GAS CHROMATOGRAPHY (GC)

Partition between stationary and mobile phase
- based on differences in volatility and structure

Suitable for:

- at least minimum volatile compounds

- at 350 °C must be at least partially in gaseous state
  (possibility of derivatisation)

ESTIMATION: ↑ M_r *and* ↑ *polarity* ⇒ ↓ *volatility*

(large nonpolar compound can be more volatile than small polar)

- thermostable compounds and nonreactive with others

- inorganic compounds are not GC amenable

GC ANALYSIS:

Injection

Split/splitless, on-column

Large volume introduction (PTV, on-column-SVE)

Separation

Theory of conventional GC

Capillary columns

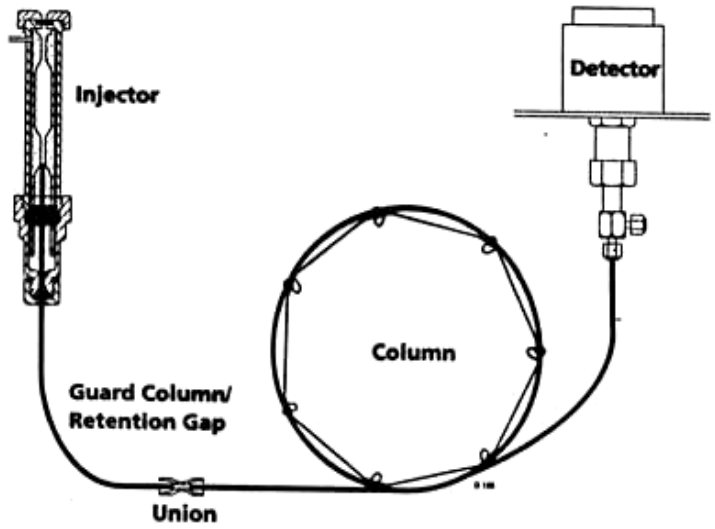
Multidimensional GC

Detection

Retention time, mass spectrum

Quantification

Peak area or height



GC SEPARATION - theory

After introduction to column molecules are **partitioning between stationary and mobile phase**, system is almost in equilibrium – distribution between stationary and mobile phase is possible to describe using $\mathbf{K_D}$

All molecules are moving only in mobile phase (with the same speed), moving of mobile phase is limiting the achievement of real equilibrium ⇒ movement of molecules to next part of stationary phase and repeated „equilibrium“

Each molecule can enter stationary phase – one enters, one stands out, the distribution between phases is kept in repeating equilibrations for each molecule

Speed of movement is given by distribution between phases

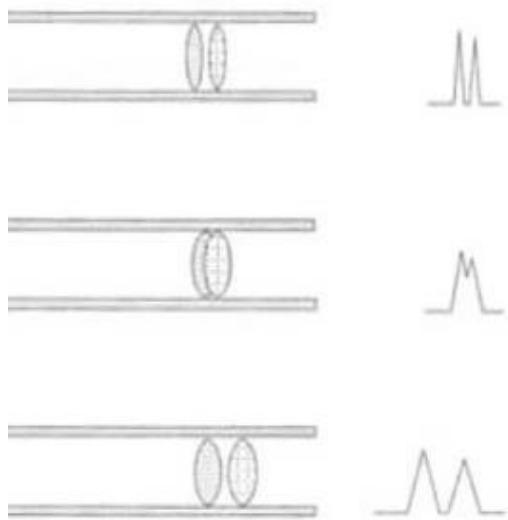
(↑ mobile phase speed ⇒ ↑ movement speed)

GC SEPARATION - theory

Separation of 2 compounds is achieved, if they have different distribution between stationary and mobile phase

IMPROVEMENT OF SEPARATION:

1. **Increasing of differences in retention times**
   (*thermodynamic aspect*) – affecting of interactions between analyte and stationary phase
   = changes of $\mathbf{K_D}$ and **temperature**
2. **Narrowing of elution bands**
   (*kinetic aspect)* – **column dimensions, mobile phase speed, diameter of sorbent particles, …**



⇒ ***narrow elution bands needs less separation than wide ones***

GC SEPARATION - theory

Major factors affecting separation

Stationary phase – higher solubility of analyte in stationary phase
⇒ higher retention

Analyte structure – differences in solubility of analytes
in stationary phase

Temperature – affects distribution of molecules between s. and m.ph.

