New Developments in Capillary Electrophoresis with focus on Bioanalysis

Lecture 5

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Lecture today

- 2D-CE
- CE-MS coupling
- More CEC

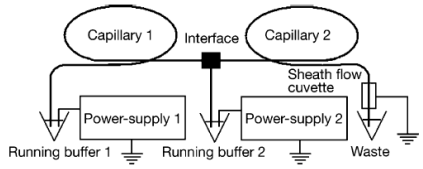
Complex Samples

- Proteomics
- Diagnostics
  - Body Fluids
- Biomarker discovery

- Large difference in concentration

2D-CE

- General Setup



2D-LC/CE

- Reversed Phase LC and CE
- CE is a fast technique suitable as the second dimension
- The two techniques are orthogonal in separation mechanism
  - Hydrophobicity
  - Mass-to-charge ratio

2D-LC/CE - Examples

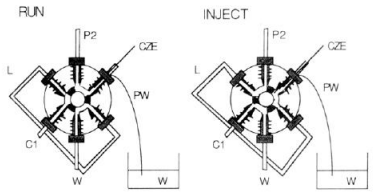


Figure 1. Two configurations of six-port, computer-controlled valve. C1 is RP HPLC column. P2 is pump 2. L is loop. CZE is capillary zone electrophoresis fused silica capillary. PW is paper wick. W is waste.

2D-LC/CE - Examples

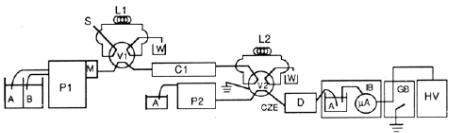
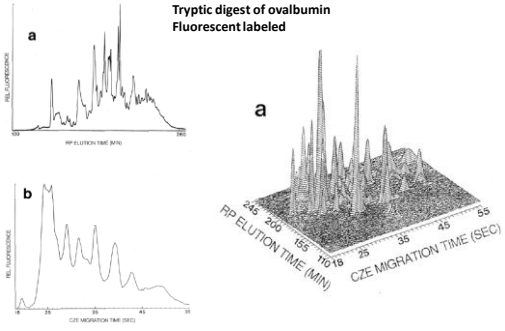


Figure 2. Schematic of 2-D LC/CZE instrumentation: A and B, buffer A and acetonitrile, respectively; P1, Brownleee microgradient syringe pump; M, 52-μL mixer; V1, Valco six-port manual injection valve; S, injection syringe; L1, 50-μL loop; C1, reversed-phase column; P2, Waters Associates Model 6000A piston pump; V2, grounded six-port electrically actuated Valco valve; L2, 10-μL loop; CZE, CZE capillary; D, fluorescence detector; IB, interlock box; μA, microammeter; GB, grounding box; HV, Spellman high-voltage power supply.

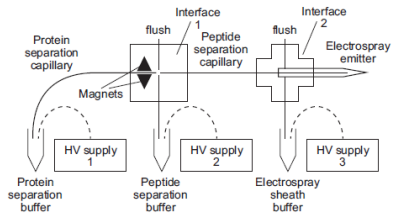
2D-LC/CE - Examples



2D-CE

- An alternative to 2D electrophoresis by slab gels for proteomic studies

2D-CE - Examples



2D-CE - Examples

- Protein Separation
- On Column Tryptic Digestion
- Peptide Separation
- Mass Spectrometry Detection
- Data Analysis

2D-CE

- Peptides from one protein introduced into the MS within a short time window
- Peptides not spread as if the proteins were digested prior to separation
- Fewer peptides are detected simultaneously as if digested just prior to introduction into MS.

2D-CE - Examples

- Use of magnetic nanoparticles
- Trypsin immobilized on nanoparticles
- Magnets used to hold the nanoparticles in the capillary
- Nanoparticles are replaced before each experiment
- Large surface-to-volume ratio

2D-CE - Examples

- Sufficient long time in microreactor
- Avoid band broadening
- Limited life time of certain microreactors, for example, monoliths
- Separation of peptides after digestion

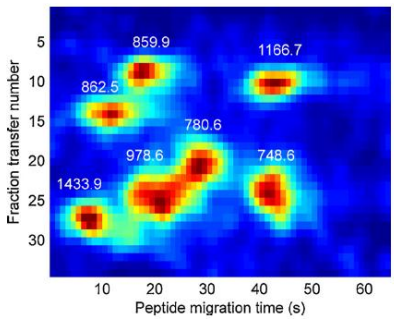
2D-CE - Examples

- Short microreactor to avoid band broadening
- The peptides are introduced to the second capillary prior to MS detection
- At the same time fresh protein is introduced into the microreactor and is digested during the peptide separation

