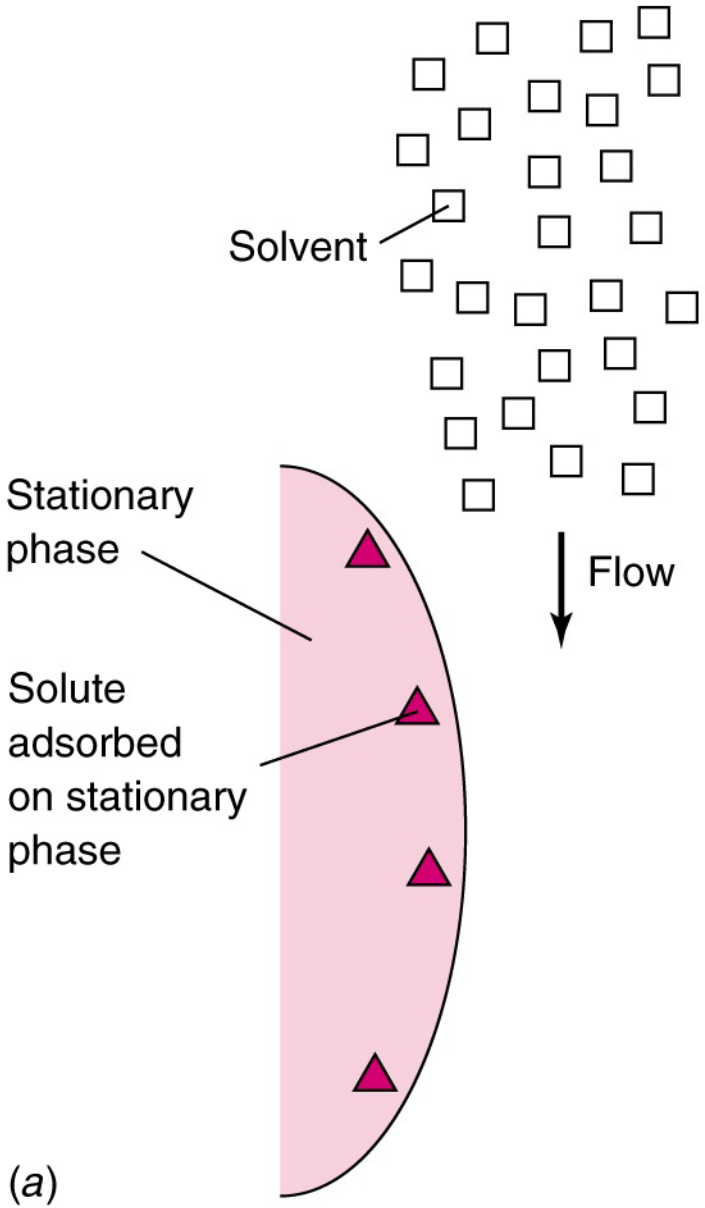
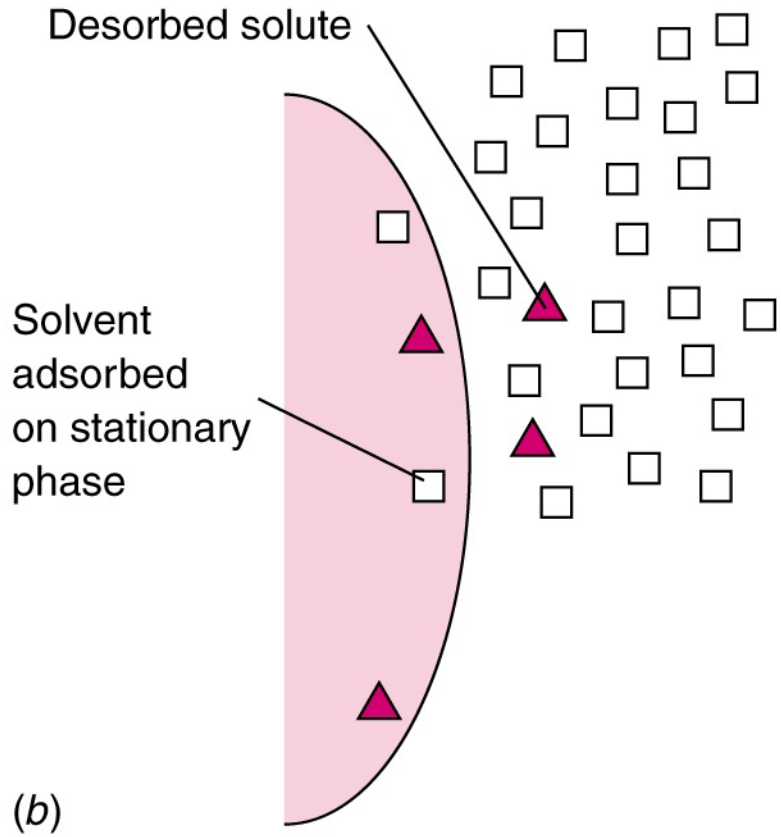
liquid chromatography!

Competition for solute adsorption on stationary phase by mobile phase solvent!