(↑ temperature ⇒ ↑ number of molecules in m.ph.
⇒ ↓ t_R and ↓ separation)

Other factors: column dimensions, carrier gas and its velocity

GC SEPARATION

Determined by: **column, temperature, linear velocity of m.ph.**

Molecules of any analyte are moving in mobile phase with the same speed and spend the same time there (t_M)

⇒ **difference in retention time (t_R)** of analytes is given by different time spent in stationary phase (reduced retention time: $t_R' = t_R - t_M$)

Capacity factor (retention factor – k or k'):

• ratio of analyte time spent in stationary ph. (t_R') and in mobile ph. (t_M)

$$k = (t_R - t_M)/ t_M$$

Measure of an analyte retention in comparison with another one
(k = 0 - no retention, k ≈ 1 - low retention, k ≈ 10 - high retention)

• alternative to retention times comparison

GC SEPARATION

Partition coefficient (or partition ratio or distribution constant - K)

- ratio of analyte molar concentration in stationary ph. and in mobile ph.
- constant for a given combination of:

  analyte, stationary phase and temperature

- analytes are divided between st.ph. and m.ph. in dependence on:

  column temperature, their structure and structure of st.ph.

$$K_D = c_S / c_M$$

$$= k\,\beta = (t_R' / t_M)\,(r / 2\,d_f)$$

$$[\beta = r / 2\,d_f = V_M / V_S \ldots \text{phase ratio}]$$

GC SEPARATION

Partition coefficient (or partition ratio or distribution constant - K)

$\mathbf{K_D = c_S / c_M} = k\,\beta = (t_R' / t_M)\,(r / 2\,d_f)$ …… $[\beta = r/2d_f = \mathbf{V_M/V_S}]$

$\mathbf{k = K_D / \beta = K_D\ 2\ d_f / r}$

- useful for ***estimation of retention changes at changes of column parameters***

- for ↑**k** is necessary ↑ $\mathbf{K_D}$ or ↓ **β**
- ↑ β ⇒ ↓ **k** (at constant K_D)

- column length, mobile ph. type and flow rate is not directly included in equation
- important relations: $t_R' = K_D\,t_M\,(2\,d_f / r) = t_M\,(2\,c_S\,d_f)/(\,c_M\,r);\ t_M = L / u$

$$\mathbf{t_R' = (2\,c_S\,d_f\ L)/(\,c_M\,r\,u)} = (c_S/c_M)\ (2d_f/r)\ (L/u)$$

K_D $1/\beta$ t_M

GC SEPARATION

Partition coefficient (or partition ratio or distribution constant - K)

***K_D x column temperature*: ↑ temperature ⇒ ↓ retention (↓ K_D)**

Constant β: *small temperature change = great retention change*

Changes are not uniformed for all analytes

– suitable temperature programming (increasing):

- **start ↑ K_D separation improvement**
- **end ↓ K_D** (higher temperature) = **narrower peaks**

↑ K_D ⇒ stronger interaction with st.ph. = ↑ t_R = more times the mass transfer = longer analysis = wider peaks

(more analyte in st.ph., where is not moving

– transfer to detector for a long time in small portions)

↓ K_D ⇒ more analyte in m.ph = **fast transfer to detector = narrow peaks**

GC SEPARATION

Selectivity factor - α

Ability of st.ph. to separate two compounds, retention ratio of two peaks

Difference (time) between tops of two peaks (no information about the quality of separation, α is the same for wide (overlaid) or narrow peaks

$$\alpha = k_2 / k_1 = (t_{R2} - t_M) / (t_{R1} - t_M)$$

- *given by functional groups, depends on specific interactions of analyte with stationary phase*

