New Developments in Capillary Electrophoresis with focus on Bioanalysis

Lecture 7

Christian Nilsson

Today´s Lecture

- Focus on metabolomic studies
- CE-MS an alternative to more traditonal techniques (LC-MS, GC-MS, NMR)
- cIEF as an alternative to slab gel IEF in pharmaceutical industry

Metabolomics

- Metabolome: Entire set of low-molecular weight compounds within a biological sample
- Metabolomics: Analysis of the metabolome
- In plants the amount of metabolites is expected to reach 1 million

Metabolomics

- Identify and quantify groups of metabolites belonging to different metabolomic pathways

Metabolomics

- Non-targeted approach
  - Measure as many metabolites as possible within a single analysis
- Targeted approach
  - Focus on analysis and quantification of a few known metabolites
- Both approaches can be combined
  - First broad approach to discover potential biomarker
  - Second approach to quantify the compounds identified in the first step

Metabolomics

- Large difference in:
  - Type of molecules
  - Concentrations
- Several internal standards often necessary
  - Each representing a class of compounds

Metabolomics

- Metabolomic fingerprint
  - Changes due to disease
  - Changes due to therapeutic treatment
- Important approach for screening of diagnostic markers for diseases

Metabolomics

- GC-MS
  - Not suitable for non-volatile, termolabile or polar compounds
  - Laborious derivatisation of metabolites often necessary

Metabolomics

- NMR
  - Rapid, non-destructive, minimal sample pretreatment
  - Limited sensitivity
  - Relatively large sample amount required (micrograms)

Metabolomics

- LC-MS
  - Now derivatization required
  - Can be used for identification and quantification of metabolites
  - Use of UPLC or monolitic LC to improve efficiency
  - Less suitable for ionic and polar compounds
    - Hydrophilic Interaction LC (HILIC) is an alternative

CE-MS in metabolomics

- Especially suitable for analysis of charged and polar metabolites
- Complementary technique to RP-LC
- HILIC-MS an alternative

Reviews:
Electrophoresis 2009, 30, 276-291
Electrophoresis 2011, 32, 52-65
Electrophoresis 2013, 34, 86-98

CE-MS for metabolomics

- No extensive sample pretreatment
- Low consumption of mobile phase and sample
- Fused silica capillaries instead of expensive LC columns
- Easier to multiplex CE compared to LC
- Low concentration sensitivity
  - Can be improved by preconcentration

CE-MS Metabolomics

- Want to achieve maximum coverage
- Have to consider pretreatment of sample
- Compounds are lost during extraction of metabolome

Sample pretreatment

- Separation of low-molecular weight compounds from larger molecules (proteins, lipids and larger peptides)
  - Ultracentrifugation
  - Precipitation
- Larger molecules can otherwise adsorb to the capillary wall and reduce reproducibility

CE-MS in metabolomics

- MS compatible buffers can be used as electrolyte for CE separation
- Have to think about both separation and detection

Sample pretreatment

Non-targeted approach

- Minimal to prevent loss of metabolites
- Extraction of metabolites from bacterias using organic solvents (hot or cold methanol, ethanol, chloroform-methanol)
- Urine can be injected directly

Sample pretreatment

Targeted approach

- Adapt procedures to target metabolites
- Remove larger compounds
- Solid Phase Extraction
  - Affinity SPE can be used

Sample pretreatment

Targeted approach

- Preconcentration of urinary nucleosides from thyroid cancer patients
- Affinity SPE column based on phenylboronic acid
  - Boronic acid used for recognition of sugars
- MEKC analysis
  - Separation buffer: 25 mM borate, 42.5 mM phosphate, pH 6.7, 200 mM SDS

Analytica Chimica Acta 2003, 486, 171-182

Sample pretreatment

Targeted approach

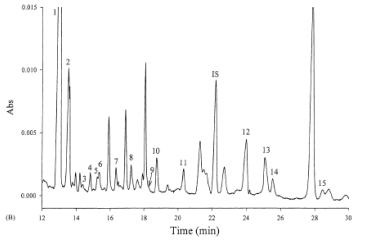
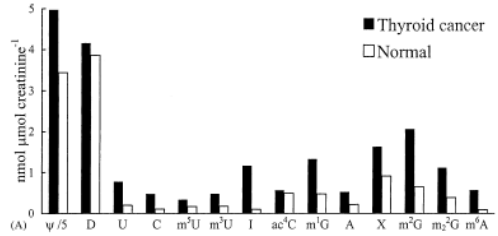


