# Lab (4):
# Column chromatography of Carotenoids (part II + III)

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# PART II: Column chromatography

- is Liquid-Solid Chromatography
- Used in separation and purification for both solids and liquids.
- Separation based on the same principle as in TLC
- Stationary phase is solid packed in column

- Components of the sample separate from each other by partitioning between the stationary phase and the mobile phase (eluent).

- Molecules with different polarity distributes by different extents, and move through the column at different rates.

Separation depends on **polarity**:

- **If stationary phase in the column is polar:**

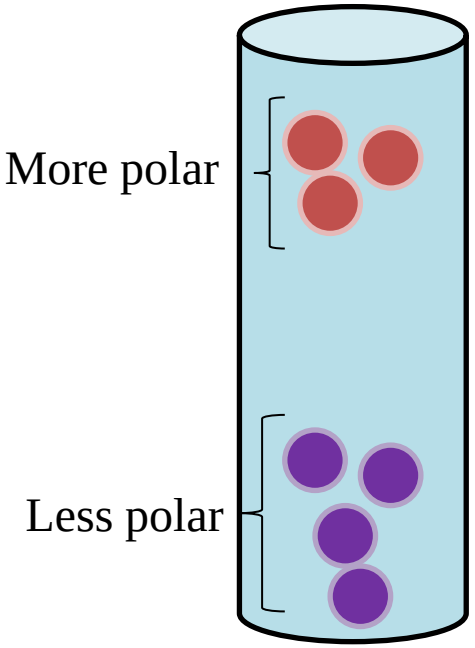
  - polar compound interacts strongly with st. phase moves slowly.
  - non-polar compound interacts strongly with st. phase moves rapidly.

- **If stationary phase is non-polar:**

  - non-polar compounds will move slowly because they are attracted to st.phase
  - more polar compound moves more quickly down the column.

**The eluent is collected in fractions.**

- depends on **polarity** of mixture components to be separated:
  - ❖ Less polar compounds ⟶ elutes first
  - ❖ More polar compounds ⟶ elutes last



# DIFFERENCES BETWEEN COLUMN CHROMATOGRAPHY AND TLC

| | TLC | Column Chromatography |
|---|---|---|
| stationary phase (adsorbent) | thin layer of silica gel ($SiO_2$) or alumina ($Al_2O_3$) on a glass, metal or plastic plate. | silica gel ($SiO_2$) or alumina ($Al_2O_3$) packed into a vertical column) |
| Sample | Mixture (sample) is applied on the bottom of the TL plate and migrates to the top of plate | Mixture (sample) is applied on the top of the column and passed through the column. (like filtration) |
| Mobile phase (solvent) or (eluent) | Solvent (eluent), rising up by capillary action | Solvent flows down through the column by gravity action or by application of air pressure. |

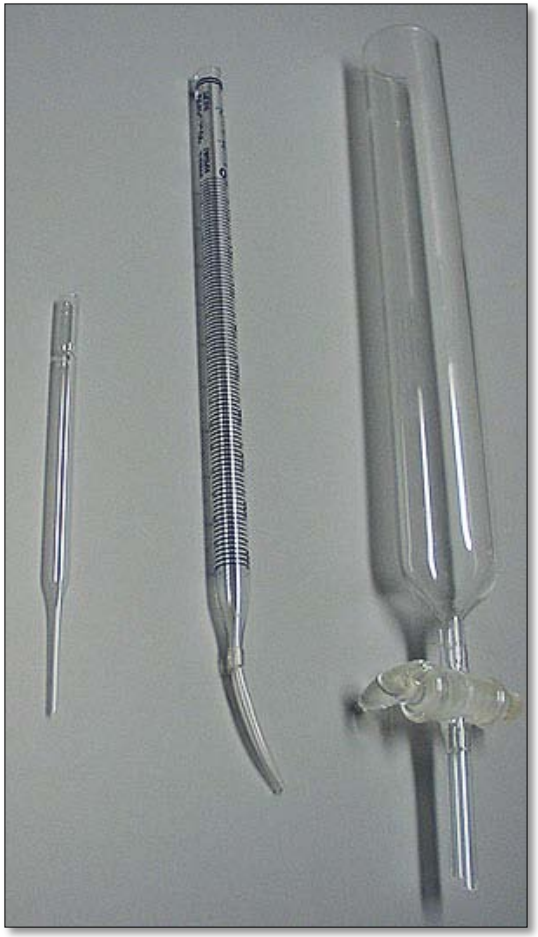
# Factors affect on column separation

- adsorbent
- polarity of the column
- polarity of the solvents
- size of the column
- rate of flow

# COLUMN

- Is **glass** or **polyethylene** vertical column or tube
- Ends with **stopcock** or **flexible tubing** attached to the bottom of the column to control the flow the solvent





tubing must not be dissolved by solvents used in separation.

