
بسم الله الرحمن الرحيم

كروماتوغرافيا الطور الغازي

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Gas Chromatography

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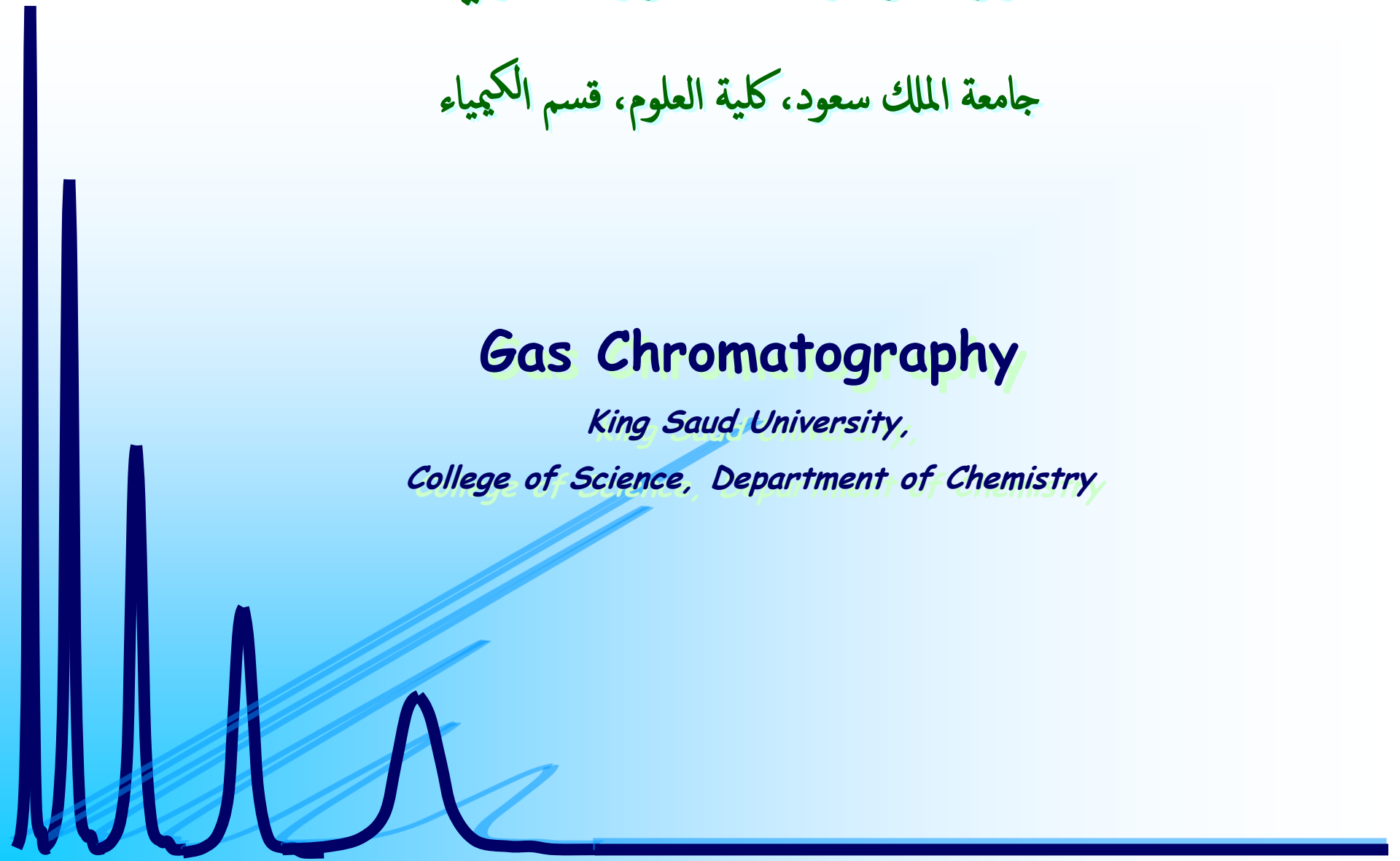


What is chromatography?

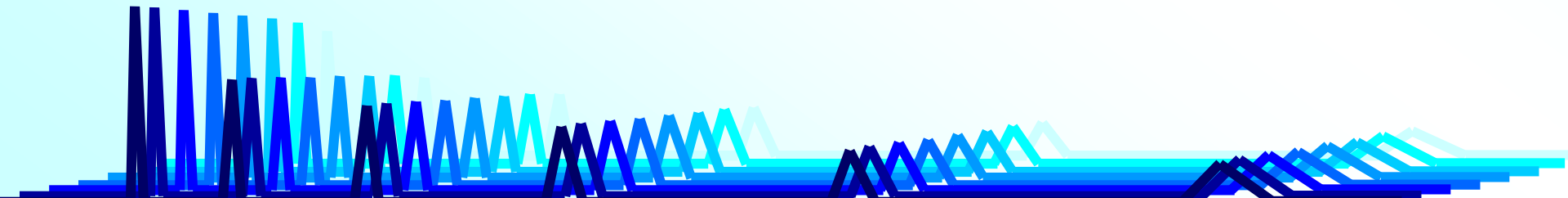
A physical method of separation

No reaction occurs during the separation process

The components partition between two phases:

- the stationary phase which is fixed and does not move
- the mobile phase which moves along the column

The separation of solutes occurs owing to their different interactions with the two phases

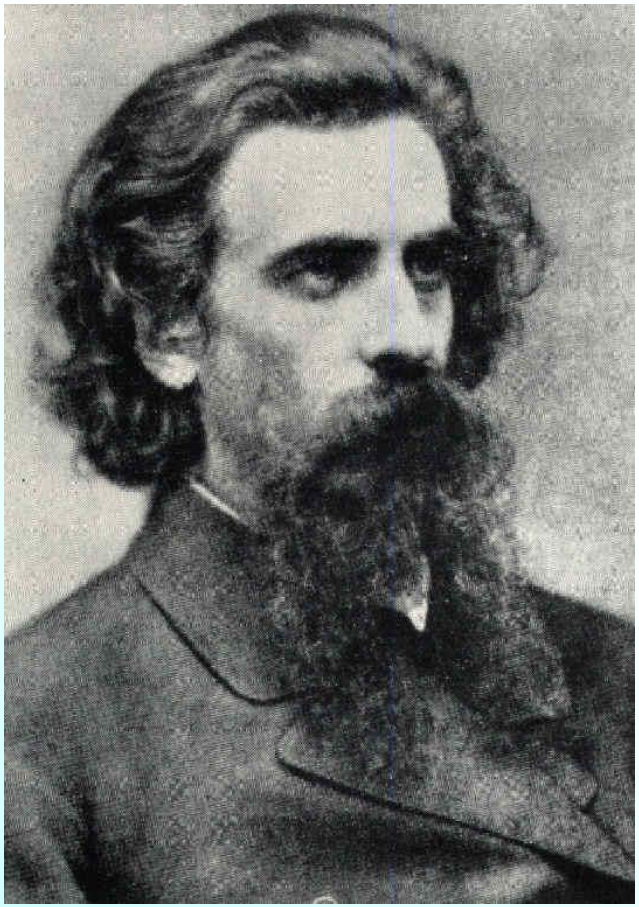


What are the main chromatographic methods?

Their classification is based on the nature of mobile phase:

- gaseous mobile phase:
  - GLC (partition chromatography)
  - GSC (adsorption chromatography)
- liquid mobile phase :
  - LLC (partition chromatography)
  - LSC (adsorption chromatography)
  - IEC (ion exchange chromatography)
  - SEC (size exclusion chromatography)
- supercritical fluid mobile phase :
  - SFC (supercritical fluid chromatography)

History

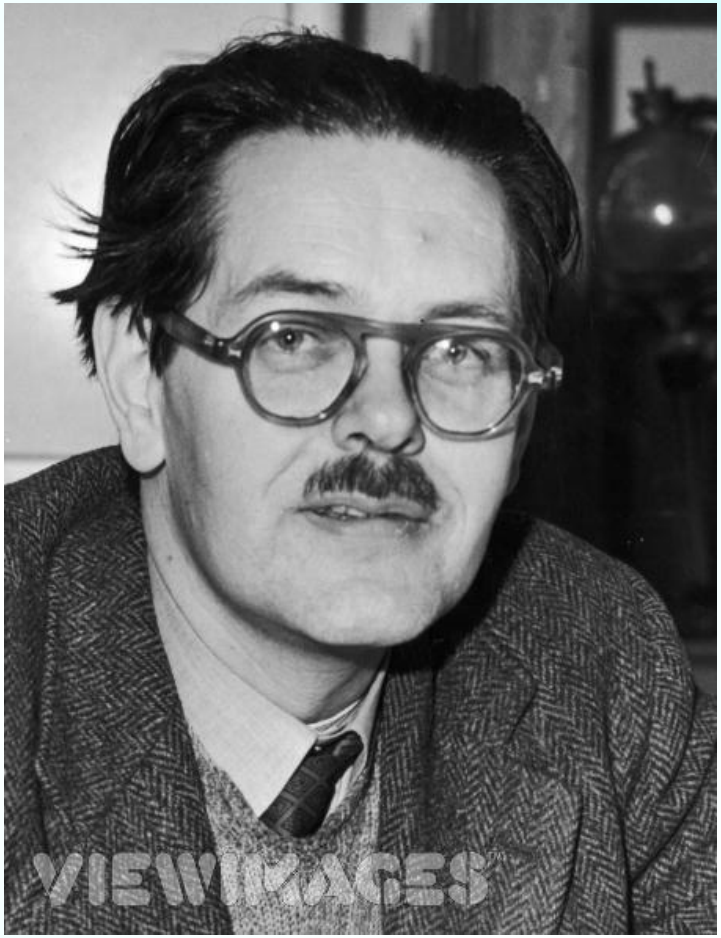


- Russian Scientist Mikhail Semenovich Tswett is credited for the discovery of chromatography. (1903)

- German student Fritz Prior is credited for developing gas chromatography. (1947)

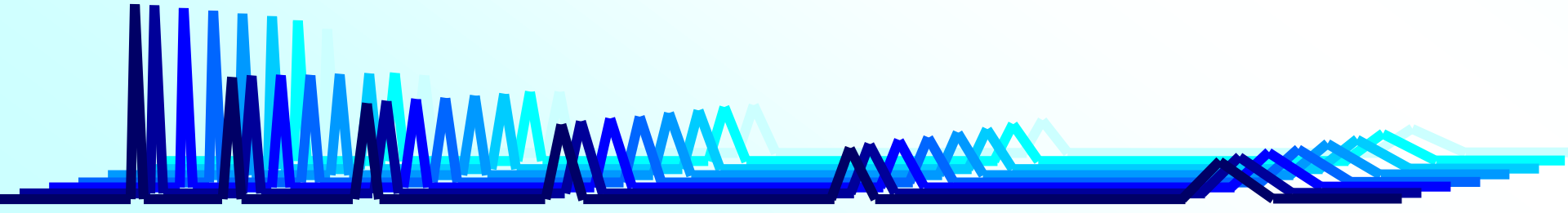
Gas Chromatography

- The father of modern gas chromatography is Nobel Prize winner John Porter Martin, who also developed the first liquid-gas chromatograph. (1950)



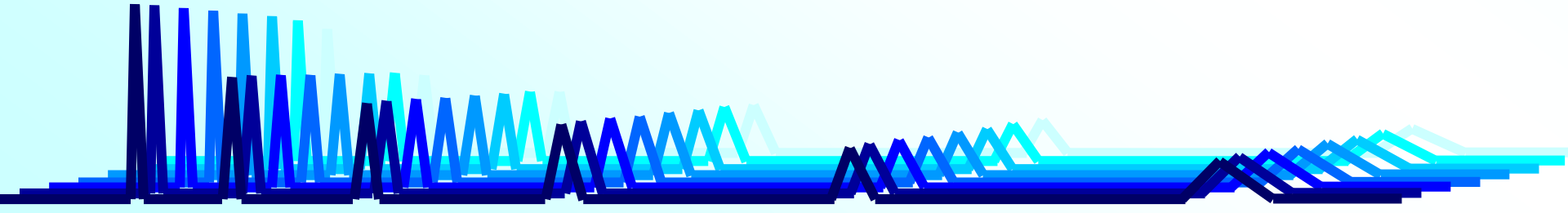
Gas chromatography principle

- Sample is vaporised and injected onto the head of a chromatographic column.
- Elution is produced by the flow of an inert gaseous mobile phase.
- Separation is based upon the partition of the analyte between a gaseous mobile phase and a liquid phase immobilised on the surface of an inert solid (GLC) at a temperature above boiling point of analyte (for various analytes: temperature programming).
- Mobile phase does not interact with molecules of the analyte.
- Eluted analytes are detected by a detector and recorded by the data system
- GC columns are either packed (with silica particles coated in stationary) or capillary in nature.



