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GAS CHROMATOGRAPHY (GC)

Partition between stationary and mobile phase

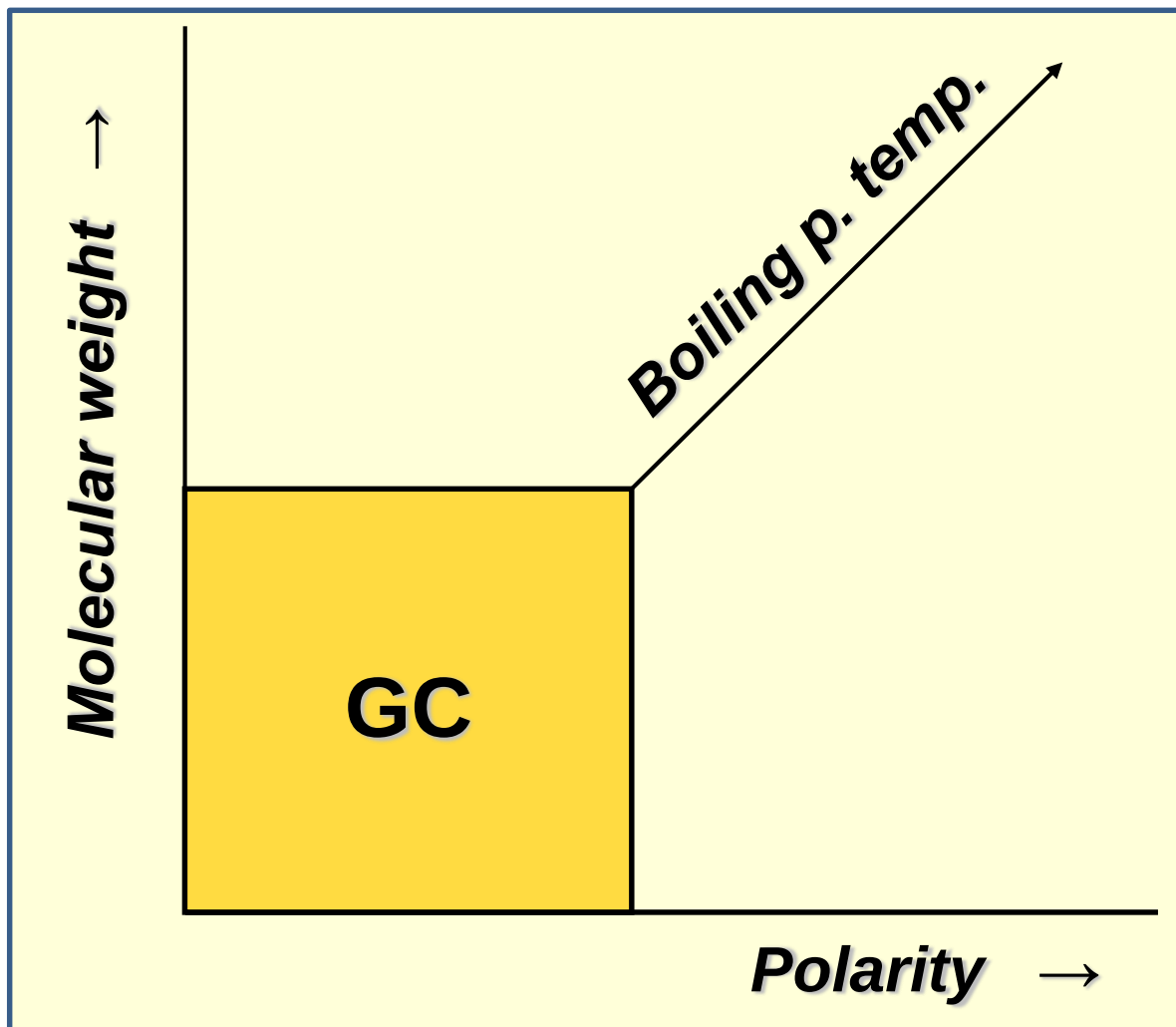
*- based on differences in volatility and structure
(separated compounds show the different chromatographic affinity)*

Suitable for:

- **thermostable compounds and nonreactive with others**
- **inorganic compounds are mostly not GC amenable
(applicable for H₂O, gases etc.)**
- **with acceptable volatility - at 350 °C it must be at least partially in the gaseous state (possibility of derivatization)**



