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On-line Pre-concentration and Pre-separation in Electrophoresis using Stationary Reaction Boundary

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Pros and cons of electromigration methods

- Zone electrophoresis + speed, - high cLOD
- Isotachophoresis + pre-concentrate, - pre-concentrate all ions
- Isoelectric focusing + pre-concentrate,
- - presence of carrier ampholytes
- Method of dreams = + pre-concentrate, + without carrier ampholytes, + low cLOD

Types of reaction boundaries

I. Simple neutralization reaction boundary – NRB
Method: Carrier ampholyte free IEF (CAF IEF)
Use: focusing of ampholytes, AA

II. Complex forming – NRB
Method: Ligand step gradient focusing (LSGF)
Use: focusing of metals

III. Asymetric – NRB
Method: no name yet
Use: acumulation of weak acids, bases, also ampholytes

Principle of the method

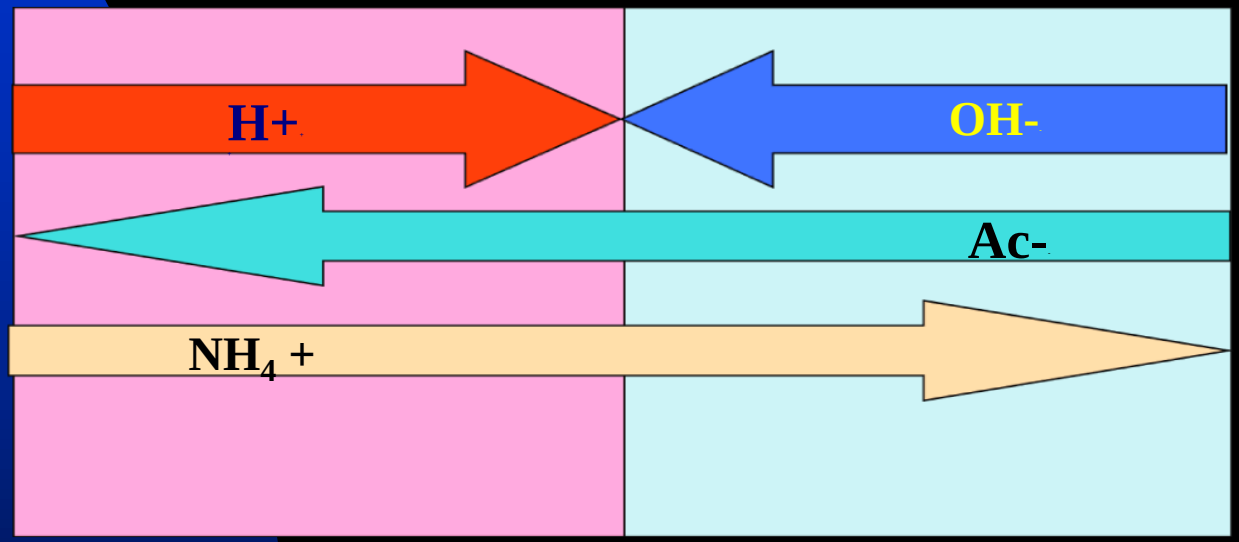
I. Simple neutralization reaction boundary

Scheme of the fluxes on neutralization reaction boundary

J_H

Acidic electrolyte:

0,01M NH_4Ac
0,01M HAc
pH=4,75



J_{OH}

Alkaline elektrolyt:

0,01M NH_4Ac
0,01M NH_4OH
pH=9,24

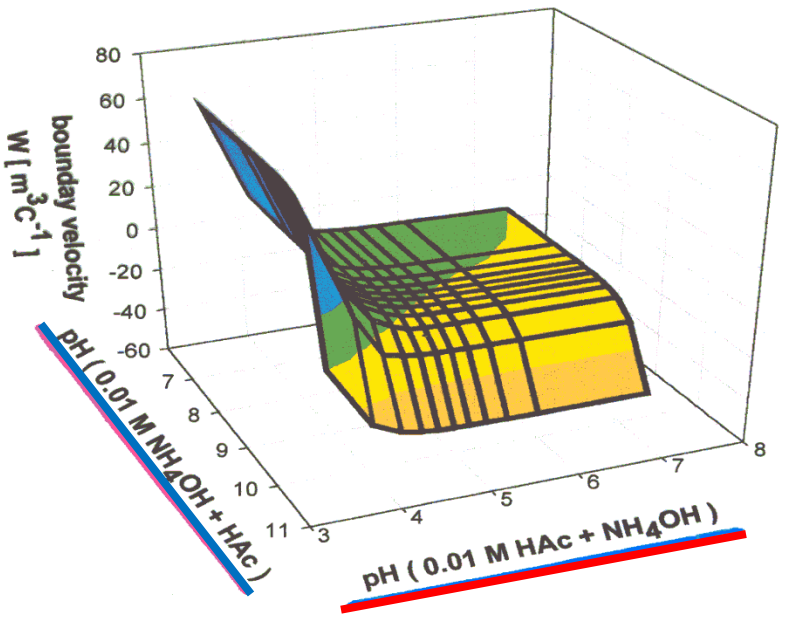
Analytical properties of neutralization reaction boundary

