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Zone sharpening of neutral solutes in micellar electrokinetic chromatography with electrokinetic injection☆

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Abstract

Zone sharpening has been used extensively to enhance both efficiency and detectability of *charged* solutes with either electrokinetic or hydrodynamic injection. In the present study, we report the zone sharpening of *neutral* solutes by sharpening the zones of charged micelles which serve as carriers for neutral (and charged) molecules. Zone sharpening with electrokinetic injection can be accomplished only when the effective electrophoretic velocity, v_{eff}, of the solute and the electroosmotic velocity are in the same direction *during the injection process*. In order to maximize the amount of solute loaded into the capillary under zone sharpening conditions, it was necessary to use a cationic mixed micelle in the *sample buffer*. The zone sharpening of a homologous series of alkylphenones was accomplished using electrokinetic injection with cationic mixed micelles of cetyltrimethylammonium bromide and dodecyldimethyl-(3-sulfopropyl)ammonium hydroxide inner salt. The *running buffer* contained *only* sodium dodecyl sulfate and phosphate buffer. As a result of the increased amount of solute loaded into the capillary without significant loss in efficiency, the limits of detection (LOD's) for the solutes studied were in some cases lowered by a factor of ten using zone sharpening. Efficiencies for heptanophenone exceeding 1 000 000 theoretical plates were generated in under 10 min on a 50-cm capillary. Such high efficiencies can be predicted from band broadening models developed for micellar electrokinetic chromatography (MEKC). If properly utilized, zone sharpening can eliminate most if not all of the band broadening associated with sample introduction, and can thus be an important tool in the fundamental study of band broadening in MEKC.

1. Introduction

The popularity of capillary electrophoresis (CE) has increased dramatically over the past 15 years. The successful application of CE, in many cases, is a result of the tremendous resolving power. The number of theoretical plates generated in a CE separation is routinely in excess of 100 000, much larger than the number of theoretical plates generated in a typical reversed-phase HPLC separation. Based on the assumption that the number of theoretical plates in CE is diffusion limited, the number of theoretical plates predicted for solutes with small diffusion coefficients (i.e., proteins) can be $>10^6$. Unfortunately, there are a number of other intracolumn and extracolumn sources of band broadening that limit the maximum attainable efficiency in CE. Intracolumn sources of band

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broadening that have been identified thus far are longitudinal diffusion [1,2], wall effects [3], hydrostatic flow [4], deviations from "plug flow" [5,6], Joule heating [7–10], and conductivity differences between the solute ions and the buffer ions [11,12]. Extracolumn sources arise from the injection and detection of the solute(s), and several authors have discussed the contribution(s) of these processes to the total plate height [4,13–15]. In particular, Deliniger and Davis [16] discussed in significant detail how the length of the injection plug affects efficiency in CE. The authors concluded that in many cases poor injection procedures (i.e., injection plugs that were too long) were the main source of low values for efficiency. The authors recommended using zone sharpening [17] to reduce the effective injection plug length and hence increase efficiency.

Zone sharpening is not a new concept in electrophoresis [18–20], but it has experienced a rebirth in the past few years in CE [21–24]. A requirement of all the experimental approaches for zone sharpening is that the solute have a non-zero mobility. In addition to increasing efficiency, zone sharpening can also be used to reduce the limit of detection (LOD) for a solute [24,25]. The short optical path length of most CE instruments results in LOD's that are typically 1 to 2 orders of magnitude lower than for comparable detectors in HPLC [26]. Therefore, any method that will lower detection limits is a valuable tool to improve overall performance of CE. Zone sharpening is, in most cases, inexpensive and relatively simple to use with commercial instrumentation and, for these reasons, its use in CE is increasing.

Neutral solutes can be separated in a CE format using micellar electrokinetic chromatography (MEKC) [27,28]. The charged micelles provide a pseudostationary phase with which neutral molecules can interact in order to achieve a separation. The band broadening mechanisms resulting from the use of micelles in the running buffer have been the subject of a limited number of studies [29,30] and currently is a subject of considerable debate. However, the sources of extracolumn variance in MEKC are very similar to those for CE [31,32].

