# Capillary Electrophoresis: an Attractive Technique for Chiral Separations

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**Cari E Sänger – van de Griend[1,2,3], Ylva Hedeland[2] and Curt Pettersson[2]**

[1] *Kantisto BV, Baarn, the Netherlands (*cari.sanger@kantisto.nl*)*
[2] *Division of Analytical Pharmaceutical Chemistry, Uppsala University, Uppsala, Sweden*
[3] *Australian Centre for Research on Separation Science ACROSS, University of Tasmania, Hobart, Australia*

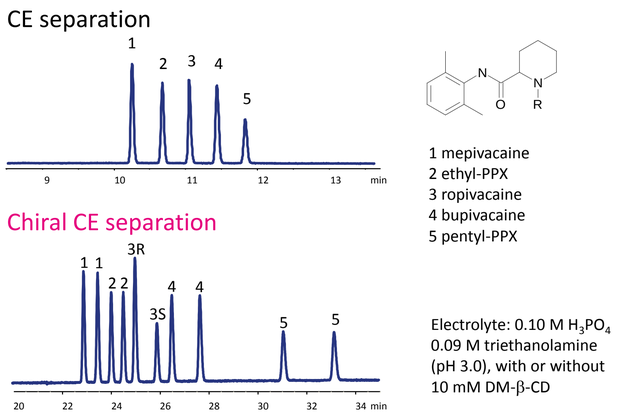


**Figure 1: Achiral and chiral separation of local anaesthetics [1, 2]**

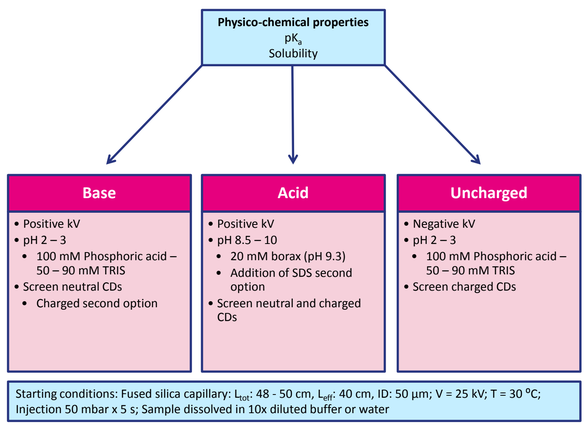


**Figure 2: Starting point for chiral CE method development**

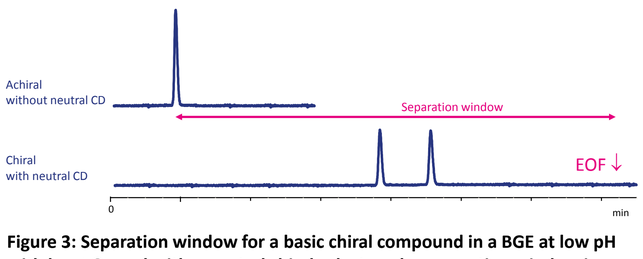


**Figure 3: Separation window for a basic chiral compound in a BGE at low pH with low EOF and with a neutral chiral selector. The separation window is between the mobility of the chiral selector (which is with the EOF) and the free mobility of the compound (which is the mobility in BGE without chiral selector).**

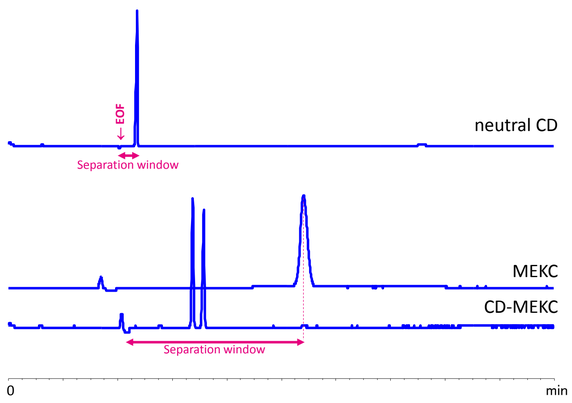


**Figure 4: Separation window for an acidic compound at a high-pH electrophoresis buffer (fast EOF) with an uncharged chiral selector in chiral CE (top) and CE-MEKC (bottom).**

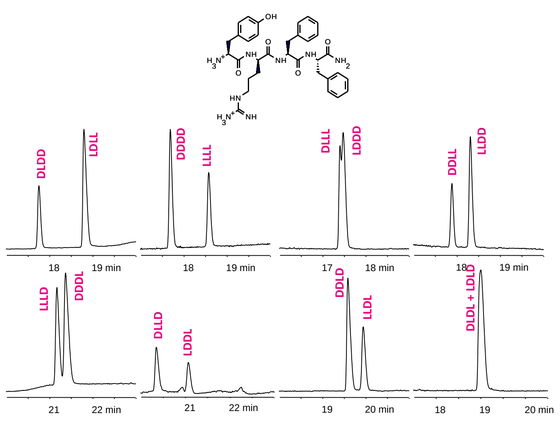


**Figure 5: Enantiomeric separation of the eight enantiomer pairs of tetrapeptide Tyr-Arg-Phe-Phe-$NH_2$. BGE consisted of 100 mM phosphoric acid, 88 mM triethanolamine (pH 3.0) and 10 mM heptakis(2,6-di-O-methyl)-β-cyclodextrin [3].**