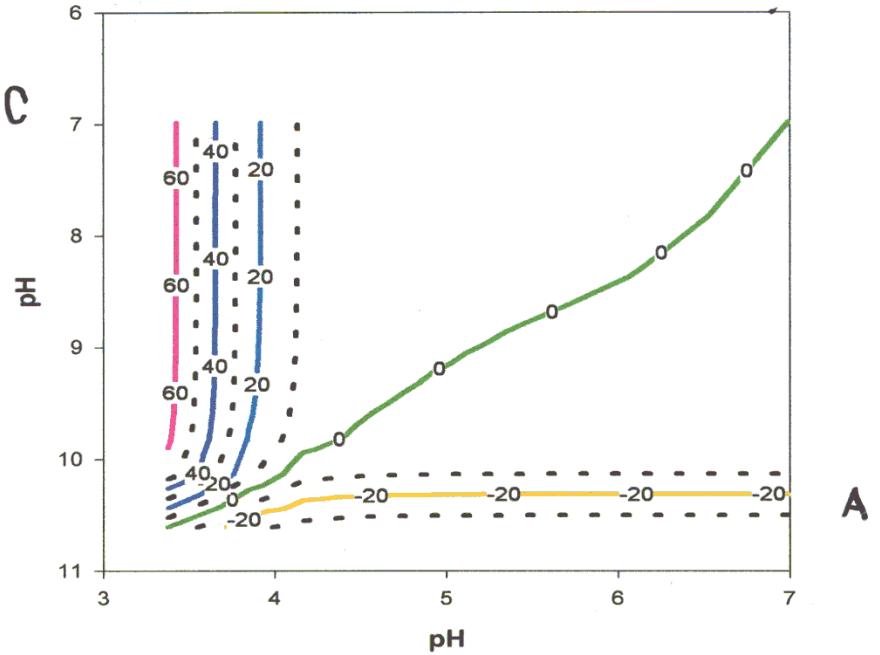
- Selectivity - focusing ampholytes
- - span of pH
- JH = JOH, boundary V=0 (cont. dosing)
- JH ≠ JOH, boundary V<> 0 (mobilization)
- Focused zones are rectangular and separated , pH=pI
- Self sharpening effect

Calculated velocity of the boundary

- Based on the composition of the adjacent electrolytes.

Calculated dependence of neutralisation boundary velocity on the composition of adjacent electrolytes- their pH





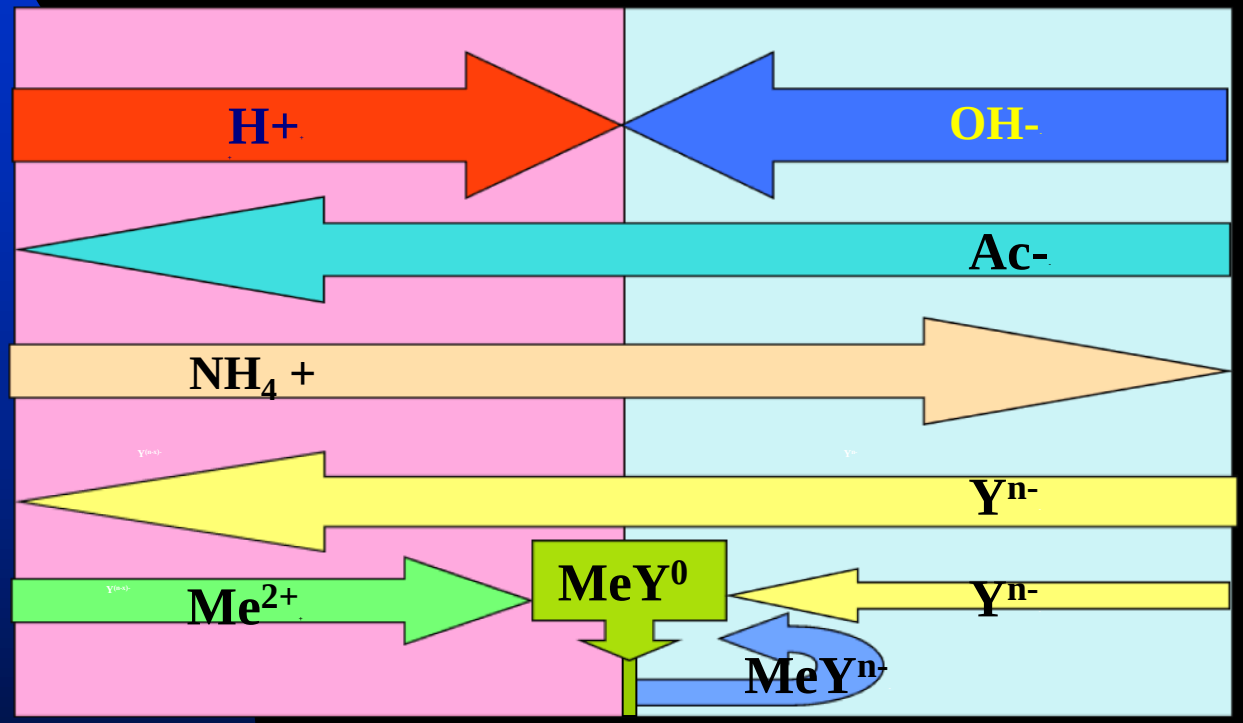
Principle of the method

II. Complex forming – NRB

Scheme of the fluxes - LSGF

Acid electrolyte

0,01M NH_4Ac
0,01M HAc
pH=4,75 + kovy



Alkaline elektrolyte:

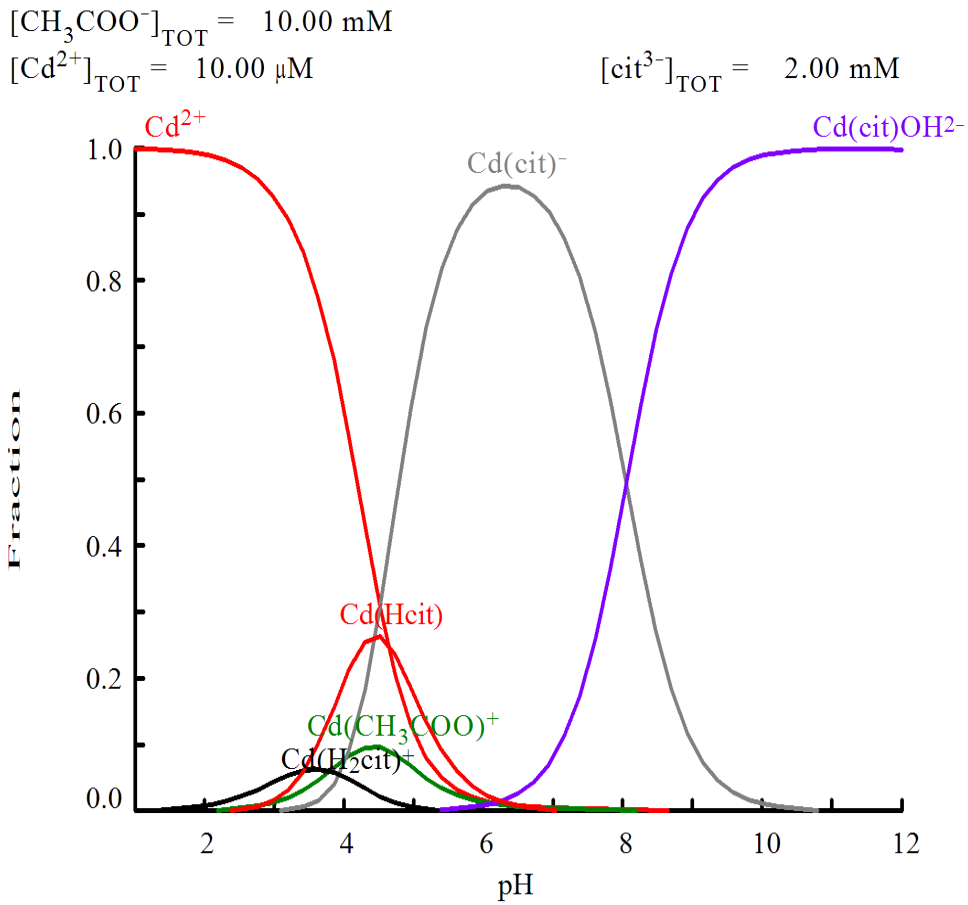
0,01M NH_4Ac
0,01M NH_4OH
0,002M $(NH_4)_3$ cit.
pH=9,24

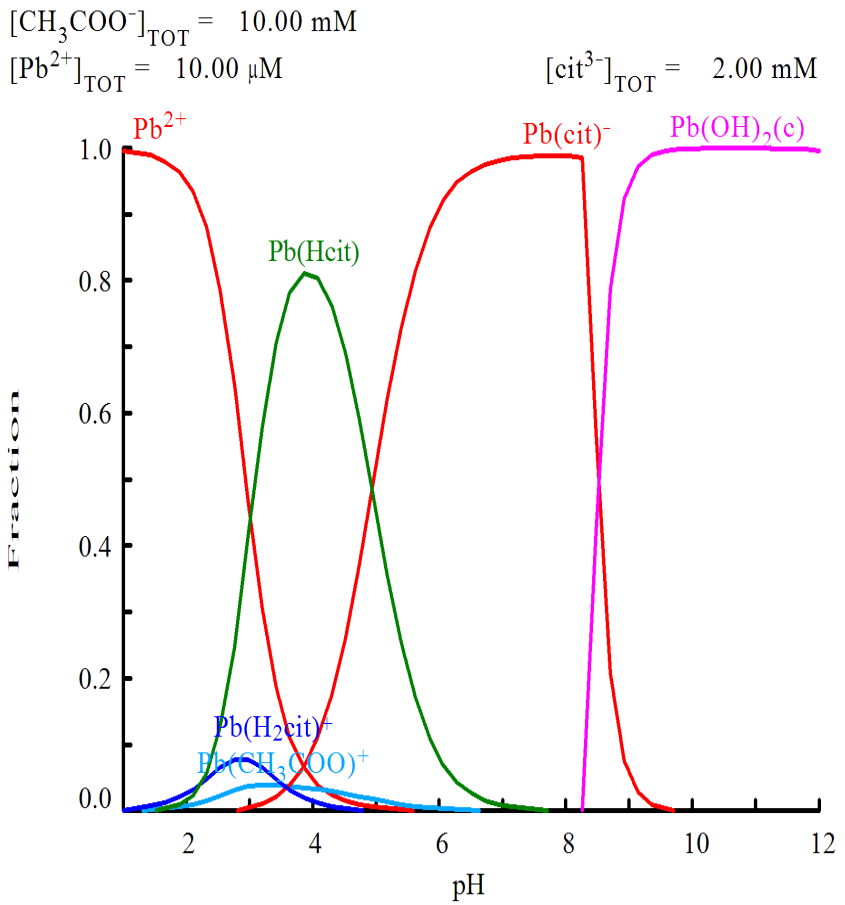
- Principle = combination of neutralization reaction boundary (NRB) and presence of convenient chelating agens.

