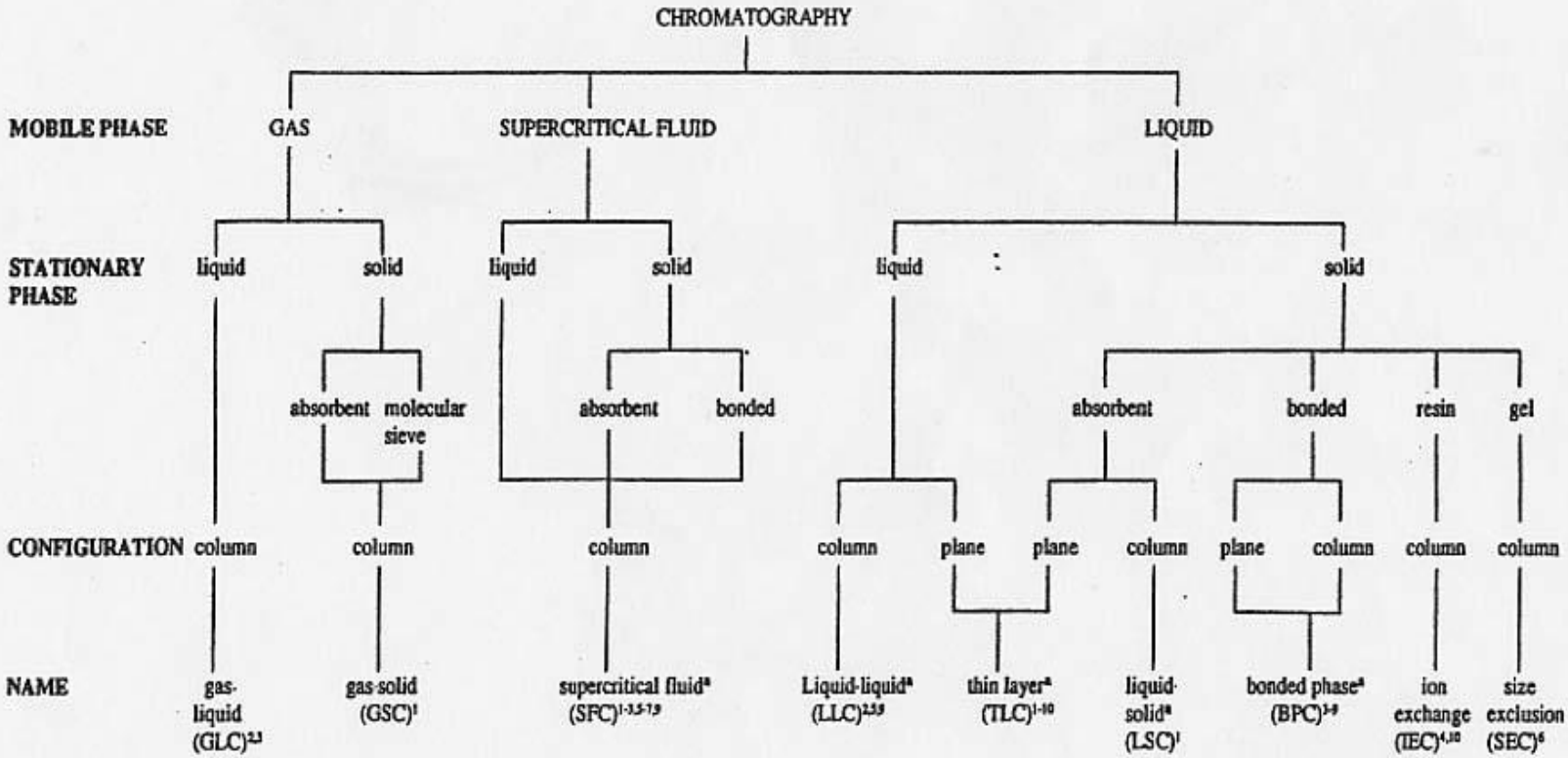
CHEMISTRY 5570

Advanced Analytical Chemistry

Lecture 13

Chromatography



[a]For these techniques the combination of mobile and stationary phase can be varied to generate either a normal phase or reversed phase system. Mechanisms which have been exploited in the various techniques are identified as: [1]adsorption, [2]partition, [3]bonded phase, [4]ion exchange, [5]ion interaction, [6]size exclusion, [7]affinity, [8]micellar, [9]chelation, [10]ion exclusion.

Fig. 1.3. Classification of chromatographic systems.

Chromatography

Gas Chromatography

Comparison with LC

Two key differences between GC and LC:

- No analyte – mobile phase interaction in GC
- Temperature is routinely changed (and always controlled) in GC

Effects of gases (vs. liquids)

- Much higher diffusivity (larger B term of van Deemter equation but very small Cm term)
- Lower viscosity of gases (backpressure is not as big an issue)
- Much lower density (capacity of column is a big issue with liquid samples)
- Gases are compressible

Chromatography

Gas Chromatography

Advantages versus LC

Main practical advantage comes from high N values (although H is usually larger) achieved with open tubular columns.

Another advantage comes from being able to use quite long columns (60 m vs. 250 mm for HPLC) because backpressure is not a major issue

Other advantages have to do with instrument cost and better detectors

Main disadvantage is for analysis of non-volatile compounds

Chromatography

Gas Chromatography

This method depends upon the solubility and boiling points of organic liquids in order to separate them from a mixture.

It is both a qualitative (identity) and quantitative (how much of each) tool.

Mobile phase - An inert gas such as helium is passed through the column as a carrier gas.

A sample is injected into a port which is much hotter than the column and is vaporized.

The gaseous sample mixes with the mobile phase and begins to travel with the carrier gas through the column.

Chromatography

Gas Chromatography

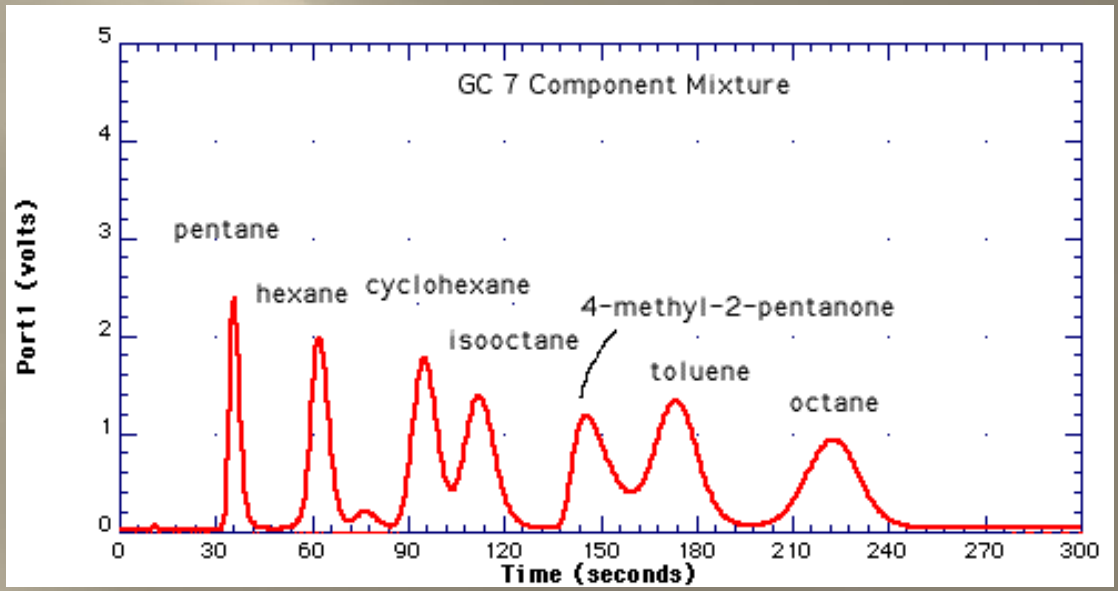
The chromatogram shows the order of elution (order of components coming off the column), the time of elution (retention time), and the relative amounts of the components in the mixture.

The order of elution is related to the boiling points and polarities of the substances in the mixture.

In general, they elute in order of increasing boiling point but occasionally the relative polarity of a compound will cause it to elute "out of order".

Chromatography

Compound	Boiling Point (^0C)
pentane	36
hexane	69
cyclohexane	80
isooctane	99
toluene	110
4-methyl-2-pentanone	117
octane	126



Chromatography

Gas Chromatography

Retention of Compounds

K value depends on:

Volatility

Polarity of analyte vs. polarity of stationary phase

Measure of volatility

Best measure is vapor pressure at temperature

Boiling point temperature is used more frequently

Depends on molecule's size and polarity

Polarity in separations

Compounds of similar polarity as stationary phase will be more retained than similar compounds of different polarity if their boiling points are the same (ether vs. acetone example)

Chromatography

Gas Chromatography

Mobile Phase

Since there is no mobile phase – analyte interaction in GC, why does the mobile phase matter?

