Gas Chromatography

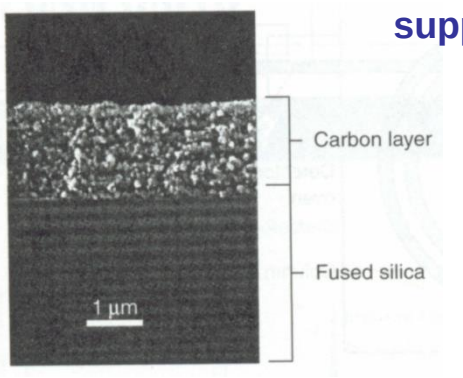
Introduction

1.) Gas Chromatography

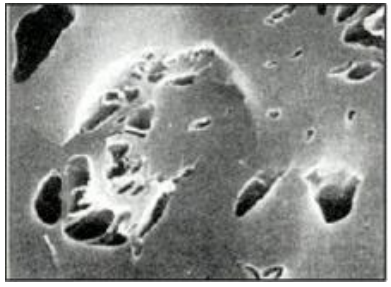
- Mobile phase (carrier gas) is a gas
  - **Usually N_2, He, Ar and maybe H_2**
  - **Mobile phase in liquid chromatography is a liquid**

- Requires analyte to be either <u>naturally volatile</u> or can be converted to a <u>volatile derivative</u>
  - **GC useful in the separation of small organic and inorganic compounds**

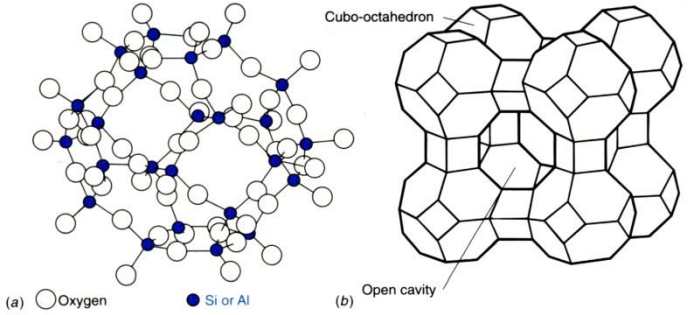
- Stationary phase:
  - ***Gas-liquid partition chromatography* – nonvolatile liquid bonded to solid support**
  - ***Gas-solid chromatography* – underivatized solid particles**
  - ***Bonded phase gas chromatography* – chemical layer chemically bonded to solid support**



Bonded phase



Magnified Pores in activated carbon



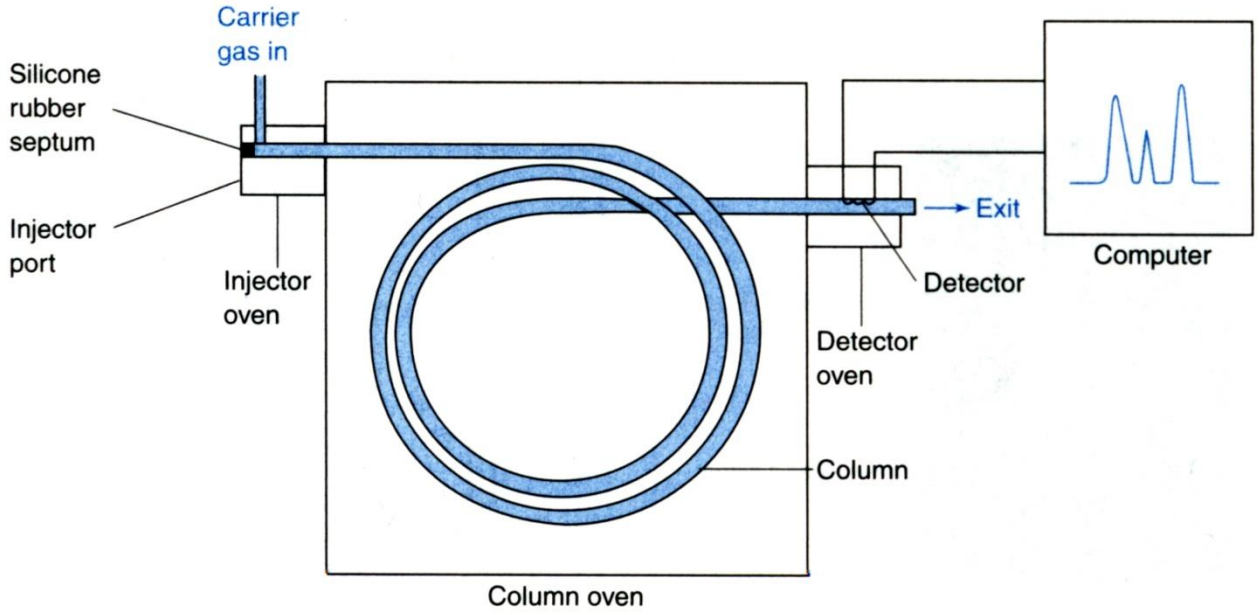
Zeolite molecular sieve

Gas Chromatography

Introduction

2.) Instrumentation

- Process:
  - **Volatile liquid or gas injected through septum into heated port**
  - **Sample rapidly evaporates and is pulled through the column with carrier gas**
  - **Column is heated to provide sufficient vapor pressure to elute analytes**
  - **Separated analytes flow through a heated detector for observation**



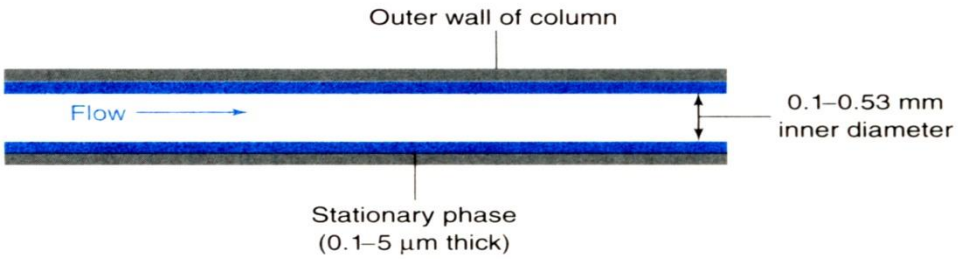
Gas Chromatography

Instrumentation

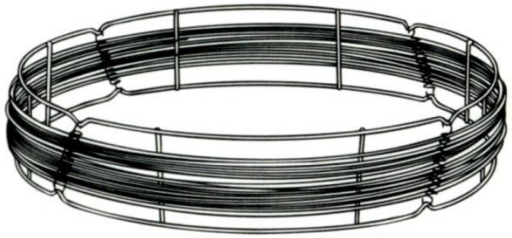
1.) Open Tubular Columns

- Commonly used in GC
- Higher resolution, shorter analysis time, and greater sensitivity
- Low sample capacity

- Increasing Resolution
  - **Narrow columns → Increase resolution**



  - **Resolution is proportional to $\sqrt{N}$, where *N* increases directly with column length**

