C27 Chromatography

(2021/03/01)

Collect:

- Column
- Mortar and pestle
- Dropper (229 mm)
- Capillary tube
- TLC plate
- Aluminum foil
- UV light (shared)

Prepare:

- Green leaves
- Beaker (30、100 mL)
- Erlenmeyer flask (50, 125 mL)
- Test tubes (20)
- Test tube rack
- Glass rod
- Funnel
- Tweezers

Chromatography

- **Objective**

  To separate and identify mixtures using column chromatography (CC) and thin-layer chromatography (TLC)

- **Principle**

  Base on the different distribution of compounds between a stationary phase and a mobile phase

- **Flow charts**

  Part I: Extraction

  Part II: Column chromatography

  Part III: Thin layer chromatography

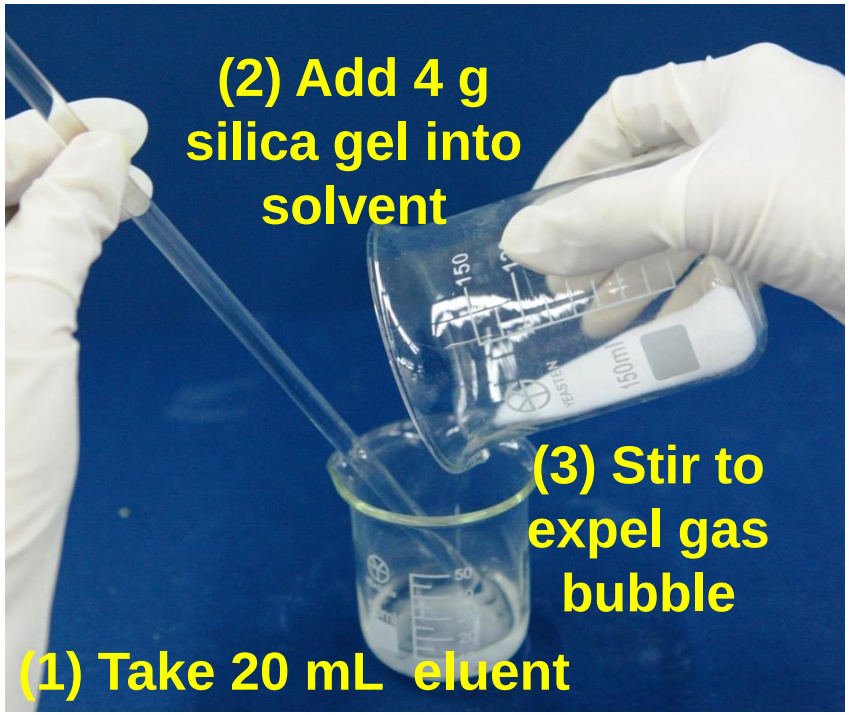
Part I: Extraction

- Cut about 1 g of green leaves to pieces
- Grind with 10 mL hexane/ethyl acetate (8 : 2, v/v) solvent
- Use dropper to transfer the extract into a graduated cylinder as the sample solution; the amount of extract solution is about 2 mL

Part II: Column Chromatography

1. Prepare silica gel slurry

- Prepare 60 mL eluent (hexane : acetone = 7 : 3)
- Pour 20 mL eluent to a beaker
- Slowly add 4 g of silica gel with stirring
- Stir the mixture with a glass rod thoroughly to free the gas bubbles



Part II: Column Chromatography

2. Slurry packing

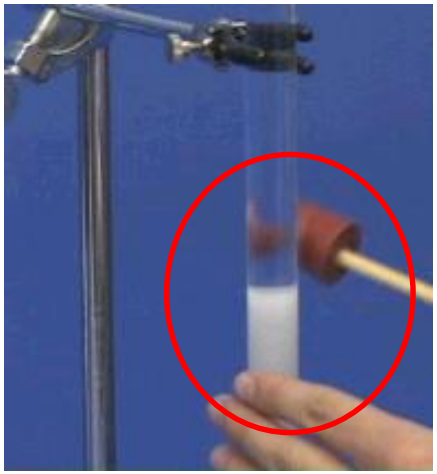
(1) Set up

- Fix the column with a clamp
- Place a funnel on top of the column
- Add 5 mL of eluent through the funnel