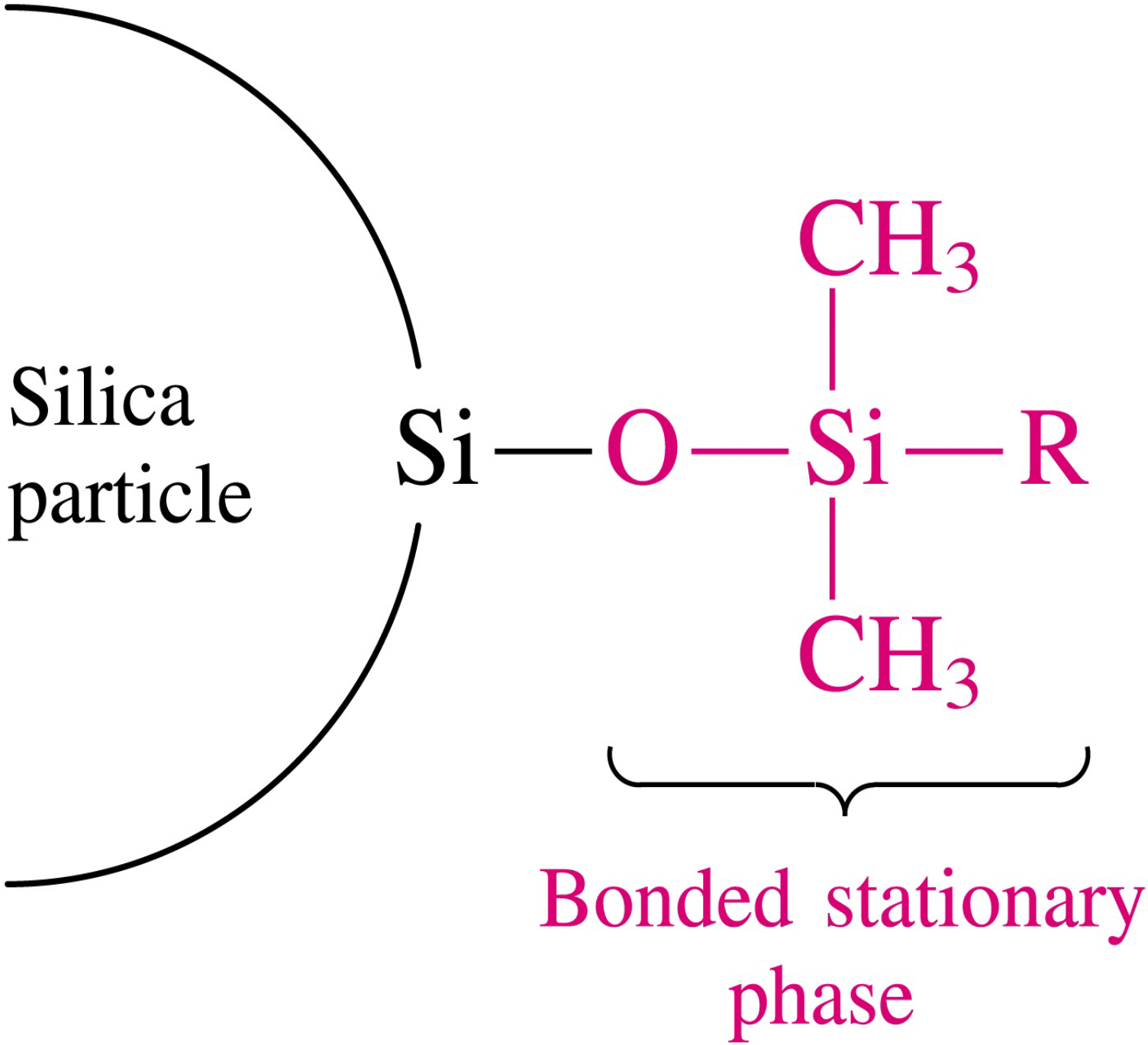


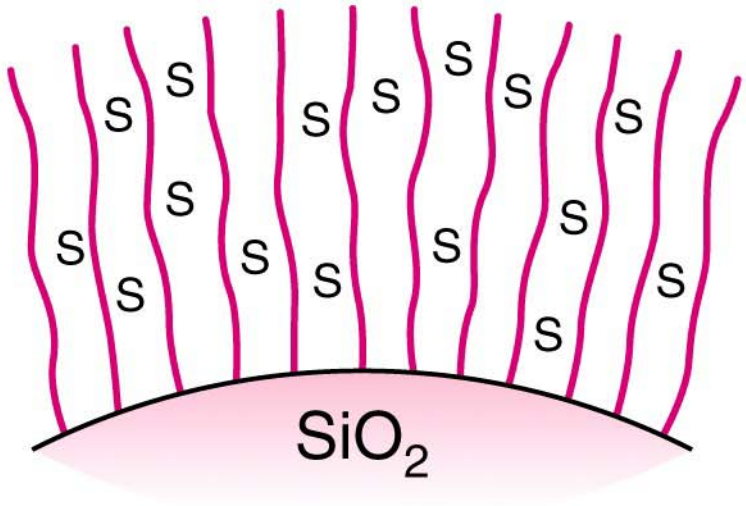
Liquid Chromatography --stationary phases!

Normal Phase Liquid Chromatography---use polar stationary phase packing (silica particles) and non-polar mobile phase
Elution order---non-polar species elute first---polar species elute last!

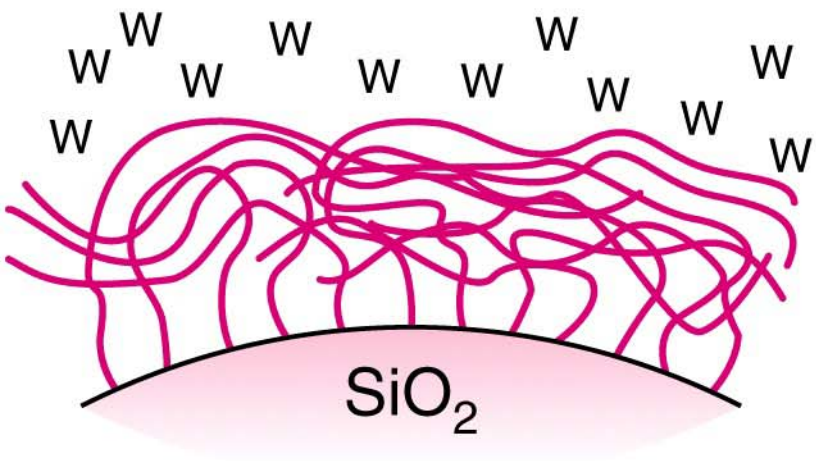
Reversed Phase (RP) Liquid Chromatography--use non-polar stationary phase (silica particles derivatized with organic coating) and polar mobile phase (water or alcohol, or acetonitrile, or mixture of these).

Eluent strength----in RP---more eluent strength when more of a less polar solvent in added to the mobile phase composition! This means K decreases for organic species (lower t_r value) by increasing eluent strength!


Silica particle
Si—O—Si—R
CH3
CH3
Bonded stationary phase

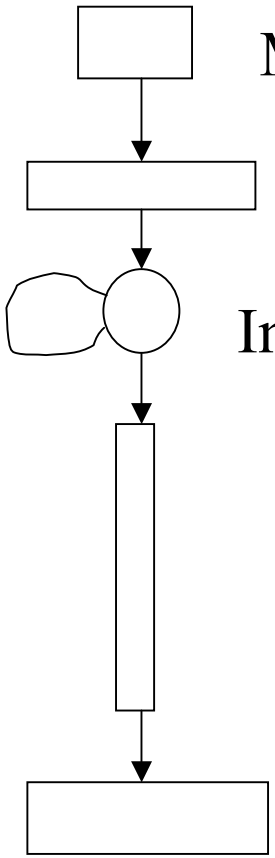


Stationary phase C_{18} chains extended straight in strongly solvating (organic) mobile phase, S



Collapsed C_{18} chains in weakly solvating (aqueous) mobile phase, W

Components of HPLC system:



