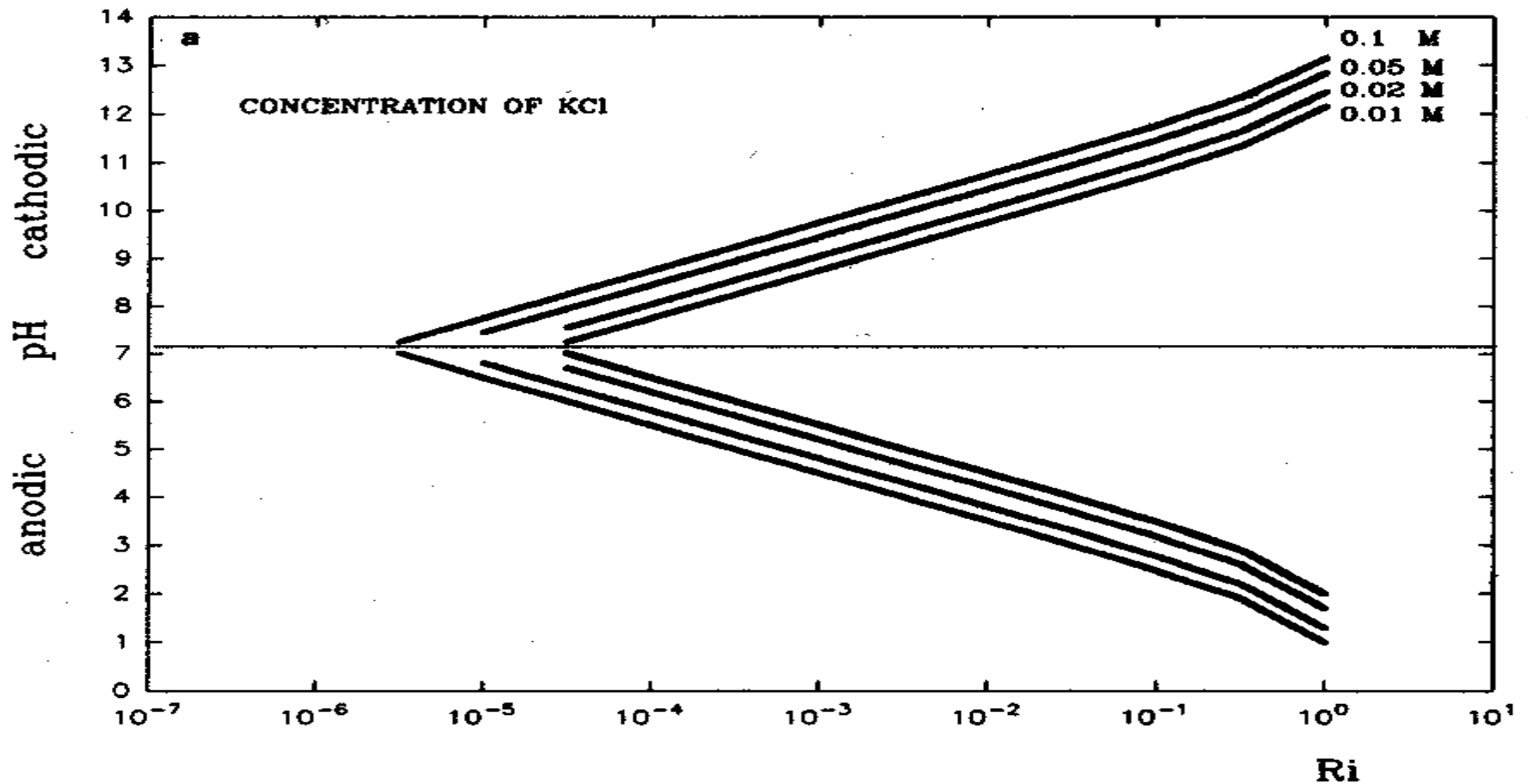




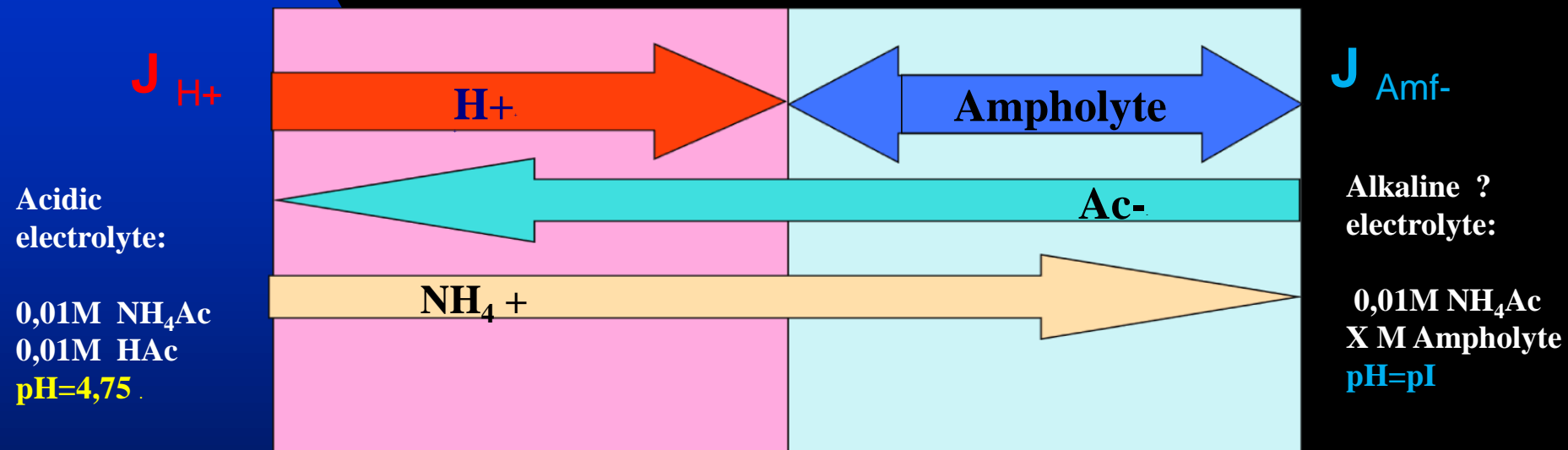
# Symmetrical and asymmetrical neutralization reaction boundary

