Lecture 9: Gas Chromatography
Over 100 detectors have been invented, but relatively few are in common use.

The criteria to consider when selecting a detector are: sensitivity, noise, minimum detectable quantity/detection limit, detection time constant or response time, and selectivity.
Instrumentation

Detectors

Common Detectors:
- Thermal Conductivity Detector (TCD)
- Flame Ionization Detector (FID)
- Electron Capture Detector (ECD)
- Alkali Flame Ionization Detector (AFID)
- Flame Photometric Detector (FPD)
- Photoionization Detector (PID)
- Mass Spectrometry Detector (MS)
Instrumentation

Detectors

Flame Photometric Detector (FPD)

Directly measure photons produced during combustion of species.

Destructive detector

Limits of detection

\(~20 \text{ pg S/sec}\)

\(~0.9 \text{ pg P/sec}\)

Linear range $10^4$
Flame Photometric Detector (FPD)

1st Flame combust and decompose solvent molecules (can quench sulfur emission).

The elected species passes into a flame or plasma inside a shielded jet which produces atoms and molecules species.

The atoms and molecules are in an excited state and when they return to the ground state, they emit at wavelengths characteristic of the atomic line or molecular band.
Instrumentation

Detectors

Flame Photometric Detector (FPD)
Instrumentation

Detectors

Flame Photometric Detector (FPD)

Specific detector for sulfur or phosphorous (sub ng levels) used mostly for pesticides.

Combustion of phosphorous and sulfur compounds – produce excited species – HPO* and S₂*, which emit at 526nm and 394nm, respectively.

Response is linear for phosphorous but non-linear for sulfur (varies as the species of the amount of sulfur present).
Detectors

Photoionization Detector (PID)

Specific detector for compounds ionized by UV. UV light is used to directly ionize the sample. The resulting current is measured.

Nondestructive

Limit of detection - 2 pg carbon/sec

Linear Range - $10^7$
Instrumentation

Detectors

Photoionization Detector

- high energy UV lamp
- exit
- polarizing electrode
- collecting electrode
- insulator
- reaction chamber
- sample in
Detectors

**Photoionization Detector**

Since only a small (very reproducible but basically unknown) fraction of the analyte molecules are actually ionized in the PID chamber, this is considered a nondestructive GC detector.

Therefore, the exhaust port of the PID *can be* connected to another detector in series with the PID. In this way data from two different detectors can be taken simultaneously, and selective detection of PID responsive compounds augmented by response from another detector.
The major challenge here is to make the design of the ionization chamber and the downstream connections to the second detector as low volume as possible so that peaks that have been separated by the GC column do not broaden out before detection.
Instrumentation

Detectors

Photoionization Detector

- Electrometer
- Oven wall
- GC column
- UV lamp
- Heated ionization chamber
- Exhaust or small volume connector to another detector
- UV transparent window
- UV opaque insulated housing
- Power supply
- Computer

ADVANCED CHROMOTOGRAFHY
Instrumentation

Detectors

Common Detectors:
- Thermal Conductivity Detector (TCD)
- Flame Ionization Detector (FID)
- Electron Capture Detector (ECD)
- Alkali Flame Ionization Detector (AFID)
- Flame Photometric Detector (FPD)
- Mass Spectrometry Detector (MS)
GC/MS Fundamentals
GC/MS Fundamentals
Process of Mass Spectrometry

Atomization
Conversion of atoms to ions (usually positive)
Separation of ions by mass-to-charge ratio (m/z)
Measurement of ion current by transducer
Atomic and molecular weights are measured in atomic mass units (amu)

The atomic weights are determined relative to carbon 12.

Carbon 12 is set to exactly 12 amu.