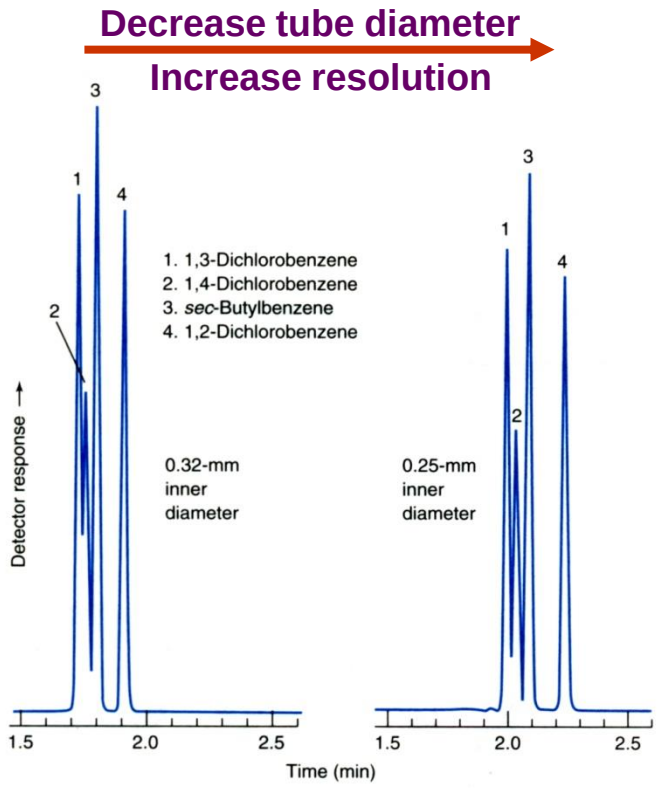


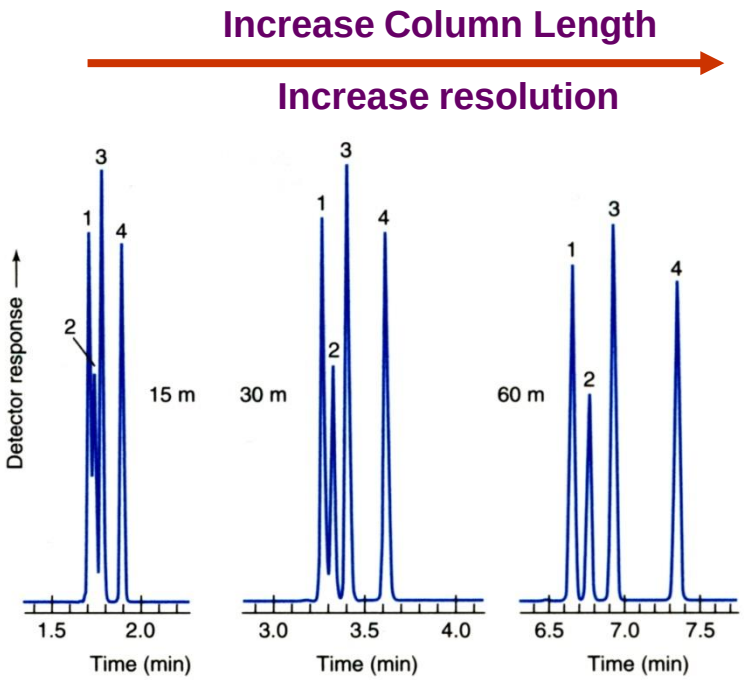
Easy to generate long (10s of meters) lengths of narrow columns to maximize resolution

Gas Chromatography

Instrumentation

1.) Open Tubular Columns
- Increasing Resolution





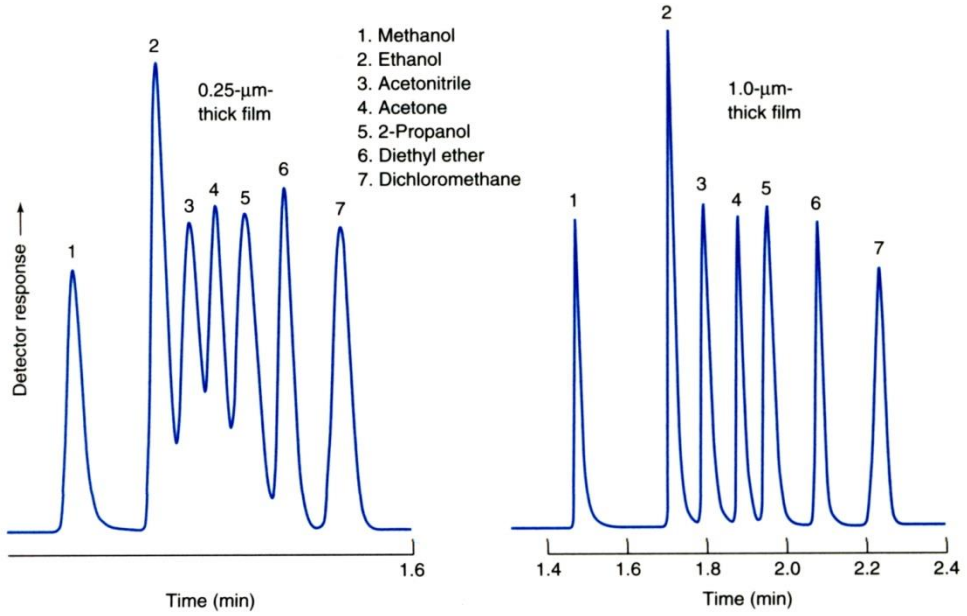
Gas Chromatography

Instrumentation

1.) Open Tubular Columns
- Increasing Resolution

Increase Stationary Phase Thickness

Increase resolution of early eluting compounds



Also, increase in capacity factor and reduce peak tailing

But also decreases stability of stationary phase

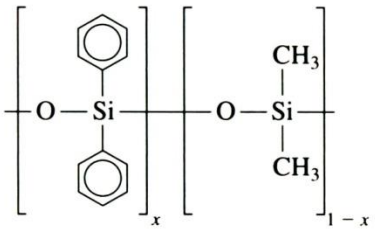
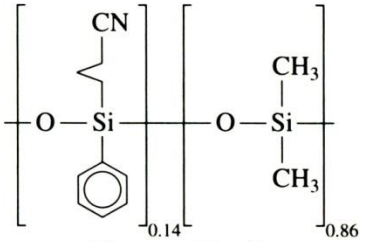
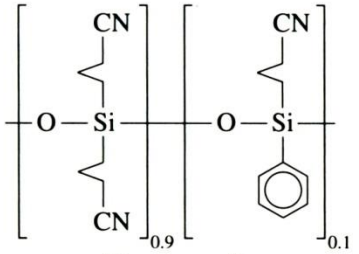
Gas Chromatography

Instrumentation

2.) Choice of liquid stationary phase:

- Based on "like dissolves like"
- Nonpolar columns for nonpolar solutes
- Strongly polar columns for strongly polar compounds
- To reduce "bleeding" of stationary phase:
  - **bond (covalently attached) to silica**
  - **Covalently cross-link to itself**

Table 24-1 Common stationary phases in capillary gas chromatography

Structure	Polarity		Temperature range (°C)
 (Diphenyl)$_x$(dimethyl)$_{1-x}$ polysiloxane	$x = 0$	Nonpolar	−60°–320°
	$x = 0.05$	Nonpolar	−60°–320°
	$x = 0.35$	Intermediate polarity	0°–300°
	$x = 0.65$	Intermediate polarity	50°–370°
 (Cyanopropylphenyl)$_{0.14}$ (dimethyl)$_{0.86}$ polysiloxane	Intermediate polarity		−20°–280°
 Carbowax (poly(ethylene glycol))	Strongly polar		40°–250°
 (Biscyanopropyl)$_{0.9}$ (cyanopropylphenyl)$_{0.1}$ polysiloxane	Strongly polar		0°–275°