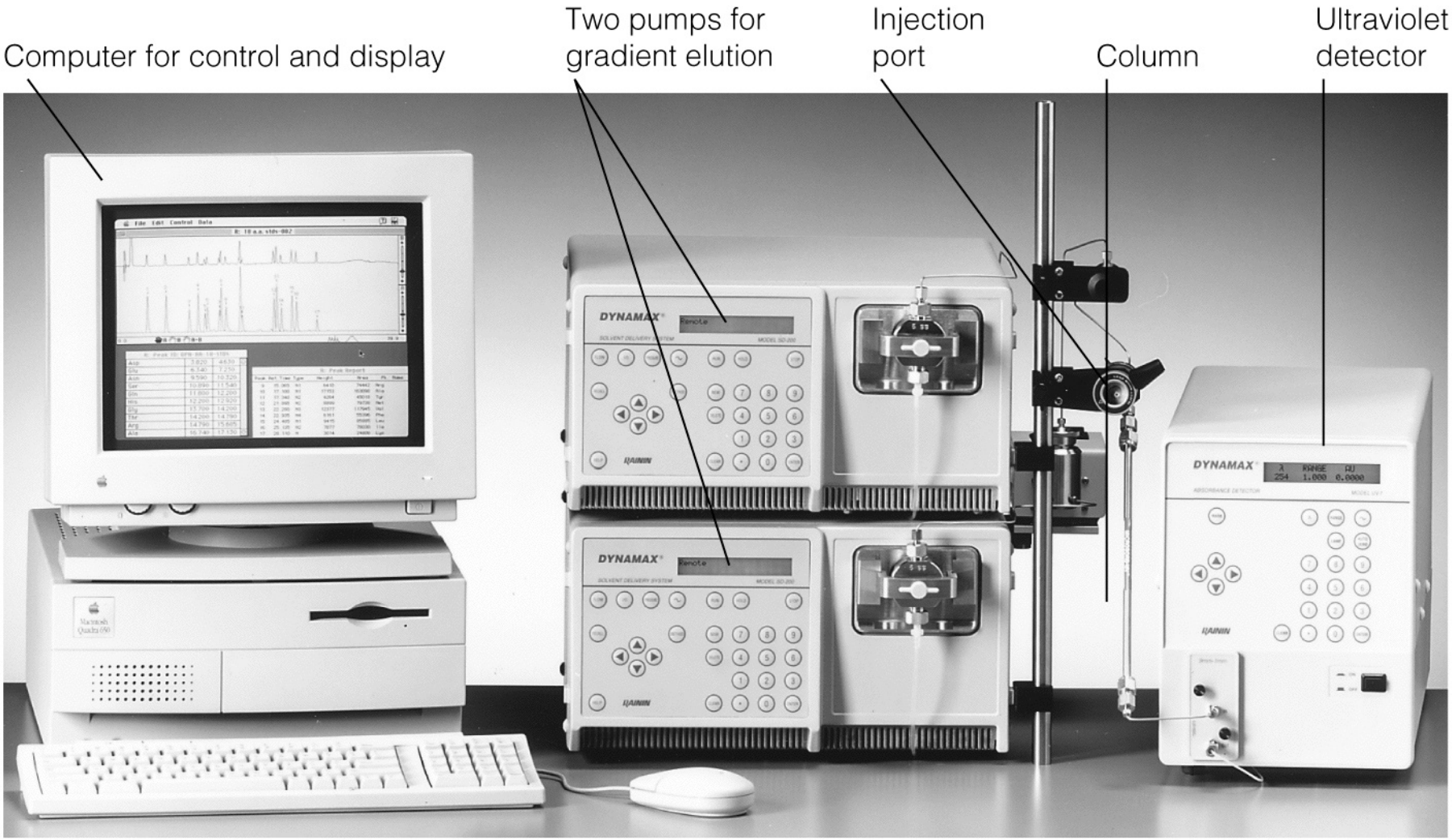
Mobile phase reservoir

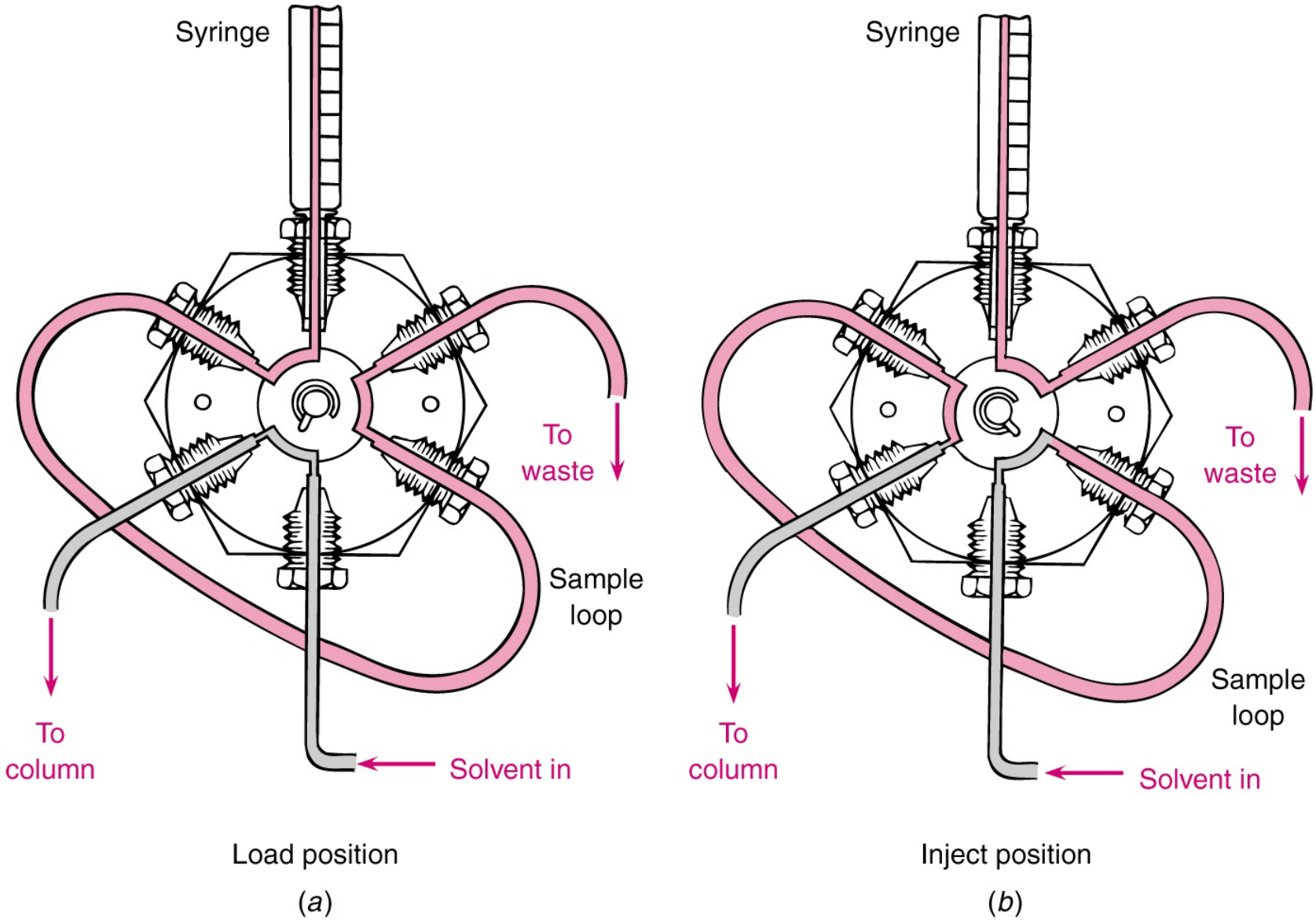
Pump--capable of forcing fluid through column with small d_p

Injection valve--25-100 μl

<u>Detector</u>--

- UV-Vis absorbance
- Refractive index
- Fluorescence
- Electrochemical
- Post-column rxn with one of above


Computer for control and display
Two pumps for gradient elution
Injection port
Column
Ultraviolet detector
DYNAMAX
Remote
DYNAMAX
Remote
DYNAMAX
RANGE
1.000
0.0000