Analytical properties of complex forming – NRB

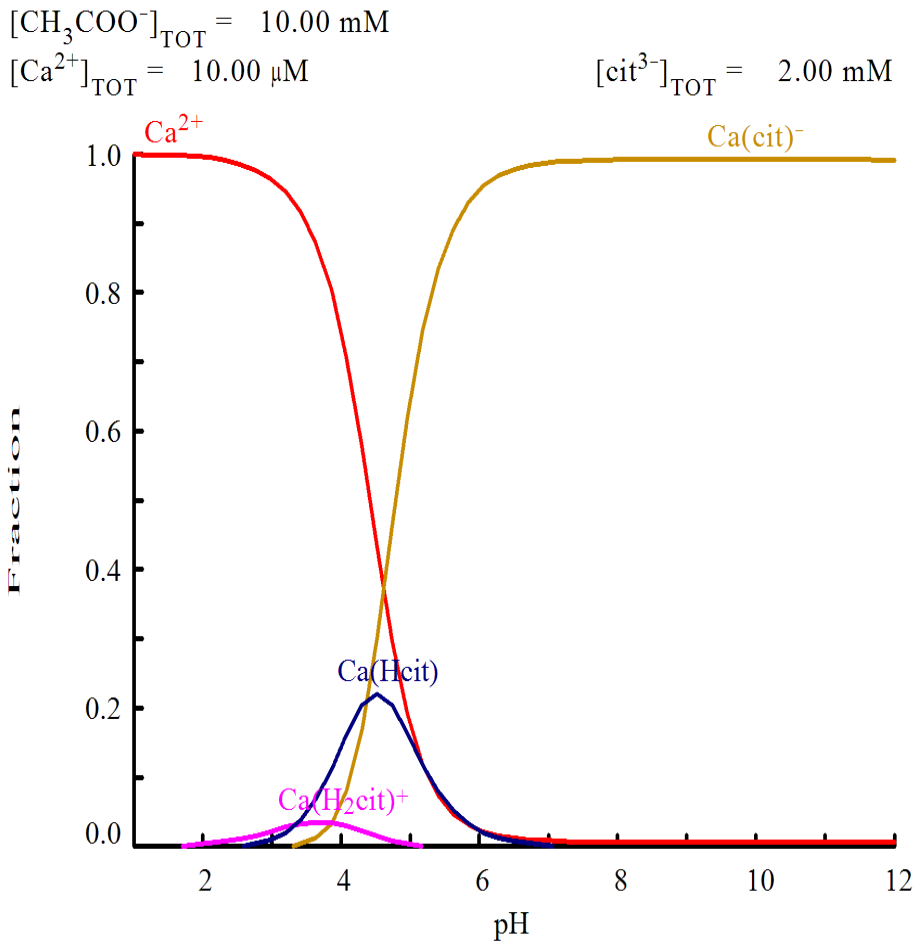
- Selectivity – nature of complexing agens
- - span of pH
- JH = JOH, boundary V=0 (cont. dosing)
- JH ≠ JOH, boundary V<> 0 (mobilization)
- Focused zones of metals are rectangular and separated according to „quasi pI“ of complex
- Self sharpening effect

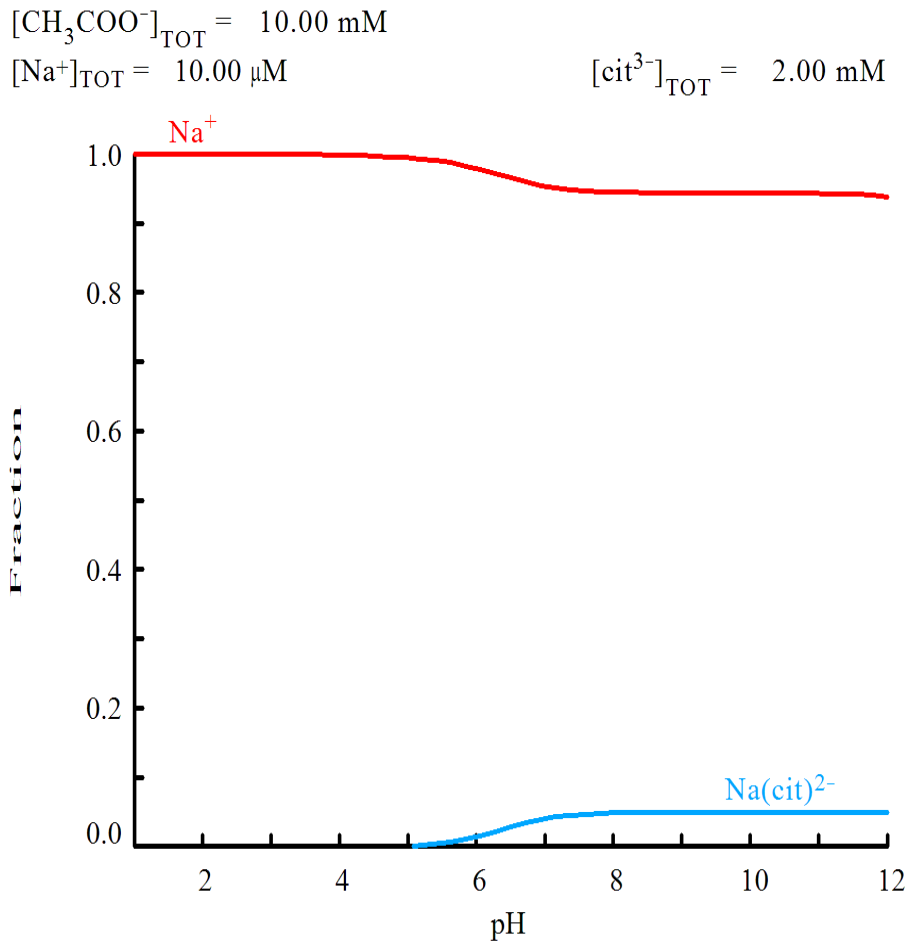
Choice a nature and conc. of complexing agens