Chromatographic terminology:

- **Solute**: constituent of a mixture to be separated by chromatography
- **Mobile phase** : fluid medium which pushes the solutes along the column
- **Stationary phase**: solid or liquid which interacts with the solutes and retains them allowing their separation
- **Support**: inert solid substrate coated with the liquid stationary phase
- **Coating**: the support with the liquid stationary phase which fill the column in partition chromatography or the solid adsorbant in adsorption chromatography
- **Chromatographic column**: the tube of variable diameter and length made with metal or glass, containing the coating
- **Elution**: transfer of the solutes in the mobile phase along the column
- **Retention**: delaying the solutes movement by their interactions with the stationary phase
- **Chromatogram**: curve obtained on the recorder giving the detector response as a function of time



How does the separation process occur?

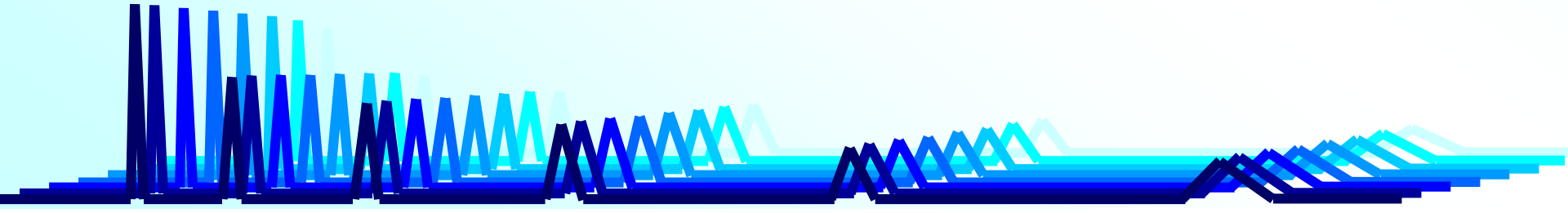
- Solutes are forced by the mobile phase to move through the stationary phase along the column

- Each species is specifically retained by the stationary phase because of interactions such as:

  adsorption on the solid surface

  relative solubility in the liquid stationary phase

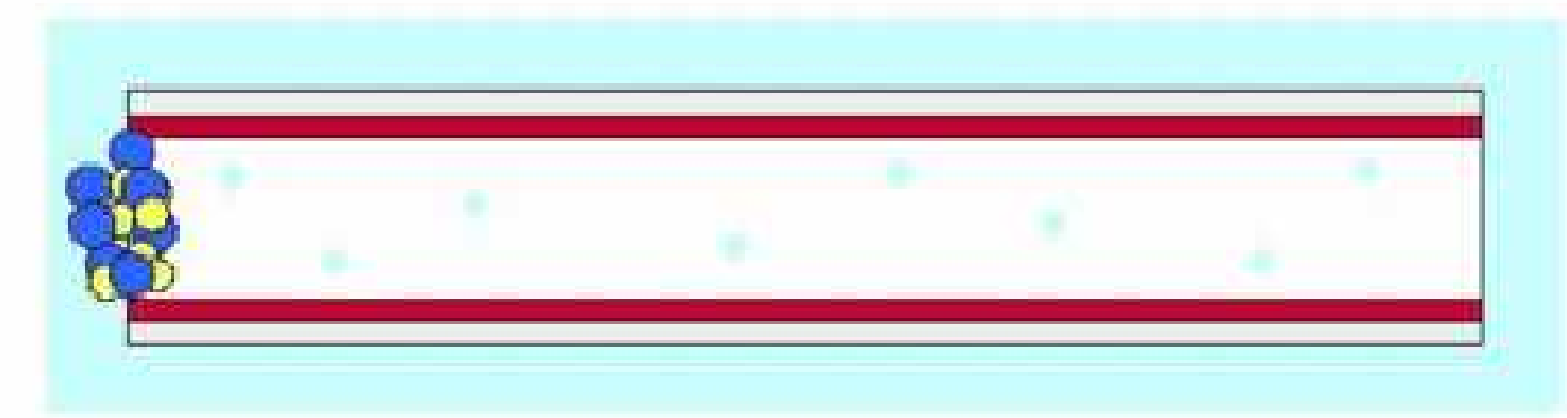
  polar interactions



Solute elution:

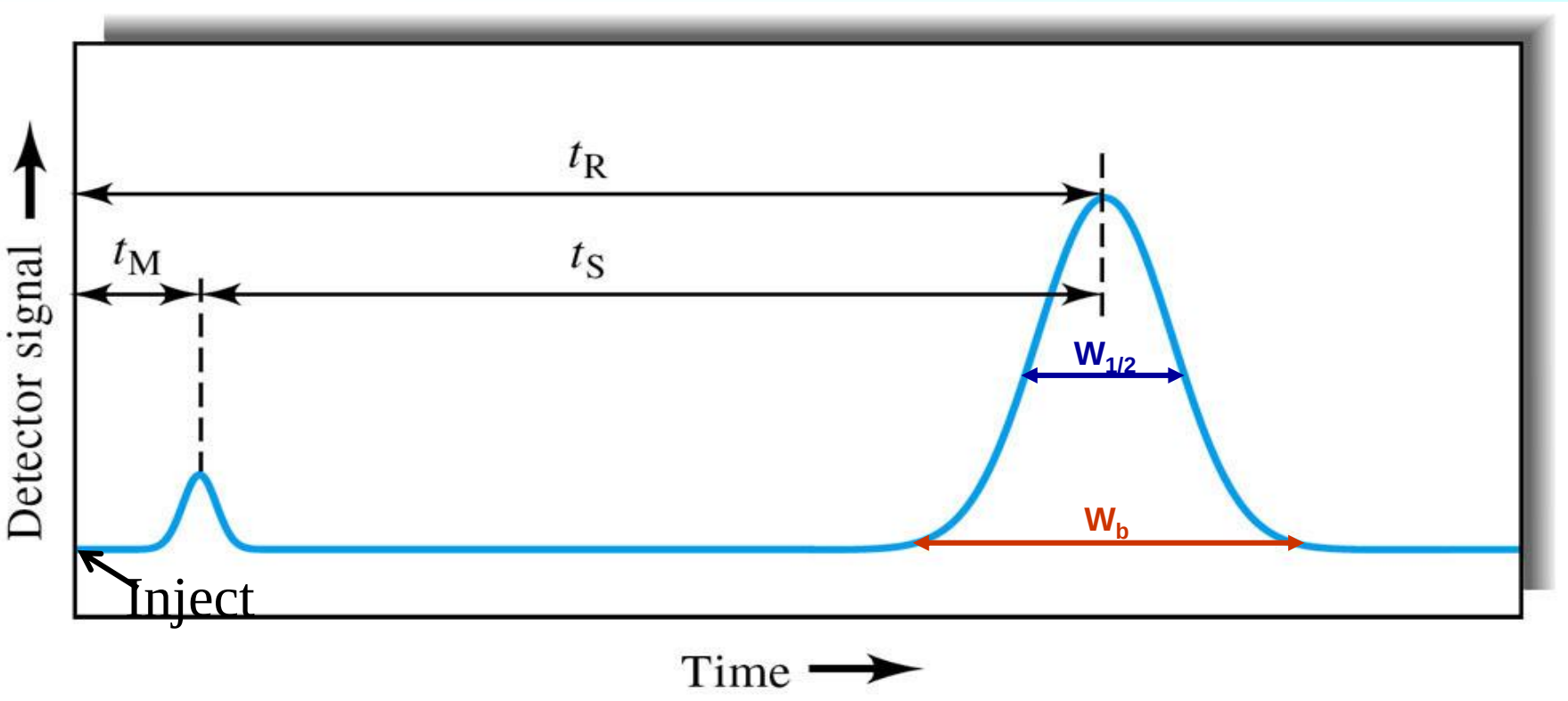
it can be explained by different competitive interactions which occur inside the column:

- solute volatility (vapor pressure)
- solute solubility in the liquid stationary phase and/or solute adsorption on the solid surfaces



Theory of Chromatography

Typical response obtained by chromatography : chromatogram concentration versus elution time

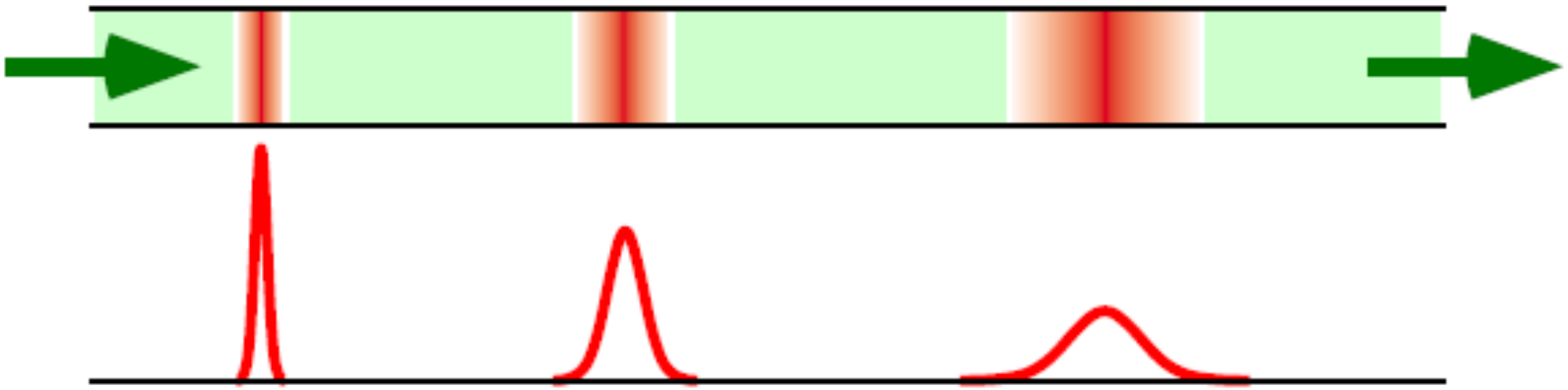


where:

t_R = retention time
t_M = void time (dead time, non-retained solute)
W_b = baseline width of the peak in time units
$W_{1/2}$ = half-height width of the peak in time units

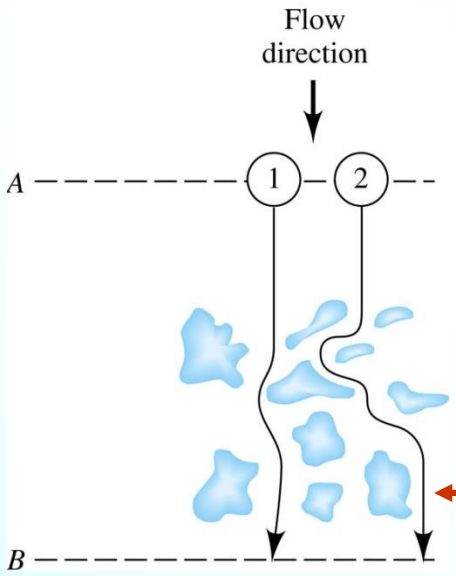
Why do bands spread?

a. Eddy diffusion
b. Mobile phase mass transfer
c. Stagnant mobile phase mass transfer
d. Stationary phase mass transfer
e. Longitudinal diffusion