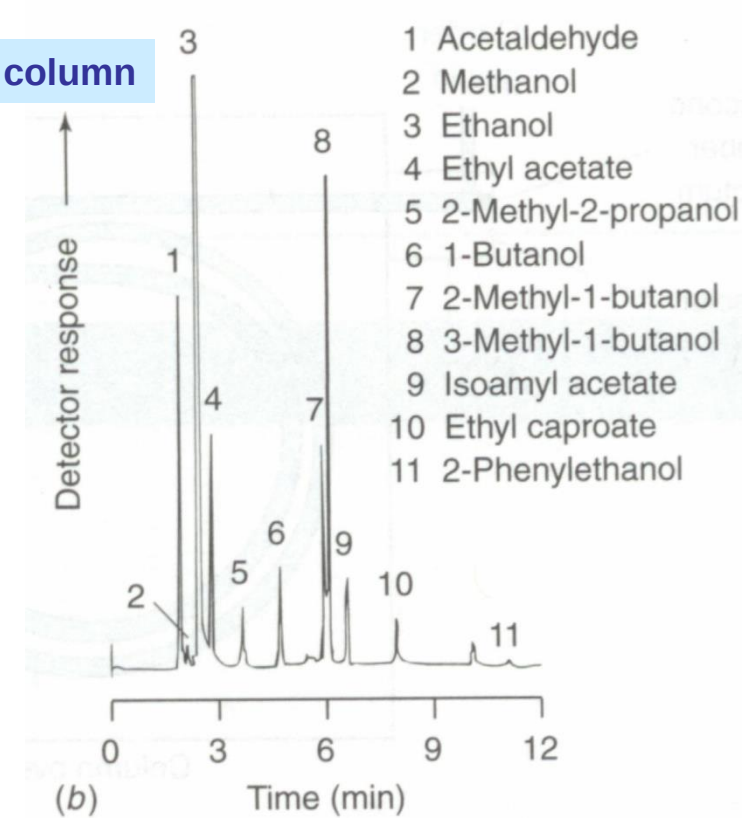
Gas Chromatography

Instrumentation

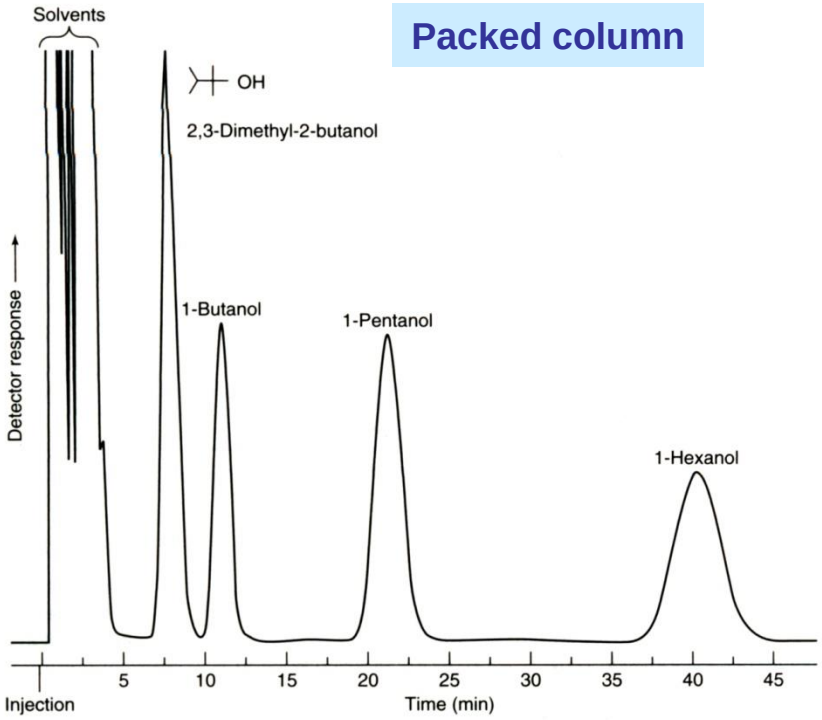
3.) Packed Columns

- Greater sample capacity
- Broader peaks, longer retention times and less resolution
  - **Improve resolution by using small, uniform particle sizes**

Open tubular column



Packed column



Gas Chromatography

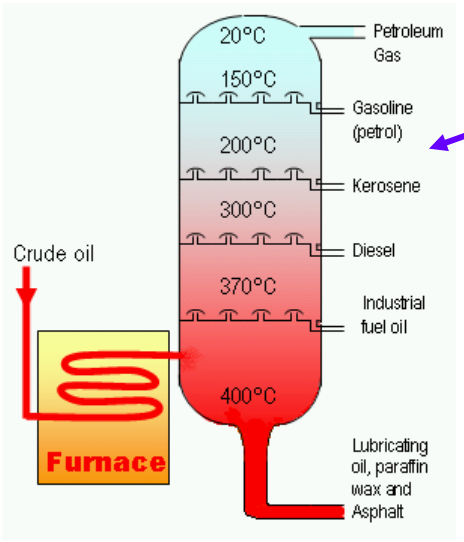
Instrumentation

3.) Packed Columns

- The major advantage and use is for large-scale or preparative purification
- Industrial scale purification maybe in the kilogram or greater range

500 L chromatography column

Oil refinery – separates fractions of oil for petroleum products



Gas Chromatography

Retention Index

1.) Retention Time

- Order of elution is mainly determined by *volatility*
  - **Least volatile = most retained**
  - **Polar compounds (ex: alcohols) are the least volatile and will be the most retained on the GC system**

Table 24-2 Polarity of solutes

Nonpolar	Weak intermediate polarity
Saturated hydrocarbons	Ethers
Olefinic hydrocarbons	Ketones
Aromatic hydrocarbons	Aldehydes
Halocarbons	Esters
Mercaptans	Tertiary amines
Sulfides	Nitro compounds (without α-H atoms)
CS_2	Nitriles (without α-atoms)
Strong intermediate polarity	**Strongly polar**
Alcohols	Polyhydroxyalcohols
Carboxylic acids	Amino alcohols
Phenols	Hydroxy acids
Primary and secondary amines	Polyprotic acids
Oximes	Polyphenols
Nitro compounds (with α-H atoms)	
Nitriles (with α-H atoms)	

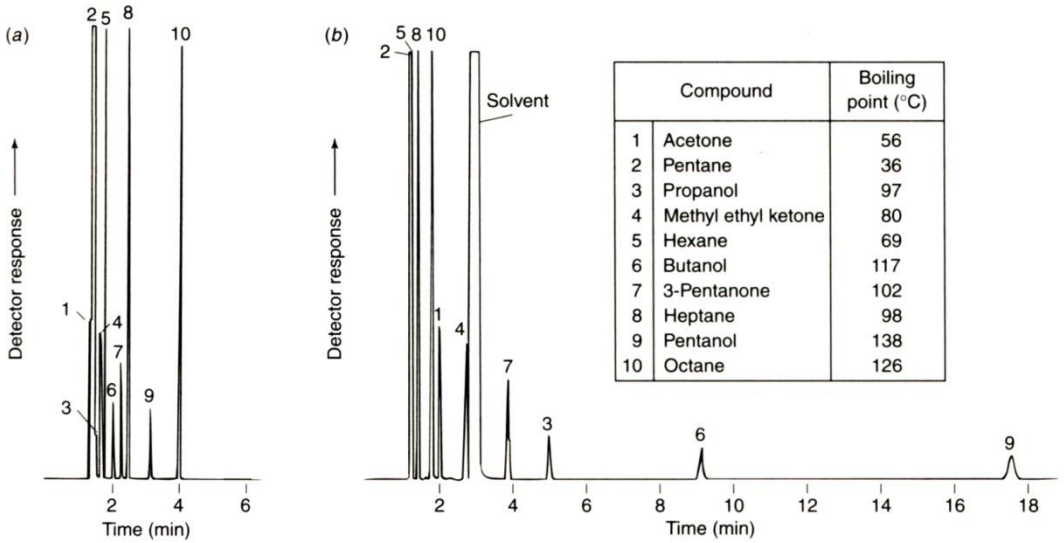
- Second factor is similarity in polarity between compound and stationary phase

Gas Chromatography

Retention Index

2.) Describing Column Performance

- Can *manipulate or adjust* retention time by changing polarity of stationary phase



	Compound	Boiling point (°C)
1	Acetone	56
2	Pentane	36
3	Propanol	97
4	Methyl ethyl ketone	80
5	Hexane	69
6	Butanol	117
7	3-Pentanone	102
8	Heptane	98
9	Pentanol	138
10	Octane	126