Expected advantages: selectivity and higher stability – extreme pH



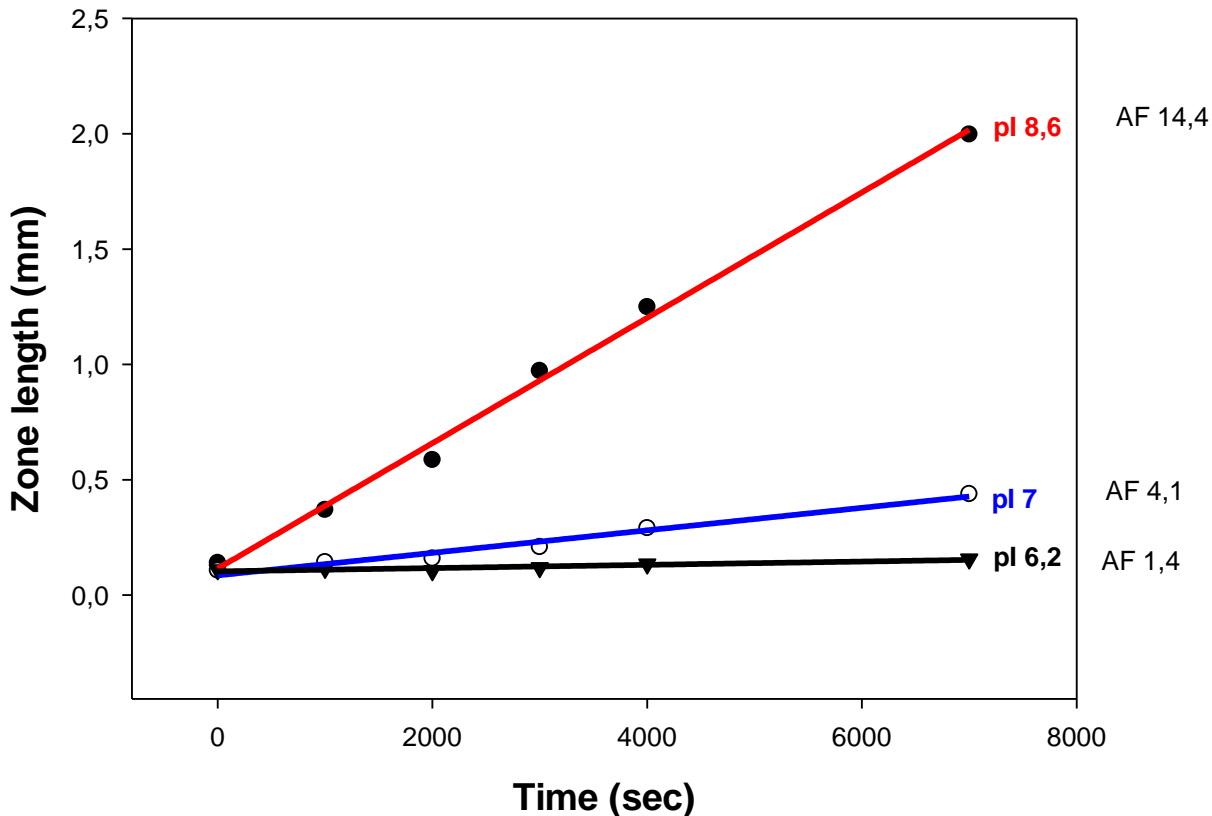
# Principle of the method

## Scheme of the fluxes on asymmetrical neutralization reaction boundary



Asymetry is reached by presence of ampholyte at pH=pI

# Accumulation of ampholytes on asymmetrical neutralization reaction boundary H<sup>+</sup>/HIS



## selectivity

DE: **0,002M HIS** + 0,01M NH<sub>4</sub>Ac + vzorek amfolytů, pH=6,95

PE: ca. 0,01M NH<sub>4</sub>OH + 0,01M NH<sub>4</sub>Ac + 1% PEG + 400ppm povrchově aktivní látky, pH=8,62

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

LE: 0,01M NH<sub>4</sub>Ac + 1% PEG + 400ppm povrchově aktivní látky, pH=6,75

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

## Electrolyte systems used for asymmetrical neutralization reaction boundary

Extreme pH

DE: 0,01 M HIS + Sample      pH=pI=7,2

PE: 0,02 M HCl + 0,005M HIS      pH=1,8

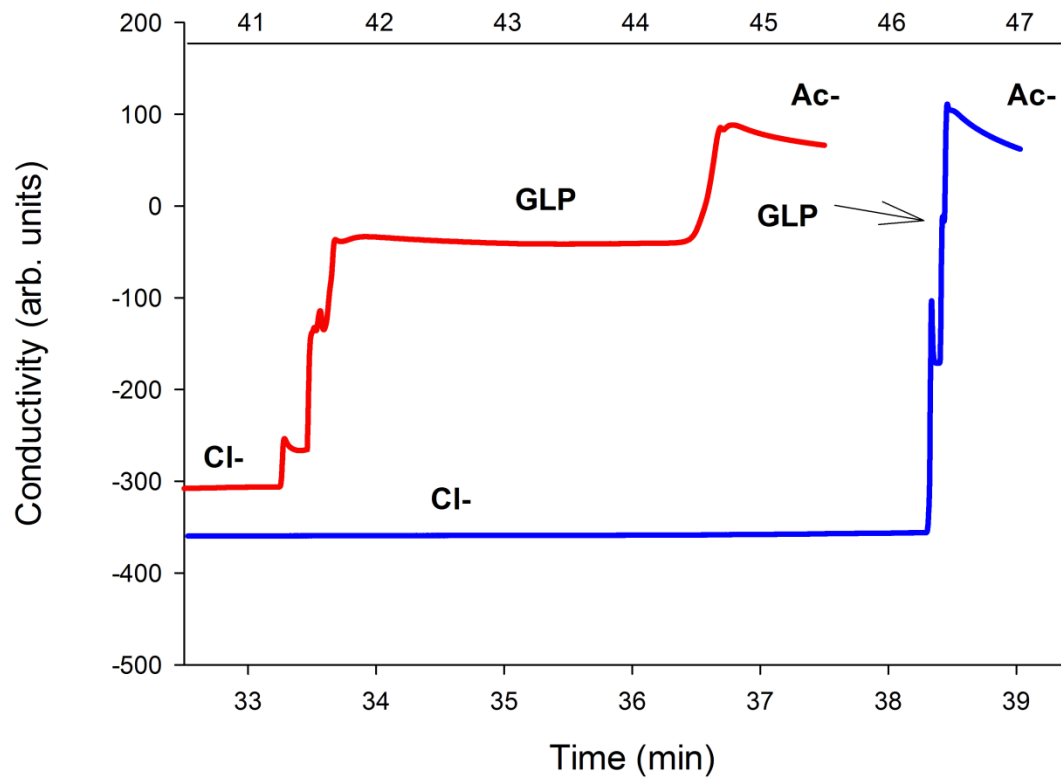
ME: 0,02 M HCl + 0,04M betaALA      pH=3,7

