Capillary Electrophoresis

Analysis of Ions and Small Molecules

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KVCV, Studiedag Ionenanalyse

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Characteristics - 1

- Electrophoresis in narrow-bore (25-150 µm id), fused silica capillaries
- High voltages (10-30 kV) and high electric fields applied across the capillary
- High resistance of the capillary limits current generation and internal heating
- High efficiency (N >10^5-10^6)
- Short analysis time (5-20 min)
- Detection performed on-capillary (no external detection cell)

Characteristics - 2

- Small sample volume required (1-50 nl injected)
- Limited quantities of chemicals and reagents required (financial and environmental benifits)
- Operates in aqueous media
- Simple instrumentation and method development
- Automated instrumentation
- Numerous modes to vary selectivity and wide application range
- Applicable to wider selection of analytes compared to other techniques (LC, TLC, SFC, cGC)

Characteristics - 3

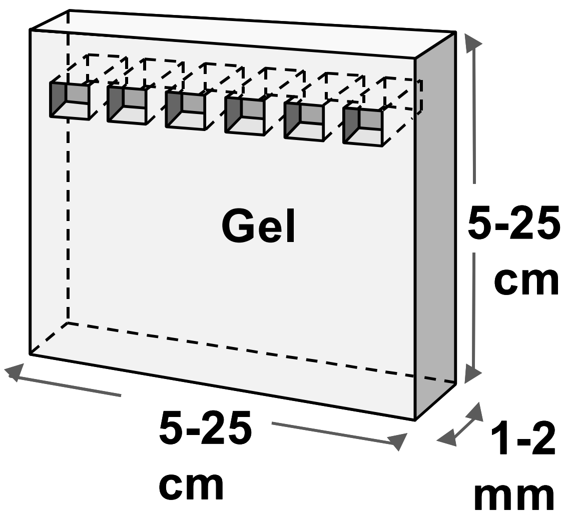
- Applicable to macro- and micromolecules
- Applicable to charged and neutral solutes
- Modern detector technology used (DAD, MS)

NEW DIMENSION IN SEPARATION SCIENCES

But ...

In order to fully exploit the possibilities of CE, thinking and reasoning in chromatographic terms should be avoided!

Slab gel to Capillary to Chip Electrophoresis

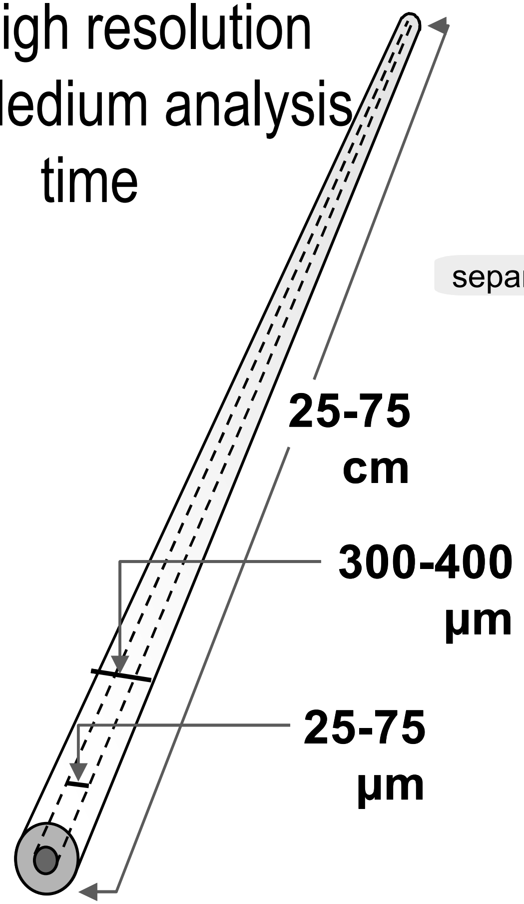


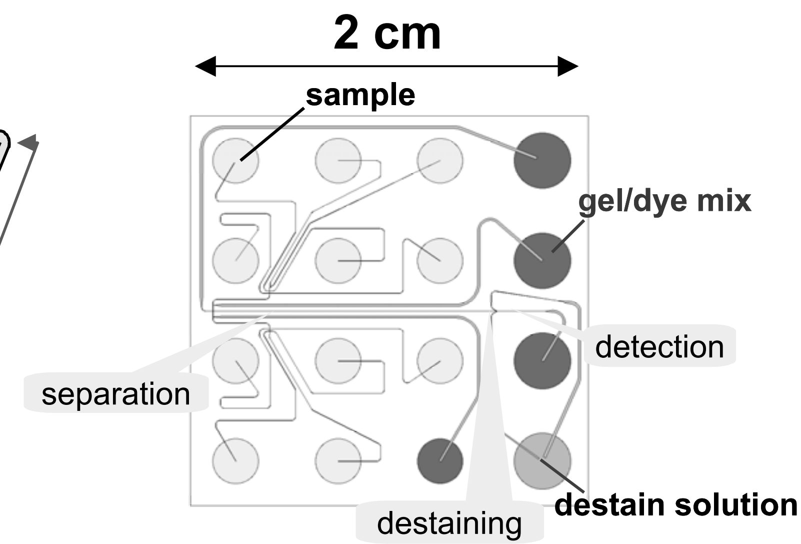
Slab Gel:

- Low resolution
- Long analysis time

Capillary:

- High resolution
- Medium analysis time





Lab-on-a-chip:

- High resolution
- Short analysis time

Modes of Operation

- ***Capillary Zone Electrophoresis*** ***(CZE)***
- *Capillary Isoelectric Focusing* *(CIEF)*
- *Capillary Isotachophoresis* *(CITP)*
- *Capillary Gel Electrophoresis* *(CGE)*
- ***Micellar Electrokinetic Chromatography*** ***(MEKC)***
- *Microemulsion Electrokinetic Chromatography* *(MEEKC)*
- *Non-Aqueous Capillary Electrophoresis* *(NACE)*
- *Chiral Capillary Electrophoresis* *(CCE)*
- ***Capillary Electrochromatography*** ***(CEC)***

Basic Principles of CE

Electrophoresis

- ***transport of charged species in a solution under the influence of an electric field***
- electrophoretic mobility (μ_{ep}) ~ f (q/r)

Electroosmosis

- ***relative movement of a liquid to a fixed charged surface caused by an electric field***
- electroosmotic mobility (μ_{eo}) ~ f (pH)
- electroosmotic flow = EOF

Electrophoresis

- Velocity of an ion in an electric field

$$v_{ep} = \mu_{ep}\,E = \mu_{ep}\,\frac{V}{L}$$

μ_{ep} = electrophoretic mobility (cm^2/Vs)

- Mobility in terms of physical parameters

$$\mu_{ep} = \frac{q}{6\,\pi\,\eta\,r}$$

(Pure spherical solutes)

q = ion charge
η = solution viscosity
r = ion radius

$$\mu_{ep} = \frac{\varepsilon\,\zeta}{6\,\pi\,\eta}$$

(More irregular shaped solutes)

ε = dielectric constant
ζ = zeta-potential

Ion velocity and mobility

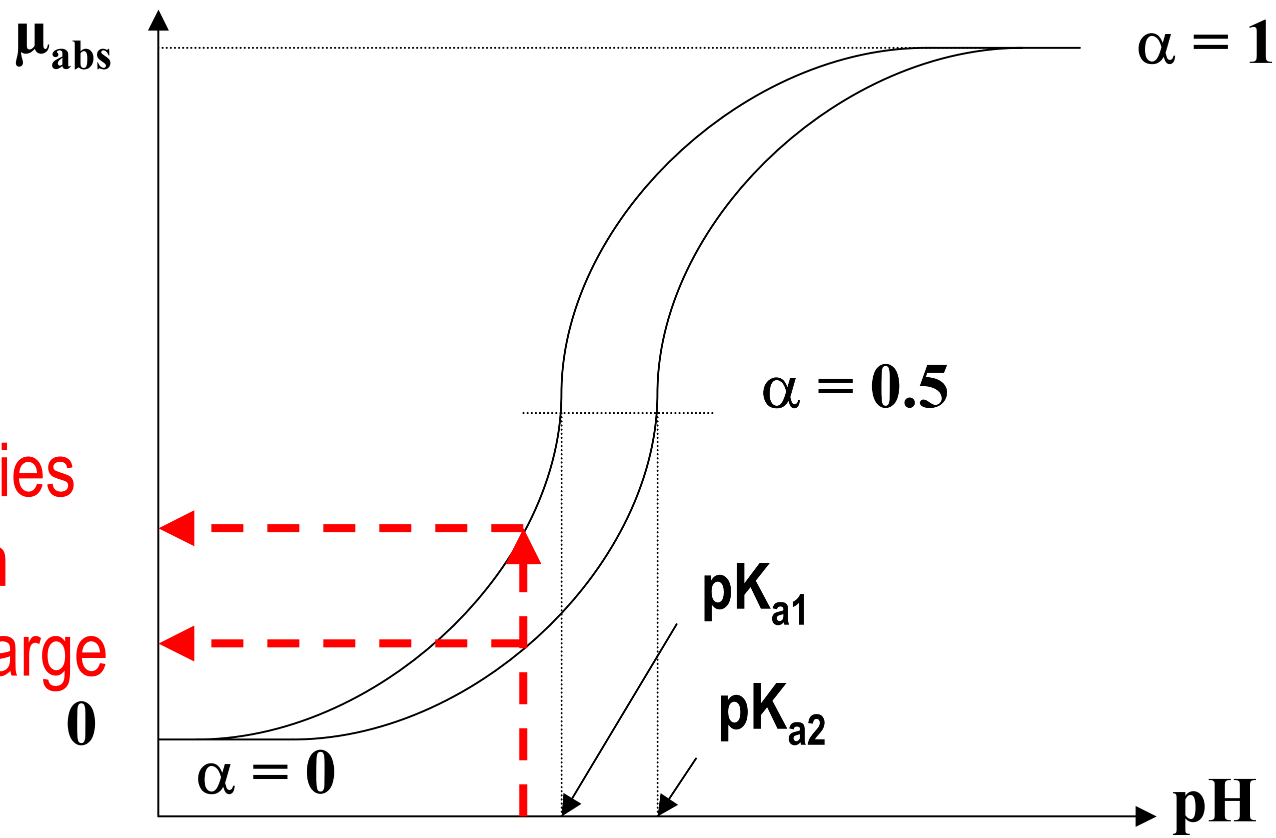
- Highly charged species = high mobility
- Minimally charged species = low mobility
- Neutral species = zero mobility
- Small species with x-charge = high mobility
- Large species with x-charge = low mobility

- **But …**

1. There is a difference between
absolute and effective mobility!
*2. The **solute radius** is in solution!*

Absolute – Effective Mobility

Different mobilities depending on pH-controlled charge



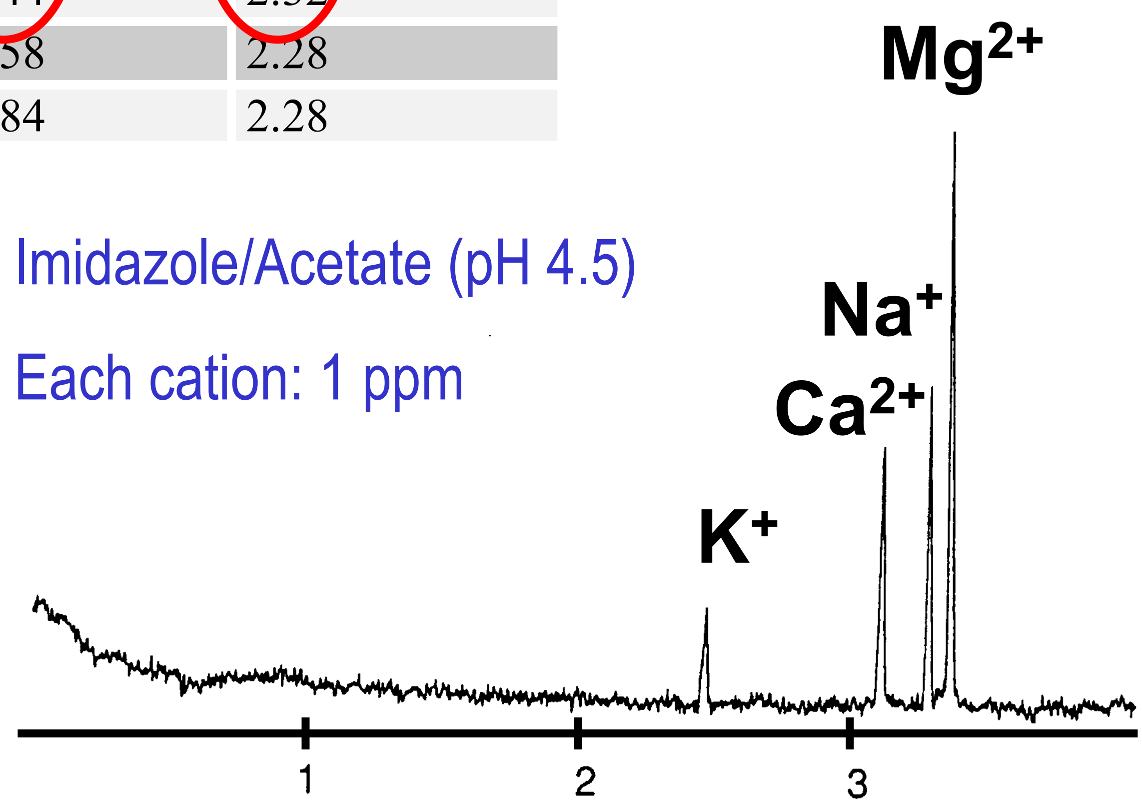
Solute Radius in Solution

Cations	μ (10^5 cm^2/V.s)	r_{ion} (crystal A°)	r_{ion} (hydrated A°)
Li	38.7	0.86	3.40
Na	50.5	1.12	2.76
K	73.5	1.44	2.32
Rb	76.5	1.58	2.28
Cs	78.0	1.84	2.28

