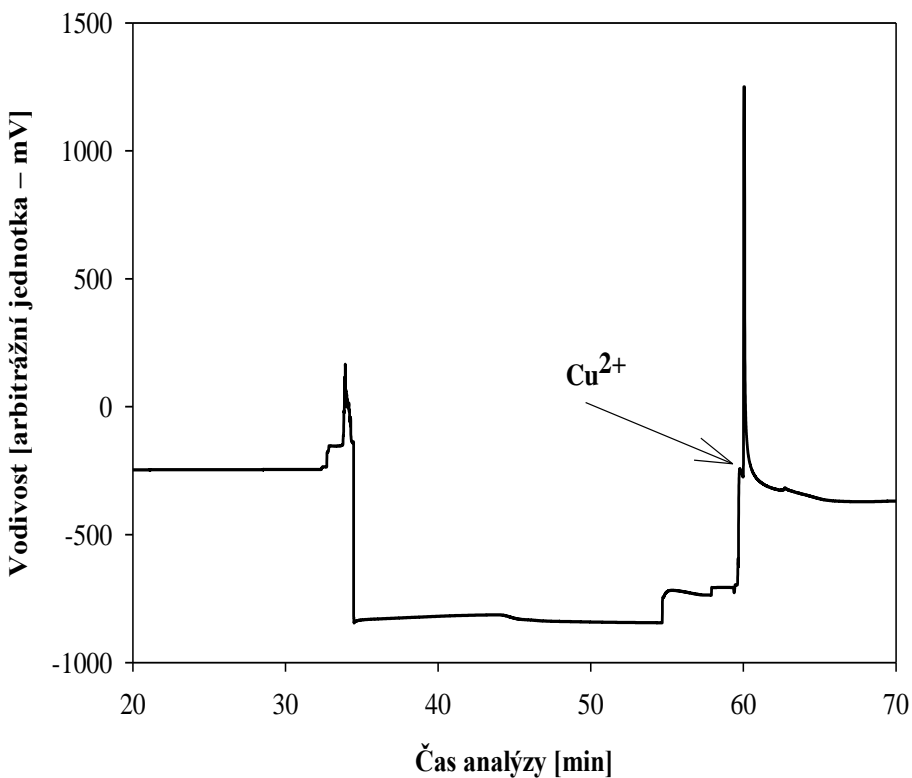


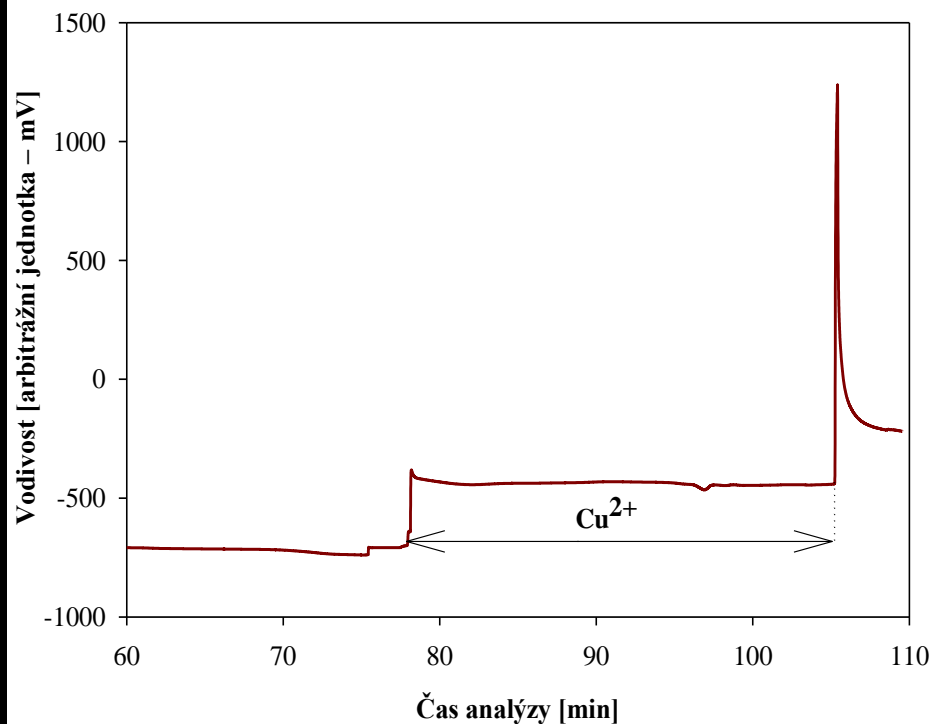
ITP analysis without and with 42min focusing in LSG sample $2 \cdot 10^{-4}$ Mol/l Cu – 77 fold accumulated

Complex forming NRB II Method LSGF-ITP

Standardní ITP analýza
(nástřik $2 \cdot 10^{-4}$ M Cu^{2+})



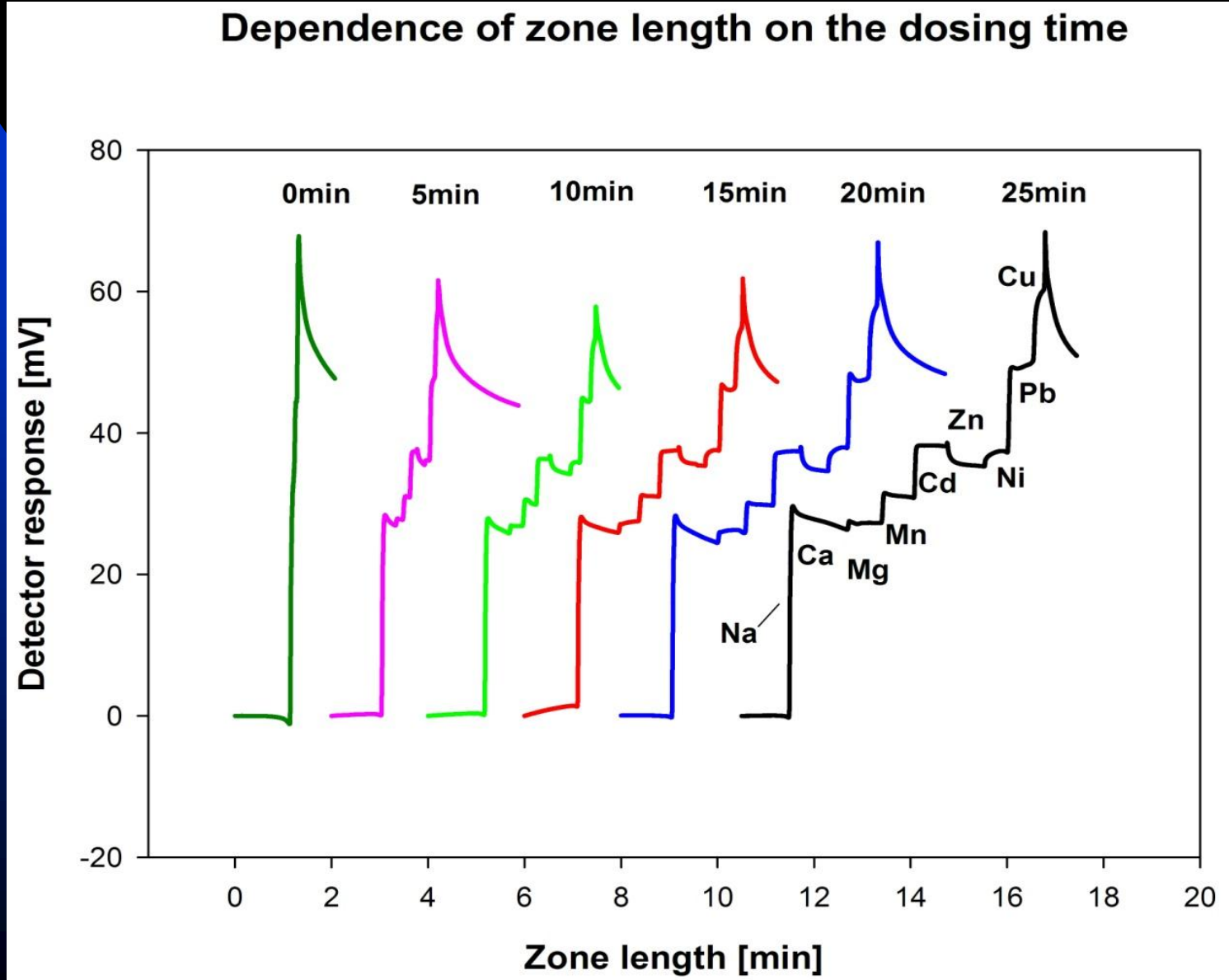
Akumulace zóny mědi z DE
($2 \cdot 10^{-4}$ M Cu^{2+} + $1 \cdot 10^{-2}$ M CH_3COOH)