- Can use these retention time differences to classify or rate column performance
  - **Compare relative retention times between compounds and how they change between columns**
- Can be used to identify unknowns

Gas Chromatography

Retention Index

2.) Describing Column Performance

- Retention index based on the difference in the number of carbons (N, n) for linear alkane and corresponding retention times (t_r'(unknown), t_r'(N),t_r'(N)):

$$I = 100\left[n + (N-n)\frac{\log t_r'(unknown) - \log t_r'(n)}{\log t_r'(N) - \log t_r'(n)}\right]$$

- Provides a means to compare the performance of different columns

Table 24-3 Retention indexes for several compounds on common stationary phases

Phase	Retention index[a] Benzene b.p. 80°C	Butanol b.p. 117°C	2-Pentanone b.p. 102°C	1-Nitropropane b.p. 132°C	Pyridine b.p. 116°C
Poly(dimethylsiloxane)	657	648	670	708	737
(Diphenyl)$_{0.05}$(dimethyl)$_{0.95}$-polysiloxane	672	664	691	745	761
(Diphenyl)$_{0.35}$(dimethyl)$_{0.65}$-polysiloxane	754	717	777	871	879
(Cyanopropylphenyl)$_{0.14}$-(dimethyl)$_{0.86}$polysiloxane	726	773	784	880	852
(Diphenyl)$_{0.65}$(dimethyl)$_{0.35}$-polysiloxane	797	779	824	941	943
Poly(ethylene glycol)	956	1 142	987	1 217	1 185
(Biscyanopropyl)$_{0.9}$-(cyanopropylphenyl)$_{0.1}$-polysiloxane	1 061	1 232	1 174	1 409	1 331

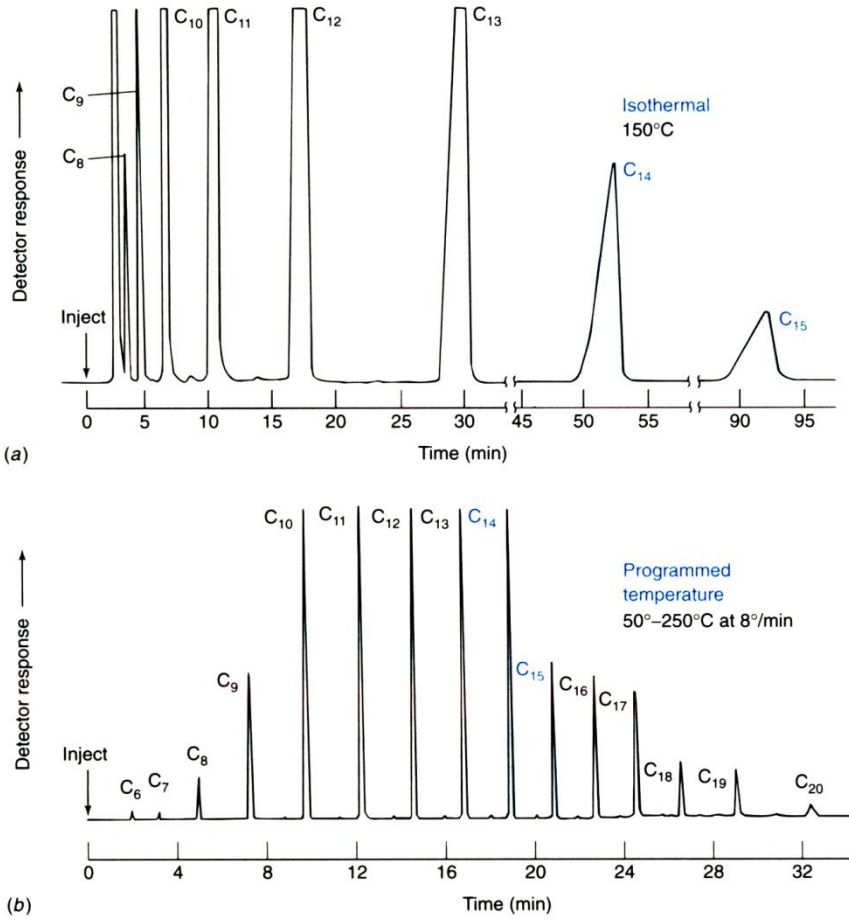
Increase in Polarity

Gas Chromatography

Temperature and Pressure Programming

1.) Improving Column Efficiency

- Temperature programming:
  - **Temperature is raised during the separation (gradient)**
  - ***increases* solute vapor pressure and *decrease* retention time**



Temperature gradient improves resolution while also decreasing retention time

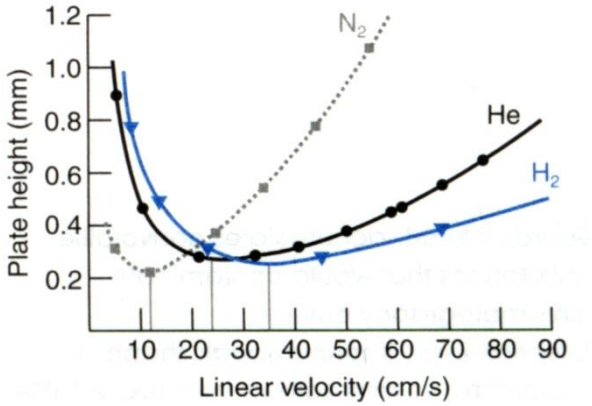
Gas Chromatography

Temperature and Pressure Programming

1.) Improving Column Efficiency

- Pressure Programming:
  - ***Increase* pressure → *increases* flow of mobile phase (carrier gas)**
  - ***Increase* flow → *decrease* retention time**

Van Deemter curves indicate that column efficiency is related to flow rate



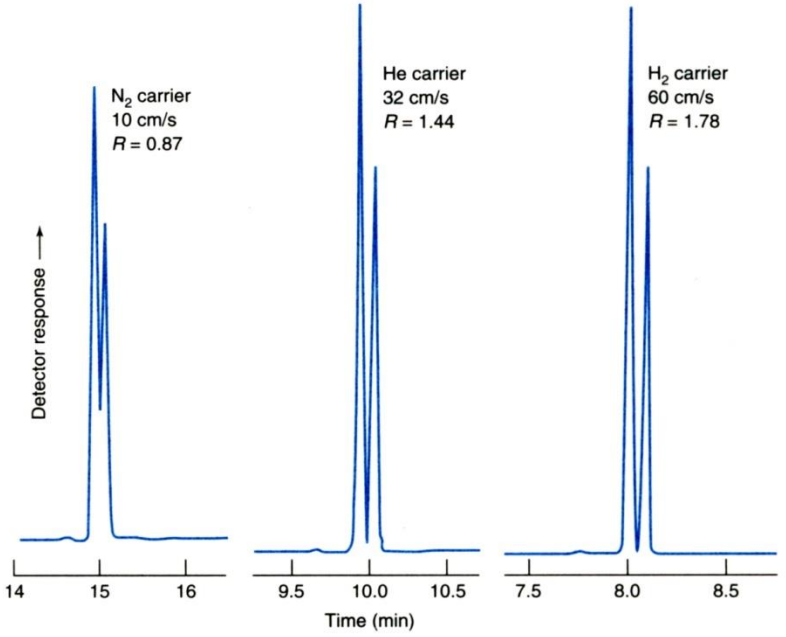
- Pressure is rapidly reduced at the end of the run
  - **Time is not wasted waiting for the column to cool**
  - **Useful for analytes that decompose at high temperatures**

