IOCB SERVICE DAYS 2023

Presentation of services by:
Research-Service Groups / Service Groups / Core Facilities

Electromigration Methods

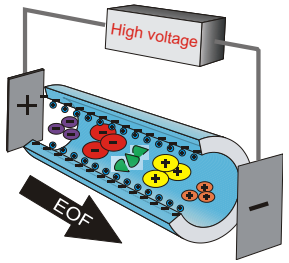
Václav Kašička

ÚOCHB AV ČR
IOCB PRAGUE

Electromigration Methods

Research-Service Group

Analytical Chemistry & Separation Sciences, cluster PHYS



People

Head: Václav Kašička

Scientists: Dušan Koval
Petra Sázelová
Veronika Šolínová
Sille Štěpánová (0.8)
Jana Jaklová (0.1)

Sci. Assist: Renáta Konášová (0.6)

PhD. Students: Maria Butnariu
Ishak Kovač (0.2)

Location

Building A, S-E wing, 2nd floor, A 2.91

ÚOCHB AV ČR, v.v.i.

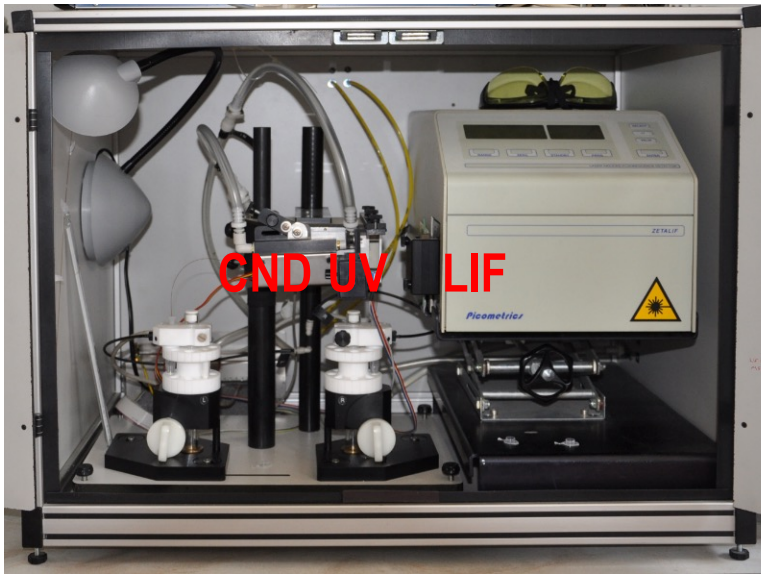
Capillary Electromigration (CE) methods

Electrophoretic:

- Zone Electrophoresis (CZE (FSCE, CGE))
- Isotachophoresis (CITP)
- Isoelectric focusing (CIEF)
- Affinity electrophoresis (ACE)

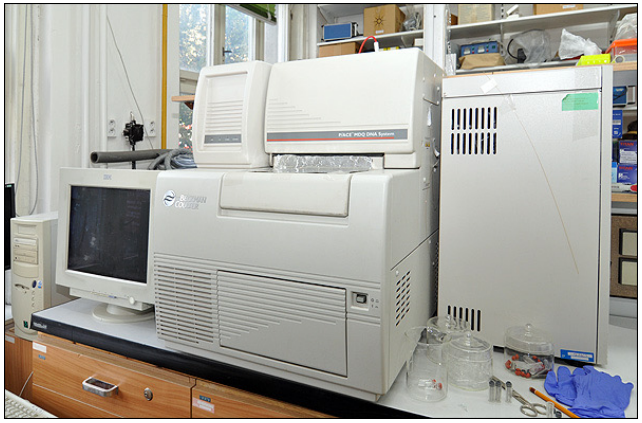
Electro-chromatographic:

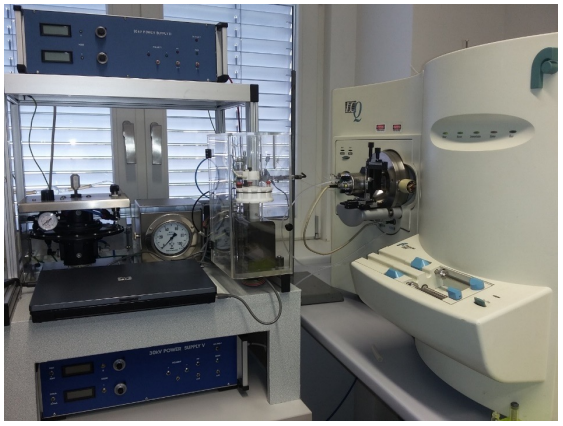
- Electrokinetic chromatography (CEKC)
- Electrochromatography (CEC)



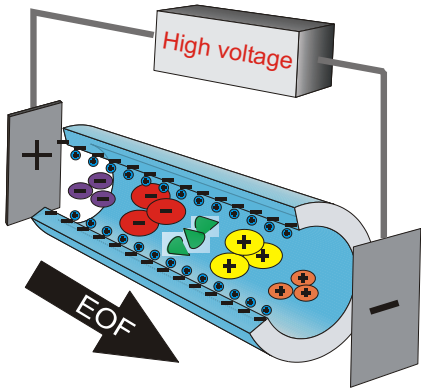
Instrumentation

- **Two CE analyzers 7100 Agilent**, fully automated, UV-vis & CND detection
- **Two CE analyzers MDQ Beckman Coulter**, fully automated, UV-vis or LIF detection
- **Two home-made CE devices**, semi-automated, CND, UV, LIF or MS detection





CE methods = high performace techniques (HPCE)



High: Separation efficiency: 10^5–10^6 theor. plates/m

Sensitivity: fmol–amol in nL–pL inj. volume; µM-nM

Typical samples: 1-100 µM, 5-20 µL, 100 µg

Speed: 5-20 min

Selectivity: charge, size, shape, mobility | affinity, hydrophobicity

Flexibility: CZE, CITP, CIEF, CGE | ACE, CEKC, CEC

Applicability: ionogenic, electroneutral, LMW, HMW, (bio)particles,

Biocompatibility: free aqueous buffered solution

Medium: Concentration sensitivity: µmol/L

Repeatability: 1–5 %

Robustness

Low: Pressure: mbar–10 bar

Running costs: 5–10 EUR / 1 m capillary

Environmental pollution: mostly in aqueous media – green methods

Provided services/collaborations (1)

Qualitative and quantitative analysis

- Quality control: determination of chemical purity degree
- Monitoring of chemical & enzymatic reactions, isolation, purification
- Determination of LMW ionic admixtures (salts)
- Determination in (bio)matrices: biofluids, tissue extracts, reaction mixtures
- Separation of complex mixtures: peptide mapping, bottom-up proteomics
- Separation of stereoisomers: chiral analysis, monitoring of racemization

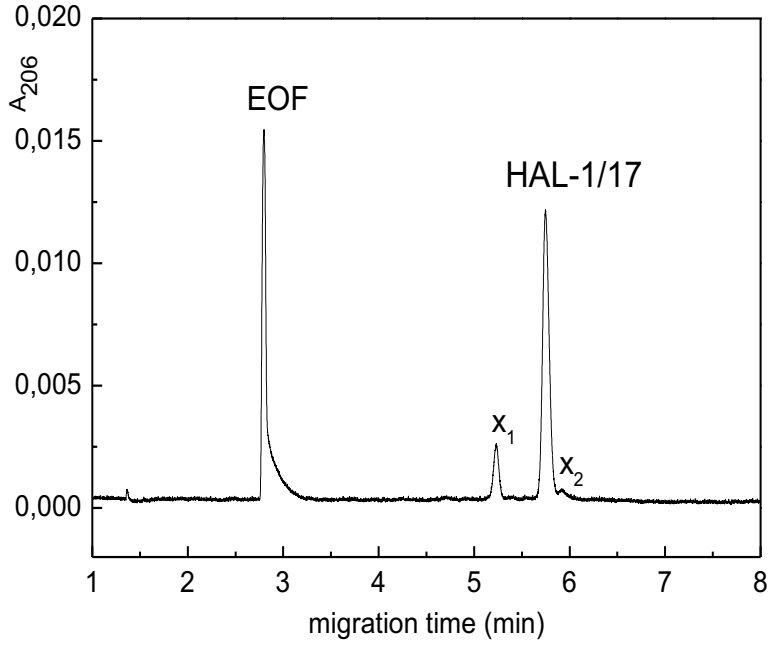
Analyzed (bio)molecules and (bio)particles:

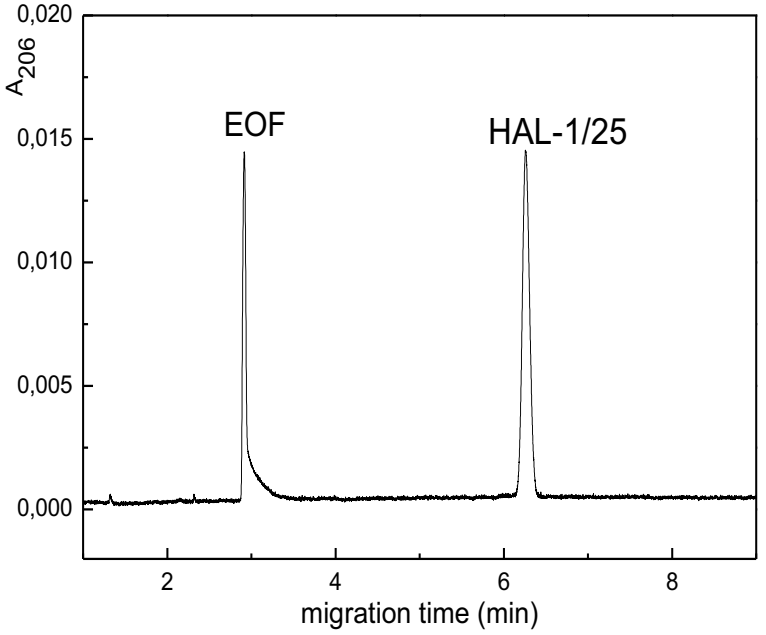
- Small organic & inorganic ions and molecules: acids, amino acids, metal ions, (nucleo)bases, nucleosides, nucleotides, monosaccharides, steroids, etc.
- Medium size (bio)molecules: peptides, oligonucleotides, oligosaccharides, helquats
- Large (bio)molecules: proteins, polynucleotides, DNA/RNA fragments, polymers
- (Bio)particles: viruses, bacteria, cells, cell organelles, nanoparticles

Soluble in: buffered solutions in water or organic solvents (MeOH, EtOH, IPA, ACN) or mixed hydro-organic solvents

CZE analysis of antimicrobial peptides

Derived from: H-Gly-Met-Trp-Ser-Lys-Ile-Leu-Gly-His-Leu-Ile-Arg-NH_2 (+4e)





Halictine analog: KMWSKILGHLIR-NH_2, +5e

Halictine fragment: WSKILGHLIR-NH_2, +4e

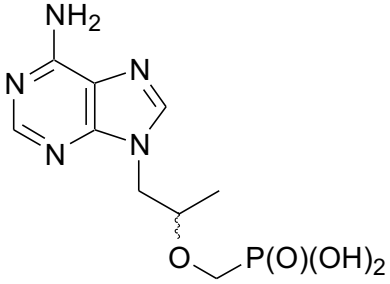
CE: home-made IOCB, capillary: **FS dynamically coated with DDAB**, 50 μm x 31/20 cm
Detection: UV-abs @ 206 nm, BGE: 25/30 mM Tris/H_3PO_4, 0.1 mM DDAB, pH 2.8, **-12 kV**, 35 μA
Sample: 100 μg peptide/100 μL BGE, hydrodynamic injection, 500 Pa, 20 s