# SIZE OF COLUMN

- Column chromatography can be small or big according to the amount of material loaded in the column.
- Longer column >>>> better separtion
- Various sizes are used
- Ordinary burette is usually used as a chromatography column.
- A pasteur pipette is also used as a chromatography column.

# SOLVENT

- THE MOST COMMON SOLVENTS ARE ARRANGED IN ORDER OF THEIR ELUTION POWERS AS :

**Water > Methanol > Ethanol > Propanol > Acetone > Ethyl Acetate > Diethyl Ether > Chloroform > Dichloromethane > Benzene > Carbon Tetrachloride > Cyclohexane > Hexane > Petroleum ether.**

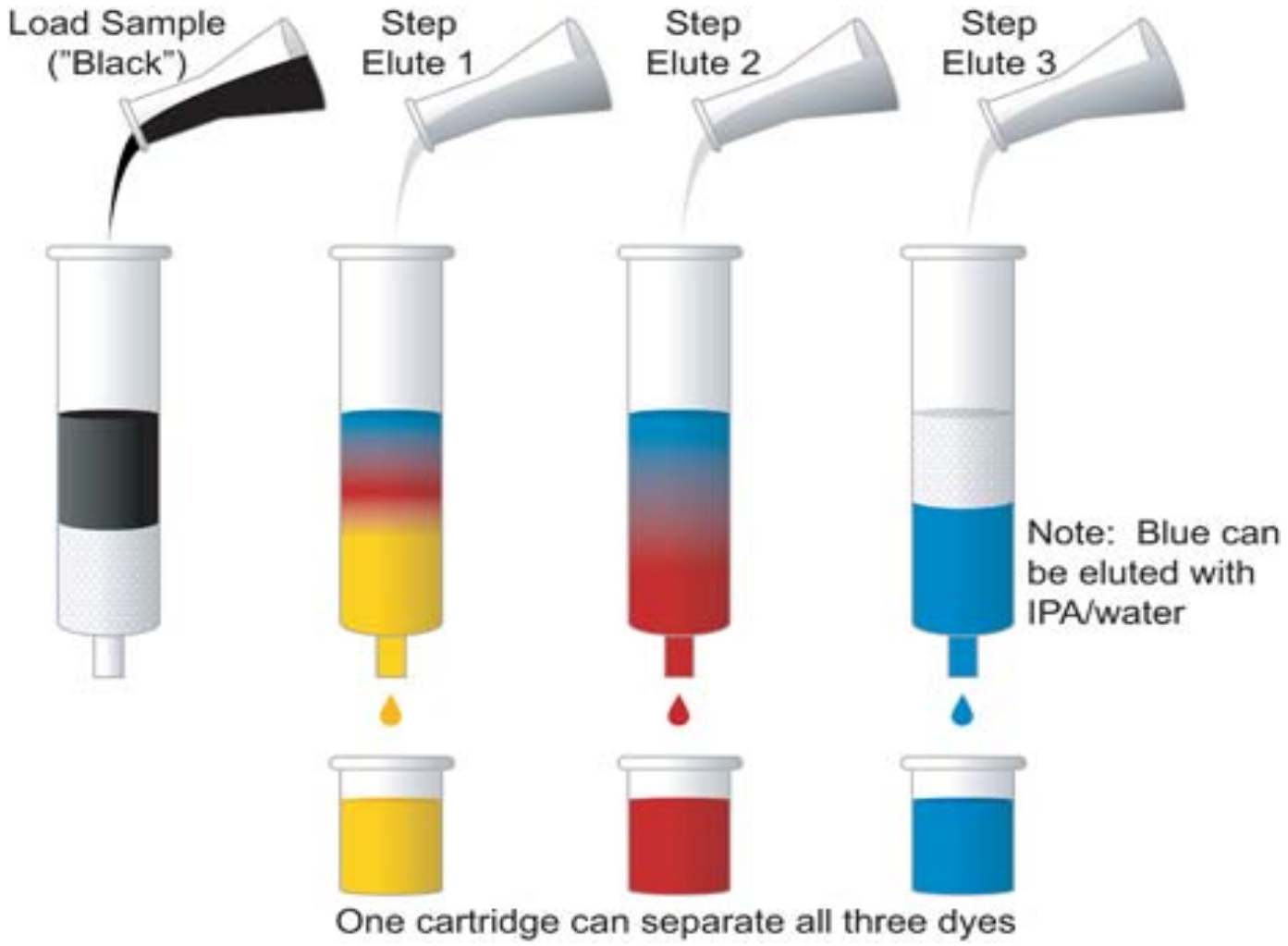
# Advantages of column chromatography

1- ability to handle large amounts of material

2- ability to change the eluting solvent throughout the course of the separation.