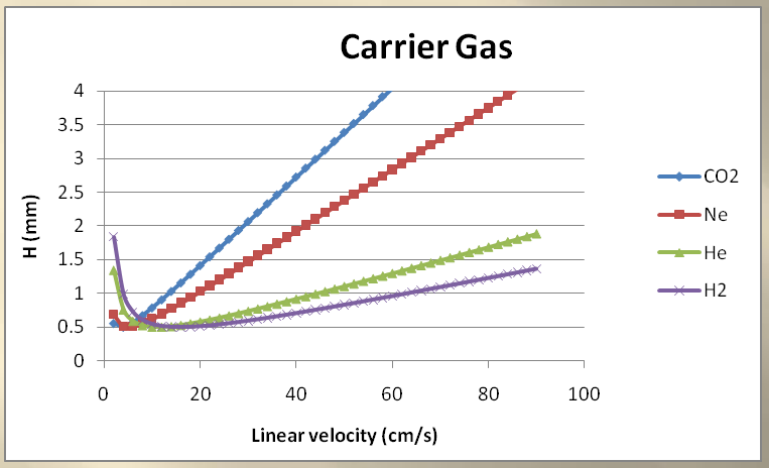
- Affects diffusion
- Smallest MW gases diffuse faster
- Hmin not affected much, but umin affected by gas chosen
- Smallest MW allows fastest runs at min. H

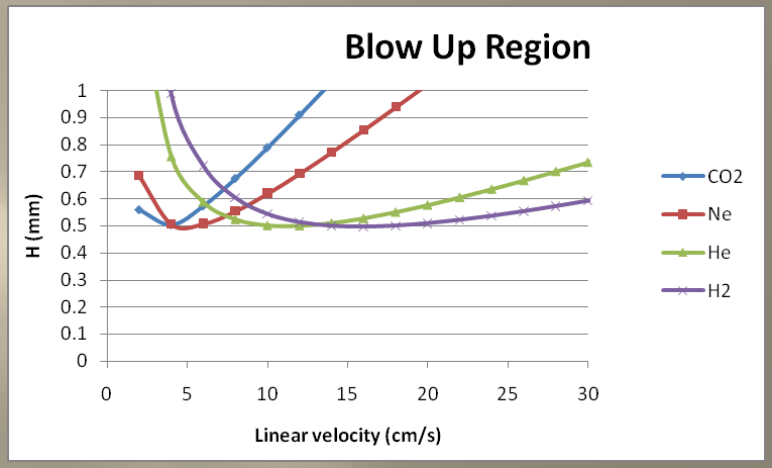
Detector requirements

- He is most common (inert, safe gas with high diffusivity for better efficiency at high flow rate)
- H2 also can be used with even better efficiency, but is less safe

Chromatography

Gas Chromatography





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Gas Chromatography

Effect of Carrier Gas Velocity

Gas flow through packed columns are generally described in terms of volumetric flow as measured at the column outlet.

Gas flow dynamics are simpler in the open tubular column and can be measured by linear velocity of the mobile phase or "average linear velocity" in cm/sec.

u – average linear mobile phase velocity

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Effect of Carrier Gas Velocity

u(cm/sec) = L(cm)/tM(sec)

tM – is called the gas hold up time

Unretained Compounds
Methane (or butane) is commonly used with FIDs
Methylene Chloride used with ECDs
Acetonitrile is used with NPDs in nitrogen mode
Methane (or butane or air) is used with TCD and MS
Vinyl Chloride is used with PID and ELCD

Chromatography

Gas Chromatography

Carrier Gas (Mobile Phase)

Carrier gas usually consist of He, N, H or a mixture of argon and methane. The function of the gas is to carry the sample through the system.

The carrier gas must be:

- inert toward the sample
- thermally stable
- cost effective
- compatible with the detector
- dry
- safe

He and N are the most popular carrier gases in GC.

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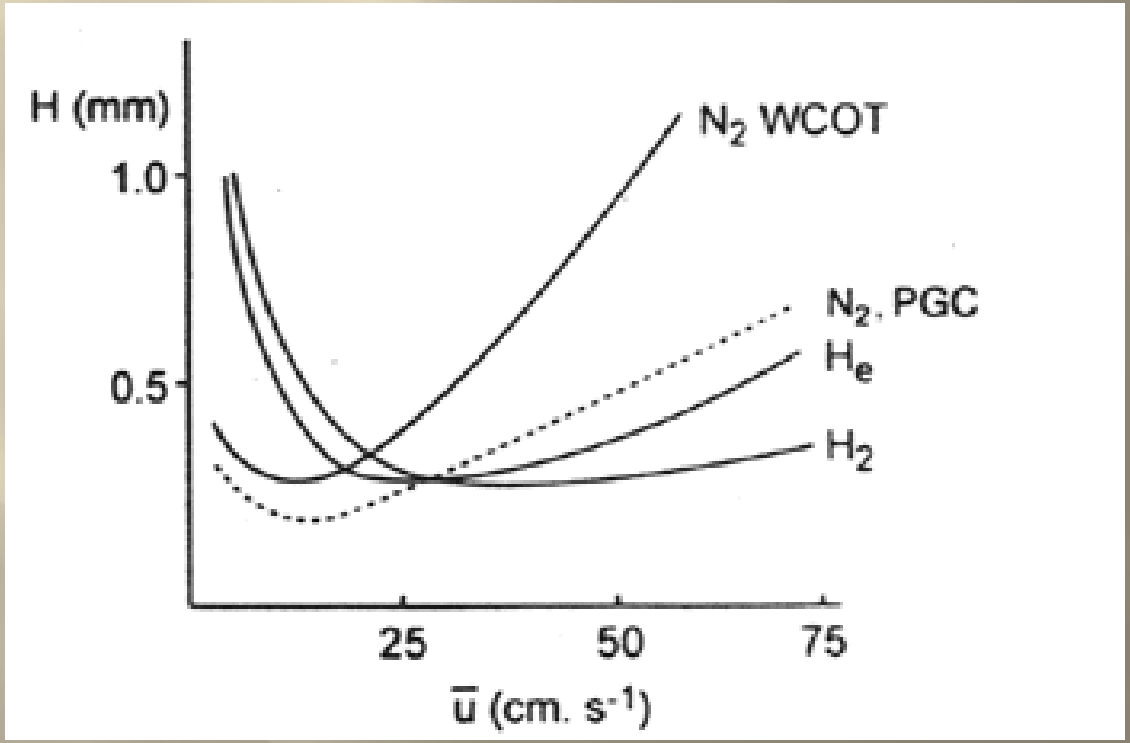
Gas Chromatography

The most efficient separations are achieved with N2 as a carrier gas. This can be seen with the following van Deemter curve:



Chromatography

Gas Chromatography

N_2 has a greater molecular weight and smaller diffusion coefficient – so lower β term, but must sacrifice analysis time. Best efficiency is at 8-10 cm/s.

H and He have better analysis time w/ a small sacrifice in efficiency.

He 16-20 cm/sec H 35-40 cm/sec

To reduce deterioration of the stationary phase and to lessen detector noise – very high purity gas needs to be used. For this reason oxygen and moisture traps in the carrier gas lines are used.

Chromatography

Gas Chromatography

Moisture traps – molecular sieves, trap water.

