Reaction boundaries and their use in electrophoresis.

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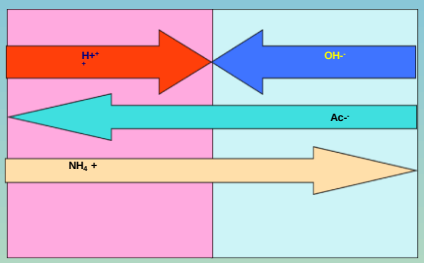
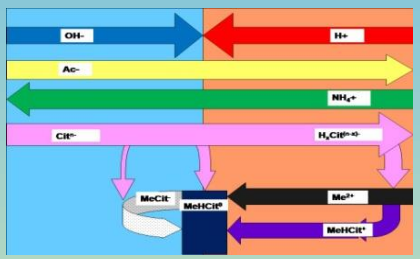
The capillary electrophoretic method was developed for the focusing and selective pre-concentration of analytes with the aim to lower cLOD.
Its consists of a three phase on-line process, where in the first step a substances are pre-concentrated and selectively pre-separated, in the second step are separated and in the third step are analysed.
All steps use a different electromigration principles and electrolytes and are set up such a way that can be arranged on-line without loosing of interesting analyte.
The corner stone of the method is the neutralization reaction boundary where accumulation of the substances takes a place. The neutralization reaction boundary can be modified to trap different types of the analytes. The accumulation is a function of dosing time, concentration and effective mobility of the analyte in the dosing electrolyte. The selectivity of the focusing is given by pH of the electrolyte, and/or by nature and concentration of the complexing /ligand/ agents in the case of the complex forming NRB.

Three type of neutralization reaction boundaties-NRB's:

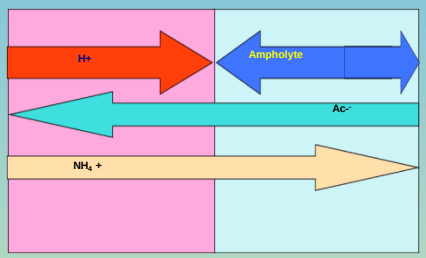
Simple – NRB
Method: Carrier ampholyte free IEF (CAF IEF)
Use: focusing of ampholytes, AA



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



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



Experimental

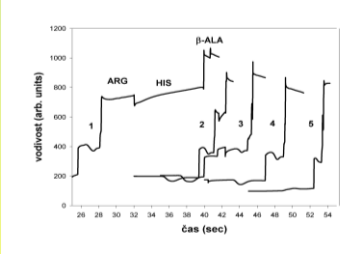
Electrolyte system

Alkaline focusing electrolyte:	Alkaline focusing electrolyte:	Alkaline focusing electrolyte:
LE/PE : 0,01M NH_4OH + 0,01M NH_4Ac + pH=9,24	LE/PE : 0,01M NH_4OH + 0,01M NH_4Ac + 0,002M sec. ammonia citrate pH=9,24	DE : 0,02M HIS+ sample acids, pH=7,4
Acidic focusing electrolyte:	**Acidic focusing electrolyte:**	**Acidic focusing electrolyte:**
DE: 0,01M HAc + 0,01-0,001M NH_4Ac + sample, pH 3,75-4,75.	DE: 0,01M HAc + 0,01-0,001M NH_4Ac + sample metals, pH 3,75-4,75.	PE: 0,02M HCl+ 0,005 M HIS, pH 1,7 ME: 0,01 M HCl+0,01 M HIS, pH 5,5
ITP analytical electrolyte:	**ITP analytical electrolyte:**	**ITP analytical electrolyte:**
LE: 0,01-0,1M HAc + 0,01M NH_4Ac, pH=4,75-4 TE: 0,03M HAc	LE: 0,01-0,1M HAc + 0,01M NH_4Ac + 0,002M sec. ammonia citrate, pH=4,75-4 TE: 0,03M HAc	LE: 0,01 M HCl + 0,01M HIS, pH=5,5 TE: 0,02 M MES

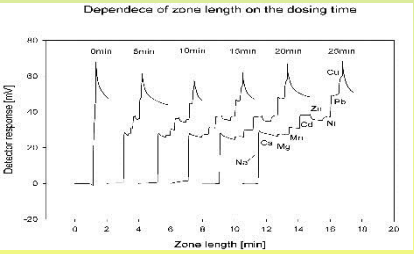
Analysis of Amino acids

Dosing time1200 sec, for different conc. of AA

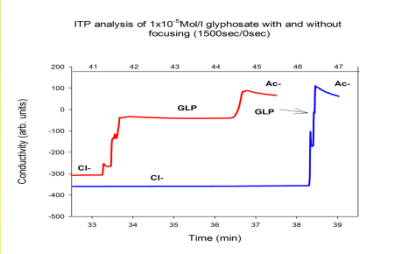
1- 2,5x10-5Mol/l
2- 2,5x10-6Mol/l
3- 2,5x10-7Mol/l
4- 2,5x10-8Mol/l
5- 2,5x10-5Mol/l
6- Without dosing.



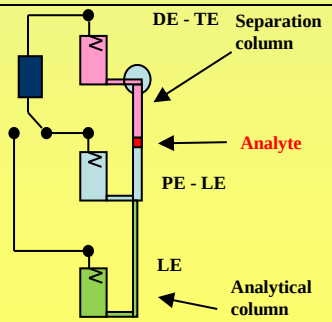
Analytical ITP record of dependence of the zone lenght on the dosing time (constant conc. of metal c= 2.10^{-5}).



Analysis of glyphosate



APPARATUS



Conclusion

Analytes were pre-concentrated, focused and analyzed using combination of NRB step focusing method with subsequent mobilization and on-line e.g. ITP analysis. A continuous dosig technique was used for the lowering of detection limit, with the dosing time of 2000-3000 sec. a cLOD can be lowered by two orders of magnitude with favourable removing bulk analyte from the sample mixture.
Such a low concentrated sample cannot by analysed by routine ITP analysis.

Acknowledgement

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