

Basic Theory

Gas Chromatography SP → Liquid

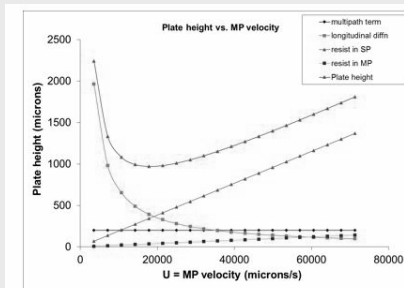
- MP → Inert gas
- ▶ No role in separation
 - ▶ Only directs analyte down column (carrier gas)

$D_m \gg D_s$

- ▶ $C_m U \sim 0$

- Flow rate
- ▶ Dictate by choice of SP (thickness, properties)
 - ▶ Modest plate height ~1mm (↑ L = ↑ N)

Theory Equations



$$A = 2\lambda d_p \quad \frac{B}{U} = \frac{2\gamma D_m}{U}$$

$$C_s U = \frac{f_s(k') d_f^2}{D_s} U$$

$$C_m U = \frac{f_m(k') d_p^2}{D_m} U \approx 0$$

Column Type

- Packed** Packed full of particles
- Put SP on particles
 - MP pushes through packed bed
- Tubing
- ▶ Glass, stainless steel, etc.
 - ▶ Inert = not part of separation

Column Type (cont)

Wall Coated Open Tubular (WCOT) Inside wall of quartz/glass tube

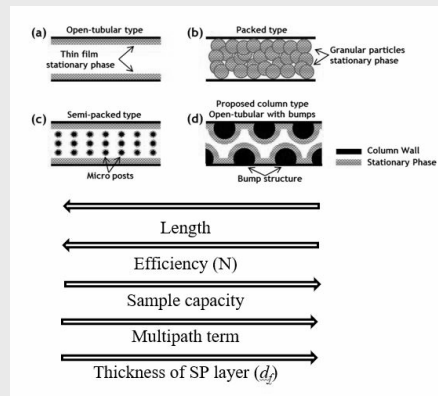
- ▶ Chemically roughen
- ▶ ↑ Surface area
- ▶ Coated with SP

Fused Silica Open Tubular (FSOT) SP coating on wall of long thin tube

- ▶ Smooth wall

Diameter ~ 75-200 μm

Column Diagram



GC Systems

Sample Introduce into injector port

- ▶ Vaporize sample
- ▶ Vaporized analyte swept into column

Mobile phase High pressure cylinders

- ▶ Use a gas flow regulator
- ▶ Regulate the pressure

Detector Detect components of the mixture being eluted off the chromatography column

- ▶ Some may require a reference flow

Oven Separation occurs

- ▶ Controlled temperature
- ▶ Fan → Circulates air and controls temperature

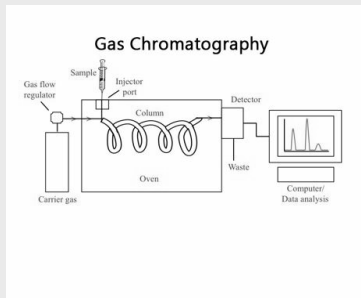


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GC System Diagram



Split Flow Injector

Sample dissolved in volatile solvent

Collect sample into syringe and inject through rubber septum

- ▶ Seals injector for analyte to go into column
- ▶ Protects from outside atmosphere
- ▶ Bad peak shapes = hole in septum

Use a heat block to "flash" sample into vapour

- ▶ ~50-100C hotter than oven
- ▶ Need to vaporize sample

GC systems design to operate with 3 main columns

- ▶ Each column has a different flow rate
- ▶ Adjust based on column used

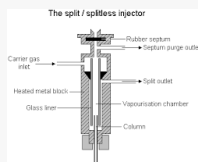
FSOT/WCOT

- ▶ Can't handle large sample mass
- ▶ Small diameter
- ▶ Limited SP
- ▶ Limited volume capacity
- ▶ Control by valve system

Split flow outlet

- ▶ Avoid overloading the column
- ▶ Packed → Set at 0 (closed)
- ▶ FSOT/WCOT → Split flow ratio → Depends on [analyte] in injection volume

