Supporting Material for

Micelle Stacking in Micellar Electrokinetic Chromatography

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I. Evidence of micelle stacking

Figure 1 shows the effect of Sudan III inclusion in the borate-cholate BGE under conditions with and without micelle stacking. Trace A is a control experiment where the BGE contains no Sudan III and the sample matrix and BGE are identical (10 mM $Na_2B_4O_7$, 80 mM sodium cholate). This electropherogram is effectively the trace expected if no injection occurred. In trace B, the BGE and sample matrix are the same as A, with the exception that Sudan III has been added to the BGE. As the sample zone migrates past the detector, the absorbance decreases due to the absence of dye in the sample. In trace C, the BGE is the same as that in A (10 mM $Na_2B_4O_7$, 80 mM sodium cholate), while the sample matrix is 25 mM $Na_2B_4O_7$. These conditions closely approximate those for sweeping conditions as described by Quirino and Terabe, where the conductivity of the sample matrix matches that of the BGE.[1, 2] Two peaks are observed in this trace. The first peak is attributed to EOF; the second is a system peak attributed to a difference between the sample matrix and BGE. In trace D, the BGE is the same as that in B, while the sample matrix is the same as that in C, 25 mM $Na_2B_4O_7$. Again, two peaks are observed; however, the second peak is now attributed to stacked cholate micelles. It is apparent that the system peak observed in C does not contribute to the signal associated with the stacked micelle band. However, it can also be seen from the slope of the vacancy peak following the stacked micelle band that the system peak does contribute to the signal in the absence of dye.