1 mol of carbon 12 weighs 12.0000 g.
For MS must know the exact mass, m.

\[ ^{12}\text{C}^1\text{H}_4 \]
\[ m = (12.000 \times 1) + (1.007825 \times 4) = 16.031 \text{ amu} \]

\[ ^{13}\text{C}^1\text{H}_4 \]
\[ m = (13.00335 \times 1) + (1.007825 \times 4) = 17.035 \text{ amu} \]
Mass – to - Charge ratio

Divide the atomic or molecular mass of an ion, m, by the charge, z, on the ion.

\[ ^{12}\text{C}^1\text{H}_4^+ \quad \text{m/z} = \frac{16.031}{1} = 16.031 \]
\[ ^{13}\text{C}^1\text{H}_4^{2+} \quad \text{m/z} = \frac{17.035}{2} = 8.518 \]

Majority of ions in MS have a single charge.
GC/MS Fundamentals

Analytes from GC column are fed into the MS ion source where the molecules are ionized. The molecular ions break apart or “fragment”. These fragments are separated according to their mass-to-charge ratio (m/z) and the intensity (ion current) of each type of ion is recorded.

Plot total ion current versus m/z – mass spectrum.
GC/MS Fundamentals

Mass Spectrometry - Ion Formation

Molecules in mass spectrometer are bombarded with electrons of sufficient energy to break the molecules into ions or neutral fragments.

The pattern of ion intensities is characteristic (fingerprint) of the original molecule.
The most popular are:
- Quadrapole
- Time-of-flight
- Double-focusing
- Ion Trap

Principle components:
- Inlet (Interface)
- Ion source
- Mass analyzer
- Ion transducer
- Pumps
- Signal processor
Figure 11-1 Components of a mass spectrometer.
Instrumentation

GC/MS Interface - Capillary Column interface

GC column is at atm pressure, while MS is under high vacuum.

Interface must efficiently convey sample components between GC and MS
Instrumentation

GC/MS Interface - Capillary Column interface

Inlet Systems
Allow introduction of representative sample into ion source with minimal loss of vacuum.
Instrumentation

GC/MS Interface - Capillary Column interface

Transfer Line

Connects column to MS port by a tube made up of fused silica. Tube must not adsorb effluent. Tube heated to within 25 °C of the column temperature. Tube volume is small to minimize band spreading.
Instrumentation

GC/MS Interface - Capillary Column interface

Direct Transfer

Column is threaded directly into MS ion source.
Instrumentation

GC/MS Interface - Capillary Column interface

Open Split

Restrictor or transfer line limits flow of GC effluent into MS.
Excess effluent swept away by purge gas (N₂ or He)
Instrumentation

GC/MS Interface - Packed Column Interface

Jet separator
Instrumentation

Other Type of Inlet Systems

Direct probe Inlet

Sample or nonvolatile liquid introduced on a probe into ionization chamber.
Probe can be heated or cooled.
Instrumentation

Direct probe Inlet

Figure 20-11  Schematic of (a) an external sample introduction system—note that the various parts are not to scale—and (b) a sample probe for inserting a sample directly into the ion source.  (From G. A. Eadon, in Treatise on Analytical Chemistry, 2nd ed., J. D. Winefordner, M. M. Bursey, and I. M. Kolthoff, Eds., Part I. Vol. 11, p. 9. New York: Wiley, 1989. Reprinted by permission of John Wiley & Sons, Inc.)
Instrumentation

Pumps

Mass Specs operates in a vacuum.

Rough pumps

Takes pressure down to $\sim 10^{-2}$ torr
Instrumentation

Pumps - Oil Diffusion Pump

Diffusion pump oil is boiled and vapor rises and condenses on chamber wall.

The first downward flow of oil vapor collides with gas from MS and compresses it in bottom of chamber.

Rough pump removes gases from bottom.

Inexpensive – but if rough pump fails oil vapor can contaminate MS.
Instrumentation

Pumps - Oil Diffusion Pump

- Water cooling coils
- Vents
- Stacks
- Heater
- To roughing pump
- Inlet
Instrumentation

Pumps - Turbo Pump

Composed of a set of fans similar to a jet engine. Alternate sets of blades rotate while others are stationary.

Blades turn at speeds over 20,000 revolutions per minute.

Molecules collide with the blades and are deflected downward and removed by rough pump.

