorbent in a volatile organic solvent (chloroform or dichloromethane) is poured over the glass plates and allowed to air-dry at room temperature. Microscope slides can be coated, two at a time, by dipping them into the slurry for some time then holding them vertically to air-dry. The jar of adsorbent must be shaken thoroughly before each use to homogenize the slurry. Three steps are involved in TLC: **spotting, developing, and visual**

Spotting. The mixture to be analyzed is dissolved in a suitable solvent (1% solution). With a drawn capillary tube, a small amount of this solution is spotted on the *TLC* plate about 1 cm from the bottom (Figure 21). The spotsshould have a diameter not larger than 1-2 mm since larger spots result in "tailing" and overlapping of close spots. Once the solvent evaporates from the spots, the plate is ready for developing.

Development of the Chromatogram. The eluent, also called developing solvent, is chosen based on the nature and polarity of the compounds being studied. It is best to choose the solvent that will give a satisfactory separation within the range of 0.2-0.8 R_f values. The plate is

placed in a developing chamber (*e.g.*, a covered beaker) containingthe solvent and lined with filter paper soaked in the solvent to help saturate the atmosphere with solvent vapors. When the solvent front reaches the finish line, the plate is removed from the beaker and placed onthe bench top to air-dry.

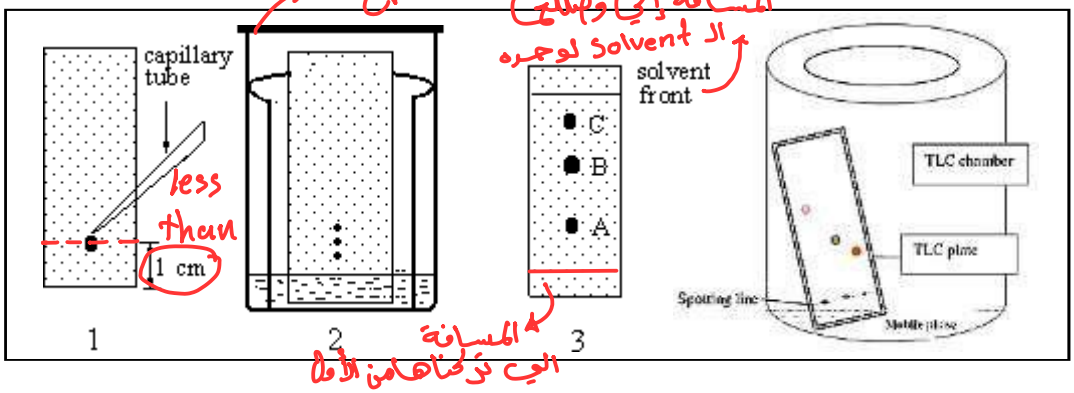


Figure 21. Steps in the TLC technique

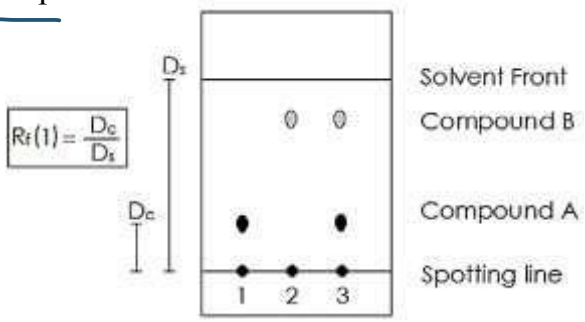
Visualization of Spots. Compounds on the plate are located according to their characteristics:

a. If the spots are colored, they can be observed in ordinary light.

b. If the compounds are colorless, they can be seen under *UV*-light or using iodine staining where they appear as dark spots on a white background.

