SEPARATION METHODS **A**

C7021
C7023

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separation methods A – **syllabus**

SEPARATION; separation methods

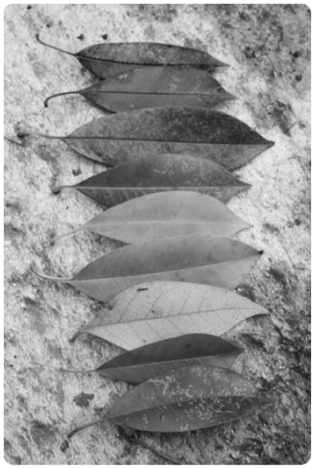
basics in separation

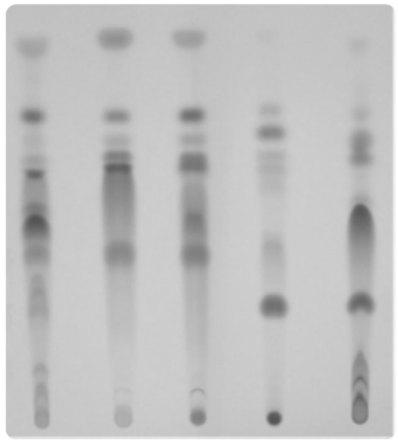
: what the separation in fact is?
: principles that allow it

preparative separation

extraction as a separation model example

: extraction L-L, S-L
: SFE, ASE, SPE, SPME, MASE, TLC, HSE





analytical separation

analytical separation methods

: liquid chromatography
:: NPLC, RPLC, LC-on-chip, HIC, HILIC, UPLC, AC, IEC, SFC, PLC
: gas chromatography
:: GC

recommended reading

J. C. Giddings, **Unified separation science**, Wiley 1991

C. F. Poole, **The essence of chromatography**, Elsevier 2003

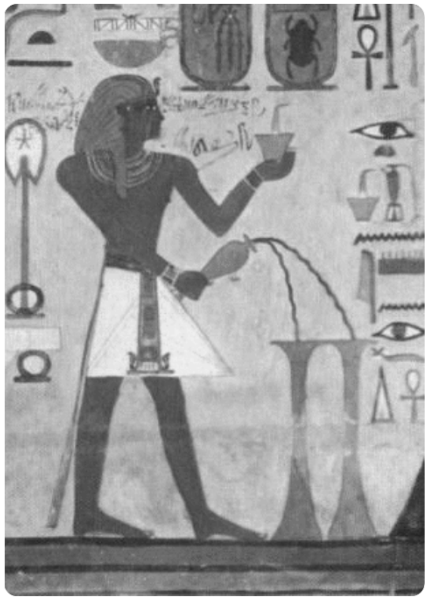
R. L. Grob *et al*., **Modern practice of gas chromatography**, Wiley 2004

G. Guiochon *et al*. (eds.), **Fundamentals of preparative and non-linear chromatography**, Elsevier 2006

J. Cazes (ed.), **Encyclopedia of Chromatography**, CRC Press 2010

L. R. Snyder *et al*., **Introduction to modern liquid chromatography**, Wiley 2010

D. Corradini (ed.), **Handbook of HPLC**, CRC Press 2011



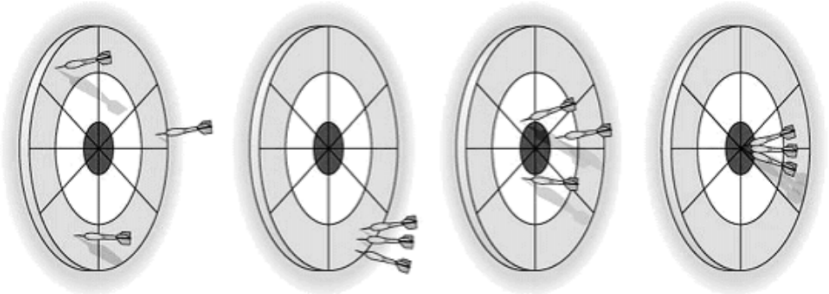
result relevance

sensitivity
: ability to exclude false negative results
:: TP / (TP + FN)

specificity
: ability to exclude false positive results
:: TN / (TN + FP)

selectivity
: ability to acquire correct positive results
:: TP / (TP + FP)

result variability



precision
: repeatability day-to-day series

accuracy
: matching „reality“; reference, normalisation

separation

segregating component(s) of a mixture of substance in space (and time)

separation ← *lat.* separatus = **SE**- *away* + **PAR**- *prepare*

(s e p a r a t i o n) **original mixture**

(s) + (e p a r a t i o n)

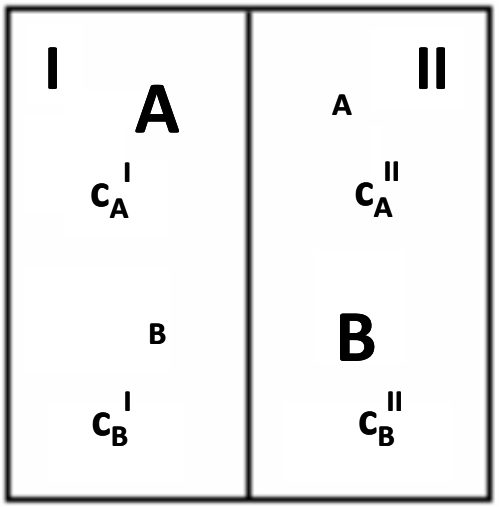
partial separation

- isolation, purification, preparation
- fractionation

(s e) + (p a) + (r a) + (t i) + (on)

(s) + (e) + (p) + (a) + (r) + (a) + (t) + (i) + (o) + (n) **complete separation**

separation of two completely miscible substances



$$\frac{c_A^I}{c_A^{II}} \gg 1$$

$$\frac{c_B^I}{c_B^{II}} \ll 1$$

$$\alpha_{(A,B)} = \frac{c_A^I / c_A^{II}}{c_B^I / c_B^{II}} = \frac{c_A^I / c_B^I}{c_A^{II} / c_B^{II}}$$

α – separation factor

$$\alpha_{(A,B)} \gg 1$$

optimal α value

it is **necessary** that there is at least **one different** physico-chemical property

a driving force is based on such property that then *transports* and *redistributes* the substances

another parameters influencing separation

: equilibrium – distribution, dissociation
: system structure – macroscopic, microscopic, molecular
: flow – hydrodynamics, hydrostatics
: mechanical processes – passing through pores

separation limitations

: **chemical**
:: equilibrium (of forms)
::: $A_1 \leftrightarrow A_2$

: **physical**
:: achievable conditions of separation
::: temperature, pressure...

:: thermodynamic aspects (1st & 2nd laws of thermodynamics)
::: **spontaneous change:** ΔS > 0 in isolated system

$$\Delta S = n \cdot R \cdot \ln \frac{V_{start}}{V_{end}}$$

diluting (ΔV≠0; ΔS>0)

(s e p a r a t i o n) → (s e p a r a t i o n)

mixing (ΔV=0; ΔS>0)

(s e p a r a t i o n) ← → (s) + (e) + (p) + (a) + (r) + (a) + (t) + (i) + (o) + (n)

separating (ΔV≠0; ΔS<0)

::: **spontaneous change:** ΔG < 0

$$\Delta G = \Delta H - T \cdot \Delta S \Rightarrow dG = V \cdot dp - S \cdot dT$$

spontaneous separation happens if
: work is done
: it is heated
: it is diluted

differential transport
: each substance somewhere/somehow else

aim in separation
: **maximise *separation*** and **minimise *dilution***
:: duel of driving forces of **separation** and **dispersion**

example 1

calculate the entropy change that follows separation of four derivatives of triptamine (5-methoxy-α-methyltryptamine, 5-methoxy-diisopropyltryptamine, 5-methoxy-dimetlyltryptamine and α-methyltryptamine), if the molar amount of each is 0.1 mmol.
after the separation, each derivative of triptamine occupies ¼ of original sample volume.

assess, whether the separation is spontaneous or not.

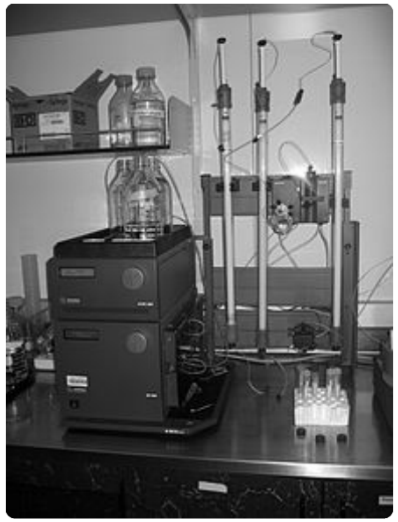
???

separation methods

there is no universal and absolute separation method
: fundamental (separation) limitations
: detection limitations

basic separation principles

: intermolecular interactions
: geometry of molecules
: external field influence



basic separation types

: analytical

:: high efficiency
:: mostly in small scale

: preparative

:: continuative
:: often in large scale

does not serve directly to analyse

preparative separation

: pre-step for instrumental analysis
: increases quality of analytical separation
: isolation of substances

:: enrichment (molar ratio of wanted component <0.1)
:: pre-concentration (<0.9)
:: purification (>0.9)

: laboratory (discrete and continual; small volumes)
: piloting (discrete and continual; large volumes)
: operating (continual; large volumes)

methods

: *discrete* – in separate/discrete steps
:: extraction, crystallisation, zone refining

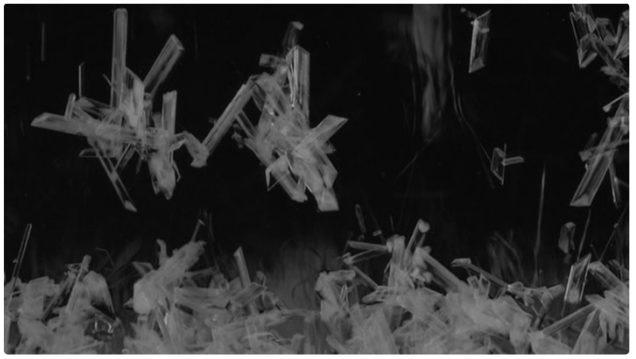
: *continuative* – in unseparated steps
:: counter-current extraction

: *continual* – in inseparable steps
:: filtration, electrolysis, distillation, chromatography

serves to analyse mixtures

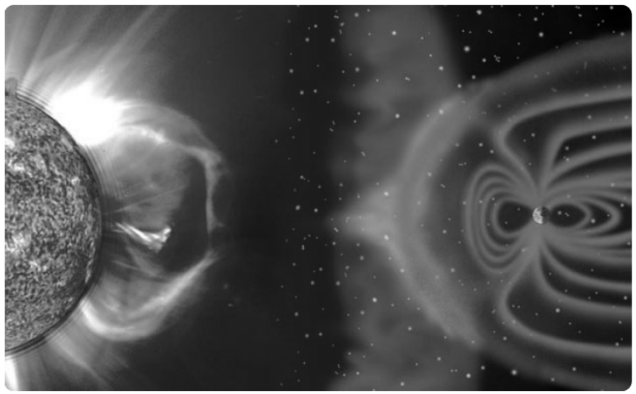
analytical separation

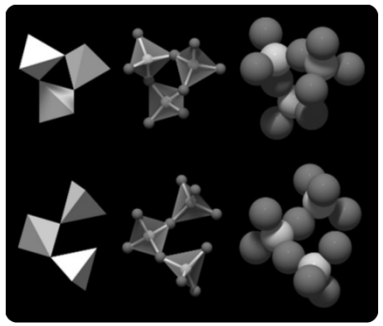
: instrumental analysis (of fully) separated components
: identification of a component
: characterisation of a component (structure, physico-chemical properties)



: distributive separation
:: chromatography

: separation in force field
:: electromigration
:: mass spectrometry
:: flow field fractionation

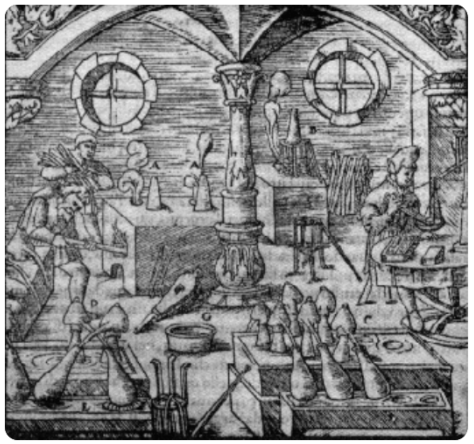




: separation based on molecule geometry

separation methods – **overview**

separation property	
volatility	distillation, sublimation
solubility	precipitation, zone refining, fraction crystallisation
distribution constant	extraction, partition chromatography (LL, GL)
dissociation constant	ion-exchange and affinity chromatography
surface activity	adsorption chromatography (LS, GS), foam separation
molecular geometry	molecular sieve
size + charge + mass	electrophoresis, flow field fractionation, mass spectrometry



two basic methods of separation

different phase distribution of components

: equilibrium – entropy influence

:: *rate* of mass transfer *through inter-phase* **isn't** controlling

: non-equilibrium

:: *rate* of mass transfer *through inter-phase* **is** controlling

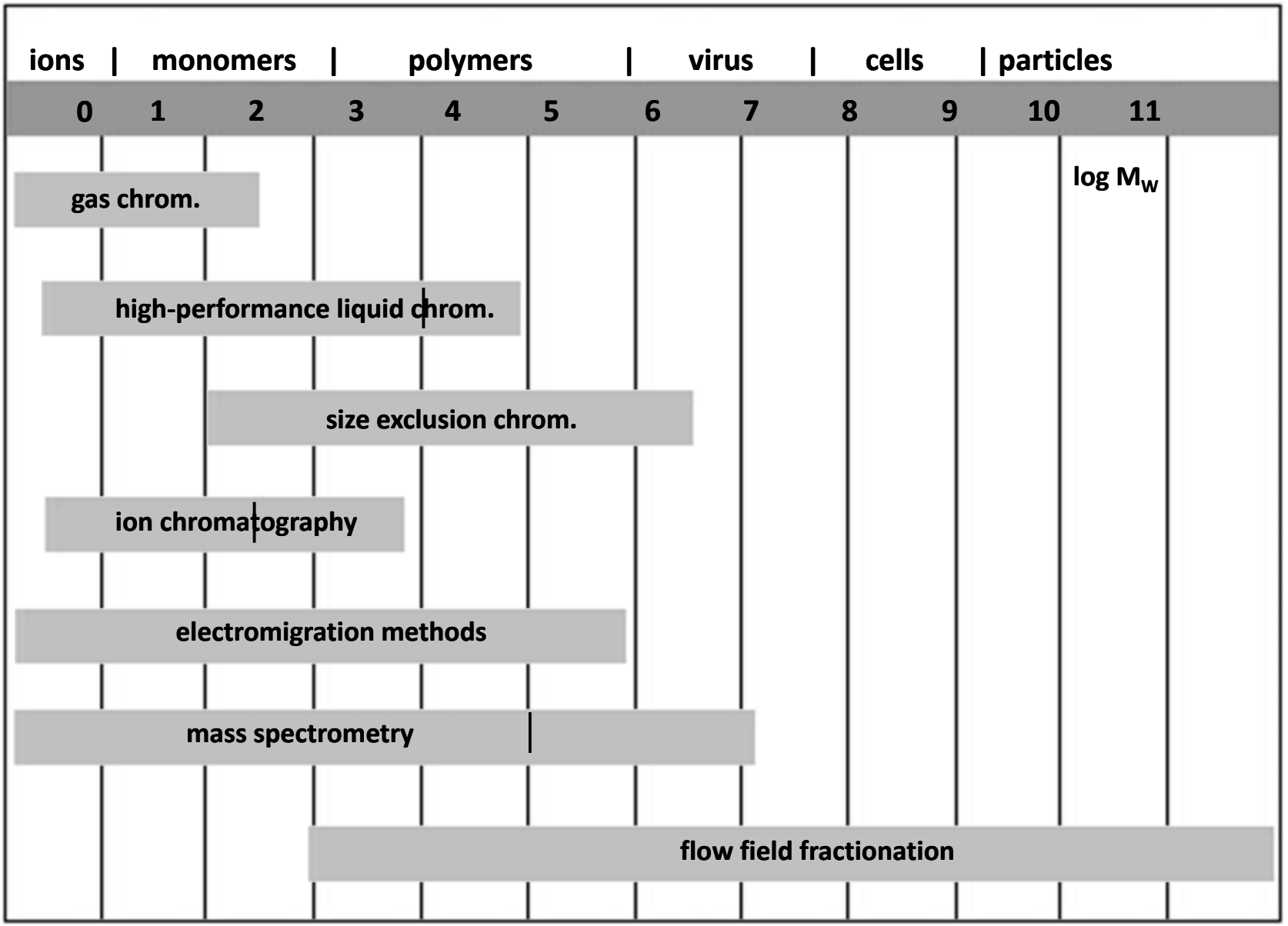
different rate of distribution of components

: through semi-permeable membrane

: in a force field

separation based on phase equilibria			
G – L	**G – S**	**L – L**	**L – S**
distillation	sublimation	extraction	precipitation
foam separation	molecular sieve	partition LC	fraction crystallisation
partition GC	adsorption GC	molecular exclusion LC	molecular sieve
			zone refining
			adsorption LC
			ion-exchange LC

separation based on differences in motion rate	
through membrane	**in field**
ultrafiltration	electromigration methods
reversed osmosis	flow fractionation
dialysis, electrodialysis	mass spectrometry, ion mobility
	ultracentrifugation
	thermodiffusion


ions | monomers | polymers | virus | cells | particles
0 1 2 3 4 5 6 7 8 9 10 11
log M_w
gas chrom.
high-performance liquid chrom.
size exclusion chrom.
ion chromatography
electromigration methods
mass spectrometry
flow field fractionation

molecular equilibrium

equilibrium in a closed system

system boundaries impermeable to mass

$$\Delta G = \Delta H - T \cdot \Delta S \Rightarrow dG = V \cdot dp - S \cdot dT$$

$\Delta G \leq 0$

spontaneous processes

water-ice 9 °C, 101.33 kPa

$\Delta G = 0$

equilibrium

water-ice 0 °C, 101.33 kPa

equilibrium in an open system

system boundaries permeable to mass

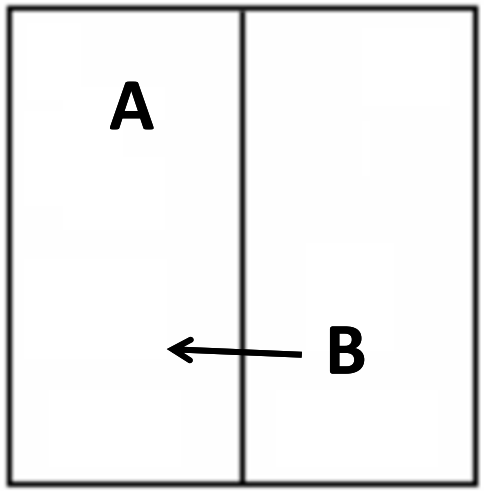
$$dG = \left(\frac{\partial G}{\partial n_B}\right)_{T,p,n_A} \cdot dn_B = \mu_B \cdot dn_B$$

μ_B – chemical potential

: change of G with amount of entering substance B

in → $dn_B > 0$

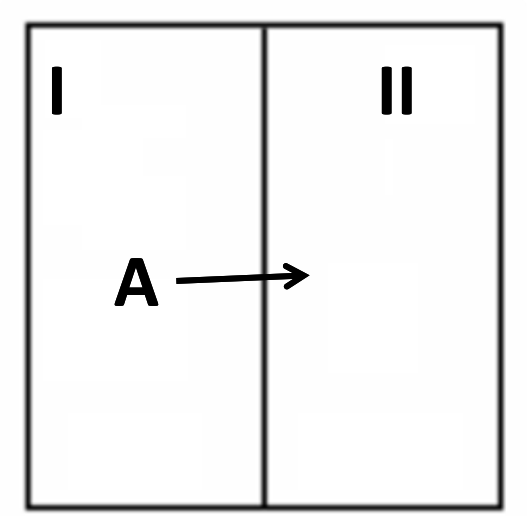
out → $dn_B < 0$



$$dG = V \cdot dp - S \cdot dT + \sum \mu_i \cdot dn_i$$

$$dG = \sum \mu_i \cdot dn_i$$

generalisation for ***i*** of substances at constant **T** and **p**



of two open systems

system of two immiscible phases creates closed system
: equilibrium sets of substance **A** between phases **I** and **II**

$$dG = 0 \qquad dG^{I} = -\mu_A^{I} \cdot dn_A \qquad dG^{II} = \mu_A^{II} \cdot dn_A$$

$$dG = dG^{I} + dG^{II} = \left(\mu_A^{II} - \mu_A^{I}\right) \cdot dn_A = 0 \quad \Rightarrow \mu_A^{I} = \mu_A^{II}$$

chemical potential of substance **A** in phase *X* (μ_A^X) depends on
: internal thermodynamic affinity of the substance to given phase; ↑ affinity ⇒ ↓ μ_A^0
: dilution of the substance (entropy of dilution influence); ~ $R \cdot T \cdot \ln a_A$

$$\mu_A = \mu_A^0 + R \cdot T \cdot \ln a_A$$

μ_A^0 – standard chemical potential (in given phase)

a_A – activity of substance A

practically in separations, we work with so-called *diluted solutions*

$$\mu_A = \mu_A^0 + R \cdot T \cdot \ln \gamma_A \cdot c_A \Rightarrow \mu_A^0 + R \cdot T \cdot \ln c_A$$

γ_A – activity coefficient of substance A

$\gamma_A \rightarrow 1$ and $c_A \rightarrow 0$

$$\mu_A = \mu_A^0 + R \cdot T \cdot \ln p_A$$

p_A – partial pressure of substance A (gas chromatography)

resulting ratio of substance A concentration in equilibrium in phases **I** and **II**

$$\Delta\mu_A^0 = \mu_A^{0,II} - \mu_A^{0,I}$$

$\Delta\mu_A^0$ controls distribution of substance between two phases

$$\left(\frac{a_A^{II}}{a_A^{I}}\right)_{dG=0} = e^{\left(\frac{-\Delta\mu_A^0}{R \cdot T}\right)} = K$$

K – distribution coefficient

$$\Delta G^0 = -R \cdot T \cdot \ln K$$

at constant pressure

$$\left(\frac{c_A^{II}}{c_A^{I}}\right)_{dG=0} = e^{\left(\frac{-\Delta\mu_A^0}{R \cdot T}\right)} = D$$

D – distribution ratio

$\gamma_A \rightarrow 1$ and $c_A \rightarrow 0$

equilibrium in open system with external field

system boundaries are permeable for energy of the field

: influences potential energy, which is additive to Gibbs free energy

$$\mathbf{dG = V \cdot dp - S \cdot dT + \sum \left(\mu_i^{int} + \mu_i^{ext}\right) \cdot dn_i}$$

$$\mathbf{dG = \sum \left(\mu_i^{int} + \mu_i^{ext}\right) \cdot dn_i}$$ generalisation for ***i*** of substances at constant **T** and **p**

$$\mathbf{dG = dG^{int} + dG^{ext} = \left(\Delta\mu_A^{int} + \Delta\mu_A^{ext}\right) \cdot dn_A = 0}$$

$\mathbf{\Delta\mu_A^{int} = \Delta\mu_A^0}$ $\Delta\mu_A^{int}$ is changed abruptly on phase boundary

$\mathbf{\Delta\mu_A^{ext} = \mu_A^{ext,II} - \mu_A^{ext,I}}$ $\Delta\mu_A^{ext}$ changes continuously in space $\mathbf{D = \left(\frac{c_A^{II}}{c_A^{I}}\right)_{dG=0} = e^{\left(\frac{-\Delta\mu_A^0 - \Delta\mu_A^{ext}}{R \cdot T}\right)}}$

separation **in external** field ***is different*** from separation **between phases**

intermolecular interaction

$$\mathbf{dG} = \left(\frac{\partial \mathbf{G}}{\partial \mathbf{n_B}}\right)_{\mathbf{T,p,n_A}} \cdot \mathbf{dn_B} = \boldsymbol{\mu_B} \cdot \mathbf{dn_B} \qquad \Delta\overline{\mathbf{G}}_{\mathbf{A}}^{\mathbf{0}} = \left(\frac{\partial \mathbf{G}}{\partial \mathbf{n_A}}\right)_{\mathbf{T,p,n}} = \Delta\boldsymbol{\mu}_{\mathbf{A}}^{\mathbf{0}}$$

enthalpy influence

$$\Delta\boldsymbol{\mu}_{\mathbf{A}}^{\mathbf{0}} = \Delta\overline{\mathbf{G}}_{\mathbf{A}}^{\mathbf{0}} = \Delta\overline{\mathbf{H}}_{\mathbf{A}}^{\mathbf{0}} - \mathbf{T} \cdot \Delta\overline{\mathbf{S}}_{\mathbf{A}}^{\mathbf{0}}$$

$\Delta\overline{G}_A^0$ – partial molar Gibbs energy
: influence of molar content change of component on system properties

$\Delta\overline{S}_A^0$ – partial molar entropy
: randomness of actual molecular surround of substance A molecules

$$\left|\Delta\overline{\mathbf{H}}_{\mathbf{A}}^{\mathbf{0}}\right| \gg \left|\mathbf{T} \cdot \Delta\overline{\mathbf{S}}_{\mathbf{A}}^{\mathbf{0}}\right|$$

within distribution of substance A between two phases

$\Delta\overline{H}_A^0$ – partial molar enthalpy
: intermolecular interactions of substance A & substance of phases I & II

$$\Delta\overline{\mathbf{H}}_{\mathbf{A}}^{\mathbf{0}} = \overline{\mathbf{H}}_{\mathbf{A}}^{\mathbf{0,I}} - \overline{\mathbf{H}}_{\mathbf{A}}^{\mathbf{0,II}}$$

$$\Delta\overline{\mathbf{H}}_{\mathbf{A}}^{\mathbf{0}} < \mathbf{0} \Rightarrow \Delta\boldsymbol{\mu}_{\mathbf{A}}^{\mathbf{0}} < \mathbf{0} \qquad \left(\frac{\mathbf{c}_{\mathbf{A}}^{\mathbf{II}}}{\mathbf{c}_{\mathbf{A}}^{\mathbf{I}}}\right)_{\mathbf{dG=0}} = \mathbf{e}^{\left(\frac{-\Delta\boldsymbol{\mu}_{\mathbf{A}}^{\mathbf{0}}}{\mathbf{R}\cdot\mathbf{T}}\right)} = \mathbf{D}$$

exponent would have positive value $\Rightarrow \mathbf{D} > \mathbf{1}$

the weaker the interaction → the lower the enthalpy

→ substance A would appear in higher amounts in phase II, because $\overline{\mathbf{H}}_{\mathbf{A}}^{\mathbf{0,I}} < \overline{\mathbf{H}}_{\mathbf{A}}^{\mathbf{0,II}}$

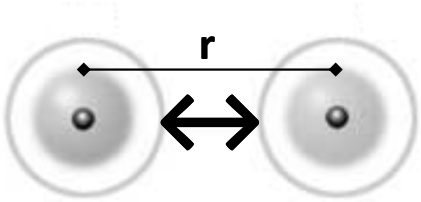
description of intermolecular interactions

intramolecular forces

: water dissociation $H_2O \rightarrow 2\ H + O$; **837** kJ·mol^{-1}

intermolecular forces

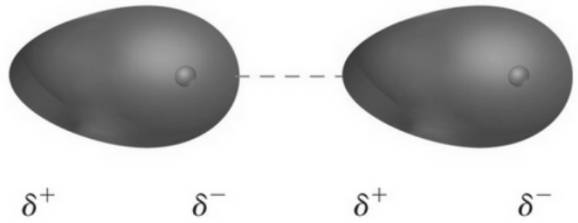
: water evaporation H_2O (*l*) → H_2O (*g*); **41** kJ·mol^{-1}



$$\mathbf{E_{intermol} = E_{attract} - E_{repulse}}$$

$$\mathbf{E} = f(\mathbf{r^{-x}})$$

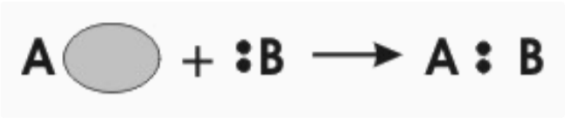
intermolecular forces = van der Waals forces = weak forces



electrostatic interactions

: ion-ion; dipole-dipole, ion-dipole, dipole-induced dipole, disperse forces (London)

:: hydrogen bridges – special case of dipole-dipole bond (take part in e.g. solvation)



Lewis interactions (theory of hard and soft acids and bases)

: co-ordination covalent, sharing of electron pairs and free binding orbitals

$$\Delta\overline{H}_A^0 = \sum_{x=\text{interaction}} E_x$$

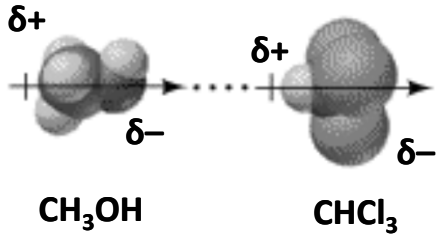
interaction types

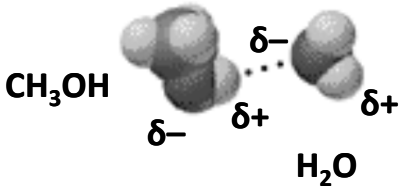
dipole-dipole (E_D)

: substances with permanent dipoles – polar

:: e.g. water, alcohols, halogen-hydrocarbons...

:: intensity depends on temperature





hydrogen bridges

: special case of dipole-dipole interaction

: strong interaction

: the most general example of Lewis interaction (soft)

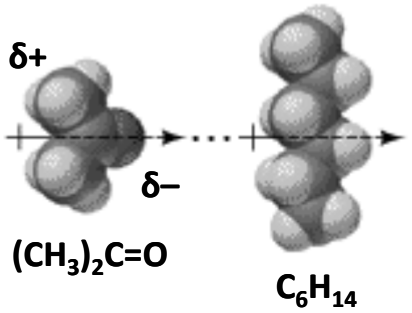
inductive (E_I)

: induced dipoles – solvation by permanent dipoles

: weak interaction

:: e.g. water, ammonium, alcohols...

:: intensity depends not on temperature



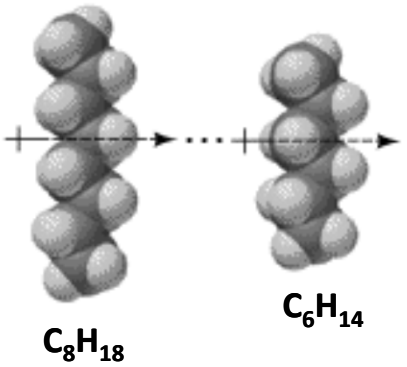
disperse (E_L)

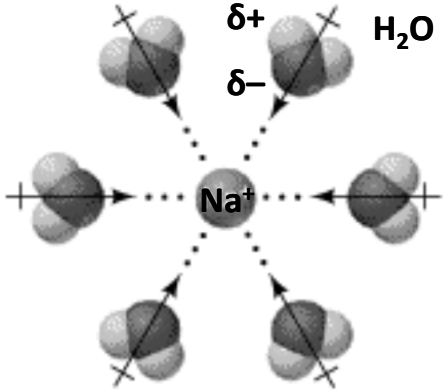
: „momentarily“ induced dipoles – primary non-polar

:: London forces

: weak interactions

:: e.g. CCl_4, benzene...





ionic (E_{AB})

: coulombic interactions between charged groups

: or between permanent dipoles and charged groups

: Lewis interactions (hard)

: strong interactions

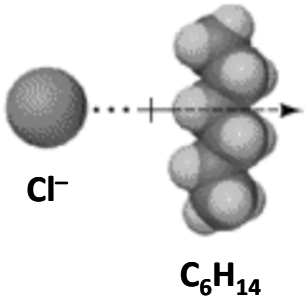
:: e.g. water, ammonium, alcohols...

inductive ionic

: interaction ion-induced dipole

: weak interactions

:: e.g. I_3^- bond



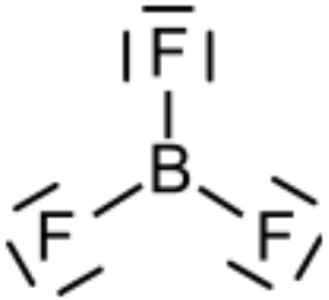
relative strength of interactions

$$E_L < E_I < E_D < E_{AB}$$

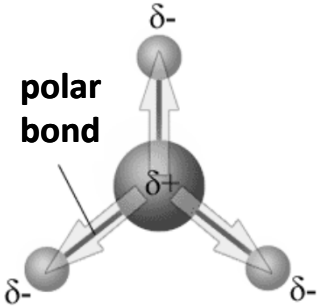
polarity description

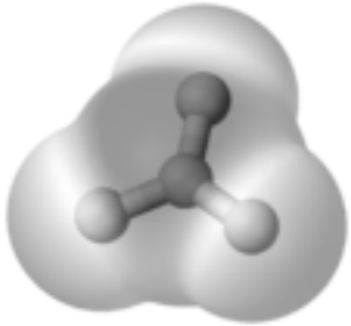
polarity

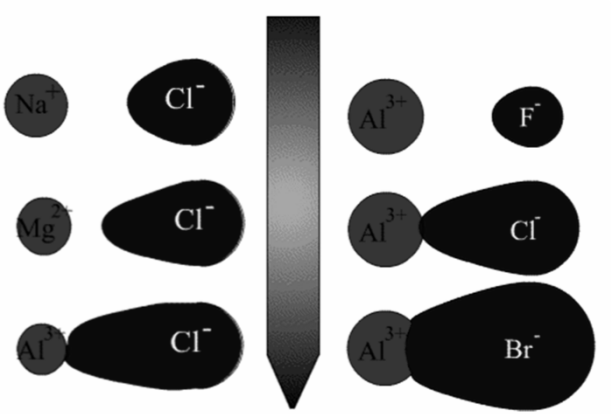
: electric charge distribution leading to molecule as electric dipole



boron trifluoride







polarisability (α)

: measure of easiness of molecular electron clouds deformation

$$\mathbf{p} = \boldsymbol{\alpha} \cdot \mathbf{E}$$

p – dipole moment, **E** – electric field intensity

$$\boldsymbol{\alpha} = \left(3\boldsymbol{\pi} \cdot \frac{\mathbf{N}}{\mathbf{4}}\right) \cdot \left(\frac{\mathbf{n^2 - 1}}{\mathbf{n^2 + 2}}\right)$$

N – Avogadro constant, **n** – refraction index

the most often used **descriptors of polarity**
: octanol-water **partition coefficient** ($D_{o/w}$; P; logP; $logP_{o/w}$)

$$\mathbf{D_{o/w} = \frac{c_A^{octanol}}{c_A^{water}}}$$

: not suitable for ionisable substances
:: octanol-water **distribution coefficient** ($K_{D,o/w}$; D)

$$\mathbf{K_{D,o/w} = \frac{a_A^{octanol}}{a_{A_{ionis}}^{water} + a_{A_{neutr}}^{water}}}$$

$$\mathbf{\log K_{D,o/w,acid} = \log D + \log\left[\frac{1}{(1 + 10^{pH - pK_a})}\right] \underset{(pH - pK_a) > 1}{\Rightarrow} \log K_{D,o/w,acid} \cong \log D + pK_a - pH}$$

$$\mathbf{\log K_{D,o/w,base} = \log D + \log\left[\frac{1}{(1 + 10^{pK_a - pH})}\right] \underset{(pK_a - pH) > 1}{\Rightarrow} \log K_{D,o/w,base} \cong \log D - pK_a + pH}$$

other interaction descriptions

relative solvent permittivity (ε)

: measure of intermolecular interactions in liquids

$$\varepsilon = \frac{\mathbf{D}}{\mathbf{E}}$$ **D** – electric induction, **E** – electric field intensity

$$\mathbf{D} = \varepsilon_0 \cdot \mathbf{E} + \alpha$$ ε_0 – permittivity of vacuum

solvents used

water miscible		water immiscible	
	ε		ε
water	78	nitrobenzene	35
methanol	32	amylalcohol	16
1-propanol	21	ethylacetate	6
pyridine	12	methyl-isobutylketon	13
dioxane	2	chloroform	5
		benzene	2
		hexane	2

Hildebrand solubility parameter (δ)

: measure of intermolecular interactions in substance it-self

$$\delta = \sqrt{\frac{\Delta E_{vap}}{V}}$$

Δ E_{vap} / V – cohesive energetic density of substance

energy necessary to evaporate a substance relative to volume

: i.e. measure of interaction between molecules

substance mixing

$$\Delta G_{mix} = \Delta H_{mix} - T \cdot \Delta S_{mix}$$

$$\Delta H_{mix} = \overline{V}_A \cdot (\delta_A - \delta_B)^2$$

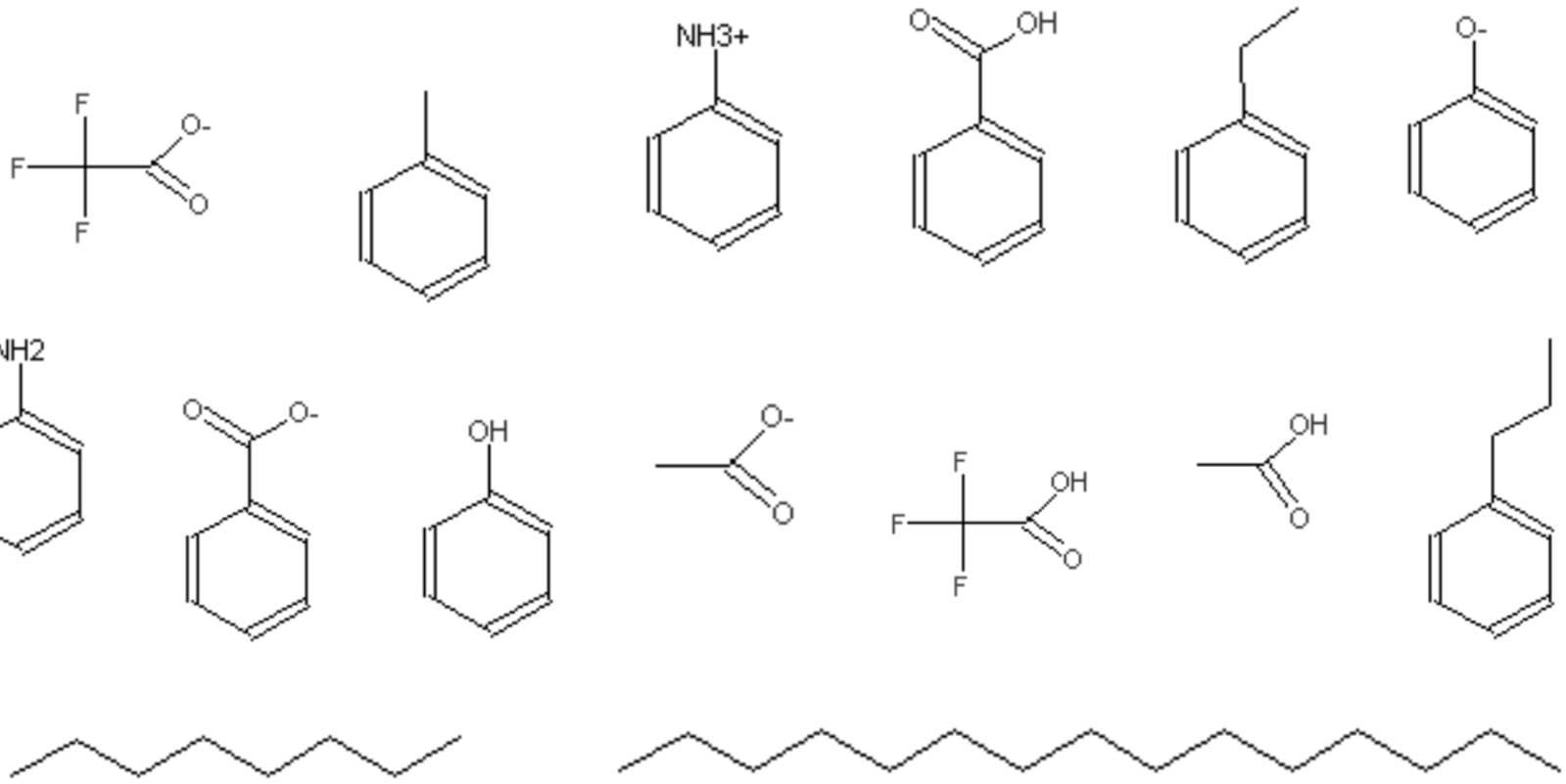
$\overline{V}_A$ – molar volume of pure dissolved substance

A – dissolved substance (*solute*)

B – dissolving substance (*solvent*)

example 2
: part 1

: sort substances by increasing the polarity



example 2 : part 2

???

molecular geometry and structure

entropy influence

$\Delta\overline{S}_A^0$: randomness of actual molecular surround of substance A molecules

i.e. how the molecule of dissolved substance „fits“ between molecules of solvent

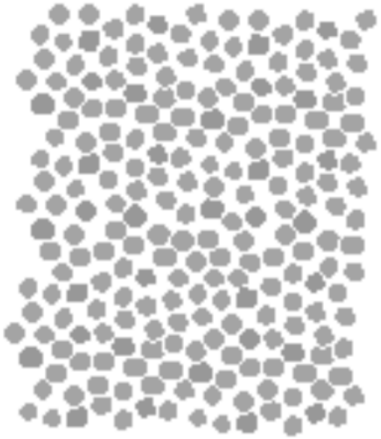
: measure of necessary re-orientation and re-positioning of molecules

$$\Delta\boldsymbol{\mu}_{\mathbf{A}}^{\mathbf{0}} = \Delta\overline{\mathbf{H}}_{\mathbf{A}}^{\mathbf{0}} - \mathbf{T}\cdot\Delta\overline{\mathbf{S}}_{\mathbf{A}}^{\mathbf{0}}$$

in most cases within distribution of substance A between two phases

$$\left|\Delta\overline{\mathbf{H}}_{\mathbf{A}}^{\mathbf{0}}\right| \gg \left|\mathbf{T}\cdot\Delta\overline{\mathbf{S}}_{\mathbf{A}}^{\mathbf{0}}\right|$$

so when does **entropy** influence the separation?

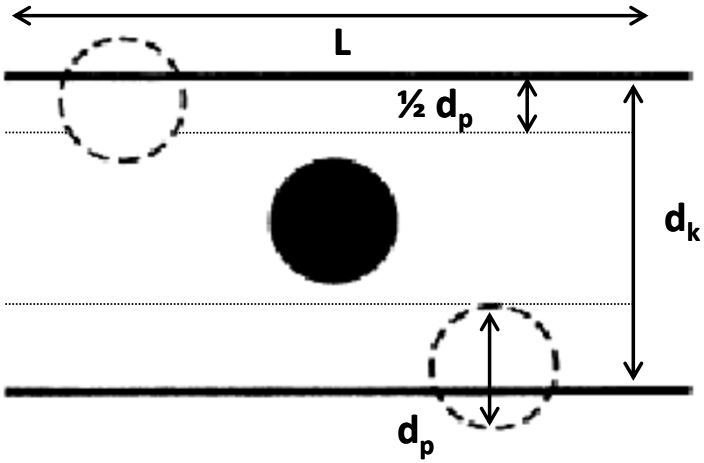


: in a case of high difference between polarity of substance A and solvent

:: re-arrangement of solvent molecules (semi-rigid structure)

: in a case of sieve effect

:: separation on porous media



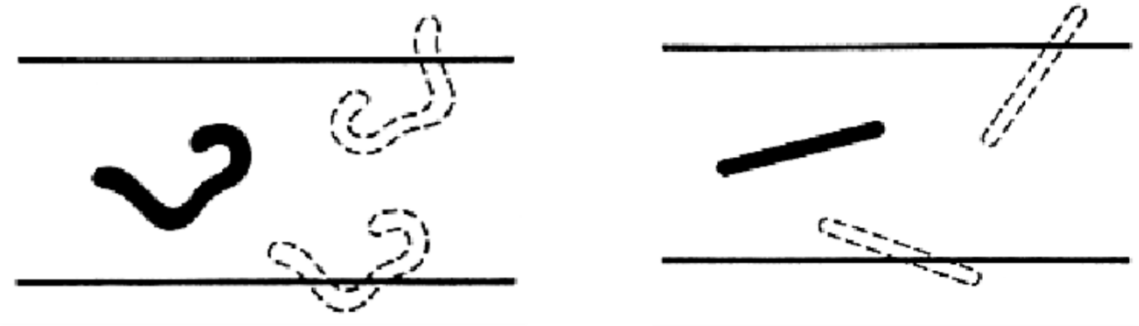
porosity influence

$$\left(\frac{c_A^{available}}{c_A^{total}}\right)_{dG=0} = D$$

d_p – particle diameter
d_k – separation channel diameter
L – separation channel length

$$D = \frac{\pi \cdot (0.5d_k - 0.5d_p) \cdot L}{\pi \cdot (0.5d_k) \cdot L} = \frac{d_k - d_p}{d_k^2} = \left(1 - \frac{d_p}{d_k}\right)^2 = \left(1 - \frac{s \cdot 0.5d_p}{2}\right)^2$$

s – channel wall surface relative to volume unit $\quad d_k = 4/s$



$$D = e^{\left(-\frac{s \cdot \bar{L}}{2}\right)}$$

$\bar{L}$ – average contour length
: for sphere equal to d_p

extraction

method based on **substance distribution** between **two immiscible phases**

phases used

: ***liquid-liquid*** (most common)
:: batch extraction – one portion of extractant
:: perforation – continuous circulation of extractant through liquid raffinate
:: continuous extraction – repeated extraction by extractant portion
::: partition chromatography – continuative variant

: ***liquid-solid*** *(leaching / infusing)*
:: maceration – infusion into one portion of extractant at ambient temperature
:: digestion – infusion into one portion of extractant at elevated temperature
:: percolation – continuous infusion of flowing extractant

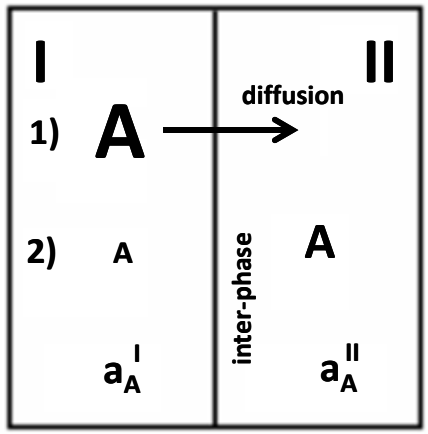
: ***liquid-gas***

: ***solid-gas***

: ***solid-supercritical fluid***

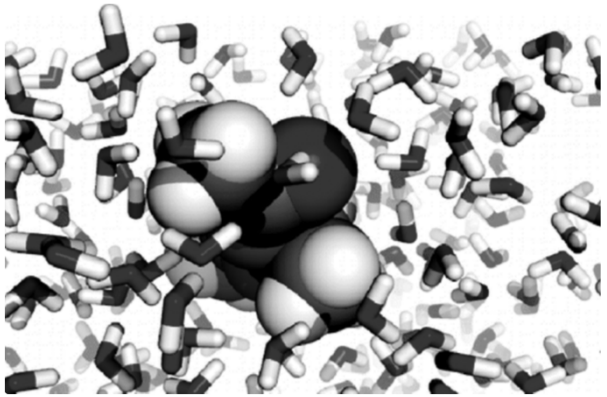
extraction liquid-liquid

equilibrium state of compound in a system **of two immiscible liquids**
: two phases; phase **I** and phase **II**



1) original state

2) after equilibration



$\mathbf{a_A}$ – activity of substance **A** in phases **I** and/or **II**

sometimes *more polar* phase (water, **w**) and phase *less* **polar** (organic, **o**)

extraction process
: transport in phase I – inter-phase transfer – transport in phase II

raffinate
: phase, which originally contained sample

extractant
: phase, into which we extract

extract
: extracted sample

menstruum
: extractant in L-S extraction

marc
: what remained left after extraction

conditions
: pH, masking agents, etc...

distribution constant (K_D)
: defines relation of separated substance to both phases

$$\mathbf{K_D = \frac{a_A^{II}}{a_A^{I}}} \qquad \mathbf{K_D = \frac{a_A^{II}}{a_A^{I}} = \lim_{[A]\to 0} \frac{c_A^{II}}{c_A^{I}}}$$

Nernst distribution law; Nernst distribution constant

distribution ratio (D)
: conditional distribution constant; depends on side reactions

$$\mathbf{D = \frac{c_A^{II}}{c_A^{I}}}$$

substitution of activity by analytical concentrations; easier determination

if the compound is present in same form in both phases $\mathbf{K_D = D \cdot \left(\gamma^{II}/\gamma^{I}\right) \Rightarrow D \approx K_D}$

γ – activity coefficients of compound in both phases

$$\mathbf{D = \frac{c_A^{II}}{c_A^{I}} = \frac{n_A^{II}/V^{II}}{n_A^{I}/V^{I}} = \frac{n_A^{II}}{n_A^{I}} \cdot \frac{V^{I}}{V^{II}} = \frac{n_A^{II}}{n_A^{I}} \cdot \frac{1}{V^{II/I}}}$$

$V^{II/I}$ – phase volumes ratio V^{II} and V^{I}

distribution constant expressed using **Hildebrand solubility parameters**

$$\mathbf{\ln K_D = -\frac{\bar{V}_A}{R \cdot T} \cdot \left(\delta^{I} - \delta^{II}\right) \cdot \left(\delta^{I} - \delta^{II} - 2\delta_A\right)}$$

distribution (mass) coefficient (D_M)

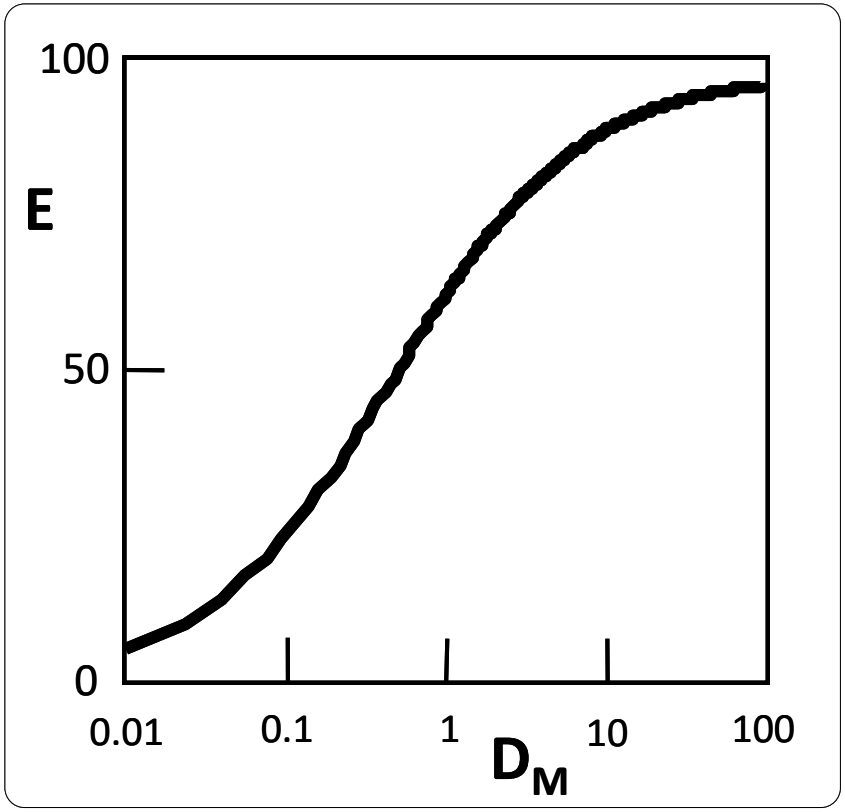
$$\mathbf{D = \frac{c_A^{II}}{c_A^{I}} \quad \Rightarrow \quad D_M = \frac{m_A^{II}}{m_A^{I}} = D \cdot \frac{V^{II}}{V^{I}} = D \cdot V^{II/I}}$$

n – molar amount of analyte in phase, **m** – weight of analyte in phase, **V** – phase volume

yield of separation (R_A)
yield of compound A

$$\mathbf{R_A = \frac{n_A^{II}}{n_A^{tot}} = \frac{n_A^{II}}{n_A^{II} + n_A^{I}} = \frac{n_A^{II}/n_A^{I}}{\left(n_A^{II}/n_A^{I}\right) + 1} = \frac{D \cdot V^{II/I}}{D \cdot V^{II/I} + 1}}$$

$$\mathbf{E_A[\%] = 100 \cdot R_A}$$



relation between $\mathbf{D_M}$ and extraction yield

$$\mathbf{E = 100 \cdot \frac{D_M}{D_M + 1}}$$

relation between **D** and extraction yield

$$\mathbf{E = 100 \cdot \frac{D \cdot V^{II/I}}{D \cdot V^{II/I} + 1}}$$

search for a such D_M, at which E would be max

: if $D_M > 1 \rightarrow E > 50$ %

: at $D_M > 10$ the increase in E is minimal

: when considering D & E relation, we have to consider $V^{II/I}$

: if we change the D value, it would change the E too

choice of extraction system

extraction system is given by ***properties of***

: **extracted substance**
: **and both participating phases**

similia similibus solvuntur
paria paribus

extractant
: non-polar
:: unpolarisable
:: polarisable
: polar
: ionic

+ salt
: increasing salt concentration means decreasing dielectric constant of water
: binds solvent in solvation sphere
: Δ ion concentration leads to extraction equilibrium shift

extracted substance

polar substance
: extractant must be solvent more polar than sample solvent

non-polar substance
: extractant must be solvent less polar than sample solvent

numeric value of **D**
might be changed by equilibria

simple extraction

$$\mathbf{D = K_D \geq 10}$$

association in organic phase

benzoic acid; water / benzene

$$\mathbf{D = K_D \cdot \left(1 + 2K_{dim} \cdot [HA]^{II}\right)}$$

$2\,(HA)^{II} \xrightarrow{K_{dim}} (HA)_2^{II}$

presence of complexation agent

I_2; CCl_4 / water + I^-

$$\mathbf{\log D = \log K_D - (\log \beta_n \cdot [I^-])}$$

$I_2 + I^- \leftrightarrow I_3^-$

$\beta_n = [I_3^-] / ([I_2] \cdot [I^-]) \Rightarrow D = [I_2]^{II} / ([I_2]^{I} + [I_3^-]^{I})$

pseudomolecular system

RCOOH; phase I / II

$$\mathbf{D = \frac{K_D}{\left(1 + \frac{1}{K_D} \cdot [H^+]\right)}}$$

$RCOO^- + H^+ \leftrightarrow RCOOH$

$K_{HA} = [RCOOH] / ([RCOO^-] \cdot [H^+])$

$K_D = [RCOOH]^{II} / [RCOOH]^{I}$

$\rightarrow D = [RCOOH]^{II} / ([RCOOH]^{I} + [RCOO^-]^{I})$

system with metal chelates

$H^+ + L^- \leftrightarrow HL$ $\qquad$ $M(H_2O)_m^{n+} + L^- \leftrightarrow ML_n + m\ H_2O$

M^{n+}; phase I / II + HL

HL, ML_n

$K_D = f$ ([H^+], chelatogenic agent)

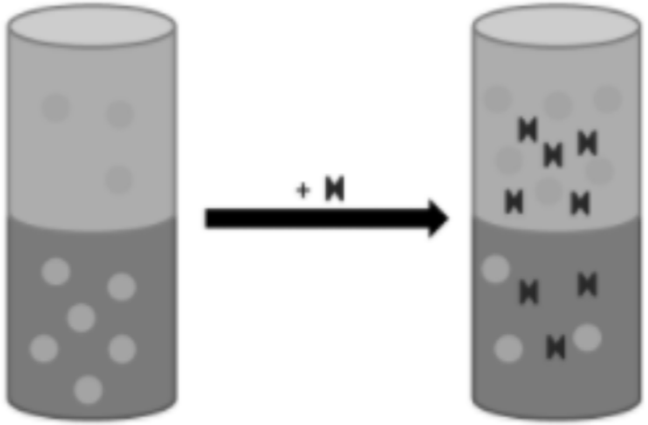
system with ionic associates

$K_D = f$ ([H^+], chelatogenic agent, ε, salting-out agent)

: liquid ion-exchangers

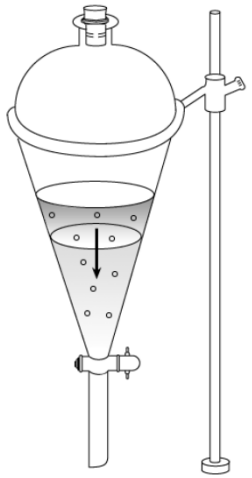
:: *ternary amines* – tri(2-ethylhexyl)amine; methyldioctylamine

: onionic systems (oxonionic ions)



process of extraction

: batch extraction
:: single and multi-step
: continuous extraction
:: for low D, continual flow of solvent in raffinate solution



phase transfer goes on on inter-phase
: maximal inter-phase area
: from each place in solution as close as possible to inter-phase

shaking the mixture of both phases in closed container

we care **not to create** *an emulsion*
: **it slows** phase separation

solvents which are mutually **immiscible**, but always **mutually soluble**
→ **change of volume** in contrast to the volume before shaking
→ *use of solvents* ***pre-saturated with the other phase***

example 3

determine the influence of other types of equilibria than distribution (dimerisation in organic phase, dissociation in aqueous phase) on extraction of benzoic acid from water to benzene. K_D = 10, K_{dim} = 0.9 $mol^{-1} \cdot l$, pK_a = 4.18, content of benzoic acid in benzene after extraction is 0.66 M.

???

extraction liquid-solid

column separation techniques, extraction on solid phase

principle: adsorption from liquid phase on a solid, then desorption

$$K_D = \frac{a_A^{II}}{a_A^{I}} \Rightarrow c_A^{II} = K_D \cdot c_A^{I} \Rightarrow n_A^{II} = f\left(c_A^{I}\right)$$

empiric formulas

sorption description – adsorption isotherm

II – stationary phase (*s*)
I – mobile phase (*l*)

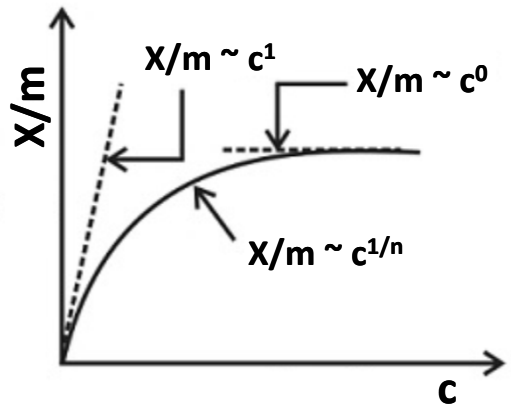
Freundlich isotherm

$$n_A^{II} = k_f \cdot \left(c_A^{I}\right)^n = k_f \cdot \left(p_A^{I}\right)^n$$

k_f – Freundlich adsorption constant
n = 0.4 – **1.0 (ideal)**

$$\frac{X}{m} = a \cdot c^{\frac{1}{n}}$$

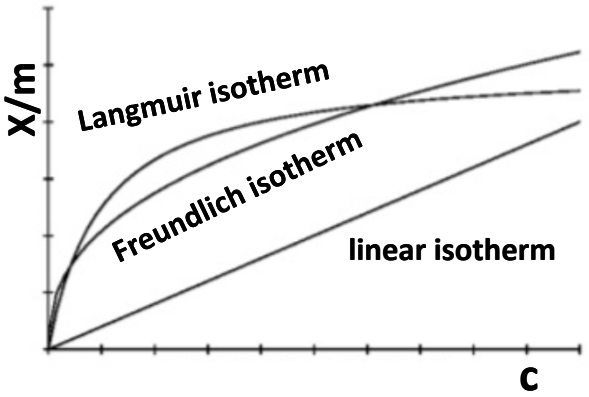
X – adsorbed quantity (g, mol)
m – weight of sorbent (g)
c – equilibrium concentration (mol·l^{-1})
a, n – constants



Langmuir isotherm

$$n_A^{II} = k_1 \cdot \frac{k_2 \cdot c_A^I}{1 + k_2 \cdot c_A^I}$$

k_2 – Langmuir adsorption constant
k_1 – maximal number of binding sites



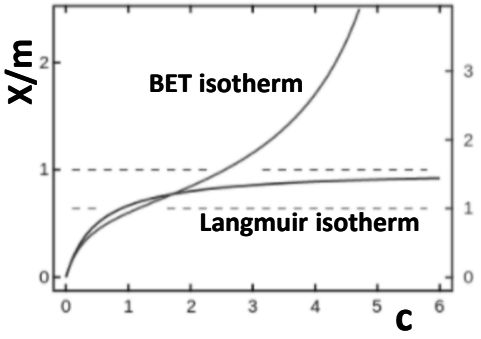
$$\frac{X}{m} = \frac{a \cdot c}{c + b}$$

X – adsorbed quantity (g, mol)
m – weight of sorbent (g)
c – equilibrium concentration (mol·l^{-1})
a – max. adsorbed quantity
b – constant depending on a sorbent kind & size, sorbed substance & solvent properties

Brunauer-Emmett-Tellerova isotherm

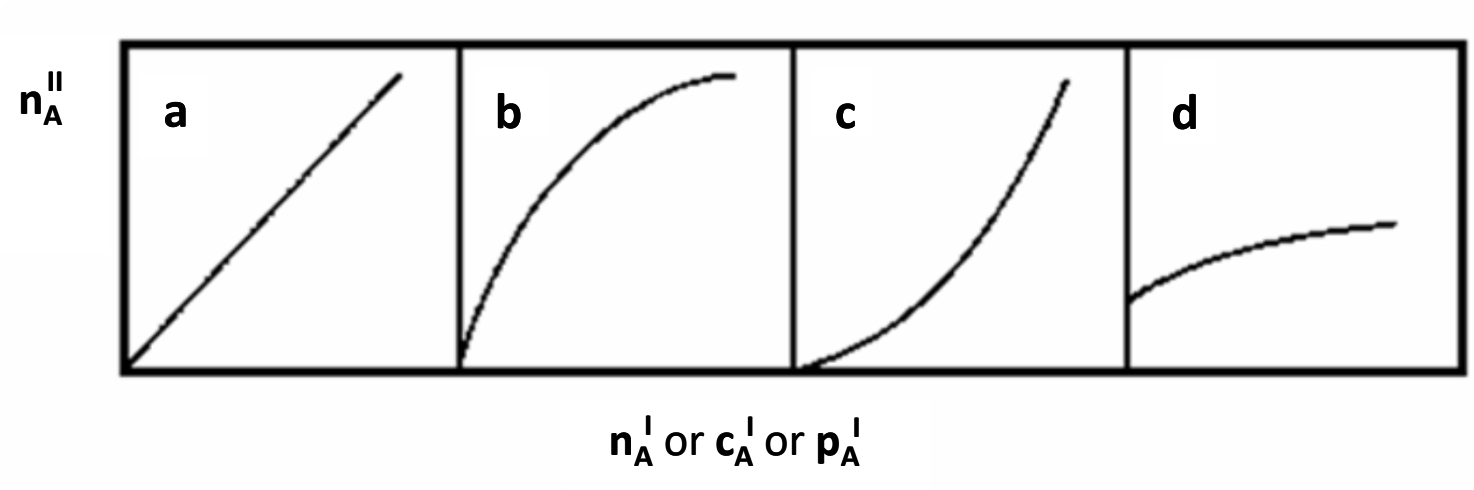
$$n_A^{II} = n_m \cdot \frac{C \cdot p_r}{(1 - p_r) \cdot (1 + p_r \cdot (C - 1))}$$

$$p_r = \frac{p_A^I}{p_A^{II}} \qquad C = e^{\left(\frac{Q_{ads} - Q_{cond}}{R \cdot T}\right)}$$

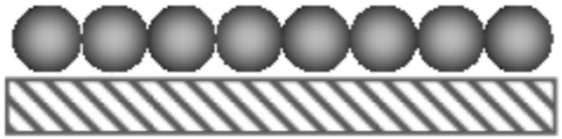


p_A^I – equilibrium vapour tension
p_A^{II} – adsorbate vapour tension
p_r – relative vapour tension
C – constant
n_m – volume of monomolecular layer of adsorbate
Q_{ads} – adsorption heat
Q_{kond} – condensation heat

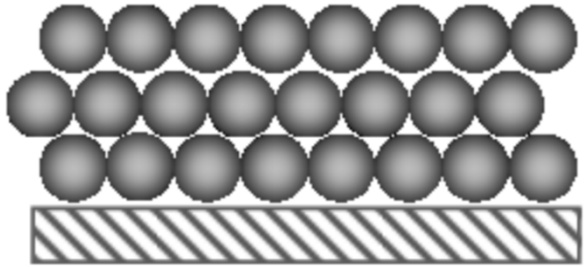
basic types of isotherms



isotherms: a – linear, **b** – Langmuir, **c** – anti-Langmuir, **d** – chemisorption



Langmuir isotherm



anti-Langmuir isotherm

description of single component extraction

separation yield (R_A)
yield of compound A

$$R_A = \frac{n_A^{II}}{n_A^{tot}} = \frac{n_A^{II}}{n_A^{II} + n_A^{I}} = \frac{n_A^{II}/n_A^{I}}{\left(n_A^{II}/n_A^{I}\right) + 1} = \frac{D \cdot V^{II/I}}{D \cdot V^{II/I} + 1}$$

remnant of substance A after extraction

$$\frac{n_A^{I}}{n_A^{tot}} = \frac{1}{1 + D \cdot V^{II/I}} \Rightarrow n_A^{I} = n_A^{tot} \cdot \left(\frac{1}{1 + D \cdot V^{II/I}}\right) \Rightarrow c_A^{I} = c_A^{tot} \cdot \left(\frac{1}{1 + D \cdot V^{II/I}}\right)$$

repeated extraction

: batch extraction
:: discrete, incontinuative
::: *small yield*

potentially solvable by phase ratio change ($V^{II/I} > 10$)
: too large disproportion of volumes is not suitable
:: problems with manipulation & mutual solubility of phases

solution
: continuous extraction
:: **repeated extraction** and fusing of organic phase thereafter

1st step

$$n_{A_1}^{I} = n_A^{tot} \cdot \left(\frac{1}{1 + D \cdot V^{II/I}}\right)$$

2nd step

$$n_{A_2}^{I} = n_{A_1}^{I} \cdot \left(\frac{1}{1 + D \cdot V^{II/I}}\right)$$

*i*th step

$$n_{A_i}^{I} = n_A^{tot} \cdot \left(\frac{1}{1 + D \cdot V^{II/I}}\right)^i$$

extraction remnant after *i*th step

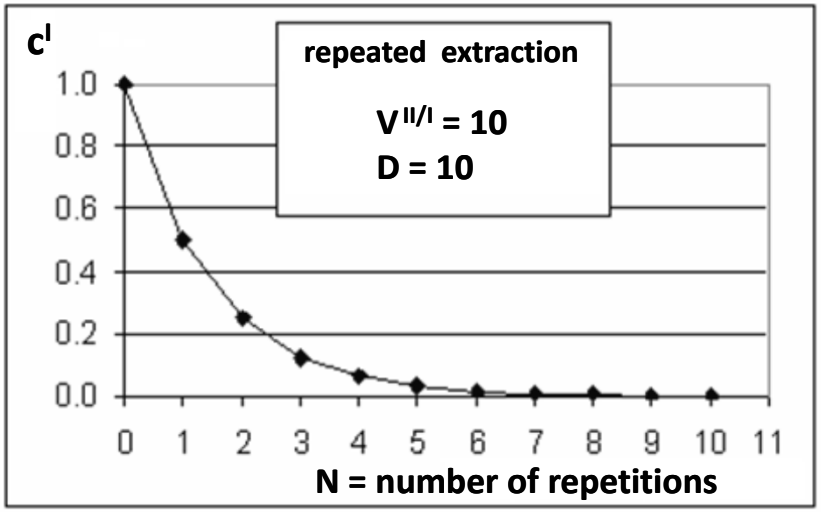
$$\frac{n_{A,i}^{I}}{n_A^{tot}} = \left(\frac{1}{1 + D \cdot V^{II/I}}\right)^i \Rightarrow n_{A_i}^{I} = n_A^{tot} \cdot \left(\frac{1}{1 + D \cdot V^{II/I}}\right)^i \Rightarrow c_{A_i}^{I} = c_A^{tot} \cdot \left(\frac{1}{1 + D \cdot V^{II/I}}\right)^i$$

extraction yield after *i*th step

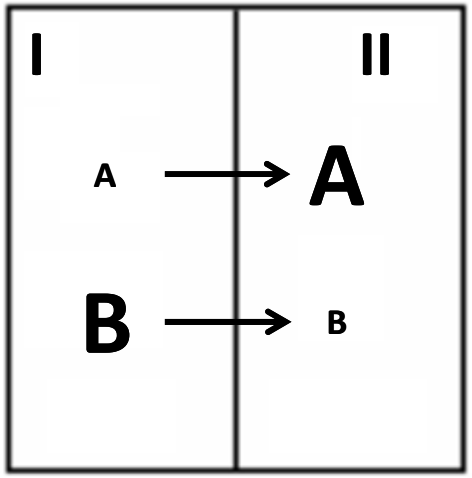
$$c_{A_i}^{I} = c_A^{tot} \cdot \left(\frac{1}{1 + D \cdot V^{II/I}}\right)^i$$

$$E_{A_i} = \left[1 - \left(\frac{100 - E}{100}\right)\right]^i \cdot 100$$

$$R_{A_i} = 1 - \left(1 - R_{A_1}\right)^i$$



description of multiple component extraction



: mixture contains at least compounds **A** and **B**
: component **A** passes to **phase II**
: component **B** stays in **phase I**

ideal separation state **A → II, B → I**

real separation **A + B → II, B + A → I**

distribution separation factor ($\alpha_{(A,B)}$)
: is measure of selectivity

$$\alpha_{(A,B)} = \frac{c_A^{II}/c_B^{II}}{c_A^{I}/c_B^{I}}$$

$\alpha_{OPTIM} = 10^6$

$$\alpha_{optim} = \frac{\left(\frac{99.9}{0.10}\right)^{II}}{\left(\frac{0.10}{99.9}\right)^{I}}$$

at what extent will the required *compound A* **be separated** *from compound B*?

$$\alpha_{(A,B)} = \frac{c_A^{II}/c_B^{II}}{c_A^{I}/c_B^{I}} = \frac{c_A^{II}}{c_A^{I}} \cdot \frac{c_B^{I}}{c_B^{II}} = \frac{D_A}{D_B}$$

check, if the extraction is plausible

$$V^{II/I} = \frac{V_{II}}{V_I} = \frac{1}{\sqrt{D_A \cdot D_B}}$$

ideal case

$D_B < 10^{-3}$; $V^{I} = V^{II}$, $\alpha_{(A,B)} = 10^6$
1. A ≈ 99.9 %, B ≈ 0.1 %

real case

D_A =10.0, D_B = 0.1, $\alpha_{(A,B)}$ =100
1. A ≈ 90.9 %, B ≈ 9.1 %

$$\alpha_{(A,B)} = \frac{D_A}{D_B}$$

$$V^{II/I} = \frac{1}{\sqrt{D_A \cdot D_B}}$$

1st extraction: in organic phase **A (90.9%)** and **B (9.1%)**
2nd extraction: in organic phase **A (99.2%)** and **B (17.4%)**
3rd extraction: ...