LE: 0,02 M HCl + 0,04M betaALA      pH=3,7

TE: 0,01 M HAc + 0,02 M HIS

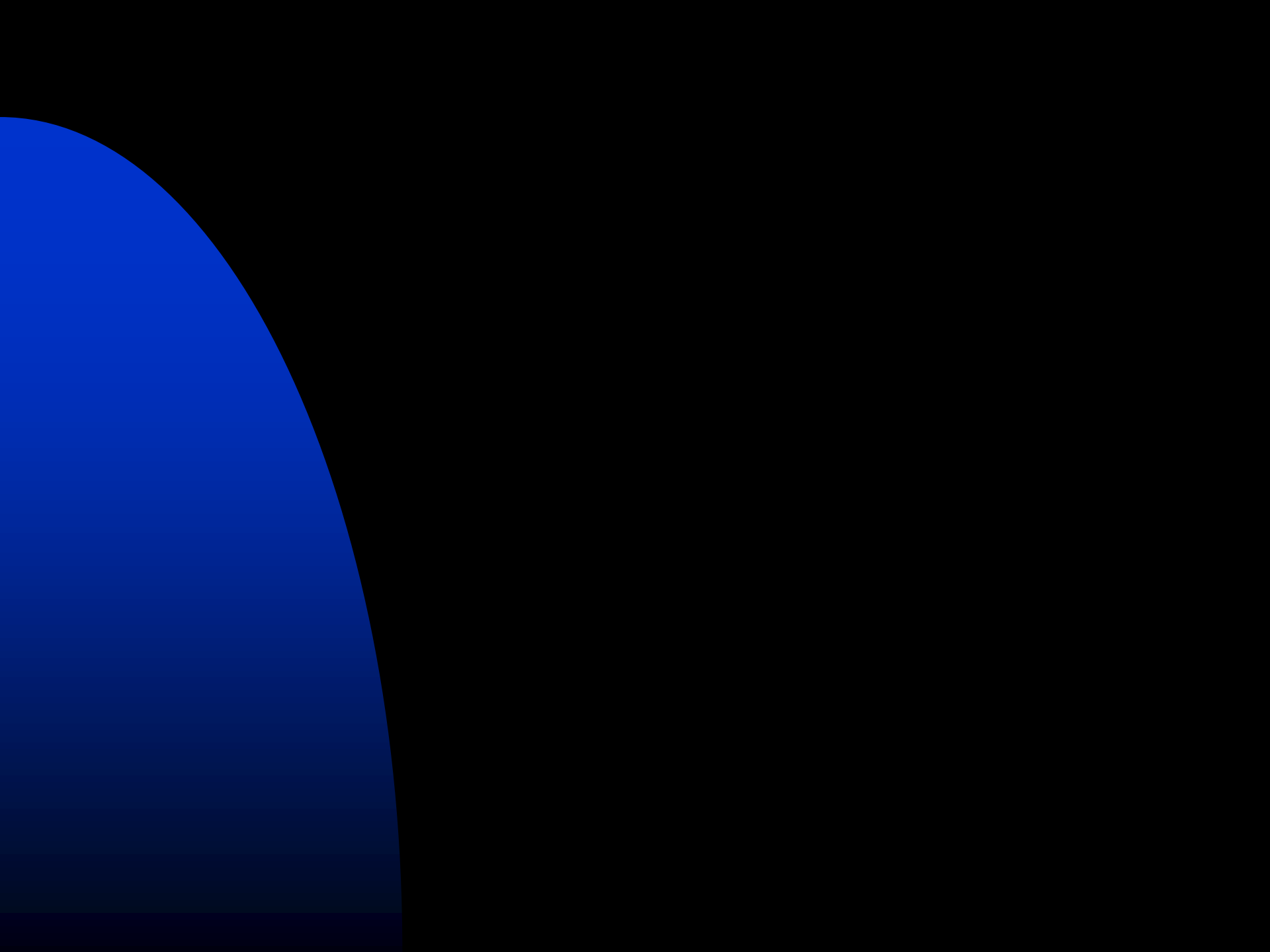
**Procedure: focusing, modification, mobilization, analysis**

