

New Developments in Capillary Electrophoresis with focus on Bioanalysis

Lecture 9

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Today

- CE of nucleic acids
- Electrophoretic preconcentration techniques

CE and Nucleic acids

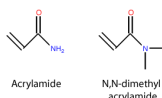
- Separation and sequencing
 - Gene analysis
- Detection of mutations
 - Disease diagnostics

DNA separation

- Use of linear hydrophilic polymers for:
 - Separation
 - To prevent adsorption to the capillary wall
 - To suppress the EOF

DNA separation

- Poly(N, N-dimethylacrylamide) (PDMA)
 - Used as linear polymer for DNA separation
- An alternative is to use star-shaped PDMA polymers
 - Lower viscosity compared to a linear polymer with the same molecular weight
 - Shorter separation time
 - Maintained resolution



J Chromatogr A, 2011, 1218, 3037-3041

DNA separation

- Linear polymers
 - Relatively high concentration necessary
 - High viscosity
 - High viscosity reduces separation speed and limits capillary refreshing
 - Viscosity depends on polymer size
 - Controlling the polymer structure to reduce viscosity

J Chromatogr A, 2011, 1218, 3037-3041

Separation of DNA

- Capillary wall coating can be avoided as PDMA adsorb to the capillary wall and reduce the interactions between the sample and capillary wall

CE for detection of SNP

- SNP = Single nucleotide polymorphism
- A single nucleotide is changed in the genome
- If the SNP is present in a gene for the protein, an amino acid can change

- Examples of CE applications

CE for detection of SNP

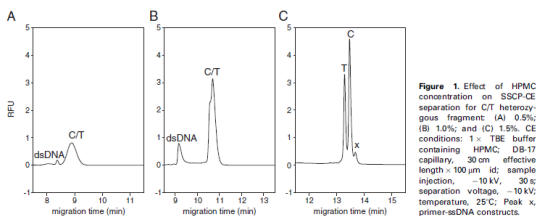
- Different types of mutations
 - Deletion: Can be separated based on length
 - SNPs: More complicated as the length are the same

CE for detection of SNP

- γ -glutamyl hydrolase gene (GGH)
 - Important for methotrexate (MTX) metabolism
 - $\text{MTX}-(\text{Glu})_n \rightarrow \text{MTX}$
 - SNP 452 C>T lower the activity of GGH and $\text{MTX}-(\text{Glu})_n$ is accumulated

Electrophoresis, 2011, 32, 2021-2027

CE for detection of SNP



Electrophoresis, 2011, 32, 2021-2027

CE for detection of SNP

- Mutation in the gene for epidermal growth factor receptor (EGFR)
- To predict the outcome of lung cancer therapy
 - Therapy designed to block the EGFR by targeting its tyrosine kinase domain
 - CE can be used to detect mutations in the tyrosine kinase domain of EGFR
 - Before using an inhibitor

J Sep Sci 2010, 33, 2349-2355

Epidermal growth factor receptor

- Start a signalling cascade when activated
- Activation of tyrosine kinase
- Important for
 - DNA synthesis
 - Cell division
 - Involved in the immune system

CE for detection of SNP

- Most frequent mutation is a deletion
 - 10-20 base pairs are deleted
- Focus on the detection of the substitution mutation

J Sep Sci 2010, 33, 2349-2355

CE for detection of SNP

- Commercial instruments with 8, 16 or 96 capillaries were used
- The EGFR gene was amplified by PCR

J Sep Sci 2010, 33, 2349-2355

CE for detection of SNP

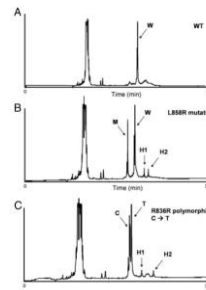
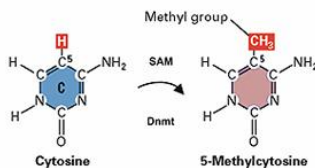


Figure 4. Comparison of peak patterns for separation of (A) wild type (WT), (B) somatic mutation EGFR exon 21 L858R and (C) silent polymorphism EGFR exon 21 R438R.

