# New Developments in Capillary Electrophoresis with focus on Bioanalysis

Lecture 8

Christian Nilsson

## Western Blotting using CE

- Drawback slab gel Western blotting
  - Manually time consuming
  - Gel preparation, separation, electro blotting, incubation
  - Sensitivity in ng range
  - Difficult to transfer large proteins from gel

Anal. Chem. 2011, 83, 1350-1355

## Western Blotting using CE

- Microscale western blotting system
- Based on Capillary Gel electrophoresis for separation of SDS-protein complexes
- Deposition on blotting membrane
- Grounding through a sheath capillary

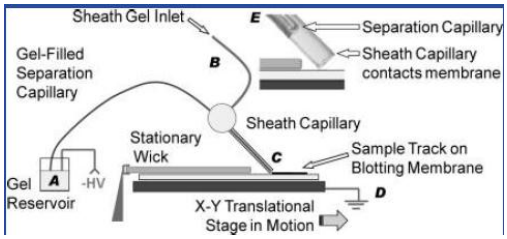
Anal. Chem. 2011, 83, 1350-1355

## Western Blotting using CE

- Translation stage to move blotting membrane past the outlet of the capillary
- Membrane moistured by methanol/buffer
- Polymer solution for sieving

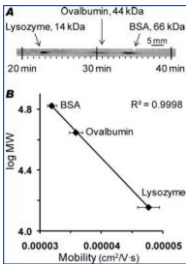
Anal. Chem. 2011, 83, 1350-1355

## Western Blotting Using CE




Anal. Chem. 2011, 83, 1350-1355

## Western Blotting Using CE




Detection limit, 10 pg

**Figure 2.** Size-dependent separation of standard FITC-labeled proteins. (A) 3 proteins, prepared in stock samples of 100−300 μg/mL. The molecular weight for unlabeled protein is noted beside each observed peak. (B) Plotting log MW as a function of mobility yields a linear plot for these FITC-labeled proteins.

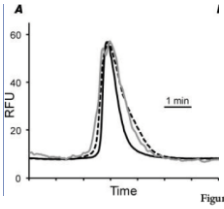
Anal. Chem. 2011, 83, 1350-1355

## Western Blotting using CE

- Faster analysis
- No electro blotting
- Automation
- Lower consumtion of reagent and sample
- Lower detection limits, 10 pg
  - Little optimization

Anal. Chem. 2011, 83, 1350-1355

## Western Blotting using CE




Band broadening ~1.7 fold

**Figure 3.** Measurements of band broadening inside sheath capillary and on membrane. (A) Comparison of peak width for on-column detection (black line), in sheath 350 $\mu$m beyond the exit of the separation capillary (dashed line), and on membrane after traveling through 500 $\mu$m of sheath (gray line). The on-column and in sheath measurements were taken from the same separation. The membrane data was from a separate injection. All separations used 150 $\mu$g/mL FITC-BSA as the sample separated at 300 V/cm with an effective capillary length of 20 cm.

Anal. Chem. 2011, 83, 1350-1355

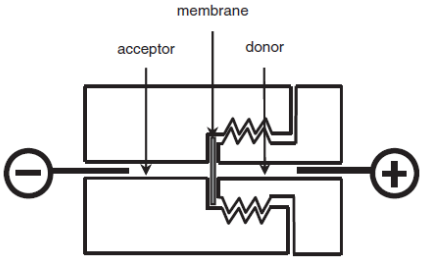
## Microelectrodialysis coupled to CE

- Pretreatment and analysis of inorganic cations in biological matrices
- No adsorption of high molecular weight compounds to the capillary wall.
- Cellulose acetate dialysis membrane with molecular weight cut-off of 500 Da
  - Can be used approximately 100 times before replaced