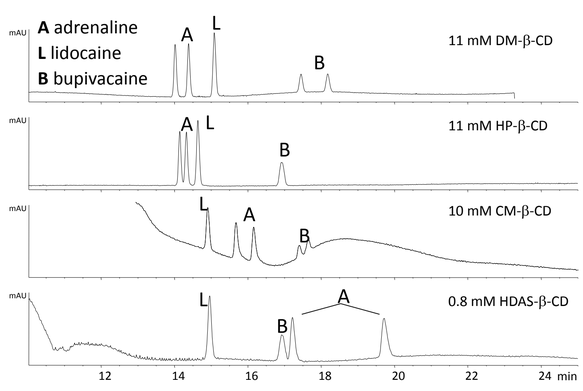


**Figure 6: Selection of cyclodextrin for the enantiomeric separation of adrenaline in local anaesthetic solutions for injection. DM-β-CD heptakis(2,6-di-O-methyl)-β-cyclodextrin; HP-β-CD hydroxypropyl-β-cyclodextrin; CM-β-CD Carboxymethyl-β-cyclodextrin; HDAS-β-CD heptakis-(2,3-diacetyl-6-sulphato)-β-cyclodextrin [4].**

## *Introduction*

*Capillary Electrophoresis (CE) separates compounds that differ in charge to hydrodynamic size ratio and is an excellent technique for the analysis of polar compounds. The technique is specifically successful for chiral separations. Chiral CE separation is achieved by adding a chiral selector to the so called background electrolyte. The enantiomers then form fast, reversible equilibria with the selector. In this poster a simple method development strategy for basic, acidic and neutral compounds is presented and illustrated with examples and common pitfalls.*

## Why chiral CE?

- Highly efficient separations at a reasonable cost
  - Screen multiple chiral selectors to a much lower cost compared to the cost for the purchase of one or more chiral columns
  - Low solvent and solute consumption
- The high efficiency in CE means that a small mobility difference between enantiomers can be sufficient for separation
- Rapid screening of optimal conditions since the enantiomeric separation is generally faster in CE and there is no need for a long equilibration time when switching to a different chiral BGE

## What is chiral CE?

Chiral CE separation is achieved by adding a chiral selector to the BGE (Figure 1). The enantiomers form fast, reversible equilibria with the selector. This means that part of the time an enantiomer migrates free in the BGE, and part of the time it migrates as an enantiomer-selector complex. The apparent mobilities of the enantiomers will change depending on the strength of the complex (i.e. the equilibrium constants) and the mobility of the enantiomer-selector complex.

## Requirements

- Either the enantiomers or the selector have to be charged
- The enantiomers of a chiral molecule need to have different affinities for the chiral selector, and/or the enantiomer-selector complexes need to differ in mobilities

## Method Development

- Starting point: Figure 2
- Basic analytes
  - Relatively simple, charged or uncharged selectors
  - Separation window large (Figure 3)
- Acidic analytes
  - With neutral selectors, the separation window is small
  - Use charged selectors or CD-MEKC (Figure 4)
- Neutral analytes
  - Charged selectors or CD-MEKC
- Optimization
  - Chiral selector concentration
  - pH, temperature, BGE composition, EOF
  - Multifactorial design
- Pitfalls chiral selector screen
  - Hard to predict proper chiral selector choice (Figure 5)
  - Many prejudices
  - Too few screened
  - Highest resolution in screen not necessarily best method situation

## Good working practice

- Describe electrolyte unambiguously: exact concentrations, appropriate volumetric glassware
- Sufficient buffering capacity
- Optimise and control temperature
- Current not too high
- Use corrected peak areas for quantitation
- Good injection practice:
  - Samples similar composition as standards
  - Samples temperature equilibrated
  - Inject at least for 3 s for better repeatability
  - Dip capillary inlet in clean vial after sample injection to reduce carry-over
  - Inject BGE plug after sample injection for better repeatability
  - Ramp application of voltage

## References

- CE Sänger – van de Griend, Y Hedeland, C Pettersson, Chromatography Today May/June 2013, 32 – 37
- CE Solutions, via www.kantisto.nl or www.sepscience.com

[1] Enantiomeric separations by capillary electrophoresis in pharmaceutical analysis, by C.E. Sänger – van de Griend, Uppsala University Library, 1999, ISBN 91-554-4561-6
[2] C.E. Sänger – van de Griend, K. Gröningsson, D. Westerlund, Chromatographia 42 (1996) 263
[3] C.E. Sänger – van de Griend, K. Gröningsson, T. Arvidsson, J. Chromatogr. A 782 (1997) 271
[4] C.E. Sänger – van de Griend, A.G. Ek, M.E. Widahl-Näsman, E.K.M. Andersson, J. Pharm. Biomed. Anal. 41 (2006) 77