Zone sharpening for charged solutes in MEKC has been reported [33] but it has also been implied that zone sharpening is not practical for neutral solutes [32,33]. This is based on the premise, that because the solutes are neutral, they posses no inherent mobility and are unaffected by typical zone sharpening conditions. We have demonstrated in a previous study [34], that although neutral solutes have no inherent mobility, in MEKC their interactions with charged micelles impart them with an effective mobility and are consequently sensitive to zone sharpening conditions with *hydrodynamic injection*. In the present study, we will show that zone sharpening for neutral solutes can be accomplished with *electrokinetic injection* in order to improve efficiency and reduce LOD's. In addition, we will discuss how zone sharpening can be used to eliminate extracolumn band broadening due to sample introduction in order to more effectively study theoretical models of intracolumn band broadening.

2. Theory

A detailed theoretical discussion of zone sharpening of neutral solutes in MEKC has been presented previously [34] and only the essential highlights will be presented here.

If the solutes are injected into a portion of the capillary in a buffer that has a lower conductivity than the buffer in the remainder of the capillary, the electric field will be larger in the buffer region of lower conductivity. As a result of the amplified electric field the solutes will migrate rapidly toward the interface between the buffer regions. Once the solutes cross the interface, the electric field is no longer amplified and the solute velocity decreases dramatically. The change in solute velocity between the two buffer regions results in the solute zone occupying a much smaller volume after crossing the interface. The conductivity difference between the two buffer regions is most easily achieved by dissolving the sample in water or very dilute running buffer.

In MEKC, solutes can interact with a charged micelle and, consequently, will have a net velocity, v_{sol}, as described below:

$$v_{sol} = v_{eff} + v_{eo} = E\mu_{eff} + E\mu_{eo} \quad (1)$$

where v_{eff} is the effective electrophoretic velocity, v_{eo} is the electroosmotic velocity, E is the electric field strength, μ_{eff} is the effective electrophoretic mobility and μ_{eo} is the electroosmotic mobility. The effective electrophoretic mobility is given by:

$$\mu_{eff} = \frac{1}{1+k} \cdot \mu_{ep} + \frac{k}{1+k} \cdot \mu_{mc} \quad (2)$$

where μ_{ep} is the absolute electrophoretic mobility of the solute, μ_{mc} is the absolute electrophoretic mobility of the micelle and k is the retention factor. If we then substitute Eq. 2 into Eq. 1 we get the following expression for the solute velocity as a function of mobility and field strength:

$$v_{sol} = E \cdot \left(\frac{1}{1+k} \cdot \mu_{ep} + \frac{k}{1+k} \cdot \mu_{mc} \right) + E\mu_{eo} \quad (3)$$

According to Eq. 3, if the electric field strength in the sample buffer region is higher than in the running buffer region, the solute velocity will be greater in the sample buffer than in the running buffer. Consequently, as the solute crosses the interface between the two buffer regions, zone sharpening will occur. It is important to note that the electroosmotic velocity is not affected in the same way as the effective electrophoretic velocity of the solute in an amplified electric field. The electroosmotic velocity is a bulk property and consequently, is the weighted average of the electroosmotic velocities in the two buffer regions [25].

Another consideration when performing zone sharpening with electrokinetic injection is the direction (i.e., sign) of the effective electrophoretic velocity. The effective electrophoretic velocity must be in the same direction (i.e., same sign) as the electroosmotic velocity during the injection process for zone sharpening to occur. If the effective electrophoretic velocity is in the opposite direction (i.e., opposite sign) as the electroosmotic velocity during the injection process, then the solutes will not cross the concentration boundary, hence zone sharpening will not occur. This is discussed in more detail at the end of this section and in the Results and discussion section.

For neutral solutes μ_{ep} is zero by definition; thus Eq. 3 reduces to the following:

$$v_{sol} = E \cdot \frac{k}{1+k} \cdot \mu_{mc} + E\mu_{eo} \quad (4)$$

The larger the retention term in Eq. 4, $k/(1+k)$, the greater the effective electrophoretic mobility of a neutral solute. And the larger the effective mobility *in the sample plug*, the more effective the zone sharpening will generally be. Given these relationships, and the fact that k is the product of the phase ratio (β) and the solute micelle water partition coefficient (P_{wm}), it is important to consider each of the latter (P_{wm}, β) when optimizing the zone sharpening effect.