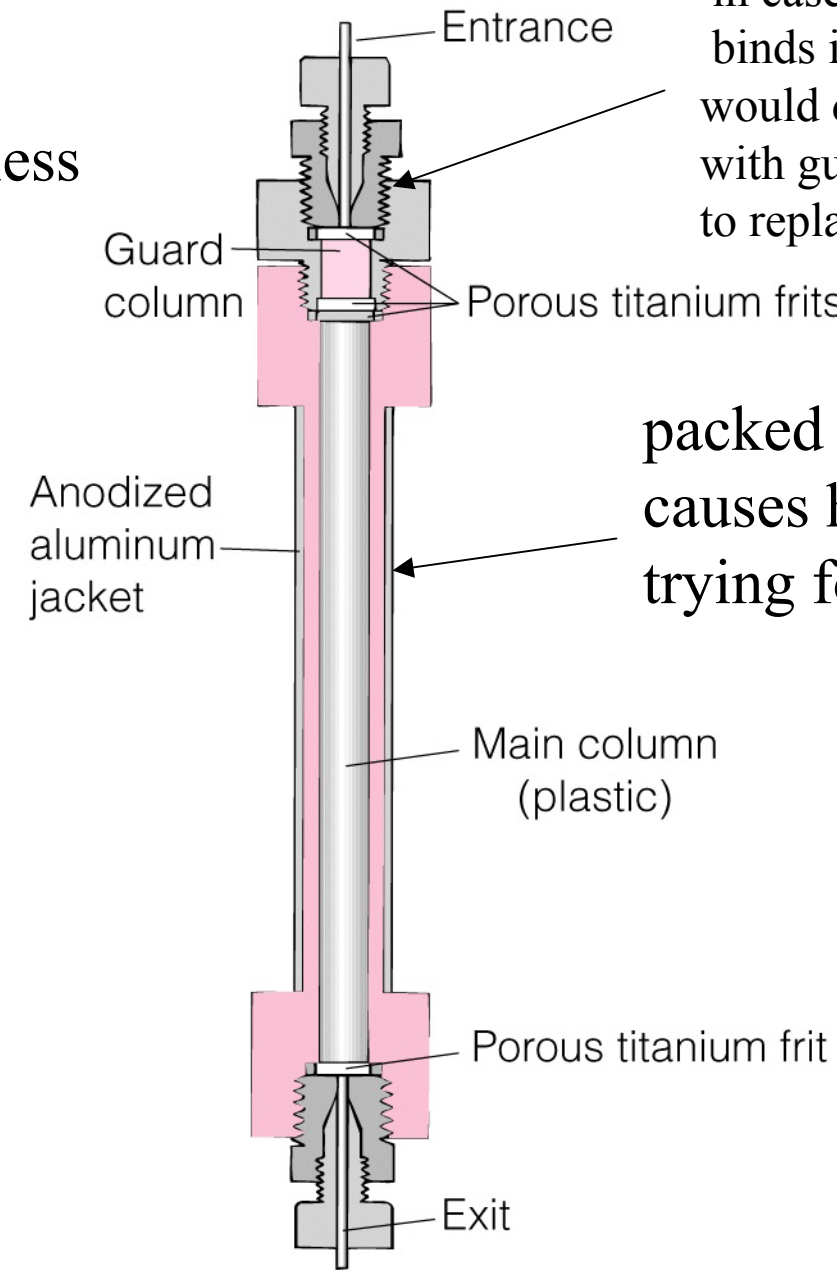


Injection loop---defines precise volume injected onto column---very reproducible from injection to injection!! (10-250 µL loops)

HPLC columns---

can also be
made of all stainless
steel!

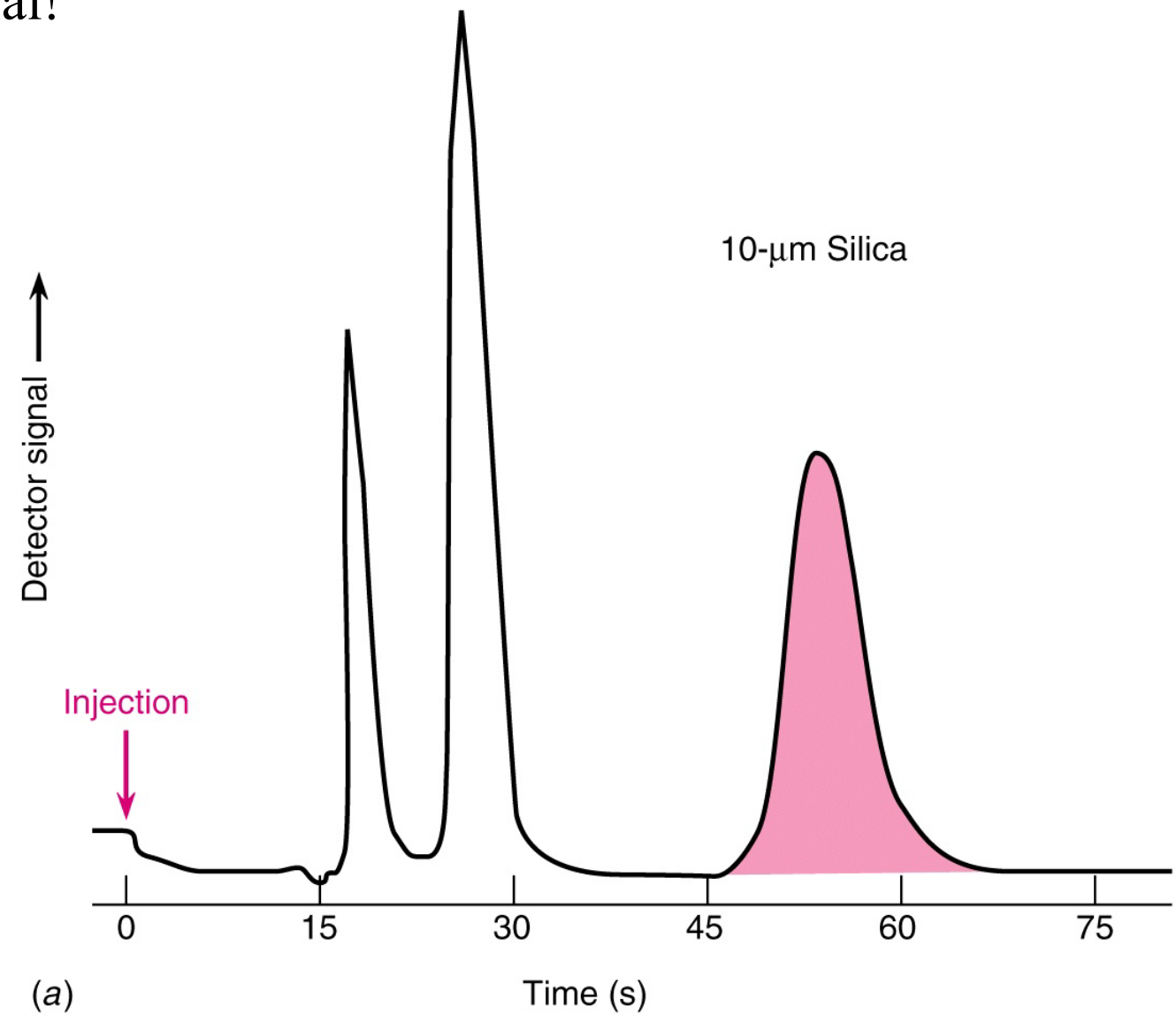
guard column---made of same packing as main column---present in case somespecies present that binds irreversibly to stationary phase-would destroy entire column---but with guard---you only need to replace this component!