Choice a nature and conc. of complexing agens

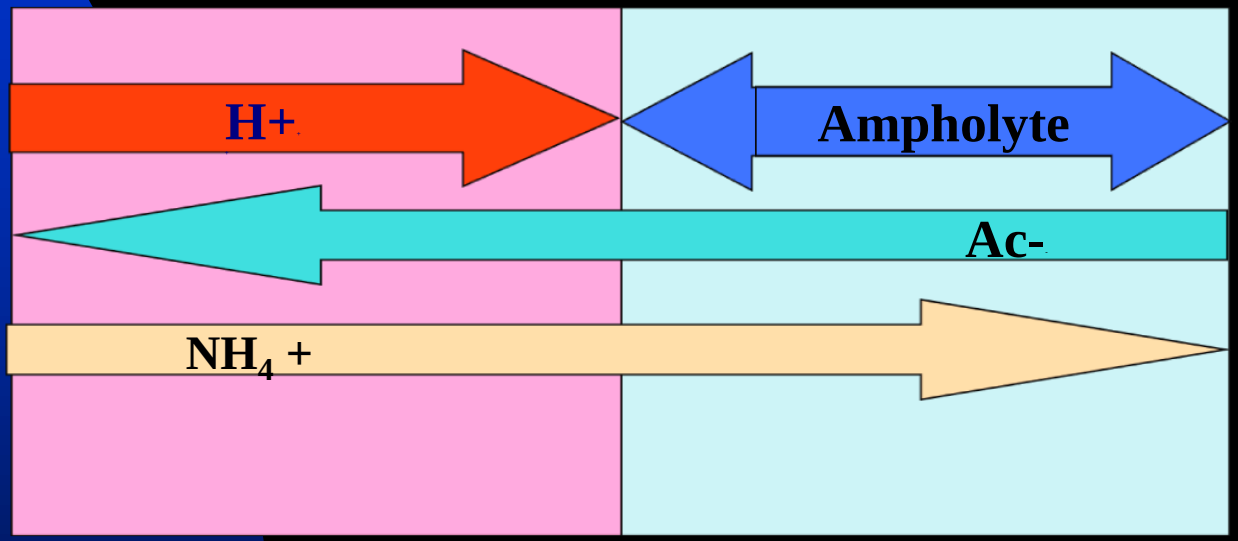




Principle of the method

III. Asymetrical NRB

Scheme of the fluxes on asymetrical neutralization reaction boundary



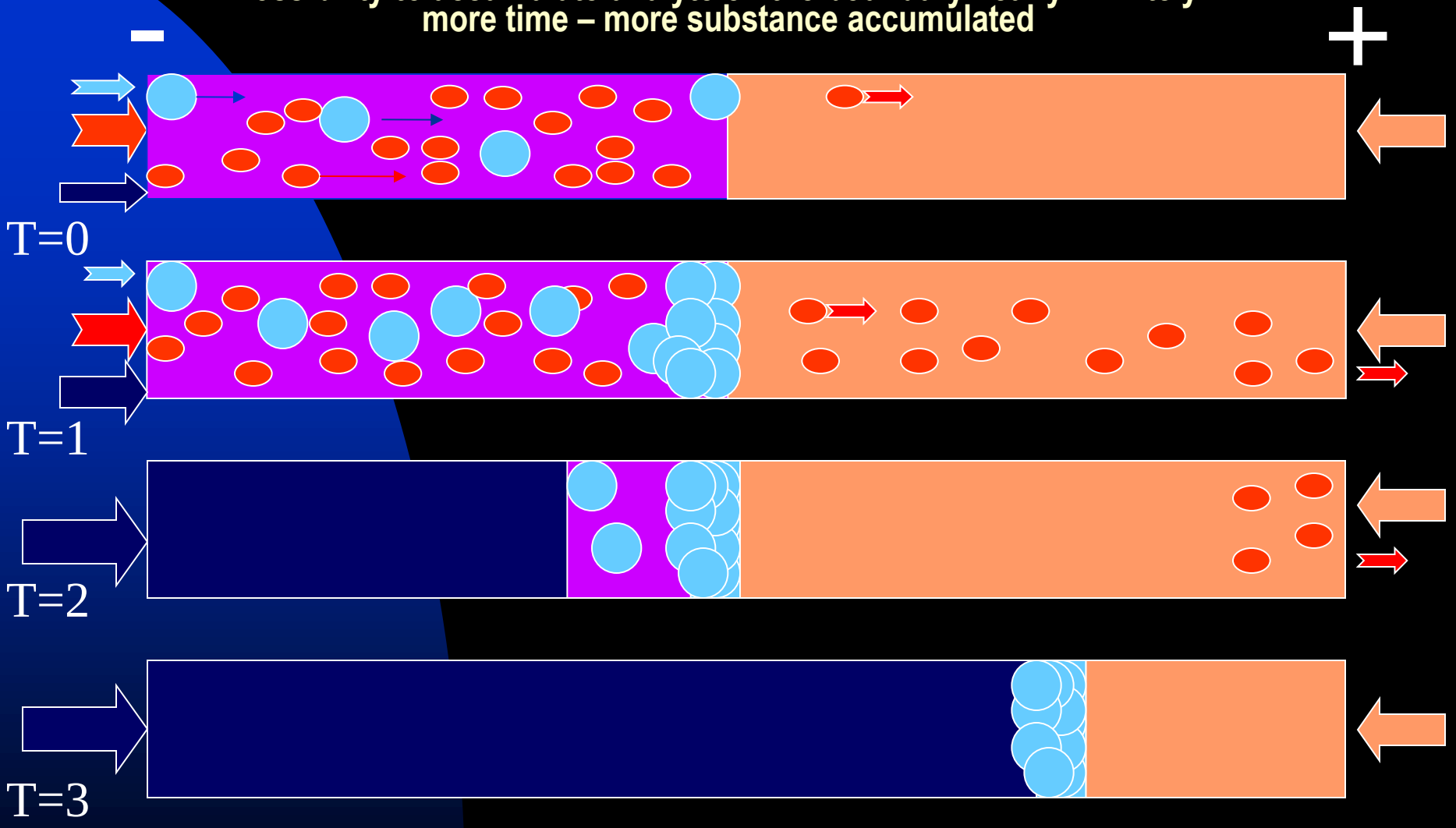
Asymetry is reached by presence of ampholyte at pH=pI

Analytical properties of asymetric NRB

- Selectivity – extreme pH
  - focusing „weak“ acids and bases
  - lower span of pH and water prod.
- JH = JOH, boundary V=0 (cont. dosing)
- JH ≠ JOH, boundary V<> 0 (mobilization)
- Zones of weak ions are immobilized but not focused – no self sharpening effect