- **necessary $\alpha > 1$, if $\alpha = 1$, then analytes is not possible to separate**

GC SEPARATION

Efficiency (number of theoretical plates – n)

↑ *efficiency* ⇒ ↓ *elution band width*

Definition based on relation between t_R and width of peak

Generally: peak width is increasing with t_R increasing

Number of theoretical plates - n

• dimensionless quantity, calculated for an analyte

$$n = 16\,(t_R / w_b)^2 = 5{,}545\,(t_R / w_h)^2$$

w_b ... *peak width at baseline,* w_h ... *peak width at half of height*

↑ n ⇒↑ efficiency ⇒↑ separation potential (narrower peaks)

GC SEPARATION

Efficiency (number of theoretical plates – n)

Important facts:

- ***n is given by retention time and shape of peak used for calculation***!
  n is higher for faster eluting analytes – for k > 5 linear dependence

Comparison must be based on the same analytes

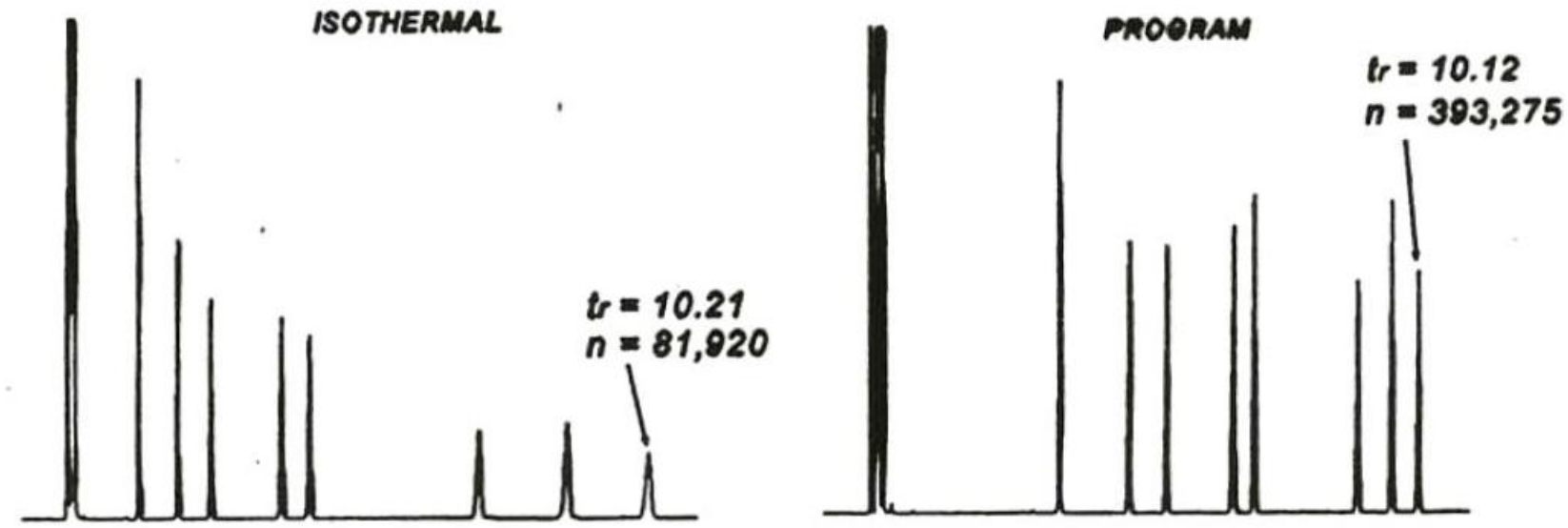
- ***n describes efficiency of the complete system***
  (injector, temperature, flow rate, ...) – not column only

Temperature programming causes peaks narrowing ⇒
n calculated for the same column under different temperatures differs

GC SEPARATION

Efficiency (number of theoretical plates – n)