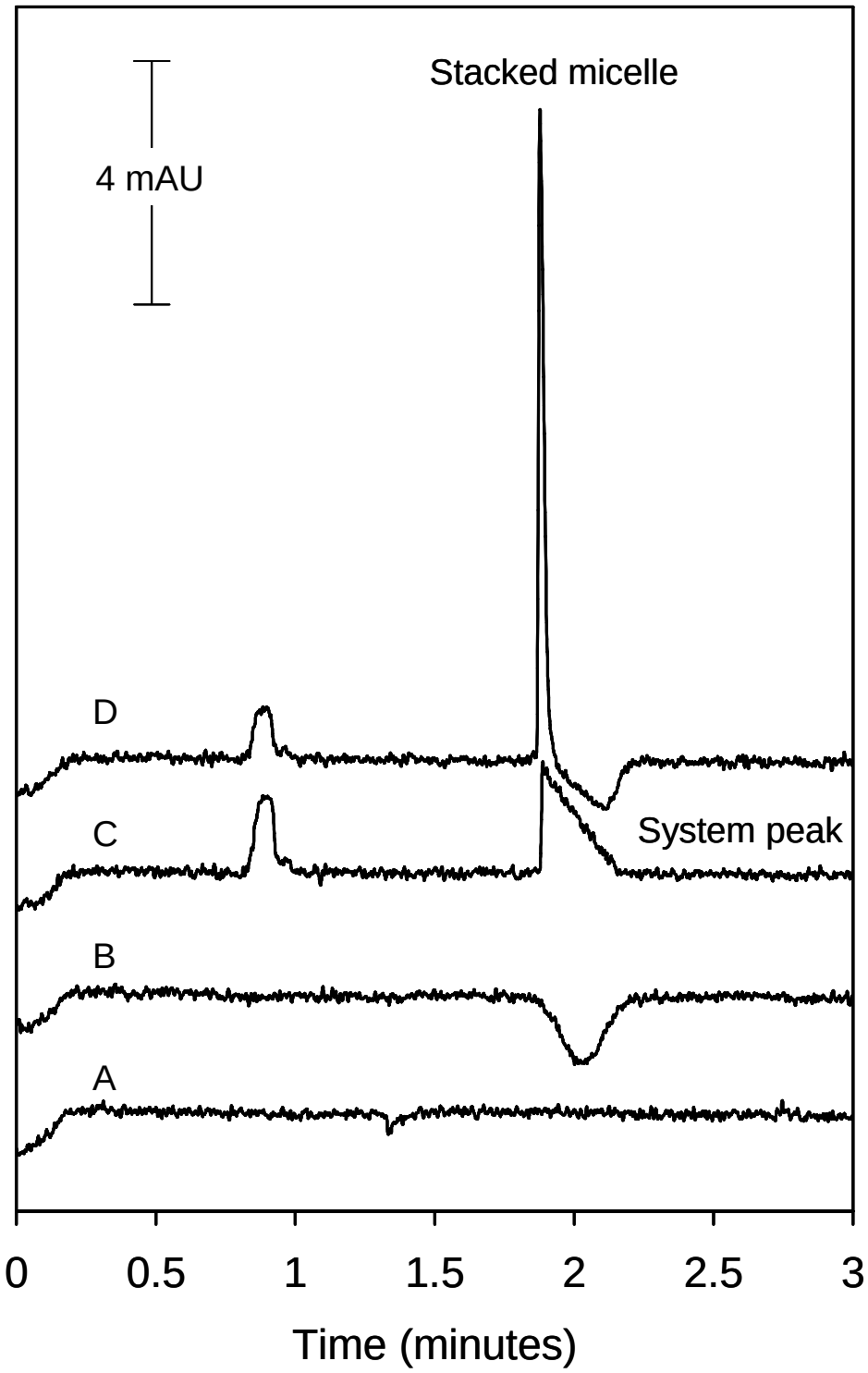

Figure 1. Evidence of micelle stacking. BGE: 10 mM $Na_2B_4O_7$, 80 mM cholate (A and C) and 10 mM $Na_2B_4O_7$, 80 mM cholate, 50 μM Sudan III (B and D). SM: 10 mM $Na_2B_4O_7$, 80 mM cholate (A and B) and 25 mM $Na_2B_4O_7$ (C and D). Experimental conditions: l_T = 60 cm (l_D = 5 cm), l_{inj} = 0.5 cm, V = 20 kV (37 μA).

II. Separation of alkaloids under conditions of maximum micelle stacking

Figure 2 shows the corresponding separations of alkaloids under conditions where the sample is prepared in each BGE as well as under the micelle stacking conditions identified in Table 1. Panels A and B show the separation of ten alkaloids in the borate-cholate and Tris-cholate BGE systems, respectively. Similarly, panels C and D show the separation of seven alkaloids in the borate-SDS and Tris-SDS BGE systems, respectively. The bottom trace in each panel shows the electropherogram obtained when the sample is prepared in each respective BGE, wherein, no micelle stacking occurs and the electric field through the capillary is homogeneous. The remaining traces in each panel show separations corresponding to sample prepared in $Na_2B_4O_7$, NaOAc, and NaCl sample matrices, bottom to top. It is apparent that no effective separation, resolving all analytes, is achieved in the 15 cm distance allotted for separations; however, all of the conditions which promote micelle stacking yield some extent of analyte preconcentration and separation.

