New Developments in Capillary Electrophoresis with focus on Bioanalysis

Lecture 10

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Today

- CE in drug development

CE in drug industry

- Liquid Chromatography dominant
- The field of LC more mature
- Limited by availability of experienced CE users

Preclinical stage

- Find new compounds for group of compounds (i.e. lead compounds) with desired therapeutic effect
- Generally small amounts of each compound
- Many compounds
- Combinatorial approch of synthesis

Passive adsorption across biological membranes

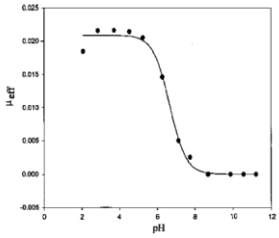
- Dissociation coefficients (pK_a)
- Partitioning behavior (e.g. octanol-water partioning)
- Solubility
- Membrane permeability

Determination of pK_a by CE

- An alternative to potentiometry or UV spectrometry
- CE
  - Smaller amount of samples
  - Less sensitive to contaminations
  - Automated
  - Chromophore necessary for UV detection

Determination of pK_a by CE

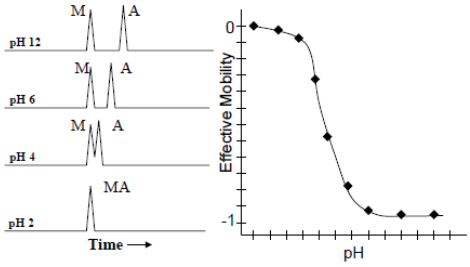
- Measuring the ionic effective mobility as a function of the pH



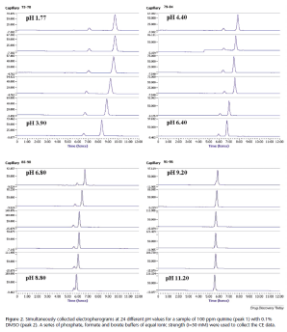
Example of 2-aminopyridine (pK_a 6.7)

A equilibrium equation is fitted to the data points

Determination of pK_a by CE



Determination of pK_a by CE



Determination of pK_a by CE

- High throughput measurements
- Commercially available 96 capillary instruments
- Commercially available kits
- Pressure-assisted CE to shorten the run times

Multiplexed CE-UV

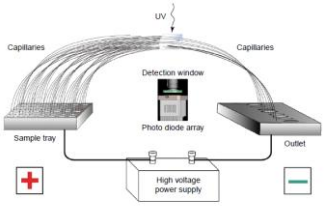


Figure 1. Schematic design of a multiplexed CE system with UV absorption detection. An array of 96 capillaries is packed side-by-side at the detection window where the capillary polyimide coating has been removed. The capillaries are arranged in an 8 x 12 format compatible to a standard 96-well plate for sample injection. The outlet ends of the capillaries are connected to a common reservoir. A deuterium lamp is used as a UV light source. The transmitted light through the capillary array detection window is collected by a flat field lens, passed through an interference filter and imaged on a photodiode array detector. UV absorption signals are monitored simultaneously for all 96 capillaries. High voltage is applied to the capillaries during electrophoresis separation.

Low Molecular weight drug candidates of possess aromatic rings or conjugated double bond structures, which make UV detection useful

Drug Discovery Today 2004, 9, 1072-1080

Multiplexed CE-UV

- Compatible with 96 well plates (8*12) at inlet side
- 96 capillaries side by side at detection window
- Can use vacuum to introduce different buffer solutions in different capillaries
- An alternative is to use multichannel microfluidic electrophoresis devices

