Chemistry 221

Experiment 11- Chromatography:

***OBJECTIVES***

1. Determining the Rf-value for *o-* and *p-*nitroaniline and 0- and p-nitrophenol by

*TLC*.

2. Identification of an unknown by determining Rf-values.

3. Separating a mixture of plant pigments by column chromatography.

*General Principles of Chromatography*

Chromatography is defined as the separation of a mixture of two or more different compounds or ions by distribution betweentwo phases, one of which is stationary and the other is moving.Various types of chromatography are possible, depending on the nature of the twophases involved: solid—liquid (column, thin-layer, and paper), liquid—liquid (high performanceliquid), and gas—liquid (vapor-phase) .

We will study two methods : Thin layer chromatography, (TLC), and column chromatography.Basically, the methods depend on the differential solubilities or adsorptivities ofthe substances to be separated relative to the two phases between which they are to bepartitioned. In practice, the compounds of interest are adsorbed onto a solid support (the stationary phase), such as alumina (aluminum oxide) or silica gel (silicon oxides). Separation is achieved by elution "washing" with a moving solvent .

The order in which the compounds are eluted will depend on how strongly they are adsorbed on the surface of the stationary phase. Alumina strongly adsorbs materials capable of forming hydrogen bonds to the basic oxygen atoms of the alumina. Compounds without the ability to form hydrogen bonds, but with substantial dipole moments, will be somewhat less strongly adsorbed due to electronic interactions between their dipoles and those of the alumina. Non-polar compounds are only very weakly adsorbed due to dipole-induced dipole interactions.

The choice of solvents used to elute the various components of the mixture from the column will depend upon the components in the mixture. For a very weakly adsorbed component a very non­polar solvent such as petroleum ether (a mixture of hydrocarbons) would be used. For more strongly adsorbed components, a more polar solvent such as ether might be used. For very strongly adsorbed components, a very polar solvent such as ethanol, water or even acetic acid might be required to displace the material from the column.

**Thin Layer Chromatography:TLC**

TLC is a simple, quick, and inexpensive procedure used to determine the number of components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the Rf of a compound is compared with the Rf of a known compound (preferably both run on the same plate)

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action.

In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are colored, visualization is straightforward. Usually the compounds are not colored, so a UV lamp is used to visualize the plates. (The plate itself contains a fluorescent dye which glows everywhere *except* where an organic compound is on the plate.)

**The Rf value**

The retention factor, or Rf, is defined as the distance traveled by the compound divided by the distance traveled by the solvent.



For example, if a compound travels 2.1 cm and the solvent front travels 2.8 cm, the Rf is 0.75:



The Rffor a compound is a constant from one experiment to the next only if the chromatography conditions below are also constant:

* solvent system
* adsorbent
* thickness of the adsorbent
* amount of material spotted
* temperature

**Experimental: TLC**

Substances to be tested: o-nitroaniline, p-nitroaniline, o-nitro phenol, p-nitro phenol

( 1% solutions in acetone)

Step 1: Prepare the developing container

The developing container for TLC can be a beaker with a watch

glass on the top. Pour solvent (ethyl acetate:chloform-10:90)

into the chamber to a depthof about 0.5 cm. Line part of the

inside of the beakerwith filter paper. Let it stand while you

prepare your TLC plate.

**Step 2:** Prepare the TLC plate

1 2 3 4

Measure ~0.8 cm from the bottom of the plate. Using a pencil,

draw a line across the plate at the 0.8 cm mark (taking care

not to disturb the adsorbent). This is the line on which you will

spot the plate.Under the line, mark lightly letters or numbers

foreach sample. Leave about 1 cm between spots.

**Step 3**: Spot the TLC plate

Dip amicrocapillary into a 1% solution of*p-*nitroaniline

1 2 3 4

in acetone and then *gently*touch the end of it onto

the proper location on the TLC plate. Don't allow the

spot to become too large. Spot the other compounds 

andthe unknown next to *p-*nitroaniline. Use a new capillary for each

compound.

**Step 4**: Develop the plate

Place the prepared TLC plate in the developing beaker,

cover the beaker with the watch glass, and leave it

undisturbed on your bench top. The solvent will rise

1 2 3 4

up the TLC plate by capillary action. Make sure the solvent level lies below the spots. Allow the solvent to move upward until it 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.

Allow the plate to dry.

**Step 5:** Visualize the spots

If there are any colored spots,circle them lightly with a pencil.

Most samples are not colored and need *Solvent Front*

1 2 3

A

S

to be visualized with a UV lamp. Hold a *Component A*

UV lamp over the plate and circle any *Component B*

spots you see. Determine R**f** values for

each component: for “A” R**f=** A/S

**Column Chromatography:**

Column chromatography is a preparative method for separating and isolating compounds from mixtures. The method is used for obtaining compounds from natural

sources or for purifying products from reaction mixtures.

