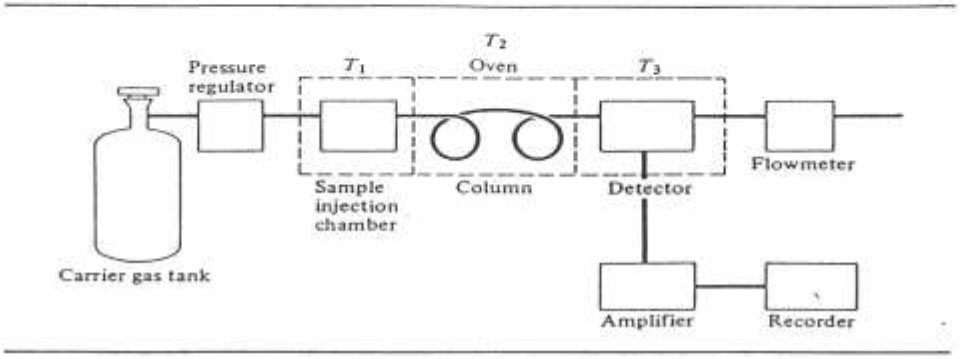
Gas Chromatography

- GSC
- GLC (packed column or open tubular column)



Sample type:

thermally stable, appreciable vapour pressure at the column temperature

- permanent gases
- most non-ionized small or medium-sized organic molecules (Typically up to C30)
- many organometallic compounds
- Not used for: macromolecules or salts

In some cases non-volatile compounds can be converted into more volatile and stable derivatives

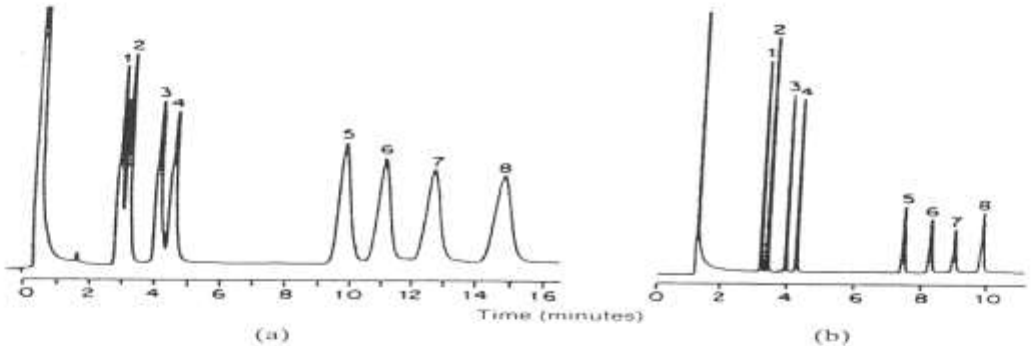
CAUTION: samples containing mixtures of volatile and non-volatile components

GC Columns:

Comparison of packed and open tubular columns.

Parameter	Column type			
	Packed	Microbore open tubular	Open tubular	Megabore open tubular
Length (m)	0.5–3	5–50	5–100	5–100
Internal diameter (mm)	2–4	> 0.1	0.18–0.32	0.53–1.00
Permeability (10^{-7} cm^2)	1–50		300–20 000	
Film thickness (μm)	1–10	0.1	0.2–2	1–5
Carrier gas average linear velocity (cm S^{-1})*	4–8	70–90	60–80	20–50
Flow rate (ml min^{-1})	40–80	0.2–0.5	0.6–4	2–50
Phase ratio, β	5–35	300–1500	80–250	25–130
Pressure drop (kPa)	70–275	70–100	14–35	7–14
Effective plates per metre	1000–2000	8000–12 000	3000–5000	1400–1800
Sample capacity (ng)	20 000	< 5	20–500	1000–15 000

*These values are optimum for hydrogen. For nitrogen the values would be about 0.3 times those shown and for helium, the values would be about 0.55 times those shown.



Efficiency comparison of (a) a packed column (1.8 m × 4 mm i.d.) and (b) an open tubular column (15 m × 0.32 mm) for the separation of chlorinated pesticides. Conditions: (a) 1.3% OV-17 + 2.1% QF-1 on Chromosorb 750, 100–120 mesh, 200°C, nitrogen carrier gas, 60 ml min^{-1}; (b) DB-5, 0.25 μm film thickness programmed from 150°C to 240°C at 5°C min^{-1}, helium carrier gas. Pesticides are: 1, α-BHC; 2, lindane; 3, B-BHC; 4, heptachlor; 5, o,p'-DDD; 6, endrin; 7, p,p'-DDD; 8, endosulfan.

Bulk chemical composition (%) of glasses used for open tubular column construction.

Component	Soda lime (soft)	Borosilicate (hard, Pyrex, Duran)	Fused quartz	Fused silica
SiO_2	68.0	81.0	99.9	99.9
Na_2O	15.5	4.0		
CaO	6.0	0.5		
Al_2O_3	3.0	2.0	100 μg g^{-1}	<1 μg g^{-1}
B_2O_3		13.0		
MgO	4.0			
BaO	1.0			
K_2O	0.5			
Fe_2O_3			100 μg g^{-1}	<1 μg g^{-1}

GSC:

preceded GLC, never achieved the same prominence:

i) adsortion isotherms are frequently nonlinear

ii) long retention times because of high surface area of adsorbents

iii) adsorbents difficult to prepare reproducibly

Advantages over GLC:

isomers, inorganic gases, low molecular mass hydrocarbons

no column bleed (higher temperatures)

Adsorbents: silica, charcoal, alumina, molecular sieves, porous polymers

Chemical type	Commercial name	Specific surface area ($m^2 g^{-1}$)	Pore diameter (nm)
Silica	Porasil B	185	15
	Porasil C	100	30
Alumina	Various	–	–
Graphitized carbon black	Carbopack C	12	–
	Carbopack B	100	–
	Carbosieve	1000	1.3
	Spherocarb	1200	1.5
Carbon molecular sieve	Carbosphere	1000	1.3
Sodium aluminium silicate	Molecular sieve 13X	700–800	1.0
Calcium aluminium silicate	Molecular sieve 5A	700–800	0.5

Porous polymer		Type*	Surface area† ($m^2 g^{-1}$)	Pore diameter (nm)	Temperature limit (°C)
Porapak	N	VP	250–350	–	200
	P‡	PS-DVB	100–200	–	250
	Q‡	EVB-DVB	500–600	7.5	250
	R	VP	450–600	7.6	250
	S	VP	300–450	7.6	250
	T	EGDMA	225–350	9	200
Chromosorb	101	PS-DVB	50	300–400	275
	102	PS-DVB	300–500	8.5	250
	103	PS	15–25	300–400	275
	104	ACN-DVB	100–200	60–80	250
	105	Acrylic ester	600–700	40–60	250
	106	PS	700–800	500	250
	107	Acrylic ester	400–500	800	250
	108	Acrylic ester	100–200	250	250

*VP, vinylpyrollidone; PS, polystyrene; DVB, divinylbenzene; EVB, ethylvinylbenzene; EGDMA, ethylene glycol dimethacrylate; ACN, acrylonitrile.