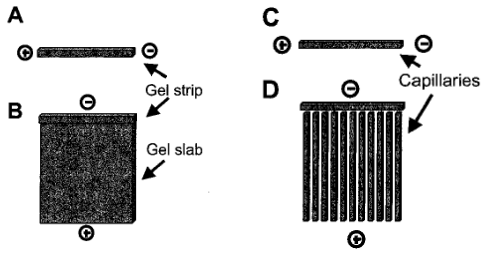
2D-CE – Examples

- Capillary Interface
  - Cross that was machined into a plexiglas plate

2D-CE - Examples



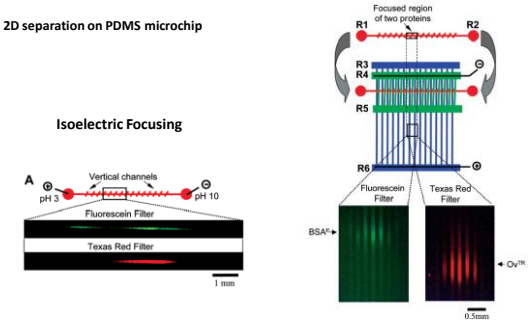
2D-CE - Examples



First dimension – One capillary

Second dimension – An array of capillaries

2D-CE - Examples



Analysis of Biofluids for Diagnostics

- Combination of techniques
  - Preconcentration
  - A combination of techniques to:
    - Separate
    - Identify
    - Quantify
- Large variation in abundance

Analysis of small proteins (below 20 kDa) and peptides in urine

- CZE coupled to ESI-MS
- High throughput
- Excellent resolution

- Biomarker often limited to the highly abundant substances

CE-MS

- 2 Review articles uploaded among study material

- Interface of CE with
  - Electrospray
  - MALDI
  - ICP

CE-MS

- Compatibility of CE electrolyte with MS detection have to be considered
- Avoid ion supression
- Use of low ionic strength
- Use of acetic or formic acid
- Use of organic solvents

Coupling of CE with ESI-MS

- Sheath Liquid
- Sheathless
- Liquid Junction Interface

CE-ESI-MS Interface

- Electrical connection for adjusting spraying potential and CE outlet potential
- Transfer of the analytes to the spray
- Continuous delivery of spray or sheath liquid

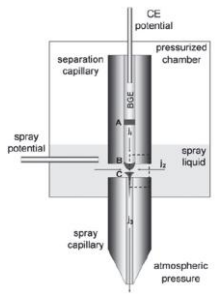
CE-ESI-MS – Sheath liquid

- The voltage is applied via a sheath liquid
- The sheath liquid is flowing outside the separation capillary and is mixing with the analytes at the spray tip

CE-ESI-MS – Sheathless interface

- Electrical contact directly via the fused silica capillary
- Lower detection limits

CE-ESI-MS – Liquid Junction



Less dilution of the analytes compared to the sheath flow techniques

CE-ESI-MS – Liquid Junction

- Narrow gap between separation and spray capillary
- The spray potential is applied to a spray liquid which is surrounding the junction
- Separation voltage is determined by the field at the inlet of the separation capillary and at the spray liquid

CE-MALDI-MS

- Off-line coupling the most common
- Separation and detection can be performed independently
- Reliable fraction collection

Applications of CE-MS

- Proteomics and Glycomics most common
  - Analysis of intact proteins
  - Analysis of digested proteins
- Useful for biology/cell studies
- High throughput, High sensitivity and resolution

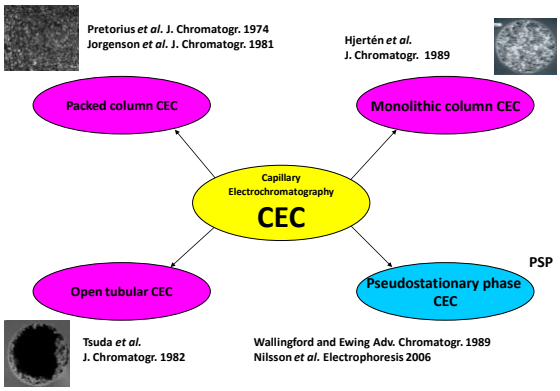
Applications of CE-MS

- Intact proteins
  - Characterization of protein isoforms in the biopharmaceutical industry
  - Impurities
  - Protein modification