EXTRACTION COMPLEATNESS vs. HIGHER CONTAMINATION!

$$k_A = \frac{n_A^{II}}{n_A^{I}} = \frac{c_A^{II} \cdot V^{II}}{c_A^{I} \cdot V^{I}} = K_D \cdot V^{II/I} \qquad \alpha_{(A,B)} = \frac{k_A}{k_B}$$

capacity factor (k)
: relation between separation factor and phase volumes

separation yield, enrichment factor ($S_{(A,B)}$)
: state in phase II: **A (99.9%)**, **B (0.1%)** ⇒ $S_{A/B}$= **10^3**

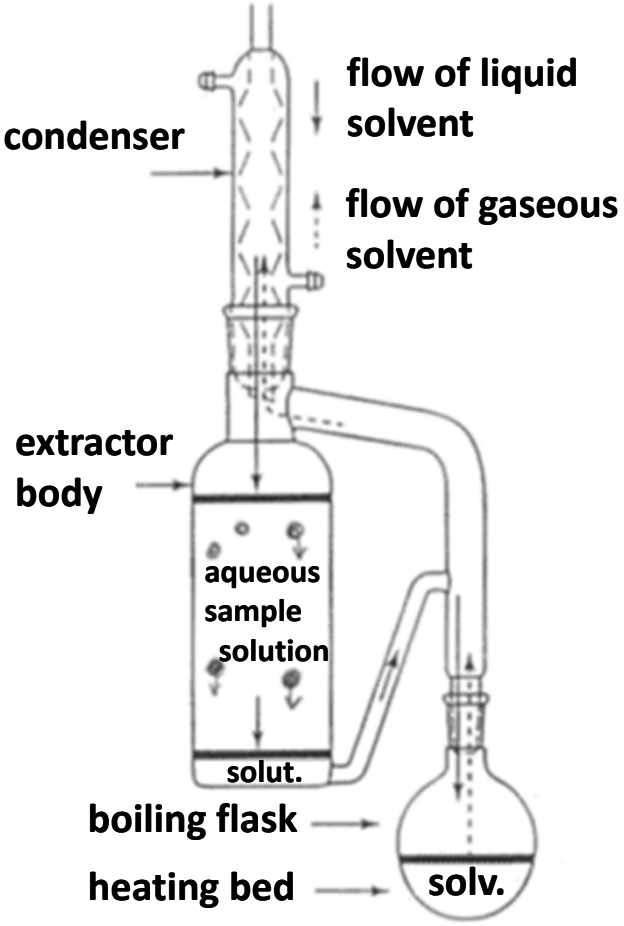
$$S_{(A,B)} = \frac{R_A}{R_B} \qquad \frac{n_A^{II}}{n_B^{II}} = S_{(A,B)} \cdot \frac{n_A^{tot}}{n_B^{tot}}$$

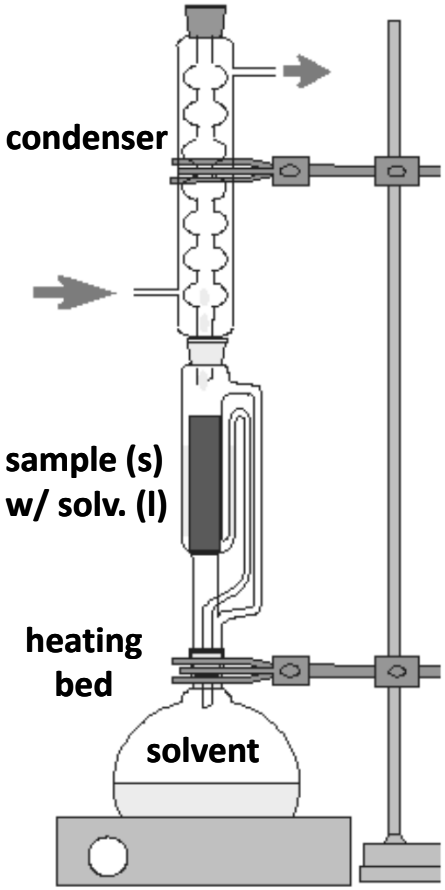
preconcentration of extract
: out of **phase I** we extract analyte into **phase II** (organic extractant)
: we **evaporate part** of the organic solvent ⇒ **$V^{II,vap} < V^{I}$**

continuative extractor

: continual L-L extraction

: raffinate lighter than extractant





Soxhlet extractor

: continual S-L extraction

: (practically) constant volume of solvent

example 4

10 ml of 0.1 M of solution of compound X is separated between organic and aqueous phase, whereas distribution coefficient D = 10. calculate, how much (in mmol) of the X will be transferred into organic phase and how much it will remain in aqueous, if we extract into a) 30 ml, b) 10 ml, c) 1 ml?

???

example 5

continuation of previous example (D = 10, c^{tot} = 0.1 M): we would like to extract as much as possible of compound X of 10 ml aqueous phase (I) into 30 ml of organic one (II). is it better to use a) one-step extraction into 30 ml or b) repeat thrice extraction into 10 ml and fuse them then?

???

we would like to extract into organic phase 90 % of compound X although the distribution ratio equals D = 1. how to do that?

example 6

if $V^{II} = V^{I}$, then $\mathbf{R_{A_1}} = \frac{\mathbf{D \cdot V^{II/I}}}{\mathbf{D \cdot V^{II/I} + 1}}$ = 0.5, i.e. E = 50 %

therefore we must choose

: **extraction into larger volume** $\mathbf{R_A} = \frac{\mathbf{n_A^{II}}}{\mathbf{n_A^{II} + n_A^{I}}} = \frac{\mathbf{1}}{\mathbf{1 + 1/(D \cdot V^{II/I})}}$

$\Rightarrow$ we need $V^{II} = V^{I} \cdot$ **9** (nine times the volume of original phase)

: **repeated extraction into same volume** $\mathbf{c_{A_i}^{I}} = \mathbf{c_A^{tot}} \cdot \left(\frac{\mathbf{1}}{\mathbf{1 + D \cdot V^{II/I}}}\right)^{\mathbf{i}}$

$\mathbf{i} = \frac{\mathbf{\log(1 - R_{A_i})}}{\mathbf{\log(1 - R_{A_1})}}$ = 3.3, i.e. at least 4 steps, i.e. three repetitions

III.

preanalytical sample preparation

chemical analysis

: ***sample preparation***

: separation

: identification, quantification

sample preparation lies mostly **in separation**
: transferring analytes from matrix into solvent useful for analysis

analysis it-self needs not to represent the biggest **problem** of all analytics
: **fundamental** can be the **sample preparation**

sample

liquid

: L-L extraction, perforation, dialysis, ultrafiltration
: microextraction on solid phase, L-S extraction

solid

: homogenisation, dissolution, evaporation / lyophilisation
: S-L extraction, Soxhlet, forced-flow leaching
: supercritical fluid extraction, microwave assisted extraction, accelerated solvent extraction, sonification assisted extraction

counter-current extraction (CCE)

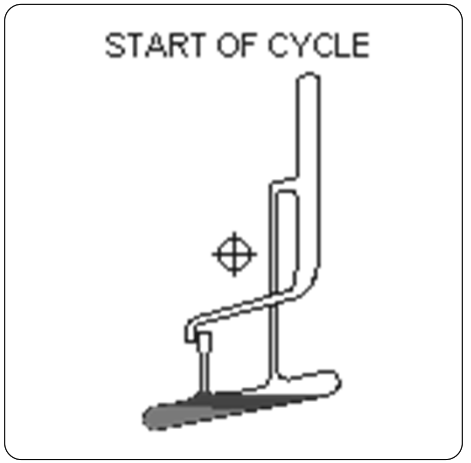
(*counter-flow separation*)

extraction L-L for the substances **A** and **B**
: $D_A > D_B$
: D_B has low value (< 1)

multiple step process, **discrete**, but *consequent,* not continuative

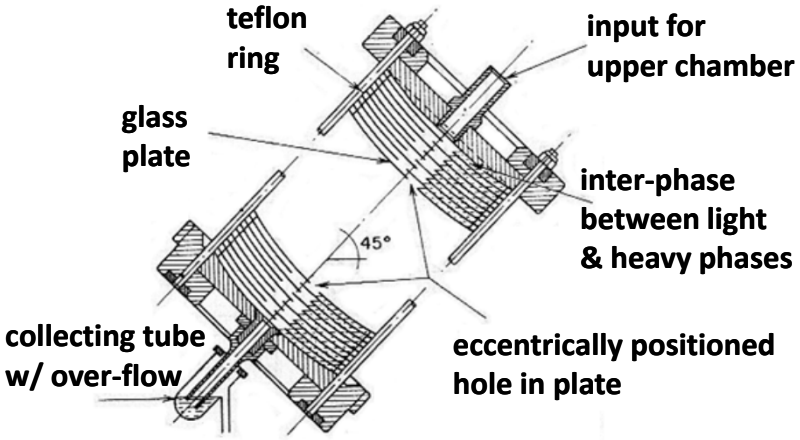
: phases are automatically mixed
: mobile phase is transferred into next compartment
:: realised by centrifugal force

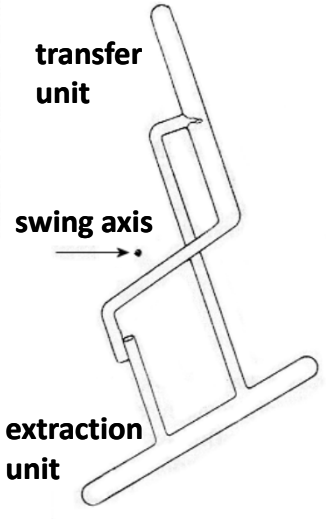
substances with **high** K_D are moving faster in the compartment system



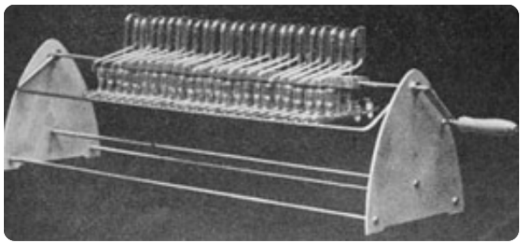
instrumentation

Ronor's column
: *side view*



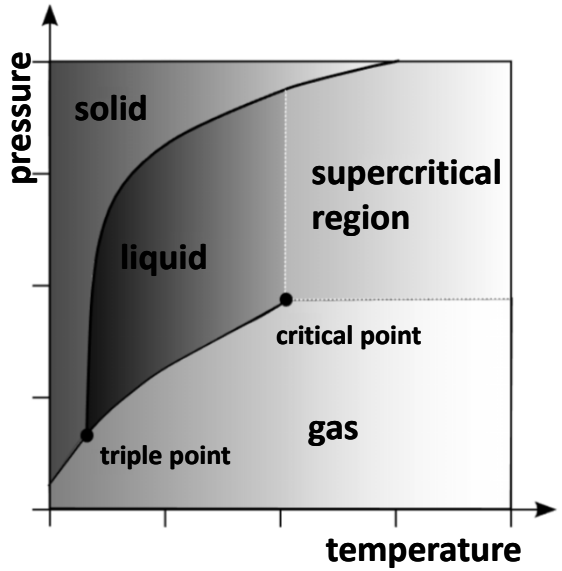


Graig-Post's extractor(1949)



supercritical fluid
: critical values (T_k, p_k)

gas in supercritical state
: *is not* **a liquid**, but ***a fluid***
:: only similar to liquid

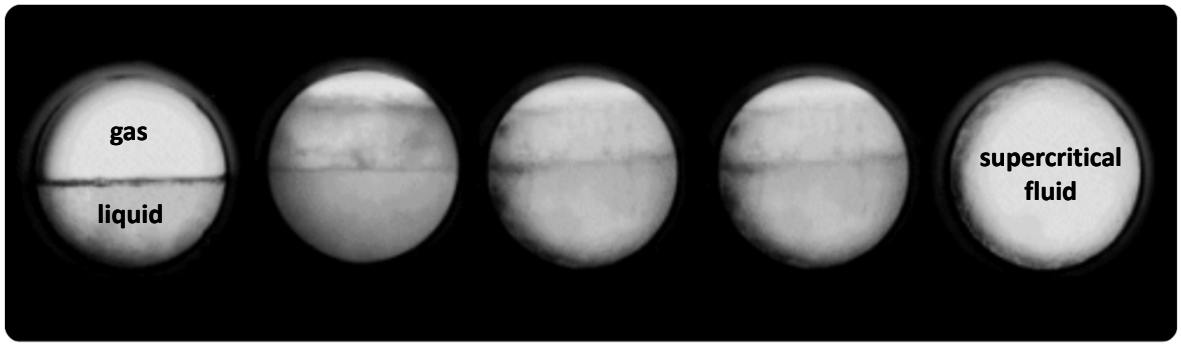


supercritical fluid extraction (SFE)

in supercritical state has the gas
: density of a liquid

properties of supercritical fluids

: viscosity
:: ***lower*** than liquids, similar as gases
: density
:: depends on pressure
: diffusivity
:: ***higher*** than liquids
: solvation abilities
:: as liquids



all these properties **are controllable**
by setting up ***pressure*** & ***temperature*** of the supercritical state
: out of one substance, fluids of many different properties

physical properties of supercritical fluids

	gas	supercritical fluid	liquid
density x10^3 [g·ml^{-1}]	0.2 – 2	470	1600 – 600
diffusion coeff. x 10^4 [cm^2·s^{-1}]	1 – 4	2 – 7	0.02 – 0.2
viscosity x 10^4 [Pa·s]	1 – 3	3 – 10	20 – 300

* elution (extraction) force **δ** is increasing w/ density

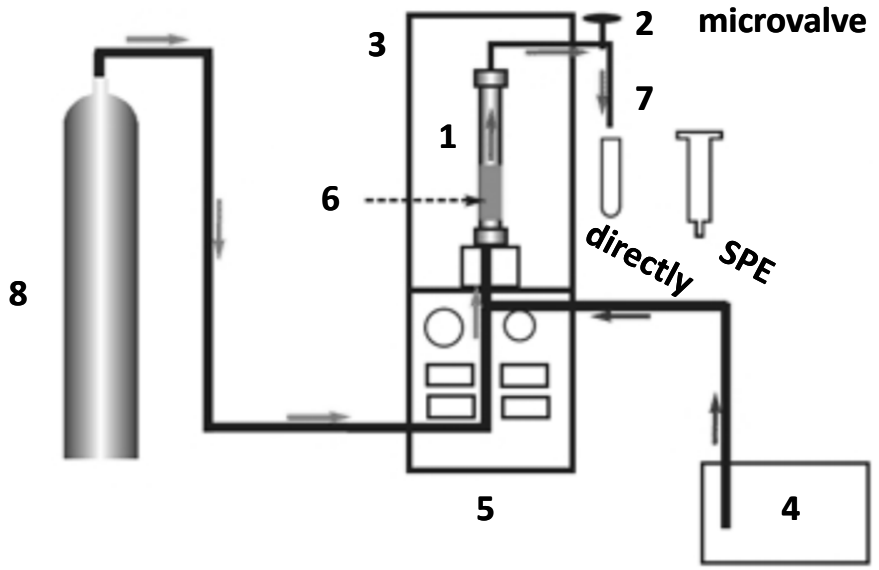
critical values for some substances in SFE

	cr. temperature [°C]	cr. pressure [MPa]	el. force δ *
CO_2	31.3	7.38	10.7
SF_6	45.5	3.77	
n-C_5H_{12}	196.6	3.39	7.2
CCl_2F_2	111.7	4.02	
$CClF_3$	28.8	3.97	7.8
N_2O	36.5	7.34	10.6
CHF_3	25.9	4.75	
$CHClF_2$	96.0	5.01	
NH_3	132.3	11.35	13.2
Xe	***16.6***	5.91	

CO_2 is very non-polar eluent, polarity (elution force) is thus possible to increase adding organic solvent : typically 5 – 10 % of methanol

scheme of SFE

1 – extraction column
2 – phase separator
3 – thermostat
4 – additive pump
5 – liquid CO_2 pump
6 – sample
7 – analyte collector
8 – CO_2 container



SFE advantages to classic extraction L-L

: 10 – 100x faster mass transfer
: direct extraction force changes by changing the density
:: by pressure or temperature
: significant reduction of the extractant volume
: some extractants are gases in normal conditions
:: easy vaporisation = **pre-concentration**

general SFE disadvantages

matrix effects (negative matrix influence)
: interactions with sample and extraction solvent

complex instrumentation
: high temperatures & pressures
: work with gases
:: restrictor
::: new technological solutions

accelerated solvent extraction (ASE)

(**PSE**, *pressurised solvent extraction*)

some disadvantages of extraction methods

: high time and solvent consumption (Soxhlet)
: low efficiency (sonification, microwaves)
: matrix effects (SFE)

extractant is liquid, sample solid

liquids in **sub-critical state**, but **under high pressure** and **temperatures** give many advantages of supercritical state eliminating **matrix effects of SFE**

high temperature influence

: higher solubility
: faster desorption kinetics (+10 °C $\Rightarrow$ 2x $\uparrow$)
: decrease in solvent viscosity
: effective solvent diffusion into matrix
:: elimination of matrix effects

high pressure influence

: solvents are under these conditions liquid
:: changed boiling point
: fast filling of the extractions cells

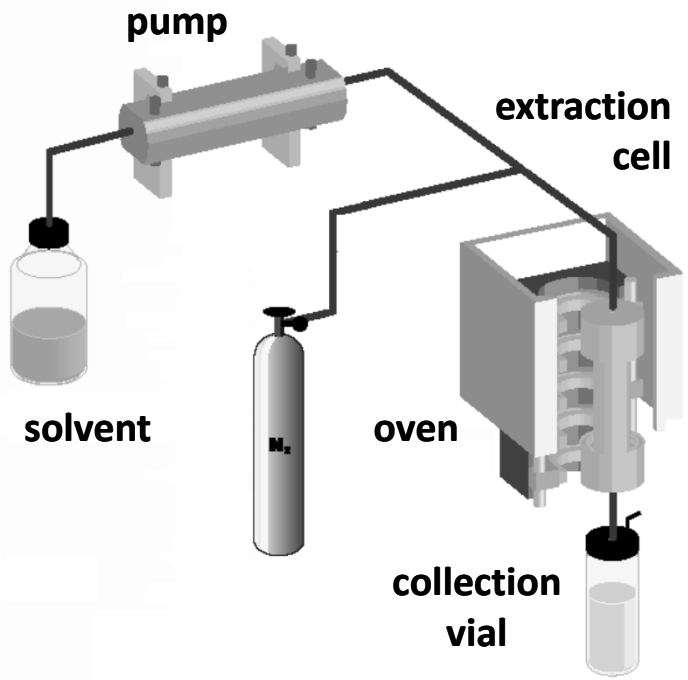
process of ASE

sample – solid or semi-solid

: water content less than 10 %
:: if it is more than 10%, add PEG

: extraction at **high pressure** & **temperature** (200 °C and 20 MPa)
: extraction cell: 10 – 33 ml
: solvent consumption: 12 – 45 ml; *ca* 110 % of sample volume
: total time of extraction (with cell filling): *max* 15 min (fast)
: connectible to HPLC

scheme of ASE



rough comparison
: 1 h on Soxhlet is equal to 1 min ASE

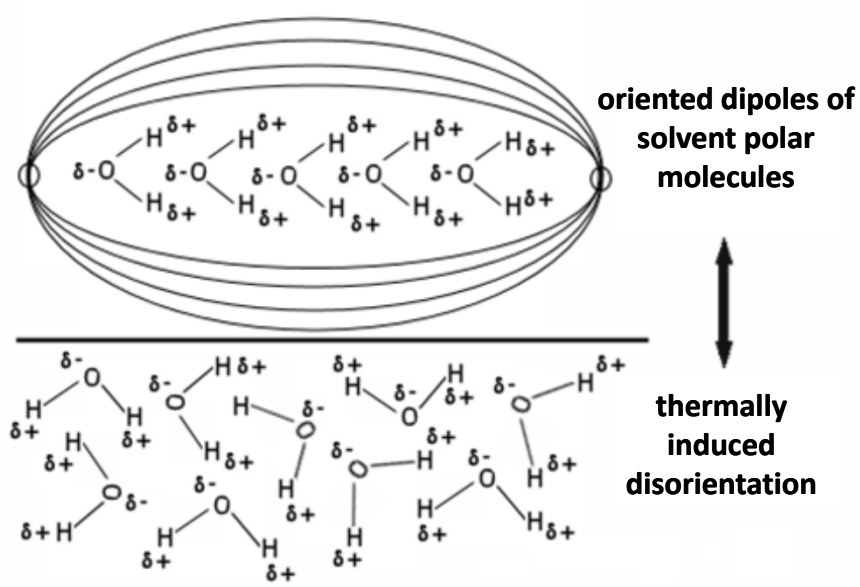
microwave assisted solvent extraction (MASE)

extraction using microwave pulses in magnetron

: **local overheating**

:: similar to ASE

extractant is liquid, sample solid



mostly **rotational** energy transfer

: *no metallic parts* – melting

speed of heating depends on

: conductivity

: heat capacity

: dielectric constant

extraction temperature T_f

$$T_f = T_i + \frac{P_{abs} \cdot t}{K \cdot C_p \cdot m} - x$$

K – conversion factor [cal]>[J]
$\mathbf{C_p}$ – heat capacity of solvent
m – weight of matrix
$\mathbf{P_{abs}}$ – absorbed energy
t – time of microwave field appl.
$\mathbf{T_i}$ – initial temperature
x – thermal losses

energy dissipation in system

$$\delta = \frac{\varepsilon''}{\varepsilon'}$$

ε' – dielectric constant
: describes polarisability of molecules in elmag field
ε" – dielectric loss factor
: describes efficiency conversion of absorbed microwave radiation into heat
δ – dissipation factor

physical properties of MASE solvents

solvent	**boiling point** [°C]	**viscosity** [mPa·s, 25 °C]	**heating speed** [$K \cdot s^{-1}$]
acetone	56	0.30	2.20
ethylacetate	77	0.43	1.78
ethanol	78	0.69	1.20
methanol	65	0.54	2.11
water	100	0.89	1.01
hexane	69	0.30	0.05

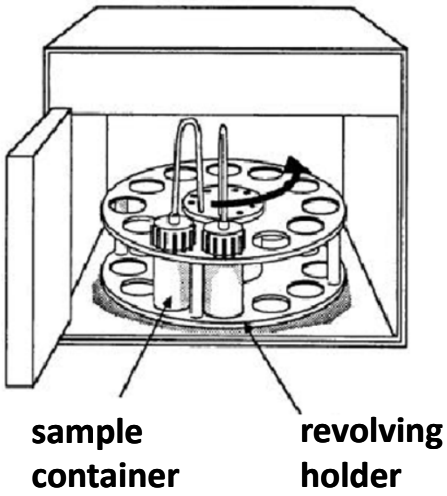
physical properties of MASE solvents

solvent	diel. constant ε' [$F.m^{-1}$]	diel. loss factor ε'' [$F.m^{-1}$]	dissipation factor δ $x10^4$
acetone	80.00	12.0	1500
ethylacetate	20.70	11.5	5555
ethanol	23.90	15.2	6400
methanol	7.00	1.6	2286
water	1.88	$1.9 x10^{-4}$	$1 x10^{-1}$
hexane	6.02	3.2	5316

MASE procedure

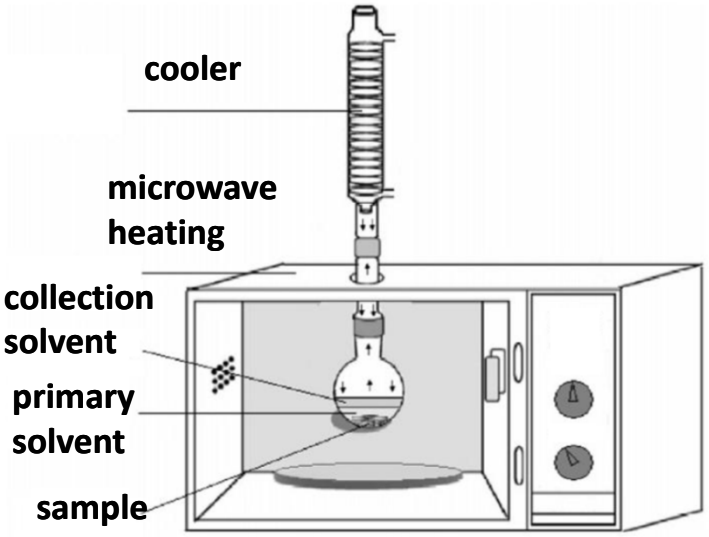
solvent absorbing microwave energy

: closed container
: heating above the boiling temperature
:: accelerated analyte extraction
:: high temperature and pressure
::: < 200 °C; ~1.2 MPa



solvent not absorbing microwave energy

: closed or open container
: cold solvent, analyte is heated
:: useful for thermolabile compounds
: possibility to use liquid CO_2 (does not absorb)
:: substitution for SFE

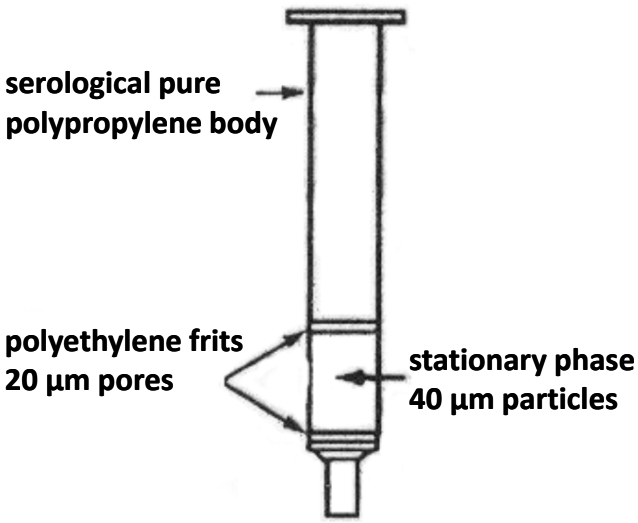


heating (10 min) / cooling (30 min) cycle
: frequency 2450 MHz
: slow, but effective

solid phase extraction (SPE)

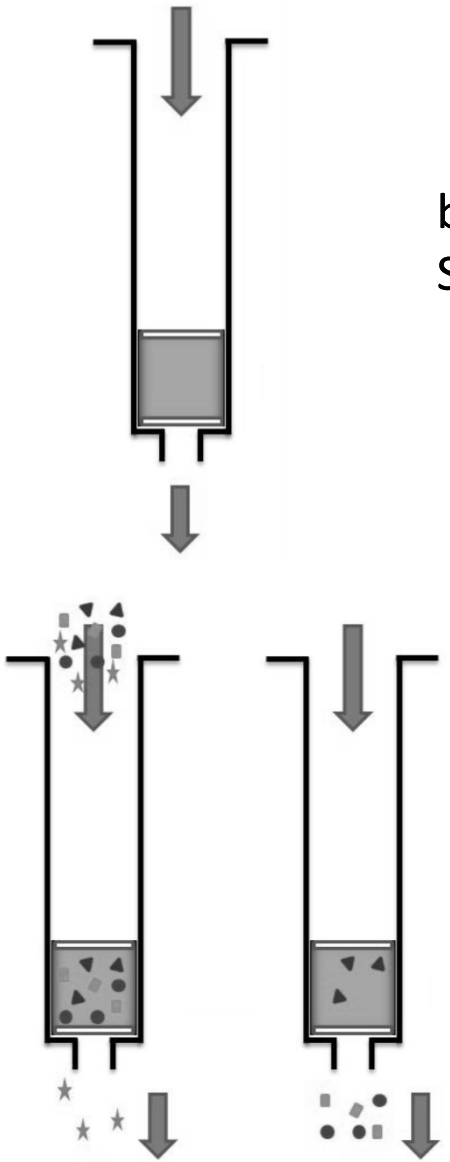
extraction L-L → **extraction L-S**

extractant is solid, sample liquid



: extraction column should not release plastic softeners and/or adsorb analyte
: frits hold macroscopic impurities (dust, fibres)

stationary phase (SP)
: same types as for HPLC (non-polar, polar *etc*.)
: graining less homogeneous, particles bigger than LC



conditioning, equilibration

before sample introduction – **important**
SP – sedimented, wet (soaked), without contamination and activated
: rinse of column by eluent
: then by sample solvent
:: *min* 2 column volumes

sample introduction

: sample is eventually diluted
:: saturation (over-saturation) of column **– sorbent capacity**
: pH is adjusted eventually
: sample flows continually, slow at 1~ 5 ml·min^{-1}

column rinsing

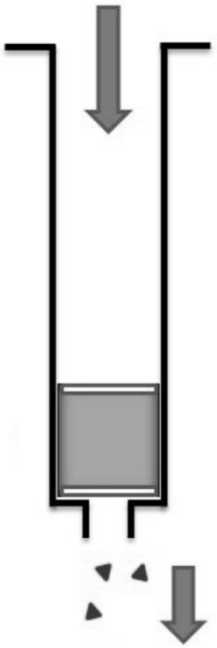
low elution force solvents to rinse the matrix (weak interactions)
: *max* 1 – 2 column volumes

column drying

not necessary, only if needed
: separation of volatile matrix components
: inert gas (e.g. N_2) or vacuum
:: dry sorbent is an aim
: *max* 1 – 10 minutes (washing out analyte)

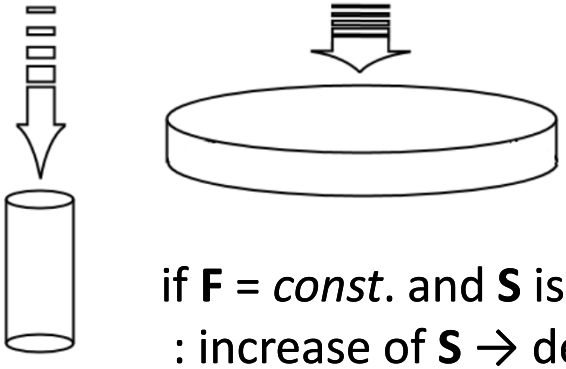
sample elution

strong eluent at moderate flow
: elutes strongly bond substances
: total volume *max* 1 – 5 column volumes
: to increase extraction efficiency – repeated elution with smaller volumes
:: effect same to repeated extraction

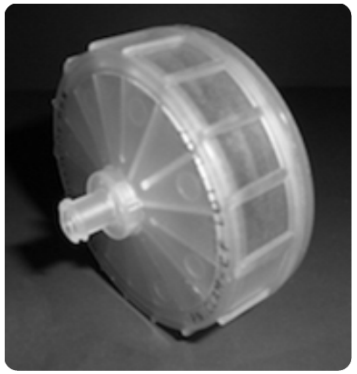


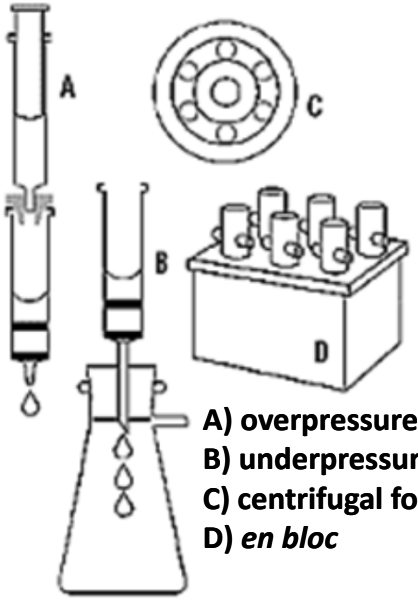
SPE arrangement

$$p = F/S$$



if **F** = *const*. and **S** is small → **p** is high
: increase of **S** → decrease of **p**

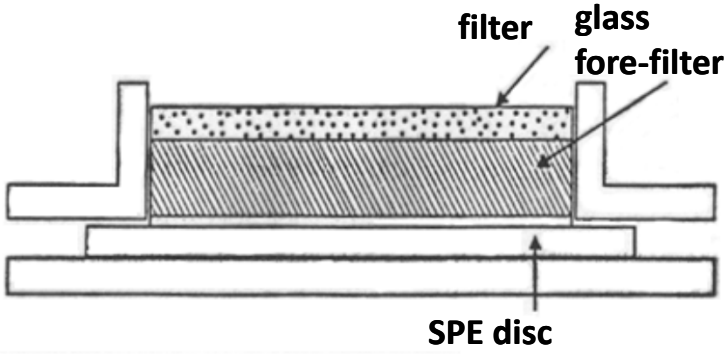




A) overpressure
B) underpressure
C) centrifugal force
D) *en bloc*

column SPE

high column & low area → high pressure & low speed



disc SPE

low column & high area → low pressure & high speed

advantages

: **lower consumption** of org. solvents → **lower price** (environment protection)
: **higher** selectivity, yield and efficiency; separation of analyte from matrix
: possible **automation** connected to flow-through system with valves

SPE *vs* extraction L-L

basically, SPE is less efficient column chromatography

: no complex manipulation by repeated extraction
: till 50 theoretical plates, i.e. fifty extraction steps
: complete separation of analyte and matrix

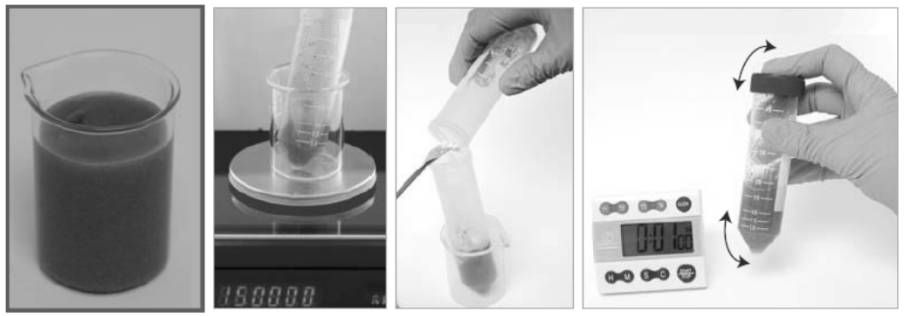
conditions of SPE method choice

method	packing	sample polarity	matrix	conditioning	elution
reversed phase	C18, C8, C2, cyclohexyl	non-polar	polar (aqueous)	MeOH > water > sample solvent	non-polar
normal phase	silica, alumina, graphite, CN, NH_2	polar	non-polar	sample solvent	polar
anex	SAX, WAX	positively charged	polar non-polar	pH = pK_a – 1-2	pH = pK_a – 1-2 proper counter-ion
catex	SCX, WCX	negatively charged	polar non-polar	pH = pK_a + 1-2	pH = pK_a + 1-2 proper counter-ion

disperse solid phase extraction (DSPE)

(**MSPD**, *matrix solid phase dispersion*)

: rubbing sample with suitable sorbent (C18)
:: sample-sorbent ratio 1 : 4
:: sample structure disruption by mechanical and hydrophobic forces
:: large inter-phase
:: matrix = new sorption phase (more complex equilibria)
: resulting mixture into column (as within SPE)
:: eventual addition of other sorbent; e.g. Florisil
::: non-polar components on C18, polar on -OH of silica
: analytes elution by suitable solvent



advantages
: isolation and purification in one step – especially in food analysis
:: saving instrumentation, time and solvents

QuEChERS (quick, easy, cheap, effective, rugged and safe; catchers)
: L-L extraction using organic solvent and solution of salts
: DSPE of resulting mixture

C. L. Arthur. J. Pawliszyn. *Anal. Chem*., 62 (1990) 21

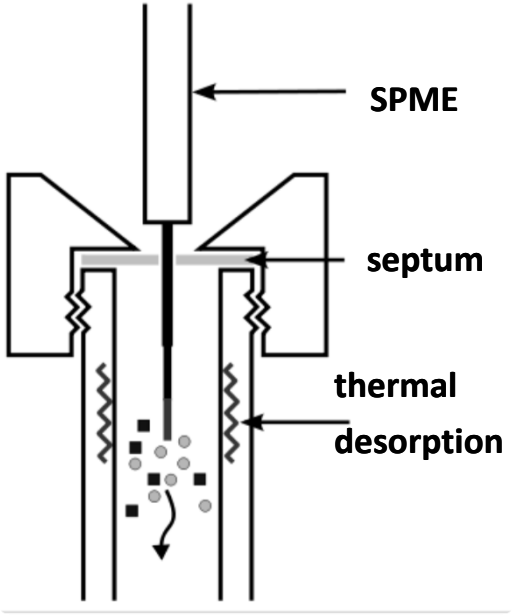
solid phase micro-extraction (SPME)

isolation of organic compounds from gaseous and liquid samples

SPE „inside out“

: adsorption and extraction goes on *on/off surface* of fibre, not inside of SPE column
:: concentration of analyte **on a fibre** sunken in matrix
::: polymer or silica covered with adsorbent

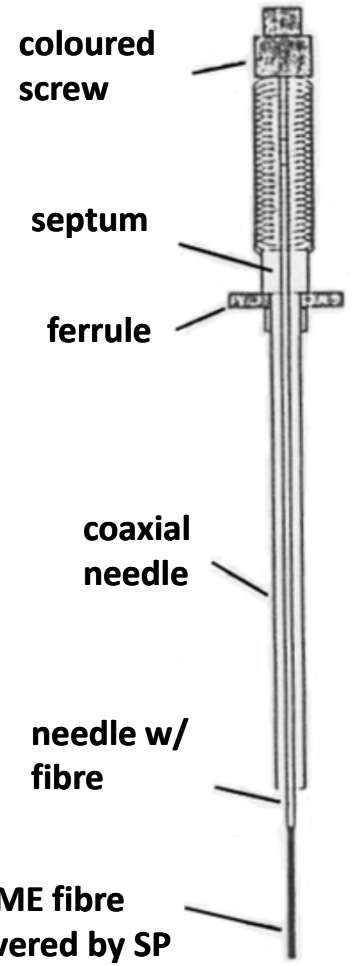
: combination of ***sample collection*** and ***concentration***
: easy, fast, sensitive
: collection in defined time
:: quantitative adsorption
: fibre with adsorbed analyte
:: desorbed in a proper volume of a solvent
:: into GC injector
::: compound thermally desorbed there



sorption rate
: 2 (G) – 30 (L) min

sensitivity
: high in connection with GC-MS
:: IT detector

SPME instrumentation



materials of SPME fibre

polydimethylsiloxane (PDMS)

polydimethylsiloxane/divinylbenzene (PDMS/DVB)

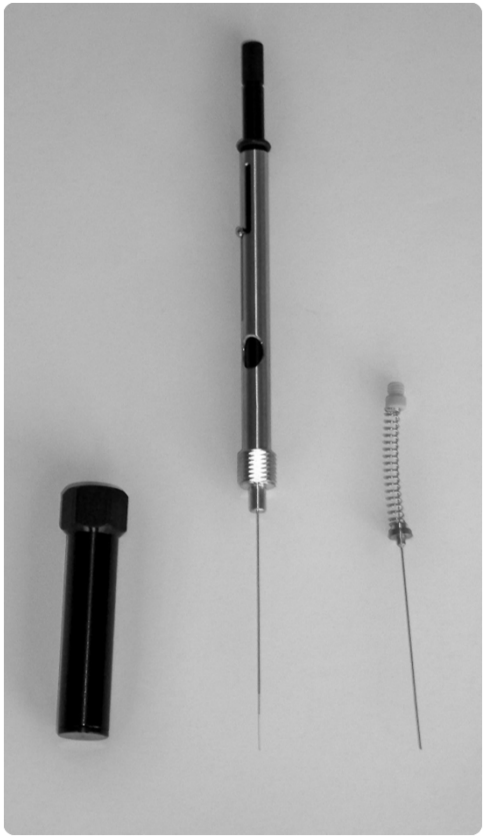
polyacrylate (PA)

carbowax/divinylbenzene (CW/DVB)

polydimethylsiloxane / carboxene (PDMS/CAR)

polydimethylsiloxane / divinylbenzene / carboxene1006 (PDMS/DVB/CAR)

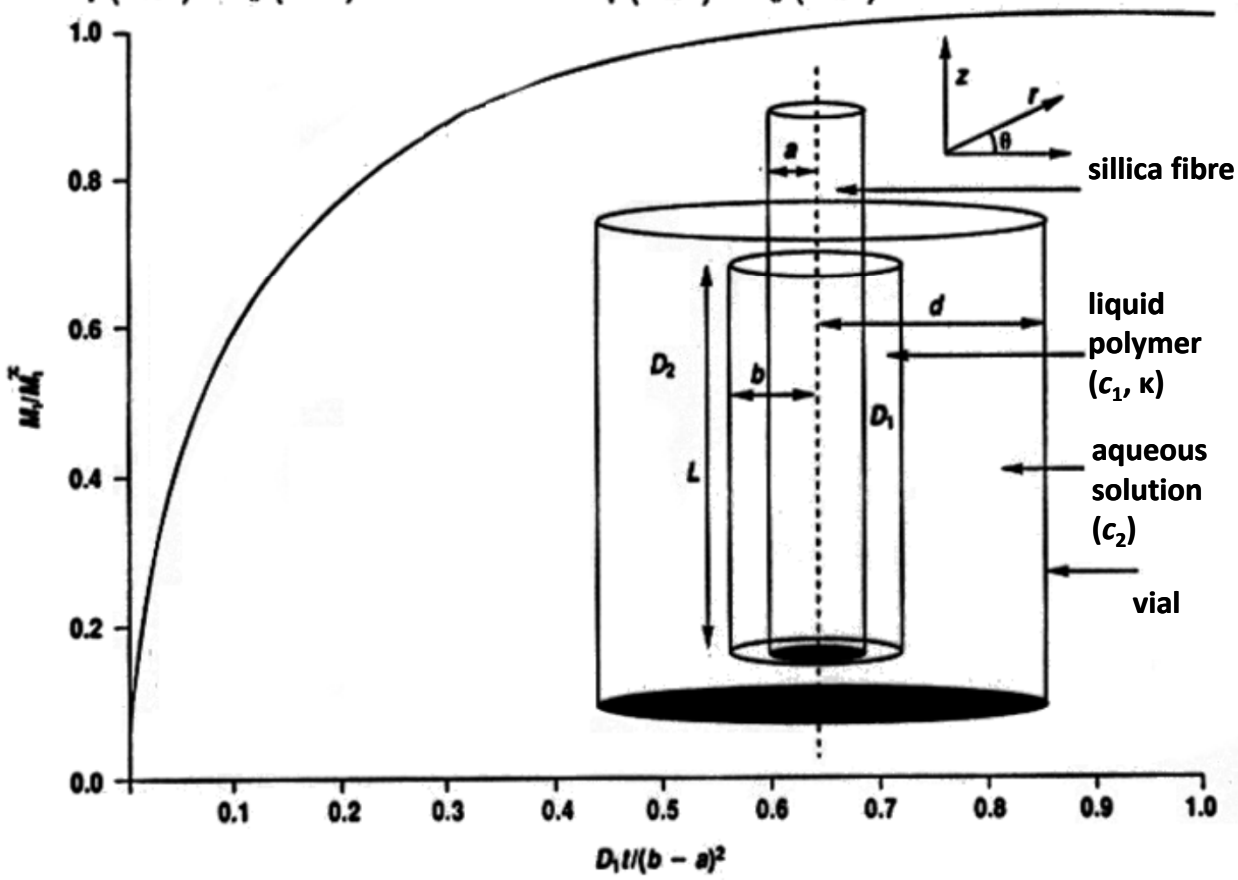
carbowax (CW/TPR)



sorption description

$$M_t = 2\pi L\left\{\kappa C_2^0\left[\frac{r^2}{2}\right]_a^b + \left(C_1^0 - \kappa C_2^0\right)\pi\sum_{m=1}^{\infty}\exp\left(-\alpha^2 D_1 t\right)\left[\gamma\left[\frac{rJ_1(\alpha_m r)}{\alpha_m}\right]_a^b - \delta\left[\frac{rY_1(\alpha_m r)}{\alpha_m}\right]_a^b\right]\right\}$$

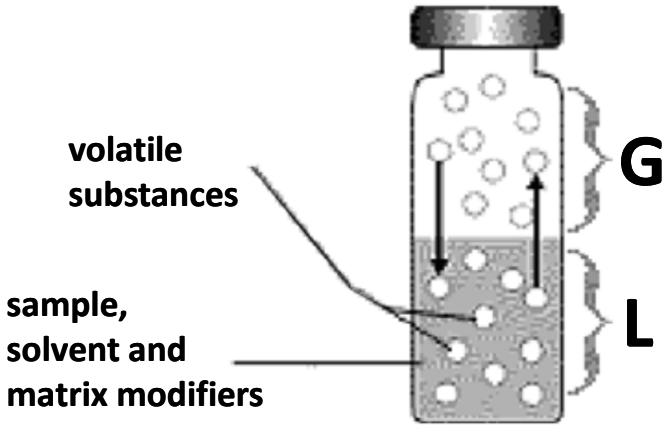
$$\delta = \frac{J_1^2(\alpha_m a)J_0(\alpha_m b)}{J_1^2(\alpha_m a) - J_0^2(\alpha_m b)} \qquad \gamma = \frac{J_1^2(\alpha_m a)Y_0(\alpha_m b)}{J_1^2(\alpha_m a) - J_0^2(\alpha_m b)}$$



headspace extraction (HSE)

headspace – unfilled space in almost full bottle, can or other container after its sealing (*Oxford English dictionary*)

: extraction method for GC
: extraction of volatile substances from non-volatile matrices



G = gas phase (*headspace*)
L = liquid phase of sample

in G sample collection – gas drawing

analyte in *G* is a gas in dynamic equilibrium above its own solution

distribution coefficient

$$K = \frac{C_L}{C_G}$$

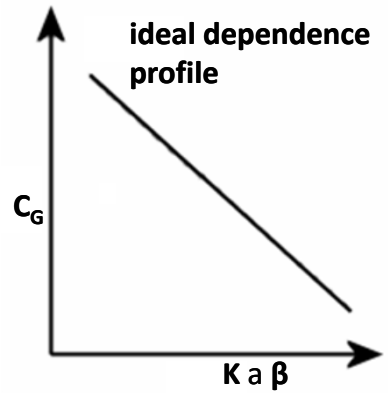
phase ratio

$$\beta = \frac{V_G}{V_L}$$

: equilibrium of sample in vial
: as much as possible of analyte should go to *G*
: analyte is taken from *g* and analysed in GC

$$C_0 \cdot V_L = C_L \cdot V_L + C_G \cdot V_G$$

$$K = \frac{C_L}{C_G} \qquad C_G = \frac{C_0}{K + V_G/V_L}$$



V_G/V_L equal for standard & sample
: else has the calibration no sense

***K* for solvents used in systems G-L**

solvent	K 40 °C	K 50 °C
cyclohexane	0.077	
n-hexane	0.14	0.015
tetrachlorethylene	1.48	
1,1,1-trichlormethane	1.65	
o-xylene	2.44	
toluene	2.82	
benzene	2.90	2.5
dichlormethane	5.65	
n-butylacetate	31.4	
ethylacetate	62.4	
methylethylketone	139.5	11
n-butanol	647	
isopropanol	825	
ethanol	1355	1150
dioxane	1618	

increasing volatility

derivatisations
: esterification, acylation, silanisation, alkylation

can be conducted *in situ* in HSE vial
: **but** contamination and pressure changes

also to suppress possible interactions in GC with inner coating of capillary
: **alcohols, acids, amines**

suppression of matrix effects

stabilisation by means of salts
: NH_3Cl, $(NH_3)SO_4$, NaCl, Na_2SO_4, K_2CO_3

modification of HSE

EPICS (equilibration partition in close system)
: two connected containers, equal concentrations, different volumes

VPT (variable phase ratio or variable volume technique)
: series of same concentrations in vials w/ different G/L ratios

MHE (multiple headspace extraction)
: dynamic gas extraction

FET (full evaporation technique)
: gas phase >> phase of analyte

TVT (total vaporisation technique)
: vaporisation at elevated temperature

VPC (vapour phase calibration)
: external gaseous standard

chromatography

theoretical fundaments of chromatography

chromatography = dynamic repeated extraction

chromatographic separation goes on in **chromatographic bed** (column or slab)
: with **stationary** (fixed) **phase** (SP) = sorbent
: and **mobile** (movable) **phase** (MP) = eluent

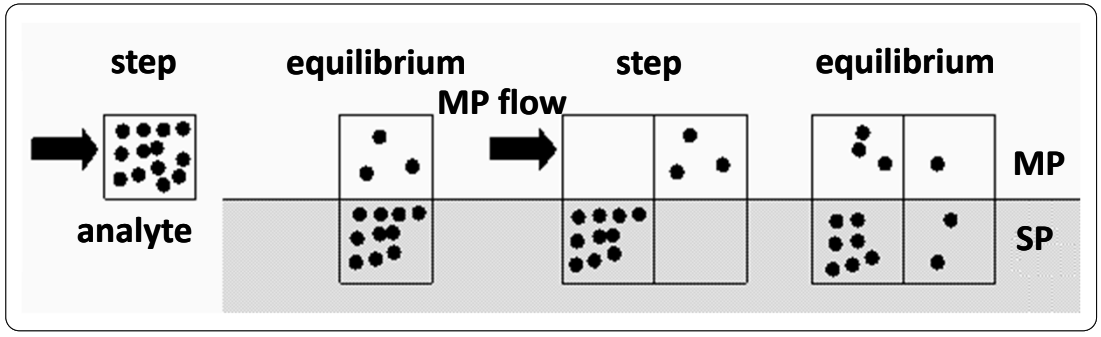
different **analytes** (separated substances) have different **affinity** to stationary phase →

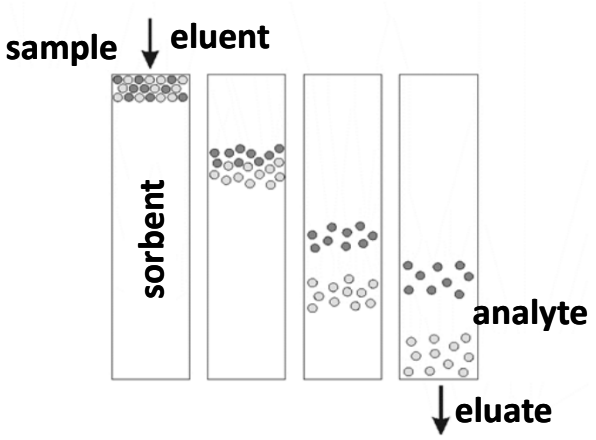
→ different **analytes** have different **distribution** between MP and SP →

→ different **analytes** are differently **retained** (time spent on SP)
differently **retarded** (total time spent in system)

basic principle

differential migration

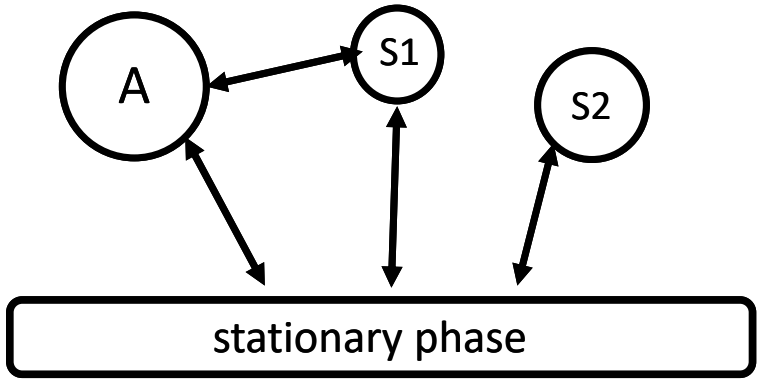




equilibrium on column

$$a_A^M + a_M^M \leftrightarrow a_A^S + a_M^S$$

$$K_A = \frac{a_A^S \cdot a_M^S}{a_A^M \cdot a_M^M}$$



a_A^S – concentration of analyte on SP surface

a_M^M – concentration of MP in eluate

a_A^M – concentration of analyte in MP

a_M^S – concentration of MP on SP surface

$$D = \frac{n_A^S / m_A^S}{n_A^M / V_A^M} \qquad K_A = e^{\left(\frac{-\Delta\mu_A^0}{R \cdot T}\right)} \qquad a_A^S = \frac{K_D}{R \cdot T} \cdot p_A \cdot \gamma_A$$

in GC

p_A – partial pressure of compound A

K_A/D decreases to ~1/2 with temperature increase of temperature by 20 °C

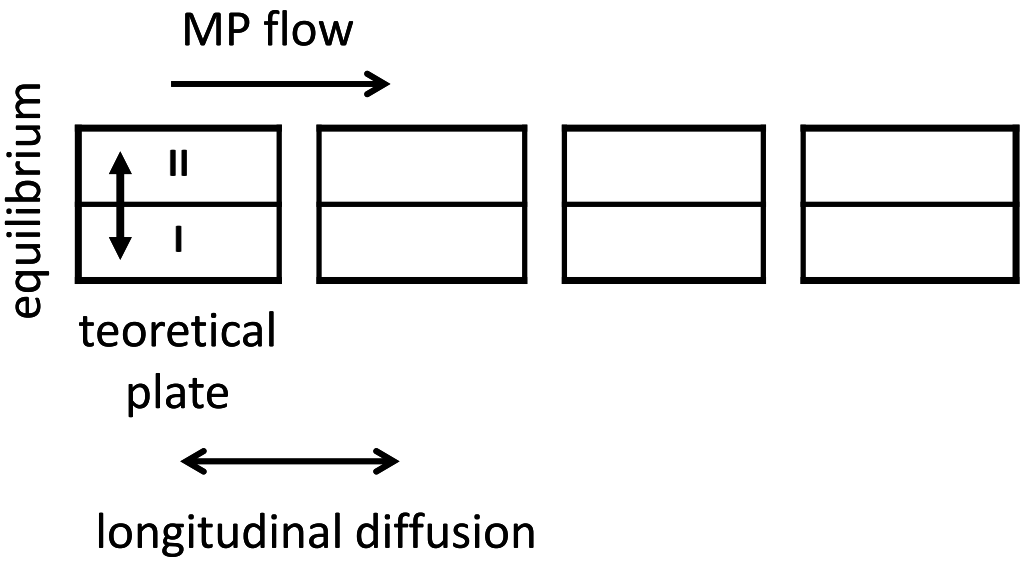
: used in GC/LC

physico-chemical description of chromatographic processes

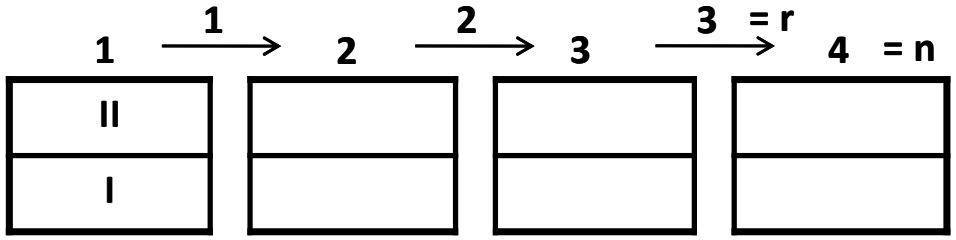
ideal linear chromatography

: is based on an idea of counter-current extraction
: column consists of ordered sections of the same volume (~ theoretical plate)
: MP flow is discontinual, stepwise, adding MP volume over whole one section at a time
: equilibrium on inter-phase is much faster than MP flow rate
: lateral diffusion is negligible
: adsorption is controlled by linear isotherm

de facto
: only point 5 can be guaranteed
: points 3 and 4 are contradictive
:: movement in all directions is of the equal rate

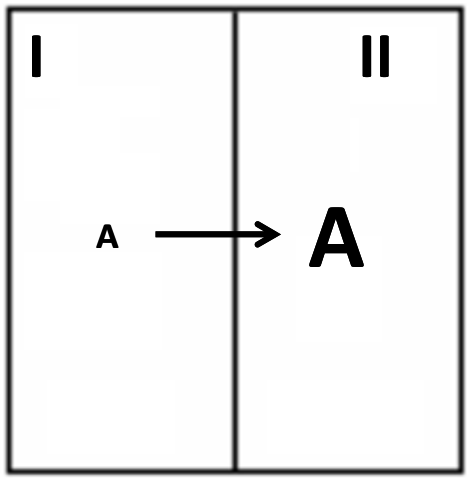


functional model based on **counter-current extraction** (*stochastic model*)



⇒

MP			
SP			

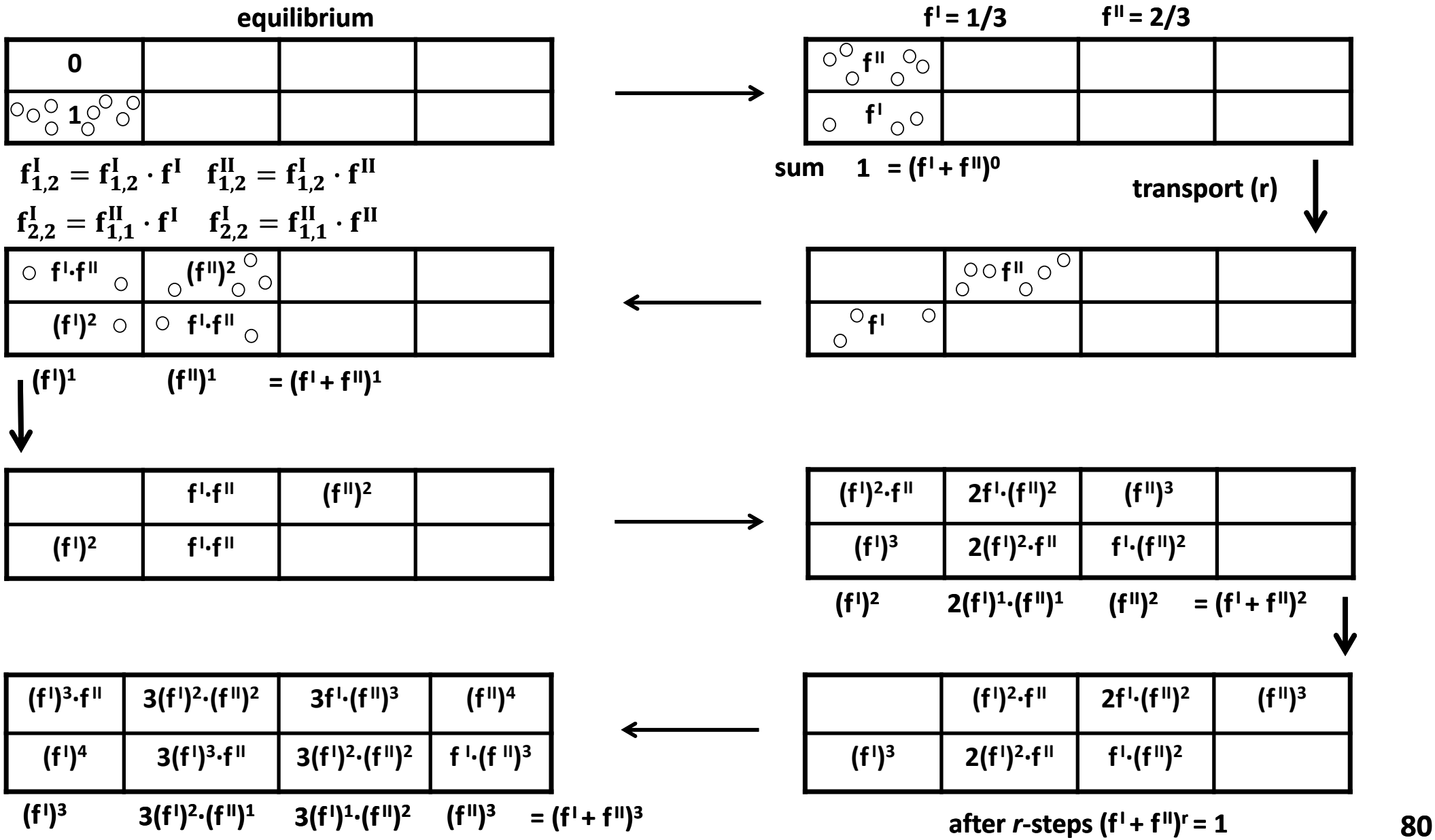


$$f_A^I = \frac{c_A^I}{c_A^{tot}} = \left(\frac{1}{1 + D \cdot V^{II/I}}\right) \qquad f_A^{II} = 1 - f_A^I$$

f_A – an aliquot of compound **A** in given phase

$$k_A = \frac{n_A^{II}}{n_A^I} = \frac{c_A^{II}}{c_A^I} \cdot V^{II/I} \qquad \text{when } V^{II/I} = 1 \qquad k'_A = \frac{f_A^{II}}{f_A^I}$$

$$f_A^I = \frac{1}{1 + k'_A} \qquad f_A^{II} = \frac{k'_A}{1 + k'_A} \qquad k_A = D \cdot V^{II/I} \qquad k'_A = D$$


equilibrium
0
1
$f^I_{1,2} = f^I_{1,2} \cdot f^I \quad f^{II}_{1,2} = f^I_{1,2} \cdot f^{II}$
$f^I_{2,2} = f^{II}_{1,1} \cdot f^I \quad f^I_{2,2} = f^{II}_{1,1} \cdot f^{II}$
$f^I = 1/3$
$f^{II} = 2/3$
f^{II}
f^I
sum 1 $= (f^I + f^{II})^0$
transport (r)
$f^I \cdot f^{II}$
$(f^{II})^2$
$(f^I)^2$
$f^I \cdot f^{II}$
$(f^I)^1$ $(f^{II})^1$ $= (f^I + f^{II})^1$
$f^I \cdot f^{II}$ $(f^{II})^2$
$(f^I)^2$ $f^I \cdot f^{II}$
$(f^I)^2 \cdot f^{II}$ $2f^I \cdot (f^{II})^2$ $(f^{II})^3$
$(f^I)^3$ $2(f^I)^2 \cdot f^{II}$ $f^I \cdot (f^{II})^2$
$(f^I)^2$ $2(f^I)^1 \cdot (f^{II})^1$ $(f^{II})^2$ $= (f^I + f^{II})^2$
$(f^I)^3 \cdot f^{II}$ $3(f^I)^2 \cdot (f^{II})^2$ $3f^I \cdot (f^{II})^3$ $(f^{II})^4$
$(f^I)^4$ $3(f^I)^3 \cdot f^{II}$ $3(f^I)^2 \cdot (f^{II})^2$ $f^I \cdot (f^{II})^3$
$(f^I)^3$ $3(f^I)^2 \cdot (f^{II})^1$ $3(f^I)^1 \cdot (f^{II})^2$ $(f^{II})^3$ $= (f^I + f^{II})^3$
$(f^I)^2 \cdot f^{II}$ $2f^I \cdot (f^{II})^2$ $(f^{II})^3$
$(f^I)^3$ $2(f^I)^2 \cdot f^{II}$ $f^I \cdot (f^{II})^2$
after *r*-steps $(f^I + f^{II})^r = 1$

into $\left(f_A^{II} + f_A^{I}\right)^r = 1$ we put $f_A^{I} = \frac{1}{1 + k'_A}$ $f_A^{II} = \frac{k'_A}{1 + k'_A}$

after **r** transports there will particular content of **A** in **n**th compartment ($f_{A_{fin}}^{II}$)

$$f_{A_{fin}}^{II} = \frac{n!}{r! \cdot (n-r)!} \cdot \left(f_A^{II}\right)^r \cdot \left(f_A^{I}\right)^{n-r} \Rightarrow \quad f_{A_{fin}}^{II} = \frac{n!}{r! \cdot (n-r)!} \cdot \frac{D^r}{(D+1)^n}$$ *solved using binomial theorem*

for **n > 20** ($r \cdot f_A^{II} \cdot f_A^{I} > 3$)
: gaussian dependence

$$f_{A_{fin}}^{II} = \frac{1}{\sqrt{2\pi \cdot \sqrt{r \cdot f_A^{II} \cdot f_A^{I}}}} \cdot e^{\left(\frac{-\left(n - r \cdot f_A^{II}\right)}{2r \cdot f_A^{II} \cdot f_A^{I}}\right)}$$

position (n_{max}) of compartment with maximal content of **A** after **r** transports

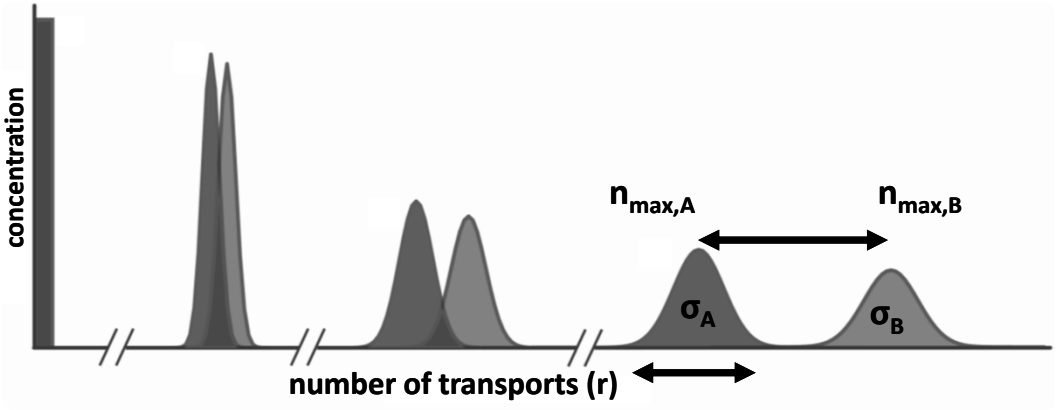
$$n_{max} = r \cdot f_A^{II} = r \cdot \left(\frac{k'_A}{1 + k'_A}\right)$$

number of compartments with compound **A**
: i.e. peak width

$$\sqrt{r \cdot f_A^{II} \cdot f_A^{I}} = \sigma = \sqrt{r \cdot \left(\frac{k'_A}{(1 + k'_A)^2}\right)}$$

therefore, **separation** is possible **only** because the **distance** between **maxima** *increases faster with increasing number of separations* rather than the **peak width**

$$\mathbf{n_{max}} = \boldsymbol{f}(\mathbf{r}) \qquad \boldsymbol{\sigma} = \boldsymbol{f}\left(\sqrt{\mathbf{r}}\right)$$



non-ideal linear chromatography

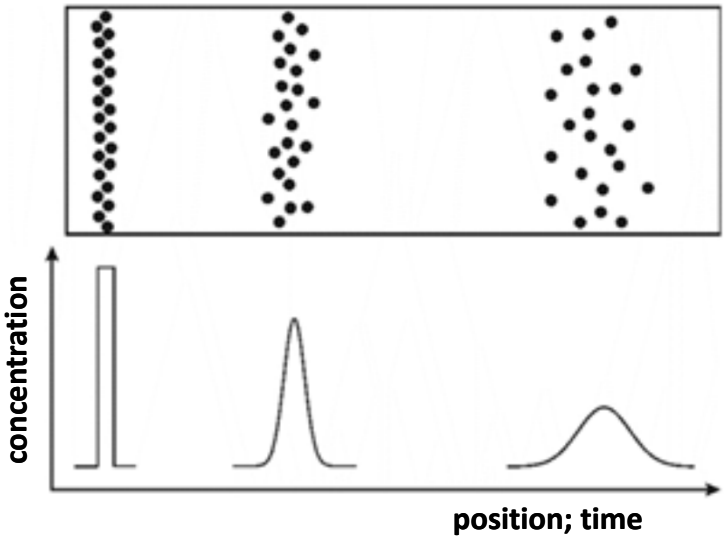
: out of the original assumptions of *ideal linear chromatography*, only **point 5** is valid
: **out of the linear part** of isotherm → **zone shape distortion**
: description of chromatographic separation (efficiency of separation process)

: **chromatographic plate theory**
:: Martin-Synge model (1941)

: **statistic (rate) theory**
:: van Deemter-Zuiderweg model (1956)

: **stochastic / kinetic (rate) theory**
:: Giddings-Eyring-McQuarrie model (1963, 1999 – Cavazzini *et al*.)

graphical illustration of separation



zone of **A** is moving through system
: signal detection of **A**, measuring its intensity ($\mathbf{I_{sign}}$)
:: ***dependence*** $\mathbf{I_{sign} = f\,(t)}$ **– chromatogram**
::: i.e. **elution curve**, **concentration profile of** analyte in zone

chromatogram analysis

function $\mathbf{I_{sign} = f\,(t)}$ gives specific shape – *gaussian peak shape*
: **chromatographic separation zone**
: **chromatographic peak**

signal intensity ($\mathbf{I_{sign,x}}$) in point **x** as a function of height ($\mathbf{I_{sign,x\,max}}$ = h) at maximum $\mathbf{x_{max}}$

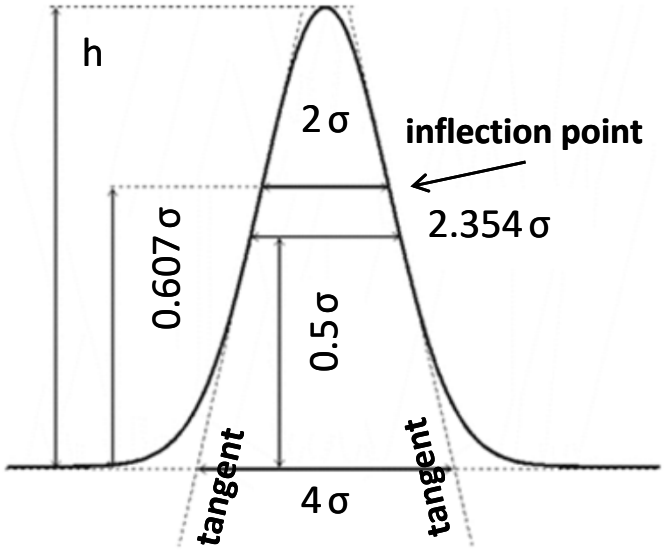
$$\mathbf{I_{sign,x} = I_{sign,x_{max}} \cdot e^{\left(\frac{-(x_{max}-x)^2}{2\sigma^2}\right)}}$$

peak width of compound **A**

: peak width between tangents at inflection points $\mathbf{w = 4\sigma}$

: peak width in half of peak height
:: **FWHM** – *full width at half maximum* $\mathbf{w_{1/2} = 2.354\sigma}$

: peak width between inflex points $\mathbf{w_i = 2\sigma}$



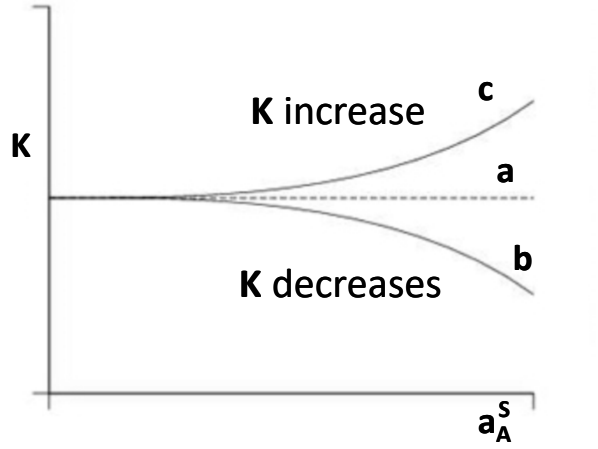
peak width is given in **temporal (**or **longevity**) units
[s, min] [mm, cm]

peak area

$$\mathbf{A = 1.064 \cdot h \cdot w_{1/2}} \qquad \mathbf{A = \frac{1}{2}(h \cdot w)}$$

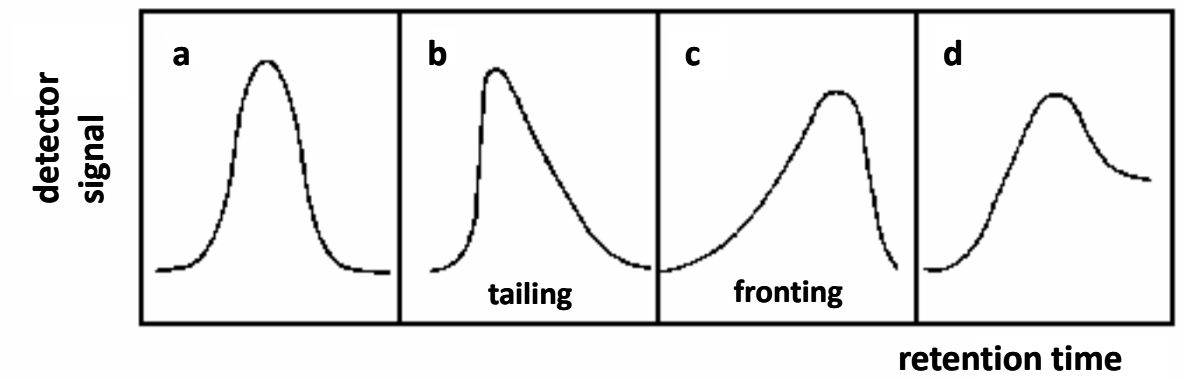
could be neglected and rectangle may be used

dependence between *K* and peak shape



$$\mathbf{K} = \frac{\mathbf{a_A^S}}{\mathbf{a_A^M}} = \boldsymbol{const.}$$

deformation of gaussian peak shape reflects adsorption



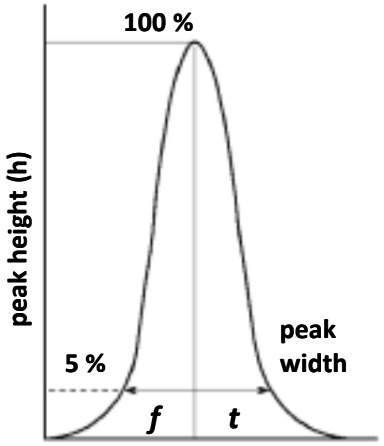
a – linear, **b** – Langmuir, **c** – anti-Langmuir, **d** – chemisorption

