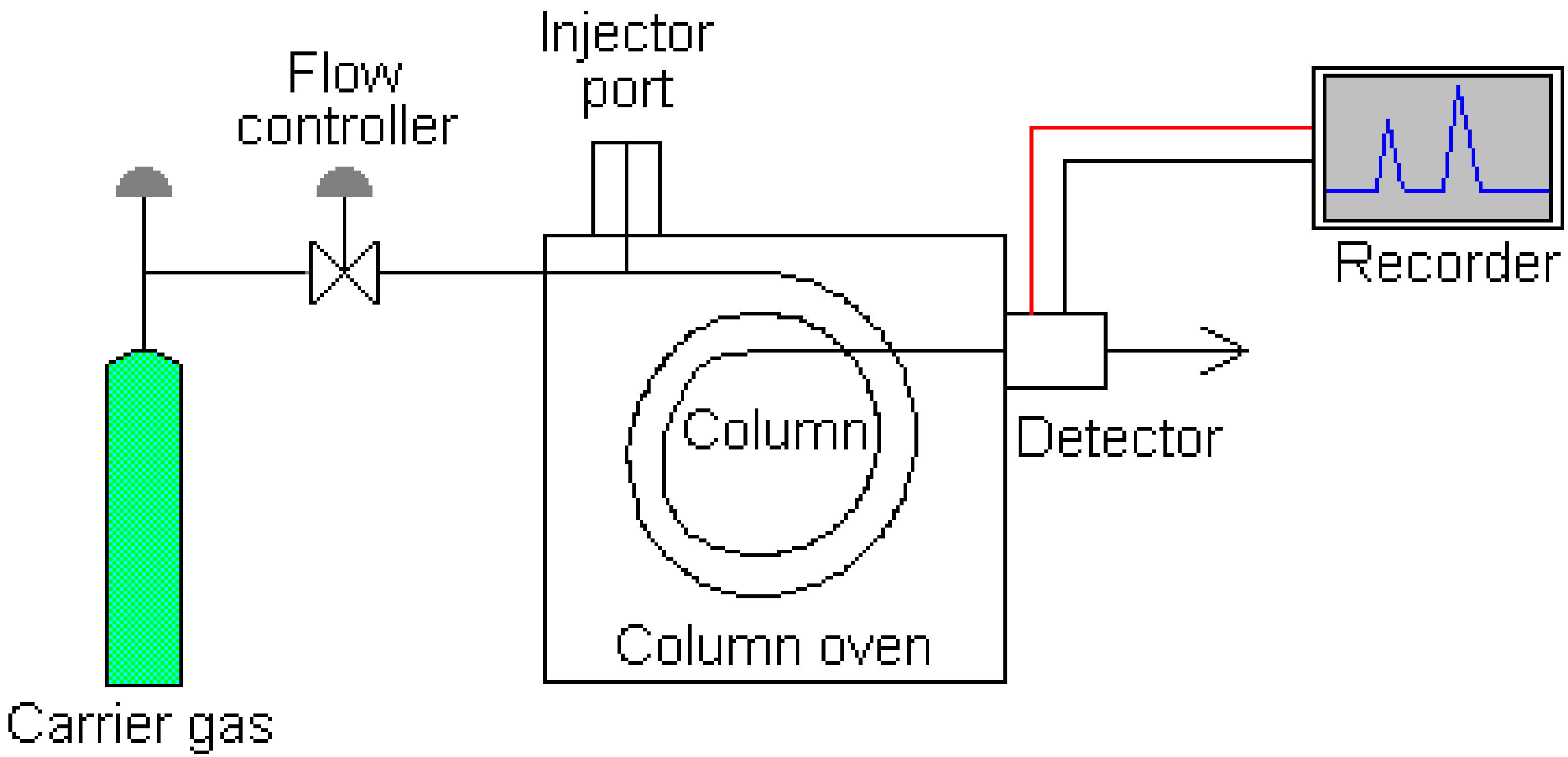
Lecture 6: Gas Chromatography

Instrumentation



Instrumentation

Columns

There are two types of columns:

Capillary

- **wall-coated open tubular (WCOT),**
- **support coated open tubular (SCOT),**
- **micropacked, fused silica open tubular (FSOT),**

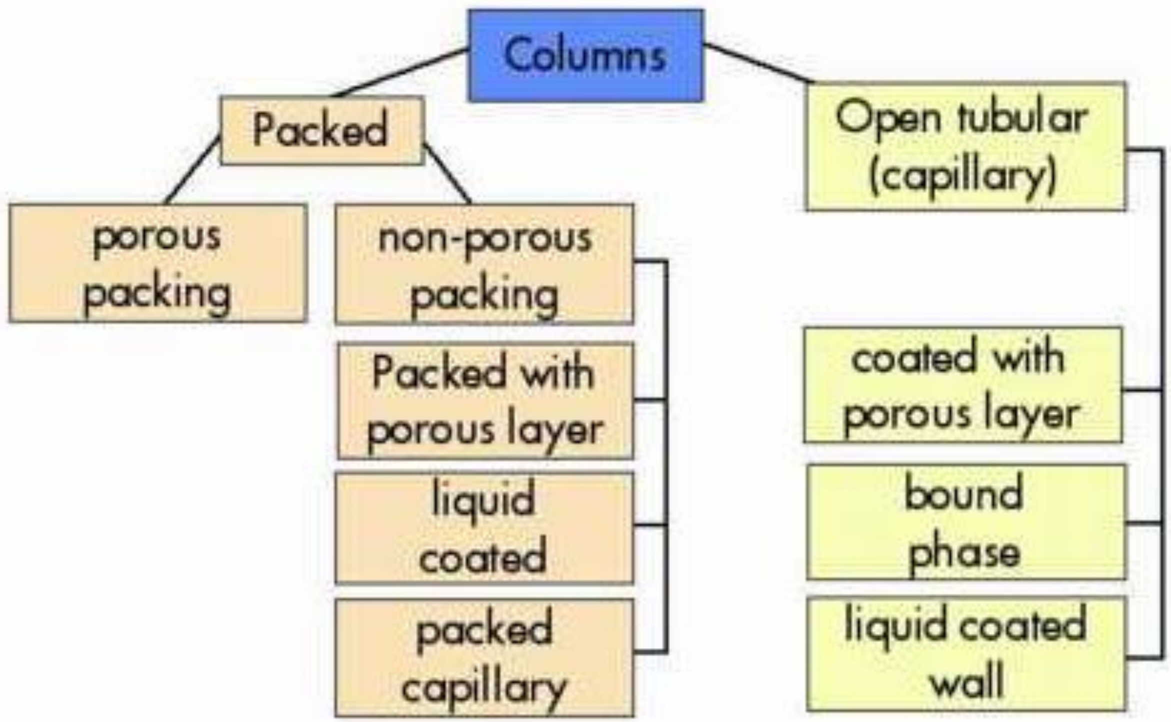
Packed column.

