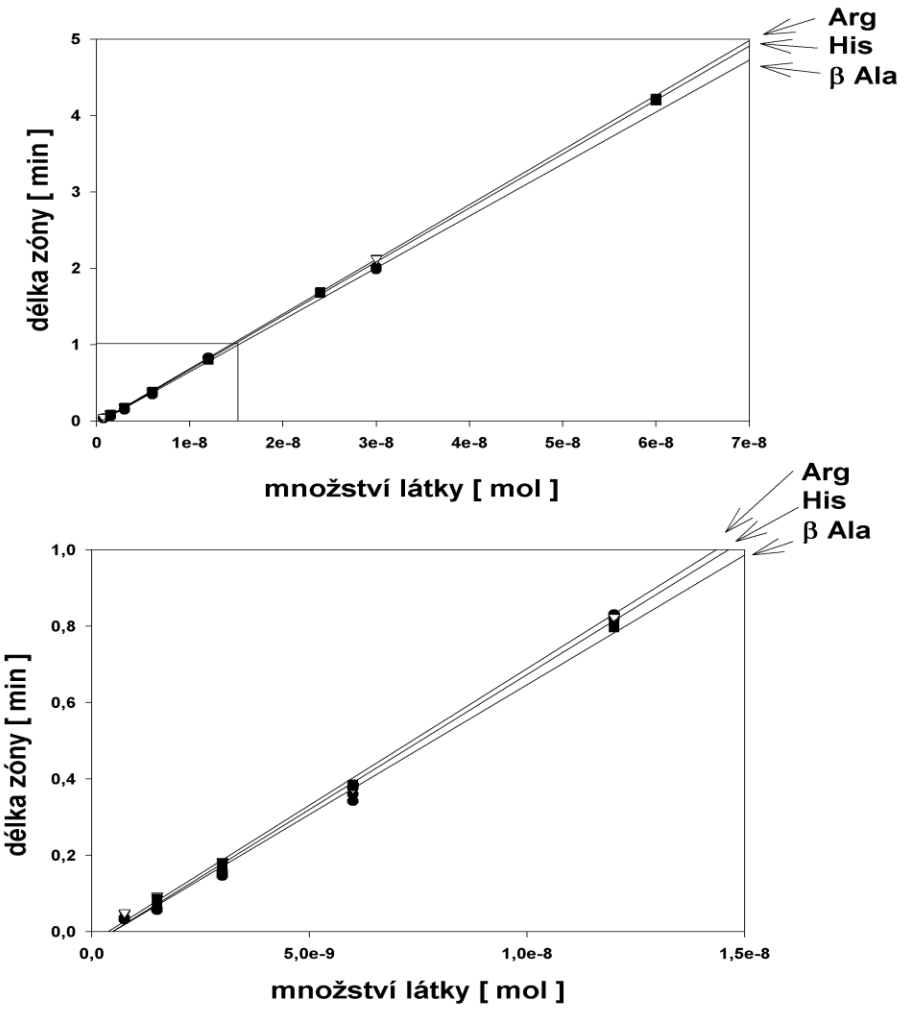
Determination of LOD of AA

Dependence of the zone length on the amount of injected AA for

ITP-ITP

cLOD 25μMol/l

LOD 750pMol



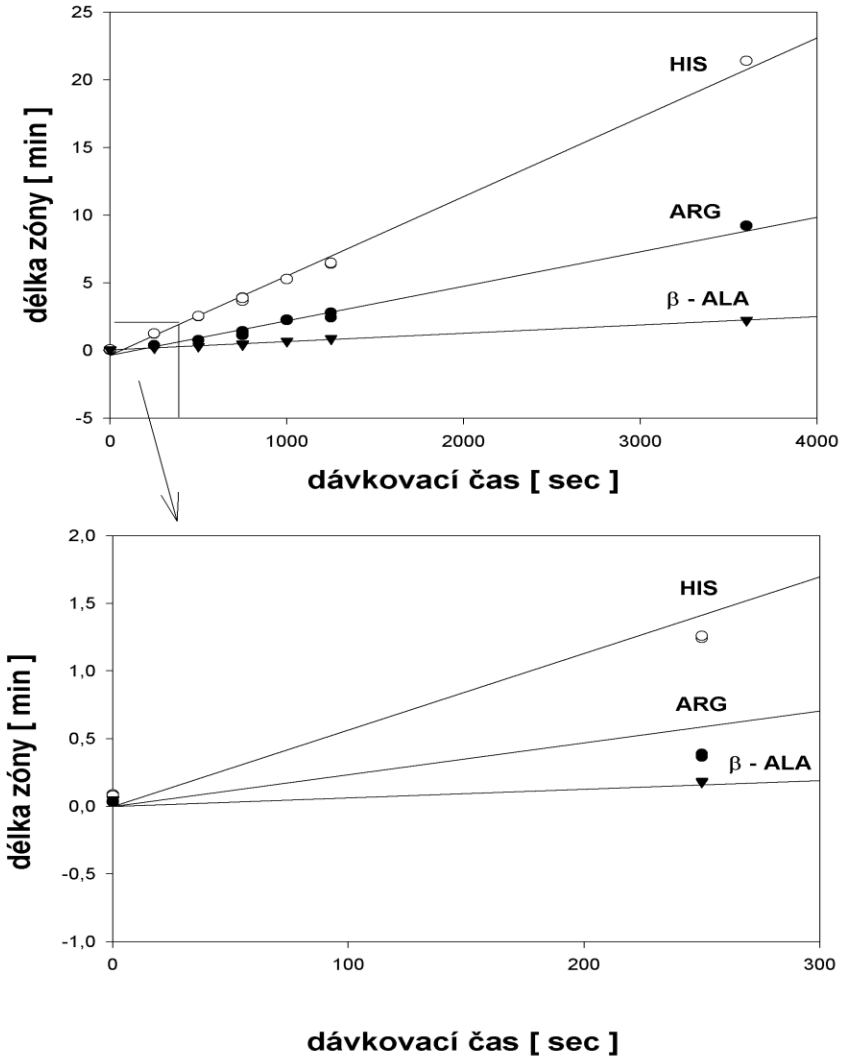
Calibration of continuous dosing of AA

Dependence of the zone length no dosing time of AA for combination CAF IEF-ITP-ITP

