New Developments in Capillary Electrophoresis with focus on Bioanalysis

Lecture 11

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Today

- Capillary Electrochromatography
  - Open tubular columns
  - Packed columns
  - Monolithic columns

Capillary Electrochromatography (CEC)

- Liquid phase separation in a column containing a stationary phase media conatining ionizable groups
- The flow is driven by electroosmosis
- The electric field across the capillary can affect the retention of the analytes

Packed column CEC

- The procedures for fabricationg packed columns came from HPLC
  - Retaining frits
  - Solvent slurry packing
- More complicated to pack capillaries than standard analytical columns

Packed column CEC

- Smith and Evans were early to demonstrate the extreme efficiency that can be achieved by CEC
  - 8 millions plate per meter
- However, the results were difficult to reproduce
- Highly polar basic compounds were anlysed using strong cation exchanger

Chromatographia 1995, 41, 197-203

Packed column CEC

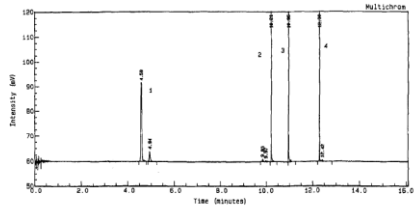


Figure 8
The separation of bendroflumethiazide from nortriptyline, clomipramine and methdilazine.
Instrument: Modified ABI 270A; Capillary: 26 cm packed, 50 cm total × 50 µm i.d.; Packing: 3 µm Spherisorb SCX; Detection: λ = 210 nm, 0.04 absorbance units full scale; Rise time: 0.2 s; Applied voltage: 30 kV; Temperature: 30 °C; Mobile phase: 70 % CH_3CN/30 % 0.05 mol/L NaH_2PO_4 apparent pH = 3.5; Injection: 2 kV for 0.5 min. **1** = bendroflumethiazide, **2** = nortriptyline, **3** = clomipramine, **4** = methdilazine. Data captured using V.G. Multichrom system.

Chromatographia 1995, 41, 197-203

Packed column CEC

- Typical packing procedure (1)
  - Attach a inline end end-frit and pack the column by pumping the slurry of beads and solvent through the capillary with high pressure
  - Flush the packed column with water to replace the solvent
  - Prepare the outlet end-frit at the desired distance from the capillary end by sintering the beads using heat over 550°C

Packed column CEC

- Typical packing procedure (2)
  - Remove the inline end-frit and flush out the extra material using reversed flow direction
  - Create the inlet end-frit by sintering the packing material
  - Remove the polyimide coating to create the detection window
  - Cut off excess capillary at the inlet side
  - Equiblirate the column with desired mobile phase

Packed column CEC

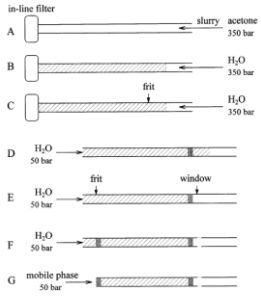


Fig. 3. Schematic view of the packing process.

Packed column CEC

- A major challenge is the fabrication of the retaining frits
- The charged particles can move in the electric field

Open tubular CEC

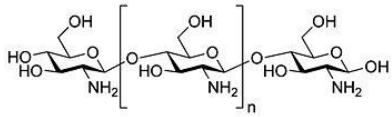
- Developed by Pretorius already in 1974
- The stationary phase is attached to the capillary wall
- Disadvantages with conventional OT-CEC
  - Low phase ratio of the stationary phase because of the small surface area
  - Relatively long distance for the analytes to reach the stationary phase material

Open tubular CEC

- Organic moieties bound to the capillary inner wall
  - The chromatographic effect is small
  - However, EOF and adsorption properties can be changed

Open tubular CEC

- Use of carboxymethyl-chitosan as stationary phase
  - A hydrophilic polysaccharide



J. Chromatogr. A, 2010, 1217, 8346-8351

OT-CEC
Chitosan as stationary phase

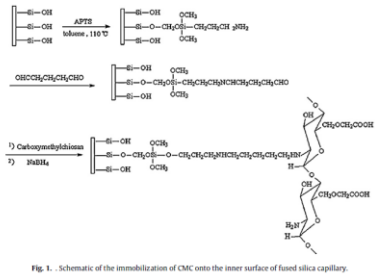
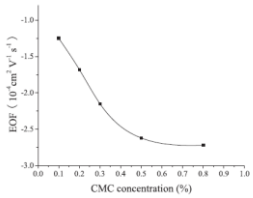


Fig. 1. . Schematic of the immobilization of CMC onto the inner surface of fused silica capillary.