Although the P_{wm} values of different solutes in a given sample may vary by as much as three orders of magnitude, for nearly all surfactant–buffer systems they will generally be the largest when no organic modifiers are present. Thus, except when an organic solvent is necessary to solubilize the analyte(s) within a sample, no organic solvents should be used in the sample buffer. And since it is generally better to keep the composition of injection and running buffers as similar as possible (while maintaining zone sharpening conditions), it would thus appear to be desirable to omit organic solvent from the running buffer as well.

The other way to maximize the effective electrophoretic mobility of a neutral solute in the sample buffer is to maximize k via β *in the sample buffer*. The phase ratio, β, in MEKC can be calculated by :

$$\beta = \frac{\bar{V}([SURF] - CMC)}{1 - \bar{V}([SURF] - CMC)} \quad (5)$$

where [SURF] is the concentration of the surfactant, CMC is the critical micelle concentration and $\bar{V}$ is the partial molar volume of the surfactant. As can be seen in Eq. 5, β can be increased by increasing the surfactant concentration or increasing $\bar{V}$. Since $\bar{V}$ is a constant for a given set of conditions, we can only increase β by increasing the surfactant concentration. If the surfactant concentration is increased with an

ionic surfactant, however, the phase ratio will increase but so will the conductivity of the buffer, and no (field amplified) zone sharpening will be possible. If the surfactant concentration is increased with a *net zero charge* (non-ionic or zwitterionic) surfactant, it is possible to simultaneously increase the phase ratio of the sample buffer while keeping its conductivity low.

Besides the phase ratio and the conductivity of the sample buffer, one additional parameter to consider is the absolute mobility of the micelle, μ_{mc}. In order for the micelle's electrophoretic mobility to be non-zero (a necessary condition for zone sharpening), its net charge must also be non-zero, and this is typically achieved by mixing a charged surfactant with the net zero charge surfactant. The mobility of these mixed micelles will depend on their composition; micelles with a high mole ratio of ionic to net zero charge surfactant will have a larger electrophoretic mobility. Obviously the maximum micelle mobility will be for solutions that contain 100% ionic surfactant and the minimum micelle mobility will be for 100% net zero charge surfactant. Mixed micelles of ionic and net zero charge surfactants will have intermediate values for the micelle mobility but will allow us to fulfill our goals with respect to the phase ratio and buffer conductivity in order to accomplish zone sharpening for neutral solutes [34].

In order to effectively utilize zone sharpening with electrokinetic injection, solutes must have an effective electrophoretic velocity ($v_{eff} = E\mu_{eff}$) in the same direction as electroosmotic flow. This requirement thus precludes the use of micelles of the same charge type in the sample buffer as in the running buffer. *In other words, under typical conditions in MEKC with anionic surfactants in the running buffer [e.g., sodium dodecyl sulfate (SDS)–phosphate, electroosmotic flow from anode to cathode], a cationic mixed micelle must be employed in the sample buffer in order to achieve zone sharpening. Conversely, if a cationic surfactant is employed in the running buffer [e.g., cetyltrimethylammonium bromide (CTAB)–phosphate, electroosmotic flow from cathode to anode], an anionic mixed micelle must be employed in the sample buffer.*

An interesting potential application of zone sharpening in MEKC during electrokinetic injection as described above (with an oppositely charged micellar system in the sample buffer) is the simultaneous zone sharpening of cationic and anionic solutes. Under similar CE conditions, such simultaneous zone sharpening is only possible using polarity switching [35], a feature not universally available on laboratory-made or commercial CE equipment. Under the presently described conditions, ions that would normally oppose electroosmotic flow and migrate away from the sample–running buffer interface (and thus not be zone sharpened) will now, because of strong interactions with oppositely charged micelles, migrate toward the sample–running buffer interface with sufficient velocity to be zone sharpened along with neutral solutes and solutes of opposite charge. Zone sharpening using oppositely charged mixed micelles may thus provide a more broadly applicable solution to the problem of simultaneous zone sharpening of anionic, cationic and neutral molecules, and may also reduce the quantitative biases that are normally observed with electrokinetic injection.