Gas Chromatography

Carrier Gas

1.) N_2, He and H_2 are typical carrier gases

- **He:**
  - **Most common and compatible with most detectors**
  - **Better resolution (smaller plate heights)**
  - **Solutes diffuse rapidly → smaller mass transfer term**
- **N_2:**
  - **Lower detection limit for a flame ionization detector**
  - **Lower resolution and solute diffusion rates**
- **H_2:**
  - **Fastest separations**
  - **Can catalytically react with unsaturated compounds on metal surfaces**
  - **Can not be used with mass spectrometers Forms explosive mixtures with air**
  - **Better resolution (smaller plate heights)**
  - **Solutes diffuse rapidly → smaller mass transfer term**



Flow rate increases N_2 < He < H_2

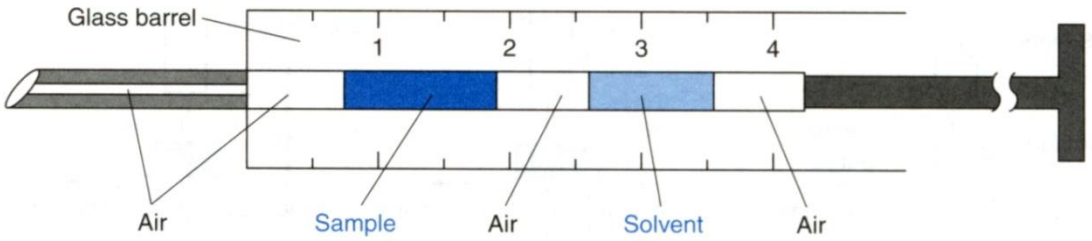
Diffusion coefficients follow: H_2 > He > N_2

Gas Chromatography

Sample Injection

1.) "Sandwich" Injection

- Separate sample with air bubbles and solvent



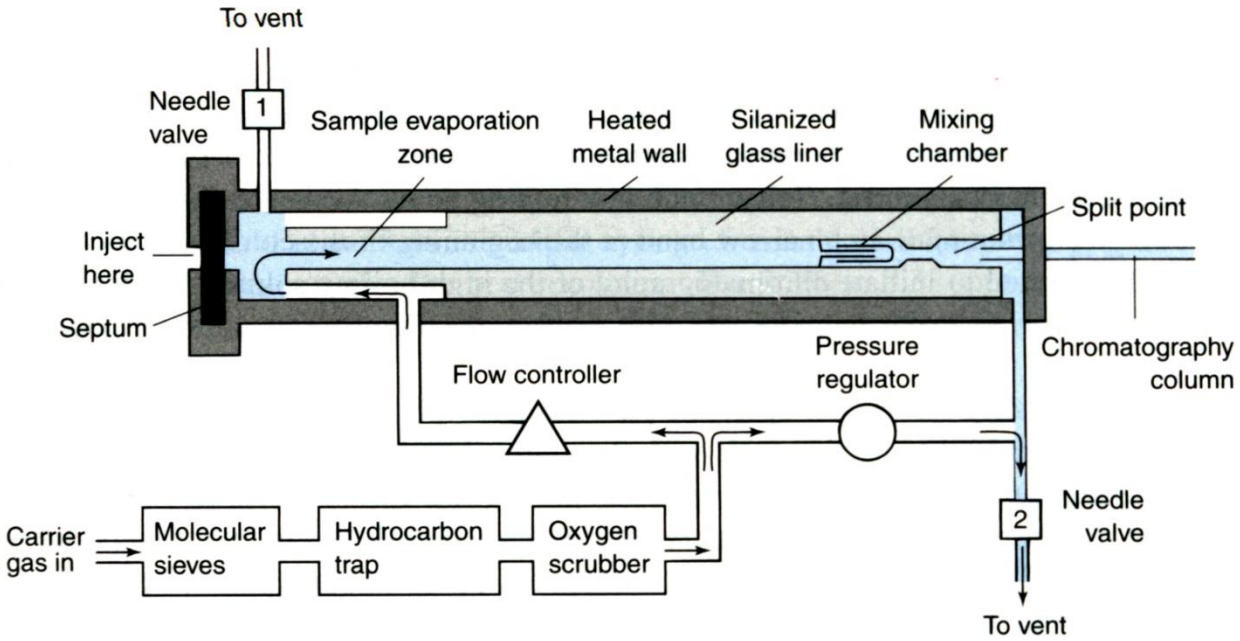
- **Air bubble prevents depletion of most volatile compounds before sample injection is complete (barrier between oven and sample during injection)**
- **Solvent is used to pushes out sample, but bubble prevents mixing**
- **Final air bubble pushes out solvent**
- **Gas-tight syringe is required for gas samples**
- **Injection volume is typically 0.1-2 μL**

Gas Chromatography

Sample Injection

1.) "Sandwich" Injection

- Injection port



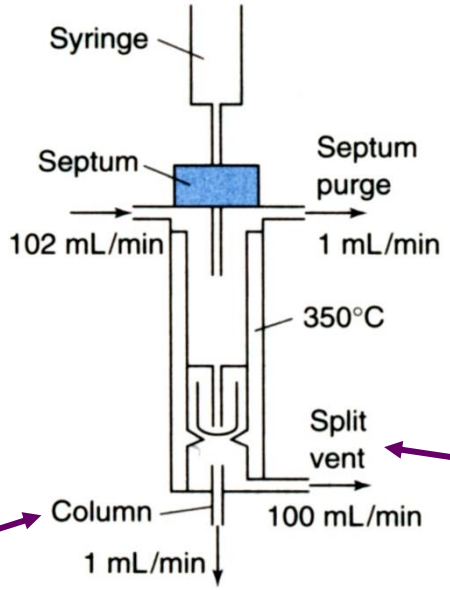
- **Inject rapidly (< 1s) through septum into evaporation zone**
- **Injector temperature is kept high (350^{o}C) for fast evaporation**
- **Rapid gas flows carries sample to mixing chamber for complete vaporization and complete mixing before entering column**

Gas Chromatography

Sample Injection

2.) Split Injection

- Delivers only 0.2-2% of sample to the column
  - **Split ratio of 50:1 to 600:1 (sample discarded)**
- For samples where analytes of interest are >0.1% of sample
  - **Best resolution is obtained with smaller amount of sample**
  - **≤ 1 μL with ≤ 1 ng of each compound (0.5 mL of gas volume)**
- Not quantitative, split not constant



Split injection

Remainder of the sample is flushed from injector port to column

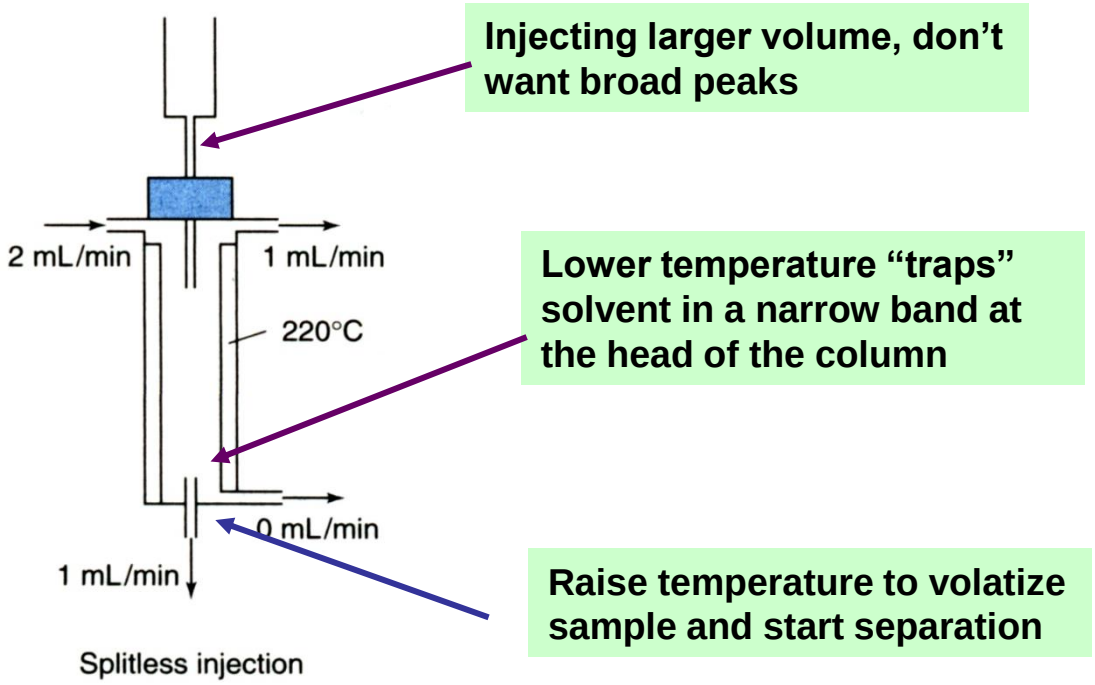
After mixing, pressure regulator controls the fraction of sample discarded