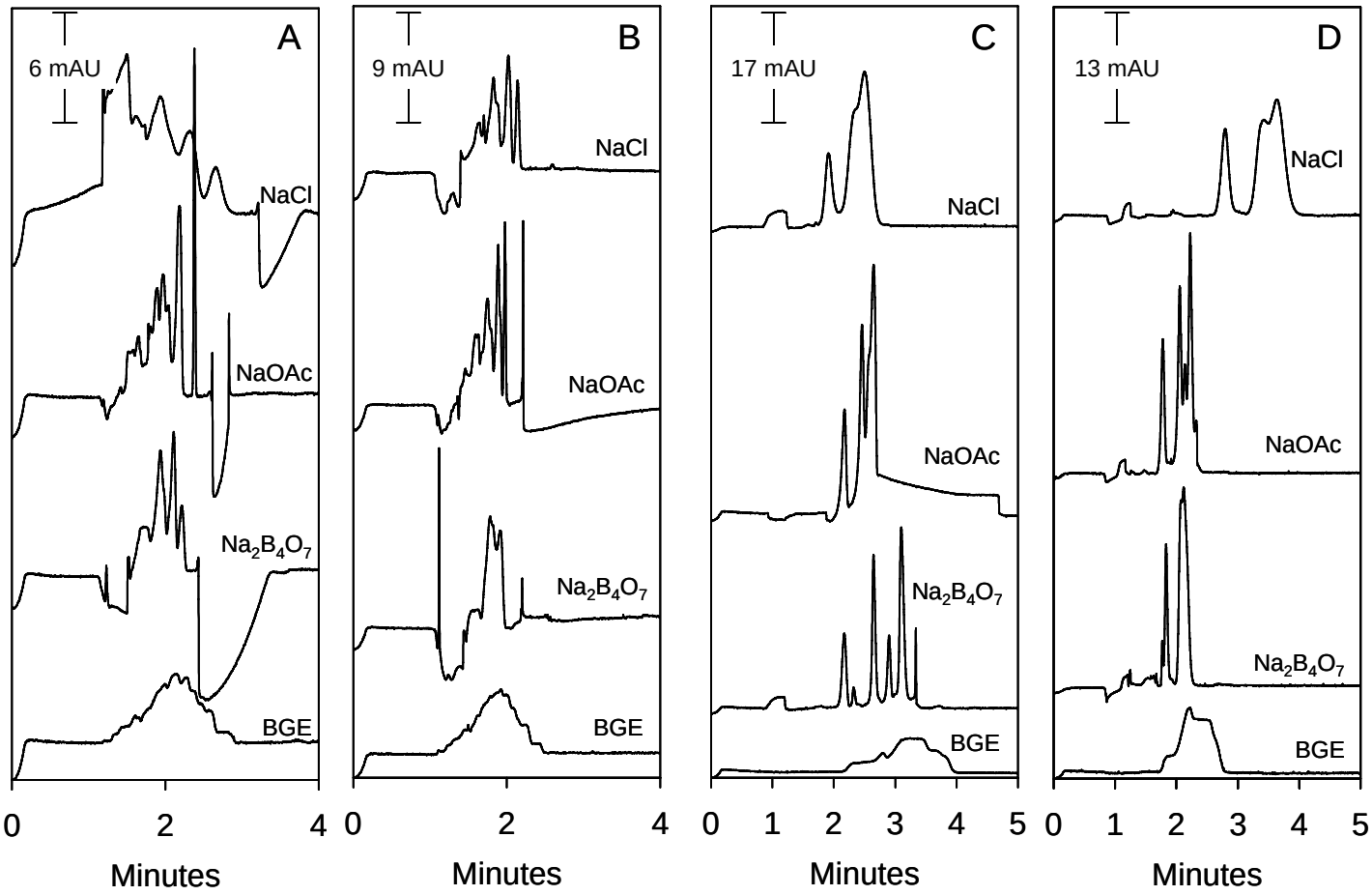

Figure 2. Effect of optimal micelle stacking condition on the separation of alkaloids. Panel A sample matrices (bottom to top): BGE = borate-cholate, 40 mM Na2B4O7, 100 mM NaOAc, and 300 mM NaCl. Panel B sample matrices (bottom to top): BGE = Tris-cholate, 11 mM Na2B4O7, 80 mM NaOAc, and 10 mM NaCl. Panel C sample matrices (bottom to top): BGE = borate-SDS, 20 mM Na2B4O7, 80 mM NaOAc, and 80 mM NaCl. Panel D sample matrices (bottom to top): BGE = Tris-SDS, 10 mM Na2B4O7, 10 mM NaOAc, and 15 mM NaCl. Experimental conditions: lT = 31 cm (lD = 15 cm), linj = 4.5 cm, V = 15 kV (56 μA). All samples prepared as indicated in the experimental section.

III. Micelle Stacking and the Separation of Alkaloids

Figure 3 shows the separation of ten alkaloids under conditions which promote micelle stacking with a NaOAc sample matrix at concentrations sufficient to achieve σ_{sample} / σ_{BGE} = 0.1, 0.5, 1, 1.5, and 2.0 relative to the borate-cholate BGE.. It is apparent from panel A that analyte enrichment and micelle stacking are achieved with each sample matrix and the effectiveness of analyte stacking improves with increasing σ_{sample} / σ_{BGE}. As shown for the $Na_2B_4O_7$ sample matrix, injected analyte separates from the stacked micelle region after less than 1 cm of separation distance. After 45 cm of separation distance (panel B), the stacked micelle region has slightly decreased in height for σ_{sample} / σ_{BGE} = 0.5, 1.0, 1.5 and 2.0. The best separation, as determined by peak symmetry and resolution of the limiting pair (yohimbine hydrochloride and thiocolchicine), occurs under conditions consistent with HSS (σ_{sample} / σ_{BGE} = 1.5).

Figure 4 shows the separation of ten alkaloids under conditions which promote micelle stacking with a NaCl sample matrix at concentrations sufficient to achieve σ_{sample} / σ_{BGE} = 0.1, 0.5, 1, 1.5, and 2.0 relative to the borate-cholate BGE. It is apparent from panel A that analyte enrichment and micelle stacking is achieved with each sample matrix and the effectiveness of analyte stacking improves with increasing σ_{sample} / σ_{BGE}. As was the case with the $Na_2B_4O_7$ and NaOAc sample matrices, injected analyte separates from the stacked micelle region after less than 1 cm of separation distance. After 45 cm of separation distance (panel B), the stacked micelle region has appreciably decreased in height for σ_{sample} / σ_{BGE} = 0.5, 1.0, 1.5 and 2.0. The best separation, as determined by peak symmetry and resolution of the limiting pair (yohimbine

hydrochloride and thiocolchicine), occurs under conditions consistent with HSS (σ_{sample} / σ_{BGE} = 2.0).