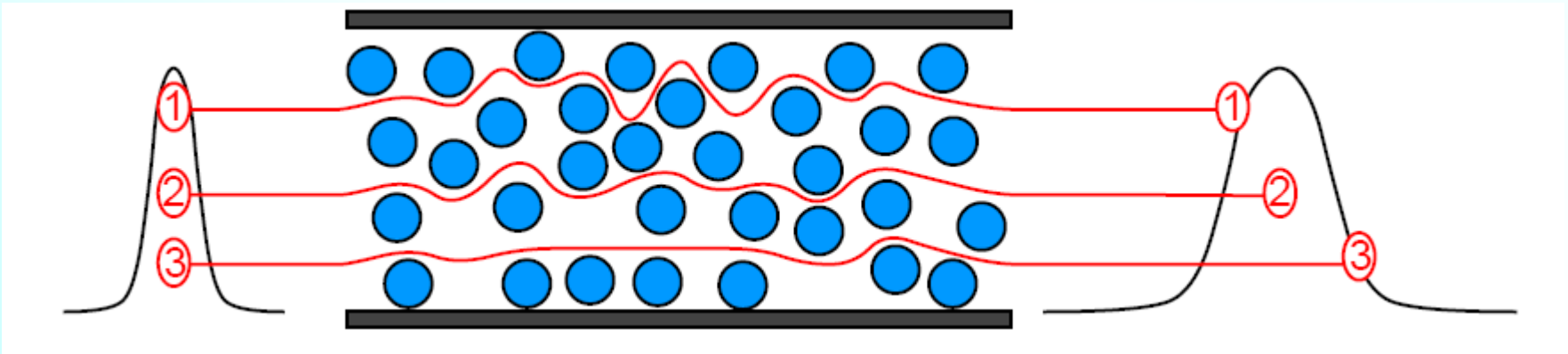
Eddy diffusion:

a process that leads to peak (band) broadening due to the presence of multiple flow paths through a packed column.



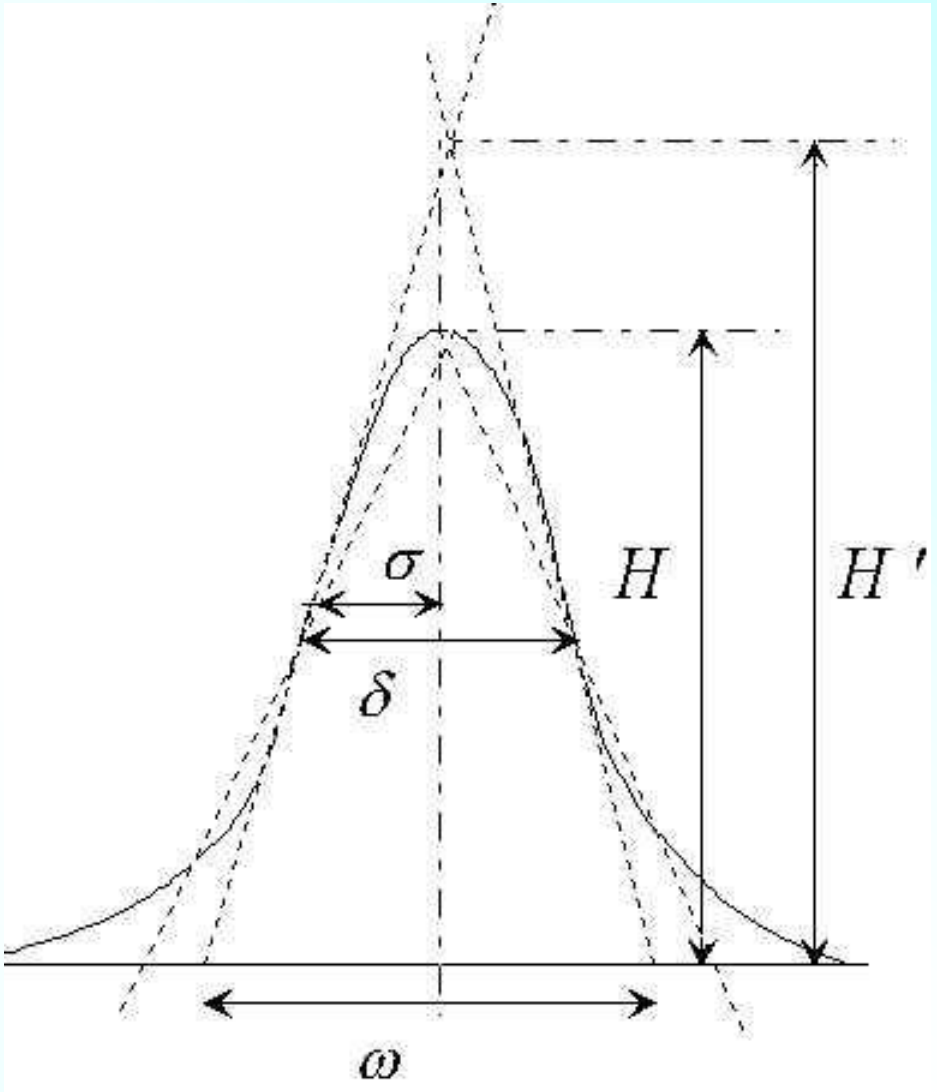
As solute molecules travel through the column, some arrive at the end sooner then others simply due to the different path traveled around the support particles in the column that result in different travel distances.

Longer path arrives at end of column after (1).



Chromatographic peak:

- supposed gaussian
- σ: standard deviation
- δ: width at half-height ($W_{1/2}$)
- ω : baseline width
- Since is often difficult to accurately evaluate the beginning and end of the peak, most equations use the width at half-height δ which can be easily measured on the chromatogram



Some fundamental equations:

- **Corrected retention time:**

$$t'_R = t_R - t_M$$

- **Capacity ratio: corresponds to a relative retention:**

$$k = t'_R / t_M = (t_R - t_M) / t_M$$

- **Column selectivity:**

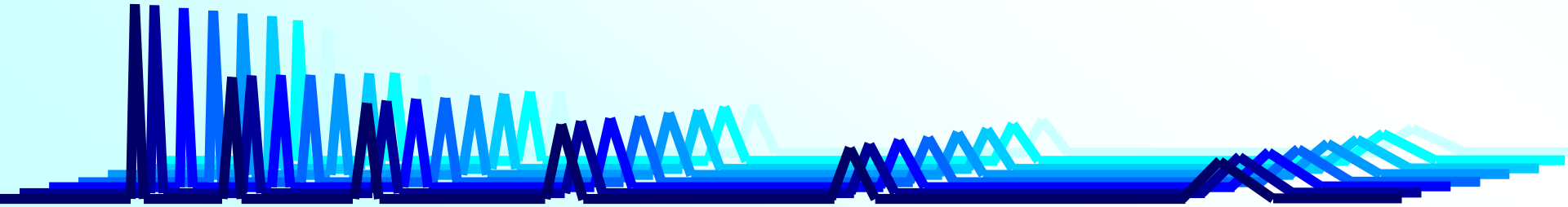
$$\alpha = t'_{R2} / t'_{R1} = k_2 / k_1 \quad (\alpha > 1 \text{ because } t'_{R2} > t'_{R1})$$

- **Resolution between two neighboring peaks:**

$$R_S = 2\,(t_{R2} - t_{R1}) / (\omega_2 + \omega_1) = 1{,}18\,(t_{R2} - t_{R1}) / (\delta_2 + \delta_1)$$

- *For two neighboring peaks, a resolution R_S higher than 1 means a complete separation (for R_S = 1, the overlapping peak surface is 2%)*

- *When R_S is less than 0.8, the separation between the two peaks is considered to be incomplete*



Column efficiency:

The chromatographic peaks being supposed gaussian, the peak broadening can be related to the separation and the column efficiency which is evaluated by the number of theoretical plates of the column N.

For a gaussian peak, N can be calculated by one of the following equations:

$$N = (t_R / \sigma)^2$$ (σ: standard deviation of the peak)

$$N = 16\,(t_R / \omega)^2$$ (ω: width at baseline)

$$N = 5{,}54\,(t_R / \delta)^2$$ (δ: width at half- height)

In order to compare columns having different lengths, one calculate the height equivalent to a theoretical plate HETP:

$$h = L / N$$ (L : column length)

h may vary from centimeters (packed columns) to several microns (high resolution capillary columns)

Van Deemter Equation :

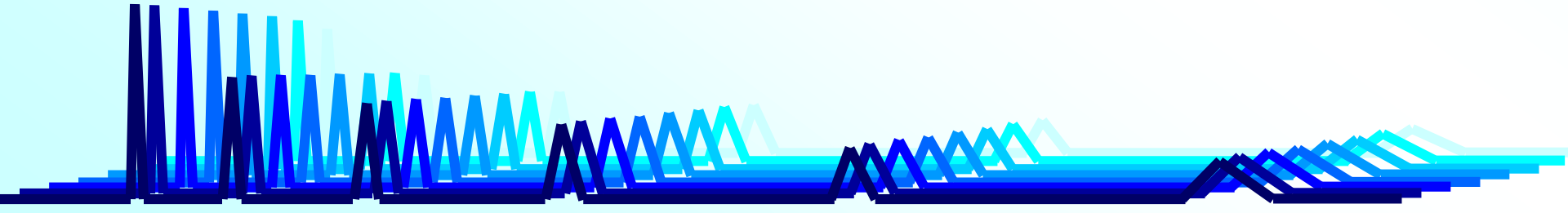
It gives the dependance of the height equivalent to a theoretical plate as a function of several parameters:

$$H = 2\lambda d_p + \frac{2\gamma D_g}{u} + \frac{8}{\pi^2}\frac{k d_f^2}{(1+k)^2 D_l} u$$

- **H** : height equivalent to a theoretical plate
- **λ** : factor characteristic of the packing column
- **d_p** : diameter of the packing particules
- **γ** : factor depending on the irregularity of interparticles space
- **D_g** : diffusion coefficient of the solute in gas phase
- **D_l** : diffusion coefficient of the solute in liquid phase
- **u** : linear velocity of the carrier gas
- **k** : capacity ratio of the studied solute
- **d_f** : thickness of the liquid stationary phase film

For packed columns, the Van Deemter equation shows three terms:

- $2 \lambda d_p$: term **A**, characteristic of the packing material
- $\frac{2 \gamma D_g}{u}$: term **B**, characteristic of the gas mobile phase
- $\frac{8}{\pi^2} \frac{k d_f^2}{(1+k)^2 D_l} u$: term **C**, characteristic of the liquid stationary phase

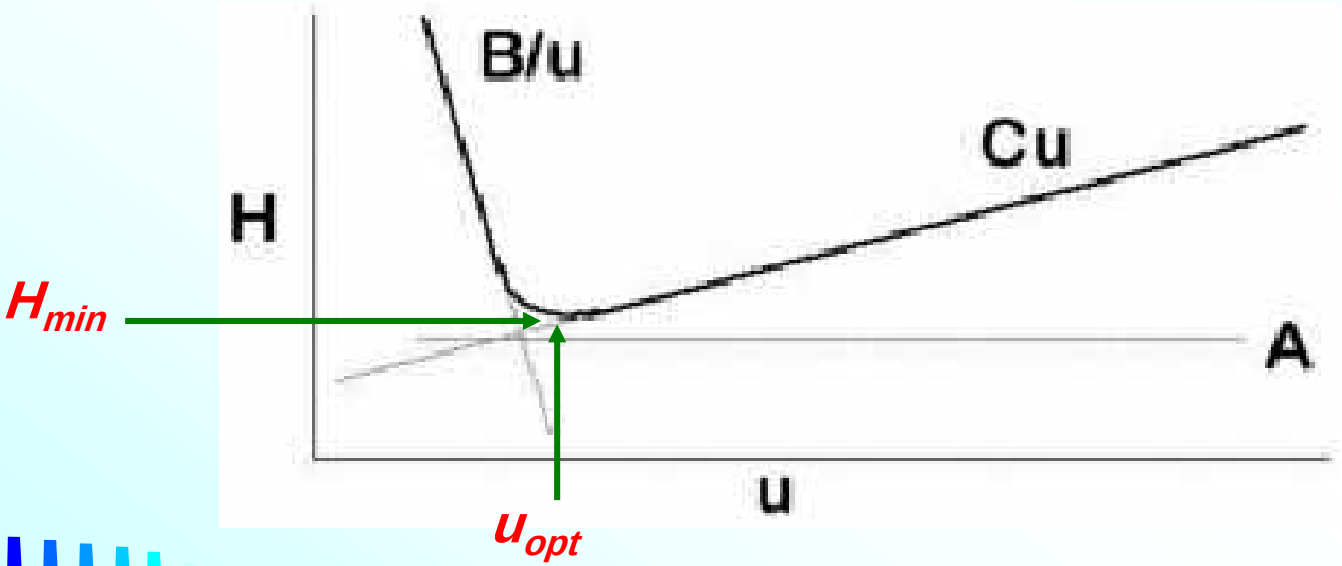


Van Deemter curve:

It allows the determination of the minimum HETP value H_{min} which corresponds to the maximum column performance obtained with the optimum velocity u_{opt}

$$H = A + B / u + C.u$$

For a packed column, this curve is as follows:

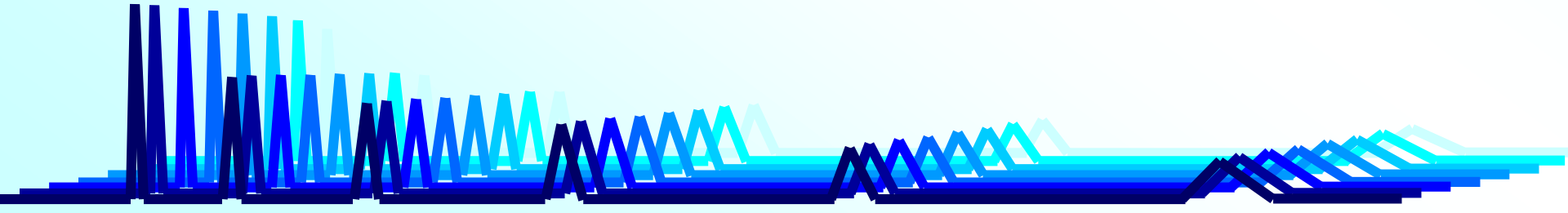


Van Deemter curve:

- It is not necessary to determine the optimum HETP for each component of the analysed mixture, we can consider a medium solute in the chromatogram to evaluate the optimum velocity

- It is often advised to select a carrier gas flowrate slightly greater than the optimum value:

  - *it allows the obtention of shorter retention times and reduction of the analysis duration*
  - *without greatly affecting the column performance*



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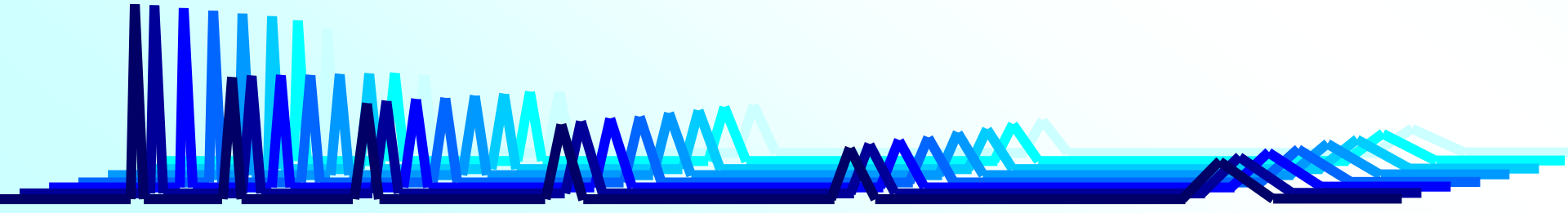


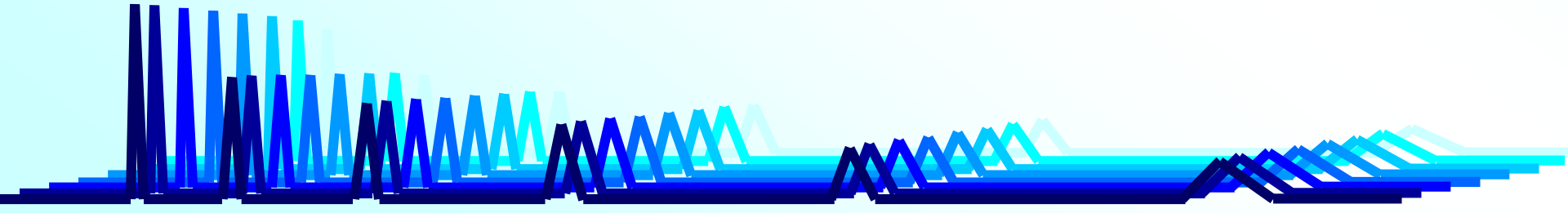
There are three terms in the van Deemter equation: the A term, the B term, and the C term, each describing a different type of contribution to the band broadening:

A Term: Multiple path term—solute molecules can take different paths through the stationary phase, so some take longer way to go through column than others. This term is independent of flow rate.

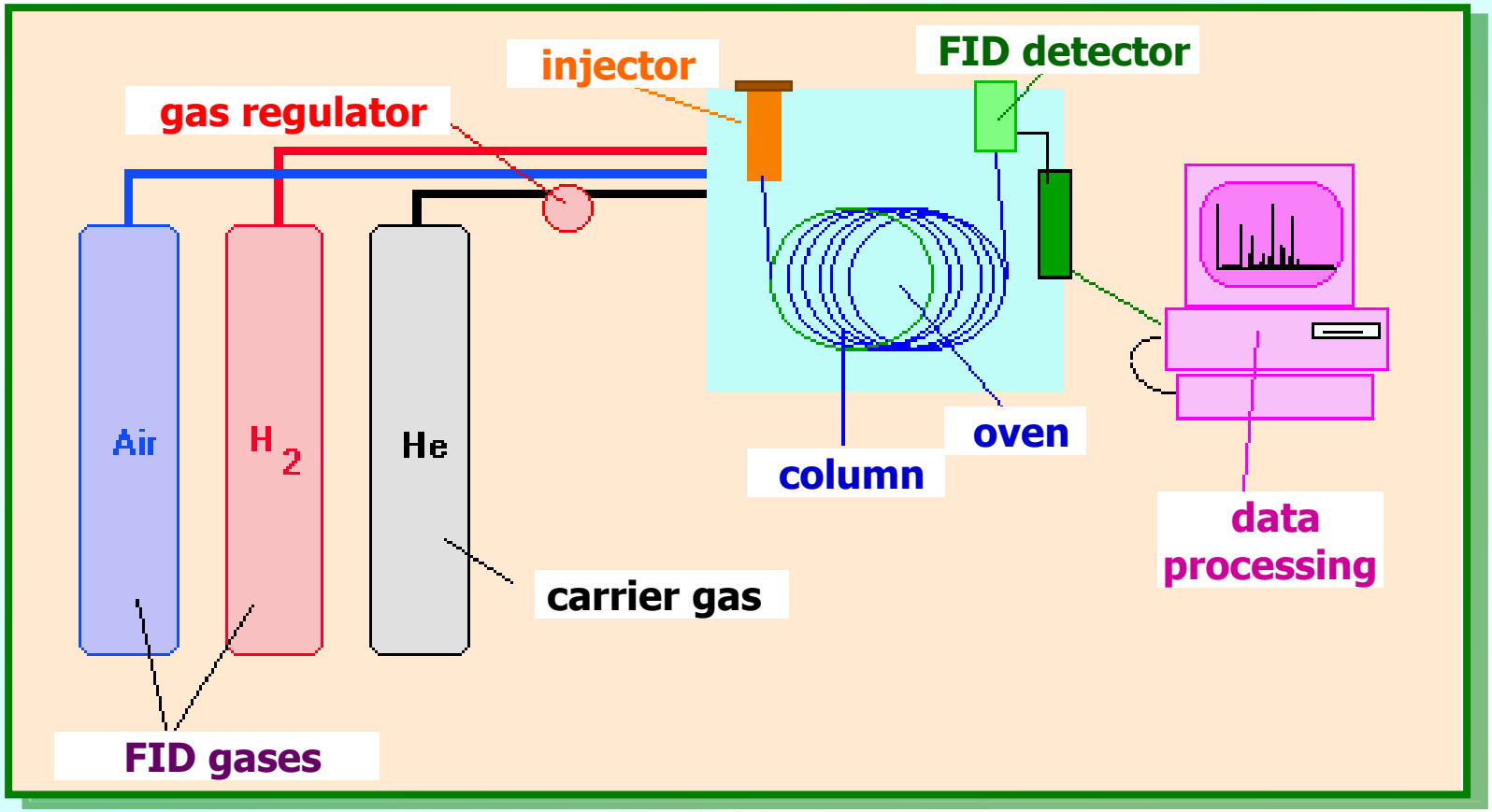
B Term: Longitudinal diffusion term—the longer time a solute stays on the column the more time it has to diffuse, therefore spreading out its concentration. This term is inversely proportional to flow rate (i.e. longer time on column with slower flow rate and vice versa).

C Term: Mass transfer term—this arises from slow equilibrium for solutes partitioning between the mobile and stationary phases. If the equilibrium is slow, while a solute molecule is in the stationary phase other molecules will have traveled down the column a certain distance. The overall result is band spreading that is proportional to the flow rate.

