LAU
School of Arts and Sciences

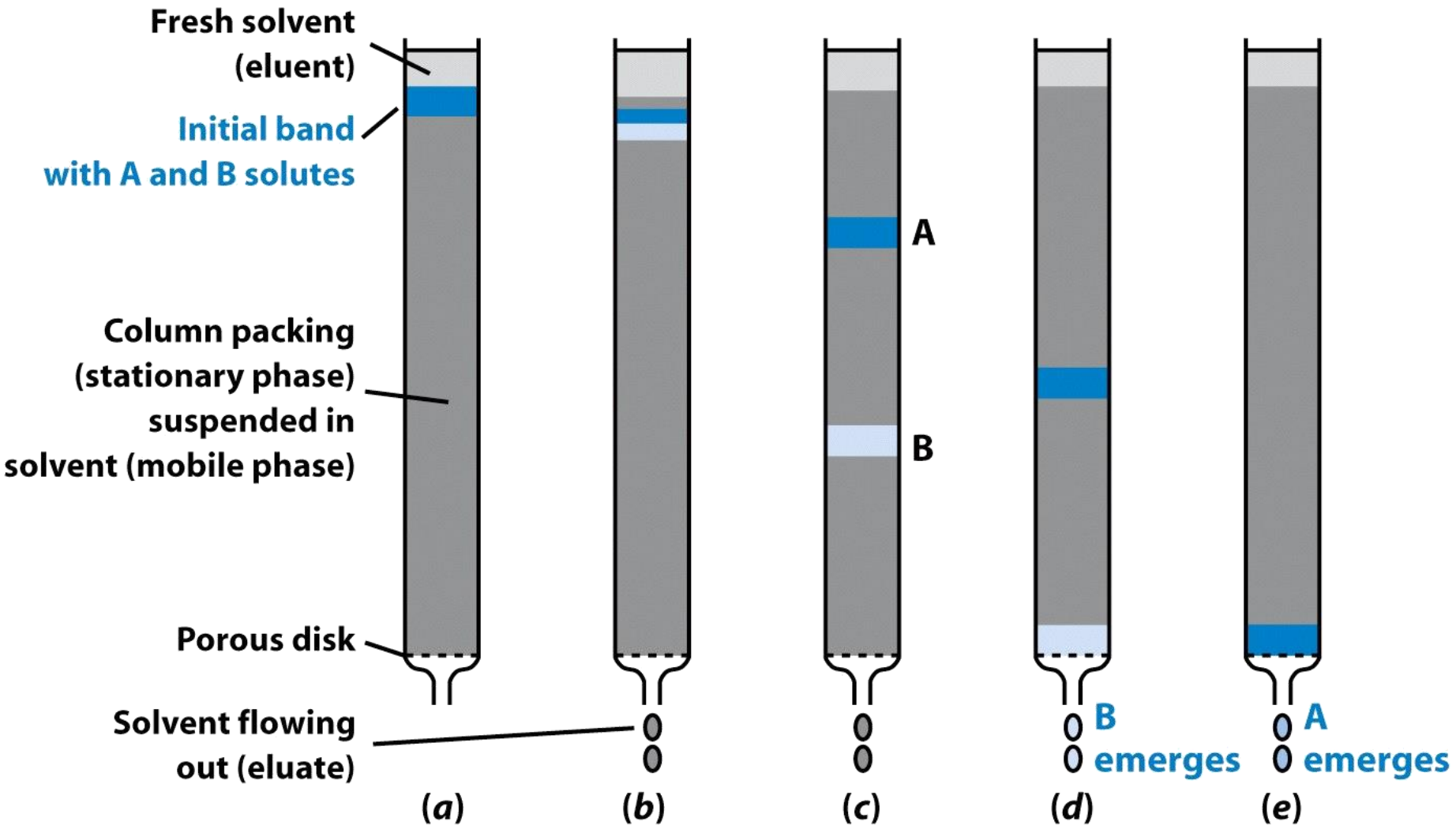
CHM 313

Exp. 10 (Part I):
CHROMATOGRAPHY
COLUMN CHROMATOGRAPHY (CC)