Temperature effect: ↑ *efficiency* ⇒ ↓ *band width*



GC SEPARATION

Efficiency: height equivalent to a theoretical plate (HETP) - h

$\downarrow h \Rightarrow \uparrow n \Rightarrow \uparrow efficiency$

- **length of column (part of column), in which equilibrium occurs once**

$$h\ (HETP) = L / n$$

- **number of theoretical plates for the defined column length**

GC SEPARATION

Resolution - R:

Measure of separation of 2 peaks - with regard to width of peak
- measure of separation quality

$$R = 1{,}18\,(t_{R2} - t_{R1}) / (w_{h1} + w_{h2})$$

R = 1.5 peaks separated without baseline between them
R < 1.5 (partial) overlay R > 1.5 separated with distance

R – given by *efficiency (peak shape)*, *selectivity (separation)* *and retention (time)*

$$R = 1/4\, n^{1/2}\,((\alpha-1)/\alpha)\,(k/(k+1))$$

GC SEPARATION

EFFICIENCY vs. RESOLUTION

a) Lower efficiency, incomplete resolution (not separated)

b) Higher efficiency, better resolution (just separated)

c) Higher efficiency and selectivity, complete resolution (separated significantly)

Analyte elution in narrow, symmetric zone
= less possibility of overlay

GC SEPARATION

Band broadening theory - Van Deemter equation

1. Eddy (turbulent) diffusion in mobile phase (H_F)

Flow between sorbent particles is different because of different distances between them (channels) ⇒ molecules of analytes move with different speed.

2. Molecular (axial or longitudinal) diffusion in mobile phase (H_L)

Analyte is concentrated at the column head in narrow zone ⇒ during elution occurs zone broadening by diffusion from higher concentration in zone centre to lower concentration at the edges of elution zone. (Fick's law). Effect decreases with ↑ u.

GC SEPARATION

Band broadening theory - Van Deemter equation

3. Resistance to mass transfer in stationary phase (H_S)

Molecules diffuse between mobile and stationary phase, with different deep ⇒ different retention. Molecules in mobile phase are faster then ones in stationary phase ⇒ band broadening.

4. Resistance to mass transfer in mobile phase (H_M)

Inconsistent mobile phase flow in capillary column (in channels between particles) – at wall (sorbent surface) is minimal X in the middle of stream maximal ⇒ molecules move with different speed. *Diffusive transfer of molecules between streams – compensation of differences*. Effect increases with ↑ u.

$$H = H_F + H_L + H_M + H_S = A + B/u + (C_S + C_M)\, u$$

$$\mathbf{H = A + B/u + C\, u}$$

GC SEPARATION

Column dimensions - Efficiency and Resolution

$$R = 1/4\, n^{1/2} ((\alpha-1)/\alpha)\, (k/(k+1))$$

$$t_R' = (2\, c_S\, d_f\, L)/(c_M\, r\, u) = (c_S / c_M)\, (2d_f / r)\, (L / u)$$

$$n = L / h; \qquad n = 16\, (t_R / w_b)^2$$

Column length: $\uparrow L \Rightarrow \uparrow n$ $\quad 2x \uparrow L \Rightarrow \uparrow$ R only by 25 - 35 %

Inner diameter: $\downarrow r \Rightarrow \uparrow n$ $\quad 4x \downarrow r \Rightarrow \uparrow$ R 2x

Film thickness of st.ph.: $\uparrow d_f \Rightarrow \uparrow$ retention

Very volatile analytes (k < 5): $\uparrow d_f \Rightarrow \uparrow$ n

Less volatile analytes (k > 5): $\uparrow d_f \Rightarrow \downarrow$ n (elution at higher T $\Rightarrow$ band broadening)

GC SEPARATION

Temperature - Efficiency and Resolution

↑ *temperature* ⇒ ↓ *retention and resolution*

Separation of members of homologous series: similar effect of temperature changes to retention of all members

Separation of significantly different analytes: temperature changes affect significantly separation process

A minimal temperature change can affect separation more significantly than the significant change of film thickness or column length