Gas chromatography equipment



Main parts of a gas chromatograph



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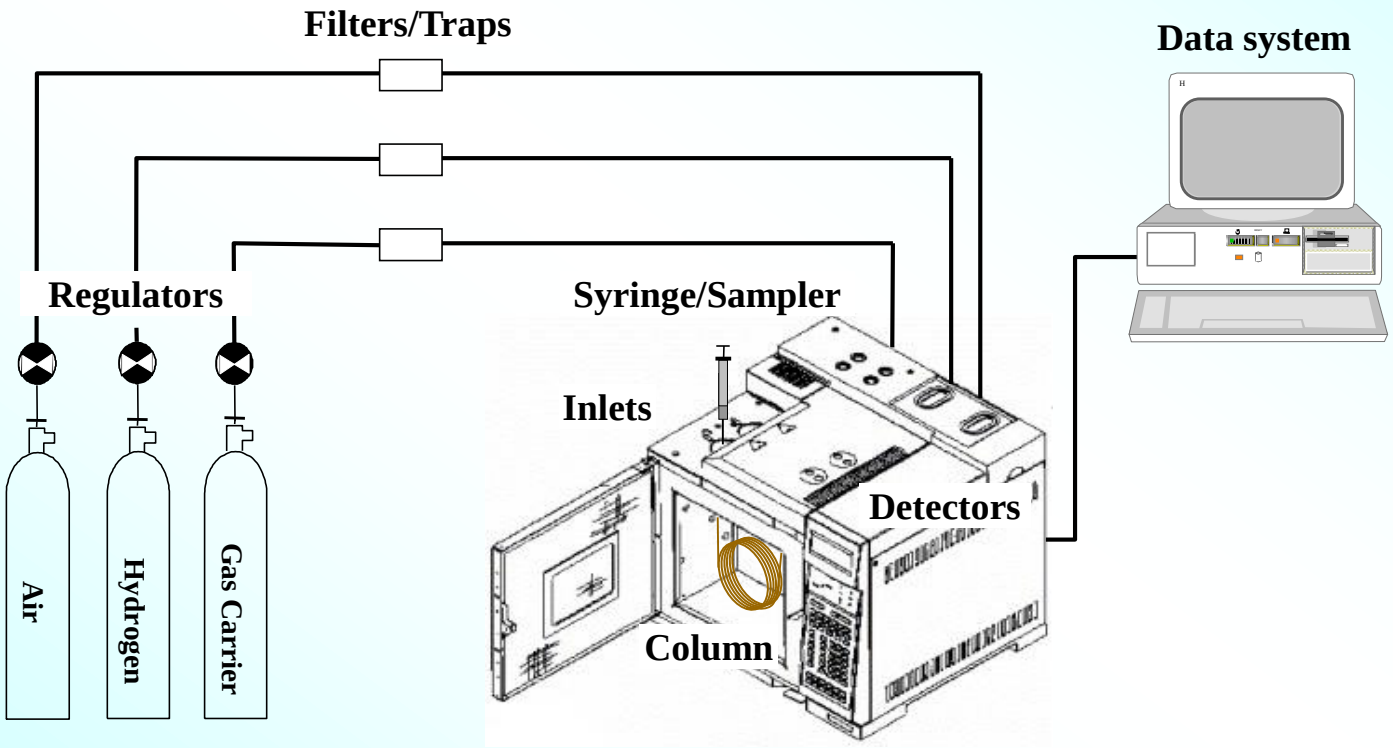
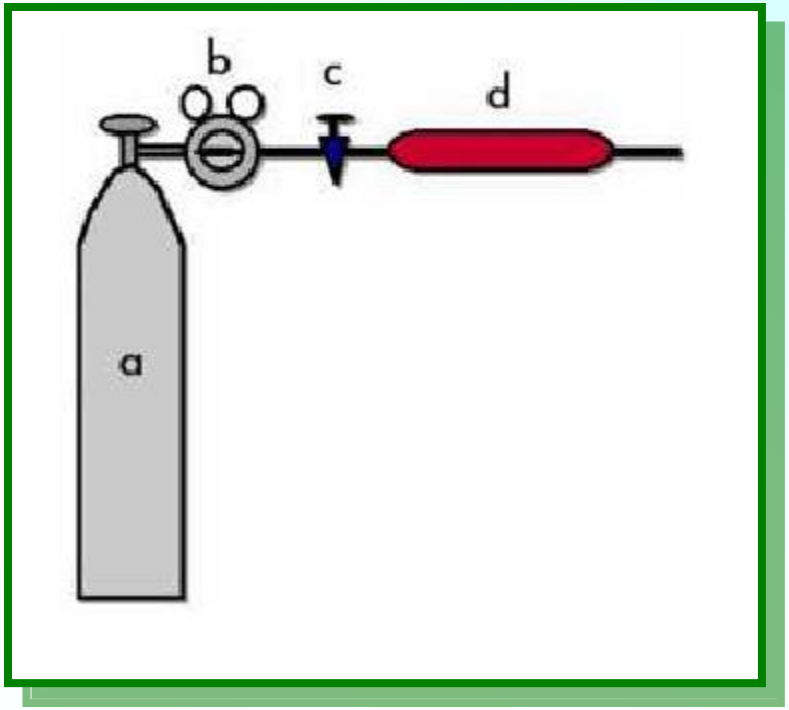


Gas supply:

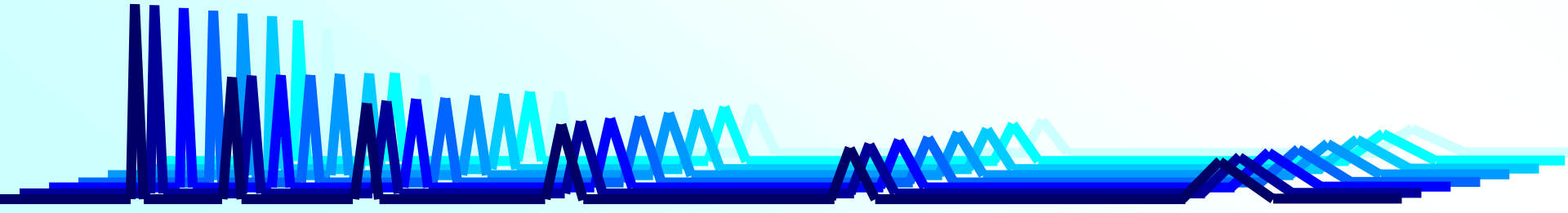
- a : compressed gas cylinder
- b : double stage pressure regulator
- c : valve
- d : gas filter (to eliminate impurities such as: water, oxygen, hydocarbons,…)



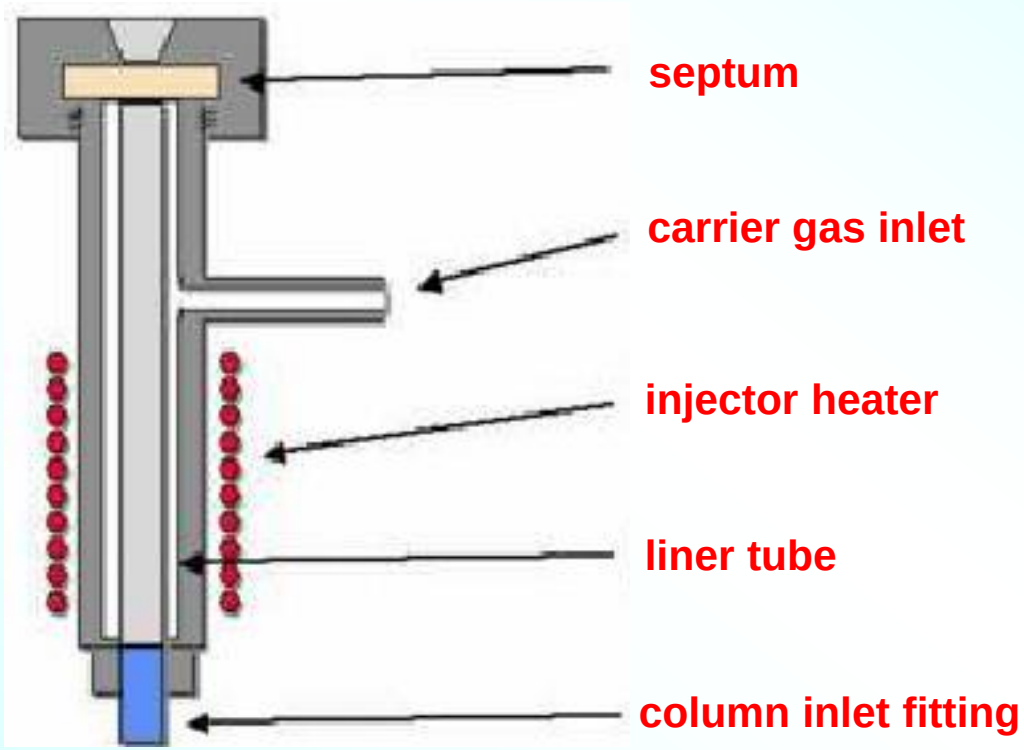
Sample injection:

It allows a rapid and simple introduction of the sample to be analysed in the gas chromatograph. There are two main injection systems, depending on the nature of sample:

- **injection port:** for introduction of liquids and solutions
- **sampling loop injection:** for introduction of gas samples



Injection port



Injection port for liquid samples:

- allows the rapid introduction and volatilisation of the liquid sample in the chromatograph
- injection port temperature: must be high enough to quickly evaporate the sample without thermal degradation
- the injection temperature is 20° higher than the boiling point of the less volatile constituent of the mixture bituellement
- to avoid condensation of the sample in the injection port, the injector temperature must be higher than column temperature:

$$T_{injector} > T_{column} \text{ (about 50°C)}$$

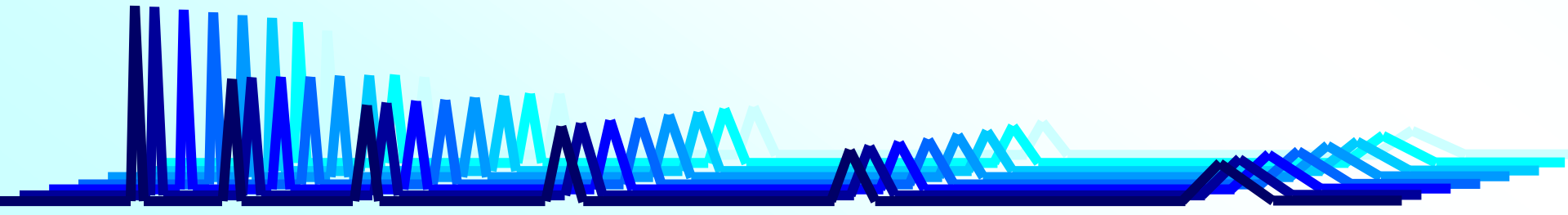
- the volume of the injection port must allow the volatilisation of the liquid sample and avoid the excessive dead volume

The syringe

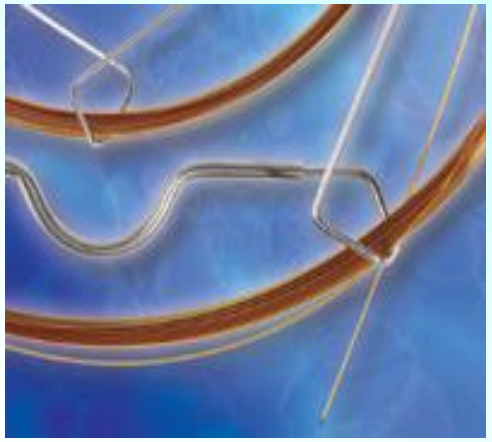
- it is used to introduce an accurate volume of the liquid or gas sample in the injector
- several syringe models are available: from 1 μl to several cm^3, with various options: fixed or removable needle, adaptor, sharp or round needle…

Columns

- Heart of the separation process.
- Vast number of materials have been evaluated.
- It is usually best to refer to various catalogs as an up to date reference.
- Can be classified by tubing diameter and packing type.



Columns in gas chromatography



Packed columns

- 2 to 4 mm I.D. and 1 to 4 meters long.
- Packed with a suitable adsorbent.
- Mostly used for gas analysis.
- Peak broadening due to zone (eddy) diffusion resulting from multitude of pathways a molecule can pass through column.

Capillary columns

- 0.1 mm to 0.5 mm I.D. and 10 m to 100 m long
- Stationary phase is coated on the internal wall of the column as a film 0.2 mm to 1 mm thick
- Sharper peaks – no Eddy diffusion.
- Up to 500,000 theoretical plates – excellent separations.
- Most popular type of column in use.

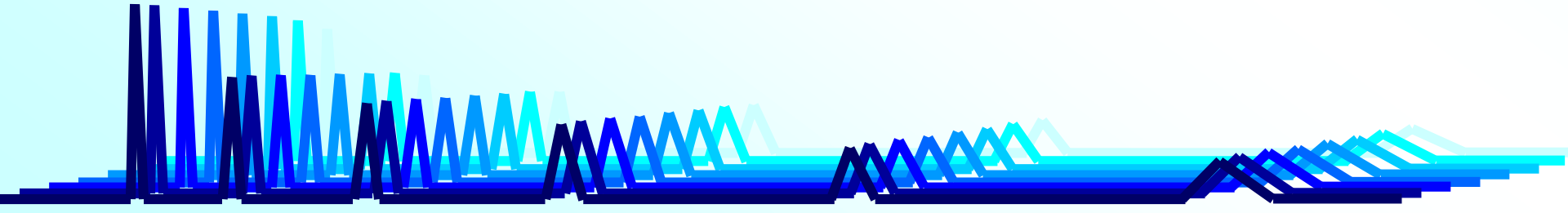
Temperature programming

The column sits in an oven.

If the temperature is held constant during the entire analysis it is isothermal.