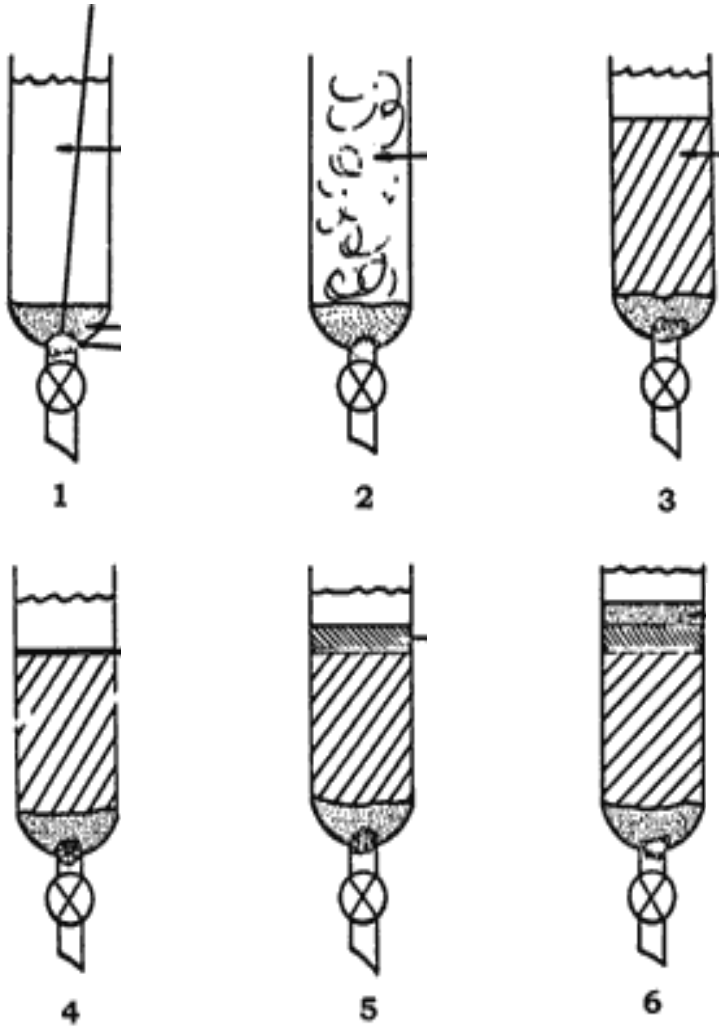
Description of Chromatography

- The sample mixture is transported in a mobile phase. The sample mixture is forced through an immiscible stationary phase (fixed in a column or on a solid surface) to which it is adsorbed or "generally attracted". Forcing the mobile phase occurs by a variety of mechanisms (capillarity, gravity, high pressure or others).
- The sample' multi-components distribute themselves between the 2 phases to varying degrees according to their affinities to these phases, i.e. strongly retained components by the stationary phase move slowly with the flow of *the mobile phase (=the eluent),* while, weakly held components *travel (=elute)* rapidly. (Other related terms: elution = development and eluate, *what are they?)*
- So, sample components separate into bands or zones that can be analyzed qualitatively and quantitatively (*analytical chromatography: ng or mg and preparative chromatogrpahy: g or Kg).*

Column Chromatography



Column Chromatography



How to prepare a column?

1. Close the chromatography tube with e. g. glass wool or a tissue ***(in your lab, it is already closed with glass frit, save yourself the trouble;-)).***
2. Add a prepared suspension a of stationary phase (*in some of the mobile phase)* and withdraw excess solvent
3. Allow sedimentation of the stationary phase.
4. When sedimentation is completed, add some sand.
5. Add the sample solution containing the mixture of compounds.
6. Adjust flow rate using the tap
7. Collect *fractions.*

