Mathematical approach by a selectivity model for the understanding of pH- and selector concentration-dependent reversal of the enantiomer migration order in capillary electrophoresis

Manuela Hammitzsch-Wiedemann, Gerhard K. E. Scriba

Friedrich Schiller University Jena, Department of Pharmaceutical Chemistry,
Philosophenweg 14, 07743 Jena, Germany

The Supporting Information contains the following additional Tables and Figures:

1. Table S-1A summarizing the systematic classification of the separation selectivity with regard to EMO in the area around the four corner points of pH and [CD] extremes for cases with equal mobilities of the diastereomeric analyte-selector complexes.
2. Table S-1B summarizing the systematic classification of the separation selectivity with regard to EMO in the area around the four corner points of pH and [CD] extremes for cases with different mobilities of the diastereomeric analyte-selector complexes.
3. Table S-2 summarizing mobilities and complexation constants used to construct the selectivity-pH-selector concentration plots shown in Figure S-1.
4. Figure S-1 showing the contour plots of the separation selectivity and peak resolution as a function of buffer pH and selector concentration for the 15 cases based on the complexation and mobility parameters listed in Table S-2.
5. Figure S-2 showing representative electropherograms of enantioseparations of Ala-Tyr at pH 2.2 and 3.8 as well as Asp-PheOMe at pH 2.2 and 3.0 at low and high concentrations of DM- β -CD.

The tables summarize a systematic classification with regard to EMO in the area around the four corner points defined by the extremes of pH and [CD]. Separation selectivity is derived from equations (17) to (20) as indicated by the respective mathematical expressions. While S is directly obtained from equations (17) and (19), equations (18) and (20) describe the first derivative $\delta S/\delta[CD]$ at $[CD] = 0$. $\delta S/\delta[CD] = 0$ refers to $S = 1$, $\delta S/\delta[CD] < 0$ refers to $S < 1$, and $\delta S/\delta[CD] > 0$ refers to $S > 1$. As sufficient for definition only the numerator of the terms derived from equations (18) and (20) is shown. A pH-dependent and [CD]-dependent reversal of EMO is indicated by a plus.

Table S-1A. Cases with equal mobilities of the diastereomeric analyte-selector complexes ($\mu_{HB^+CD1} = \mu_{HB^+CD2}$).

Case	Constellation of mobility and complexation parameters as well as resulting separation selectivity								EMO reversal			
	Low pH				High pH				pH-dependent		[CD]-dependent	
	High [CD] eq (17)	Low [CD] eq (18)			High [CD] eq (19)	Low [CD] eq (20)			High [CD] eq (17) → (19)	Low [CD] eq (18) → (20)	Low pH eq (18) → (17)	High pH eq (20) → (19)
I	$\frac{\mu_{HB^+CD1}}{\mu_{HB^+CD2}} = 1$	S = 1	$\mu_{HB^+} \cdot (-K_{+1} + K_{+2}) + \mu_{HB^+CD1} \cdot K_{+1} - \mu_{HB^+CD2} \cdot K_{+2} = 0$	S = 1	$\frac{\mu_{HB^+CD1}}{\mu_{HB^+CD2}} \cdot \frac{K_{+1} \cdot K_{n2}}{K_{+2} \cdot K_{n1}} < 1$	S < 1	$\mu_{HB^+} \cdot (-K_{n1} + K_{n2}) + \mu_{HB^+CD1} \cdot K_{+1} - \mu_{HB^+CD2} \cdot K_{+2} < 0$	S < 1	–	–	–	–
II									–	–	–	–
III									–	+	–	–
IV									–	–	–	–
V									–	+	–	+
VI									–	–	–	+
VII									–	+	–	–

Table S-1B. Cases with different mobilities of the diastereomeric analyte-selector complexes ($\mu_{\text{HB}^+\text{CD1}} \neq \mu_{\text{HB}^+\text{CD2}}$).

