Gas Chromatography

Chromatography: Separate analytes in a mixture with a resolution ≥1.5 in the shortest amount of time and detection of separated components.

- Gas supplies usually have traps to remove any water, oxygen, hydrocarbons or other "contaminants" from compressed gases
- Instruments can have multiple injectors, detectors or columns
- Injectors and detectors temperatures controlled
- The GC oven has a large fan and a vent door for rapid cooling/heating.
- Data collection and integration system

- The analyte (GC) – necessarily in gas phase. Partitions between the mobile phase (carrier gas) and the liquid stationary phase (predominant) inside capillary column or on particles inside a packed column
- Some packed-column GC uses non-coated solid stationary phases; gas-solid adsorption chromatography

Achieve separation by using suitable;

- columns with proper stationary phase, diameter of column, stationary phase loading, β, and column length.
- injection modes to optimize the of loading and separation of the sample mixture
- temperature (or pressure) programs for the column
- detector that is suitable for the analyte(s) of interest

Early practice of gas chromatography was done with packed columns. Such columns are still used for preparative chromatography as they can handle larger amounts of materials.

Chromatography (analytical) is practiced with capillary columns, which are open tubular columns.

packed (preparative, larger capacity, low resolution)
capillary (analytical, smaller capacity, high resolution)

Example
Stationary Phase: Bonded; poly(50% n-octyl/50% methyl siloxane)
Temp. Limits: -60 °C to 280 °C (isothermal or programmed); ≤0.32 mm I.D.
Open Tubular Columns

WCOT, SCOT: s.p. is coated as a film.

PLOT

Porous
Carbon layer

Fused silica

WCOT specifically is the primary type of capillary GC column for quantitative analysis:

Higher resolution and shorter analysis time and allows greater ability to discriminate between components.

Capacity is small, is of less concern for analytical purposes as long as sufficient analyte is available for detection; pg/mL (ppt) to µg/mL (ppb).

- Column constructed of fused silica tubing
- Polyamide coating gives it strength
- Liquid stationary phases coated or chemically bonded to the inner wall of capillary
- Column diameters 0.10 - 0.53 mm, length 30-60m

Column:

Characterized by Stationary phase (mobile phase, LC) materials inert particle size \( d_p \) and s.p. loading which is proportional to the s.p. film thickness \( d_f \) and the column length \( L \).

Temperature range of operation (GC); s.p. dependent, Bonded – thermally stable.

Mobile phase - carrier gas (GC), usually an 'inert'gas, primarily pushes the material through the column. In LC; m.p. is a solvent, should be compatible with the analytes and influences the equilibration process in partitioning etc.
The column suitability for analysis is determined by s.p/m.p system in LC and, s.p. in GC.

**Use a s.p. with similar ‘polar’ characteristics as the analytes to effect a retention.**

Polar analytes require polar stationary phases to be retained in the s.p.

Non-polar analytes require less/non-polar stationary phases.

Analytes generally are a mixture of polar and non-polar components – compromise necessary.

<table>
<thead>
<tr>
<th>Length vs. resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter vs. resolution</td>
</tr>
<tr>
<td>Stationary phase thickness vs. resolution</td>
</tr>
<tr>
<td>Sample capacity v. resolution</td>
</tr>
</tbody>
</table>

Sample capacity: the amount of sample that can be injected onto a column without overloading. Often expressed as grams of sample per gram of packing.

Overloading is defined as the point at which the sample mass injected makes the column efficiency N, decrease by 10% from its normal value; sometimes called sample loading.

**Table 24-2**  
**Polarity of solutes**

<table>
<thead>
<tr>
<th>Non-polar</th>
<th>Weak intermediate polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated hydrocarbons</td>
<td>Ethers</td>
</tr>
<tr>
<td>Olefinic hydrocarbons</td>
<td>Ketones</td>
</tr>
<tr>
<td>Aromatic hydrocarbons</td>
<td>Aldehydes</td>
</tr>
<tr>
<td>Halocarbons</td>
<td>Ketones</td>
</tr>
<tr>
<td>Mercaptans</td>
<td>Tertiary amines</td>
</tr>
<tr>
<td>Sulfides</td>
<td>Nitro compounds (without α-H atoms)</td>
</tr>
<tr>
<td>C3</td>
<td>Nitrites (without α-H atoms)</td>
</tr>
</tbody>
</table>