Open tubular is also capillary.

Columns determine selectivity and efficiency of the sample.

Instrumentation

Columns



Instrumentation

Columns

Packed columns contain a finely divided, inert, solid support material (commonly based on diatomaceous earth) coated with liquid stationary phase. Most packed columns are 1.5 – 10 m in length and have an internal diameter of 2 – 4 mm.

Greater sample capacity compared to capillary columns

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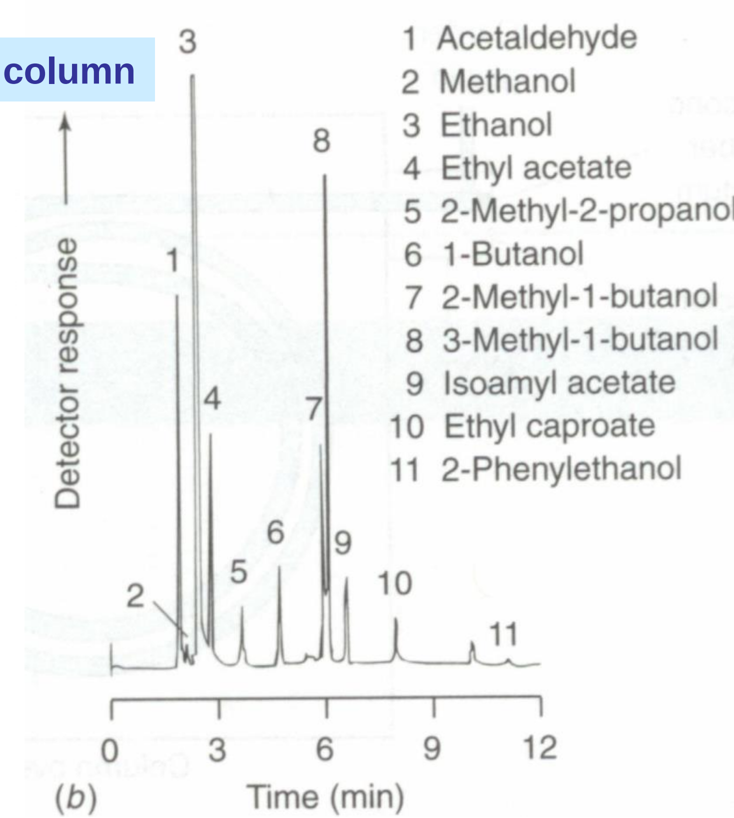
CHEM5390

Gas Chromatography

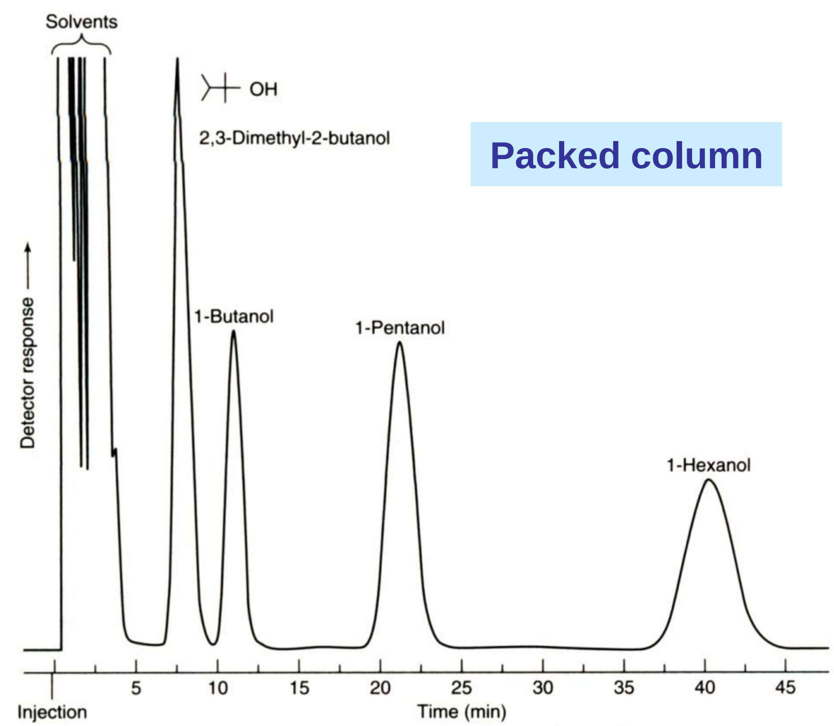
Packed Columns

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

Open tubular column



Packed column



Gas Chromatography

Types of Columns

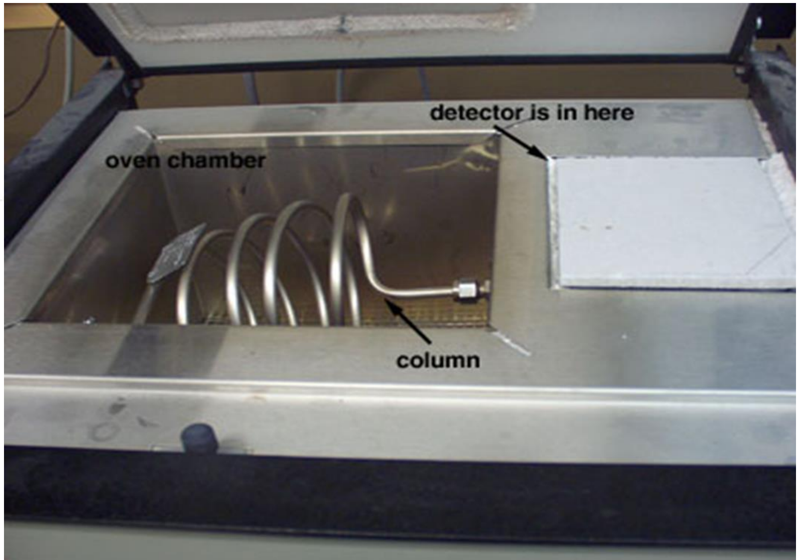
<u>Packed Columns</u>

Older type of column

Both solid and liquid stationary phase

Best column for preparatory GC and for use with thermal conductivity detectors

Sometimes used for very specific applications (low production volume less of an issue)



Gas Chromatography

<u>Packed Columns</u>

For large-scale or preparative purification on an Industrial scale - purification may be in the kilogram or greater range.