Inorganic cations

Imidazole/Acetate (pH 4.5)

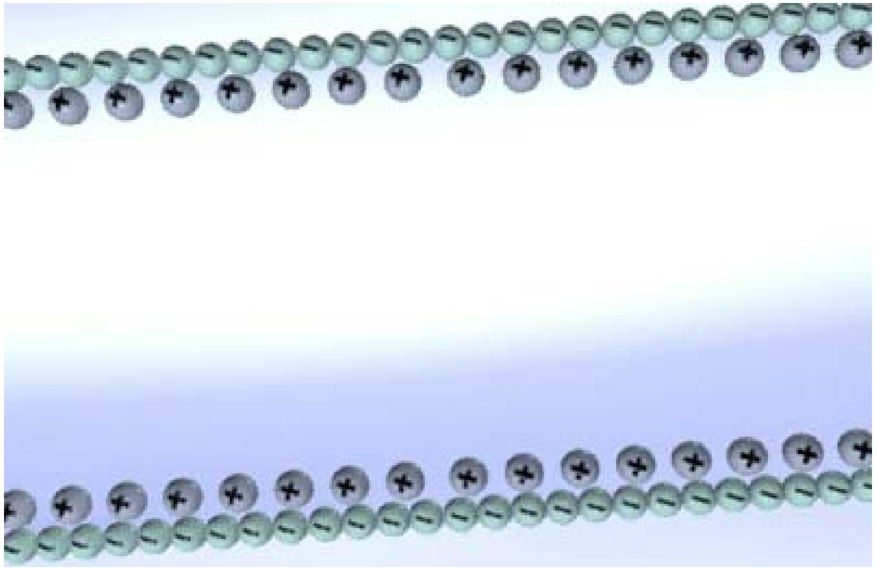
Each cation: 1 ppm



Electroosmosis

Dissociation of silanol groups capillary wall at pH > 2

- Negatively charged wall (SiO^-)
- Positively charged solution
- E $\Rightarrow$ bulk flow towards cathode = EOF



EOF

- Velocity of EOF (liquid-flow) in an electric field

$$v_{eo} = \mu_{eo} E$$

μ_{eo} = electroosmotic mobility

- Mobility in terms of physical parameters

$$\mu_{eo} = \frac{\varepsilon \zeta}{4 \pi \eta}$$

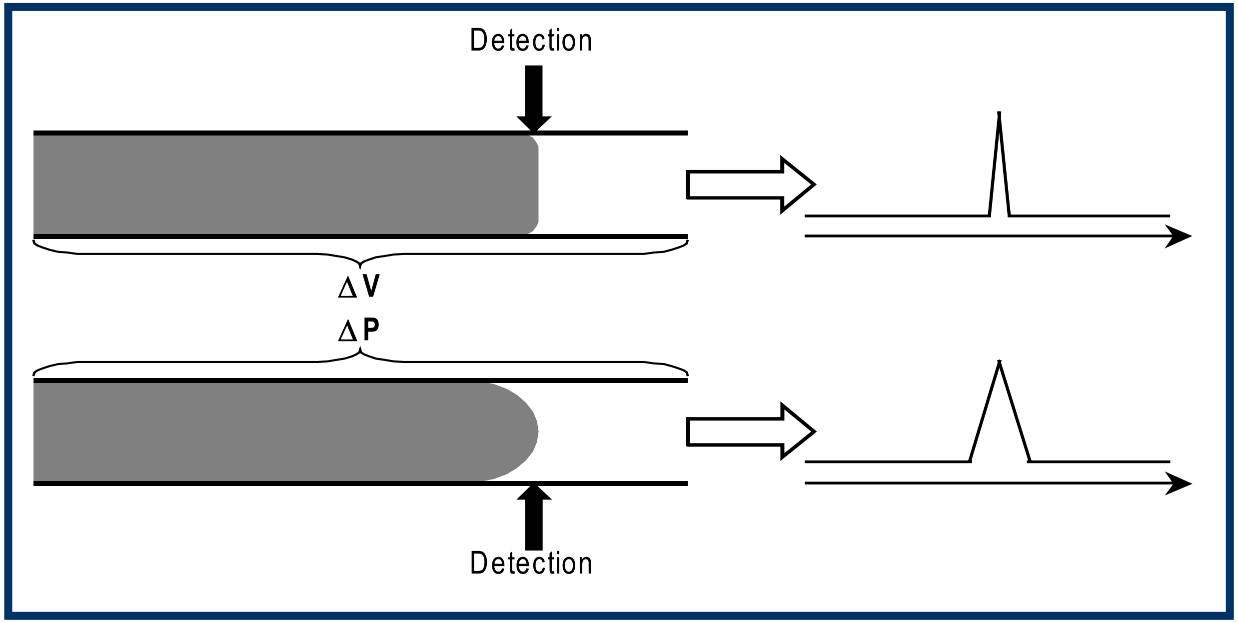
η = solution viscosity
ε = dielectric constant
ζ = zeta-potential

Control of EOF

Electric field	*EOF proportional change*
Buffer pH	*Decrease at low pH, increase at high pH*
Ionic strength and buffer concentration	*Decrease ζ-potential and EOF*
Temperature	*Changes η 3% per °C*
Organic modifier	*Decrease ζ-potential and η*
Surfactant	*Adsorbs to capillary wall (change EOF !)*
Neutral hydrophylic polymers	*Adsorbs to capillary wall*
Covalent coating	*Stability?*




LC vs CE flow profile



Electrodriven flow (EOF)
flat flow profile

Pressure driven flow (laminar flow)
parabolic flow profile

Less contribution of flow to dispersion of solutes

Potentially more efficiency

Mobility

The interplay of μ_{ep} and μ_{eof}

The observed or total mobility = sum of the mobility by electrophoresis and electroosmosis

$$\mu_{tot} = \mu_{ep} + \mu_{eof}$$

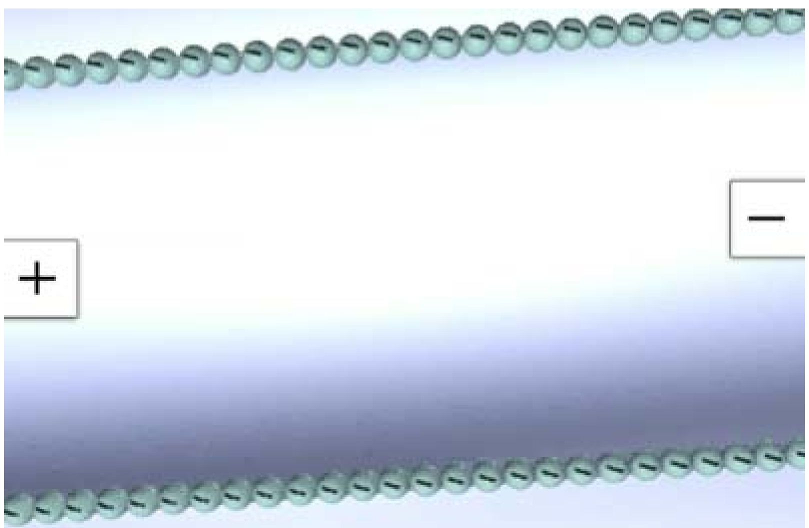
and

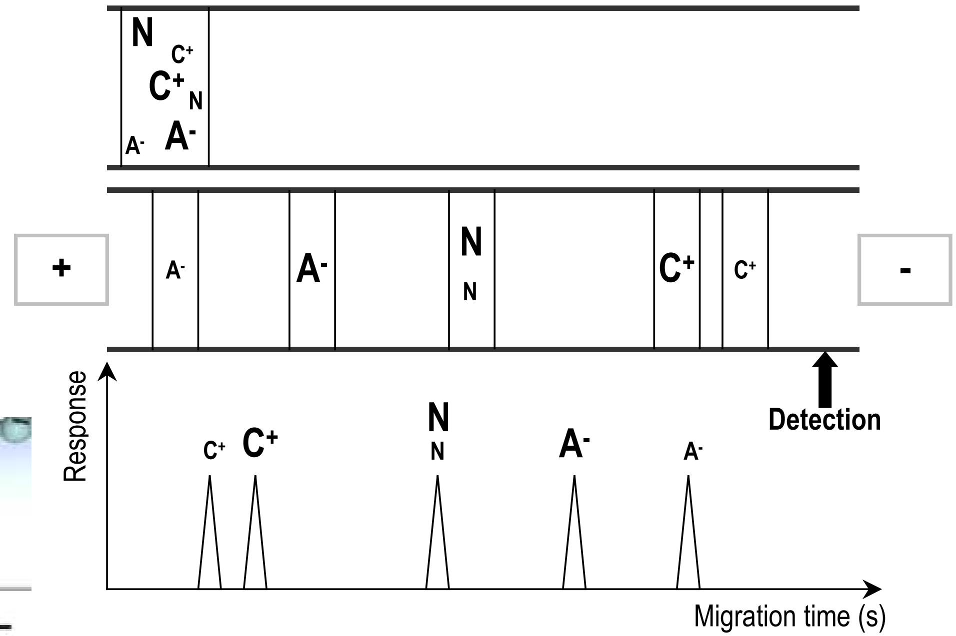
$$v_{tot} = v_{ep} + v_{eof}$$

Elution order CZE - Size

Normal polarity

= Injector at anode (+ to -)





Elution order CZE - Charge

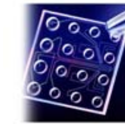
Lets consider 3 cations, 3 neutrals and 3 anions with nearly the same mass, but with different charges.

C_2^{1+}	N_1	A_2^{1-}
C_2^{2+}	N_2	A_2^{2-}
C_3^{3+}	N_3	A_3^{3-}

As function of the pH (5-9) some of them can move downstream!

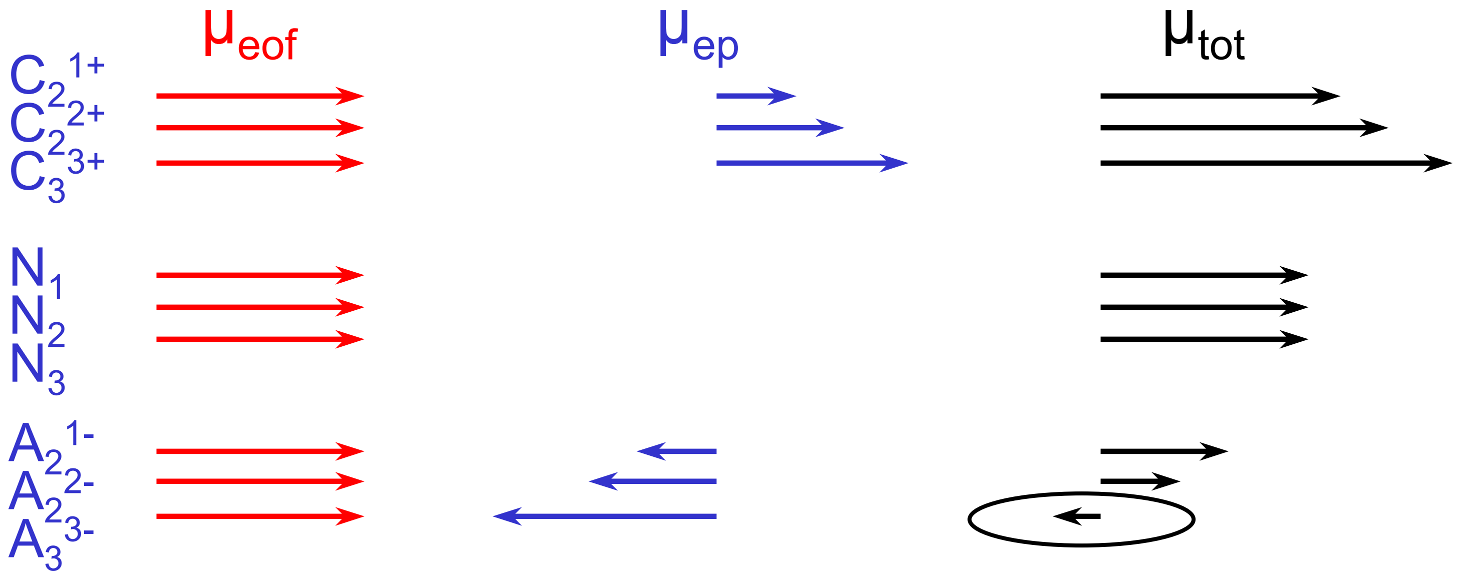
Upstream is in the direction of the EOF
Downstream is in the opposite direction of the EOF