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

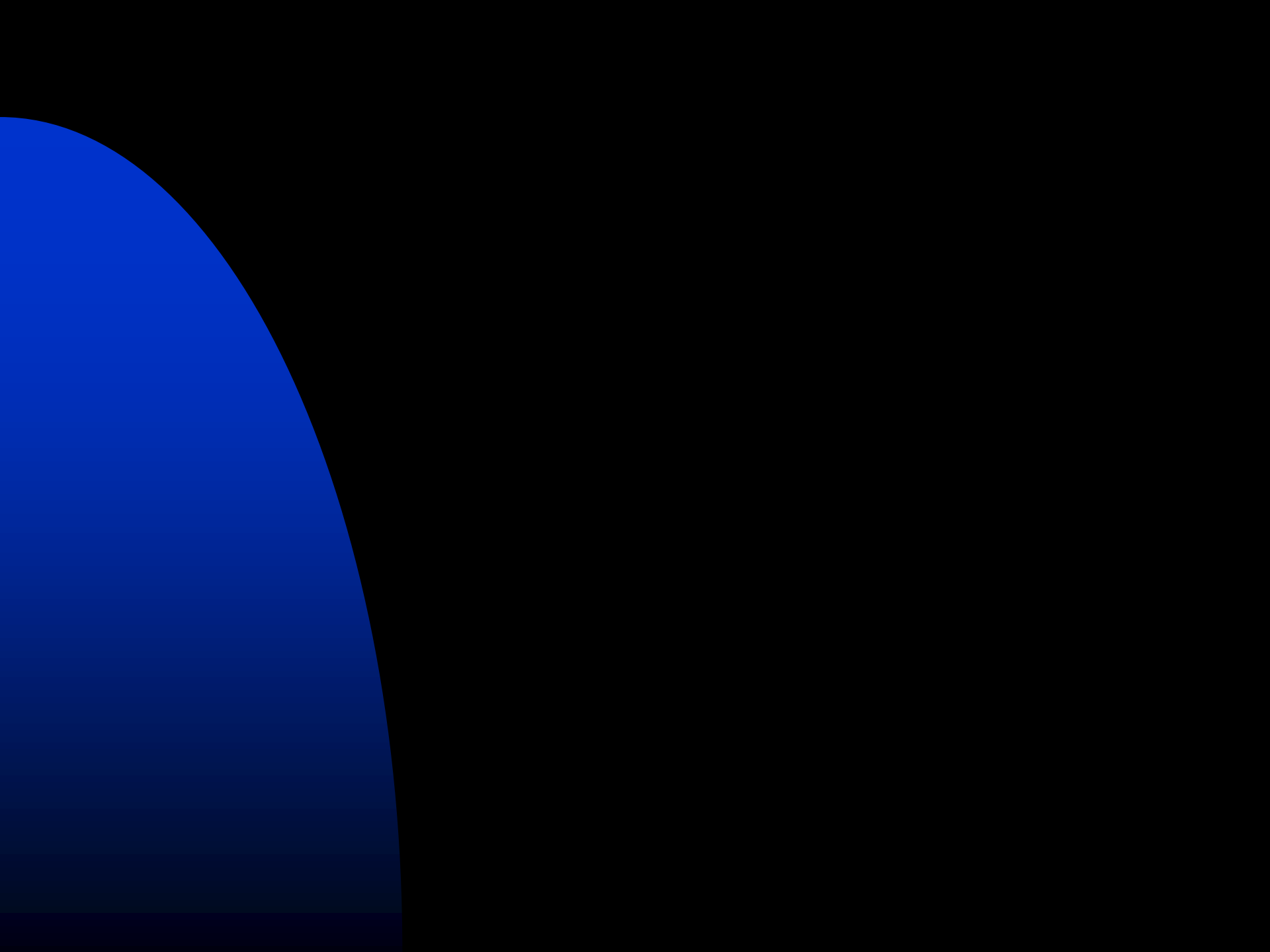


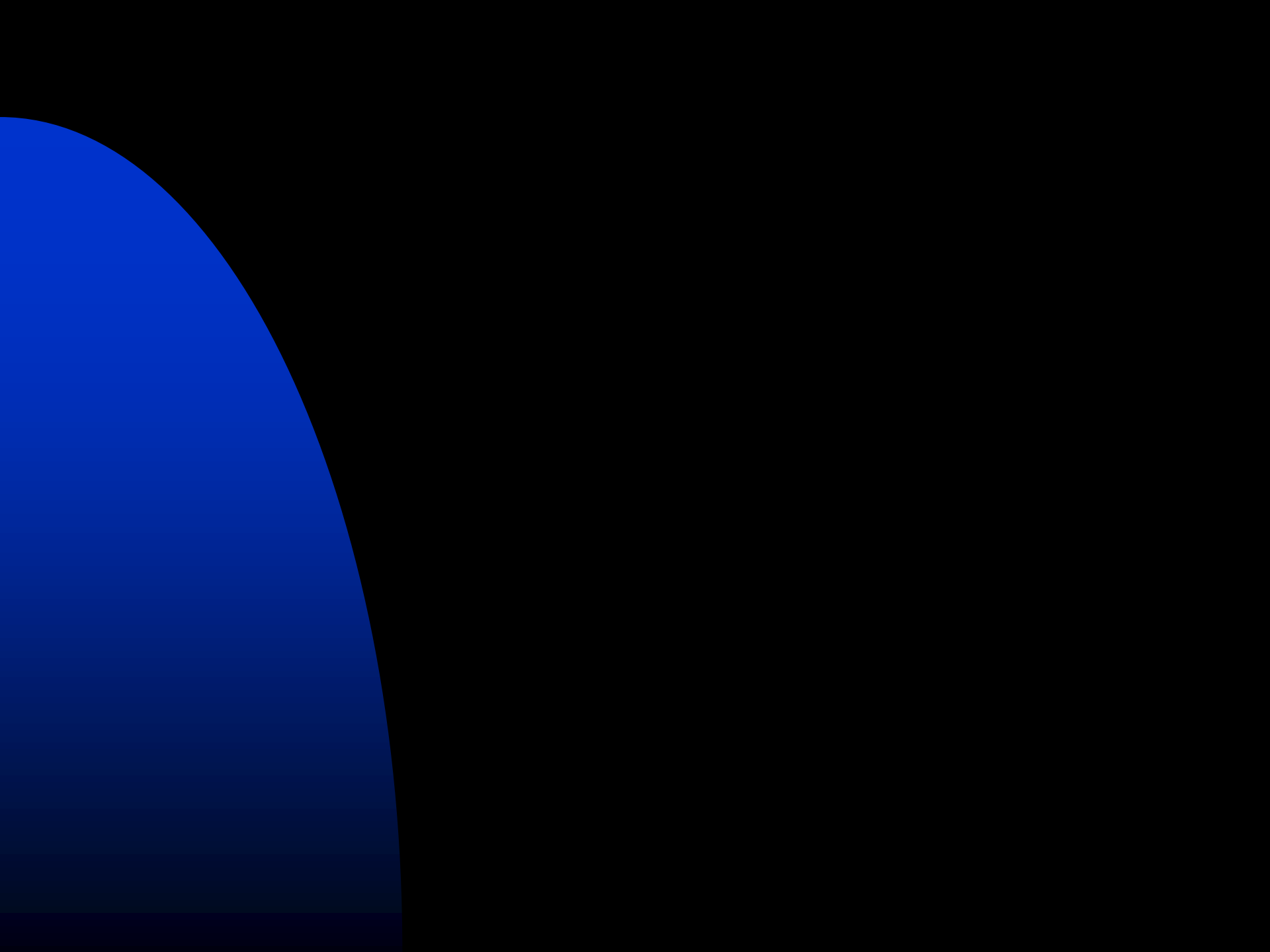
**Conclusion for focusing on  
asymmetrical neutralization reaction  
boundary**