- Help in elution of components that strongly bound to stationary phase.
- Solvents changes include: changes in polarity, pH or ionic strength.
- The last two are used largely in biological separations.

3- efficient separation: due to ability in varying the stationary phase and changing solvent


Load Sample
("Black")
Step
Elute 1
Step
Elute 2
Step
Elute 3
Note: Blue can
be eluted with
IPA/water
One cartridge can separate all three dyes

# Lab Practice

- In this experiment the carotenoids separating according to the differences in polarity

  Beta carotene is non polar

  lycopene is more polar

In this lab:

- Stationary phase (adsorbent): alumina (polar)
- Mobile phase (eluent): petroleum ether (less polar)

  petroleum ether+ chloroform(5:1) more polar
- Sample: carrot extract (β-carotene)

  tomato extract (lycopene + β-carotene)


meritnation

- **β-CAROTENE WILL ELUTE FIRST THEN LYCOPENE**

- β-carotene (non-polar) will held to the alumina (polar) more weakly therefore, move through the column more faster

- Lycopene (polar) will held to the alumina (polar) more tightly therefore, move through the column more slowly.

- We needs to increase the polarity of solvent to allow lycopene to move faster and elute from the column

# PART III: Column chromatography

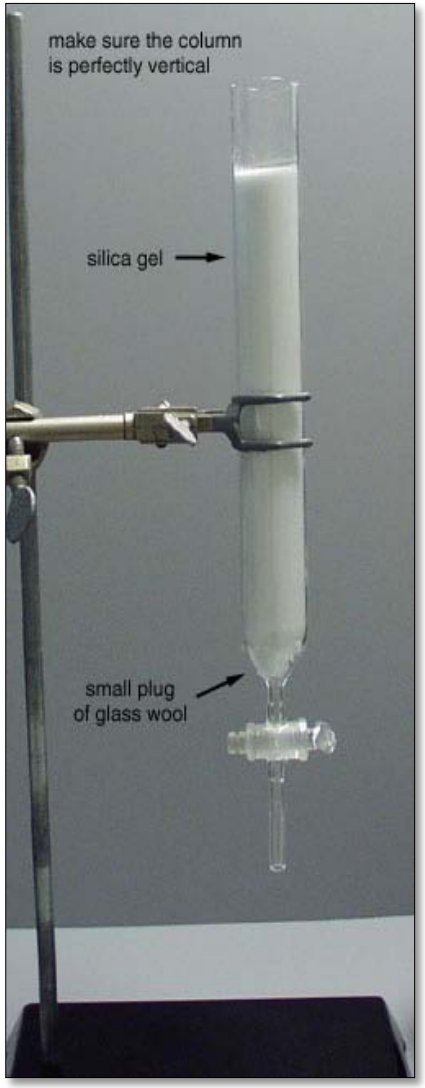
## Procedure

### 1- Prepare the column

- Plug the bottom of the column: by placing a very small glass wool with long glass rod, until all entrapped air is forced out.

  (plug: allows the solvent to pass and keep the solid alumina from getting out of the column)

- Clamp the column to a ring stand in vertical position.



**2- Prepare of the slurry**

- Prepare slurry (Mixture of a solvent and stationary phase
  in beaker: (30g alumina + double volume of petroleum ether 60ml)

**3- Packing the column**

- By mixing the slurry and adding it in the column
- Tapping the column to avoid air bubbles formation
- Open the stopcock to allow solvent to get out
- Re-add solvent again on the top of column to avoid alumina drying (solvent must be 1cm above st.phase)

**4- Adding the sample (carotene extract)**

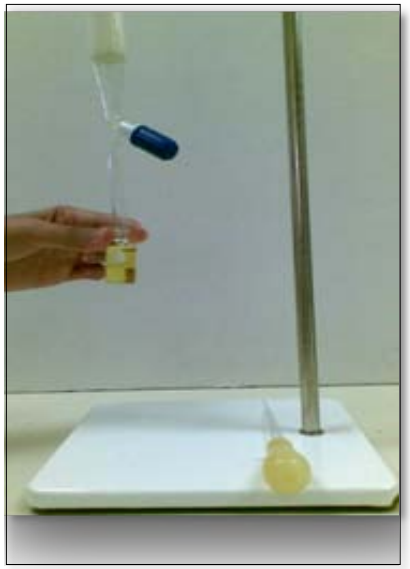
- Open stopcock and allows solvent to get out
- add 1 or 2 ml (use pipette)of the sample extract on the column