Fig. 1. Capillary electropherograms of nucleoside standard mixture (A) and nucleosides extracted from pooled urine (B). Conditions: uncoated fused silica capillary (370 mm × 50 μm i.d., 500 mm to detector window) maintained at 20 °C, 25 mM borate–42.5 mM phosphate buffer (pH 6.7)–200 mM SDS, 15 kV hydrodynamic injection for 4 s at 3.4 kPa and UV detection at 210 nm. Peaks: 1: pseudouridine; 2: dihydrouridine; 3: uridine; 4: cytidine; 5: 5-methylcytidine; 6: 5-methyluridine; 7: inosine; 8: N^4-acetylcytidine; 9: guanosine; 10: 1-methylguanosine; 11: adenosine; 12: xanthosine; 13: N^2-methylguanosine; 14: N^2,N^2-dimethylguanosine; 15: N^6-methyladenosine; IS: 3-deazauridine.

Analytica Chimica Acta 2003, 486, 171-182

Sample pretreatment

Targeted approach



Analytica Chimica Acta 2003, 486, 171-182

CE-MS in Metabolomics

- Non-targeted metabolomics
  - Analysis both at high and low pH to improve coverage
  - Identification based on molecular weight

Comparsion of CE-MS and UPLC-MS

- CE-MS
  - Use of triple layer coating of polybrene-dextran sulfate-polybrene
  - Profiling of human urine
  - Analysis of urine from 30 females and 30 males
    - Compared to analysis with reversed phase UPLC-MS
    - Differences in profile between genders

Mol. Biosyst. 2011, 7, 194-199

Comparsion of CE-MS and UPLC-MS

- CE-MS
  - Different compounds was used for gender classification by CE-MS and UPLC-MS
  - CE: Highly polar compounds with no retention in reversed phase UPLC
  - CE: A m/z value in the range of 50-150 compared to >150 in UPLC.

Mol. Biosyst. 2011, 7, 194-199

Comparsion of CE-MS and UPLC-MS

Table 2 Summary of the metabolites identified by PB-DS-PB CE-TOF-MS that showed significant differences between female and male urine samples. Concentration of these metabolites was decreased in female urine samples

Name	Molecular formula	m/z observed	m/z calculated	Error/mDa	Migr. time[b] observed/min	Migr. time[b] standards/min
Methylhistidine	$C_7H_{11}N_3O_2$	170.1080	170.0924	15.6	17.6	17.6
Glutamic acid	$C_5H_9NO_4$	148.0850	148.0532	31.8	10.9	10.8
Pyroglutamic acid	$C_5H_7NO_3$	130.0630	130.0426	20.4	9.1	ND[a]
Hypotaurine	$C_2H_7NO_2S$	110.0802	110.0196	60.7	17.1	ND[a]
Threonine	$C_4H_9NO_3$	120.0912	120.0582	33.0	15.2	15.1
Methionine	$C_5H_{11}NO_2S$	150.0920	150.0511	40.9	15.7	15.7
Methylnicotinamide	$C_7H_8N_2O$	138.0728	138.0715	1.3	12.1	12.2
Proline betaine	$C_7H_{13}NO_2$	144.1160	144.1019	14.1	12.0	11.9

[a] ND, not determined (no standard available). [b] Migr. time = migration time.

Only glutamic acid retained in Reversed phase UPLC

Mol. Biosyst. 2011, 7, 194-199

CE-MS applications

CE-MS in Metabolomics

- Optimisation of Electrolyte and Sheath liquid
  - Anionic metabolytes
  - Frequently relatively low sensitivity in negative ionization mode
  - Improved sensitivity
  - Use of triethylamine in electrolyte and sheath liquid

Electrophoresis 2011, 32, 3016-3024

CE-MS in Metabolomics

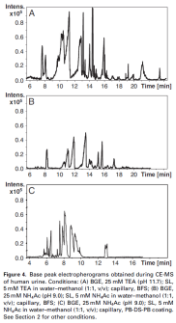


Figure 4. Base peak electropherograms obtained during CE-MS of human urine. Conditions: (A) BGE, 25 mM TEA (pH 11.7); SL, 5 mM TEA in water–methanol (1:1, v/v); capillary, BFS; (B) BGE, 25 mM NH_4Ac (pH 9.0); SL, 5 mM NH_4Ac in water–methanol (1:1, v/v); capillary, BFS; (C) BGE, 25 mM NH_4Ac (pH 9.0); SL, 5 mM NH_4Ac in water–methanol (1:1, v/v); capillary, PB-DS-PB coating. See Section 2 for other conditions.