Electrophoresis 2011, 32, 464-471

## Microelectrodialysis coupled to CE

- Less than 1 µl of sample is needed




Electrophoresis 2011, 32, 464-471

## Microelectrodialysis coupled to CE

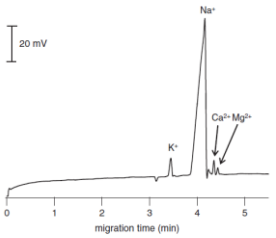



**Figure 2.** Separation of inorganic cations in the optimized BGE solution. CE conditions: $L_{tot/eff}$: 50/42 cm, separation voltage: +15 kV, hydrodynamic injection from 30 cm for 30 s, BGE: 12.5 mM maleic acid, 15 mM L-Arg and 3 mM 18-crown-6, pH 5.5.

Electrophoresis 2011, 32, 464-471

## Microelectrodialysis coupled to CE

**Table 1.** Analytical parameters of the developed CE-$C^4D$ method for determination of inorganic cations in biological fluids, $n = 6$

| Ion | RSD (%) M.T. | RSD (%) P.A. | Calibration range (mM) | $r^2$ | LOD (µM) |
|---|---|---|---|---|---|
| $NH_4^+$ | 0.36 | 1.18 | 0.04–0.4 | 0.9988 | 0.5 |
| $K^+$ | 0.41 | 0.79 | 0.05–0.5 | 0.9988 | 0.66 |
| $Na^+$ | 0.4 | 1.31 | 0.2–2 | 0.9957 | n.a. |
| $Ca^{2+}$ | 0.42 | 2.46 | 0.02–0.2 | 0.9992 | 2 |
| $Mg^{2+}$ | 0.43 | 1.18 | 0.01–0.1 | 0.9981 | 1 |

M.T., migration time; P.A., peak area; n.a., not available; concentration of $Na^+$ was kept constant at 2 mM in all LOD measurements.

**Table 2.** Repeatability and recovery values of electrodialytic pretreatment of standard solution and biological fluid samples in µED system

| | $K^+$ | $Na^+$ | $Ca^{2+}$ | $Mg^{2+}$ |
|---|---|---|---|---|
| *Repeatability (RSD) values in %, n = 6* | | | | |
| Standard solution | 2.0 | 2.8 | 3.8 | 5.2 |
| Human plasma | 3.6 | 2.3 | 6.8 | 8.2 |
| Human serum | 6.4 | 6.1 | 11.8 | 6.8 |
| Whole blood | 4.9 | 3.7 | 8.9 | 6.9 |
| *Recovery values in %, n = 6* | | | | |
| Standard solution | 110.0 | 102.4 | 98.7 | 96.3 |
| Human plasma | 101.5 | 105.7 | 101.5 | 103.0 |
| Human serum | 98.2 | 99.2 | 99.1 | 98.4 |

Electrophoresis 2011, 32, 464-471

## Use of additives to CE/CEC

- Since the Terabe introduced micelles as additive for CE, a large amount of different additives have been used
- Follows the development in material science and nanotechnology

## Use of additives in CE/CEC

- Requirements for nanoparticles to be used

**Table 1.** Required properties of nanoparticles for use in PSP-CEC

| |
|---|
| Be able to form stable suspensions in a wide range of electrolytes |
| Provide desired selectivity in interaction with analytes |
| Be charged, *i.e.*, not to coelute with EOF |
| Show equal velocity to prevent peak-broadening |
| Show small mass transfer resistance |
| Not disturb detection |
| Be small to provide high surface area to improve sample capacity |

## Use of additives in CE/CEC

$$H_{tot} = H_l + H_m + H_{ap(m)} + H_T + H_{ep(m)} \quad (1)$$

where $H_l$ is the longitudinal diffusion, $H_m$ is the adsorption/desorption kinetics, $H_{aq(m)}$ is the intermicelle mass transfer, $H_T$ is the radial temperature gradient, and $H_{ap(m)}$ is the dispersion due to different mobilities of the micelles.