Analytical records of metal mixture at different dosing times – constant conc. of metals 2×10^{-5} Mo/l

Complex forming NRB II

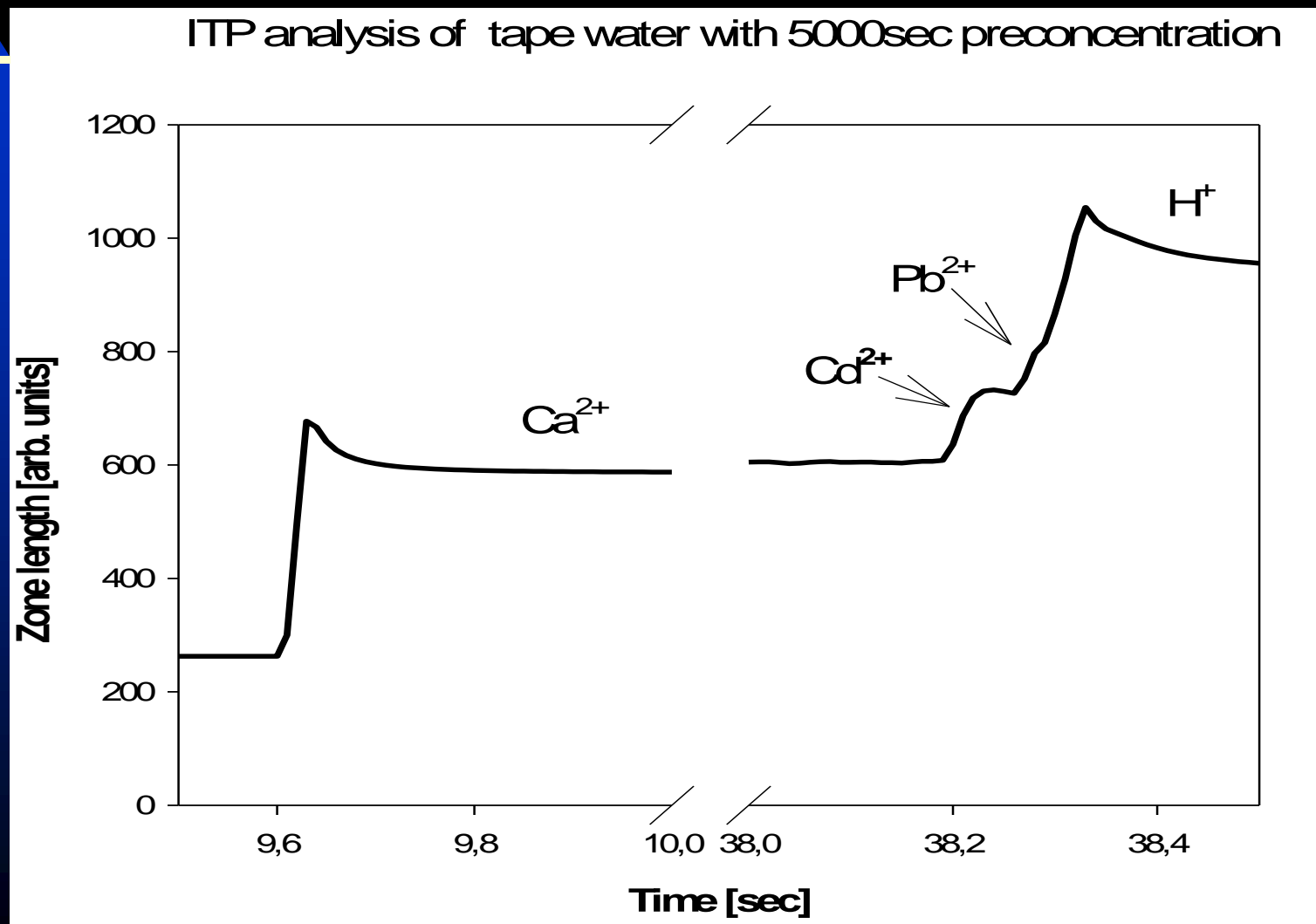
Method LSGF-ITP



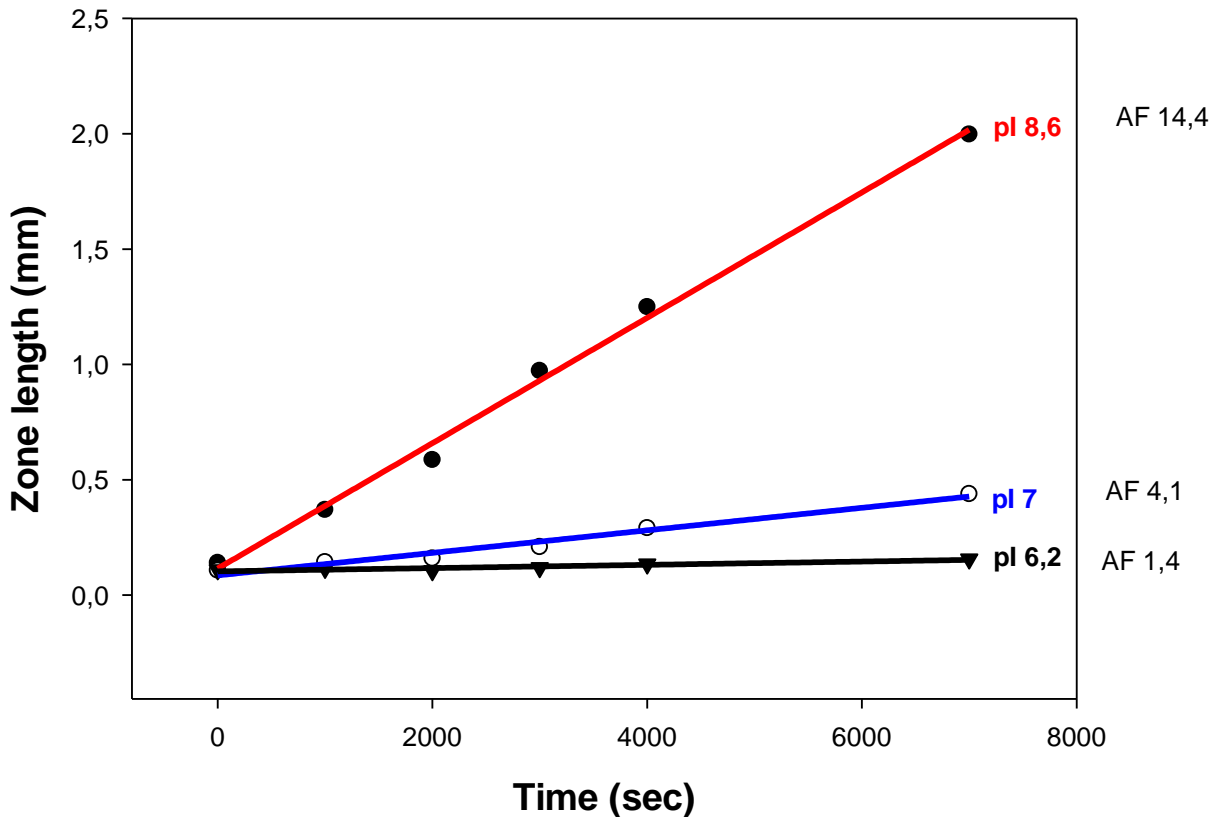
ITP analysis of tape water 5000 sec focusing in LSG found $1,0 \times 10^{-8}$ Mol/l Cd, $0,9 \times 10^{-8}$ Mol/l Pb