Gas Chromatography

Sample Injection

2.) Splitless Injection

- Delivers ~80% of sample to the column
- For trace analysis, where analytes of interest are < 0.01% of sample
  - **Large volume (~2 μL) injected slowly (2s)**
- No mixing chamber or split vent
  - **Injection temperature is lower (220°C)**
  - **40°C *below* the boiling point of the solvent**



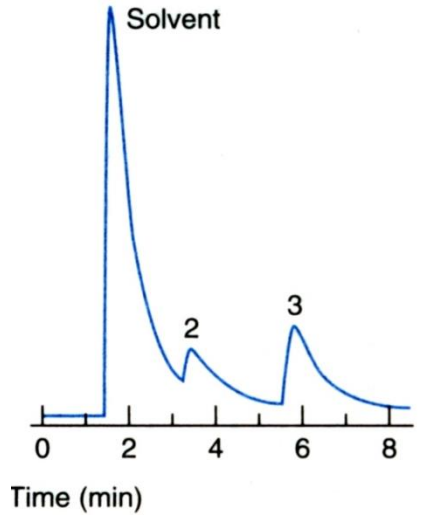
Gas Chromatography

Sample Injection

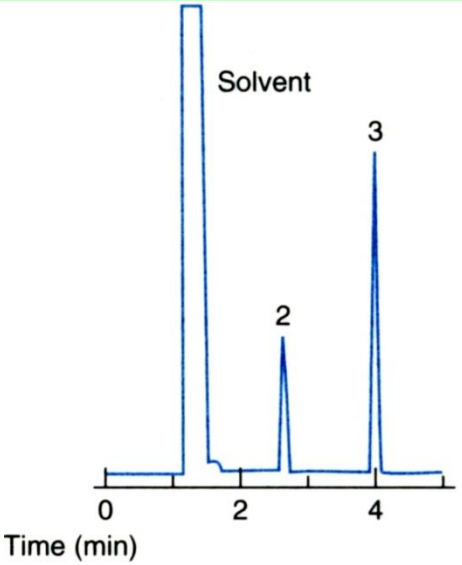
2.) Splitless Injection

- "Solvent trapping" significantly improves the performance of splitless injections
  - **Initial lower temperature of column during injection keeps larger volume into a narrow band**
  - **Chromatography is initiated by raising column temperature**
  - **<u>Cold trapping</u> – condense solutes in narrow band at the beginning of column by using an initial temperature 150°C below boiling points of solutes of interest**

Without "Solvent trapping"



With "Solvent trapping"

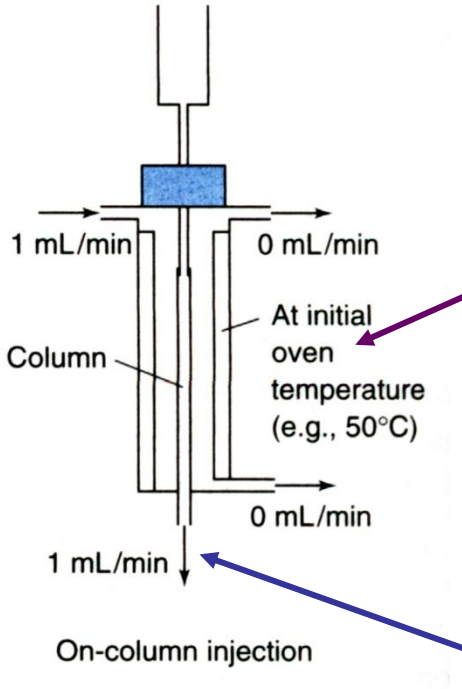


Gas Chromatography

Sample Injection

3.) On-column Injection

- Delivers ~100% of sample to the column
- For samples that decompose above their boiling points
- Solution injected directly on column
  - **Warming column initiates chromatography**



Lower initial column temperature to prevent sample decomposition

Raise temperature to volatize sample and start separation

Gas Chromatography

Detectors

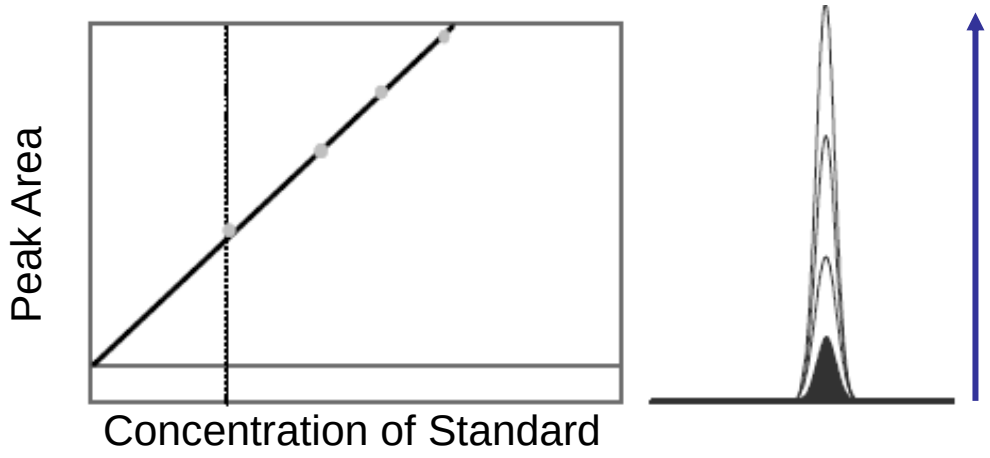
1.) Qualitative and Quantitative Analysis

- Mass Spectrometer and Fourier Transform Infrared Spectrometers can identify compounds as part of a GC system
  - **Compare spectrum with library of spectra using a computer**

- Compare retention times between reference sample and unknown
  - **Use multiple columns with different stationary phases**
  - **Co-elute the known and unknown and measure changes in peak area**

- *The area of a peak is proportional to the quantity of that compound*

$$\textbf{Area of Gaussian peak} = 1.064 \times \textit{peak height} \times w_{1/2}$$



Peak area increases proportional to concentration of standard if unknown/standard have the identical retention time → same compound