3. Experimental

Either of two Quanta 4000 CE systems (Waters Chromatography, Milford, MA, USA) with UV detection at 254 nm (slit dimensions 75 μm × 1000 μm) were used throughout the study. The sample was loaded into the capillary by either electrokinetic injection with an applied voltage of 2.5 kV or hydrodynamic injection (siphoning using a height difference between the buffers reservoirs of ca. 9.5 cm) for appropriate lengths of time. Data were collected at a rate of 20 Hz and processed on a Macintosh IIci (Cupertino, CA, USA) computer using a MacLab 4-channel A/D converter with appropriate vendor software (ADInstruments, Milford, MA, USA). The capillary tubing was 50 μm I.D. × 370 μm O.D., purchased from Polymicro Technologies, (Tucson, AZ, USA) and cut to 50 cm in length (injection to detector). The capillaries were activated [36] and electroosmotic velocities were

measured [37] using previously published methods. Applied voltages and operating currents appear in the text where appropriate. Liquid levels in the buffer reservoirs were kept as even as possible to reduce band broadening due to hydrodynamic flow [4]. The homologous series of alkylphenones was obtained as a kit from Aldrich (Milwaukee, WI, USA). In addition, dodecyldimethyl-(3-sulfopropyl)ammonium hydroxide inner salt (SB-12), CTAB, HPLC-grade water and acetonitrile were also obtained from Aldrich. SDS was purchased from Sigma (St. Louis, MO, USA) and used as received. The running buffer was 50 m*M* SDS and 20 m*M* phosphate buffer (pH 7.2). The composition of the various sample buffers appears in the text where appropriate. Buffers were prepared by weighing the proper amounts of surfactant and pipetting the proper amount of 100 m*M* phosphate buffer stock solution. The latter was prepared by dissolving the appropriate amount of NaH_2PO_4 in water and adjusting the pH to 7.2 with 1 *M* NaOH.

3.1. Calculations

Variances were calculated by the following:

$$\sigma^2 = \left(\frac{W_{0.10}}{4.2919}\right)^2 \quad (6)$$

where $W_{0.10}$ is the width (in time units) of the peak at 10% of its height. The number of theoretical plates was calculated using:

$$N = \left(\frac{t_r}{\sigma}\right)^2 \quad (7)$$

where t_r is the migration time. We did not use the Foley–Dorsey equation [38] in this study because the asymmetry values provided by the commercial software were unreliable; the Gaussian-based equation above (Eq. 6), although less accurate than the Foley–Dorsey equation, is considerably more accurate than all other commonly used Gaussian-based equations (half-height, inflection points, etc.), and is sufficient for our purposes here. The efficiencies reported are those measured directly from the chromatogram. In some cases the relatively large detector window in our instrument may have significantly biased the observed efficiency. Therefore, the efficiency values were corrected for the band broadening due to the detector [39], in order to give the reader an idea of the magnitude of *N* that could be expected with a shorter, more appropriate detector window. Finally, the values used for t_{mc} (the time required for micelles to migrate from the capillary inlet to the detector) were calculated using the iterative procedure of Bushey and Jorgenson [40].

4. Results and discussion

In order to achieve zone sharpening for neutral solutes with electrokinetic injection it was necessary to use mixed cationic micelles of CTAB and SB-12. The SB-12 serves as the net zero charge surfactant used to increase the phase ratio without increasing conductivity and the CTA^+ surfactant ion is used to impart an overall positive charge to the micelle. Using this approach to zone sharpening, the efficiency (*N*) increased by a factor of between 2 and 5 over controls for solutes with $k > 30$ and decreased by a factor of 2 for solutes with $k < 30$. The increased *N* and the increased amount of solute injected into the capillary using this approach, lead to significantly reduced LODs for solutes with $k > 30$. We are currently investigating the use of zone sharpening with electrokinetic injection to simultaneously zone sharpen anionic, cationic and neutral solutes.