The amount of compounds that was detected was more than doubled by the use of TEA

Probably due to less ion suppression with TEA in the buffer compared with ammonium acetate

Electrophoresis 2011, 32, 3016-3024

Simultaneous detection of amino acids and carboxylic acid by CE-MS

- Improving the coverage in a single run
- Amino acids
- Carboxylic acids (for example: glycerate, lactate, fumate, succinate, malate, citrate)

- Acidic electrolyte (1M Formic acid)
- Uncoated capillary
- Normal polarity

Anal. Chem. 2010, 82, 9967-9976

Simultaneous detection of amino acids and carboxylic acid by CE-MS

- A high sheath gas flow pressure was used (20 psi)
- The gas flow caused a liquid suction throw the capillary reducing the migration time of the carboxylic acids
- The polarity is changed from positive to ngative during the CE run to detect both amino acids and carboxylic acids

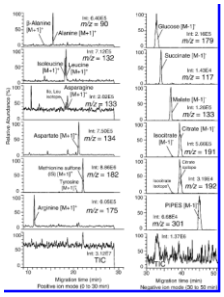
Anal. Chem. 2010, 82, 9967-9976

Simultaneous detection of amino acids and carboxylic acid by CE-MS

- The high sheath gas pressure might cause band broadening
- However, it was possible to separate most of the compound evaluated in the described study

Anal. Chem. 2010, 82, 9967-9976

Simultaneous detection of amino acids and carboxylic acid by CE-MS



Analysis of pineapple leaf as an example

The ionization mode is changed after 29 min

Anal. Chem. 2010, 82, 9967-9976

Single Cell Metabolomics

- Want to get a better understanding of cell functions
- Investigate cell-to-cell differences
- Low molecular weight compounds that are produced in one cell can be found in many other cells, which complicate predictions
- This is also true for macromolecules (such as proteins and DNA) but to less extent

Single neuron detection

- Single cell is an emerging field in MS metabolomics
- See [Current Opinion in Biotechnology 2013, 24, 95-104] for a review of single cell metabolomics in general

Anal. Chem. 2011, 83, 6810-6817

Single neuron detection

- CE-MS for single neuron analysis
- Home-made sheath liquid interface with a flow rate of 750 nl/min
- 6 nl from each neuron extract was injected into the capillary using a 500 nl stainless steel sample vial
- More than 300 compounds were detected

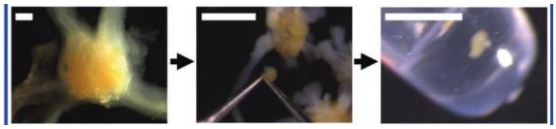
Anal. Chem. 2011, 83, 6810-6817

Single neuron detection

- 6 different types of neuron were compared
- Could compare the metabolyte levels of the different neurons
- Could see chemical similarities among some neurons and other had more distinct features
- The described platform is adapted to other nanoliter samples

Anal. Chem. 2011, 83, 6810-6817

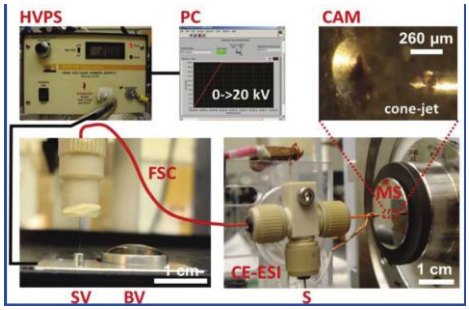
Single neuron detection



White bars = 1 mm.
Extraction of the intracellular analytes from a single neuron

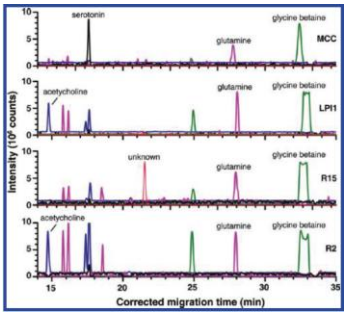
Anal. Chem. 2011, 83, 6810-6817

Single neuron detection



Anal. Chem. 2011, 83, 6810-6817

Single neuron detection



Comparsion among four different types of neurons

Anal. Chem. 2011, 83, 6810-6817

MS couplings

CE-MS with platinum ESI spray needle

- Improvement of sheath flow CE-MS for anionic metabolites
  - Using platinum ESI spray needle instead of stainless steal needle
  - Negative ionization mode
  - CE in reversed polarity due to positively charged capillary coating