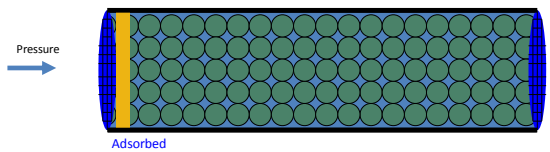
Applications of CE-MS

- Peptides
- Biomarkers
  - Large differences in concentrations
- Tryptic digests of proteins

Nanoparticle-based CEC

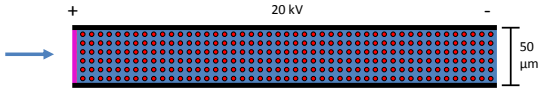


Conventional Liquid Chromatography (LC)



- Complicated packing procedures
- Need for retaining frits
- Sample matrix can modify column
  - Fouling
  - Reduced reproducibility

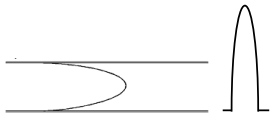
Pseudostationary phase – Capillary electrochromatography



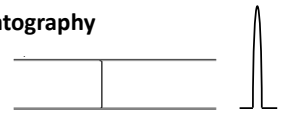
- Small particles (nanoparticles) ~5-100 nm

Large surface area → Enhanced mass transfer → Increased efficiency

- **Liquid Chromatography**
  - Pressure-driven flow
  - 1-5 mm column diameter
  - 2-5 µm particle diameter



- **Capillary Electrochromatography**
  - Electro-driven flow
  - ~50 µm column diameter
  - 10-100 nm particle diameter

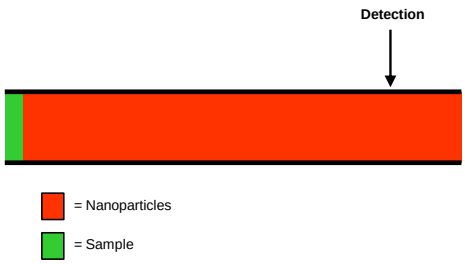


Nanoparticle-based PSP-CEC

- **No packing or retaining frits**
- **One-time use of stationary phase**
  - No carry over
  - Minimal column regeneration
  - Low consumption of nanoparticles
  - Can start a new separation before the previous one finished
- **Small particles (sub-micron)**
  - High surface-to-volume ratio
  - High efficiency

Detection ???

Continuous Full Filling

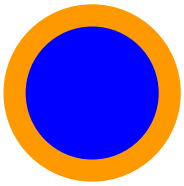


Detection

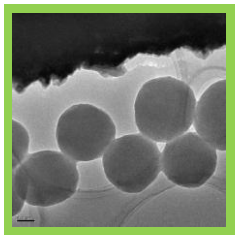
- Orthogonal ESI-MS
- Laser Induced Fluorescence (LIF)
- UV
- Other…

Separation of Small Molecules

Dextran-coated Polymer Nanoparticles



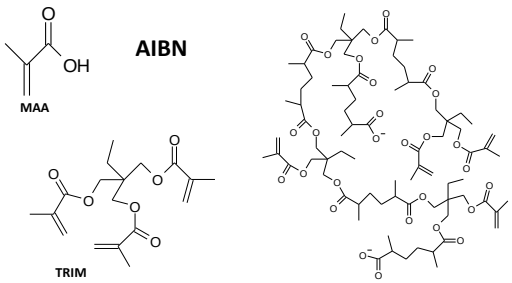
- Hydrophobic core
- Hydrophilic surface