Complex forming NRB II

Method LSGF-ITP



Accumulation of ampholytes on asymmetrical neutralization reaction boundary H⁺/HIS



selectivity

DE: **0,002M HIS** + 0,01M NH₄Ac + vzorek amfolytů, pH=6,95

PE: ca. 0,01M NH₄OH + 0,01M NH₄Ac + 1% PEG + 400ppm povrchově aktivní látky, pH=8,62

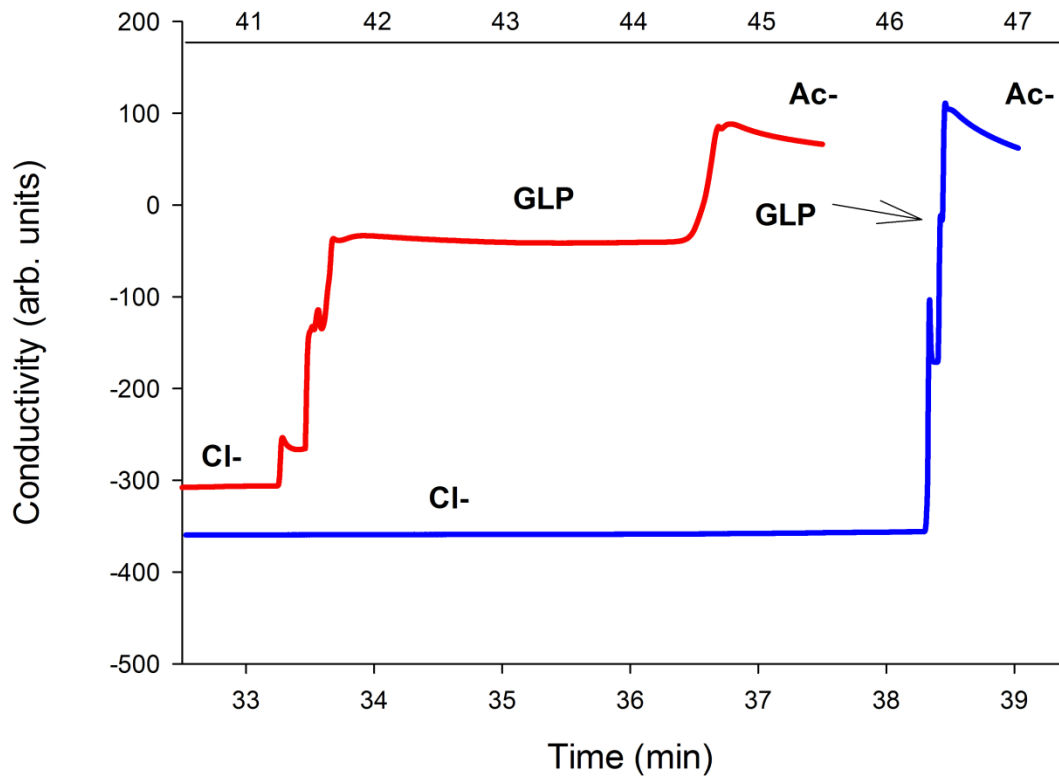
TE: 0,03M HAc, pH=3,12

LE: 0,01M NH₄Ac + 1% PEG + 400ppm povrchově aktivní látky, pH=6,75

Sample: LMW ampholyte dyes pI=8.6+7+6.2

Accumulation of glyphosate /RoundUp/ on asymmetrical neutralization reaction boundary H^+/HIS

ITP analysis of 1×10^{-5} Mol/l glyphosate with and without focusing (1500sec/0sec)