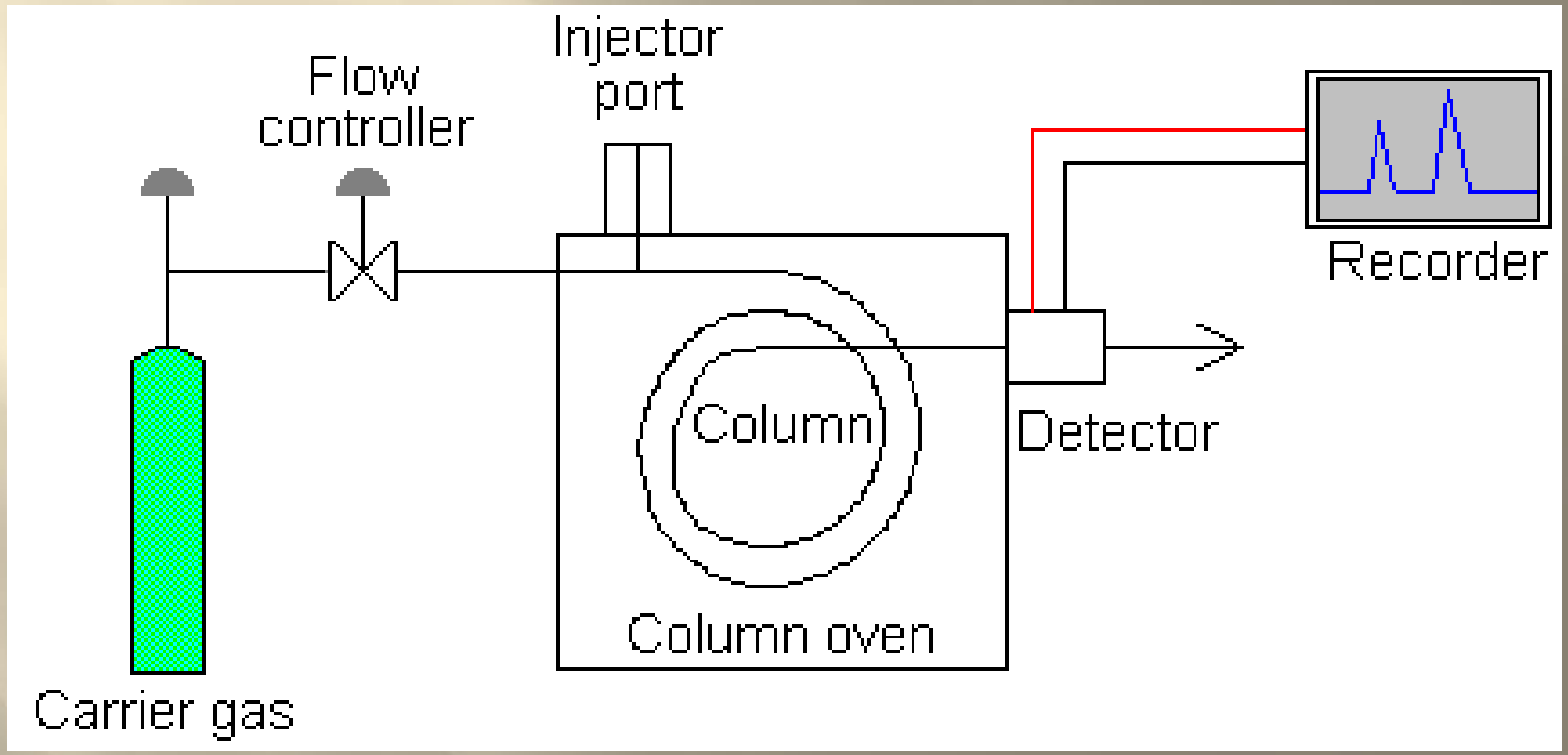
Hydrocarbon traps – activated charcoal, traps organics

Oxygen traps – metal catalyst

(For multiple traps – order from gas source – moisture, hydrocarbon, oxygen, indicating oxygen)

Chromatography

Gas Chromatography – Injection Methods



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Injection ports

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapour - slow injection of large samples causes band broadening and loss of resolution.

The most common injection method is where a microsyringe is used to inject sample through a rubber septum into a flash port at the top of the column.

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Injection ports

The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample.

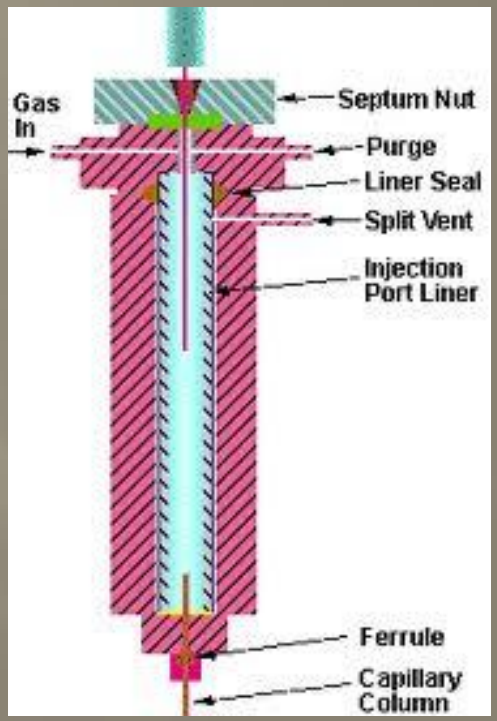
For packed columns, sample size ranges from tenths of a microliter up to 20 microliters.

Capillary columns, on the other hand, need much less sample, typically around 10-3 mL, split/splitless injection.

Chromatography

Injection ports

Purpose of port is to flash evaporate your sample and introduce it into the column.



Chromatography

Sample Injection

Peak widths are influenced by the efficiency of the injection process. The shorter the length of the column occupied by the injected sample, the shorter the band as it begins and completes the chromatographic process.

The critical function of the injection process is to introduce the sample so that it occupies the shortest possible length of column.

Chromatography

Sample Injection - Factors that affect Injection

-Transfer of the sample from the syringe to the inlet (greater concern in heated injectors)

-Transfer of the sample from the inlet to the column (inlets have active sites)

-Length of the column initially occupied by the injected sample (all modes)

-Rate and efficiency of sample vaporization in the inlet (split and splitless)

-Speed of sample transport from the inlet to the column (split, splitless, and PTV)

-Completeness of sample transport from the inlet (all modes)

-Homogeneity of temperature and phase ratio for sample band

Chromatography

Sample Injection

Syringe Technique

Universal method of introduction is with a microsyringe through a septum.

Reproducibility with gas samples is poor since the volume of gas is temperature dependent – use of a sampling valve increases precision. Most samples are injected as liquids.

Overall packed columns are relatively forgiving of poor technique because of large sample size.

Discrimination - selective loss of some sample components during injection.

Open tubular columns are more demanding on technique.

Chromatography

Sample Injection

Syringe Technique

Several techniques are used to inject the sample:

A vaporizing injection can retain the sample in the needle when introduced into the injection port. Most common and simple but poorest method. Variations include hot needles, solvent flush (or air), cold needles and filled needles.

Chromatography

Sample Injection

Syringe Technique

Hot needle - sample in barrel only (temperature allowed to equilibriate).
Cold needle - sample in barrel only (immediate injection).
Filled needle - sample in barrel and needle.
Solvent flush - solvent + sample + solvent or solvent + air + sample + air.

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Sample Injection

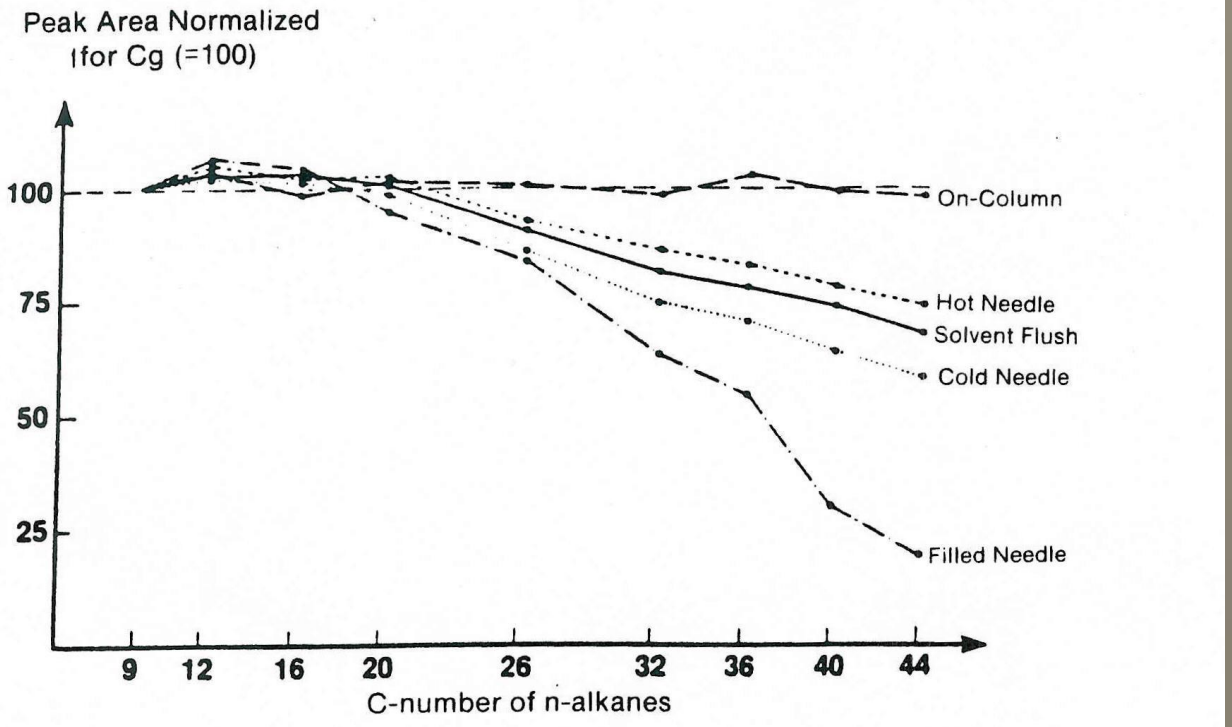

Fig. 3.5. Discrimination of *n*-alkanes obtained by different injection techniques using a split injection (1 μl; split ratio 1:15) and injection port temperature of 350°C. Methods of syringe handling are described in the text. Reproduced with permission from Grob and Neukom (1979). *J. High Resol. Chromatogr., Chromatogr. Comm.*, **2**: 15.