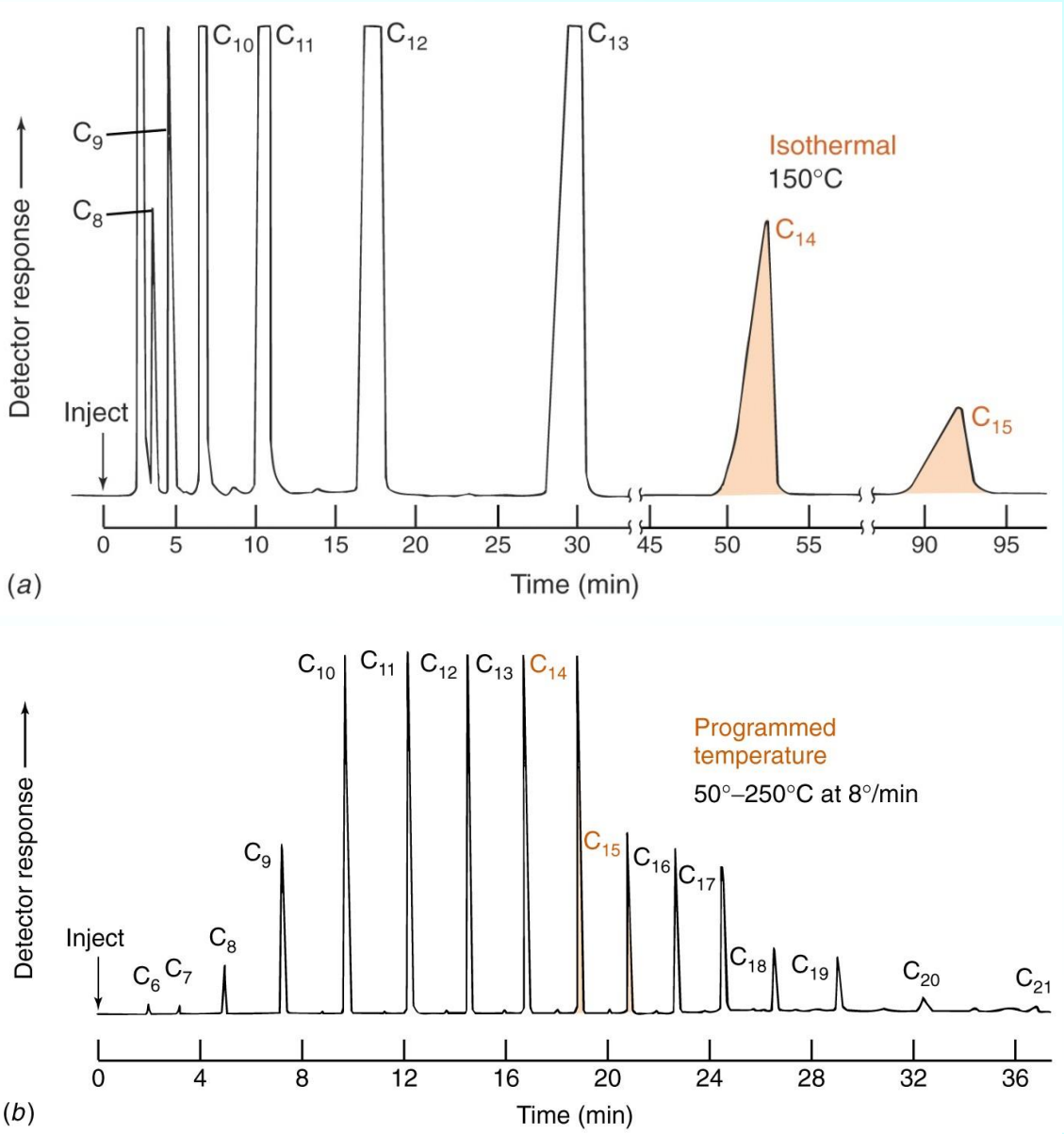
If you vary the temperature during the analysis, you typically use a temperature program.

Why bother?

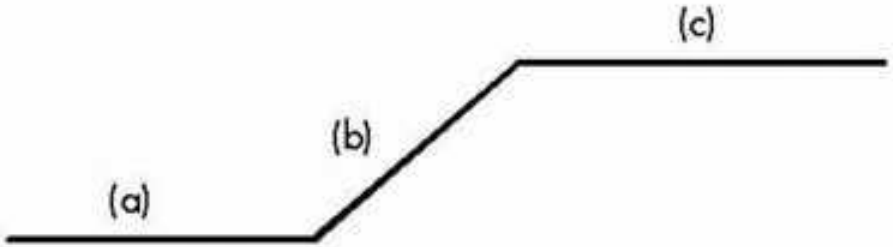


Comparison of isothermal and programmed temperature separation

Temperature gradient improves resolution while also decreasing retention time



A temperature program



a - initial temperature and time

b - ramp (°C/min)

c - final hold time and temperature

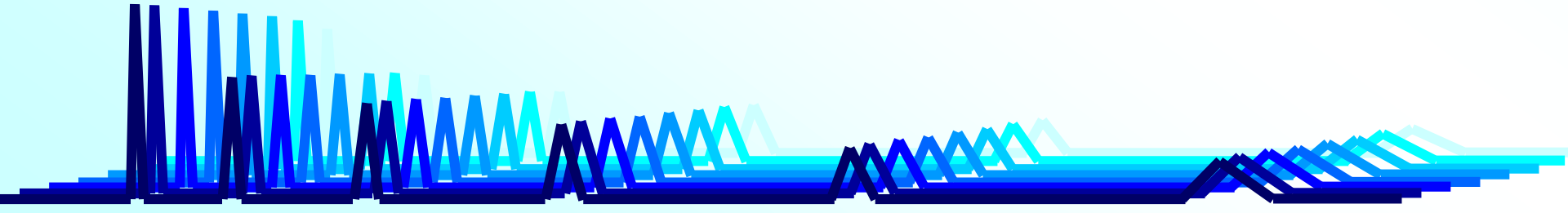
Some GCs will allow for a more complex program.

Detectors

Each can be roughly classified based on

Destructive	vs.	nondestructive
General	vs.	some discrimination
	vs.	very discriminating

Let's start by reviewing some general concepts such as detection limit and sensitivity.



Characteristics of ideal GC detector

- Good stability and reproducibility.
- Linear response to analytes that extends over several orders of magnitude.
- Similarity in response toward all analytes.
- Temperature range from room temperature to 400°C.
- A short response time that is independent of flow rate.
- Non-destructive.
- High reliability and ease of use.
- No one detector exhibits all of these characteristics

Thermal conductivity detector

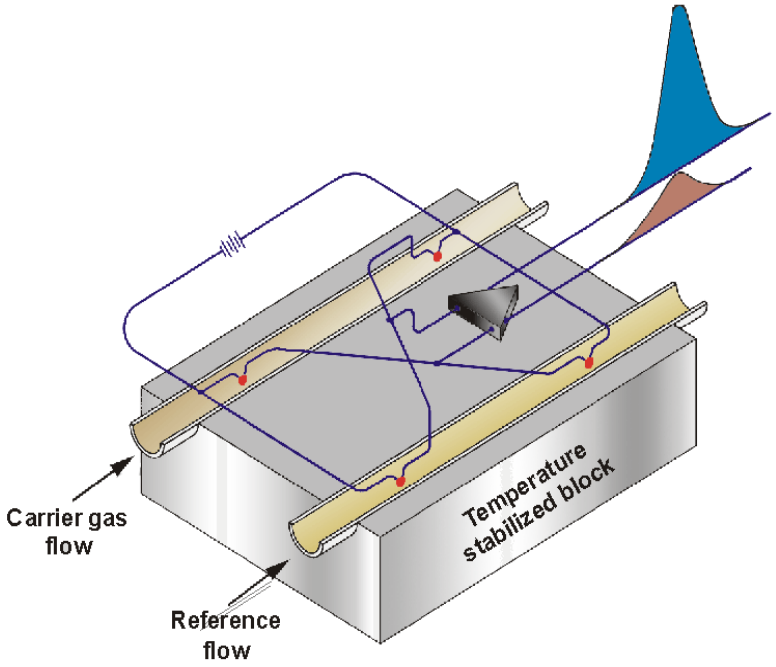
- General purpose
- Nondestructive
- Limit of detection ~ 400 pg/ml carrier
- Linear range ~ 10^6

Mode of detection

Change in resistance of a wire based on variations in the thermal conductivity of the gas evolving from a column.

Thermal Conductivity Detector

- Exploits the changes in the thermal conductivity of a gas stream brought about by the presence of analyte molecules.
- The resistance of either a heated platinum wire or a heated semiconductor thermistor gives a measure of the thermal conductivity of the gas.
- Twin detector pairs are typically incorporated into two arms of a Wheatstone bridge.
- In the presence of a relatively small concentration of analyte a large decrease in thermal conductivity of carrier gas occurs resulting in a temperature rise in detector.
- Thermal conductivities of He and H^2 are ~ 6 – 10 times higher than most organic compounds. Necessitates the use of these gases as carrier gas.
- Linear range of 10^5 and is suitable for organic and inorganic samples.
- Non-destructive and allows collection of sample after detection but low sensitivity ~ 10^{-8} g/s analyte/gas



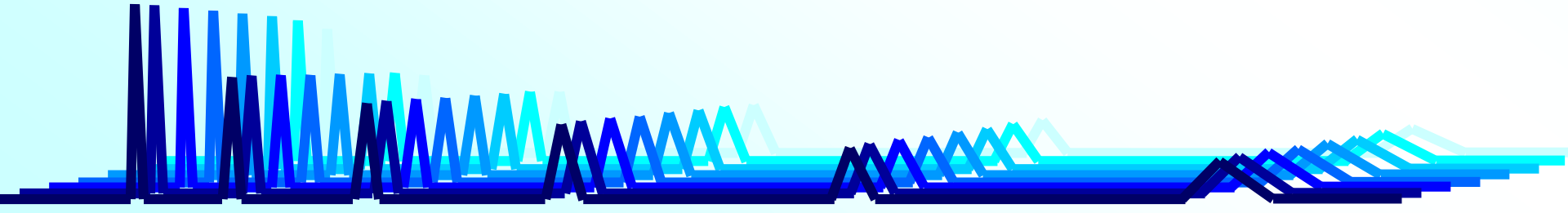
Flame ionization detector

- Specific - sample must be combustible
- Destructive
- Limit of detection ~ 5 pg carbon / second
- Linear range ~ 10^7

Mode of detection

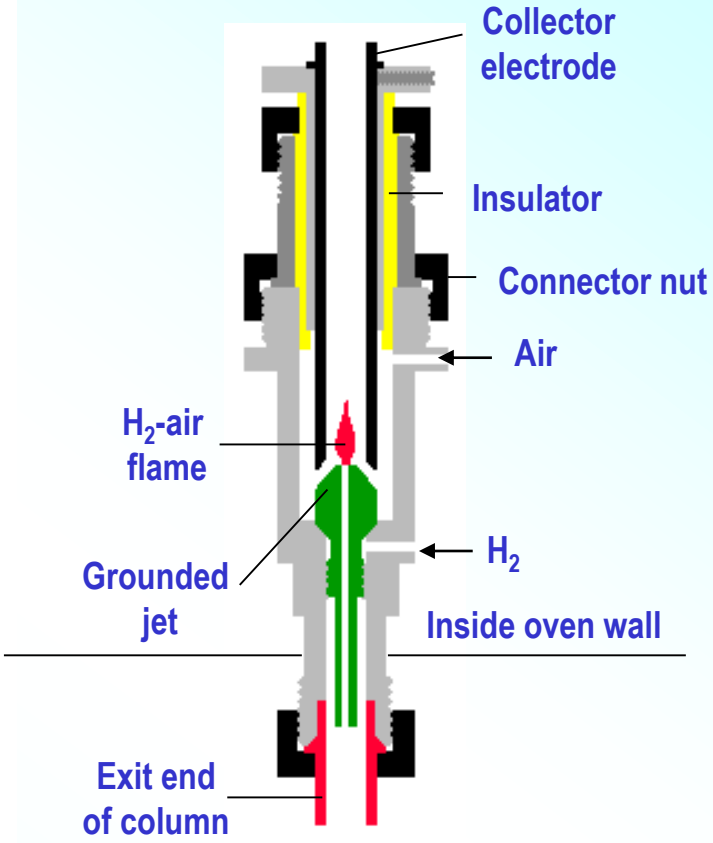
Production of ions in a flame result in a current that can be measured.