MODE of continuous dosing for all types of NRB

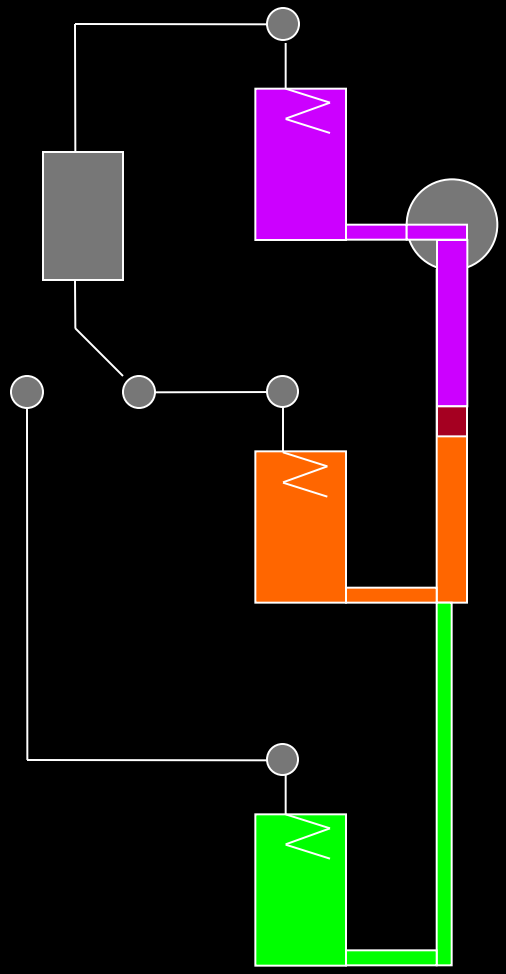
Possibility to accumulate analyte on the boundary nearly infinitely – more time – more substance accumulated



$pH_1 > pI_A < pH_2$ $pH_1 > pI_B > pH_2$

Experimental arrangement I,II

- Equipment



DE - TE

Separation column

Analyte

PE - LE

CAF IEF - ITP

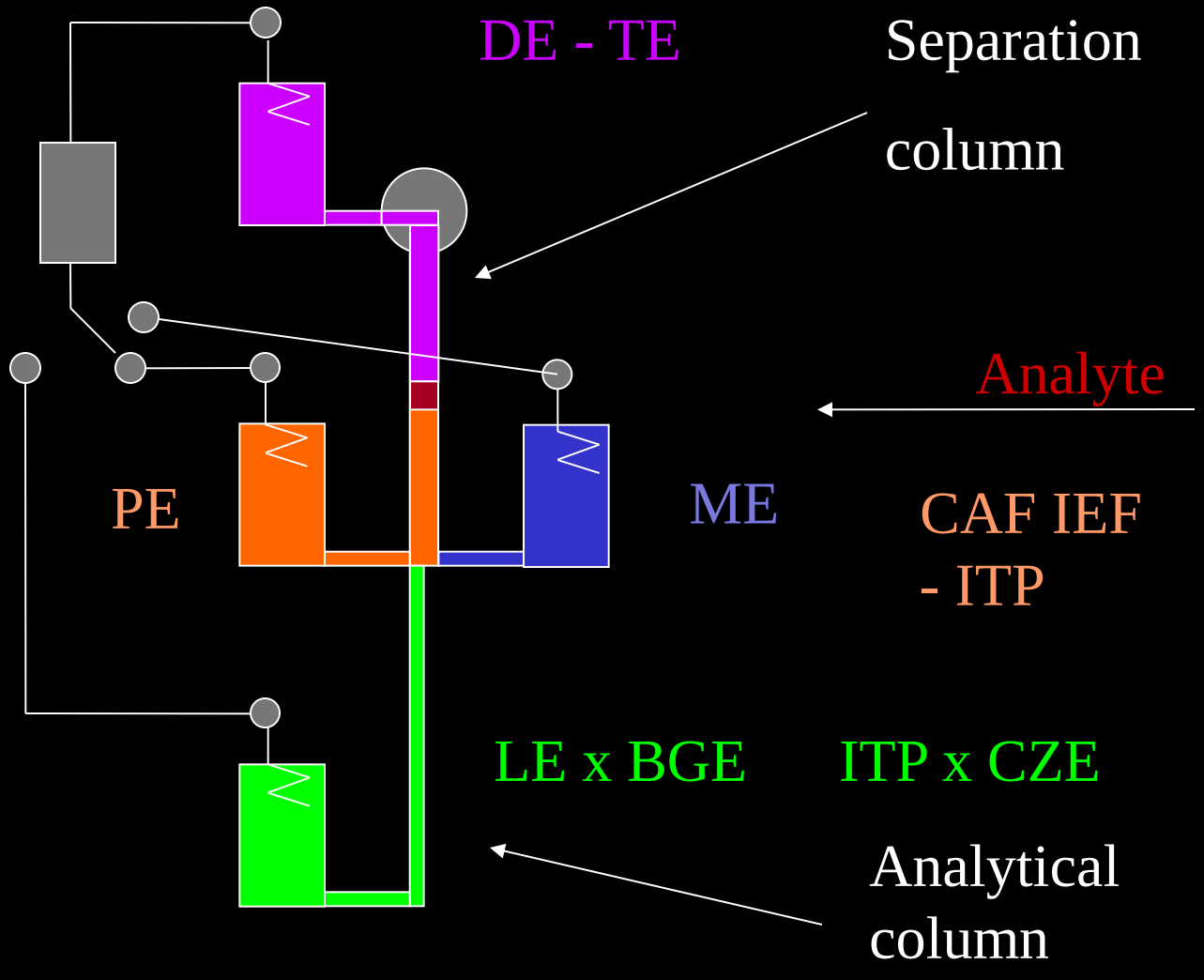
LE x BGE

ITP x CZE

Analytical column

Experimental arrangement III

- Equipment



Electrolyte systems used

- Stability-buffers, high purity, selectivity

- Simple NRB I. : Ammonia acetate pH 4,7-9,24

- Complex forming NRB II : Ammonia acetate ca. pH 4,7-9,24
- alkali earth metals + Cresolphthalexone
- heavy metals + Citrate
- copper + PAR + Citrate