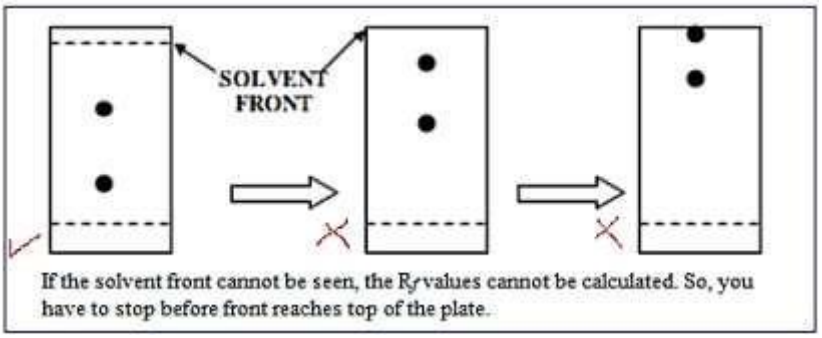
c. Colorless spots may also be located with an indicator. Most organic compounds form complexes with iodine giving dark brown spots whenthe plate is exposed to iodine vapor.

Sulfuric acid may also be used to make colorless spots visible. Most organic compounds turn black when sprayed with sulfuric acid.



Notes:

o Allow the plate to develop until the solvent is about half a centimeter below the top of the plate. Remove the plate from the beaker and immediately mark the solvent front with a pencil.



o A pure substance produces one spot on the TLC plate while an impure substance produces two or more spots.

OBJECTIVES

1. Determining the R_f-value for *o*- and *p*-nitroaniline by *TLC*.
2. Separating a mixture of two dyes by paper chromatography.
3. Determining the constituents of an analgesic drug by *TLC*.

Having done this experiment, you will have seen the use of *TLC* and paper chromatography in the separation of mixtures and in the characterization of organic compounds.

EXPERIMENTAL

MATERIALS NEEDED	Glassware: 2 Microscope slides,, capillary tubes, beaker (200 mL), Petri dish with cover, filter paper, UV lamp. Chemicals: stock solutions (1% in acetone) of *o*-nitrophenol, and *p*-nitrophenol, green dye, 5 mL dichloromethane, 20 mL isoprophyl alcohol, 1 mL methanol, 6 mL benzene, 3 mL ether, 1 mL acetic acid and analgesic tablet (Remin, Revanin, Paracetamol or Excedrin), stock solutions (5% in acetone) of aspirin, phenacetin, salicylamide, caffeine, acetaminophen.

TLC EXAMINATION OF ISOMERIC NITROANILINES

Prepare the developing chamber by placing a filter paper inside a 200mL beaker and adding 5 mL dichloromethane. Cover the beaker while preparing the *TLC* plates as described before. Dip a capillary tube into a 1% solution of *p*-nitroaniline in acetone and touch it to the *TLC* plate at the origin.

After the solvent has evaporated from the spot, place the slide in the developing chamber. When the solvent front has reached the finish line, remove the slide and allow the solvent to evaporate. Locate the center of the spot and calculate the R_f-value. Repeat the procedure with *o*-nitroaniline.

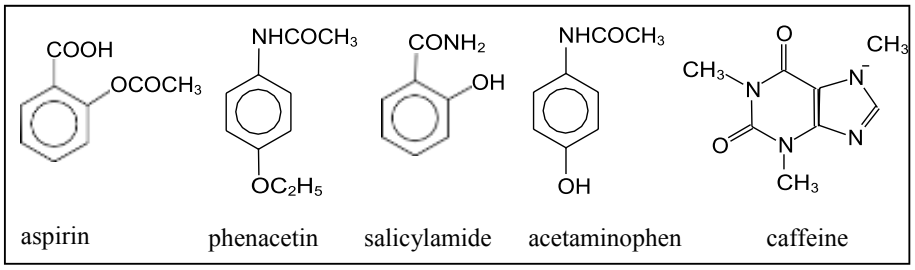
On a new slide, place the spots of *p*-nitroaniline and *o*-nitroanilineside by side at the origin so that the two compounds run parallel on the same slide. Calculate the R_f-value for each. Repeat using amixture of the two compounds in addition to the single compounds as references. Place one spot of the mixture in the middle and a spot of

each isomer on either side of the mixture. Note the resolution of the mixture into two spots and compare the R_f-values obtained for the mixture and for the individual compounds.