Case	Constellation of mobility and complexation parameters as well as resulting separation selectivity							EMO reversal			
	Low pH			High pH				pH-dependent		[CD]-dependent	
	High [CD] eq (17)		Low [CD] eq (18)	High [CD] eq (19)		Low [CD] eq (20)		High [CD] eq (17) → (19)	Low [CD] eq (18) → (20)	Low pH eq (18) → (17)	High pH eq (20) → (19)
VIII	$\frac{\mu_{\text{HB}^+\text{CD1}}}{\mu_{\text{HB}^+\text{CD2}}} < 1$	$S < 1$	$\mu_{\text{HB}^+} \cdot (-K_{+1} + K_{+2}) + \mu_{\text{HB}^+\text{CD1}} \cdot K_{+1} - \mu_{\text{HB}^+\text{CD2}} \cdot K_{+2} \leq 0$	$S \leq 1$	$\frac{\mu_{\text{HB}^+\text{CD1}}}{\mu_{\text{HB}^+\text{CD2}}} \cdot \frac{K_{+1} \cdot K_{n2}}{K_{+2} \cdot K_{n1}} \leq 1$	$\mu_{\text{HB}^+} \cdot (-K_{n1} + K_{n2}) + \mu_{\text{HB}^+\text{CD1}} \cdot K_{+1} - \mu_{\text{HB}^+\text{CD2}} \cdot K_{+2} \leq 0$	$S \leq 1$	–	–	–	–
IX					$\mu_{\text{HB}^+} \cdot (-K_{n1} + K_{n2}) + \mu_{\text{HB}^+\text{CD1}} \cdot K_{+1} - \mu_{\text{HB}^+\text{CD2}} \cdot K_{+2} > 0$	$S > 1$	–	+	–	+	
X					$\frac{\mu_{\text{HB}^+\text{CD1}}}{\mu_{\text{HB}^+\text{CD2}}} \cdot \frac{K_{+1} \cdot K_{n2}}{K_{+2} \cdot K_{n1}} > 1$	$\mu_{\text{HB}^+} \cdot (-K_{n1} + K_{n2}) + \mu_{\text{HB}^+\text{CD1}} \cdot K_{+1} - \mu_{\text{HB}^+\text{CD2}} \cdot K_{+2} \leq 0$	$S \leq 1$	+	–	–	+
XI						$\mu_{\text{HB}^+} \cdot (-K_{n1} + K_{n2}) + \mu_{\text{HB}^+\text{CD1}} \cdot K_{+1} - \mu_{\text{HB}^+\text{CD2}} \cdot K_{+2} > 0$	$S > 1$	+	+	–	–
XII			$\mu_{\text{HB}^+} \cdot (-K_{+1} + K_{+2}) + \mu_{\text{HB}^+\text{CD1}} \cdot K_{+1} - \mu_{\text{HB}^+\text{CD2}} \cdot K_{+2} > 0$	$S > 1$	$\frac{\mu_{\text{HB}^+\text{CD1}}}{\mu_{\text{HB}^+\text{CD2}}} \cdot \frac{K_{+1} \cdot K_{n2}}{K_{+2} \cdot K_{n1}} \leq 1$	$\mu_{\text{HB}^+} \cdot (-K_{n1} + K_{n2}) + \mu_{\text{HB}^+\text{CD1}} \cdot K_{+1} - \mu_{\text{HB}^+\text{CD2}} \cdot K_{+2} \leq 0$	$S \leq 1$	–	+	+	–
XIII						$\mu_{\text{HB}^+} \cdot (-K_{n1} + K_{n2}) + \mu_{\text{HB}^+\text{CD1}} \cdot K_{+1} - \mu_{\text{HB}^+\text{CD2}} \cdot K_{+2} > 0$	$S > 1$	–	–	+	+
XIV					$\frac{\mu_{\text{HB}^+\text{CD1}}}{\mu_{\text{HB}^+\text{CD2}}} \cdot \frac{K_{+1} \cdot K_{n2}}{K_{+2} \cdot K_{n1}} > 1$	$\mu_{\text{HB}^+} \cdot (-K_{n1} + K_{n2}) + \mu_{\text{HB}^+\text{CD1}} \cdot K_{+1} - \mu_{\text{HB}^+\text{CD2}} \cdot K_{+2} \leq 0$	$S \leq 1$	+	+	+	+
XV						$\mu_{\text{HB}^+} \cdot (-K_{n1} + K_{n2}) + \mu_{\text{HB}^+\text{CD1}} \cdot K_{+1} - \mu_{\text{HB}^+\text{CD2}} \cdot K_{+2} > 0$	$S > 1$	+	–	+	–