Split Flow



Requirements of SP

Requirements of SP (cont)

Prefer a low volatile solvent → Don't want SP to vaporize in oven

Thermal Stability SP in column

Don't want thermal breakdown products

Inert/Reactive Don't want analyte to react with SP

Only want to interact

Stationary Phase

Siloxane Polymer Low volatility

Thermally stable bond

Contains a silicone backbone

- ▶ Close to inert
- ▶ Can be derivatized

Add pendant functional groups

- ▶ Tune selectivity/solubility/retention
- ▶ Adjust polarity

Non-Polar Poly(dimethyl)siloxane (PDMS)

- ▶ Good quality

Fluorocarbons

Polar Can replace dimethyl/methyl groups

- ▶ CN, CO, OH

Phenyl Groups (Benzene Ring) Non-polar

πe^- → delocalized

When approach by polar molecules

- ▶ e^- reorganized → Induced dipole interactions
- ▶ Can behave polar with polar molecules (vice versa)

Chiral Moiety Chiral-chiral interactions on SP

Rise to selectivity of 1 enantiomer over another

Solvent Must dissolve analyte

Bad SP

- ▶ Unretained
- ▶ Affects $R'_f \sim 0$
- ▶ No separation

Volatility A substance with high volatility is more likely to exist as a vapour

A substance with low volatility is more likely to be a liquid or solid



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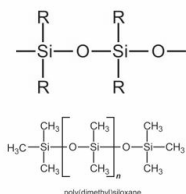
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Siloxane Polymer



Minimize Loss of SP

Bonded Phase Process of the SP polymer is attached to

- ▶ Silica support particle
- ▶ Wall of a capillary

A liquid-liquid chromatography method in which a stationary phase is covalently bound to a carrier particle

Cross-Linked Phase Polymer attached to wall

Polymer cross-linked with each other

- ▶ Critical for separation

Produce more rigidity, hardness and ↑ Melting point

- ▶ Formation of covalent bonds

Issue Most SP are non-polar and silica support surface are polar

- ▶ Not much interaction

Uses phases to prevent issue of contact

Use silane reaction to bond/cross-link

Silane Reaction Use to anchor/bond silicones to silica surfaces

- ▶ In packing materials (particles)
- ▶ FS capillaries

Use to deactivate silanols

Same chemistry for polymerization and cross-linking

Minimize Loss of SP (cont)

Silanol

- ▶ Very polar
- ▶ Expose on surface of silica
- ▶ Disagree with SP polarity
- ▶ Competition for polar analyte

Deactivation chemistry

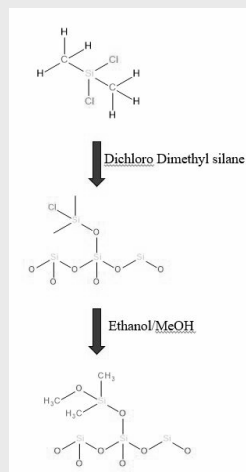
- ▶ Use dichloro dimethyl silane
- ▶ Use ethanol/MeOH
- ▶ Create less polar surface

Inertness of Column

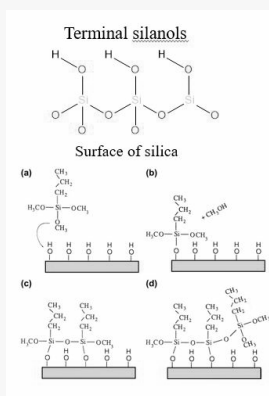
Residual silanols

- ▶ React strongly to polar compounds
- ▶ Produce tailing peaks
- ▶ Undesirable interactions in column

Deactivation of Silanol



Silane Reaction Mechanism



Controlling Retention

Retention Controls resolving power (R')

- ▶ R' depends on K'
- ▶ K' depends on separation conditions

Want all peaks to fall in "ideal range" of retention

- ▶ 1-10

MP is inert

- ▶ Only function to control retention
- ▶ Equilibrium constant = thermodynamic property

Temperature

- ▶ Alter overall retention

Type of SP

- ▶ Alter selectivity

Impact of Different Temperature Isothermal separation

- ▶ A thermodynamic process, in which the temperature of the system remains constant

