Lecture 7: Gas Chromatography
Instrumentation

- Carrier gas
- Flow controller
- Injector port
- Column oven
- Detector
- Recorder
Instrumentation

Columns

- Packed
  - porous packing
  - non-porous packing
    - Packed with porous layer
    - liquid coated
    - packed capillary
- Open tubular (capillary)
  - coated with porous layer
  - bound phase
  - liquid coated wall
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.
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
Instrumentation

Columns

Stationary Phases (GLC)

Categories of stationary phases
- non-polar
- polar
- specialty phases
Instrumentation

Columns

Stationary Phases (GLC)

Hydrocarbons – non-polar – have been used as non-polar stationary phase. Most have a high molecular weight for low volatility. Examples: Squalane \((\text{C}_{30}\text{H}_{62})\), Apolane C87, long-chain \(n\)-alkanes, Apiezon L - not used much.
Columns

Stationary Phases (GLC)

Alkylsilicone Phases – can be non-polar or polar.
Polymers based on silicon-oxygen-silicone backbone – most widely used group of stationary phases. Differences in these stationary phases differ mainly in the degree of substitution on the silicon backbone.
Instrumentation

Columns

Stationary Phases (GLC)

Standard polysiloxanes are characterized by the repeating siloxane backbone. Each silicon atom contains two functional groups. The type and amount of the groups distinguish each stationary phase and its properties.

\[
\text{[O-Si]}_n\text{[O-Si]}_n
\]

\[
R = \begin{align*}
\text{CH}_3 & \quad \text{methyl} \\
\text{CH}_2\text{CH}_2\text{CH}_2\text{CN} & \quad \text{cyanopropyl} \\
\text{CH}_2\text{CH}_2\text{CF}_3 & \quad \text{trifluoropropyl} \\
\text{phenyl} & 
\end{align*}
\]
Columns

Stationary Phases (GLC)

The most basic polysiloxane is the 100% methyl substituted. When other groups are present, the amount is indicated as the percent of the total number of groups. For example, a 5% diphenyl-95% dimethyl polysiloxane contains 5% phenyl groups and 95% methyl groups. The "di-" prefix indicates that each silicon atom contains two of that particular group. Sometimes this prefix is omitted even though two identical groups are present. If the methyl percentage is not stated, it is understood to be present in the amount necessary to make 100% (e.g., 50% phenyl-methyl polysiloxane contains 50% methyl substitution).
Cyanopropylphenyl percent values can be misleading.

A 14% cyanopropylphenyl-dimethyl polysiloxane contains 7% cyanopropyl and 7% phenyl (along with 86% methyl).

The cyanopropyl and phenyl groups are on the same silicon atom, thus their amounts are summed.
Columns

Stationary Phases (GLC)

For select stationary phases, a low bleed or "ms" version is available. These stationary phases incorporate phenyl or phenyl type groups into the backbone of the siloxane polymer. These types of stationary phases are commonly called arylenes. The phenyl group strengthens and stiffens the polymer backbone which inhibits stationary phase degradation at higher temperatures. This results in lower column bleed and, in most cases, higher temperature limits.
### Instrumentation

#### Stationary Phases (GLC)

<table>
<thead>
<tr>
<th>Chemical structure</th>
<th>Classification</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% dimethyl silicone</td>
<td>Non-polar</td>
<td>Boiling point separations (solvents, petroleum products, pharmaceuticals)</td>
</tr>
<tr>
<td><img src="image" alt="Chemical structure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% dimethyl, 5% phenyl silicone</td>
<td>Non-polar</td>
<td>Boiling point separations (aromatics, flavours, aromatic hydrocarbons)</td>
</tr>
<tr>
<td><img src="image" alt="Chemical structure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>86% dimethyl, 7% phenyl, 7% cyanopropyl silicone</td>
<td>Intermediate polarity</td>
<td>Pesticides, alcohols</td>
</tr>
<tr>
<td><img src="image" alt="Chemical structure" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Instrumentation