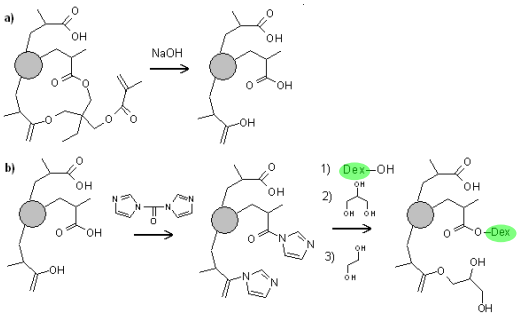
TEM

Average Diameter 600 nm

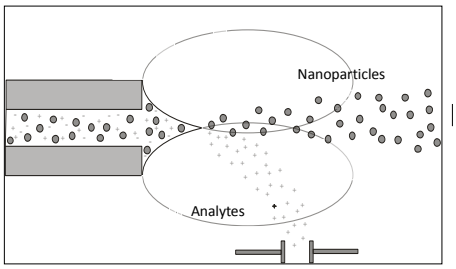
Polymerisation



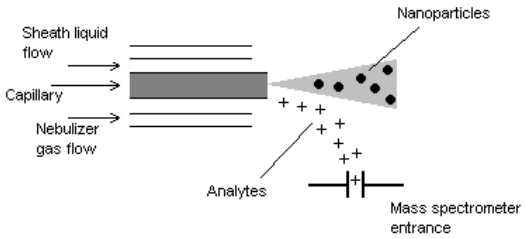
Surface modification



Orthogonal electrospray interface



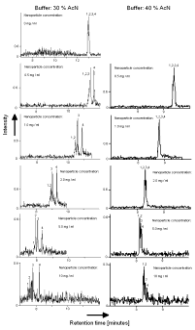
Ortogonal electrospray interface



Conclusions RP-CEC

- Nanoparticles could be used for separation with an electrolyte with low acetonitrile concentration
- It was possible to use high amount of nanoparticles in continuous full filling without contamination of the mass spectrometer

Continuous full filling RP-CEC

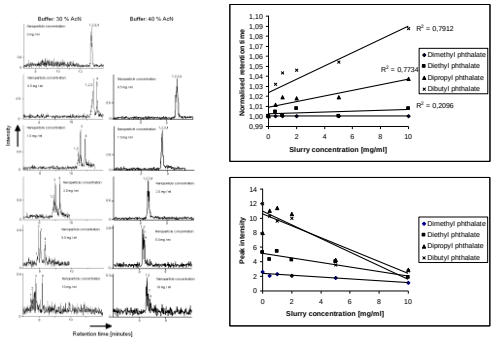


30 kV

Electrolyte: Acetonitrile and 10 mM ammonium acetate, pH 5,6 (30:70 and 40:60 v/v)

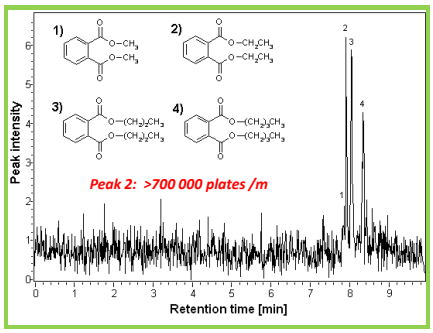
Nanoparticle slurry: 0; 0,5 1,0; 2,0, 5,0 and 10,0 mg/ml.

Continuous full filling RP-CEC



Separation of Small Molecules

Reversed Phase



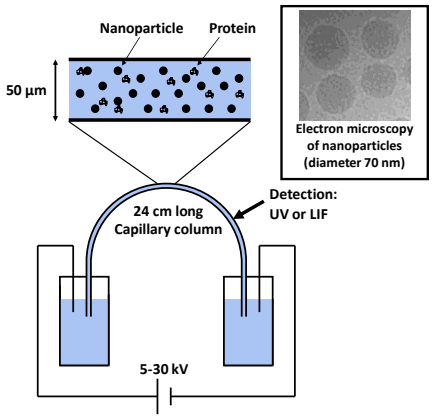
Conclusions RP-CEC

- Separation was performed with high peak numbers.
  - High concentration of acetonitrile.
  - High concentration of nanoparticles.
- Low consumption of nanoparticles
  - 10 µl nanoparticle slurry per effective hour of separation

Future improvements

- Further optimisation of acetonitrile concentration, nanoparticle concentration and pH.
- Use of other nanoparticles.
- Use of combinations of nanoparticles.
- Use of smaller nanoparticles.
- Start a new separation before last one finished

Separation of Proteins



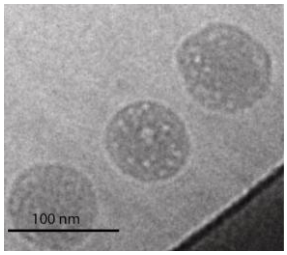
Protein CE/CEC

- Capillary Wall Adsorption
- Solutions to the problem
  - –Buffer pH (high or low)
  - –Salt or Zwitterionic Additives
  - –Static or Dynamic Coatings
  - –Nanoparticles?

