GC cont'd

Requirements for Stationary Phases:

1. Low volatility---b.p. > 200^{o} C
2. Thermal Stability
3. Chemically inert--no rxns with solutes
4. Desirable Solvent Characteristics--i.e. appropriate K values for solutes being separated and detected
   - use polar phases to separate polar solutes
   -use non-polar phases to optimize separation of non-polar solutes

Examples---

Squalane---$C_{30}H_{62}$ ---good for separating hydrocarbons---max operating temp-- 150^{o}C

Carbowax-20-- a polyethylene glycol--
$HO\text{-}(CH_2\text{-}CH_2\text{-}O)_nCH_2CH_2OH$
good for amines, alcohols, etc.---operates to 250^{o}C

Some fundamentals---must make corrections for changes in volume of gas due to temperature and pressure--when reporting retention volumes in GC!

Flow rate usually measured at ambient temperature--but column temperature is usually much higher--

Therefore: $F_c = F_a (T_c/T_a)$ where T is in ^oK

F is in ml/min

Also---since gas is compressible--must correct for Pressure drop across column--since V_r is not simply F_c x t_R in GC; volume of gas in column is not the volume measured outside with flow meter

avg column pressure = p = $\frac{2}{3}\left(\frac{p_i^3 - p_o^3}{p_i^2 - p_o^2}\right)$

where p_i = inlet pressure

p_o = outlet pressure (1 atm)

compressibility factor = j

$$j = \frac{p_o}{p} = \frac{3}{2}\left[\frac{\left(p_i/p_o\right)^2 - 1}{\left(p_i/p_o\right)^3 - 1}\right]$$

Therefore--- for GC;