Chromatography

Sample Injection

Split Injection

First open tubular column injection technique used.

Sample is injected using solvent flush or hot needle technique and after evaporation and mixing with carrier gas, the sample is split into two unequal portions – the smaller one passes to the column while the rest is vented to waste.

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Sample Injection

Split Injection

Dynamic Splitting

Evaporation, mixing and splitting of the sample in a flowing stream – most common.

Why split? – capillary columns have a limited sample capacity.

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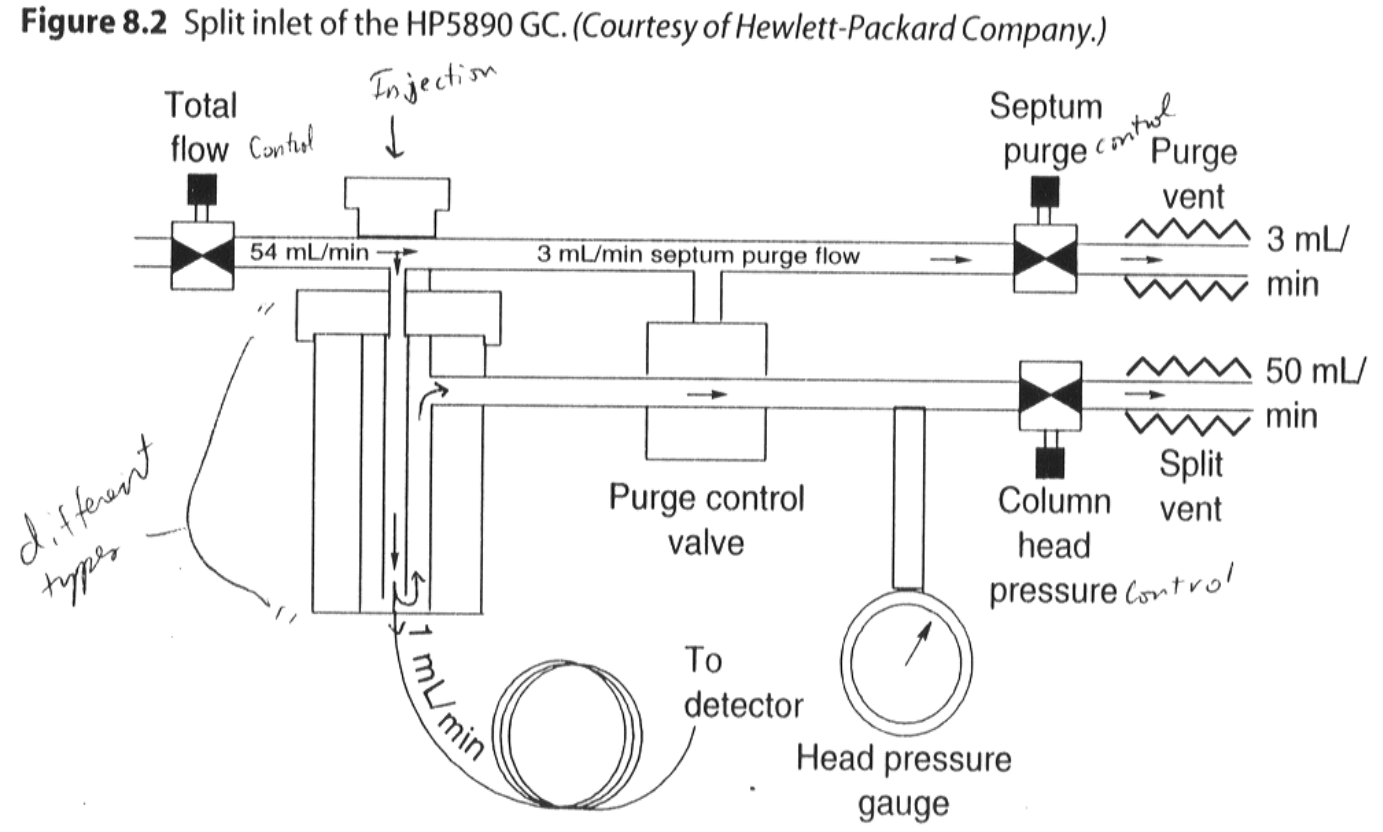
Sample Injection - Split Injection



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Sample Injection

Split Injection

Total carrier gas flow into the inlet is split into three portions as it passes the inlet port.

1st portion – 1 to 3 ml/min – passes past the septum to sweep away contaminates from the septum to purge vent.

2nd and 3rd portion then flow into the inlet of the injection port.

At the bottom of the injection port a split occurs
a very small portion flows down into the column
~ 1 ml/min
the rest flows out the split vent

Chromatography

Sample Injection

Split Injection

So ~90% of the sample is thrown away, however for quantitative analysis the proportion of the sample analyzed must be known.

$$\text{Split Ratio} = \frac{\text{split vent flow + column flow}}{\text{column flow}}$$

Split ratios range from 10:1 to 500:1.

For our figure:

$$\text{Split Ratio} = \frac{50+1}{1} = 51{:}1$$

Chromatography

Sample Injection

Split Injection

The smaller the column the higher the split ratio used to keep from overloading the column.

Disadvantage: split inlets have a high probability of suffering from sample discrimination. So it is important to select the appropriate liner. (Variables – molecular size, polarity of analytes, injected volume, diameter of split liner, viscosity)

For a split system quantitation is actually not very reliable unless the operator is an expert, because of all the different variables involved.

Chromatography

Liners

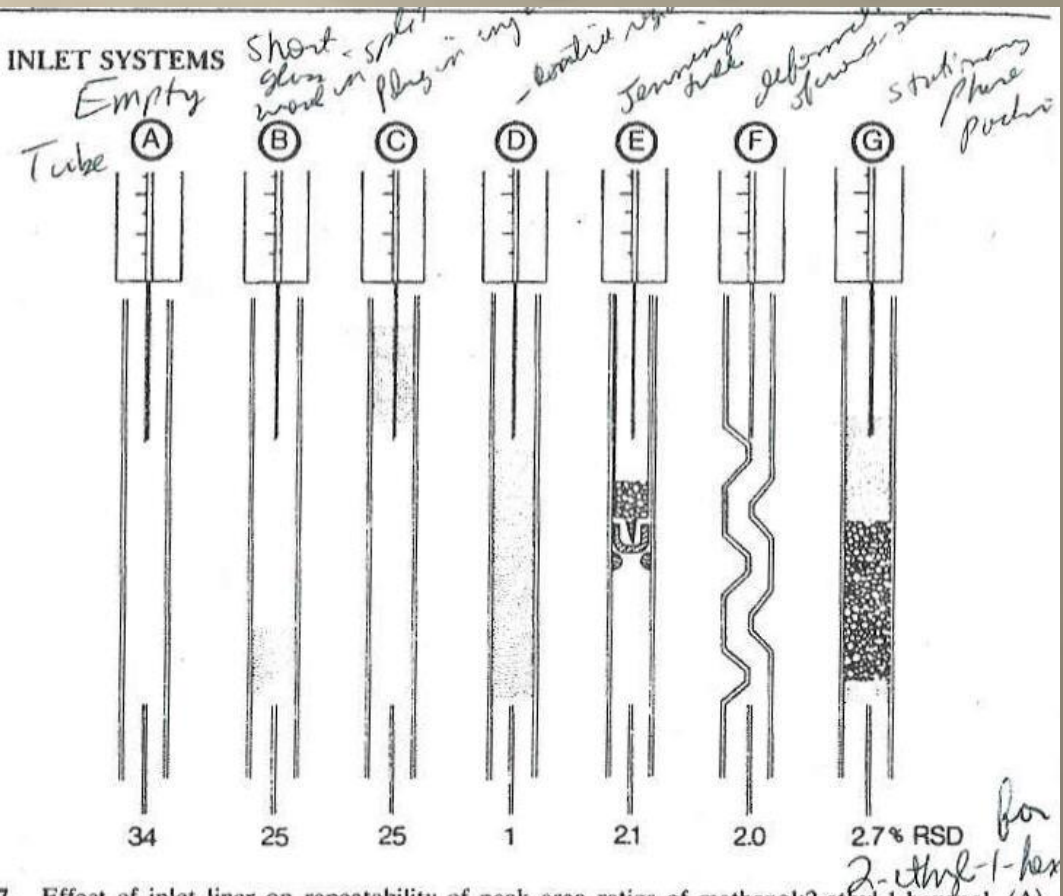
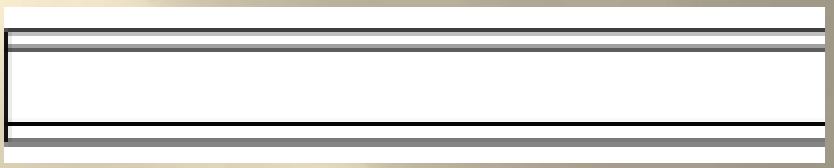


Fig. 3.7. Effect of inlet liner on repeatability of peak area ratios of methanol:2-ethyl-1-hexanol. (A) empty tube, (B) short glass wool plug in the splitting region, (C) short glass wool plug in the injection region, (D) long and tight glass wool plug, (E) Jennings tube, (F) deformation of cross-section, (G) chromatographic support packing. Reprinted with permission from Schomburg *et al.* (1977). *J. Chromatgr.*, **142**: 87.