J. Chromatogr. A, 2010, 1217, 8346-8351

OT-CEC

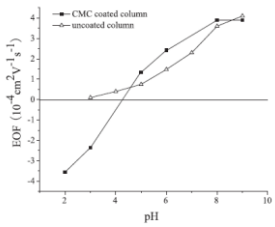
- Effect of chitosan concentration on EOF



J. Chromatogr. A, 2010, 1217, 8346-8351

OT-CEC

- The EOF can be normal or reversed depending on the pH



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OT-CEC

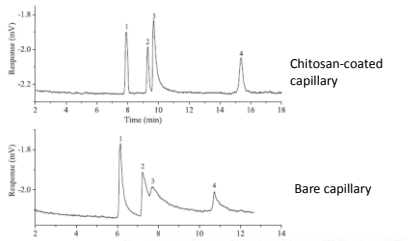


Fig. 6. Separation of opium alkaloids on (a) CMC-modified column and (b) bare fused-silica capillary. Conditions: columns: CMC-modified capillary column: 60.0 cm total length (40.0 cm effective length) × 50 μm i.d. capillary; bare fused-silica capillary: 52.0 cm total length (32.0 cm effective length) × 50 μm i.d. capillary; mobile phase: 50 mM phosphate buffer at pH 6.0; applied voltage: −15 kV; wavelength: 214 nm. Peaks: 1, morphine; 2, thebaine; 3, narcotine; 4, papaverine.

J. Chromatogr. A, 2010, 1217, 8346-8351

OT-CEC

Separation of basic proteins

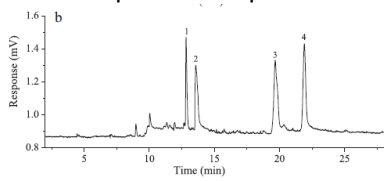


Fig. 5. Electrochromatogram of four proteins in CMC-modified column at (a) pH 2.5 and (b) pH 3.0. Conditions: column: 57.0 cm total length (37.0 cm effective length) × 50 μm i.d.; mobile phase: 20 mM phosphate buffer (pH 2.5 or pH 3.0); applied voltage: +15 kV; wavelength: 214 nm. Peaks: 1, trypsin; 2, ribonuclease A; 3, cytochrome C; 4, lysozyme.

Open tubular CEC

- Use of etched capillaries
  - Etching to increase the surface area
- Chemical modification of etched capillaries
- The etching procedure will affect the EOF
  - Nitrogen and fluoride from the etching reagent are incorporated into the surface
  - The surface that is generated is more biocompatible (with less tendency for adsorption)
  - The nitrogen alter the electroosmotic behavior of the capillary . At low pH the EOF will be reversed

OT-CEC

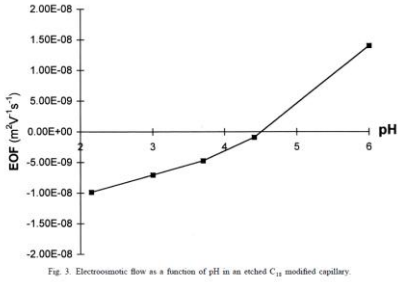


Fig. 3. Electroosmotic flow as a function of pH in an etched C_{18} modified capillary.