A make-up gas may be required to maintain an optimum flow - capillary columns



Flame Ionisation Detector

- Most organic compound pyrolyse in H_2-air flame and produce ions and electrons.
- A potential of a few hundred volts is applied across the burner tip and a collector electrode located above the flame.
- The resulting current is amplified and proportional to the number of carbon atoms in the flame.
- General detector for GC. However, carbonyl, alcohol, halogen and amine groups yield few electrons. Also insensitive to H_2O CO_2 SO_2 NO_X.
- Large linear response range (~ 10^7) and low noise (once detector has settled). Needs to be burning 24 hours before analysis.
- Exhibits very high sensitivity ~ 10^{-13} g/s of analyte/second

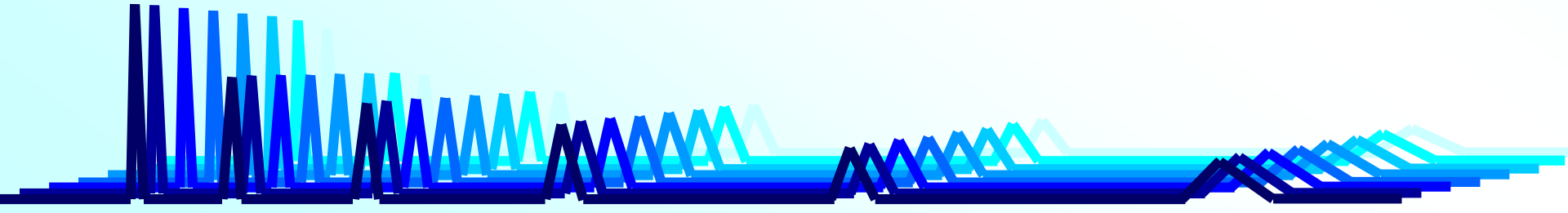


Flame ionization detector

Compounds with little or no FID response

noble gases	NH_3	CS_2
NO_x	CO	O_2
H_2O	CO_2	N_2

perhalogenated compounds
formic acid
formaldehyde



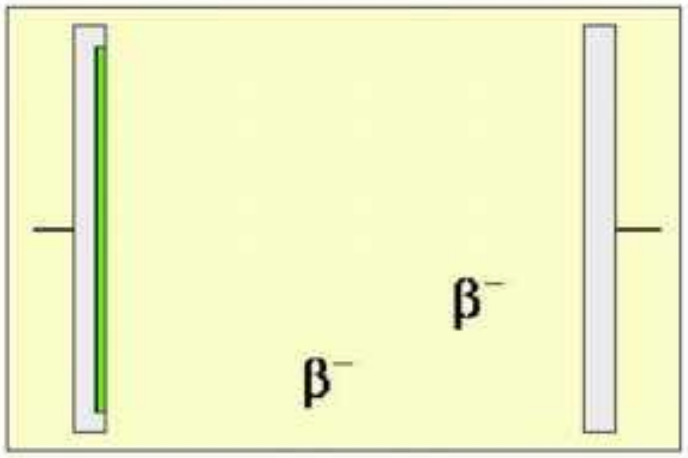
Electron capture

- Specific - sample must contain a gas phase electrophore
- Non-destructive
- Limit of detection ~ 0.1 pg Cl / second
- Linear range ~ 10^4

Mode of detection

Absorption of β particles by species containing halogens, nitriles, nitrates, conjugated double bonds, organometallics.

Electron capture

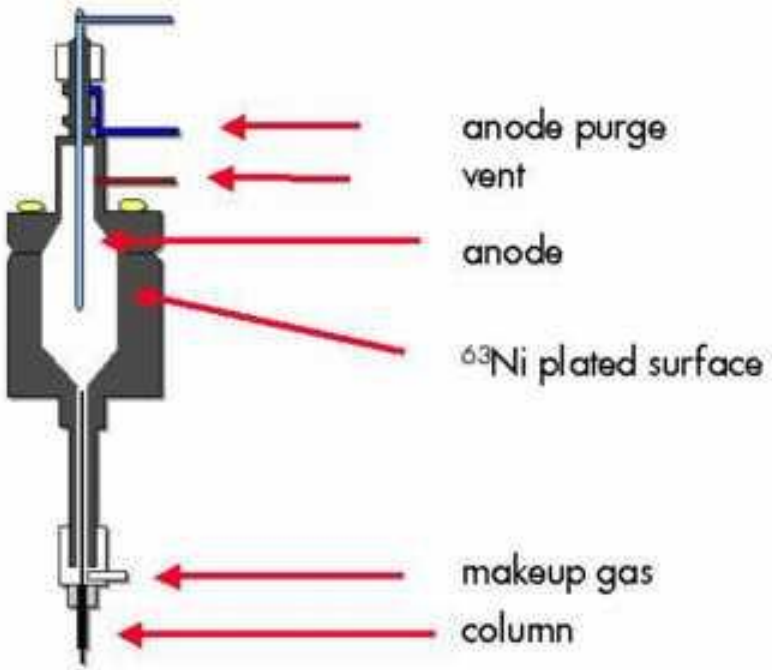


β^- are emitted by an ^{63}Ni source.

Electrophores will absorb β^-, reducing the current.

This is the basis for the response

Electron capture

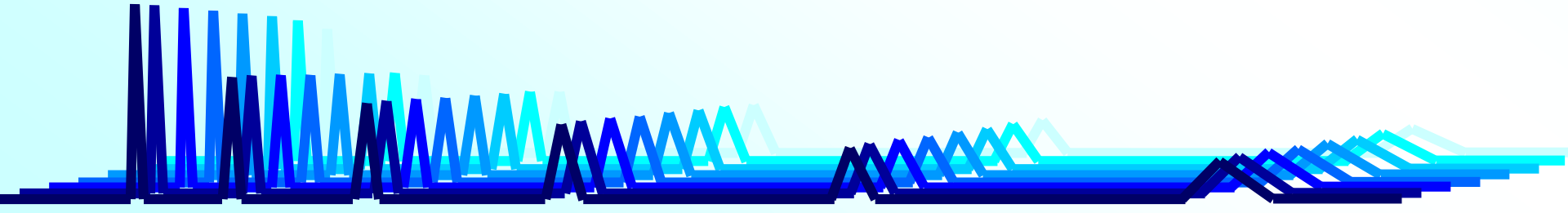


Electron capture detector

Provides excellent trace analysis of
- halogenated compounds
- nitro group compounds
- eluents with conjugated double bonds

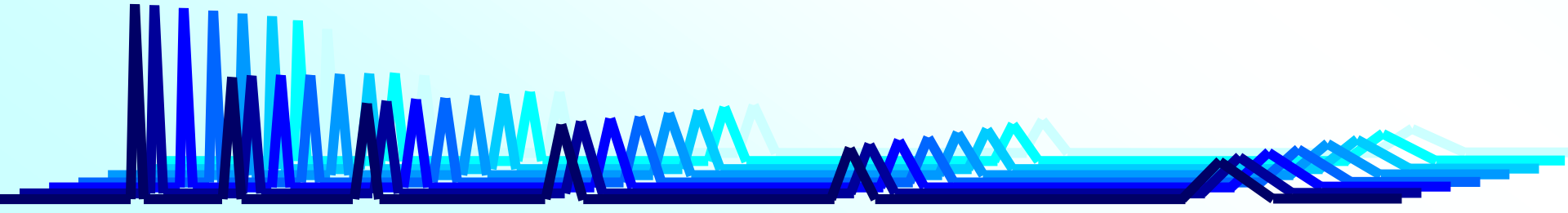
Most common use is environmental analysis of organochlorine pesticides

Major problem - detector is radioactive. Requires regular area testing and must be licensed.



Packed vs. capillary columns

	Packed	Capillary
length, M	0.5 - 5	5 - 100
ID, mm	2 - 4	0.1 - 0.7
flow, ml/min	10 - 60	0.5 - 15
head pressure, psig	10 - 40	3 - 40
total plates	4000	250,000
capacity	10μg/peak	100ng/peak
film thickness, μm	1 - 10	0.1 - 8



Capillary columns

Major differences

- Smaller ID
- Longer
- No packing
- Smaller sample capacity

This all adds up to allow components to remain on the column longer while still retaining good peak shape.

Improved sensitivity

Because peaks remain narrower, the sensitivity is improved.

Packed

Capillary

Both peaks have an area of 5000 units.

Because the capillary peak is higher, you get a better S/N.

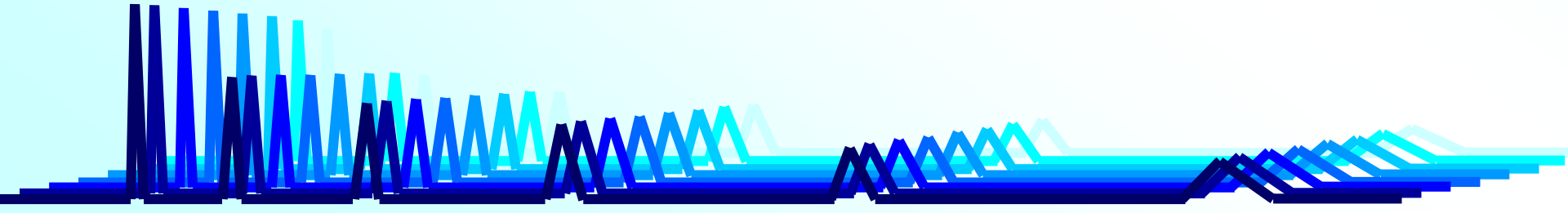
Instrumental considerations

To account for capillary work, the injection and detection systems must be modified.

- Flows and sample amounts are smaller
- Peaks are much narrower

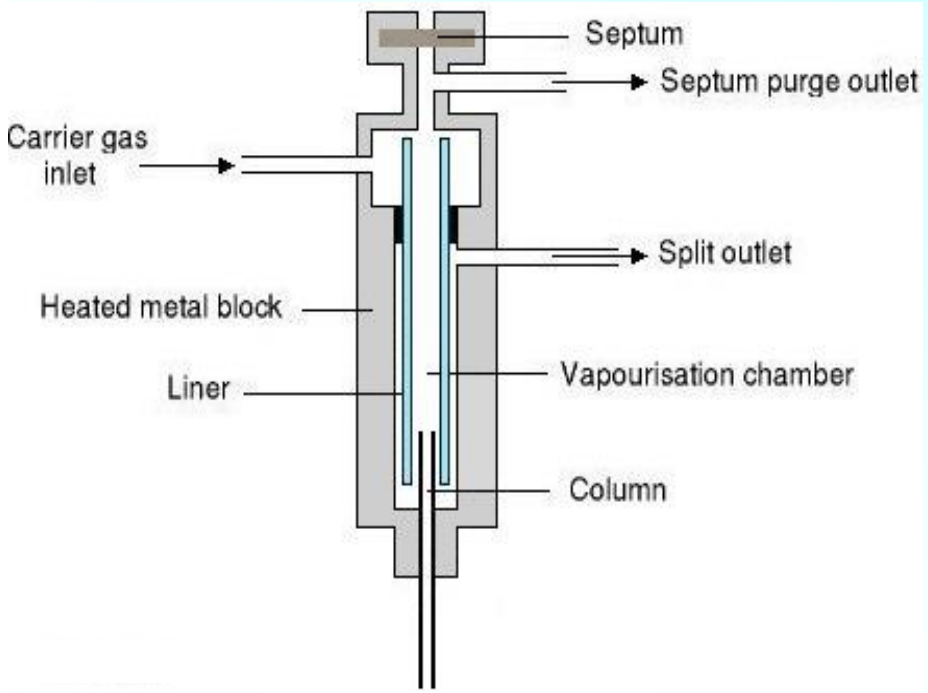
Detectors - must be redesigned to reduce 'dead' volumes.

Injectors - modified to account for low column capacity and alternate injection methods.