GLC:

liquid stationary phase retained on a solid support (packed columns) or the column wall (open tubular columns)

Solid Supports:

retains stationary phase

provides a large interface between the mobile phase and stationary phase

Characteristics: inert, thermally stable, large surface area, mechanically strong, uniform pore and particle size

Diatomaceous earths (diatom skeleton deposits consisting of silica and metallic impurities)

PTFE beads, glass beads, charcoal, porous silica

Table 3.10 Physical properties of selected solid supports.

Trade name	Specific surface ($m^2 g^{-1}$)	Pore diameter (μm)	Packed density ($g ml^{-1}$)	Maximum loading (% w/w)
Chromosorb W	1.0	0.9	0.24	15
Chromosorb P	4.0		0.47	30
Chromosorb 750	0.5–1.0		0.36	7
Anakrom	1.0–1.4	1.0		
Gas Chrom Q		Data unavailable		
Supelcoport		Data unavailable		
Glass beads	0.04–0.36			0.5
Chromosorb T	7–8		0.49	20

Table 3.11 Support treatments.

AW or A	Acid washed
NAW or U	Non-acid washed (or untreated)
DMCS or S	Dimethyldichlorosilane treated
AW-DMCS	Acid washed and dimethyldichlorosilane treated
HMDS	Hexamethyldisilazane treated
HP or Q	High-performance (high quality AW DMCS treated)

Table 3.12 Mesh sizes of solid supports in gas–liquid chromatography.

Mesh size (ASTM sieve)	Nominal screen size (mm)
40–60	0.42–0.25
60–80	0.25–0.18
80–100	0.18–0.15
100–120	0.15–0.125

Stationary Phases:

Similar liquids used in packed and open tubular columns (cross-linked, bonded)

300 stationary phases available
1000 stationary phases described in the literature

Ideal properties:

- low vapour pressure
- thermal and chemical stability
- low viscosity
- nonreactive
- -80°C to 450°C
- dissolving power

Non-polar stationary phases:

contain no functional groups capable of specific interactions, e.g. H-bonding, dipole interactions, only dispersive forces are involved
Components separate according to their volatility (elution order follows their b.p.)

Polar stationary phases:

contain functional groups capable of specific interactions with sample components
Elution order depends on a combination of volatility and specific polar-polar interactions

Non-polar stationary phases:

excellent solvents for non-polar solutes

e.g. alkanes are selectively retained as compared to polar solutes of similar b.p.

Hydrocarbons:

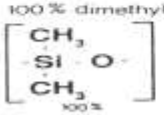
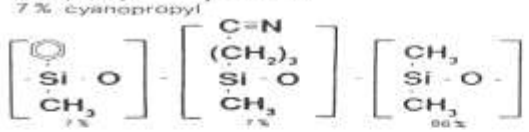
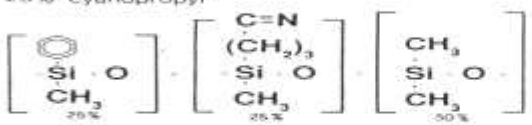
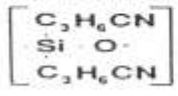
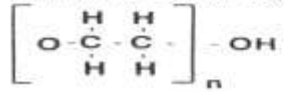
high molecualr mass discrete hydrocarbons

e.g. squalane or Apolane C87 or mixtures of long-chained *n*-alkanes such as Apiezon L

Important reference phases (not widely used in open tubular columns).

Alkylsilicone phases:

Polymers based on Si-O-Si backbone

Chemical structure	Classification	Uses
100 % dimethyl silicone 	Non-polar	Boiling point separations (solvents, petroleum products, pharmaceuticals)
95 % dimethyl, 5 % phenyl silicone 	Non-polar	Boiling point separations (aromatics, flavours, aromatic hydrocarbons)
86 % dimethyl, 7 % phenyl, 7 % cyanopropyl silicone 	Intermediate polarity	Pesticides, alcohols
50 % dimethyl, 25 % phenyl, 25 % cyanopropyl silicone 	Polar	Triglycerides, phthalate esters
100 % cyano propyl silicone 	Polar	Fatty acid methyl esters, carbohydrates
polyethylene glycol 20 M 	Polar	Flavours, fatty acid methyl esters, acids, amines

Trademark	Company
BP	Scientific Glass Engineering (SGE)
DB	J & W
OV	Ohio Valley
CP	Chrompack
SE	General Electric
DC	Dow Corning
SP	Supelco
SPB	RSL Belgium/Alltech
Superox	RSL Belgium/Alltech

Polar stationary phases:

Substituted silicone phases:

Prepared by substituting polar trifluoropropyl or cyano groups for the methyl groups of the dimethylsilicones

Wide range of polarities

Trifluoropropyl group: high dipole moment, strong e-acceptor properties, high selectivity for analytes containing lone-pair electrons, e.g. NO, CO, OH groups

Cyano group: strongly attracts electrons, π-bonded groups (olefins, carbonyl groups, phenyl rings, and esters)

Secondary effect: phase can tolerate higher temperature

Ester phases:

polymeric esters, poly(diethyleneglycol succinate) (DEGS), poly(diethyleneglycol adipate) (DEGA) limited applications

Used for resolving esters with different degrees of unsaturation, not geometric isomers

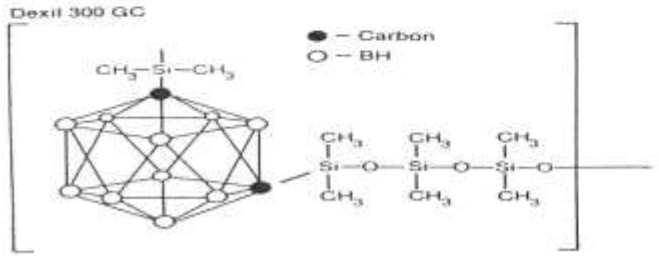
Limited chemical and thermal stability

Polyether phases:

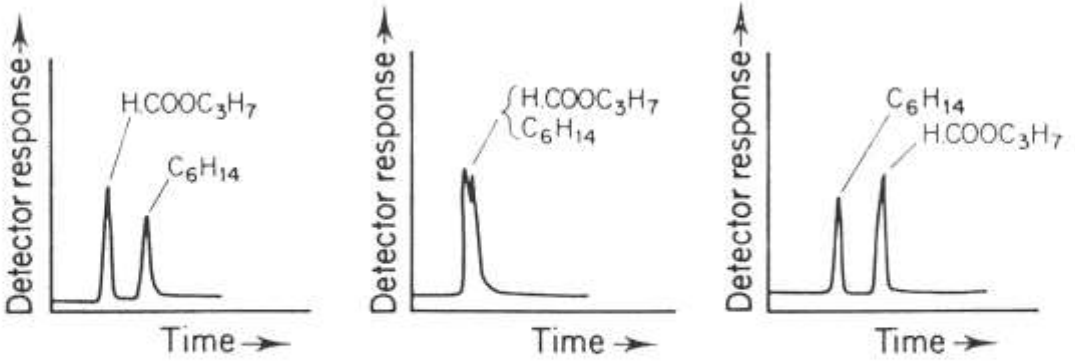
-(CH_2CH_2-O)- (polyethylene glycols, polyoxiranes)