GC SEPARATION

Carrier gas - Efficiency and Resolution

- **inert, no influence on sorption – desorption, selectivity**

- type and velocity affect efficiency and analysis time

Linear velocity

- u (cm/s) – velocity of carrier gas moving through column
- dissimilar across a column – average value is used

Van Deemter curve

- **relation of u and efficiency expressed as h (HETP)**

$$h_{min} = A + B / u + C u$$

GC SEPARATION

Carrier gas - Efficiency and Resolution

Golay equation: $h_{min} = B / u + C u$

Wall Coated Open Tubular column - WCOT (capillary c.): A = 0

- **low *u*** = small velocity of analyte in column = more phase interactions **(more separation)**

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high longitudinal diffusion
(separated zones overlaying)

- **high *u*** = minimal longitudinal diffusion

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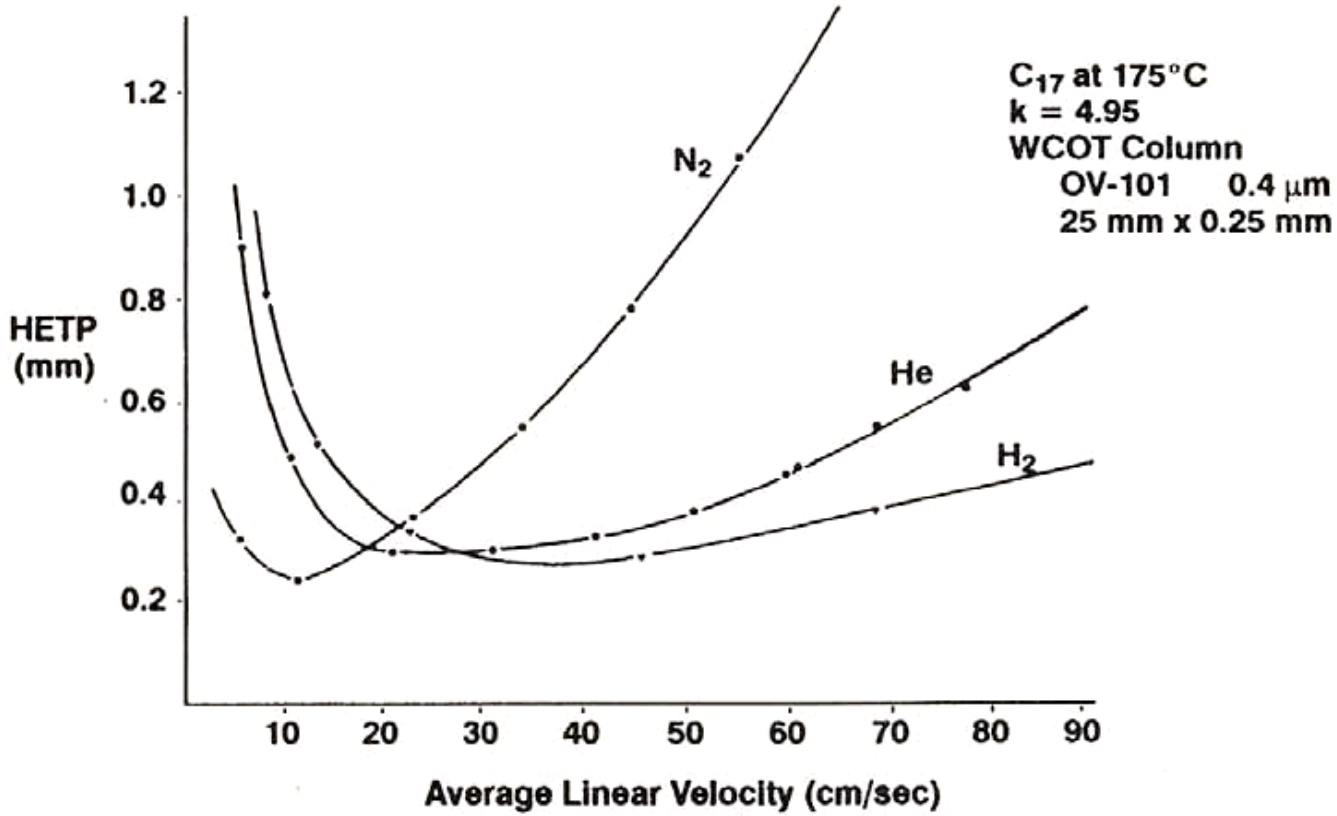
limited phase interactions