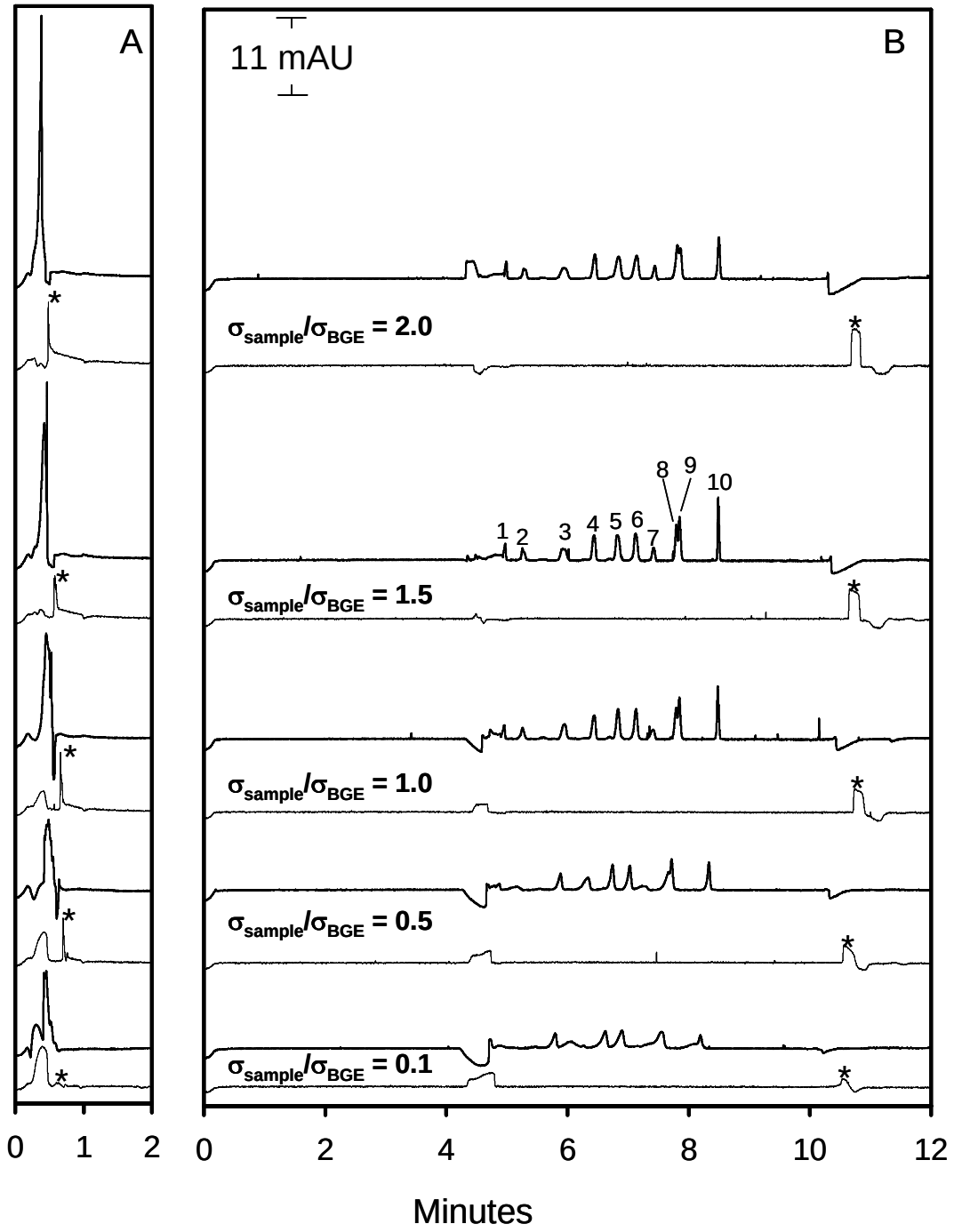

Figure 3. Effect of relative conductivity and separation distance on the separation of 10 alkaloids in NaOAc sample matrix. Experimental conditions: L_T = 60 cm, effective length = 1 cm (A) and 45 cm (B), l_{inj} = 20 mm, V = 25 kV, UV-absorbance at 254 nm, BGE = 10 mM $Na_2B_4O_7$, 80 mM cholate, sample matrix (bottom to top) = NaOAc at 0.1, 0.5, 1, 1.5 and 2.0 σ_{sample} / σ_{BGE}. All samples prepared as indicated in the experimental section. Separation of the alkaloids (dark trace); extent of micelle stacking (light trace) as determined by the presence of 50 μM Sudan III in the BGE.