- ▼ Temperature = ▼ Thermal energy available
- ▶ Less thermal energy
- ▶ Analyte spends more time in SP
- ▶ More time in column
- ▶ Clearer separation

- ▼ Temperature = ▲ Resolution = ▲ Overall time
- ▶ Can become excessive
- ▶ Needs to adjust separation as it proceeds

▼ Temperature → Favors SP

▲ Temperature → Favors MP

Different Ramp Rates Altered t_R and resolution independently

Controlling Retention (cont)

Adjust temperature during course of separation

Resolution improves under better retention conditions for the analyte

Change in gradient steep → Improves separation

- ▶ Shorten separation time
- ▶ Increase resolution
- ▶ As a function of temperature

Round-Up of T Programming

Powerful tool for controlling K'

Directly affects distribution constant

▲ Temperature = ▼ K'

Ramped (gradient) temperature is used to adjust K'

Make GC less intuitive

- ▶ ▼ $R = \frac{dK'}{dT}$ (general)
- ▶ ▲ $R = \frac{dK'}{dT}$ (T programming)
- ▶ $K' = f(T)$

Separation limited by $\Delta T/\Delta t$ (ramp rate)

Column lifetime is shorter at higher temperature

Other Factors

$K' = K(V_s/V_m)$

- ▶ SP thickness
- ▶ Total mass of SP

FSOT columns

- ▶ Calculate phase ratio (V_s/V_m)



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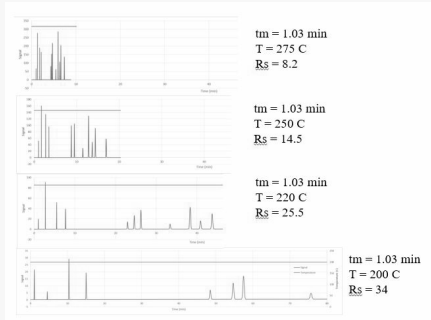
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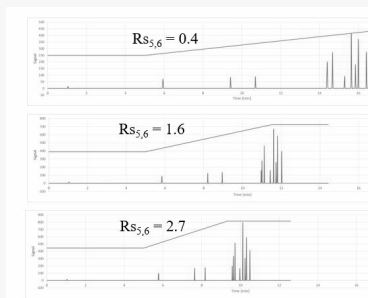
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Graphs with Different Temperature



Ramp Rate Graphs



GC Detectors

- Requirements**
- Sensitivity
 - ▶ 10^{-8} - 10^{-15} g analyte/s
 - ▶ Packed → All sample used → Decrease efficiency = broader peaks
 - ▶ FSOT → Split flow injector (5-10% sample used) → Increase efficiency = narrow peaks

Stability

- ▶ Noise on baseline → Smooth → Detect the smallest peaks → Minimal DL
- ▶ Drift → No baseline (goes up and down)

LDR

- ▶ 5-8 orders of magnitude

GC Detectors (cont)

- Can accept MP over a wide temperature range
 - ▶ T Programming → Improves separation
 - ▶ Immune to T change
 - ▶ Compensate T change → Require reference gas flow

Fast response and independent of T

Simple to use, maintain, repair

Selective/Universal

- ▶ Detect analyte of interest (S)
- ▶ Detect all species (U)

Non-destructive

Flame Ionization Detector (FID)

Analyte elute from column

- ▶ Mix with H₂ gas
- ▶ Combusted

Reduced carbons

- ▶ Produce ions that alter conductivity of flame and alter current

Signal proportional to # of reduced carbons

- ▶ Mass sensitive

Oxidized and e⁻ capturing species

- ▶ No-little signal
- ▶ Cannot be oxidized further

Non-combustible gasses

- ▶ No signal
- ▶ Already oxidized

High sensitivity

- ▶ 10^{-13} g/s
- ▶ use FSOT/WCOT

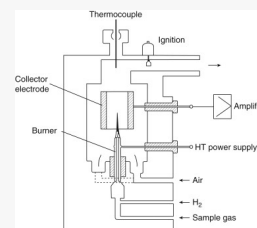
Large LDR

- ▶ 7 orders of magnitude

Destructive

No reference flow

FID Diagram



Electron Capturing Detector (ECD)