**Strong intermediate polarity**  
**Strongly polar**

| Alcohols | Polyhydroxy alcohols |
| Carboxylic acids | Amino alcohols |
| Phenols | Hydroxy acids |
| Primary and secondary amines | Polymeric acids |
| Osmes | Polyphersols |
| Nitro compounds (with α-H atoms) | Nitrites (with α-H atoms) |

*Adapted from H. M. McNair and R. E. Reutter, Basic Gas Chromatography (Palo Alto, C.A.: Noyes, Instrument Division, 1976).*

Thick films: increases column bleeding
**Capillary columns**

Increased length leads to longer separation times; band broadening problems arise if too long.

Stationary phase thickness and column diameter increases leads to increased sample capacity and can provide increased resolution; tradeoffs - longer analysis time and more column bleed.

Thick stationary phases bleed more - contaminate MS detection system.

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**Packed Columns**

They have greater sample capacity vs. open tubular columns but generates broader peaks, longer retention times and lower resolutions.

Useful for preparative work.

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**Capillary vs. Packed Columns**

- **Capillary Columns:**
  - Higher R
  - Smaller H; high N
  - Fast
  - Greater sensitivity
  - Analytical
  - Smaller sample capacity
  - Higher cost/column
  - Columns fragile

- **Packed Columns**
  - Low R
  - Larger R, low N
  - Slow
  - Greater sample capacity
  - Lower cost
  - More rugged
  - Preparative

---

**Column selection GC:**

Select a stationary phase that would retain ('dissolve') the analytes of interest.

Stationary phase polarity – analyte polarity; (like dissolves like).

Selectivity to individual analytes determines the quality of separation (ability to discern the components) measured in terms of relative retention \( \alpha_{AB} \).

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**Order of elution (GC):**

Lesser retained analytes elute earlier.

*Given similar molecular characteristics, more volatile analytes (low b.p., high vapor pressure) elutes earlier; i.e. low molecular masses elutes earlier.*

Consider polarity, molecular mass, b.p./vapor pressure of analytes *in concert with* the 'polarity' of the phase(s) to determine the order of elution.
Intermolecular Forces (attractive):

IMFs determine the retention times. GC – s.p. LC – both phases. This is a functional/practical classification.

1. H bonding
2. Ion-ion
3. Ion-dipole
4. Dipole-dipole
5. Acid-base type
6. Conformational interactions
7. Pi-pi attractions
8. London forces (weakest but always present)

Retention times changes with the ‘polarity’ of the stationary phase (GC in particular).

Kovat’s Retention Index:

A logarithmic scale that relates the corrected retention time $t_r'$, of an analyte to those of linear alkanes in a given column (s.p.)

Linear alkanes (or any homologous series) elutes in the increasing order of their molecular masses.

Kovat’s Retention index (RI) by definition for an alkane = 100n, where n = # C atoms

- RI value for n-alkanes i.e. linear homologues.

Experimentally plot of $\log(t_r')$ vs RI is linear.

As a general rule; RI is larger if the s.p. retains the analyte longer.
For an analyte eluting from a column (injected with linear homologues) between the smaller homologue (n) and N larger homologue (N carbons) has an RI of:

\[ RI = I = 100 \left[ n + (N - n) \frac{\log(t_r) - \log(t_{r,n})}{\log(t_{r,N}) - \log(t_{r,n})} \right] \]

RI indicates the appearance of a given analyte in the chromatogram in relation to straight chain compounds.

Different stationary phases (GC) give different RI values for the same analyte because different stationary phases will have different retentive characteristics.

However, in any column order of elution of a homologous series is the same, 

lowest MW/m.p./b.p. elutes first, ....

Column (stationary phase) characterization:

Based on ‘polarity’ (operational IMFs) of the stat. phase.

Polarity of a stationary phase is expressed with RIs of probe/index compounds in the s.p. of interest.

RI of probe compounds are measured on the s.p. of interest and on a totally non polar phase – squalene (IMFs - dispersion forces only).

For probe/index compound: \( (R_{sp} - R_{squalene}) = \) McReynold’s Constant is calculated.