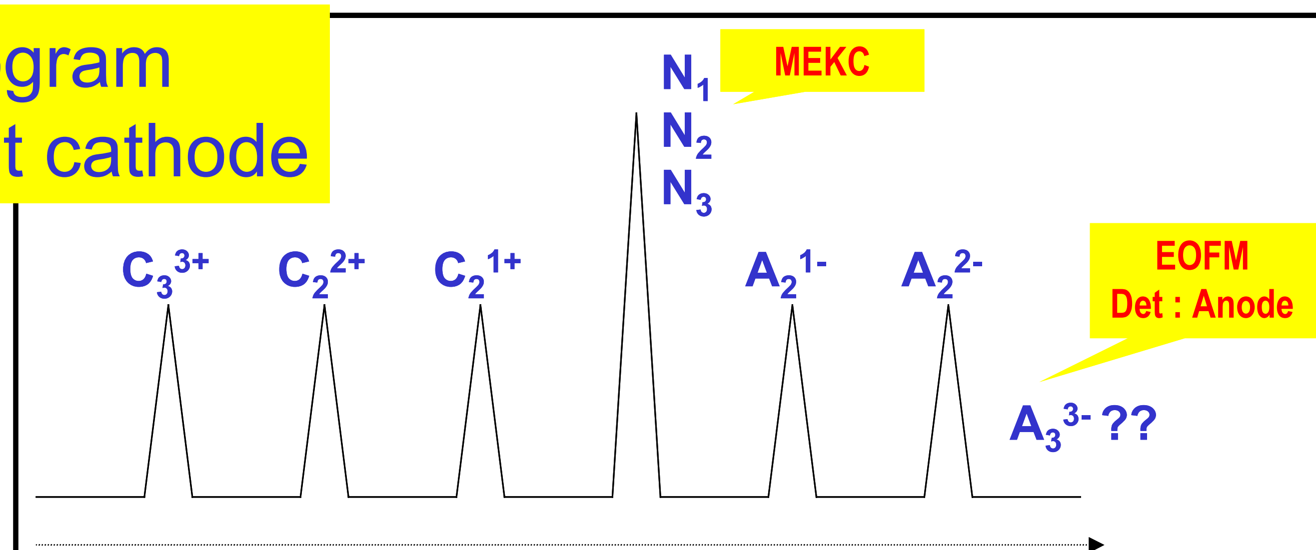



Elution order CZE - Charge

pH 7



Electropherogram
- Detection at cathode



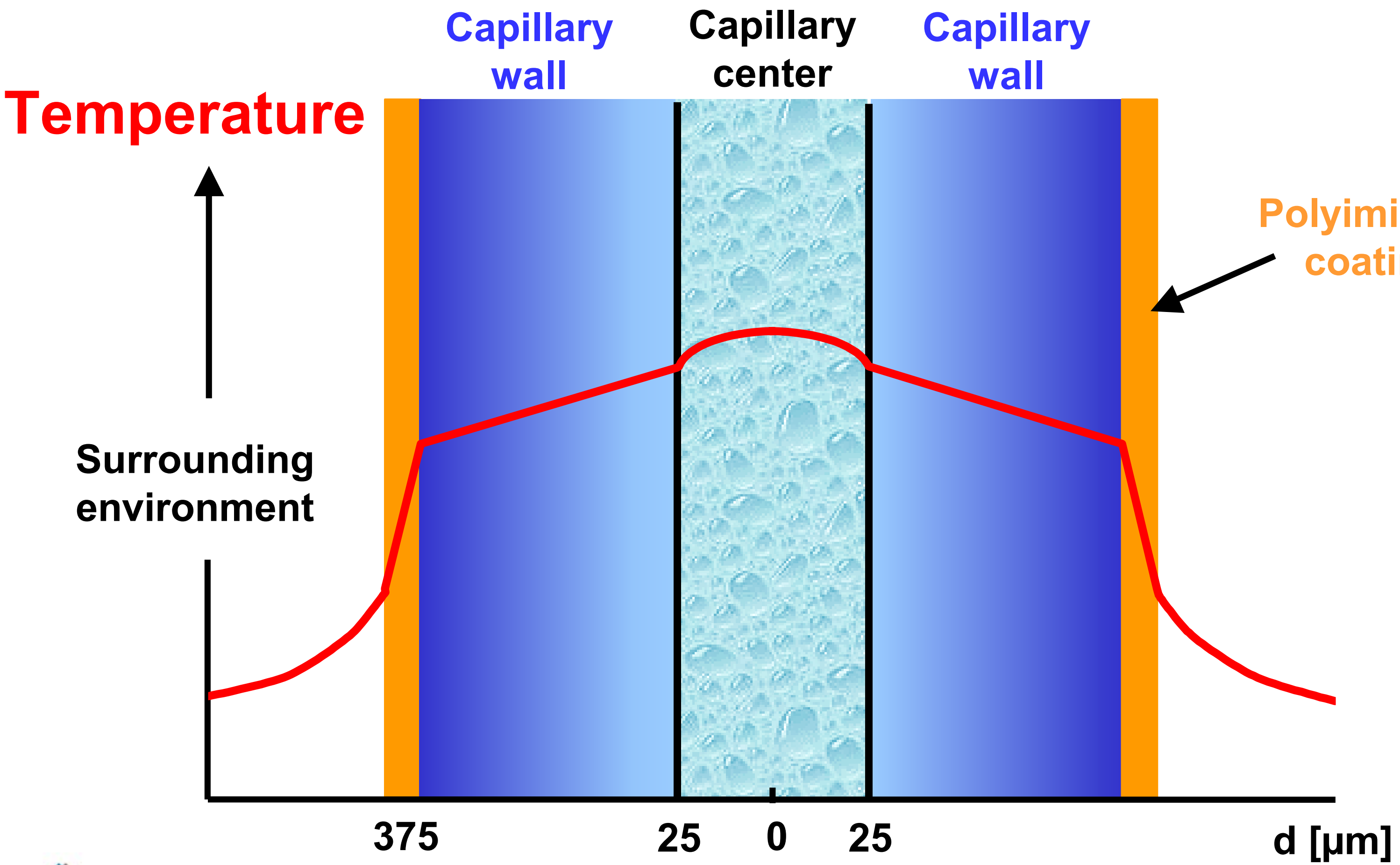
Zone broadening

Factors affecting efficiency (dispersion):

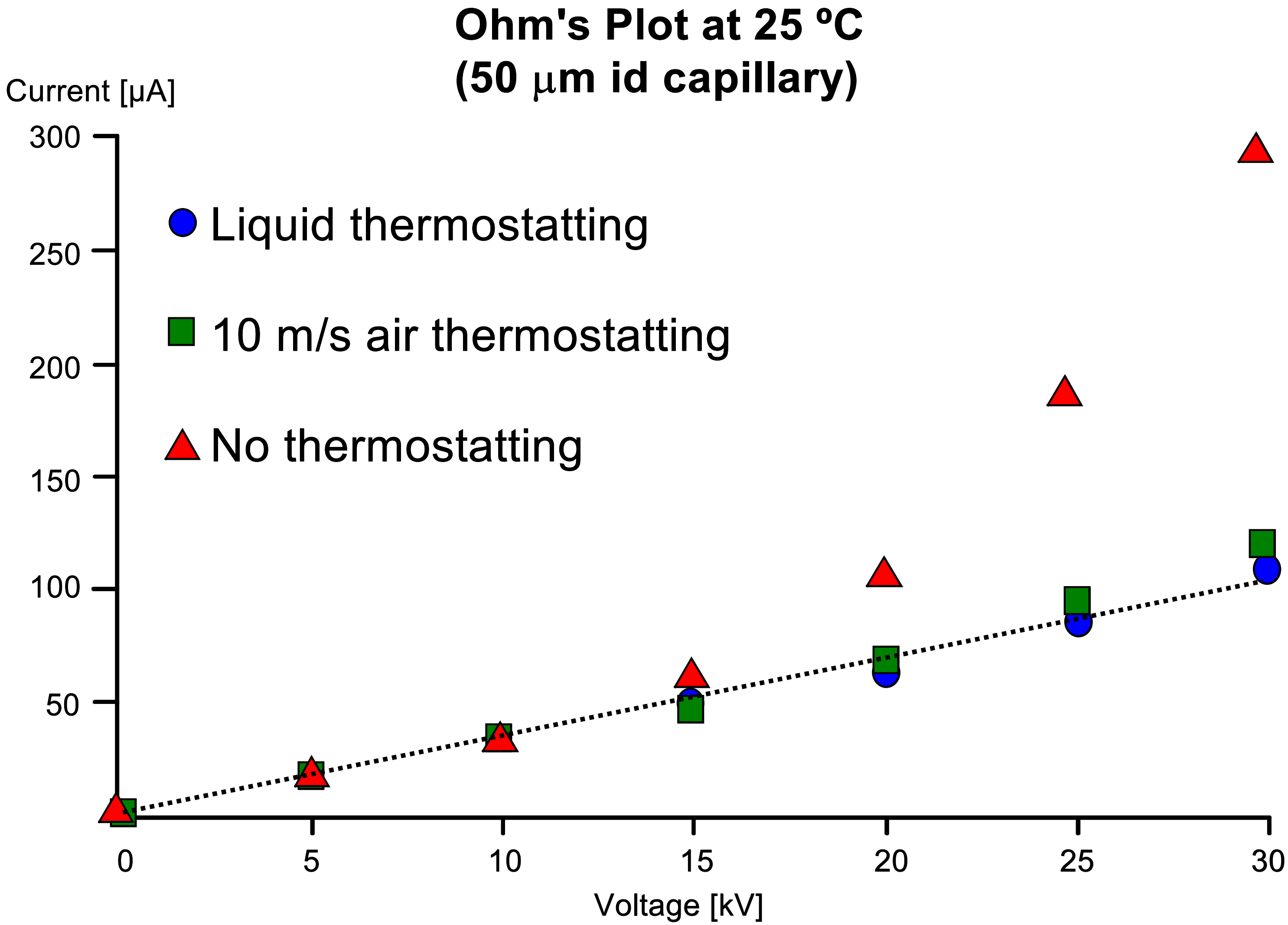
$$\sigma^2{}_t = \sigma^2{}_{LDif} + \sigma^2{}_{inj} + \sigma^2{}_{temp} + \sigma^2{}_{ads} + \sigma^2{}_{det} + \sigma^2{}_{electrodispersion} + \ldots$$

- Longitudinal diffusion
- Injection length
- Joule heating
- Adsorption on the capillary wall (solute-wall interactions)
- Detector cell size
- Mismatched conductivities of sample and buffer ions (electrodispersion)