Anal. Chem. 2009, 81, 6165-6174

CE-MS with platinum ESI spray needle

- Stainless steel needle showed oxidation and corrosion due to electrolysis
- Iron oxides precipitate and plugged the capillary outlet
  - Shorter capillary life time

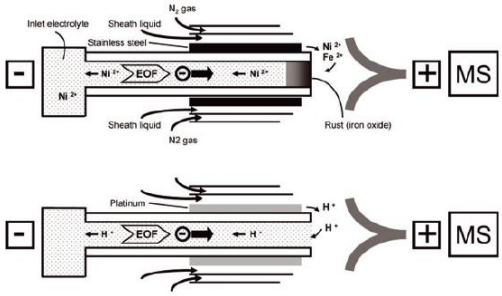
Anal. Chem. 2009, 81, 6165-6174

CE-MS with platinum ESI spray needle

- Many anionic metabolites formed complexes with iron oxides and nickel ions from the stainless steel tip.
- Metal-metabolite complexes caused ionization suppression and reduced sensitivity
- Platinum is not oxidized by electrolysis

Anal. Chem. 2009, 81, 6165-6174

CE-MS with platinum ESI spray needle



Anal. Chem. 2009, 81, 6165-6174

CE-MS with platinum ESI spray needle

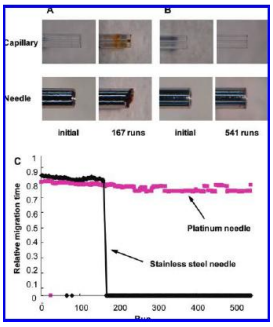
Nickel(II)- and Iron(II)-anion complexes formed using a stainless steel needle

$[Fe(II)\text{-citrate}]^-$ $[Ni(II)\text{-citrate}]^-$

$[Ni(II)\text{-GTP}]^{2-}$ $[Fe(II)\text{-CoA}]^{2-}$

$[Ni(II)\text{-GTP}]^-$

CE-MS with platinum ESI spray needle



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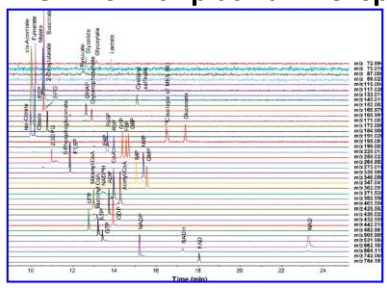
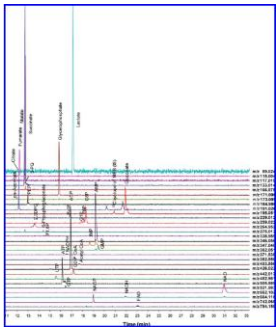


Example of analysis of metabolytes from:

-Glycolysis
-Pentose phosphate pathway
-Tricarboxylic acid cycle

Anal. Chem. 2009, 81, 6165-6174

CE-MS with platinum ESI spray needle



Metabolites in mouse liver

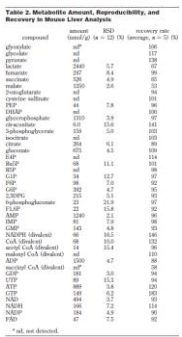
Quantification of compounds from the central metabolic pathways

32 compounds detected

Extraction
-Liver tissue was put in methanol
-Homogenized , 2 min
-300µl was mixed with 500µl water and 200µl cloroform
-Ultracentrifugation, 15000 rpm, 15 min
-Aqueous layer was filtered to remove proteins

Anal. Chem. 2009, 81, 6165-6174

CE-MS with platinum ESI spray needle



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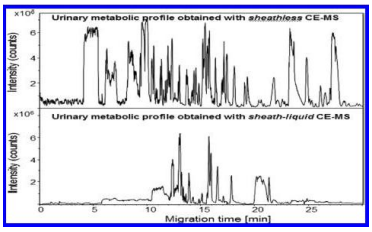
CE-MS with porous sheathless MS connection