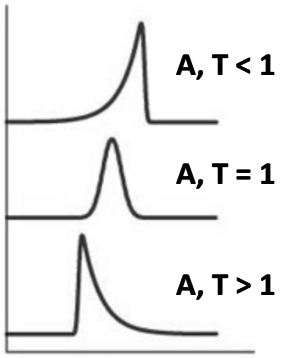
deformation of peak we observe through its **asymmetry**

asymmetry measure $\mathbf{A = t/f}$

symmetry measure $\mathbf{S = 1/A}$

tailing factor $\mathbf{T = (f + t)/2f}$
: asymmetry measure USP
(*United States Pharmacopeia*)





$$\mathbf{A = 2T - 1}$$

sometimes ***f*** and ***t*** are measured at 10 % h mutual relation of **A** and **T**

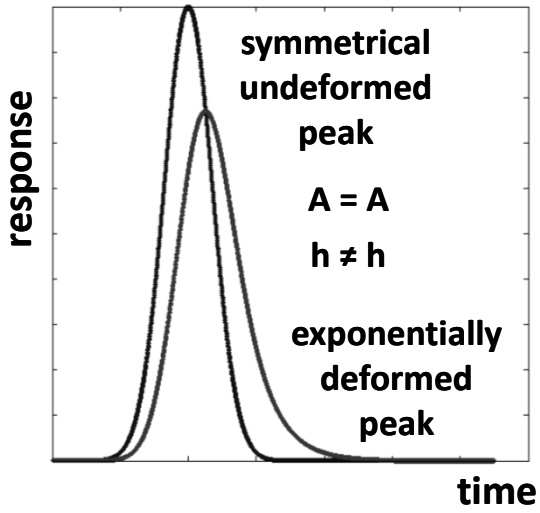
non-ideal peak shape

complex separation process

: impossible to establish exact mathematical (analytical) model

:: combination of Gauss function and exponential function (t^n)

::: allows to express deformations of ideal Gauss curve



$$I_{sign,t} = \frac{A}{\tau \cdot \sqrt{(2\pi \cdot \sigma_t^2)}} \cdot e^{\left[-\frac{(t_{R_{max}} - M_1 - t')^2}{2\sigma_t^2}\right]} \cdot e^{\left(-\frac{t'}{\tau}\right)} dt'$$

τ – exponential element

:: summary asymmetry contribution

t' – auxiliary variable of integration

integration of model elution curve

: **statistical moments of** separation zone (**M'$_n$** & **M$_n$**)

nth normal momentum

$$M'_n = \frac{\int_0^\infty t^n \cdot I_{sign,t}\, dt}{\int_0^\infty I_{sign,t}\, dt}$$

n ≥ 1

nth central momentum

$$M_n = \frac{\int_0^\infty (t - M'_1)^n \cdot I_{sign,t}\, dt}{\int_0^\infty I_{sign,t}\, dt}$$

n ≥ 2

zero momentum $\mathbf{M_0 = A}$

number of theoretical plates $\mathbf{N = \frac{M'^2_1}{M_2} = \frac{t^2_R}{\sigma^2}}$

other important statistical moments $\mathbf{M'_1 = t'_R}$ $\mathbf{M_2 = \sigma^2}$

$\mathbf{M_3 = 0}$ only for an ideal Gaussian peak
: peak symmetry S (*skew*)
:: $M_3 > 0$ – tailing

$\mathbf{S = \frac{M_3}{\sqrt{M_2^3}}}$

$\mathbf{M_4 = 0}$ only for an ideal gaussian peak
: vertical peak deformation E (*excess*)
:: $M_4 > 0$ – sharper than a Gaussian profile

$\mathbf{E = \frac{M_4}{M_2^2} - 3}$

$\mathbf{\frac{\tau}{\sigma} = 0}$ **τ = 0** – no peak deformation; **w** – 100 %; **h** – 100 %

$\mathbf{\frac{\tau}{\sigma} = 1}$ **w** – 124 %; **h** – 78 %

$\mathbf{\frac{\tau}{\sigma} = 4}$ **w** – 215 %; **h** – 40 %

e_R – error of retention time estimation

$$\mathbf{e_R = M'_1 - t'_R = \tau \cdot \left[1 - \sqrt{\frac{\sigma}{2\tau}}\right]}$$

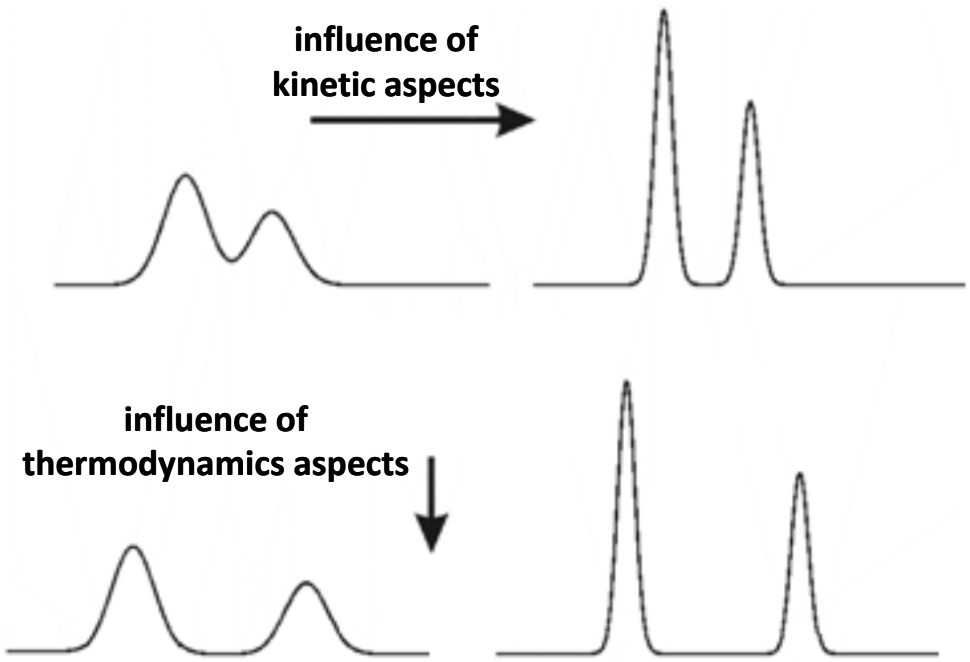
e_σ – error of peak width estimation

$$\mathbf{e_\sigma = 100 \cdot \frac{M_2 - \sigma^2_{exp}}{M_2}}$$

$\mathbf{\sigma_{exp} \sim w^2, w^2_{1/2}}$

sample moving through column

: analytes **are separated** (*retention time)*
: zones of analytes **get broader**
:: separation vs dilution



thermodynamic aspects of separation

influences extent of **interaction between SP & analyte**
: eventually SP & MP

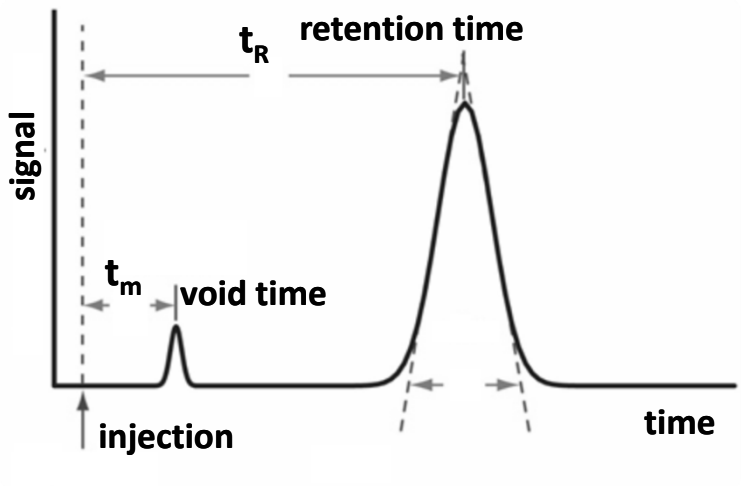
kinetic aspects of separation

influences on **broadening of zones A** during separation (*peak width*)

thermodynamic* and *kinetic aspects of separation mutually ***coincide***
→ they influence ***resolution*** of peaks

thermodynamics of chromatography – analyte distribution

thermodynamic aspects of separation



chromatographic system description

stationary phase volume	V_S [ml/cm^3]
mobile phase volume	V_M [ml]
mobile phase flow rate	F_M [ml·min^{-1}]
linear mobile phase flow rate	**u** [cm·min^{-1}]
length of column	**L** [cm]
column cross-section	**A** [cm^2]

$$V_M = F_M \cdot t_m$$

$$u = \frac{L}{t_m}$$

$$u = \frac{F_M}{A}$$

retention quantities measurable characteristics of analyte retention

i-th analyte retention volume	$V_{R,i}$ [ml]
i-th analyte retention time	$t_{R,i}$ [min]
void column volume	V_m [ml]
void retention time	t_m [min]

$$V_{R_i} = F_M \cdot t_{R_i}$$

$$V_M = F_M \cdot t_m = V_m$$

retention time

: total time of **A** spent **in separation column**

retention volume

: MP volume gone through column in retention time

void column time

: retention time of inert **A**, moving with a front of MP

:: all compounds stay in a system for at least t_m

::: $t_R > t_m$; total time of **A** spent **in mobile phase**

void column volume

: eluent gone through the column in void time

:: elution time of un-retained (inert) substance

::: interstitial volume of column (V_M)

adjusted retention quantities

adjusted retention time $t'_{R,i}$ [min]

adjusted retention volume $V'_{R,i}$ [ml]

$$t'_{R_i} = t_{R_i} - t_m \qquad V'_{R_i} = V_{R_i} - V_m$$

$$V'_{R_i} = F_M \cdot t'_{R_i}$$

MP moves through column in a constant flow rate

: all molecules of **A** spent **in mobile phase** the same (void) time

: **retention time** includes **void time + adjusted retention time**

:: **A** spends **adjusted retention time** on **stationary phase**

relations between retention values and distribution constant

$$t_{R_A} = \frac{V_{R_A}}{F_M} = \frac{V_m + K_{D_A} \cdot V^S}{F_M} \qquad t'_{R_A} = \frac{V'_{R_A}}{F_M} = \frac{K_{D_A} \cdot V^S}{F_M}$$

$$V_{R_A} = V_m + K_{D_A} \cdot V^S \qquad V'_{R_A} = K_{D_A} \cdot V^S$$

distribution constant

$$K_A = \frac{a_A^S}{a_A^M} \Rightarrow D = \frac{n_A^S}{n_A^M} \cdot \frac{V^I}{V^{II}}$$

separation factor

$$\alpha_{(A,B)} = \frac{k_A}{k_B} = \frac{t'_{R_A}}{t'_{R_B}} = \frac{K_A}{K_B}$$

retention (capacity) factor (capacity ratio)

serves also to compare separation
: k_A in given system of SP and MP = ***const*.**

$$k_A = \frac{n_A^S}{n_A^M} = K_A \cdot \frac{V^S}{V^M}$$

$$k_A = \frac{t_{R_A} - t_m}{t_m} = \frac{t'_{R_A}}{t_m} = \frac{t'_{R_A}}{(t_{R_A} - t'_{R_A})}$$

$$k_A = \frac{V_{R_A} - V_m}{V_m} = \frac{V'_{R_A}}{V_m}$$

$$\frac{t'_{R_A}}{t_{R_A}} \Rightarrow \frac{k_A}{(k_A + 1)}$$

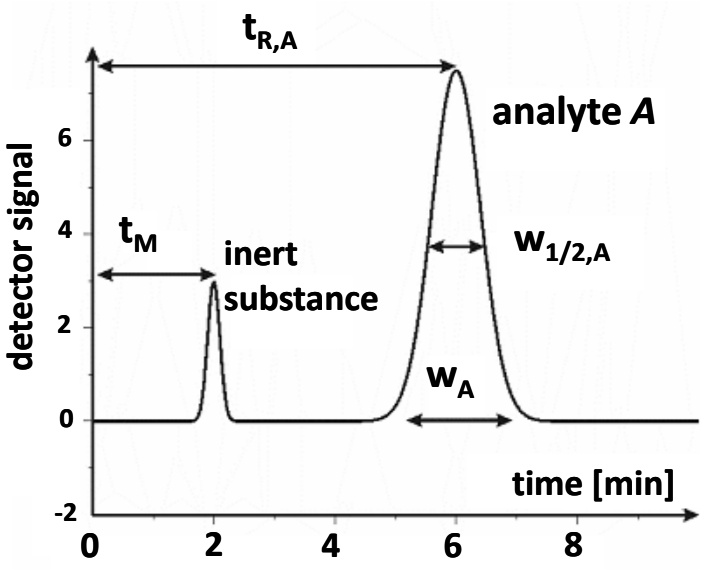
$$k_A = \frac{t'_{R_A}}{t_m} \Rightarrow t_{R_A} = \frac{L}{u} \cdot (1 + k_A)$$

K_A and **k_A** do characterise **selectivity**, i.e. **retention** / **retardation** of on column

$k_A > 1$ → adequate retention, better separation quality

kinetic aspects of separation

zones of **A** do **broaden** during analysis
: **consequence of** *non-ideal linear chromatography*



efficiency of chromatographic column

characterises zone **broadening** of substance **A**

number of theoretical plates of column

Martin-Synge model
: measure of column efficiency

$$\mathbf{N = \frac{t_R^2}{\sigma^2} = \left(\frac{t_R}{\sigma}\right)^2}$$

$$\mathbf{w = 4\sigma \Rightarrow N = \left(\frac{t_R}{w/4}\right) = 16\left(\frac{t_{R_A}}{w_A}\right)^2}$$

relation of theoretical plate number, column length & σ
: σ – standard deviation of peak position

$$\mathbf{w_{1/2} = 2.354\sigma \Rightarrow N = 5.545\left(\frac{t_{R_A}}{w_{1/2_A}}\right)^2}$$

$$\mathbf{N = \left(\frac{L}{\sigma}\right)^2}$$

effective number of theoretical plates of column

$$N' = N \cdot \left(\frac{k}{1+k}\right)^2$$

: measure of column efficiency for higher ***k*** values
: mostly in GC

$$N' = 16 \cdot \left(\frac{t'_{R_A}}{w_A}\right)^2 = 5.545 \cdot \left(\frac{t'_{R_A}}{w_{1/2_A}}\right)^2$$

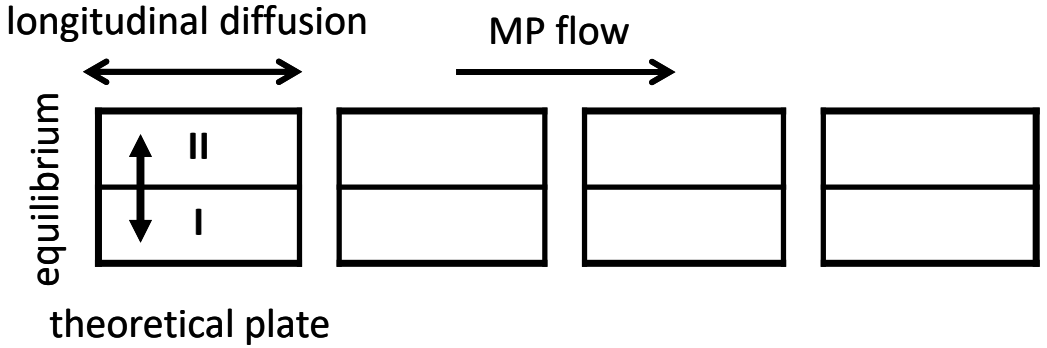
height equivalent of the theoretical plate (*HEPT*)

: comparison of column with different length

σ^2 – mean of squared deviation of peak position

$$H = \frac{L}{N} = \frac{L}{16} \cdot \left(\frac{w_A}{t_{R_A}}\right)^2 = \frac{L}{5.545} \cdot \left(\frac{w_{1/2_A}}{t_{R_A}}\right)^2$$

$$H = \frac{\sigma^2}{L}$$



$$\sigma = \sqrt{r \cdot \left(\frac{k'_A}{(1+k'_A)^2}\right)}$$

r – transport number (~H), k'$_A$ ~ distribution ratio

molecular diffusion

mass transfer against the concentration gradient (ΔS>0)

2nd Fick law

$$\frac{\partial c}{\partial t} = D_m \cdot \frac{\partial^2 c}{\partial z^2} - u \cdot \frac{\partial c}{\partial z}$$

Einstein equation

$$t_D = \frac{l}{2D_m}$$

$\partial c/\partial t$ – increase of analyte concentration on unit area at time unit,
$\partial c/\partial z$ – convective transport (on ***z*** axis) at rate **u**, D_m – diffusion coefficient,
l – diffusing particles trajectory, t_D – time needed to travel its distance

$$c = \frac{1}{2(\pi \cdot D_m \cdot t)^{1/2}} \cdot e^{\left(\frac{-x^2}{4D_m \cdot t}\right)}$$

solution of 2nd Fick law

$$\sigma^2 = 2D_m \cdot t$$

Eistein-Smoluchowski equation

σ^2 – diffusing compound zone width

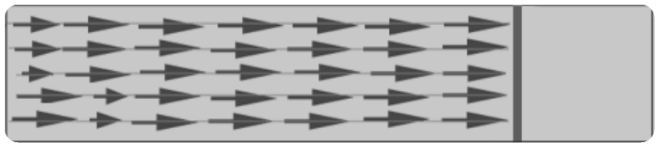
convection in open channel

$$R_e = \rho \cdot u \cdot \frac{d_k}{\eta}$$

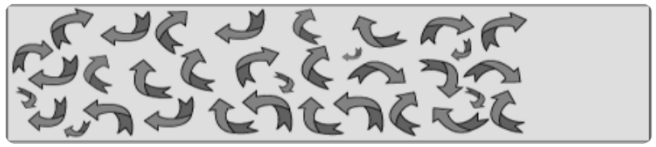
R_e – Reynolds number
: marks convection type

ρ – liquid density, **u** – linear flow rate, d_k – channel diameter, **η** – viscosity

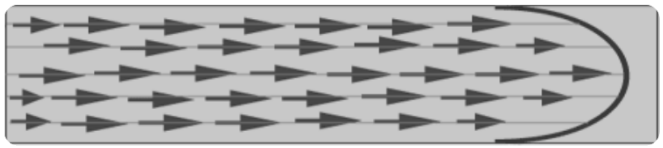
: ideal (piston) convection – $R_e \sim \infty$
:: unfeasible in praxis



: turbulent convection – $R_e \geq 2100$
:: basically better than laminar



: laminar (parabolic) convection – $R_e < 2100$
:: most often in praxis



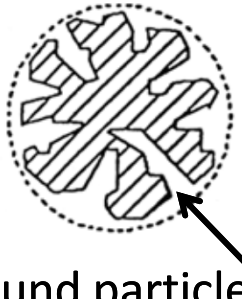
$$u_z(r) = \left[\left(a^2 - r^2\right)/4\right] \cdot \frac{\Delta p}{\mu \cdot L}$$

[pwah-ZAY]

Hagen-Poisseuille equation

$u_z(r)$ – forward flow rate of liquid in column axis in distance **r** from this axis, **a** – column diameter, **Δp** – pressure difference between input and output of column, **μ** – dynamic viscosity of liquid, **L** – length of column

convection between porous particles



: turbulent convection – $R_e \geq 100$
: turbulent + laminar convection – $R_e = 1 \div 100$
: laminar (parabolic) convection – $R_e < 1$

three types of space
: stationary phase – unreachable
: stagnant mobile phase – in pores and around particle
: moving mobile phase

$$\xi_{ip} = \frac{V_{ip}}{V_{col}}$$ ξ_{ip} – intra-particle porosity, V_{ip} – pore volume, V_{col} – column volume **ξ_{ip} ~ 0.4**

$$\xi_{ep} = \frac{V_{ep}}{V_{col}}$$ ξ_{ep} – inter-particle porosity, V_{ep} – moving MP volume, V_{col} – column volume **ξ_{ep} ~ 0.4**

$$\xi_{tot} = \frac{V_m}{V_{col}}$$ ξ_{tot} – total porosity, V_m – void volume, V_{col} – column volume

ξ_{tot} ~ 0.8
: *ca* 80 % of column is SP

average flow rate

$$\bar{u} = \Delta p \cdot \frac{B_0}{\xi_{ip} \cdot \eta \cdot L}$$

Darcy equation

specific coefficient of permeability

$$B_0 = \frac{d_p^2 \cdot \xi_{ep}^3}{180 \cdot (1 - \xi_{ep})^2}$$

Kozeny-Carman equation

premises
: laminar convection
: $\xi_{ep} \leq 0.5$

Δp – pressure difference between inlet and outlet of column, d_p – particle diameter

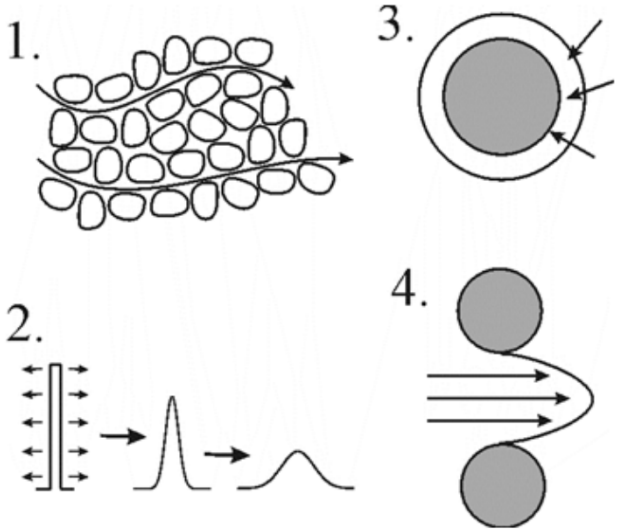
reasons for zone broadening

eddy (turbulent) diffusion
: different molecules must run different distances

longitudinal molecular diffusion
: molecules run from a place of **higher** concentration to places w/ **lower**

mass transfer resistance in stationary phase
: different molecules diffuse different deeply into SP

mass transfer resistance in mobile phase
: flow rate profile of MP inside of the channel is parabolic



influence of MP flow rate on zone broadening

height equivalent of theoretical plate (H) depends on linear MP flow rate (u)

$$\mathbf{H} = \boldsymbol{f}(\mathbf{u})$$

$$\mathbf{H} = \mathbf{H_V} + \mathbf{H_P} + \mathbf{H_S} + \mathbf{H_M}$$

individual contributions

eddy diffusion

$$H_V = \frac{(\sigma^2)_V}{L} = A\ (const.)$$

not dependent on **u**

Einstein-Smoluchowski equation

$$D_E = \lambda \cdot d_p \cdot u \qquad \sigma^2 = 2D_m \cdot t = 2D_m \cdot \frac{L}{u} \qquad H_V = \frac{(\sigma^2)_V}{L} = A = 2\lambda \cdot d_p \cdot L$$

D_E – formal effective diffusion coefficient

λ – empirical SP homogeneity factor
d_p – average SP particle size

longitudinal molecular diffusion

$$H_P = \frac{(\sigma^2)_P}{L} = \frac{B}{u}$$

$$H_P = \frac{(\sigma^2)_P}{L} = \frac{B}{u} = \frac{2D_m}{u}$$

$$H_P = \frac{(\sigma^2)_P}{L} = \frac{B}{u} = \frac{2D_m}{\left(u \cdot \left(1 + \frac{\xi_{ip}}{\xi_{ep}}\right)\right)} = \frac{2D_m \cdot \gamma}{u}$$

$B = 2D_m$ **open channel**

$B = 2\gamma \cdot D_m$ **column with particles**

ξ_{ip} – intraparticle porosity, ξ_{ep} – interparticle porosity, **γ** – MP resistance (labyrinth / tortuosity) factor, D_m – analyte diffusion coefficient in MP

mass transfer resistance in MP

$$\mathbf{H_M = C_M \cdot u}$$

$$\mathbf{H_V + H_M = A + C_M \cdot u = \left(\frac{2\lambda \cdot d_p^{(1+x)}}{D_m^x} \right) \cdot u^x}$$

$\mathbf{d_p}$ – average particle size, $\boldsymbol{\lambda}$ – eddy diffusion coefficient (0.5 ÷ 1.5), **x** – system constant (0 ÷ 0.33; x = 0 for GC, x = 0.33 for LC)

mass transfer resistance in SP

$$\mathbf{H_S = C_S \cdot u} \qquad \mathbf{H_S = (C_{M\rightarrow S} + C_{S\rightarrow M}) \cdot u}$$

out of MP into SP

$$\mathbf{H_{M\rightarrow S} = C_{M\rightarrow S} \cdot u = f(k) \cdot \left(\frac{d_p^2}{D_{MP}} \right) \cdot u}$$

$$\boldsymbol{f(k) = \frac{Q \cdot \xi_{ep} \cdot k^2 \cdot \left(1 + \frac{\xi_{ip}}{\xi_{ep}} \right)}{\xi_{ip} \cdot (1+k)^2}}$$

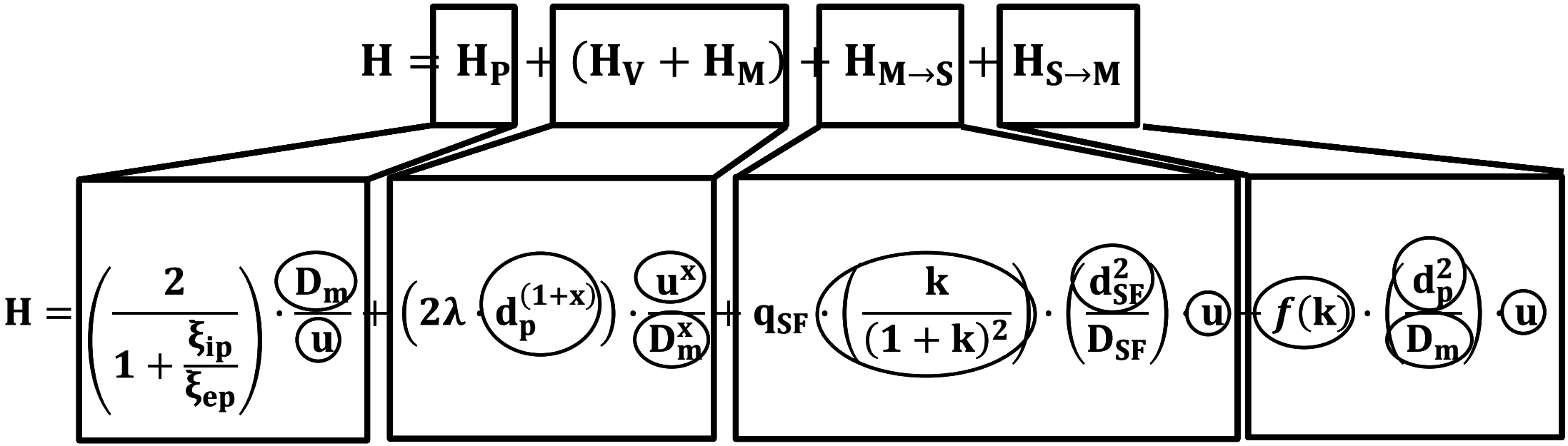
$\boldsymbol{f}$ **(k)** – function proportional to capacity factor, $\mathbf{D_{MP}}$ – diffusion coefficient in MP, $\mathbf{d_p}$ – SP particle diameter, **Q** – shape coefficient (1/30 for sphere)

out of SP to MP

$$\mathbf{H_{S\rightarrow M} = C_{S\rightarrow M} \cdot u = q_{SP} \cdot \left(\frac{k}{(1+k)^2} \right) \cdot \left(\frac{d_{SP}^2}{D_{SP}} \right) \cdot u}$$

$\mathbf{q_{SP}}$ – constant of SP active surface shape (2/3 for thin layer), $\mathbf{D_{SP}}$ – diffusion coefficient in SP, **k** – capacity ratio, $\mathbf{d_{SP}}$ – thickness of SP active surface

$$H = A + \frac{B}{u} + (C_S + C_M) \cdot u = A + \frac{B}{u} + C \cdot u$$



van Deemter equation (van Deemter, Zuiderweg, Klinkenberg)

$$H = A + \frac{B}{u} + (C_S + C_M) \cdot u = A + \frac{B}{u} + C \cdot u$$

low flow rate (**C·u** is small)
: **H** depends on **B/u**
high flow (**B/u** is small)
: **H** is directly proportional to **C·u**

for GC

Knox equation

for LC

$$h = A \cdot \sqrt[3]{\nu} + \frac{B}{\nu} + C \cdot \nu \qquad h = \frac{H}{d_p} \qquad \nu = u \cdot \frac{d_p}{D_m}$$

reduced parameters
: dimensionless

Golay equation

$$H = \frac{B}{u} + C \cdot u$$

A → 0 for open tubular columns (OTC)

Giddings equation

$$H = \frac{A}{1 + \frac{E}{u}} + \frac{B}{u} + C \cdot u$$

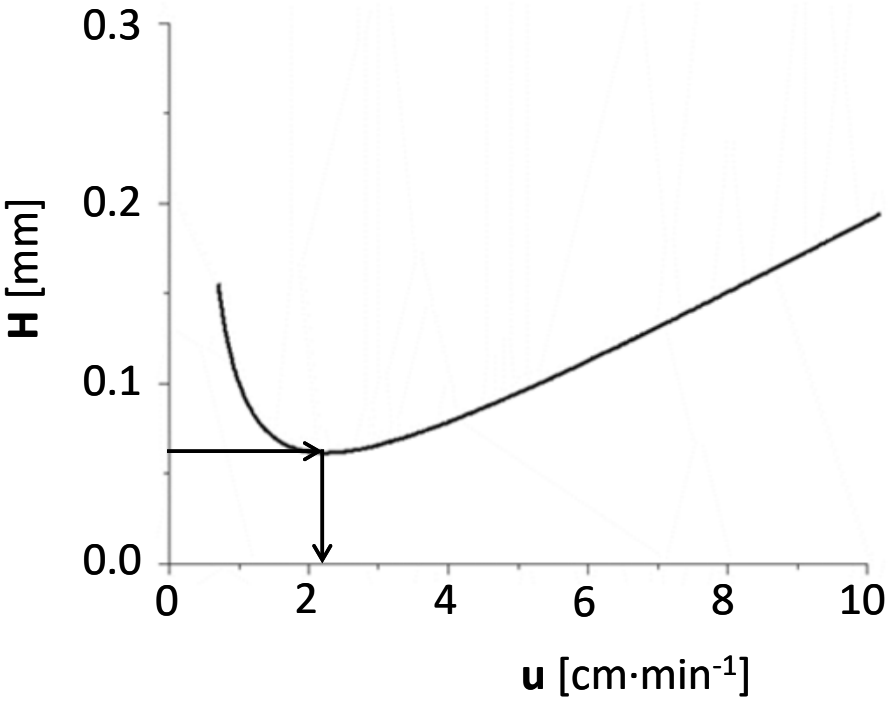
includes in term **E** diffusivity of MP
u >> E → van Deemter, **u << E** → 1st term = 0

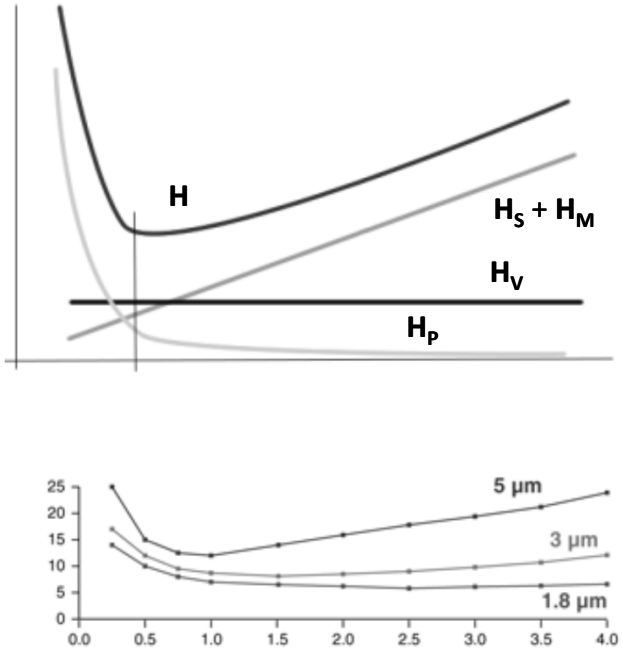
Huber(-Hulsman) **equation**

$$H = \frac{A}{1 + \frac{E}{\sqrt{u}}} + \frac{B}{u} + C \cdot u + D \cdot \sqrt{u}$$

term **D** – turbulent mixing
√u >> E → similar to van Deemter equation

van Deemter curve





$$\frac{\partial H}{\partial u} = -\frac{B}{u^2} + C = 0$$

$$H_{opt} = A + 2\sqrt{B \cdot C}$$

$$u_{opt} = \sqrt{\frac{B}{C}}$$

curve minimum ≈ optimal flow rate
: given column shows **the highest efficiency**
:: minimal zone broadening of analytes

resolution

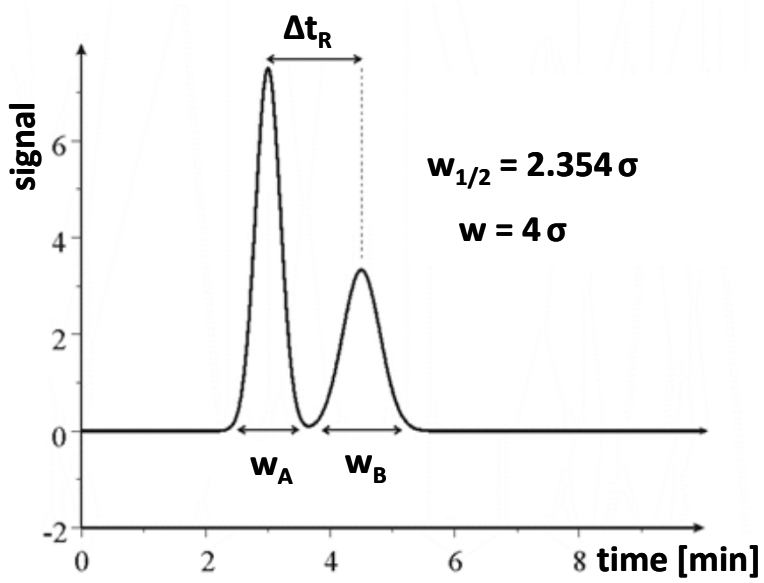
resolution characterises
: measure of relative separation
: measure of mutual overlap of two neighbouring peaks

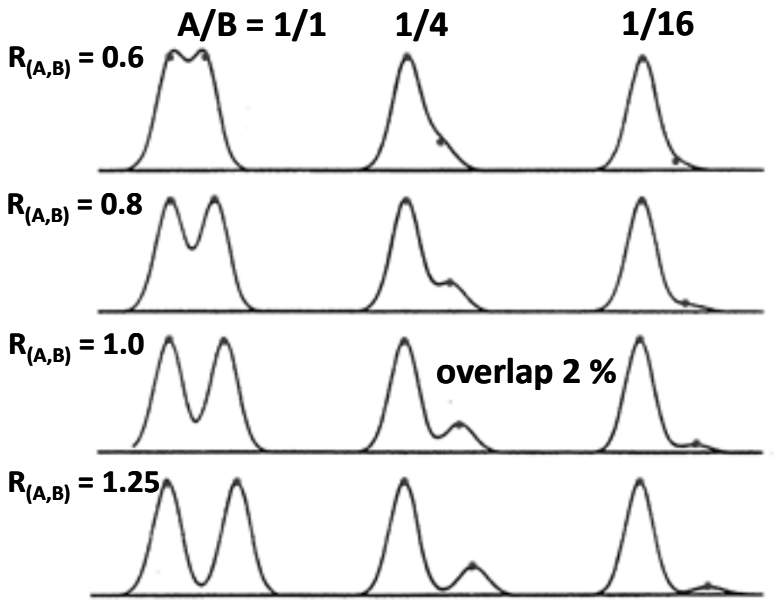
$$R_{(A,B)} = \frac{2\left(t_{R_B} - t_{R_A}\right)}{w_A + w_B} = \frac{2 \cdot \Delta t_R}{w_A + w_B} \approx \frac{x_2 - x_1}{4\sigma}$$

US Pharmacopoeia (USP)

$$R_{(A,B)} = \frac{1.18\left(t_{R_B} - t_{R_A}\right)}{w_{1/2_A} + w_{1/2_B}}$$

European Pharmacopoeia (Ph.Eur.), EDQM, Council of Europe





$R_{(A,B)} > 1.5$ – complete separation, at $R_{(A,B)} = 1.5$ the overlap is **0.1 %**

resolution factors

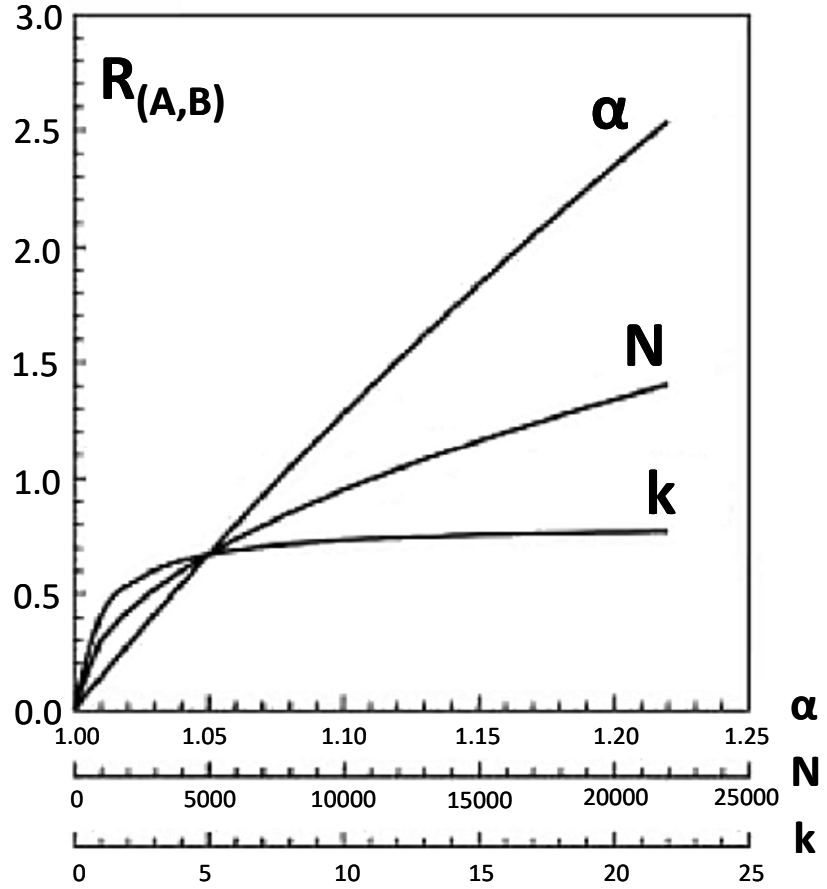
let us presume for two neighbouring peaks

: $N_A \approx N_B$

: $\alpha > 1$

$$\mathbf{R_{(A,B)}} = \frac{\sqrt{\mathbf{N}}}{\mathbf{4}} \cdot \frac{\boldsymbol{\alpha}_{\mathbf{(A,B)}} - \mathbf{1}}{\boldsymbol{\alpha}_{\mathbf{(A,B)}}} \cdot \frac{\mathbf{k_B}}{\mathbf{1} + \mathbf{k_B}}$$

efficiency *selectivity* *capacity*

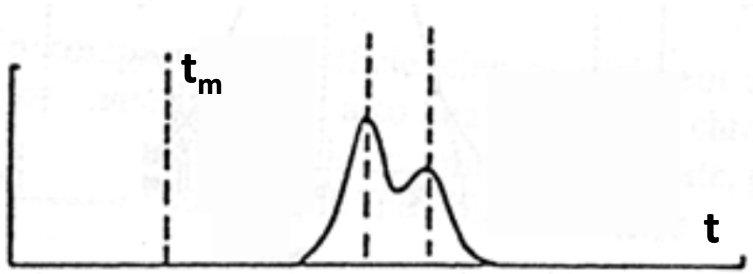


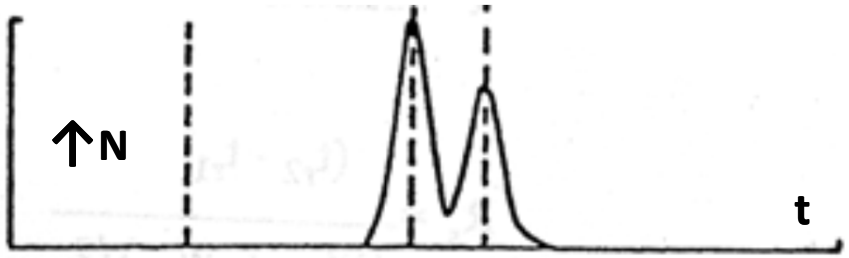
capacity factor (practically k ≈ 3 to 10)
: amount of SP in column
: change of SP or MP
: temperature (in LC less important)

efficiency factor
: mobile phase flow rate
: column length
: grain size, temperature, viscosity

selectivity factor (very important)
: stationary phase change
: mobile phase change

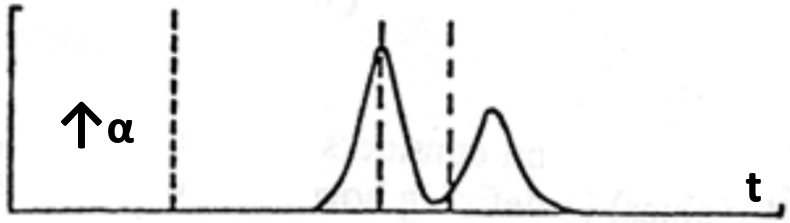
chromatographic separation
: model **initial state**

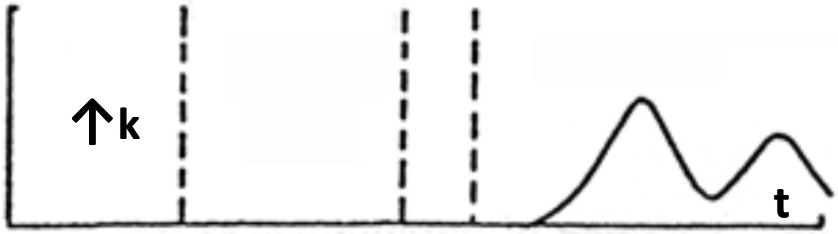




influence of change of theoretical plates number

influence of selectivity change





influence of capacity factor change

peak capacity

ability to separate

: number of well-resolved (**R** > x) peaks (**n**) during separation (t_{R0} to t_{Rmax})

general view

$$n = 1 + \int_{t_{R_0}}^{t_{R_{max}}} \frac{1}{w} \cdot dt = 1 + \int_{t_{R_0}}^{t_{R_{max}}} \frac{1}{4\sigma} \cdot dt \qquad \sigma = \frac{t_{R_0}}{\sqrt{N}} \cdot (k+1)$$

w – base peak width, **k** – capacity factor

$$n = 1 + \int_{t_{R_0}}^{t_{R_{max}}} \frac{\sqrt{N}}{4} \cdot \frac{1}{k+1} \cdot \frac{dt}{t} \quad \Rightarrow \quad n = 1 + \frac{\sqrt{N}}{4} \cdot \frac{1}{k+1} \cdot \ln \frac{t_{R_{max}}}{t_{R_0}}$$

N *is not constant* for different analytes, thus in different t_R: **N** = ***f*** **(t_R)**

$$n = 1 + \frac{\sqrt{N}}{4} \cdot \ln \frac{t_{R_{max}}}{t_{R_0}}$$

simplified view

$$\frac{1}{k+1} \approx 1$$

$$n = \frac{t_{r_{max}}}{w_{last} - w_{first}} \cdot \ln \frac{w_{last}}{w_{first}}$$

formula with efficiency

$$n = \frac{t_{R_{max}}}{\sum_{i=1}^{j} w_i / j}$$

the most general formula

basic chromatography arrangement

: **column arrangement** (in tube, in capillary)
: **planar arrangement** (inter cellulose fibres of e.g. paper)

chromatographic analysis is conducted mostly in diluted analyte solutions

$\Rightarrow$ linear region of **sorption isotherm**

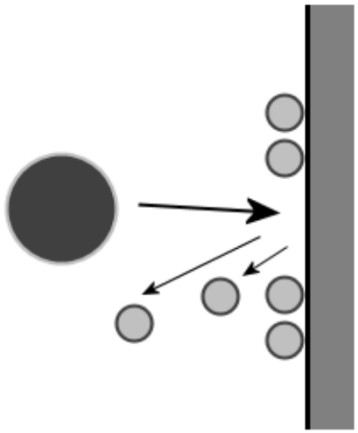
basic principles of chromatography

: **adsorption**
:: sorption and desorption of analyte in phase system G-S or L-S

: **partition**
:: distribution of analyte between two phases G-L or L-L

: **ion-exchange**
:: exchange of ion and counter-ion in system of phases L-S

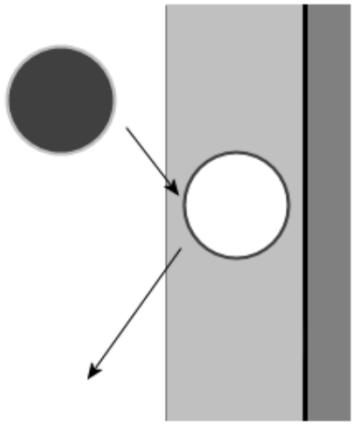
: **affinity**
:: specific binding of two molecules through weak interactions



adsorption chromatography
LSC, GSC

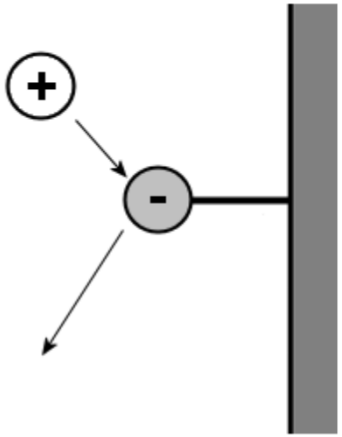
different adsorption of molecules **A** between solid surface of SP & fluid mobile phase MP (L, G)

$$\mathbf{K_D} = \frac{\mathbf{a_A^S}}{\mathbf{a_A^M}}$$ ***S*** – SP, ***M*** – MP



partition chromatography
LLC, (GLC)

different distribution of analyte molecules (**A**) between two completely immiscible fluids (LL, GL)



exchange distribution of ions **X** and counter-ions **Y** between surface of (ion) exchanger **R** and mobile phase solution

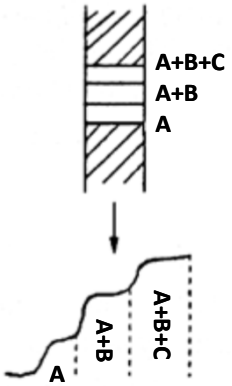
exchange chromatography
EC

$$\mathbf{K_E} = \frac{[\mathbf{R^-}] \cdot [\mathbf{X^+}] + [\mathbf{Y^+}]}{[\mathbf{R^-}] \cdot [\mathbf{Y^+}] + [\mathbf{X^+}]}$$

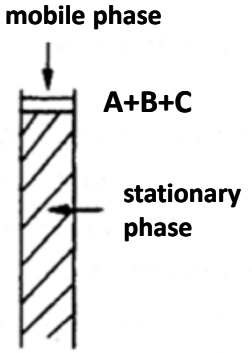
R $^-$ (***R*** $^+$) – anex, (catex)

X $^+$, ***Y*** $^+$ (***X*** $^-$, ***Y*** $^-$) – ion and counter-ion

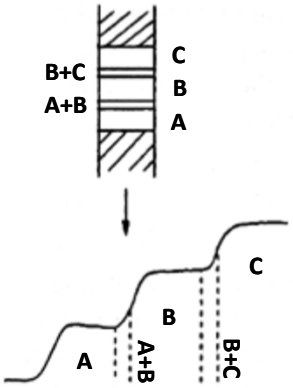
basic chromatographic techniques

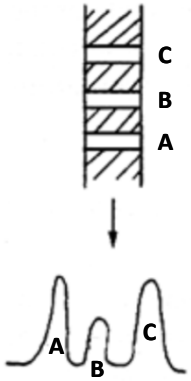


: **frontal**
:: continual introduction of mixture with constant concentration
use
: measurement of adsorption isotherms
: industrial preparative separation
:: isolation of major mixture component



: **displacement**
:: introduction of mixture, eluted just after change of MP
use
: pre-concentration (SPE), preparative separation, affinity & ionex chromatography





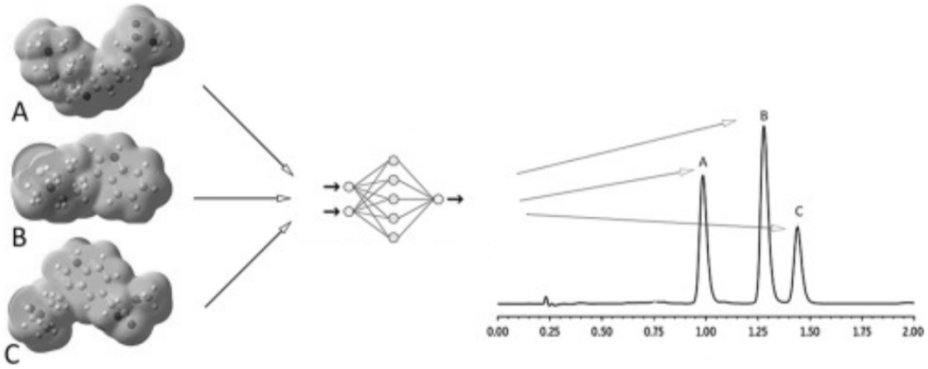
: **elution**
:: single introduction of mixture into continuously flowing MP
use
: partition and adsorption liquid chromatography

study and description of chromatographic separation

quantitative structure-retention relationship (**QSRR**)

complex modelling of retention properties **numerically** (*hard modelling*) asks for alternative ways
: **semi-approximation** (*semi-hard*)
: **approximation** (*soft modelling*) method of retention properties modelling

approaches within separation relations modelling



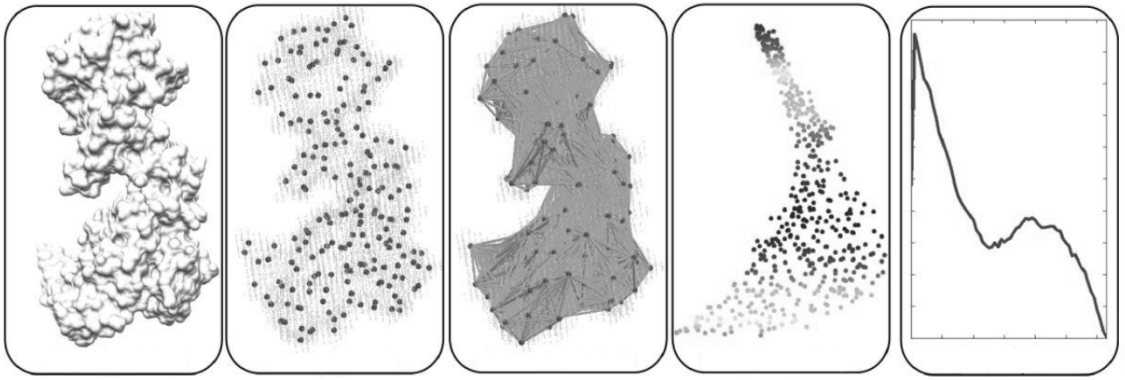
: models including structural descriptors
: models including solvation effects
:: reduced linear solvation energy relationship (LSER)
: models including distribution factors
:: correlation between retention analyte in RPLC system and its distribution coefficient
::: e.g. in system n-octanol / water or hexadecane (l) / hexadecane (g)

methods of relations modelling

: combination of multilinear regression and artificial neural networks (MLR-ANN)
: comparative analysis of molecular fields (CoMFA)

descriptors of separated analyte

: allow ***to describe*** **system** and **interaction** at least **semi-empirically**
: are used within development of separation methods



:: angular momentum
:: total energy
:: polarisability
:: ionisation potential
:: dipole moment
:: subpolarity – ability of analyte to create polar interaction with SP
:: stericity – geometry of molecule
:: hydrophobicity
:: HOMO/LUMO energies of molecular orbitals
:: free Gibbs energy of adsorption

important factors in regard to prediction of separation optima

retention
: polarisability of analyte and subpolarity; LUMO energy

selectivity
: hydrophobicity

calculations of some complex descriptors

geometry and interaction properties

: semi-empirical quantum-chemistry models – AM1, MNDO and PM3

holistic descriptor (hydrophobicity, stericity and electric properties)

: weighted holistic invariant molecular descriptors (WHIP)

$$\mathbf{log(1/C) = a \cdot \pi + b \cdot \sigma + c \cdot Es + d}$$

C – molar concentration of molecules in given state (= property)

π – hydrophobicity index, **σ** – electric property index, **Es** – stericity index

free Gibbs energy of adsorption

$$\mathbf{\Delta G_{ads} = -R \cdot T \cdot log(k/\Phi)} \qquad \mathbf{\Phi = V^S/V^M}$$

Δ(**Δ**G_{ads}) – difference of free Gibbs energy of adsorption of two separated analytes

capacity and selectivity factors

$$\mathbf{\alpha = k_B/k_A} \qquad \mathbf{\alpha = e^{[(1/R \cdot T) \cdot \Delta(\Delta G_{ads})]}}$$

semi-empirical models used

solvation parameter model

: Poole

:: valid to LLC, GLC or chemically bound SP

::: interaction solvent-solute (solvation)

$$\log k = c + m \cdot V_x + r \cdot R_2 + s \cdot \pi_2^H + a \cdot \sum \alpha_2^H + b \cdot \sum \beta_2^H$$ **LC**

$$\log k = c + r \cdot R_2 + s \cdot \pi_2^H + a \cdot \sum \alpha_2^H + b \cdot \sum \beta_2^H + I \cdot \log L^{16}$$ **GC**

descriptors of solute *(Kamlet-Taft parameters)*

: R_2 – interaction between π-systems of solvent and solute

: π_2^H – dipole-dipole interactions

: $\Sigma\alpha_2^H$ – acidity of hydrogen bridges (donor)

: $\Sigma\beta_2^H$ – basicity of hydrogen bridges (acceptor)

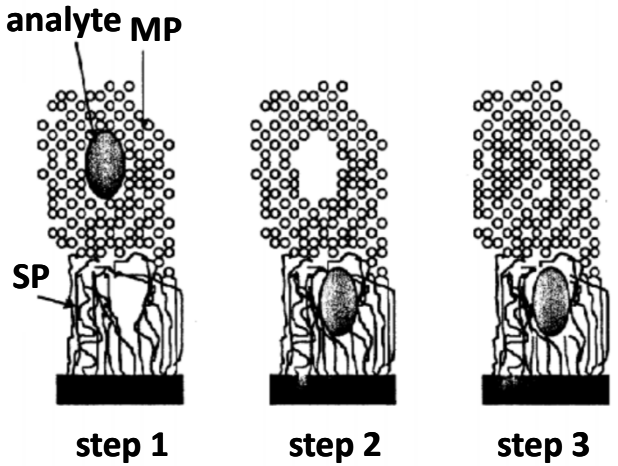
: $\log \cdot L^{16}$ – $K_{D,g\text{-}l}$ of solute in hexadecane (London forces)

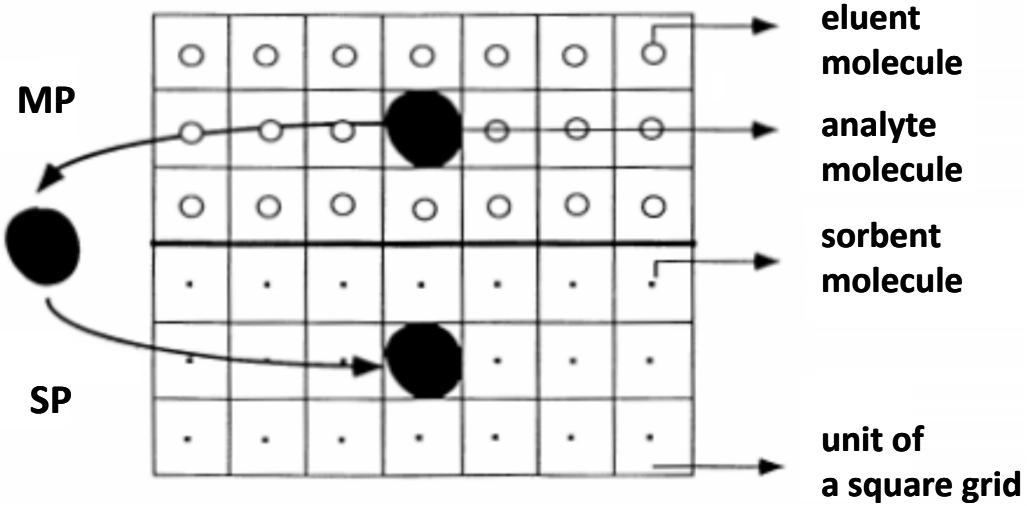
: V_x – molecular volume (McGowan method)

system descriptors – c, m, r, s, a, b, l (dependent on SP, MP & temperature)

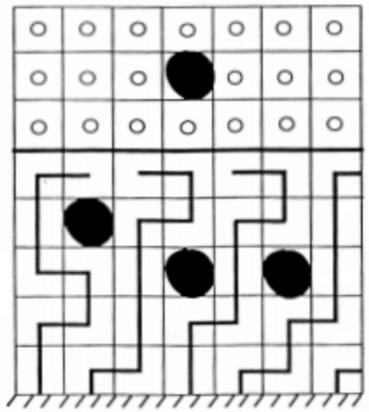
solvatophobic model
: Horváth

partition and displacement model (*cavity model*)
: Jaroniec; Dill
: creating cavity in SP
: transfer of analyte into SP
: closing cavity in MP





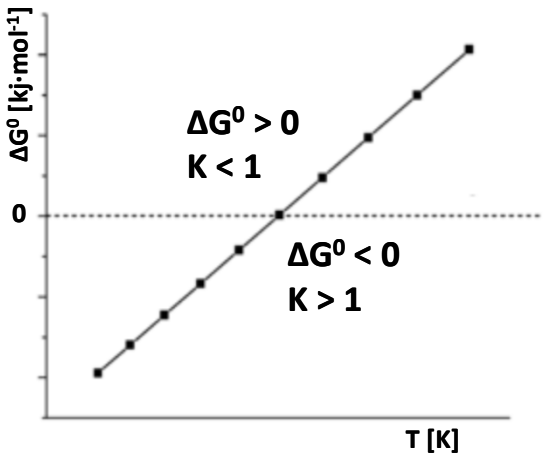
lattice model
: Martire & Boehm, Dill



phenomenological model
: LePree and Cancino

van't Hoff's plots retention phenomena dependence on temperature

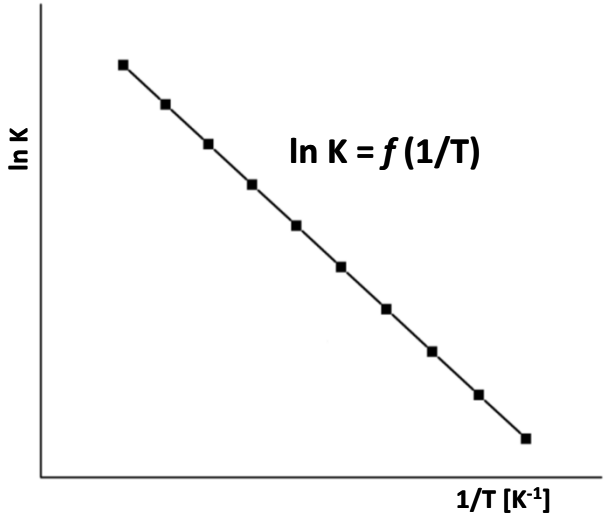
$$\Delta G^0 = \Delta H^0 - T \cdot \Delta S^0 \qquad \Delta G^0 = -R \cdot T \cdot \ln K$$



$$\ln K = -\frac{\Delta H^0}{R \cdot T} + \frac{\Delta S^0}{R} = \ln\left(k \cdot \frac{V^M}{V^S}\right)$$

$$\ln k = -\frac{\Delta H^0}{R \cdot T} + \frac{\Delta S^0}{R} - \ln\frac{V^M}{V^S} \sim$$

$$\sim -\frac{\Delta H^0}{R \cdot T} + \frac{\Delta S^0}{R} + \ln\frac{V^S}{V^M}$$



$$a \sim -\frac{\Delta H^0}{R}$$

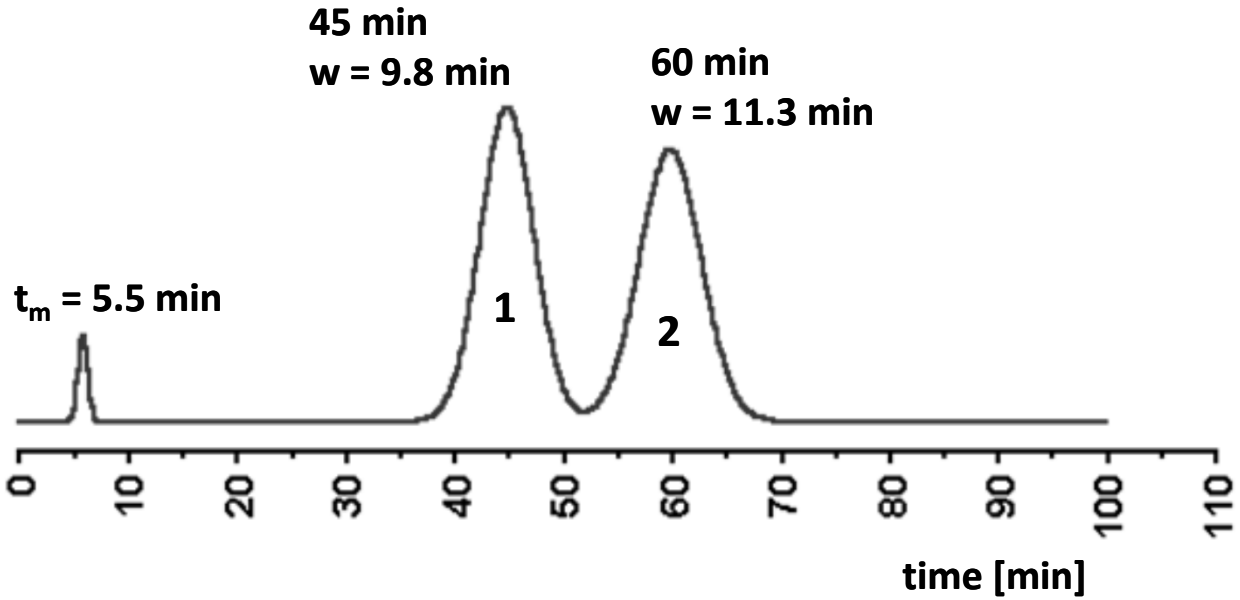
$$b \sim -\frac{\Delta S^0}{R}$$

: determining exo- / endo-thermicity of a process
:: slope value
: linear vs. non-linear curve
:: phase transitions in stationary phase (qS > qL)
: „breaks“ in line
:: analyte pK_a changes (-0.03 K^{-1})

$$\ln k_2 - \ln k_1 = \ln\frac{k_2}{k_1} = \ln\alpha = -\frac{\Delta\Delta H^0}{R \cdot T} + \frac{\Delta\Delta S^0}{R}$$

example 7 : part 1

injecting two-componential mixture onto 150 mm column C18 (=strong non-polar SP), with MP methanol : water 7:3 (v/v), MP flow-rate 0.5 ml·min^{-1}, we obtained following chromatogram:



: calculate resolution, capacity factor and H of *component 2*

: which component has higher affinity to SP and how long it stayed on it during separation?

: how would the separation parameters change (t_R, $R_{(A,B)}$, N, k, α), if we change MP for 100% methanol?

example 7 : part 2

???