Stationary Phases (GLC)

86% dimethyl
7% phenyl
7% cyanopropyl
\{\text{silicone}\}

Intermediate polarity
Pesticides, alcohols

50% dimethyl
25% phenyl
25% cyanopropyl
\{\text{silicone}\}

Polar
Triglycerides, phthalate esters
Instrumentation

**Columns**

**Stationary Phases (GLC)**

Polyether Phases – polar phases – based on polyethylene glycol, \( HO-CH_2-CH_2-[O-CH_2-CH_2]_n-OH. \) Marketed under the trade name, carbowax or superox.

Polyethylene glycols stationary phases are not substituted, thus the polymer is 100% of the stated material. They are less stable, less robust and have lower temperature limits than most polysiloxanes. With typical use, they exhibit shorter lifetimes and are more susceptible to damage upon over heating or exposure to oxygen.
Instrumentation

Columns

Stationary Phases (GLC)

Polyether Phases – polar phases – based on polyethylene glycol,
HO-CH$_2$-CH$_2$-[O-CH$_2$-CH$_2$]$_n$-OH.
Marketed under the trade name, carbowax or superox.

Advantage - Average molecular weight of 20,000, so can be used at high temperatures.
Disadvantage is that it reacts with even trace amounts of oxygen at high temperatures, as well as decomposing to acetaldehyde and acetic acid.
Instrumentation

Stationary Phases (GLC)

100% cyano propyl silicone

\[
\begin{array}{c}
\text{C}_3\text{H}_6\text{CN} \\
\text{Si-O-} \\
\text{C}_3\text{H}_6\text{CN}
\end{array}
\]

Polar
Fatty acid methyl esters, carbohydrates

Polyethylene glycol 20 M

\[
\begin{array}{c}
\text{H-H} \\
\text{O-C-C-} \\
\text{OH} \\
\text{H-H}
\end{array}
\]

Polar
Flavours, fatty acid methyl esters, acids, amines

Fig. 3.17. Structure of polysiloxane and polyethylene glycol stationary phases.
Instrumentation

Columns

Stationary Phases (GLC)

High-temperature phases – substituted silicone-carborane copolymers. Stable up to 450°C - 500°C. Their advantage is in their low stationary bleed – for use with highly sensitive detectors.
Instrumentation
Stationary Phases (GLC)
High-temperature phases

Dexil 300 GC

- Carbon
- BH

Fig. 3.18. A silicone-carborane copolymer used as a high temperature stationary phase.
Instrumentation

Columns

Stationary Phases (GLC)

Chiral Phases - used to distinguish between enantiomers of optically active compounds.

α,β,γ – cyclodextrins – composed of six or more D(+)glucose units bonded through a α-(1,4) – glycosidic linkage.
Instrumentation

Columns

Stationary Phases (GLC)
Stationary Phases

Dodecanethiol MPNs, in which the monolayer is dodecanethiol linked to the gold nanoparticle

Journal of Chromatography A Volume 1060, Issues 1–2, 10 December 2004, Pages 225–236
Columns

Temperature Limits

Columns have lower and upper temperature limits.

If a column is used below its lower temperature limit, rounded and wide peaks are obtained (i.e., loss of efficiency).

No column damage has occurred; however, the column does not function properly. Using the column at or above its lower limit maintains good peak shapes.
Columns

Temperature Limits

Columns have lower and upper temperature limits. Upper temperature limits are often stated as two numbers. The lower one is the isothermal temperature limit. The column can be used indefinitely at this temperature and reasonable column bleed and lifetime are realized. The upper number is the temperature program limit. A column can be maintained at this temperature for 10-15 minutes without severely shortening column lifetime or experiencing excessively high column bleed. Exceeding the upper temperature limits may damage the stationary phase.
Temperature Programming

The column sits in an oven. If the temperature is held constant during the entire analysis it is isothermal. If you vary the temperature during the analysis, you typically use a temperature program.