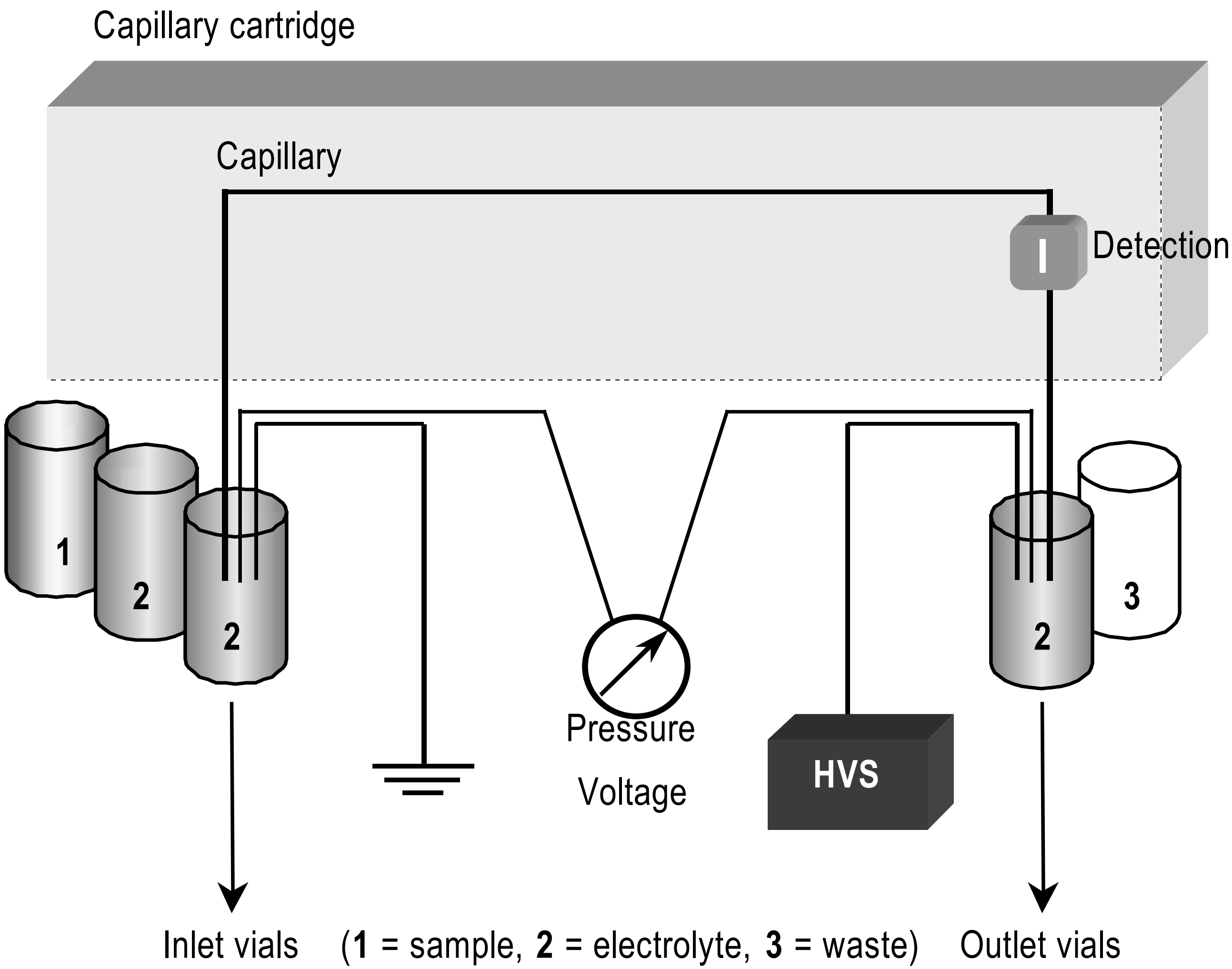
Joule heating and temperature gradient across the capillary



Capillary thermostatting



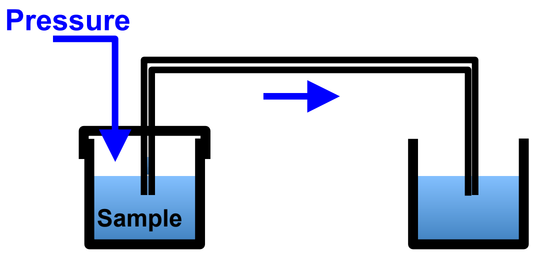
CE instrumentation



Basic components

- Column
- Injector
- Thermostat
- HVPS
- Detector
- Autosampler
- Data/software system

Sample injection methods

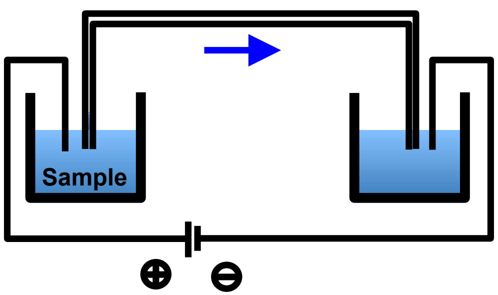


Hydrodynamic
(Pressure)

Hydrodynamic (ΔP)

$$V_{inj} = c\,\Delta P\,t$$

Non discriminating



Electrokinetic

Electrokinetic (ΔV)

$$Q_{inj} = c\,\Delta V\,t$$

Discriminating

Injection amounts

	ID	V_{inj}	q_{inj} (moles)	q_{inj} (mw = 100)
LC	4.2 mm	***10 µl***	10^{-8}	1µg
µLC	320 µm	100 nl	10^{-10}	10 ng
CZE	75 µm	***4.4 nl***	$4x10^{-12}$	440 pg
	50 µm	***2.0 nl***	$2x10^{-12}$	200 pg
	25 µm	490 pl	$5x10^{-13}$	49 pg

C_{inj} = 1 mM = 0.01 % solution if mw = 100




Detection

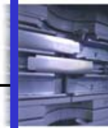
- UV-Vis: direct, indirect
- Multi wavelength: DAD
- Laser Induced Fluorescence (LIF)
- MS: electrospray (AP-ESI)

Application area

Pharmaceutical	Reaction intermediates, purity validation, stability, final product testing, ion analysis, counter-ions (includes low MW, charged and neutrals, chirals)
Bioscience	Peptides, proteins, DNA, carbohydrates
Foods	Inorganic cations/anions, organic acids, amino acids, carbohydrates
Environmental Chemical	Pesticides, PAHs, inorganic ions, transition metals, surfactants, dyes, polymers
Forensic	Drugs of abuse, explosive residue, gun powder residue

CE modes

Free solution	Separation media
CZE (capillary zone electrophoresis)	**CGE** (capillary gel electrophoresis)
CIEF (capillary isoelectric focussing)	**MEKC** (micellar electrokinetic chromatography)
CITP (capillary isotachophoresis)	**CEC** (capillary electrochromatography)




Applications CE

- **CZE**, Capillary zone electrophoresis
  - Separation by **electrophoresis** in free solution

- **MEKC**, Micellar electrokinetic chromatography
  - Separation by **electrophoresis and distribution** between mobile and **pseudostationary phase**

- **CEC**, Capillary electrochromatography
  - Separation by **electrophoresis and distribution** between mobile and **stationary phase**

CZE - Applications

Capillary Zone Electrophoresis (CZE)

Applications : ...

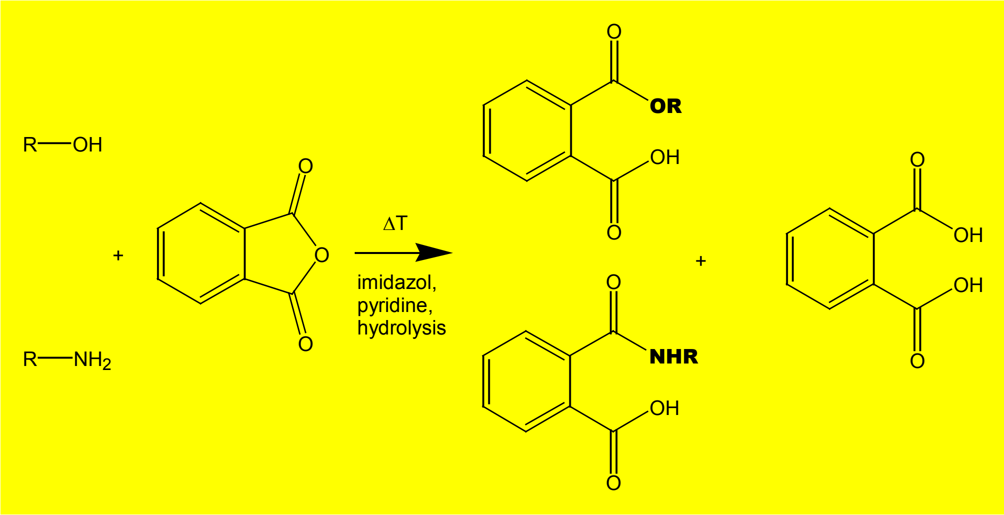
ENDLESS

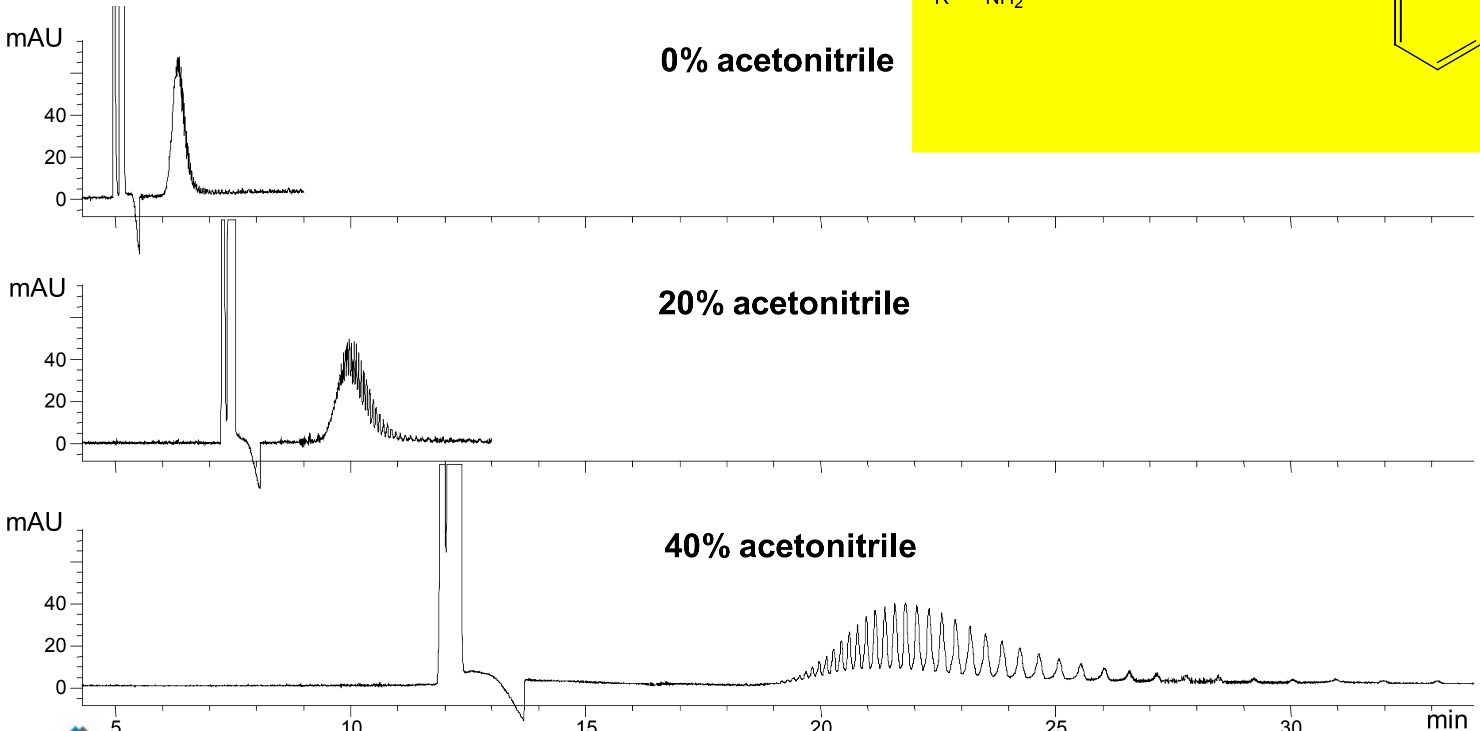
... but charge required!

