

Key Distance Learning Module IV: Separation of Anionic Compounds Using Micelle Electrokinetic Capillary Chromatography

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Materials

The buffer, 3-[cyclohexylamino]-1-propanesulfonic acid (CAPs), dimethylformamide, n-decanophenone, indoprofen, sulindac, and indomethacin may be replaced. Ideally, the lab should include a series of charged analytes of similar structure, but different hydrophobicity, a neutral marker, and a micelle marker to allow the students to use critical thinking and laboratory skills to determine capacity factor. We selected CAPs because it buffers at pH 10 and provides a low background current. You may choose other systems, for example other charged nonsteroidal anti-inflammatory drug. Before asking students to use different chemicals, we recommend you perform the experiments outlined in Learning Module IV with your substitute set. If you replace the sodium dodecyl sulfate with another surfactant, or change the pH of the running buffer, you may need to optimize the separation parameters to obtain an acceptable migration time.

Procedures: The Module

1. Outline the experiments you must do to determine the retention factor (capacity factor) for 3 NSAIDs. To determine the retention factor (k') for 3 NSAIDs, several steps must be followed. First, the free-zone migration times for the NSAIDs and a neutral marker (neutral, hydrophilic compound such as dimethylformamide) must be determined in replicate runs. Next, the MEKC migration times for the NSAIDs, a neutral marker, and a micelle marker (neutral, highly hydrophobic compound such as n-decanophenone) must be determined in replicate runs. All migration times should be determined using the same bare-fused silica capillary, and the length to the detection window (L_w), the total length (L_t), and separation voltage (V_{sep}) must be known. The run buffer should be the same concentration with respect to the buffering species (CAPs) and pH for both the free-zone and MEKC trials. From the free-zone runs, you should be able to determine the free-zone electrophoretic mobility for each NSAID (μ_{eph_NSAID}) using the following equation:

$$\mu_{apparent_NSAID(CE)} = [L_w \times L_t] / (t_{NSAID(CE)} \times V_{sep})$$

$$\mu_{eof\ free\ zone} = [L_w \times L_t] / (t_{eof\ marker_DMF} \times V_{sep})$$

$$\mu_{eph_NSAID} = \mu_{apparent_NSAID(CE)} - \mu_{eof\ free\ zone}$$

From the MEKC runs, the electroosmotic mobility for each trial (μ_{eof_MEKC}) is determined using the following equation:

$$\mu_{eof_MEKC} = [L_w \times L_t] / (t_{eof\ marker(MEKC)} \times V_{sep})$$

By combining these two terms, along with the migration times of the eof marker (t_{eof_MEKC}), the NSAID (t_{NSAID_MEKC}), and the micelle marker (t_{n-dec_MEKC}), all of which are obtained from the MEKC trials, the retention factor (k') is calculated for each NSAID using the equation below.

$$k' = \frac{t_{NSAID_MEKC} \left(1 + \frac{\mu_{eph_NSAID}}{\mu_{eof_MEKC}} \right) - t_{eof_MEKC}}{t_{eof_MEKC} \left(1 - \frac{t_{NSAID_MEKC}}{t_{n-dec_MEKC}} \right)}$$

t_{NSAID_MEKC}	=	MEKC migration time NSAID
μ_{eph_NSAID}	=	calculated CE electrophoretic mobility of NSAID
μ_{eof_MEKC}	=	MEKC electroosmotic mobility (DMF)
t_{eof_MEKC}	=	MEKC migration time DMF
t_{n-dec_MEKC}	=	MEKC migration time n-decanophenone

The retention factor for each NSAID should be determined separately for each run. The mean value for k' for each NSAID should be reported as the mean value for replicate runs. It is important to note that under optimized conditions, all three NSAIDs can be separated during a single MEKC trial, giving individual migration times for each NSAID. However, for the free-zone trials, each NSAID must be analyzed separately because of the similarity in migration times under free-zone conditions.

- Determine the retention factor (capacity factor) for indoprofen, sulindac, indomethacin. These values should be reported as mean values determined from replicate runs. For the MEKC runs, the running buffer is 25 mM CAPs, 100 mM SDS buffered to pH 10. For the capillary electrophoresis runs you will perform, the running buffer is 25 mM CAPs, buffered to pH 10. You should use a ~25 micron inner diameter fused silica capillary ~42 cm in total length, ~32 cm to the window, 20,000 V.