Gas Chromatography

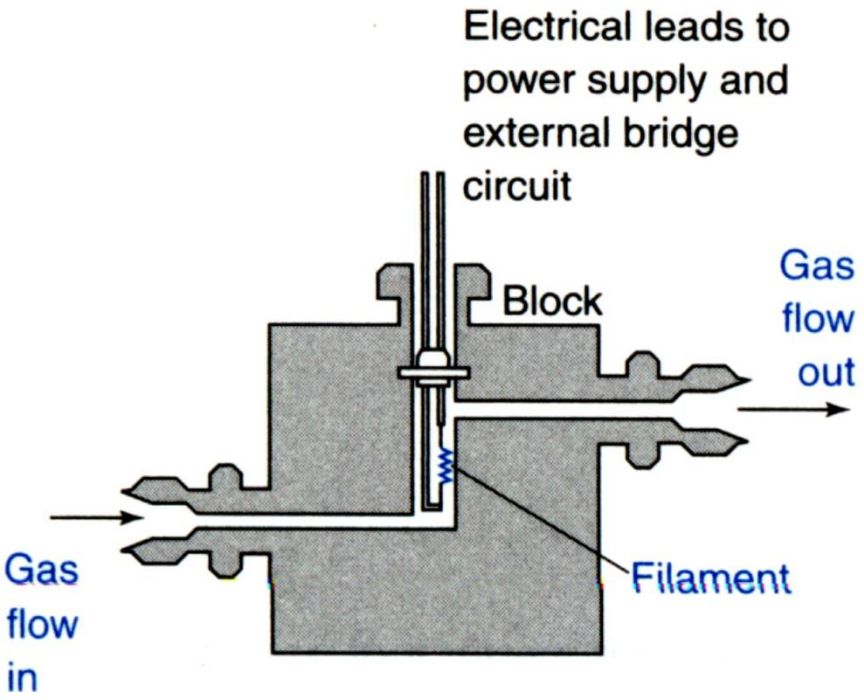
Detectors

2.) Thermal Conductivity Detector

- Measures amount of compound leaving column by its ability to remove heat
  - **He has high thermal conductivity, so the presence of any compound will lower the thermal conductivity increasing temperature of filament**
- As heat is removed from filament, the resistance (R) of filament changes
  - **Causes a change in an electrical signal that can be measured**
- Responds to *all* compounds (universal)
  - **Signal changes in response to flow rate of mobile phase and any impurities present**
  - **Not very sensitive**

Ohm's Law: V =IR

Based on Ohm's law, monitored potential (V) or current (I) Changes as resistance (R) of filament changes due to presence of compound

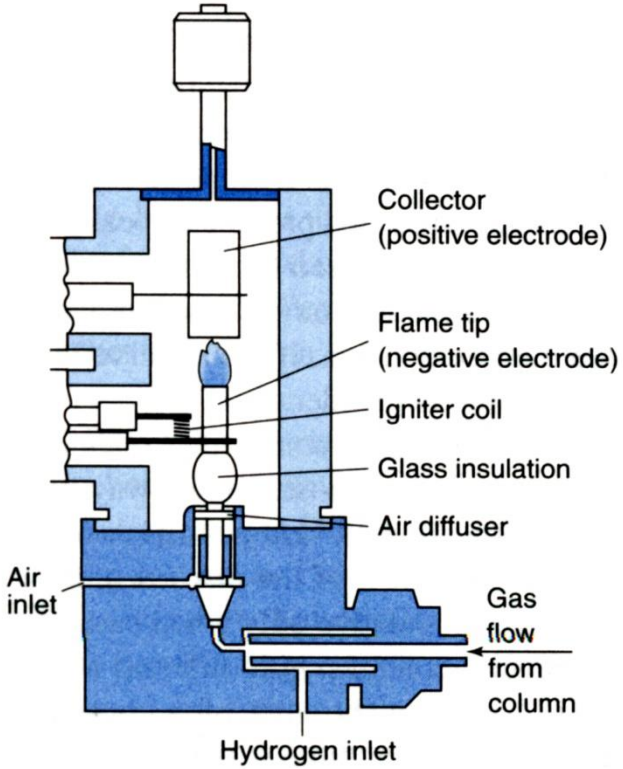


Gas Chromatography

Detectors

3.) **Flame Ionization Detector**

- **Mobile phase leaving the column is mixed with H_2 and air and burned in a flame**
  - **Carbon present in eluting solutes produces CH radicals which produce CHO^+ ions**
  - **Electrons produced are collected at an electrode and measured**
- **Responds to almost all organic compounds and has good limits of detection**
  - **100 times better than thermal conductivity detector**
  - **Stable to changes in flow rate and common mobile phase impurities (O_2, CO_2,H_2O,NH_3)**



$$CH + O \longrightarrow CHO^+ + e^-$$

Burn sample and measure amount of produced electrons

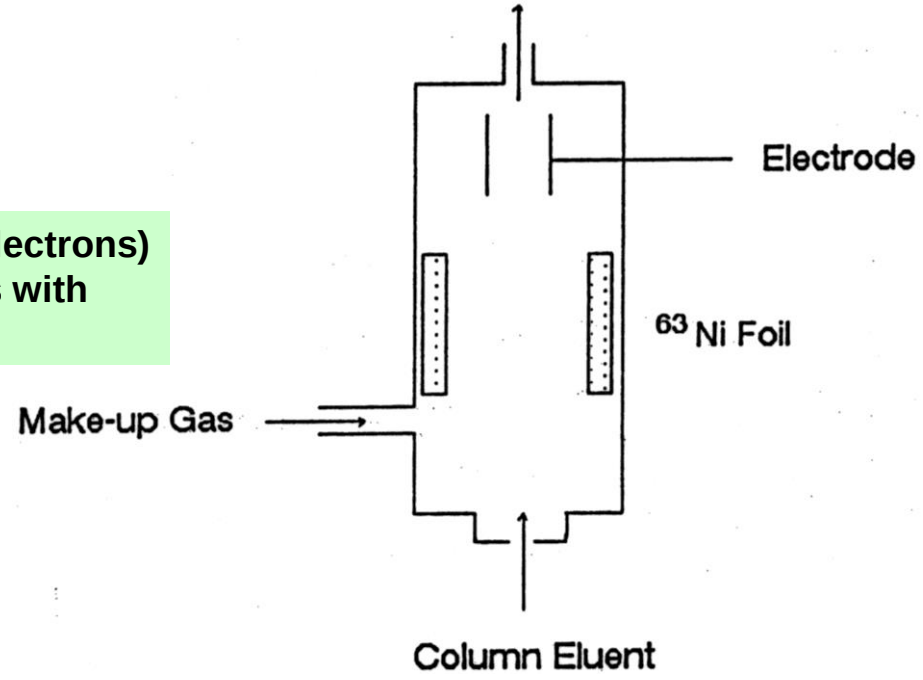
Gas Chromatography

Detectors

4.) Electron Capture Detector

- Sensitive to halogen-containing and other electronegative compounds
- Based on the capture of electrons by electronegative atoms
  - **Compounds ionized by β-rays from radioactive ^{63}Ni**
- Extremely sensitive (~ 5 fg/s)

Steady current (flow of electrons) disrupted by compounds with high electron affinity

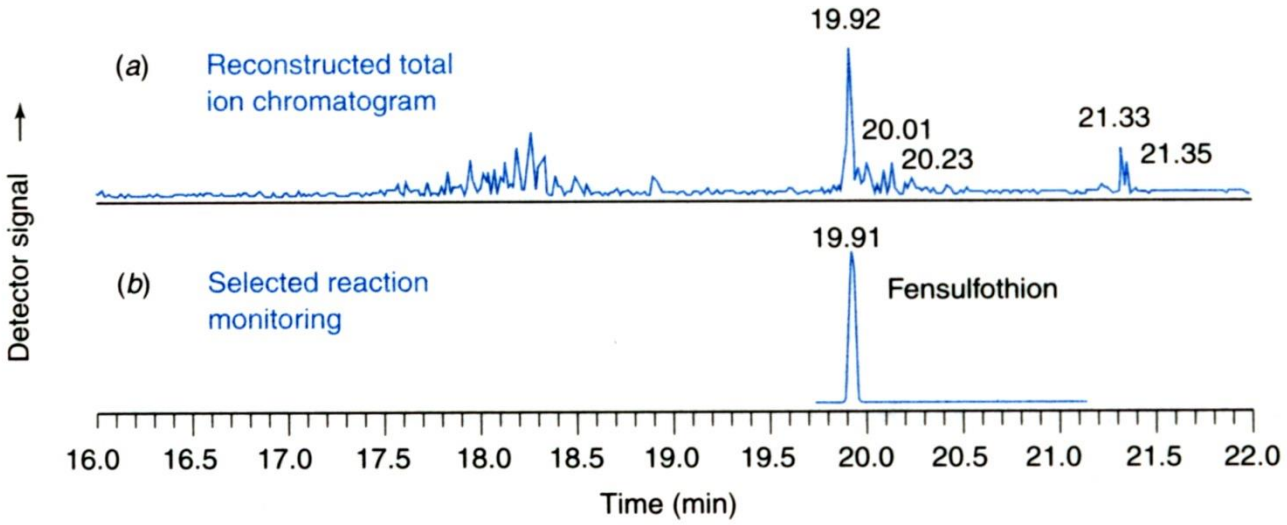


Gas Chromatography

Detectors

5.) Mass Spectrometry

- Detector of Choice → *But Expensive!*
- Sensitive and provides an approach to identify analytes
- Selected ion monitoring – monitor a specific mass/charge (mz) compared to scanning over the complete spectra
  - **Simplifies complex chromatogram**
  - **Increases sensitivity by 10^2-10^3**