Open tubular CEC

- Etching procedure (50 µm ID capillary) (1)
  - Fill the capillary with concentrated HCl, heat overnight (80°C)
    - To remove impurities from the capillary wall
  - Flush with water, acetone and diethyl ether
  - Dry capillary with nitrogen
  - Etching with ammonium hydrogen difluoride (5%) in methanol, wait one hour, remove methnol by nitrogen

J. Chromatogr. A 1996, 736, 255-264

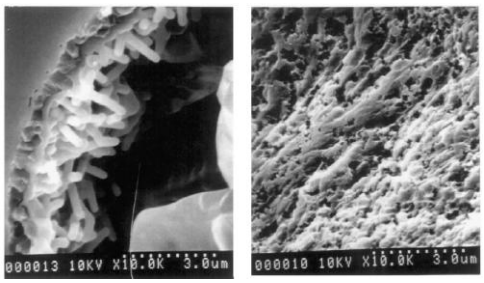
Open tubular CEC

- Etching procedure (50 µm ID capillary) (2)
  - Methnol is used to assure uniform distribution of the ethcing agent during drying.
  - Heating to 300-400°C for 3-4 hours in a GC oven
  - The temperature and time determines the morphology of the surface

J. Chromatogr. A 1996, 736, 255-264

Open tubular CEC

SEM of etched silica capillaries



J. Chromatogr. 2000, 887, 31-41

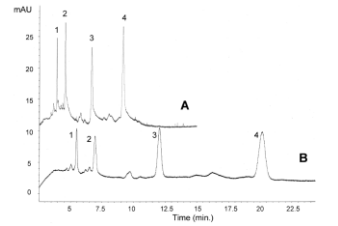
OT-CEC

- Chemically modified etched capillaries
  - The surface area can be enhanced 1000 times
  - Silica material can extend 5 µm into the capillary and thereby decrease the distance for the analyte to reach the stationary phase
  - Can be coupled with MS
    - Rinse the column after formation, to avoid leakage when coupled to MS

Anal. Chem. 2007, 79, 4942-4949

OT-CEC

- Protein separation in etched and non-etched capillaries



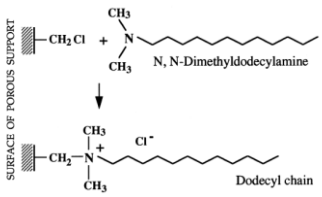
OT-CEC

- Separation of proteins and peptides
- Porous-layer open tubular (PLOT) column
- A crosslinked polymer layer on the wall of the capillary
- Polymerization of vinylbenzyl chloride and divinylbenzene in presence of 2-octanol

J. Chromatogr. A, 1999, 858, 91-101

OT-CEC

- Functionalisation of PLOT column



J. Chromatogr. A, 1999, 858, 91-101

OT-CEC

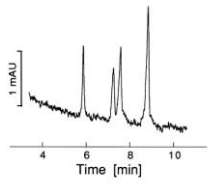
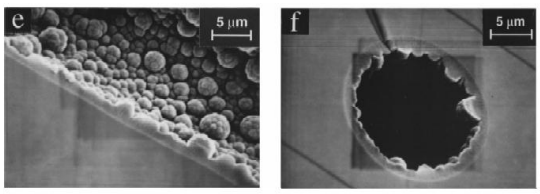


Fig. 6. Electrochromatogram of four basic proteins obtained under isocratic elution conditions by using a PLOT column. Column, 47 cm (effective length 40 cm)×20 μm fused-silica capillary, with a ca. 2 μm thick polymer layer having dodecyltrimethylammonium functions at the surface as the stationary phase. Mobile phase, 20% (v/v) acetonitrile in 20 mM aqueous sodium phosphate, pH 2.5. Applied voltage, −30 kV. Peaks: (1) α-chymotrypsinogen A, (2) ribonuclease A, (3) lysozyme, (4) cytochrome c. Mobility of EOF measured with DMSO, $\mu_{ep} = -3.46 \cdot 10^{-8}$ m^2 V^{-1} s^{-1}; migration time of DMSO, 3.10 min. Theoretical plate numbers for each protein are shown in Table 1.

Use of PLOT column

OT-CEC

- SEM of PLOT column



J. Chromatogr. A, 1999, 858, 91-101

Monolithic CEC columns

- Composed of in situ prepared polymers
- Porous properties and surface chemistry can be controlled
- Used in LC as well as CEC
- First used by Hjertén in 1989

J. Chromatogr. 1989, 473, 273-275

Monolithic CEC

- Monolithic columns solve most of the problems with the other types of CEC (open tubular and packed column
- No retaining frits
- No movement of particles
- Easier to prepare
- Possible to form molecularly imprinted monoliths by the same procedure

Monolithic CEC

- Early methods to produce monolithic columns involved the fixation of an already packed capillary column
- A heated wire can be drawn along the capillary to achieve sintering of the beads
- The capillary can be heated to to 360°C in presence of sodium bicarbonate solution
- The interstitial volume can be filled with sol gel structures

Monolithic CEC

- The described methods solve the problem with column stability
- However, retaining frits still needed to be fabricated

- Instead the monolithic CEC column can be fabricated by in situ polymerization

Monolithic CEC

- The length of the column can easily be adjusted
  - UV initiated polymerization can be used instead of thermial initiated polymerization. A mask can be used.