For particle systems, the total band-broadening ($H_{tot}$) is described by Eq. (2)

$$H_{tot} = H_l + H_m + H_{aq(p)} + H_T + H_{ap(p)} \quad (2)$$

where $H_{aq(p)}$ = interparticle mass transfer, and $H_{ap(p)}$ = dispersion due to different mobilities of the particles.

According to Eq. (2), band-broadening can be decreased by using particles with the same mobilities (most likely particles with similar properties). Also adsorption/desorption kinetics can be improved by controlling the surface properties and porosity of the particles and/or by changing the composition of the electrolyte. To improve interparticle mass transfer, particle concentration can easily be varied.

## Use of additives – Silica particles

- Early use of particles as pseudostationary phase
- Use of reversed phase particles (1.5 µm)
- Surfactants used to coat the particles to form stable suspensions
- A partial filling approach was used due to the light scattering at the particles
- Nine phenol derivates were separated

J. Chromatogr. A 1994, 688, 283-292

## Use of additives – Silica particles

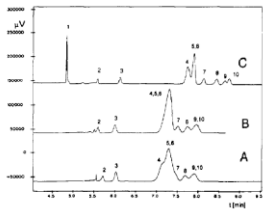



Fig. 5. Comparison of the separation of nine phenols using (A) buffer, (B) SDS and (C) 0.1 g of particles in 10 ml of buffer. Conditions: buffer, 10 m*M* sodium tetraborate–5 m*M* sodium phosphate (pH 9.5); SDS concentration, 4 m*M*; particle amount, 0.1 g of RP-18 (1.5 µm) in 10 ml of buffer with SDS; capillary, 59 cm to detector, 77 cm total length, 75 µm I.D.; injection, hydrodynamic, 50 mbar, 12 s; analyte concentration, 0.9 m*M* of each phenol; separation, 20 kV; detection, UV at 206 nm.

**UV detection**

The particles were relatively big which caused problem with suspension stability and thereby separation reproducibility.

Could be improved by sonicating the particle suspension every hour

The mobility of the particles was larger and in the opposite direction compared to the EOF

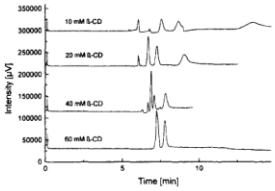
## Use of additives – Silica particles

- Improvement of method
- Fluorescence detection was used circumvent the complications with light scattering of the particles when UV detection is used
- Smaller particles was used (500 nm in diameter)

J. Chromatogr. A, 1997, 768, 320-324

## Use of additives – Silica Particles

- Addition of cyclodextran or urea to the mobile phase was necessary to prevent "too" strong binding of the analytes to the particles, causing assymmetric peaks and band broadening




## Use of additives – Silica particles

- Particles with a diameter of 500 nm
- Covalent modification of the particles to introduce carboxylic groups
- Fluorescence detection
- Migration window optimized by changing the pH. The widest window was observed at pH 7.0

Bächmann, K., Göttlicher, B., *Chromatographia* 1997, *45*, 249–254.

## Use of additives – Silica Particles

- The main factors for reducing the plate numbers were concluded to be:
  - Mass transfer resistance at the particle surface
  - Different velocity of the particles

Bächmann, K., Göttlicher, B., *Chromatographia* 1997, *45*, 249–254.

## Use of additives – Molecular micelles

- Molecular micelles are micelles that are covalently linked
- They have a zero CMC
- They are stable in presence of organic solvent

## Use of additives – Molecular micelles

- Amino acid-based molecular micelles have been used for enantioseparation of eight β-blockers
- Possible with UV as well as MS detection