An ionization detector

- ▶ response is based upon the ability of molecules with certain functional groups to capture electrons generated by the radioactive source

Radioactive source → ^{63}Ni

- ▶ Emits beta-particles

When disintegration occurs

- ▶ Large energy release
- ▶ Beta particle emission
- ▶ Impacts any filler gas and/or MP present in detector and ionize it

Use a N₂ make-up gas

- ▶ Get ionized by high energy
- ▶ Ionized N₂ gas → Pass an electric current through detector cell

In absence of analyte with e⁻ capturing groups

- ▶ A constant current established through the detector

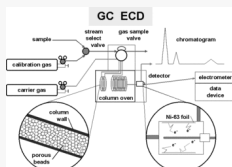
When analyte with e⁻ capturing groups enters cell

- ▶ Quench some ionization
- ▶ Reduce conductivity of gas = reduce current in cell

Selective detector

- ▶ analytes with a high electron affinity
- ▶ Sensitive for species that can disrupt ionization of N₂ gas
- ▶ Pesticides → halides, peroxides, nitro groups

ECD Diagram



Thermal Conductivity Detector(TCD) (cont)

Modest sensitivity ~ 10^{-9} to 10^{-10} g/ml

- ▶ Less sensitive than FID

Modest LDR

- ▶ Very short linearity

Non-destructive

Require a reference flow

Basic Theory

Based on ability of the gas exiting the column to absorb heat

Contains thin filament

- ▶ electrically heated
- ▶ As heat capacity of gas changes (MP vs MP+analyte), so does the T of the filament

Resistance of thin filament

- ▶ T changes the resistance
- ▶ Resistance changes the current of the circuit
- Current is VERY sensitive to T

Reference

Flow (Type 1)

To compensate for the T of MP coming from the oven

- ▶ T is changing with T programmed elution
- ▶ left section of diagram

Equation

$$V_{out1} = V_{applied} * (R_{ref} / (R_{column} + R_{ref}))$$

If $R_{column} = R_{ref}$

- ▶ two resistors are "balanced"
- The signal from the column is coming from the MP
- ▶ $V_{out1} = (1/2)V_{app}$

Thermal Conductivity Detector(TCD)

Properties Signal proportional to change in heat capacity

- ▶ Difference between MP and MP+analyte are relatively small

Universal detector

- ▶ Detect solvent as well
- ▶ Undersirable → Solvent order of magnitude is more concentrated than analyte
- ▶ Result in large solvent peaks and small analyte peaks
- ▶ If analyte is not well retained → Interfered by solvent



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Thermal Conductivity Detector(TCD) (cont)

If $R_{column} \neq R_{ref}$

- ▶ Analyte's heat capacity changes T
- heating or cooling of filament
- ▶ V_{out1} increases as analyte elutes
- As R_{column} gets closer to 0, V_{out1} gets closer to V_{app}

Reference Flow (Type 2) Opposite concept as reference flow type 1

- ▶ right section of diagram

Equation

▶ $V_{out2} = V_{applied} * (R_{column}/(R_{column} + R_{ref}))$

If $R_{column} = R_{ref}$

- ▶ two resistors are "balanced"
- The signal from the column is coming from the MP
- ▶ $V_{out1} = (1/2)V_{app}$

If $R_{column} \neq R_{ref}$

- ▶ Analyte's heat capacity changes T
- Heating or cooling of filament
- ▶ V_{out2} decreases as analyte elutes
- As R_{column} gets closer to 0, V_{out2} gets closer to 0

Reference Flow (Type 3) Type 1 and Type 2 TCD operating together

- ▶ With separate power supplies

If $R_{column} = R_{ref}$

- ▶ two resistors are "balanced"
- The signal from the column is coming from the MP
- ▶ $V_{out1} = (1/2)V_{app}$

Thermal Conductivity Detector(TCD) (cont)

If $R_{column} \neq R_{ref}$

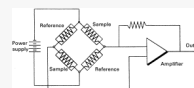
- ▶ As analyte elutes
- V_{out1} increases
- V_{out2} decreases
- ▶ V_{out1} and V_{out2} have same magnitude, opposite signs
- Taking the difference of the two will double the V measured
- Double the signal for the same effort