OPTIMAL LINEAR VELOCITY
– compromise to these opposed phenomena

GC SEPARATION

Carrier gas - Efficiency and Resolution

Golay equation: $h_{min} = B / u + C u$



GC SEPARATION

Carrier gas - Efficiency and Resolution

NITROGEN: the best efficiency (small h)

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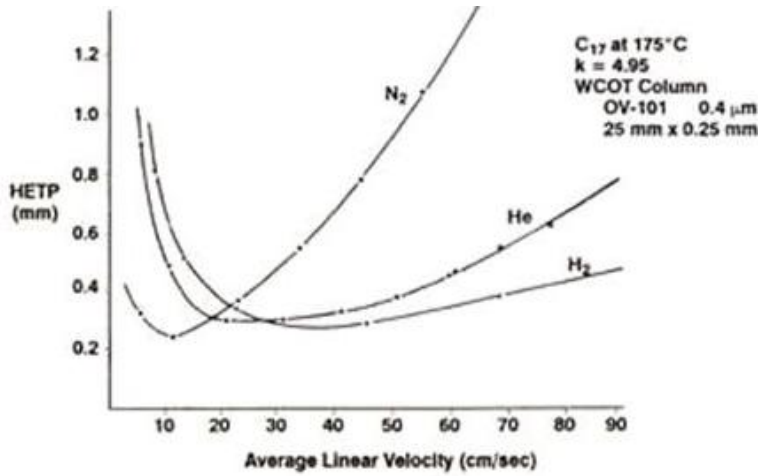
- **optimal *u* – *too low*** = high retention times
- **very steep curve** = small velocity change
  = big efficiency change
- **for inconstant flow:** many of analytes eluted with non-optimal linear velocity
- **cheap**



GC SEPARATION

Carrier gas - Efficiency and Resolution

HELIUM: efficiency a little lower compare to nitrogen

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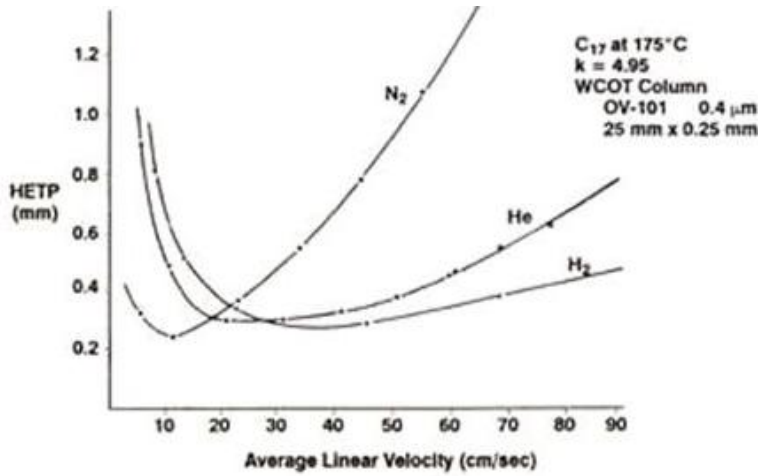
- **optimal *u* higher** = similar resolution for shorter time
- **not so steep curve** = lower loss of efficiency at higher velocity
- **expensive**



GC SEPARATION

Carrier gas - Efficiency and Resolution

HYDROGEN: efficiency between nitrogen and helium

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- optimal *u* at high values = decreasing of retention times without loss of efficiency

- very flat curve

- price between N_2 and He

- best one for capillary columns, risk of explosion