ANALYSIS OF ANALGESIC DRUGS BY TLC

In many non-prescription analgesic drugs such as Remin, Revacod, Revanin, Paracetamol, Excedrin etc.., the active ingredient is one or more of the compounds listed below. The constituents of an analgesic drug may be qualitatively determined by *TLC* using suitablereference samples. Introduce a small piece of an analgesic tablet intoa test tube, add 1 mL of methanol and stir well. Allow the insoluble material to settle and use the supernatant liquid for spotting on the *TLC* plate.

Make dilute methanolic solutions of the reference compounds and spot as many of them as possible on the same plate. Use a solvent mixture of benzene: ether: acetic acid (2:1:0.3) to develop the chromatograms. Examine the developed plates under ultraviolet light and determine the composition of the analgesic tablet.



aspirin phenacetin salicylamide acetaminophen caffeine

PAPER CHROMATOGRAPHIC ANALYSIS OF A DYE

In this experiment green food coloring (composed of a yellow and a blue component) will be examined by paper chromatography using a Petri dish (10 cm in diameter) as a developing chamber (figure 22).

Locate the center of a circular piece of filter paper by folding it in half. Make sure that the filter paper has a diameter slightly larger than that of the Petri dish. Using a melting point capillary tube with both sides open, apply a small spot of food coloring at the center of the paper.

Punch a small hole at the center and through it insert a small strip of filter paper rolled together to make a wick (Figure 22). Put 20-30 mL of the developing solvent (isopropyl alcohol - water 2:1) into the Petri disk and rest the filter paper on the rim of the dish making sure that the wick dips into the solvent.

Cover the paper with the Petri dish cover and leave the chromatogram to develop undisturbed for 10 minutes until the colors separate into distinctcircles. Remove the paper chromatogram and allow to air-dry.Calculate the R_f-values for the yellow and the blue dye.

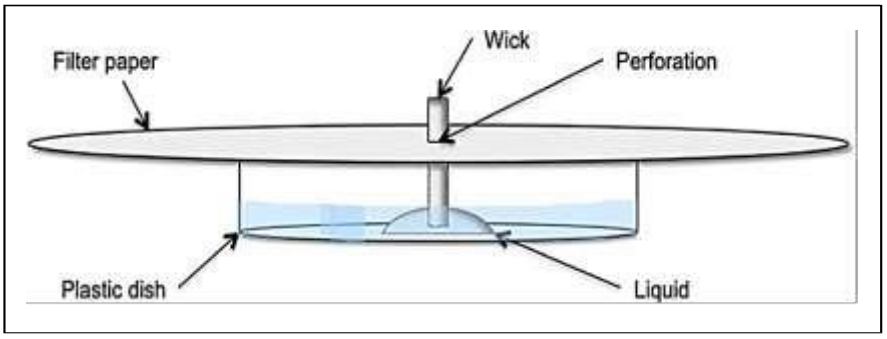
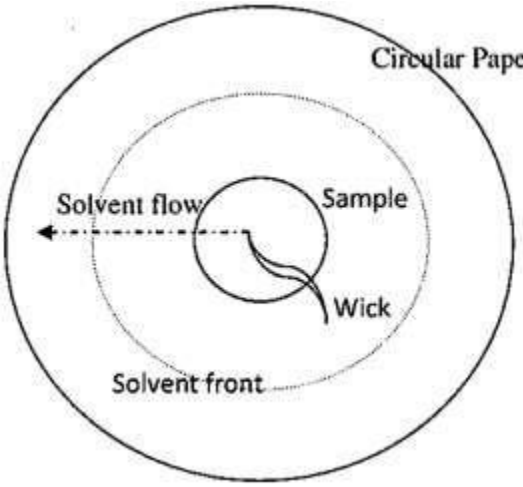


Figure 22. Paper chromatography using a Petri dish



Useful links

Thin Layer and Paper Chromatography
https://www.youtube.com/watch?v=w65S1dqrSAY

Paper chromatographic analysis of a dye

- Paper chromatography is used to separate mixtures of soluble substances. These are often coloured substances such as food colourings, inks, dyes or plant pigments.
- is a technique used for the separation of compounds based on the differential solubility in the stationary phase and mobile phase (solvent).