As in previous studies we wanted to maximize the difference in the conductivity of the sample and running buffer to achieve a large amplification of the electric field. This meant first reducing the concentration of the ionic species in the *sample buffer* by reducing the phosphate buffer to 1 m*M*, and the CTAB to 15 m*M*. The phase ratio in the sample buffer was increased by using 75 m*M* SB-12. Further reductions in the phosphate concentration may result in insufficient buffering capacity; further reductions in the concentration of CTAB will result in mixed micelles with a mobility too small to be useful [34]. The next step was to maximize the conduc-

tivity of the *running buffer* without creating large currents and significant Joule heating effects. The resulting running buffer was 50 m*M* in SDS and 20 m*M* in phosphate. For an applied voltage of 25 kV (≈450 V/cm), the operating currents were less than 60 μA. (Note that the cationic mixed micelle is only in the sample buffer and the running buffer contains only SDS and phosphate buffer.)

Shown in Fig. 1 is a comparison of the separation of alkylphenones (acetophenone through heptanophenone) with "proper" electrokinetic zone sharpening (Fig. 1A, "appropriate" mixed micelles, i.e., micelles that swim downstream), without electrokinetic zone sharpening (Fig. 1B, control), and with "improper" electrokinetic zone sharpening (Fig. 1C, "inappropriate" mixed micelles, i.e., micelles that try to swim upstream). Peak heights for all the solutes obtained under proper conditions were larger than the corresponding ones obtained with the control or under improper conditions. The largest differences in the peak heights between the zone sharpened chromatogram (Fig. 1A) and control (Fig. 1B) are for the solutes with the largest k, and consequently the largest effective mobility (Eq. 2). Fig. 1C illustrates why electrokinetic zone sharpening should not be performed with micelles that oppose electroosmotic flow. The direction and magnitude of the solute velocity under these conditions is such that the amount of solute entering the capillary is reduced; not surprisingly this effect is most noticeable for solutes with the largest k.

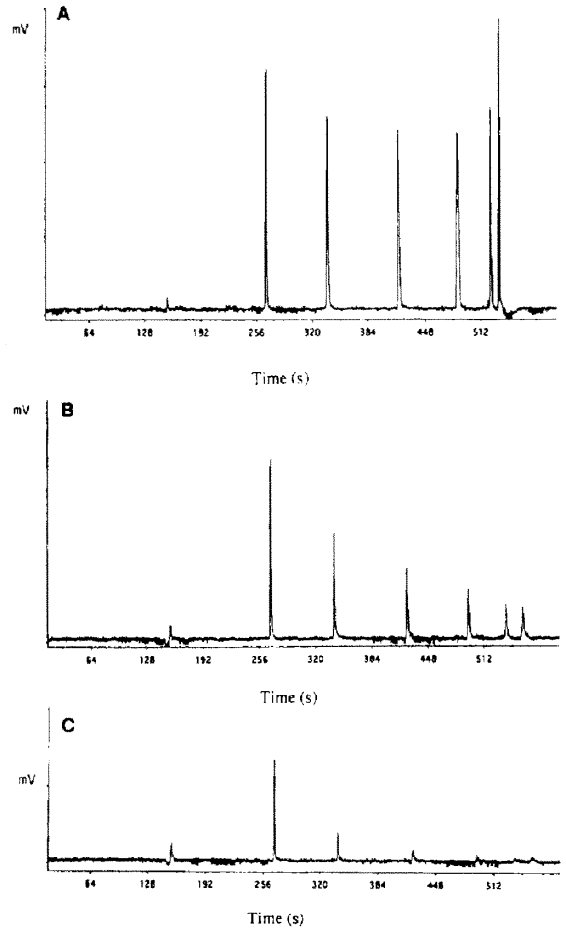

Fig. 1. Comparison of the MEKC separation of alkylphenones (acetophenone through heptanophenone) after a 10-s, 2.5-kV electrokinetic injection with (A) appropriate zone sharpening (CTAB–SB-12 mixed micelles), (B) no zone sharpening (sample buffer = running buffer) and (C) inappropriate zone sharpening (SDS–SB-12 mixed micelles). Other conditions as in the Experimental section.