(2) Pack

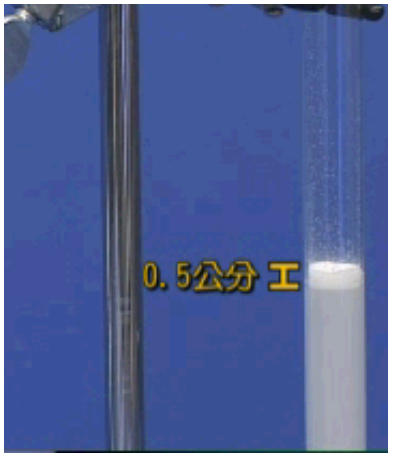
- Stir and pour the slurry into the column quickly

(3) Tap



- Tap the wall of the column gently to flatten the top of the column and pack the column more tightly

(4) Add

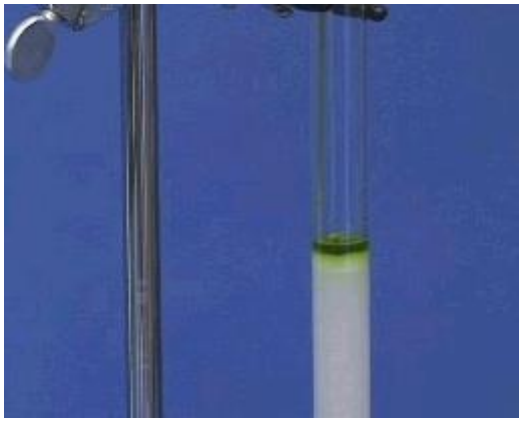


- Add a layer of Na_2SO_4 of 0.5 cm thickness at the top when the eluent reaches the top of the silica gel
- Add more eluent if needed

Part II: Column Chromatography

3. Apply sample and start eluting

(1) Apply sample

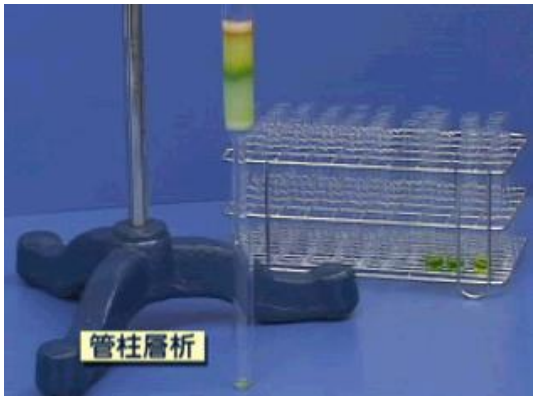


- Let the surface of the eluent reaches the top of the stationary phase
- Apply the sample solution circularly with a dropper to the top of stationary phase gently to form a small layer

(2) Rinse

- Let sample solution submerge into the surface of the silica gel completely
- Add small layer of eluent with dropper to drain into the column until the column just dries

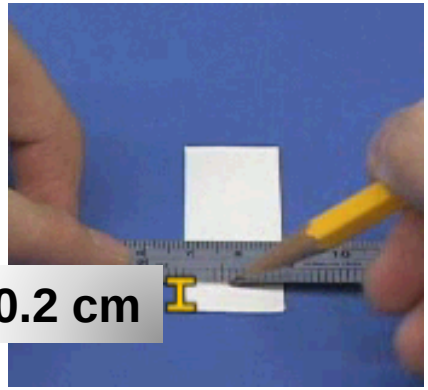
(3) Elute and collect

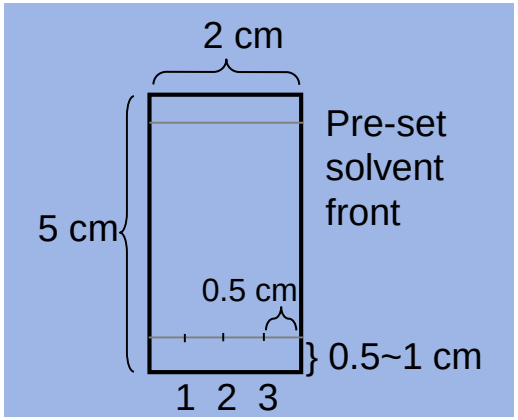


- Continue adding eluent to start the chromatography
- Collect the first colored band in the test tube for one milliliter per tube