- Improvement of sensitivity with sheathless detection



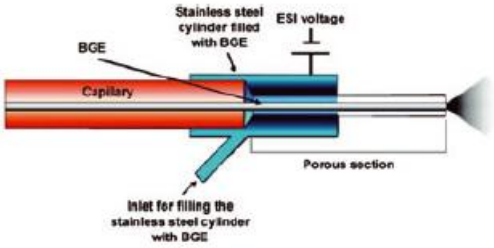
Analytical Chemistry 2012, 84, 885-892

CE-MS with porous sheathless MS connection

- Sheathless MS interface
- Polyimide coating was removed at the outlet side of the capillary
- HF was used to etch the capillary wall to thickness of about 5 µm
- The etched part that now is conductive is insert into a ESI needle
- The ESI needle is filled with electrolyte

Analytical Chemistry 2012, 84, 885-892

CE-MS with porous sheathless MS connection



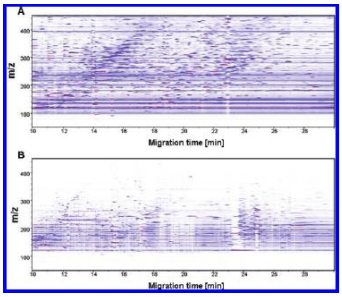
Analytical Chemistry 2012, 84, 885-892

CE-MS with porous sheathless MS connection

- The described connection is useful for narrow capillaries and low flow nano-ESI-MS
- The analytes are not diluted as with a sheath flow connection
- Improved coverage of the urinary metabolome

Analytical Chemistry 2012, 84, 885-892

CE-MS with porous sheathless MS connection



~900 compounds is detected instead of ~300 compounds with a sheath flow interface

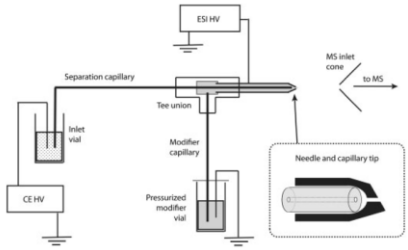
Analytical Chemistry 2012, 84, 885-892

Flow-through microvial interface

- The capillary is inserted in a stainless steel hollow electrospray emitter
- The small volume between the capillary end and the inner wall of the electrode tip act as a flow-through micro vial (outlet vial)
- Addition of a chemical modifier solution at a low flow rate is possible
- Ensure stable flow to the tip with a minimum of sample dilution

Electrophoresis 2010, 31, 1130-1137

Flow-through microvial interface



Electrophoresis 2010, 31, 1130-1137

Flow-through microvial interface

- Amino acid analysis at low pH (pH 3.1)
- Uncoated capillary
- 5-fold improvement of the detection limits compared to a conventional sheath liquid interface

Electrophoresis 2010, 31, 1130-1137

Flow-through microvial interface

Table 1. LODs and linearity of response for amino acid analysis with different interface configurations[40]

Amino acid	Decoupling interface		Sheath-flow interface		Improvement over sheath-flow interface	
	R^2	LOD (μmol/L)	R^2	LOD (μmol/L)	LOD (fold improvement)	$S/N^{[b]}$ (fold improvement)
Ala	0.9980	0.7	0.9981	3.8	5	11
Arg	0.9993	0.1	0.9702	0.4	3	3
Asn	0.9954	1.7	0.9793	7.4	4	5
Asp	0.9995	0.6	0.9963	2.3	4	3
Cys-Cys	0.9995	1.0	0.9813	10.9	11	6
Gly	0.9998	1.1	0.9977	6.3	6	4
Glu	0.9979	0.3	0.9950	1.3	4	4
Gln	0.9996	0.2	0.9939	1.1	4	6
His	0.9940	0.4	0.9992	2.4	6	6
Ile	0.9998	0.1	0.9800	0.4	4	6
Lys	0.9994	0.2	0.9731	0.4	2	2
Met	0.9998	1.2	0.9956	2.3	2	2
Phe	0.9985	0.1	0.9946	2.4	17	13
Pro	0.9996	0.9	0.9816	2.4	3	6
Ser	0.9989	3.4	0.9967	5.3	2	4
Thr	0.9995	1.7	0.9946	2.6	2	5
Trp	0.9998	0.1	0.9938	0.2	3	4
Val	0.9992	0.3	0.9904	1.3	5	6