Estimation of compound suitability for GC



TECHNICAL REALIZATION OF GC

Injection - injector

Split/splitless, on-column, pulse techniques

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

Desorption techniques

Separation – column

Packed and capillary columns

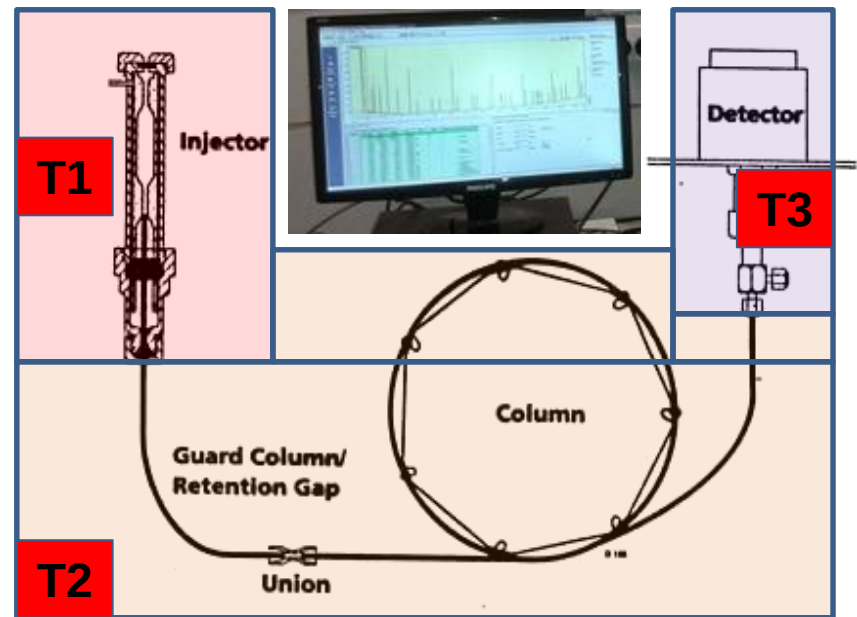
Parallel and multidimensional GC

Detection - detector

Retention time, spectrum

Quantification - software

Detector response - peak area or height



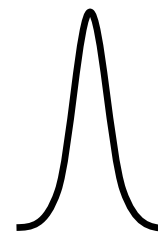
After introduction to column molecules are **partitioning between stationary (SP) and mobile phase (MP)**, system is almost in equilibrium – distribution between SP and MP is possible to describe using distribution constant K_D

All molecules are moving only in mobile phase, all molecules go through the same path at different times according to their different affinity to the stationary phase.

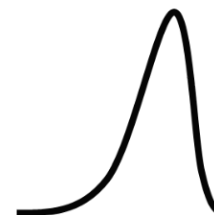
Each molecule can enter stationary phase – one enters, one exits, the distribution between phases is kept in repeating equilibrations for each molecule.

If the position is occupied, the molecule travels further until it finds a free space. Another molecule may enter the released site. With a large number of molecules oversaturation of column can appear. Typical consequences of the column oversaturation is either fronting or tailing of peaks, in the extreme case, a significant reduction of retention times.

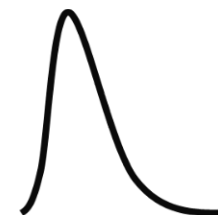
NORMAL



FRONTING



TAILING



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

IMPROVEMENT OF SEPARATION

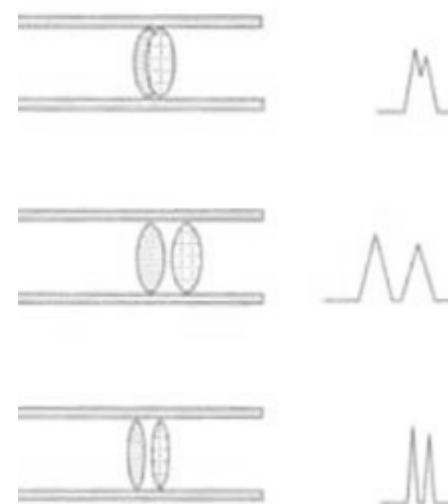
1. Increasing of differences in retention times

(*thermodynamic aspect*) – affecting of interactions between analyte and stationary phase
= changes of K_D and **temperature**

2. Narrowing of elution bands

(*kinetic aspect*) – **column dimensions**,
film thickness of SP, **mobile phase speed**

*narrow elution bands needs
less separation than wide ones*



MAJOR FACTORS AFFECTING SEPARATION

Stationary phase – **higher solubility (affinity)** of analyte in SP
⇒ **higher retention**