Dosing speed is

226, 132, 36 nMol/As for

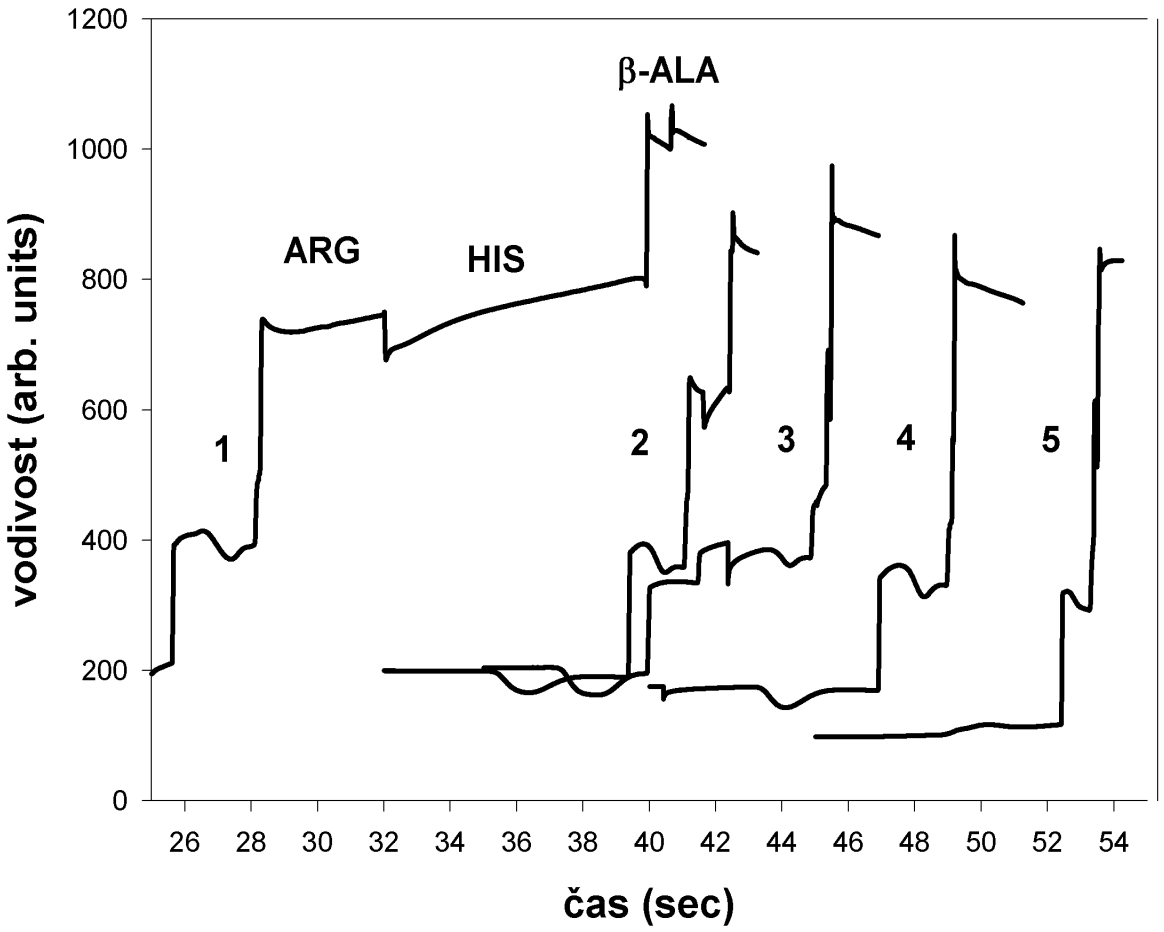
His, Arg, b-Ala.



Analysis of model mixture AA with and without dosing.

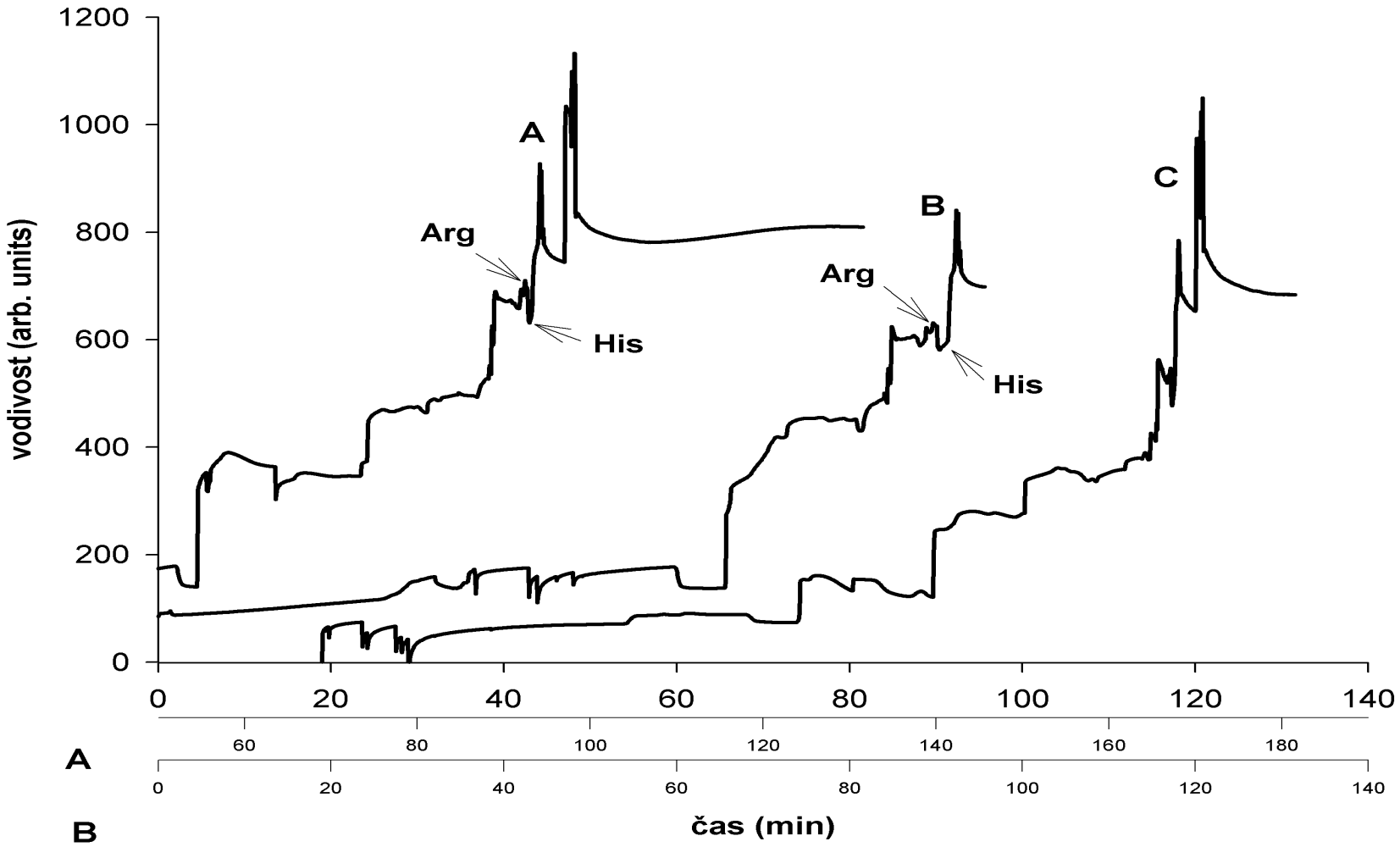
- Dosing time1200 sec, for different concentration of AA

- 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

- A-cont. dosing 2000s, B-sample spiked with 25μmol AA,
- C –sample without cont. dosing.



Conclusion for focusing of ampholytes and amino acids

- The method was suggested, developed and verified for the determinations of amino acids AA.
- We reached the cLOD 250 nmol/l , this is about two orders of magnitude better than in classical and combined methods.
- Concentration factor is 10^7 **per** 1000 sec.
- The analysis of real sample (leaves of betula pendula) was performed, with results of 0,86 μg arginine and 1,4μg of histidine per gramm of dry matter.

- Amount of AA serves for the evaluation of the health of forests in the poluted area of the country.