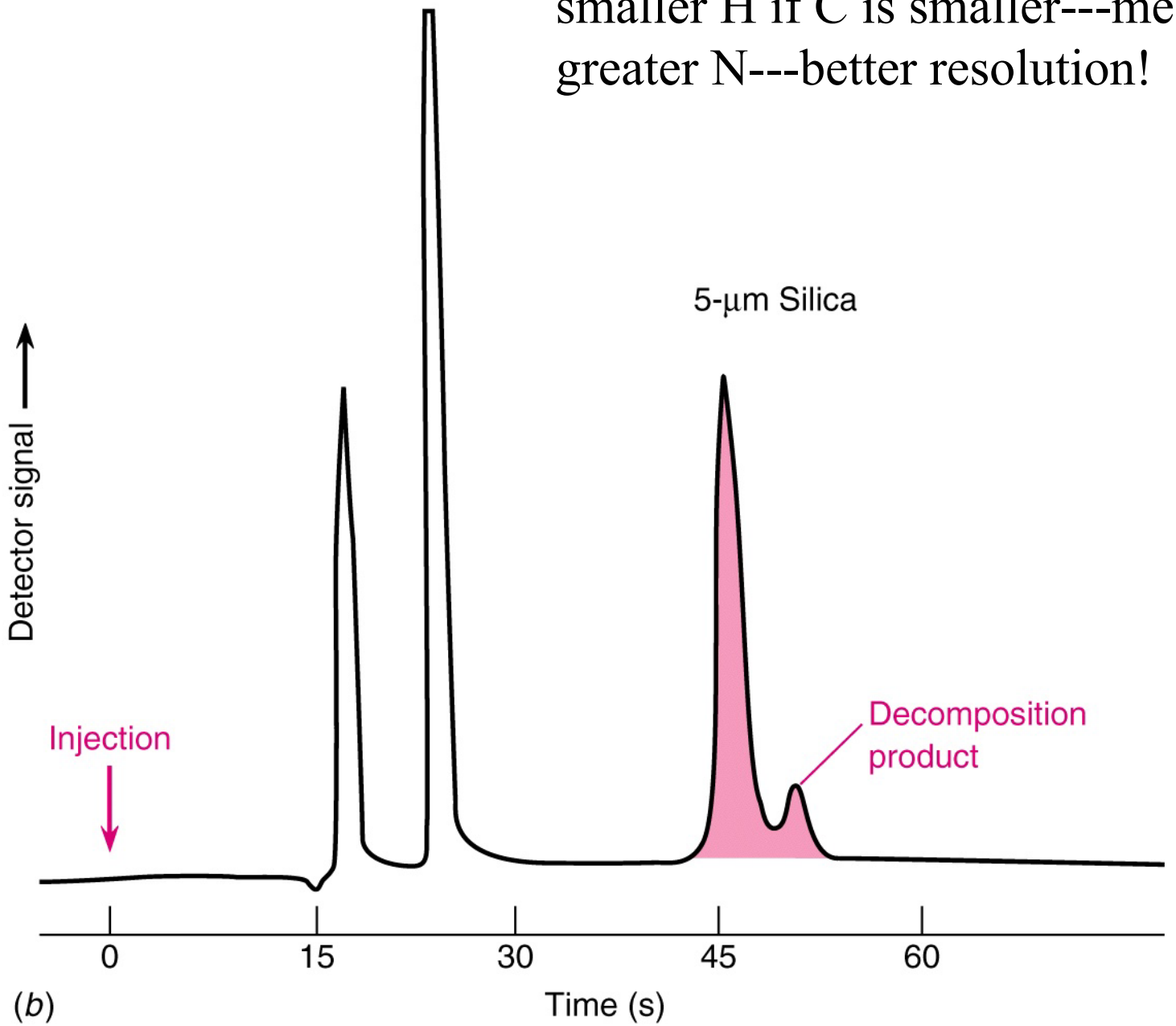
packed with small particles-causes high pressure when trying for force liquid through

Effect of size of packing material on separation!!---smaller is better

C-term of van-Deemter equation depends on d_p ---diameter of packing material!



smaller H if C is smaller---means greater N---better resolution!