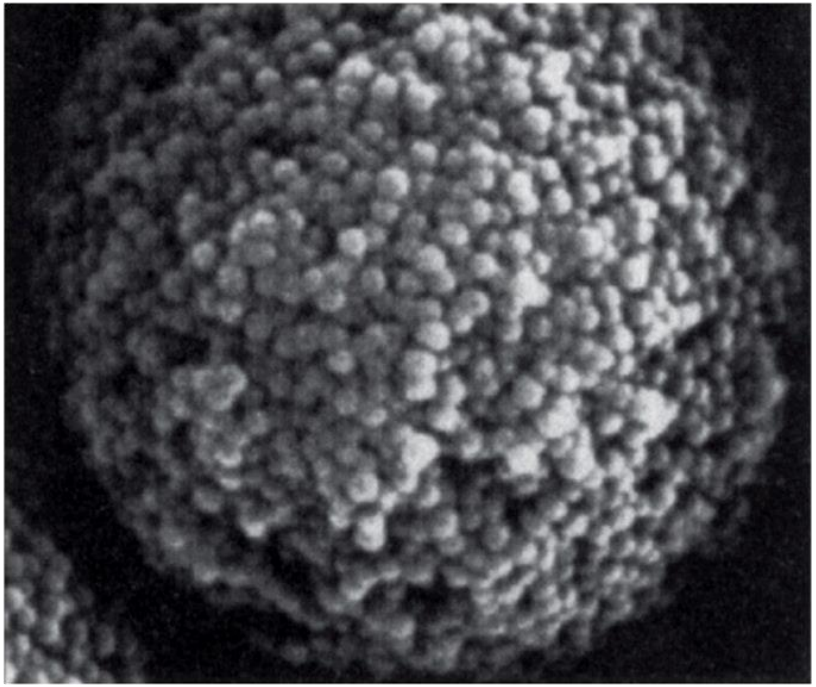
Demonstration*: *Column Chromatography.flv*

**** Do some critical thinking and find out the reasons why in the lab you will obtain a better separation than the student in this video clip.***

What Is the Secret? SILICA”

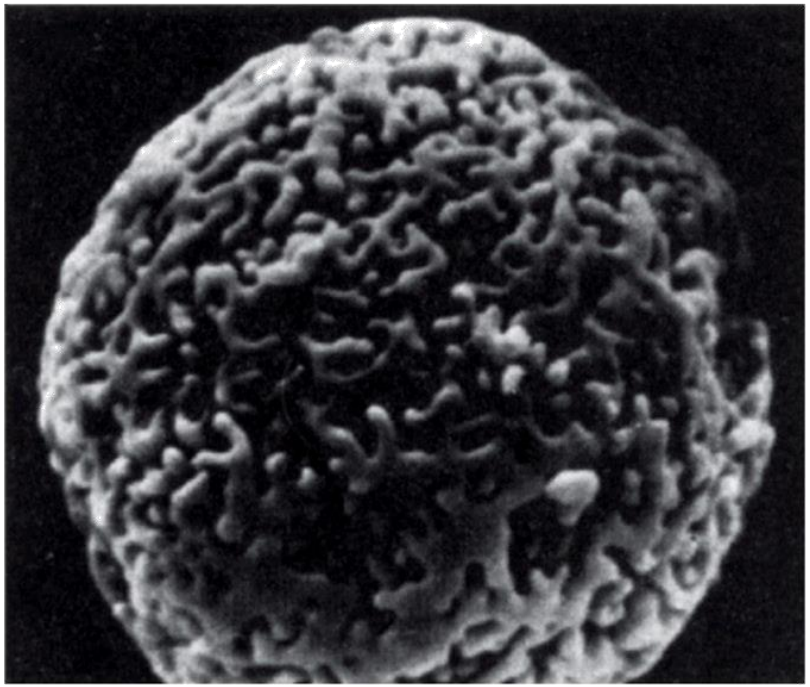
Electron micrographs of silica particles used in chromatography, *nominal* pore size 10 nm. Most native or bare silica should not be used above pH 8 where they dissolve nor at pH <3 where the surface groups are protonated, ***which groups???***

Spherical particle, **porosity 50%,** surface area **150 m^2 / g !!**



(a)