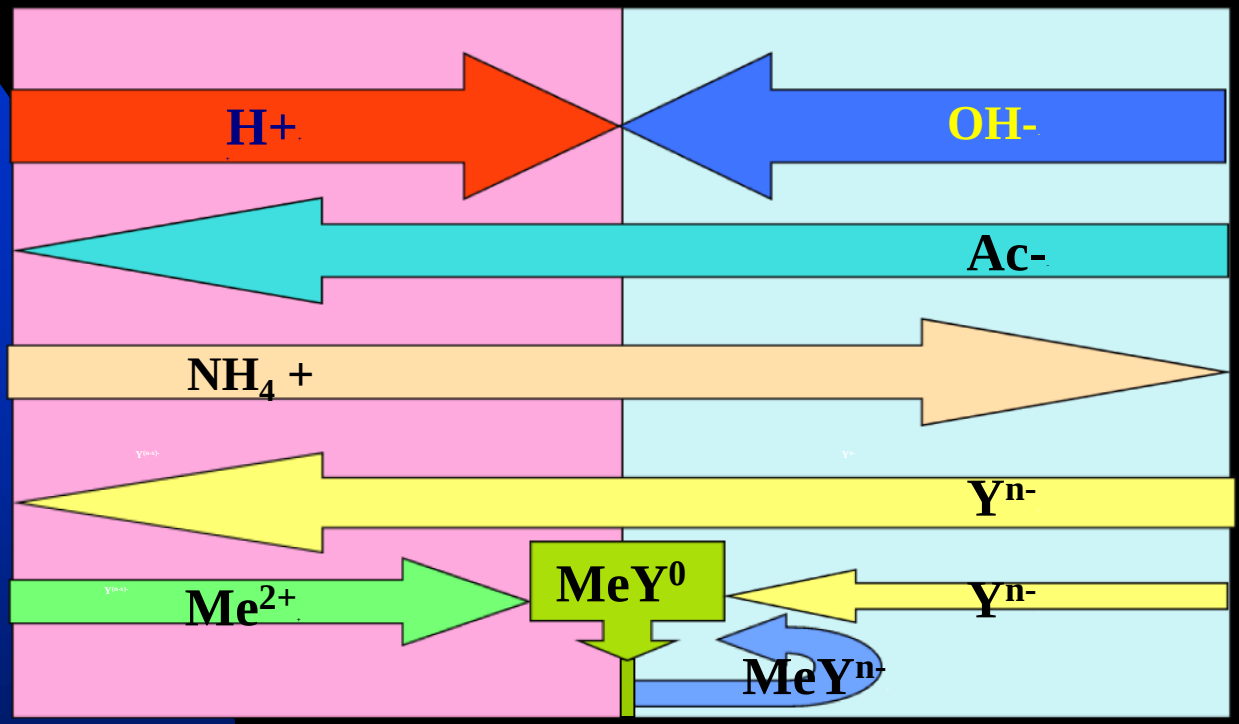
Fokusing of metals in ligand step gradient

Focusing of metals in ligand step gradient - LSG

Jan Pospíchal
Eliška Glovinová

Principle of the method

Scheme of the fluxes - LSG



Acid electrolyte

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

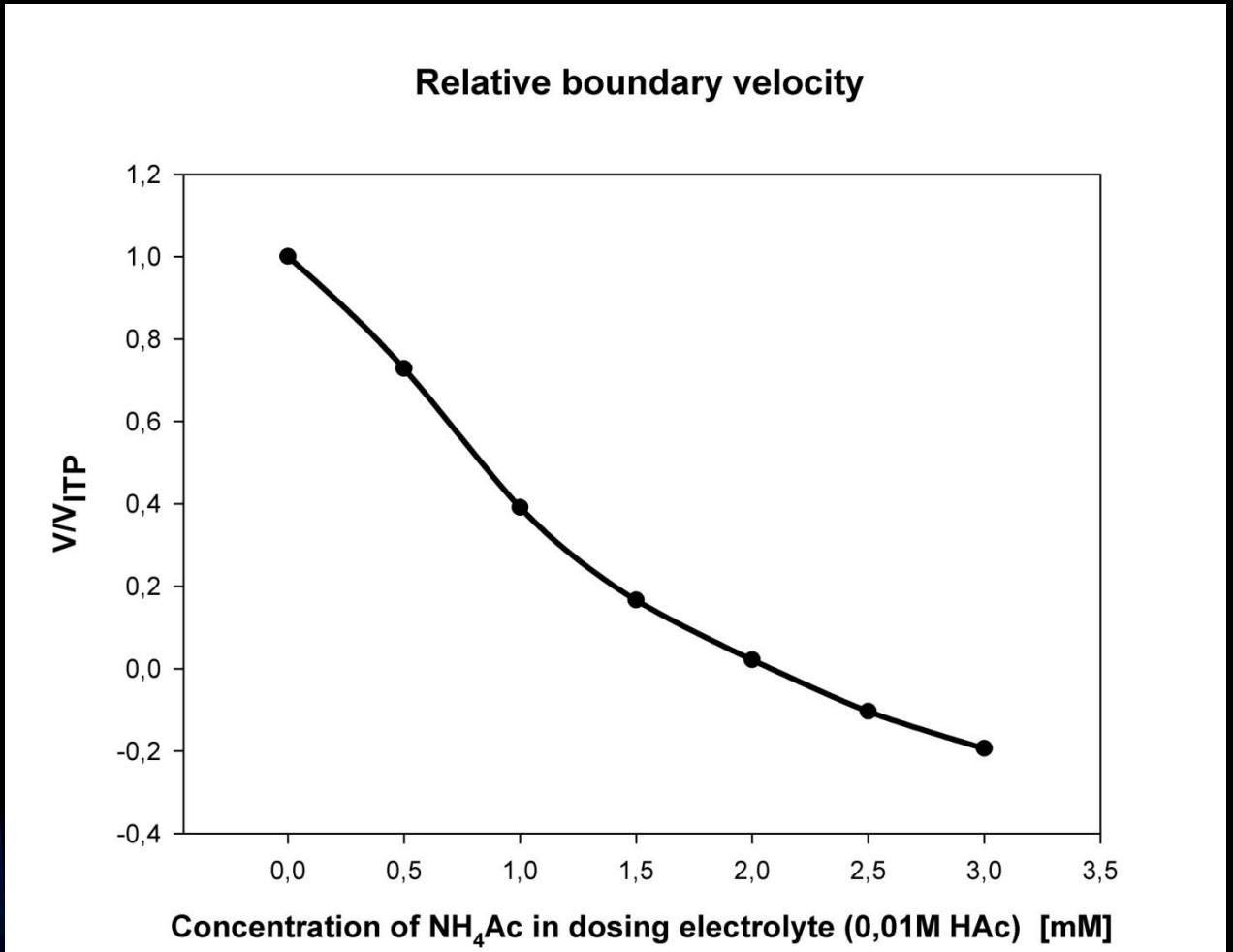
Alkaline elektrolyte:

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.

Choice of electrolyte comp. pH-exp.