500 L chromatography column

Gas Chromatography

<u>Open Tubular Columns</u>
More commonly used in GC

Much better analytical performance (large N values)

Most common is wall coated format (WCOT)
Variety of diameters (0.25 to 0.53 μm most common) allow high resolution vs. easier injection

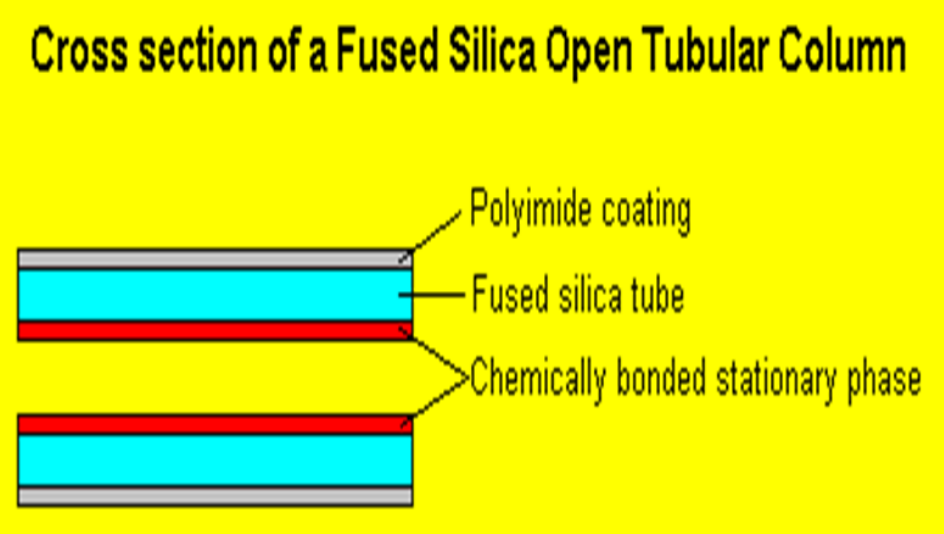
Stationary phases are mainly bonded of varying amounts of polarity

Good reliability

Disadvantages: harder to make and much less sample capacity

Gas Chromatography

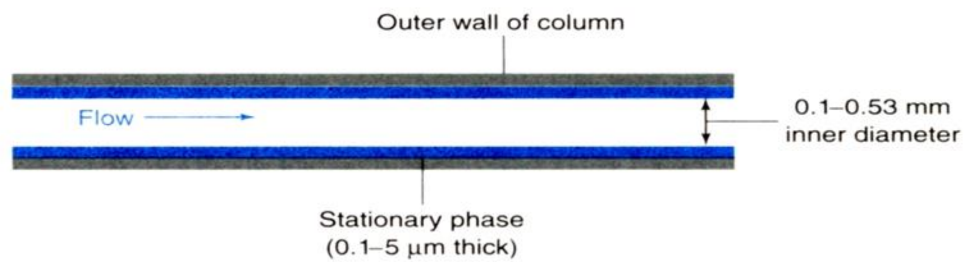
Open Tubular Columns - capillary



Gas Chromatography

Open Tubular Columns - capillary

- Increasing Resolution
  - Narrow columns → Increase resolution



  - Resolution is proportional to square of N, where *N* increases directly with column length – lengths usually available from 7 m to 60 m

Instrumentation

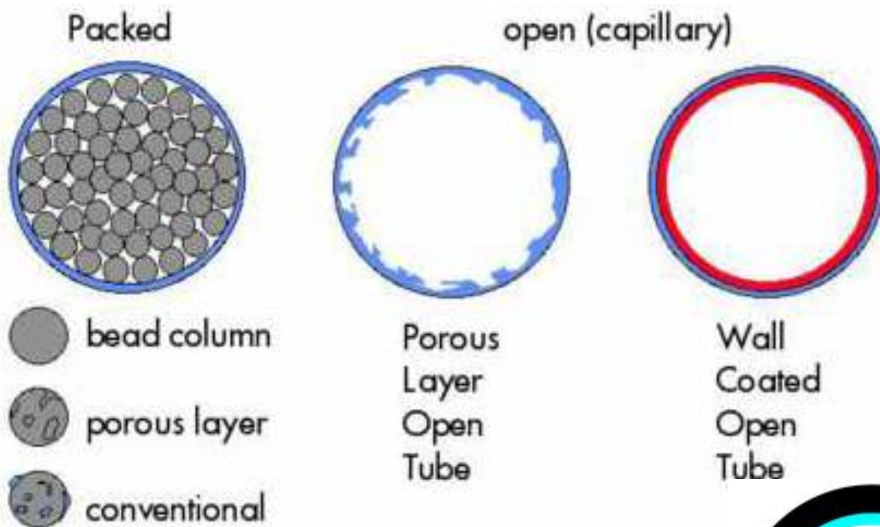
Columns

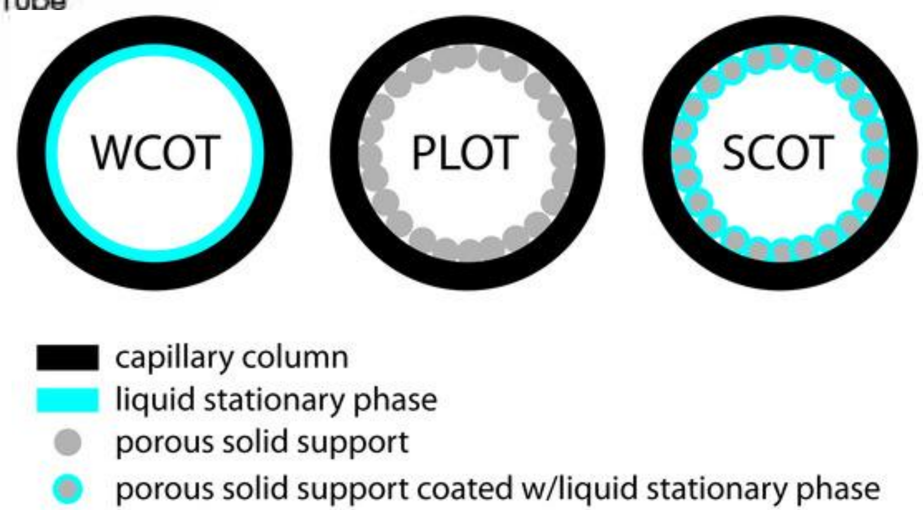
Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase.

In support-coated columns, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed.
SCOT columns are generally less efficient than WCOT columns.
Both types of capillary column are more efficient than packed columns.