CZE – Low MW Polymers

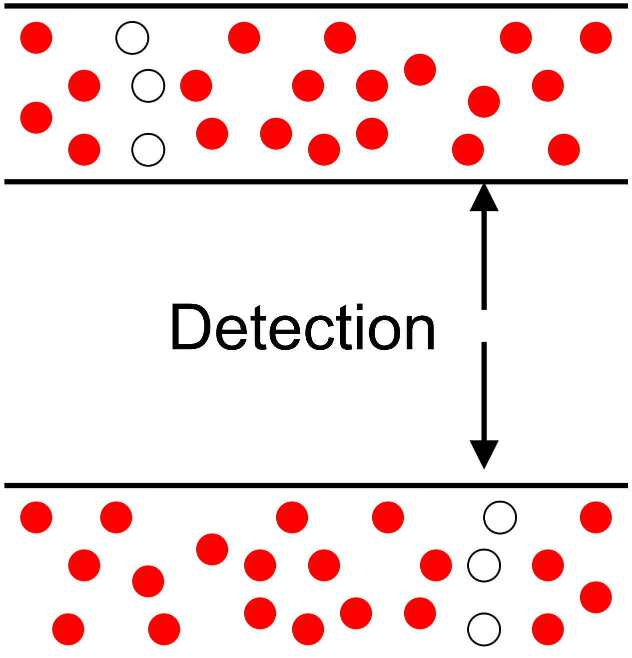
Derivatized PEG 2000 (2 mM)

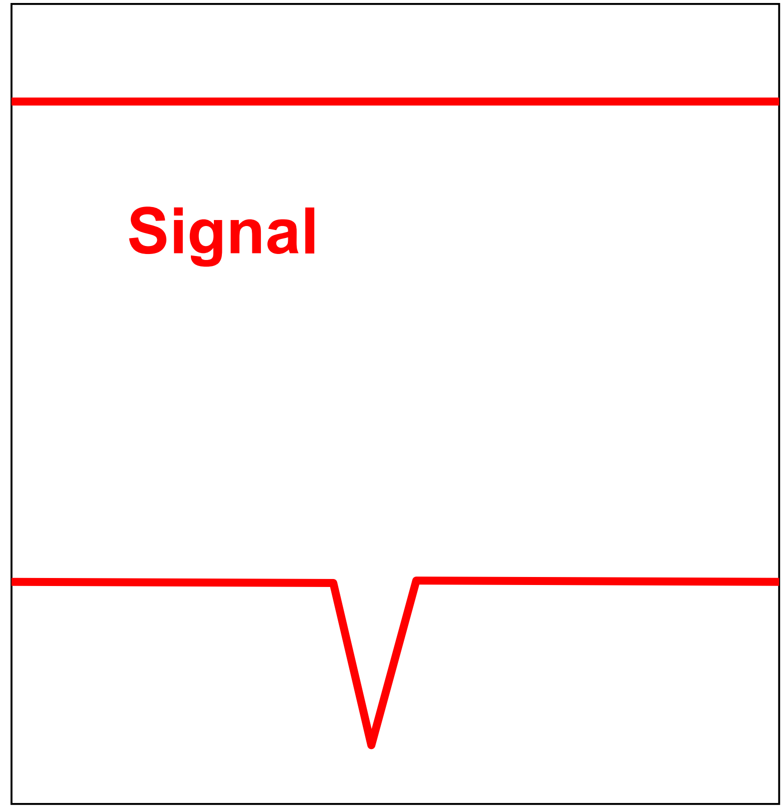
Buffer: 210 mM Tris pH 8.7
in 0, 20, and 40% ACN





Indirect detection

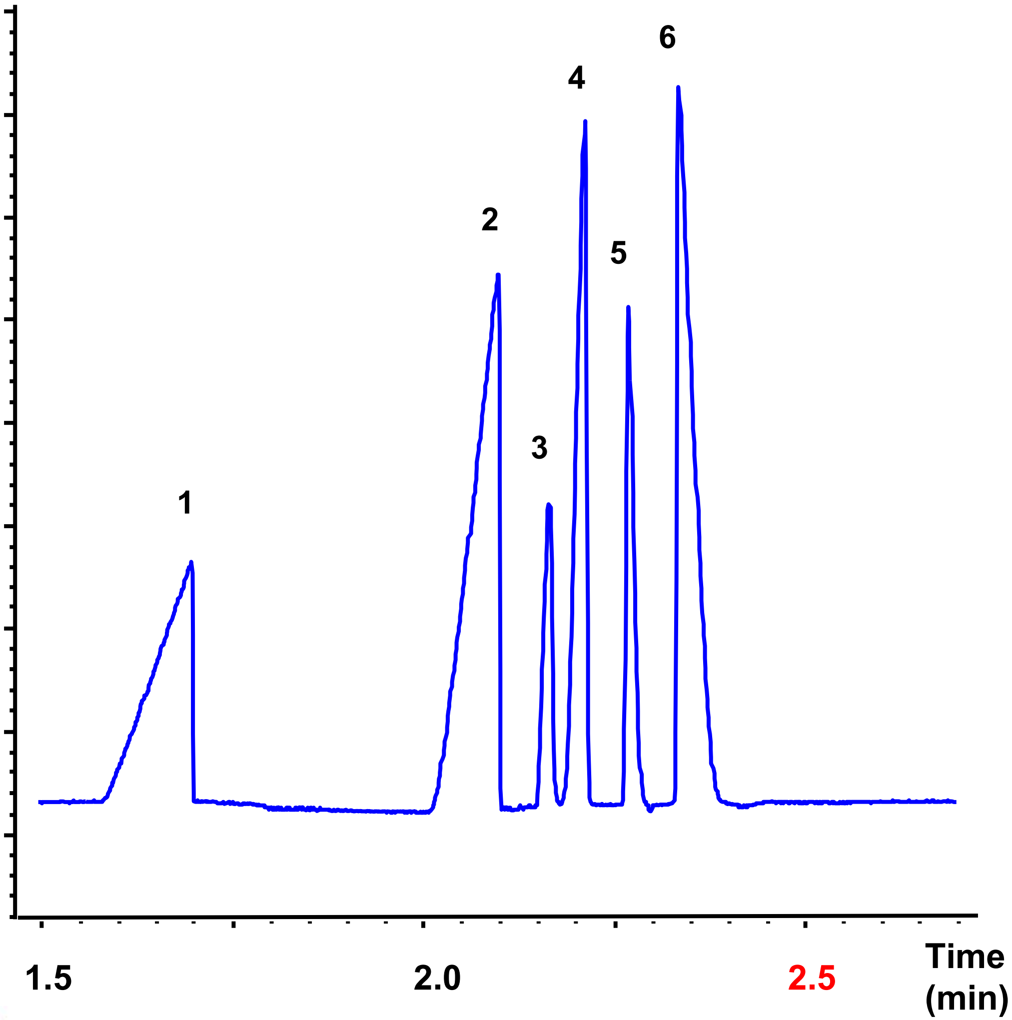




● = BGE (background electrolyte), UV absorbing

o = Analyte ion, UV inactive

Peak shape cations (indirect UV)

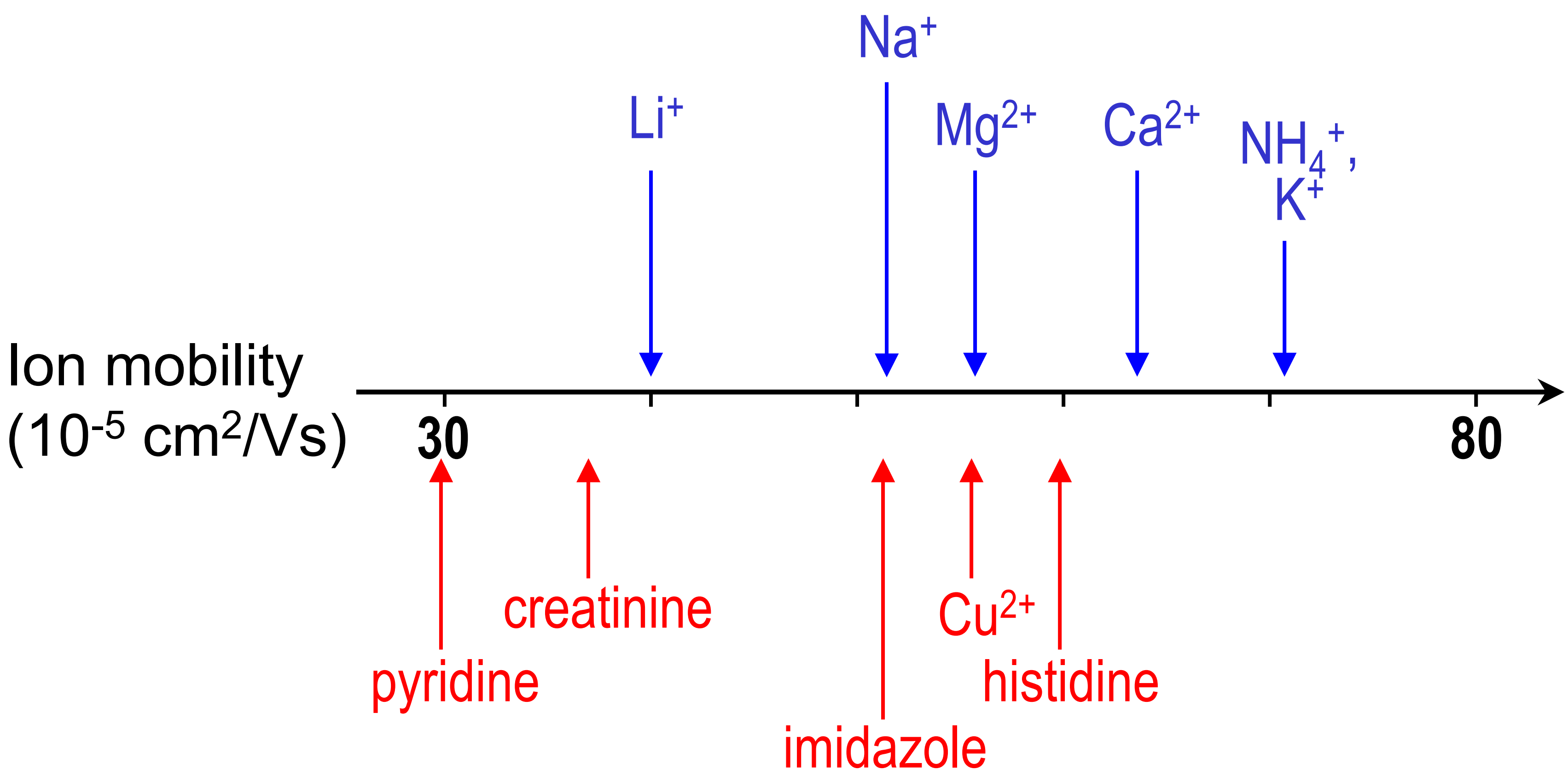


[Salt] = 100 ppm

1 =K^+
2 =Na+
3 =Ba^{2+}
4 =Ca^{2+}
5 =Mg^{2+}
6 =Li^+

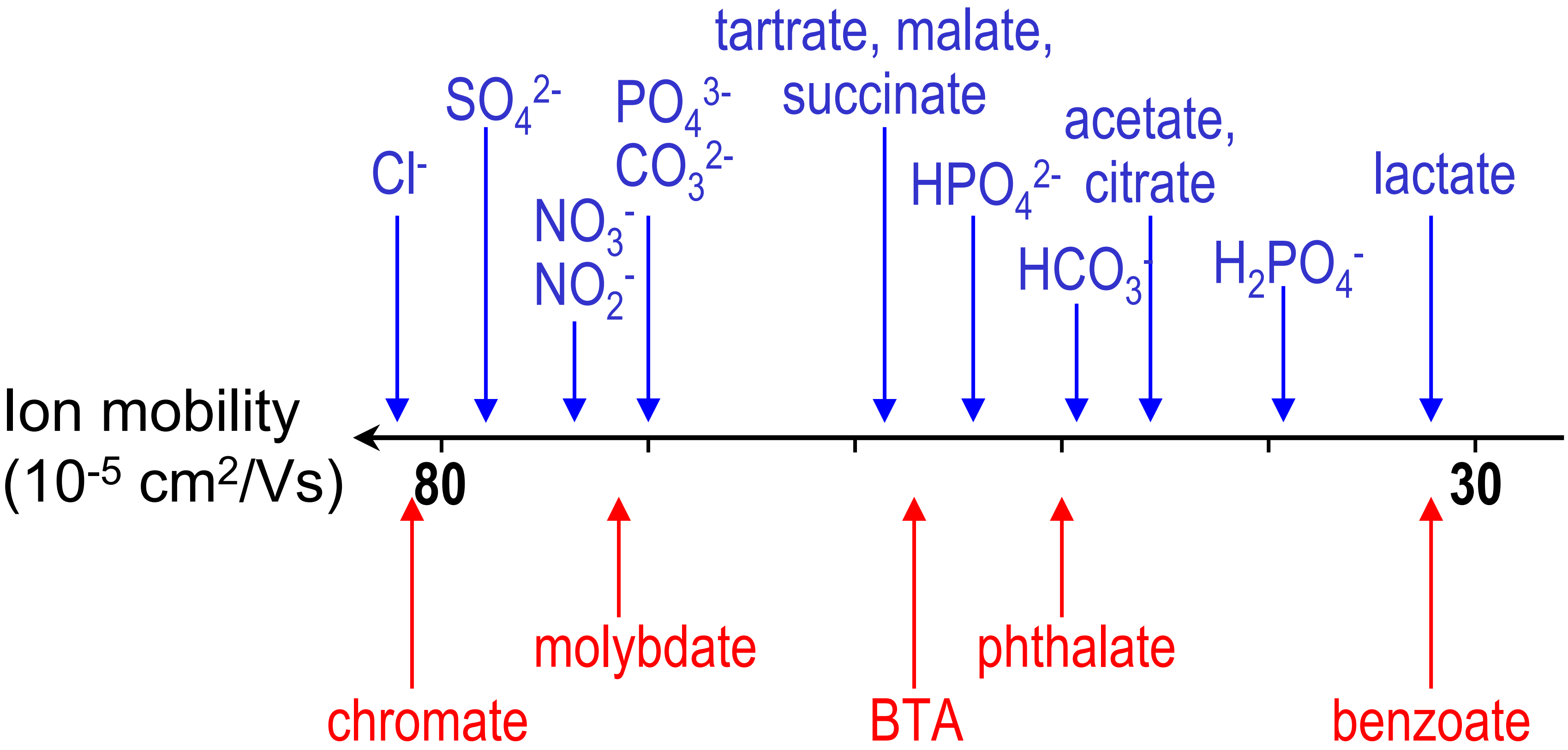
Buffer:
5 mM p-amino pyridine, pH 5.8
Conditions:
Positive Polarity
L = 56/64.5 cm
ID =75 µm
Temp 35 °C
Indirect UV Detection

Choice BGE - Cations



Reference: V. Pacáková et al., Electrophoresis 24 (2003), 1883-1891.