V^o_R = corrected retention volume = $jV_R = jF_ct_R$

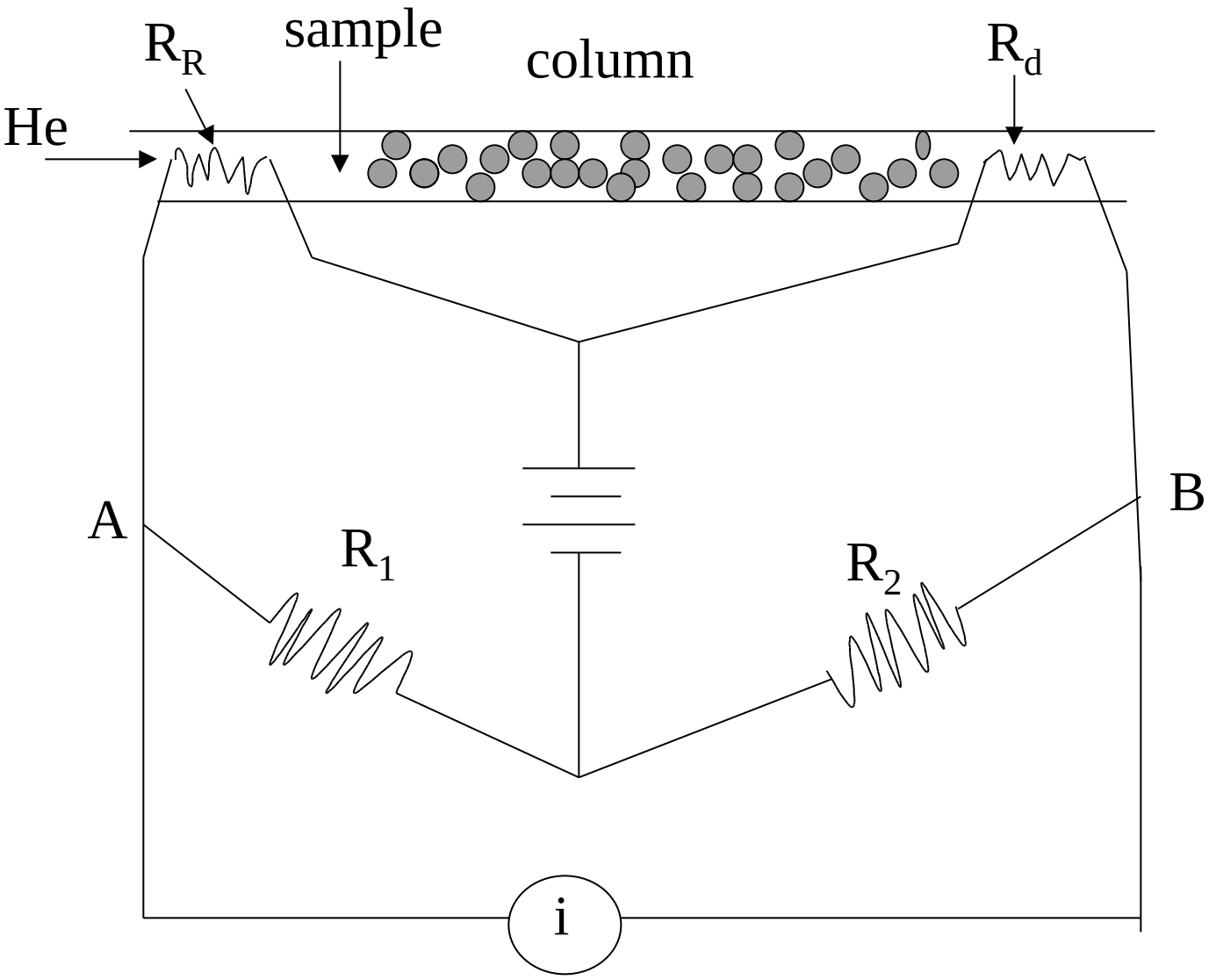
Detectors in GC---Most common--

1. Thermal conductivity--TCD
2. Flame ionization
3. Electron Capture
4. Mass Spectrometer

TCD Detector-based on change in thermal conductivity of gas stream as solute is eluted--Thermal conductivity of He or Hydrogen gas is 6-10 x that of most organics --N_2 and CO_2 cannot be used with TCD since thermal conductivities are similar to organics

Utilize platinum or tungsten filament wire in wheatstone bridge arrangement---as current passes through filament it heats up---and has given resistance at this temperature. When solute elutes in carrier gas, the temperature of the wire increases--and resistance of wire increases or decreases-depends on temperature coefficient of conductor!

typical configuration---used matched pair of filaments---one before sample introduction and one at end of column---



When $R_1 = R_2$ and $R_R = R_d$-----no current flow-when R_d changes---then voltage drop across AB-get current---flow--Want R_R and R_d to be matched filaments---change He flow rate--will effect temperature equally---

Advantages of TCD--

- Simple
- Rugged--used in field instruments
- Inexpensive
- Non-selective---can be good and bad
- Non-destructive---good for preparative GC

Disadvantage--not sensitive; 10 ng/ml gas

Flame Ionization Detector---FID

--use hydrogen/air flame to pyrolyze solutes---
form ions and free electrons in flame---that can carry current in gas phase of flame-current increases as solute species pass through detector---
See figure---
get larger current for larger organics that can fragmen into more ions and electrons than smaller organics

Advantages-

- Very sensitive---0.1 fg/ml
- Large linear response---7 orders of magnitude
- Can use any carrier gas--

Disadvantages

- Destructive to sample
- Requires cylinders of gas--not for field use

Electron Capture Detector---

Sample passed over β−emitter (high energy e^-)

^{63}Ni foil---

$He + \beta \longrightarrow He^+ + e^-$

-carrier gas is ionized by beta particles---

-free electrons produced in gas phase gives current when electric field is applied

-used pulsed field 1-3 μsec duration--

-then wait100-200 μsec between pulses to give chance for electrons to be captured by electro-negative atoms--

Get decrease in current measured when solute with electronegative atoms passes through detector-- halogens, phosphorous, silicon, nitro groups--