Analyte structure – differences in solubility of analytes
in stationary phase

Temperature – affects distribution of molecules
between SP and MP

↑ temperature ⇒ ↑ number of molecules in MP
⇒ ↓ t_R and ↓ separation)
⇒ narrower elution zones
with lower retention times

Other factors: **column dimensions, carrier gas and its velocity**



Capacity factor (retention factor – k or k')

It depends on: column SP and dimensions, temperature, MP linear velocity

Molecules of any analyte pass through the same length (volume) of column, which is the so-called dead (elution) volume expressed as the retention time of an analyte passing through the system without delay at time t_0 (or t_M)

⇒ **reduced retention time t_R'** expressing the time spent in SP is obtained for a given analyte as the retention time t_R reduced by the dead retention time t_0

⇒ $t_R' = t_R - t_0$, the retention times of the analytes thus differ by the difference in the times spent in the SP

⇒ **definition of capacity factor:**

ratio of times spent by analyte in SP t_R' and in MP t_0

$$k = t_R' / t_0 = (t_R - t_0) / t_0$$

A measure of retention of a compound in SP compared to another one

$k = 0 \Rightarrow$ no retention, $k \approx 1 \Rightarrow$ low retention, $k \approx 10 \Rightarrow$ high retention

- characterization of GC separation in terms of speed



Distribution constant (Partition coefficient or ratio - K_D)

Distribution constant definition

- ratio of molar concentration of analyte in SP (c_S) and in MP (c_M)
- expresses the distribution of analyte between SP and MP
- constant for the given analyte, SP and temperature

Analytes are partitioned between SP and MP depending on the column temperature, their structure and the nature of the SP (chromatographic affinity).

$$K_D = c_S / c_M = k \beta = (t_R' / t_0) (r / 2 d_f)$$

$$\beta = r / 2 d_f = V_M / V_S \text{ is phase ratio}$$



Distribution constant – important relations

$K_D = c_S / c_M = k \beta = (t_R' / t_0) (r / 2 d_f)$, $\beta = r / 2 d_f = V_M / V_S$ is phase ratio,

$$\Rightarrow k = K_D / \beta = K_D 2 d_f / r$$

- useful for the estimation of the impact of changes in column parameters on column retention and efficiency during method optimization

- for $\uparrow k$ is necessary $\uparrow K_D$ or $\downarrow \beta$ vice versa $\uparrow \beta \Rightarrow \downarrow k$ (at constant K_D)

$\beta = r / 2 d_f$ $\Rightarrow \uparrow \beta \approx \uparrow r$ i.e. $\uparrow$ column radius or $\approx \downarrow d_f$ i.e. $\downarrow$ SP thickness

Relationship containing column length and MP (carrier gas type and flow rate)

$$t_R' = t_0 (2 c_S d_f) / (c_M r) ; t_0 = L / u$$

$$\Rightarrow t_R' = (2 c_S d_f L) / (c_M r u) = \underbrace{(c_S / c_M)}_{K_D} \underbrace{(2 d_f / r)}_{1/\beta} \underbrace{(L/u)}_{t_0}$$



Distribution constant – practical aspects

Influence of temperature on K_D

$\uparrow$ temperature $\Rightarrow \downarrow K_D \Rightarrow \downarrow$ retention

- small change in temperature = large change in retention (c. β)

$\uparrow K_D \Rightarrow$ stronger connection with SP $\Rightarrow \uparrow t_R$

- multiple mass transfer = longer analysis = more of analyte in SP, where it does not move $\Rightarrow$ analyte is introduced into detector for a long time in the broaden zone $\Rightarrow$ wide peak

$\downarrow K_D \Rightarrow$ more of analyte in MP = $\downarrow t_R$

- fast transfer to detector = narrow peak

K_D changes are not the same for all analytes - suitable temperature programming is necessary

- at the beginning of the analysis $\uparrow K_D$ (lower temperature) - improves separation

- at the end of the analysis $\downarrow K_D$ (higher temperature) = narrower peaks



Separation factor or selectivity - α

Ability of SP to separate 2 compounds

- retention ratio of the two peaks
 $\approx$ the distance between the tops of two peaks
- no information about the quality of elution zone (peak shape)
- the same for wide (overlaid) or narrow peaks

$$\alpha = k_2 / k_1 = (t_{R2} - t_0) / (t_{R1} - t_0)$$

- determined by functional groups
 $\approx$ depends on specific interactions of analytes with SP
- if > 1 , compounds can be separated (sometimes only partially)
- if ≤ 1 , compounds cannot be separated in the given system