Table S-2. Model parameters used for the construction of the contour plots shown in Figure S-1. Further parameters used for the calculations: $\mu_{\text{HB}^+} = 18.0 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1} \text{ V}^{-1}$ and $\text{pK}_{\text{a}} = 4.0$. The pK_{a} values of the complexes, $\text{pK}_{\text{a/c1}}$ and $\text{pK}_{\text{a/c2}}$, were calculated from the respective parameters. All values were chosen in such a way to allow a clear illustration of the cases.

Case	$\mu_{\text{HB}+\text{CD1}}$ [$10^{-9} \text{ m}^2 \text{ s}^{-1} \text{ V}^{-1}$]	$\mu_{\text{HB}+\text{CD2}}$ [$10^{-9} \text{ m}^2 \text{ s}^{-1} \text{ V}^{-1}$]	K_{+1} [L/mol]	K_{+2} [L/mol]	K_{n1} [L/mol]	K_{n2} [L/mol]	$\text{pK}_{\text{a/c1}}$	$\text{pK}_{\text{a/c2}}$
I	8.0	8.0	175	175	190	160	3.96	4.04
II	8.0	8.0	190	160	171	144	4.05	4.05
III	8.0	8.0	190	160	19	16	5.00	5.00
IV	8.0	8.0	190	160	210	140	3.96	4.06
V	8.0	8.0	190	160	10.5	7	5.26	5.36
VI	8.0	8.0	190	160	1850	1650	3.01	2.99
VII	8.0	8.0	190	160	18	17	5.02	4.97
VIII	8.0	8.5	190	160	171	144	4.05	4.05
IX	8.0	8.5	190	195	1900	2025	3.00	2.98
X	8.0	8.5	190	160	1900	1800	3.00	2.95
XI	8.0	8.5	190	160	80	100	4.38	4.20
XII	8.0	8.5	160	190	16	19	5.00	5.00
XIII	8.0	8.5	160	190	144	171	4.05	4.05
XIV	8.0	8.5	160	190	14	21	5.06	4.96
XV	8.0	8.5	160	190	150	200	4.03	3.98

Case I **Case II** **Case III** **Case IV** **Case V** **Case VI** **Case VII** **Case VIII** **Case IX**

Figure S-1. Contour plots of the enantiomeric selectivity, S , and the peak resolution, R_S , as a function of pH and selector concentration in the absence of an EOF using the mobility and complexation parameters listed in Table S-2. S was calculated according to equation (8), R_S was calculated according to Rawjee and Vigh (*Anal. Chem.* **1994**, 66, 619-627) using the operator dependent parameters: voltage $U = 20$ kV, effective and total length of the capillary $l_{\text{eff}} = 40$ cm and $l_{\text{tot}} = 50$ cm, temperature $T = 293.15$ K. White zones $S = 1$ and $R_S = 0$, dark zones $S \neq 1$ and $R_S \neq 0$, respectively, the darker the color, the higher S or R_S .

Ala-Tyr

Asp-PheOMe

Figure S-2. Representative electropherograms of separations of the DD- and LL-enantiomers of Ala-Tyr and Asp-PheOMe using DM- β -CD as chiral selector. Experimental conditions: 40/50.2 cm fused-silica capillary, 50 μ m ID; electrolyte solutions: pH 2.2: 60 mM sodium phosphate buffer; pH 3.0: 40 mM sodium phosphate buffer; pH 3.8; 30 mM sodium aspartate buffer; 20°C, 25 kV.