Table 4.1. Free Zone Data

$L_w = 31.2$ cm, $L_t = 41.2$ cm, $V_{sep} = 20,000$ V, pH 10 CAPS

	TRIAL 1	TRIAL 2	TRIAL 3	
Indoprofen migration time (s)	100.4 ₄	100.2 ₉	100.0 ₁	
μ app ($\text{cm}^2\text{V}^{-1}\text{s}^{-1} \times 10^{-4}$)	6.39 ₉	6.40 ₉	6.42 ₇	
DMF migration time (s)	76.7 ₅	76.7 ₅	76.7 ₅	
μ eof ($\text{cm}^2\text{V}^{-1}\text{s}^{-1} \times 10^{-4}$)	8.37 ₄	8.37 ₄	8.37 ₄	
Sulindac migration time (s)	97.0 ₉	97.0 ₈	97.0 ₈	
μ app ($\text{cm}^2\text{V}^{-1}\text{s}^{-1} \times 10^{-4}$)	6.62 ₀	6.62 ₀	6.62 ₀	
DMF migration time (s)	77.1 ₉	76.7 ₅	77.1 ₈	
μ eof ($\text{cm}^2\text{V}^{-1}\text{s}^{-1} \times 10^{-4}$)	8.32 ₆	8.37 ₄	8.32 ₇	
Indomethacin migration time (s)	97.0 ₂	97.0 ₂	97.0 ₉	
μ app ($\text{cm}^2\text{V}^{-1}\text{s}^{-1} \times 10^{-4}$)	6.62 ₅	6.62 ₄	6.62 ₀	
DMF migration time (s)	77.7 ₅	77.7 ₅	76.7 ₅	
μ eof ($\text{cm}^2\text{V}^{-1}\text{s}^{-1} \times 10^{-4}$)	8.26 ₆	8.26 ₆	8.37 ₅	
μ eph $\text{cm}^2\text{V}^{-1}\text{s}^{-1} (\times 10^{-4})$				AVE \pm SD
Indoprofen	-1.97 ₅	-1.96 ₅	-1.94 ₇	-1.96 ₃ \pm 0.01
Sulindac	-1.70 ₇	-1.75 ₄	-1.70 ₇	-1.72 ₃ \pm 0.03
Indomethacin	-1.64 ₁	-1.64 ₂	-1.75 ₄	-1.67 ₉ \pm 0.07

Table 4.2a. MEKC DATA (100 mM SDS)
 $L_w = 31.2$ cm, $L_t = 41.2$ cm, $V_{sep} = 20,000$ V, pH 10 CAPS 100 mM SDS

	Migration Time						
	Trial						
Marker	1	2	3	4	5	6	
DMF	110.7 ₅	109.0 ₈	107.1 ₆	107.1 ₄	109.0 ₆	109.1 ₂	
n-dec	303.2 ₇	298.8 ₁	296.5 ₃	298.8 ₁	298.8 ₁	300.9 ₁	
MEKC μ eof $\text{cm}^2\text{V}^{-1}\text{s}^{-1}$ ($\times 10^{-4}$)	5.80 ₃	5.89 ₂	5.99 ₈	5.99 ₉	5.89 ₃	5.89 ₀	
	Migration Time						
	Trial						
Analyte	1	2	3	4	5	6	
Indoprofen	180.0 ₉	177.2 ₇	175.2 ₃	176.0 ₂	177.2 ₄	178.0 ₃	
Sulindac	214.7 ₉	209.9 ₃	208.0 ₀	209.2 ₁	211.1 ₄	211.4 ₇	
Indomethacin	258.7 ₂	251.8 ₇	250.5 ₉	252.9 ₄	252.1 ₂	255.2 ₁	
	k'						
Analyte	1	2	3	4	5	6	AVE \pm SD
Indoprofen	0.1 ₉	0.2 ₁	0.2 ₄	0.2 ₆	0.2 ₁	0.2 ₂	0.2 \pm 0.0 ₃
Sulindac	1.2 ₅	1.2 ₂	1.2 ₈	1.3 ₁	1.2 ₆	1.2 ₅	1.3 \pm 0.0 ₃
Indomethacin	4.4 ₉	4.1 ₄	4.4 ₁	4.5 ₆	4.1 ₈	4.4 ₃	4.4 \pm 0.2

Below is data collected for running buffer comprised of 150 mM SDS rather than 100 mM. Note how the retention factor changes as the amount of surfactant increases. The increase is understandable if you think of how the total number of moles of analyte will change as the moles of surfactant (or micelles) changes. Using this information you might devise a new learning module where you compare the effects of surfactant concentration.

Table 4.2b. MEKC DATA (150 mM SDS)									
$L_w = 31.2$ cm, $L_t = 41.2$ cm, $V_{sep} = 20,000$ V, pH 10 CAPS 150 mM SDS									
	Migration Time								
	Trial								
Marker	1	2	3	4	5	6	7		
DMF	116.4 ₉	114.2 ₆	116.4 ₉	117.7 ₉	117. ₈	116.4 ₉	116.4 ₉		
n-dec	359.6 ₇	357.8 ₁	359.6 ₇	359.6 ₇	358.8 ₃	358.1 ₇	359.6 ₇		
MEKC μ eof $\text{cm}^2\text{V}^{-1}\text{s}^{-1}$ ($\times 10^{-4}$)									
	5.51 ₇	5.62 ₅	5.51 ₇	5.51 ₇	5.45 ₆	5.51 ₇	5.51 ₇		
	Migration Time								
	Trial								
Analyte	1	2	3	4	5	6	7		
Indoprofen	219.7 ₄	217.1 ₉	218.7 ₂	218.7 ₉	218.7 ₃	217.1 ₉	217.1 ₉		
Sulindac	268.9 ₂	266.0 ₈	267.8 ₀	267.8 ₅	266.0 ₇	266.0 ₅	266.0 ₈		
Indomethacin	318.0 ₈	314.5 ₆	317.8 ₁	316.0 ₇	316.0 ₆	313.9 ₅	316.0 ₇		
	k'							AVE	
Analyte	1	2	3	4	5	6	7		
Indoprofen	0.55 ₄	0.60 ₅	0.53 ₅	0.53 ₆	0.48 ₄	0.51 ₁	0.50 ₈	0.53 \pm 0.04	
Sulindac	2.3 ₃	2.4 ₀	2.2 ₈	2.2 ₈	2.1 ₁	2.2 ₂	2.1 ₉	2.3 \pm 0.0 ₉	
Indomethacin	7.78 ₀	7.70 ₅	7.71 ₆	7.32 ₃	7.19 ₆	7.08 ₇	7.32 ₃	7.4 \pm 0.3	