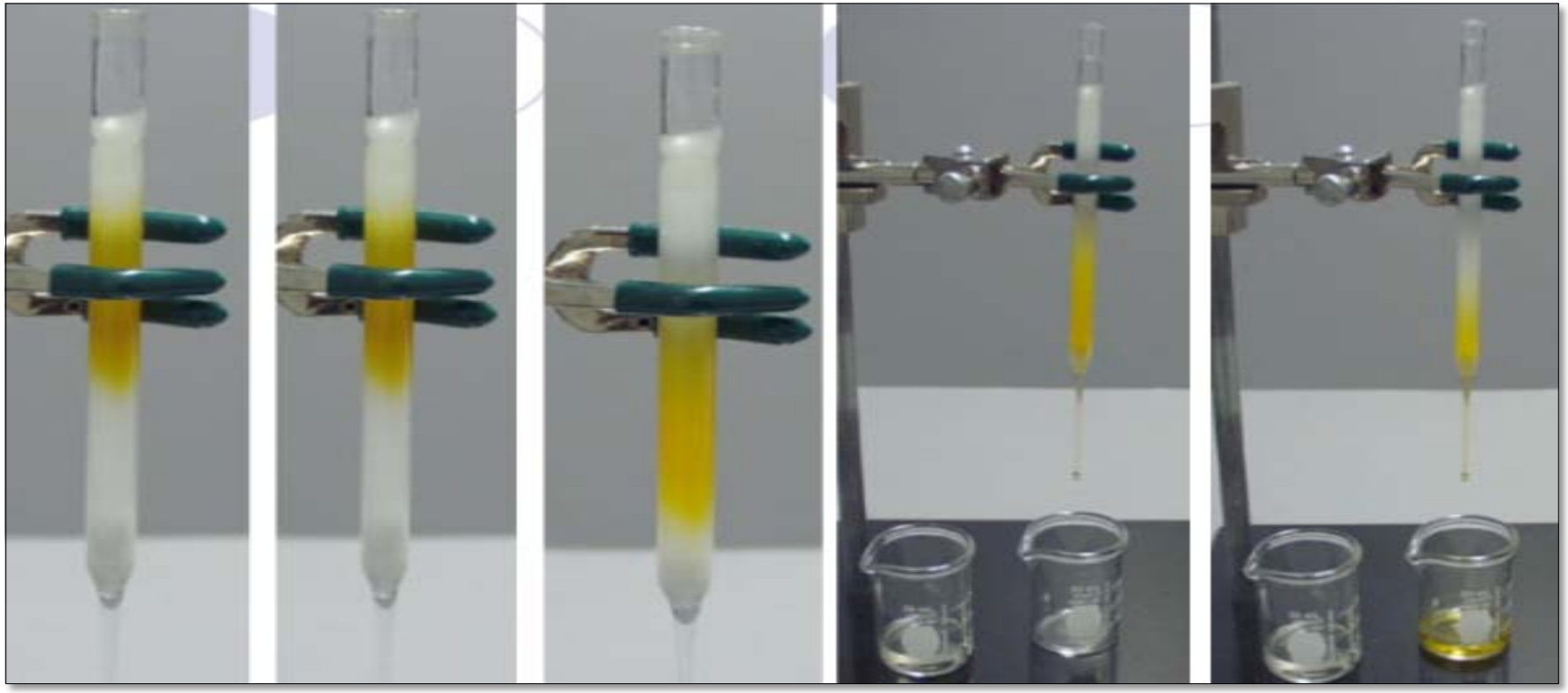
( note: add sample slowly and on the wall side of the column to avoid st. phase distribution)

- After sample move and enter st.phase add enough amount of solvent for elution

## 5- Sample collection

- Collect each fraction (color) in separate beaker





# PART III: TLC analysis of sample fractions

- Stationary phase: Silica gel TL plates
- Mobile phase (solvent): Benzen-cyclohexan 1:9
- Sample:

**- carrot:**

1- extract (before column)

2- β-carotene (after purification)

**- tomato:**

1- extract (before column)

2- β-carotene (after purification)

3- lycopene