Conclusion

Simply NRB – CAF IEF

- Reached 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

Complex forming NRB – LSGF

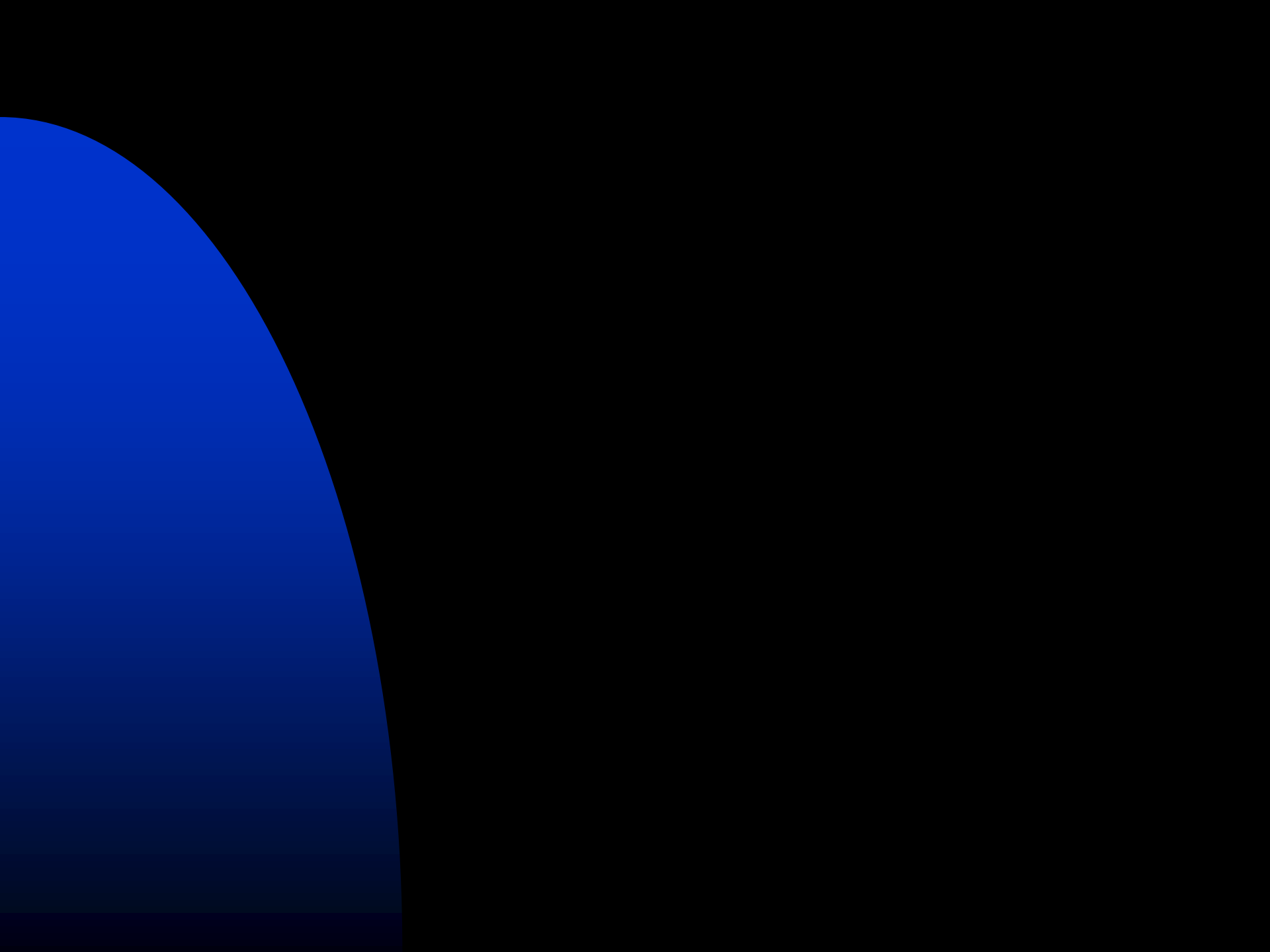
The reached pre-concentration degree ranged 9-229x.
2000s