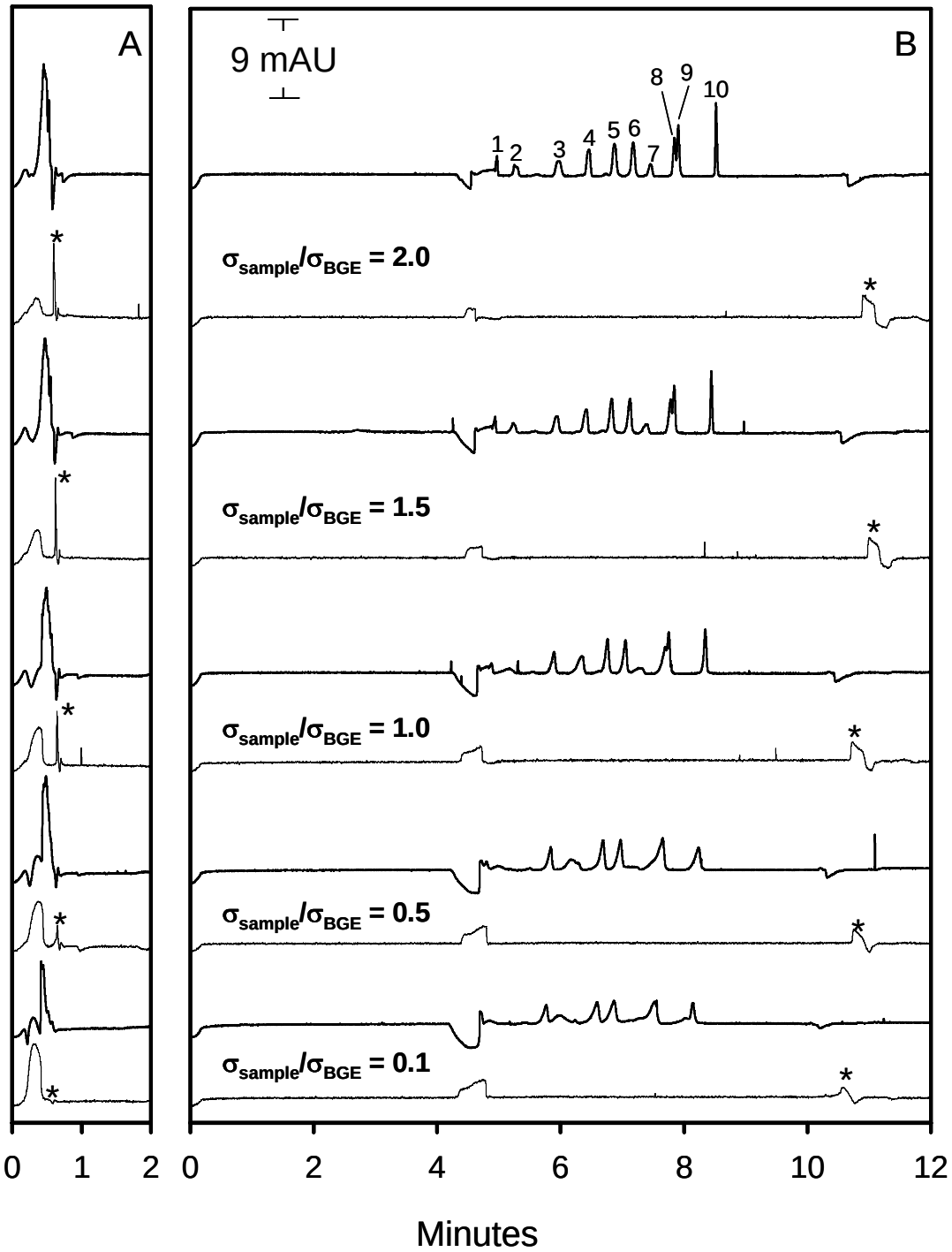

Figure 4. Effect of relative conductivity and separation distance on the separation of 10 alkaloids in NaCl sample matrix. Experimental conditions: LT = 60 cm, effective length = 1 cm (A) and 45 cm (B), linj = 20 mm, V = 25 kV, UV-absorbance at 254 nm, BGE = 10 mM Na2B4O7, 80 mM cholate, sample matrix (bottom to top) = NaCl at 0.1, 0.5, 1, 1.5 and 2.0 □sample / □BGE. All samples prepared as indicated in the experimental section. Separation of the alkaloids (dark trace); extent of micelle stacking (light trace) as determined by the presence of 50 □M Sudan III in the BGE.

IV. Relationship between l_{inj} and l_{sweep}

Terabe and coworkers have indicated that analyte preconcentration efficiency should follow $l_{sweep} = l_{inj} / (1+k)$ regardless of micelle stacking due to destacking via electrodispersion.[3, 4] Upon closer inspection this equation it is obvious that *k* is assumed constant regardless of whether micelle stacking or destacking occurs; and that a plot of l_{sweep} vs. l_{inj} should be linear with a slope of 1/(1+k). Therefore, analytes that are weakly retained by the micelle (smaller k) elute earlier and should demonstrate more positive slopes than analytes that are strongly retained by the micelle (larger k). Moreover, because is assumed constant, the slope of l_{sweep} vs. l_{inj} should also be positive and constant.