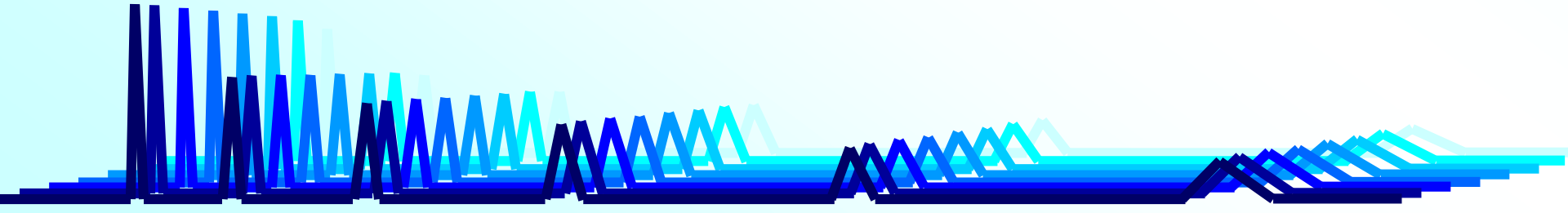
Split – Splitless Injection

- Septum purge outlet prevents components of previous injections from entering the column and minimizes the effect of septum bleed (low flow rate ~3 ml/min).
- The sample is injected into the liner region where it is completely vaporised. Mostly glass liners – zero dead volume
- The sample volume is then split between the column and the split outlet. Split injection is employed to dilute the sample and prevent column overloading. Typically 1:100 split ratios are employed with 99% of sample being vented to atmosphere.

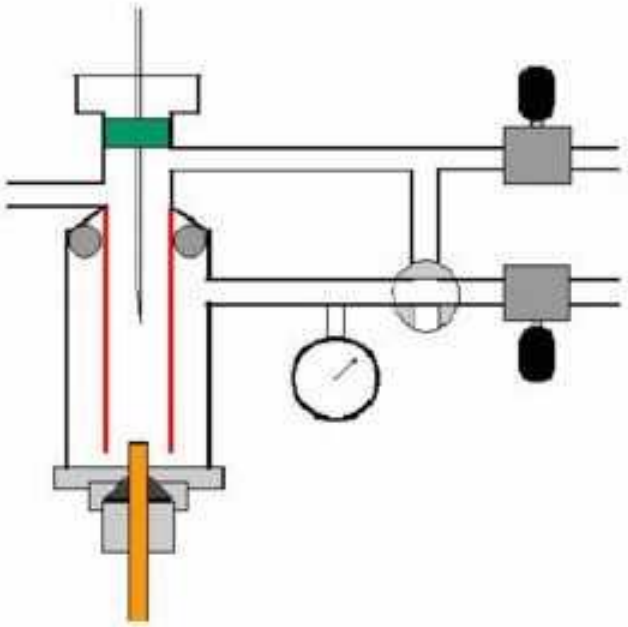


Split injection

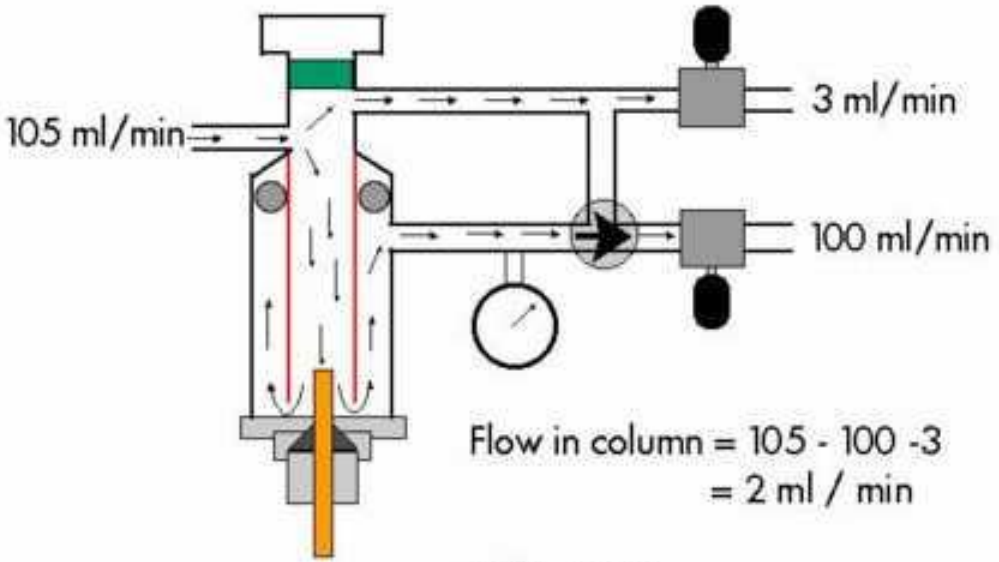
- It would be difficult to introduce volumes much less than 1 µl directly.
- One way to accomplish the same thing is to split the sample after injection - reducing the total load entering the column.
- After volatilization and mixing with carrier gas, most of the flow can be directed out the split vent.
- Only a fraction of the sample actually enters the column.



Split injection



Split mode



Flow in column = 105 - 100 -3
= 2 ml / min

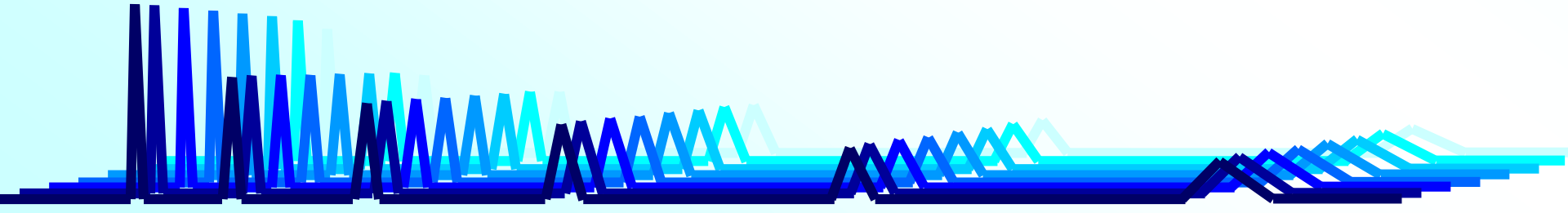
Split = 50:1

Calculation of the split ratio

$$\text{Split ratio} = \frac{\text{split vent flow + column flow}}{\text{column flow}}$$

Split vent flow can be measured directly at the split vent with a bubble meter.

Determining the column flow is a bit more difficult.



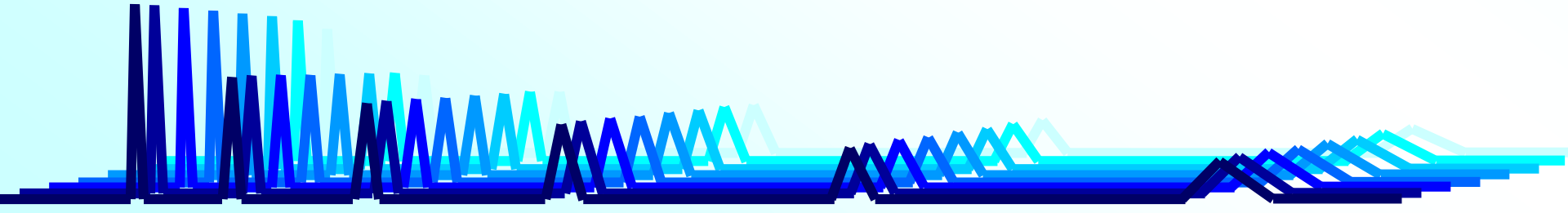
Splitless injection

Split injection is best when you have relatively high levels of the eluents of interest.

For trace analysis, splitless injection can be used.

Two approaches:

- Splitless - total sample enters column
- Splitless/Split - Grob injection
  - Attempt to selectively remove solvent



Splitless/Split

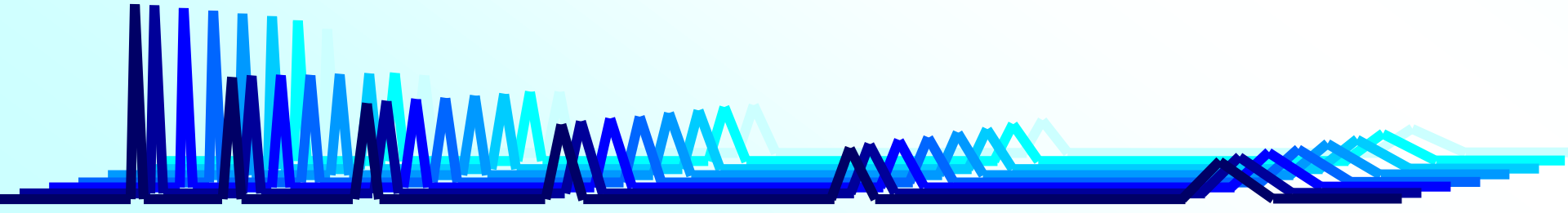
Developed by Grob so it's sometimes called a Grob injection.

Two step process

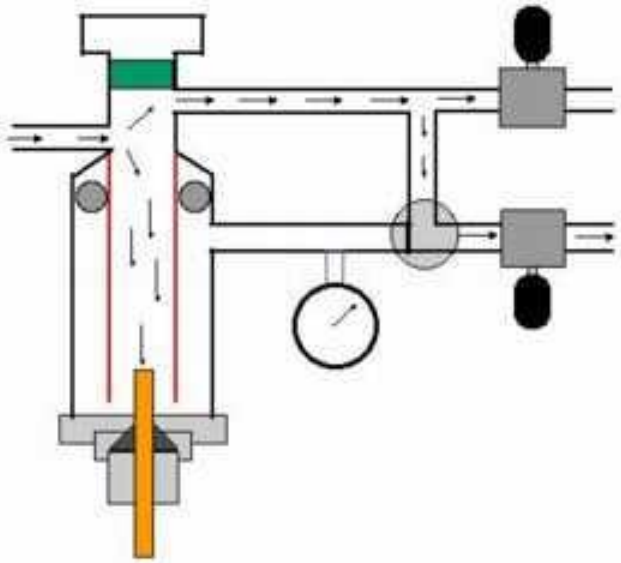
Initial injection under splitless conditions

Change to a split mode after a fixed period of time - purge time.

Goal is to introduce the majority of the sample components but not the solvent.



Step one - purge off



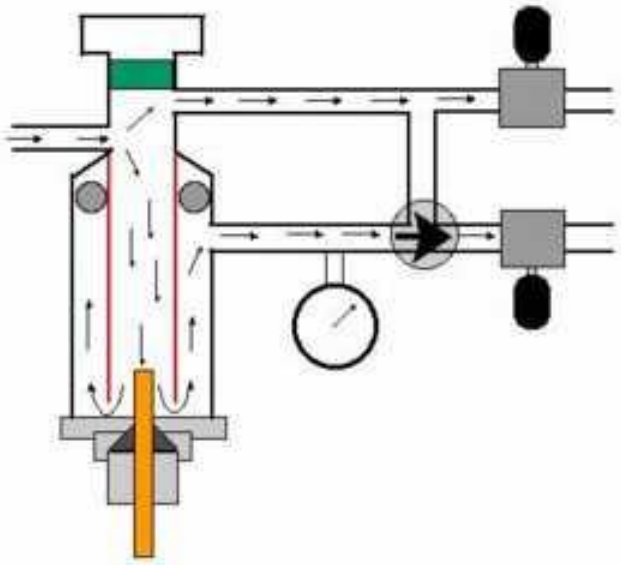
Initially the purge is off.

All flow entering the injection port will go into the column.

The normal 'split' flow bypasses the liner.

This is done to help maintain the column head pressure

Step two - purge on



After a fixed period of time, the purge is turned back on - split mode.

Any remaining material is essentially flushed from the injection port.

Advantages and disadvantages of GC

- Fast analysis
  - typically minutes (even sec.)
- High resolution
  - Record: N~1.3 x 10^6
- Sensitive detectors (easy ppm, often ppb)
- Highly accurate quantification (1-5 % RSD)
- Automated systems
- Non-destructive
  - allows online coupling to mass spectometry
- Small sample (μL)
- Reliable and relatively simple
- Low cost (~€20,000)

- Limited to volatile samples
  - Temperature limited to ~ 380°C
- Needs P_{vap} ~ 60 Torr at that temperature
- Not suitable for thermally labile samples
- Some samples may require extensive preparation (derivatization)
- Requires spectroscopy (usually MS) to confirm peak identify