CE of enantiomers of acyclic nucleotide phosphonates

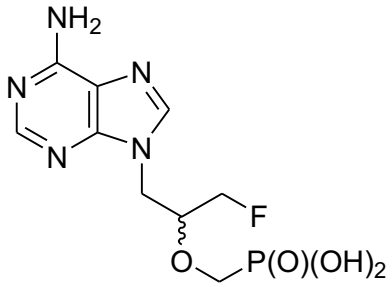
BGE: 30 mM $Na_2B_4O_7$ + NaOH, pH 10.0

Chiral selector: 20 mg/mL β-cyclodextrin

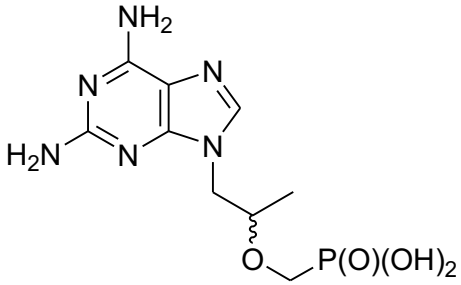
(*R,S*)-PMPA

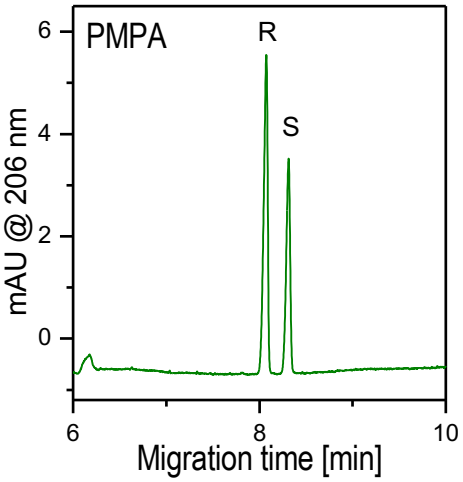


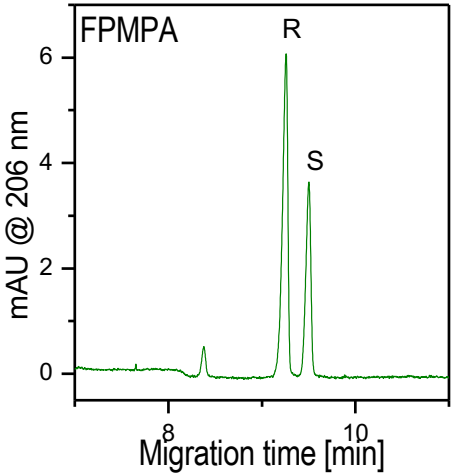
(*R,S*)-FPMPA

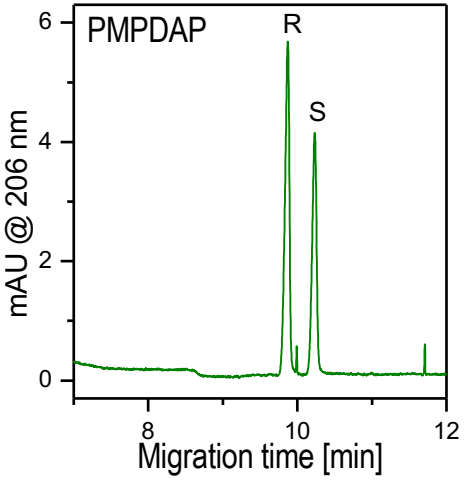


(*R,S*)-PMPDAP





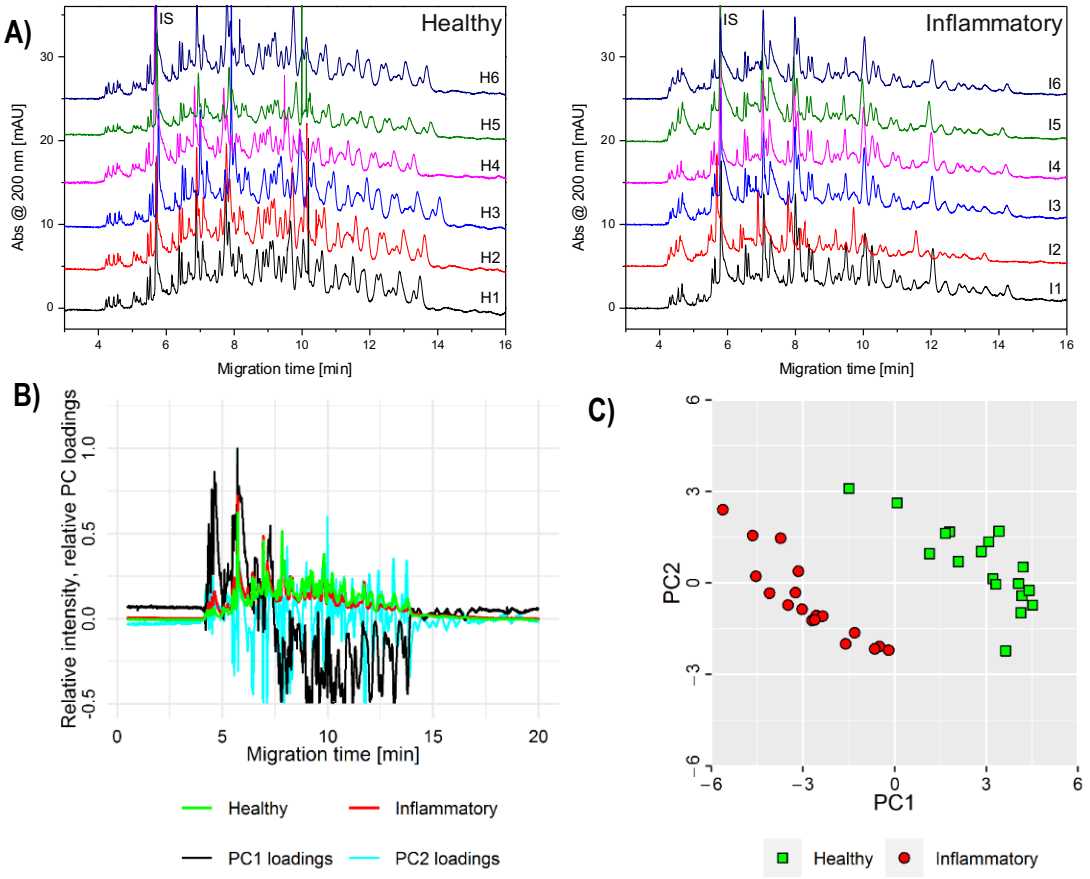




Fused silica capillary 50/375 µm, 39.2/29.0 cm, 15 kV, 66.5 µA, 20°C, inj. 6.9 mbar, 5 s

CE separations of complex peptide mixtures

A) **CE-UV profiles** of tryptic peptides of proteins of **healthy (left)** & **inflammatory (right) bone tissues**
B) Averaged normalized CE-UV profiles and relative loadings of the first two principal components
C) PCA scores plot of healthy and inflammatory tissue samples