: **extraction L-L**
: **extraction L-S**

liquid chromatography

: **mobile phase** (**MP**, liquid or supercritical fluid)
: **stationary phase** (**SP**, solid matter or thin layer of liquid on solid carrier)

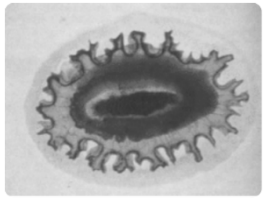
contact area (*max*), where the sorption/desorption of analyte happens
: liquid flows between particles (~ μm) of sorbent

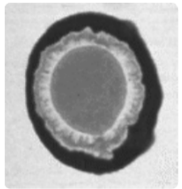
LC history

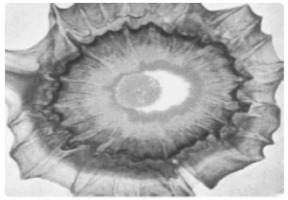
1834 Friedlieb Ferdinand Runge

: chemist
: he discovered the method of ***capillary migration***
:: *Chemische Produktionsfabrik Oranienburg*

F. F. Runge, *Farbenchemie I* (1834)

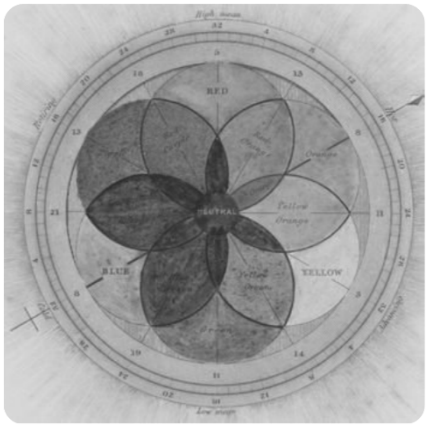






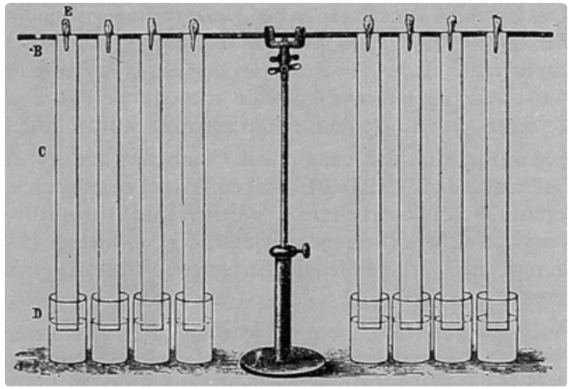
1835 George Field

: chemist
: worked in dye chemistry



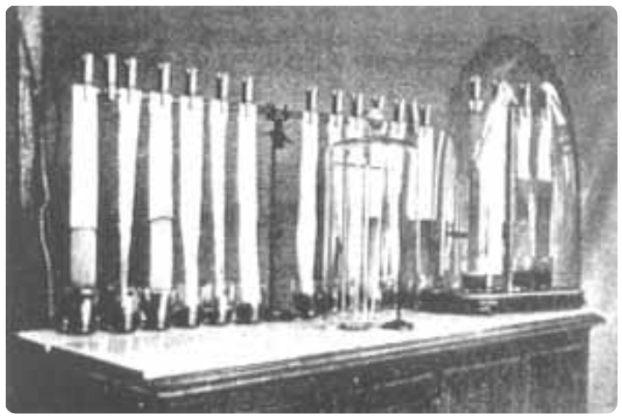
he introduced new terms
: **chromatography** – studies on colours in painting
:: Greek ***τό χρώμα*** (colour) a ***γράφειν*** (to write)
: **chromatograph** – colour preparation apparatus
: **chromatology** – colour studies and analysis

1861 **Christian Friedrich Schönbein**



: chemist
: lecture on use of capillary migration (*Haarröhrchenanziehung*)
:: arrangement with hanging paper strip
::: water moves quicker than colours, and with these, each differently

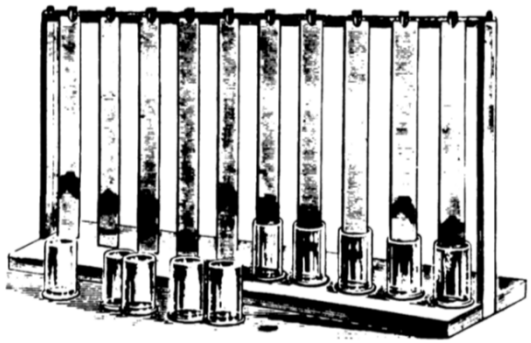
1897 **David Talbot Day**



: geologist & petrologist
: he used column to study influence of geological layers on oil

D. T. Day, *Proc. Am. Phil. Soc.*, 36 (1897) 112
W. C. Mendenhall, *Science*, 17 (1903) 1007

1900 **Christoph Friedrich Goppelsröder**

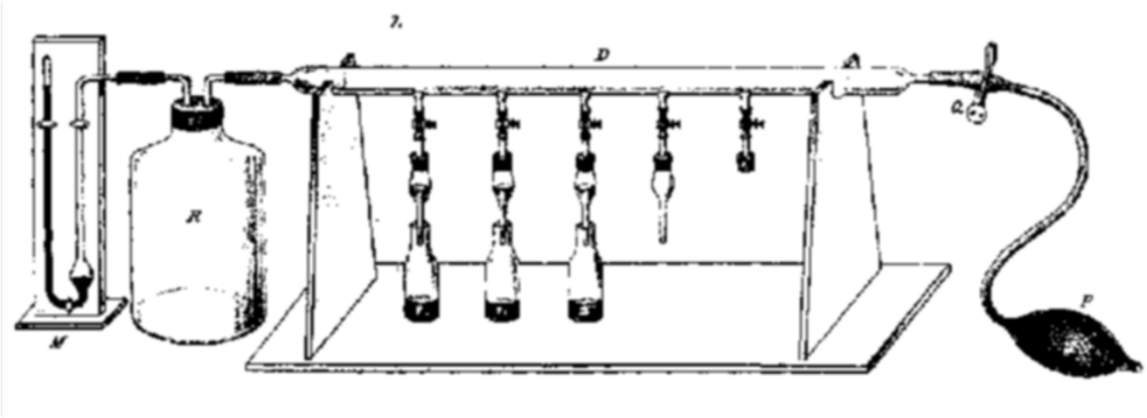


: chemist
: described capillary migration as *adsorption analysis*
:: inspiration by Schönbein's lecture
:: used for analysis of (plant) colourants

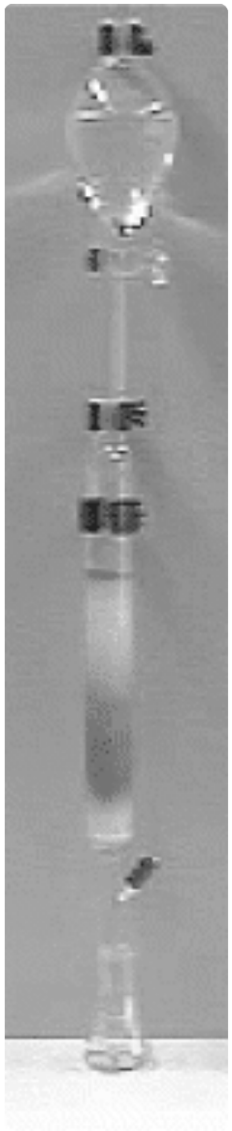
1903

Mikhail S. Tswet
(*rus*. Михаил Семёнович Цвет, *it*. Michail S. Tswett)

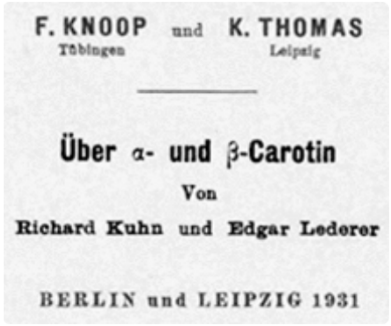
: botanist
: separation of chloroplast pigments of different plant extracts
:: glass column filled with $CaCO_3$ using organic solvents
:: ***chromatographic adsorption analysis*** *(on column)*



M. Tswett, *Trav. Soc. Nat. Varsovie*, 6 (1903) 14
M. Tswett, *Ber. Dtsch. Botan. Ges*., 24 (1906) 316
M. Tswett, *J. Chem. Educ*., 44 (1967) 238



1931 **Kuhn** and **Lederer** : re-discovery of LC
first application – carotenoid separation


F. KNOOP und K. THOMAS
Tübingen Leipzig

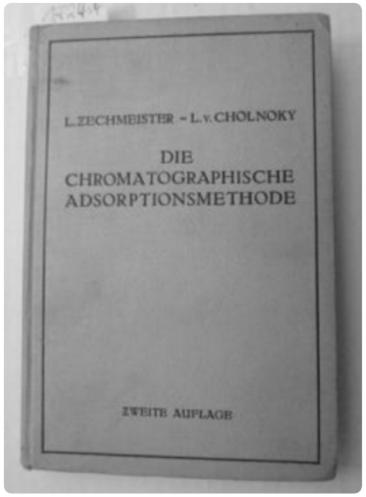
Über α- und β-Carotin

Von

Richard Kuhn und Edgar Lederer

BERLIN und LEIPZIG 1931

1936 **Zechmeister** and **von Cholnoky** –
book *Die chromatographische Adsorptionsmethode*


L. ZECHMEISTER – L. v. CHOLNOKY

DIE
CHROMATOGRAPHISCHE
ADSORPTIONSMETHODE

ZWEITE AUFLAGE

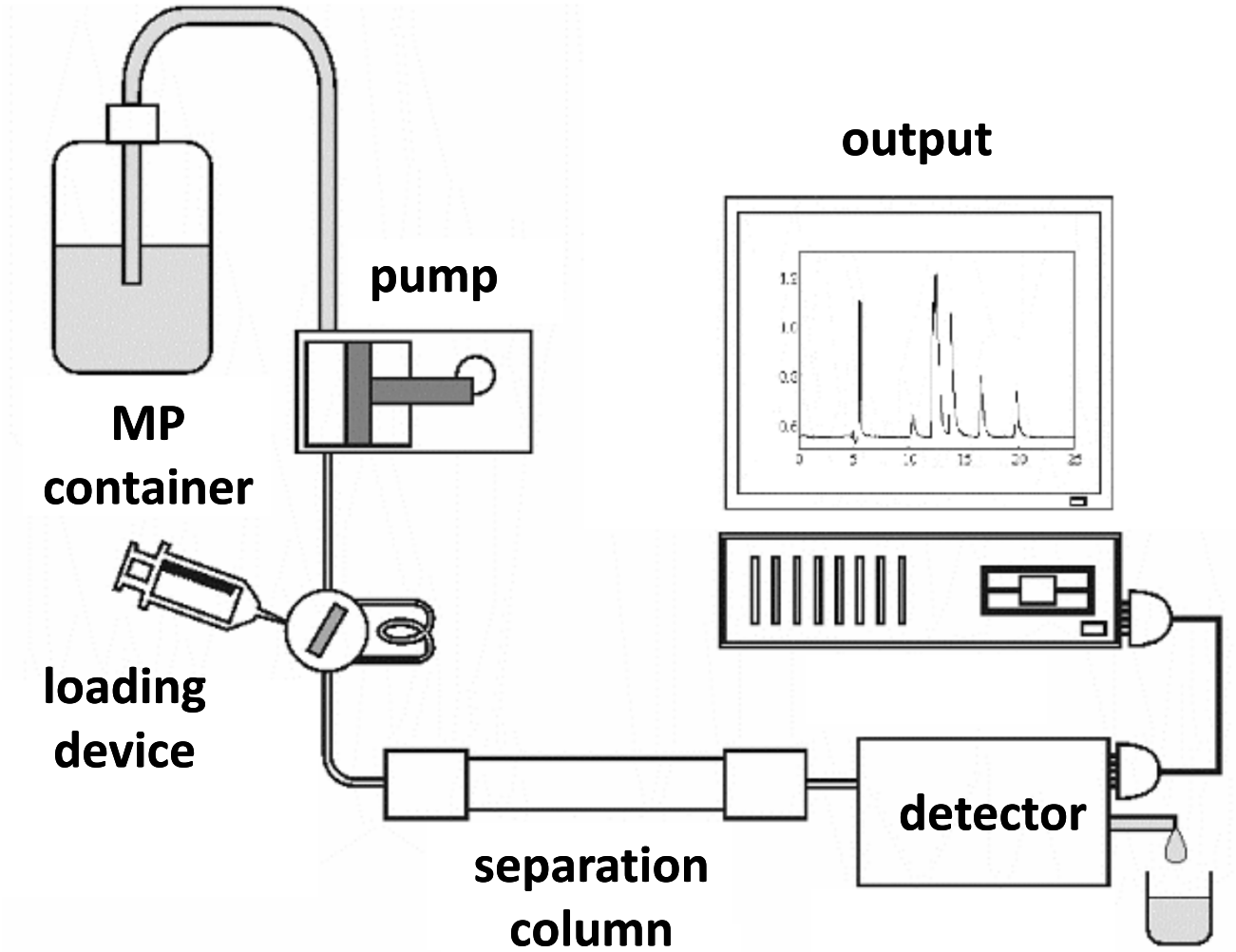
M. Steiger and T. Reichstein, *Helv. Chem. Acta*, 31 (1938) 546
A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, 35 (1941) 1358
R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, 38 (1944) 224
A. Tiselius, *Science*, 94 (1941) 145

1940-1949 **Martin** and **Synge** – separation rate is limited by diffusion rate
: of dissolved analyte from liquid phase
: separation of small molecules, namely amino acids (AA in wool)

since 1965 **Halasz**, **Horvath**, **Kirkland** *et al.*, **Regnier** *et al.*
: high performance liquid chromatography (HPLC)

contemporary : micro-, nano-column LC; capillary LC
: monolithic columns, LC-on-chip
: separation at very high pressures

liquid chromatography arrangement



MP delivery

: undisturbed and stabile flow of liquid
: 0.001 – 10 ml·min^{-1}
: pressure up to 40 MPa (400 bar, 395 atm, 5800 psi)
: resistance to MP (influence of e.g. salts)
: pressure and flow control
: thermostating possibility

psi (*pound per square inch*)
1 psi = 6 894.75729 Pa

atmosphere
1 atm = 1.01325 bar

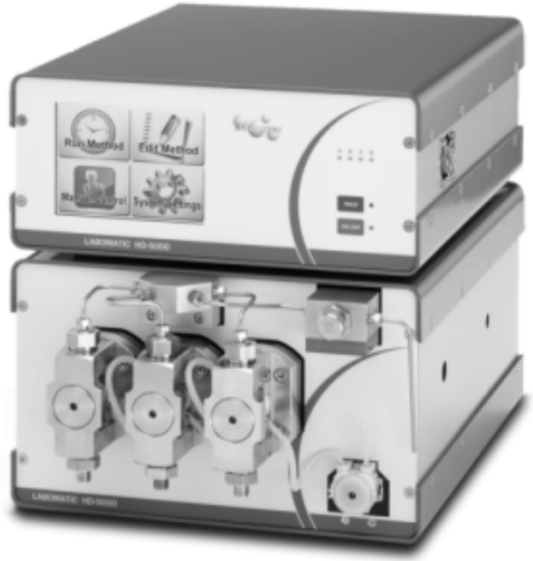
standard pressure
101325 Pa

Torr, mmHg (*Torricelli*)
1 Torr = 133.3224 Pa

bar (*βάρος*)
1 bar = 100 kPa

basic pump types according to flow rate

: high flow rate > 10 ml·min^{-1} (perfusion separation, monoliths, affinity separation)
: conventional 0.2 – 10 ml·min^{-1}
: low flow rate < 0.2 ml·min^{-1} (micro- and capillary columns)



constant pressure pump

only for column filling with SP

constant flow pump

analytical use
: single-action piston (syringe) pumps
: double-action piston (reciprocating) pumps

syringe pump

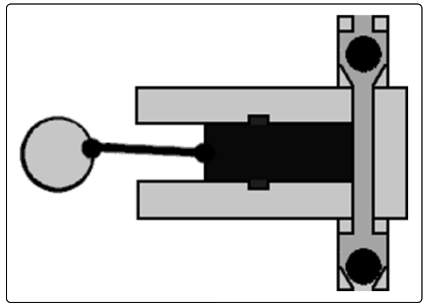
(+) easy and robust
: high pressures

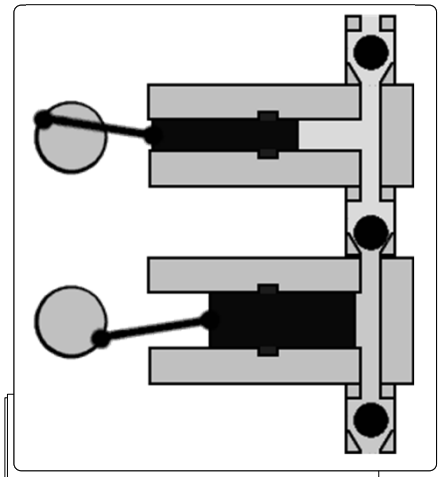
(–) small reservoir
: limited use in gradient
: problems with MP degassing

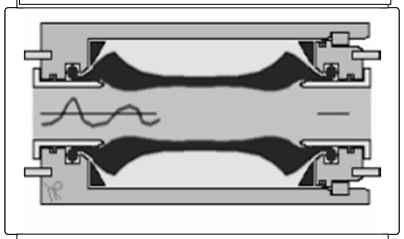
reciprocating pump

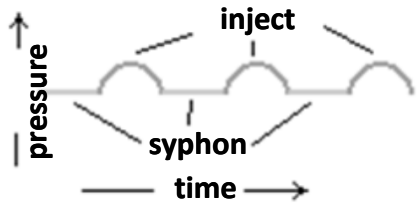
(+) versatility (isocratic, gradient)
: high scale of flow-rates

(–) pressure pulses

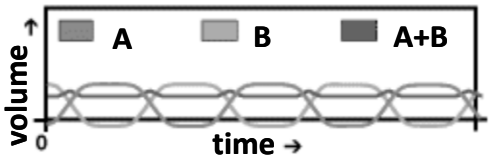








asks for pulse dampener



MP filtering

removal of macroscopic impurities

metal filter at MP inlet

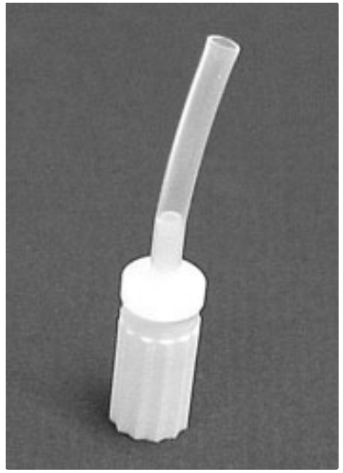
filtration apparatus

: vacuum

: fine filters

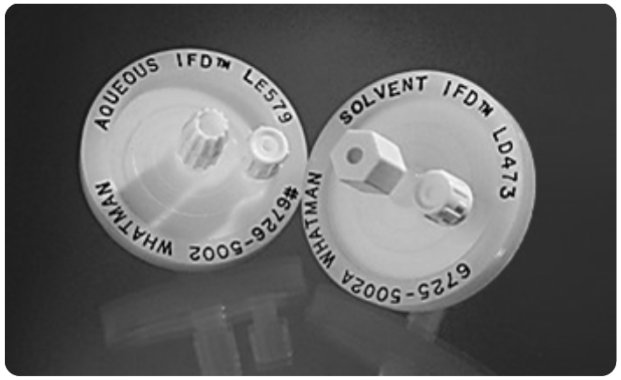
:: 0.45 µm for HPLC

:: 0.20 µm for UPLC



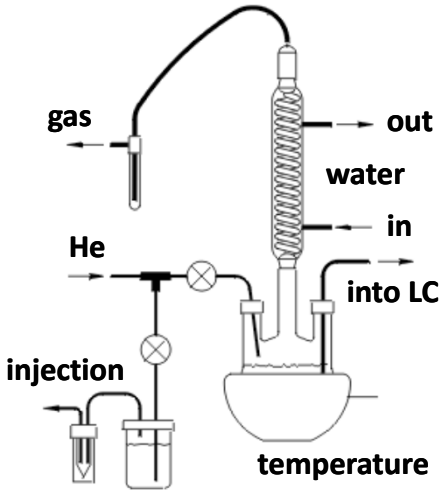
plastic filter at MP inlet

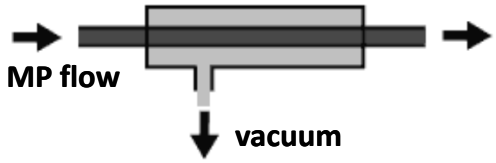
: He degassing



MP degassing

removal of gases dissolved in MP
: dangerous expansion of bubbles (caisson disease)



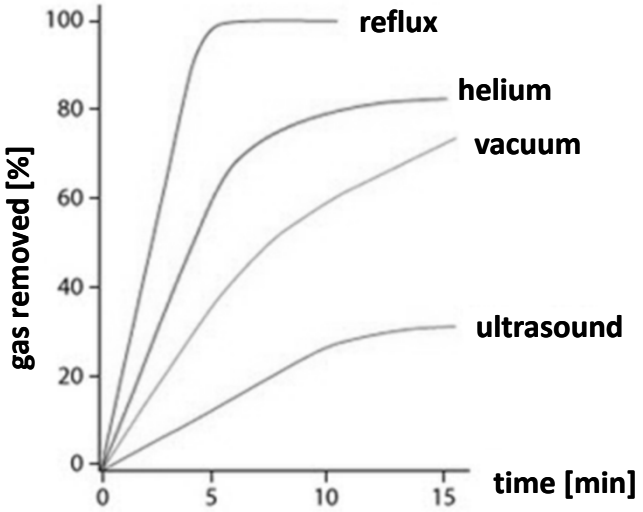


: boiling
:: ideal and unpractical
::: also as **reflux** (4 min, – 100 % of gas)
:: boiling under low pressure

: inert gas bubbling (He)
:: 10 min, – 80 % of gas

: vacuum filtration
:: 10 min, – 60 % of gas
:: in-line membrane degassing

: ultrasound sonication
:: 30 min, – 30 % of gas
:: optimal forestep for vacuum filtration



elution and elution force

: parameters of polarity — interaction of **A** and SP, MP

: parameters of selectivity influences

so-called **eluotropic (LSC**; increasing polarity**)** & **mixotropic (LLC**; decreasing polarity**) order**

elution force of MP (e) $$\log K_D = \log(V_{ads} \cdot m_{SP}/V_m) + \alpha \cdot (S^0 - A \cdot e)$$

K_D – distribution constant of analyte, V_a – adsorbed layer volume, m_{SP} – weight of SP, V_m – void volume, α – adsorbent activity parameter, S^0 – free energy of solute adsorption, **A** – adsorption cross-section of solute, **e** – elution force

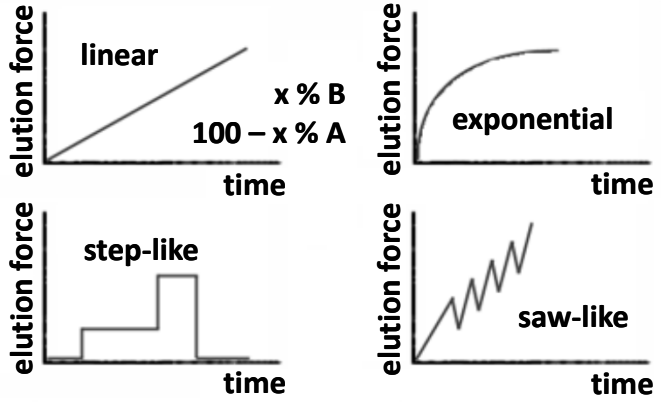
change of retention factor by change of elution force ($e_1 \rightarrow e_2$) $$\log k_1/k_2 = \alpha \cdot A \cdot (e_1 - e_2)$$

NP: hexane + isopropanol
RP: water + methanol/acetonitrile

eluent – MP liquid coming into column
eluate – MP liquid coming out of column
effluent – liquid flowing out (of column)

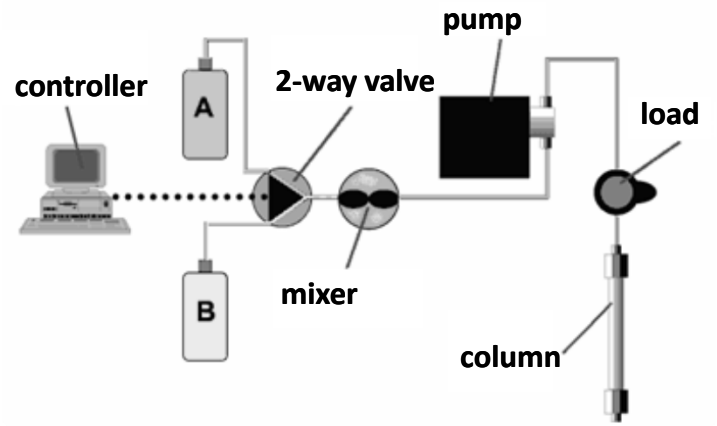
implementation of elution force

: **isocratic**
:: elution force remains constant during elution
: **gradient**
:: elution force is changing (increasing) during elution

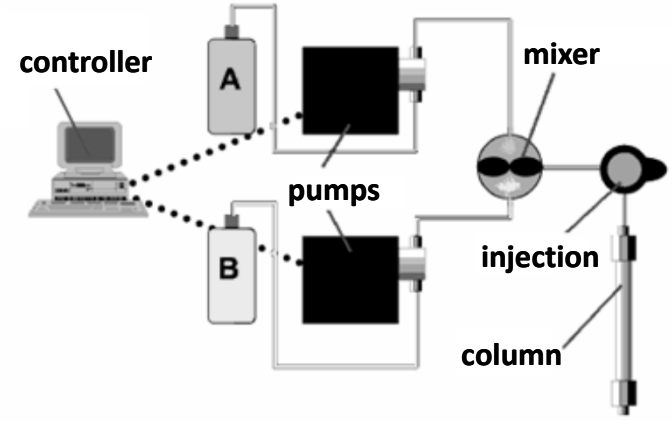


$$e_{AB} = e_A^0 + \log\left[x_B \cdot 10\alpha \cdot A_B \cdot \left(e_B^0 - e_A^0\right) + 1 - x_B\right]/\alpha \cdot A_B$$

e_A^0 & e_B^0 – elution forces of solvents A and B, x_B – molar ratio of B, α – parameter of adsorbent activity, A_B – adsorption profile of solute B

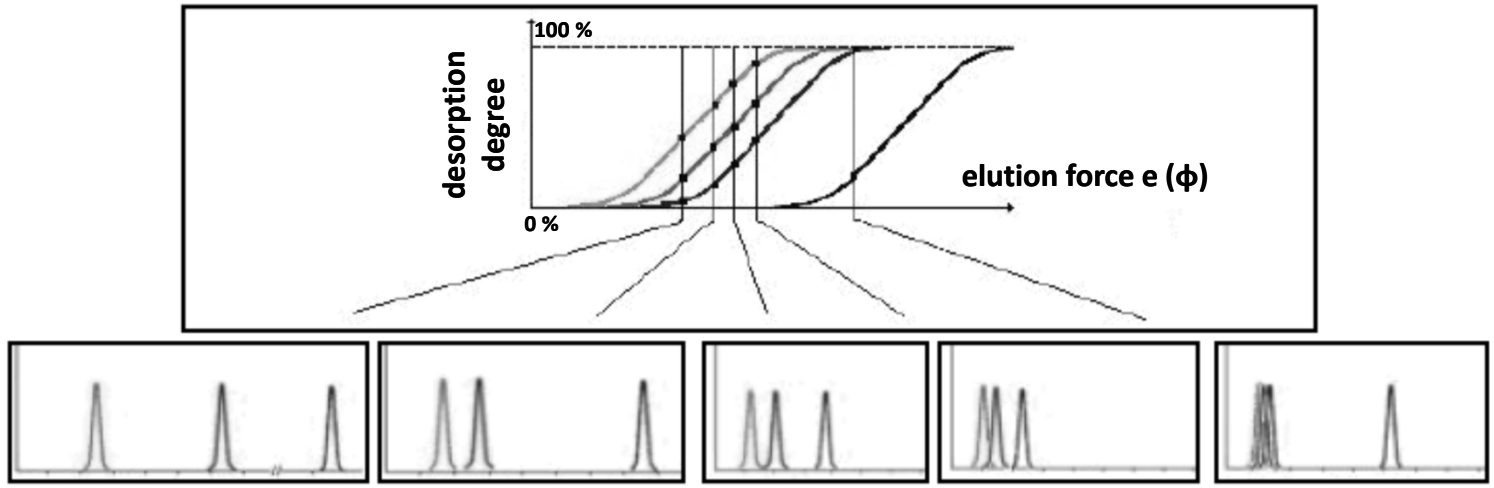


low-pressure gradient

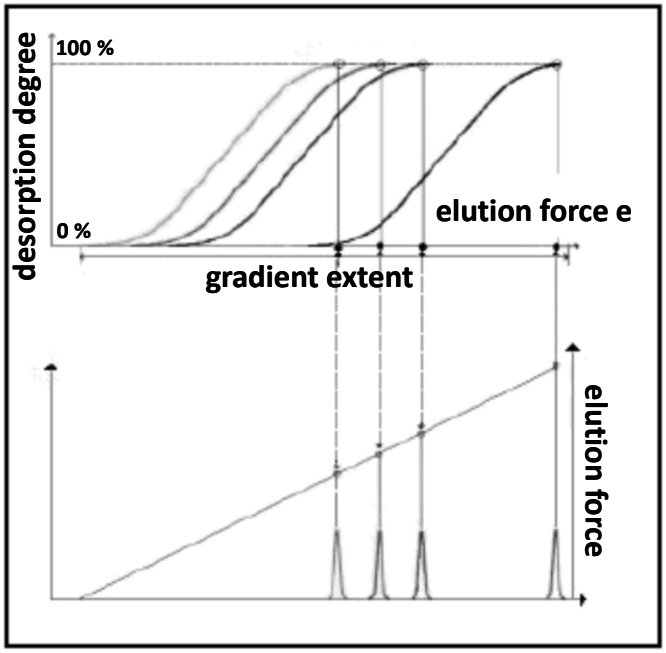


high-pressure gradient

isocratic
or
gradient?



isocratic elution



gradient elution

: shorter analyses
: higher resolution
: higher peak capacity
: high content of organic phase at the end of gradient keeps column

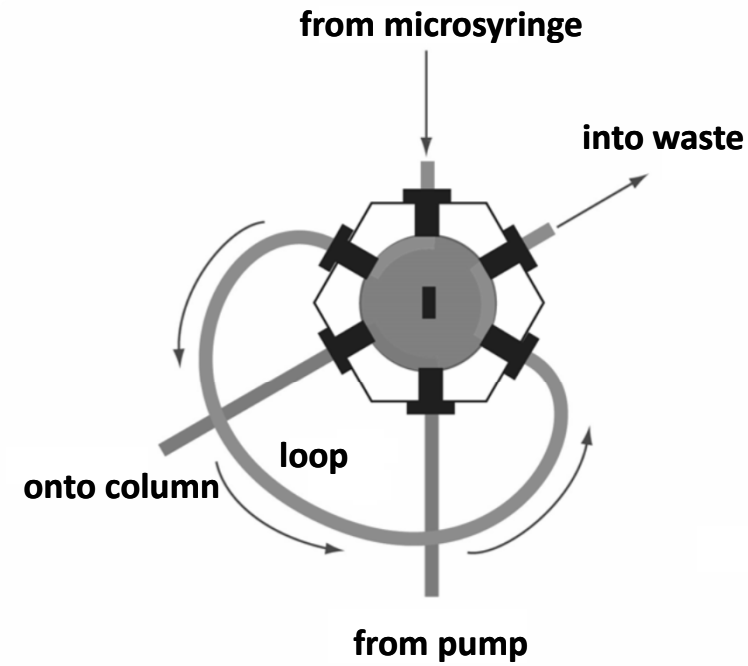
: *expensive*
: *re-equilibration after each measurement*

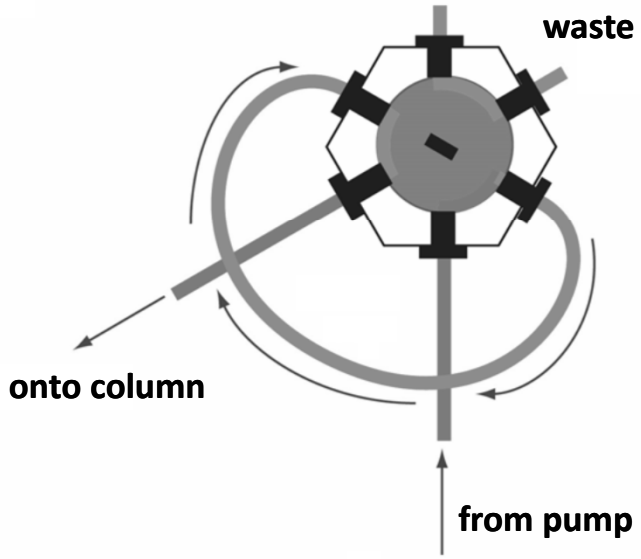
injection device

loading of **A** onto column without interrupting of MP flow

manual injection

(six-way) valve

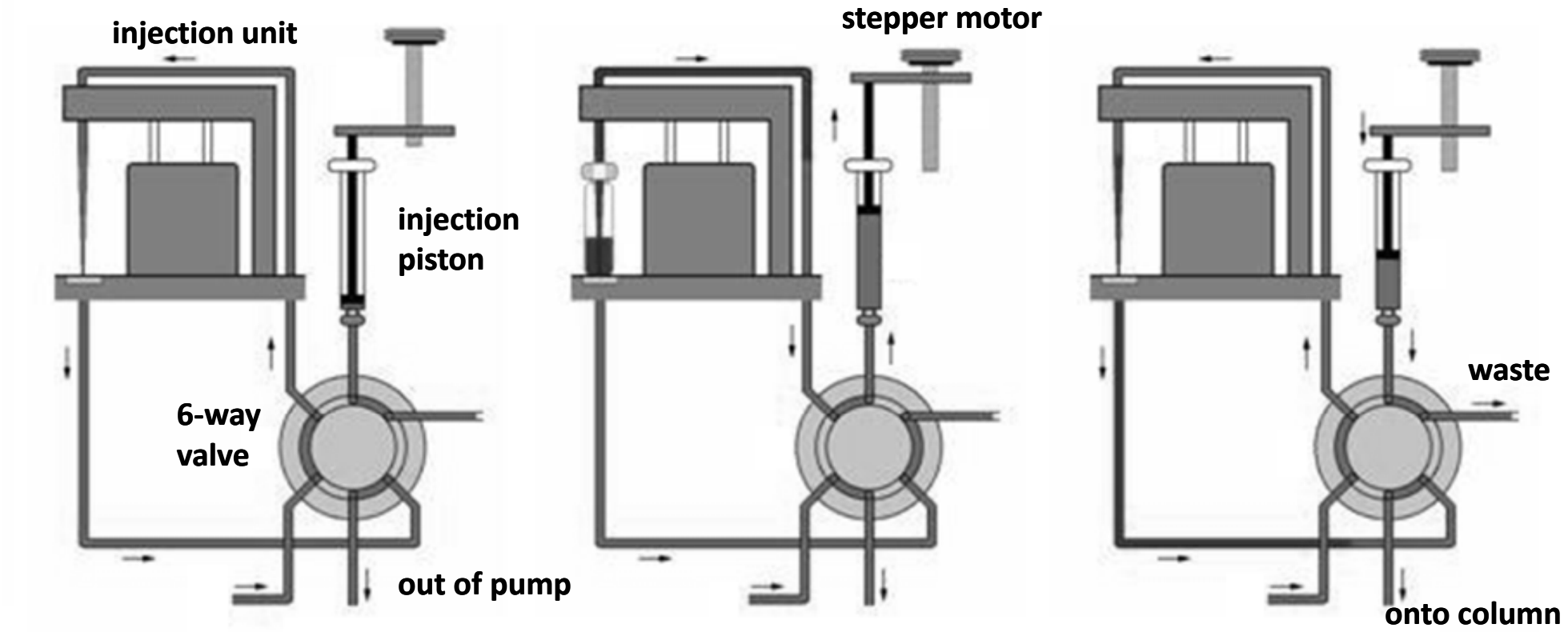




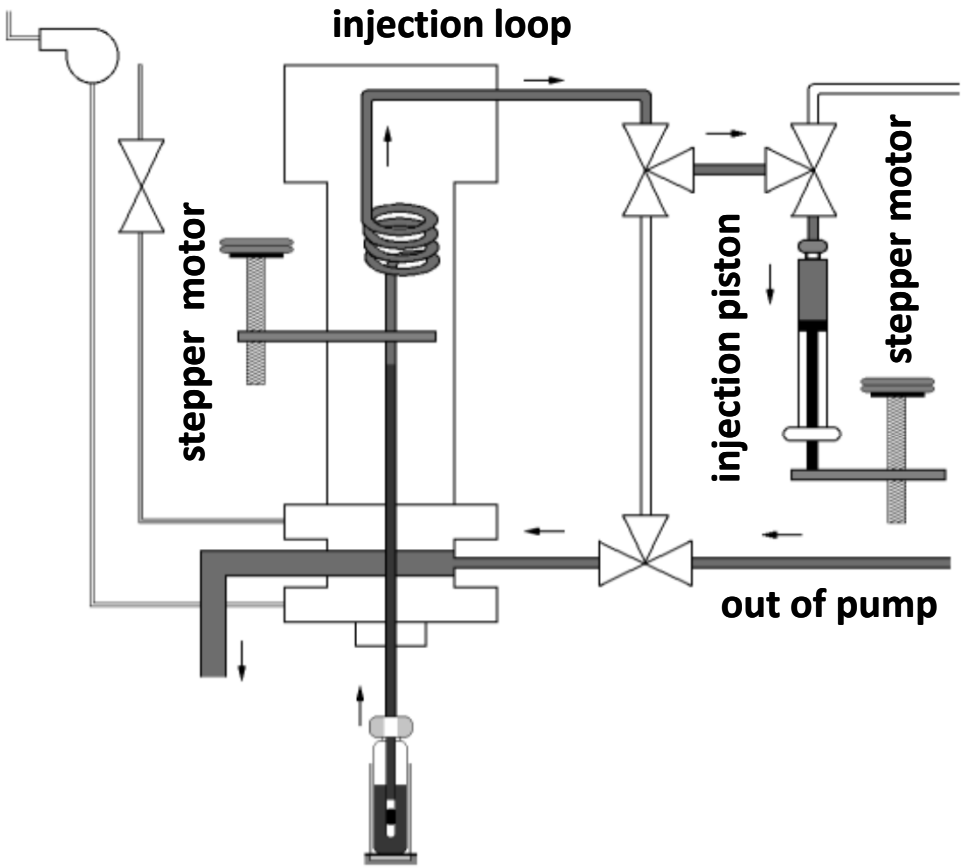
automatic injection

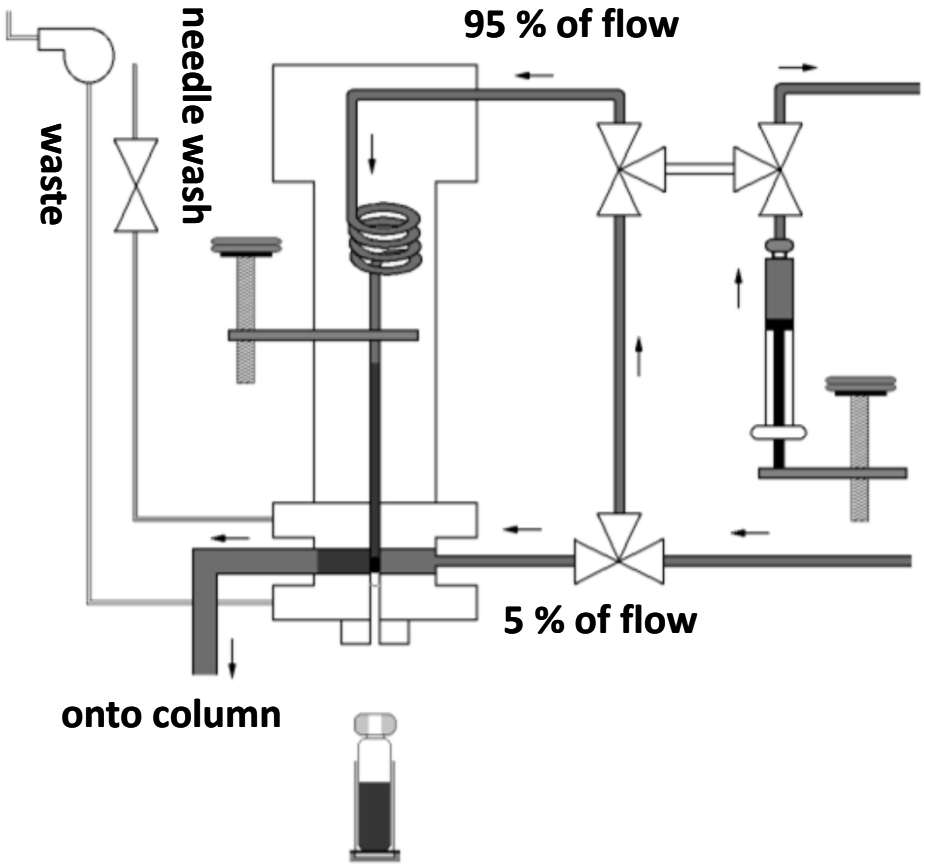
(*autosampler*)

solution with six-way valve (Agilent)



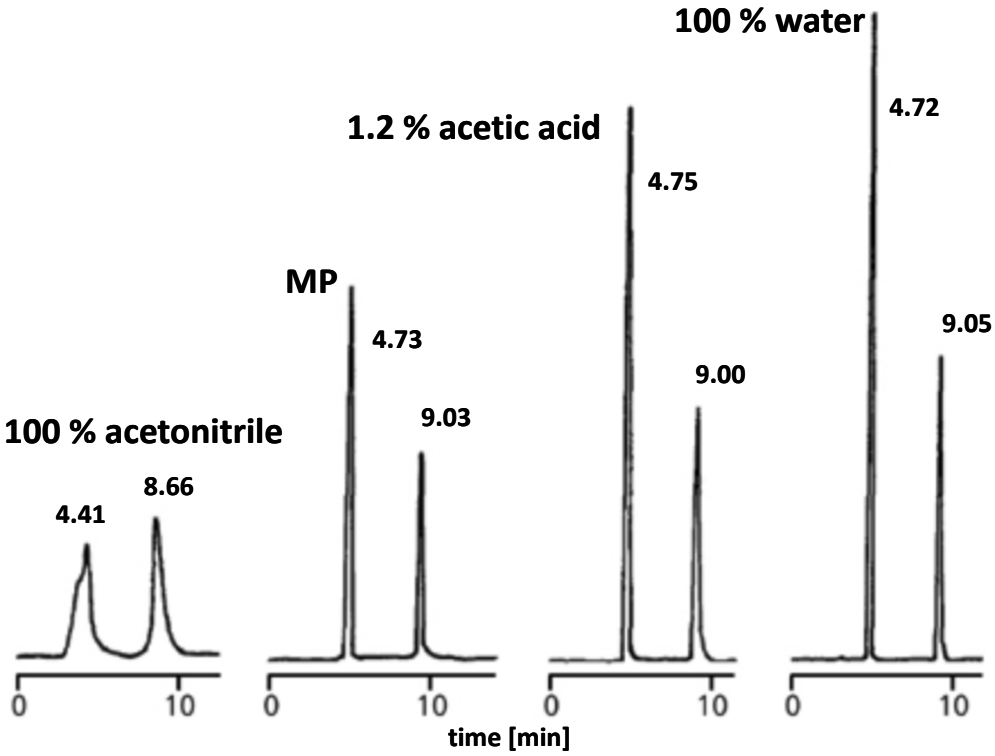
solution with multiple valves – 3x three-way and 1x two-way (Waters)

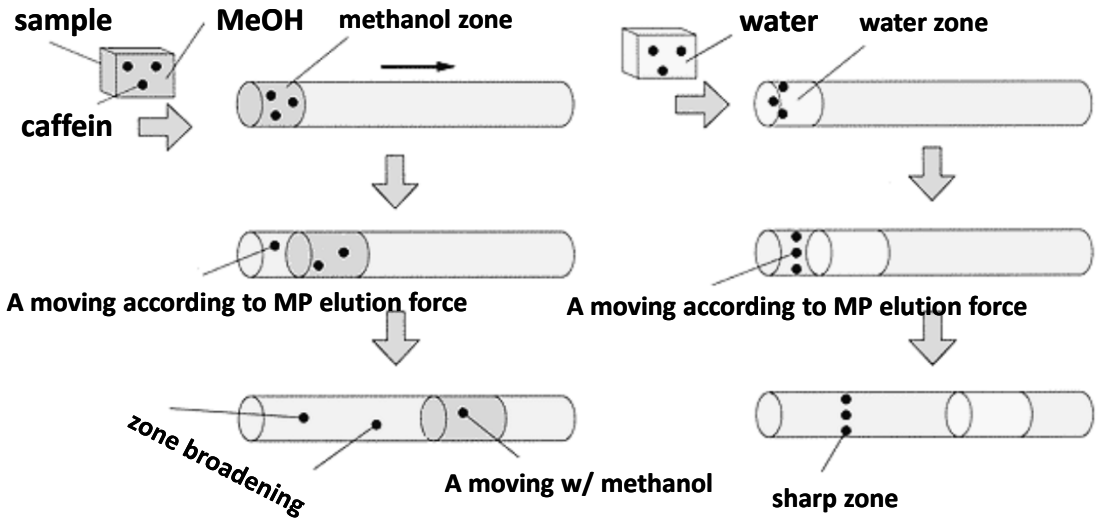




sample should be injected in a suitable solution regarding MP

: strong eluent causes zone broadening, weak analyte focusation in zone



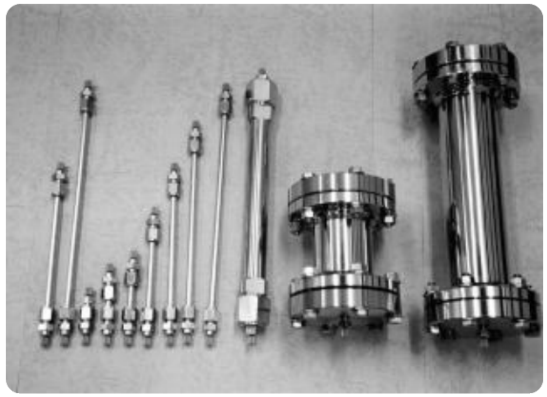


caffein & salicylamide; column C8, 4.6 x 150 mm

isocratic elution: 18:81:1 acetonitrile-water-acetic acid (v/v)

separation column

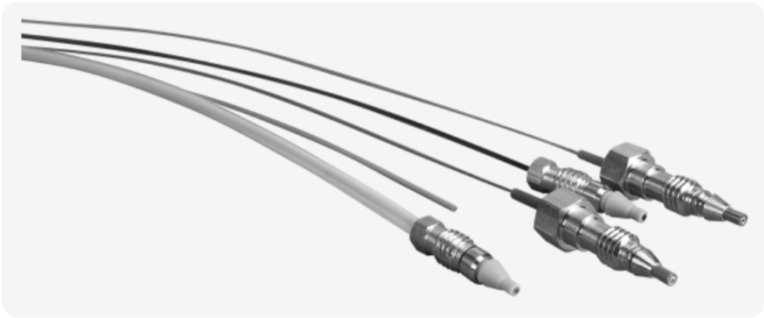
container w/ SP included into flow of MP

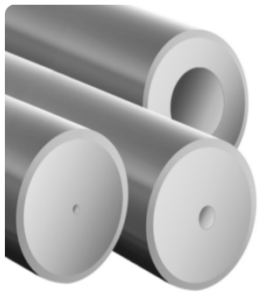


: stable SP
:: in flow (terminal frits)
:: undisturbed SP column
:: robust cover

tubular / column
: preparative (necessary capacity)
: analytical

length: 250 – 5000 mm
diameter: 4.6 – 1000.0 mm





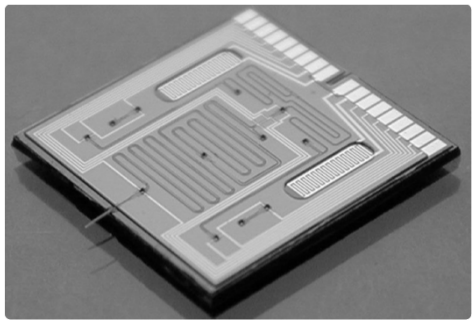
microfluidics

capillary

i.d.
~ 300 µm – micro-column
~ 75 µm – nano-column

length: 10 – 250 mm
diameter: 1.0 – 4.6 mm

length: 50 – 2000 mm
diameter: 0.075 – 1.000 mm

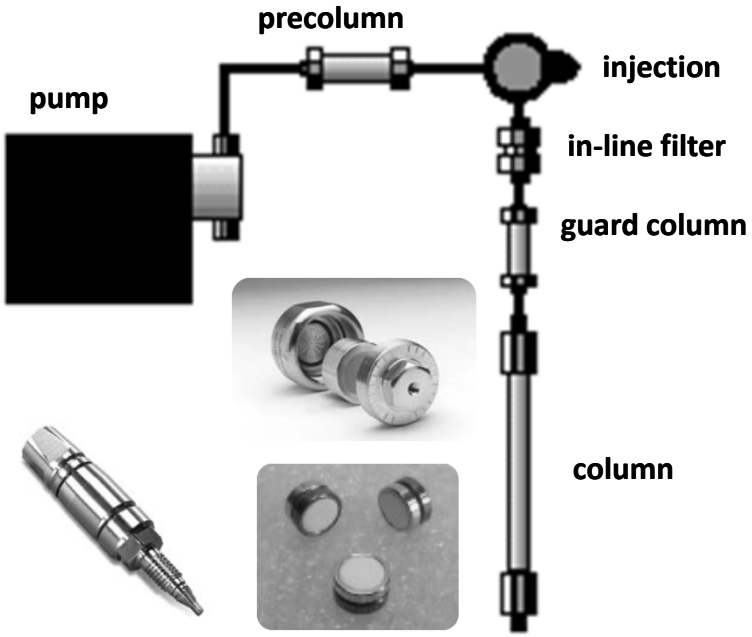


chip

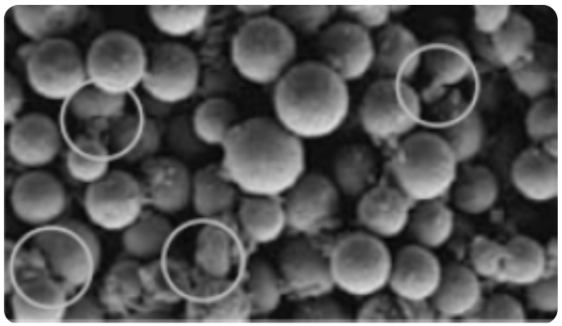
SP guarding **precolumns**

precolumn
: silica (free -OH)
: guarding pH changes

guard column
: SP same as column
: preconcentration

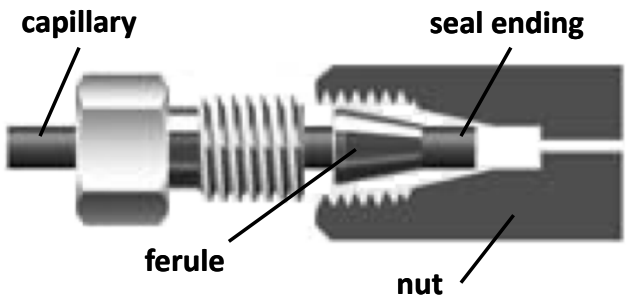


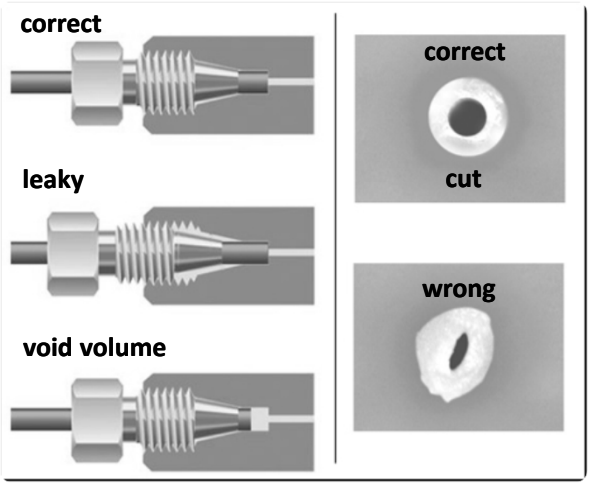
in-line filter
: filters MP



connections

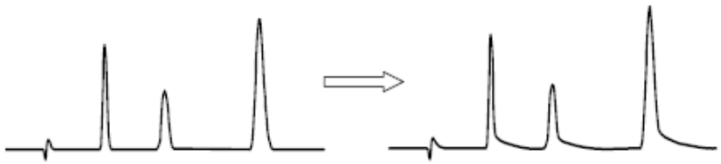
pressure resistant
: unified coil of screw thread





capillaries

conducting MP between parts of instrumentation



void volume in capillary connections

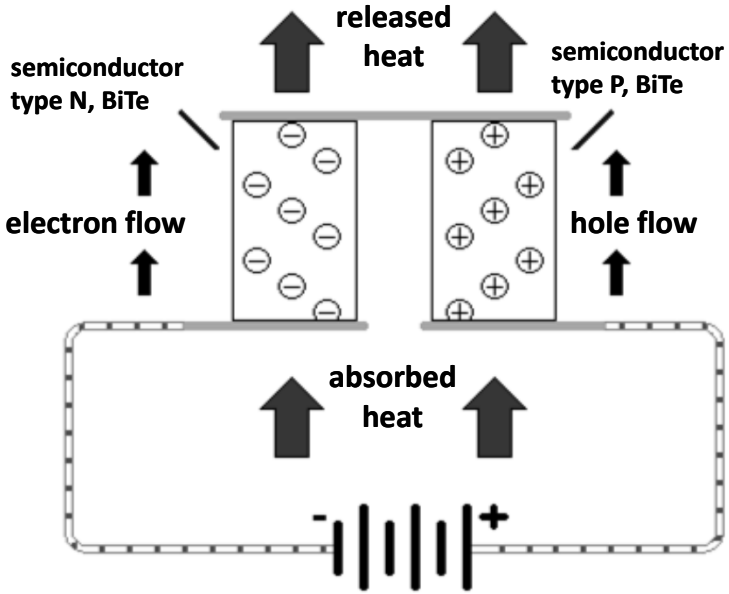
stainless steel, PEEK (polyether ether ketone), teflon
: minimal volume – additive to void volume
: inertness and proper diameter (Aris-Taylor equation))

column oven

keeps stable temperature

temperatures > 40 °C
: influence quality of separation

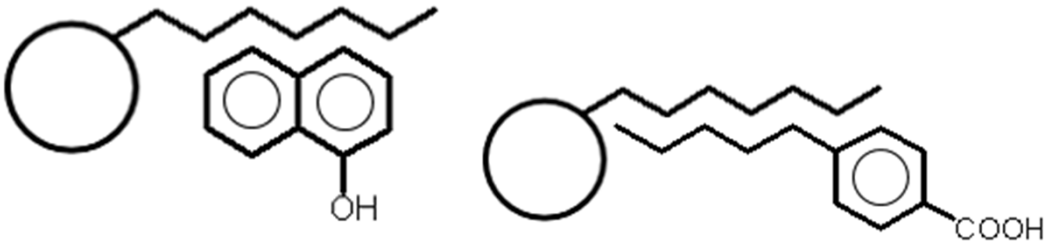
Peltier cooler



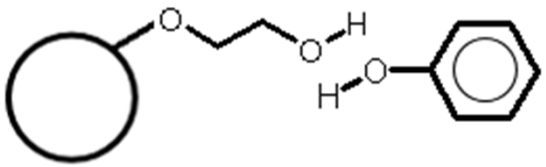
flow of electrons and holes transports the heat

interaction types (generally)

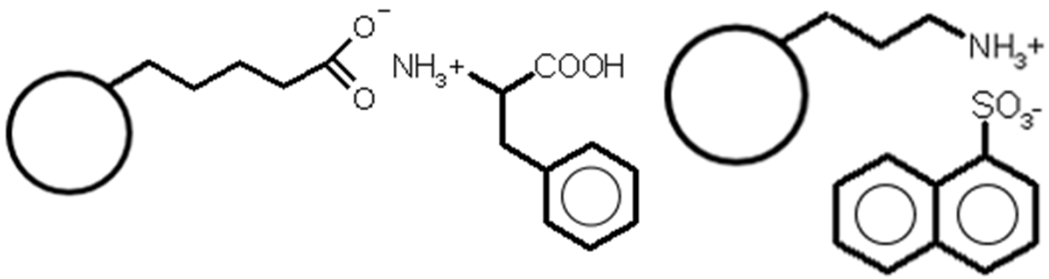
van der Waals forces



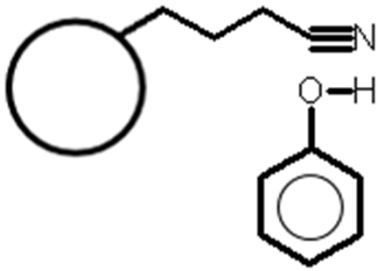
hydrogen bond



electrostatic interaction



interaction dipole-dipole

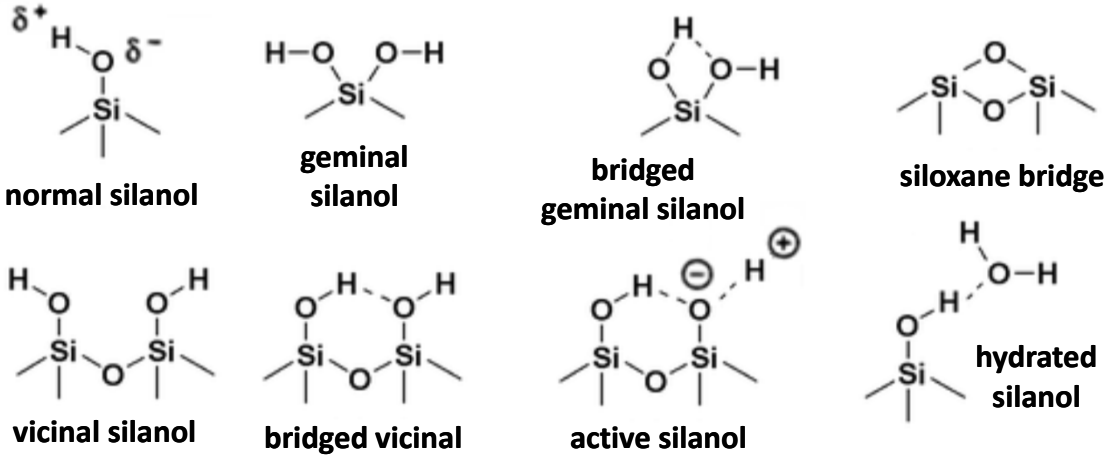


overview of main SP carriers

silica $SiO_2 . (H_2O)_x$, by hydratation, silicate acid is created

: tempering to 150 °C ⇒ water removal = *activation* of silica
: labile over pH 8

: interaction with silanol groups
: silica surface is mildly acidic
:: it has proton-donor properties



alumina Al_2O_3, resp. $Al(OH)_3$

: similar properties to silica
: modifications – amount of water bound, crystal structure
: *activation* by drying [$Al(OH)_3 \Rightarrow AlO(OH) \Rightarrow Al_2O_3$]
: at high water content (15%) separation effects appear
:: besides of **proton-donoring** hydroxyls appear on the surface also **proton-accepting centres**

other carriers, polar and non-polar

polystyrene-divinylbenzene, activated carbon, polyamide, fluorisil ($MgSiO_3$), ZrO_2, porous glass, kaolinite, MgO, $CaCO_3$, $CaSO_4$, infusorial earth, cellulose, $Ca_3(PO_4)_2$ [$Ca_5(OH)(PO_4)_3$]

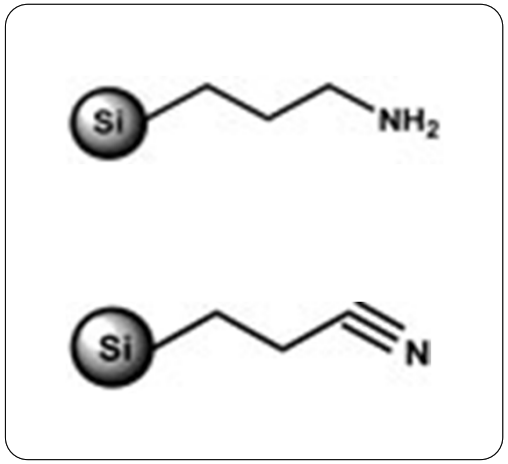
column filling

: solid – particles directly of SP material
: bound – SP bound (physically or chemically) to carrier

carrier material – reactive

solid polar SP **(LSC)**
: silica (silicon oxide) – polar, acid, basic compounds
: fluorisil (magnesium silicate) – polar, strongly acidic, basic compounds
: alumina (aluminium oxide) – polar, basic, acidic compounds
: organic polymers (styrene-divinylbenzene)

chemically bound polar SP **(LSC)**
: cyanopropyl (–CN)
: aminopropyl ($–NH_2$)
: N-propylethylene diamine (PSA)

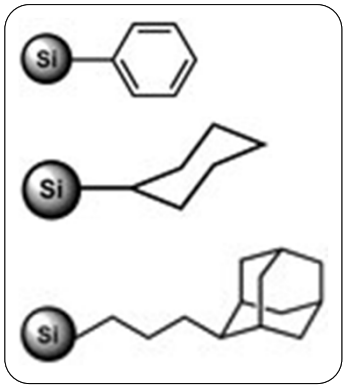


physically bound polar SP **(LLC)**
: dimethyl sulphoxide
: water
: propane-1,3-diol
: ethane-1,2-diamine

solid non-polar SP

: activated carbon – almost non-polar, non-polar compounds

: organic polymers (polymethyl methacrylates)



chemically bound non-polar SP **(LSC)**

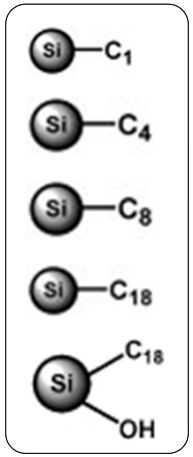
: ethyl (C2)

: octyl (C8)

: octadecyl (C18)

: cyclohexyl (CH)

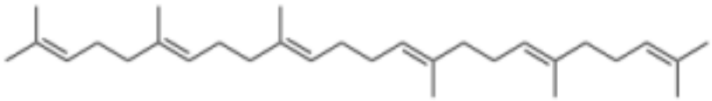
: phenyl (PH)



physically bound non-polar SP **(LLC)**

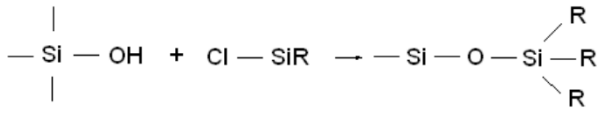
: ethane-1,2-diol

: squalane



chemically bound non-polar SP

: reaction of silica with alkylsilanes (R ~ 1 – 18 C atoms)

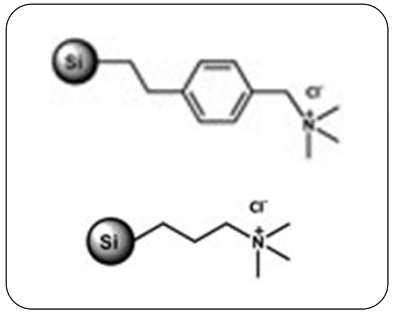


solid ion-exchange SP

: zeolites (aluminium silicates)

: clays

: organic polymers (polystyrenes, acrylates)



anexes

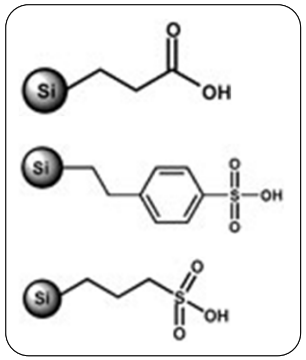
chemically bound ion-exchange SP

: anion-exchangers (anex)

:: $R\text{-}NH_3^+$, $R\text{-}CH_2N^+(CH_3)_3$

: cation-exchangers (catex)

:: $R\text{-}COO^-$, $R\text{-}SO_3^-$



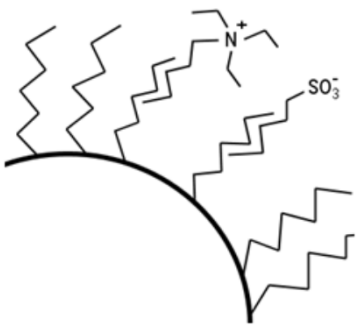
catexes

physically bound ion-exchange SP

: ion-pairing

: uses combination of **chemically bound non-polar SP** and **ion pairing agents**

:: surface active substances (surfactants)



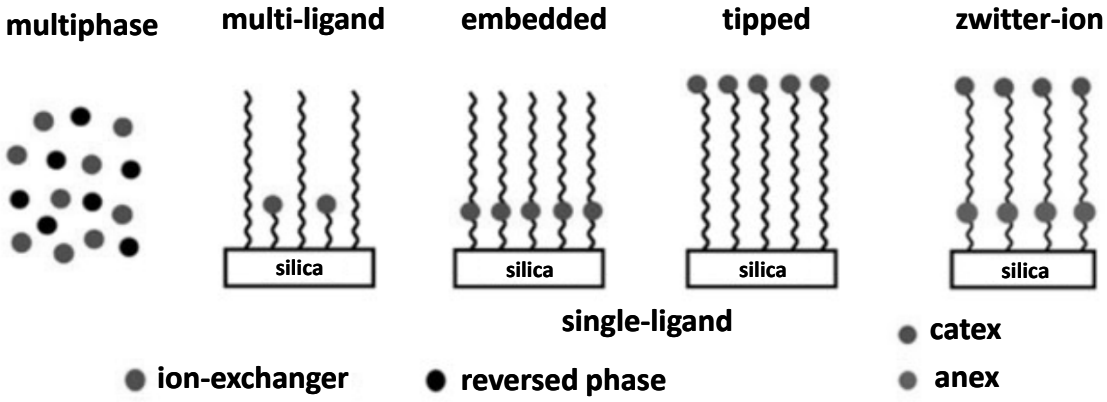
quasi-anex : quaternary amines (TEA, TBAH)

quasi-catex : sulphonic acids (pentane sulphonic acid)

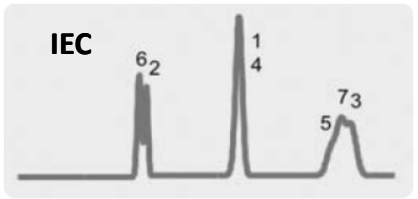
mixed mode chromatography (MMC)

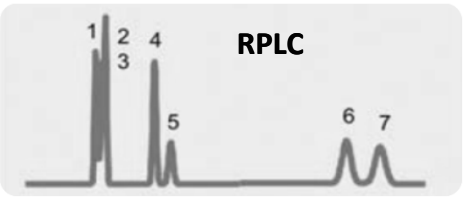
: 1986 F. Ringier; AEC-HIC combination
: 1998 A. Štrancar; CLC monoliths
: 1999 J. R. Yates; SCX-RPLC

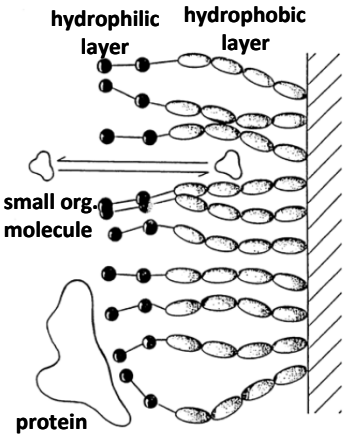
: higher selectivity
: higher loading capacity
: one MMC column in cyclic system for 2D-LC

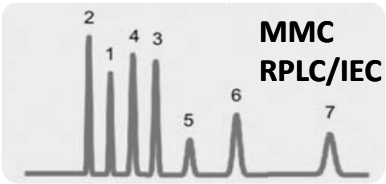


: the most used combinations
:: IEC/HIC, IEC/RPLC, HILIC/RPLC, HILIC/IEC, SEC/IEC









shielded stationary phases

: shielding against negative SP influences
: multi-modal separation
:: restricted access material SP (RAM)

respective mobile phases

polar SP + non-polar basic MP

chromatography with **normal phases** (*SP polar*)
elution force increases in following order
: pentane, benzene, chloroform, acetone, acetonitrile, ethanol, methanol, water

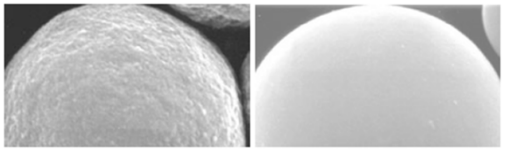
ion-exchanging MP

solutions of inorganic acids and bases with defined ionic strength and given pH

non-polar SP + polar basic MP

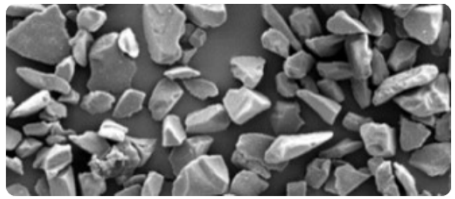
chromatography with **reversed phases** (*SP non-polar*)
elution force increases in following order
: water, methanol, ethanol, acetonitrile, isopropanol, tetrahydrofuran

particle sorbent



basic types of sorbents

shape: spherical (regular shape)
size for different column types
: *analytical* 1.5 – 8.0 µm
: *preparative* > 10 µm

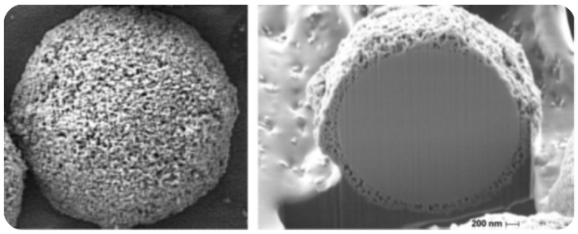


: pores (FPP, *fully porous particles*)
:: historically older, negative influence on kinetic aspects

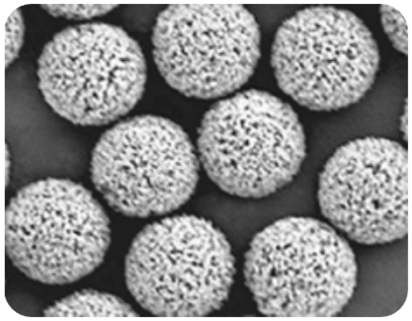
: smooth surface
:: difficult manufacturing, more useful kinetic properties

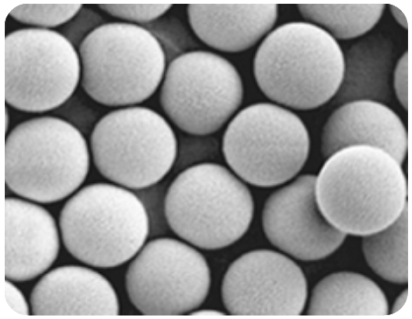
: superficial differentiation (SPP, *surface porous particles*)
:: half-way between particle and monolithic sorbent
::: core-shell, poroshell, halo

core: 1.7 – 4.5 µm
shell: 0.25 – 1.00 µm
shell pores: ~ 30 nm

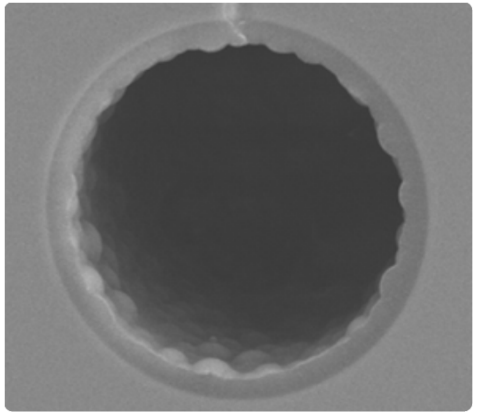


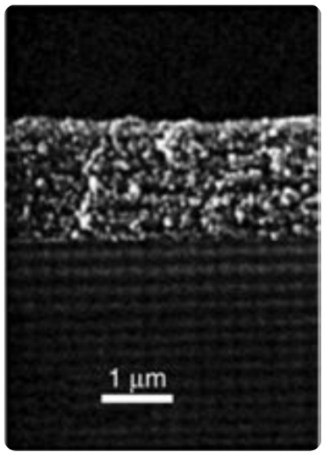
: pore volume
:: $cm^3 \cdot g^{-1}$ (< 1); relative pore volume
: surface area
:: $m^2 \cdot g^{-1}$ (50 – 500)
: pore size
:: nm (5 – 50); 1 Å = 0.1 nm
::: < 10 nm ~ < 3000 Da
::: 10 – 30 nm ~ 3 – 10 kDa
::: > 30 nm ~ > 10 000 Da





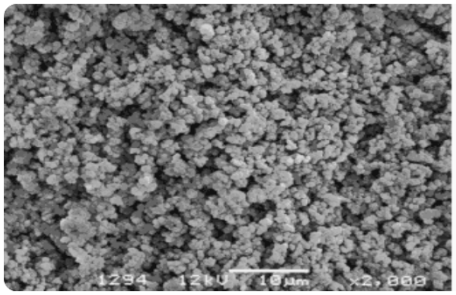
with porous layer (*porous layer open tube*, PLOT)

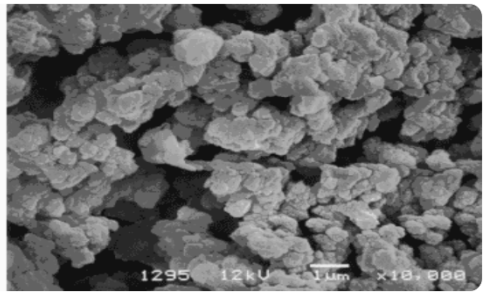


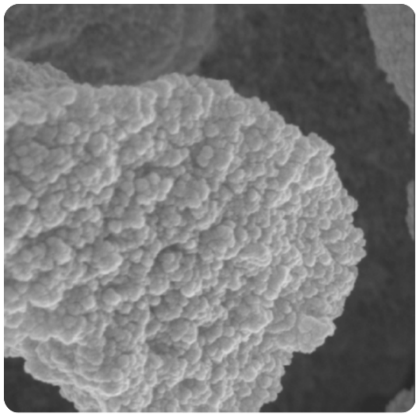


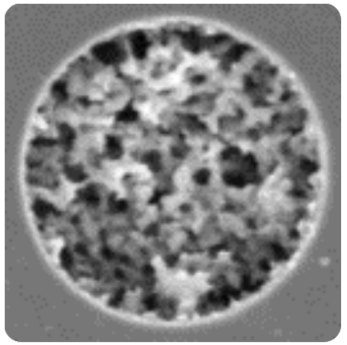
only capillary columns (i.d. 70 µm; 2 µm layer)
: low pressure (70 MPa) within long columns (50 cm)

monolithic sorbent (*silica-rod*, ML)









macropores: ~1500 nm; mezopores: ~ 10 nm, micropores < 2 nm

porosity of ML SP almost 85 %; vs. particle SP ø 5 µm with porosity *max* 60 %

high flow rate at **low** pressure; ***large effective area*** →

fast separation: seconds or minutes
high resolution and *high* capacity

disadvantage: difficult manufacturing

material ***monomer*** **+ polymerisation agent + porogenic compounds**

ML-SP based on silica

tetramethoxysilane (TMOS)
tetraethoxysilane (TEOS) + acetic acid + polyethylenglycol (PEG)

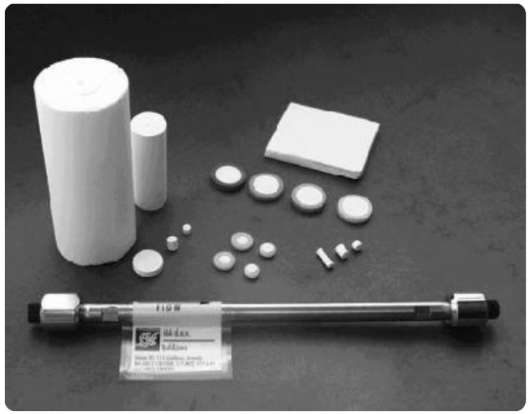
ML-SP based on organic materials

styrene-divinylbenzene (S-DVB)
methacrylates
vinyl-derivatives (vinylpyrrolidone, vinyl acetate)

isooctane
tetrahydrofuran
decanol

outer stabilisation
: PTFE (polytetrafluoroethylene), PEEK (poly(ether-ether-ketone))

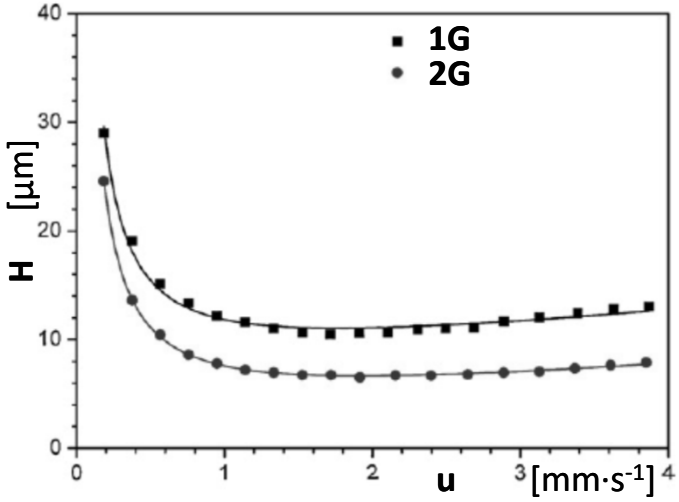
design: disc, tube, filled capillary

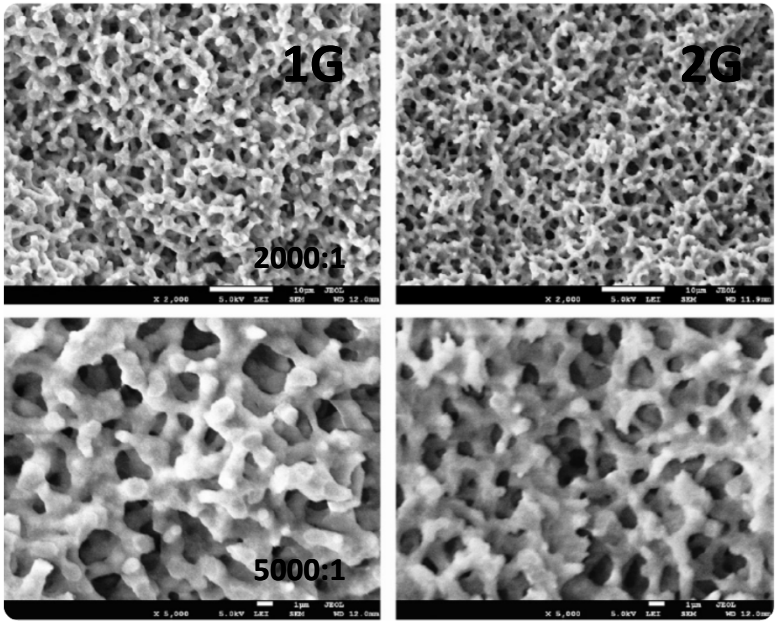


silica based monoliths of the first & second generation

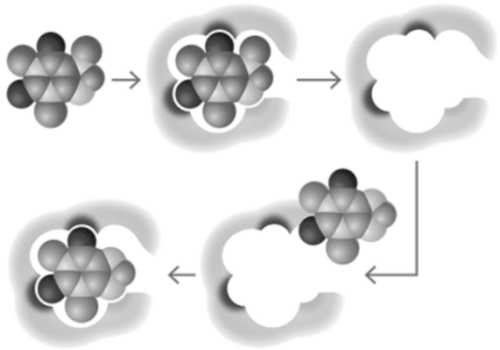
: mezopores 11-12 nm (1G) > 14-16 nm (2G)
: macropores 1.8-2.0 µm (1G) > 1.1-1.2 µm (2G)
: decreasing SP heterogeneity