Instrumentation

Columns





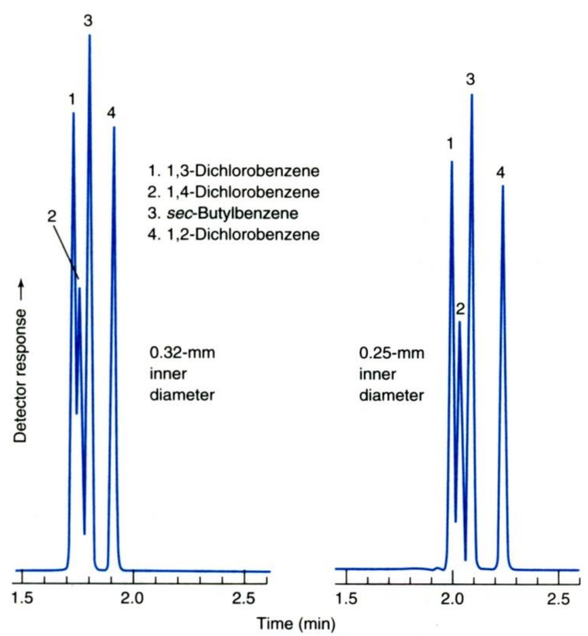
Gas Chromatography

Open Tubular Columns - capillary

- Increasing Resolution
  - Narrow columns → Increase resolution

Decrease tube diameter

Increase resolution



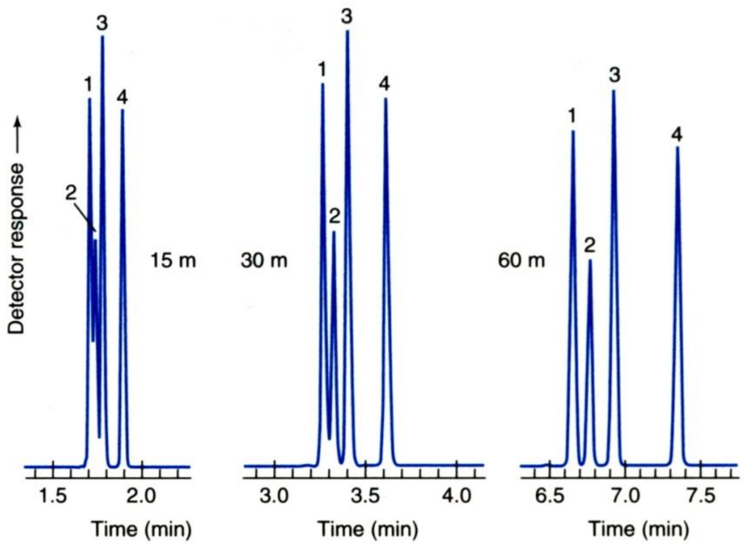
Gas Chromatography

Open Tubular Columns - capillary

- Increasing Resolution
  - Resolution is proportional to square of N, where *N* increases directly with column length – lengths usually available from 7 m to 60 m

Increase Column Length

Increase resolution



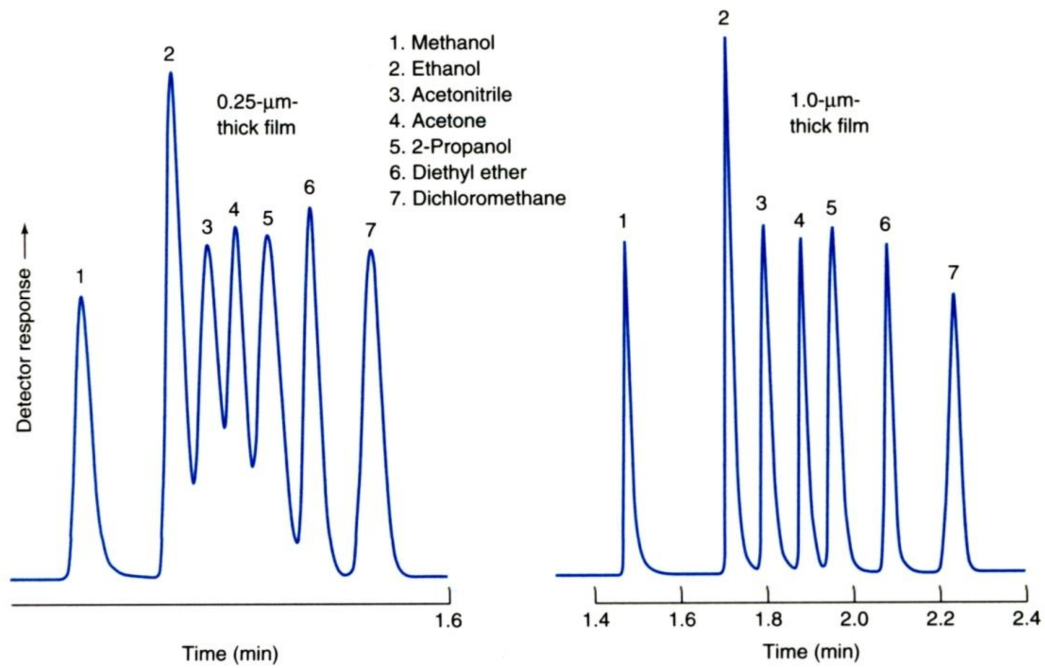
Gas Chromatography

Open Tubular Columns - capillary

- Increasing Resolution

Increase Stationary Phase Thickness

Increase resolution of early eluting compounds



Instrumentation

Column Materials

•Column tubing supports the stationary phase.

•Needs to be:
-chemically inert (to prevent sample decomposition)
-thermally stable
-robust
-flexible – form a coil to fit into oven

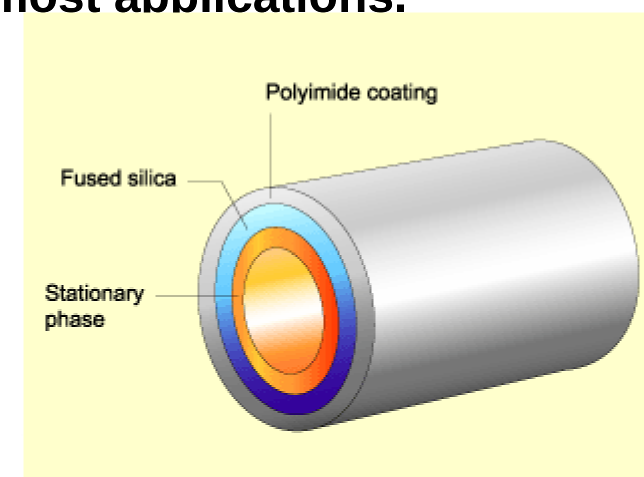
Instrumentation

Column Materials

Early columns were metal
(stainless steel, Cu, Al, Ni) but these were too reactive.

Glass is popular – but fragile

Fused silica columns – weak and subject to atmospheric corrosion. Use a protective outer Al or polymer sheath. These columns are used for most applications.