Tradename: Carbowax, Superox

Specialty stationary phases:



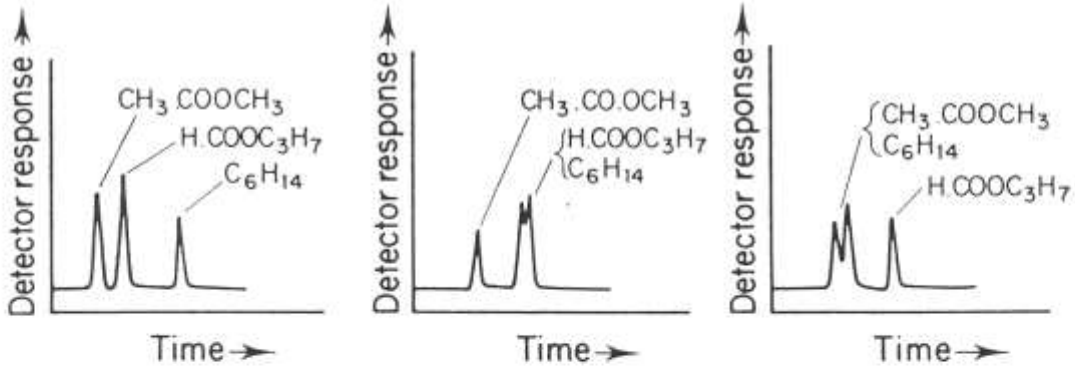
A silicone–carborane copolymer used as a high temperature stationary phase.



(i) Stationary phase = squalane

Stationary phase = silicone oil

Stationary phase = PEG-S



(ii) Stationary phase = squalane

Stationary phase = silicone oil

Stationary phase = PEG-S

Mobile Phase:

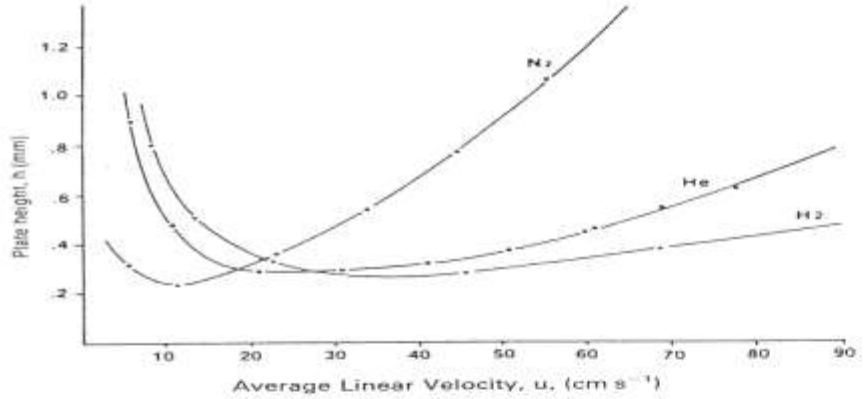
nonreactive towards analyte, nonflammable, cheap

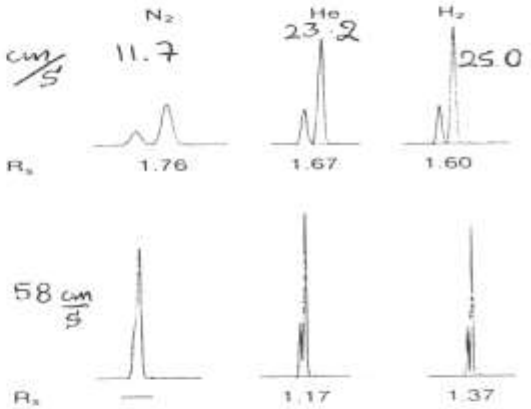
Choice of mobile phase is determined by practical constraints of cost, availability, inertness, detector compatability, etc.

Can influence resolution and analysis time (differences in solute diffusion rates for various gases)

Physical properties (at 273 K and 101 kPa) and applications of gases used in gas chromatography.

Gas	Thermal conductivity (10^{8} W m^{-1} K^{-1})	Viscosity (10^{-7} Pa.s)	Density (kg m^{-3})	Application
Hydrogen	16.75	84	0.0899	Carrier and burner gas
Helium	14.07	186	0.1785	Carrier gas
Nitrogen	2.39	166	1.2505	Carrier gas
Argon	1.67	212	1.7839	Carrier gas
Neon	4.56	298	0.8999	Carrier gas
Oxygen	2.43	192	1.4289	Burner gas
Air	2.39	171	1.2928	Burner gas





Oxygen and moisture traps

Carrier gas regulation

Typical values of flow rate, pressure and average linear gas velocity for different sized open tubular columns using hydrogen as carrier gas.

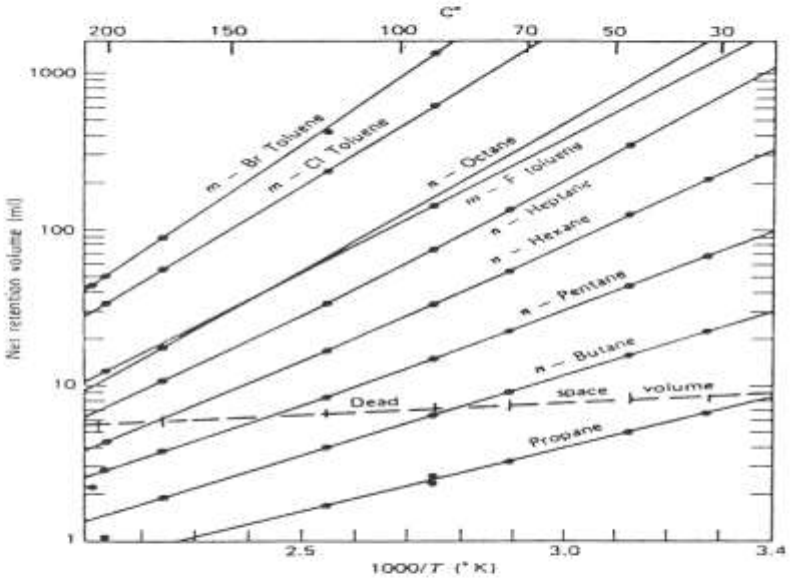
Column i.d. (mm)	Film thickness (μm)	Film length (m)	Flow rate (ml min^{-1})	Average linear velocity (cm s^{-1})	Pressure (kPa)
0.10	0.10	12	0.2–0.5	38	80
0.22	0.5	12	0.8–2.0	36	35
0.32	0.5	12	1.7–4.0	34	17
	2.0	12	1.7–4.0	28	17
	0.5	25	1.7–4.0	28	35
0.53	1.0	12	3–50	28	7
	5.0	12	3–50	13	7
	5.0	25	3–50	13	14

Column Temperature:

Accurate temperature (+/- 0.1 ^{O}C) results in reproducible retention times(stds 0.01-0.10%)

Rates of temperature rise: 0.25-40 ^{O}C/min

tR decrease as T increase because K is temperature dependent in accordance to:
logp^{O}= - ΔH/2.3RT + constant (Clausius-Clapeyron Equation)