- Asymetrical NRB III. : Ammonia acetate + HIS pH 6,95-8,62
  HCl+HIS/ βALA pH 1,70-7,20

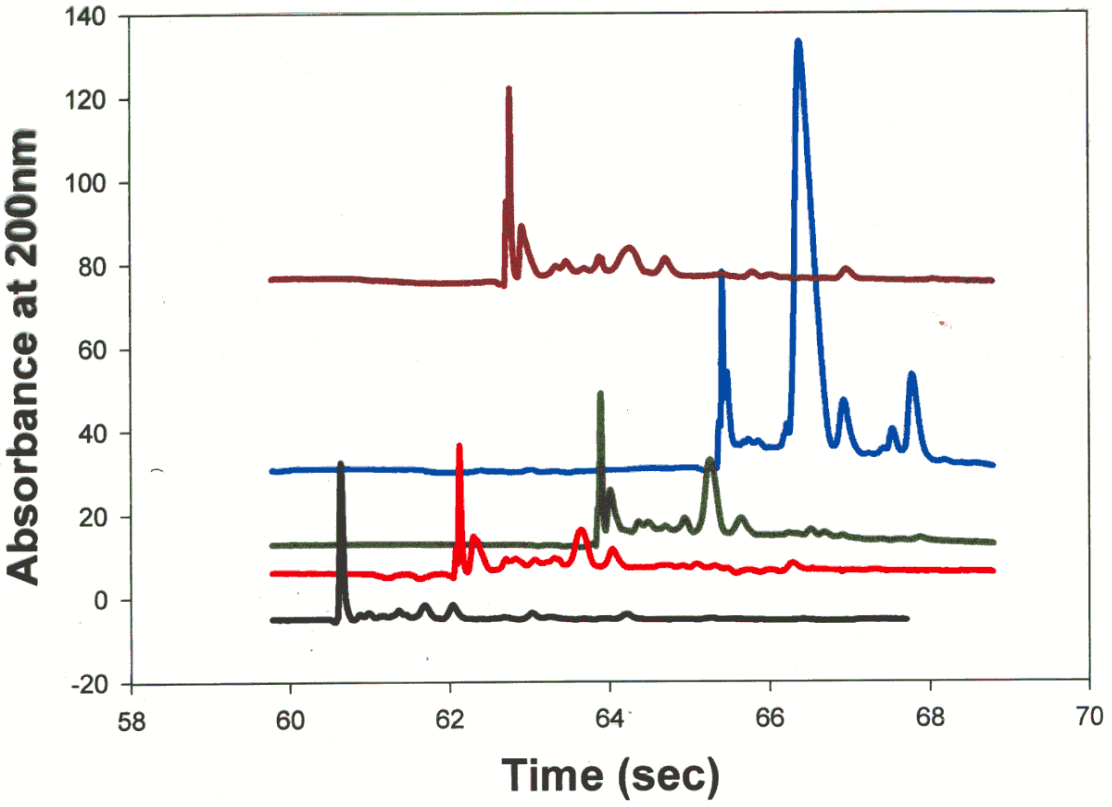
Results

Simple NRB I

Method CAF IEF -CZE

- Introduction to the electrophoresis
- Principle of the method
- Analytical properties
- Choice of the electrolyte system
- Procedure of the focusing
- Results
- Conclusion

CZE analysis - 1000 sec. of continuous dosing at different concentrations of ampholyte



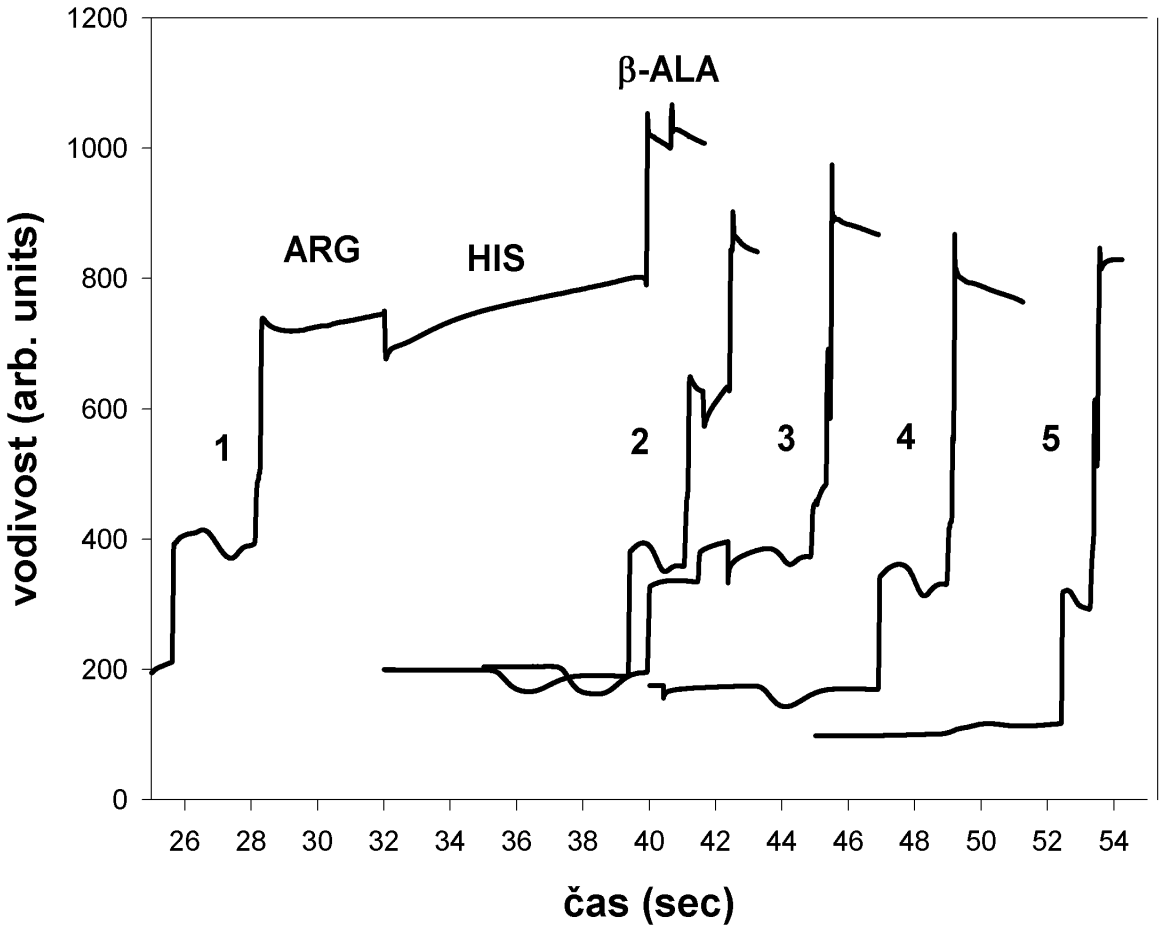
Analysis of model mixture AA with and without dosing.