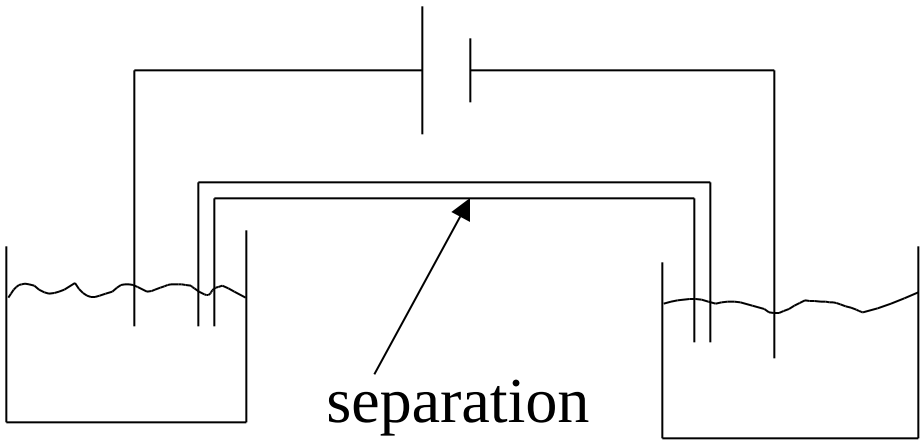
sensitivity highly dependent on # of electronegative atoms in solute---can be even more sensitive than FID in some cases---

Can form derivative of compound with fluorinated species--to make solute highly detectable by ECD-

$CF_3CH_2OH + RCOOH \rightarrow$ ester with F atoms

Mass Spec---with GC, just leak small amount of effluent into ionization source---for capillary columns--volume is low, all the gas can enter--
if using packed column, then you may use splitter to decrease amount of gas entering MS system--

Electrophoresis---separation of species based on movement in electrical field---
-carried out with paper, gels or capillaries (20-200μm)



solute moves according to charge and size--

distance travels = d = μt(E/S)

where μ=electrophoretic mobility
t = time
E/S= field strength=volts/distance between electrodes

d/t = velocity = $v = \mu(E/S)$

for two different solutes:

Δv = difference in velocity = $(\mu_A - \mu_B)(E/S)$

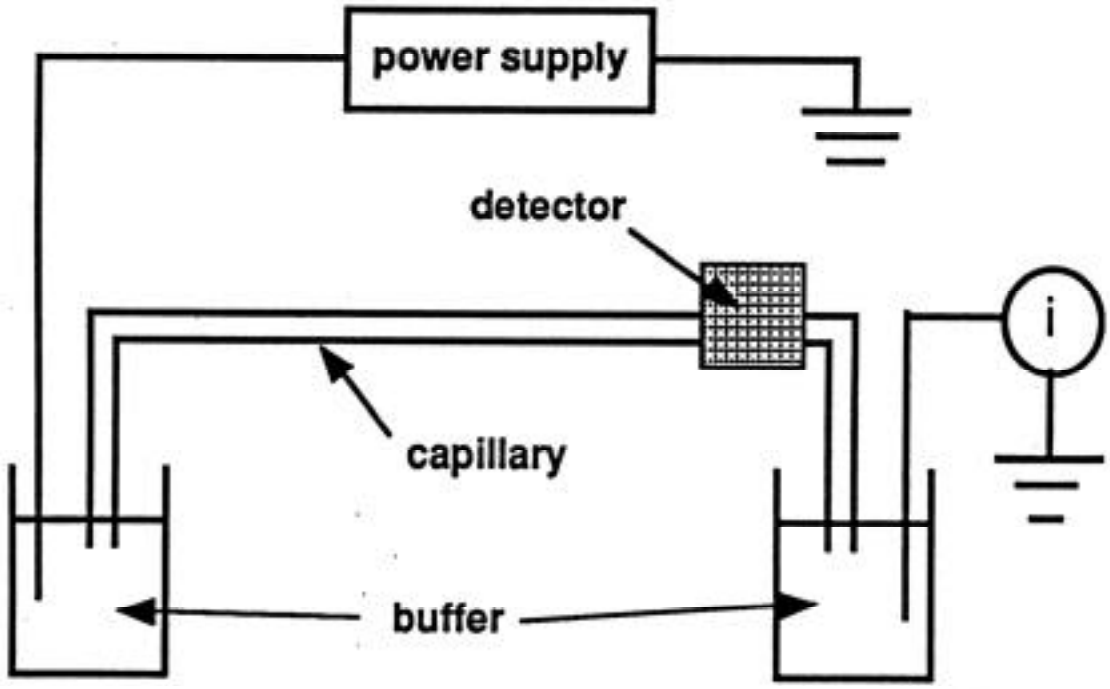
You get bigger difference in velocity if you increase E/S; field strength---also incrasing E/S speeds up separation time minimizing band broadening due to diffusion

