Requirements for Stationary Phases:
1. Low volatility---b.p. > 200° 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°C
Carbowax-20--- a polyethylene glycol--
   HO-(CH_{2}-CH_{2}-O)_{n}CH_{2}CH_{2}OH
   good for amines, alcohols, etc.---operates to 250°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: \( F_c = F_a \left( \frac{T_c}{T_a} \right) \) where \( T \) is in °K

\( F \) is in ml/min

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

The average column pressure is:

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

where \( p_i = \) inlet pressure

\( p_o = \) outlet pressure (1 atm)

compressibility factor = \( j \)

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

\[
j = \frac{3}{2} \left[ \left( \frac{p_i}{p_o} \right)^3 - 1 \right]
\]
Therefore--- for GC;

\[ V^o_R = \text{corrected retention volume} = jV_R = jF_c t_R \]

### 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--\( \text{N}_2 \) and \( \text{CO}_2 \) 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 fragment
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 $\beta$–emitter (high energy e$^-$)

$^{63}$Ni foil---
He + $\beta$-----> 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 $\mu$sec duration--
then wait100-200 $\mu$sec between pulses to give chance for electrons to be captured by electronegative 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--
$\text{CF}_3\text{CH}_2\text{OH} + \text{RCOOH} \rightarrow$ 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)

\[
\text{distance travels } = d = \mu t (E/S)
\]

where \( \mu \)=electrophoretic mobility

\( t = \text{time} \)

\( E/S= \text{field strength}=\text{volts/distance between electrodes} \)
d/t = velocity = v = \mu (E/S)

for two different solutes:
\[ \Delta v = \text{difference in velocity} = (\mu_A - \mu_B) (E/S) \]

You get bigger difference in velocity if you increase E/S; field strength---also increasing 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 volume; 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:

[Diagram of the process]
Can separate both positive and negatively charged species---owing to electroosmotic flow!

Indeed: velocity of solute \( v = (\mu_e + \mu_{eo}) \frac{E}{S} \)
\( \mu_e \) = standard electrophoretic mobility
\( \mu_{eo} \) = electroosmotic mobility---due to movement of solvent in capillary
If \( \mu_e \) is negative---can still get solute to come out to detector--if \( \mu_{eo} \) 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