<table>
<thead>
<tr>
<th>Index compound</th>
<th>Measures IMF of type</th>
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<tr>
<td>Benzene</td>
<td>Aromatic, olefinic; x'</td>
</tr>
<tr>
<td>1-butanol</td>
<td>Electron attractor; y'</td>
</tr>
<tr>
<td>2-pentanone</td>
<td>Electron repeller; z'</td>
</tr>
<tr>
<td>1-nitropropane</td>
<td>Nitro, nitrile; u'</td>
</tr>
<tr>
<td>Pyridine</td>
<td>Pyridine; s'</td>
</tr>
</tbody>
</table>

McReynold’s Constant for probe compound:

\[ \Delta I = (R_{sp} - R_{squalene}) \]

McReynold’s constant measures the retention of a probe in a s.p., over that in squalene. It is a measure of ‘a specific type of polarity’ of the s.p.

McReynold’s constant measures the ability to retard analytes of specific ‘polar’ characteristics on the stationary phase.

Behaves like an (hypothetical) alkane with 8.36 C atoms.
Probe compounds and what they measure:

‘polarity’ – arises from the intermolecular forces certain chemical moieties are capable of exerting.

Example: \( x' = \Delta I_{\text{benzene}} = (R_{I_{\text{sp}}} - R_{I_{\text{squalene}}})_{\text{benzene}} \)

Total (effective) retardation ‘polarity’ of stationary phase:

\[ \sum \Delta I_i = x' + y' + z' + u' + s' \]

Average polarity of a s.p.:

\[ \bar{P}_R = \frac{1}{5} \sum \frac{\Delta I_i}{\Delta I_{squalene}} \times 100 \]

Utility of McReynold’s Constants - Examples:

Alcohol + ether; very similar boiling points. Column to elute alcohol before ether?

z’ (ether); y’ (alcohol). SP2401 or OV210

Ether before alcohol? OV275 or SP2340

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<td>Nitro, nitrile</td>
</tr>
<tr>
<td>Pyridine, s’</td>
<td>pyridine</td>
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Benzene, cyclohexane; boiling points - very similar.

OV101 NO
OV225 Reasonable time frame
OV275 Too long

Almost every column in the ‘short list’ is technically Usable here. Consider elution times.
Bonded Columns (HPLC)

In C8 and C18 columns the stationary phase is a thin film of non-polar liquid phase (mono molecular layer) that has been chemically anchored to an inert material (Silica particles).

Chemically linked to the silica particles surface by reaction with the polar silanol groups on the stationary phase surface and renders them less polar or non-polar.

C8 columns have silica particles attached to C8 carbon units while C18 is coated with C18 moieties. C18 columns are more hydrophobic.

Modes of Separation: GC

Isothermal = constant temperature
Gradient = varying temperature

Column Temperature

- temperature too high, causes co-elution
  • poor resolution but faster separation
- temperature is too low, longer elution times
  • adequate resolution, but a separation that takes very long
- compromise temperature or program

Temperature Programming: GC

- The “simplest” way to alter the separation in GC is to alter the temperature/program in the oven. Pressure programming of the carrier gas is less common.
- By altering the temperature, rate of equilibration of analytes changes, increases differences in the capacity factor (k' ↔ K):
  - they spend more or less time in the stationary phase
  - the greater the difference in the times between analytes can be fastened and vice versa.
**GC Carrier Gases** (mobile phase)

- Helium (availability in relatively pure state, but availability issues are cropping up)
- Nitrogen (requires oxygen and water traps)
- Hydrogen
  - normally used in FID (fuel for the flame)
  - rarely used in as a carrier gas - safety concerns
  - current developmental work has addressed the safety issues

$$k' = \frac{n_{i,s}}{n_{i,m}} = \frac{C_{i,s}V_i}{C_{i,m}V_m} = \left(\frac{K_iV_i}{V_m}\right) = \frac{K_i}{\beta}$$

\[ K_i = \frac{C_{i,k}}{C_{i,m}} \]

“Inert” gases (do not react/interact with analytes)
- Function:
  - sweep the sample through the column
  - protect the column from oxygen exposure (especially) at high temperatures
  - assist with the function of the detector

Typical plot

- High $u$ without compromising $H$.
- Well packed; small $d_p$ distribution lowers $A$
- Better particle morphology, low bonded phase density (reduce mass transfer delays) reduces slope, $C$

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Samples are injected through a septum:
- keeps oxygen out of the column
- provides a seal - carrier gas pressure up at the head of the column
- The injector is usually lined with a de-activated glass liner

**GC Injection**

- On-Column Injection:
  - used widely in packed-column GC, less in capillary GC
  - sample - deposited directly on the column
    - Good for thermally unstable compounds
    - Good for quantitative analysis at low concentrations
      - entire sample reaches the detector
  - Smaller injections (Capillary GC)

- Splitless Injection:
  - Sample - vaporized in the injector and ALL of the sample is swept onto the column by the carrier gas
  - Relatively small samples (≤10μL)
  - Sample spends a large amount of time in the injector
  - Best for trace (1 - 100 ppm range) concentrations of high boiling point analytes in low boiling point solvents
    - extra time in the injector helps volatilize the analytes.