Multiplexed CE-UV

Table 1. Summary of commercial multiplexed CE systems and their applications

Manufacturer	System	No. of capillaries	Detection	Applications
Amersham Biosciences www.amershambiosciences.com	Megabase 500	48	LIF	DNA sequencing, genotyping
	Megabase 1000	96		
	Megabase 4000	384		
Applied Biosystems www.appliedbiosystems.com	ABI 3730	48,96	LIF	DNA sequencing, genotyping
	ABI 3730xl	96		
	ABI Prism 3100	4,16		
Beckman Coulter www.beckman.com	CEQ 8800	8	LIF	DNA sequencing, genotyping
	CEQ 8000			
CombiSep www.combisep.com	CePRO 9600	96	UV	pK_a, log P, chiral, purity, SDS-protein sizing, DNA sizing, peptide mapping, absorption amino acid analysis, CIEF, oligonucleotide QC
Genteon www.genteon.com	Capella 400	384	LIF	Genotyping, DNA sizing, oligonucleotide QC
Spectrumedix www.spectrumedix.com	SCE series	24,96,192,384	LIF	Genetic analysis, DNA sequencing, DNA/protein gel shift
	REVEAl series			Mutation discovery
	Ident series			Genotyping
	HTS series			Protein analysis
Caliper Technologies www.caliperls.com	Caliper 3000 HTS	4–12 channels Microfluidic device	LIF	Enzyme assay

Determination of pK_a by CE-MS

- Pressure-assisted CE
- A series of 10 volatile buffers covering pH 2.5-10.5
- High throughput screening
- Higher sensitivity than CE-UV
- Can be used for non-UV-absorbing compounds

Rapid Communications in Mass Spectrometry 2003, 17, 2639-2648

Determination of pK_a by CE-MS

- Simultaneous measurement of more than 50 compounds in less than 150 min

Rapid Communications in Mass Spectrometry 2003, 17, 2639-2648

Determination of distribution coefficients

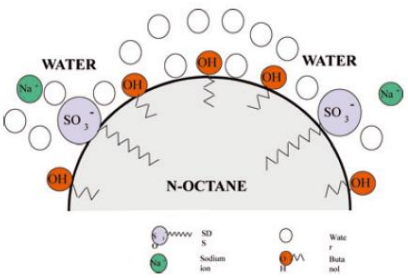
- Methods based on:
  - Micellar Electrokinetic Chromatography (MEKC)
  - Microemulsion Electrokintetic Chromatography (MEEKC)
- Separation based on hydrophobicity
- Compared to standard compounds of known hydrophobicity
- Separation of uncharged compounds
  - High pH for weak bases
  - Low pH for weak acids

Microemulsion Electrokinetic Chromatography

- Use of a microemulsion as electrolyte
  - Oil-in-water or water-in-oil microemulsion
  - Stable clear emulsion
  - Water, hydrocarbon and surfactant, co-surfactant

Electrophoresis 2013, 34, 159-177

Microemulsion Electrokinetic Chromatography



Comparsion between MEEKC and MEKC

- Enhanced solubilization capacity and separation of MEEKC
- Analytes can partition more effectively into the microemusion droplets compared to the more rigid micelles
  - Higher rate of mass transfer give more efficient separation

Analysis of pharmaceutical counterions

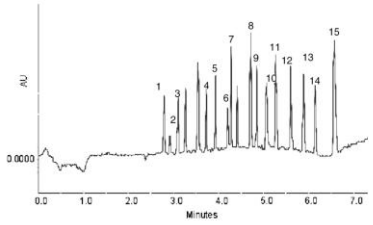


FIGURE 2 Separation of 15 acids used as pharmaceutical salt-forming agents, peak assignment: 1. Hydrochloric, 2. Nitric, 3. Sulfuric, 4. Tartaric, 5. Malic, 6. Citric, 7. Succinic, 8. Acetic, 9. Lactic, 10. Phosphate, 11. Propionic, 12. Butyric, 13. Pentanoic, 14. Hexanoic, and 15. Octanoic (taken from reference 21. With permission.).