Parameter	Capillary	Packed
Efficiency (plates/m)	2 000 – 4 000	500 - 1 000
Sample size (ng)	10 - 75	10 – 1 000 000
Relative pressure	Low	High
Relative speed	Fast	Slow
Chemical inertness	Best	Poorest
Flexible column	Yes	No

Instrumentation

Columns

Column Activity leads to:

-tailing or skewed peaks

-partial or total disappearance of a solute

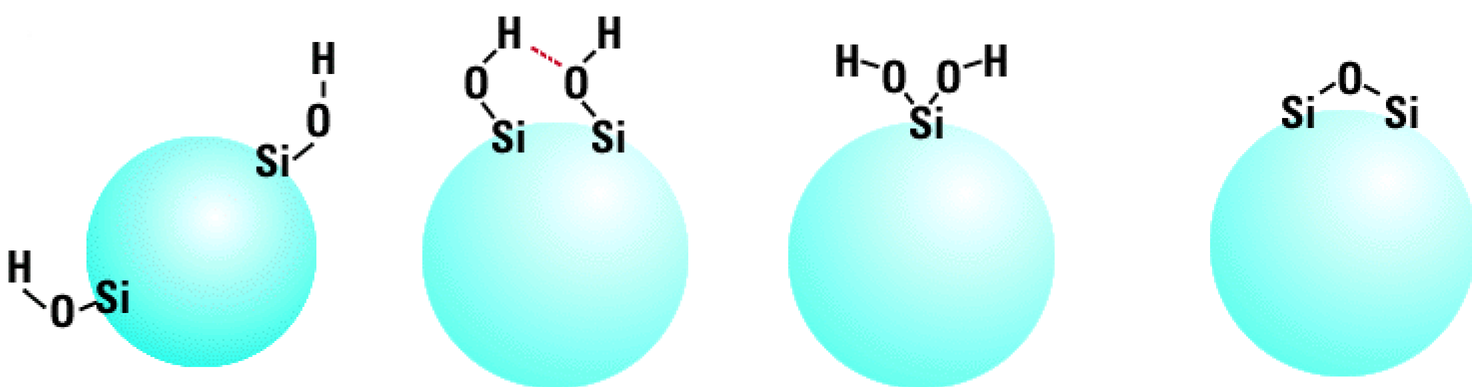
reversible peak adsorption
non-reversible peak adsorption
breakdown

The surface properties of the column – catalytic or sorptive are important to consider to eliminate column activity.

Instrumentation

Columns

Various surface groups attributed to the silica surface have been identified:



Isolated Silanol | **Vicinal Silanol** | **Geminal Silanol** | **Siloxane**

Instrumentation

Columns

Surface hydroxyl groups can act as proton donors in hydrogen bonding interactions

-very strong sorptive sites for molecules with localized high electron density.

Surface siloxane bridge can act as

-proton acceptors functioning as sorptive sites for molecules such as alcohols

Instrumentation

Columns

Metallic contamination

Metal ions present on the surface of soda lime or borosilicate glasses can function as

-Lewis acid sites absorbing molecules that have regions of localized high electron density such olefins, aromatic compounds, alcohols, ketones and amines. (Lewis acids – a species that accepts an electron pair)

Instrumentation

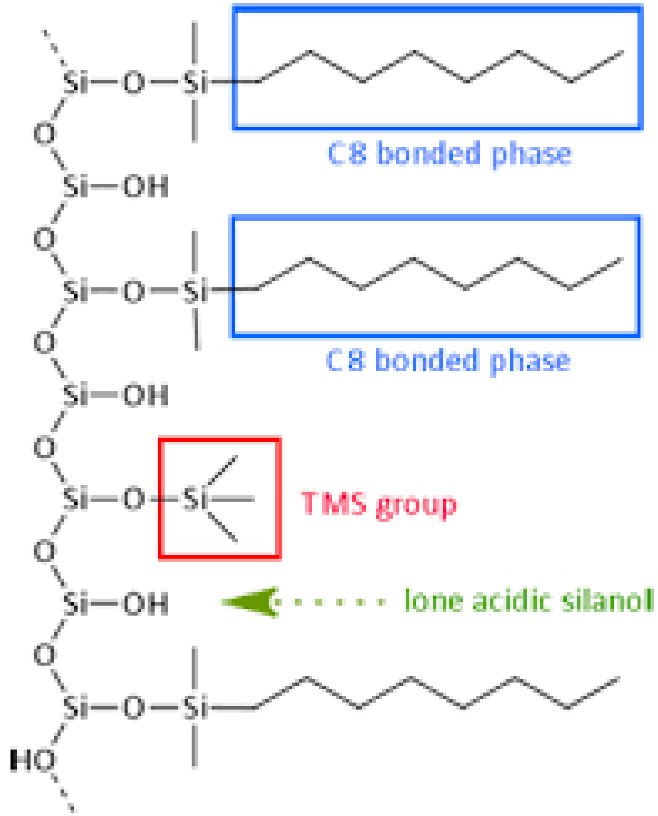
Columns

Columns are deactivated before use. One method deactivates reactive hydroxyl groups by silanizing or silylating the inner surface of the column.
Typical derivatization reactions are:

- Silylation
- Acylation
- Alkylation
- Esterification

Instrumentation

Columns

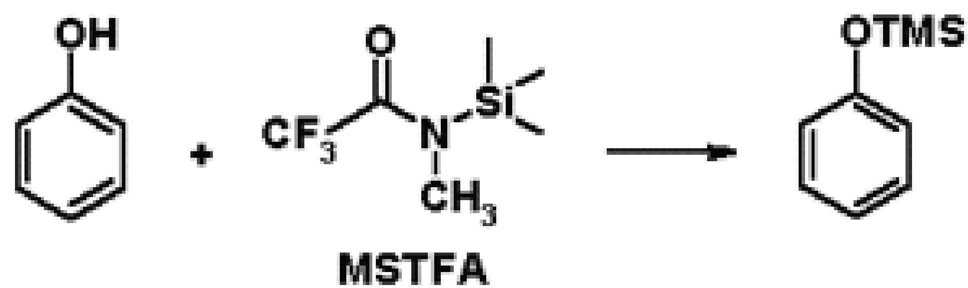


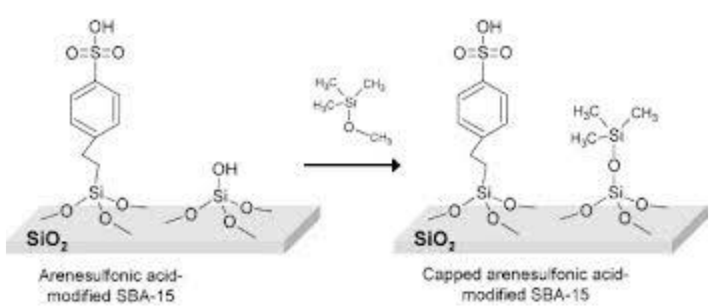
Columns are deactivated before use. Remember that these reactions are used to take care of a "problem" – but can just as well be used to "create" new stationary phases… we will cover this more later.