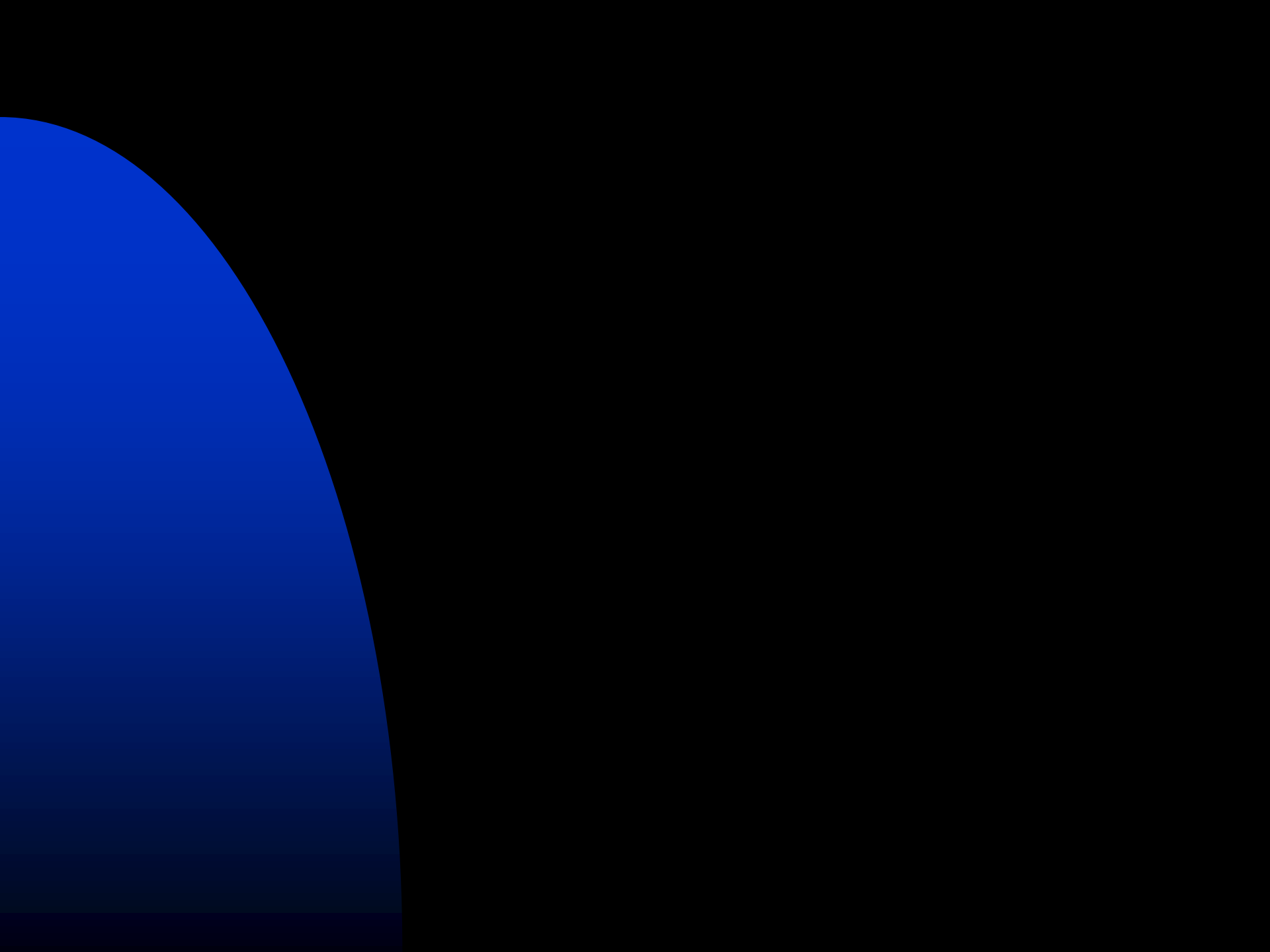
Asymmetrical NRB

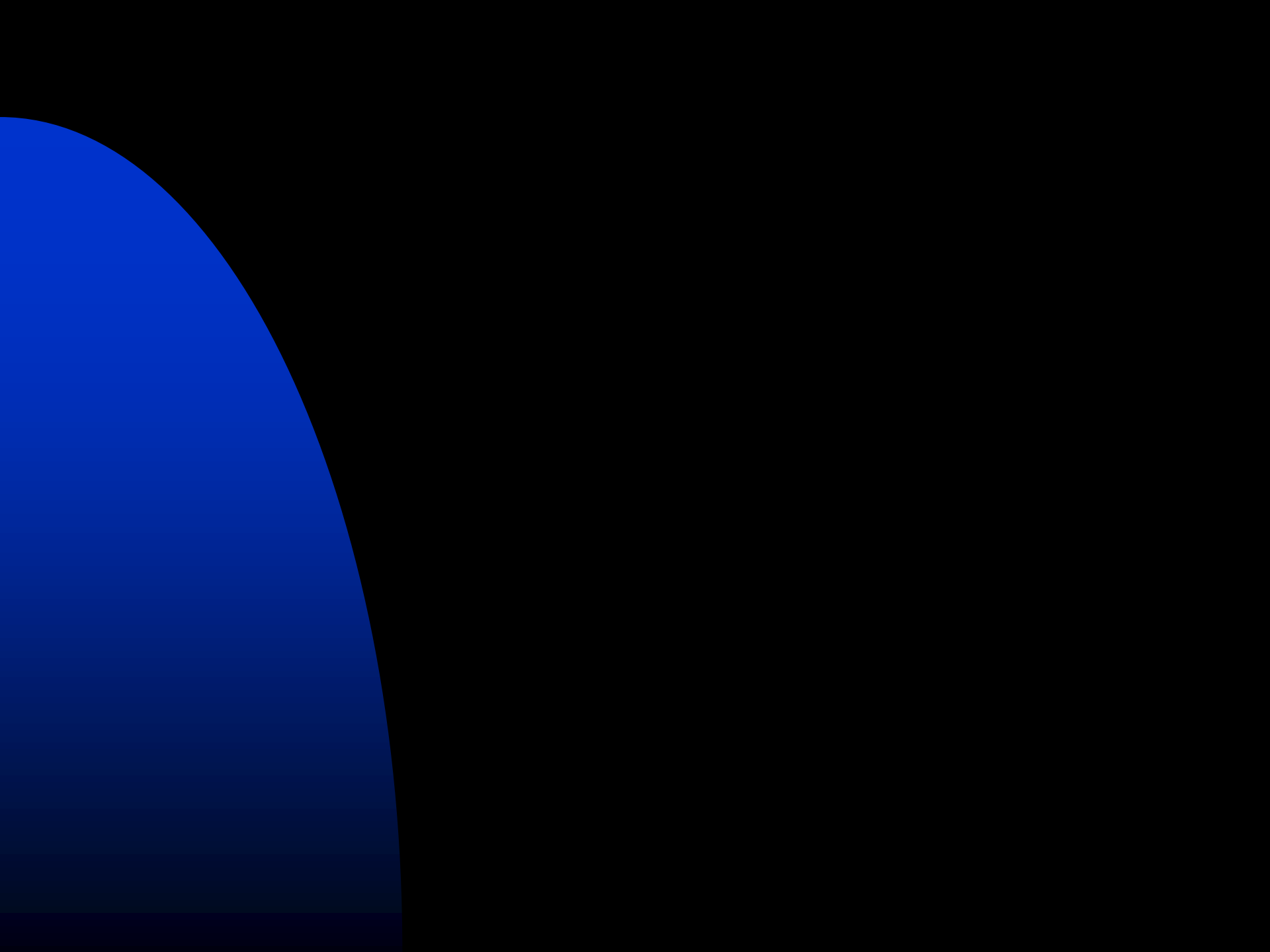
The reached pre-concentration degree for GLP 180x for
1500s

Aknowledgement

- This work was supported by MSMT project
 - CZ 1.07/2.2.00/28.0302







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

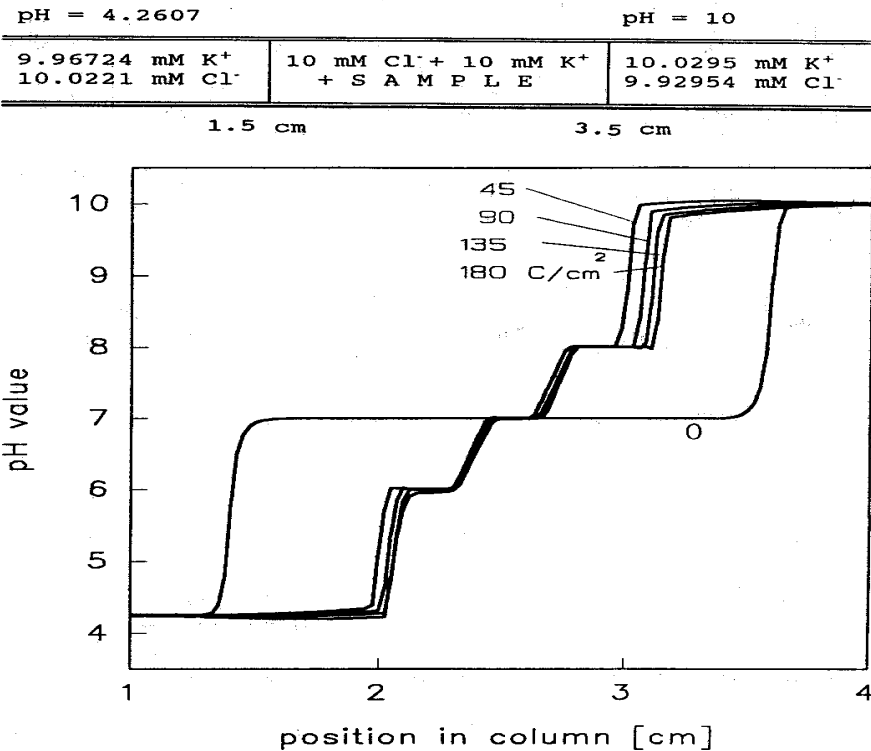
• Focusing of heavy metals

- DE = 0,01 M NH_4Ac + 0,01 M HAc (Real sample $2 \cdot 10^{-4}$ M NH_4Ac + $1 \cdot 10^{-5}$ M HAc) + kovy pH=4,7
- PE =0,01M NH_4Ac + 0,01 M NH_4OH + 0,002 M $(\text{NH}_4)_3\text{Cit}$ + 1%PEG6000, pH = 9,2
- LE =0,01 M NH_4Ac +0,1 M HAc + 0,002 M $(\text{NH}_4)_3\text{Cit}$ + 1%PEG6000
- TE =0,03 M HAc

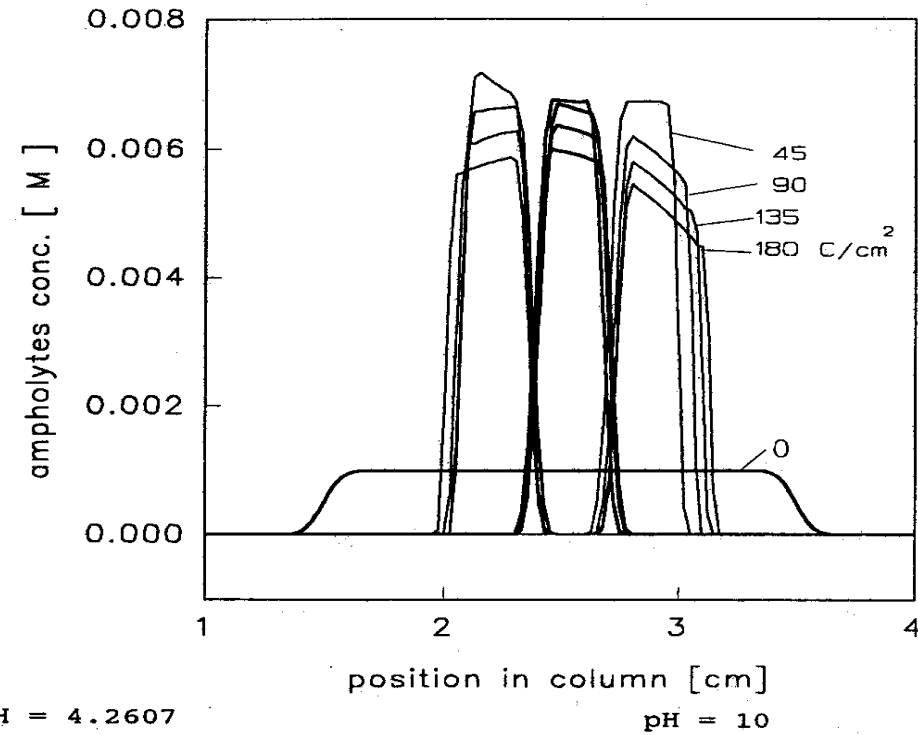
Properties of ampholyte zones

Simulated longitudinal pH profile of three ampholytes (pI=6,7,8). Developed stationary zones of pH=pI.