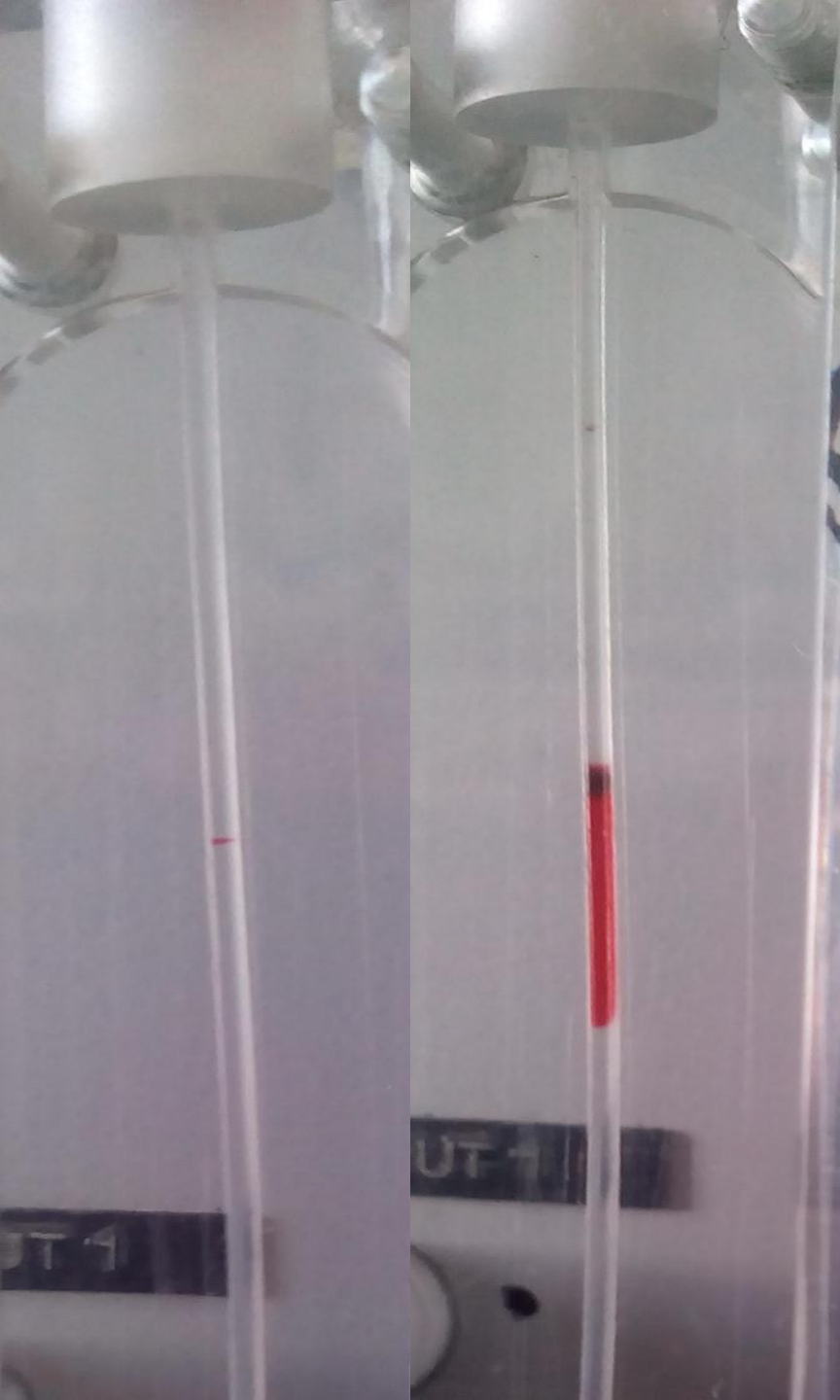
**Conclusion for focusing on asymmetrical  
neutralization reaction boundary**











# Focusing pre-concentration for electrophoresis

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

# Electromigration methods origin

1800 - Alessandro Volta - voltaic pile – battery - DC

1800- Nicholson - decomposed water- electrolysis

1856- Weideman – reported migration of ions

1948 – Tiselius – Nobel prize for electrophoresis of proteins

Ionic mobility  $\mu$  velocity of ion in the uniform electric field ( $30 \times 10^{-5} \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$ )

Effective mobility  $\mu_{\text{ef}} = \mu \cdot \alpha$

# Electromigration methods

Separation methods based on the different migration velocity of analytes in solution by influence of the electric field

$$v = E \cdot \mu_{ef} \quad \mu_{ef} = f(\text{pH}, \text{cL})$$

Zone electrophoresis

ZE

Isotachopheresis

ITP

Isoelectric focusing

IEF

Moving boundary el.

MBE

- Introduction to the electrophoresis

- Principle of the method

- Analytical properties

- Choice of the electrolyte system

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- Conclusion

# Zone electrophoresis

BGE

$E = \text{konst}$ ,  $\text{pH} = \text{konst}$ ,  $V_A = E \cdot \mu_A$   $V_B = E \cdot \mu_B$ ,

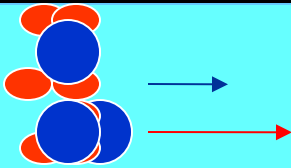


$\mu_A > \mu_B$ ,  $V_A > V_B$

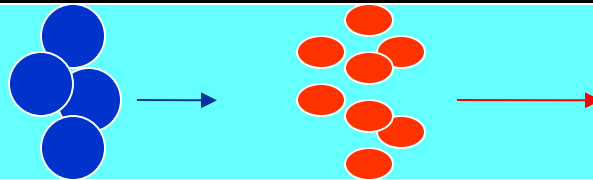
A

B

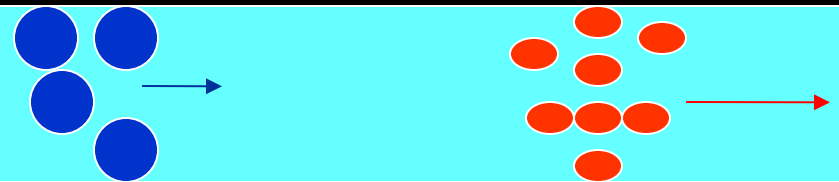
T=0



T=1



T=2



# Zone electrophoresis

BGE

$E = \text{konst}$ ,  $\text{pH} = \text{konst}$ ,  $V_A = E \cdot \mu_A$   $V_B = E \cdot \mu_B$ ,



$\mu_A > \mu_B$ ,  $V_A > V_B$

A

B

Quantity is measured as a area of gaussiann peak

Quality is measured as a migration time from the beginning of the analysis in the cappilary techniques. (the gell techniques as a RF factor relative migration distance)