Choice BGE - Anions



Reference: V. Pacáková et al., Electrophoresis 24 (2003), 1883-1891.

Organic Acids

- Capillary wall is normally negatively charged (silanol)
- Wall needs to be positively charged
  (quaternary ammonium salts, formation of a double layer at capillary wall)
- Reversed polarity
- Acids and EOF move in the same direction

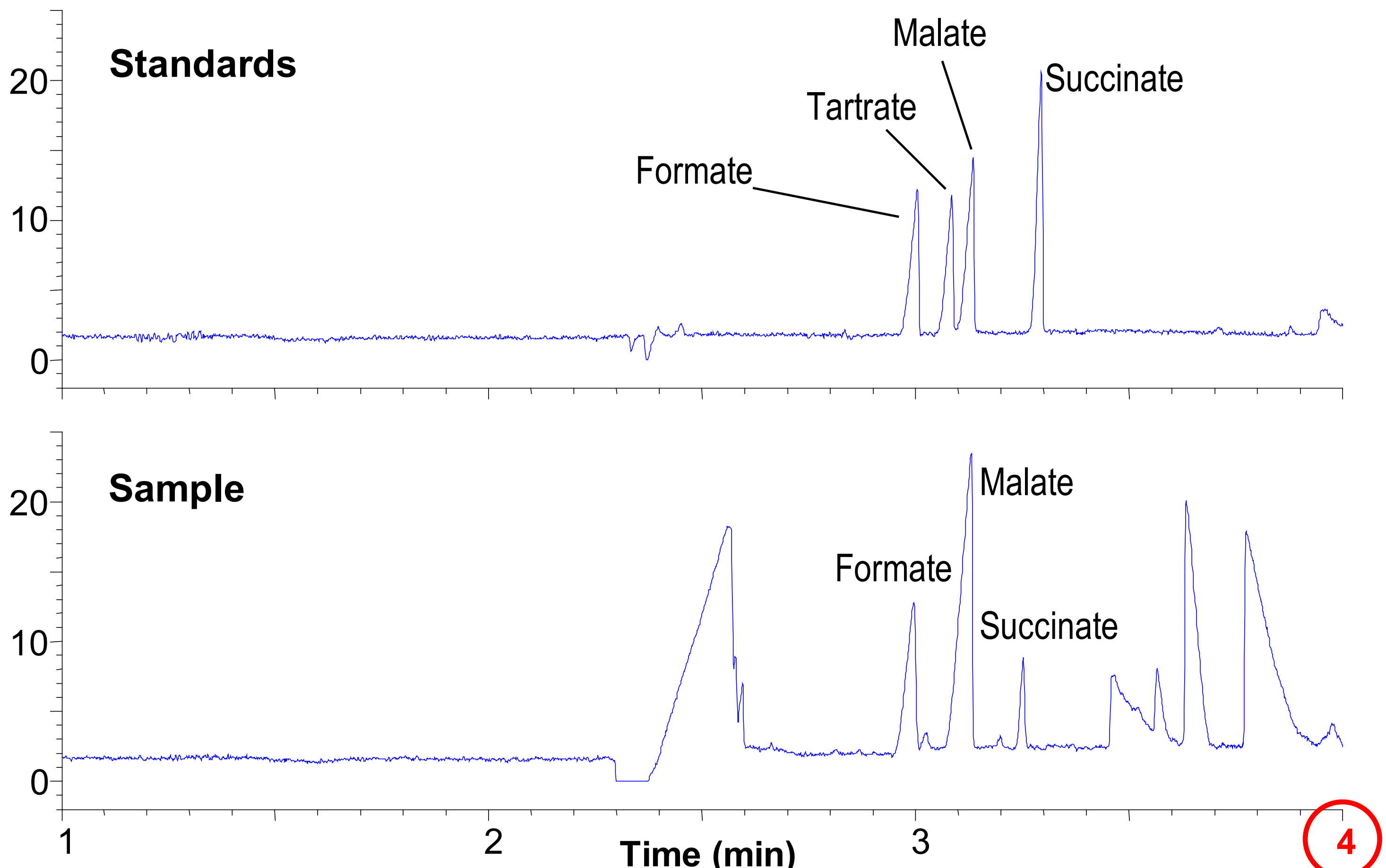
Short analysis time!!

Analysis of Organic Acids

Standards: ca. 20 ppm each

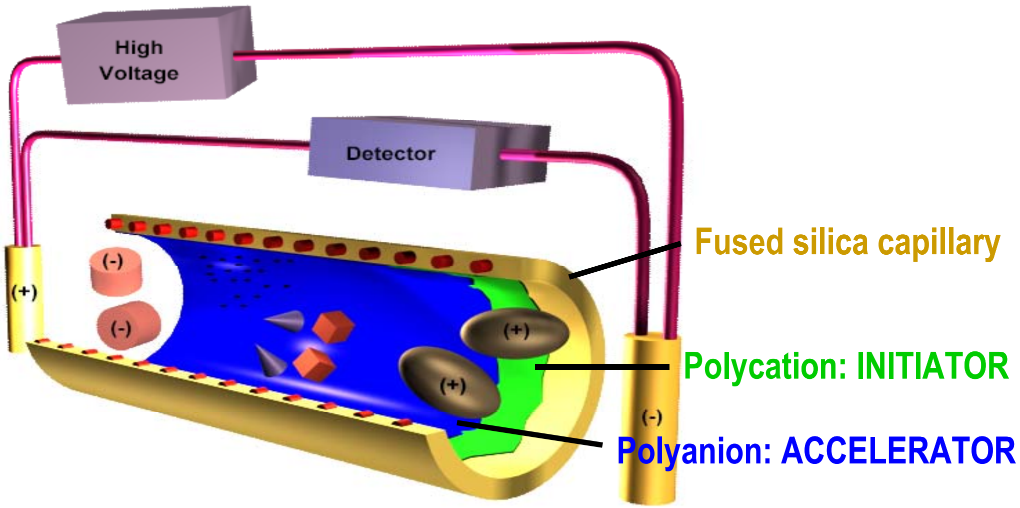
Sample: filtrate of sugar refinerey juice, diluted 1/100 in water

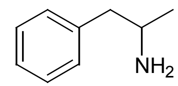
mAU



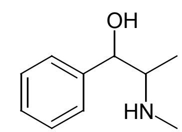
Reference: Lalljie S., Vindevogel J., Sandra P., J. Chromatogr. A, 652 (**1993**) 563-569.

Dynamic coating for fast and reproducible CE analyses

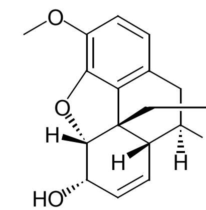




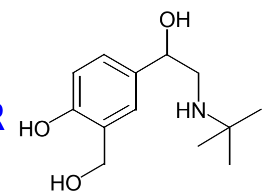
Amphetamine



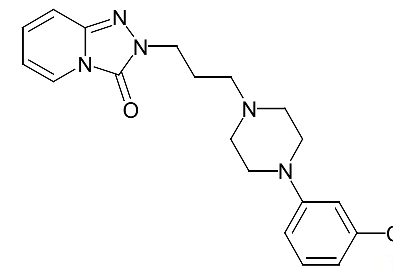
Ephedrine



Codeine



Salbutamol



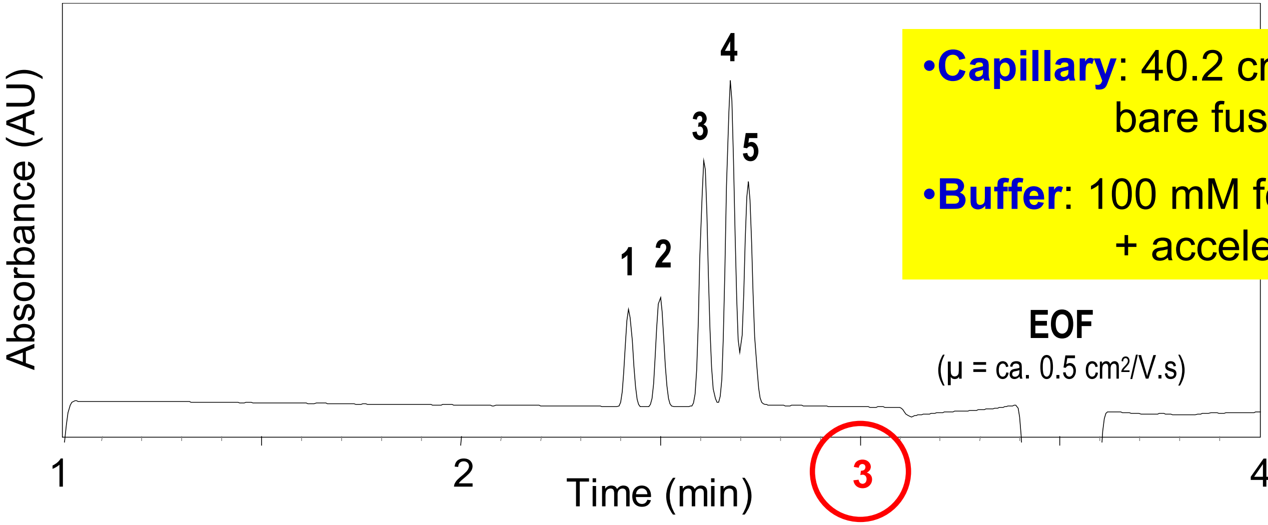
Trazodone

Ceofix™ (Analis)

Wall coated with high amount of negative charges (accelerator)

High and stable EOF at low pH

CE-DAD Basic Drugs



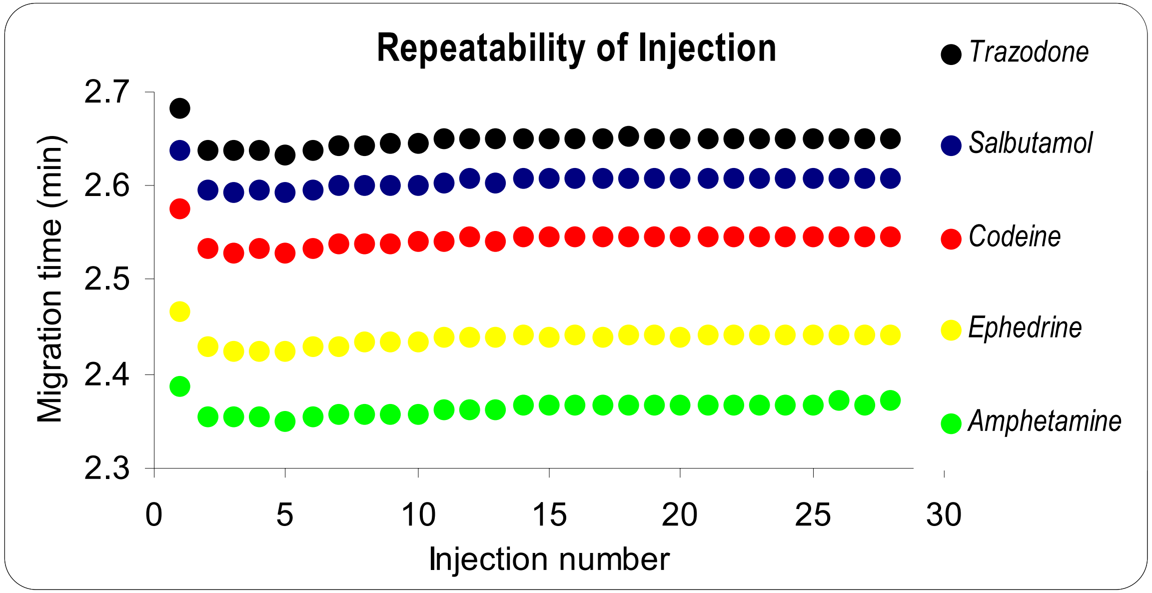
•**Capillary**: 40.2 cm (L_{tot}), 30.2 cm (L_{eff})
bare fused silica, 75 µm I.D.