: decreasing total porosity 3.5 ml·g^{-1} (1G) > 2.9 ml·g^{-1} (2G)
: decreasing surface area 320 m^2·g^{-1} (1G) > 250 m^2·g^{-1} (2G)
: increasing number of theoretical plates 50 000 m^{-1} (1G) > 155 000 m^{-1} (2G)



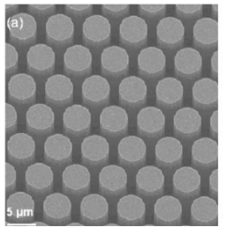


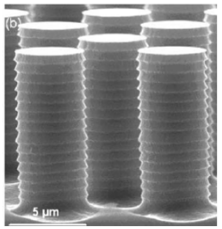
molecularly imprinted polymers (MIP)

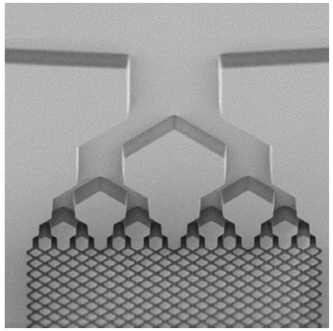


new type of SP
: suitable for chiral or affinity separation
: similar preparation to ML

chip (*lab-on-chip, LC-on-chip*)







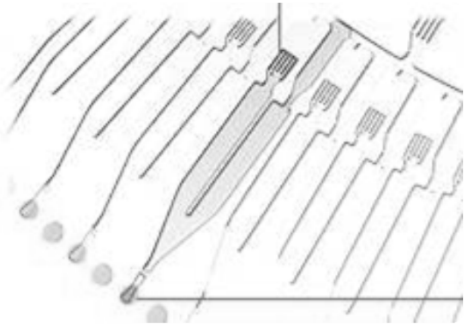
structures etched into silicon (Si) plate

advantages: analysis speed, size, low sample consumption (< nl)

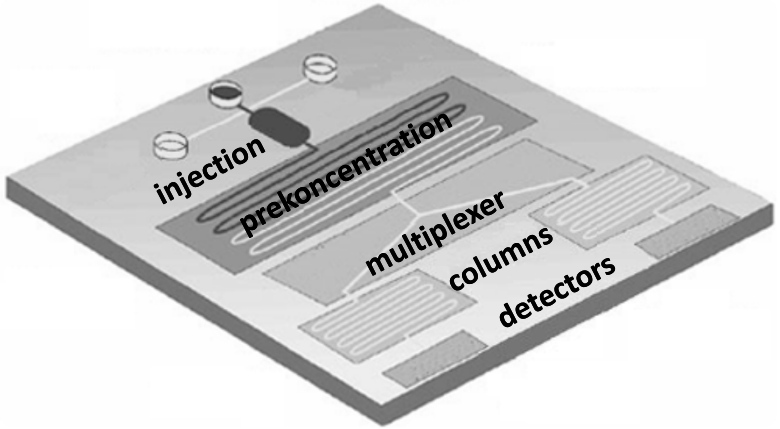
disadvantages: too small volumes (surface tension, electrostatic interactions)

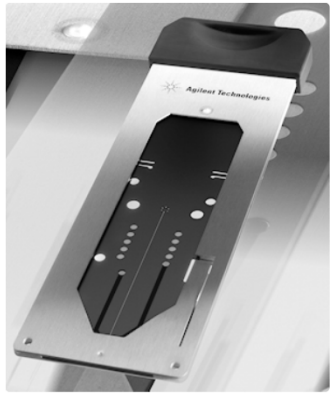
use: pre-separation and sample preparation for MS

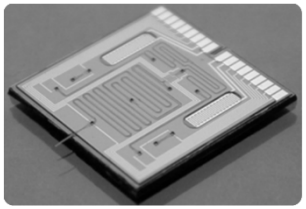
: proteolysis, desalting
: pre-concentration and desalting for ESI-MS



MP delivery: centrifugal force, electrostatic force



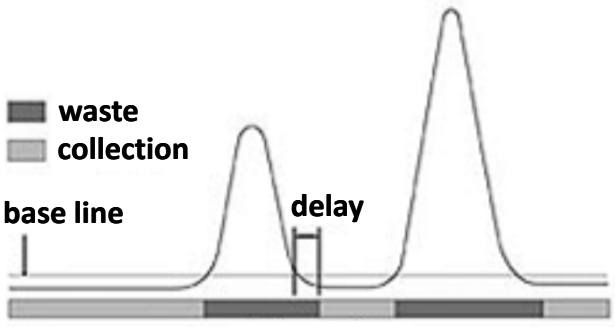




detectors

allow to gain **information on separated analytes** → **signal** (signal intensity)
: speciality – ***MP recycling***; eluate without sample with properties of pure MP

range of detected analytical information
: ***universal detector***
: ***non-selective detector***
:: **presence** (absorbance at one wavelength)
: ***selective detector***
:: **identity** (UV-Vis spectrum, mass spectrum, redox potential)
:: **structure** (mass spectrum, NMR spectrum)



detector hyphenation
: high quality of detection (UV-Vis + MS)

influencing the nature of analyte by detection
: ***non-destructive detector***
:: no chemical change of detected analyte
: ***destructive detector***
:: detected analyte irreversibly changed

fast response
: if slow (slower than MP flow)
:: signal distortion, low sensitivity

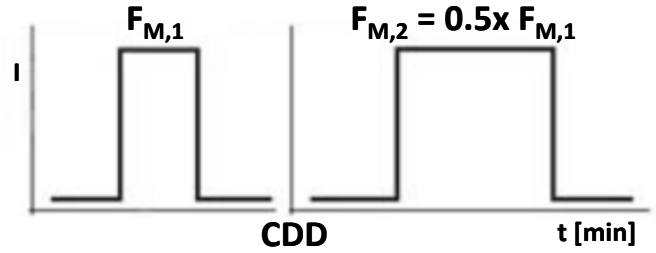
signal stability
: unstable signal
:: loss of (quantitative) information

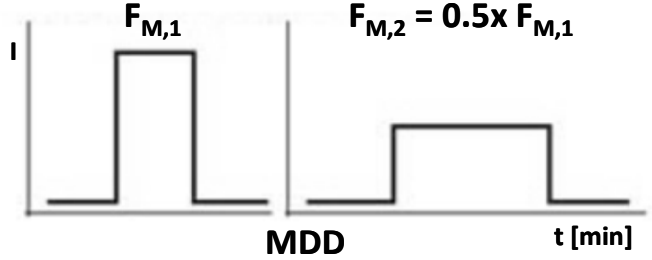
selectivity, sensitivity, linearity

concentration dependent detector (CDD)

: **dm/dV** (component mass concentration in effluent) independent on intake of component in detector

:: at F_M change, peak area height changes, while height remains the same





mass dependent detector (MDD)

: **dm/dt** (component mass flow in effluent) dependent on intake of component in detector

:: at F_M change, also peak height changes, but area remains the same

detector response

$$\mathbf{R} = f(\mathbf{c_A})$$

R – detector response

$$\mathbf{R_{CCD}} = \mathbf{S} \cdot \mathbf{c_A}$$

S – detector sensitivity

$$\mathbf{R_{MDD}} = \mathbf{S} \cdot \frac{\partial \mathbf{m}}{\partial \mathbf{t}}$$

m – mass of analyte

dynamic range

concentration range, in which change in concentration causes signal intensity change

linear dynamic range $\log \mathbf{R} = \mathbf{k} \cdot \log(\mathbf{c_A})$

linearity is given as slope value (**k**) in range 0.98 to 1.02 or c_A = ± 5 %

elution curve area

$$A_{CDD} = \int_{t_1}^{t_2} R \cdot dt = S \cdot \int_{t_1}^{t_2} c_A \cdot dt = S \cdot c_A \cdot \int_{t_1}^{t_2} dt = S \cdot c_A \cdot \Delta t = S \cdot \frac{n}{V} \cdot \Delta t = S \cdot \frac{n}{F_m}$$

F_m – flow rate

F_m must be constant for quantitative use of elution curve area

$$A_{MDD} = \int_{t_1}^{t_2} R \cdot dt = S \cdot \int_{t_1}^{t_2} \frac{dm}{dt} \cdot dt = S \cdot \int_{t_1}^{t_2} dm = S \cdot m$$

elution curve area is independent on F_m

extra-column contributions to zone broadening in LC

$$\sigma_{tot}^2 = \sigma_{col}^2 + \sigma_{inj}^2 + \sigma_{con}^2 + \sigma_{det}^2$$

σ_{tot}^2 – total zone broadening

$$\sigma_{inj}^2 = \frac{V_{inj}^2}{X^2} \qquad \sigma_{det}^2 = \frac{V_{det}^2}{Y^2}$$

σ_{inj}^2 – broadening given by injection volume; X ≈ 1-12
: dependent on injector shape

σ_{det}^2 – broadening given by detector cell volume; Y ~ X

$$\sigma^2_{con} = \pi \cdot d_t^4 \cdot L \cdot \frac{F_m}{384 \cdot D_m}$$

σ_{con}^2 – broadening given by length and diameter of capillaries
: Aris-Taylor equation

d_t – capillary diameter, **L** – capillary length
F_m – flow rate, D_m – diffusion coefficient

$$\sigma^2_{con} = \frac{u \cdot r_t^2 \cdot L}{24 D_m}$$

r_t – capillary diameter
u – linear flow rate

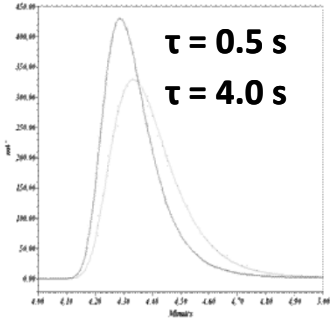
time constants

of detector (τ_{det}) $\sigma^2_{tot} = \sigma^2_{Gauss} + \tau^2$

for t_R ~ min and **n** = 10 000 is $\tau \approx 0.6$ s (column 3.9x150 mm and F_m = 1.5 ml·min^{-1})
: detectors should generally have $\tau < 1$s, at best around 0.1 s

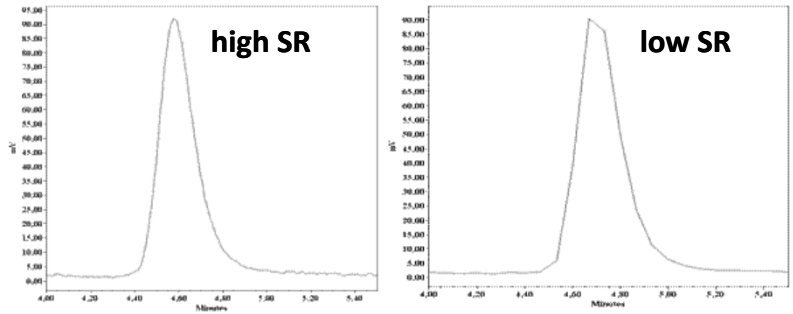
of A/D converter ($\tau_{A/D}$)

$$\sigma^2_{A/D} = \frac{\tau_{A/D}}{\sqrt{12}}$$



sampling (τ_{SR})

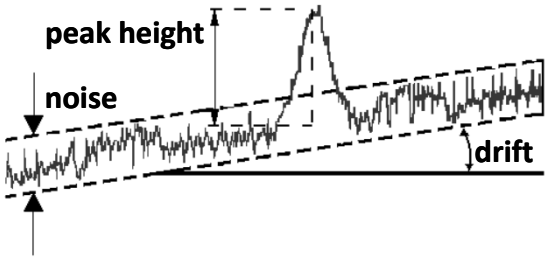
influences the peak depiction
: **SR** – sampling rate



other factors

detector noise

noise drift

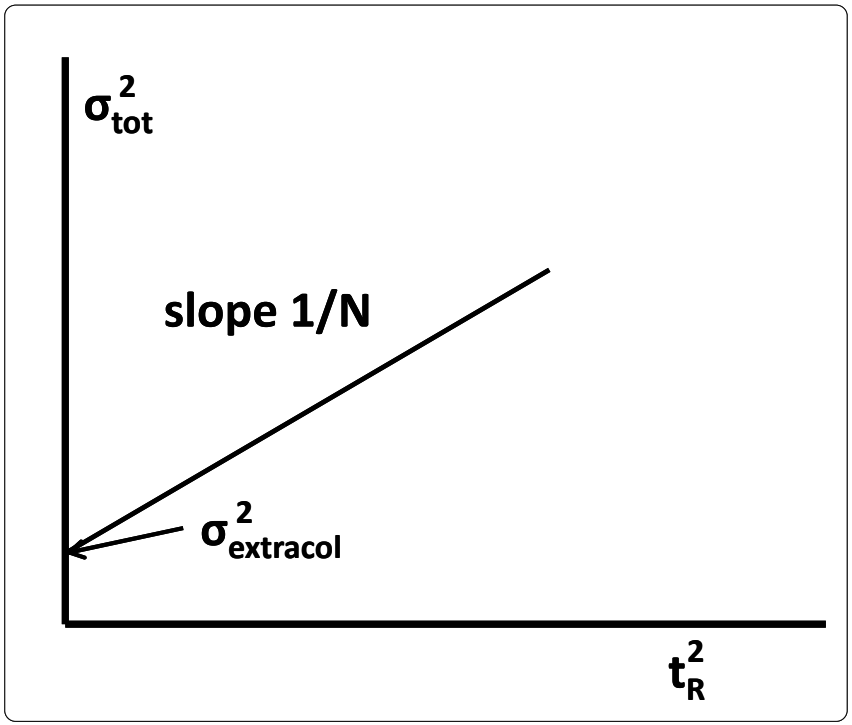


determination of extra-column contributions to zone broadening

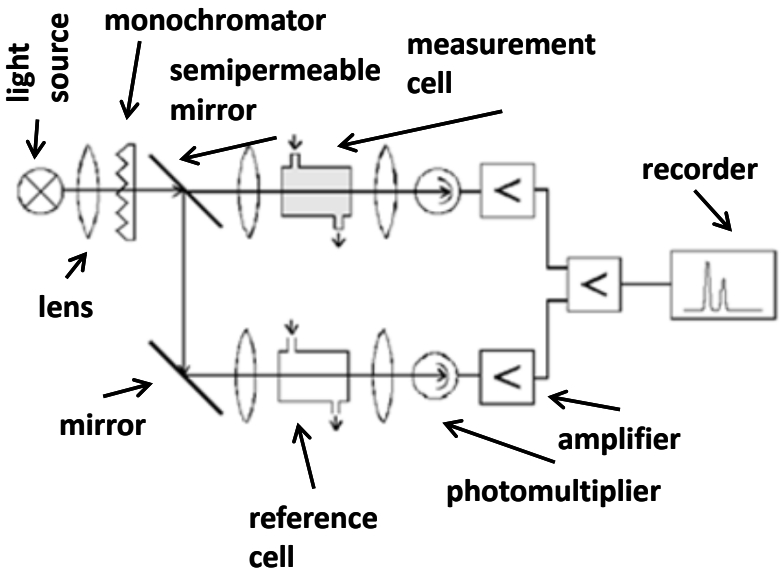
$$\sigma_{tot}^2 = \sigma_{col}^2 + \sigma_{extracol}^2$$

$$N = \left(\frac{t_R}{\sigma_{col}}\right)^2$$

$$\sigma_{tot}^2 = f(t_R^2) = \frac{t_R^2}{N} + \sigma_{Gauss}^2$$



absorption photometric detector



CDD

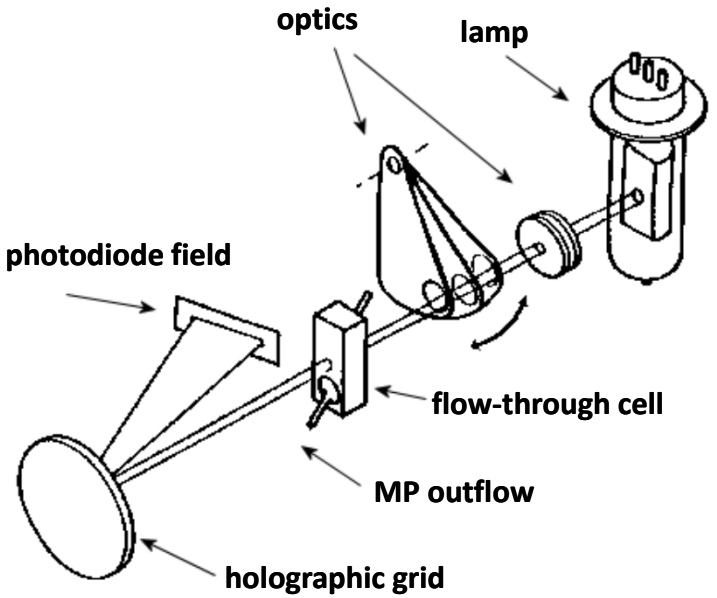
signal: light absorbance (B-L-B law)

: base line drift at gradient elution

: eluate transparency at measuring wavelengths

: optic path length is important (detection Z-cell)

diode array detector (DAD)



PDA – photodiode array

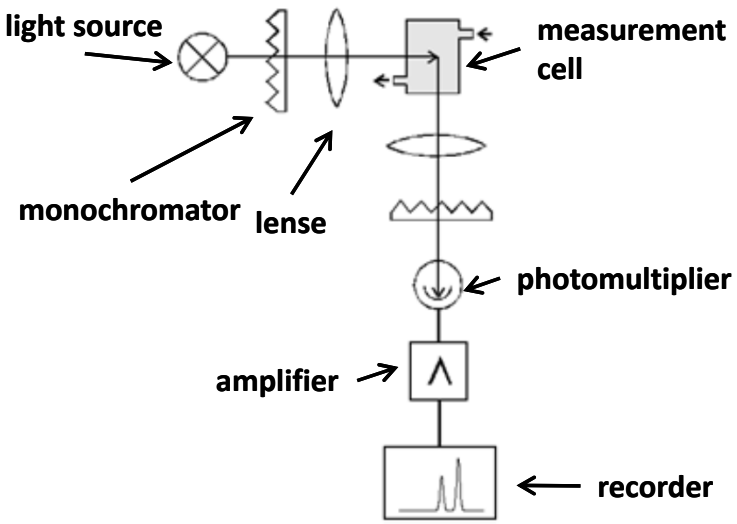
absorbance

: noise 10^{-4}

: dynamic range 10^{5}

: sensitivity 10^{-9} M

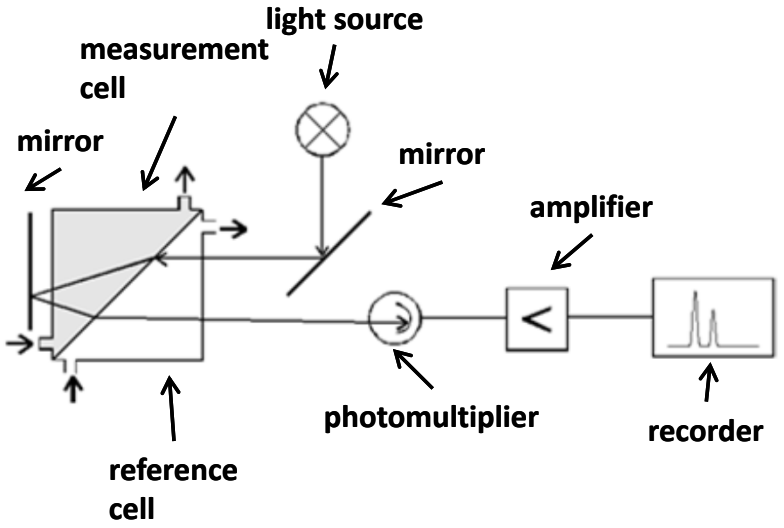
fluorometric (fluorescence) detector



fluorescence
: noise 10^{-5}
: dynamic range 10^{4}
: sensitivity 10^{-11} M

refraction index
: noise 10^{-7}
: dynamic range 10^{4}
: sensitivity 10^{-6} M

refractometric detector



CDD
signal: emission of wavelength λ_2 after excitation at λ_1
: $\lambda_2 > \lambda_1$

$$\Phi_{emis} = k \cdot \Phi_{excit} \cdot \varepsilon_{\lambda} \cdot c \cdot l$$

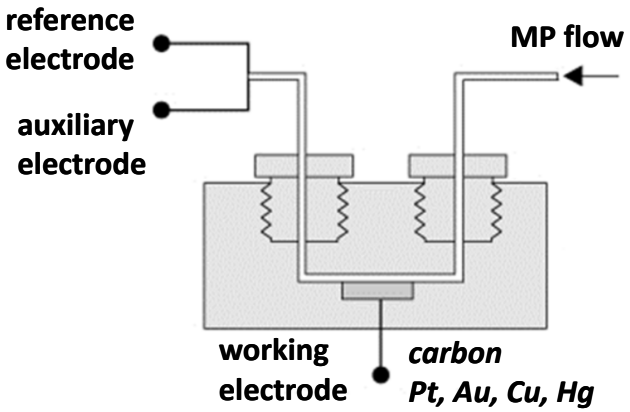
: limited linearity $\Phi = f(c)$ for higher concentrations
: detector shielding from excitation radiation
: optical path length is also important

CDD
signal: light refraction angle (universal detector)

: low sensitivity
:: depends on difference in sample & MP refraction
: high temperature influence
: improper for gradient elution
: for substances w/o other detection properties

electrochemical detector

CDD (amperometry)
MDD (coulometry)
signal: current coming from redox substance passing the measuring cell



coulometry
: noise 10^{-5}
: dynamic range 10^{7}
: sensitivity 10^{-14} M

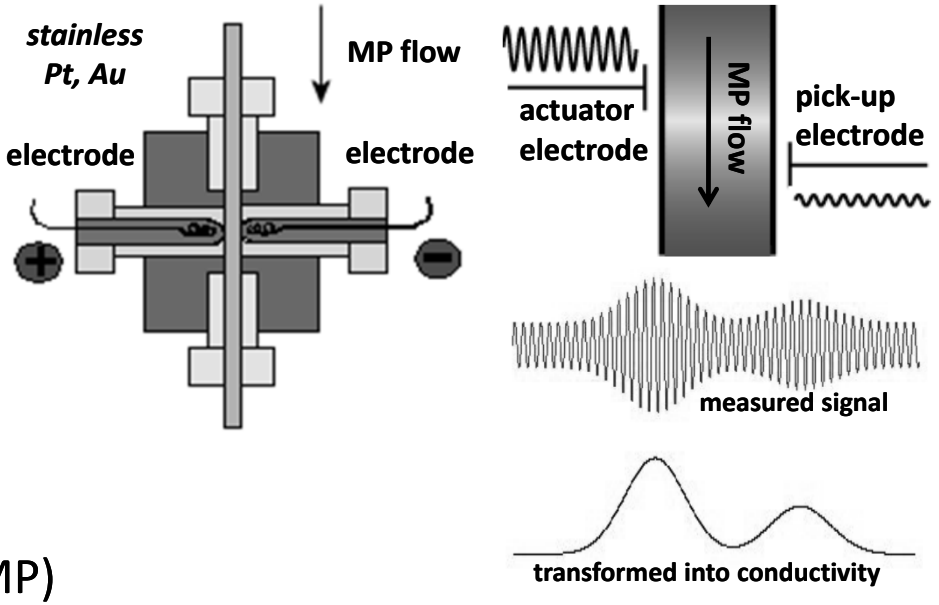
amperometry
: noise 10^{-4}
: dynamic range 10^{6}
: sensitivity 10^{-12} M

: high sensitivity and response rate (temperature); selective detector – sugars
: electrode passivation and consequent cleaning; MP – conductive (so no NP-HPLC)
: destructive detector

conductivity detector

CDD
signal: current coming from charged substance passing measuring cell

: AC voltage (X polarisation)
: lower sensitivity; unspecific detector
: MP – non-conducting (no buffers in MP)

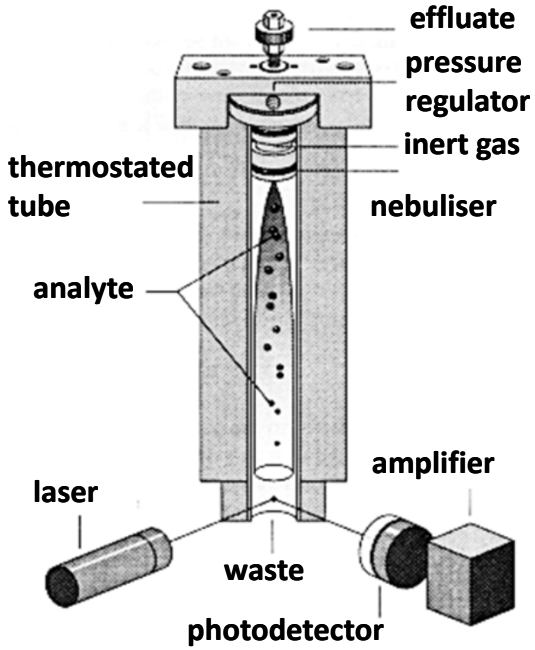


contactless conductivity detection

conductivity
: noise 10^{-3}
: dynamic range 10^{6}
: sensitivity 10^{-9} M

light-scattering detector

ELSD – *evaporative light scattering detector*



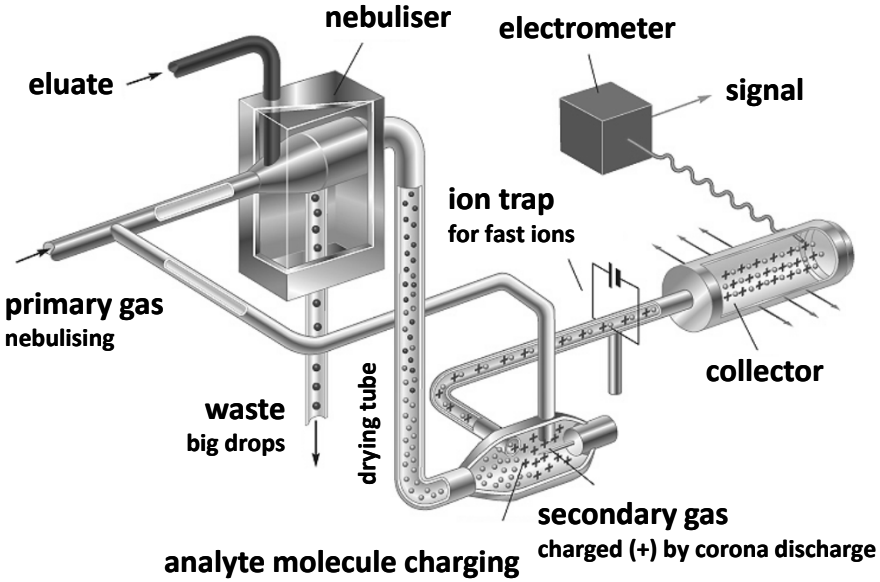
MDD
signal: light scattering

scattering
: noise 10^{-8}
: dynamic range 10^{4}
: sensitive 10^{-8} M

: volatile MP additives
:: e.g. ammonium acetate
: high sensitivity; universal detector
: also for gradient elution

corona charged aerosol detector

CAD – *corona charged aerosol detector*



MDD
signal: cationic current

charged particles flow
: noise 10^{-6}
: dynamic range 10^{5}
: sensitivity 10^{-9} M

: volatile MP additives
:: e.g. ammonium acetate
: high sensitivity
: also for gradient elution

mass spectrometry

MDD
signal: charged particles flow
: connecting LC and MS
:: (soft) ionisation

: improper for quantitation
: high sensitivity and selectivity
: universal detector
: structural information

ion count
: noise 10^{-8}
: dynamic range 10^2 – 10^4
: sensitivity 10^{-18} M

matrix assisted laser desorption/ionisation (MALDI)

RPLC and NPLC
mixing effluate with matrix
: discrete points or continuous trace
: off-line or in-line (endless band)

atmospheric pressure photoionisation (APPI)

RPLC and NPLC
dopant: acetone, toluene, hexane

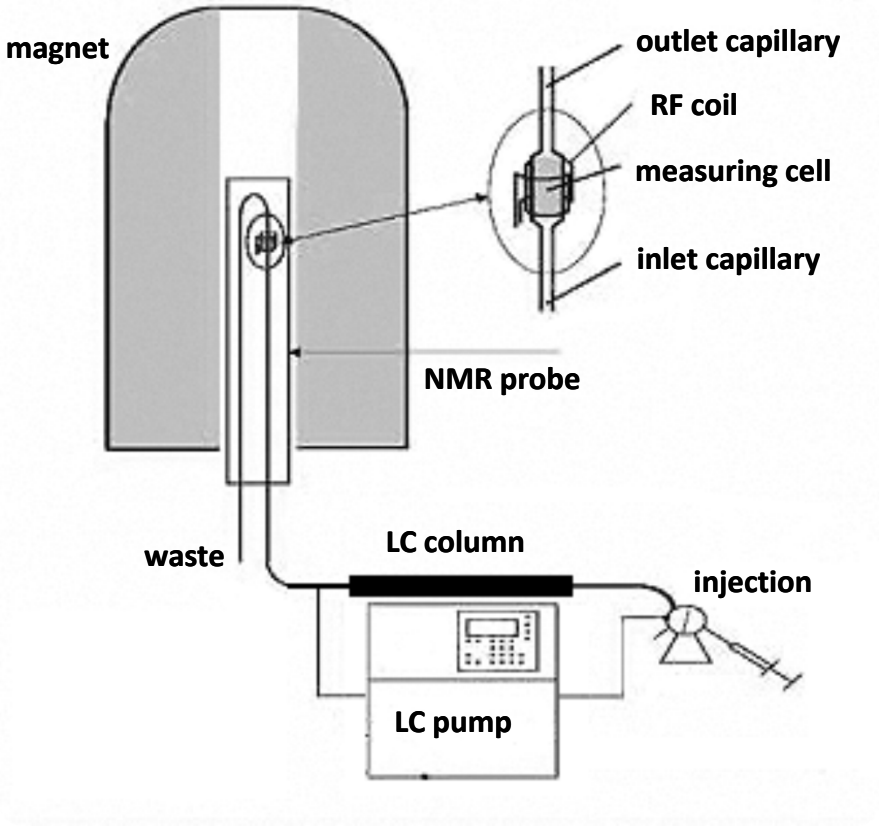
electrospray ionisation (ESI)
: multiply charged ions

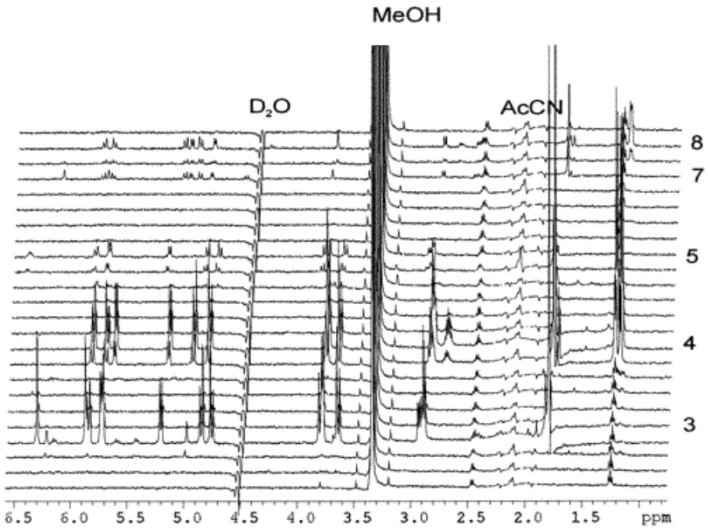
RPLC, HILIC
1:1 MeOH + strong acid (formic)

atmospheric chemical photoionisation (APCI)
: not so soft ionisation

polar organic MP (HILIC, RPLC)
volatile component
: ammonium trifluoroacetate

nuclear magnetic resonance





record of LC-NMR

: deuterated solvents
: polar organic MP (RPLC)
:: HTLC, SWLC

nuclear spin
: noise 10^{-2}
: dynamic range 10^4
: sensitivity 10^{-7} M

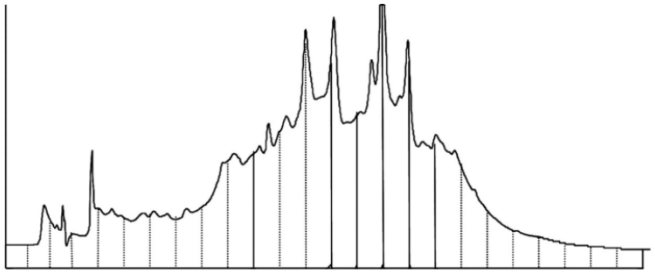
CDD
signal: spin of nuclear particles in magnetic field

: structural information
: very expensive (1 l D_20 ~140 EUR, 1 l AcN ~15 EUR)

collection of fractions

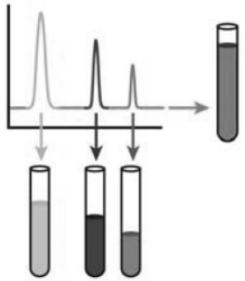
allows to isolate part of separated sample (fraction)
: separation into groups – fractionation

controlled valve preceding waste outlet
: leading liquid into collection vials



collection of fractions
: in defined time periods
:: mixed separation zones
:: does not require detector *in-line*

: at defined change of signal intensity in time
:: collection of „pure“ zone



necessity to know the capillary volume between detection cell and outlet

sample derivatisation

chemical derivatisation of sample before entering detection system

: **increasing sensitivity** or **allowing detection** at all
: **increasing resolution** or **allowing separation** at all
: **supressing unwanted sorption** of substance on column

derivatisation
: change of separated substance elution
:: separation efficiency and analysis time

pre-column derivatisation

: uses often autosampler
:: derivate must be chemical individual and should be stable enough
:: reaction must go on quantitatively and selectively
:: reaction need no go on at high rate
:: reaction with side products, under mild reaction conditions (pH, temperature)
::: no pre-separation
:: good separation of main product from side ones; different detection properties

post-column derivatisation

: one-step derivatisation (w/ creation of derivatisation agent *in situ*)
: two-step derivatisation
:: reaction need not result in definitive chemical individual
:: reaction need not to be quantitative; good reproducibility necessary
:: reaction must go on at high rate, even under extreme conditions (pH, temperature)
:: reaction agent surplus → dilution of MP by agent
:: reaction may not be selective, side products of reaction are of no harm
:: sample is separated in unchanged form
:: expensive; special instrumentation and reactors, but automatic

on-column derivatisation

: more common in GC
or electromigration

definition of chromatographic system in LC

MP **mobile phase composition**

isocratic: buffer concentration, % content of organic, pH, eventually I

gradient: gradient profile – A – water component; B – organic component
: e.g. 20 % A – 80 % B; 5 min 5 % A – 95 % B, 5 min 100 % B

flow rate / pressure (ml·min^{-1} / MPa) **temperature** (20 – 60 °C)

load (*X* µl)

SP **stationary phase type**

trade mark, type, particle size (Nucleosil100, C-18, 5 µm)

column (length, inner diameter)

length *x* inner diameter (250x4 mm)

detector basic characteristic according to type

During II-RPLC runs, mobile phases with 5 to 30 mM ammonium acetate pH 6–8 and methanol content between 13 and 15% (v/v) were used. All measurements were carried out at 25 °C and all chromatographic data for analytes studied were evaluated at 254 nm. Particular conditions in detail for each experiment are given in the text below and/or in the captions of relevant figures or tables.

Chromatographic experiments were performed using a liquid chromatograph Shimadzu 10AVP system (Kyoto, Japan) consisting of a SCL-10AVP system controller, two LC-10AVP pumps, a GT-154 degasser, a CTO-10ASVP column oven with Rheodyne 7120 injection valve (20 µl sample loop) and a SPD-M10AVP photodiode-array detector. A Luna C18(2) 250 × 3,0 mm, 5 µm (Phenomenex, USA) column was used along with a guard column Security Guard C18 4 × 2,0 mm (Phenomenex, USA). Data were collected and evaluated using VP-Class software (version 6.13 SP2).

analytical information in chromatogram

qualitative information

retention time

≈ retention factor, distribution constant

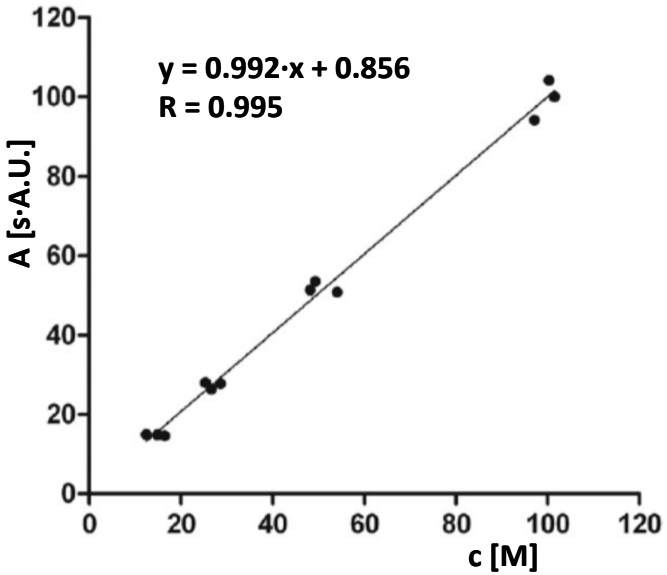
: compound identification (*standard method*)

spectroscopic detectors

: UV-Vis spectra

: MS spectra (ESI / APCI; Qq / IT / o-TOF)

: NMR spectra (^{1}H, ^{13}C)



quantitative information

peak area

≈ compound amount (concentration)

: occasionally peak height

calibration curve method

: correct method

$$\mathbf{A = k \cdot c^{x}}$$ calibration curve

: **x** = 1 ⇒ calibration line

$$\mathbf{\log A = x \cdot \log c + \log k}$$ linearisation

c – at least three orders of concentration

standard addition method (spiking)

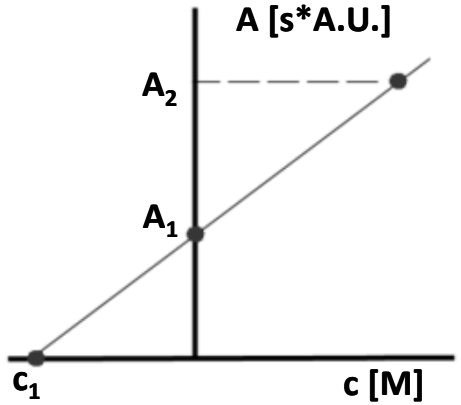
assumes linear calibration dependence

$$c_1 = \frac{V_S}{V_1} \cdot \frac{c_S}{\frac{A_2}{A_1} \cdot \frac{(V_1 - V_S)}{V_1} - 1}$$

A_1 – analyte peak area, unknown concentration c_1
A_2 – analyte peak area of unknown concentration c_1 after addition of standard of known concentration c_S
V_1 – sample volume, V_S – standard solution volume

checking the calibration dependence linearity
: the second addition of standard

dilution influence
: signal may decrease after addition
:: graphical solution is then not possible



internal standard method

uses presence of substance of constant/defined concentration in chromatogram

absolute quantitation

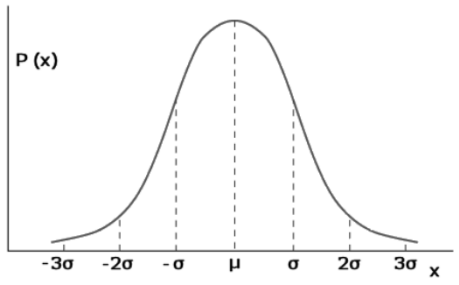
$$c = (A/A_{IS}) \cdot c_{IS}$$

relative quantitation

$$\Delta c = (A_2 - A_1)/A_{IS}$$

number of in parallel measured values
statistical evaluation
: ≥ 7 according to Student
: 3 – 7 according to Dean-Dixon

data normality test (D'Agostino-Pearson test)



accuracy and precision

μ – real value
σ – x_i value distribution around **μ**
P(x) – probability density

***μ* estimation**
average

$$\bar{x} = \frac{1}{n} \cdot \sum_{i=1}^{n} x_i$$

for *n = 3*
median

$$\tilde{x} = x_2$$

measurement of retention time or **peak area**

***σ* estimation**
standard deviation

$$s = \sqrt{\frac{1}{n} \cdot \sum_{i=1}^{n} (x_i - \bar{x})^2}$$

(*SD*)

***σ* estimation** Dean-Dixon

$$s_R = k_n \cdot R$$

where $R = x_{max} - x_{min}$

R – span
$\mathbf{k_n}$ – Dean-Dixon coefficient

relative standard deviation

$$s_r = \frac{s}{\bar{x}} \cdot 100$$

(*RSD*)

outlying results
Grubbs, T-test

$$T_i = \frac{|\bar{x} - x_i|}{S_n}$$

$$s = \sqrt{\frac{1}{n} \cdot \sum_{i=1}^{n} (x_i - \bar{x})^2}$$

if $T_i < T_\alpha$ (tabul.)
result is not outlying

Q-test acc. to Dean-Dixon

$$Q_i = \frac{x_i - x_{i-1}}{R}$$

i = 2 or **n**

if $Q_i < Q_\alpha$ (tabul.)
result is not outlying

---

result identity
Student t-test

for $n_A = n_B = n$

$$t = \frac{|\bar{x}_A - \bar{x}_B| \cdot \sqrt{(n-1)}}{\sqrt{(s_A^2 + s_B^2)}}$$

if $t < t_\alpha$ (tabul.)
results are identical

for $n_A \neq n_B$

$$t = \frac{|\bar{x}_A - \bar{x}_B|}{\sqrt{(s_A^2/(n_A - 1) + s_B^2/(n_B - 1))}}$$

$s_A^2 \geq s_B^2 \rightarrow F_A = s_A^2/s_B^2$
$s_B^2 > s_A^2 \rightarrow F_B = s_B^2/s_A^2$

if $F_{A\ or\ B} > F_\alpha$ (tabul.)
difference is significant

$$t_\alpha = \frac{t_1 \cdot s_A^2/(n_A - 1) + t_2 \cdot s_B^2/(n_B - 1)}{s_A^2/(n_A - 1) + s_B^2/(n_B - 1)}$$

t_1 is t_α for $v_1 = (n_A - 1)$
t_2 is t_α for $v_2 = (n_B - 1)$

Lord u-test

$$u = \frac{|\bar{x}_A - \bar{x}_B|}{R_A + R_B}$$

if **u or U** $< u_\alpha$ or U_α (tabul.)
results are identical

Moore U-test

$$U = \frac{|\bar{x}_A - \bar{x}_B|}{R_A + R_B}$$

if $F_{A\ or\ B} < F_\alpha$ (tabul.)
difference is insignificant

---

confidence interval

$$L = \bar{x} \pm s \cdot \frac{t_\alpha}{\sqrt{n}}$$

L according to Dean-Dixon

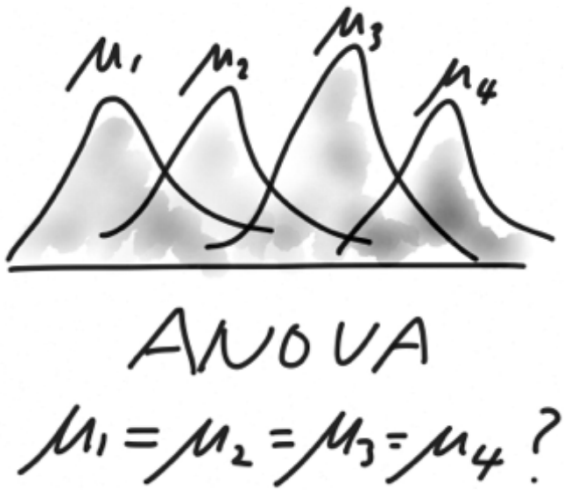
$$L = \bar{x} \pm K_n^\alpha \cdot R$$

μ value lies in interval **L**
with probability **1-α** (95 – 99%)

K_n^α – Dean-Dixon coefficient for **α**

repeatability and reproducibility **analysis of variance (ANOVA)**

comparing **more data sets (>2)** of retention times or areas
: day-to-day repeatability (*inter-day repeatability*)
: reproducibility, inter-laboratory tests



test on **data homoscedasticity** (same **σ** value in frame of individual days)
: if not suited → non-parametric **Kruskal-Wallis ANOVA**

null hypothesis H_0
: average values of individual samples are same ($\mu_0 = \mu_1 = \mu_2 \ldots \mu_k$)

: we need to know, if the influence of some factor (*different day of measurement*), which assumes different values, on our studied quantity (t_R) is statistically relevant

in case of **null hypothesis H_0 invalidity** additive tests
: **Bonferroni test** (tests on identity of averages)
: **Leven test** (tests on identity of variance)

programmes used – **Statistica, M$ Excell, SPSS, R…**

analyte concentration determination

linear regression
: <u>x</u> value is not subjected to error

Y = a·X + b

$$a = \frac{(\sum x)\cdot(\sum y) - n\cdot\sum(x\cdot y)}{(\sum x)^2 - n\cdot\sum x^2}$$

$$b = \frac{1}{n}\cdot\left(\sum y - a\cdot\sum x\right)$$

standard deviations

$$s_{yx} = \sqrt{\frac{\sum(y_i - Y_i)^2}{n-2}}$$

$$s_a = \frac{s_{yx}}{\sqrt{\sum(x_i - \bar{x})^2}}$$

$$s_b = s_{yx}\cdot\sqrt{\frac{1}{n} + \frac{\bar{x}^2}{\sum(x_i - \bar{x})^2}}$$

outlying results
Grubbs, T-test

$$T = \frac{|Y_i - y_i|}{s_{yx}}\cdot\sqrt{\frac{n}{n-2}}$$

segment significance

$$t = \frac{|b|}{s_b}$$

t_α for ν = (n-2)
insignificant $t < t_\alpha$

correlation coefficient

$$r_{xy} = \frac{n\cdot\sum(x\cdot y) - (\sum x)\cdot(\sum y)}{\sqrt{[n\cdot\sum x^2 - (\sum x)^2]\cdot[n\cdot\sum y^2 - (\sum y)^2]}}$$

confidence interval

$$L_b = b \pm s_b\cdot t_\alpha$$

$$L_a = b \pm s_a\cdot t_\alpha$$

for regression parameters

for given **x**

$$L = Y \pm t_\alpha\cdot s_{yx}\cdot\sqrt{\frac{1}{n} + \frac{(X-\bar{x})^2}{\sum(x_i - \bar{x})^2}}$$

for individual value

$$L = Y \pm t_\alpha\cdot s_{yx}\cdot\sqrt{\frac{1}{n} + \frac{(X-\bar{x})^2}{\sum x_i^2 - (\sum x_i)^2/n}}$$

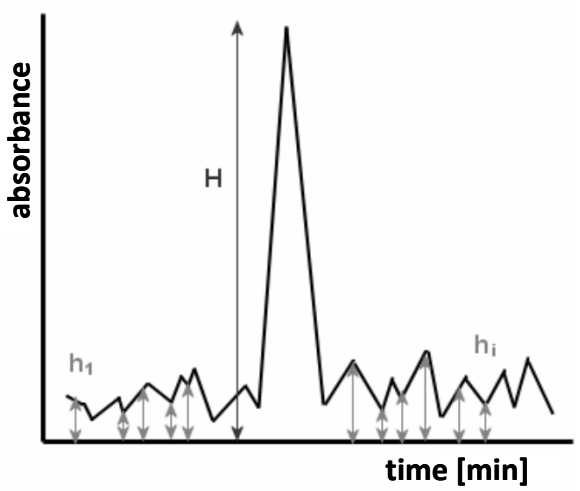
limit of detection **(LOD)** : the lowest relevant detectable amount

optimally: by sequential dilution till disappearance of the observable signal

from value s_0 of background x_0

s_0 – standard deviation of $\bar{x}_0$

$$\mathbf{LOD} = \bar{\mathbf{x}}_\mathbf{0} + \mathbf{w} \cdot \mathbf{s}_\mathbf{0}$$



$$\bar{\mathbf{x}}_\mathbf{0} = \sum_{\mathbf{i=1}}^{\mathbf{n}} \mathbf{h_i}/\mathbf{n} = \mathbf{0}$$ in HPLC

$$\mathbf{LOD} = \mathbf{3} \cdot \frac{\mathbf{c}}{\mathbf{H}} \cdot \mathbf{s}_\mathbf{0}$$

c – known, low concentration

H – *height* of peak; noise has no area

from linear regression, b = 0

$$\mathbf{LOD} = \mathbf{3} \cdot \frac{\mathbf{s}_\mathbf{0}}{\mathbf{a}}$$

from linear regression, b ≠ 0

$$\mathbf{LOD} = \frac{(\mathbf{b} + \mathbf{3} \cdot \mathbf{s}_\mathbf{b})}{\mathbf{a}}$$

from confidence interval

$$\mathbf{LOD} = \mathbf{t}_\alpha \cdot \mathbf{s}_\mathbf{a} = \mathbf{t}_\alpha \cdot \mathbf{s}_\mathbf{yx} \cdot \sqrt{\frac{\mathbf{1}}{\mathbf{n}} + \frac{\bar{\mathbf{x}}^\mathbf{2}}{\sum(\mathbf{x_i} - \bar{\mathbf{x}})^\mathbf{2}}}$$

upper limit of detection **(ULOD)**

: the highest relevant detectable amount

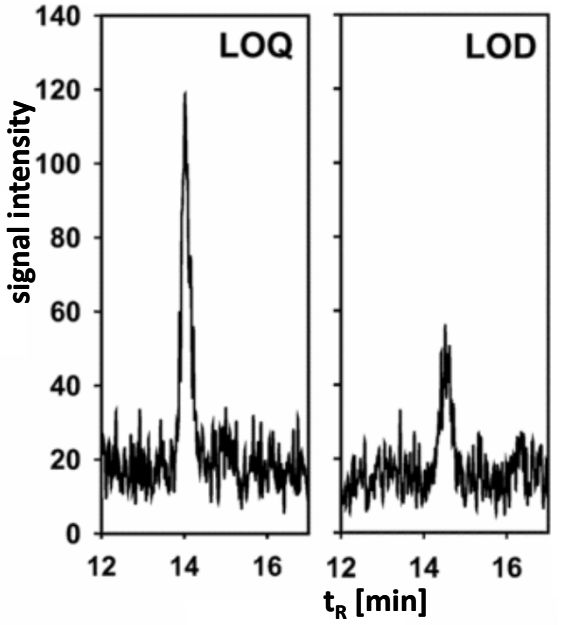
$$\mathbf{ULOD = 100 - w \cdot s_0}$$

:: higher content than **99.85 %** is not possible to determine directly

limit of quantification **(LOQ)**

: the lowest relevant determinable amount

$$\mathbf{LOQ = 10 \cdot \frac{s_0}{a}} \qquad \mathbf{LOQ = \frac{(b + 10 \cdot s_b)}{a}}$$



method detection limit **(MDL)**

the lowest determinable concentration on a level of significance α = 0.01 in a sample with given matrix

: uses a low concentration
: includes of steps of sample preparation
: includes matrix

consists of two steps

: first measured concentration; calculate s_{x1}

$$\mathbf{MDL} = t_{n,\alpha} \cdot s_{x_1}$$

here we can end and calculate **MDL**
but better to approve the result:
: next concentration, different from first, but similar; calculate s_{x2}

$$\mathbf{MDL} = t_{n_1+n_2,\alpha} \cdot s_{x_{12}}$$

: conduct *F-test on identity* of both sets
if $F < F_\alpha$ calculate s_{x12} and **MDL**

$$s^2_{x_{12}} = \frac{n_1 \cdot s^2_{x_1} + n_2 + s^2_{x_2}}{n_1 + n_2}$$

where **n** is number of degrees of freedom

approach is burdened by presumption of same dispersion all over the concentration scale

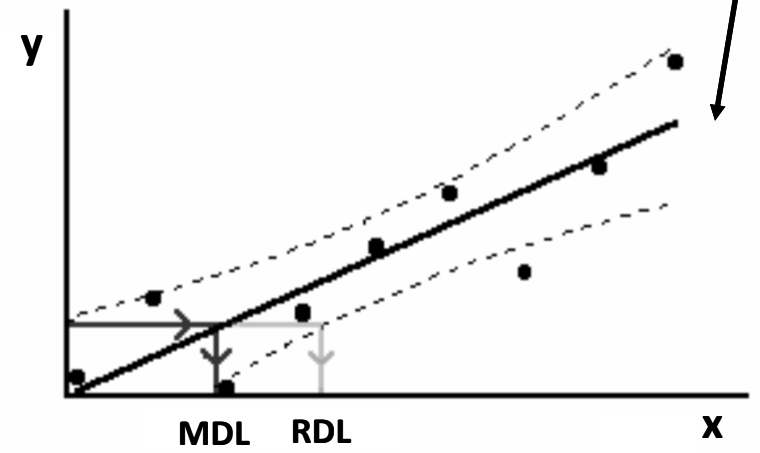
method of a use of standard deviation estimation of segment *b*

$$\mathbf{MDL = b + t_{\alpha} \cdot s_b}$$ for b ≠ 0

method according to Hubaux-Vos

works with confidence interval of linear regression of calibration dependence
: to construct the calibration curve using linear regression
: to *calculate* MDL as a $\mathbf{x_{MDL}}$ value of confidence interval for **x = 0** or to *determine it graphically*

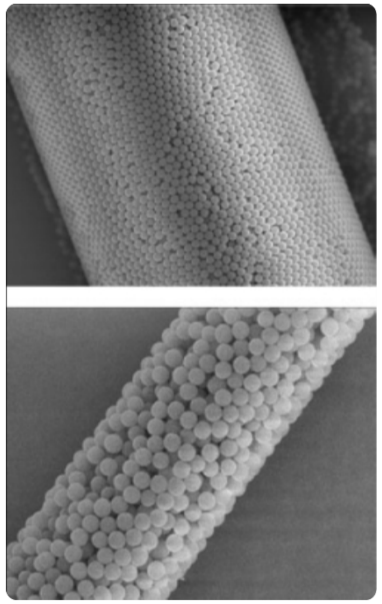
$$\mathbf{MDL = \frac{1}{a} \cdot t_{n,\alpha} \cdot s_{yx} \cdot \sqrt{\frac{1}{n}}}$$



RDL (reliable detection limit)
: the highest estimation of detection limit

evaluation of separation efficiency

check and assurance of reproducible conditions for separation



stationary phase is manufactured in series

we check : physical state
: chemical state
: topology

reproducibility of SP
: manufacturer-to-manufacturer
: batch-to-batch

parameters of material
: pore volume, pore size, surface area, carbon content, modified surface coverage, end-capping

separation parameters
: resolution, selectivity, void volume, efficiency

other properties
: inertness, hydrophobicity, metal ions influence, longevity

test measurements in LC

testing method

: method of performance measurement
: standard of good behaviour, which is comparable
: universal test of good behaviour

what must the test fulfilled

: simplicity
: illustrativeness
: instructiveness

observable separation parameters (one analyte)

: N – number of theoretical plates, $\mathbf{t_R}$ – retention time, **Δp** – pressure change

index of good behaviour π

$$\boldsymbol{\pi = N^2/(t_R \cdot \Delta p) = (N/t_R) \cdot (N/\Delta p)}$$

$\pi \in (10^3 – 10^5)$, $[t_R]$ = s, [Δp] = MPa
increase of **η** or **k'** → **decrease** of **π** w/ constant N
η – viscosity MP, **k'** – capacity factor

separation impedance E

increasing E
→ behaviour deterioration

$$\boldsymbol{E = \left(\pi \cdot \eta \cdot (1 + k')\right)^{-1}}$$

$$\boldsymbol{E = t_R \cdot \Delta p \cdot \left(N^2 \cdot \eta \cdot (1 + k')\right)}$$

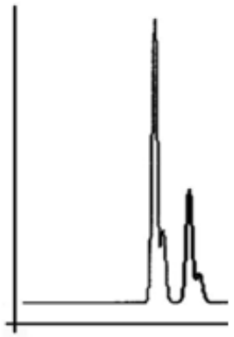
$$\boldsymbol{E = H^2/K = h^2 \cdot \Phi}$$

h – reduced height of theoretical plate
Φ – column resistance parameter

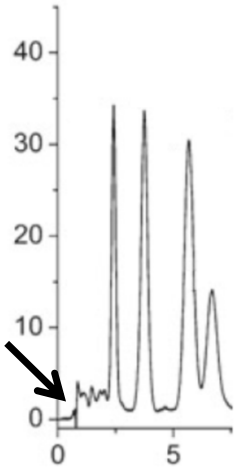
void volume determination

non-interacting substance
: runs through the system along with front of MP

normal phase – metaxylene
reversed phase – thiourine, aceton
ionex – uncharged or same charge substance



column damaged by pressure,
non-additive void volume



so-called system peak

column testing

efficiency

testing mixture: contains series of substances with increasing retention

normal phase – metaxylene, nitrobenzene
reversed phase – aceton/uracil, benzene, toluene, naphthalene
catex – uracil, cytosine
anex – uridine, uridine monophosphate

in dependence on time (at constant flow rate) ***we observe***
: retention times of components
: number of theoretical plates
: symmetry of peaks
: selectivity factor

other properties of SP reversed phase

inertness ***state of free silanols/end-capping***

testing mixture: pyridine, phenol, toluene
MP: acetonitrile : water 1:1

positive outcome: pyridine (charged) is eluted before phenol
negative outcome: pyridine is eluted after phenol or is broadening

hydrophobicity ***sensitivity to methylene groups***

testing mixture: butylbenzene, amylobenzene
MP: acetonitrile : water 4:1

positive outcome: high selectivity (**α**)

sensitivity to metal ions ***influence of heavy metal ions***
(DERT, *dihydroxynaphthalene efficiency ratio test*)

testing mixture: 2,7-dihydroxynaphthalene, 2,3-dihydroxynaphthalene
MP: acetonitrile : water 1:1

positive outcome: 2,3-dihydroxynaphthalene (chelator) is not broadening
: asymmetry ratio of both peaks should be ~ 1.0

longevity ***test on β-blocker separation***

testing mixture: pindol, metoprolol, propranolol
MP: 25 mM P, pH = 9.75, AcN/MeOH 35:10:55

we observe: selectivity (**α**), resolution and peak asymmetry
: difficult separation; complex influence of column state

principles of SP storage ***column care guide***

: regular testing (before measurement day and after) and records about it

: MP filtering and degassing

: not to expose column to harming conditions
:: not to overload column by high pressure
:: extreme pH only for a short period of time (max hours)
:: max one night with MP w/ content of organic phase lower than 60 % AcN (70 % MeOH)
:: after measurement on border limits (pH, salts, temperature) wash thoroughly
::: 5 % organic phase and then keeping MP

column revitalisation
: turn column up-side down, MP flow from the other side
: 5 % AcN, 60 % AcN, 100 % IPA or THF, 60 % AcN

COLUMN STORAGE

- Column storage conditions affect column lifetime
- Never store columns with buffers
- Flush with 5 column volumes of mobile phase without buffer to remove any buffers or salts

Storage Conditions for Silica-Based HPLC Columns:

TABLE 6

Column Type	Storage Solvent
Reversed Phase C18, C12, C8, C4, C2, C1, Phenyl, PFP	65 % Acetonitrile/ 35 % Water
Normal Phase Silica, CN, NH_2, PAC, Diol Alumina	Isopropanol or Hexane
Ion-Exchange SAX, SCX, WAX, WCX	Methanol*
Size-Exclusion Diol	0.05 % NaN_3 in water or 10 % methanol
HILIC Luna HILIC	80 % Acetonitrile/ 20 % Water

*Flush column with 50 mL HPLC grade water prior to storage solvent

5

VII. basic modes of liquid chromatography

counter-current liquid chromatography
(CCC)

normal-phase liquid chromatography
(NP-HPLC, NPLC)

reversed-phase liquid chromatography
(RP-HPLC, RPLC)
: ion-pairing (IP-RPLC)
: ultra-performance (UPLC)
: high-temperature (HTLC)
: ultra-performance at elevated temperature (ET-UPLC)

hydrophilic interaction liquid chromatography
(HILIC)

hydrophobic interaction liquid chromatography
(HIC)

ion-exchange liquid chromatography
(IEC)
: ion exclusion chromatography (IXC)
: chromatofocusing (CF)

affinity chromatography
(AC)
: with immobilised metal ion (IMAC)

super-critical fluid chromatography
(SFC)

perfusion chromatography
(PLC)

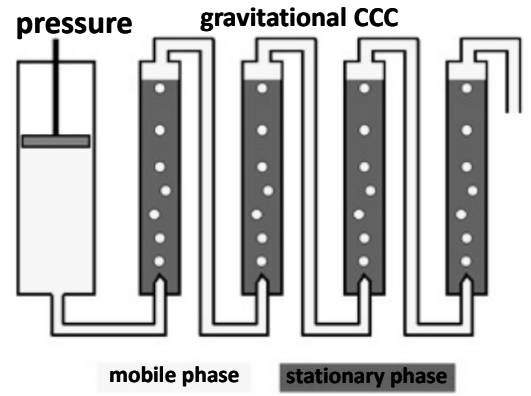
chiral chromatography
(XLC)

planar chromatography
(TLC)

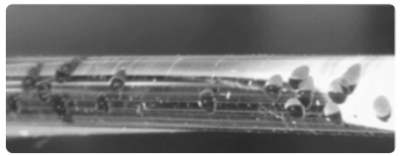
mode	% of use
RPLC	36
IEC	18
NPLC	10
SEC	10
HILIC	8
XLC	7
HIC	2
AC	2

counter-current chromatography (CCC)

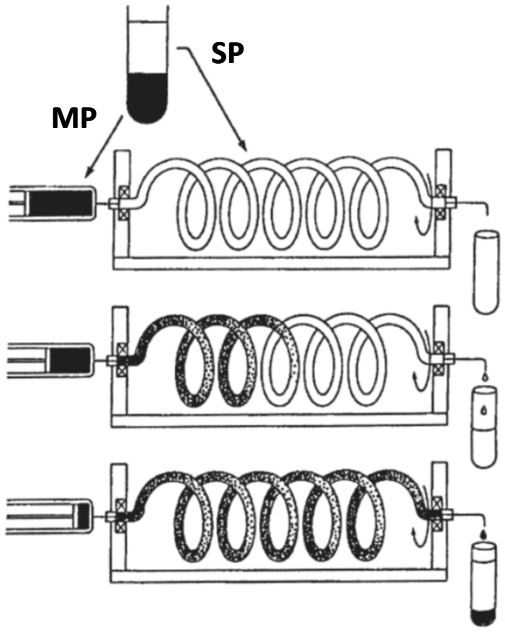
method was discovered in 1949
: both phases are realised by immiscible liquids of different density
: **gravitational** & **centrifugal** arrangement



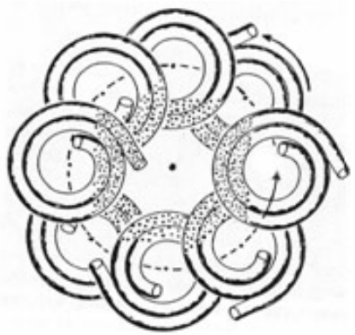
centrifugal counter-current chromatography (CCCC)



: hydrodynamic equilibrium with variable acceleration field
:: Archimedean screw principle – biaxial rotation
: system pressure max 2.5 MPa
: high separation efficiency for low K_D & short elution times
: low SP stability & worse repeatability
:: SP retention depends on angular velocity & flow rate

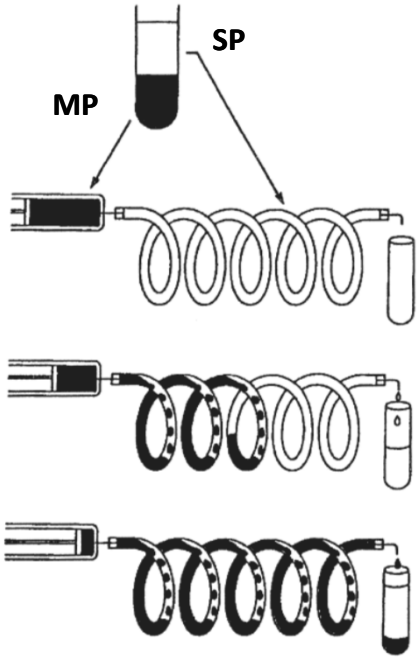


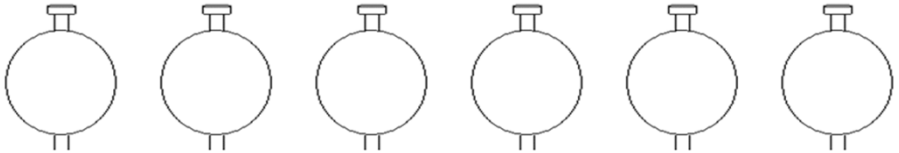
high speed CCC (HSCCC)
: the most frequent contemporary variant of CCC
:: **ω** up to 280G; 200 g of material per day
: three columns on planetary arrangement (*J-type*)



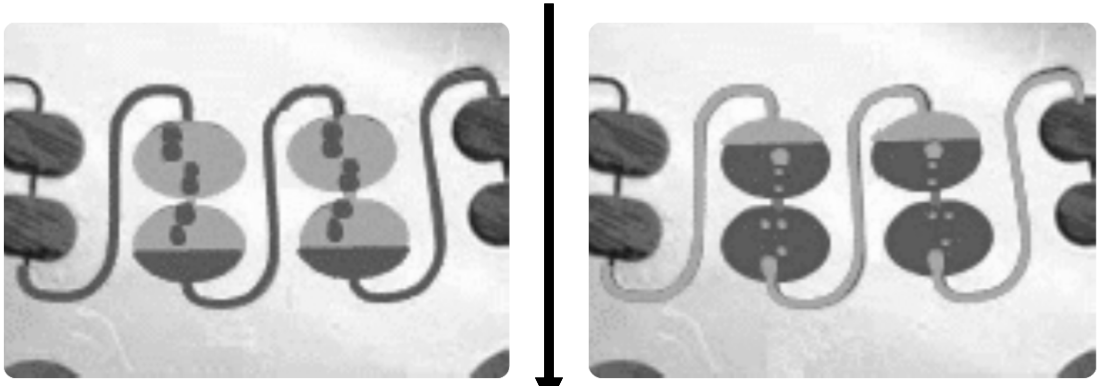
centrifugal partition chromatography (CPC)

: hydrostatic equilibrium with constant acceleration field
  :: achieved by flowing MP, not SF
: centrifugal force at monoaxial rotation holds liquid SP
: MP is pushed though it
  :: system pressures *ca* 4 GPa (900G)
: low separation efficiency, but still usable
  :: high flow rates & high capacity (~ 30 kg per day)
: very quick wear of expensive rotation seals





descending mode (MP heavier) ascending mode (MP lighter)



F_G

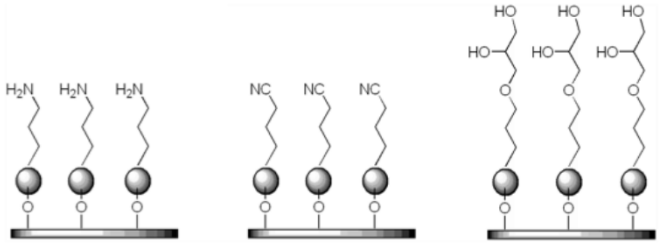
normal-phase high-performance liquid chromatography (NP-HPLC)

stationary phase

: silica

: modified silica (amido-, amino-, cyano-)

: polymer SP (polymethyl methacrylates, divinylbenzene)



mobile phase

: organic solvents

:: n-hexane, heptane, chloroform, alcohols

: additives modify selectivity

:: *ion-pairing NPLC* (IP-NPLC)

:: diethanolamine, trifluoracetic acid

:: (monovalent) salts – LiCl, NaCl, $AgNO_3$

aqueous normal phase LC (ANP)

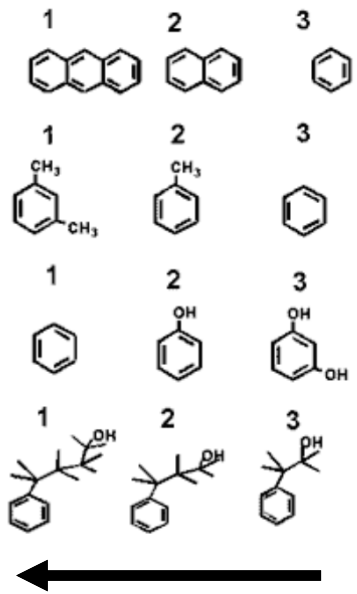
: special SP (hydride Si-H, sometimes Si-COOH or Si-alkyl)

:: works also as a RPLC

: mobile phase contains also water

:: > 60 % of organic phase

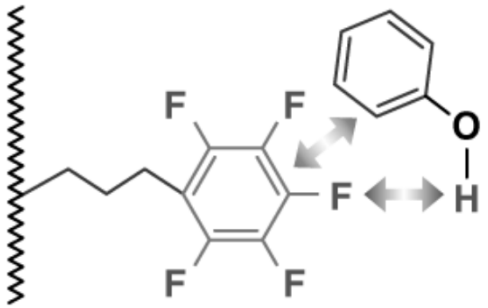
increasing retention on NP

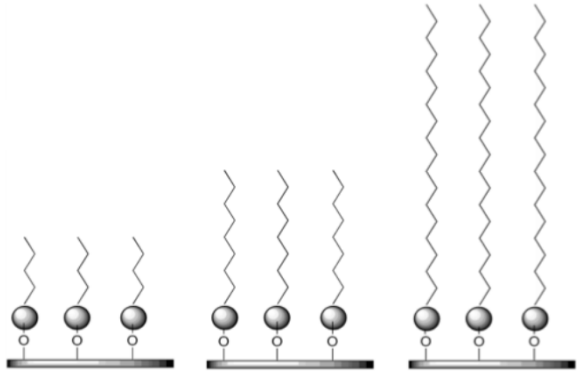


increasing retention on RP

reversed-phase high-performance liquid chromatography (RP-HPLC)

1950 – RPLC description

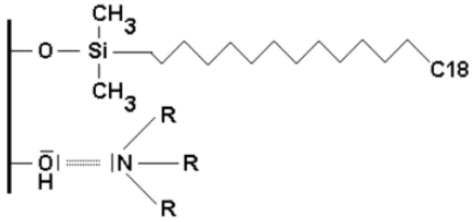




stationary phase

: (C1, C2, C4,) **C8, C12, C18, C30, phenyl, perfluorophenyl (PFP)**

:: + variations (*polar group spacing*)