Simulated longitudinal concentration profile of three ampholytes (pI=6,7,8).



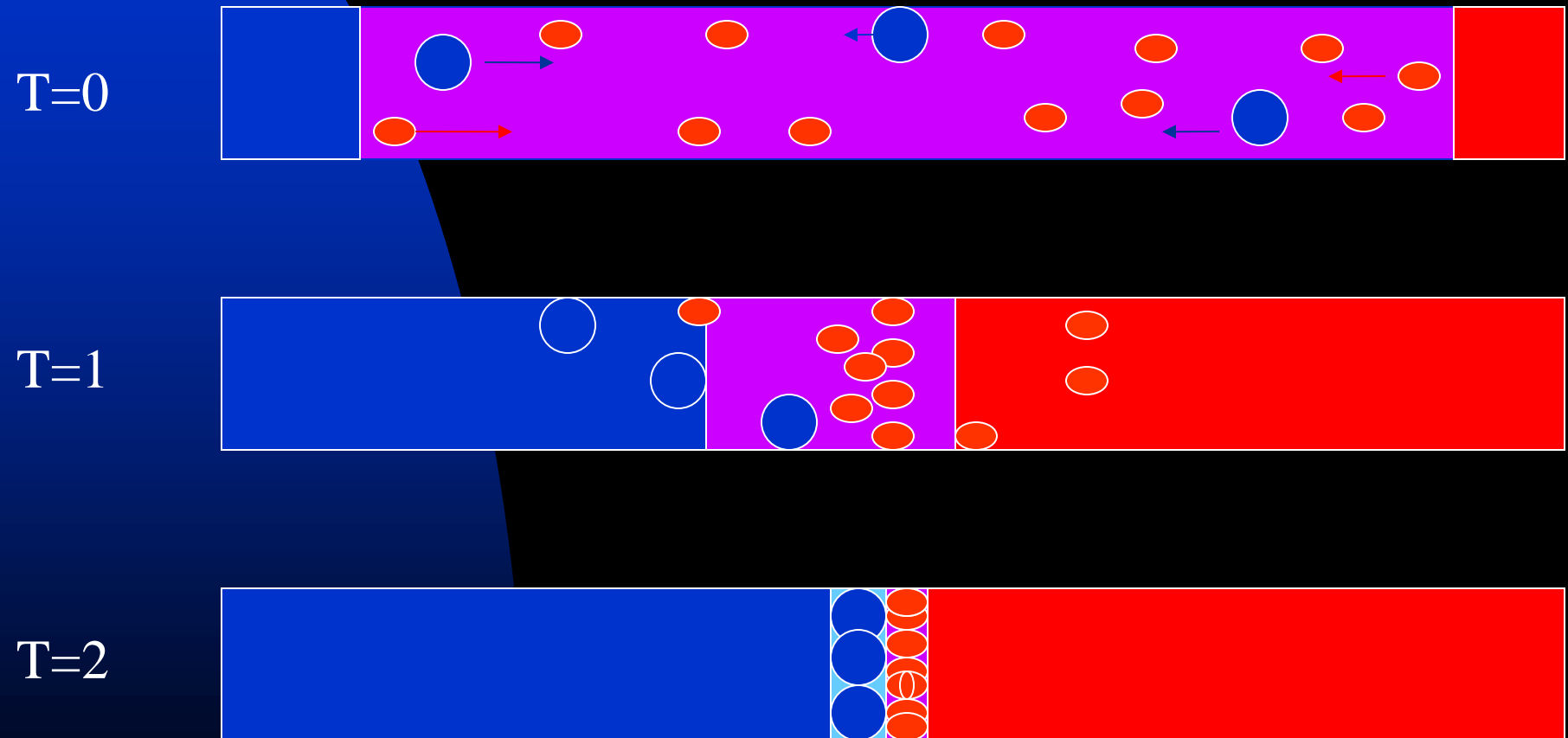
Focusing of three ampholytes. Top. Concentration profiles, Bottom. pH profiles. Sample: 1 mM ampholyte pI = 6, 1 mM ampholyte pI = 7, 1 mM ampholyte pI = 8. $pK_1 = 4.5$, $pK_2 = 7.5$, $\mu^0 = \mu^+ = \mu^- = 30 \times 10^{-9} \text{ m}^2/\text{V}\cdot\text{sec}$. $pK_1 = 5.5$, $pK_2 = 8.5$, $\mu^0 = \mu^+ = \mu^- = 30 \times 10^{-9} \text{ m}^2/\text{V}\cdot\text{sec}$. $pK_1 = 6.5$, $pK_2 = 9.5$, $\mu^0 = \mu^+ = \mu^- = 30 \times 10^{-9} \text{ m}^2/\text{V}\cdot\text{sec}$. Current density 100 A/m² meshpoints per column 200.



Isoelectric focusing without carrier ampholytes CAF IEF

$E \neq \text{konst}$, $\text{pH} = f(x)$, $V_A = E_A \cdot \mu_A$, $V_B = E_B \cdot \mu_B$,

$\mu_A > \mu_B$, $V_A = V_B = 0$, $\text{pH}_A = \text{pI}_A$, no carrier ampholytes



Choice of the electrolyte system

$$J_{\text{H}^+} = J_{\text{OH}^-} \quad w = 0$$

$$J_{\text{H}^+} \neq J_{\text{OH}^-} \quad w \neq 0$$

- Buffered electrolytes
- Dependence $w = F (\text{pH}_\text{H}, \text{pH}_\text{OH})$
- must be known
- Mobilization $V \text{ sample} \geq W$
- Correct migration $\mu \text{ sample} > \mu_{\text{OH}^-}, \text{H}^+$

- Introduction to the electrophoresis

- Principle of the method

- Analytical properties

- Choice of the electrolyte system

- Procedure of the focusing

- Results

- Conclusion

Electrolytes

PARAMETR	PRIMARY ELECTROLYTE LE-PE	DOSING ELECTROLYTE DE	TERMINATING ELECTROLYTE TE	BACKGROUND ELECTROLYTE BGE-CZE
Cation	NH_4^+	NH_4^+	H^+	H^+
Conc. Mol/l	0,015	0,005	0,01	0,05
Anion	CH_3COO^-	CH_3COO^-	CH_3COO^-	CH_3COO^-
Conc. Mol/l	0,005	0,015	0,01	0,05
pH	9,24	4,74	3,3	7
Conc. %	2			2
Sample		pI marker		