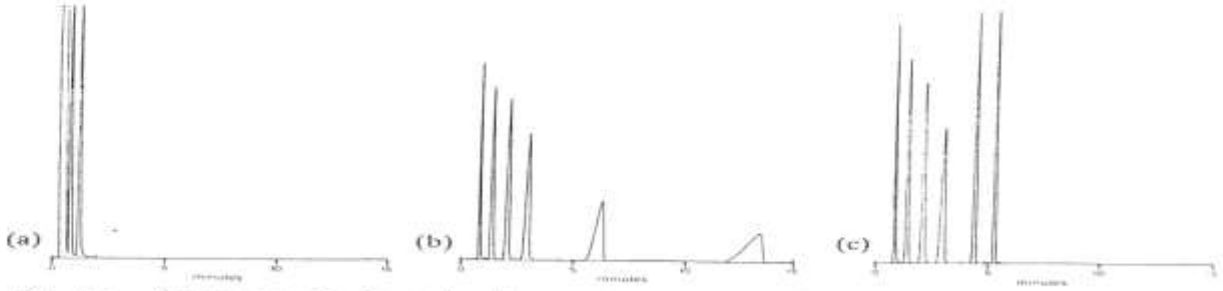


Fig. 3.23. Comparison of isothermal and temperature-programmed separation of an equimolar mixture of ethanol, butan-2-ol, 2-methylpropanol, butan-1-ol, pentan-1-ol and hexan-1-ol using a PTV injection (0.1 μl) in split mode (1:100) and flame ionization detection. Column temperatures: (a) and (b), isothermal at 105°C or 50°C, respectively and (c) 50°C isothermal for 3 min then programmed to 140°C at 10°C min^{-1}. In chromatogram (b) the effects of exceeding column sample capacity are seen as a fronting peak. Note that use of a higher column temperature in chromatogram (a) enhances the sample capacity.

GC Sample Introduction

Syringe techniques (<1 μl solution, 50-500 μl gas)

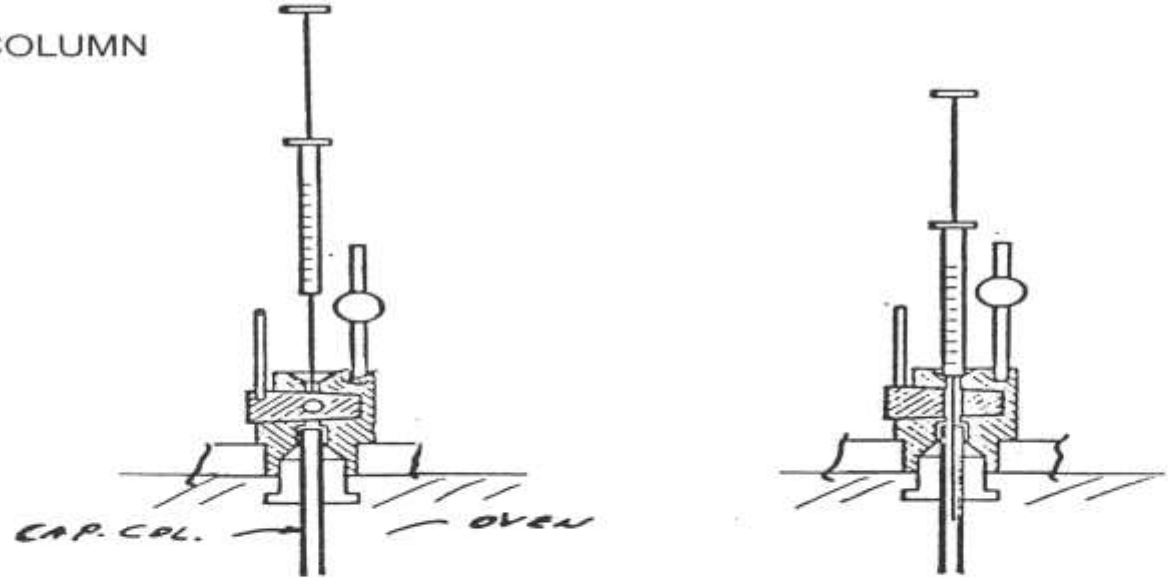
a. direct on-column
b. Split-splitless (via septum).

Probe techniques:

a. solids injection
b. pyrolysis probe

Purge and trap

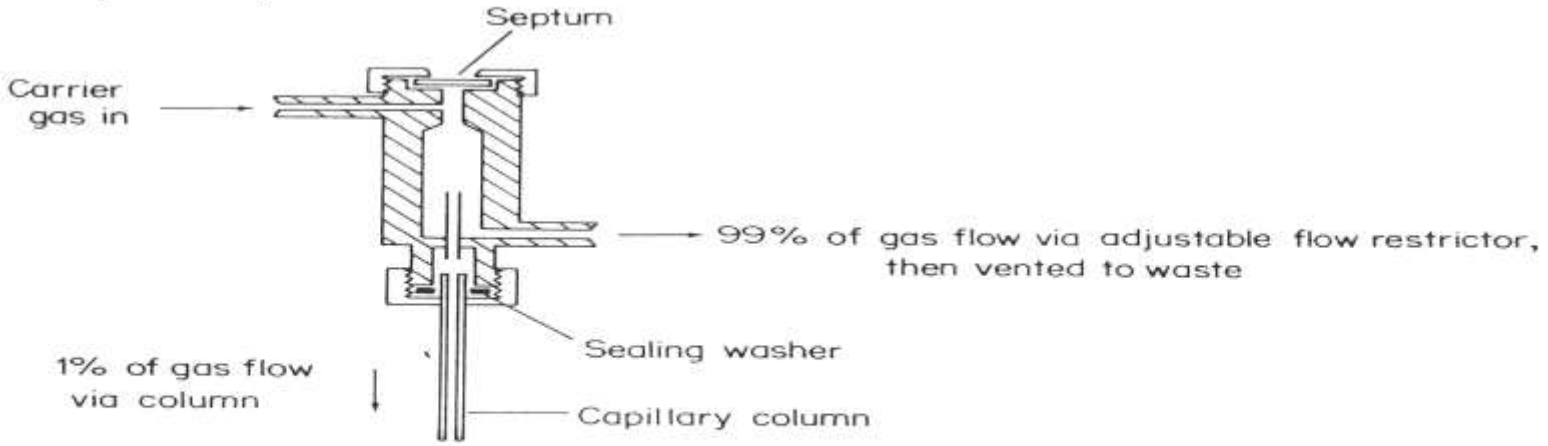
ON-COLUMN



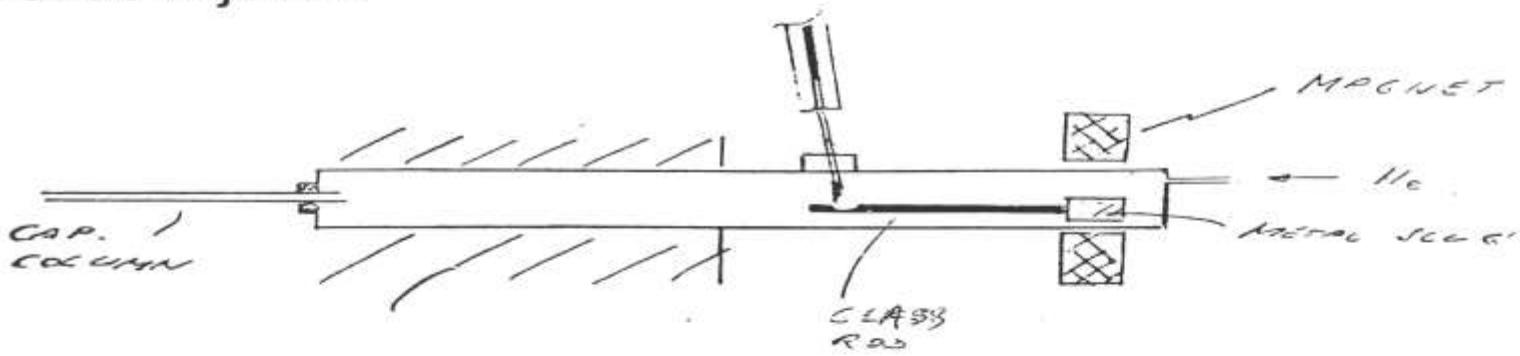
Inject about 1 μl solution
oven temperature about 20 ^{o}C < b.p. of solvent (solvent removal in about 10 min)
High efficiency

Not applicable for low boiling compounds

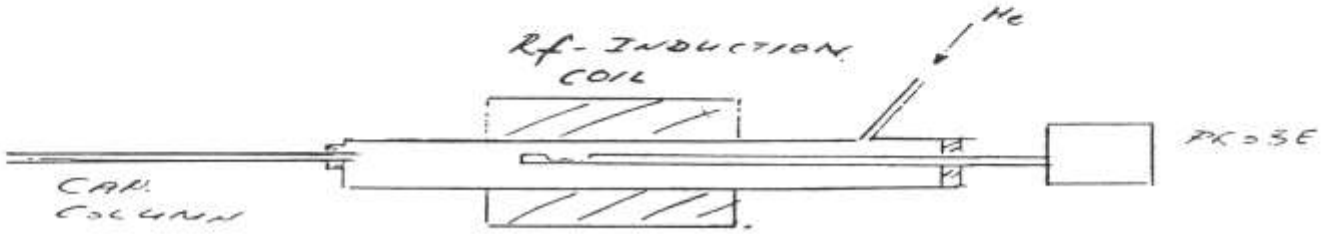
Split-Splitless



Solids Injector



Pyrolysis Probe



Detectors for GC

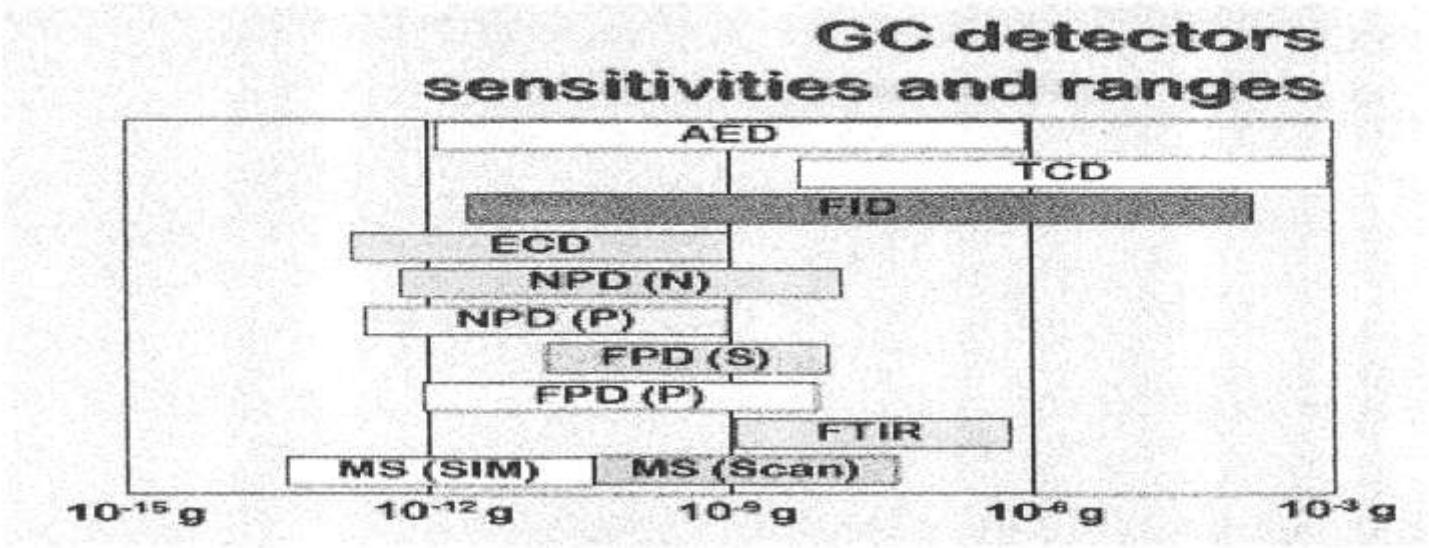
Over 100 detectors for GC, only a few in common use

Important characteristics (listed in random order):

a. High sensitivity
b. Low limit of detection
c. Large linear dynamic range
d. Universal or selective response
e. Inexpensive to purchase and operate, reliable and easy to operate
f. Capable of providing information on the identity of the solute

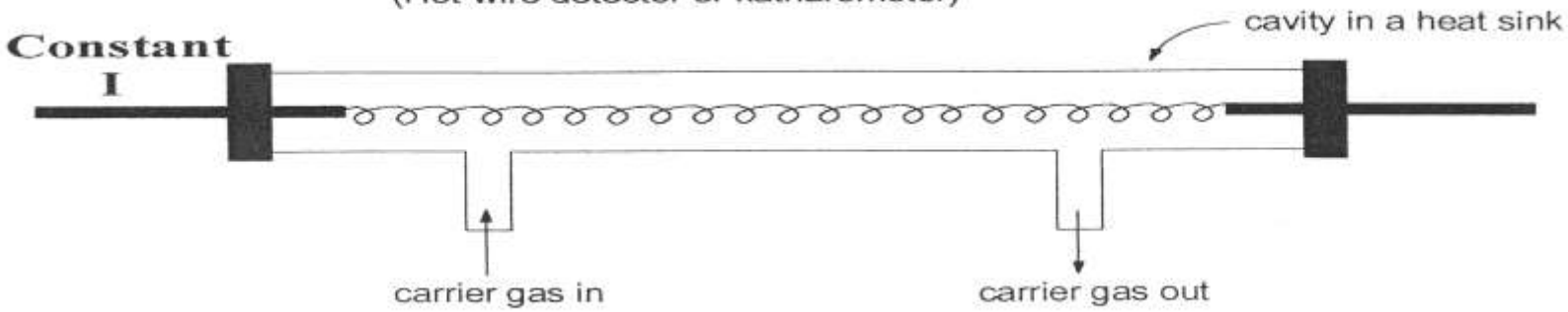
Classification of the most common gas chromatographic detectors.