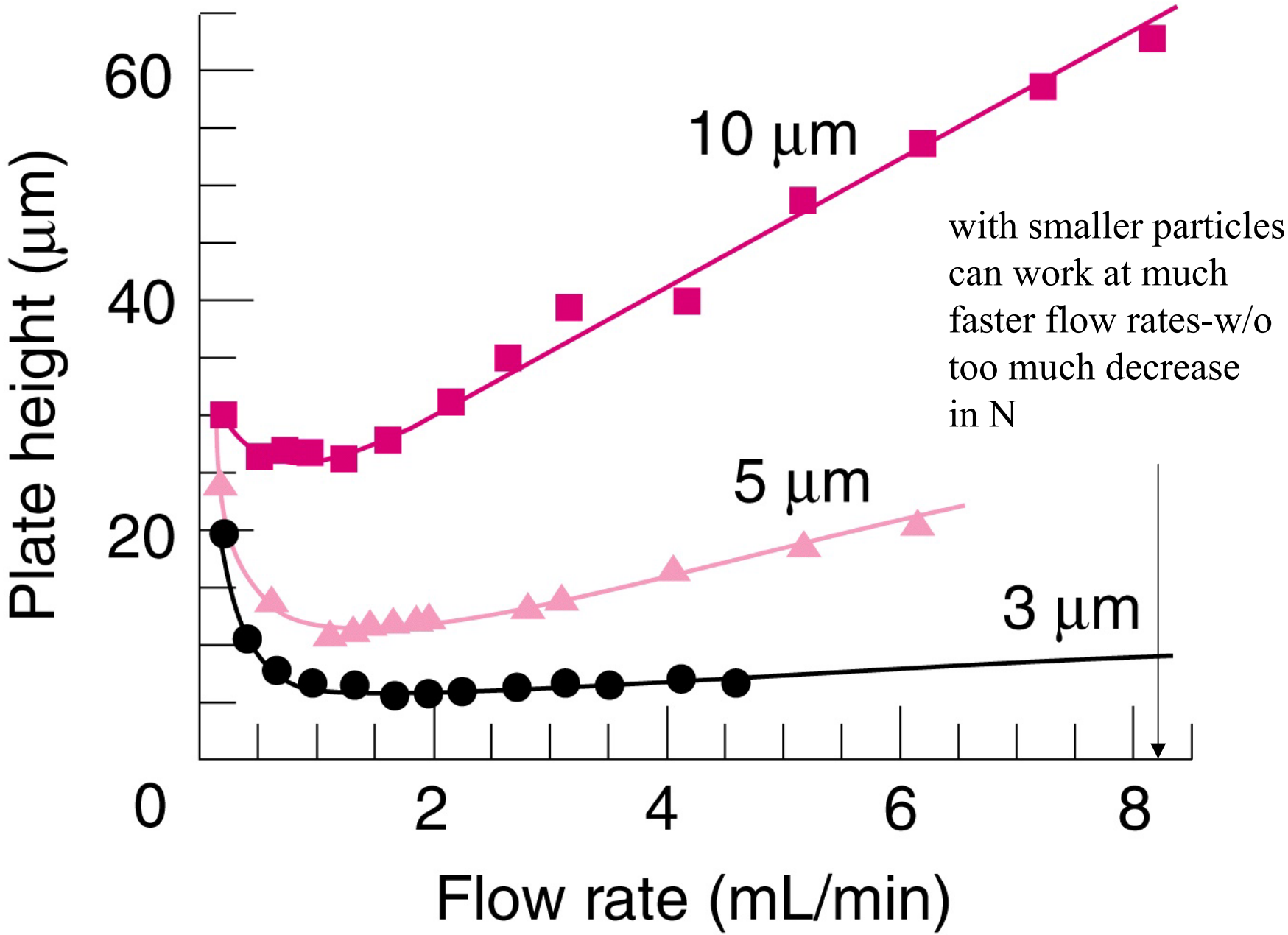
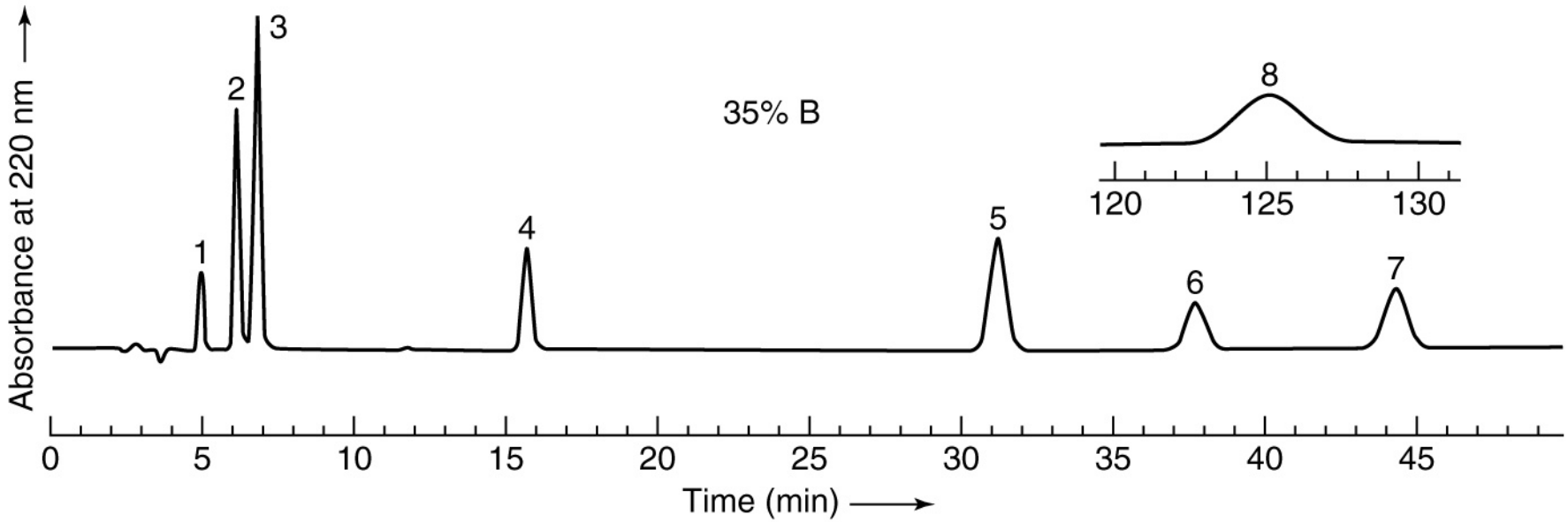
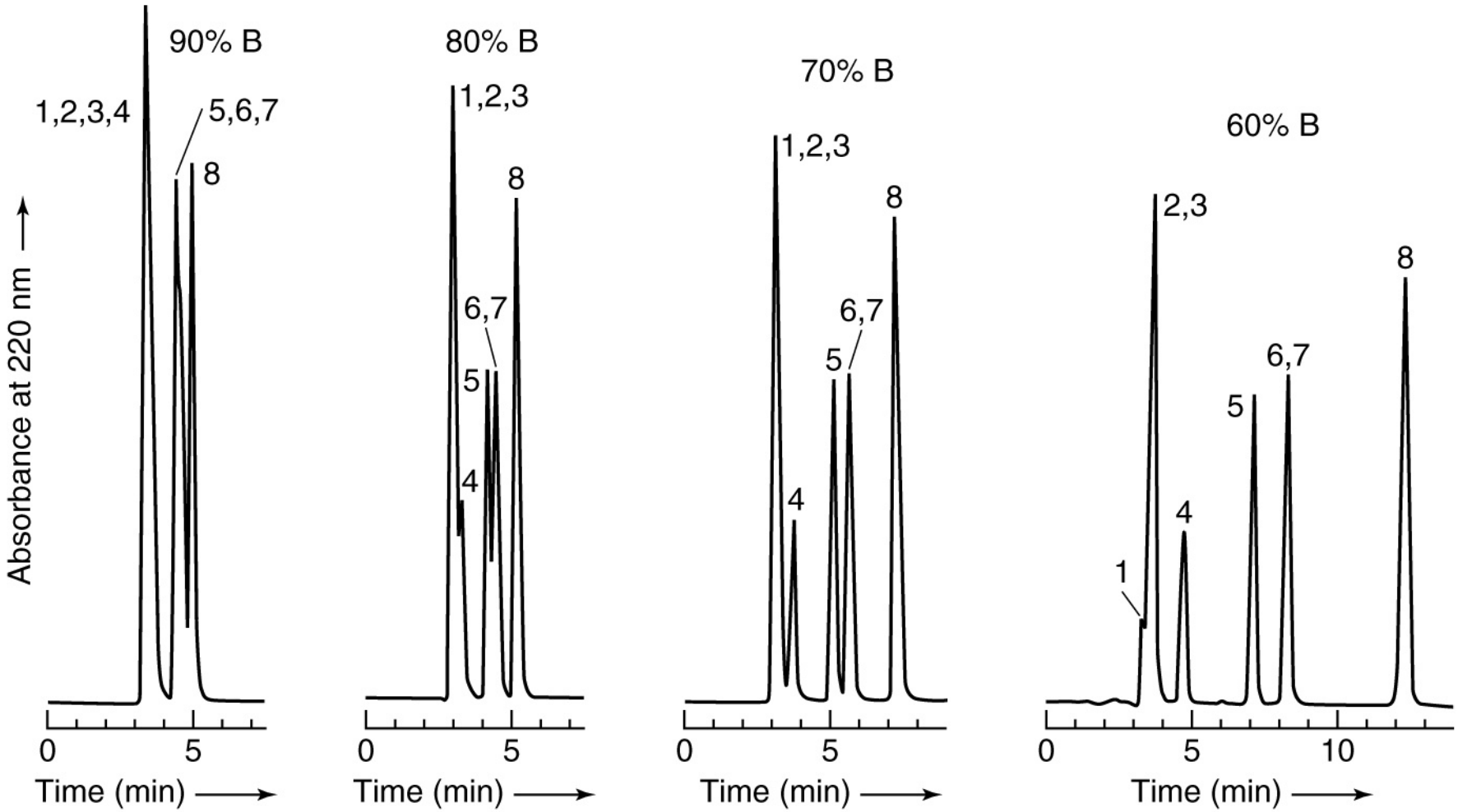


Plate height (µm)
60
40
20
0
2
4
6
8
Flow rate (mL/min)
10 µm
5 µm
3 µm
with smaller particles
can work at much
faster flow rates-w/o
too much decrease
in N