Chromatography

Liners

Straight 

Function: Low surface area for less activity

Recommended for: Volatiles

Advantages

- Simple to use
- Least expensive
- Low activity

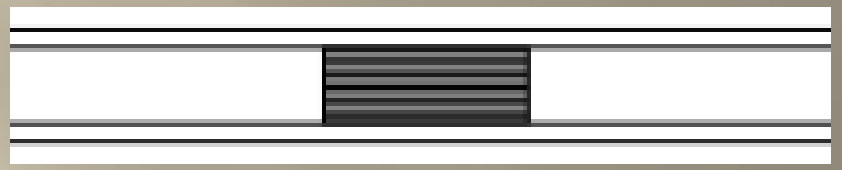
Disadvantages

- Possible inlet discrimination
- More frequent seal maintenance from exposure to sample contamination
- Possible inconsistency if sample injection bypasses split ratio

Chromatography

Liners

Glass Wool (middle)



- **Function:** Traps non-volatiles; mixes sample; vaporizes sample above the column
  Recommended for: Dirty samples, volatiles, high initial oven temperatures

Advantages

-Reduces seal contamination and maintenance

-More reproducible results

-Can help focus analytes

-Can help focus analytes

Disadvantages

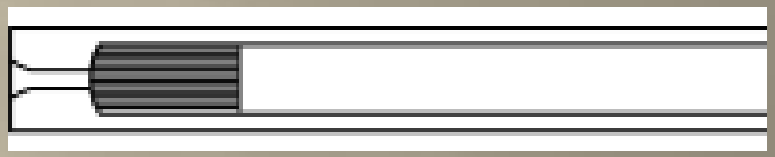
- Higher surface area that can become active

- Glass wool can become dislodged

Chromatography

- **Liners**

Glass Wool (bottom)



- **Function:** Traps non-volatiles; mixes sample; vaporizes sample above the column
  Recommended for: Dirty samples

Advantages

-Reduces seal contamination and maintenance

- More reproducible results
- Can provide higher responses than wool in middle

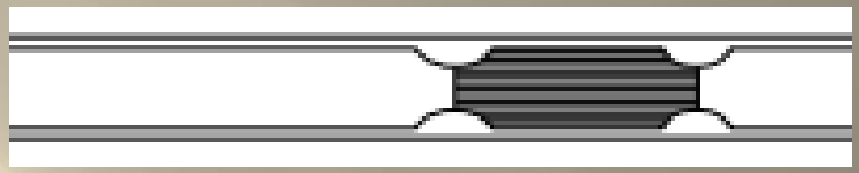
Disadvantages

- Higher surface area that can become active
- Glass wool can become dislodged

Chromatography

- **Liners**

Glass Wool (top)



- **Function:** Keeps glass wool in place; wipes syringe needle clean

  Recommended for: Pressure pulsed injections, dirty samples, volatiles, high initial oven temperatures

Advantages

-Reduces seal contamination and maintenance

-More reproducible results

-Can help focus analytes

-Extends column life

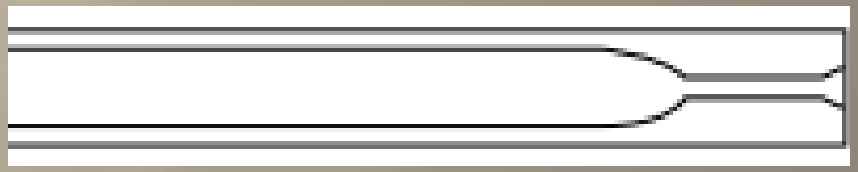
Disadvantages

- Higher surface area that can become active

Chromatography

Liners

Taper/Gooseneck (top)



- **Function:** Limits the expansion of the solvent to the inlet
- **Recommended for:** Water injections

Advantages

-Allows for larger injection volumes

- Decrease backflash

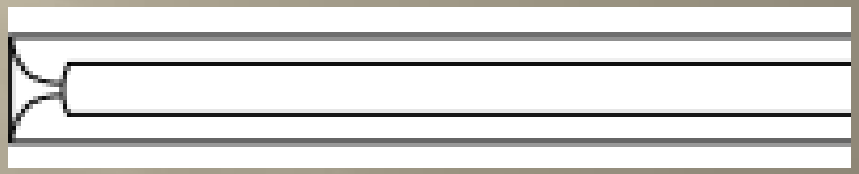
Disadvantages

- Higher risk of needle breakage
- Increased cost
- Cannot self-pack with glass wool

Chromatography

Liners

Taper/Gooseneck (bottom)



- **Function:** Directs flow onto column; low surface area
- **Recommended for:** Pesticides (without wool), semi-volatiles (with wool)

Advantages

-Reduces seal contamination and maintenance
- Improved sensitivity
- Lower activity

Disadvantages

- Increased cost

Chromatography

Liners

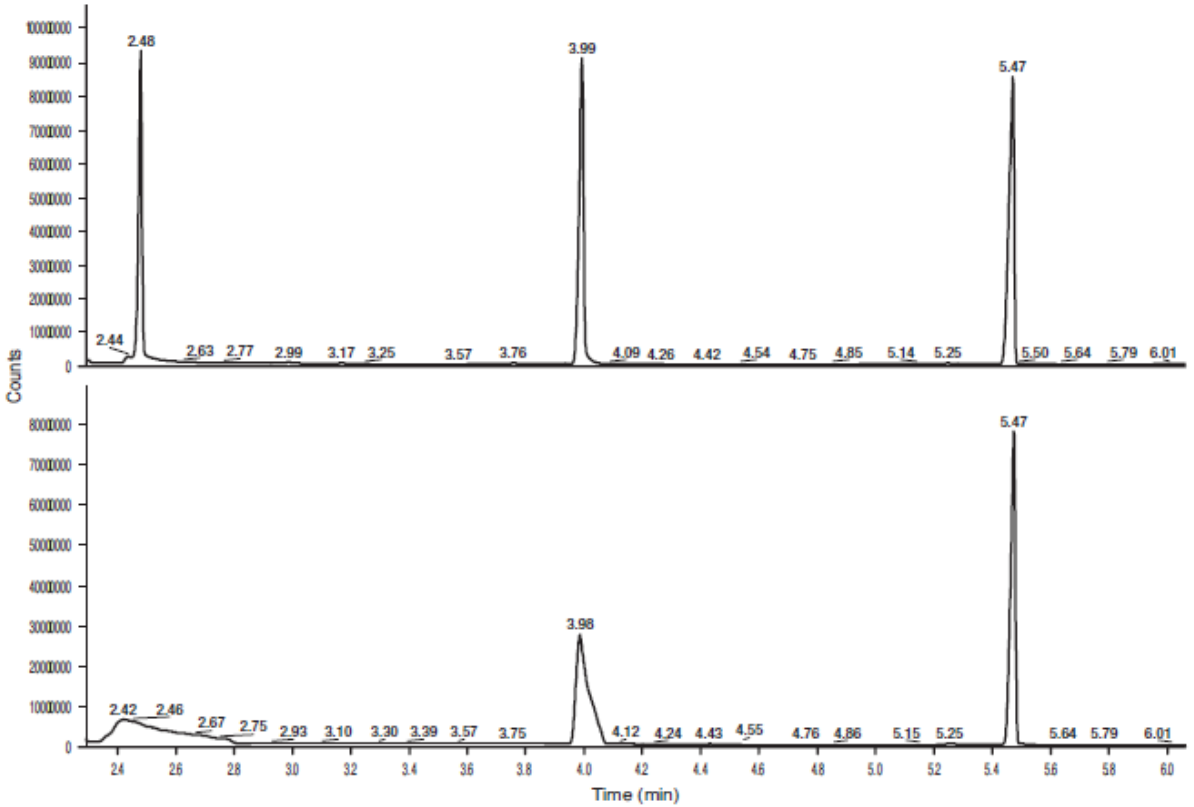


Figure 3: The effect of using the wrong liner in splitless mode (n-alkanes). Top chromatogram, splitless (correct) liner, bottom chromatogram split (incorrect) liner

Chromatography

Sample Injection

Liners

- For high boiling temperature solutes – a packed liner works well – increases the liner's heat capacity and assists in sample vaporization.
- For labile solutes – packed liners can contribute to solute decomposition.
- For very volatile solutes – packed liner will increase band broadening because of the multipath diffusion through the packing – unpacked liners are better.