Introduction to the electrophoresis

Principle of the method

Analytical properties

Choice of the electrolyte system

Procedure of the focusing

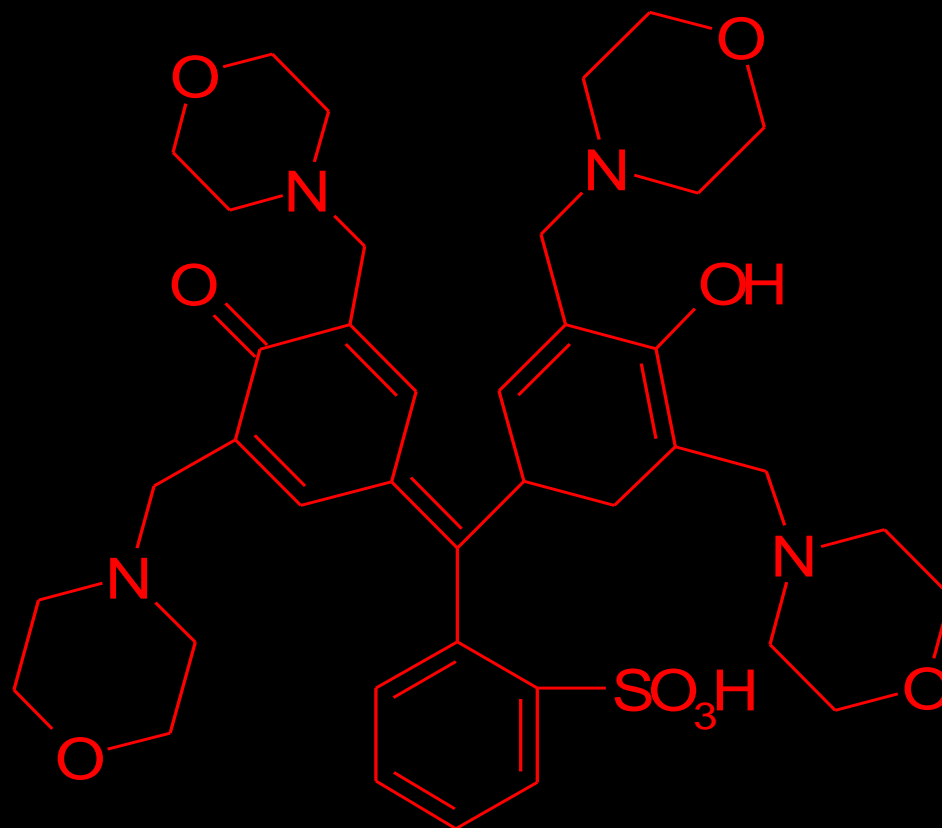
Results

Conclusion

Model substance for the verification of the method

pI marker pI = 7.4

Mw = 679 g/mol (Dr. K. Šlais, UACH
AVČR, Brno)



■ Introduction to the electrophoresis

■ Principle of the method

■ Analytical properties

■ Choice of the electrolyte system

■ Procedure of the focusing

■ **Results**

■ Conclusion

Calibration curve for ITP-CZE combination

Dependence of peak area on the amount of injected ampholyte for combination ITP-CZE

Concentration detection limit cLOD $1\mu\text{Mol/l}$

Detection limit 10pMol

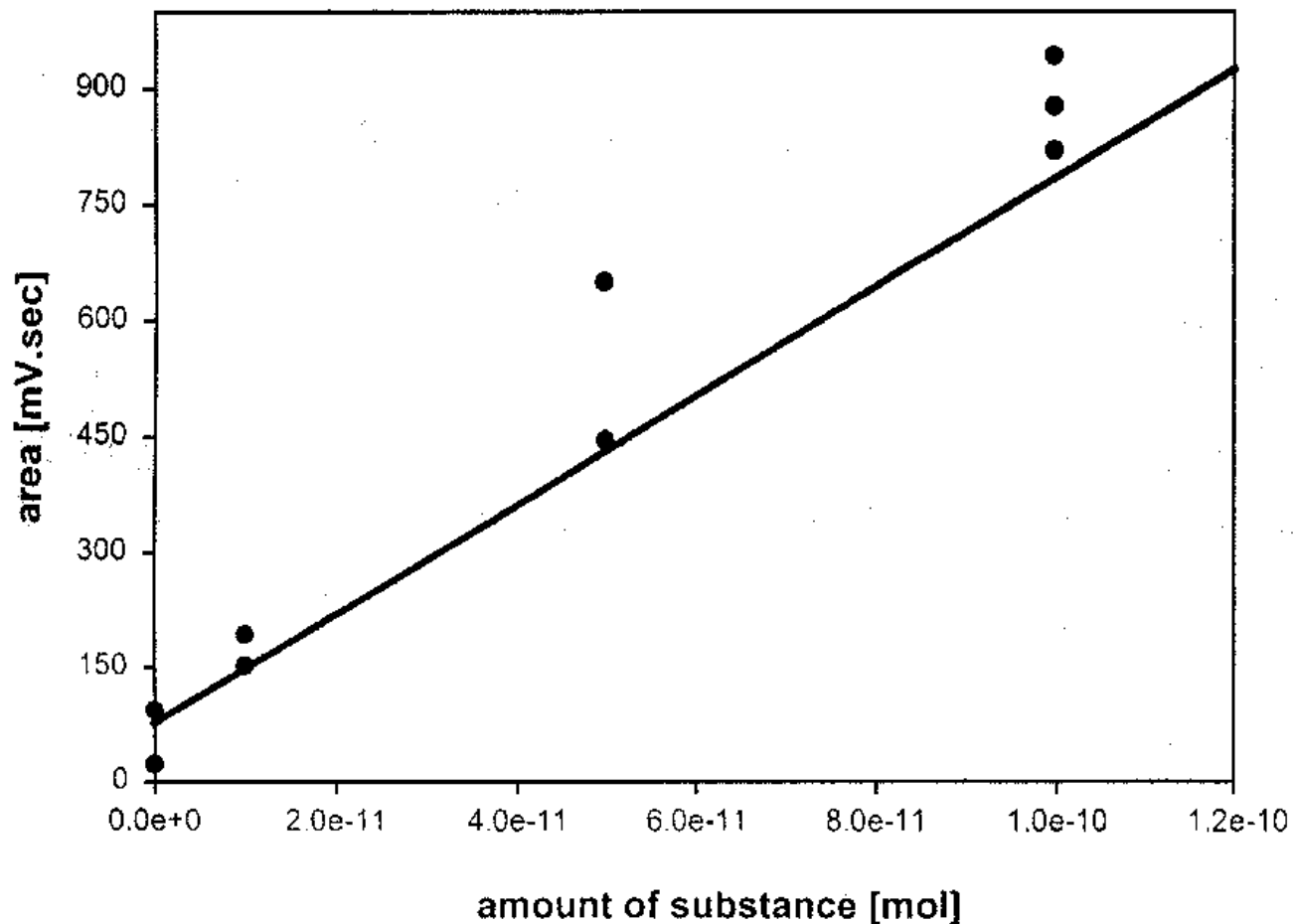


Fig. 3. Dependence of peak area on the amount of injected ampholyte for the ITP-CZE combination. LE=0.005 M NH_4Ac +0.01 M NH_4OH , TE=0.01 M HAc, BGE=0.05 M HAc.