Efficiency $\approx$ performance

Expressed as a number of theoretical plates – n (N)

$\uparrow$ efficiency $\Rightarrow \downarrow$ width of the elution zone $\Rightarrow \uparrow$ separation potential

narrower elution zones = higher peak capacity

- dimensionless parameter calculated for 1 analyte, i.e. does not characterize the separation of 2 compounds
- relationship between retention time and peak width

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

w_b – peak width at the baseline, w_h - peak width at half height

Efficiency = number of theoretical plates – n (N)

- *n is given by the compound selection for the calculation, i.e. by retention time and shape of peak used for calculation!*
- *n is higher for earlier eluted compounds and with increasing of their retention times decrease (elution zones are widening)*
- *correct characterization of the column - peak with $k > 5$ (linear dependence of n and k)*
- *column efficiency comparison - peaks with identical k value*
- *calculated n actually describes the efficiency of the whole analytical system and method (injection, carrier gas, flow rate, column temperature etc.)*
- *narrowing of peaks by temperature programming - fundamental influence on the n value*

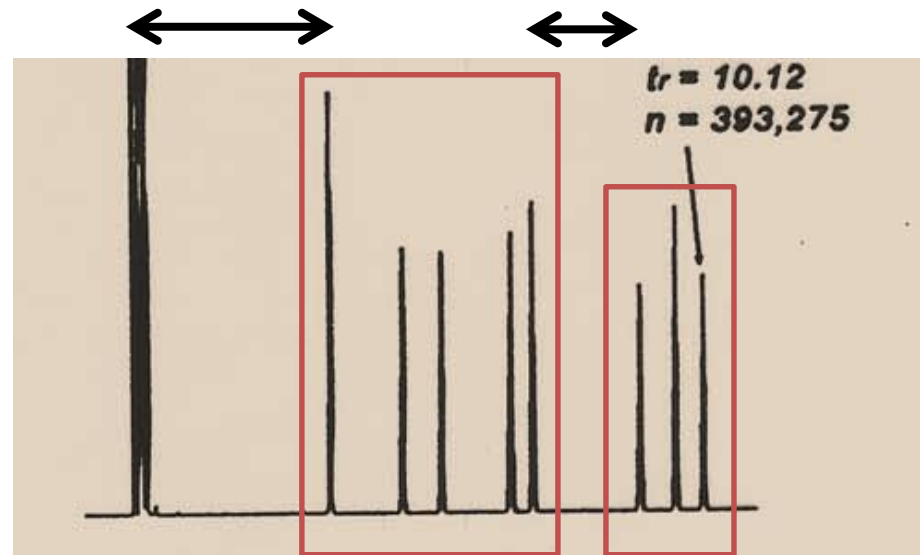
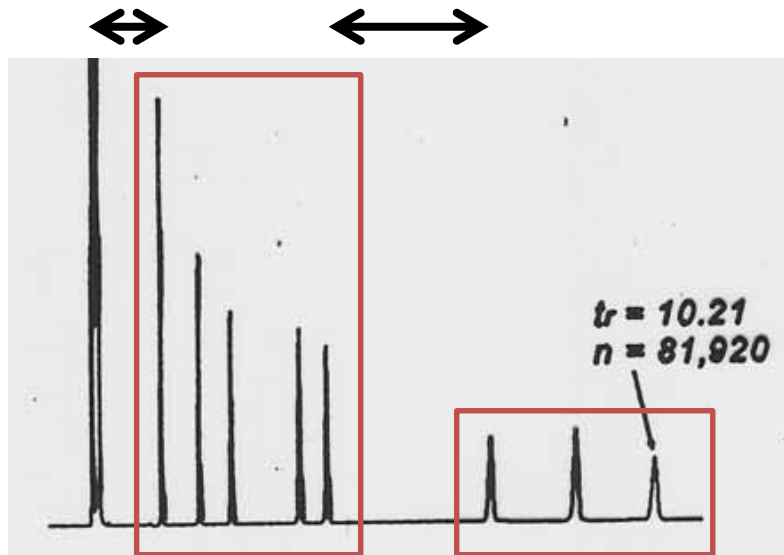
Efficiency (number of theoretical plates) – temperature effect

↓ width of the elution zone ⇔ ↑ efficiency

isothermal

vs.