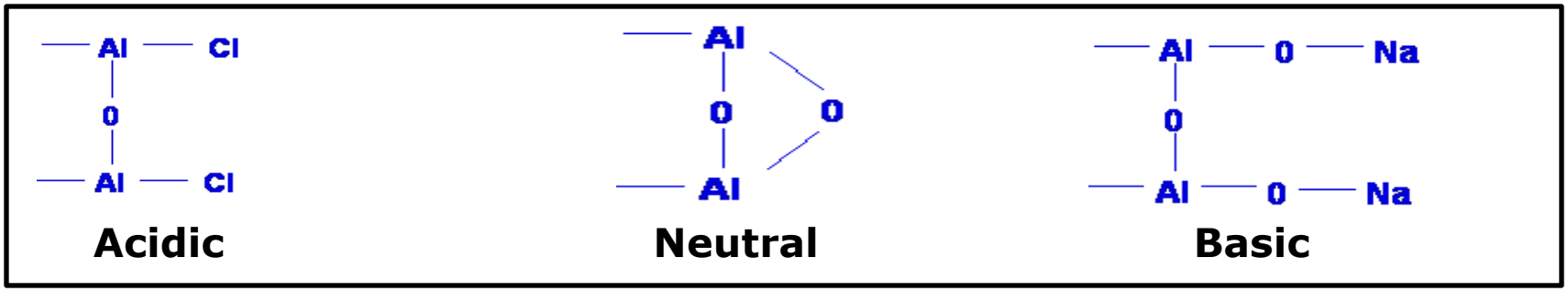
Sponge-like structure, **porosity 70%,** surface area **300 m^2 / g !!!!**



(b)

Stationary Phase: Alumina Al_2O_3

- More frequently in column chromatography than in TLC.
- Alumina is quite sensitive to the amount of water which is bound to it: the higher its water content, the less polar sites it has to bind organic compounds.
- The structure of alumina is complicated and somewhat uncertain and can be changed very significantly by heat treatment. The interactive groups on the surface of neutral alumina, in contact with aqueous solvent, are largely hydroxyl groups with similar interactive properties to the hydroxyl groups on the surface of silica gel.
- Different types according to manufacturing process: neutral, acidic or alkaline surface



Mobile Phases

Gases (He, N_2) are used in gas chromatography (called "carrier gas"), **liquids** are used in any other chromatographic technique (TLC, HPLC,…)

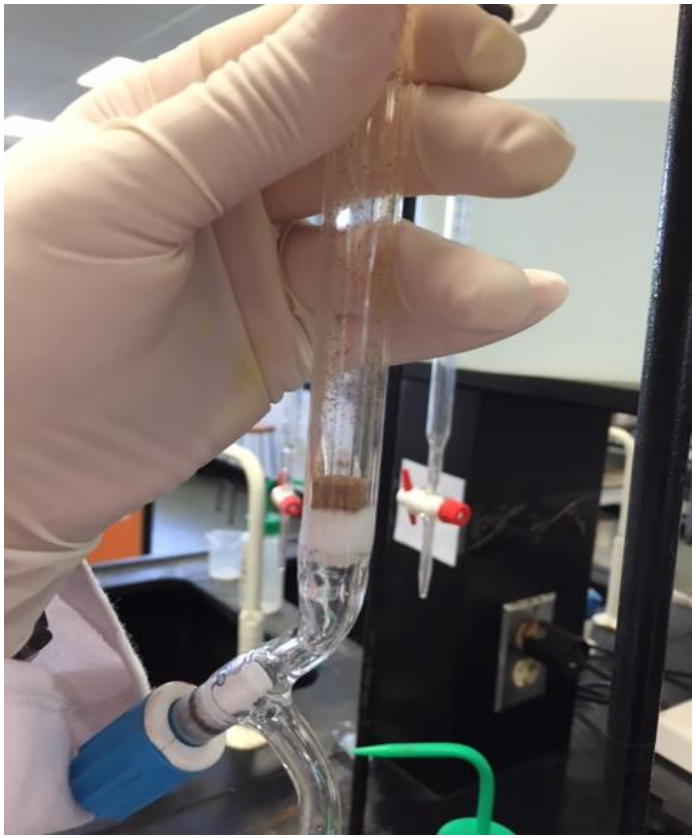
➢ Pure solvents (Analytical grade):

- Generally all solvents can be used for chromatography, but they must be immiscible with ST phase used to avoid dissolving or damaging it.
- Any chemical reactions with analytes must be avoided to keep the integrity of the chemical nature of the sample.
- Can consist of 2,3… or more pure solvents mixed together, where buffers or other salt solutions can be added for certain chromatographic purposes.