Essentially column chromatography is an upside-downversion of TLC .

Instead of having a thin layer of adsorbent attached to a solid support, a column is filed with a larger amount of adsorbent and the mixture is loaded ontop of it.

While TLC relies on capillary forces for moving the solvent, in column chromatography an eluent is allowed to percolate through the column by gravity. As the eluent is moving down the column it carries the soluble compounds with it. Compounds having strong interactions with the adsorbent move more slowly than compoundshaving weaker interactions with the adsorbent. Under the right conditions the compounds

will separate in distinctive bands and each band will come out of the

columnindividually. The bands are collected and the solvent is

evaporated to give a clean compound.

Microscale Column: {Dry Packing}:

Clamp aPasteur pipet (dropper) vertically. Break most of the tipof the

dropper. Use a glass rod or a piece of wire to push a small ball of cotton

in the dropper. Take care not to plug the column totallyby tamping the

cotton too hard.

The desired amount of adsorbent( ~ 4 g) is added slowly, and the

dropper is tapped constantly, until the level of adsorbent has reached

the desired height.

When the column is packed, added solvent is allowed to run throughthe

adsorbent until the entire column is moistened. The solvent is not added

until justbefore the column is to be used.

We will be using approximately 2 g ofalumina for this column chromatography. Use the same 70:30 mixture of petroleum ether/ethyl acetate asthe solvent in preparing your column.

Place your plant extract on the column,***{You will be provided with spinach extract}***

Use a wash bottle containing70:30 mixture of petroleum ether/ethyl acetate to elutethe column. Keep eluting the until the yellow/orangeband of β-carotene has been collected.

 Try to collect all of this band in the smallest fractionpossible. After theβ -carotene has been collected, use pure chloroform or ethyl acetate as your developingsolvent to collect your chlorophylls.



**Experimental Procedure:**Extraction and isolation Plant pigments:

**To save time, you do not need to do this. We will provide the plant extract**

1-Weigh a 10 g sample of spinach, or some other plant leaves (squeeze out the water first!) into a 100 mL beaker. Add 25 mL of acetone and mix the mixture vigorously until the leaves lose most of their color to the acetone solution.

2- Place a plug of glass or cotton wool in a short stem funnel, and filter your

mixture through this plug into a 125 mL separatory funnel. Press the spinach pulp with a spatula,and rinse the leaves with an additional 5 mL of acetone.

3-Add 30 mL of hexane and 30 mL of saturated aqueous sodium chloride to this separatory funnel, shake this mixture vigorously with occasional venting, and drain off the aqueous layer. Wash the organic layer two more times, using 30 mL of water each time. You should drain off the aqueous layer after each wash with 30 mL of water!

4-If you have any emulsions during these washings, they may be broken by adding a small amount of saturated aqueous sodium chloride.

5-Transfer your green hexane solution to a 50 mL Erlenmeyer flask and dry with anhydrous magnesium sulfate.

6-Filter this solution into a clean, dry 50 mL Erlenmeyer flask.

7- Evaporate the solvent by placing the solution in a 100 mL beaker and heating the solution on a hot plate (use a boiling chip ) in the hood until only 3-5 mL of the dark green solution remains.

Questions:

1. Predict the relative order of elution of a mixture of triphenylmethanol, benzoic acid and methyl benzoate from a silica gel column using chloroform as an eluting solvent-(Which compound will come out of the column first ?). Explain your answer.

2. A mixture composed if biphenyl, benzoic acid, and benzyl alcohol is spotted on a TLC plate and developed in a dichloromethane-cycloheaxane solvent mixture. Predict the relative Rf values for the three components in the sample.

3.A student spots an unknown sample on a TLC plate. After developing in hexanes/ethyl acetate 50:50, he/she saw a single spot with an Rf of 0.55. Does this indicate that the unknown material is a pure compound? What can be done to verify the purity of the sample?

4.An unknown compound, X, was analyzed by TLC using two differenteluting solvent mixtures. Three standard compounds, G, H, and I, wereused. The *Rf*values for the analyses are given below. Which of the threestandards is most likely to be compound X? Explain your reasoning.

|  |  |
| --- | --- |
| 5:1 Hexane/Acetone | 3:1 Hexane/Ethyl acetate |
| Compound | Rf | Compound | Rf |
| G | 0.35 | G | 0.55 |
| H | 0.65 | H | 0.95 |
| I | 0.65 | I | 0.85 |
| X | 0.65 | X | 0.95 |