## Use of additives – Molecular micelles

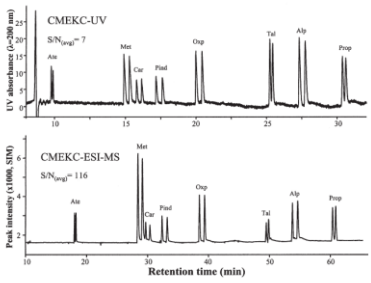



**Figure 7.** Electropherograms illustrating simultaneous UV and MS detection of β-blockers. Electrolyte: 15 mM poly-L-SUCL, 25 mM each of $NH_4OAc$ and triethylamine (TEA) (pH 8.0). Injection: hydrodynamic, 50 mbar for 1 s. Capillary: total length 120 cm, 50 µm ID. Detection: MS, sheath liquid composition, 40 mM $NH_4OAc$ in 80% v/v methanol (pH 8.0); sheath liquid flow rate, 5 L/min; drying gas temperature, 200°C; acquisition, positive mode; $V_{cap}$ = 2.5 kV; fragmentor voltage, 85 V; SIM 8 ions at eight different *m/z* (249, 250, 260, 266, 267, 268, 293, and 364). Reproduced from [45], with permission.

## Additives in CE/CEC – DNA analysis

- Conventional DNA analysis in CE is based on using a physical gel (i.e. polymer solution)
  - Limited by the high viscosity of solution
  - Replenishment cumbersome
- An alternative is to use additives in the gel:
  - Nanoparticles
  - Carbon nanotubes

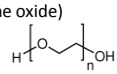
## Additives in CE/CEC – DNA analysis

- Separation of DNA is possible with lower polymer concentration in presence of nanoparticles

## Additives in CE/CEC – DNA analysis

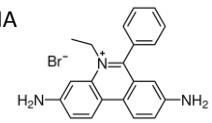
**Gold nanoparticles**

- Gold nanoparticles
  - Relatively easy to prepare
  - Need to be stabilized
    - For example by polymers, e.g. poly (ethylene oxide)
  - Have affinity for thiols (R-SH)




## Additives in CE/CEC – DNA analysis

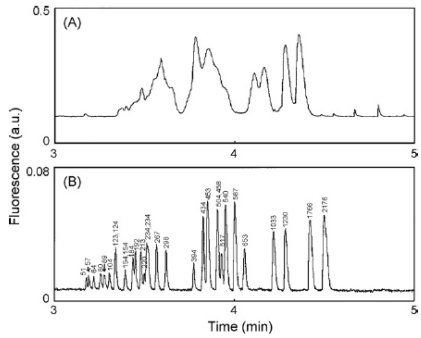
- Separation of DNA using PEO-coated gold nanoparticles
- A continuous full filling approach is used
  - The capillary is filled with nanoparticles
- Laser induced fluorescence as detection
- Intercalation of Ethidium bromide to detect DNA




## Additives in CE/CEC – DNA analysis

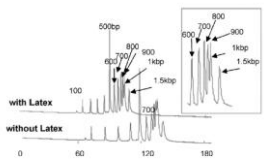
- Lower viscosity of gold nanoparticle suspensions
- The capillary was dynamically coated with PVP to suppress EOF and prevent interactions between DNA and capillary wall
- A suggested separation mechanism involve that DNA temporary interwines with the PEO on the gold nanoparticles

## Additives in CE/CEC – DNA analysis



## DNA analysis on chip using nanoparticles

PEG-coated polystyrene latex nanoparticles was used as PSP in MCEC for separation of dsDNA (10 bp to 3 kbp), by Tabuchi et al. [97]. Fig. 10 illustrates the separation of DNA in the range of 100–1500 bp. Low viscosity gels are normally used for separation of large DNA. However, the addition of nanoparticles as obstacles in the gel, also enable separation of small DNA in the low viscosity gel. A polymer solution in combination with nanoparticles was used to separate large and small DNA, simultaneously. The PEG-coated nanoparticles were prepared by emulsion polymerization of styrene in the presence of PEG macromonomers. Separation was improved due to the PEG on the nanoparticle surface. Nanoparticles of different sizes (80 nm, 110 nm and 193 nm) were mixed with hydroxyl propyl methyl cellulose buffer solution for improved separation compared to separations in conventional hydroxyl propyl methyl cellulose buffer. Separation of a fairly wide range of DNA (10 bp to 2 kbp) was improved, with higher efficiencies for smaller nanoparticles. Fast filling of the separation media was possible due to its low viscosity.