Calibration for continuous dosing

Dependence
of the peak
area on the
time of the
dosing -
combination
CAF IEF
ITP-CZE

cLOD

10nMol/l za
1000 s

Dosing speed

19,88
nMol/Asec

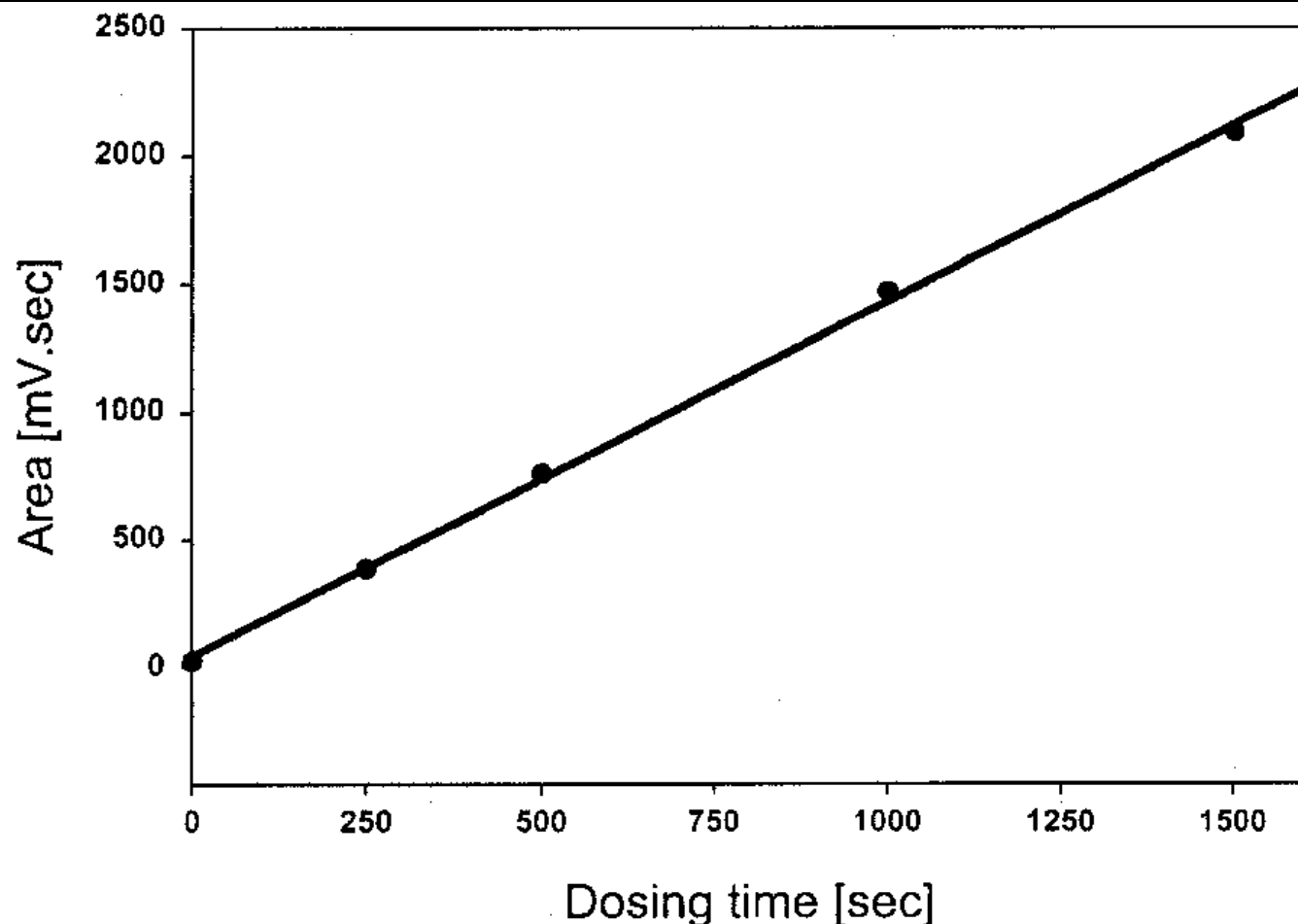
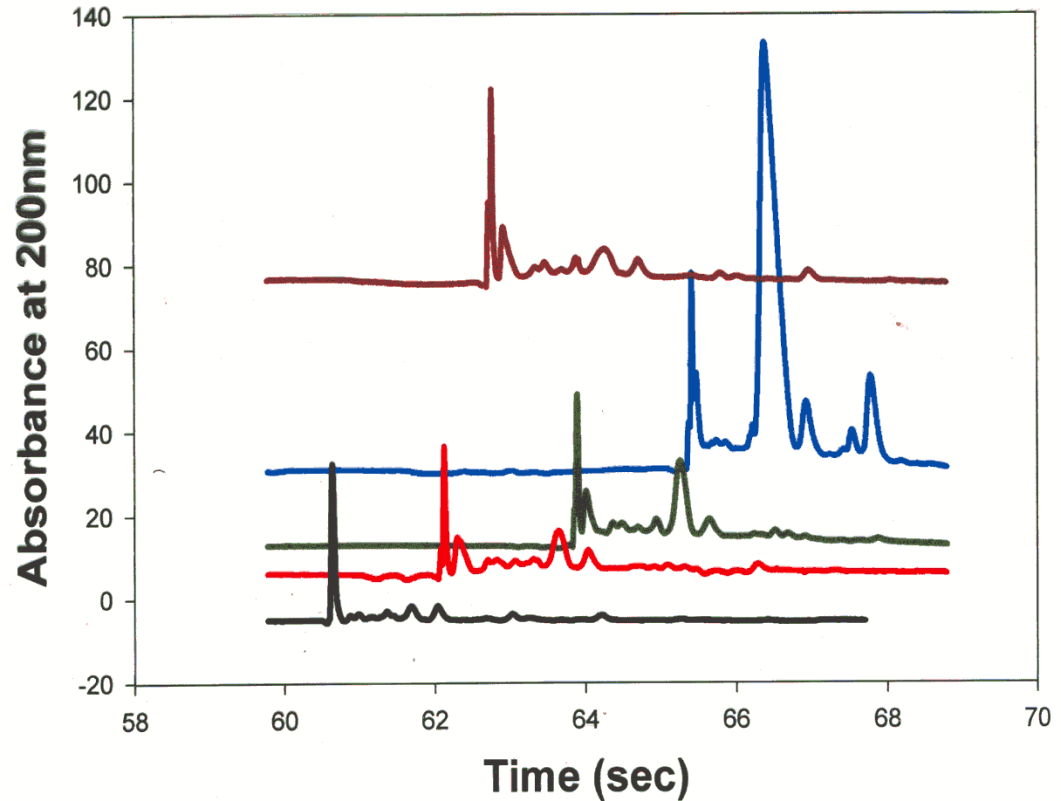


Fig. 6. Dependence of the peak area of the ampholyte on the dosing time for the CAF-IEF-ITP-CZE combination. LE=0.005 M NH₄Ac+0.01 M NH₄OH, TE=0.01 M HAc, DE=0.005 M NH₄Ac+0.01 M HAc, BGE=0.05 M HAc.

Analytical CAF IEF - CZE results

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



- Ampholyte 10⁻⁶ mol/l
- Ampholyte 10⁻⁷ mol/l
- Ampholyte 10⁻⁸ mol/l
- Ampholyte 10⁻⁹ mol/l special dosing.....
- Blanck run

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

Elektrolytes for AA

- Calculated velocity of the boundary for electrolyte KAc.
- Larger pH span in the alkaline region.