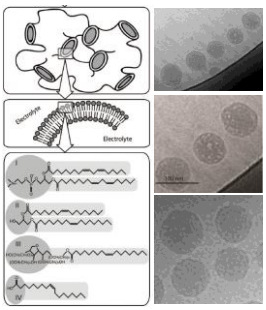
...in bare silica capillary
...tricine, zwitterionic, low µA
...at neutral pH

Lipid Nanoparticles

~70 nm diameter

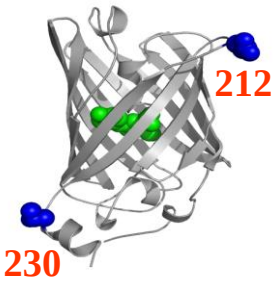


Lipid-based liquid crystalline nanoparticles



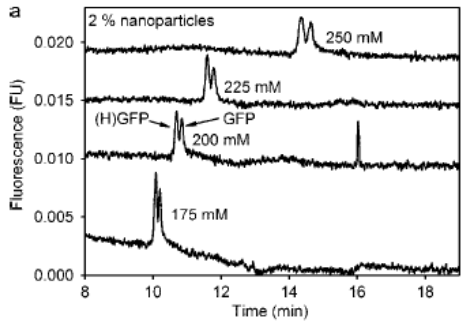
- **Average diameter 70 nm**
- **Bicontinuous Cubic Phase**
- **Porous (100 Å)**
- **Protein compatible**
  - – Membrane proteins
  - – Drug delivery
- **Easy to prepare**
  - – One-step procedure

Green fluorescent protein (GFP) Mutants

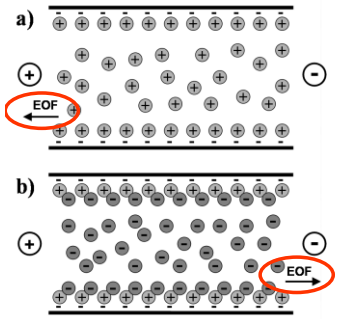


26 kDa
238 amino acids
pI=5.7

Protein Separation



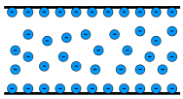
PSP- CEC cationic and anionic porous lipid based nanoparticles



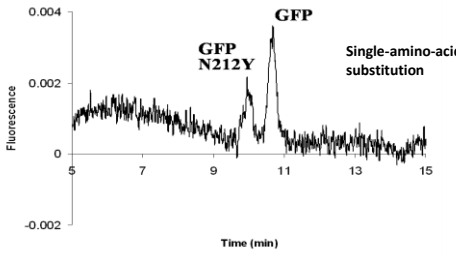
Cationic

Cationic + Anionic

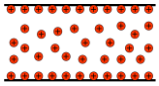
Protein Separation



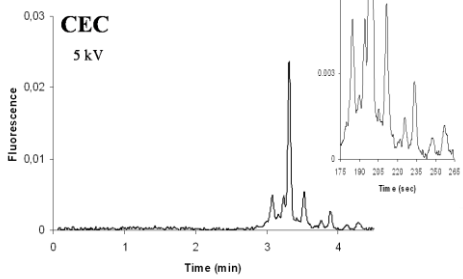
High resolution



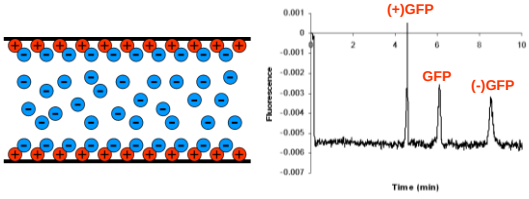
Protein Separation



High efficiency

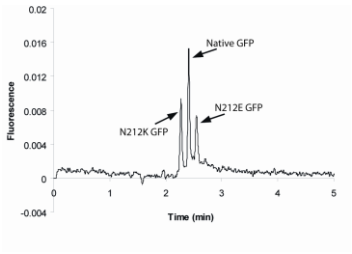


Separation of Proteins



Transfer to chip format

GFP variants **In Topas® Capillary**



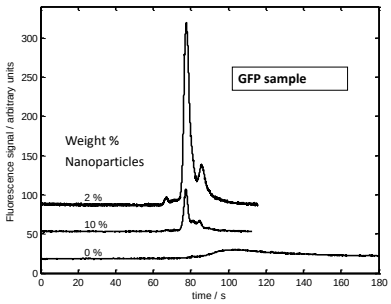
With nanoparticles

Microchip electrophoresis of bacteria using lipid-based liquid crystalline nanoparticles

Zhi-Fang Wang, Shuang Cheng, Shu-Li Ge, Jin-Kun Zhu, Huan Wang, Qi-Ming Chen*, Qing-Jiang Wang*, Pin-Gang He, Yu-Zhi Fang

Department of Chemistry, East China Normal University, Shanghai 200062, China

Separation on polymer chip



Future of nanoparticle CEC?

- Separation with UV detection
- Separation of membrane proteins
- Analysis of biological nanoparticles