EXPERIMENT 6
CHROMATOGRAPHY
Report Sheet

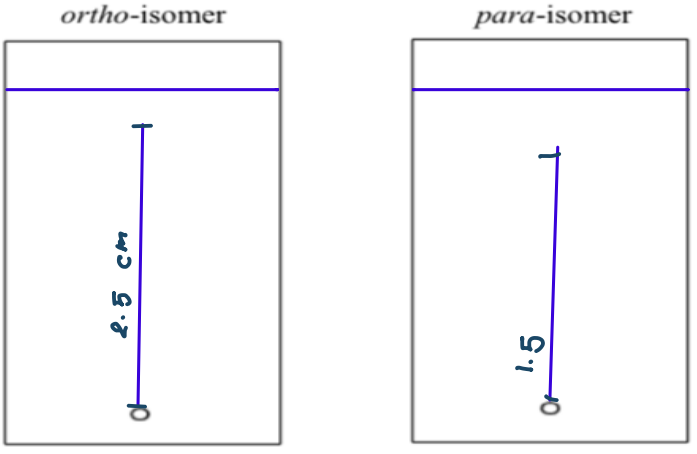
Group number		Section no:	

➢ **OBJECTIVES:**

- Determining the Rf-value for ortho-isomer & para-isomers By TLC.
- Seperating a mixture of 2 dyes by paper chromatography.

➢ **TLC EXAMINATION OF ISOMERIC NITROANILINES:**

Represent your observations (showing the distance for the solvent and the samples):



Find the retardation factor for the *ortho*–isomer and *para*–isomer (show your detailed calculations):

R_f (ortho) = $\frac{2.5}{4.2} = 0.595$

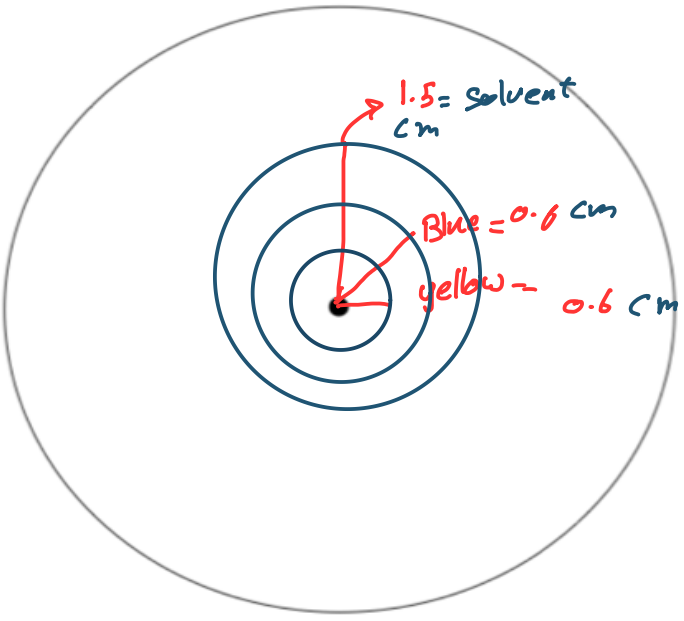
R_f (para) = $\frac{1.5}{4.2} = 0.357$

Which compound is more strongly adsorbed on silica, *ortho*– or *para*–nitroaniline?

ortho

➢ PAPER CHROMATOGRAPHIC ANALYSIS OF A DYE:

Represent your observations (showing the distance for the solvent, the yellow and blue sample):



Find the retardation factor for the blue and yellow dye (show your detailed calculations):

R_f (the yellow dye) = $\frac{0.6}{1.5} = 0.4$

R_f (the blue dye) = $\frac{0.9}{1.5} = 0.6$

Which dye would be more soluble in n-propyl alcohol, the blue or the yellow? How can you tell?

- yellow, because it can form H-Bond with the solvent "isopropyl alcohol"