•**Buffer**: 100 mM formic acid + trimethylamine
+ accelerator

RSD (*n*=28):

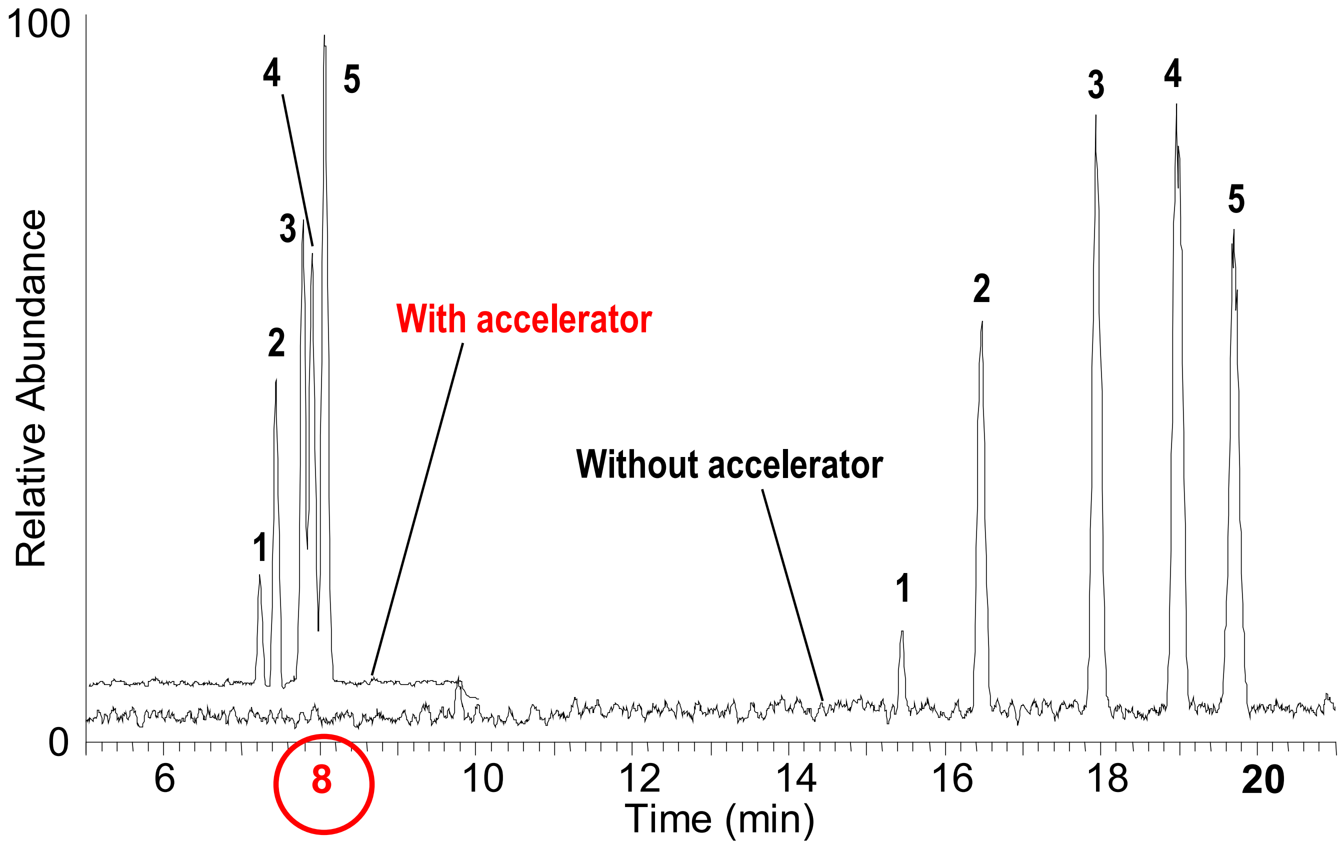
•Migration time: **< 0.25%**

•Area: **< 2.40%**



CE-MS Basic Drugs

- **Capillary**: 93.5 cm (L_{tot})
- **Buffer**: 100 mM formic acid + 1 mM TFA (+ accelerator)

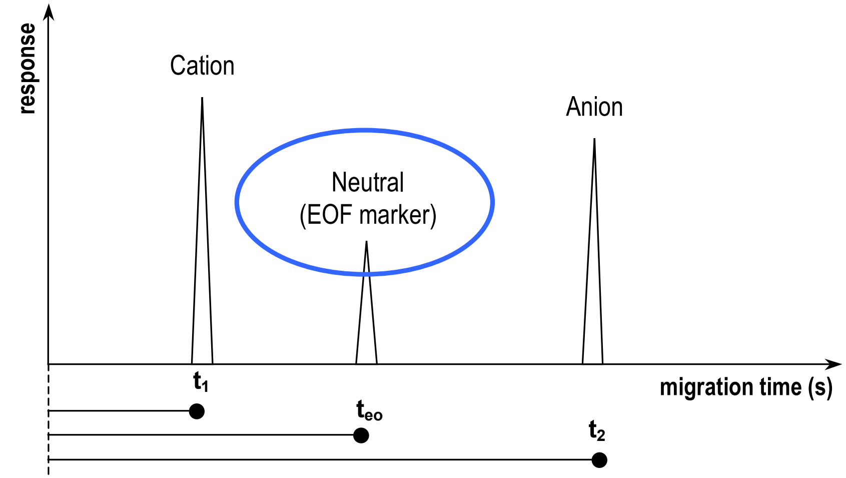


MEKC - Applications

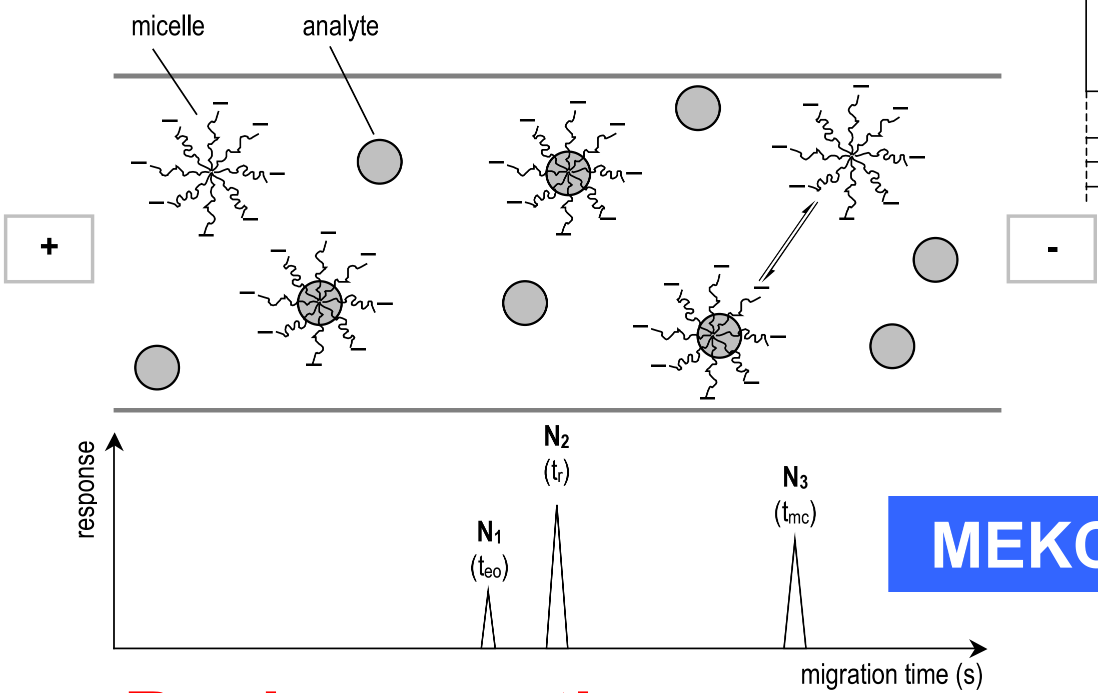
ENDLESS

Neutral <u>and</u> charges species

MEKC - principle



CZE



MEKC

Basic operation:

High pH, SDS micelles, Positive polarity

Surfactants MEKC

		CMC (mM)	Aggregation number
Anionic	SDS	8.2	62
Cationic	DTAB	14	50
	CTAB	1.3	78
Non Ionic	Octylglucoside	---	---
	n-Dodecyl-b-D-maltoside	0.16	---
	Triton X-100	0.24	140
Zwitterionic	CHAPS	8	10
	CHAPSO	8	11
Bile Salt	Cholic acid	14	2 – 4
	Deoxycholic acid	5	4 – 10
	Taurocholic acid	10 –15	4



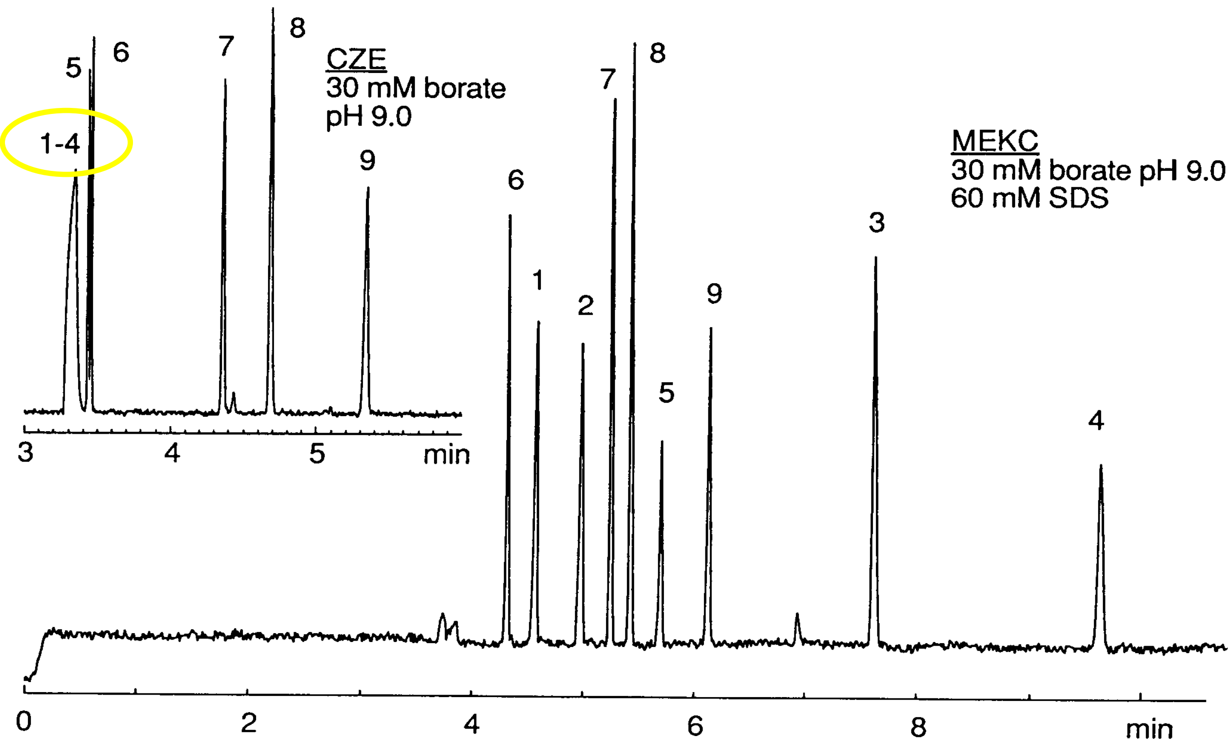

MEKC - Resolution

Factors affecting resolution in MEKC through

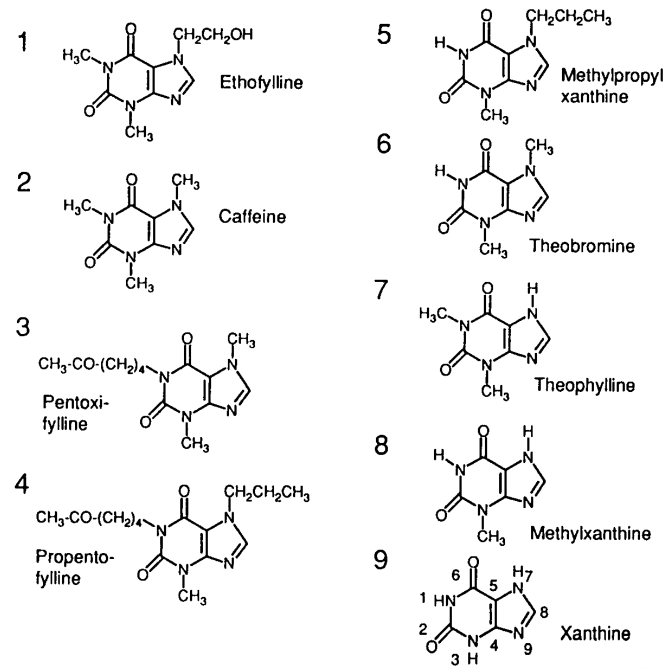
N, α, k, K, β and t_0 / t_{MC}

- ***pH***
- ***Buffer*** *type, concentration, strength*
- ***Micelles*** *type, concentration*
- ***Organic modifier*** *short or long chain*
- ***Wall coating***
- ***Temperature***
- ***Applied voltage***