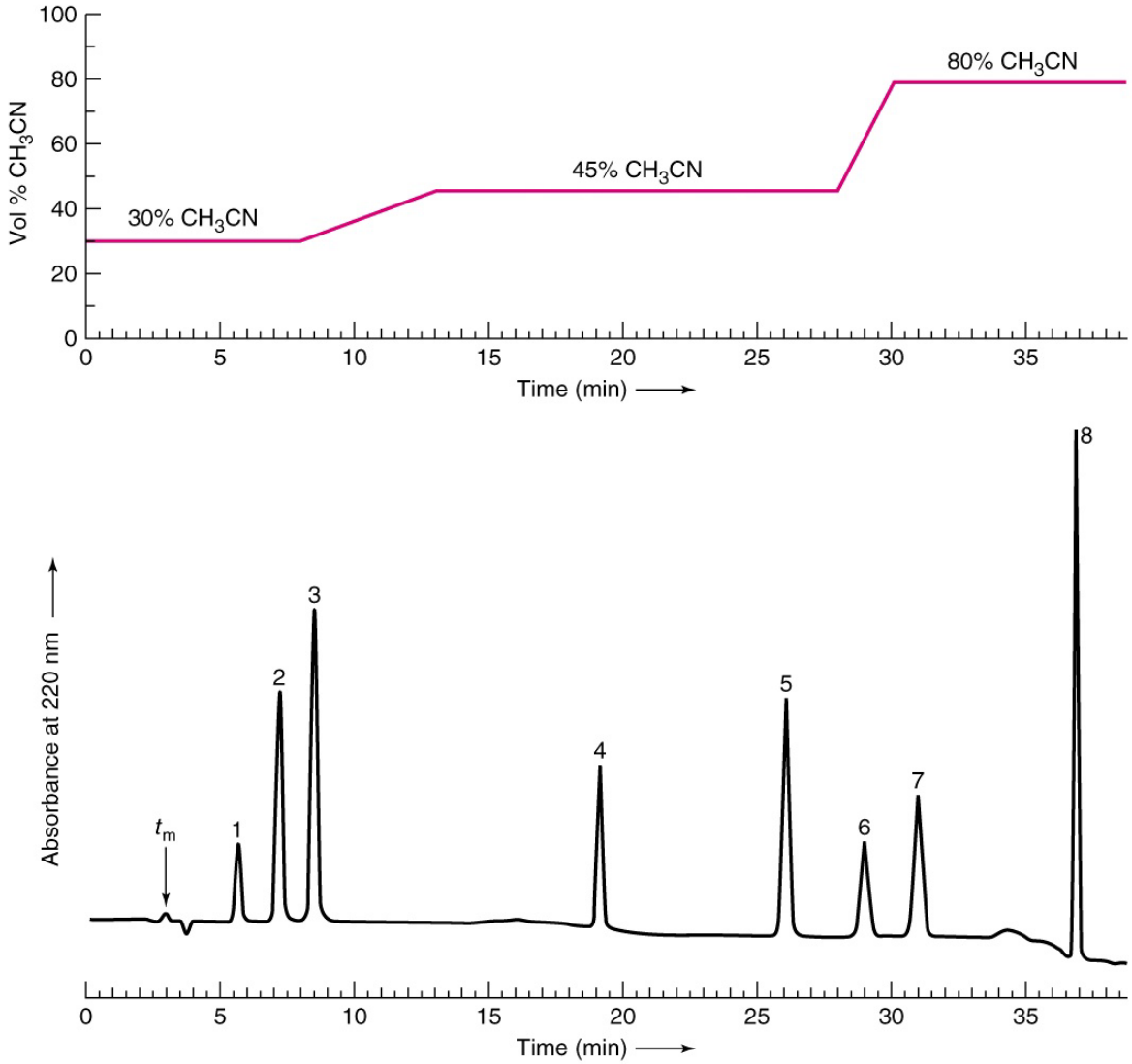
solutes--separated by **isocratic** mobile phase conditions----same mobile phase composition throughout the separation!---A is aqueous buffer---B is acetonitrile!



solutes with large K values---elute very very late----slows analysis time!!--


90% B
1,2,3,4
5,6,7
8
80% B
1,2,3
8
6,7
5
4
70% B
1,2,3
8
6,7
5
4
60% B
2,3
8
6,7
5
4
1
Absorbance at 220 nm
0
5
10
Time (min)

solution to problem---Use **Gradient Elution**---ala temperature programming in GC---change K for longer retention solutes--by changing mobile phasecomposition during separation!



Detectors---in LC---

most often use UV-vis absorption---with low dead volume detector!

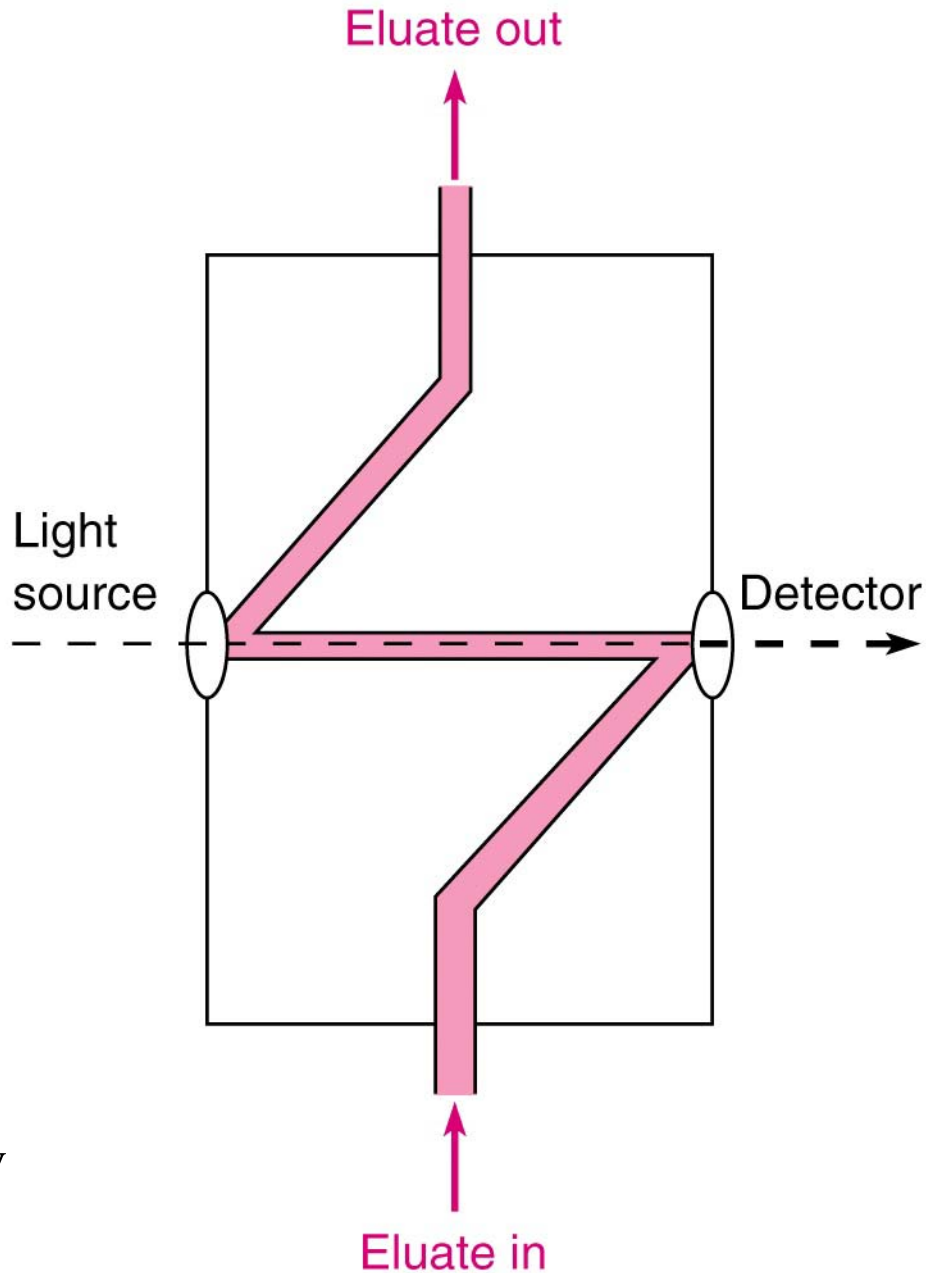
diode array to obtain full spectrum of each solute

or fixed wavelength---!