Instrumentation

Pumps - Turbo Pump
Instrumentation

Ion source

• ionizes and fragments molecules
• form the ions into a focused beam
• introduce the beam into the mass analyzer

Two common modes for ionization: Gas phase and Desorption
Instrumentation

<table>
<thead>
<tr>
<th>Basic Type</th>
<th>Name and Acronym</th>
<th>Ionizing Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas phase</td>
<td>Electron impact (EI)</td>
<td>Energetic electrons</td>
</tr>
<tr>
<td></td>
<td>Chemical ionization (CI)</td>
<td>Reagent gaseous ions</td>
</tr>
<tr>
<td></td>
<td>Field ionization (FI)</td>
<td>High-potential electrode</td>
</tr>
<tr>
<td>Desorption</td>
<td>Field desorption (FD)</td>
<td>High-potential electrode</td>
</tr>
<tr>
<td></td>
<td>Electrospray ionization (ESI)</td>
<td>High electrical field</td>
</tr>
<tr>
<td></td>
<td>Matrix-assisted desorption/ionization (MALDI)</td>
<td>Laser beam</td>
</tr>
<tr>
<td></td>
<td>Plasma desorption (PD)</td>
<td>Fission fragments from $^{252}$Cf</td>
</tr>
<tr>
<td></td>
<td>Fast atom bombardment (FAB)</td>
<td>Energetic atomic beam</td>
</tr>
<tr>
<td></td>
<td>Secondary ion mass spectrometry (SIMS)</td>
<td>Energetic beam of ions</td>
</tr>
<tr>
<td></td>
<td>Thermospray ionization (TS)</td>
<td>High temperature</td>
</tr>
</tbody>
</table>
Ion sources are classified as:

**Hard sources**
- Impart sufficient energy to analyte molecules to promote to a highly excited energetic state.
- Relaxation involves rupture of bonds, producing fragment ions with m/z ratios less than the molecular ion.

**Soft sources**
- Causes little fragmentation, producing few ions.
Instrumentation

Figure 20-2  Mass spectrum of 1-decanol from (a) a hard source and (b) a soft source.
Instrumentation

Ion source - Electron ionization

The electron ionization ion source consists of a small chamber called the ion volume.

A beam of electrons with an energy of 70 electron volts passes through the volume.

Molecules from the GC interact with the electron beam and are ionized.
Instrumentation

Ion source - Electron ionization

Molecular and fragment ions are focused into a beam by a series of electronic lenses and sent to the mass analyzer.

Ions produced are accelerated through electrostatic plates which then goes to the mass analyzer.
Instrumentation

Ion source - Electron ionization

Instrumentation

Electron-impact source (gas phase source)

Produce a complicated fragmentation spectra, that can be useful for compound identification.

Collision product peaks:
Sometimes peaks are produced at higher mass numbers than that of the molecular ion.
Usually occurs where collision transfers a H atom to the ion to give a (M + 1)+ peak.
### Instrumentation

#### TABLE 20-2 Some Typical Reactions in an Electron-Impact Source

<table>
<thead>
<tr>
<th>Reaction Type</th>
<th>Reaction Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular ion formation</td>
<td>$\text{ABCD} + e^- \rightarrow \text{ABCD}^{++} + 2e^-$</td>
</tr>
<tr>
<td>Fragmentation</td>
<td>$\text{ABCD}^{++} \rightarrow \text{A}^+ + \text{BCD}^*$</td>
</tr>
<tr>
<td></td>
<td>$\quad \rightarrow \text{A}^* + \text{BCD}^+ \rightarrow \text{BC}^+ + \text{D}$</td>
</tr>
<tr>
<td></td>
<td>$\quad \rightarrow \text{CD}^* + \text{AB}^+ \rightarrow \text{A}^* + \text{B}^+$</td>
</tr>
<tr>
<td></td>
<td>$\quad \rightarrow \text{AB}^* + \text{CD}^+ \rightarrow \text{D}^* + \text{C}^*$</td>
</tr>
<tr>
<td>Rearrangement followed by fragmentation</td>
<td>$\text{ABCD}^{++} \rightarrow \text{ABDC}^{++}$</td>
</tr>
<tr>
<td></td>
<td>$\quad \rightarrow \text{BC}^* + \text{AD}^+$</td>
</tr>
<tr>
<td></td>
<td>$\quad \rightarrow \text{AD}^* + \text{BC}^*$</td>
</tr>
<tr>
<td>Collision followed by fragmentation</td>
<td>$\text{ABCD}^{++} + \text{ABCD} \rightarrow (\text{ABCD})^+ + \text{BCD}^* + \text{ABCD}A^+$</td>
</tr>
</tbody>
</table>
Instrumentation