MEKC - Xanthines



Nonionic **Ionic**



High efficiency: ***N = 130 000 – 400 000***

MEKC – Beer Bitter Acids

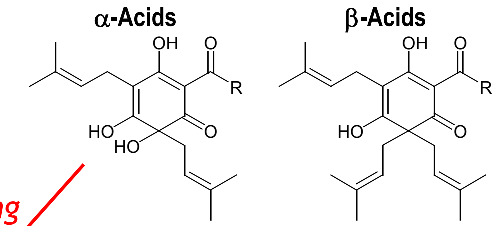
- **Present in hops**
  Humulones, lupulones
  Tasteless
  (Nearly) not in beer

- **Present in beer**
  Isohumulones
  Bitter tasting
  Lightstruck flavour

- **Reduced iso-a-acids**
  Dihydro-, tetrahydro-isohumulones
  Added to beer for taste
  and foam stability
  ('Reinheitsgebot')

R =		Prefix:
	$-CH(CH_3)_2$	co-
	$-CH_2CH(CH_3)_2$	-
	$-CH(CH_3)CH_2CH_3$	ad-

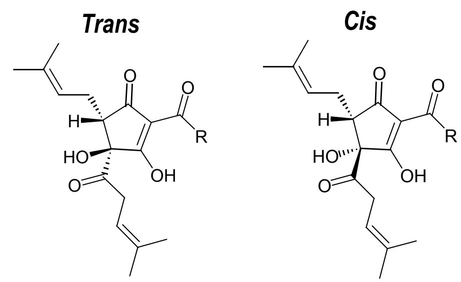
#6
Hop acids (A, B)



A1 = cohumulone
A2 = adhumulone
A3 = humulone
B1 = colupulone
B2 = adlupulone
B3 = lupulone

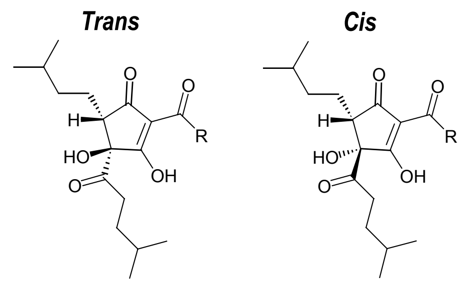
Isomerization during brewing

#6
Iso-α-acids (IAA)



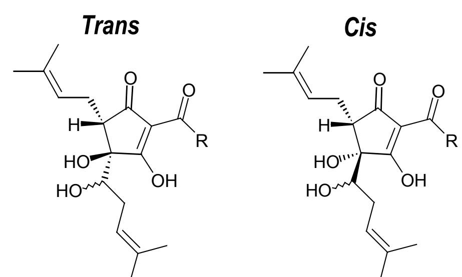
IAA1 = *cis*-isocohumulone
IAA2 = *trans*-isocohumulone
IAA3 = *cis*-isoadhumulone
IAA4 = *cis*-isohumulone
IAA5 = *trans*-isoadhumulone
IAA6 = *trans*-isohumulone

#6
Tetrahydro-iso-α-acids (TH)



TH1 = *cis*-TH-isocohumulone
TH2 = *trans*-TH-isocohumulone
TH3 = *cis*-TH-isoadhumulone
TH4 = *cis*-TH-isohumulone
TH5 = *trans*-TH-isoadhumulone
TH6 = *trans*-TH-isohumulone

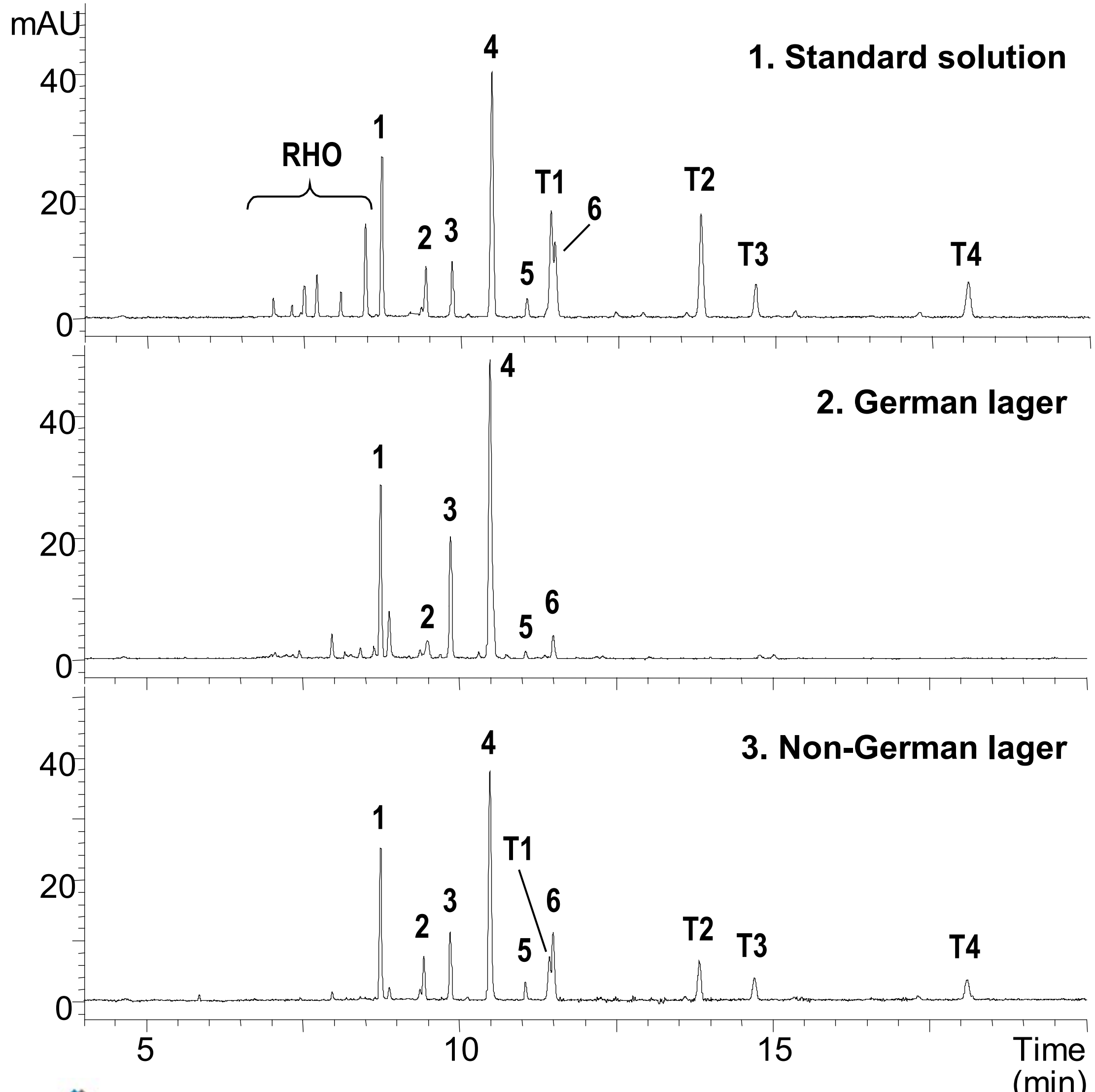
#12
Dihydro-iso-α-acids (DH)



DH1 = *cis*-DH-isocohumulone
DH2 = *cis*-DH-isocohumulone
DH3 = *cis*-DH-isoadhumulone
DH4 = *cis*-DH-isohumulone
DH5 = *cis*-DH-isoadhumulone
DH6 = *cis*-DH-isohumulone

Possible number of relevant acids in beer = 24

MEKC – Beer Bitter Acids



SBSE-LD extracts

(*Stir Bar Sorptive Extraction*
– *Liquid Desorption*)

Detection of added (non-natural) reduced Iso-alpha-acids (Rho and T)

Buffer:

phosphate pH 10.2, SDS

CEC - Applications

- **Stationary phases:** C18, C8, Phenyl, C18/SAX mixed phase
- **N in CEC vs HPLC**
  - N_{CEC} higher (flat vs laminar flow profile)
  - No restrictions in column length and particle size

$N \sim L/2d_p$

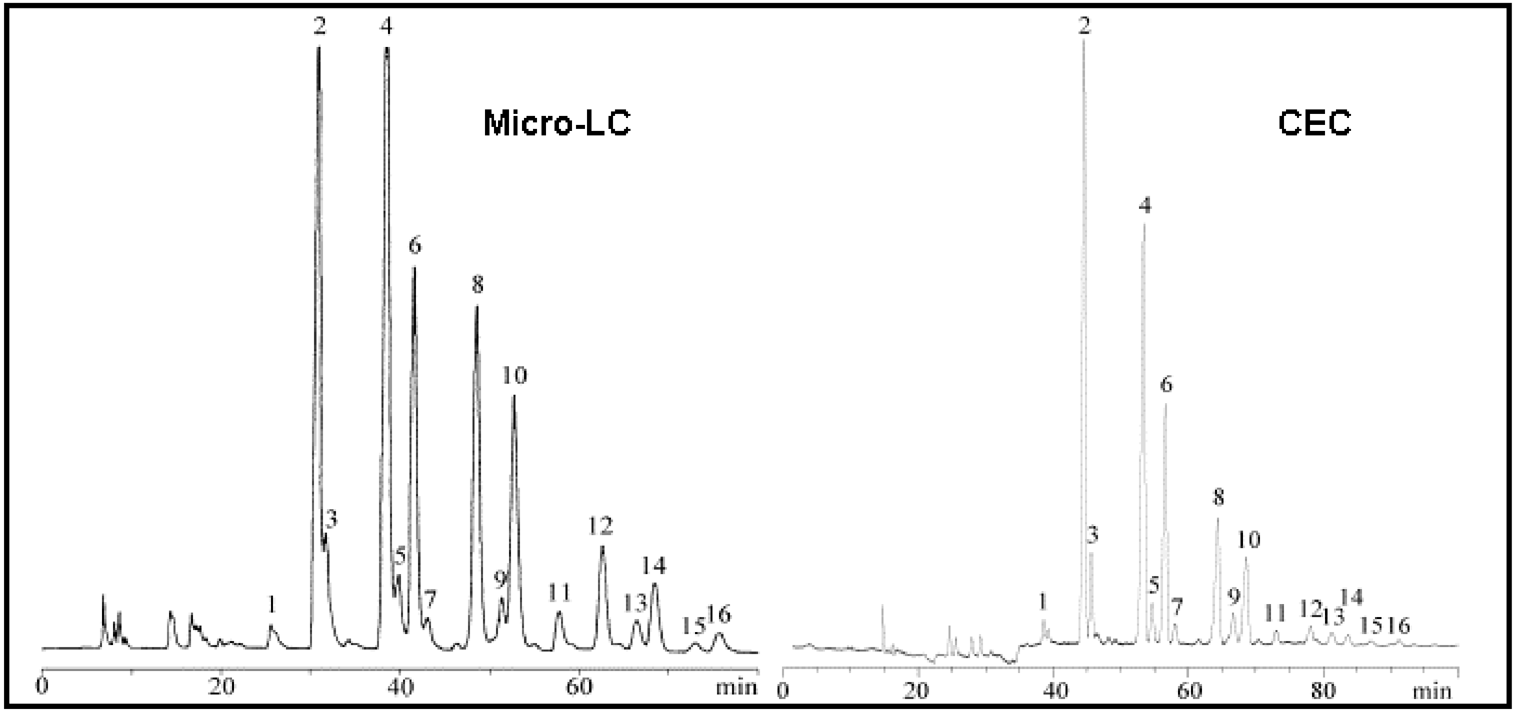
HPLC:	L = 100-250 mm, d_p = 3-10 µm
CEC:	L = 250-750 mm, d_p = 1-3 µm

- **Separation**: combination of partitioning and electrophoresis (additional selectivity compared to HPLC)

CEC – Triglycerides in Corn Oil

Micro-LC: Column: 2x250 mm x 1 mm I.D., C18, 5 µm;
mobile phase: ACN/IPA/n-hexane (57/38/5); detection: ELSD

CEC: Column: 400(485) mm x 100 µm I.D., C18, 3 µm;
mobile phase: ACN/IPA/n-hexane (57/38/5)-**50 mM NH_4OAc**; detection: UV 200 nm



1 = LLLn
2 = LLL
3 = OLnL
4 = OLL
5 = OLnO
6 = PLL
7 = POLn
8 = OLO
9 = SLL
10 = PLO
11 = PLP
12 = OOO
13 = SLO
14 = POO
15 = PLS
16 = POP