Figure 5 shows the effect of injection length (l_{inj}) on peak width (l_{sweep}) for colchicoside, yohimbine, and emetine. Panels A and B illustrate the trends for the borate-cholate BGE with optimized NaOAc and NaCl sample matrices, respectively. Similarly, panels C and D illustrate the trends for the Tris-cholate BGE with optimized NaOAc and NaCl sample matrices, respectively. For each sample matrix in the borate-cholate BGE it is apparent that, for colchicoside, a less positive slope is observed at shorter l_{inj} (< 4 cm) than at longer l_{inj} (> 4 cm). This indicates that as l_{inj} increases, colchicoside experiences a gradual increase in k that eventually stabilizes by l_{inj} = 4 cm. It is also evident in the borate-cholate BGE that yohimbine and emetine exhibit negative slopes at shorter l_{inj} (< 4 cm) and shallow, positive slopes at longer l_{inj} (> 4cm). Whereas the shallow slope at short l_{inj} for colchicoside indicates a gradual increase in k, the negative slopes for yohimbine and emetine indicate that these analytes experience more rapid increases in *k* at short l_{inj} values that eventually stabilize by l_{inj} = 4 cm.

Similarly, in the Tris-cholate BGE, colchicoside exhibits a less positive slope at shorter l_{inj} (< 2 cm) than at longer l_{inj} (> 2 cm), indicating a gradual increase in *k* that stabilizes more rapidly than in the borate-cholate BGE (~ 2 cm). For yohimbine and emetine in the Tris-cholate BGE, a similar trend as that for colchicoside is observed in the NaCl sample matrix; however, in the NaOAc sample matrix, a negligible slope is observed over the entire range of l_{inj}. This trend intimates that analytes experience *k* values that continue to increase monotonically with l_{inj}. These data illustrate that the time-dependence of the growing stacked micelle region is manifest in a separation via the extent of analyte enrichment. Moreover, it is demonstrated that the presumption of a constant *k* value is only reasonable with relatively large injection lengths, where analyte is afforded sufficient time to interact with the stacked micelle region to experience relatively stable *k* values.

Even though the extent of micelle stacking is significantly less in SDS-containing systems, the effect of injection length on *k* is no less realized. Figure 6 shows the effect of injection length (l_{inj}) on peak width (l_{sweep}) for colchicoside, thiocolchicoside, and strychnine. Panels A and B illustrate the trends for the borate-SDS BGE with optimized $Na_2B_4O_7$ and NaCl sample matrices; whereas panel C illustrates these trends with a $Na_2B_4O_7$ concentration such that $\sigma_{sample} / \sigma_{BGE} = 1.0$ (sweeping). Panels D – F illustrate the trends for the Tris-SDS BGE with optimized $Na_2B_4O_7$, NaOAc and NaCl sample matrices. Although the extent of micelle stacking is substantially less in SDS-containing BGE than in cholate-containing BGE, these data further demonstrate that the value of *k* experienced by individual analytes generally increases with short l_{inj} and tends to plateau after about 2 cm, regardless of electronic conditions (FAS, sweeping, or HSS).

It is interesting to note that in Figure 6A, initially a negative slope is observed for each analyte, followed by an increasing slope observed for colchicoside after l_{inj} = 1 cm, a zero slope observed for thiocolchicoside after l_{inj} = 2 cm, and a less negative slope observed for strychnine after l_{inj} = 3 cm. These data indicate that colchicoside experiences a relatively stable k value for $l_{inj} > 1$ cm, that thiocolchicoside experiences a monotonically increasing k value for $l_{inj} > 2$ cm, and that the k value experienced by strychnine is increasing less rapidly after $l_{inj} > 3$ cm, than at $l_{inj} < 3$ cm. Moreover, these trends illustrate the effect of retention order on the extent of analyte enrichment. Complimentary analyte-specific trends are observed in Figures 6B and 6E. In each of these figures, analytes that are weakly retained by the micelle will rapidly enter and exit the growing stacked micelle region, experiencing marginal benefit from the gradually increasing localized micelle concentration. However, analytes that are strongly retained by the micelle will experience greater enrichment from the growing stacked micelle region. Thus, the extent of analyte enrichment from micelle stacking is intrinsically linked to the extent of interaction with the micelle.