As in a previous study it was observed (results not shown) that as the injection time was increased, the apparent k of the solutes increased. We have attributed this effect to the excess surfactant introduced into the running buffer from the sample buffer. During the zone sharpening process, the CTAB–SB-12 micelles cross the interface between the two buffer regions and the surfactant monomers will then be distributed among the "pure" SDS micelles, thus increasing β and therefore k. Consequently, this type of surfactant system may not be suitable for thermodynamic studies.

In addition to increasing the amount of solute injected into the capillary under zone sharpening conditions, there were changes in efficiency for the various solutes in compared to controls. Fig. 2 shows the trends in efficiency as a function of retention factor for a 10-s hydrodynamic injection, 10-s electrokinetic injection and 10-s electrokinetic injection with simultaneous zone sharpening. For solutes with $k > 30$, the efficiency was increased by factors of 2–5, consistent with the results obtained for zone sharpening after hydrodynamic injection [34]. For solutes with $k < 30$, the efficiency with electrokinetic injec-

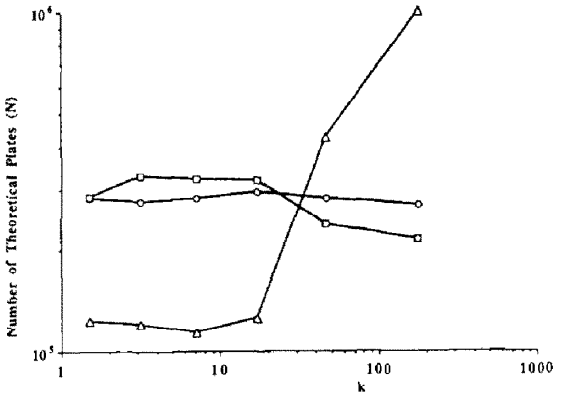

Fig. 2. Experimentally measured dependence of efficiency on retention factor for three different conditions: hydrodynamic injection (○) and electrokinetic injection (□) *without* zone sharpening; and electrokinetic injection *with* zone sharpening (△). In all cases, the injection time was 10 s. Other conditions as in the Experimental section.

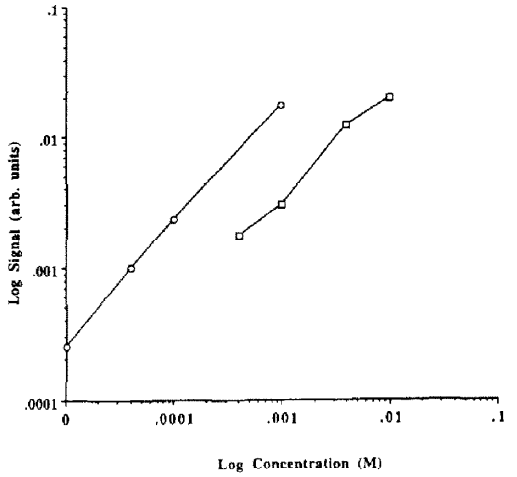

Fig. 3. Comparison of the detectability of hexanophenone with (○) and without (□) zone sharpening. Experimental conditions as in Fig. 1. There were no differences in the amount of noise in chromatograms with and without zone sharpening.

tion zone sharpening was about half that of the controls, in contrast to the slightly higher efficiencies obtained with zone sharpening after hydrodynamic injection [34]. The lower efficiency for solutes with $k < 30$ will result in approximately a 30% reduction in their resolution, which may be tolerable in cases where improvements in detection limits are needed for high-k solutes. In an attempt to improve the overall enhancement in efficiency, a small water plug was injected into the capillary before the zone sharpening step [23], but this had an insignificant effect for all of the solutes studied. We are currently developing a computer model which may provide some insight as to the mechanism of the reduced efficiency when using zone sharpening with electrokinetic injection for solutes with $k < 30$.