<http://www.youtube.com/watch?v=1J-tTN3P1AY>

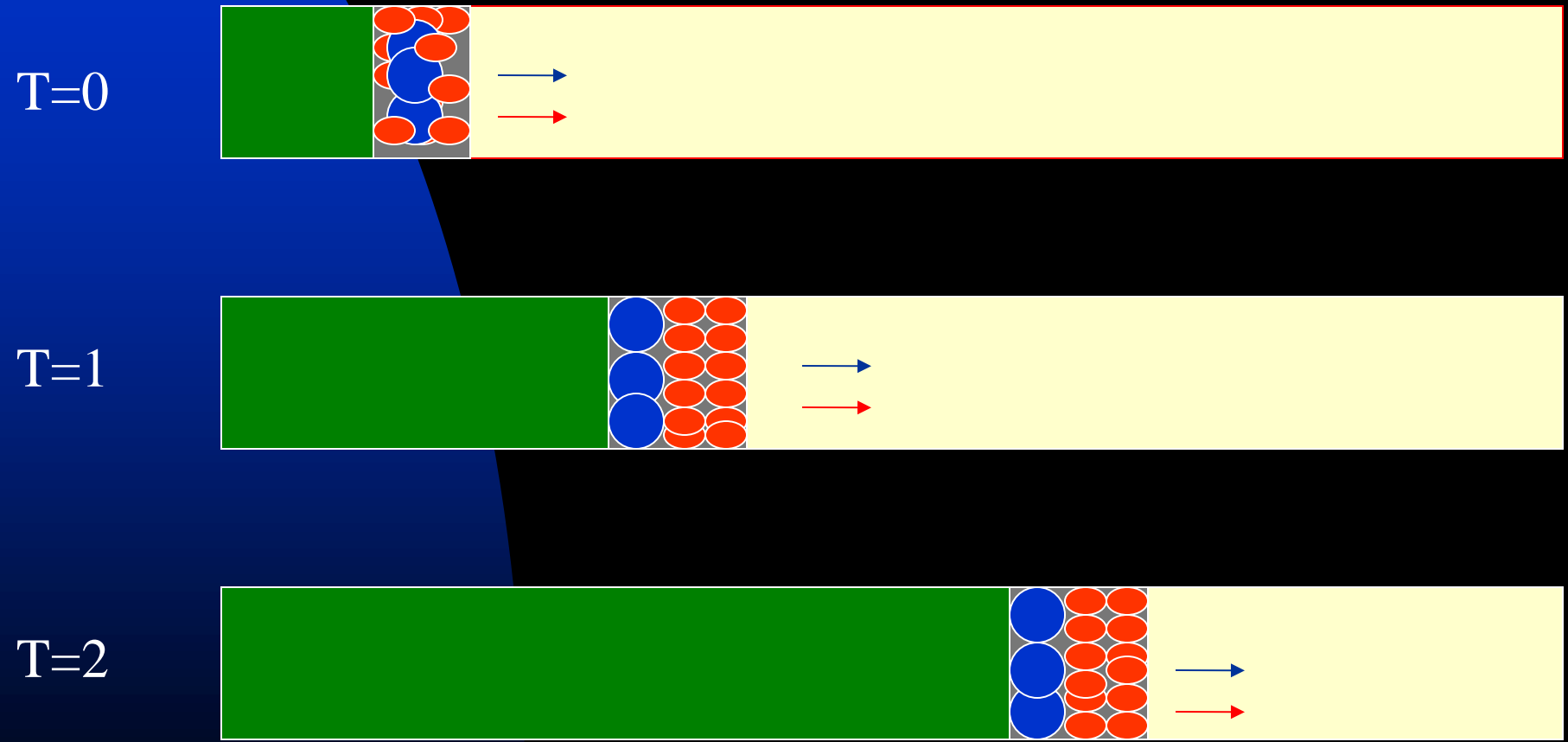


# Isotachopheresis

$E \neq \text{konst}$ ,  $I = \text{konst}$ ,  $\text{pH} \neq \text{konst}$ ,  $V_A = E_A \cdot \mu_A$ ,  $V_B = E_B \cdot \mu_B$ ,  $\mu_L$   
 $> \mu_A > \mu_B > \mu_T$ ,  $E_L < E_A < E_B < E_T$ ,  $V_A = V_B$

TE

LE



# Isotachopheresis

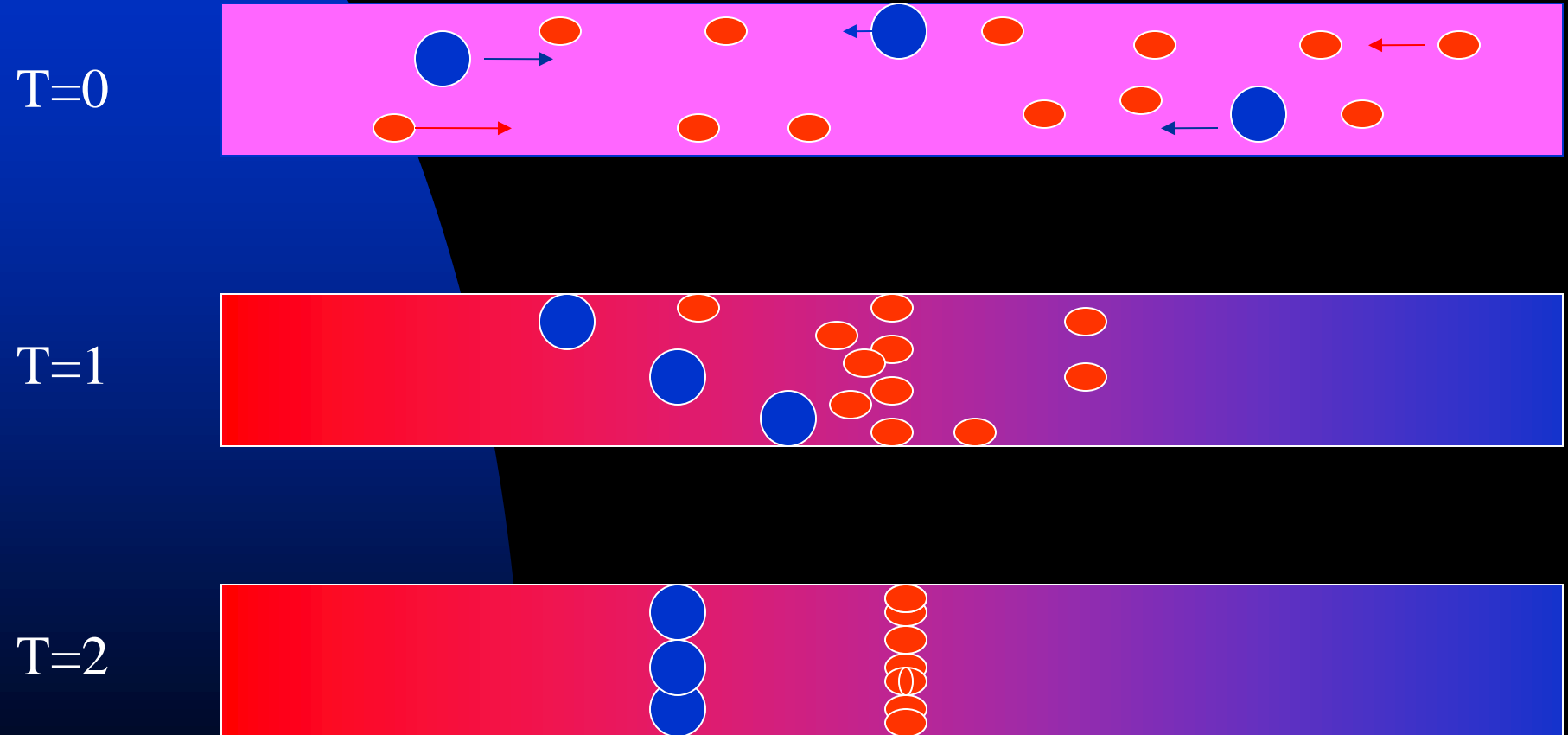
$E \neq \text{konst}$ ,  $I = \text{konst}$ ,  $\text{pH} \neq \text{konst}$ ,  $V_A = E_A \cdot \mu_A$ ,  $V_B = E_B \cdot \mu_B$ ,  $\mu_L$   
 $> \mu_A > \mu_B > \mu_T$ ,  $E_L < E_A < E_B < E_T$ ,  $V_A = V_B$