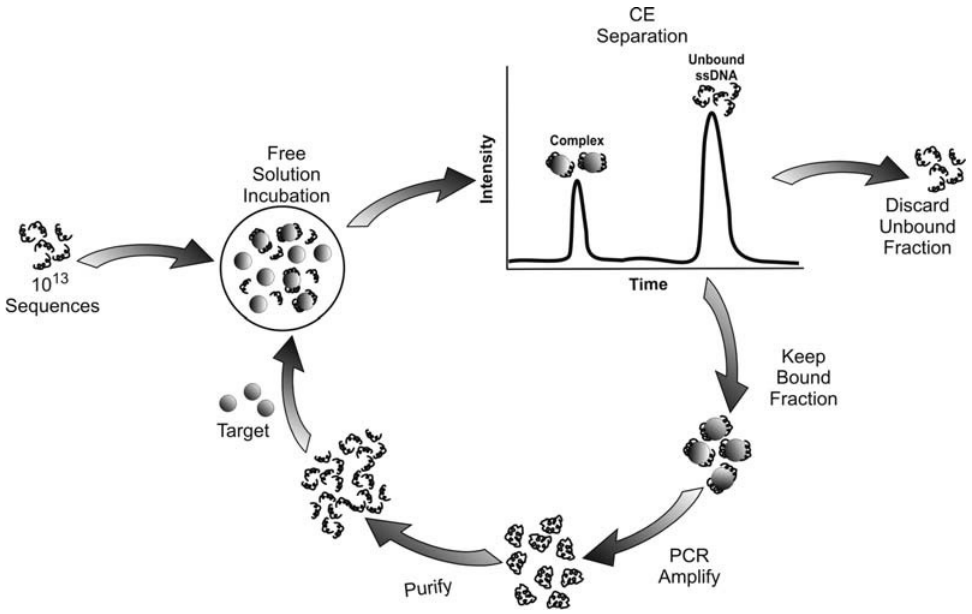


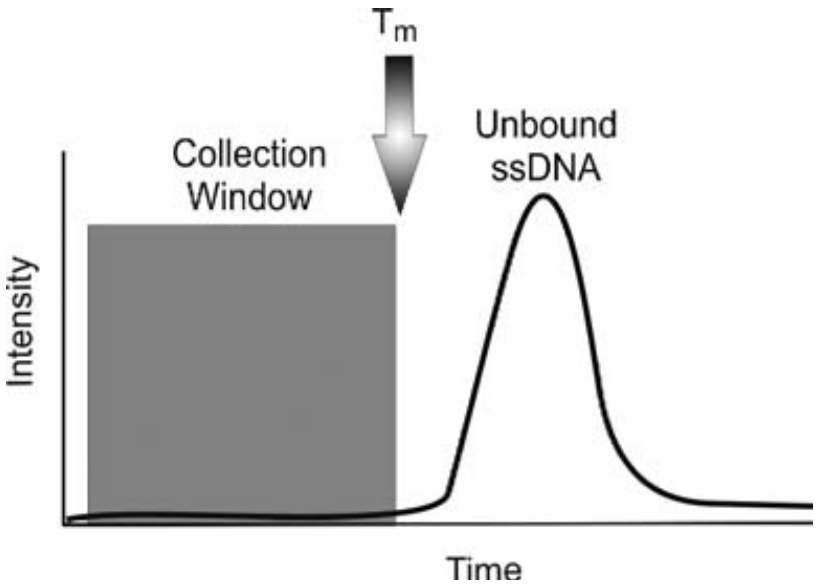
FS capillary: 50/375 µm id/od, 30/40 cm effect./total length, UV det. @ 200 nm
BGE 5: 55 mM H_3PO_4, 14 mM Tris, pH 2.01; 12 kV, 20°C, IS, internal standard (0.2 mM tyramine)

Provided services/collaboration (2)

Microscale isolation: ng – µg scale isolation (selection of binding aptamers)

CE-SELEX (CE – Systematic Evolution of Ligands by Exponential Enrichment)





Scheme of the CE–SELEX process. A random sequence DNA library is incubated with the target. Sequences bound to the target are separated and isolated by CE, PCR amplified and made single stranded, generating a new pool suitable for further rounds of enrichment.