For solutes that are effectively zone sharpened, detection limits can significantly reduced by zone sharpening during electrokinetic injection. Fig. 3 shows that for a given concentration of hexanophenone, the detector signal (peak area) obtained with zone sharpening is approximately six times that obtained under non-zone-sharpening conditions. Since the zone sharpening had no effect on the amount of noise, if we approximate the LOD as ca. 3 times S/N, the LOD for hexanophenone is $2 \cdot 10^{-5}$ M and $1.5 \cdot 10^{-4}$ M with and without zone sharpening, respectively. Since at present the effectiveness of zone sharpening is positively correlated with solute retention factor, reductions in detection limits via zone sharpening conditions will be largest for highly retained solutes ($k > 30$) and smaller for other solutes ($k < 30$).

Another potential application of zone sharpening is the validation of a proper theoretical model for intracolumn band broadening in MEKC. At present there are two band broadening models for neutral solutes in MEKC [29,30], and for $k > 1$ and a given set of operating conditions, both predict a decrease in variance as k increases. Shown in Fig. 4 are theoretical curves calculated from each of these models (A and B) and three sets of experimental data obtained in our laboratory (C, D and E) that are representative of band broadening due to injection and/or intracolumn phenomena.

For sample injection (hydrodynamic or electrokinetic) without zone sharpening (Fig. 4, curve C), the variance is relatively constant over a wide range of retention factors. This suggests that the injection variance, the largest source of *extra*column band broadening, is obscuring the

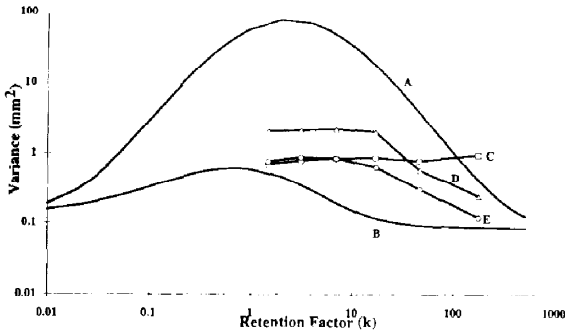

Fig. 4. Comparison of theory and experiment for the dependence of peak variance on the retention factor in MEKC. Theoretical curves were calculated using the band broadening models proposed by Davis [29] (A) and Terabe et al. [30] (B). Parameters used in the calculations: $D_{aq} = 8.5 \cdot 10^{-4}$ mm²/s, $D_{mc} = 5.0 \cdot 10^{-5}$ mm²/s, $t_0/t_{mc} = 0.25$, micelle residence time (t_{mc}) = $1 \cdot 10^{-5}$ s for $0 \leq k \leq 10$ and $1 \cdot 10^{-4}$ s for $k \geq 10$, and $v_{eo} = 3.5$ mm/s. Experimentally measured peak variances are for hydrodynamic injection (SDS micelles) without zone sharpening (C), electrokinetic injection (CTAB–SB-12 micelles) with zone sharpening (D) and hydrodynamic injection (SDS–SB-12 micelles) with zone sharpening (E). Other experimental conditions as in Fig. 1.

trends in intracolumn band broadening that would otherwise be observed. Further evidence of this is provided by curves D and E of Fig. 4, which represent total measured variances after most (if not all) of the electrokinetic or hydrodynamic injection variance, respectively, has been eliminated by zone sharpening. As can be seen, the variances of curves D and E decrease as the retention factor increases, in better agreement with the theoretically predicted trends of curves A and B than the data obtained without zone sharpening (curve C). Zone sharpening may thus be a valuable tool in the development and validation of a comprehensive band broadening model in MEKC for neutral and charged solutes.