programmed temperature



Height equivalent to a theoretical plate (HETP) – h

$\downarrow h \Rightarrow \uparrow n \Rightarrow \uparrow \text{efficiency}$

One theoretical plate = part of the column, in which occurs once the equilibration

- number of theoretical plates - total or per meter of column length

$$\mathbf{n = L / h \text{ (HETP)}}$$

**- change of the theoretical plates number in time $\Rightarrow$ checking after a defined number of analyzes = officially acceptable evidence of changes of column properties – e.g. for accredited analyzes
 $\Rightarrow$ shortening vs. column replacement**



Resolution - R

The degree of separation between two peaks with respect to their width - a measure of the quality of the compounds separation

R is given by efficiency ($n \approx$ peak shape), SP selectivity (α) and retention time (k)

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

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



$R < 1.5$ (partial) peak overlap; lower n and α



$R = 1.5$ peaks separated with zero spacing between them



$R > 1.5$ peaks separated with spacing between them; higher n and α

Effects on elution zones (peak shape) - broadening and other deformations

Theory developed for packed columns (subsequently simplified for capillary columns)

1. Eddy (turbulent) diffusion in MP ($H_F \approx A$ (m))

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

2. Molecular longitudinal diffusion in MP ($H_L \approx B$ (m² s⁻¹))

Analyte is concentrated at the column head in narrow zone $\Rightarrow$ 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 $\uparrow u$ [m s⁻¹].

3. Resistance to mass transfer in SP ($H_S \approx C (s)$)

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

4. Resistance to mass transfer in MP ($H_M \approx C (s)$)

Inconsistent mobile phase flow in capillary column (in channels between particles) – at wall (sorbent surface) is minimal X in the middle of stream maximal $\Rightarrow$ molecules move with different speed. *Diffusive transfer of molecules between streams – compensation of differences.* Effect increases with $\uparrow u$ [$m s^{-1}$].

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

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

Selection of parameters to achieve the required resolution

1. Temperature programming, pressure programming, different column lengths and diameters, different SP thicknesses
 - effect on peak width, retention time (elution order may change), peak capacity, peak distribution on the chromatogram
2. Selection of stationary phase - difference in character and retention, difference in selectivity - there is a change in the elution order, different effect on different analytes - application of parallel chromatography on two different SP
3. Selection of mobile phase - different carrier gases, differences in optimum flow rates



Column dimensions - efficiency and resolution

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

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

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

Length: $\uparrow L \Rightarrow \uparrow n$ $2x \uparrow L \Rightarrow \uparrow R$ but only about 25 - 35 %

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

SP film thickness: $\uparrow d_f \Rightarrow \uparrow \text{retention}$

Highly volatile compounds ($k < 5$): $\uparrow d_f \Rightarrow \uparrow n$

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



Temperature - efficiency and resolution

↑ temperature ⇒ ↓ retention

The effect on resolution is not unequivocal, but it is significant.

Temperature affects not only retention but also peak width, i.e. efficiency.

Temperature programming plays an important role.

Either linearly or in different ramps and gradients - the order of elution of analytes (according to the significance of influences – b.p.t., affinity) and resolution can also be influenced.

SEPARATION OF MEMBERS OF HOMOLOGOUS SERIES

Temperature changes have a similar effect on retention of all analytes

SEPARATION OF COMPOUNDS OF DIFFERENT CHARACTER

Temperature changes have a major effect on the separation

A minimal change in temperature may have a more significant effect on the separation than a change of SP film thickness or column length



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)
- $h_{\min} \approx$ the most theoretical levels, i.e. the highest efficiency

$$h = A + B / u + C u$$

Golay's equation for capillary columns - does not contain A (sorbent)

$$h = B / u + C u$$

↓ **u** = low analyte velocity in column = more mass transfers MP $\Leftrightarrow$ SP
 $\Rightarrow$ large longitudinal diffusion (zones overlap)

↑ **u** = minimal longitudinal diffusion
 $\Rightarrow$ only a limited number of mass transfers

OPTIMAL LINEAR VELOCITY

it is therefore a compromise for these opposing phenomena.