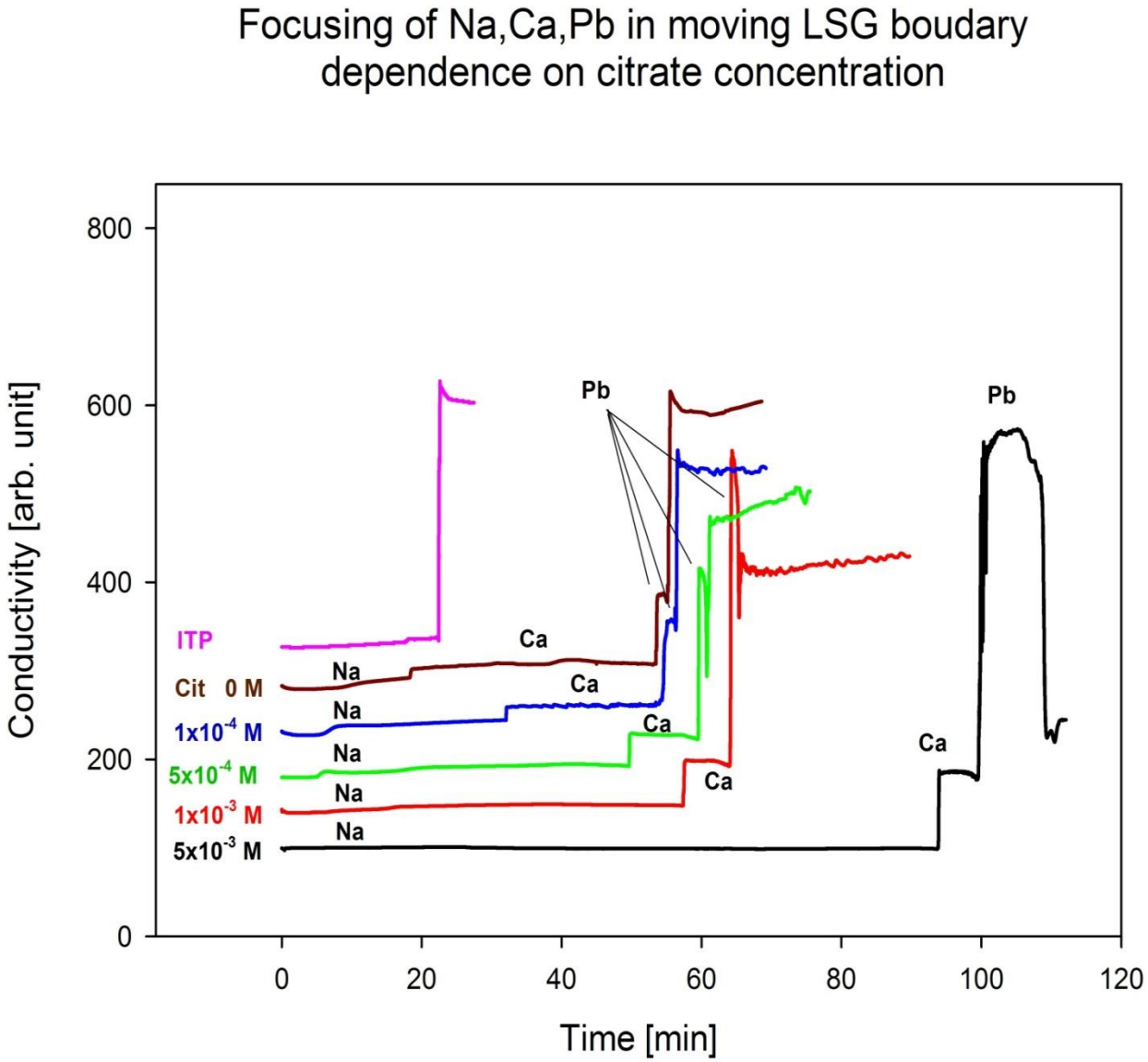
Measured velocity of the boundary – dependence on the composition of acidic DE, for contant composition of alkaline ellyte-PE-PE
(0,01 M NH_4Ac + 0,01 M NH_4OH + 0,002 M $(NH_4)_3cit$)
+ acidobasic indicator



Experimental evaluation of proper conc. of complexing agens using MBE

DE
0,01M Hac
0,001 M NH_4Ac
10^{-5} M Na, Ca, Pb
pH=3,83

PE
0,01 M NH_4Ac
0,01 M NH_4OH
0,002 M $(NH_4)_3$ cit +
1%PEG6000
pH = 9,3



Electrolyte systems used

- Focusing of alkali earth metals
- DE = 0,01 M NH_4Ac + 0,03M HAc +kovy pH=3,83
- PE/LE =0,02 M NH_4OH + 0,005 M KFX+ 400ppm Triton + 1%PEG6000 , pH = 8,9
- TE =0,03 M HAc

- Focusing of heavy metals
- DE = 0,01 M NH_4Ac + 0,01 M HAc (Real sample 2.10^{-4} M NH_4Ac + 1.10^{-5} M HAc) + kovy pH=4,7
- PE =0,01M NH_4Ac + 0,01 M NH_4OH + 0,002 M $(NH_4)_3Cit$+ 1%PEG6000, pH = 9,2
- LE =0,01 M NH_4Ac +0,1 M HAc + 0,002 M $(NH_4)_3Cit$ + 1%PEG6000
- TE =0,03 M HAc

Electrolyte systems used

- Focusing of copper
- DE = 0,001 M NH_4Ac + 0,01 M Hac + X M Cu pH=3.7

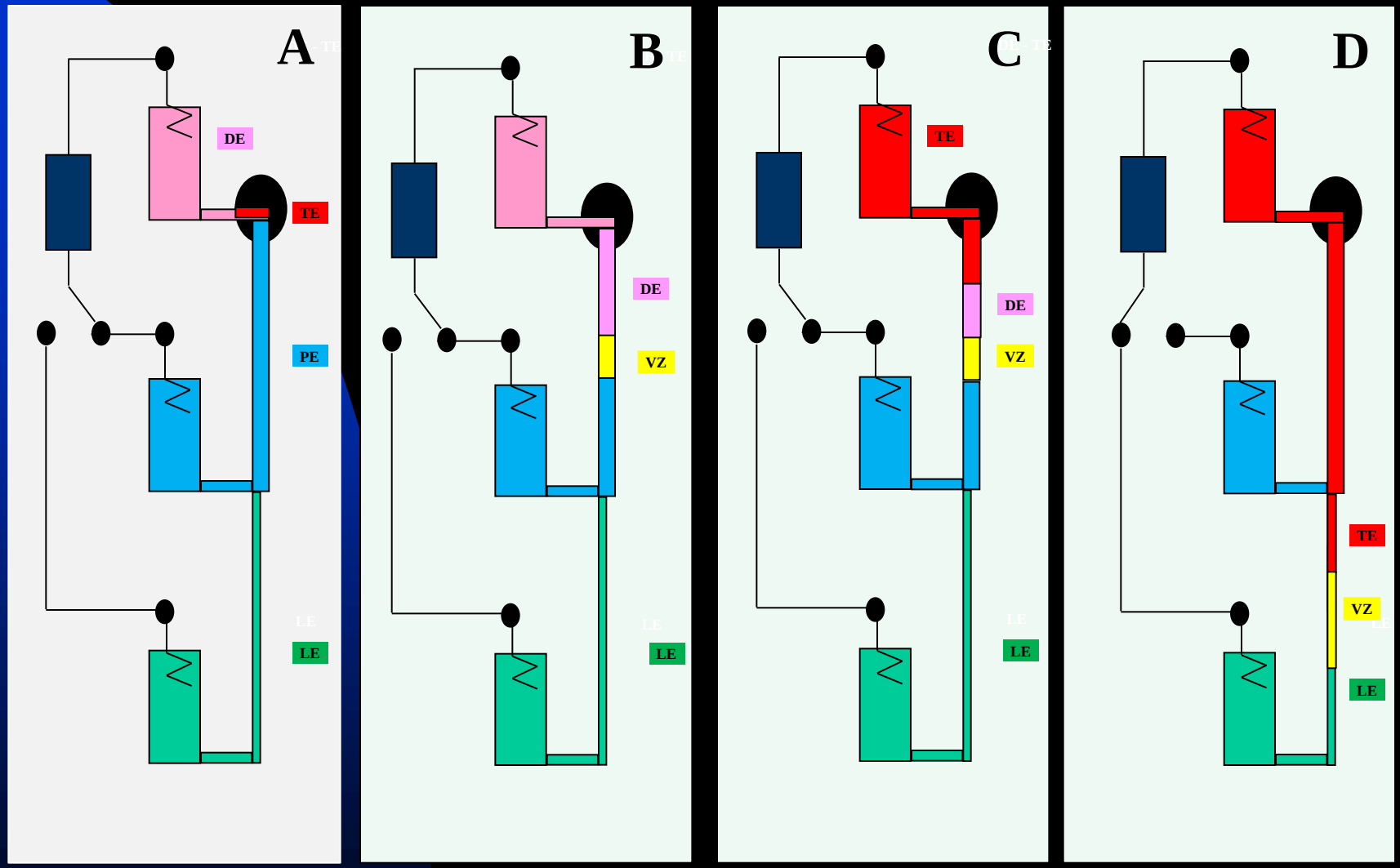
- PE =0,01M NH_4Ac + 0,01 M NH_4OH + 0,00005 M PAR +
- 0,005 M $(NH_4)_3Cit$ + 0,0001 M BKP pH = 9,2