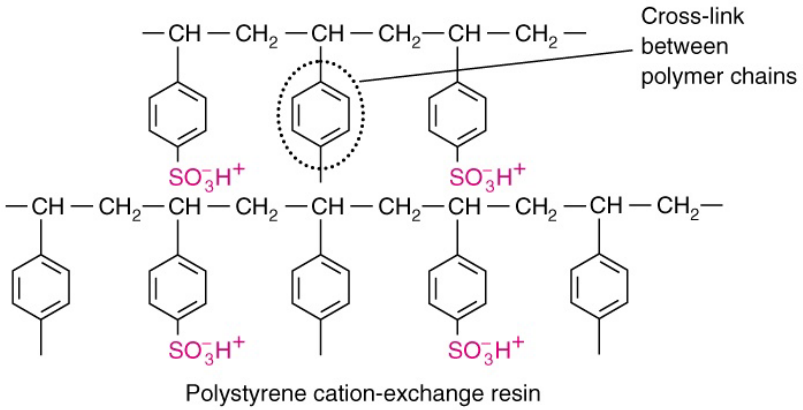
also--refractive index detectors

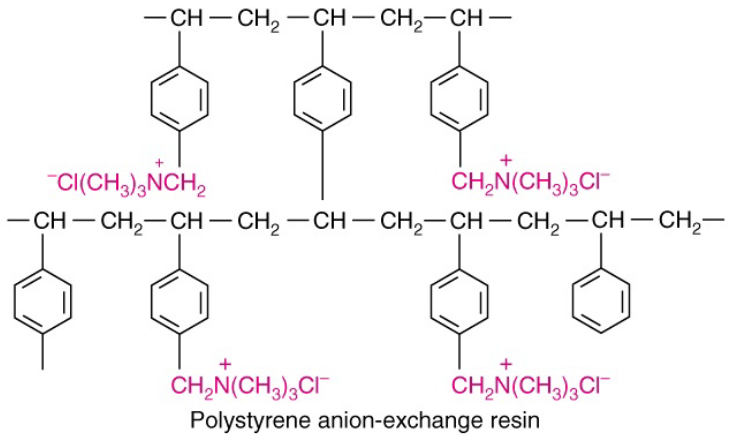
Can also use electrochemical detectors (including conductivity, amperometric potentiometric, etc.)

And---LC-MS; use electrospray MS system as detector!



Ion-Exchange chromatography---also an HPLC method---K based on electrostatic interaction of ions with charged stationary phases!--

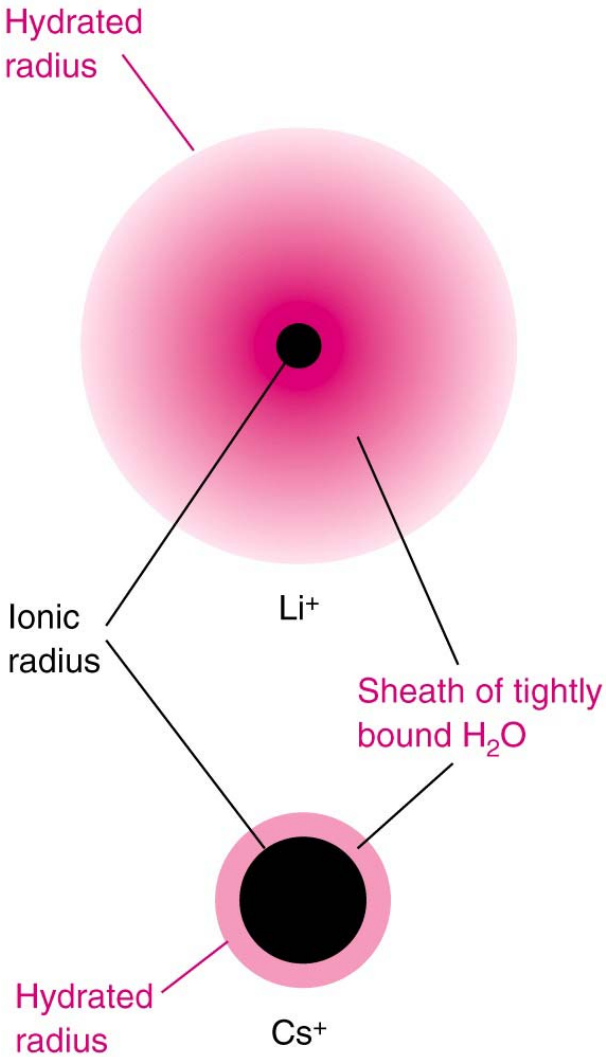


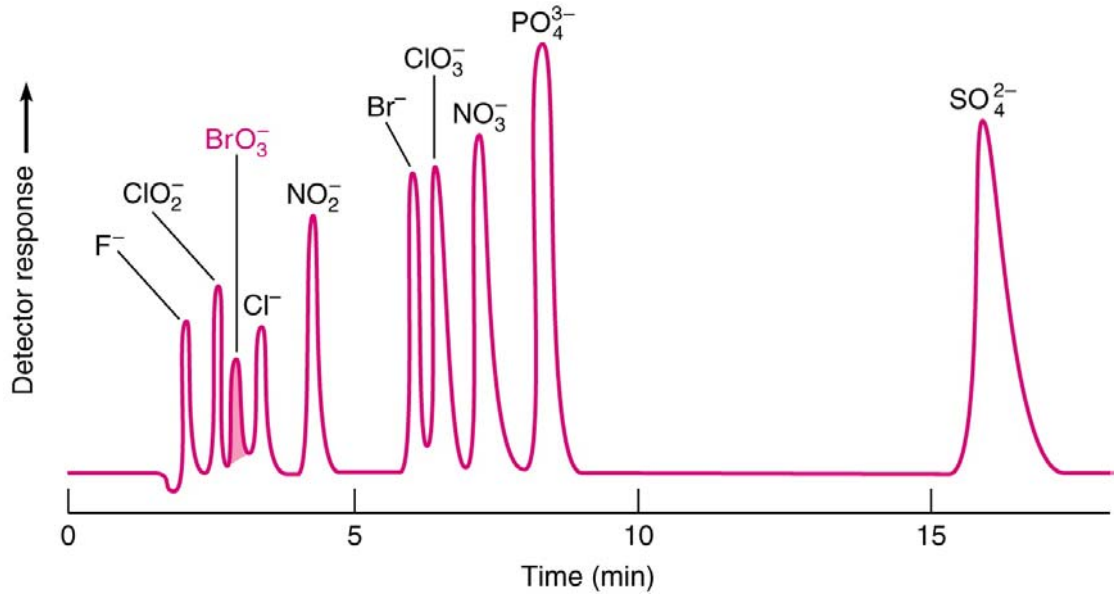


<u>in case of cation exchange column---</u>need to keep fixed concentration of given cation in mobile phase---usually by using acidic pH of mobile phase!

ion-<u>exchange equilibrium:</u>

$R\text{-}SO_3^-H^+ + M^+_{mob} \text{ <-------> } R\text{-}SO_3^-M^+ + H^+_{mob}$





<u>for cation exchange:</u>

t_r for $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$

larger ions are retained longer (with same charge and e-config)---K larger!

and trivalent > divalent > monovalent!

suppressor---
forms neutral
species from
mobile phase
components---
so background
conductivity is
low!!