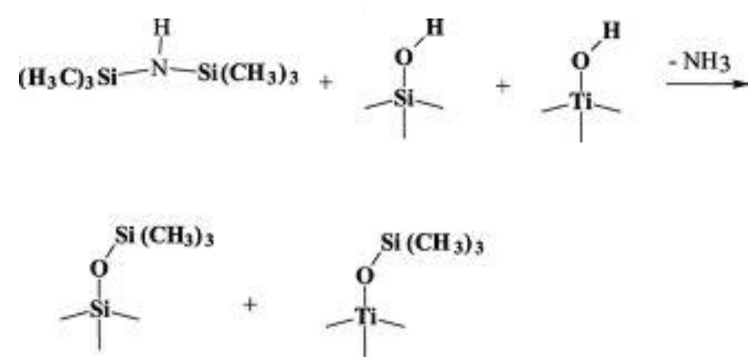
Instrumentation

Columns

Silylation - It involves the replacement of an acidic hydrogen on the compound with an alkylsilyl group, for example, $-SiMe_3$. The derivatives are generally less polar, more volatile and more thermally stable.







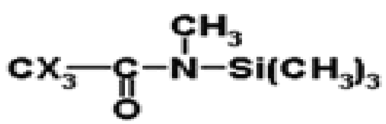
Instrumentation

Columns

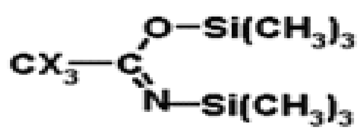
Various silanizing reagents can be used. The most common reagents for silylation are the trimethylsilyl (TMS) reagents.

$(CH_3)_3SiCl$
Trimethylchlorosilane (TMCS)

$(CH_3)_3SiNHSi(CH_3)_3$
Hexamethyldisilazane (HMDS)



X=H, N-methyl-N-(trimethylsilyl)acetamide (MSTA)
X=F, N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA)



X=H, N,O-bis-(trimethylsilyl)acetamide (BSA)
X=F, N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA)

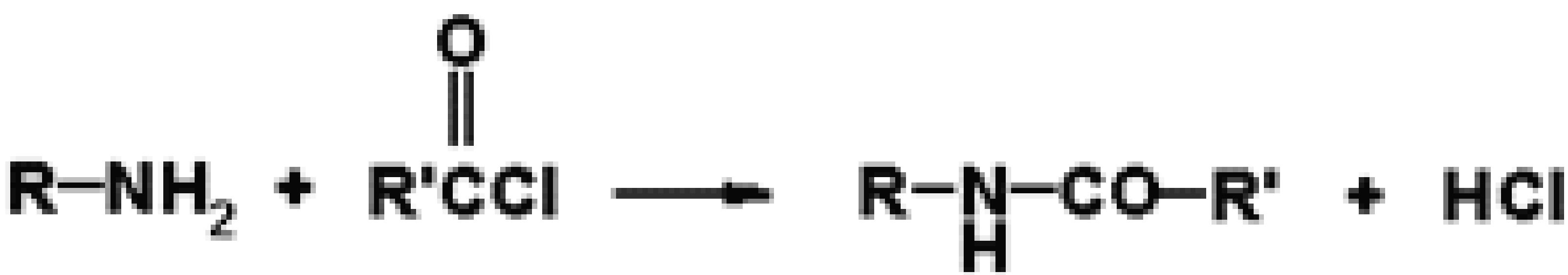
$(CH_3)_3Si{-}N(C_2H_5)_2$
N-trimethylsilyldiethylamine (TMSDEA)

N-trimethylsilylimidazole (TMSIM)

Instrumentation

Columns

Acylation - Acylation is one of the most widely used derivatization procedures for gas chromatography. Acylation reduces the polarity of compounds that contain active hydrogens (-NH, -OH, -SH amino, hydroxyl and thiol groups) into amides, esters, or thioesters by using carboxylic derivatives.

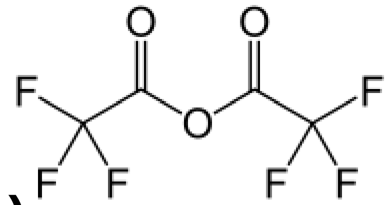


Instrumentation

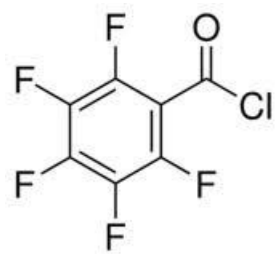
Columns

The most widely used acylating reagents is acetic anhydride, $(CH_3CO)_2O$.

Acylating reagents



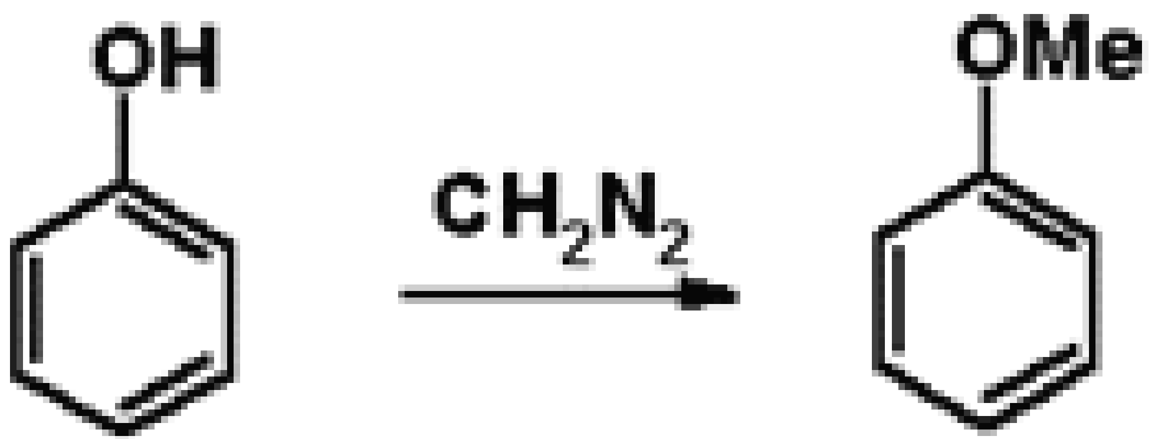
Trifluoroacetic anhydride (TFAA)
Pentafluoropropionic anhydride (PFPA)
Heptafluorobutyryl anhydride (HFBA)
Pentafluorobenzoyl chloride