J Sep Sci 2010, 33, 2349-2355

Determination of methylated DNA

- Methylation in eukaryotic DNA
 - Only 5-methylcytosine?
 - Suggested that methyladenine is present in eukaryotic cells but its function is unknown



Determination of methylated DNA

- DNA methyl transferase catalyse the reaction
- Cytosine methylation in eukaryotes is associated with gene silencing
- CE to get a deeper understanding of the biological functions of methylations

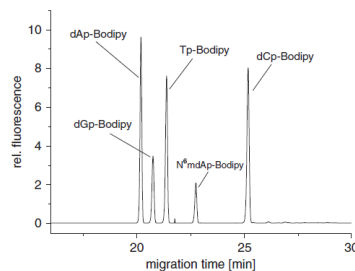
Determination of methylated DNA

- CE-LIF has been proved to be significantly faster and more sensitive than more traditional method such as HPLC-UV
- The DNA is hydrolyzed prior to fluorescent labeling
- A fluorescent dye is used for detection
 - Fluorescent dye: BODIPY FL EDA
 - Bind covalently to the phosphate group of the nucleotide
 - Attomol level of detection

Electrophoresis 2010, 31, 3548-3551

Determination of methylated DNA

Analysis of methylated adenine from bacterias



Electrophoresis 2010, 31, 3548-3551

Micelle to solvent stacking

- New online sample concentration technique for charged analytes

J Chromatogr A 2010, 1217, 6290-6295

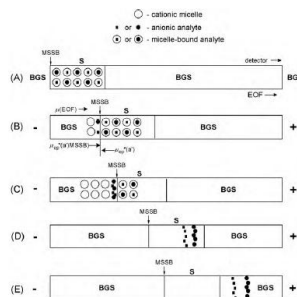
Micelle to solvent stacking

- The micelles and analytes are opposite charged
 - Cationic micelles
 - Separation of anions
 - Methanol as organic solvent

Micelle to solvent stacking

- In sample: Micelles + Samples
- In electrolyte: Organic solvent
- In the sample plug the analyte bind to the micelles
- When the micelles reach the micelle to solvent boundary the affinity of the analytes for the micelles will be significantly lower due to the organic solvent

Micelle to solvent stacking



10-fold enhancement of the sensitivity

Later more than 100-fold enhancement of detection has been achieved [J Chromatogr A, 2010, 1217, 175-178]

J Chromatogr A 2010, 1217, 6290-6295

Sweeping combined with micelle to solvent stacking

- For separation of organic anions
- Generally micelle to solvent stacking can be used for either only anions or only cations

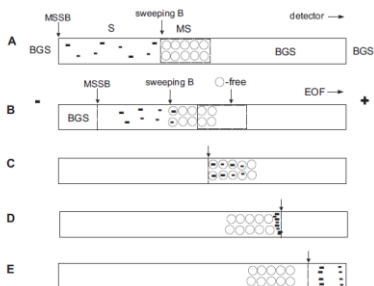
J Chromatogr 2011, 1218, 1004-1010

Sweeping combined with micelle to solvent stacking

- Positively charged capillary coating
- Electrolyte with organic solvent
- 1. Injection of micelle solution of cationic solvent
- 2. Injection of sample

J Chromatogr 2011, 1218, 1004-1010

Sweeping combined with micelle to solvent stacking



2-3 times improvement compared to micelle to solvent stacking alone

J Chromatogr 2011, 1218, 1004-1010

Sweeping combined with micelle to solvent stacking

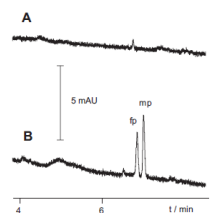


Fig. 6. Sweeping-MMS of blank (A) and herbicides spiked (B) waste water sample. Spiked sample before extraction contained 1 µg/ml of fenprop (fp) and 2 µg/ml of mecoprop (mp). S injections=30 s, MS injections=15 s. Other conditions are similar to that in Fig. 3B. Explanation in the text.

J Chromatogr 2011, 1218, 1004-1010