- LE =0,02 M NH_4Ac +0,01 M HAc + 0,0002 M SPADNS
- TE =0,03 M HAc

sedmdesátinásobného naakumulování mědi během 42

Analytical procedure

A) Kolona naplněná elektrolyty B) fokusace v LSG (mod IEF?) C) Mobilizace zon (mod MBE-ITP) D) Analýza zon (mod ITP)



Results

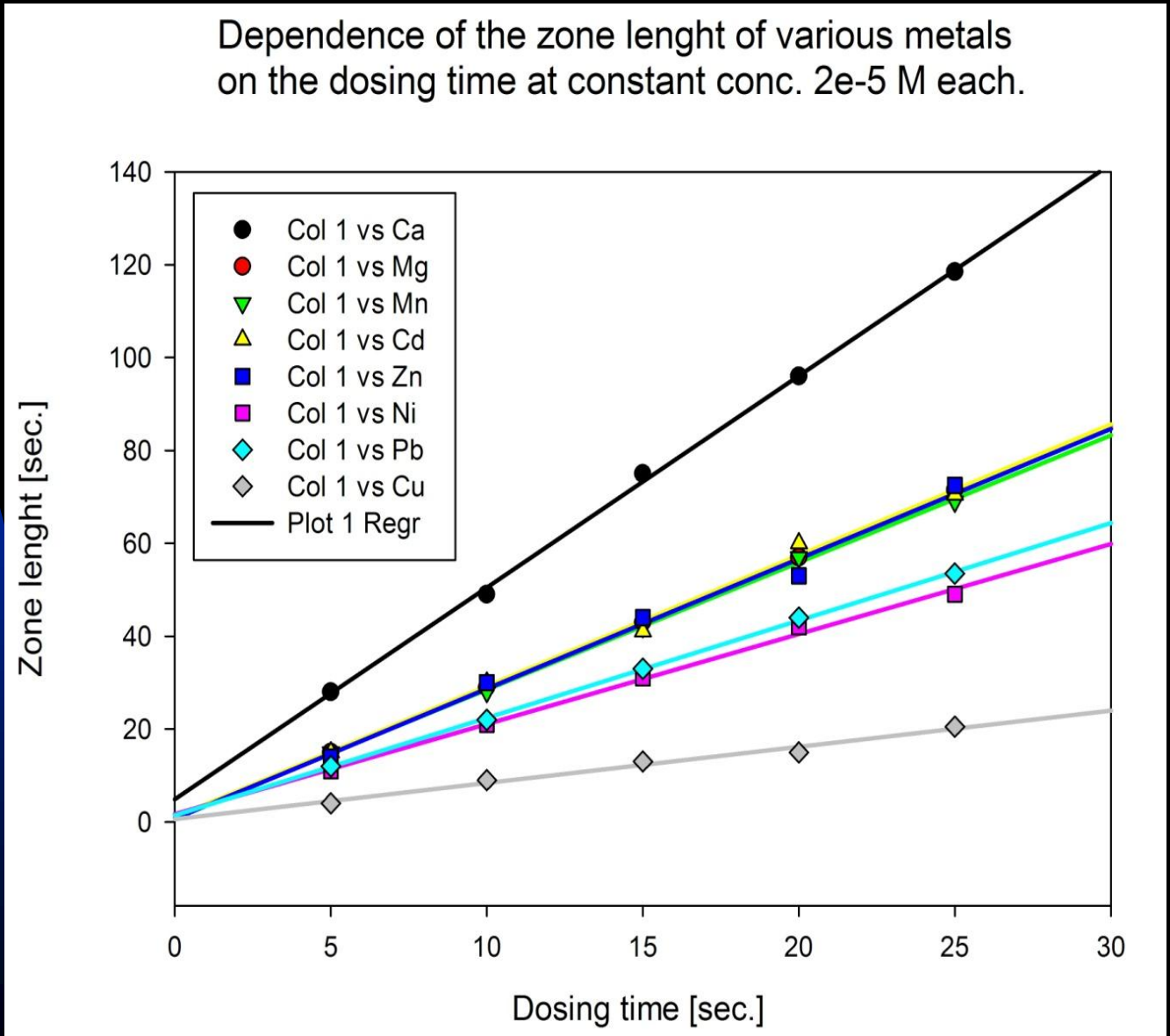
Dependence of zone length of metal on the dosing time – at constant conc. of metals $2x10^{-5}$ Mol/l