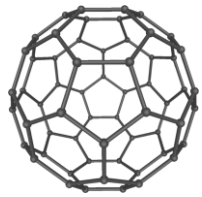

[97] M. Tabuchi, Y. Katsuyama, K. Nogami, H. Nagata, K. Wakuda, M. Fujimoto, Y. Nagasaki, K. Yoshikawa, K. Kataoka, Y. Baba, Lab Chip 5 (2005) 199.

## Additives in CE/CEC – Carbon nanostructures

- For example:
  - Fullerenes
  - Carbon nanotubes
  - Carbon nanohorns
- Low solubility in aqueous buffers
- Can be solved by:
  - Oxidization of surface
    - Sonication in sulfuric and nitric acid
    - Carboxylic group on surface
  - Addition of surfactants (i.e. SDS)

## Additives for CE/CEC - Fullerenes

- Discovered in 1985
- Commercially availible with different surface chemistries



## Additives for CE/CEC - Fullerenes

- SDS used to solubilize fullerenes in water
- Use of fullerene-SDS complexes for separation polycyclic aromatic hydrocarbons (PAHs)
- Similar separation mechanism as MEKC
- Fullerenes enhance separation

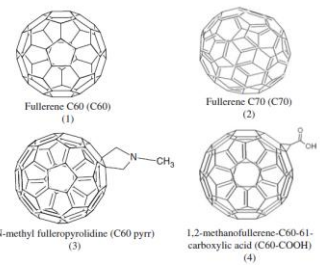
J. Chromatogr,. 2000, 873, 257-267

## Nonaqueous CE of Fullerenes

- Separation of different variants of fullerenes
  - C60 and C70 fullerenes as well as C60 variants

Anal. Bioanal. Chem. 2012, 404, 307-313

## Nonaqueous CE of Fullerenes

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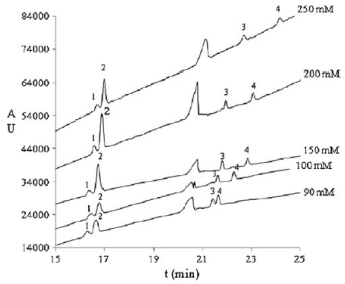
## Nonaqueous CE of Fullerenes

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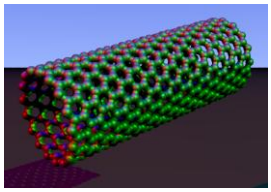
## Additives in CE/CEC – Carbon nanotubes

- Diameter of a few nm up to a few tens of nm.
- Length up to several micrometers

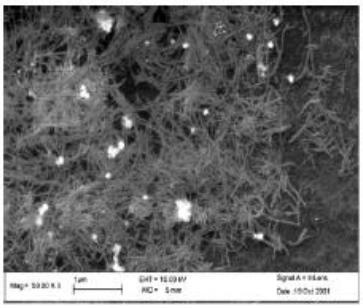


## Additives in CE/CEC – Carbon nanotubes

- Separation of homologues of caffeine and theobromine
- Distinct changes in the separation occur at a certain concentration
- The nanotubes formed sieving networks in the capillary that acted as pseudostationary phase
- The nanotube network prevented the diffusion of the analytes to the capillary wall and thereby minimized the adsorption to the capillary wall

Electrophoresis 2003, 24, 4181-4188

## Additives in CE/CEC – Carbon nanotubes



**SEM**

Electrophoresis 2003, 24, 4181-4188

## Additives in CE/CEC – Carbon nanotubes

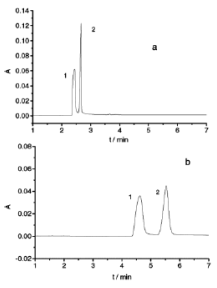



**Figure 3.** Electropherograms of 2 mM theobromine (peak 1) and 2 mM caffeine (peak 2). Run buffer: 12.5 mM sodium borate (pH 9.18) (a) without c-SWNT and (b) with 0.05 mg·mL$^{-1}$ c-SWNT solution. Applied voltage, 15 kV; UV detection, 214 nm.