Instrumentation

Columns

Alkylation- **Alkylation is the replacement of a active hydrogen in R-COOH, R-OH, R-SH, and R-NH_2 with an alkyl group or, sometimes aryl group.**



Instrumentation

Columns

Alkylating reagents

- Alkyl halide (eg. pentafluorobenzyl bromide, PFBBr, 3,5-ditrifluoromethylbenzyl bromide)
- Diazoalkane (eg. diazomethane)
- N,N-Dimethylformamide dialkyl acetal
- 3-alkyl-1-p-tolyltriazene
- Fluorosulfonate
- Trimethylanilinium hydroxide (TMAH)
- Trialkyloxonium fluoroborate
- Alkyl trichloroacetimidate

Columns

Functional group	Derivatization
-OH (hydroxyl group) in primary, secondary and tertiary alcohols; phenols; carbohydrates)	Silylation Acylation Benzoylation Alkylation Dansylation Reaction with Dis-Cl Reaction with FDNB Reaction with NBD-Cl Ion-pair formation
-COOH (carboxylic acids)	Esterification Silylation Ion-pair formation
-C=O (carbonyl group) in aldehydes and ketones	Oxime formation Oxime formation and silylation Ketal/acetal formation Hydrazone formation Schiff's base formation Silylation

Columns

Functional group	Derivatization
-NH2 (amino group) in primary amines, amino acids, amino sugars	Acylation Benzoylation Silylation Treatment with CS2 Thiourea formation Schiff's base formation 2,4-Dinitrophenylation Sulphonamide formation Carbamate formation Treatment with pyridoxal Treatment with NBD-Cl Alkylation Ion-pair formation
-NH-R (amino group) in secondary amines, imino acids, substituted amino sugars	Acylation Benzoylation Silylation 2,4-Dinitrophenylation Sulphonamide formation Treatment with NBD-Cl Ion-pair formation
-NH2 and -COOH in amino acids	Silylation Esterification + Acylation
-NO2 (nitro compounds)	Chromatograph without derivatization

Gas Chromatography

Conditioning of Columns (stationary phases)

Conditioning for at least 10 h at about 20 °C, above or just close to the upper working temperature of the column

Conditioning must be done below the maximum temperature limit for the stationary phase

Instrumentation

Columns

Stationary Phases

Selection of stationary phase affects k and α values.

Main concerns of stationary phase are:
- polarity,
- functional groups,
- maximum operating temperature, and
- column bleed (loss of stationary phase due to decomposition)

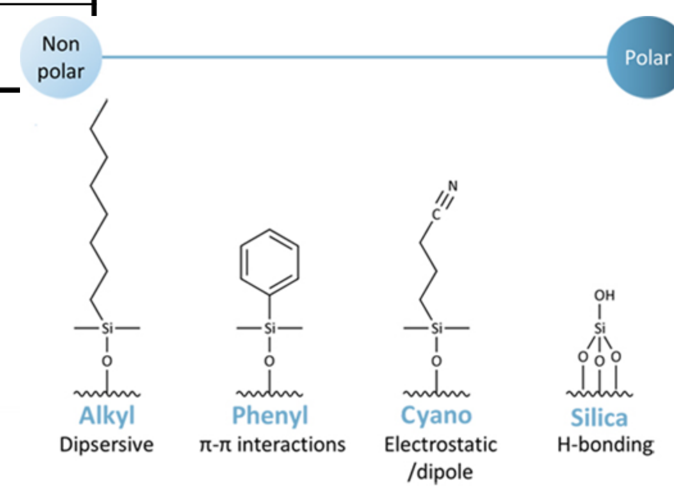
More polar columns suffer from lower maximum temperatures and greater column bleed

Instrumentation

Columns

Stationary Phases (examples)

Type	Functional Groups	Polarity
OV-1	methyl	Non-polar
OV-17	50% methyl/50% phenyl	Somewhat polar
OV-225	Cyanopropyl, methyl, and phenyl	More polar
carbowax	Ether groups	polar

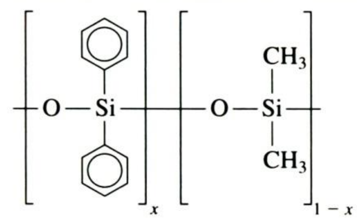
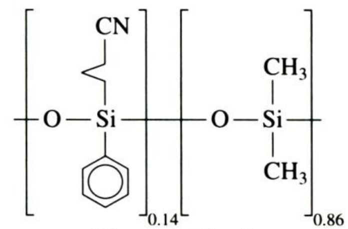
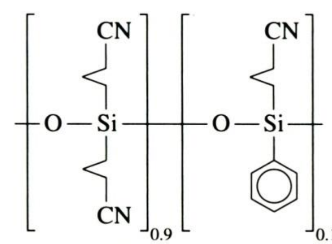


Instrumentation

Columns

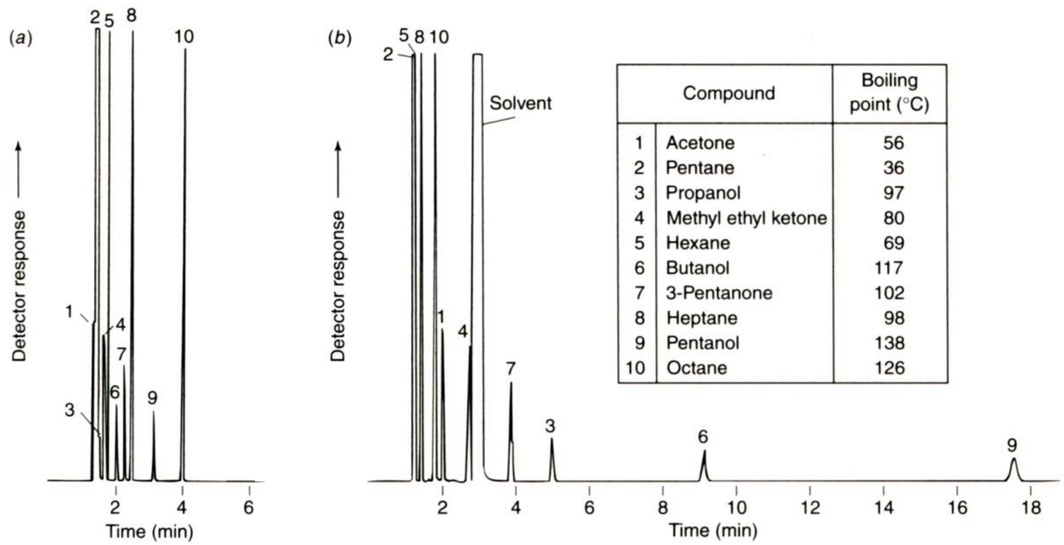
Stationary Phases (examples)