With homologues, the retention time increases exponentially with the number of carbon. As $t_R$ increases, width increases and the height decreases, making detection impossible after a few peaks have eluted.
Instrumentation

Columns

Temperature Programming

General steps to create a program assuming that the separation is possible.
- Determine initial temperature and time based on best possible separation of first few peaks.
- Repeat 1 for the last few peaks to find the best final temperature and time.
- Experiment with various ramps to account for the rest of the components.
Column capacity is the maximum amount of a solute that can be introduced into a column before significant peak distortion occurs. No damage occurs if a column is overloaded.
Instrumentation

Columns
Capacity

Column capacity is related to stationary phase polarity, film thickness, column diameter and solute retention. Higher column capacities are obtained for solutes that are similar in polarity to the stationary phase. For example, a polar column has a higher capacity for a polar solute than a non-polar solute.

Thicker film and wider diameter columns have higher capacities.
Columns

Stationary Phase Film Thickness
Stationary phase loadings were 20-30% w/w prior to 1970’s.
Now loadings are in the order of 1-3%.

Film thickness, $d_f$, has a direct effect on the retention, sample capacity and elution temperature.

For best column efficiency, the film thickness is kept as thin as possible in order to reduce resistance to mass transfer in the stationary phase, $C_s$. 
### Instrumentation

#### Columns

#### Column Capacity in ng

<table>
<thead>
<tr>
<th>Film Thickness (µm)</th>
<th>Column Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.18-0.20</td>
</tr>
<tr>
<td>0.10</td>
<td>20-35</td>
</tr>
<tr>
<td>0.25</td>
<td>35-75</td>
</tr>
<tr>
<td>0.50</td>
<td>75-150</td>
</tr>
<tr>
<td>1.00</td>
<td>150-250</td>
</tr>
<tr>
<td>3.00</td>
<td>400-600</td>
</tr>
<tr>
<td>5.00</td>
<td>1000-1500</td>
</tr>
</tbody>
</table>
Instrumentation

Columns

Film Thickness Selection

1. For 0.18-0.32 mm I.D. columns, a film thickness of 0.18-0.25 µm is average or standard (i.e., not thin or thick) and used for most analyses.

2. For 0.45-0.53 mm I.D. columns, a film thickness of 0.8-1.5 µm is average or standard (i.e., not thin or thick) and used for most analyses.

3. Thick film columns are used to retain and resolve volatile solutes (e.g., light solvents, gases). Thick columns are more inert and have higher capacities. Thick film columns exhibit higher column bleed and decreased upper temperature limits.

4. Thin film columns are used to minimize the retention of high boiling, high molecular weight solutes (e.g., steroids, triglycerides). Thin columns are less inert and have lower capacities. Thin film columns exhibit lower column bleed.
Instrumentation

Columns

Column Diameter Selection

1. Use 0.18-0.25 mm I.D. columns when higher column efficiencies are needed. 0.18 mm I.D. columns are especially well suited for GC/MS systems with low pumping capacities. Smaller diameter columns have the lowest capacities and require the highest head pressures.

2. Use 0.32 mm I.D. columns when higher sample capacity is needed. They often provide better resolution of earlier eluting solutes for splitless injections or large injection volumes (>2 µL) than 0.25 mm I.D. columns.

3. Use 0.45 mm I.D. columns when only a Megabore direct injector is available and higher column efficiency is desired. Well suited for high carrier gas flow rate situations such as with purge & trap and headspace samplers.

4. Use 0.53 mm I.D. columns when only a Megabore direct injector is available. Well suited for high carrier gas flow rate situations such as with purge & trap and headspace samplers. 0.53 mm I.D. columns have the highest sample capacities at constant df.
Instrumentation

Columns

Column Length

Column length influences three parameters:
- efficiency,
- retention (analysis time) and
- pressure.
Columns

Column Length

Column efficiency (N) is proportional to column length.
Resolution is a square root function of the theoretical plate number. For example, doubling column length (thus efficiency) theoretically increases resolution by 1.41 times (closer to 1.2-1.3 times in real practice).