Quantity is measured as a length of the rectangular zone

Quality is measured as E or conductivity of the zone

# Isoelectric focusing

$E \neq \text{konst}$ ,  $\text{pH} = \text{fct}(X)$ ,  $V_A = E_A \cdot \mu_A$ ,  $V_B = E_B \cdot \mu_B$ ,  $\mu_A > \mu_B$ ,  $V_A = V_B = 0$ ,  $\text{pH}_A = \text{pI}_A$ , carrier ampholytes.



# Isoelectric focusing

$E \neq \text{konst}$ ,  $\text{pH} = \text{fct}(X)$ ,  $V_A = E_A \cdot \mu_A$ ,  $V_B = E_B \cdot \mu_B$ ,  $\mu_A >$   
 $\mu_B$ ,  $V_A = V_B = 0$ ,  $\text{pH}_A = \text{pI}_A$ , carrier ampholytes.

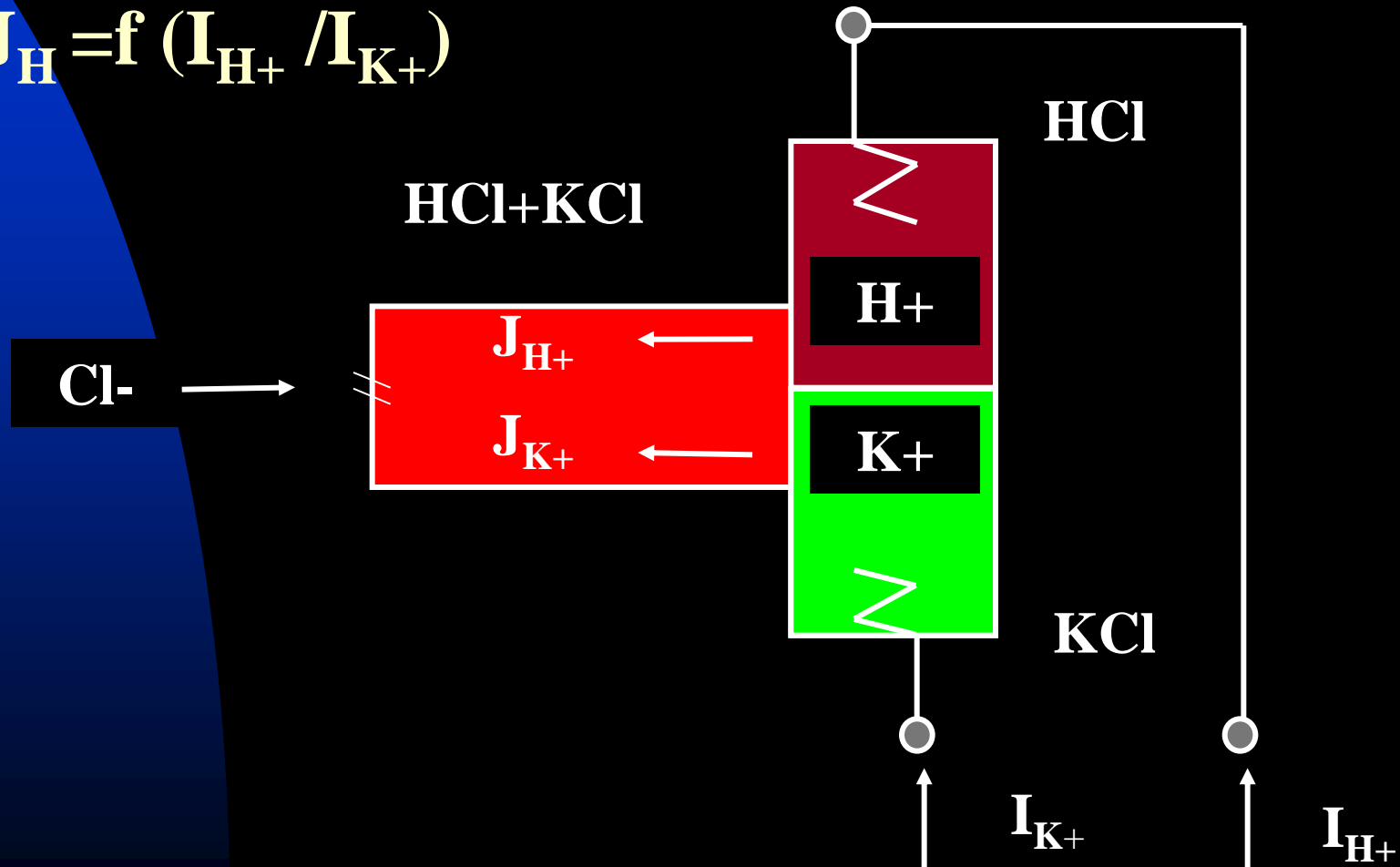
Quantity is measured as a area of gaussiann peak