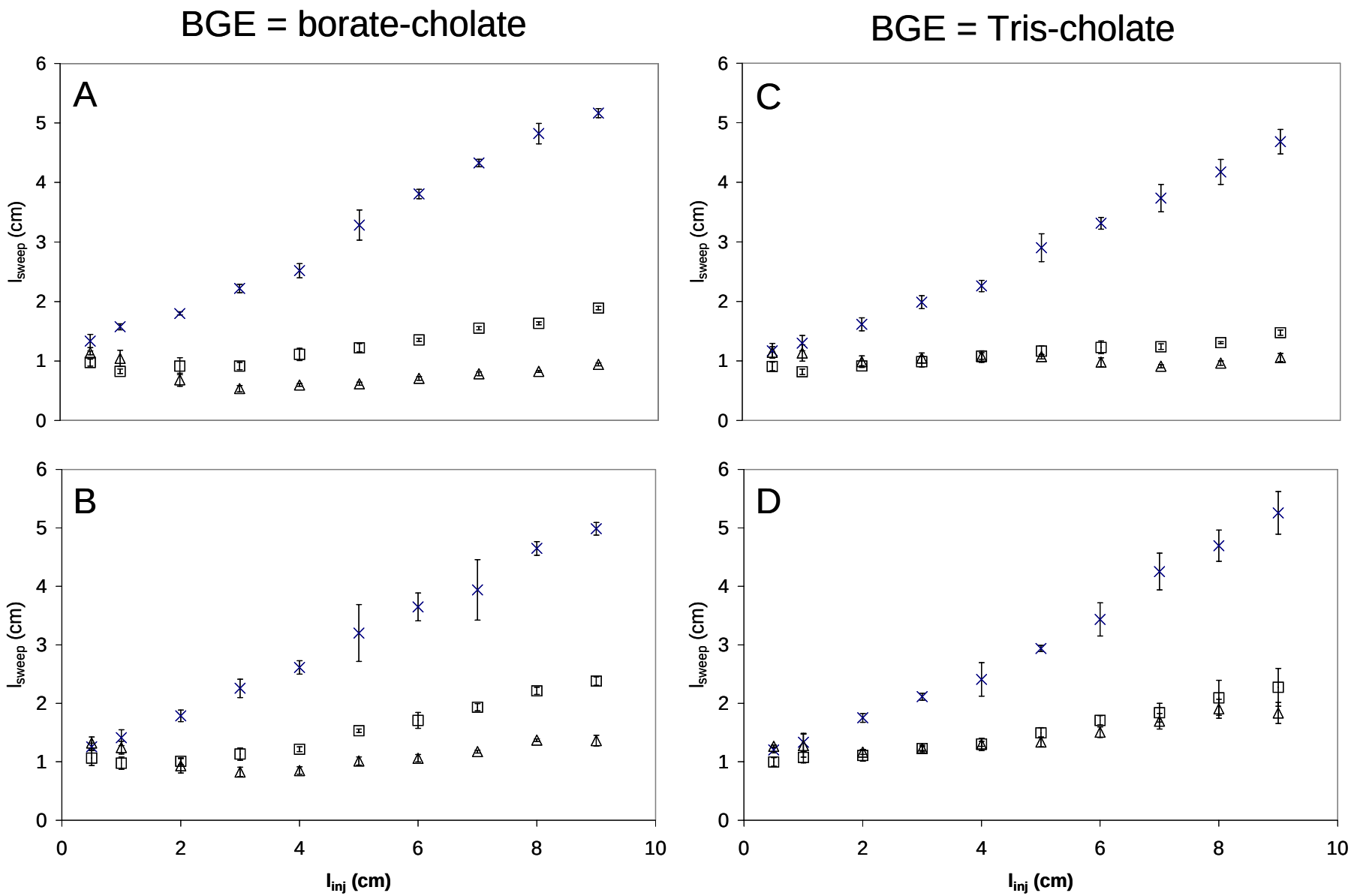

Figure 5. Effect of injection length (l_{inj}) and sample matrix on peak width (l_{sweep}) for colchicoside (×), emetine (□), and yohimbine (△) in borate-cholate and Tris-cholate BGE systems. Panel A: BGE = 10 mM $Na_2B_4O_7$, 80 mM cholate, SM = 100 mM NaOAc (σ_{sample} / σ_{BGE} = 1.6). Panel B: BGE = 10 mM $Na_2B_4O_7$, 80 mM cholate, SM = 90 mM NaCl (σ_{sample} / σ_{BGE} = 2.1). Panel C: BGE = 10 mM Tris, 80 mM cholate, SM = 80 mM NaOAc (σ_{sample} / σ_{BGE} = 1.4). Panel D: BGE = 10 mM Tris, 80 mM cholate, SM = 70 mM NaCl (σ_{sample} / σ_{BGE} = 4.1). All samples prepared as indicated in the experimental section. Experimental conditions: l_T = 60 cm, l_D = 45 cm, l_{inj} = 0.5 - 9.0 cm, V = 25 kV (56 μA).