Longer columns are used when peak separation is small and high column efficiency (i.e., narrow peaks) is needed.
Columns

Column Length

Longer columns are used when peak separation is small and high column efficiency (i.e., narrow peaks) is needed.
Instrumentation

Columns

Column Length

Solute retention is proportional to column length for isothermal temperature conditions.

Column head pressure is nearly proportional to column length. Pressure is usually not an issue unless the column has a very small or large diameter.

Long, small diameter columns require extremely high head pressures.
**Columns**

**Column Length Selection**

1. Start with 25-30 meter columns when the best length is unknown.

2. 10-15 meter columns are well suited for samples containing very well separated solutes or very few solutes. Shorter lengths are used for very small diameter columns to reduce head pressures.

3. 50-60 meters should be used when resolution is not possible by other means (smaller diameter, different stationary phase, change in column temperature). Best suited for complex samples containing a large number of solutes. Long columns have long analysis times and higher cost.
Instrumentation

Columns

Stationary Phase Selection
Usually historically based – do what someone else did.

Selecting the Stationary Phase
Consider:
• polarity
• temperature limits
• column efficiency
• detector compatibility.
Non-polar phases usually exhibit superior lifetimes – so best to select least polar phase needed for a particular separation. Most difficult factor to access is the ability of a phase to produce a good separation. In theory selection is based on knowing the types of interactions.
Types of interactions:
- London or dispersive forces (weak, non-specific). Temporarily induced dipole. As molecular weight increases dispersive forces increase (electrons further from nucleus)
- Dipole-dipole interactions (polar molecules)
- Acid – Base interactions (proton sharing or electron transfer) (hydrogen bonding)
Instrumentation

Columns

Types of interactions

- Dispersion is the dominant interaction for all polysiloxane and polyethylene glycol stationary phases.
- Dispersion can be simplified into the concept of volatility. The more volatile a solute, the faster it elutes from the column (i.e., shorter retention time).
Boiling points are fairly valid when dealing with compounds with similar structures, functional groups or homologous series. When dealing with compounds with mixed functional groups, the boiling point simplification often fails. If compound boiling points differ by more than 30°C, they usually can be separated by most stationary phases (there are exceptions). If compound boiling points differ by less than 10°C, the boiling point simplification becomes less certain and more likely to be in error (except for compounds in a homologous series).
Columns

Types of interactions

- If the stationary phase is capable of dipole interaction, it enhances its power to separate solutes whose dipole moments are different. Only some stationary phases are able to exploit this interaction.

- Polyethylene glycols, and cyanopropyl and trifluoropropyl substituted polysiloxanes readily undergo the dipole interaction; methyl or phenyl substituted groups do not undergo a dipole interaction.
Instrumentation

Columns

Types of interactions

- Empirical results have shown that dipole interaction stationary phases are well suited for samples containing compounds that have base or central structures to which different groups are attached in various positions.

- Examples include substituted aromatics, halocarbons, pesticides and drugs.
Instrumentation

Columns

Types of interactions
- The hydrogen bonding interaction occurs if there is hydrogen bonding between the solute molecules and the stationary phase.
- It is the difference in the strength of the hydrogen bonding that is critical.