Quality is measured as a position in the column gel

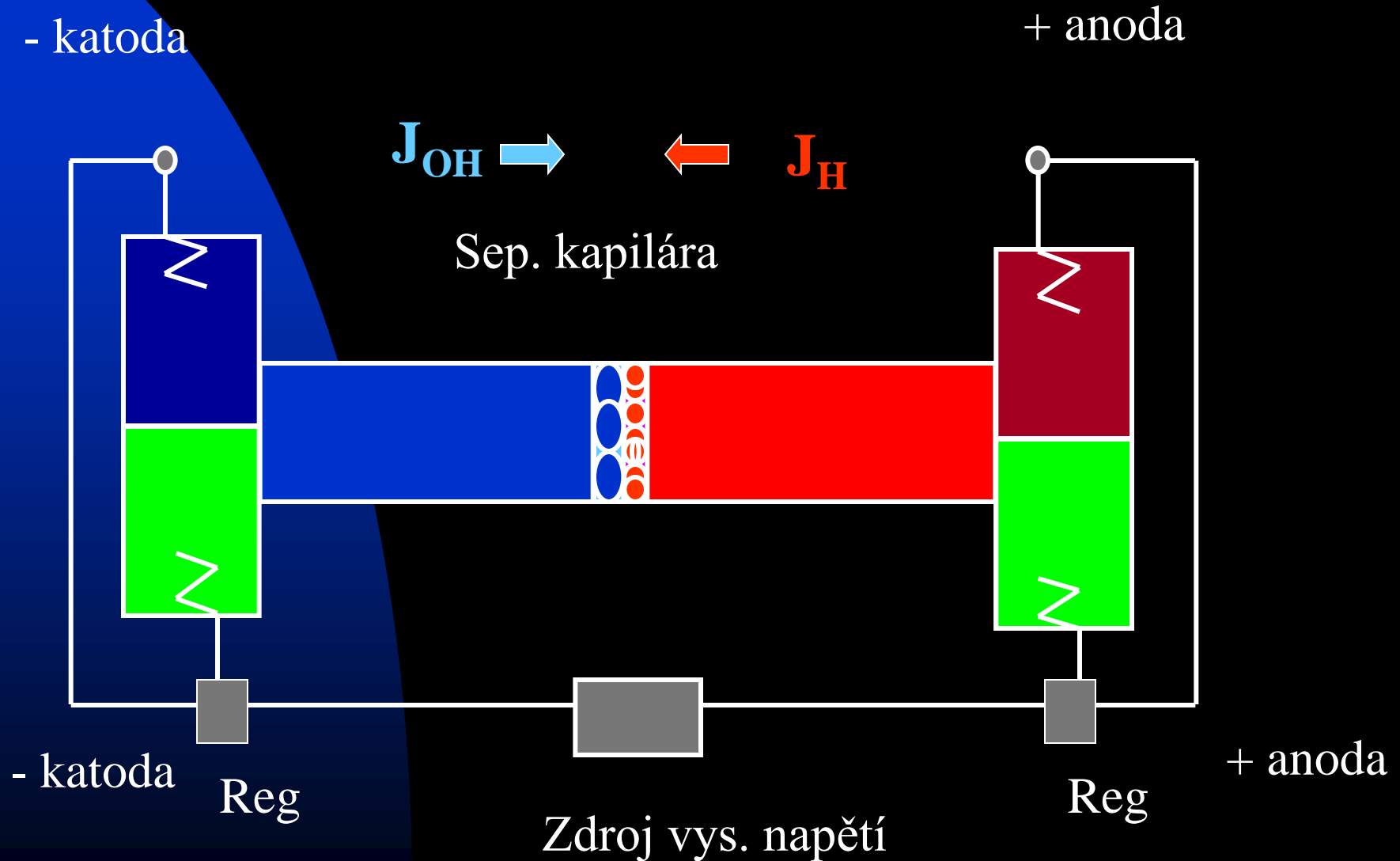
# Izoelektrická fokusace bez nosných amfolytů CAF IEF

Aby rozhraní i zony stály, musí být  $J_H = J_{OH}$

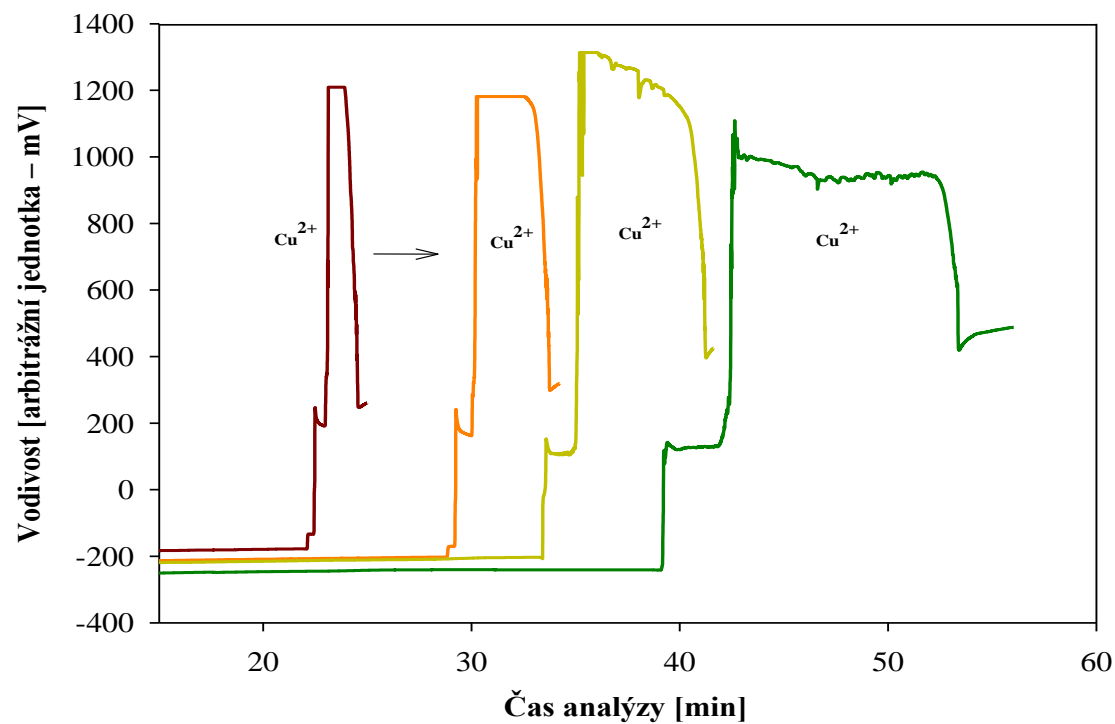
$$J_H = f(I_{H^+} / I_{K^+})$$



# Izoelektrická fokusace bez nosných amfolytů CAF IEF



**Závislost akumulace délky zóny mědi na koncentraci octanu amonného  
v DE ( $7.10^{-5}$  M  $\text{Cu}^{2+}$  +  $1.10^{-2}$  M  $\text{CH}_3\text{COOH}$  + X M  $\text{CH}_3\text{COONH}_4$ )**



- přídavek 0 M octanu amonného
- přídavek  $1.10^{-4}$  octanu amonného
- přídavek  $5.10^{-4}$  octanu amonného
- přídavek  $1.10^{-3}$  octanu amonného