**Injection modes**
• Split Injection:
  – injection is split, with only a fraction of the sample (usually 1% - 20%) actually makes it to the column
  – the most common method of injecting samples onto small diameter, open-tubular columns.
  • Even for injections 20 μL, only a fraction (which is adjustable) makes it on to the column
  – Not for analytes mixtures with a wide range of boiling points
  • some may be swept out the split vent before they are volatilized

Modern capillary GCs come with a Split/Splitless injectors standard.

Switch between modes by changing the split vent gas flow and using a different injection liner.

Splitless injection – trace analysis, split valve is closed, most of the sample is introduced on the column. @ flow rate ~1 ml/min. transfer to the column takes a long time. Thus peak tailing.

Splitless time - split valve opened after 20 - 120s, sample transfer stops. Yet long transfer can be long, results in increased peak width.

Solvent trapping - sample injected with the column at a temperature <20°C the boiling point of solvent. After ~30s (splitless time) increase the temperature fast 20°C above solvent boiling point. Fast transfer from gas to liquid and again to the gas phase sharpens the elution band.
GC Capillary Columns:

\[ H_{\text{column}} = \frac{2\gamma D_u}{u} + \left( \frac{q k}{(k+1)^2} + \frac{1+6k+11k^2}{24(k+1)^2} \right) \frac{D_u}{D} \]

GC Detectors

- Numerous types of detectors available
- Requirements:
  - sensitive to the analytes of interest
  - compatible with the column, carrier gas, solvent, etc.
  - rugged
  - useful linear range
- have its own temperature control.
- Measures response as a voltage or a current.

### Table 24-4

<table>
<thead>
<tr>
<th>Gas</th>
<th>Thermal conductivity ( \kappa/(m \cdot K) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>H_2</td>
<td>0.170</td>
</tr>
<tr>
<td>He</td>
<td>0.044</td>
</tr>
<tr>
<td>N_2</td>
<td>0.021</td>
</tr>
<tr>
<td>O_2</td>
<td>0.003</td>
</tr>
<tr>
<td>Ar</td>
<td>0.094</td>
</tr>
<tr>
<td>C_2H_2</td>
<td>0.094</td>
</tr>
<tr>
<td>C_3H_6</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Carrier gas has a thermal conductivity.
- The presence of analyte molecules in the carrier gas alters (lowers) the thermal conductivity of the gas.
- Second filament to act as a reference (the carrier gas is split).
- Increased sensitivity with decreasing temperature (detector), flow rate and applied current.
- Universal detector

The detector temperature selection; a balance between sensitivity, filament life and boiling points of analytes. Low temperatures give high sensitivity and longer filament life. High temperature assures gaseous state of the analytes.
**FID**

\[ CH + O_2 \rightarrow CHO^+ + e^- \]

- Destructive detector
- Analytes containing C burn in a hydrogen-oxygen flame and produce ions
- CHO\(^+\) ions are collected on a cathode and the current they produce results in the signal
- Detect carbon containing compounds
- A makeup gas may be required

**ECD**

Electrons travel between a \(^{63}\)Ni electrode and a collector electrode.

As analytes with “electron capturing ability” pass through the cell, the flow of electrons is reduced.

The *change in current*, due to reduced flow of electrons, proportional to the analyte conc.

Very sensitive to halogens, nitriles, carbonyls, nitro compounds, pesticides, herbicides, and PCBs in environmental samples.

Non-destructive detector.

**FPD: Flame Photometric Detector**

sample exits the analytical column into a hydrogen diffusion flame.

Ions and atoms (excited) produced by organic compounds during combustion

When compounds are burned in the FPD flame, they emit photons of distinct wavelengths

photons that are within the frequency range of the filter specifications can pass through the filter to the PMT. The PMT generates a proportional analog signal (current)

uses band pass filters over the PMT to selectively detect compounds containing S and P.