Simple NRB I

Method CAF IEF -ITP

- Dosing time1200 sec, for different concentration of AA

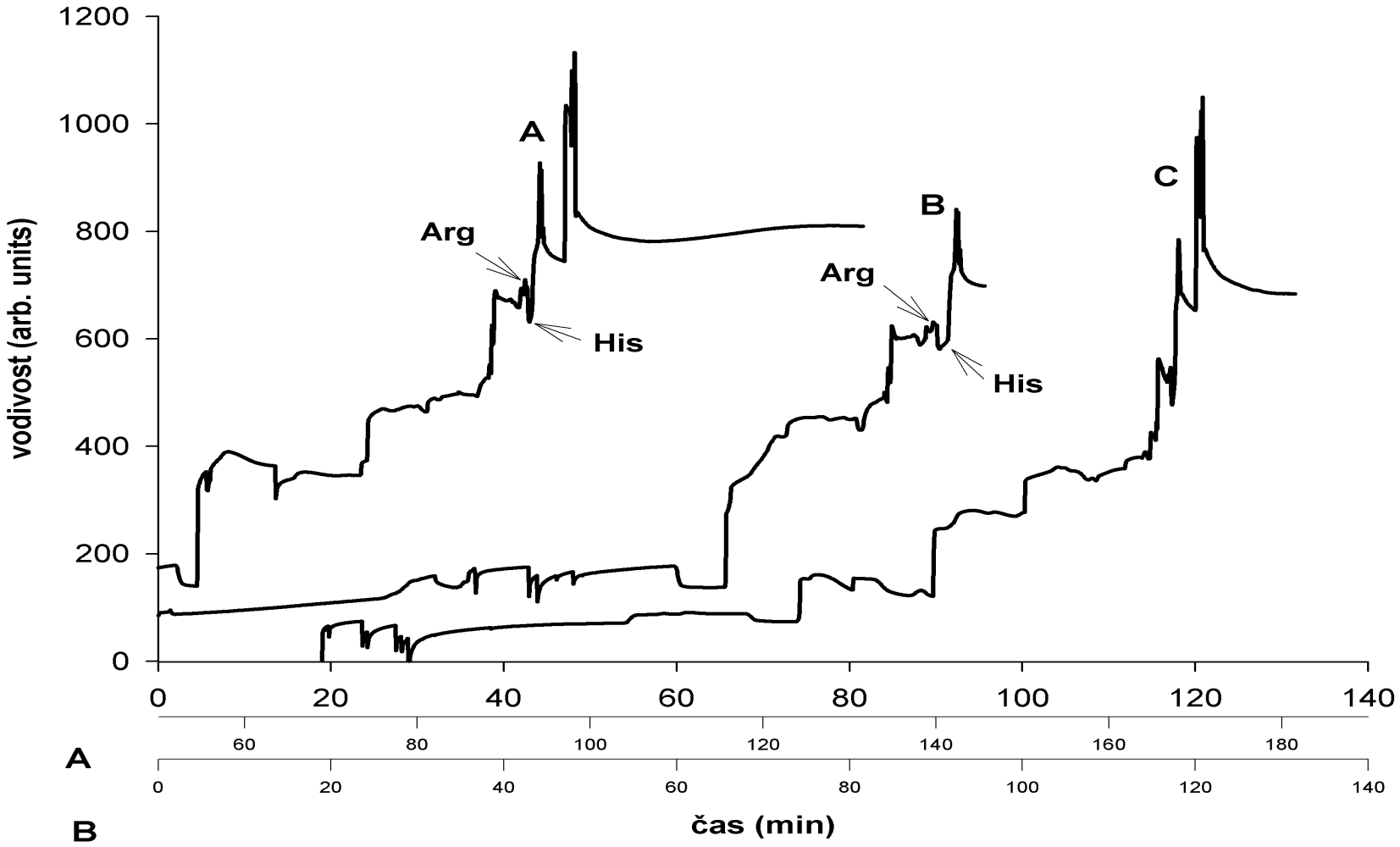
- 1- 2,5x10-5Mol/l
- 2- 2,5x10-6Mol/l
- 3- 2,5x10-7Mol/l
- 4- 2,5x10-8Mol/l
- 5- 2,5x10-5Mol/l
- Without dosing.



Analysis of real sample of AA

Simple NRB I Method CAF IEF -ITP

- A-cont. dosing 2000s, B-sample spiked with 25μmol AA, C –no dosing.



ITP analysis of supernatant abowe $BaSO_4$ without preconcentration (A) with preconcentration 2000s v LSG (B)

Complex forming NRB II

Method LSGF-ITP