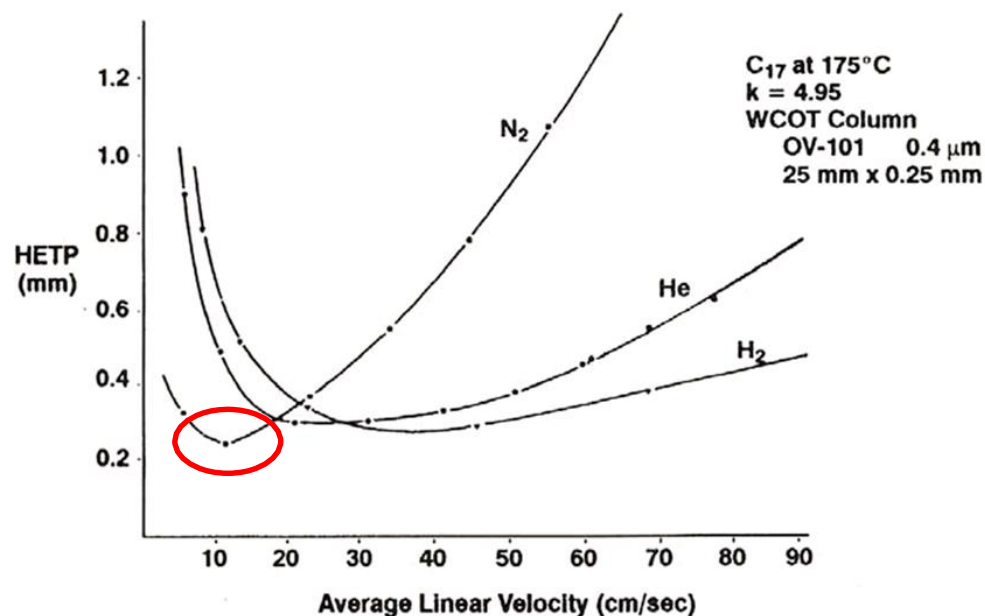
Highest efficiency ($h_{\min}$)

Optimal value of u - low (small flows) and very narrow range
 $\Rightarrow$ high retention times

Curve is very steep $\Rightarrow$ small increase in velocity
 $\Rightarrow$ big change in efficiency

When programming temperature, constant flow is recommended.

- cheap and affordable
- possibility of production using generator



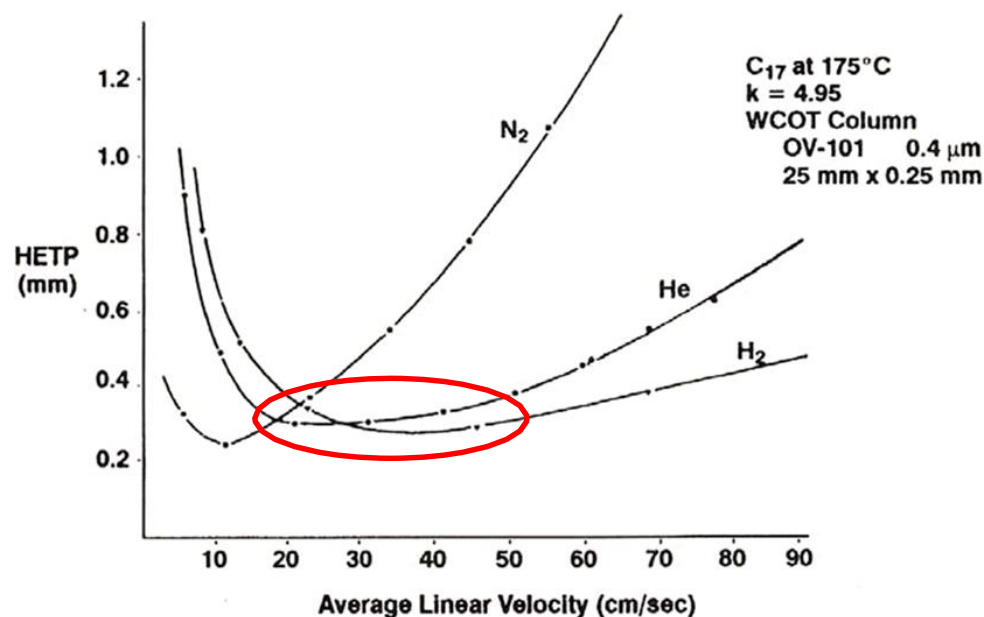
Acceptably high efficiency ($h_{\min}$) - lower than for N_2

Optimal value of u - high enough and larger range

⇒ lower retention times - the curve is not so steep

⇒ velocity increase = small change in efficiency

- expensive and limitedly available
- price increases



High efficiency ($h_{\min}$) – between N_2 and He

Optimal value of u - high and large range

⇒ lower retention times - the curve is flat

⇒ velocity increase = minimum change in efficiency

- reasonable price and availability
- possibility of production using generator
- risk of explosion