- The polymerization mixture can be prepared using a wide variety of monomers allowing an almost unlimited choice of polymer morphology and surface chemistry

Monolithic CEC

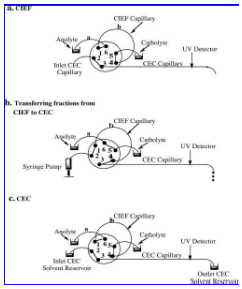
- EOF is not as dependent on the pore size as a mechanically pumped flow
- By using monoliths with small pores improving the analyte mass transfer
- Will not give problem with large pressure drop

Monolithic CEC

- CEC as one dimension in 2D separation
- CIEF followed by CEC for separation of proteins

Journal of Proteome research 2006, 5, 2001-2008

Monolithic CEC



Journal of Proteome research 2006, 5, 2001-2008

Monolithic CEC

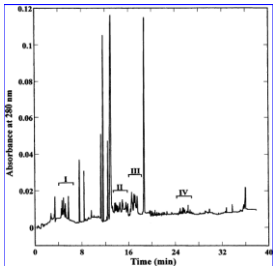


Figure 6. CIEF electropherogram of albumin-depleted human serum using HPC-coated fused-silica capillary, 45 cm (to detector), 51 cm (total length) × 50 μm i.d. Injection by filling the whole capillary column with sample. Sample was dissolved in 2% (v/v) Pharmalyte in water; anolyte, 1% v/v acetic acid in water, pH 2.5; catholyte, 1% v/v ammonium hydroxide in water, pH 10.7; focusing for 15 min at 20 kV; gravity mobilization by elevating the anode reservoir by 5 cm. Wavelength, 280 nm.

Journal of Proteome research 2006, 5, 2001-2008

Monolithic CEC

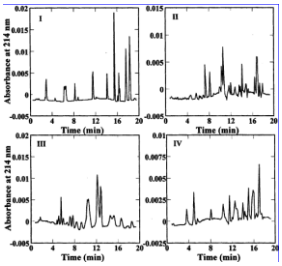


Figure 7. Analysis of albumin-depleted human serum by CIEF–CEC 2D separation platform. After CIEF separation of sample as in Figure 6, further resolution of the focused zones were achieved using CEC in the 2D separation platform. Heart cuts I, II, III, and IV are corresponding to zones I, II, III, and IV shown in Figure 6.

Journal of Proteome research 2006, 5, 2001-2008

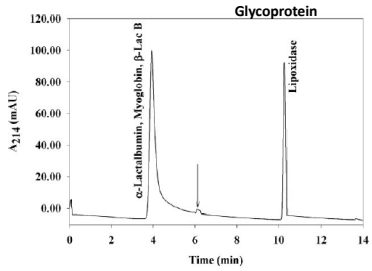
Monolithic CEC

- CEC in affinity mode
- Immobilize lectins for separation of glycoproteins
- For recognition of N-glycans

- Lectins: Sugar binding proteins. Sits on the cell surface and is responsible for recognition

Electrophoresis 2006, 27, 1020-1030

Monolithic CEC



Lectin-based CEC

Electrophoresis 2006, 27, 1020-1030

Monolithic CEC

- Modification of the surface chemistry of a monolith
- A monolith can be prepared with an ”optimal” morphology/ pore structure
- In a second step the surface can be modified to achieve the requested interaction properties and a suitable EOF

Monolithic CEC

- Protein separation in monolithic CEC
- As with conventional CE proteins tend to adsorb to the surface
- One common way to solve the adsorption problems is to use an acidic buffer to ensure that the proteins are positively charged and interact less with the positively charged surface

Anal. Chem. 2004, 1044, 3-22