Relative Hydrogen Bonding Strengths

<table>
<thead>
<tr>
<th>Strength</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong</td>
<td>Alcohols, carboxylic acids, amines</td>
</tr>
<tr>
<td>Moderate</td>
<td>Aldehydes, esters, ketones</td>
</tr>
<tr>
<td>Weak to None</td>
<td>Hydrocarbons, halocarbons, ethers</td>
</tr>
</tbody>
</table>
### Instrumentation

### Columns

### Stationary phase interaction

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Dispersion</th>
<th>Dipole</th>
<th>Hydrogen Bonding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>Strong</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Phenyl</td>
<td>Very Strong</td>
<td>None</td>
<td>Weak</td>
</tr>
<tr>
<td>Cyanopropyl</td>
<td>Strong</td>
<td>Very Strong</td>
<td>Moderate</td>
</tr>
<tr>
<td>Trifluoropropyl</td>
<td>Strong</td>
<td>Moderate</td>
<td>Weak</td>
</tr>
<tr>
<td>PEG</td>
<td>Strong</td>
<td>Strong</td>
<td>Moderate</td>
</tr>
</tbody>
</table>
Columns

Column Selection

Selection process takes experience (many years of use).
Easiest to remember “like dissolves like.”

i.e.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Phase-Choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol (polar)</td>
<td>Polar</td>
</tr>
<tr>
<td>Alkane (non-polar)</td>
<td>Non-polar</td>
</tr>
<tr>
<td>Xylenes (non-polar)</td>
<td>Polar (highlights the slight difference in polarity among the isomers)</td>
</tr>
</tbody>
</table>
Each chromatographic setup will vary to some degree.

Retention times for a known set of species can be hard to reproduce from one lab to another or even one instrument to another.

Retention indexing helps to standardize your results.
Instrumentation

Columns

Column Selection

Methods to characterize stationary phases (Chromatographic systems):

Kovats Index/Rohrschneider Constants/McReynolds Constants

Use the relative retention of a solute – plot of the logarithm of the adjusted retention time versus Kovats retention index, I (linear).
Instrumentation

Columns

Method is based on results of a homologous series.

Plot of the logarithm of the adjusted retention time versus Kovats retention index, I (linear).
The Kovat index value for any material can be found by assigning the retention index \((I)\) of an \(n\)-alkane to equal a value 100 times its carbon number.

- \(n\)-octane = 800
- \(n\)-decane = 1000
- \(n\)-dodecane = 12000, etc.

An \(I\) value of something different than \(n\)-alkane can be determined by “spiking” the mixture with \(n\)-alkanes. Plot \(\log t'\) vs. \(I\) (or use equation) and \(I\) of solute is determined.
Instrumentation

Columns

Equation:

\[
I_x = 100Z + \frac{100(\log t_{r,x}' - \log t_{r,z}')}{\log t_{r,z+1}' - \log t_{r,z}'}
\]

where,

- Z - carbon # of n-alkane.
- \( t_{r,x}' \) - adjusted retention time of component under consideration.
- \( t_{r,z}' \) - adjusted retention time of alkane eluting before x
- \( t_{r,z+1}' \) - adjusted retention time of alkane eluting after x.
Instrumentation

Columns

All that is really being done is to normalize each component compared to n-alkanes. It assumes that you are dealing with either identical or at least very similar columns or packings.

\[
RI_{(target)} = 100 \left( \frac{t_{S_{target}} - t_n}{t_{n+1} - t_n} + n \right)
\]
Methods to evaluate a wide range of phases are based on measuring performance for a set of representative substances. Can then tell if two phases should give comparable performance or if a phase is better for specific functional groups.

McReynolds Constants or Rohrschneider Constants
Use a series of reference compounds. McReynolds used 10 probe solutes – each with a different functionality to measure a specific interaction with a liquid phase. He measured I for the 10 compounds on over 200 phases, including squalene (serves as a reference). Calculated ΔI value for each probe = \( I_{\text{liquid phase}} - I_{\text{squalene}} \).
As $\Delta I$ increases for a probe on a given liquid phase, the degree of a specific interaction associated with the probe increases.

The probes are assigned symbols of $X'$, $Y'$, $Z'$, $U'$, $S'$ (Rohrschneider Constants) and McReynolds expanding it adding $H'$, $J'$, $K'$, $L'$, $M'$.