Ion source
Electron-impact source (gas phase source)

Advantages:
- Convenient
- Sensitive - Produce high ion currents
- Fragmentation

Disadvantages:
- Fragmentation
- Only for volatile samples
Chemical ionization (Cl) takes place in an ion source similar to the EI source. The main difference is a moderate pressure (~1 torr) of reagent gas is maintained in the source.

Cl is a two step process:
- the electron beam interacts with the reagent gas and ionizes it.
- the resulting reagent ions collide with sample molecules to produce analyte ions.
Instrumentation

Ion source - Chemical Ionization

CI is a soft ionization process and compared to EI produces fewer fragment ions.

Methane is often used as a CI gas. Electrons from the beam react with methane and produce, $\text{CH}_4^+$, $\text{CH}_5^+$ and $\text{C}_2\text{H}_5^+$ ions.
Instrumentation

Ion source - Chemical Ionization

Most common reagent is methane which gives $\text{CH}_4^+$, $\text{CH}_3^+$, and $\text{CH}_2^+$ under electron bombardment.

$$\text{CH}_4^+ + \text{CH}_4 \rightarrow \text{CH}_5^+ + \text{CH}_3$$

$$\text{CH}_3^+ + \text{CH}_4 \rightarrow \text{C}_2\text{H}_5^+ + \text{H}_2$$

Besides methane, isobutene and ammonia are common reagent CI gases.
Instrumentation

Ion source - Chemical Ionization

The produced ions interact with the analyte molecules by four basic mechanisms:
- Charge transfer
  \[ \text{CH}_4^+ + \text{RH} \rightarrow \text{RH}^+ + \text{CH}_4 \]
- Proton transfer
  \[ \text{CH}_5^+ + \text{RH} \rightarrow \text{RH}_2^+(M+1)+ + \text{CH}_4 \]
- Hydride Abstraction
  \[ \text{C}_2\text{H}_5^+ + \text{RH} \rightarrow \text{R}^+(M-1) + \text{C}_2\text{H}_6 \]
- Addition
  \[ \text{C}_2\text{H}_5^+ + \text{RH} \rightarrow \text{C}_2\text{H}_5:RH^+(M+29) \]
Field-Ionization Sources (Gas phase source)

Ions are formed under large electric fields ($10^8$ V/cm).

The fields are produced by applying high voltages (10-20 kV) to emitters made up of fine tips with diameters less than 1 mm.

The emitter is often a W wire that is coated carbon dendrites.
Ion source

Field-Ionization Sources

The emitters are mounted 0.5-2 mm from the cathode, the sample diffuses above the microtips and are ionized by quantum mechanical tunneling mechanism. The electrons of the analyte are extracted by the microtips. Ionization occurs but very little fragmentation.

Figure 20-5  Photomicrograph of a carbon microneedle emitter. (Courtesy of R. P. Lattimer, BF Goodrich Research and Development Center.)
Field-Ionization Sources

![Diagram of field-ionization source]

**Figure 20-6** Mass spectra for glutamic acid: (a) electron-impact ionization, (b) field ionization, and (c) field desorption. (From H. D. Becket, A. Heindrich, and H. U. Winkler, Int. J. Mass Spec. Ion Phys., 1970, 3, App. 11. With permission.)
Instrumentation

Ion source

Matrix-Assisted Laser Desorption/Ionization (desorption source)
MALDI was developed in 1988.