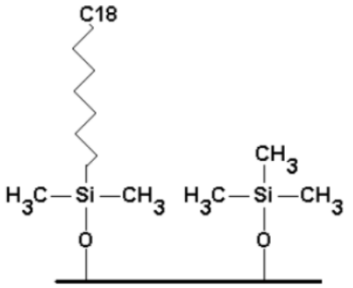


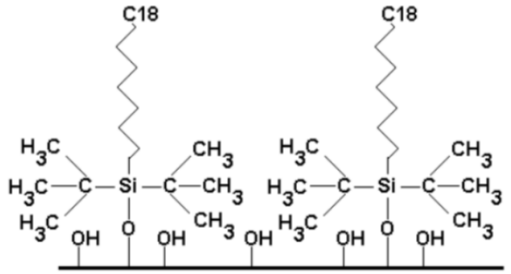
stable surface

: *controlled modification shields silica*

end-capping of free silanols

: organic amides, trimethylsiloxanes

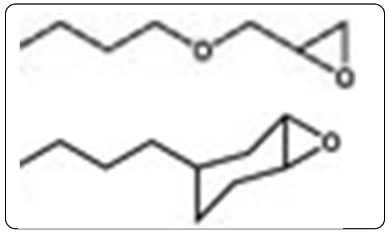




secondary silanolisation

: polar sample, non-polar eluent

: **we add modifier into MP**



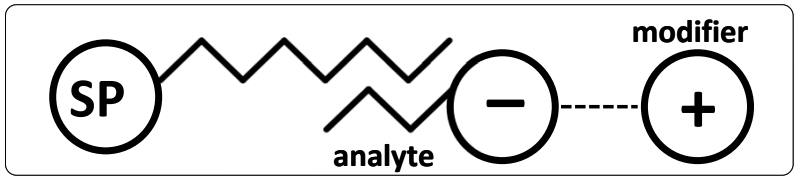
easy adaptation of SP on different LC type

:: *chiral selector*

::: **chiral** LC

:: *pH change*

::: **ion-suppression** RP-HPLC



:: *sizable ion*

::: **ion interaction** RP-HPLC

:: *surfactant*

::: **micellar** RP-HPLC

(*c_{tens} > CMC*; *micellar liquid chromatography MLC*)

::: **ion-pairing** RP-HPLC

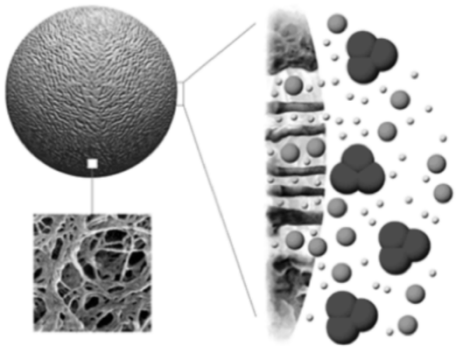
(*quasi-ionex*)

: **so-called non-interaction mode of RPLC**

:: *non-interaction conditions* – very high elution force

::: **size/molecular exclusion** LC (separates sccording to size)

:::: sieving effect



mobile phase

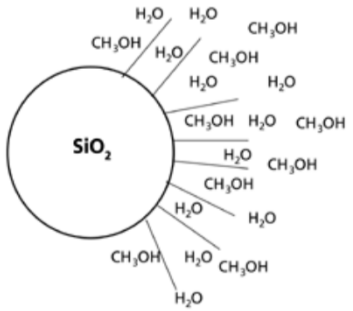
water – in RP **low elution** force

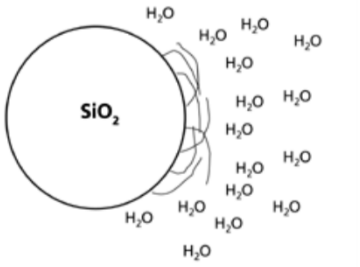
increasing portion of organic phase ⇒ **decreasing** retention time (**decreasing** surface tension)

: **organic** phase must be **100% miscible with water**

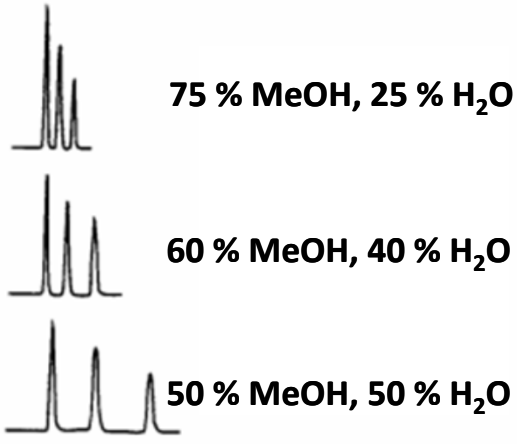
: **onto column** always **min 5%** or **max 95 % organic** phase in water

:: SP dewetting





dewetted SP



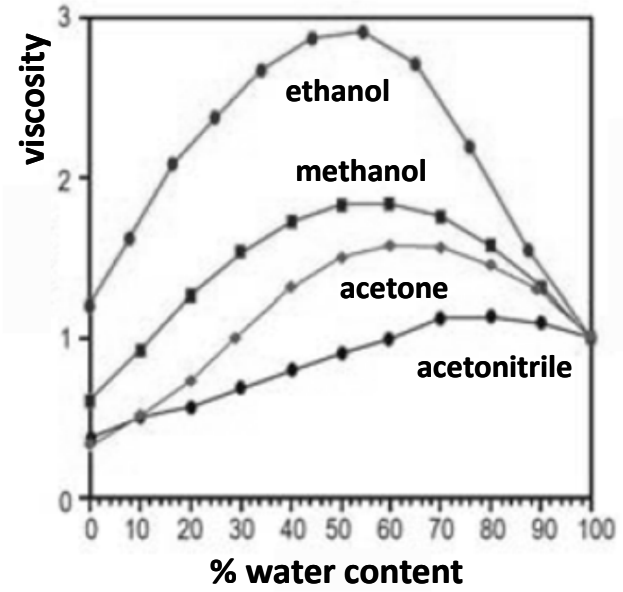
solvent	↓ *k* with ↑ content in 10%
water	–
dimethyl sulphate (DMS)	**1.5 x**
ethylene glycol	**1.5 x**
acetonitrile (AcN)	**2.0 x**
methanol (MeOH)	**2.0 x**
acetone	**2.2 x**
dioxane	**2.2 x**
ethanol (EtOH)	**2.3 x**
tetrahydrofuran (THF)	**2.8 x**
isopropanol	**3.0 x**

content of organic component changes MP viscosity

: different polarity of both components
: increasing of pressure in system

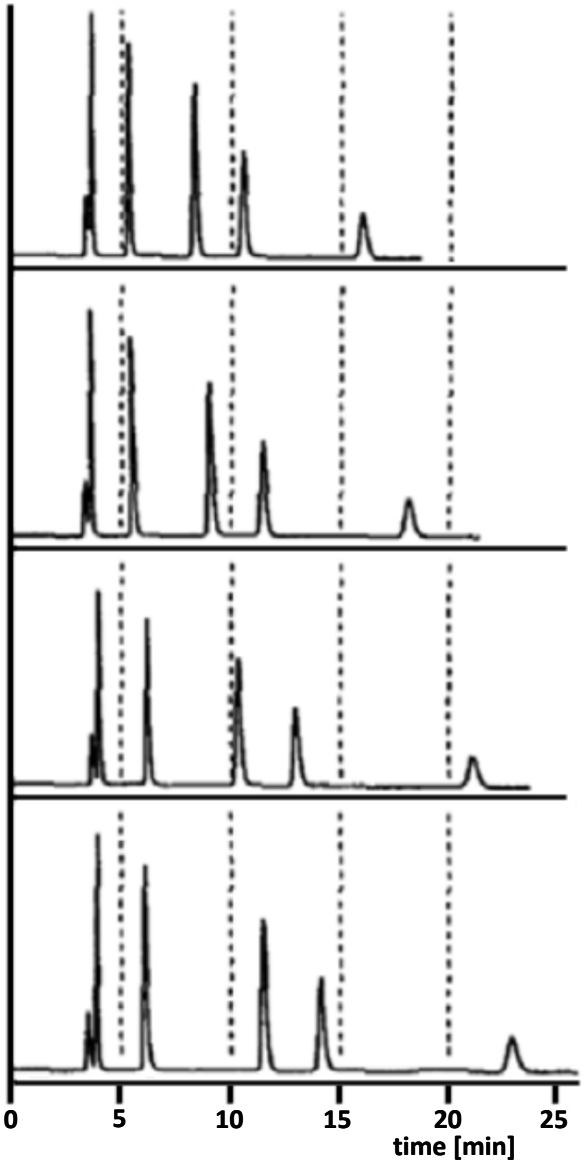
problem within gradient elution
: increased temperature decreases viscosity

MP composition influence on its viscosity



solvent	viscosity [mP·s] at 20 °C
hexane	0.29
acetone	0.32
acetonitrile	0.34
THF	0.46
methanol	0.54
DMF	0.80
ethanol	1.08
isopropanol	1.90

MP preparation influence on separation efficiency

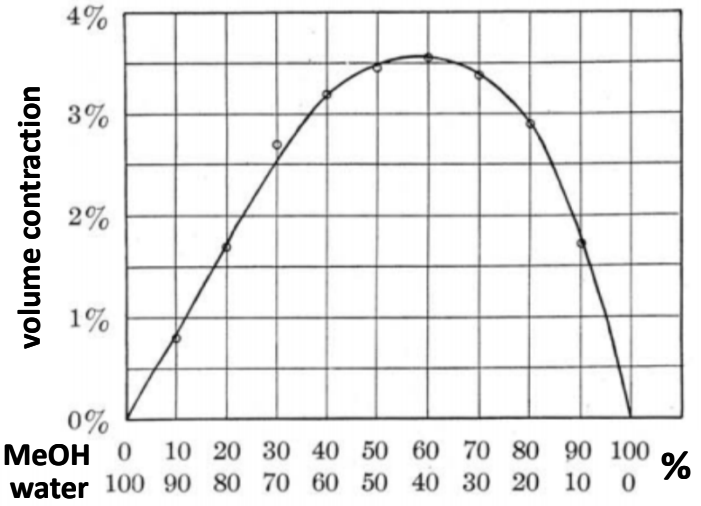


volumetric flask (1 l) w/ 400 ml of water
: filled with MeOH up to scale line
: 635 ml of MeOH → 1.59 : 1.00

mixing 400 ml of water and 600 ml of MeOH
: 400 ml of water → 1.50 : 1.00
:: resulting volume 965 ml

high-pressure mixing of MP
: A – water 0.4 & B – MeOH 0.6 ml·min^{-1}
: ~ 1.45 : 1.00

volumetric flask (1 l) w/ 600 ml of MeOH
: filled with water up to scale line
: 435 ml of water → 1.38 : 1.00

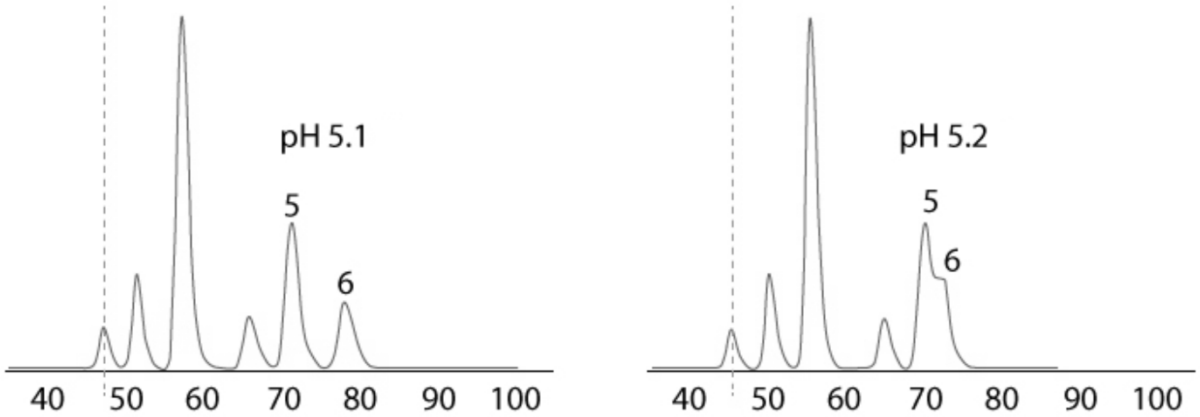


pH influence

influences **ionisable** analytes (organic acids and bases) and **polarisable** analytes

in RP – **suppress** ionisation any time it is possible

it is necessary to adjust pH of aqueous constituent of MP
: change in 0.1 pH has a strong influence on retention



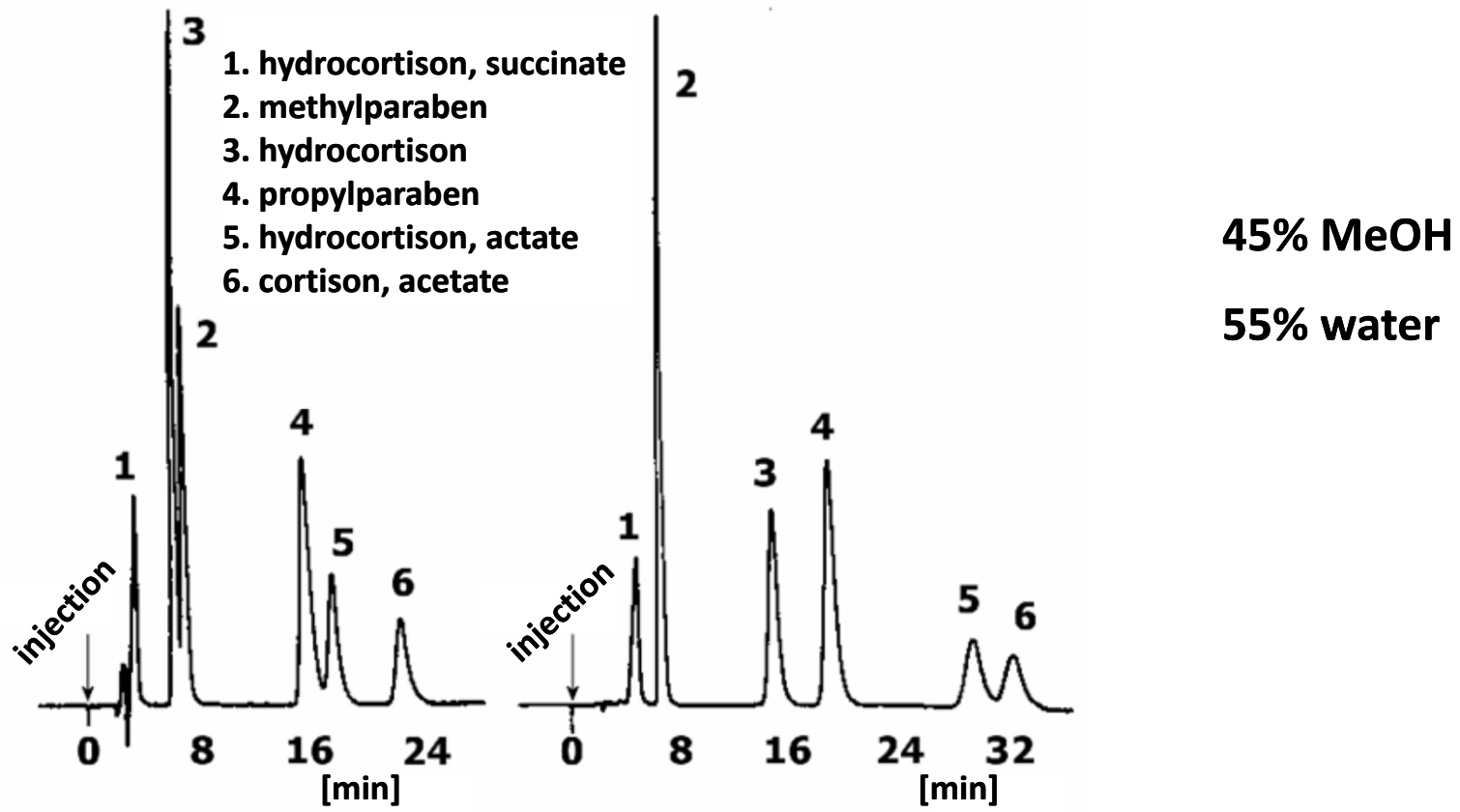
aqueous MP constituent
: buffer (reproducible pH)
:: concentration 50 mM and lower (system ***salinisation***)
:: always check ***solubility*** in given MP (influence of org. phase)

addition of ***neutral salt*** **increases** surface tension a that **increases** retention

selectivity choice of MP according to elution force and interaction type (Snyder's triangle)

30% AcN

70% water



45% MeOH

55% water

300x4.6 mm C-18, 1.5 ml·min^{-1}, detection 254 nm, 10 mg load

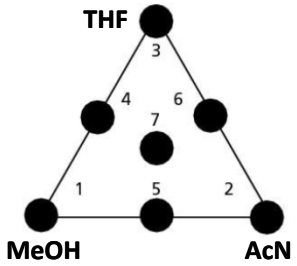
change in content and type of organic phase means **change** of retention

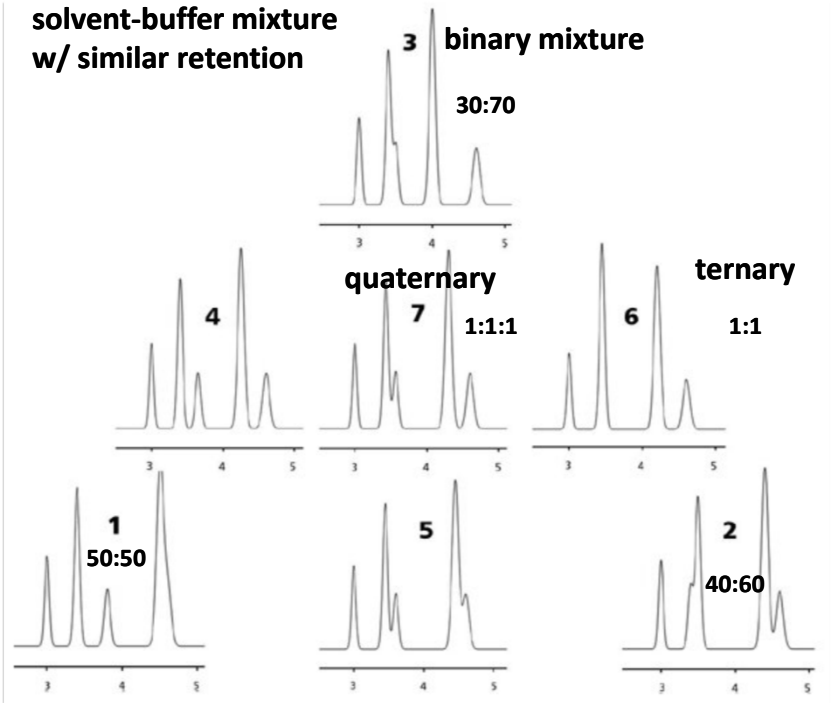
Snyder's triangle

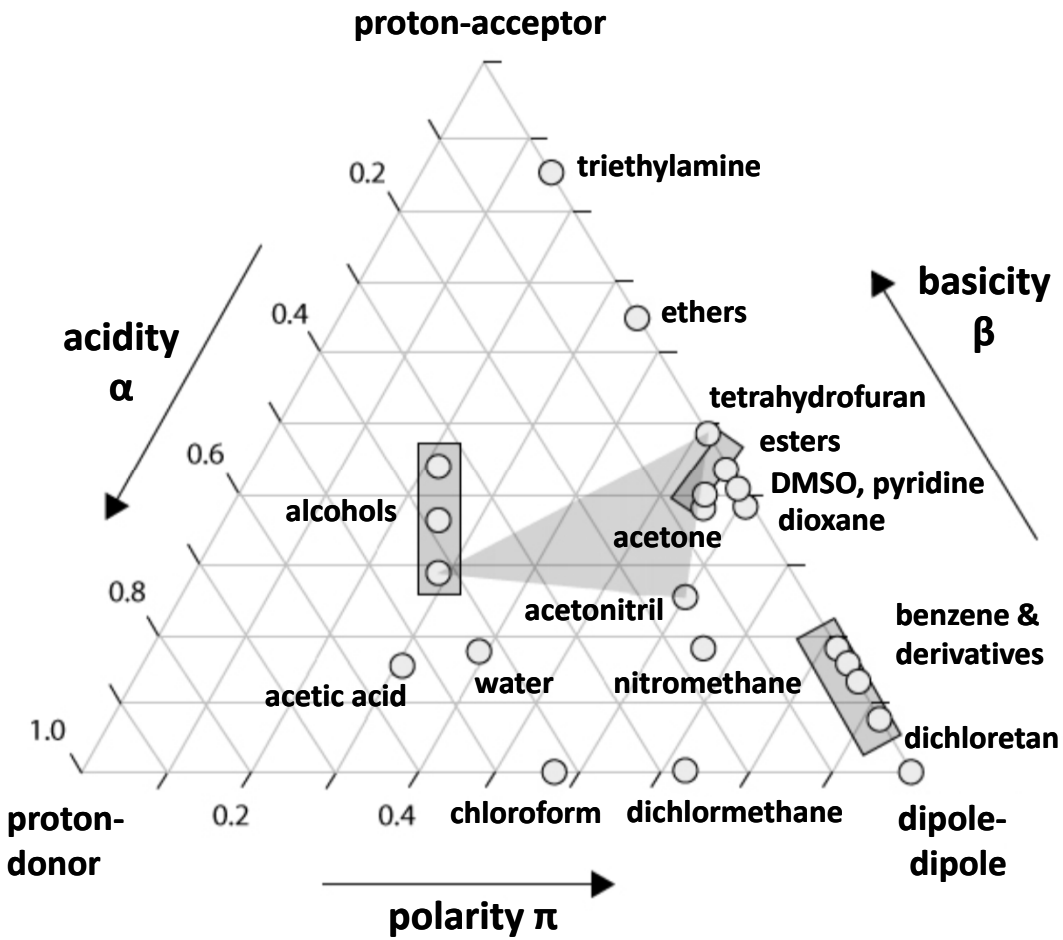
for interactions on RP

type of organic phase
: favours different interactions

content of organic phase
: amplifies given interaction







$$\log k = \log k_0 - m \cdot \varphi$$

Soczewiński-Wachtmeister (semi-logarithmic) equation

m – individual parameter of each analyte in system
log k_0 – logarithm of extrapolated *k* value for water as MP
ϕ – voluminal ratio of organic phase

A – ideal separation

B – high elution force

C – low elution force

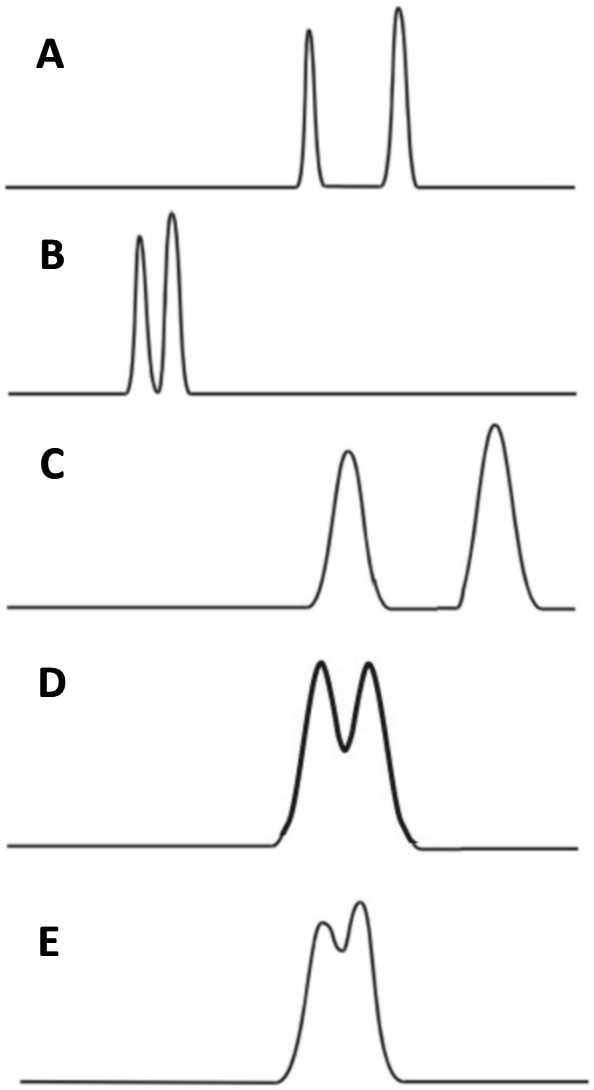
D – improper column or flow rate

E – improper column or flow rate

$$\log k = \log k_0 - m \cdot \log \varphi$$

Snyder & Soczewiński (logarithmic) equation

isocratic elution mode



$$\log k = \log k_0 - a \cdot (\varphi_0 + b \cdot t)$$

gradient elution mode

gradient steepness *b*

$$b = (t_m \cdot \Delta\varphi \cdot a)/t_G = (V_m \cdot \Delta\varphi \cdot a)/t_G \cdot F_m = (\Delta\varphi/t_G) \cdot (V_m \cdot a/F_m)$$

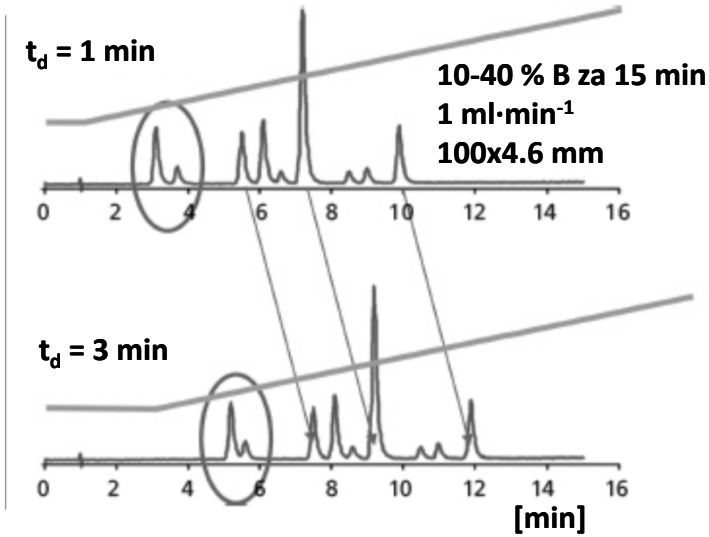
t_G – time length of gradient
$\Delta\phi$ – change of voluminal ratio of organic phase

retention time dependence on gradient steepness and profile

$$t_R = (t_m/b) \cdot \log(2.3 \cdot k_0 \cdot b + 1) + t_m + t_d$$

t_d – hold-up or dwell time of gradient

$t_d >> 0 \Rightarrow$ separation begins at k_0 (isocratic) and $b_{pract} < b_{theor}$



increasing *the flow rate* **causes** *the gradient steepness* **to reduce**

the gradient steepness can be used to *alter* **retention**, but also **selectivity**

resolution within gradient elution

$$R_{(A,B)} = \frac{\sqrt{N}}{4} \cdot \left(\alpha_{(A,B)} - 1\right) \cdot \frac{k''}{1 + k''}$$

average capacity factor within gradient elution

$$k'' = 1/\left(1.15 \cdot b + (1/k_0)\right)$$

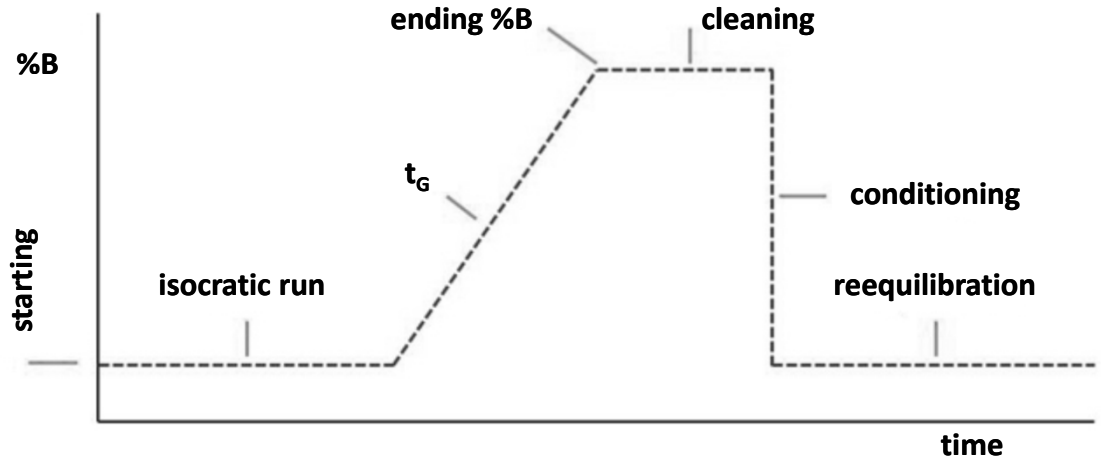
capacity factor within gradient elution

under $t_d = 0$

$$k = (1/b) \cdot \log(2.3 \cdot k_0 \cdot b + 1)$$

peak capacity within gradient elution

$$n = 1 + \frac{t_G}{\sum_{i=1}^{j} w_i / j}$$



thin-layer chromatography (TLC)

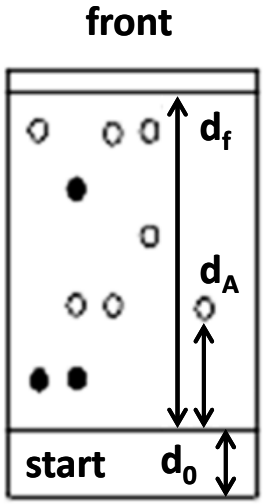
1938 – TLC (Izmailov and Shreiber)
1944 – paper chromatography (PC; Consden *et al*.)

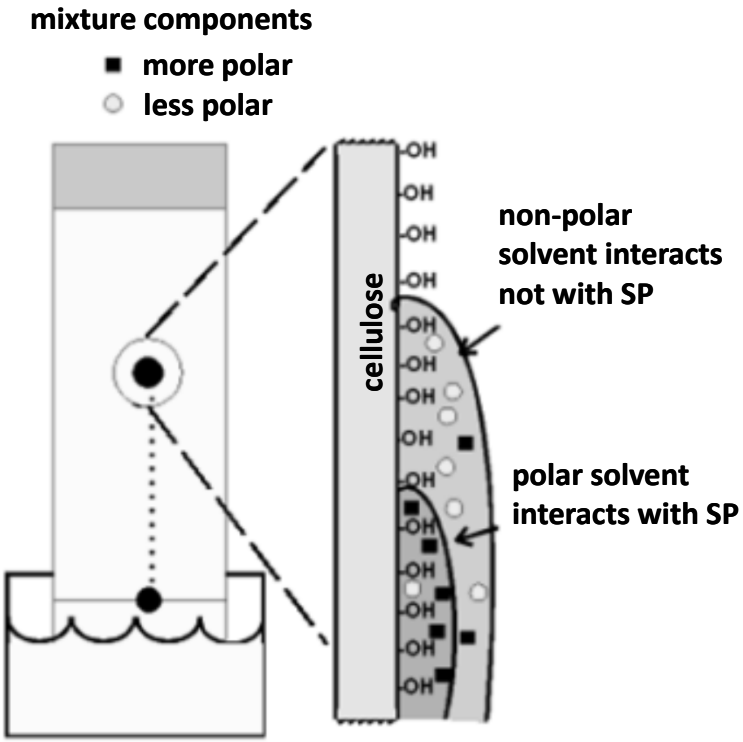
arrangement
: vertical (ascendant, descendant)
: horizontal (annular TLC)

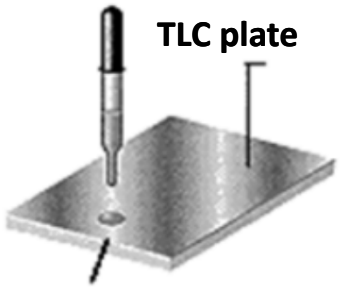
$$R_f = \frac{d_A}{d_f}$$

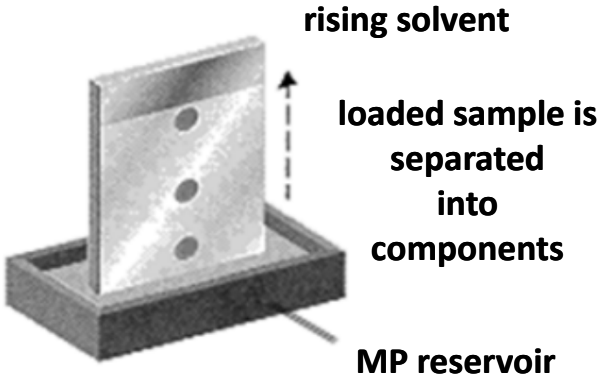
retardation factor
: analyte identification

reached distance
d_f – MP front
d_A – analyte
d_0 – edge-to-start
: no separation
:: but a MP wicks



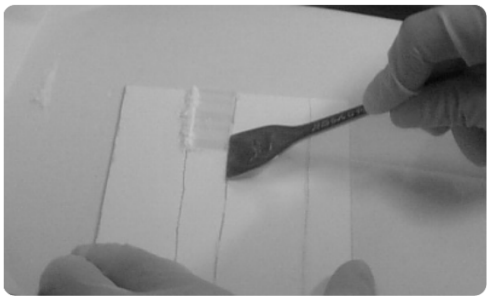






analysis and **preparation**

: *scratching off SP layer, elution*
: *direct elution from SP*

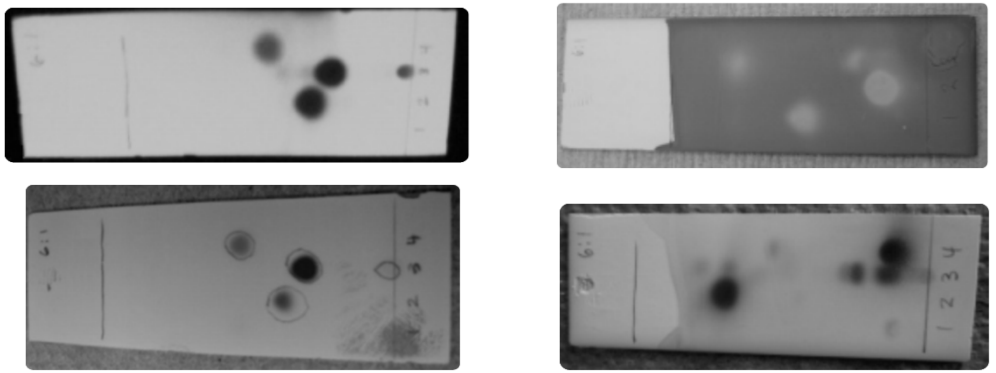


stationary phases (SP)
: silica (SiO_2) – *on carrier*
RP-18, chiral RP-18, NH_3^-, CN^-
: alumina (Al_2O_3) – *on carrier*
: cellulose – paper
: polyamide 6 (polycaprolactam)

visualisation

: sulphuric a./heat: *destructive*
:: burned spots
: cerium staining: *destructive*
:: dark-blue spots (polar compounds)
: iodine staining: *semi-destructive*
:: iodine adsorption, not stable
: UV irradiation: non-destructive
:: base is green, dark spots

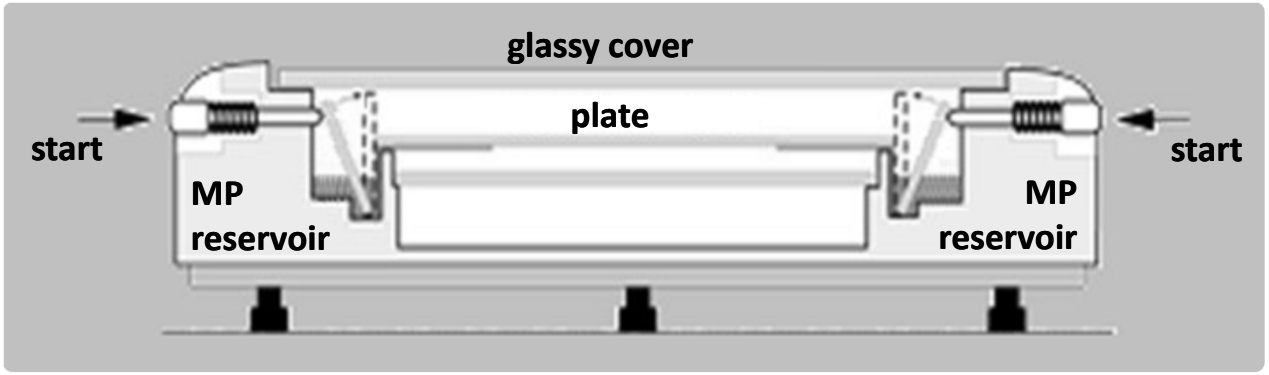
imbuing
: sinking of TLC plate into solution
:: heating for staining fixation

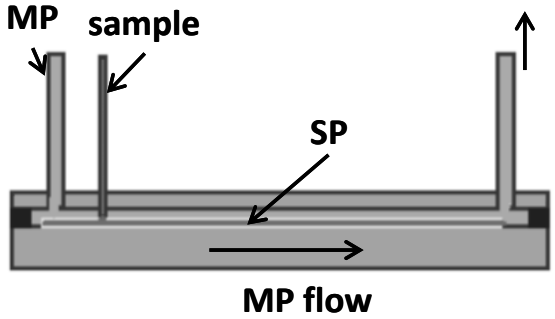


: different agents for different analyte classes are used
:: aminoantipyrin/$K_3Fe(CN)_6$ (aryls), $AgNO_3/H_2O_2$ (halogenhydrocarbons), ninhydrin (amines), $FeCl_3$ (amides), dithizone (metal ion), anisaldehyde (sugars), $SbCl_3/SbCl_5$ (lipids)...

high performance thin-layer chromatography (HPTLC)

: horizontal arrangement
: MP forced-flow
: MP over-pressured



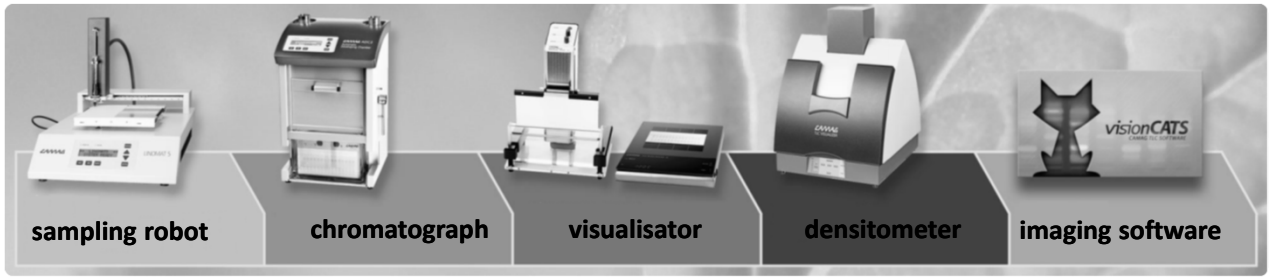


$$k = \frac{1 - R_f}{R_f} = \frac{d_f}{d_A} - 1$$

$$N = 16 \cdot d_A \cdot \frac{d_f}{w_A^2}$$

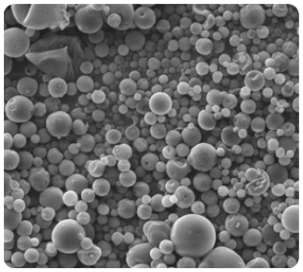
w_A – zone width

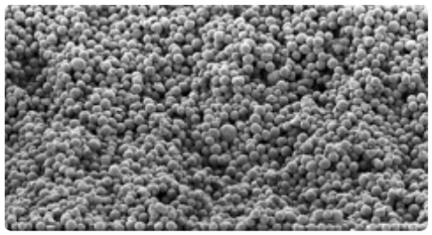
: mobile phase is in reservoirs
:: *blue* in Figure
: separation start by their opening
: MP out of the rises on porous plates



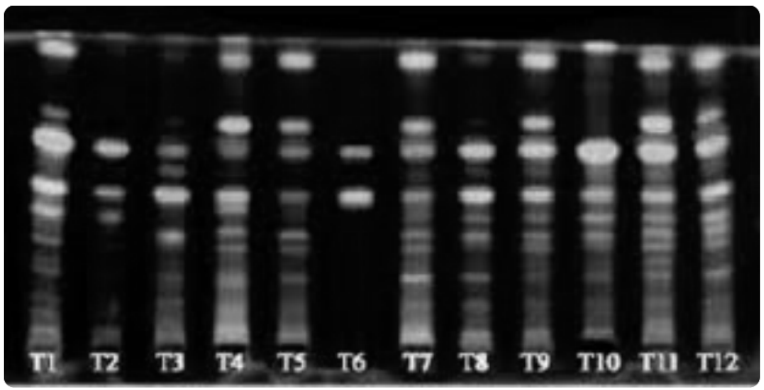
HPTLC *vs*. TLC

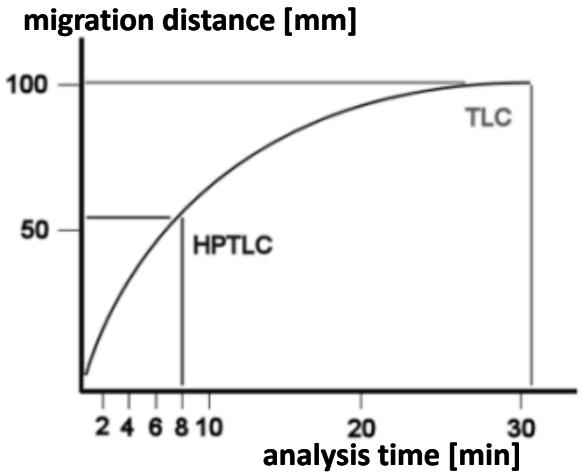
thinner layer of sorbent – 0.20 *vs*. 0.25 mm
: small grain diameter – 7 *vs*. 12 – 20 µm and low polydispersity of grain
:: lower longitudinal diffusion, 10x lower limit of detection
:: better price/output ratio
: higher resolution on shorter runs $\mathbf{d_f}$
:: 50 mm *vs*. 100 – 120 mm ⇒ faster analysis
: better optical properties for densitometry



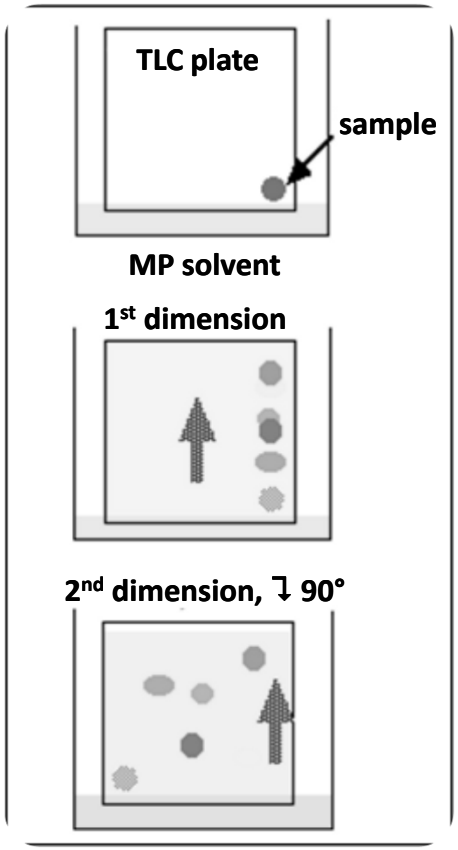


disadvantages
: smaller sample input than TLC (1 / 10 till 1 / 15)
: higher demands on sample quality – purity
: technical background for data evaluation
:: good densitometer & imaging software





2D TLC



(HP)TLC separation description

$$d_f = \sqrt{\chi \cdot t_f}$$

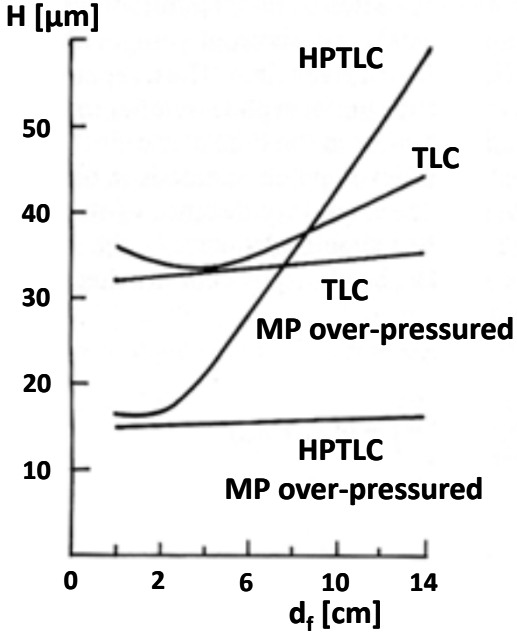
χ – system constant
t_f – MP flow time to front

height equivalent of theoretical plate

$$H = a \cdot \frac{\left(d_f^{2/3} - d_0^{2/3}\right)}{d_f - d_0} + b \cdot (d_f + d_0)$$

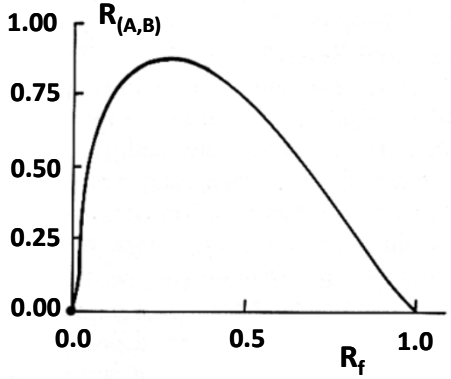
$$a = \frac{3}{2} \cdot A \cdot d_p \cdot \sqrt[3]{\left(\frac{d_p}{2D_m}\right)} \qquad b = \frac{2D_m}{\chi \cdot R_f}$$

d_p – particle diameter
D_m – diffusion coefficient

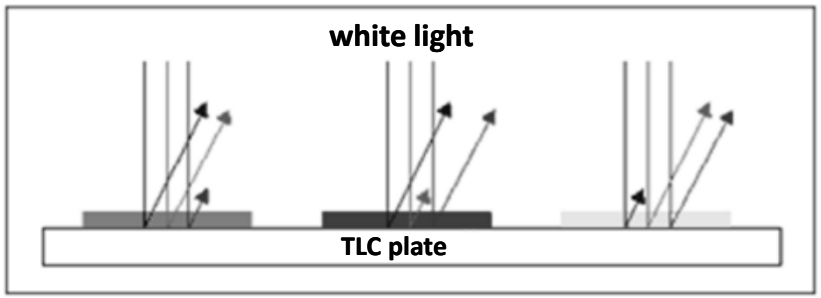


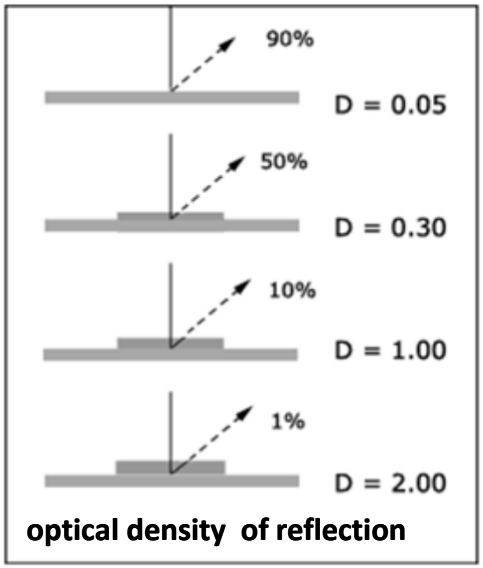
resolution

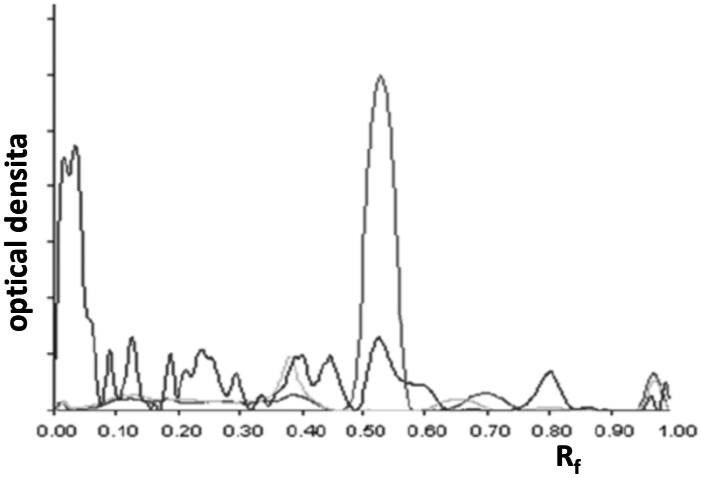
$$R_{(A,B)} = \sqrt{\frac{N}{1+k}} \cdot (\alpha - 1) \cdot \left(\frac{k}{1+k}\right)$$

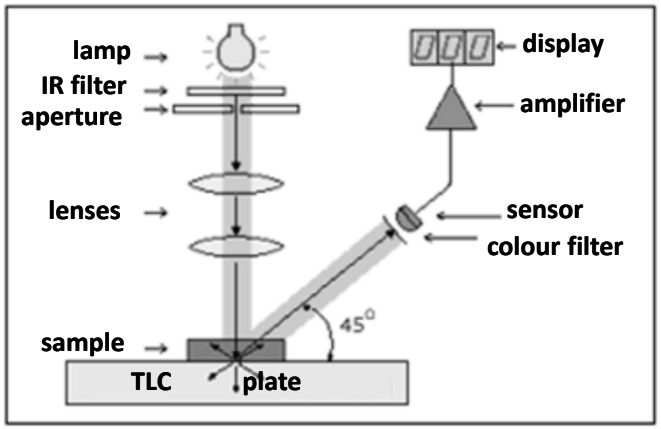


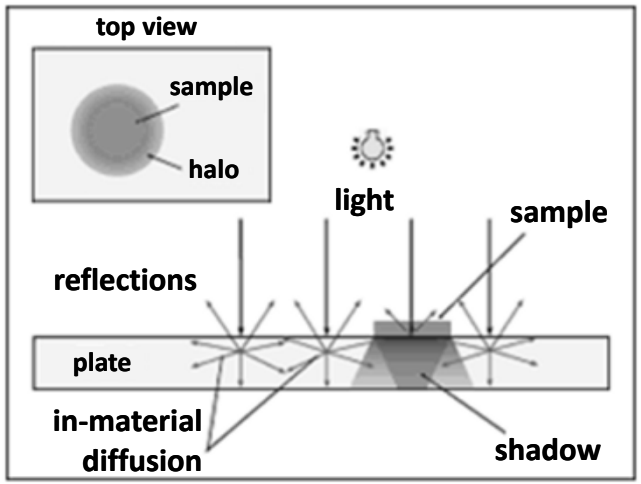
reflexive densitometric detection

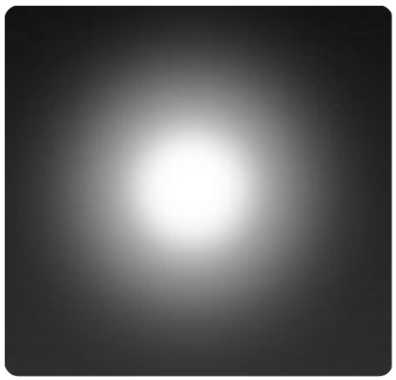












$$\mathbf{D = \log(100/R)}$$

D – optical density
R – reflexivity %

ultra-high performance liquid chromatography (UPLC)

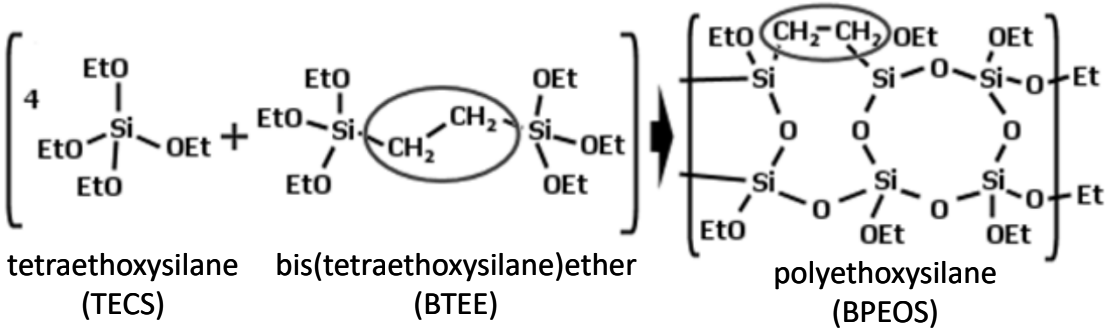
2001 – new subtype of RPLC

: SP with particle size 1.3 – 1.7 µm (*sub-two micron*)
:: pressure up to 0.2 GPa *(10x higher than by HPLC)*
:: at same MP flow rate, analyses are **3x faster**

competition to monolithic columns

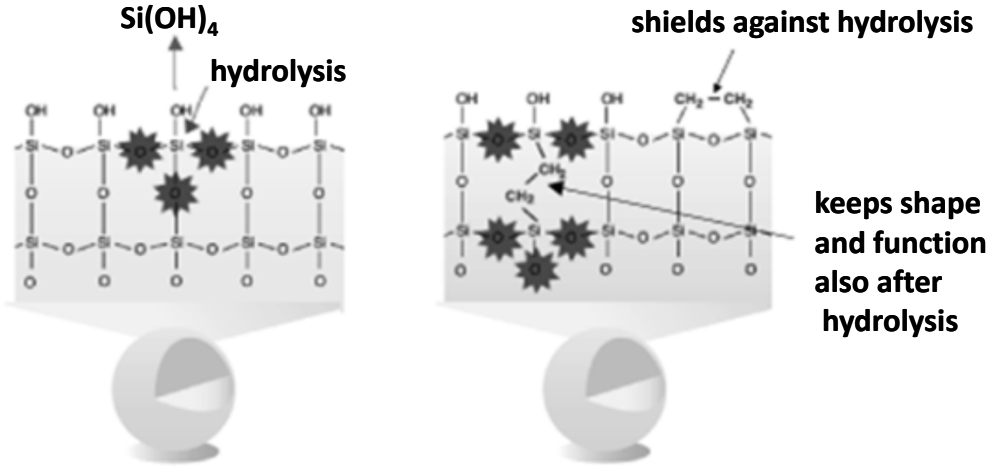
new SP

BEH withstands very high pressures
: high range of pH 1 – 12



tetraethoxysilane (TECS) | bis(tetraethoxysilane)ether (BTEE) | polyethoxysilane (BPEOS)

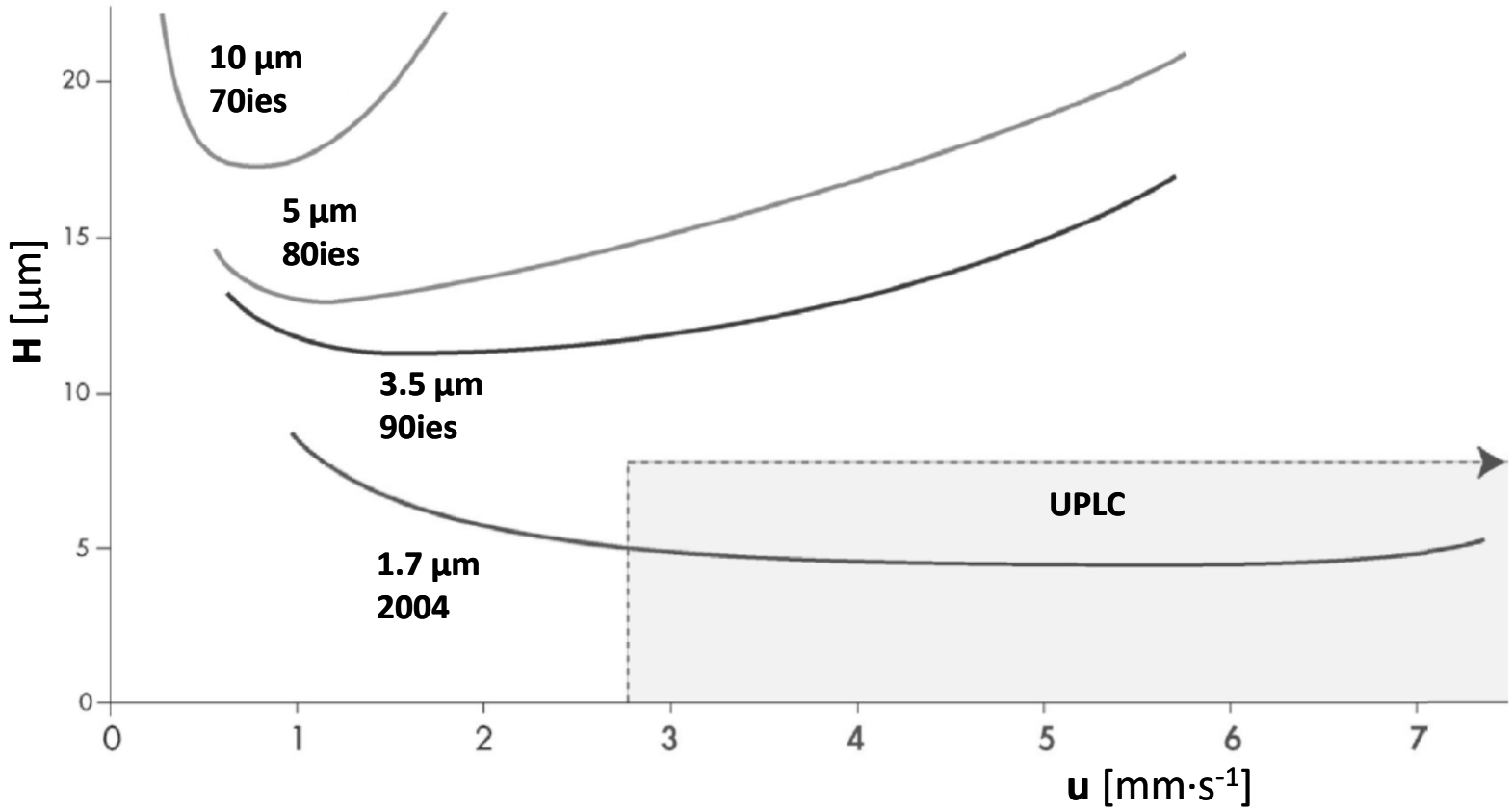
bridged ethylsiloxane/silica hybrid (BEH)



stability at pH > 10 ~300 h
: normal SP based on silica ~40 h

development of separation efficiency

from HPLC to UPLC

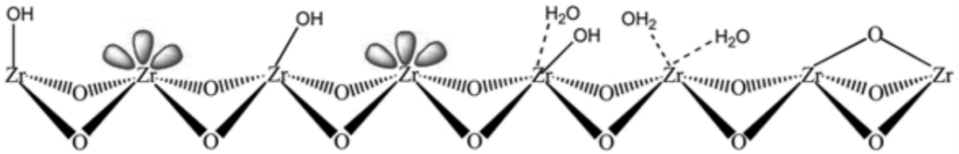


high temperature liquid chromatography (HTLC)

superheated (water liquid) chromatography
(SWC, SWLC)

subcritical water
: **change** of retention thermodynamic between **low** (15 – 55 °C) & **high** temperatures (125 – 200 °C)
:: **change** of distribution constant **K** leads to **change** of capacity factor **k**
::: how to **change** distribution constant **K?** → **increasing temperature**
:: substitution of mild polar MP based on acetonitrile-water mixture by pure water

use of HTLC presumes existence of respective SP with **very stable carrier**
: solution – **zircon**-particles covered by *polybutadiene*, *polystyrene* or *carbon*
:: SP *Discovery*
:: thermal stability up to 250 °C



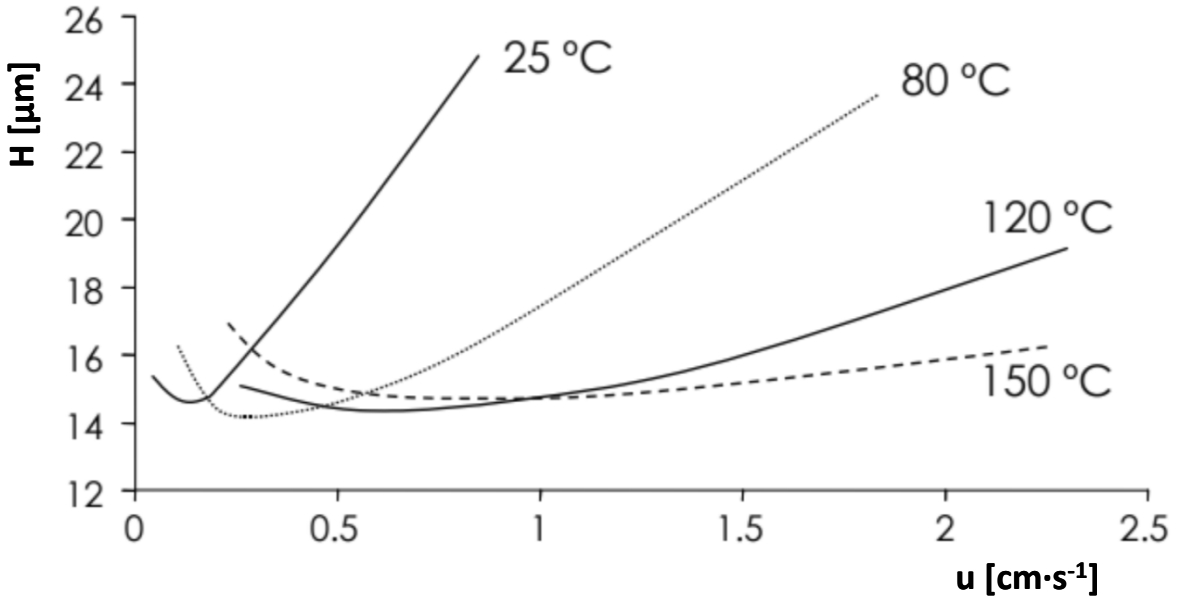
HTLC ideal for on-line **^{1}H-NMR** detection by means of **D_2O**
: so-called green chromatography

elevated temperature ultra-HPLC (ET-UPLC)

2003 – combination of **UPLC** and **HTLC**

decreasing MP viscosity after increase of temperature, high pressure is compensated
: **u** (80 °C) = **2.6 · u** (25 °C), pressure = *const*.
:: **u** – linear flow rate
: SP ø1 µm, pressure 180 MPa, temperature 90 °C
:: **N ~ 420 000 m^{-1}**

UPLC reaches **N ~ 200 000 m^{-1}**
RPLC reaches **N ~ 10 000 – 25 000 m^{-1}**
CZE reaches **N > 1 000 000 m^{-1}**



hydrophilic interaction liquid chromatography (HILIC)

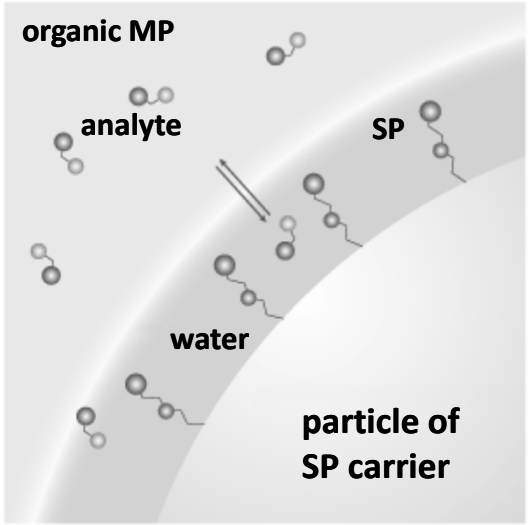
chromatography on **„reversed reversed phase“**
for very polar analytes or substances with many interacting groups

$$\log k = \log k_0 - m \cdot \log \varphi_{pol}$$

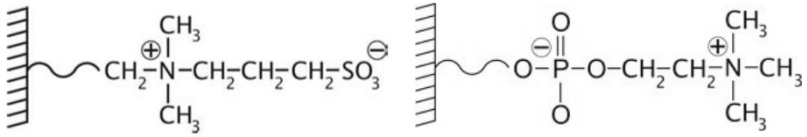
presumed retention mechanism

primary interactions
: distribution between organic and aqueous phases

secondary interactions
: in aqueous phase between aqueous phase and SP
:: hydrophilic (electrostatic)
:: ionic



stationary phases



monolithic and **polymer SP**
: **polyfunctional polymer**
:: polymethyl methacrylate

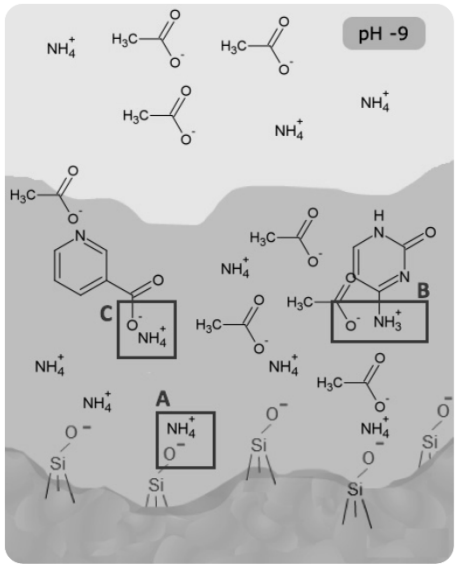
: charged – strong electrostatic interactions (silica, aminopropyls)
: neutral polar – no electrostatic interactions (diols, amides)
: zwitter-ions – weak electrostatic interactions (sulphoalkyl betain, phosphatidylethanolamine)

mobile phase

: organic component – max 97%
:: min 3% water on SP hydratation sublayer

: salt content – ammonium salts
:: for low pH formiate, for high pH hydroxide
:: regulates pH and also ionic strength
:: defines interaction types

ionic interactions in HILIC
A – ion pair with SP
B – ion pair with sample anion
C – ion pair with sample cation



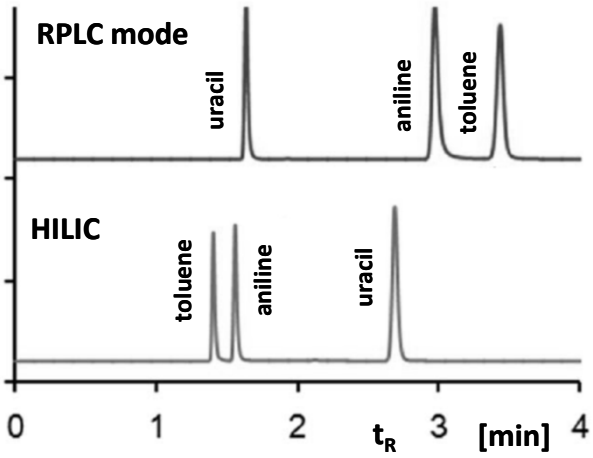
advantages
: orthogonal to RPLC (substitution to NPLC)
: advantageous for connection to MS due to high organic content

disadvantages
: complex and not yet precisely known retention mechanism

RPLC mode *dual separation mode*

: organic component < 50 %
:: on SP hydrophobic sublayer appears

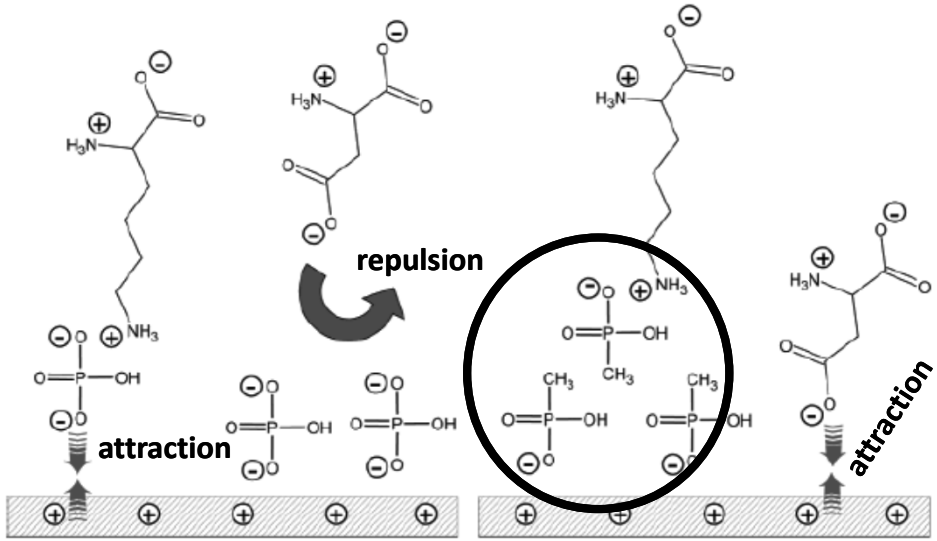
: requires specific SP type
:: mixed-mode stationary phase

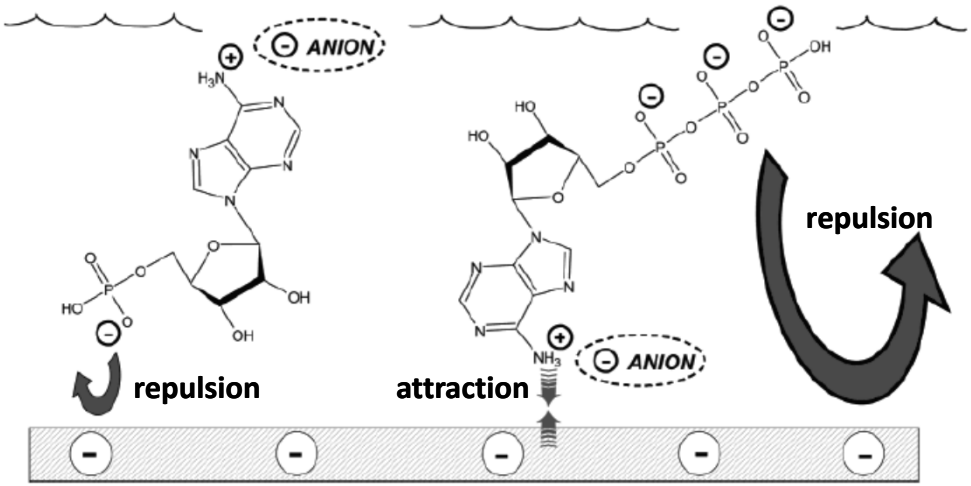


electrostatic repulsion hydrophilic liquid chromatography (ERLIC)

HILIC mode

uses repulsion of the same charges of SP and analyte
: increases chances of other polar groups to influence retention
:: coulombic interactions have higher chemical potential than polar ones
:: organic component content in MP > 70 %
:: increasing influence of salts and molecule spatial orientation

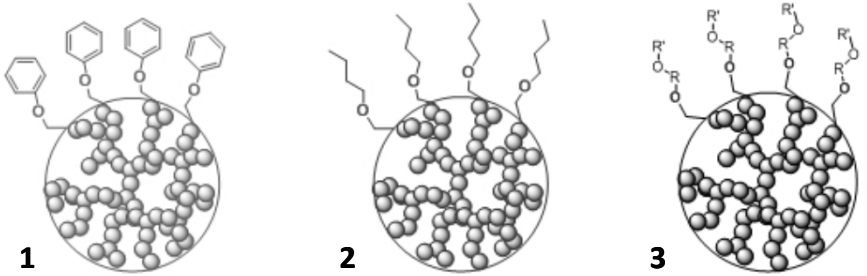




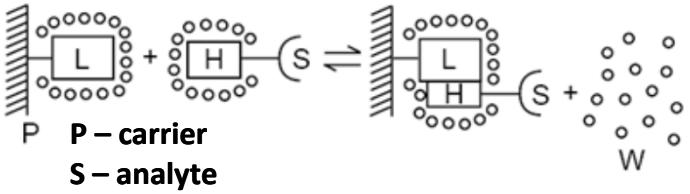
hydrophobic interaction liquid chromatography (HIC)

technique for separation of macromolecules
: ***it is not*** RP-HPLC

uses stimulated interactions of *hydrophobic surface parts* of macromolecule with SP
SP: carrier (agarose, dextran) modified by *small non-polar* group



1 – phenyl (Phe), 2 – octyl (C8), 3 – butyl (C4)



P – carrier
S – analyte
L – SP modification
H – hydrophobic analyte region
W – water molecules

$$\ln k = \ln k_0 + a \cdot c_{sl}$$ c_{sl} – concentration of chaotrope

sample is loaded in solution with ***ammonium sulphate*** (chaotropic agent)
: **w/ increasing** entropy of water **increases** strength of hydrophobic interactions
: stabilisation of proteins