Detector	Response	Optimal detection limit	Linear range	Classification
TCD	Organic and inorganic solutes	10^{-9} g ml^{-1}	10^{4}	Concentration, nondestructive
FID	All organic solutes except formic acid and formaldehyde	10^{-12} g ml^{-1}	10^{7}	Mass flow-rate; destructive
ECD	Halogenated and nitro compounds	10^{-15} mol ml^{-1}	10^{3}–10^{4} (pulsed)	Concentration; nondestructive
AFID	P- or N-containing solutes	N: 10^{-14} g s^{-1} P: 10^{-13} g s^{-1}	10^{3}–10^{5}	Mass flow-rate; destructive
FPD	P- or S-containing solutes	S: 10^{-10} g s^{-1} P: 10^{-12} g s^{-1}	S: 10^{3} P: 10^{5}	Mass flow-rate; destructive



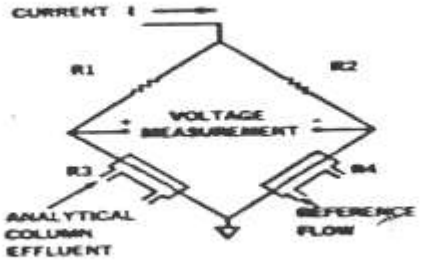
Thermal Conductivity Detector (TCD)

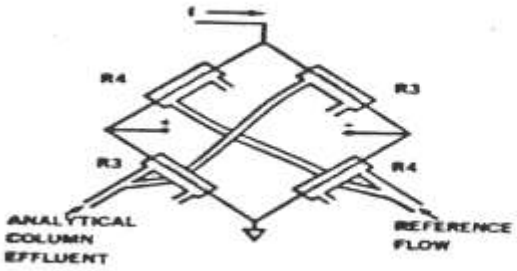
(Hot-wire detector or katharometer)



Resistance (R) depends on the Temperature of the filament.
Ohm's Law: V=I.R

Wheatstone Bridge





Relative thermal conductivities of selected compounds.

Compound	Relative thermal conductivity
Carrier gases	
Helium	100.0
Nitrogen	18.0
Hydrogen	128.0
Argon	12.5
Carbon dioxide	12.7
Typical analytes	
Ethane	17.5
n-Butane	13.5
iso-Butane	14.0
Benzene	9.9
Ethanol	12.7
Acetone	9.6
Chloroform	6.0
Ethyl acetate	9.9

Used for:

inorganic gases, eg hydrogen, oxygen, nitrogen, CS_2, water

Sensitivity depends on:

- detector cell design
- difference in TC of the carrier gas and the carrier gas plus analyte; H_2 and He are common carrier gases, N_2 used only in special cases
- Heating the filament to a higher temperature with a power supply will improve sensitivity
- carrier gas flow rate changes
- temperature of surroundings; lower temperature provides improved sensitivity

CAUTIONS: acids, halogenated compounds, and oxygen cause damage to the filament.

Other interesting points:

most widely used, nondestructive, inexpensive, reasonable rugged.

Examples:

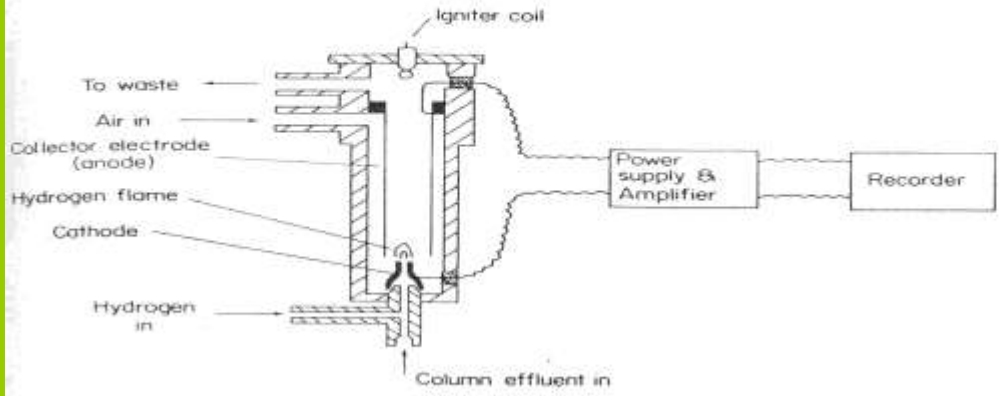
Which carrier gases would you use for the following analyses (GC-TCD)

- determination of traces of H_2 in air
- mixture of propanone and propan-2-ol
- determination of Ar in air

Flame Ionization Detector (FID)

(General detector for organics)

Mode of Detection: Production of ions in a flame result in a current that can be measured

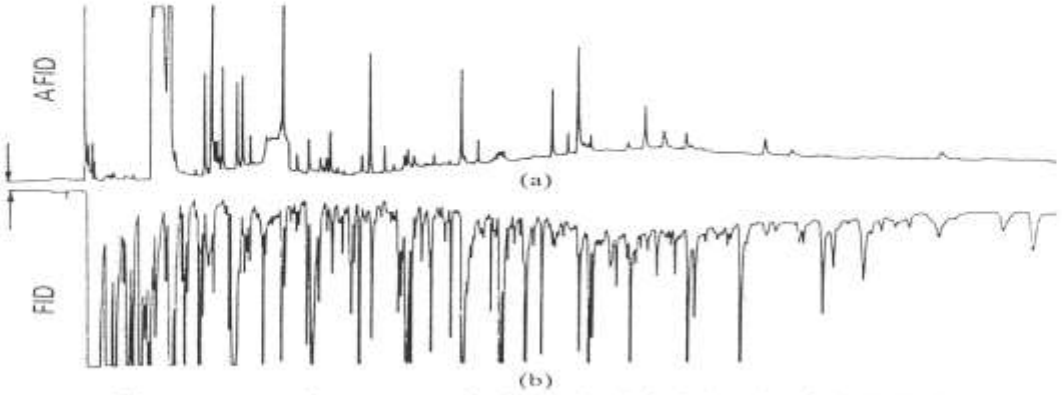


Air/H_2 approx. 10:1
He/H_2 approx. 1:1
30-60 ml/min

Applications:

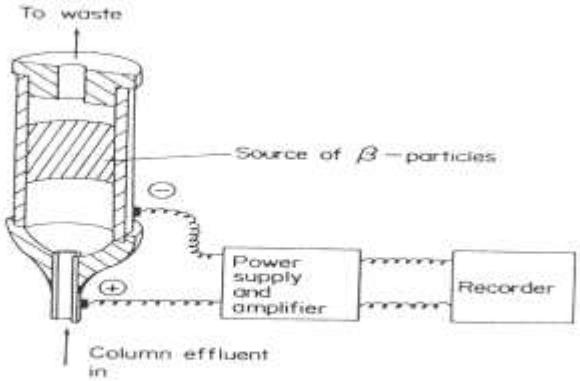
Used for the detection of organic compounds (gives similar responses, which are approximately proportional to the total mass of the carbon and hydrogen in the analyte).
Reduced response for compounds with a large proportion of oxygen.
Virtually no response for inorganic compounds: H_2O, CO, CO_2, N_2, O_2, CS_2, noble gases

Alkali Flame Ionization Detector (AFID)



Chromatograms of a serum sample obtained with dual detection. (a), alkali flame ionization detector; (b), flame ionization detector. Reproduced with permission from F. Hsu *et al.* (1980). *J. High Resol. Chromatogr., Chromatogr. Comm.*, 3: 648.