Time calibration curve
Complexes are quantitatively broken

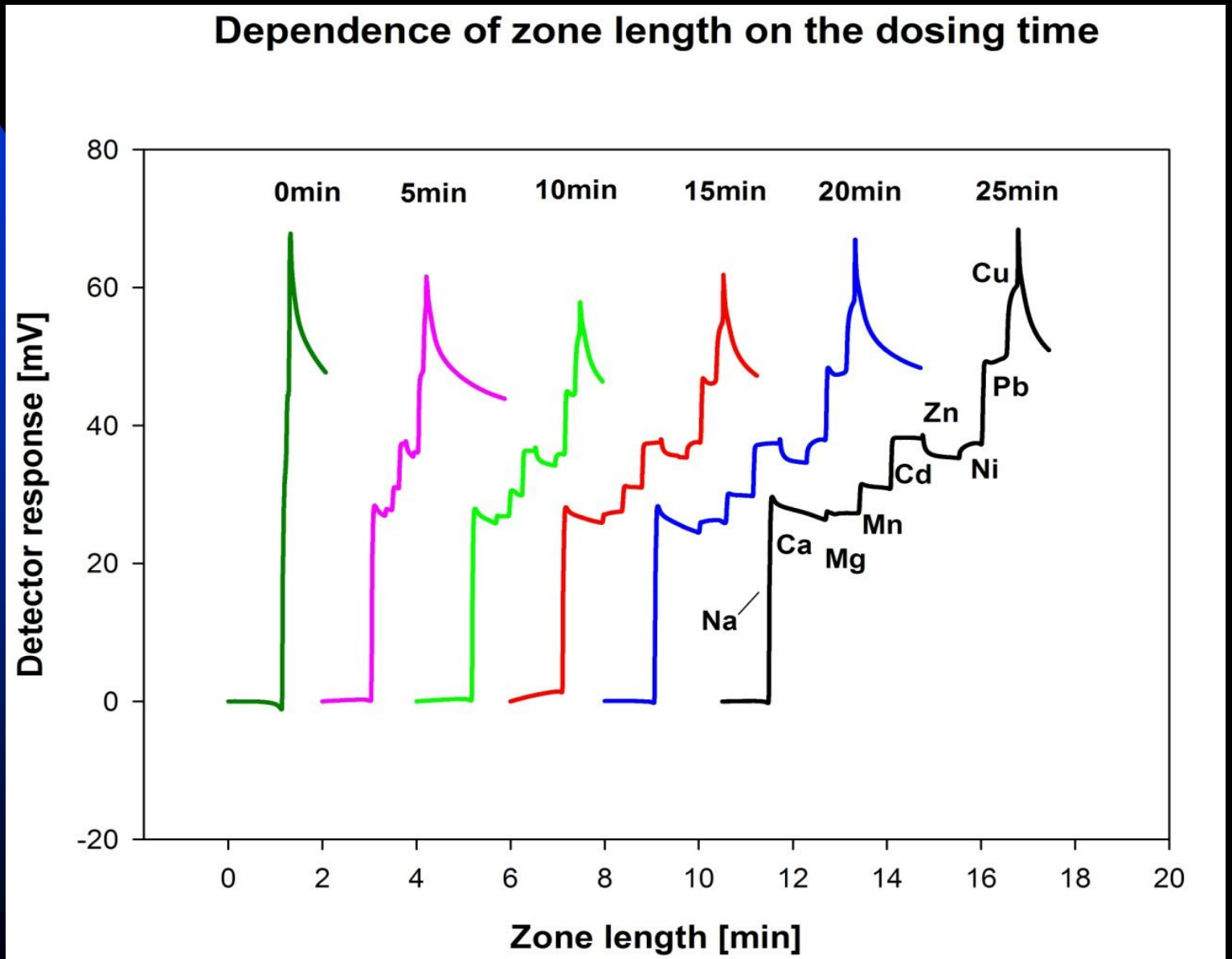
PE = 0,01 M NH_4Ac + 0,01 M NH_4OH + 0,002 M $(NH_4)_3$ cit + 1%PEG6000 + $2x10^{-5}$ M kovy
pH = 9,3

DE = 0,01 M NH_4Ac + 0,01M HAc + kovy pH=3,83
LE =0,01 M NH_4Ac + 0,1 M HAc + 0,002 M $(NH_4)_3$ cit + 1%PEG6000 , pH = 4