High throughput screening and lab on chip

- HTS to identify chemical hits against a therapeutic target to detect lead candidates
- Tradtional HTS method use radioactive or fluorescent labeling
  - Label may affect the result

High throughput screening by CE

- Screening for enzyme inhibitor
- Enzymes common therapeutic drug targets
- L-glutamic dehydrogenase (GLDH) used as model enzyme
- Inline enzyme bioreactor in capillary column
- GLDH immobilized on gold nanoparticles
- The gold nanoparticles was absorbed on the inner wall of the capillary at the inlet

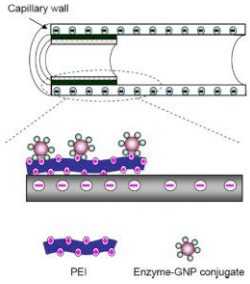
Analytical Biochemistry 2011, 411, 88-93

High throughput screening by CE

- Method used to screen plant extracts

Analytical Biochemistry 2011, 411, 88-93

High throughput screening by CE



The capillary surface is pretreated with a polyelectrolyte

The enzyme is absorbed based on ionic binding

Analytical Biochemistry 2011, 411, 88-93

High throughput screening by CE

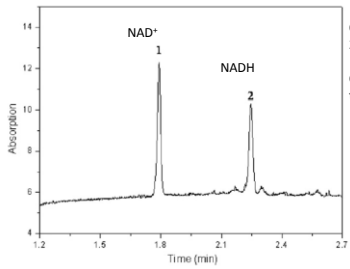
- Enzyme loading will be much greater if meditated by gold nanoparticles compared to in free solution
  - Enzyme enriched on GNPs that possess a high surface-to-volume ratio
- Enzyme-Nanoparticle conjugates much more stable than enzyme-polyelectrolyte conjugates
  - Thiol groups present in proteins bind to the gold nanoparticles strongly

Analytical Biochemistry 2011, 411, 88-93

Nanoparticle enhancement

- Examples of other application
  - Immobilization of trypsin on nanoparticles to enhance the trypsin activity
  - Immobilization of cyclodextrin on nanoparticles to enhance chiral separation

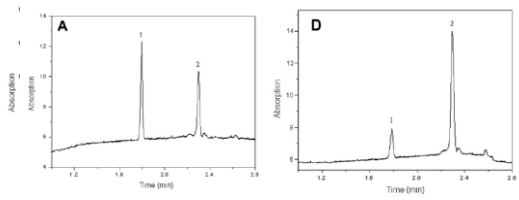
High throughput screening by CE



Quantification of NAD^+ at 254 nm

Conversion of glutamate to α-ketoglutarate

High throughput screening by CE



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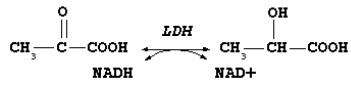
Perphenazine (a known GLDH inhibitor

Other methods for high throughput screening

- Immobilization of enzymes on silica particles that were packed into LC columns
- Enzyme bioreactors based on entrapment of enzymes in sol-gel-derived monoliths in CE
- Immobilized enzymes on magnetic nanoparticles

Enzyme purification and characterization



Produced to recover NADH
During hard work / Shortage of oxygen

Enzyme purification and characterization

- Genetic modification to make purification easier
- Examples:
  - Modification to make target enzyme more resistant to heat
  - Attachment of Histidin tag to allow IMAC

Example of enzyme purification

- Production of enzyme in E.coli. Using a plasmid containing target enzyme (genetic modified)
- Destroy the cell membrane
  - Sonication or lyzosyme
- Heat treatment
- IMAC purification

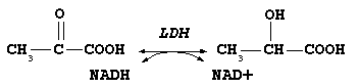
IMAC

- Immobilized metal ion affinity chromatography
- Metals such as Cupper and Nickel have affinity for histidine
- Imidazole for elution
- Phosphorylated peptides or proteins can be used purified by e.g. Zink

Affinity Chromatography

- Lectins for purification of glycoproteins
- Antibodies
- Aptamers

Enzyme purification and characterization



Spectrophotometry to determine LDH amount indirectly by measuring NAD+ concentrations

CE Enzyme Assays

- Pre-capillary enzyme assay
  - Initiation of reaction
  - Termination of reaction
  - Analysis of product(s) and or reactant(s) by CE
- In-capillary enzyme assay
  - The enzyme, reactant and product have different mobility
  - Low consumption of reactants, enzymes and cofactors

CE Enzyme Assays