Electron Capture Detector (ECD)



Sources: ^{3}H/foil
^{63}Ni (β particles)
Mobile phase: N_2, Ar (5-10% CH_4)

Source ^{63}Ni emits high energy β particles (67 keV)
Each β particle may generate 100-1000 thermal e^- with the carrier gas

$$N_2 + \beta \rightarrow N_2^+ + e^-$$

e^- are monitored at the anode (50 V across the chamber)
Electrophilic analyte molecule can capture a thermal e^-

$$AB + e^- \rightarrow AB^- \quad \text{nondissociative capture}$$

$$AB + e^- \rightarrow A + B^- \quad \text{dissociative capture}$$

As a result the fast moving e- are replaced by slow moving analyte ions, so the system loses e^- and the current is reduced

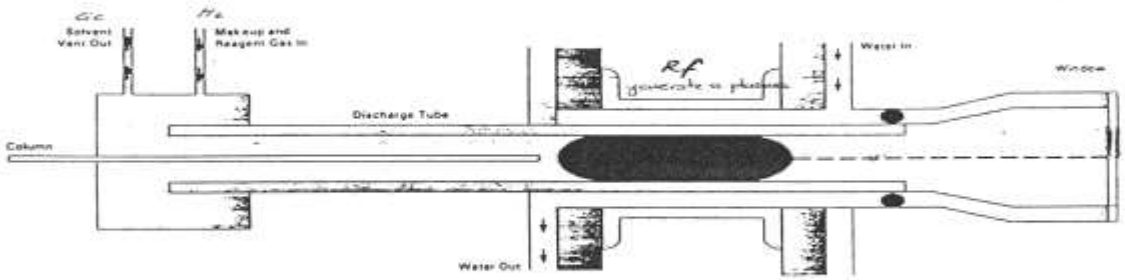
Temperature effect: increase in T favours dissociative reaction; decrease in T favours nondissociative reaction

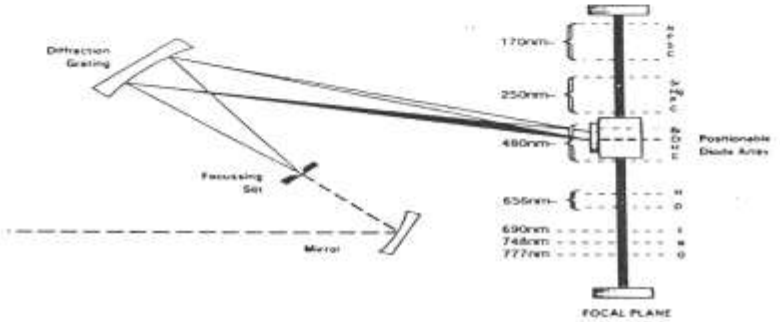
Relative response of the ECD and FID to selected analytes.

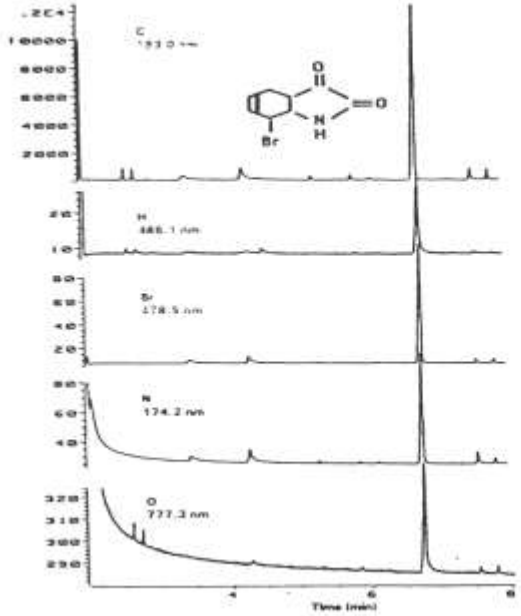
Analyte	Relative response of the ECD compared with FID
Halogenated organics	10^4
Organometallic compounds	10^3
Aromatic compounds	10^2
Conjugated unsaturated compounds	10^1
Non-conjugated compounds	10^{-4}

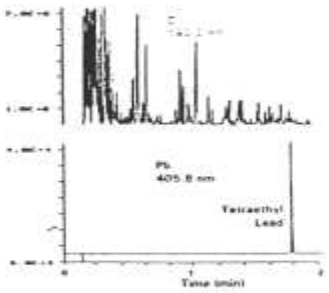
Atomic Emission Detection (AED)

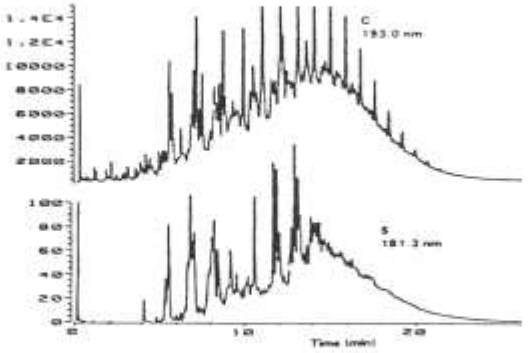
GC → Analyte Molecules —(Plasma Excitation)→ ATOMS* —(hv, (λ))→ ATOMS











Qualitative and Quantitative Analysis in Chromatography

Qualitative Analysis
- Retention parameters
- Chemical Methods
- Peak Trapping
- Selective detectors
- Coupled techniques

Retention parameters

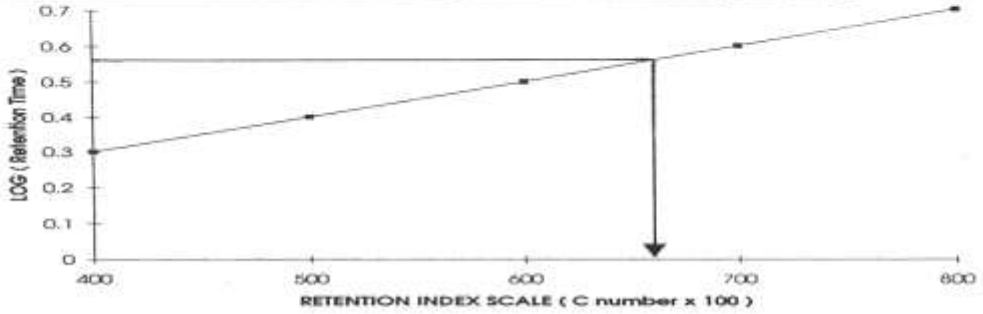
t_R

Disadvantages:
- → requires standards in order to compare t_R
- → several other compounds may have the same t_R

- Repeat analyses using a second column with a stationary phase with markedly different polarity
- Spiking a sample in order to assist in confirming the identity of a peak

Relative retention

Retention indeces for gas chromatography



$$I = n \times 100 + 100 \times \left[\frac{\log t_{r,u} - \log t_{r,n}}{\log t_{r,n+1} - \log t_{r,n}} \right]$$

Table 9.2 Comparison of chromatographic behaviour of a test mixture containing dimethyl phthalate (peak 1), di-*n*-butyl phthalate (peak 2) and pyrene (peak 3) on different chemically bonded ODS-silica packings using a methanol-water (90:10) mobile phase [12, p. 278].