MP serving for elution (KSCN and $KClO_4$) has **kosmotropic effect**
: disrupts interactions, but does not denature sample
: addition of **alcohol** *decreases* surface tension of water and thus strongly desorbs (cleaning)

ion exchange chromatography (IEC)

displacement LC

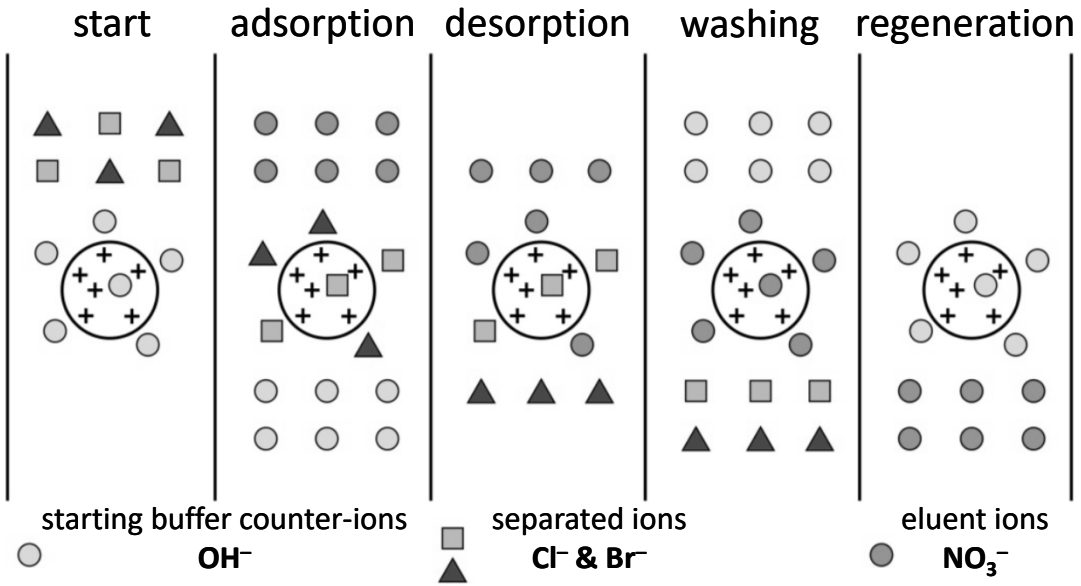
ion exchange – ions retained on solid surface (insoluble) are exchanged for ions contained in around flowing solution by means of contact with carrier

D. T. Day – clays and zeolites have ion exchanging properties

1935 – the first synthetic ionex

1950-1959 – start of intense IEC development

exchanging ion bound strongly than the eluting ion by higher concentration



polymer porous

microporous spheres, diameter *ca* 10 µm

: polystyrene-divinylbenzene

sample: amino acids, peptides and saccharides

stationary phase

dowex, amberlite,
Bio-Rex, chelex...

pellicular bound

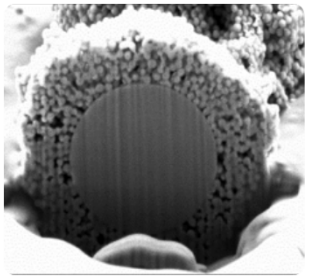
silica, glass, with polymeric coverage; particles must be porous

: hydrophilic polymer coating

slow diffusion *in polymer particle* is substituted w/ **fast diffusion** *in thin layer of polymer*

sample: proteins, nucleic acids and other big charged molecules

SP surface enlargement

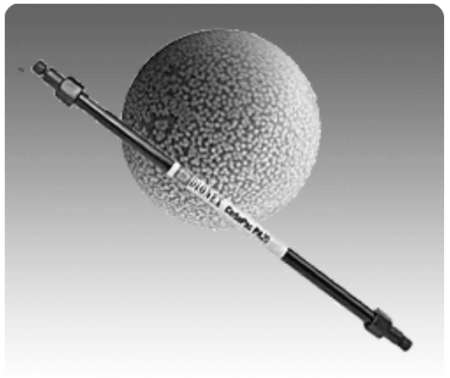


: particle (5 µm) w/ R-SO_3^- anchored particles (0.1 µm) w/ active -SO_3^- & anchoring -R_3N^+

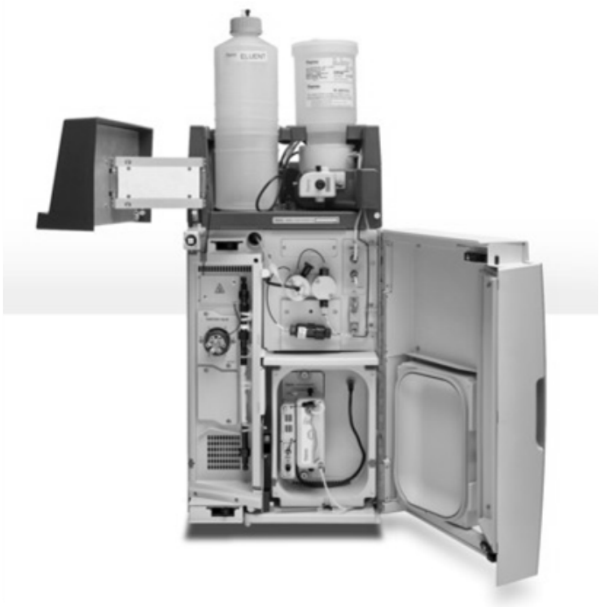
: particle (5 µm) w/ R-SO_3^- rich on caverns *(quasi-monolith)*

: particle (4.5 µm) of highly cross-linked polymer w/ surface layer active groups (*core-shell*)

high-performance ion-exchange chromatography (HPIEC)



: small particles (4 – 6 µm) of cross-linked polymer (PSDVB)
:: CarboPac™ carrier + MicroBead™ membrane with R-NH_3^+ or R-SO_3^-
:: the higher the cross-linking, the smaller analytes
::: very high load capacity
: often combined with *pulse amperometric detector* (PAD)



HPAEC, high-performance anion-exchange chromatography

HPCEC, high-performance cation-exchange chromatography

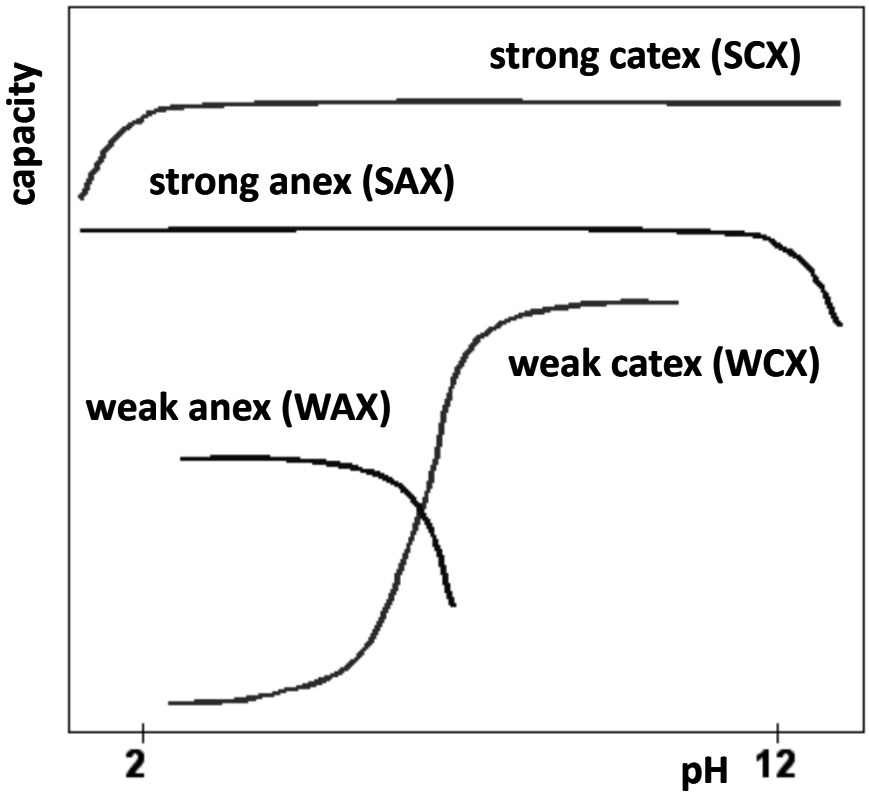
ion-exchanger capacity

quantitative measure of ion-exchanging ability (of counterions)

strong acid	$-SO_3^- H^+$	**strong base**	$-NH(CH_3)_3^+ OH^-$
weak acid	$-COO^- H^+$	**weak base**	secondary or tertiary amines

total capacity

amount (mols) of potentially ionisable groups related to 1 gram of dry ionex



actual capacity

amount of groups eventually exchangeable under given experimental conditions
: the amount is pH dependent

ionic interaction types

ionic complexes with neutral molecules

e.g. reaction of saccharide with borate

$$\text{saccharide} + B(OH)_4^- \rightarrow [\text{saccharide}\cdot\text{borate}]^-$$

complexes with ligands

they change relative retention or fully properties of ions
: cation could be thus separated on anex

$$Fe^{3+} + 4\,Cl^- \rightarrow FeCl_4^-$$

neutralisation reactions

ion-exchangers in acidic or basic forms are base of IEC

$$HA + R^+OH^- \rightarrow R^+A^- + H_2O$$
$$B + R^-H^+ \rightarrow R^- + BH^+$$

ligand exchange

used within catexes conditioned with Ni^{2+} or Cu^{2+}
: separation of amino acids and other bases

$$RM\cdot L + X \rightarrow RM\cdot X + L$$

RM – metal / ion-exchange ion pair
L – ligand, forming complex with metal M
X – analyte-ligand

mobile phase

: solubility (salts, buffers)
: retention by elution force
: separation

typical MP
: aqueous solutions of salts, buffered and modified water-miscible organic solvents
:: methanol, acetonitrile, *etc*.

elution force and selectivity
: concentration of ions in buffer and of other salts
:: **increasing** ionic strength **means increasing** elution force
: pH
: type and concentration of organic solvents

cation exchange

$x\ RSO_3^-H^+\ (SP) + M^{x+}\ (MP) \rightarrow (RSO_3^-)_xM^{x+}\ (SP) + x\ H^+\ (MP)$

anion exchange

$x\ RN(CH_3)_3^+OH^-\ (SP) + A^{x-}\ (MP) \rightarrow (RN(CH_3)_3^+)_xA^{x-}\ (SP) + x\ OH^-\ (MP)$

distribution equilibrium

$$Ca^{2+}\,(MP) + 2H^{+}\,(SP) \rightarrow Ca^{2+}\,(SP) + 2H^{+}\,(MP) \qquad K' = \frac{[Ca^{2+}]^{SP} \cdot [H^{+}]^{MP}}{[Ca^{2+}]^{MP} \cdot [H^{+}]^{SP}}$$

$[Ca^{2+}]^{SP}$ and $[H^{+}]^{MP}$ molar concentration in SP
: concentration has values 0 – max, when all binding sites are occupied with one compound

retention controlled by ***pH*** of MP

retention on anex increases with increase of pH (pH : 1 → 14), ***conversely on catex***

retention decreases with increasing concentration of ***organic solvent***
: effect is higher for less polar solvents

change in selectivity is achievable by adding different solvents
: methanol, ethanol, acetonitrile and dioxane

other factors influencing retention

small changes of temperature easily influence the selectivity
: useful if no other method leads to results

temperature 15 – 60 °C
: change of MP viscosity with higher temperature ⇒ better separation
: separation at 50 – 60 °C are advantageous (if column allows)
: biochemical separations (enzymes) at 4 °C

elution eluting ion in surplus in both phases

elution of Ca^{2+} by H^+ ions $[Ca^{2+}]_{SP} << [H^+]_{SP}$ $[Ca^{2+}]_{MP} << [H^+]_{MP}$ concentration of H^+ is constant in both phases

SP affinity to Ca^{2+} in regard to H^+
: generally the higher K, the higher affinity

$$\mathbf{K'} = \frac{[\mathbf{Ca^{2+}}]_{\mathbf{SF}} \cdot [\mathbf{H^+}]_{\mathbf{MF}}}{[\mathbf{Ca^{2+}}]_{\mathbf{MF}} \cdot [\mathbf{H^+}]_{\mathbf{SF}}} \approx \frac{[\mathbf{Ca^{2+}}]_{\mathbf{SF}}}{[\mathbf{Ca^{2+}}]_{\mathbf{MF}}} \qquad \frac{[\mathbf{H^+}]^{\mathbf{2}}_{\mathbf{SP}}}{[\mathbf{H^+}]^{\mathbf{2}}_{\mathbf{MP}}} = \boldsymbol{const.}$$

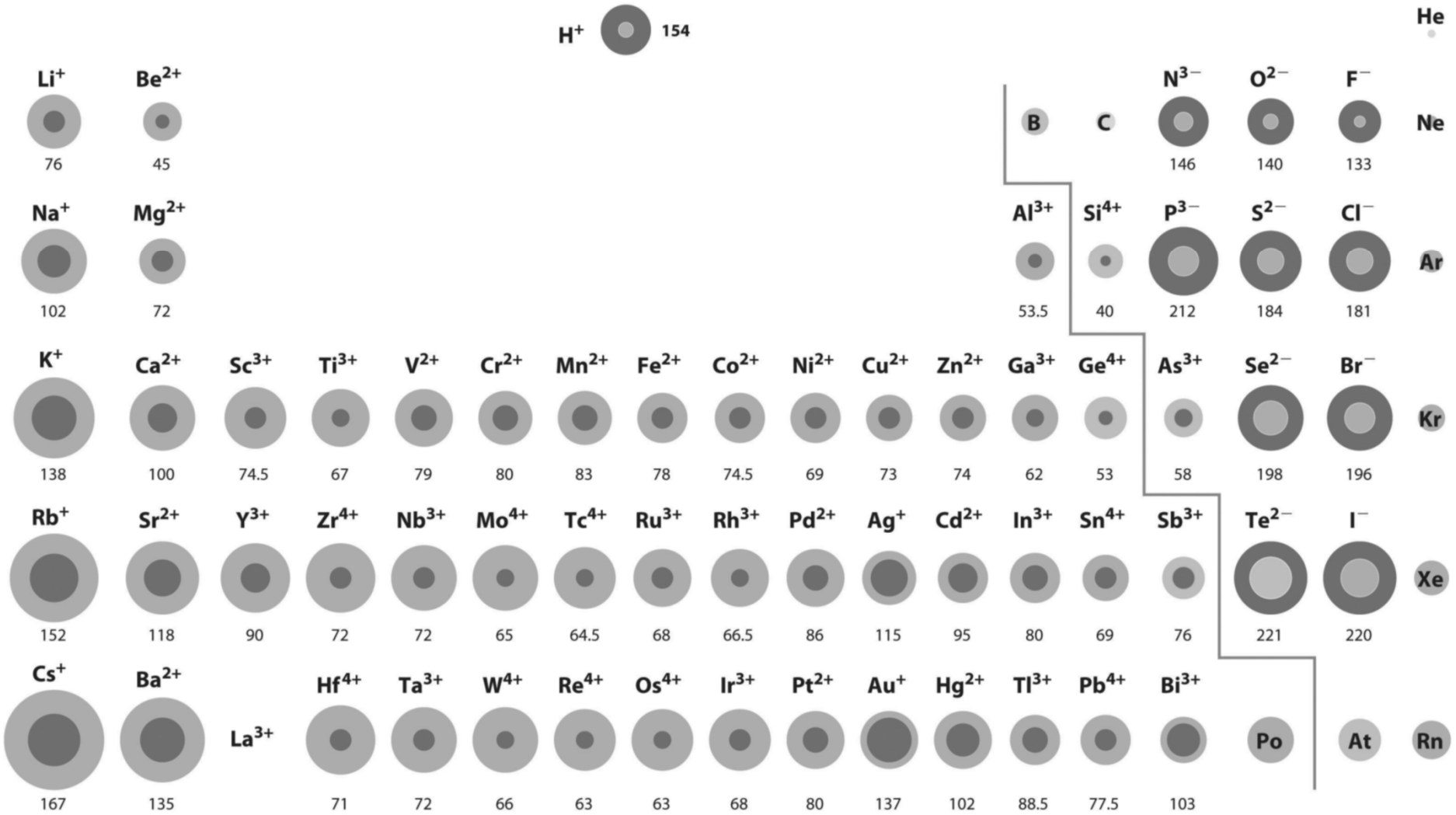
ion affinity to SP *Hofmeister ion series*

bigger ions have higher affinity than smaller, polyvalent ions have higher affinity than monovalent

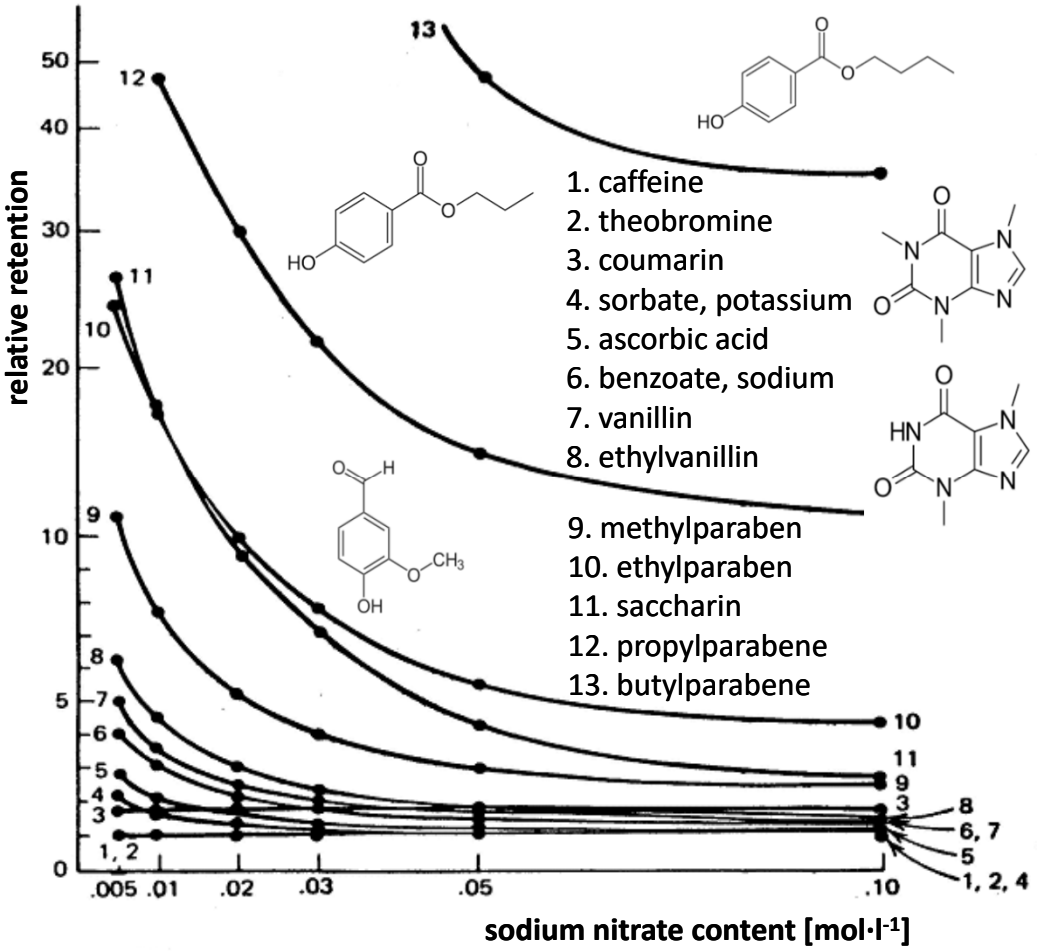
for a ***typical catex***, **K** decreases with ion diameter in order
: monovalent
$Ag^+ > Cs^+ > Rb^+ > K^+ > NH_4^+ > Na^+ > H^+ > Li^+$
: divalent
$Ba^{2+} > Pb^{2+} > Sr^{2+} > Ca^{2+} > Ni^{2+} > Cd^{2+} > Cu^{2+} > Co^{2+} > Zn^{2+} > Mg^{2+} > UO_2^{2+}$

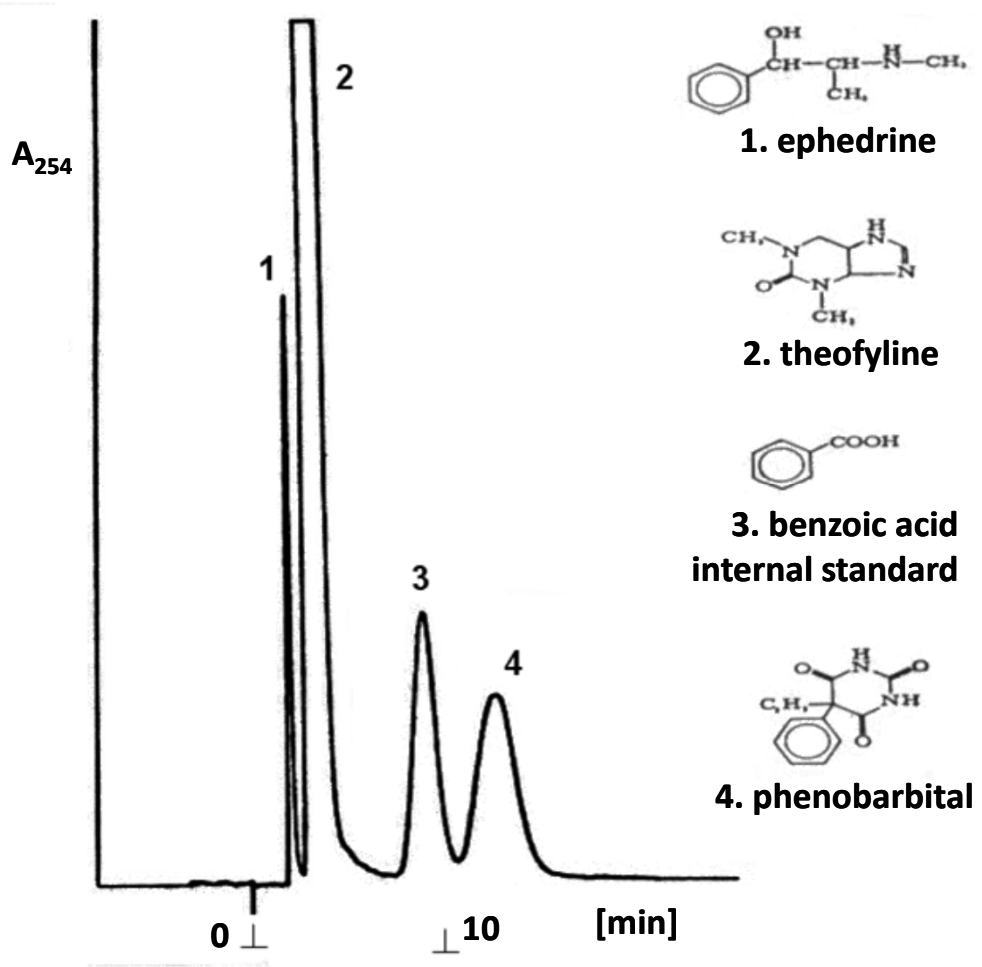
for ***anex*** in this order
citrate > SO_4^{2-} > oxalate > I^- > NO_3^- > CrO_4^{2-} > Br^- > SCN^- > Cl^- > formate > acetate > OH^- > F^-

atomic and ionic radii



influence of ionic strength on separation





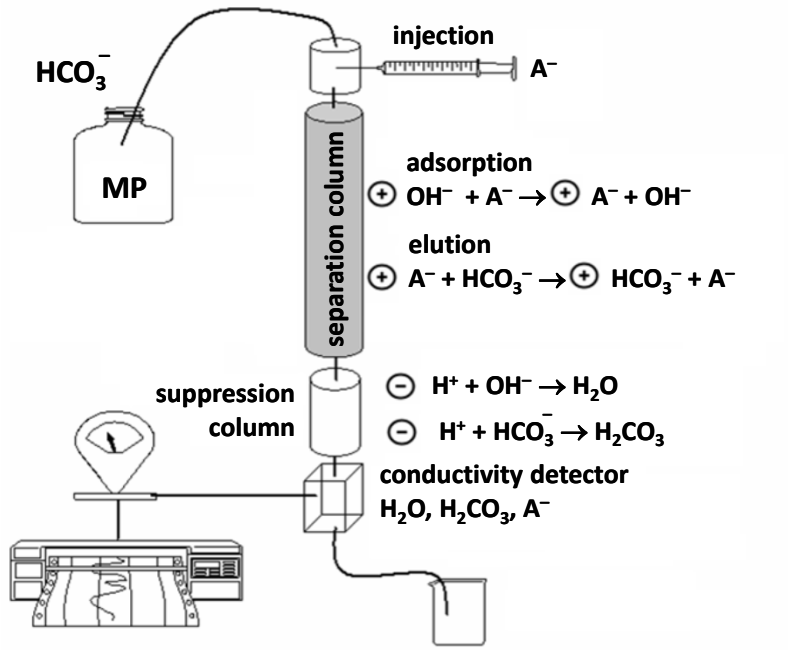
Zipax-SAX w/ 1% TBAOH, 0.01 M borate, pH 9.2, 24 °C

Zipax-SAX anex, 0.01 M $NaNO_3$, pH 5.7, 37 °C

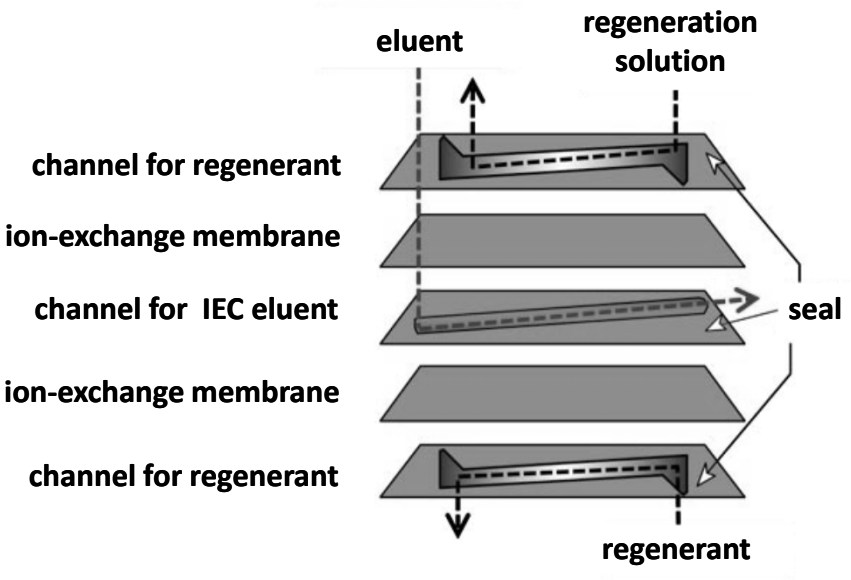
IEC conductivity suppression

two ion-exchange columns and conductivity detector
: MP conductivity suppression

suppression column (Dionex)



suppression micromembranes



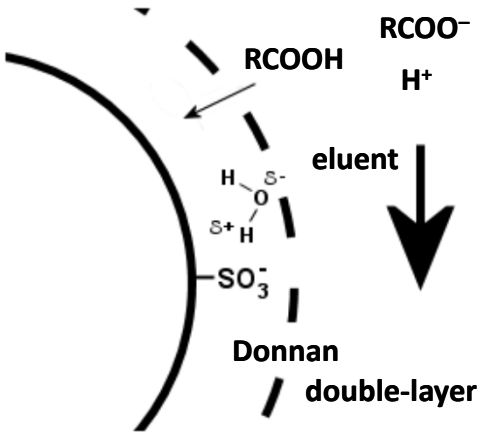
ion exclusion chromatography (IXC)

analyte **separation** based on **difference** in **distribution ratio** between two *liquid phases*
: MP and liquid immobilised on SP
: similar to *size exclusion chromatography* (SEC)
:: Donnan exclusion
: separation of classes of *uncharged species* (ions are excluded)
:: weak acids and bases, hydrophilic substances (saccharides, alcohols)

: **MP** – according to analyte
:: water or strong acid (heptafluorobutyric acid); for weak acids
:: addition of organic solvent hastens separation (up to 40% AcN)

: **SP** – **WCX** are used, eventually WAX
:: Donnan equilibrium

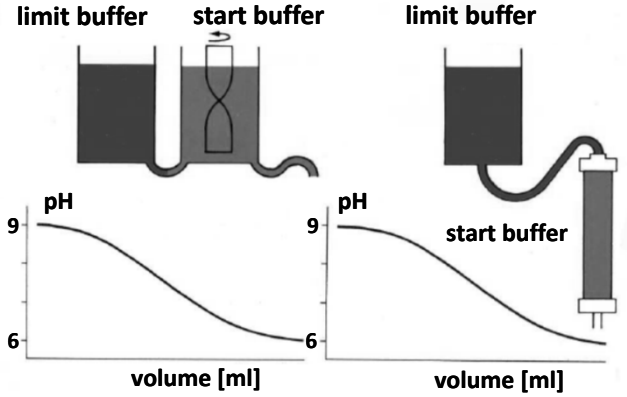
the lower polarity, ***the higher retention***
: homologous lines are separated with increasing acidity
: diacids have lower retention
: double bond or aromatic ring are causing higher retention

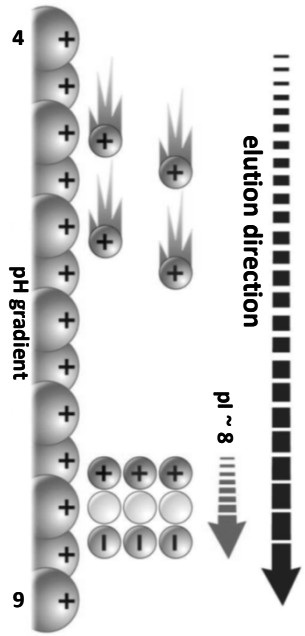


chromatofocusing (CF)

variant of IEC – **1978 Sluyterman**

separation of analyte based on **difference** in **isoelectric point** value (pI)
: it uses buffer abilities of charged ionex groups
:: column with anex is equilibrated by buffer of higher value than the highest pI
:: onto column, sample is inserted, with the starting buffer
::: compounds at pH above pI are negative and chatcheed on the top of column
::: compounds at pH below pI turn positive, migrate & bind in zone pH > pI
::: compounds with the highest pI are eluted first
::: separation at Δ pI = 0.05 resolution





mobile phases
: ampholytes
:: mixture of oligopeptides w/ different but close pK_a
::: titration curve is almost linear – pH gradient
:::: large number of small step in range of these pK_a values
: Pharmalyte 8 – 10.5 / Polybuffer 96 / Polybuffer 74

stationary phases
: PBE 96 (*Polybuffer exchange 96*) based on Sepharose

affinity chromatography (AC)

1987 – P. Cuatrecasas, M. Wilchek, Wolf prize for discovery of AC

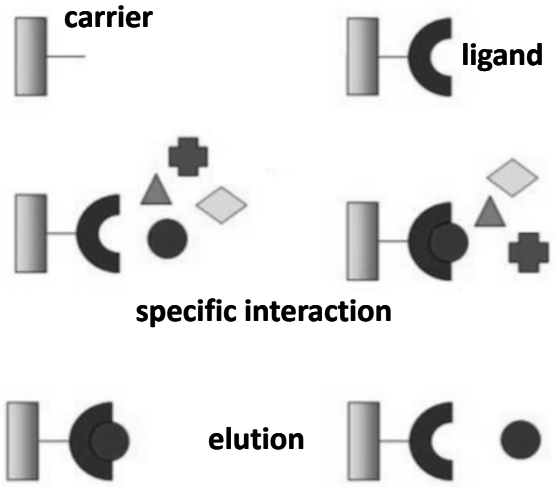
specific interaction of immobilised ligand with analyte

very strong and specific interaction – **multiple interaction**

quasi-displacement chromatography

involved are **weak interactions**
: Coulombic
: disperse
: van der Waals

in praxis **natural biogenic systems** are suitable



nucleic acid ↔ nucleic acid
: complementary chain
nucleic acid ↔ protein
: histone, polymerase, binding protein

protein ↔ protein
: antibody, antigen
protein ↔ peptide
: tag
protein ↔ low mass compound
: substrate, inhibitor, coenzyme, hormone, synthetic analogues

$K_D = 10^{-4} – 10^{-8}$ M

solid carrier

: no interaction with analyte
: high amount of reactive groups
: mechanical stability
: porous
: homogenous

stationary phase

low-pressure LC

: cellulose (not homogenous)
: polystyrene (not homogenous, strong hydrophobic, less pores)
: PVA (volume change on rehydration and ionic strength)
: dextran (less pores)
: agarose (melts, sensitive to chemical influence /Gua, urea/)

high-pressure LC, FPLC

: spheron – methacrylate (hydrophobic)
: silasorb – SiO (sensitive to above pH 8)

spacer

: sufficiently long
:: steric hindrances, interactions with spacer, spacer aggregation
: HMDA or more methylene bridges or hydrophilic spacers (polyGly *etc*.)

ligand

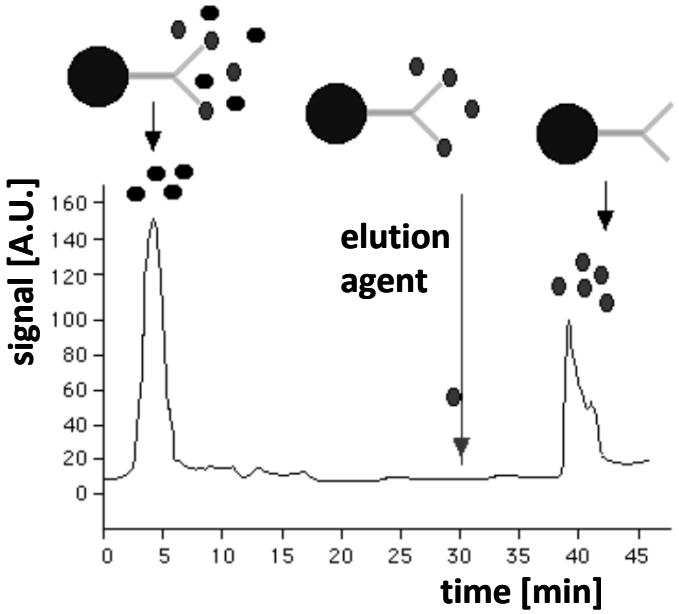
: covalently bound to carrier

AC separation procedure

conditioning
: regeneration, cleaning, equilibration

sample loading
: sorption

elution
: elution force
:: disruption of weak bond



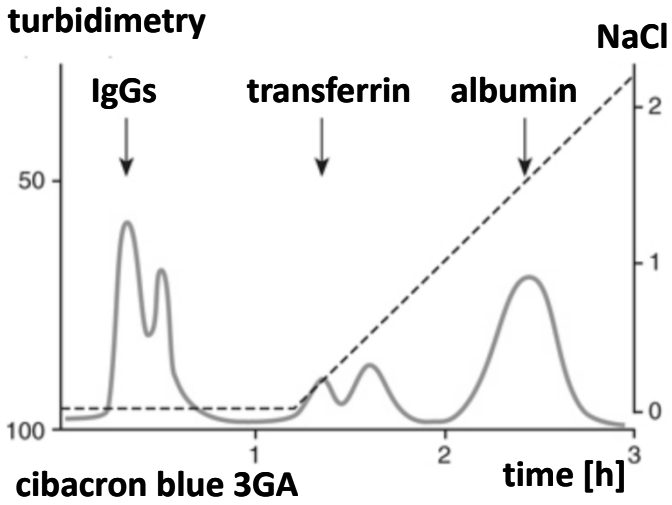
change of analyte conformation

: Δt, ΔpH, ΔI, Δε
:: *solutions of salts, acids, bases, organic solvent*

: specific agents
:: allosteric effects

displacement of analyte

: low mass (free) molecular ligand



isolation of analyte

selective separation
: very complex mixtures without many clean-up steps

use of AC

analysis of ligand binding to analyte

dissociation constants of complex analyte – anchored ligand (K')

: **K'** value must be in a range $1x10^{-6}$ – $5x10^{-3}$ mol·l^{-1}
:: bond to immobilised ligand is generally weaker than to free ligand

$$\mathbf{1/(V_R - V_0) = K'/(V_0 - V_M) \cdot c_L}$$

: V_M – void column volume, V_R – elution volume of studied analyte
: V_0 – el. volume of unretained analogue of analyte (M_r)
: c_L – concentration of bound ligand

dissociation constant determination of complex biopolymer – free ligand (K)

principle – **competitive elution**
: free and anchored ligands *competitively* bind analyte

: c_L' – concentration of competitive ligand

$$\mathbf{1/(V_R - V_0) = K'/(V_0 - V_M) \cdot c_L' + [K' \cdot c_L]/[(V_0 - V_M) \cdot c_L' \cdot K]}$$

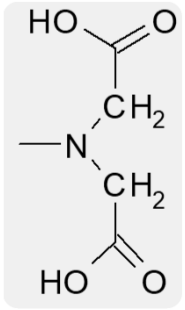
real systems – more binding sites for ligand & different binding sites w/ different affinity

immobilised metal ion affinity chromatography (IMAC)

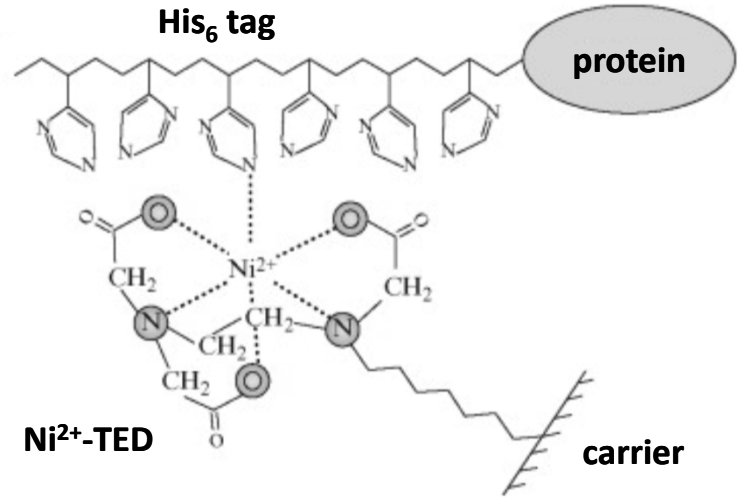
carrier + chelating ligand + **metal ion**

chelating ligands

IDA – iminodiacetae
TACN – 1,4,7-triazocyclonan
NTA – nitrilotriacetate
TREN – *tris*-(2-aminoethyl)-amine
TED – *tris*-(carboxymethyl)-ethylenediamine



IDA



metal ions

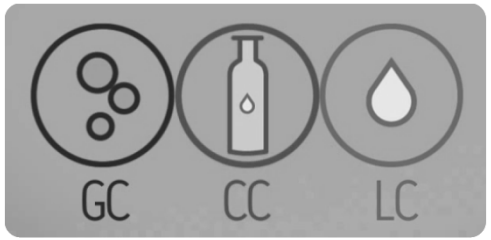
: Ag^{+}, Al^{3+}, Ca^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Eu^{3+}, Fe^{3+}, Hg^{2+}, La^{3+}, Mn^{2+} Nd^{3+}, Ni^{2+}, Yb^{3+}, Zn^{2+}, Ga^{3+} ...

bond chelating ligand – metal ion

enough strong (not to be washed out during separation)
: but the stronger the bond is, the weaker the interaction with macromolecule

supercritical fluid chromatography (SFC)

ultraperformance convergence chromatography™ **(UPC²)**



***SFC** vs*. **HPLC**
: fast separation
: no use of organic solvents
: high number of H, great ratio S / N, high resolution

***SFC** vs*. **GC**
: higher resolution
: analysis of thermolabiles (lower temperatures)

1958 – Lovelock, method proposal
1962 – Klesper, Corwin and Turner, procedure
1980 – first commercial apparatus
2011 – Waters introduced UPSFC/UPC²
:: connectible with MS

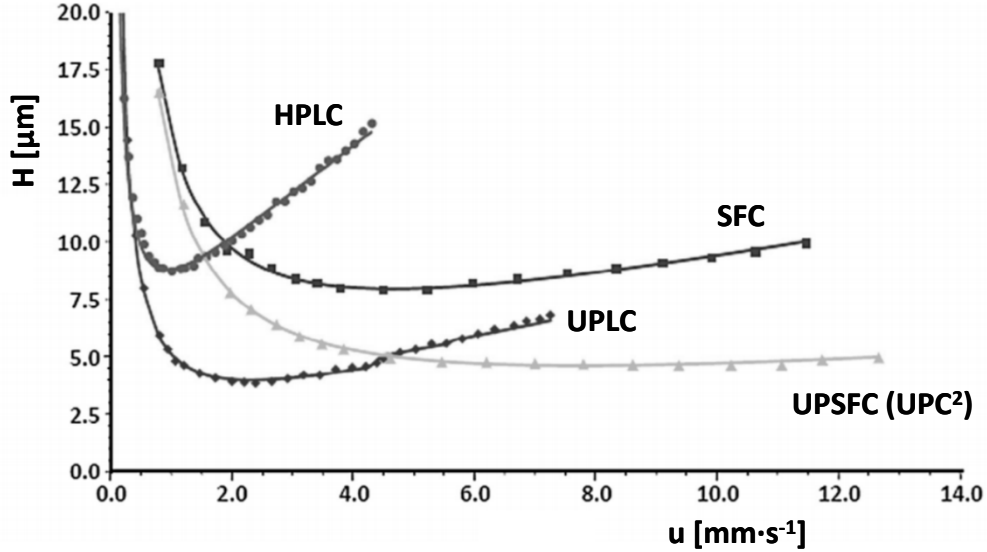
increase of pressure ⇒ **increase of** density ⇒ **increase of** solvation force
: **pressure gradient** in SFC ~ gradient in HPLC and temperature gradient of GC

increase of solubility (Δ) ⇒ **increase of** density ⇒ **decrease of** retention

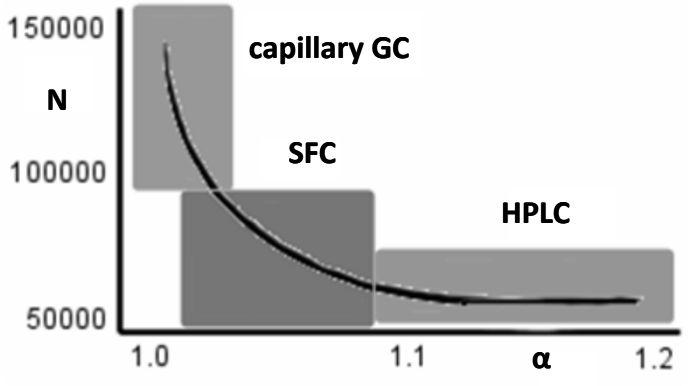
$$\Delta = 1.25 \cdot \sqrt{p_k \cdot (\rho_{SF}/\rho_{liq})}$$

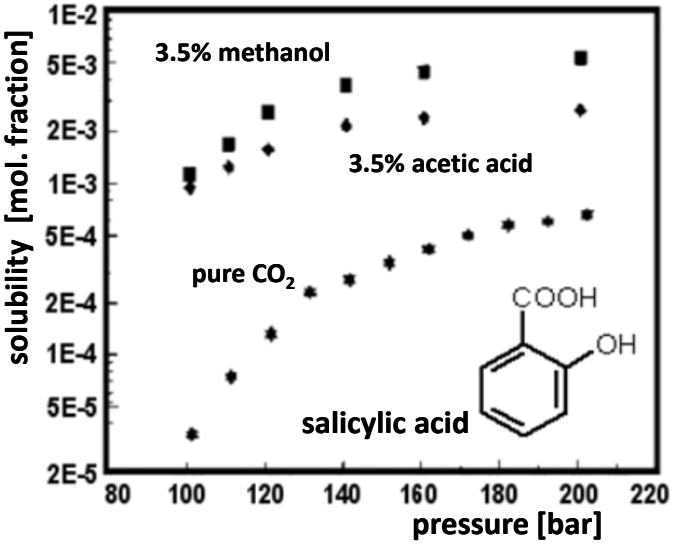
supercritical CO_2 ~ heptane
: SFC ~ NPLC
: additives for moderating polarity
:: MeOH, HAc

van Deemter curves comparison



comparison of SFC with HPLC & GC

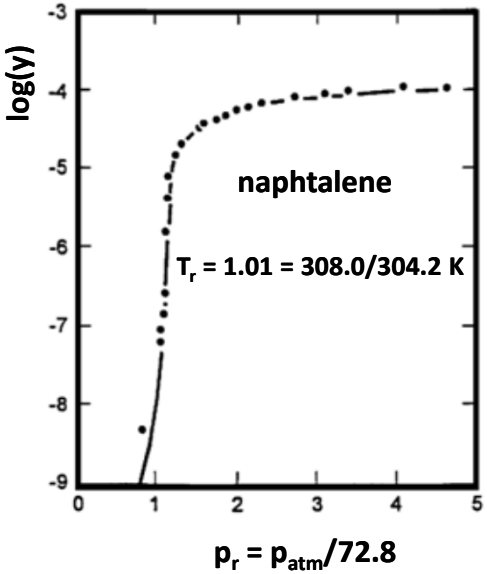




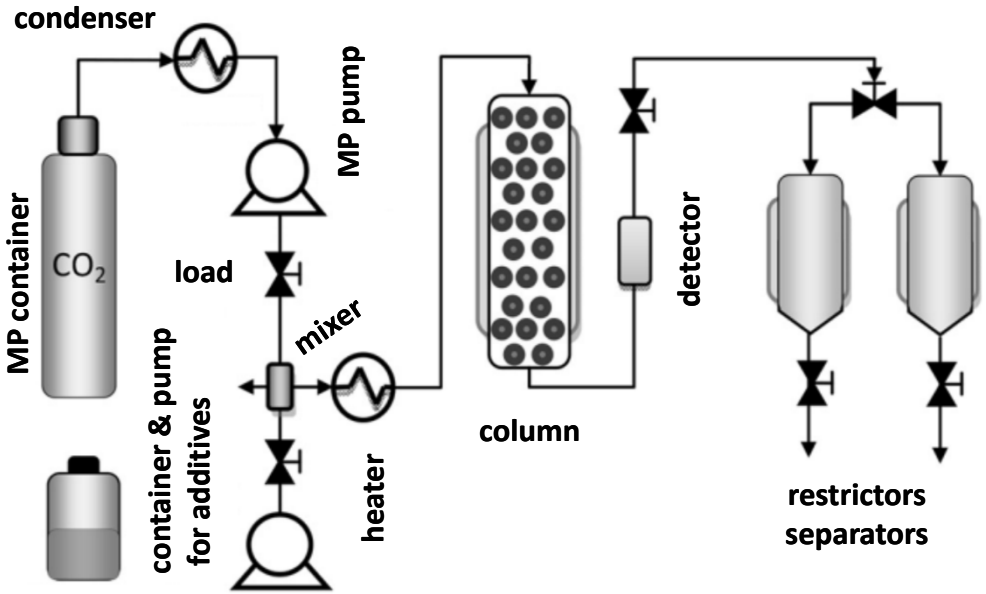
solubility in CO_2

: solubility of additives

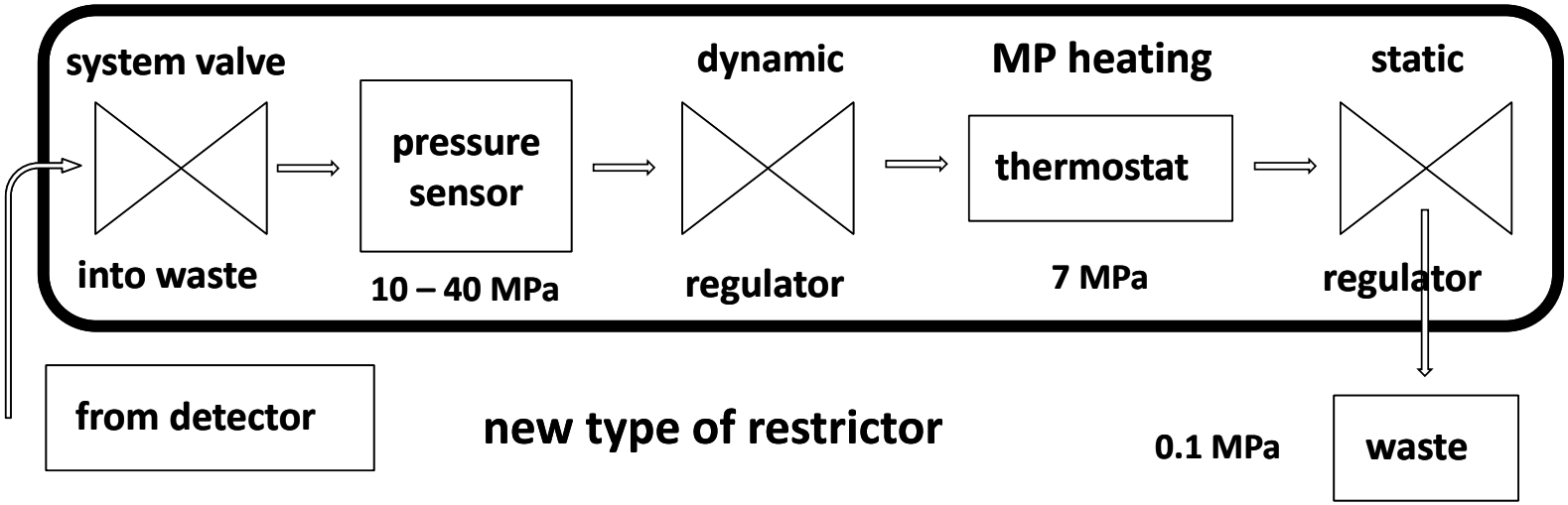
: influence of pressure



SFC procedure

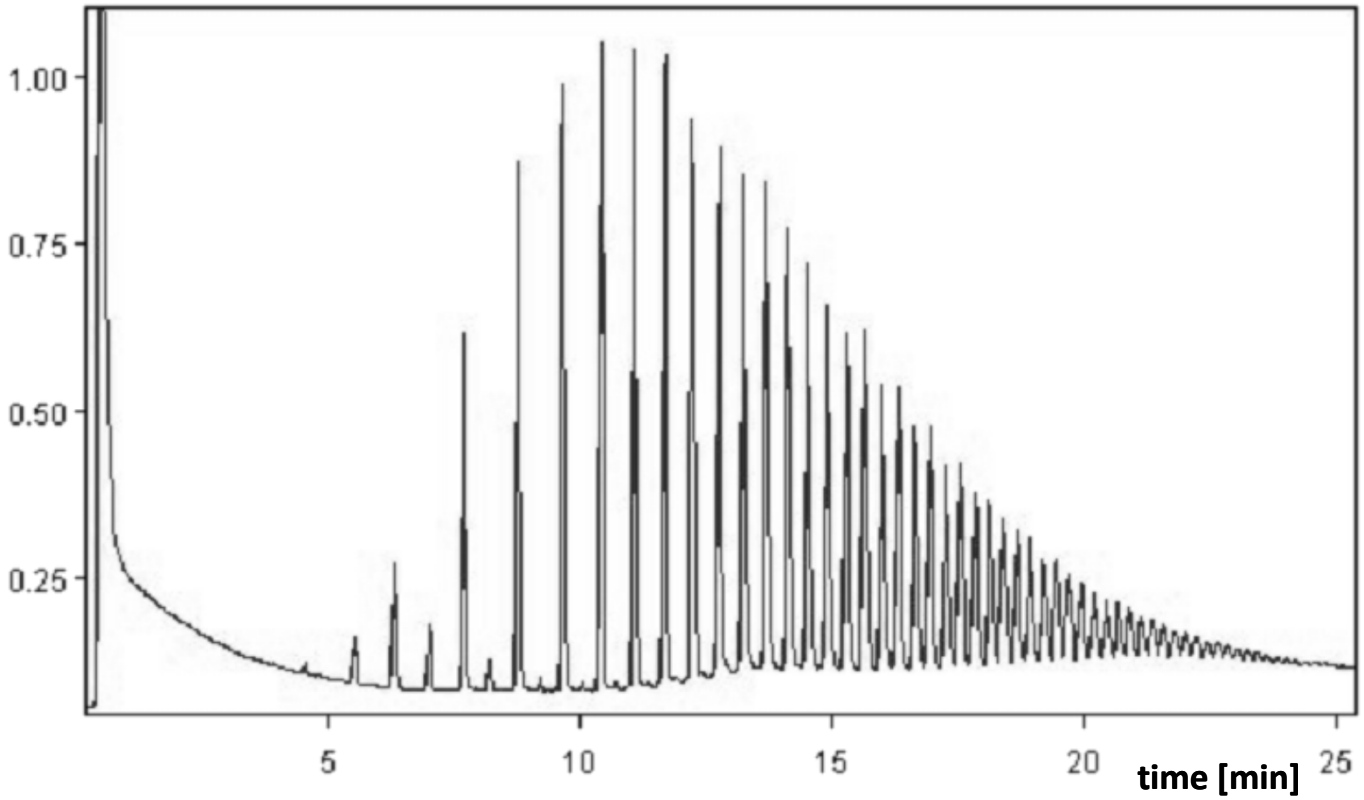


detectors used for **LC** and **GC**



application of SFC

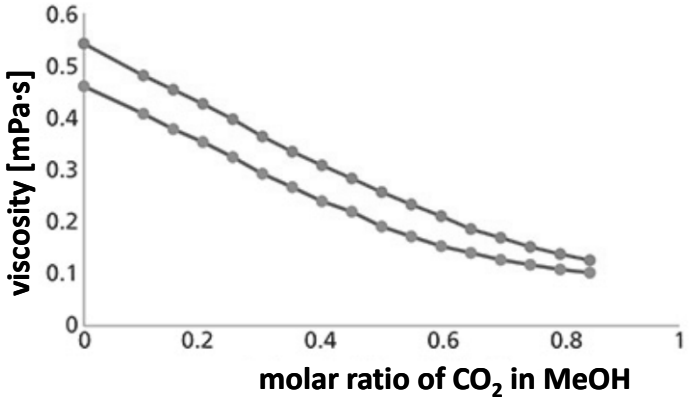
silicon oil (Lukoil) in CH_2Cl_2, injection 60 nl, linear pressure change 8 – 36 MPa (30 min)
detector: FID, 350 °C. restrictor *Integral*, column diameter 320 mm, L=145 mm, SP 5 µm C18



enhanced fluidity liquid chromatography (EFLC)

exploitation of low viscosity mobile phases
: mixing liquid and supercritical fluid
  :: no phase separation should happen (L & G)
: transitional mode between LC & SFC (UPC^2)
: applicable to RPLC, NPLC, HILIC and even SEC

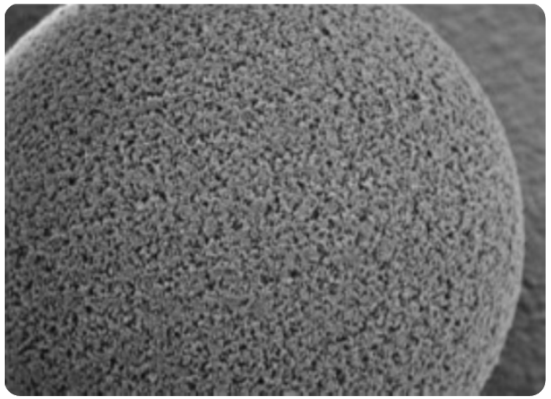
subcritical fluid chromatography
: intermediate between SFC and EFLC
: keeping supercritical pressure, but subcritical temperature
  :: even closer to liquid than supercritical liquid (density, diffusion coefficient)
  :: no phase separation easily happens



within RP-EFLC at molar fraction of CO_2 0.3
: decreasing analysis time
  :: ~ 2x **decrease**
: increasing separation efficiency
  :: ~ 2.5x **decrease** of viscosity **causes** 2x **increase** in separation efficiency

perfusion chromatography (PLC)

newer method (since beginning of 90'ies)



SP particles have large **lateral pores** (POROS media)

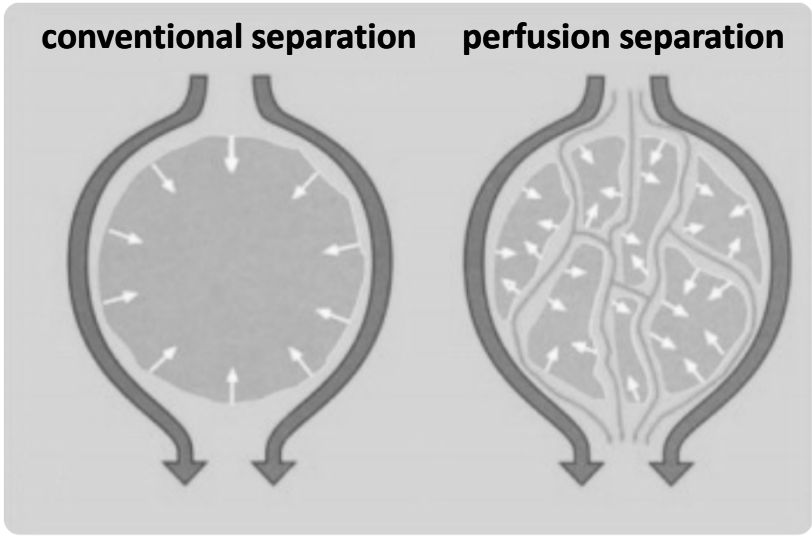
analyte carried by **convective flow** of MP and enters **the particle interior**

lateral pores are mutually connected by **short diffusion pores**
: cross-linked structure with large space inside for interaction of **A** and carrier

SP material

poly(styrene-divinylbenzene) polymer

particle size	10, 20, 50 µm
lateral pores	0.6 – 0.8 µm
diffusion pores	0.05 – 0.15 µm



principle

MP flow creates across the particle a **pressure gradient**
& causes convective flow through lateral pores (*perfusion*)
: molecules of analyte **in contact** not only **with particle surface**, but also **with binding sites interior**
: convective transport is much **higher** than **diffusion transport** controlled by concentration gradient

effect of perfusion happens only **at certain flow-rates** >1000 $cm.h^{-1}$

modern HPLC and FPLC – small analytical columns POROS
: 4.6 x 100 mm, volume 1.7 mL, flow-rate 10 $mL \cdot min^{-1}$

systems for **perfusion chromatography** generate pressures up to 20 MPa and high flow-rates

PLC *vs*. **LC**
: high capacity independent on flow-rate
: high resolution independent on flow-rate
: fast separation
:: 10 – 100x LC, in order of minutes

IX. multidimensional chromatography (mD-LC)

enhancing quality of separation by serial connection of different LC modes
: increasing the peak capacity (***i*** = number of dimension)

: separation in space
:: slab techniques (e.g. TLC)

: separation in time
:: column techniques

: **heart-cutting**
:: in following dimensions, separation of certain fractions; LC-CL $\mathbf{n_{2D} = n_{1.D} + m \cdot n_{2.D}}$

: **comprehensive**
:: in following dimensions, separation of the whole eluate; LC×LC $\mathbf{n_{mD} = \prod n_{i.D}}$

connection problems

: incompatibility of solvents between modes
:: *miscible solvents*

: zone broadening between columns because of valves, loops and detector
:: *second separation dimension must be focusing*

: need for much faster separation in 2nd dimension than in the 1st
:: *technical solution of eluate transfer from one dimension to other*

A – miscible solvents
B – immiscible solvents

interphase between dimensions

continuous connection for A

1D: long micro-column with low flow rates
2D: short or monolithic column with high flow rates
interface: 10-way valve; system of two loops transports eluate from 1D to 2D

continuous connection for A

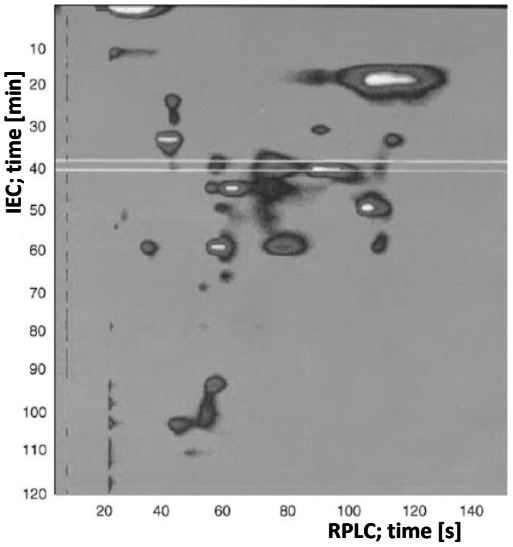
1D: long micro-column with low flow rates
2D: *several* short or monolithic columns connected in-parallel
interface: 6-way valve; loopless transport of a fraction from 1D to free 2D

discontinuous connection for A

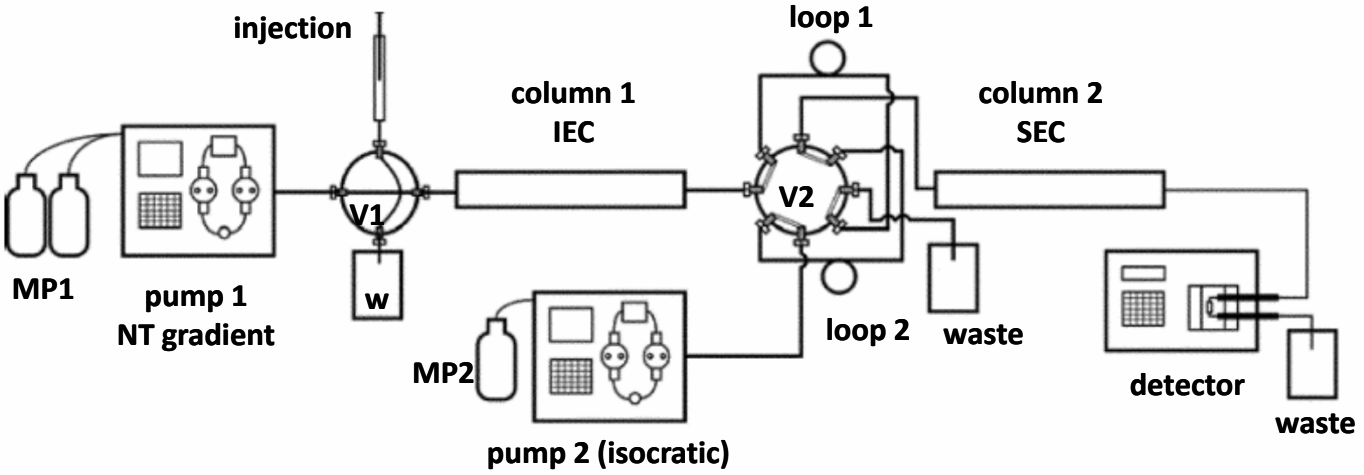
1D: long micro-column with low flow rates
2D: short or monolithic column with high flow rates
interface: 6-way valve; through loop a fraction transported from 1D to free 2D

discontinuous connection (A + B)

1D: any column
2D: any column
interphase – 6-way valve
: through *capture column* (RAM SP type) fractions are moved from 1D to 2D
: through *fraction collector* fractions are moved from 1D to 2D
: through *evaporation loop* fractions are moved from 1D to 2D
:: MP1 removed from sample and new solubilisation in MP2



record from 2D LC (IEC-RP)



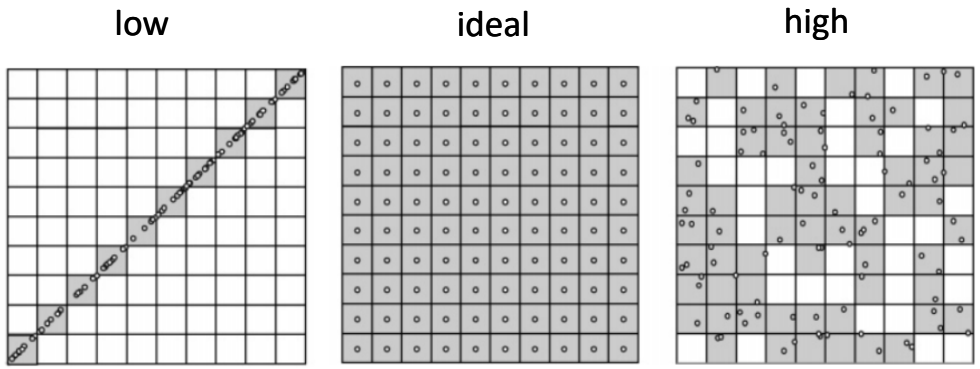
combined dimensions

1D : HILIC**; 2D :** RP
1D : IEC**; 2D :** SEC, RP
1D : SEC**; 2D :** IEC, RP
1D : AC**; 2D :** RP

dimension complementarity (*orthogonality, **O***)

to choose modes of both following dimensions so, that the separation selectivity would be maximal

complementarity (othogonality)measure

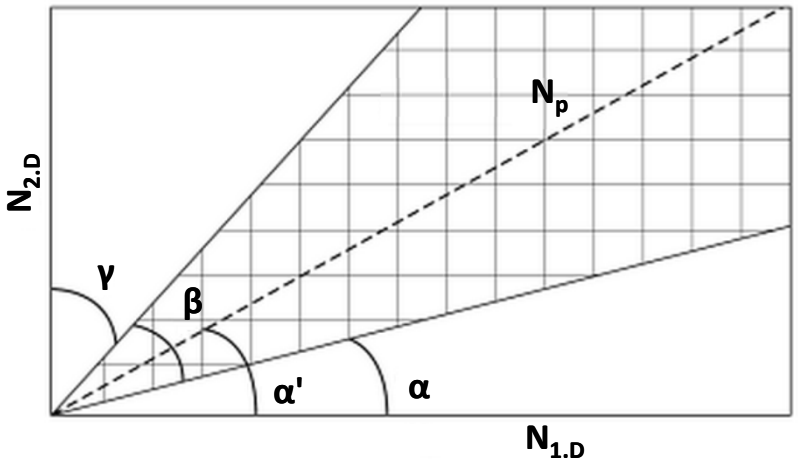


$$\mathbf{O} = \frac{\sum \mathbf{bin} - \sqrt{\mathbf{n_{max}}}}{\mathbf{0.63 \cdot n_{max}}}$$

bin – a position with value in 2D histogram

$\mathbf{n_{max}}$ – max peak capacity

: sum of all bins in histogram

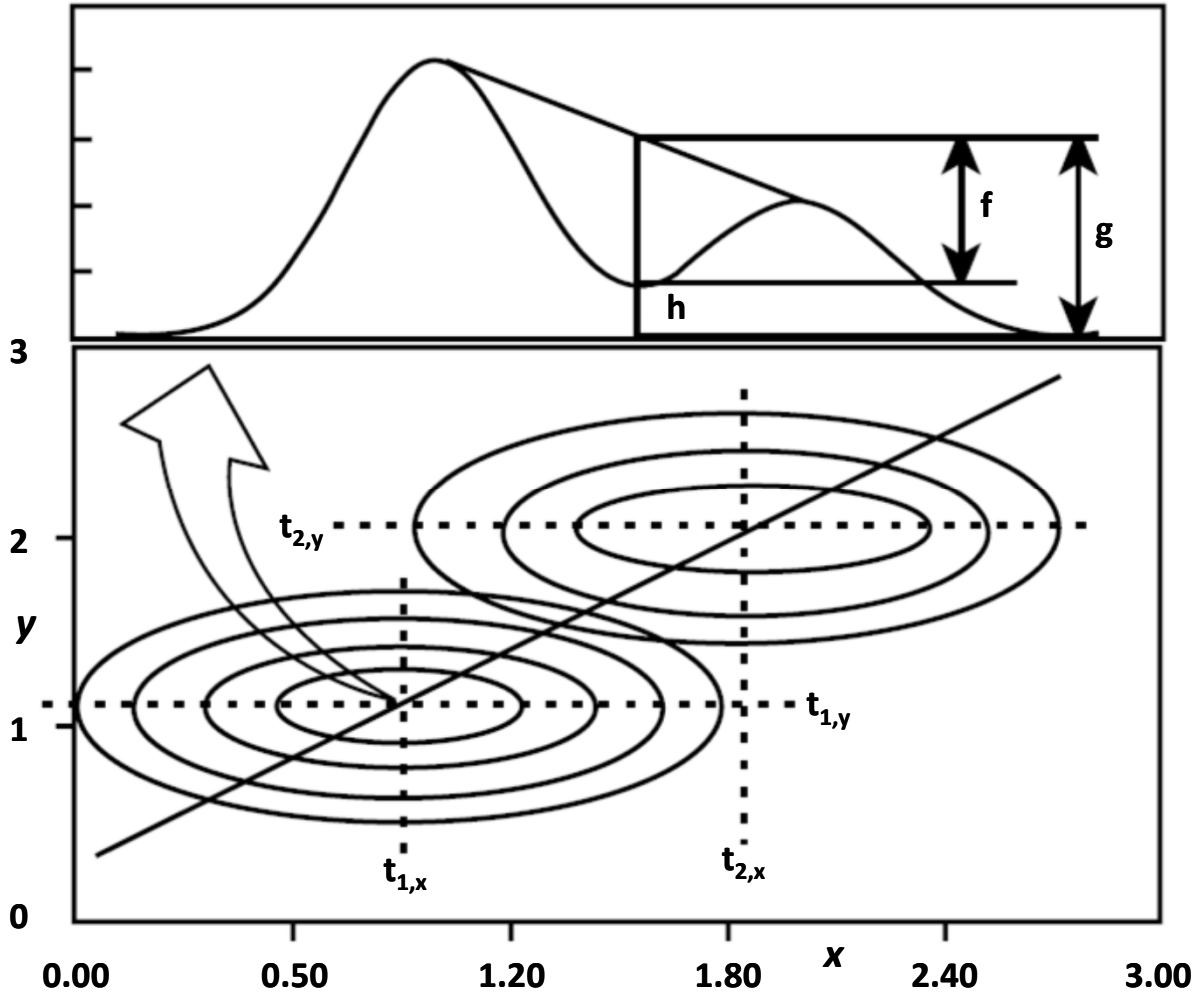


$$\mathbf{N_p = N_{teor} - \left[N_{2.D}^2 \cdot \tan(\alpha) + N_{1.D}^2 \cdot \tan(\gamma)\right]}$$

$\mathbf{N_p}$ – effective area; practical peak capacity

β – so-called spreading angle

resolution in 2D LC



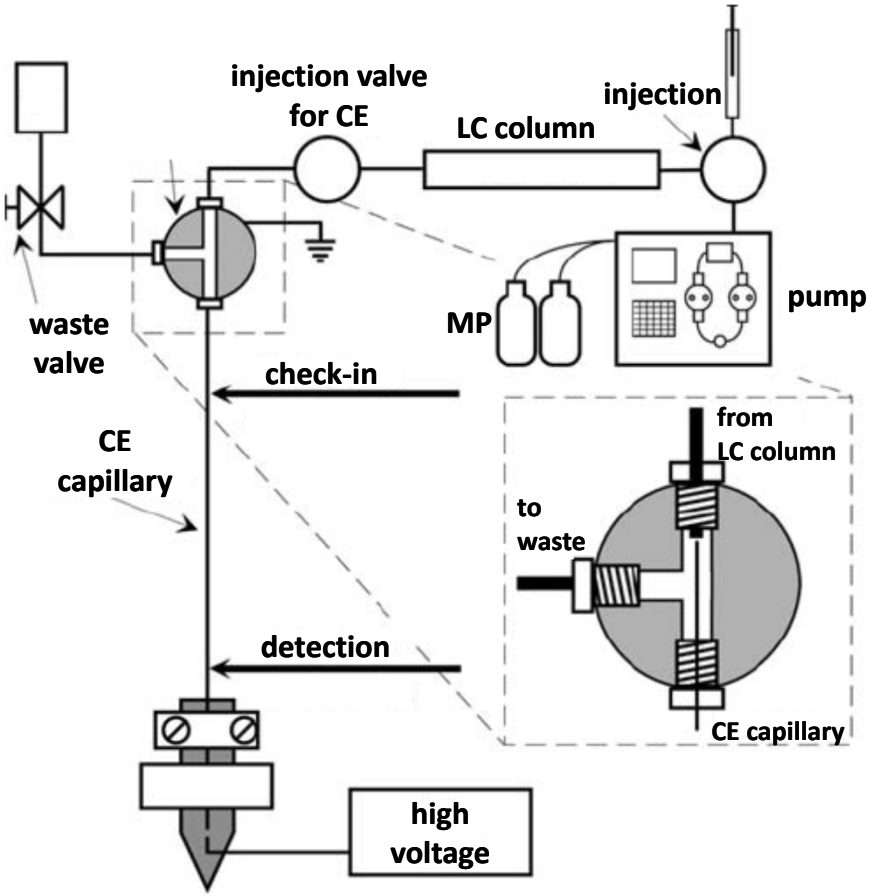
$$R_{(A,B)} = \sqrt{-0.5 \cdot \ln\left(\left(1 - (f/g)\right)/2\right)}$$

combined / hyphenated techniques

enhancing quality of separation by connecting different separation techniques

hyphenated techniques

1D : LC**; 2D :** CE
1D : CE**; 2D :** LC
1D : LC**; 2D :** MS



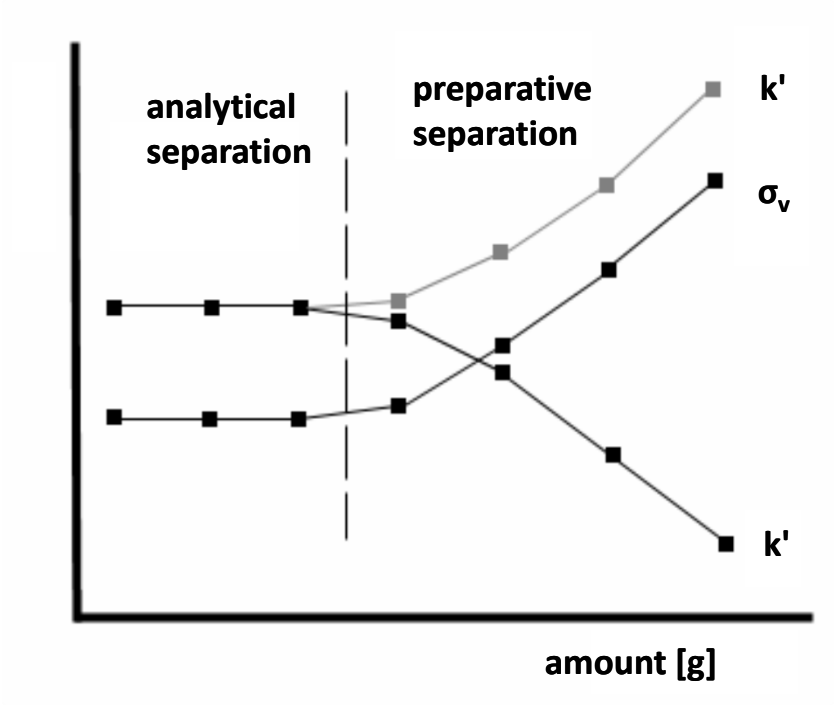
limited compatibility of principles

: requires *special interphases*

preparative chromatography

isolation and **purification** by means of LC

in extent of µg up-to **kg** – purification of enzymes up-to industrial scale



non-linear part of adsorption isotherm

according to substance type

: increasing load [g] leads to **decrease** of k'
:: asymmetric peaks, strong tailing
::: **concentration overloading**

: increasing load [g] leads to **increase** of k'
:: asymmetric peaks, strong fronting
::: **volume overloading**

separation optimisation: yield, purity, speed

enlargement of system dimensions (*scale-up*)

positives: still symmetric peaks
negatives: size of column and solvent consumption

methods of preparative LC

optimisation: flow rate; amount of sample

separation parameters conversion

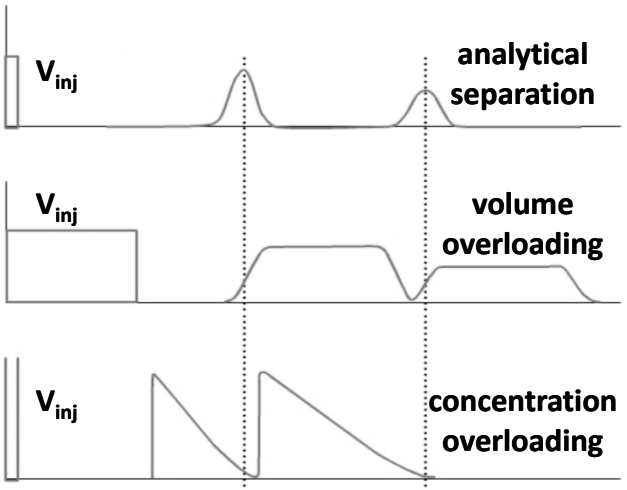
$$\frac{F_{M_1}}{F_{M_2}} = \frac{r_1}{r_2}$$

F_M – voluminal flow rate
r – column diameter

$$\frac{x_1}{\pi \cdot r_1^2} = \frac{x_2}{\pi \cdot r_2^2} \cdot \frac{L_2}{L_1}$$

x – max volume loaded
L – column length

system overloading



volume overloading

: at bad sample solubility in MP
: rectangular peak shape
: linear adsorption isotherm
: controlled by column diameter
: small SP particle size needed

concentration overloading

: at good sample solubility in MP
: triangular peak shape
: non-linear adsorption isotherm
: controlled by selectivity
: small influence of SP particle size

SP used and system parameters

optimisation on analytical column – **same SP** as in preparative mode

stationary phase

: critical is an extent of **coverage** by active layer (**mol·m^{-2}**)
:: controlled by the particle diameter
::: 5 µm for *poorly* separated mixtures
::: 7 – 10 µm for *well* separated mixtures

column diameter [mm]	for α < 1.2 [mg]	for α > 1.5 [mg]
4.6	2 – 3	20 – 30
9.4	10 – 20	100 – 200
21.2	50 – 200	500 – 2000
30, 50	> 200	> 2000

it is important to provide **appropriate capillary diameter**

$$\sigma^2 = \frac{\pi \cdot r^4 \cdot F_M \cdot L}{24 \cdot D_m}$$ ***Aris-Taylor equation***

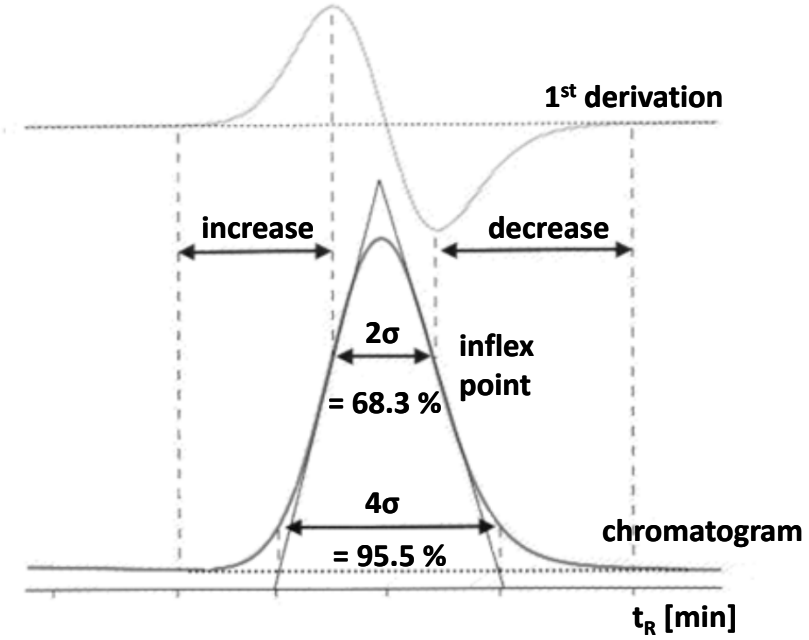
σ^2 – zone broadening, **F_M** – voluminal flow rate, **L** – column length,
D_m – diffusion coefficient, **r** – capillary diameter

fraction collection **detection controlled**

UV-Vis 1st peak derivation is used

good signal filtering
: noise smoothing (Savitzky-Golay)

sharp peaks
: cause lower losses by peak identification
: important to minimise post-column broadening
: not too long capillaries
: fast connection of PC and fraction collector



MS monoisotopic peak of analyte is used

mobile phase

: suitable spectroscopic properties
: **volatility**, boiling point (substance isolation from fraction)
: viscosity (too high pressure)
: purity
: solubility
: **price** (acetonitrile > heptane > acetone > methanol)

volatile buffers

buffer	pH
trifluoroacetate	< 1.5
ammonium formate	3.0 – 5.0
pyridinium formate	3.0 – 5.0
ammonium acetate	3.8 – 5.8
ammonium carbonate	5.5 – 7.5; 9.3 – 11.3
ammonium	8.3 – 10.3

X. gas chromatography

: extraction G-L
: extraction G-S

: **mobile phase** (**MP**, gas)
: **stationary phase** (**SP**, liquid, solid, thin layer of liquid on carrier)

GC history

1941

Synge and **Martin** – theoretic principles of GC
...very refined separations of volatile substances should be possible in a column in which permanent gas is made to flow over gel impregnated with a non-volatile solvent ...