Chromatography

Sample Injection

Splitless Injection

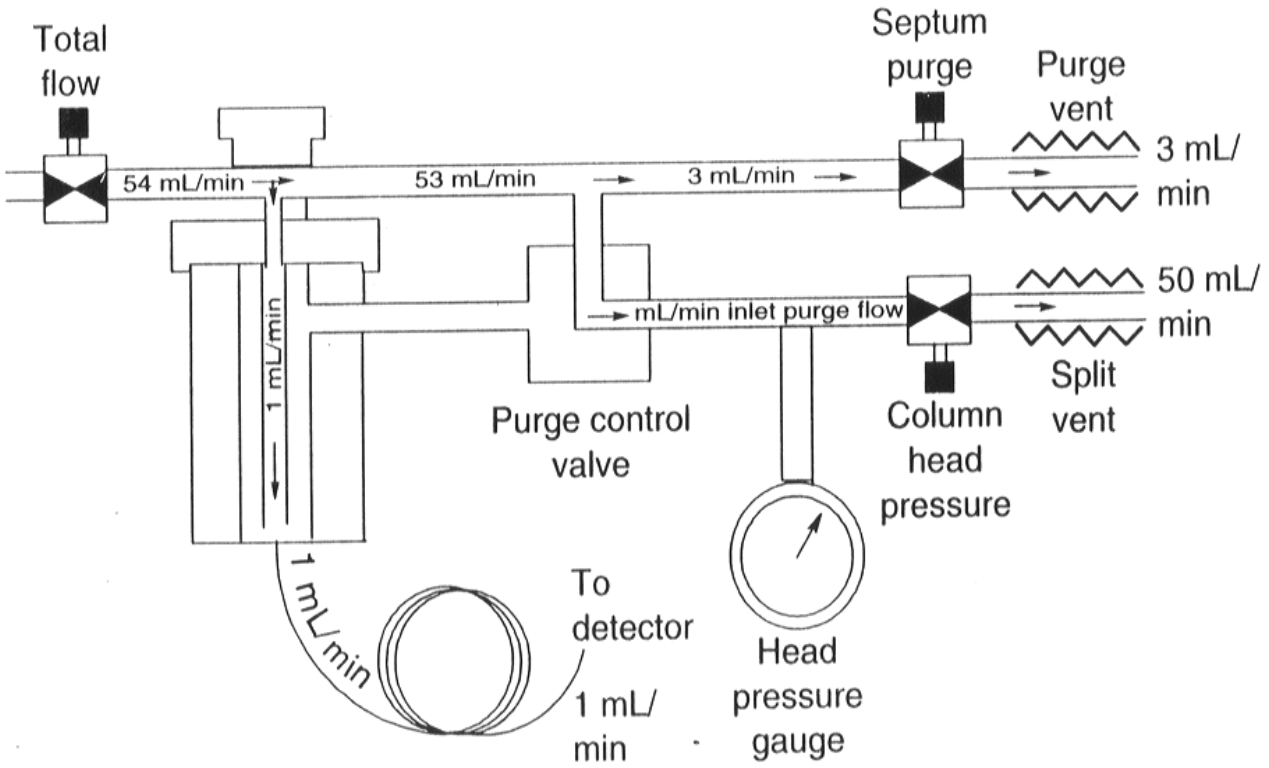
- For ultratrace analysis, very complex samples with wide range of boiling points – splitless injection is better.

- There are two types
  - evaporation and trapping (PTV – programmed temperature vaporizer)
  - cold-on-column injection or direct on column

Chromatography

Sample Injection - Splitless Injection

Figure 8.3 Splitless configuration. *(Courtesy of Hewlett-Packard Company.)*



Chromatography

Sample Injection

Splitless Injection

- For splitless – a large volume (1-5 ml) of dilute sample is introduced. The carrier gas velocity is lower for splitless than for split.
- Residence time of sample in injection port liner is longer for splitless mode (15 s) than for split mode (less than 1 sec).

Chromatography

Sample Injection

Splitless Injection

- Splitless work best for bonded phase columns since a high solvent load is placed on the column.
- Cold-on-column injection reduces solute decomposition and discriminatory effects if the injection is rapid. One disadvantage is that non-volatile material in the sample will enter the column and remain there.
- So the programmed temperature vaporizer (PTV) was developed using a cold split/splitless injection port, which can be rapidly heated. Non-volatile materials remain in the inlet and discrimination from the needle is minimized since the port is initially cold.

PTV is a universal injection system – program – split/splitless.

Chromatography

Sample Injection

Programmed Temperature Vaporizing (PTV)

PTV is a universal injection system that can be used in split, splitless or direct mode.

The initial inlet temperature is below the boiling point of all components including the solvent.

After the sample is injected, the temperature of the inlet is programmed to increase at a high rate.

Linearity up to C28 hydrocarbon.

Chromatography

Sample Injection

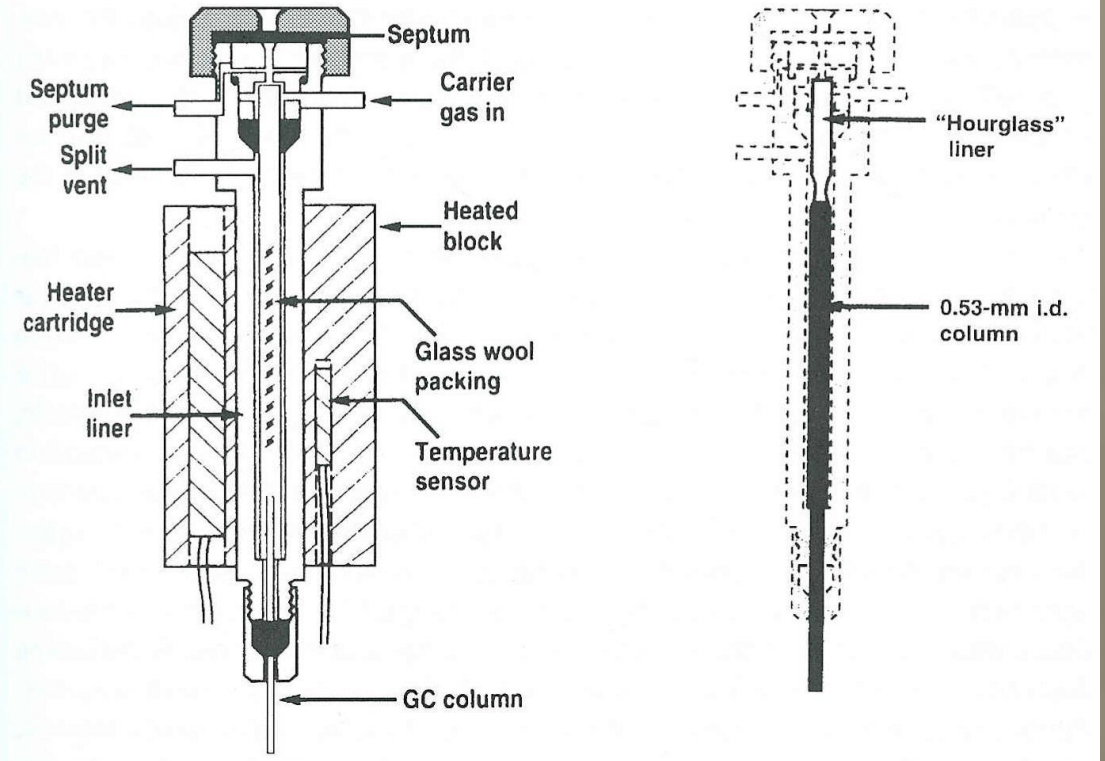


Fig. 3.7. Left, a Schematic representation of a PTV injector configured for split/splitless injection. The cooling coils were omitted for clarity. Right, the same injector modified for on-column PTV injection. The glass insert helps guide the needle directly into the column. *Taken from, J. Hinshaw, LC–GC, **10**:748 (1992)* with permission.

Chromatography

Sample Injection

Purge-and-Trap Method

Effective way for sampling and analyzing low levels of volatile organic compounds from matrices such as drinking water, waste water, soil, and sludge.

Preferred method for evaluation of water purity in the US using EPA methods. One method can quantitate 82 analytes at a detection limit of 0.1 ppb in a single analysis.

Chromatography

Sample Injection

Purge-and-Trap Method

A sample (liquid) is purged for a specific time and temperature with a purge gas, usually helium.