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References

[1] C. Schwer and E. Kenndler, *Chromatographia*, 33 (1992) 331–335.
[2] E. Kenndler and C. Schwer, *J. Chromatogr.*, 595 (1992) 313–318.
[3] P.D. Grossman and J.C. Colburn (Editors), *Capillary Electrophoresis: Theory and Practice*, Academic Press, New York, 1992.
[4] E. Grushka, *J. Chromatogr.*, 559 (1991) 81–93.
[5] F. Foret, M. Deml and P. Boček, *J. Chromatogr.*, 452 (1988) 601–613.
[6] H.K. Jones, N.T. Nguyen and R.D. Smith, *J. Chromatogr.*, 504 (1990) 1–19.
[7] E. Grushka, R.M. McCormick and J.J. Kirkland, *Anal. Chem.*, 61 (1989) 241–246.
[8] W.A. Gobie and C.F. Ivory, *J. Chromatogr.*, 516 (1990) 191–210.
[9] J.M. Davis, *J. Chromatogr.*, 517 (1990) 521.
[10] J.H. Knox and I.H. Grant, *Chromatographia*, 24 (1990) 135.
[11] S. Hjertén, *Electrophoresis*, 11 (1990) 665–690.
[12] P.D. Grossman, in P.D. Grossman and J.C. Colburn (Editors), *Capillary Electrophoresis: Theory and Practice*, Academic Press, New York, 1992, pp. 3–43.
[13] K. Otsuka and S. Terabe, *J. Chromatogr.*, 480 (1989) 91–94.
[14] X. Huang, W.F. Coleman and R.N. Zare, *J. Chromatogr.*, 480 (1989) 95–110.
[15] E.V. Dose and G. Guiochon, *Anal. Chem.*, 64 (1992) 123–128.
[16] S. Deliniger and J. Davis, *Anal. Chem.*, 64 (1992) 1749.
[17] R.L. Chien and D.S. Burgi, *Anal. Chem.*, 64 (1992) 489A–496A.
[18] S. Hjertén, S. Jerstedt and A. Tiselius, *Anal. Biochem.*, 11 (1965) 219–223.
[19] H. Haglund and A. Tiselius, *Acta Chem. Scand.*, 4 (1950) 957–962.
[20] L. Ornstein, *Ann. N.Y. Acad. Sci.*, 121 (1964) 321.
[21] F.E.P. Mikkers, F.M. Everaerts and T.P.E.M. Verheggen, *J. Chromatogr.*, 169 (1979) 1–10.
[22] F.M. Everaerts, T.P.E.M. Verheggen and F.E.P. Mikkers, *J. Chromatogr.*, 169 (1979) 11–20.
[23] R.L. Chien and D.S. Burgi, *J. Chromatogr.*, 559 (1991) 141–152.
[24] D.S. Burgi and R.L. Chien, *J. Microcol. Sep.*, 3 (1991) 199–202.
[25] R.L. Chien and D.S. Burgi, *Anal. Chem.*, 64 (1992) 1046–1050.
[26] M. Albin, P.D. Grossman and S.E. Moring, *Anal. Chem.*, 65 (1993) 489A–496A.
[27] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111–113.
[28] S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834–841.
[29] J.M. Davis, *Anal. Chem.*, 61 (1989) 2455–2461.

[30] S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 61 (1989) 251–260.
[31] D.E. Burton, M.J. Sepaniak and M.P. Maskarinec, *Chromatographia*, 21 (1986) 583–586.
[32] P. Sandra and J. Vindevogel, *Introduction to Micellar Electrokinetic Chromatography*, Hüthig, Heidelberg, 1992.
[33] R. Szucs, J. Vindevogel, P. Sandra and L.C. Verhagen, *Chromatographia*, 36 (1993) 323–329.
[34] K.R. Nielsen, E.S. Ahuja and J.P. Foley, *Anal. Chem.*, (1994) in press.
[35] R.L. Chien and D.S. Burgi, *J. Chromatogr.*, 559 (1991) 153–161.
[36] E.L. Little and J.P. Foley, *J. Microcol. Sep.*, 4 (1992) 145–154.
[37] E.S. Ahuja, E.L. Little and J.P. Foley, *J. Liq. Chromatogr.*, 15 (1992) 1099–1113.
[38] J.P. Foley and J.G. Dorsey, *Anal. Chem.*, 55 (1983) 730–737.
[39] K.R. Nielsen and J.P. Foley, *Anal. Chem.*, (1994) submitted for publication.
[40] M.M. Bushey and J.W. Jorgenson, *J. Microcol. Sep.*, 1 (1989) 125–130.