1952

James and **Martin** : practical introduction of GC
: separation of volatile fatty acids

1963

GC-MS – first hyphenated technique

1980

capillary columns in GC – distinctive separation improvement

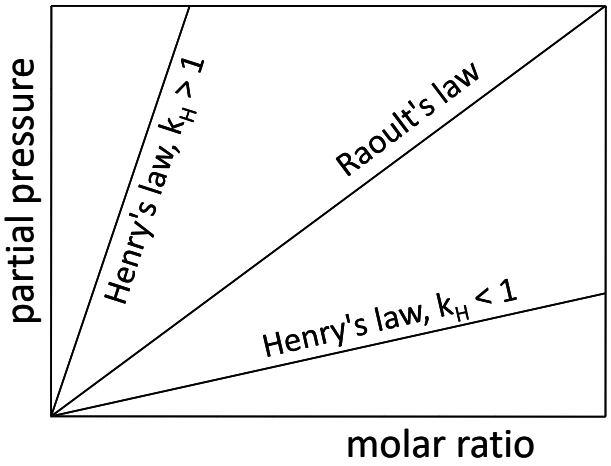
gas is **compressible** (liquid not)

principal differences between GC and LC

Raoult's law

$$p_A = p_A^0 \cdot x_A$$

x_A – molar ratio of **A** in mixture
p_A^0 – pressure of saturated vapours of **A**



Henry isotherm

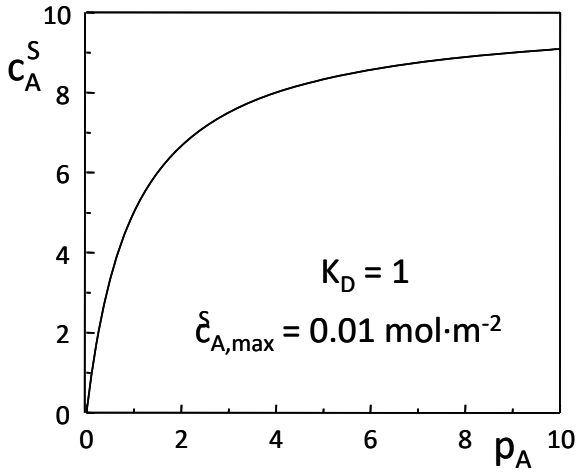
$$c_A^S = k_H \cdot p_A$$

low concentrations of **A**, non-ideal solution
k_H – Henry's constant
p_A – partial pressure of **A** over mixture

Langmuir isotherm

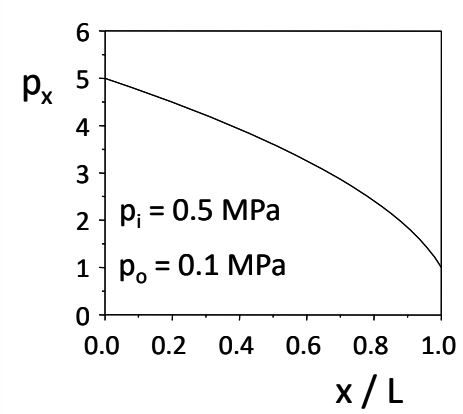
$$c_A^S = c_{A_{max}}^S \cdot \frac{K_D \cdot p_A}{1 + K_D \cdot p_A}$$

c_{max}^S – maximal bound concentration on SP

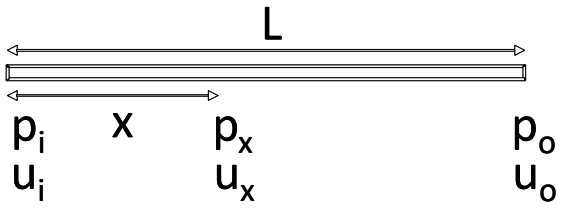


distribution constant is strongly dependent of **vapour pressure** and **volatility of analyte**

linear flow rate of carrier gas (MP)

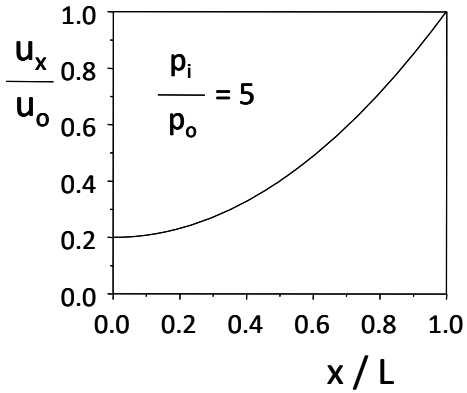


pressure gradient profile on column



L – column length
p – gas pressure
u – linear flow rate
indices: **i** – on inlet
x – in point x of length
o – on outlet

value profile of linear flow rate



keeping constant flow rate
: solely by column (input pressure checked; regulation by metal membrane)
: pneumotoric serial resistance (capillary + needle valve)
: constant mass flow (feed back)

average linear MP flow rate

$$\bar{u} = \frac{B_0 \cdot (p_i - p_o)}{\eta \cdot \varepsilon \cdot L}$$

B_0 – specific permeability of column [m^2]
$(p_i\text{-}p_o)$ – pressure gradient [Pa]
η – dynamic viscosity [Pa·s]
ε – sorbent inner porosity
L – column length [m]

compressibility factor

$$\bar{u} = j \cdot u_o \qquad j = \frac{3}{2} \cdot \frac{\left(\frac{p_i}{p_o}\right)^2 - 1}{\left(\frac{p_i}{p_o}\right)^3 - 1}$$

j – compressibility factor

retention quantities

retention volume / time of *i*-th analyte — $V_{R,i}$ [ml], $t_{R,i}$ [min] — $V_{R_i} = F_M \cdot t_{R_i}$

void volume / time of column — V_m [ml], t_m [min] — $V_m = F_M \cdot t_m = V_M$

corrected retention volume / time — $V'_{R,i}$ [ml], $t'_{R,i}$ [min] — $t'_{R_i} = t_{R_i} - t_m$

$$V'_{R_i} = F_M \cdot t'_{R_i} \qquad V'_{R_i} = V_{R_i} - V_m$$

net retention volume — V_N [min] — $V_N = F_M \cdot t'_{R_i} \cdot j = V'_{R_i} \cdot j$

corrected retention volume adjusted to carrier gas compressibility

specific retention volume — V_h [ml·g^{-1}] or V_p [ml·m^{-2}]

w_L – amount of immobilised SP (L)
S – SF area (S)

net retention volume
related to 1 g or 1 m^2 SP and to 0 °C

$$V_h = \frac{273.15 \cdot V_N}{w_L \cdot T_k} \qquad V_p = \frac{273.15 \cdot V_N}{S \cdot T_k}$$

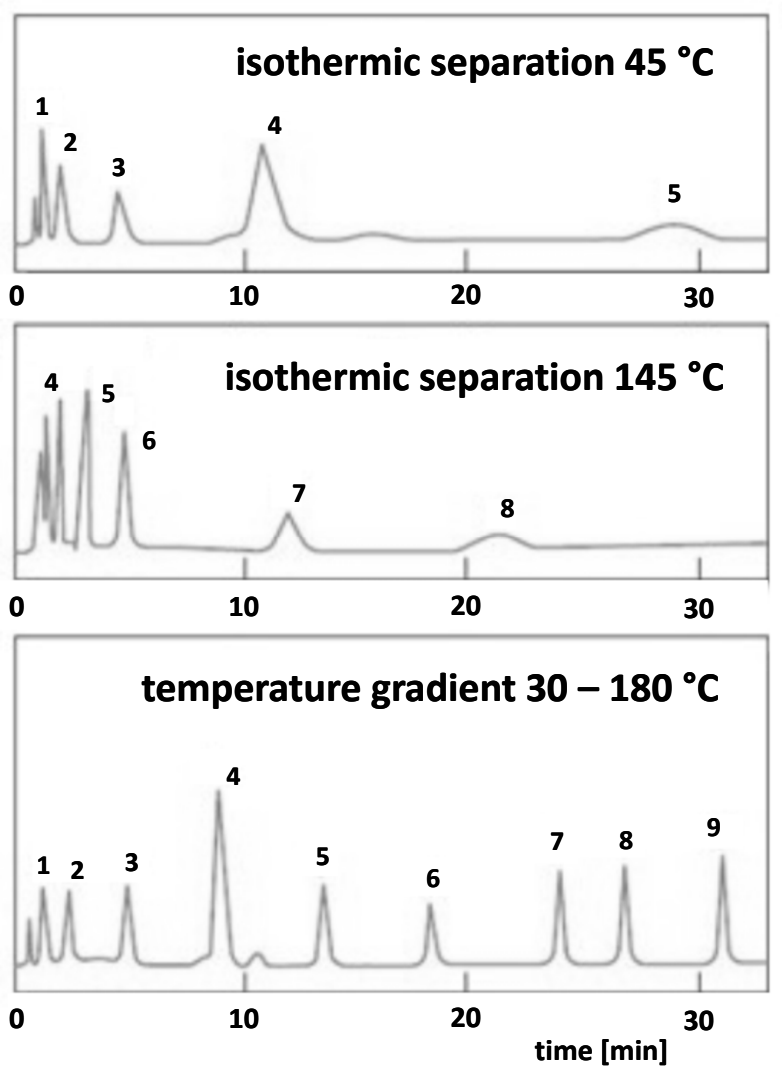
temperature influence

T_{col} greater than T_{boil}
while T_{inj} greater or equal to T_{col}
while T_{det} greater than T_{col}

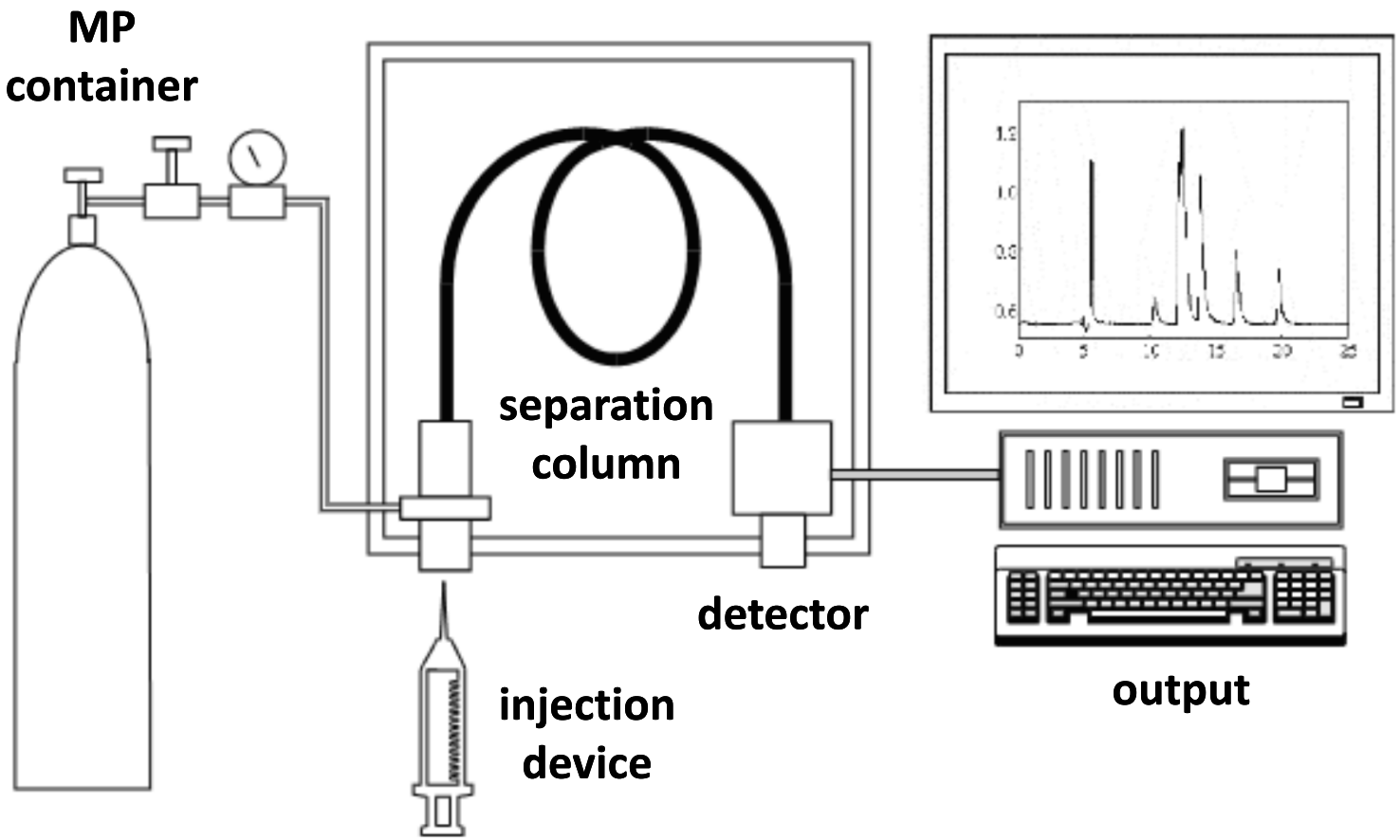
T_{inj} – injection head temperature
T_{col} – column thermostat temperature
T_{det} – detector temperature

: **higher** T_{col} leads to faster analysis
: **higher** T_{col} demands **higher** MP pressure on column inlet
:: keeping **u** through column

isothermic analysis: T_{col} = *const.*
analysis with temperature gradient: $T_{col2} - T_{col1} > 0$



GC arrangement



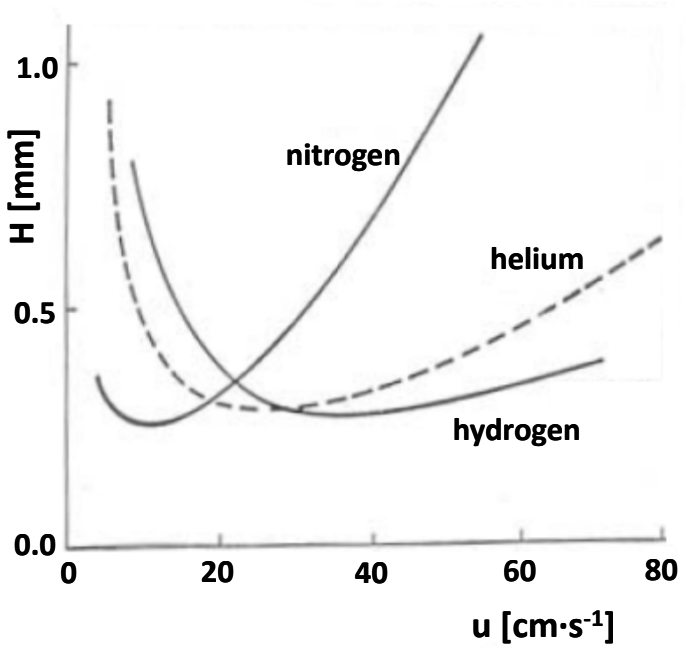
MP delivery

: 0.5 – 400 ml·min^{-1}
:: HP-GC 1200 ml·min^{-1}
: pressure up to 400 kPa
:: HP-GC 1 MPa
: pressure and flow control
: thermostating

gas sources
: pressure containers
: compressor
: electrolyser

advanced flow control
(AFC)

advanced pressure control
(APC)



carrier gas

H_2 (hydrogen) **+** high thermal conductivity, low viscosity
– high diffusivity, explosive

N_2 (nitrogen) **+** cheap, safe
– low thermal conductivity

He (helium) **+** combines advantages of N_2 & H_2
– expensive

Ar (argon) especially for ECD

must be *chemically inert* – always necessary to remove **humidity** and **O_2**

purity – pre-set guard column with molecular sieve

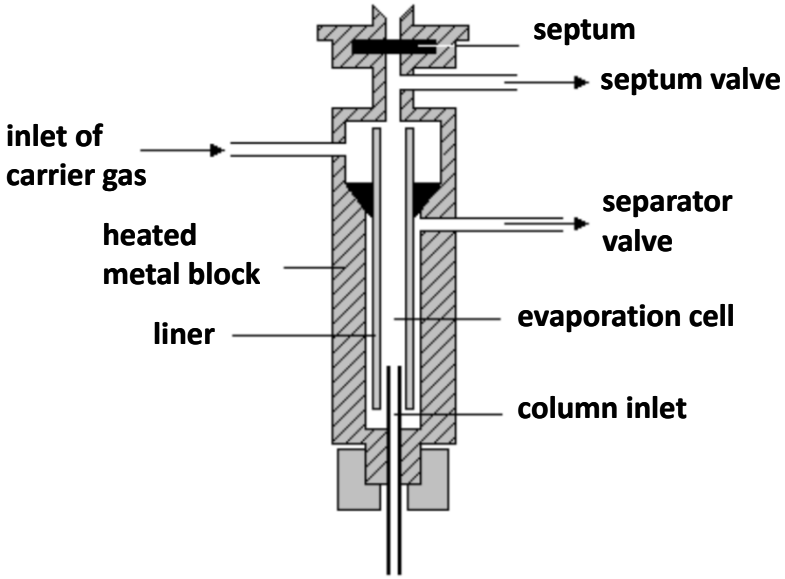
injection device

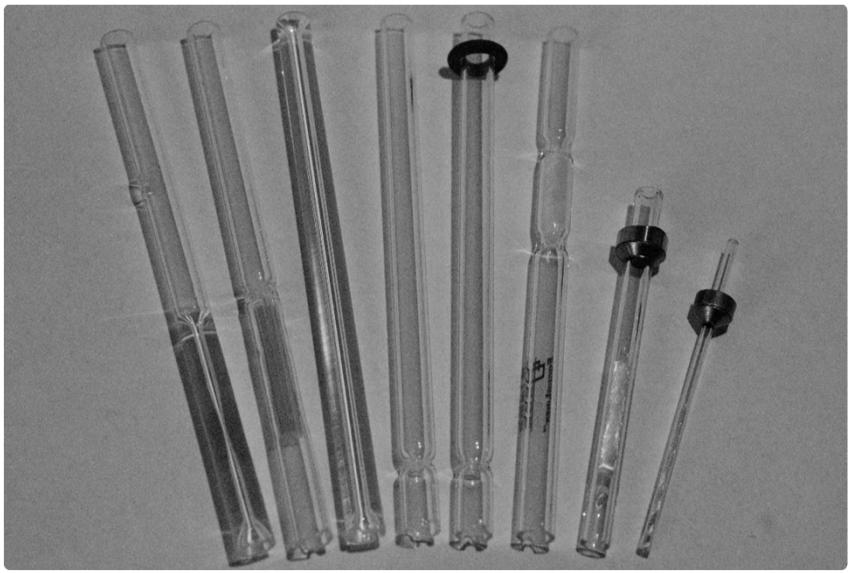
loading of **A** onto column
: more difficult than by LC

tubular columns	~ 200 µg
capillary columns	max 20 µg, opt 1 µg

specially within capillary columns, inject *small volume* and do it *quickly*
: slowly and large volume (overload) means broad zones and resolution loss

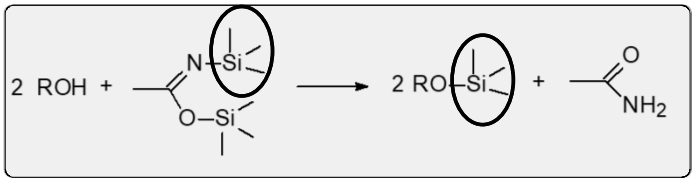
liner of injector
: heat evaporation and sample vapours mixing with carrier gas





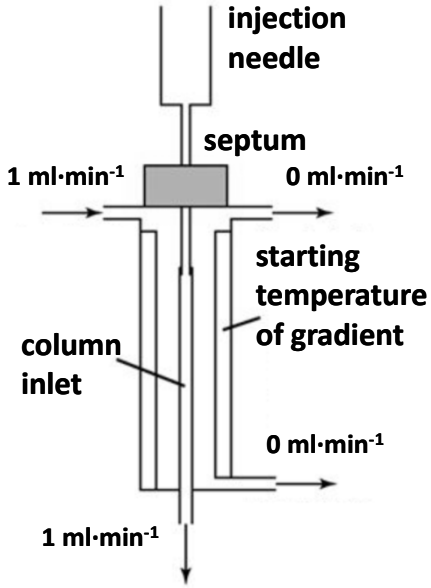
sample evaporation

necessity to (quickly) transform liquid and solid samples into gaseous state
: without changing the nature of sample
: requires *heated space* on the beginning of the column
:: sometimes gasification on-column



volatility increment
: chemical derivatisation
:: *silylation* (N,O-bis(trimethylsilyl)acetamide), *silanisation* (dimethylchlorsilane), *acetylation* (acetanhydride)

on-column injection

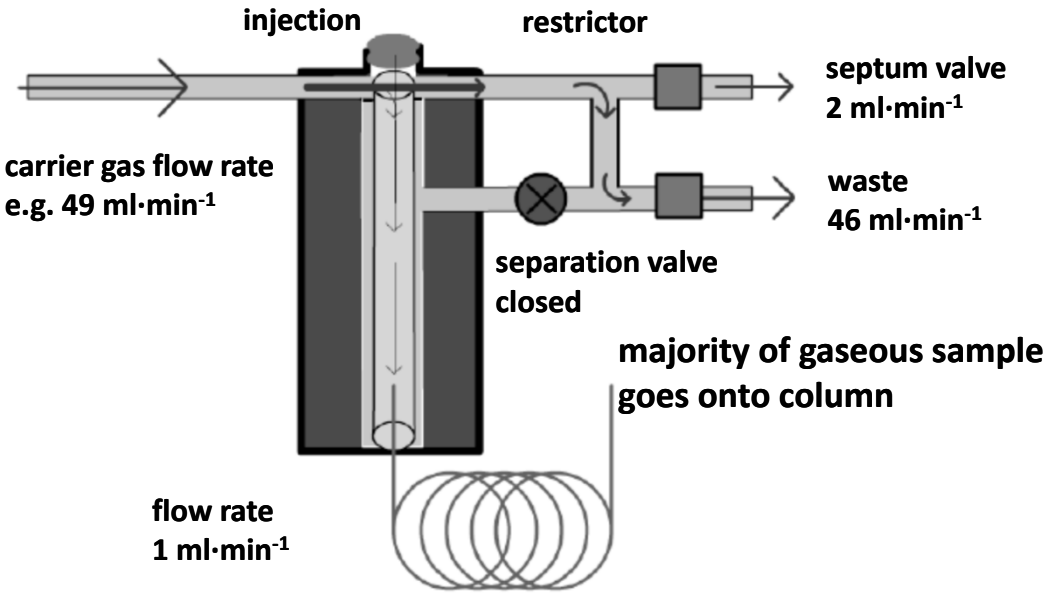


: similar to splitless injection
:: after certain time, the valve is open & rest of the sample is washed out
: injects precise amount
: no evaporation during injection, until in the temperature gradient on column
:: selective evaporation of compounds with lower boiling temperatures
: instrumentally demanding
:: necessity to restrict the pressure losses within injection
: overloads column with liquid (1 µl for 50 cm of column)
:: peak broadening (solution similar applies as to splitless injection)

splitless injection

: suitable for classical packed columns
:: and diluted samples

: after a time without splitting, the valve is open
:: meanwhile the sample is loaded on column
::: 10 – 40 s, opt 20 s (*splitless time*)
:: the rest of the sample is washed out



advantages

: majority of the sample goes onto column
:: suitable for trace analysis

disadvantages

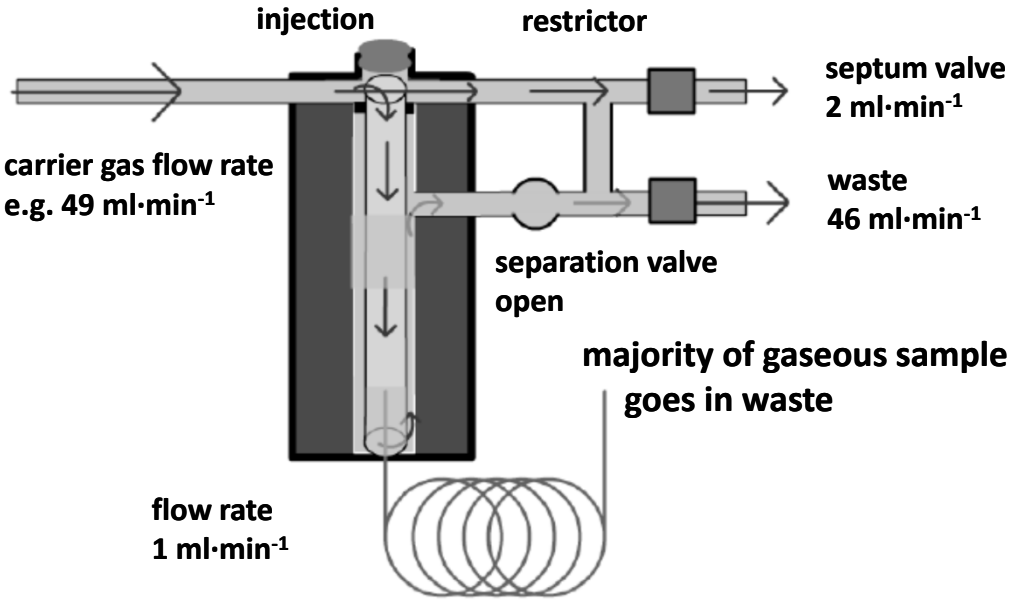
: slow mass transfer onto column
:: zone broadening
::: a need for re-concentration

split injection

today, the most used way of injection

$$S = \frac{F_M}{F_S \cdot F_M}$$

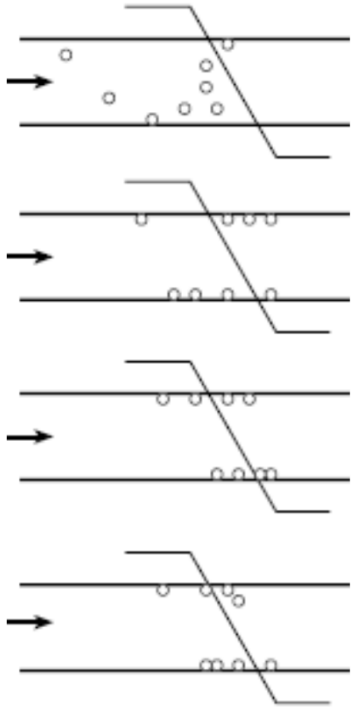
S – degree of sample splitting
$\mathbf{F_M}$ – column flow rate
$\mathbf{F_S}$ – splitter flow rate



advantages
: injection of a *small volume*
:: sharp zones & low column polution

disadvantages
: unsuitable for trace analysis
: depends on the splitter geometry

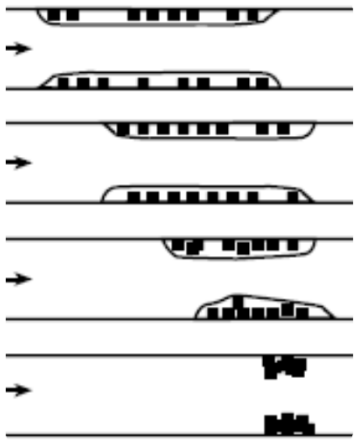
sample **re-concentration**
: prevents zone broadening within direct and splitless injection



cold trapping

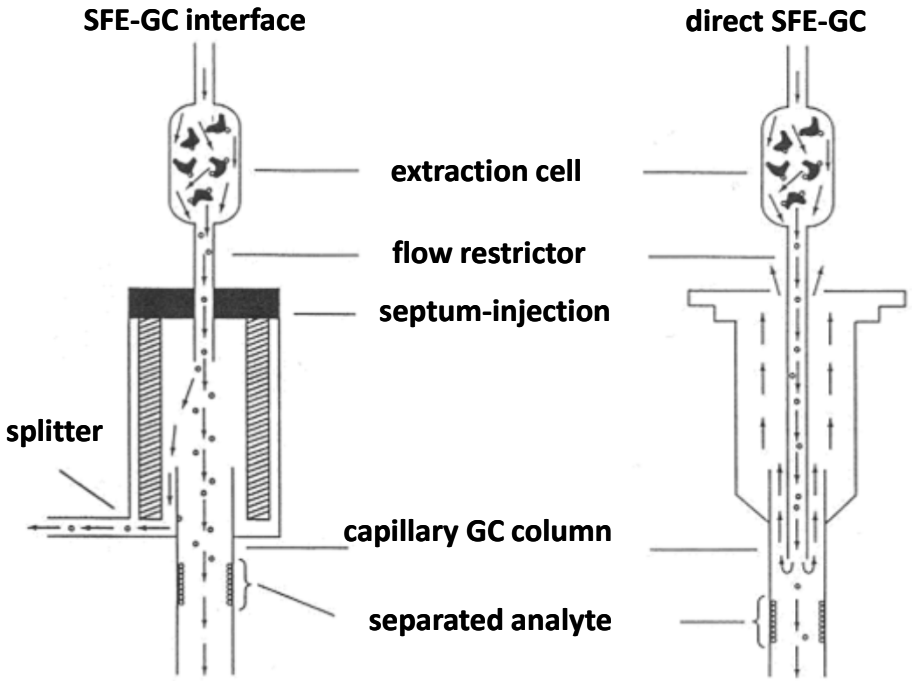
: first few *cm* of column has negative temperature gradient
:: ~ 250 °C (injection) decreases to 40 °C in capture region
::: *ca* about 150 °C lower than the compound with the highest T_{boil}
: mobility of components with high T_{boil} is thus zero
: and thus their re-concentration is achieved

solvent effect



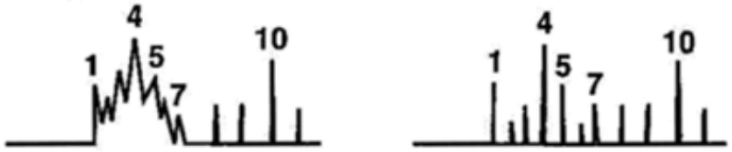
: first few *cm* of column has negative temperature gradient
:: ~ 250 °C (injection) decreases in capture region to *ca* 20 °C bellow solvent T_{boil}
: sample components with low T_{boil} condensate with solvent
: from the created thin film, the solvent is slowly evaporating
: and thus re-concentrate the components with low T_{boil}

hyphenation of SFE with GC
(cold-trapping)



separation by means of cold-trapping

1. T_{col} in time (t = 0) ≤ 25 °C
2. d_f ≥ 2 µm SP

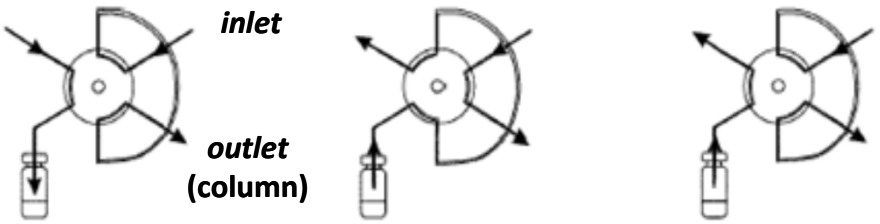


a) w/o utilisation **b) w/ utilisation**

separation of supercritical fluid from sample increases quality GC analysis

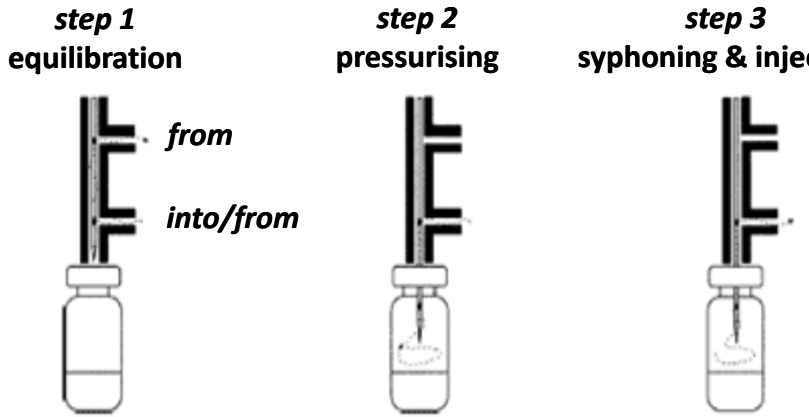
gas injection

pressure valve



hyphenation HSE-GC

injection in system with pressure equilibrium



separation column

packed tubular
: analytical
: preparative

length: 0.5 – 10.0 m
diameter: 1 – 6 mm

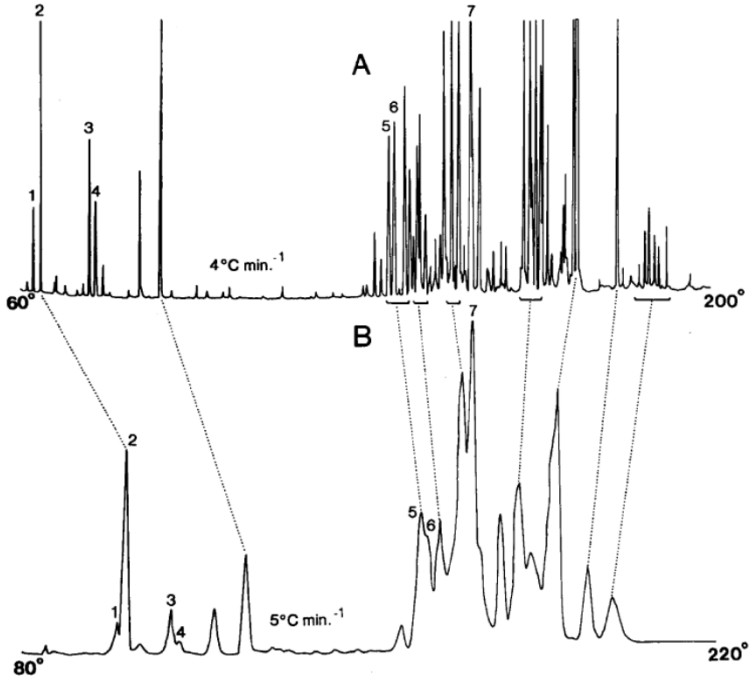
length: 2 – 6 m
diameter: > 6 mm

capillary
: open
: filled

length: 10 – 100 m
diameter: 0.1 – 0.5 mm

length: 0.5 – 50.0 m
diameter: 0.3 – 1.0 mm

≡ 0.10 – minibore
< 0.25 – narrow bore
≡ 0.32 – wide bore
≡ 0.45 – high speed megabore
≡ 0.53 – megabore



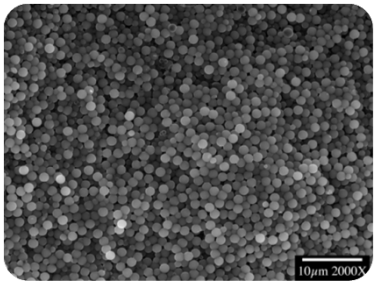
GC separation of calamus oil components
A – 50 m capillary column
B – 4 m tubular column

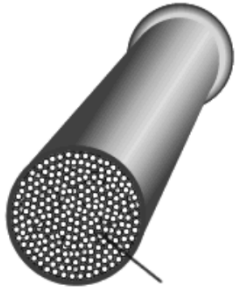
packed tubular columns

cover
: glass, steel, copper, polymers

basic type of sorbents

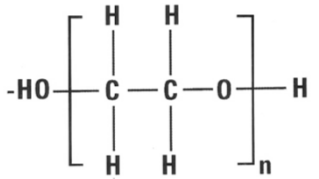
carriers
fine, solid and inert material (spherical silica)
: active centres (silanols and siloxanes) cause tailing of more polar components
:: suppression – *silylation*
: serves directly as SP (GSC)
: or is covered by thin liquid phase film (GLC)




solid SP

adsorbents
: *unspecific* (activated carbon)
: *specific* (silica, alumina, molecular sieves etc.)

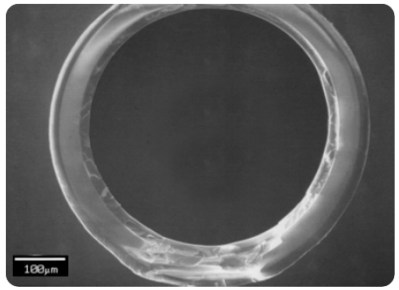
non-polar
: methylated polysiloxane, squalene, apolane C-87



mildly polar
: phenylated polysiloxane

strongly polar
: polysiloxane with CH_2-CH_2-CN, -CH=CH-CN, Carbowax 20M (PEG-based)

capillary columns

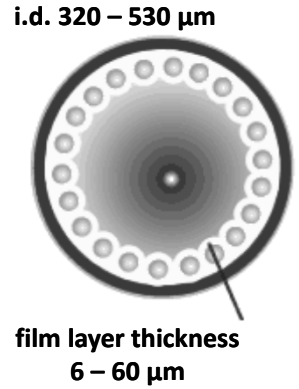


quartz
: surface enlargement by etching
: polyimide cover increases mechanical stability

SP universal non-polar silicon phases or immobilised Carbowax

fused silica open tubular (FSOT)

thin wall with outer polyimide cover (GSC)

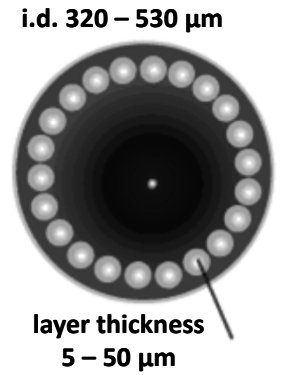


wall-coated open tubular columns (WCOT)

liquid SP anchored directly on the capillary wall (GLC)

support-coated open tubular columns (SCOT)

carrier is on capillary wall, SP is on it (GLC)



porous-layer open tubular columns (PLOT)

layer of solid active sorbent on an inner capillary wall (GSC)

column thermostat

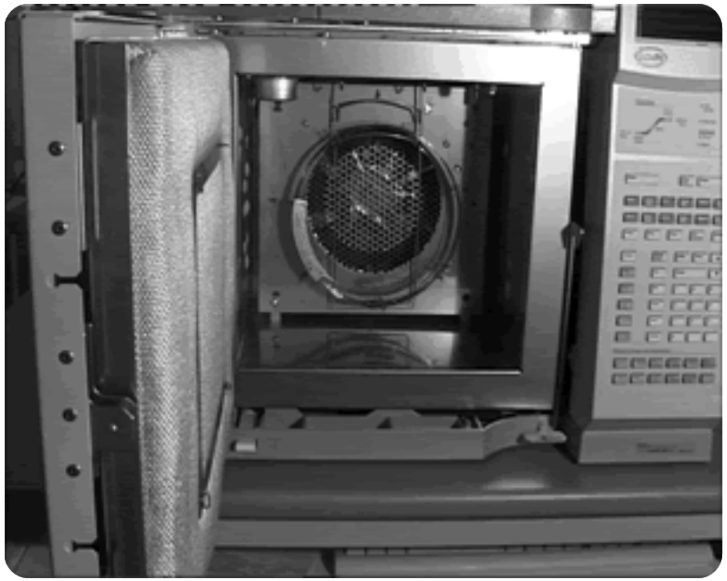
importance of temperature of GC

: evaporation of liquid or solid sample
: kinetic aspects of separation

kept with precision of 0.1 °C
: thermostat range (T_{lab} + 4 °C) – 450 °C

optimal loading temperatures
: T_{boil} of component with highest value + 30 – 50 °C

optimal column temperature around T_{boil} of analyte
: column temperature greater or equal to T_{boil} , thus t_R = 2 – 30 min
: minimal temperature means better resolution, but higher t_R

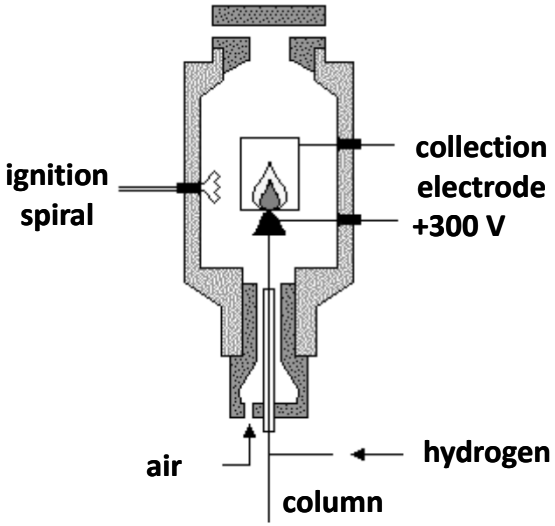


wide range of T_{boil} of separated components
: requires *temperature programme / column gradient*
:: temperature change during experiment
:: temperature may be increased gradually or in steps

detectors **detected compound is volatile, in gaseous state**

concentration dependent detector (CDD)
: dilution with carrier gas decreases sensitivity

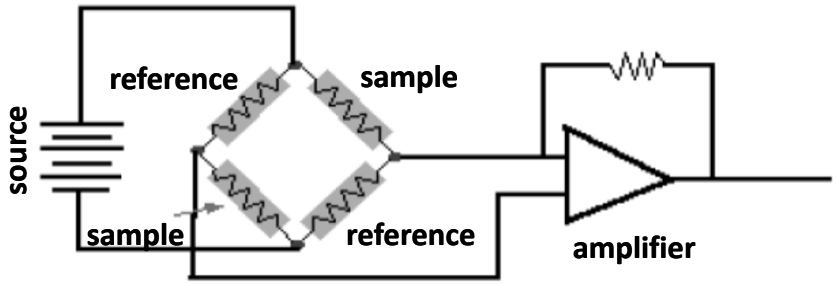
mass dependent detector (MDD)
: carrier gas interferes not, depends on introduction rate into detector



flame ionisation detector FID

MDD
signal: current created by pyrolysis of carbon sample

ion current
: noise 10^{-13}
: dynamic range 10^{7}
: sensitivity 10^{-9} M



thermal conductivity detector

TCD
catharometer

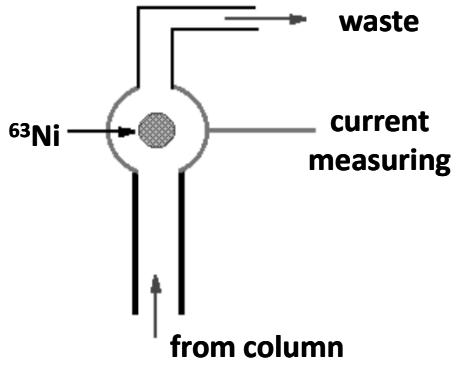
differential thermal conductivity
: noise 10^{-5}
: dynamic range 10^{6}
: sensitivity 10^{-8} M

CDD
signal: sample molecules change (decrease) thermal conductivity of carrier gas
: carrier gas must have high thermal conductivity (He, H_2...)
: temperature dependent, universal

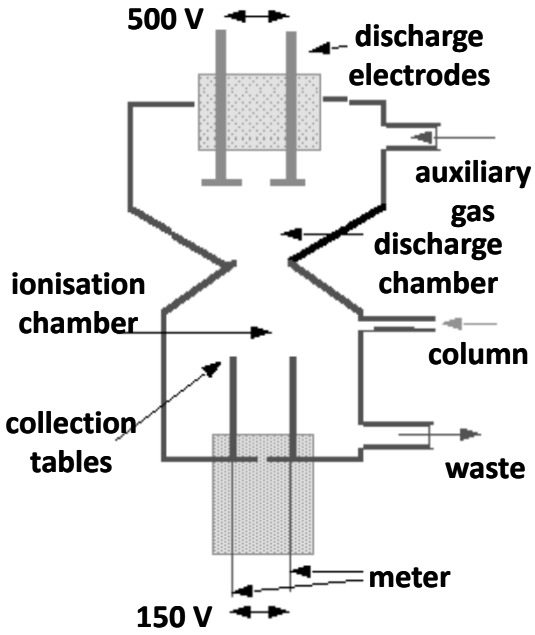
electron capture detector

ECD

ion current
: noise 10^{-12}
: dynamic range 10^{5}
: sensitivity 10^{-13} M



MDD
signal: analyte molecules decrease current generated by β-emitter
: halides, nitrites, cyano-compounds, peroxides, anhydrides, organometals



helium ionisation detector HID

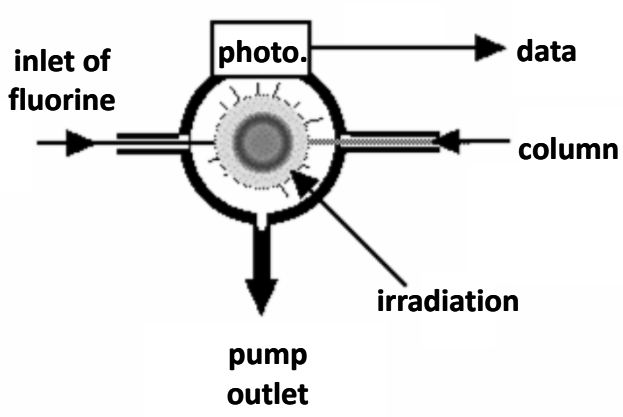
MDD

signal: auxiliary gas is ionised first (He, Ar),
its ions then secondary ionise sample molecules

ion current
: noise 10^{-14}
: dynamic range 10^{6}
: sensitivity 10^{-12} M

chemoluminiscence detector

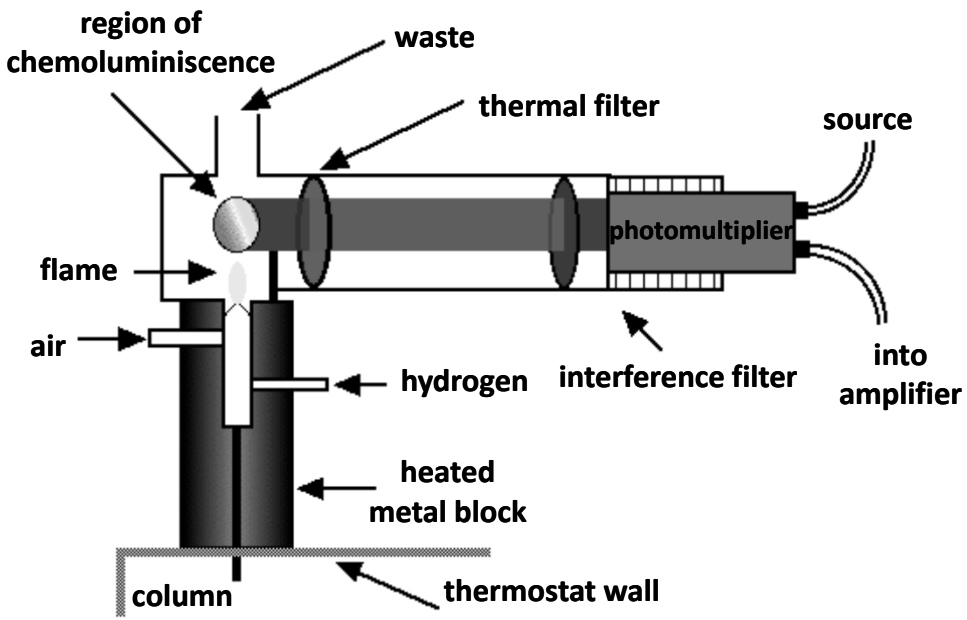
chemiluminiscence
: noise 10^{-13}
: dynamic range 10^{4}
: sensitivity 10^{-11} M



CDD

signal: reaction of F (strong oxidant) with analyte

flame photometric detector FPD



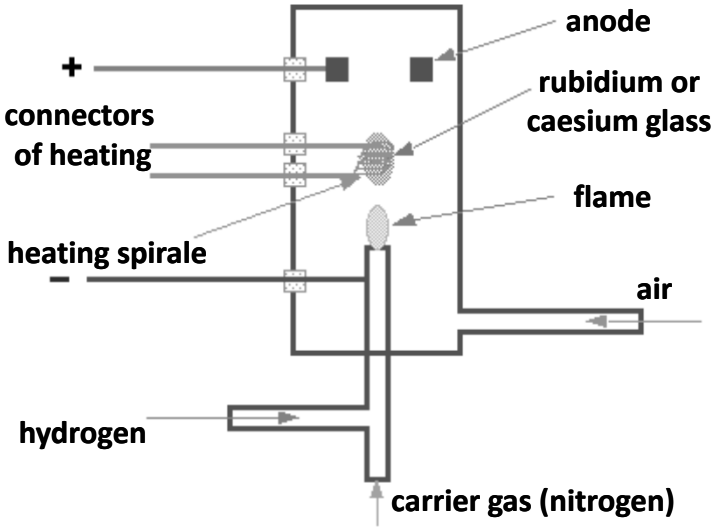
chemiluminiscence
: noise 10^{-12}
: dynamic range 10^{7}
: sensitivity 10^{-10} M

MDD
signal: chemoluminiscence
: selective S (394 nm), P (526 nm)

nitrogen phosphorus detector

NPD – thermoionisation detector

ion current
: noise 10^{-12}
: dynamic range 10^{6}
: sensitivity 10^{-10} M



MDD
signal: Rb/Ce glass thermoionisation electron emission enhanced by N or P presence

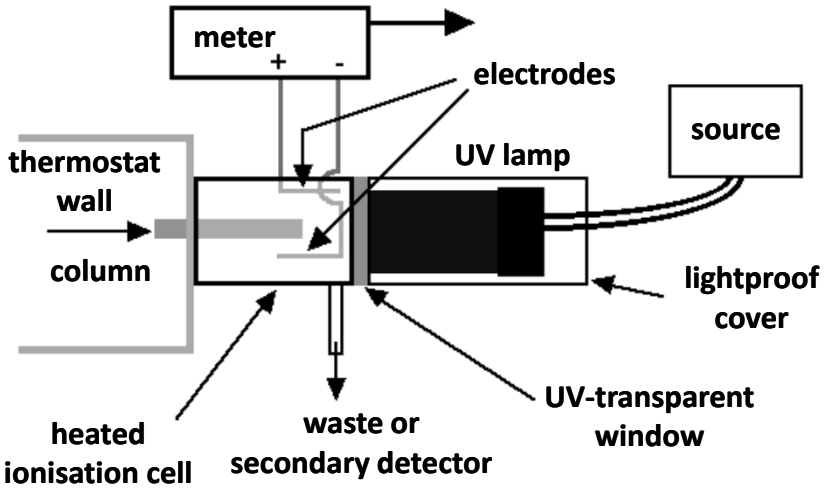
photoionisation detector

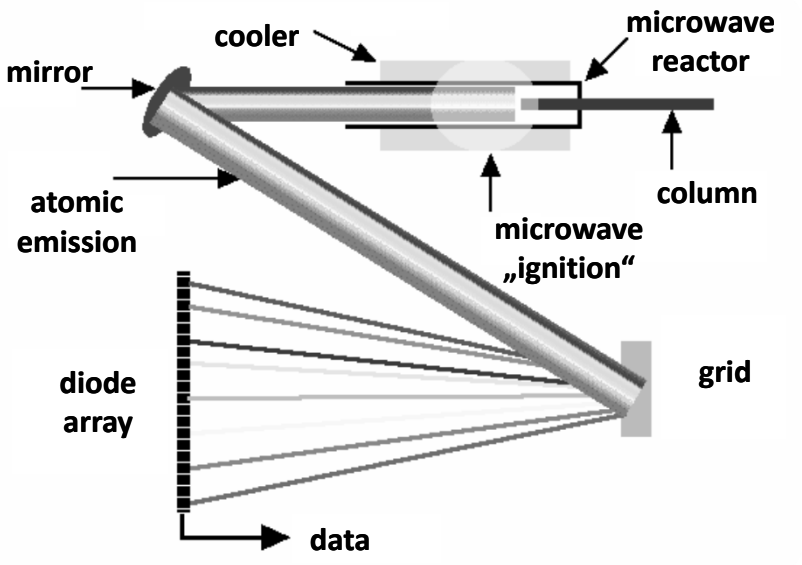
PID

MDD

signal: UV-irradiation ionisation

ion current
: **noise** 10^{-13}
: **dynamic range** 10^{7}
: **sensitivity** 10^{-11} M





atomic emission detector

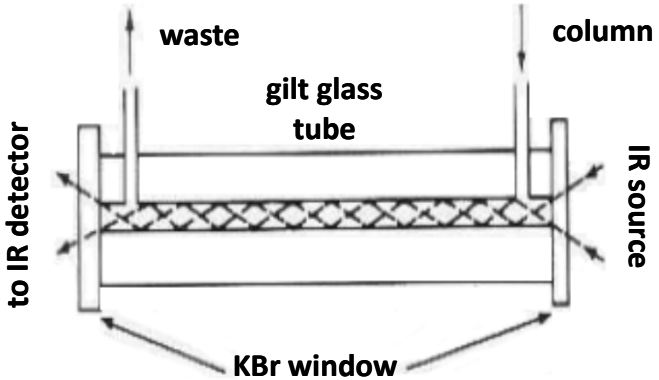
AED

MDD

signal: microwave induced plasma
: selective according to chosen emission wavelength
: very expensive

atomic emission radiation
: **noise** 10^{-14}
: **dynamic range** 10^{4}
: **sensitivity** 10^{-11} M

infrared detector IRD



CDD
signal: IR absorbance

absorption of infrared radiation
: noise 10^{-12}
: dynamic range 10^{5}
: sensitivity 10^{-10} M

mass spectrometric detector MS

GC-MS interface
: gaseous state, splitter

MDD
signal: ion count
universal

ionisation
: electron ionisation (EI)
: chemical ionisation (CI)

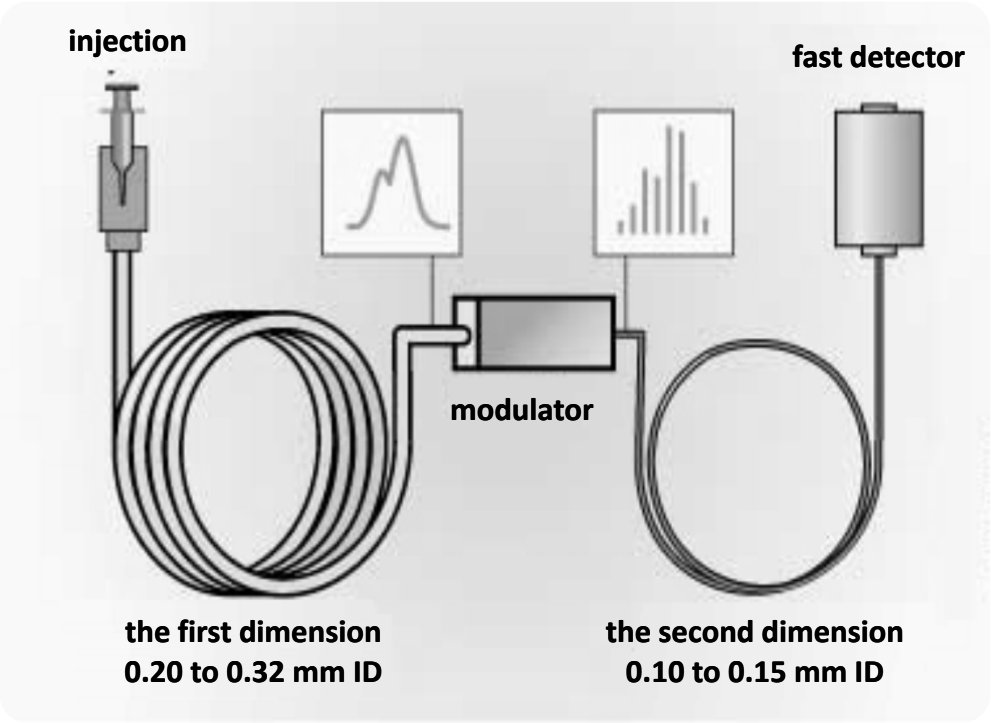
analysers
: quadrupole (Q, Qq)
: ion trap (IT)
: magnetic sector
: time-of-flight (TOF)

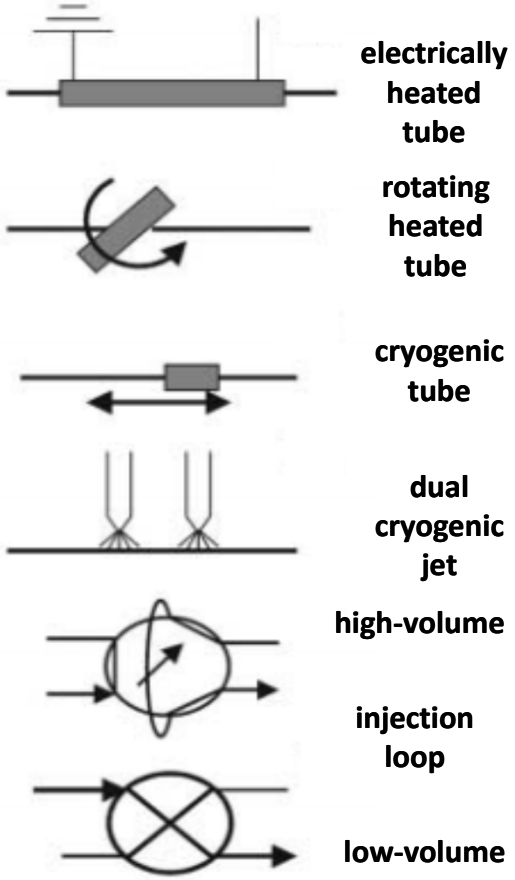
ion-count
: noise 10^{-14}
: dynamic range 10^{3}
: sensitivity 10^{-15} M

multi-dimensional gas chromatography (2D-GC)

peak capacity in 2D-GC

$$n_{2D} = \frac{4}{\pi} \cdot n_{1.D} \cdot n_{2.D}$$





modulators

: allow transfer between dimensions

definition of chromatographic system in GC

MP **carrier gas type**

flow / pressure (ml·min^{-1} / kPa)

injection (*X* µl)
injection type (event. splitting rate)

SP **stationary phase type**

Reoplex 400 (3 m x 3 m I.D.); packing, 5% on Chromosorb G HP, 80 – 100 mesh; Carrier gas: N_2; 30 mℓ/min., exhaust split 1 : 9; detector FID.
Temperature programmes:
C_6 – 1: 50° – 200°C/min.
C_6 – 2: 100°C isothermal
C_6 – 3: 100° – 200°C at 2°C/min.

length, inner diameter, manufacturer, SP type, film thickness
25m x 0.32 ID J&W DB-5 DF – 1.0

temperature gradient profile
initial temperature and its period, temperature increase; inlet temperature

(e.g. 130 °C 1 min, 130 – 250 °C at 5 ° C·min^{-1}, 250 °C 5 min; 250 °C)

detector basic characteristics according to type

analytical information in chromatogram

qualitative information

retention time
: compound identification (*standard method*)

spectroscopic detectors
: UV-Vis spectra
: MS spectra (ESI / APCI; Qq / IT / o-TOF)
: NMR spectra (^{1}H, ^{13}C)

quantitative information

peak area ≈ amount (concentration) of compound
: because of narrow peaks frequently only height

retention time formulation

specific retention volume (V_p) $$\mathbf{V_p = \frac{273.15 \cdot F_M}{S \cdot T_{col}}}$$

relative retention time ($r_{A,B}$)
: comparison with internal standard $$\mathbf{r_{A,B} = \frac{t'_{R_A}}{t'_{R_B}}}$$

Kovats retention indices ($r_{A,B}$)
: linear dependence of retention time logarithm of homologues on carbon number

internal normalisation method

: all components are eluted
:: solvent does not count
: all they have same response factor

$$\mathbf{c_{\%} = A_{\%,j} = \frac{100 \cdot A_j}{A_{tot}}}$$

external standard method
internal standard method
standard addition method

test measurements in GC

column testing

in dependence on time ***we observe***
: normalised retention times of components
: height of peaks
: symmetry of peaks

efficiency ***testing mixture for uncoated carriers***

n-decane, 1-aminoacetate, 3,5-dimethylpyrimidine, n-dodecane, 1-aminodecane, 2,6-dimethyl-aniline, N,N-dicyclohexylamine, 1-aminododecane and n-heptadecane

MP – H_2, $\mathbf{T_{initial}}$ = 40 °C, $\mathbf{T_{terminal}}$ = 180 °C

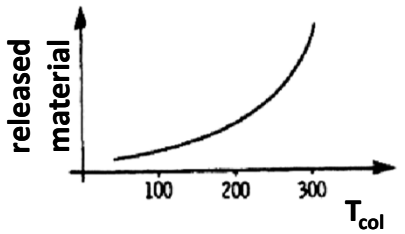
testing mixture for coated carriers **(Grob test)**

methyl decanoate, methyl undecanoate, methyl dodecanoate, n-decane, n-undecane, n-dodecane, 1-octanol, nonanal, 2,3-butanediol, 2,6-dimethylaniline, 2,6-dimethyl-phenol, dicyclohexylamine, 2-ethylhexanoic acid

MP – H_2 or He, $\mathbf{T_{initial}}$ = 40 °C, $\mathbf{T_{terminal}}$ = 100 °C, resp. 175 °C

thermostability

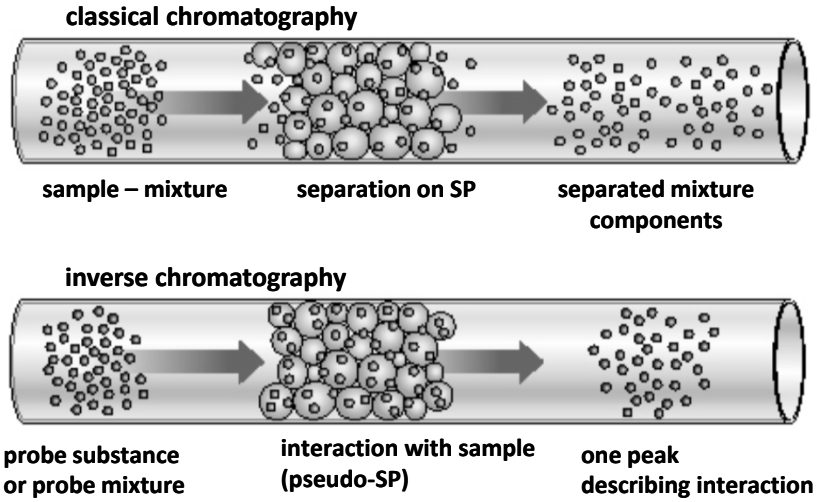
column bleeding
: decomposition of polymer materials in system
:: SP material, septum



$n\text{-}C_{22}$
MP – He, $\mathbf{T_{initial}}$ = 40 °C, $\mathbf{T_{terminal}}$ = 300 °C

inverse chromatography (IC)

inverse (gas/liquid) chromatography



study of **termodynamic properties of materials** (pseudo-SP)
: granular or fibrous p-SP
: **infinite dilution IC** (IC-ID)
:: small probe amount, elimination of their mutual interaction
:: for sruface properties, transition temperatures, solubility
: **finite concentration IC** (IC-FC)
:: monolayer on p-SP, sometimes even more
:: for desorption isotherms & surface inhomogeneities

$$\Delta G_a^0 = f(\chi_T)$$

ΔG_a^0 – change of free adsorption energy
χ_T – probe molecular descriptor

: combination of frontal & elution chromatography
:: probe in column until equilibrium
::: probe & studied material
:: load of pseudo-MP creates **vacancies**
:: regions without probe
: resulting chromatogram
:: inverse towards elution method record