The volatile analytes are swept by the purge gas to a trap and absorbed.

The trap material (usually Tenax - 2,6-diphenylene-oxide polymer resin) is an absorbant material.

After a specified trapping time, the trap is rapidly heated and the analytes are desorbed and swept into the GC column by the carrier gas.

Chromatography

Purge-and-Trap Method

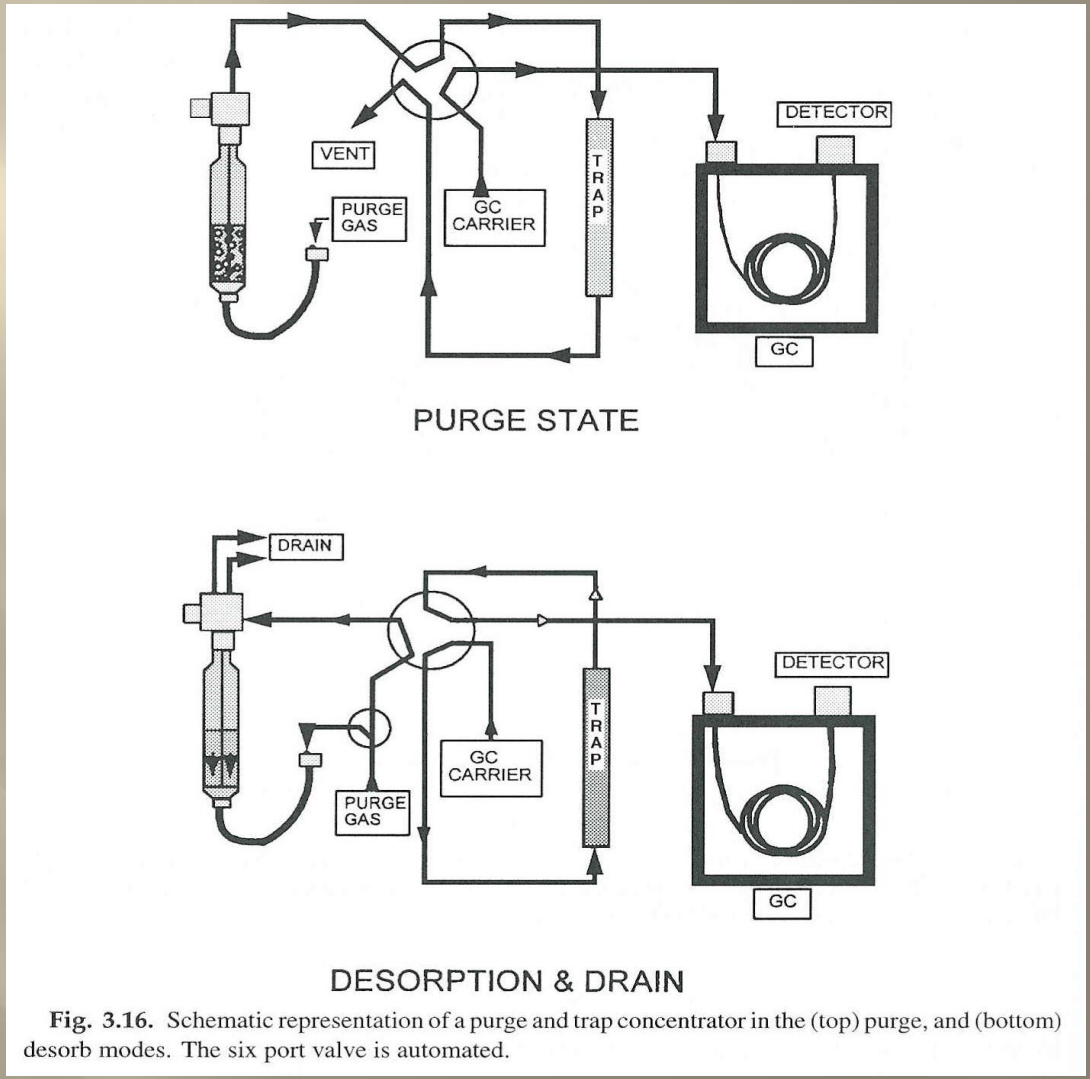


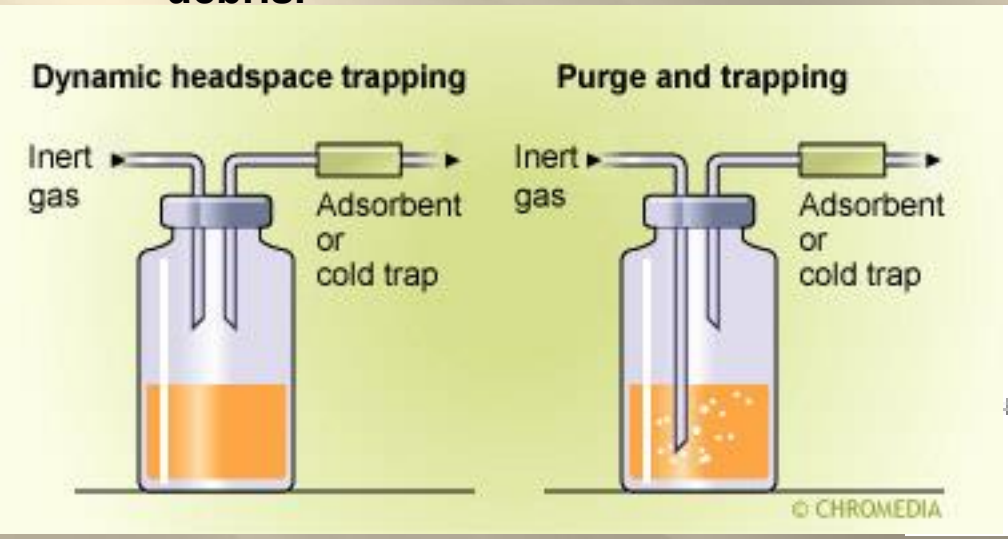
Fig. 3.16. Schematic representation of a purge and trap concentrator in the (top) purge, and (bottom) desorb modes. The six port valve is automated.

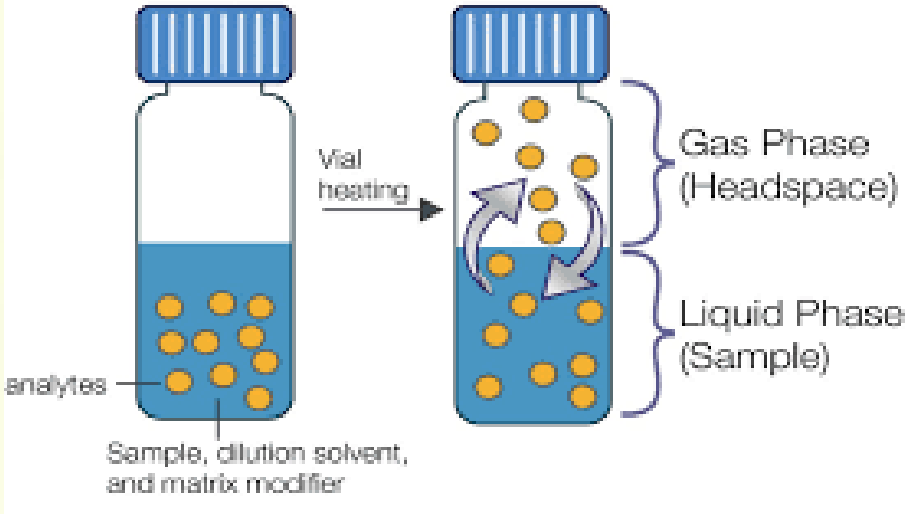
Chromatography

Headspace Sampling Method

Headspace sampling is a separation technique in which volatile material may be extracted in the headspace above a liquid or solid and injected into a gas chromatograph for analysis.

This is the main method used in forensic analysis of blood alcohol and arson/fire debris.