Gas Chromatography

Detectors

6.) Other Detectors

- Respond to limited class of analytes
- Modification of previous detectors
- Nitrogen-Phosphorous detector
  - **Modified flame ionization detector**
  - **Extremely sensitive for compounds containing N and P**
  - **Important for drugs and pesticides**

- Flame photometric detector
  - **Measures optical emission from P (536 nM) , S (394 nM), Pb, Sn, and other select elements after passing sample through flame (flame ioniation detector)**

- Photionization detector
  - **Uses a ultraviolet source to ionize aromatic and unsaturated compounds, electrons produced are measured (Electron capture detector)**

- Sulfur/nitrogen chemiluminescence detector
  - **Collects exhaust of flame ionization detector**
  - **S and N converted to SO and NO**
  - **Mix with O_3 form excited state of SO_2 (emits blue light) and NO_3**

Gas Chromatography

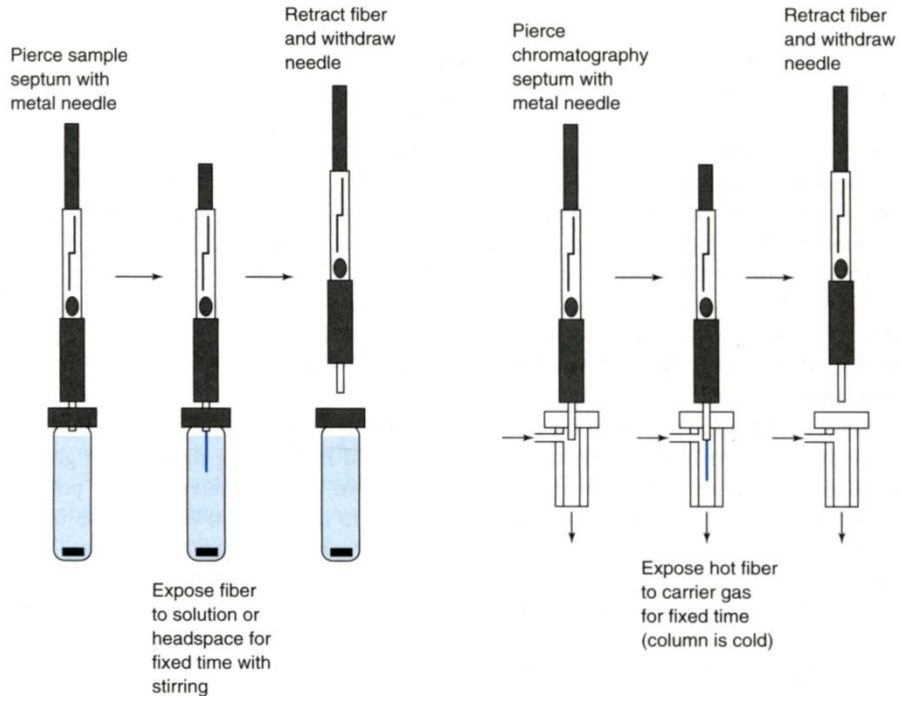
Sample Preparation

1.) Transform sample into form suitable for analysis

- Extraction, concentration, removal of interfering species or chemically transforming (derivatizing)

2.) Solid-phase microextraction

- Extract analytes from complex mixture *without* solvent
- Uses a fused-silica fiber coated with stationary phase
  - **Stationary phase similar to those used in GC**
- Expose Fiber to sample to extract compounds and then inject fiber into GC to evaporate analytes



Gas Chromatography

Sample Preparation

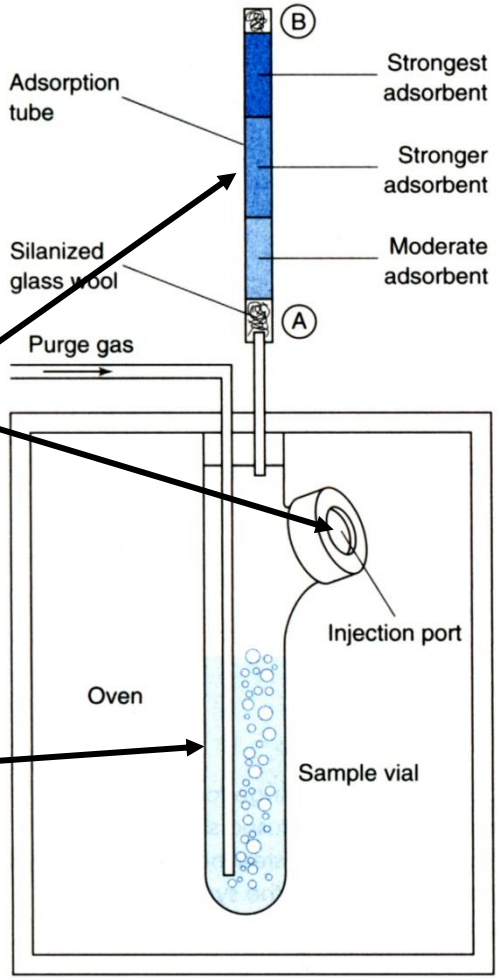
3.) Purge and Trap

- Removes volatile analytes from liquids or solids concentrates sample and transfer to GC
- Goal is to remove 100% of analyte

Connect port to GC

An on Heat column to 200°C to transfer analytes to GC

Bubble purge gas (He) through heated sample to evaporate analytes



Gas Chromatography

Method Development in GC

1.) How to Choose a Procedure for a Particular Problem

- Many Satisfactory Solutions
- The order in which the decision should be made should consider:
  1. **Goal of the analysis**
  2. **Sample preparation**
  3. **Detector**
  4. **Column**
  5. **Injection**

- Goal of the analysis
  - **Qualitative vs. quantitative**
  - **Resolution vs. sensitivity**
  - **Precision vs. time**
  - **Interest in a specific analyte**

- Sample preparation
  - **Cleaning-up a complex sample is essential**
  - **Garbage in → garbage out**

- Choosing the Detector
  - **Detect a specific analyte(s) or everything in the sample**
  - **sensitivity**
  - **Identify an unknown (MS, FTIR)**

Gas Chromatography

Method Development in GC

1.) How to Choose a Procedure for a Particular Problem

- Selecting the Column
  - **Consider stationary phase, column diameter and length, stationary phase thickness**
  - **Match column polarity to sample polarity**
  - **To improve resolution, use a:**
    - **a. Longer column**
    - **b. Narrower column**
    - **c. Different stationary phase**

- Choosing the Injection Method
  - **Split injection is best for high concentrated samples**
  - **Splitless injection is best for very dilute solutions**
  - **On-column injection is best for quantitative analysis and thermally instable compounds**