Commercial name	Carbon load (%)	Relative retention		
		Peak 1	Peak 2	Peak 3
Partisil 10 ODS	5	1.0	8	22
Hypersil ODS	9	1.4	13	26
Spherisorb ODS	7	2.0	13	28
Partisil 10 ODS 3	10	0.9	16	31
μBondapak C_{18}	10	13	31	45
Zorbax ODS	15	1.9	16	50
Spherisorb S5 ODS 2	10	2.0	20	49
LiChrosorb RP18	–	1.5	18	65
Partisil 10 ODS 2	15	0.7	25	66
Nucleosil 5 C_{18}	–	1.5	17	50

Chemical Methods

- → pyrolysis GC
- → derivatisations
  - deuterated reagents for GC-MS and LC-MS (mass shift);
  - pre-column derivatisation of analytes with reactive functional groups (peak shift)
- → analyte abstraction (analyte elimination)
- → post-column derivatisation

On-line instrumental Methods

Coupled Techniques

Quantitative Analysis

Detector Specifications

→ SENSITIVITY

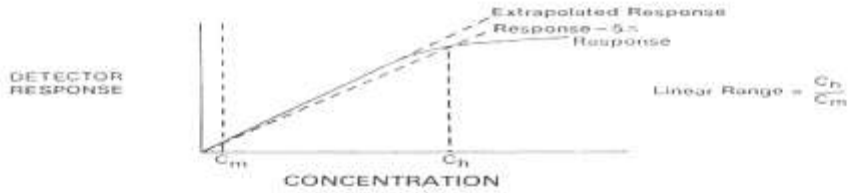
The sensitivity is a measure of the magnitude of the signal generated by the detector for a given amount of analyte.

The sensitivity of a:

concentration-type detector is expressed as signal/concentration, e.g. mV/(concentration) or mV ml g^{-1}

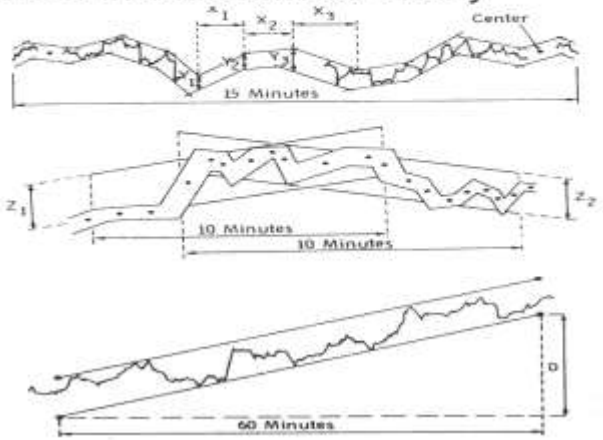
mass flow-rate-type detector is expressed as signal/ absolute analyte mass reaching the detector per unit time, e.g. mV s g^{-1}

<u>Linear dynamic range</u> of detector is defined as the range of the sample amount over which the sensitivity of the detector is constant to within 5%.



→ NOISE

Noise is the random perturbation in signal produced by a detector in the absense of any sample.

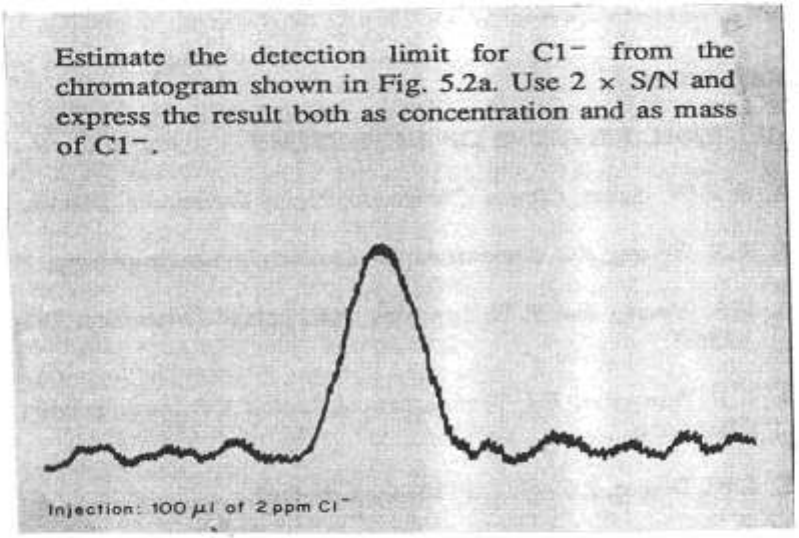


➡LIMIT OF DETECTION (MINIMUM DETECTABLE QUANTITY)

Limit of detection is the minimum quantity of analyte for which the detector will give a visible response. Usually defined as S/N=3

Expressed in concentration units for a concentration-type detector and in g s^{-1} for a mass flow-rate detector.

Limit of determination (S/N=10), represents the smallest peak that can be confidently quantified with accuracy.



➡TIME CONSTANT and RESPONSE TIME

These are measures of the speed of responce of a detector. They are defined as the time (usually in ms) a detector takes to respond to 63.2% and 98%, respectively, of true value following a sudden change in signal.

➡SELECTIVITY OF RESPONSE

Calibration Procedures

Normalisation

Normalisation is achieved by dividing the area of each peak by the total area for all peaks in the chromatogram and multiplying by 100.

Method assumes: i)each sample component gives rise to a peak in the chromatogram and ii) detector response is equivalent for all compounds (response factors: ratio of the peak area per unit mass)

Normalisation is commonly used for analysis that are performed repeatedly on very similar samples.

External calibration

This method requires precise control of the analytical technique, particularly the size of the injected sample.

More widely used for HPLC than GC.

Internal standard

- The internal standard should resemble the analyte as closely as possible in terms of physical and chemical properties. It must not react with any component of the sample.
- The internal standard must not be a normal constituent of the sample.
- The internal standard should be incorporated into the sample in exactly the same way as the analyte. This ideal can rarely be achieved in practice.
- In general, the analyte and internal standard should elute close together with baseline resolution. There are exceptions to this requirement where the two can be distinguished by the detection system as, for example, with isotopically labelled samples.
- The internal standard and analyte should respond to the detection system in a similar manner and be present in nearly equal concentrations.

Substance used as internal standards include analogues, homologues, isomers, enantiomers, and isotopically labeled analogues of the analyte.

Advantages: not necessary to know the volume of the sample injected, corrects for sample loss during sample preparation.

Standard addition

CASE 3. Determination of nicotinic acid in instant coffee

An aqueous extract of instant coffee (1.000g in 50ml) was filtered using a 0.45 μm filter and subjected to clean-up on a reversed-phase cartridge column. The eluent was injected by a valve injector (30.μl) onto a reversed-phase column with a UV detector.

Peak height data for nicotinic acid standards and unknown coffee samples.

Sample	AU
Nicotinic acid standards ($mg\ l^{-1}$)	
5	0.0109
10	0.0219
15	0.0326
20	0.0437
25	0.0543
30	0.0658
Coffee samples	
1	0.0170
2	0.0209