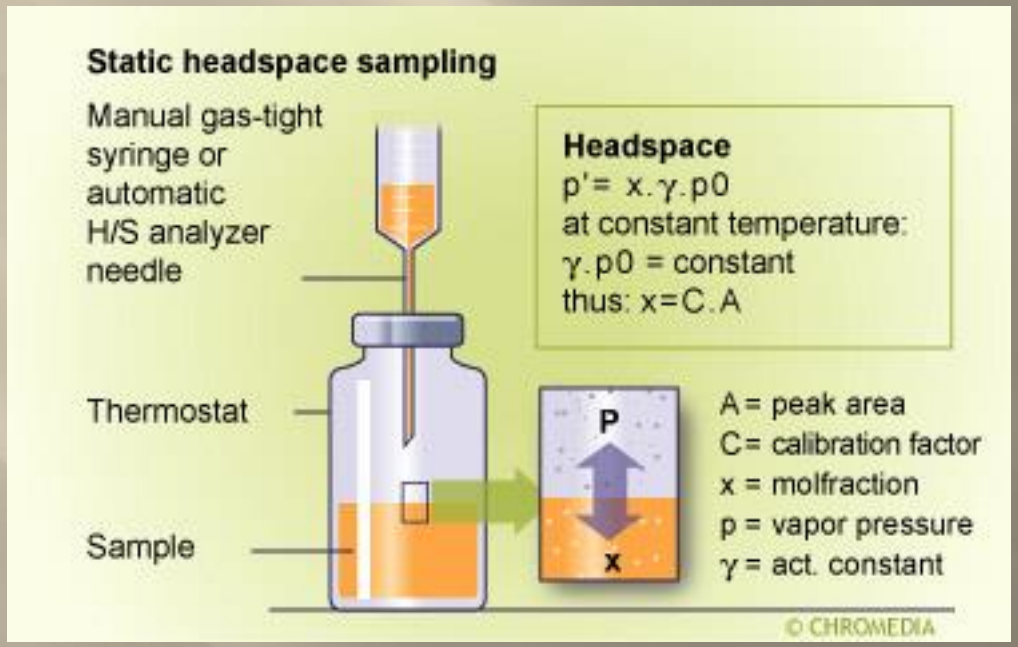




Chromatography

Headspace Sampling Method

Static headspace sampling is the simplest method, particularly when carried out manually with a gas-tight syringe. The sample is thermostatted and allowed to reach equilibrium. The headspace sample is small in comparison with the total headspace volume.



Chromatography

Headspace Sampling Method

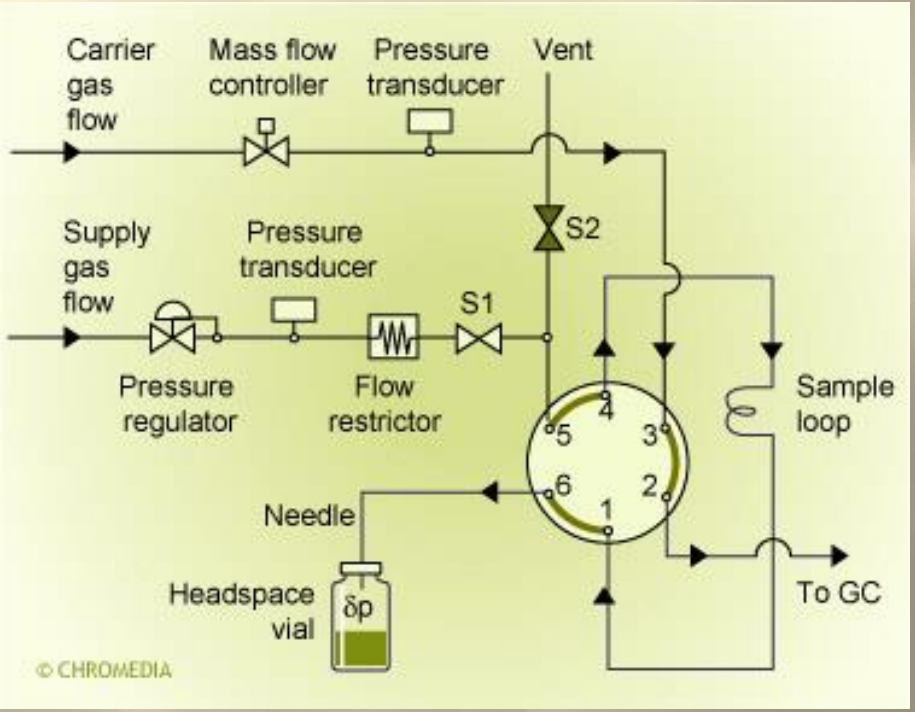
Automatic headspace are based on static headspace where samples are contained in thermostatted vials and headspace samples are taken automatically by penetration of a septum by a needle. The concentration of each component in the headspace is related its concentration in the liquid or solid sample according to Henry's law.

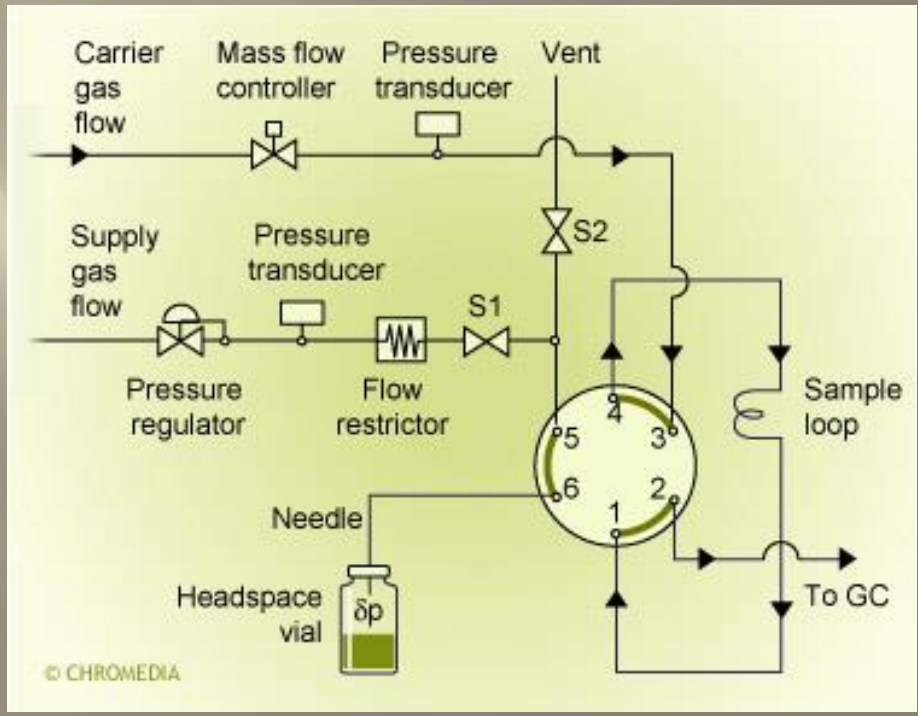
The sample vial is heated during a fixed period of time. After that the sample vial is pressurized. By switching the valve, the headspace vapour flows to the sample loop and injection takes place by switching the 6-port valve again.

Chromatography

Headspace Sampling Method

Automatic headspace





Chromatography

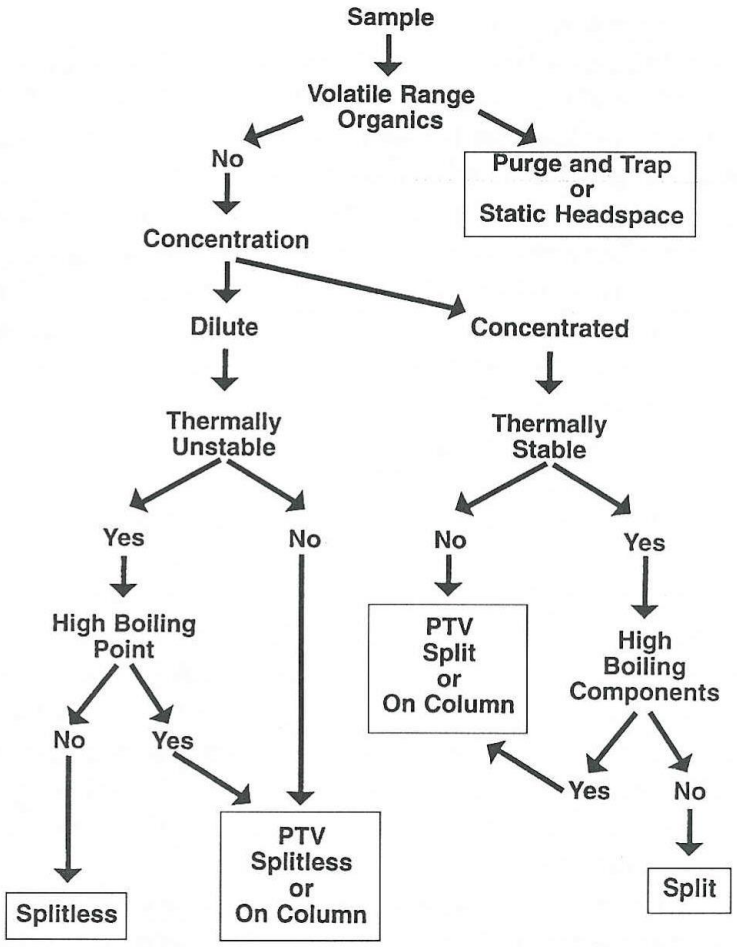


Fig. 3.19. A sample-based flow chart for selecting the injection mode. As an example, if the sample did not contain volatile range organics, was reasonably concentrated, thermally stable, and lacked high boiling components, the injection mode of choice is split.

Assignment

- Test II

Chem 5570