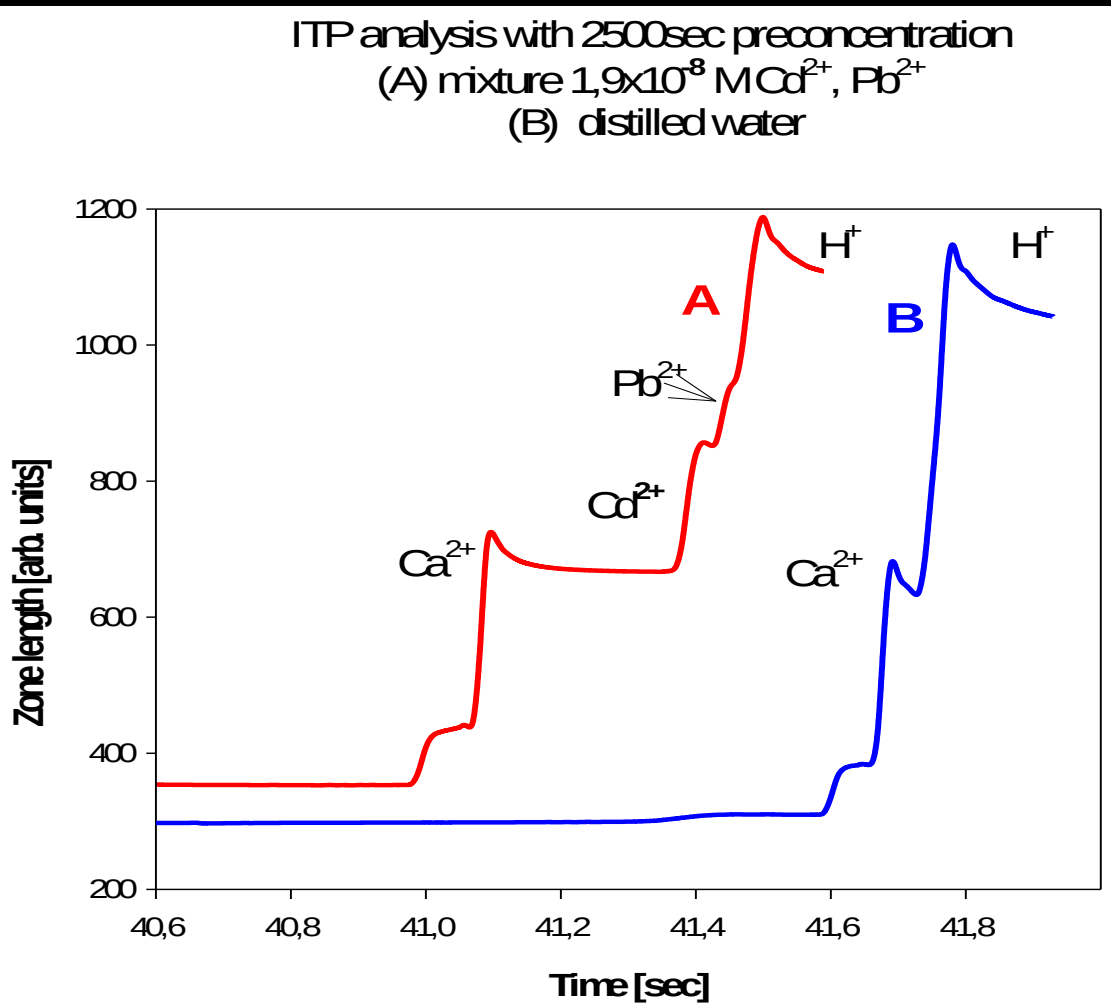
TE =0,03 M HAc



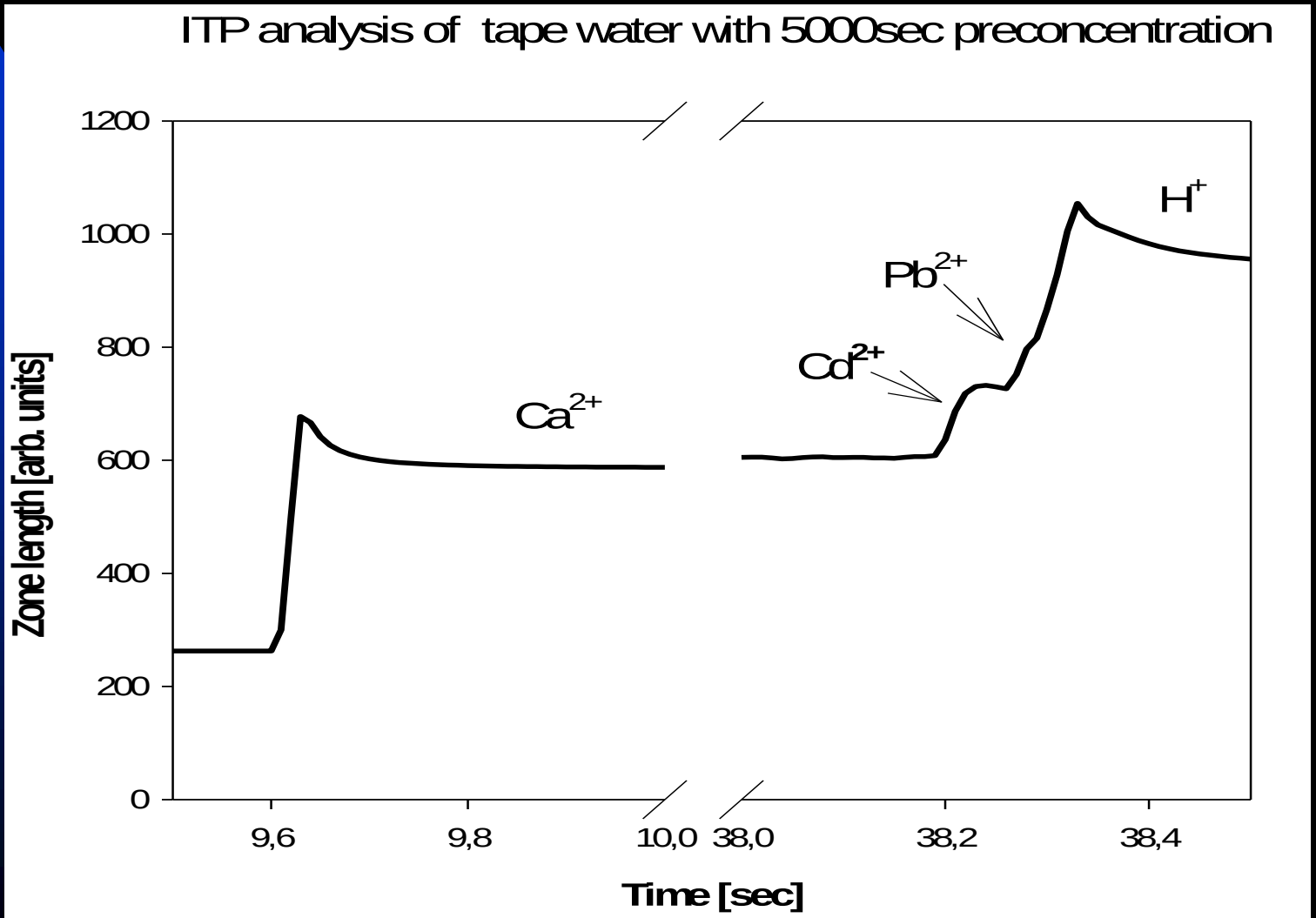
Analytical records of metal mixture at different dosing times – constant conc. of metals $2x10^{-5}$ Mol/l



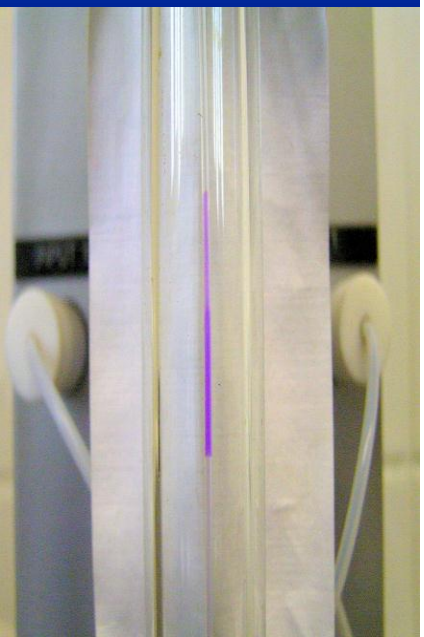
ITP analysis with 2500 sec focusing in LSG (A) sample mixture 1,8.10^{-8} Mol/l Cd, Pb (B) sample distilled water

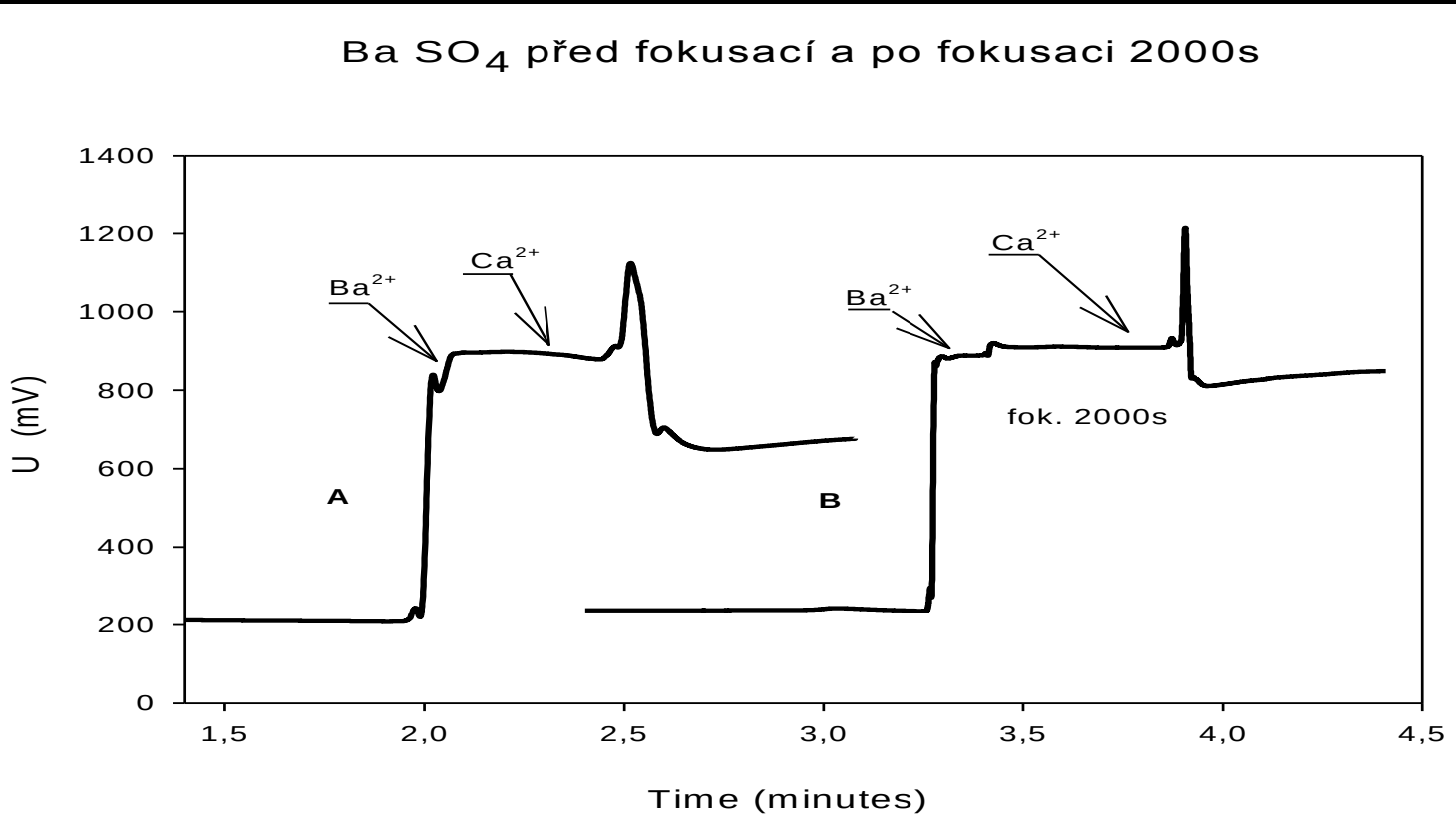


ITP analysis of tape water 5000 sec focusing in LSG found $1{,}0 \times 10^{-8}$ Mol/l Cd, $0{,}9 \times 10^{-8}$ Mol/l Pb