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°
$\left[CH_2CH_2-O \right]_n$ Carbowax (poly(ethylene glycol))	Strongly polar		40°–250°
 (Biscyanopropyl)$_{0.9}$ (cyanopropylphenyl)$_{0.1}$ polysiloxane	Strongly polar		0°–275°

Stationary Phases (examples)

- 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

Instrumentation

Columns

Stationary Phases (GSC)

GSC of inorganic gases is the one area where packed columns are still used almost exclusively. Main adsorbents for packed columns are based on:

- silica
- charcoal
- alumina
- molecular sieves

Instrumentation

Columns

Stationary Phases (GSC)

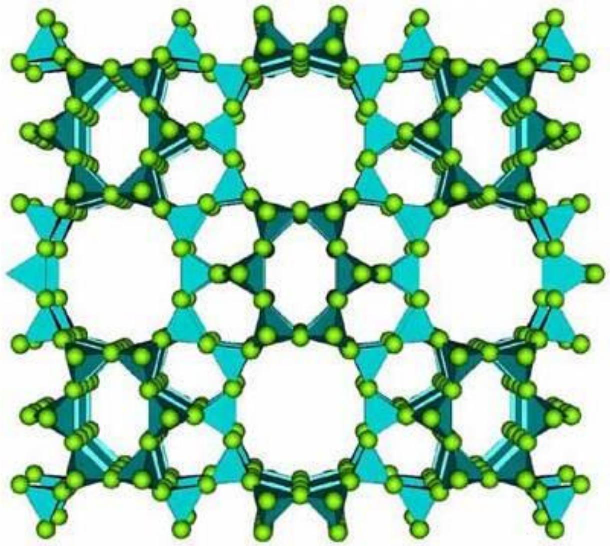
- **Porous silica is used with a variety of surface areas and pore diameters (separate low molecular mass hydrocarbons).**

- **Alumina has a variety of surface areas – but different selectivities due to Lewis acid sites associated with aluminum ions on the surface. To modify selectivity and improve efficiency, both silica and alumina can be coated with a non-volatile liquid.**

Instrumentation

Stationary Phases (GSC)

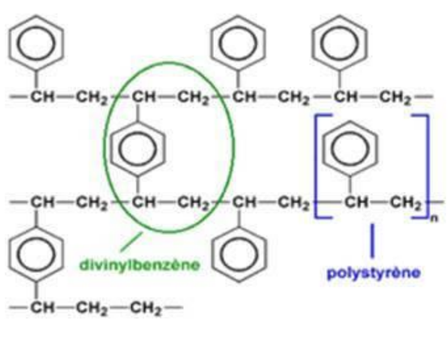
- **Molecular sieves are artificially prepared zeolites (i.e. Na, K, Ca – aluminosilicates) – small pore diameter used to separate low molecular weight molecules and hydrocarbons.**

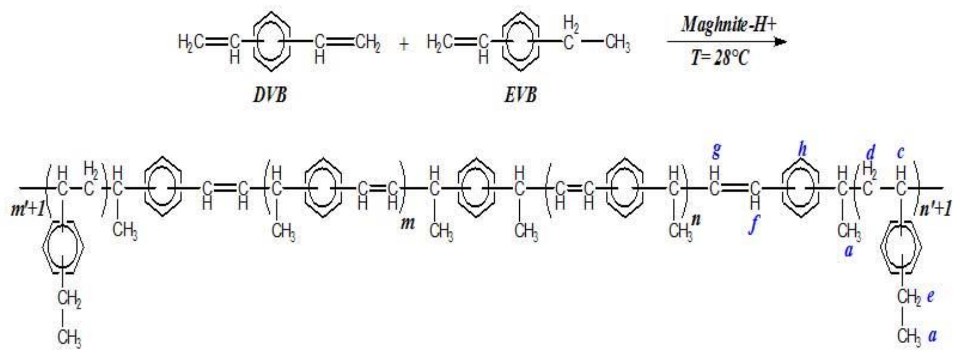


Instrumentation

Stationary Phases (GSC)

- **Porous polymers – good for separating polar analytes. Usually consist of copolymers of divinylbenzene (DVB), CH_2=CH-(benzene ring)-CH=CH_2, with another aromatic olefin (ethylene CH_2=CH_2).
Easy to use and has superior performance and unique separating abilities.
The nature of the separation is adsorption, but the mechanism is unclear.**





Scheme 1: Polymerization of DVB

Instrumentation

Columns

Stationary Phases (GSC)

Adsorbents for GSC

Chemical Type	Specific Surface Area (m^2g^{-1})	Pore Diameter (nm)
Silica	~ 100 – 185	15 – 30
Alumina	-	-
Carbon Black	10 – 1000	1 – 2
Molecular Sieves	700 – 1000	0.5 – 2.0
Porous Polymers	~ 100 – 800	5 – 500

Instrumentation

Columns

Stationary Phases (GLC)

Liquid Phase – required properties

- low vapor pressure
- thermal and chemical stability
- high viscosity
- nonreactive toward sample components
- wide operating temperatures (-80 to 450°C)
- reasonable solvent properties

Gas Chromatography

Columns (stationary phases) Examples for GLC

100% dimethyl polysiloxane

Non-polar, Stable to 360°C

Uses: solvents, waxes, petroleum and pharmaceutical samples

5% diphenyl - 95% dimethyl polysiloxane

Non-polar, Stable to 360°C

Uses: flavors, environmental samples, aromatic hydrocarbons

Carbowax® PEG

Polar, Stable to 250°C

Uses: fatty acids, flavors, acids, amines, solvents, xylene

50% cyanopropylphenyl - 50% phenylmethyl polysiloxane

Polar, Stable to 260°C

Uses: fatty acids, carbohydrates

Gas Chromatography

Columns (stationary phases) Examples for GLC

20% diphenyl - 80% dimethyl polysiloxane
Slightly polar, Stable to 310°C
Uses: volatile compounds, alcohols

6% cyanopropylphenyl - 94% dimethyl polysiloxane
Slightly polar, Stable to 280°C
Uses: volatile compounds, insecticides, residue solvents in pharmaceutical products

14% cyanopropylphenyl - 86% dimethyl polysiloxane
Intermediately polar, Stable to 280°C
Uses: pesticides, Aroclors, alcohols, oxygenates

65% diphenyl - 35% dimethyl polysiloxane
Intermediately polar, Stable to 370°C
Uses: triglycerides, rosin acids, free fatty acids

Assignment

- Read Chapter 3: Principles and Practice of Modern Chromatographic Methods, Peter E. Jackson, Academic Press.
- Read Chapter 3: Analytical Gas Chromatography, Walter Jennings, Academic Press. – it is available as an ebook at the UNT Libraries.