Combustion of sulfur compounds forms SO.

Reaction of SO with ozone ($O_3$) is chemiluminescent.

The reaction mechanism:

$$S\text{-compound} + O_3 \rightarrow SO + Other \text{Products}$$

$$SO + O_3 \rightarrow SO_2 + O_2 + h\nu (300-400 \text{ nm})$$

The light ($h\nu$) passes through an optical filter and is detected by a photomultiplier tube. The current generated in the PMT is proportional to the concentration of the analyte.

**MS**

The GC eluate is a mixture separated into segments of pure substances with each analyte segment mixed with the mobile phase.

These are introduced into an ion source of a MS, blasted with electrons, which cause them to break into pieces and turn into positively charged molecular ions and fragmented ions (ion source).

Ions are directed to travel through a ‘filter’ where the ions, based on ‘masses’ are filtered and ‘detected’. The filter continuously scans through the range of masses as the stream of ions come from the ion source.

Ionized analyte generally produce many smaller ions apart from the molecular ion. In TIC the current generated by all such ions from analytes is measured, which would be proportional to the amount/concentration of analyte.

A plot of TIC vs t is equivalent to the chromatogram.
A given molecule generates many ion fragments. Every segment of the chromatographic eluate would generate ions. A plot of the total ion current (TIC) measured as a function of time would be the chromatogram (total ion chromatogram).

Two other modes of detection:
   a. selected ion (monitoring - SIM) current is detection
   b. selected reaction monitoring (later)

makes MS a very sophisticated and versatile detection system.

TICs are plots of summed current from all the ions generated at the ionization stage of the eluting analytes. The plot resembles a normal chromatogram.

SIM chromatograms are generated by selecting a specific ion or a narrow range of specific ions generated at the ionization stage from the eluting analytes. This mode is more sensitive and generates a cleaner background.

Therefore many eluting peaks will not be shown on the plot.
MS allows qualitative analysis of analytes using the full scan of all the ions produced during the ionization process (mass spectrum).

Analysis of the mass spectra of each analyte and/or comparison of spectra from MS libraries to experimental spectra accomplishes the identification of analytes.

### Headspace Analysis

G = the gas phase - headspace and lies above the condensed sample phase.

S = the sample phase - the sample phase contains the analytes; liquid or solid in combination with a diluent or a matrix modifier.

Sample phase is introduced into the vial and the vial is sealed, volatile components diffuse into the gas phase until the headspace has reached a state of equilibrium. The sample is then taken from the headspace with a gas sampling syringe.

It is most suited for the analysis of the very light volatiles in samples that can be efficiently partitioned into the headspace gas volume from the liquid or solid matrix sample.

The technique is the preferred method for the analysis of gases and very light volatiles which cannot be analyzed by other techniques such as P&T and Thermal Desorption.

### Headspace Vial

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### Purge-Trap & Thermal Desorption

The technique is the preferred method for the analysis of gases and very light volatiles which cannot be analyzed by other techniques such as P&T and Thermal Desorption.
Purge and Trap (P&T) Thermal Desorption is routinely used for the analysis of volatiles in environmental samples as well as food samples, through the proper selection of adsorbent resins, as Tenax TA.

Water can be eliminated from being introduced into the GC.

This is important for the analysis of high water content samples such as food products and water samples.

Chemical Derivatization (prior to analysis);

Gas chromatography is for the separation of volatile compounds which are thermally stable.

GC not always possible (biomedical and environmental interest) particularly for those of high molecular weight and/or molecules containing polar functional groups.

Derivatization used when analytes are not sufficiently volatile, tail significantly (too strongly attracted to the stationary phase) and thermally unstable (decompose).

Chemical Derivatization would;

- increase the volatility by decreasing the polarity of compounds.
- reduce thermal degradation of samples by increasing their thermal stability may reduce tailing. Enable selective detection of analytes
- increase specific detector response by incorporating functional groups which lead to higher detector signals, e.g. CF₃ groups for ECD(GC); fluorophores to enable fluorometric detection (LC), chromophores to enable UV-VIS detection etc (LC).
Column Bleed: At high temperatures s.p. may vaporize into the carrier gas, resulting in column “bleed.”

Fensulfothion (nominal mass 308 Da)