However, if you use conventional gels or paper to do electrophoresis--increasing E/S will result in Joule heating (more current) --and this heating of sample will greatly increase diffusion, enhancing band broadening;

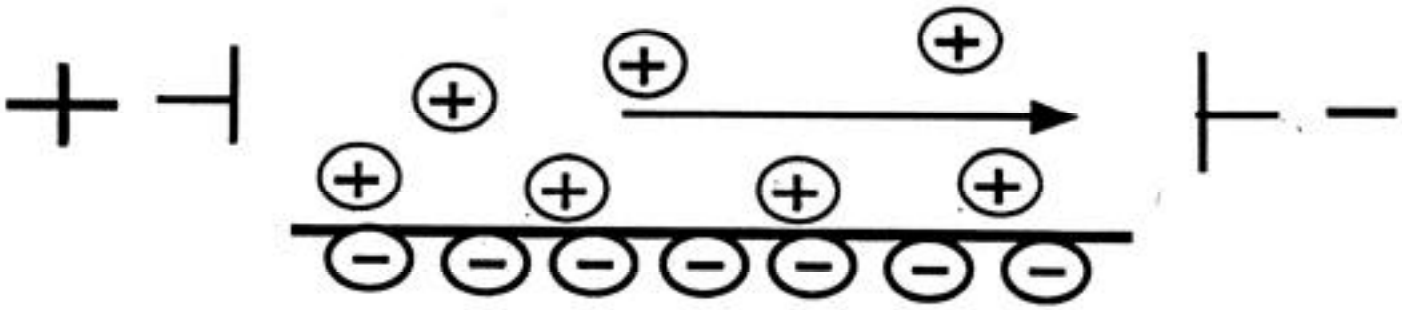
Solution: use tiny capillaries instead of large planar separation medium---this way you have large surface area to separation volumn; joule heat will dissipate more quickly----yielded modern capillary electrophoresis!

Use 20-30,000 volts; get million theoretical plates in 100 cm capillary; yet fast analysis time

Capillary Electrophoresis



Blow up of capillary wall:



Can separate both positive and negatively charged species---owing to electroosmotic flow!

indeed: velocity of solute = v = $(\mu_e + \mu_{eo})$ (E/S)

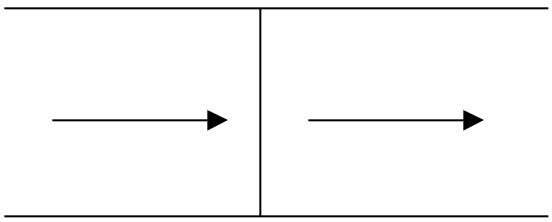
μ_e =standard electrophoretic mobility

μ_{eo} = electroosmotic mobility---due to movement of solvent in capillary

if μ_e is negative---can still get solute to come out to detector--if μ_{eo} is appreciable!

electroosmotic flow--due to wall of capillary being charged--usually negative; counterions are cationic in solution----solution flows toward negatively charged electrode! get bulk flow--

Key is that flow is a flat profile if capillary is narrow



when you pump solution through tube--you usually get parabolic flow---which contributes more to band broadening!

modes of operation:

- CZE--electrolyte solution in capillary
- capillary electrokinetic chromatography--use micelles in solution to allow separation of neutrals
- capillary gel electrophoresis--used for sieving--based on size and charge---used for DNA separations and sequencing