Electrophoresis 2010, 31, 1130-1137

Capillary Isoelectric Focusing of Protein Isoforms

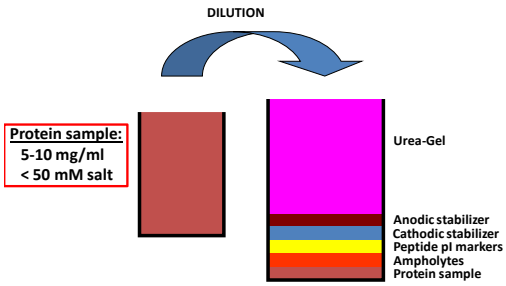
Outline

- **Background – Capillary Isoelectric Focusing**
  - Sample Preparation
  - Sample Injection
  - Focusing
  - Mobilization
- **Results**
- **Conclusions**

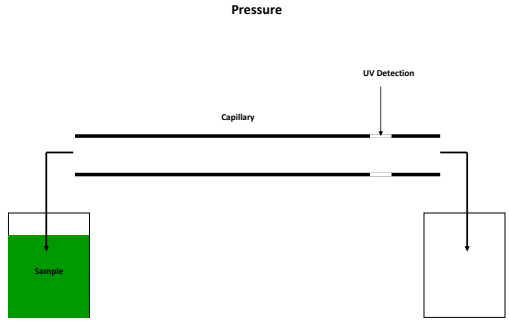
Capillary Isoelectric Focusing

- Separation based on isoelectric point
- Capillary Electrophoresis Equipment
- Carrier ampholytes to establish pH gradient

cIEF – Sample Preparation

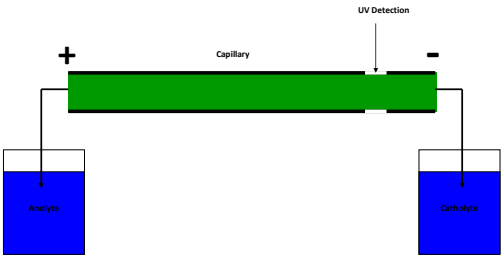


cIEF - Injection



cIEF - Focusing

Applied Voltage: 25 kV



cIEF - Focusing

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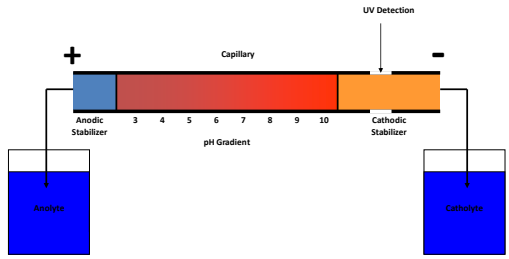
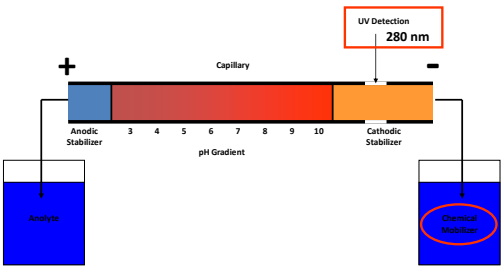
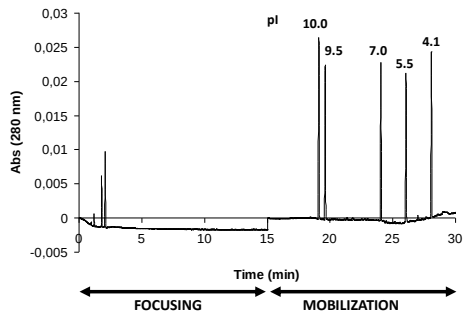


cIEF - Mobilization

Applied Voltage: 30 kV



Results: Peptide markers



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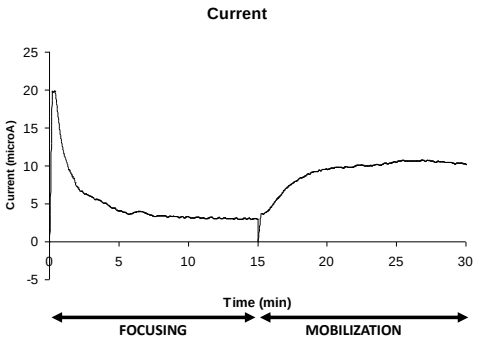
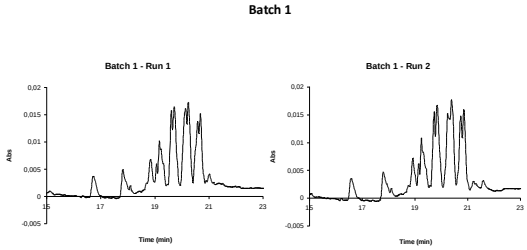