For squalene all $= 0$
### Instrumentation

#### Columns

<table>
<thead>
<tr>
<th>Group</th>
<th>Substance</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>aromatic, olefinic</td>
<td>benzene</td>
<td>X'</td>
</tr>
<tr>
<td>alcohols, phenols, acids</td>
<td>1-butanol</td>
<td>Y'</td>
</tr>
<tr>
<td>ketones, ethers, esters, aldehydes</td>
<td>methyl-n-propyl ketone</td>
<td>Z'</td>
</tr>
<tr>
<td>nitro, nitriles</td>
<td>nitropropane</td>
<td>U'</td>
</tr>
<tr>
<td>bases, aromatic hetrocyclics</td>
<td>pyridine</td>
<td>S'</td>
</tr>
</tbody>
</table>
Squalane is used as the reference material and all other packings are normalized to it.

<table>
<thead>
<tr>
<th>Packing</th>
<th>TMAX</th>
<th>X'</th>
<th>Y'</th>
<th>Z'</th>
<th>U'</th>
<th>S'</th>
</tr>
</thead>
<tbody>
<tr>
<td>squalane</td>
<td>150</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SE-30</td>
<td>350</td>
<td>15</td>
<td>53</td>
<td>44</td>
<td>64</td>
<td>41</td>
</tr>
<tr>
<td>OV-7</td>
<td>350</td>
<td>69</td>
<td>113</td>
<td>111</td>
<td>171</td>
<td>128</td>
</tr>
<tr>
<td>Carbowax 20M</td>
<td>250</td>
<td>322</td>
<td>536</td>
<td>368</td>
<td>572</td>
<td>59</td>
</tr>
</tbody>
</table>
### Instrumentation

**TABLE 3.4 Probes Used in the McReynolds and Rohrschneider Classifications of Liquid Phases**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>McReynolds Probe</th>
<th>Rohrschneider Probe</th>
<th>Measured Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>X'</td>
<td>Benzene</td>
<td>Benzene</td>
<td>Electron density for aromatic and olefinic hydrocarbons</td>
</tr>
<tr>
<td>Y'</td>
<td>n-Butanol</td>
<td>Ethanol</td>
<td>Proton donor and proton acceptor capabilities (alcohols, nitriles)</td>
</tr>
<tr>
<td>Z'</td>
<td>2-Pentanone</td>
<td>2-Butanone</td>
<td>Proton acceptor interaction (ketones, ethers, aldehydes, esters)</td>
</tr>
<tr>
<td>U'</td>
<td>Nitropropane</td>
<td>Nitromethane</td>
<td>Dipole interactions</td>
</tr>
<tr>
<td>S'</td>
<td>Pyridine</td>
<td>Pyridine</td>
<td>Strong proton acceptor interaction</td>
</tr>
<tr>
<td>H'</td>
<td>2-Methyl-2-pentanol</td>
<td>—</td>
<td>Substituted alcohol interaction similar to n-butanol</td>
</tr>
<tr>
<td>J'</td>
<td>Iodobutane</td>
<td>—</td>
<td>Polar alkane interactions</td>
</tr>
<tr>
<td>K'</td>
<td>2-Octyne</td>
<td>—</td>
<td>Unsaturated hydrocarbon interaction similar to benzene</td>
</tr>
<tr>
<td>L'</td>
<td>1,4-Dioxane</td>
<td>—</td>
<td>Proton acceptor interaction</td>
</tr>
<tr>
<td>M'</td>
<td>cis-Hydrindane</td>
<td>—</td>
<td>Dispersion-interaction</td>
</tr>
</tbody>
</table>
Assignment

• Read Chapter 2: Principles and Practice of Modern Chromatographic Methods, Peter E. Jackson, Academic Press.
• Read Chapter 3: Principles and Practice of Modern Chromatographic Methods, Peter E. Jackson, Academic Press.