Provided services/collaboration (3)

Phys-chem. & biochem. characterization

$$m_{eff} = \frac{v}{E} \doteq \frac{q}{6\pi\eta r}$$

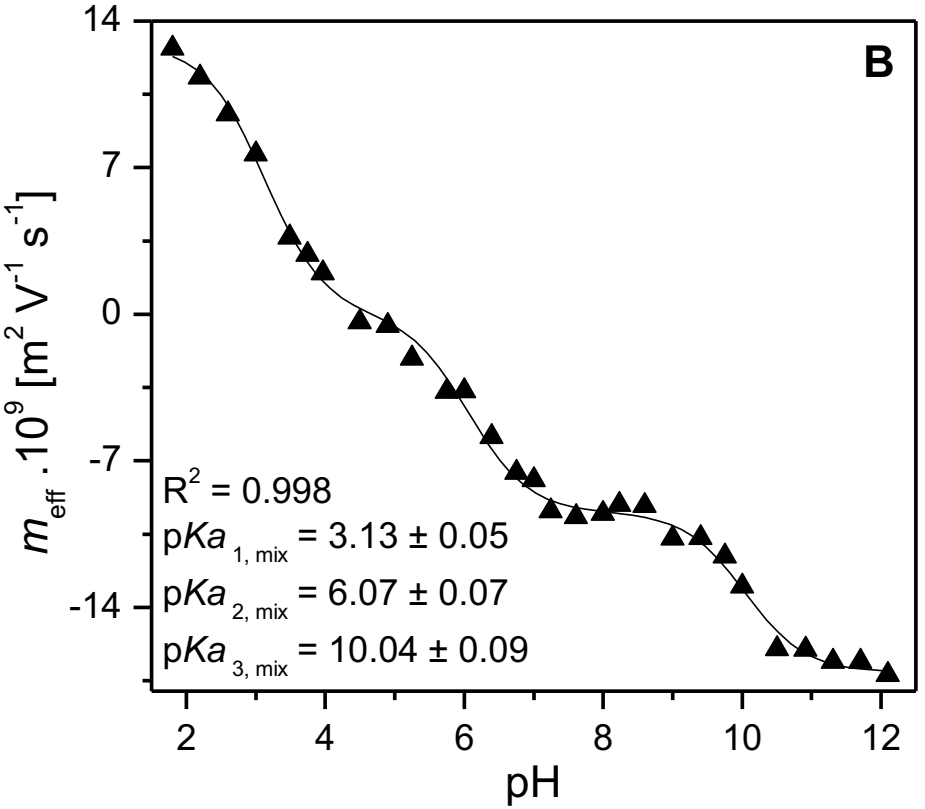
$$m_{eff} = \frac{L_{tot} L_{eff}}{U_{sep}} \left(\frac{1}{t_{mig}} - \frac{1}{t_{eof}} \right)$$

- Effective, actual & limiting (absolute) electrophoretic mobilities
- Effective charges
- Stokes radii
- Relative molecular masses (SDS-CGE)
- Diffusion coefficients
- Isoelectric points
- Acidity (ionization) constants
- Association (binding) constants of complexes
- Interconversion barriers of enantiomers
- Rate constants of chem. & enzymatic reactions
- Gibbs energy, enthalpy, entropy

Determination of acidity constants (pKa) of trivalent peptide by CE

pGlu-His-Trp-Ser-Tyr-Gly-Leu-OH
pKa2 pKa3 pKa1

$$m_{eff} = \frac{m_{P^+} 10^{pK_{a,1}^{mix} - pH} + m_{P^-} 10^{pH - pK_{a,2}^{mix}} + m_{P^{-2}} 10^{2pH - pK_{a,2}^{mix} - pK_{a,3}^{mix}}}{10^{pK_{a,1}^{mix} - pH} + 10^{pH - pK_{a,2}^{mix}} + 10^{2pH - pK_{a,2}^{mix} - pK_{a,3}^{mix}} + 1}$$



CONCLUSIONS

Do you need:

- High-sensitive analysis
- High-efficient separation
- Microscale isolation
- Phys-chem. & biochem. characterization

of your (bio)molecules?

Do not hesitate to contact us:

Building A, S-E wing, A 2.91
ext. 239, kasicka@uochb.cas.cz
electromigration.group.uochb.cz/en

Since ca. April 2023:
T.G. Masaryk Water Research Institute
Podbabská 2582/30, 160 00 Praha 6