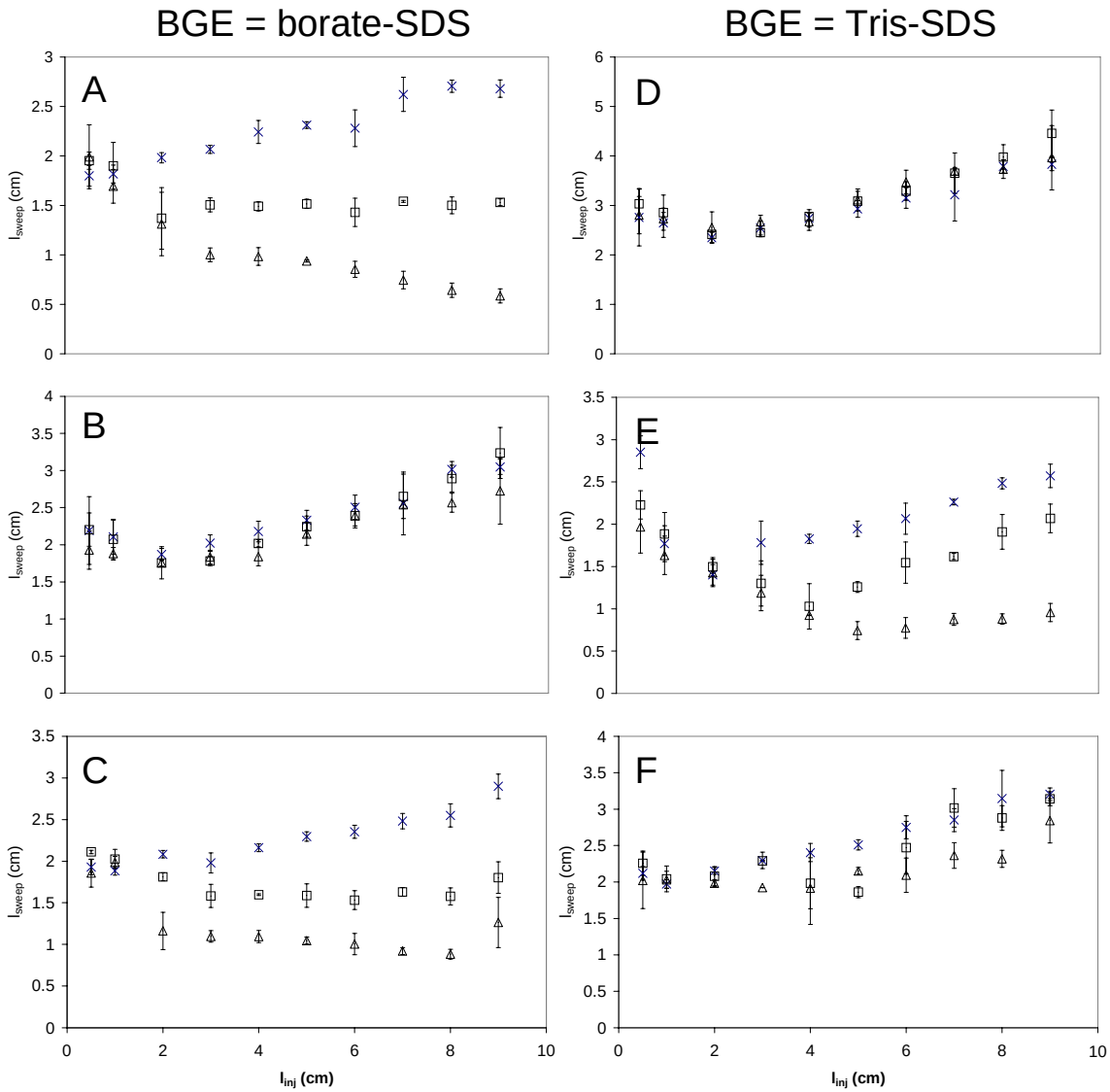

Figure 6. Effect of injection length (l_{inj}) and sample matrix on peak width (l_{sweep}) for colchicoside (×), thiocolchicoside (□), and strychnine (△) in borate-SDS and Tris-SDS BGE systems. Panel A: BGE = 10 mM $Na_2B_4O_7$, 80 mM SDS, SM = 10 mM $Na_2B_4O_7$ (σ_{sample} / σ_{BGE} = 0.8). Panel B: BGE = 10 mM $Na_2B_4O_7$, 80 mM SDS, SM = 40 mM NaCl (σ_{sample} / σ_{BGE} = 1.8). Panel C: BGE = 10 mM $Na_2B_4O_7$, 80 mM SDS, SM= 15 mM $Na_2B_4O_7$ (σ_{sample} / σ_{BGE} = 1.0). Panel D: BGE = 10 mM Tris, 80 mM SDS, SM = 10 mM $Na_2B_4O_7$ (σ_{sample} / σ_{BGE} = 1.2). Panel E: BGE = 10 mM Tris, 80 mM SDS, SM = 10 mM NaOAc (σ_{sample} / σ_{BGE} = 0.5). Panel F: BGE = 10 mM Tris, 80 mM SDS, SM = 15 mM NaCl (σ_{sample} / σ_{BGE} = 1.1). All samples prepared as indicated in the experimental section. Experimental conditions: l_T = 60 cm, l_D = 45 cm, l_{inj} = 0.5 - 9.0 cm, V = 25 kV (56 μA).

References

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