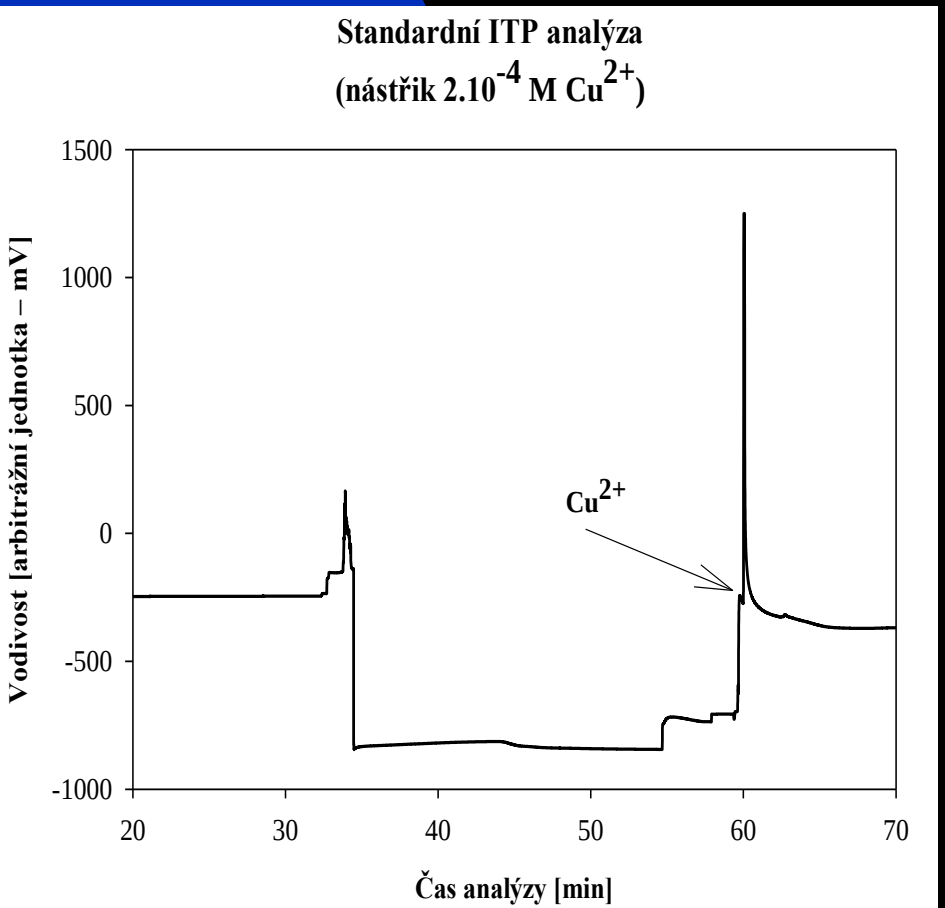


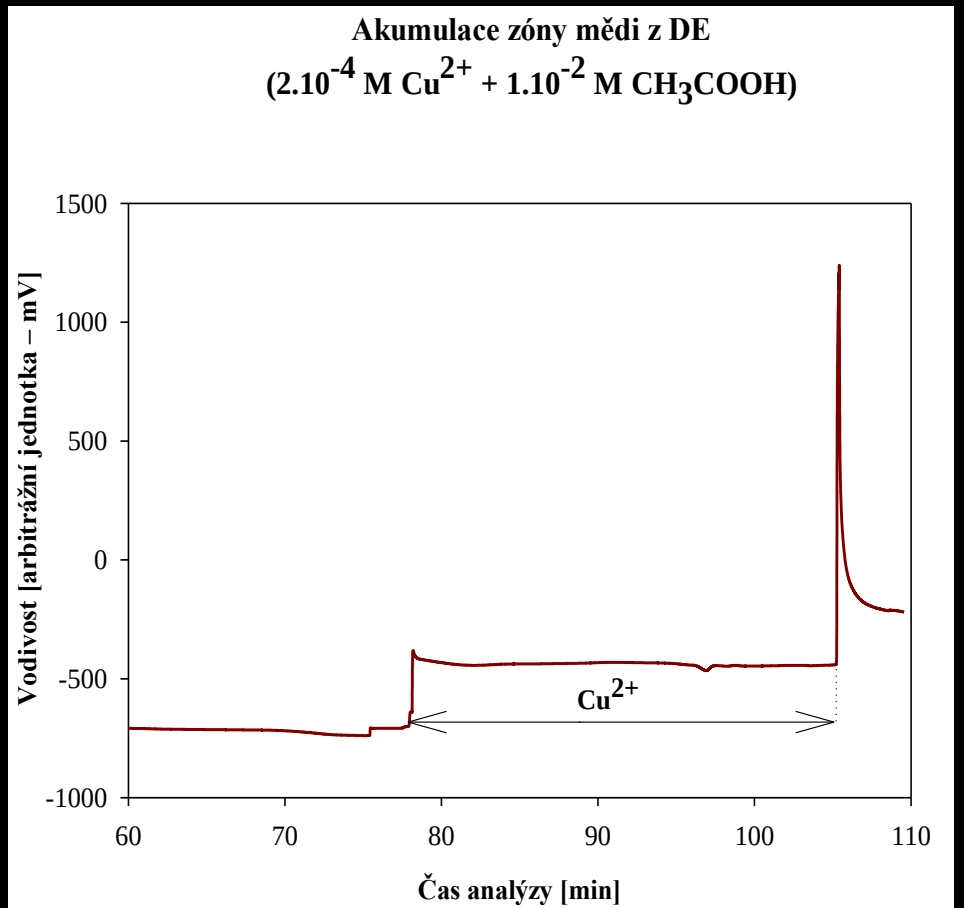
ITP analysis of supernatant abowe $BaSO_4$ without preconcentration (A) with preconcentration 2000s v LSG (B)





ITP analysis without and with 42min focusing in LSG sample 2.10^{-4} Mol/l Cu – 77 fold accumulated





Conclusions - LSGF

We developed and verified new method for the pre-concentrationand pre-separation of heavy metals in the ligand step gradient.

Used ligands

KFX - alkaline earth metals
Citrate - heavy metals
PAR+citrate - copper

The reached pre-concentration degree ranged 9-229x.

Method is convenient as a pre-concentration step before ITP or CZE analysis.