Reference Flow (Type 4) Same as Type 3, but with a single power supply

Wheatstone bridge

- ▶ Name of this circuit
- ▶ Common approach for detecting VERY small change in resistance
- Advantage: Doubles the signal magnitude

TCD Diagram



GC-MS

Properties Versatile

- ▶ Provide identification power

Have to run known standards (spiked)

Electron beam ionization

- ▶ M^+ and fragments

Excellent DL

- ▶ Depending on instrument and analyte
- ▶ ~ 2-20 picog injected

Concentration DL in sample

- ▶ Depends on sample work-up

Long LDR

- ▶ Dependent on instrument
- ▶ 4-6 orders of magnitude



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GC-MS (cont)

Selective

- ▶ Less interferences
- ▶ Filters out MP signal

Destructive

Expensive

Basic Quadruple MS

Theory ▶ Contains 2 positive and 2 negative poles

Movement of M^+

- ▶ M^+ travels in a sinusoidal path
- ▶ If M^+ is too light or too heavy, it is kicked out of quadrupole
- b/c they are not really able to respond to polarity change
 - ▶ How to fix this
- Quickly change the frequency and voltage of the poles
- Can quickly scan through all m/z ratio to obtain mass spectrum

Spectrum generated

- ▶ Total ion current (TIC)
- Easiest way
- Sum of all ion signals that passes through
- Acts as a universal detector: does not filter out MP signal
- Tells you how many species are present
- ▶ Extracted mass spectra
- Take a slice of TIC peak and see its fragments

GC-MS (cont)

Isotopes

- ▶ Parent ion
- Most prominent and heaviest
- ▶ Isotopes
- Daughter peak from most prominent peaks
- can provide more info depending on its ratio with parent peak
- ▶ Isotopically labelled analytes
- Replacing parts of molecule with deuterium
- Produces a known mass higher than the original mass
- Compare spectrum with original

Positive identification

- ▶ Compare experimental spectrum with the "real" analyte spectrum
- ▶ 3 steps
- Correct mass of molecule?
- Correct set of fragments?
- Correct fragment intensities?

Quantitation

- ▶ Usually multiple ions monitored/measured
- Validate ratio of peaks at the correct m/z ratio

Column bleed

- ▶ SP is boiling and bleeding out
- ▶ Leads to a rise in baseline
- Not good
- ▶ How to fix it
- ▶ Running at low T
- Purchase column made specifically for MS (\$\$\$)



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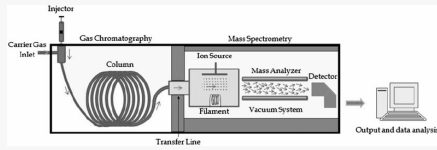
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GC-MS Diagram



Key Factors and Applications (cont)

Food and Beverages

- ▶ Wine/alcohol
- ▶ Pesticides

Environmental

- ▶ Pesticides
- ▶ PAH and industrial solvents
- ▶ Oil/hydrocarbon spills

R&D

- ▶ Organic synthesis
- ▶ Catalysis (monitor products)

Industrial

- ▶ Feedstock
- ▶ Off gassing

Key Factors and Applications

When will GC be useful

1. Analyte
 - ▶ Needs to be volatile
 - ▶ Not proteins → Unstable at high temperature
 - ▶ Silation reaction → Produce volatile products (Risk of contamination, loss, produce new products)
 - ▶ Needs to be stable → Stable enough to transit the column

2. High enough concentration to detect
 - ▶ Packed columns: great sample capacity but low resolving power and resolution
 - ▶ FSOT: lower capacity (split flow) but high resolving power and resolution
 - ▶ Detectors: Has a good sensitivity

3. Does sample require high R' separation
 - ▶ Depends on the type of detector
 - ▶ Universal = high R
 - ▶ Selective = low R

4. Generally faster than LC

Applications

Anti-doping and forensics

BAC (Crime/forensics labs)

Pharmaceuticals

- ▶ Process control
- ▶ Quality control
- ▶ Research and development



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