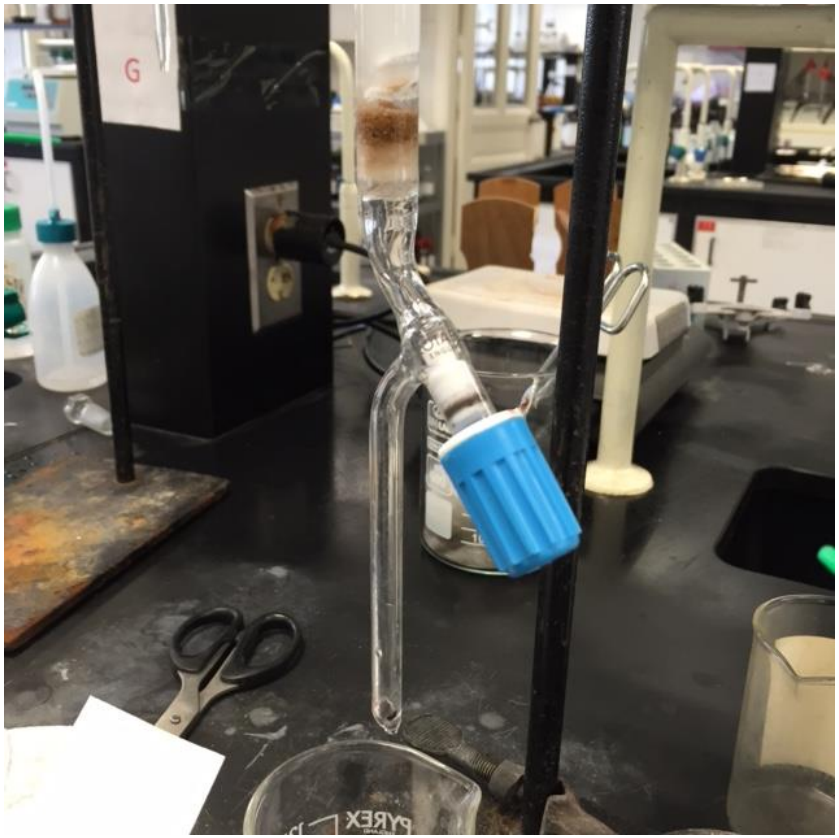
Isocratic elution	Gradient elution
The composition of the mobile phase is **constant** during chromatographic experiment	The composition of the mobile phase **changes** during chromatographic experiment

Preparing CC

1cm Cotton then 2cm Sand



Add Ethanol & Silica gel (or Alumina) by Dry or Wet method- Stationary phase



Stationary phase: contained in a column

Mobile phase: liquid, passes through column (gravity or pressure)

Make sure that Ethanol&Silica mixture is well packed, no bubbles.

The mixture must be slurry.

Open the stop cock & allow excess solvent to drain out.

CC- Separation of two dyes: Fluorescein sodium (red) & Methylene blue (dark green)

- Prepare a mixture of the two dyes by mixing them on a glass watch
- Dissolve the sample mixture in a minimum amount of solvent
- Transfer it to the column through the funnel
- Open the stopcock so the components of the mixture will run down the column forming two separate colored bands (you can add pressure to increase the rate of flow)
- Keep adding solvent, don't let it dry
- Collect each fraction in a separate flask

NB: Green band is the Fluorescein sodium (up) & Yellow band is the Methylene blue (down)

Column cleaning:

- Shake the column to suspend the silica gel or alumina
- Quickly invert the column over a waste container and shake out packing
- Rinse out remaining silica gel or alumina with water
- Rinse with Acetone
- Return the column clean & dry