Part III: Thin Layer Chromatography

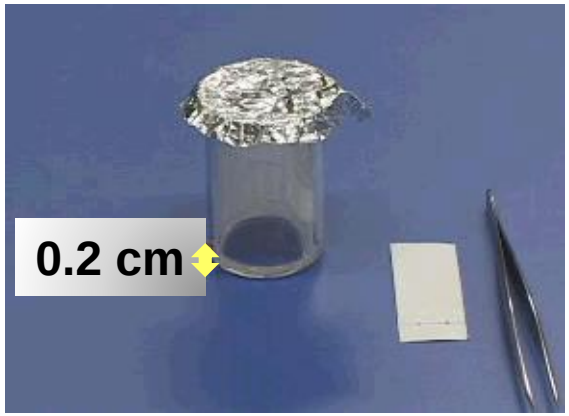
1. Spotting





- Draw a line at a position about 0.5 cm above the bottom of the TLC plate with a pencil
- Fill the capillary tube with the original extract (r) and touch to the TLC plate briefly
- Place two spots of the fractions to the plate using another capillary tube

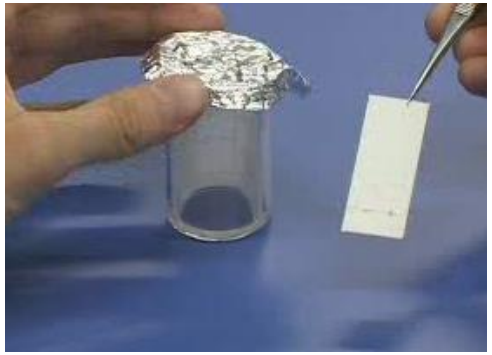
2. Development chamber



- Add developing solvent (hexane/acetone = 7 : 3) to a 30 mL beaker to about 0.2 cm high
- Attach a cut filter paper to the inside wall of the beaker and cover the beaker with aluminum foil to reach liquid-vapor equilibrium

Part III: Thin Layer Chromatography

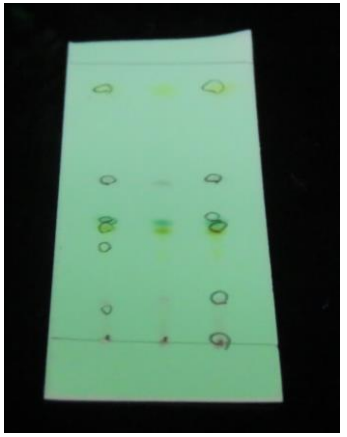
3. Developing



- Place the plate into the developing chamber with the tweezers
- Check the height of the spots should be higher than the surface level of the developing solvent
- Cover the beaker with aluminum foil and allow the solvent to advance up

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

4. Recording



- Take the TLC plate out when the solvent front is 0.5 to 1.0 cm from the top of the plate
- Record the position of the solvent front and the distance it has traveled with a pencil
- Locate the center of each spot and measure the subsequent distances they traveled
- Calculate their values of R_f

Notice

Brief report

- Put on the disposable mask to avoid inhalation of small particulates of silica gel
- Apply the eluent constantly to avoid the level of eluent lower than the stationary phase that may lead to cracking of stationary phase
- The capillary tube should be touched to the TLC plate very briefly and gently while spotting
- Touch the used capillary tube to paper towel to draw the sample out, then rinse with clean solvent several times to reuse it
- Reverse the column to drain the adsorbent and apply pressure to force the solid out of column after experiment
- Discard the organic waste, TLC plates, capillary tubes, and silica gel to the waste bins

Manipulating of Column Chromatography

- Prepare silica gel slurry with stirring to free air bubbles
- Add portions of eluent to free the gas in fritted glass of column
- Gently tap the column while filling the column to free any trapped air bubbles and pack the column more tightly and uniformly
- Add a layer of Na_2SO_4 of 0.5 cm thickness at the top when the eluent reaches the top of the silica gel
- Apply the sample when the surface of the eluent reaches the top of the stationary phase
- The sample should be introduced uniformly and symmetrically, without disturbing the column sorbent
- Apply the eluent constantly to avoid the level of eluent lower than the stationary phase that may lead to cracking of stationary phase
- Recycle the used silica gel to designated waste bin

Manipulating of Thin-Layer Chromatography

- The sample should be diluted with the solvent before spotting to avoid tailing
- Spotting should be carried out with a capillary tube with diameter smaller than 1 mm to control the sample spot to within a diameter of 2 mm
- Draw the line and spot the sample gently to avoid damaging the plate
- Place the plate into the developing chamber with the tweezers, set it in the center of the chamber, and avoid touching the sides
- The starting line should be higher than the surface level of the eluent to prevent the sample from dissolving in it
- Cover the beaker with aluminum foil to reach liquid-vapor equilibrium
- Take the plate out when the solvent front is 0.5 to 1.0 cm from the top of the plate
- Mark the solvent front immediately with pencil after developing
- Do not shine the UV light directly on skin or eyes while observing the sample points