An aqueous/alcohol solution of the sample is mixed with a large excess of a radiation absorbing matrix material.

Solution is evaporated on the surface of a metal inserted into MS and then hit with a pulse laser beam.

This sublimes the sample as ions into a time-of-flight spectrometer.
Instrumentation
Matrix-Assisted Laser Desorption/Ionization
<table>
<thead>
<tr>
<th>Matrix</th>
<th>Analytes</th>
<th>Wavelength, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitropyridines:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Amino-4-methyl-5-nitropyridine</td>
<td>Proteins, oligonucleotides</td>
<td>355</td>
</tr>
<tr>
<td>2-Amino-5-nitropyridine</td>
<td>Oligonucleotides</td>
<td>355</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>Proteins, glycoproteins, oligonucleotides</td>
<td>266, 220–290</td>
</tr>
<tr>
<td>Benzoic acid derivatives:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,5-Dihydroxybenzoic acid</td>
<td>Proteins</td>
<td>266, 337, 355, 2940</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>Proteins</td>
<td>266</td>
</tr>
<tr>
<td>2-Aminobenzoic acid</td>
<td>Proteins</td>
<td>266, 337, 355</td>
</tr>
<tr>
<td>2-(4-Hydroxyphenylazo) benzoic acid</td>
<td>Proteins, gangiosides, polymers</td>
<td>266, 377</td>
</tr>
<tr>
<td>2-Pyrazinecarboxylic acid</td>
<td>Proteins</td>
<td>266</td>
</tr>
<tr>
<td>3-Aminopyrazine-2-carboxylic acid</td>
<td>Proteins</td>
<td>337</td>
</tr>
<tr>
<td>Cinnamic acid derivatives:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>Proteins, oligonucleotides</td>
<td>266, 337, 355, 488</td>
</tr>
<tr>
<td>Sinapinic acid</td>
<td>Proteins, industrial polymers</td>
<td>337, 355</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>Proteins, oligonucleotides</td>
<td>266, 337, 355, 10600</td>
</tr>
<tr>
<td>α-Cyano-4-hydroxy cinnamic acid</td>
<td>Proteins, oligosaccharides</td>
<td>337</td>
</tr>
<tr>
<td>3-Nitrobenzyl alcohol</td>
<td>Proteins</td>
<td>266</td>
</tr>
<tr>
<td>3-Nitrobenzyl alcohol with rhodamine 6G</td>
<td>Proteins</td>
<td>532</td>
</tr>
<tr>
<td>3-Nitrobenzyl alcohol with 1,4-diphenyl-1,3-butadiene</td>
<td>Proteins</td>
<td>337</td>
</tr>
<tr>
<td>3-Hydroxypicolinic acid</td>
<td>Oligonucleotides, glycoproteins</td>
<td>266, 308, 355</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>Proteins</td>
<td>2940, 10600</td>
</tr>
</tbody>
</table>
Ion source

Electrospray Ionization (Desorption source)

ESI/MS developed in 1984, now most important technique for analyzing biomolecules with MW > 100,000 daltons.

Can also be used to characterize inorganic species and synthetic polymers.
Ion source

Electrospray Ionization (Desorption source)

Solution of sample is pumped through a stainless steel capillary needle at few ml/min.

A few kV is applied between the needle and a surrounding cylindrical electrode.

The charged spray from the needle enters a desolvating capillary that evaporates the solvent and desorbs ions into ambient gas.
**Instrumentation**

**Ion source**

Figure 20-8  Apparatus for electrospray ionization.  *(From J. B. Fenn et al., Science, 1989, 246, 65.)*
Figure 20-9  Typical electrospray mass spectra of proteins and peptides. The numbers above the peaks represent the molecular charge associated with each peak.  (From R. D. Smith et al., Anal. Chem., 1990, 62, 887.)
Assignment

- Read Chapter 3: Principles and Practice of Modern Chromatographic Methods, Peter E. Jackson, Academic Press.