Electrophoresis 2003, 24, 4181-4188

## Additives in CE/CEC – Carbon nanohorns

- Diameter of approximately 2 nm
- Length 30-50 nm, which is substantially shorter than the carbon nanotubes
- Conical ends
- Forms flower-like structures with a diameter of 80-100 nm, with a large surface area.

## Additives in CE/CEC – Carbon nanohorns

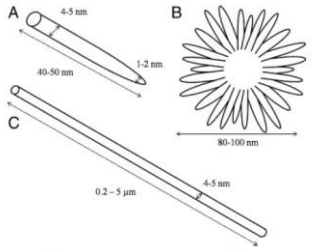
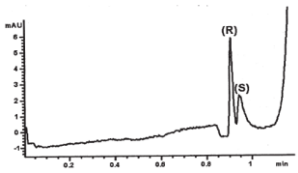
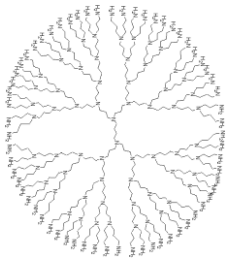



**Figure 1.** Schematic representation of the relative dimensions of (A) single-walled carbon nanohorn, (B) dahlia flower-like aggregate of single-walled carbon nanohorns and (C) single-walled carbon nanotube. Reprinted from [33] with permission.

## Example of other additives in CE/CEC

- Molecular imprinted polymer nanoparticles
- Dendrimers



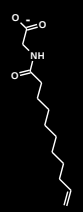



## Template Orientation
### at o/w Interface Using a Functional Surfactant

Template

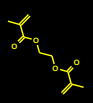
(S)-propranolol

Functional surfactant



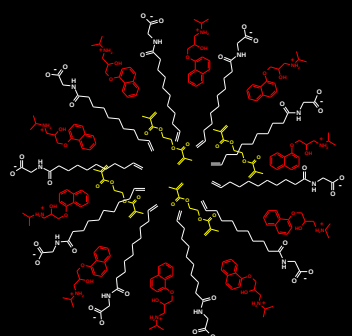
*N*-undecenoyl glcinate

Cross-linker

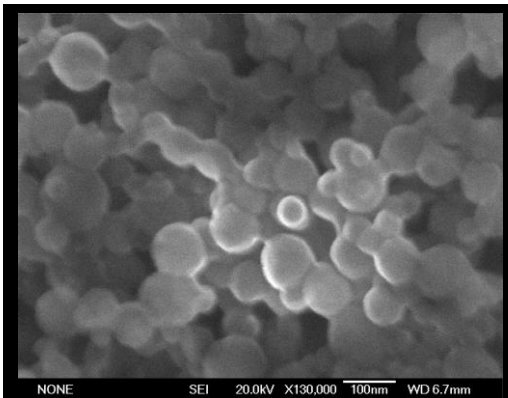


EDMA

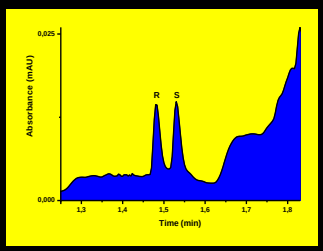
MIP Nanoparticle Synthesis



(Mini-emulsion Strategy)




## Monoclonal Imprints!!!




**CEC Separation of Racemic mixture of Propanolol**

Nanoparticles; hydrodynamic injection 0.5psi for 10 s,
sample injection 3 s 6 kV, electrolyte 10 mM phosphate/20% acetonitrile,
16 kV, T, 30 ºC. L, 30 cm