Results: 3 batches of proteins



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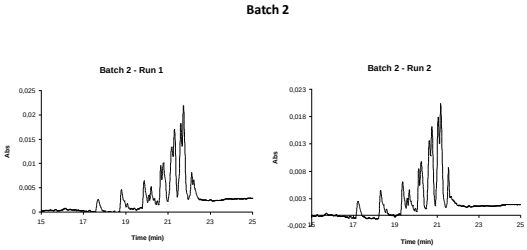
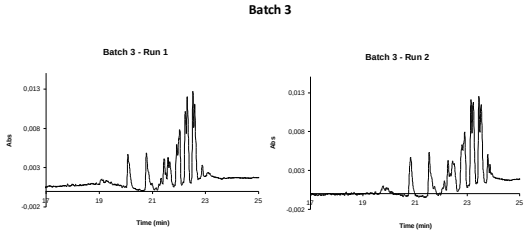
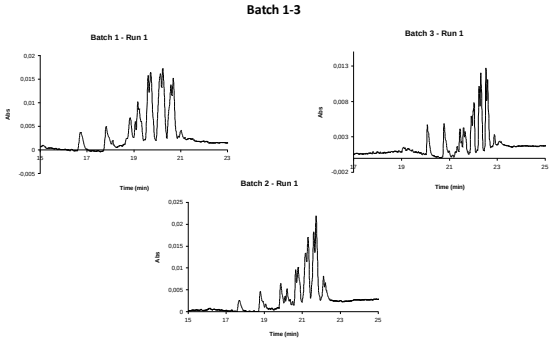


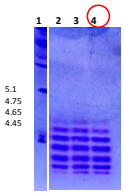
Results: 3 batches of proteins



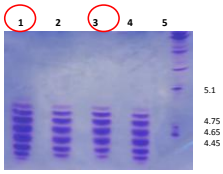
Results: 3 batches of proteins



Results: Gel IEF of proteins

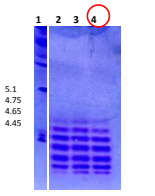


Legend:
1.PI marker.
2.Ref. material 09800031 14 µg.
3.DS 08800050 14 µg.
4.DS 09PD80010 14 µg.

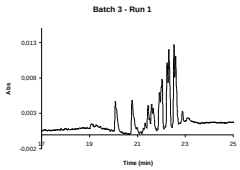


Legend:
1.2192-122 (Tox 2) 20 µg.
2.2192-121 (Undiluted Tox 2) 15 µg.
3.1178-160 (Tox 1) 15 µg.
4.1178-139 (STD) 15 µg.
5.PI marker.

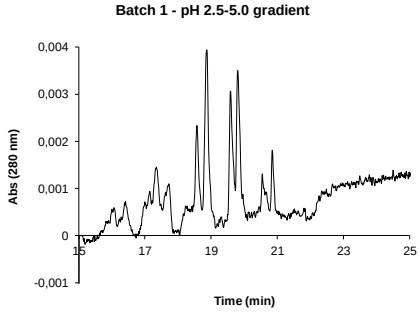
Results: Gel IEF of proteins



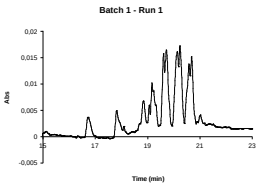
Legend:
1.PI marker.
2.Ref. material 09800031 14 µg.
3.DS 08800050 14 µg.
4.DS 09PD80010 14 µg.

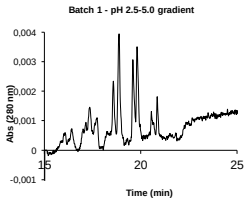


Results: rFSH – Narrow pH gradient



Results: rFSH – Narrow pH gradient





Conclusions

- High Resolution
- Reproducibility
- Old CE equipment used