Micellar Electrokinetic Capillary Chromatography Theory Based on Electrochemical Parameters: Optimization for Three Modes Of Operation

ARTICLE *in* ANALYTICAL CHEMISTRY · DECEMBER 1990

Impact Factor: 5.64 · DOI: 10.1021/ac00223a013

3 AUTHORS, INCLUDING:

Kiumars Ghowsi
Islamic Azad University-Majlesi Branch
9 PUBLICATIONS **125** CITATIONS
SEE PROFILE

Joe P. Foley
Drexel University
108 PUBLICATIONS **3,261** CITATIONS
SEE PROFILE

(9) Glinkhammer, G.; Elderfield, H.; Hudson, A. *Nature* **1983**, *305*, 185-188.
(10) Date, A. R.; Gray, A. L. *Spectrochim. Acta, Part B* **1985**, *40B*, 115-122.
(11) Doherty, W.; Vander Voet, A. *Can. J. Spectrosc.* **1985**, *30*, 135-146.
(12) Doherty, W. *Spectrochim. Acta, Part B* **1989**, *44B*, 263-280.
(13) Longerich, H. P.; Fryer, B. J.; Strong, D. F.; Kantipuly, C. J. *Spectrochim. Acta, Part B* **1987**, *42B*, 75-92.
(14) Lichte, F. E.; Meier, A. L.; Crock, J. G. *Anal. Chem.* **1987**, *59*, 1150-1157.
(15) Hirata, T.; Shimizu, H.; Akagi, T.; Sawatari, H.; Masuda, A. *Anal. Sci.* **1988**, *4*, 637-643.
(16) Peppard, D. F.; Mason, G. W.; Maier, J. L.; Driscoll, W. J. *J. Inorg. Nucl. Chem.* **1957**, *4*, 334-343.
(17) Peppard, D. F.; Moline, S. W.; Mason, G. W. *J. Inorg. Nucl. Chem.* **1957**, *4*, 344-348.
(18) Peppard, D. F.; Mason, G. W.; Moline, S. W. *J. Inorg. Nucl. Chem.* **1957**, *5*, 141-146.
(19) Peppard, D. F.; Mason, G. W.; Sironen, R. J. *J. Inorg. Nucl. Chem.* **1959**, *10*, 117-127.
(20) Kimura, K. *Bull. Chem. Soc. Jpn.* **1960**, *33*, 1038-1046.
(21) Kimura, K. *Bull. Chem. Soc. Jpn.* **1960**, *34*, 63-68.
(22) Shabani, M. B.; Akagi, T.; Shimizu, H.; Masuda, A. Paper presented at the 3rd Surrey Conference on Plasma Source Mass Spectrometry, July 1989.
(23) Shabani, M. B.; Akagi, T.; Shimizu, H.; Masuda, A. Paper presented at the 38th Annual Meeting of the Japan Society for Analytical Chemistry, Oct 1989.
(24) Thomas, N. E.; Burkhart, L. E. *J. Inorg. Nucl. Chem.* **1974**, *36*, 1369-1376.
(25) Mason, G. W.; McCarty, S.; Peppard, D. F. *J. Inorg. Chem.* **1962**, *24*, 967-977.
(26) Tachikawa, K. M. S. Dissertation, Tokyo University, 1990.

RECEIVED for review June 29, 1990. Accepted September 13, 1990. This work was supported in part by a Grant-in-Aid for Fundamental Scientific Research from the Ministry of Education, Science, and Culture, Japan.

Micellar Electrokinetic Capillary Chromatography Theory Based on Electrochemical Parameters: Optimization for Three Modes of Operation

Kiumars Ghowsi, Joe P. Foley, and R. J. Gale*

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

Two new, fundamental equations for micellar electrokinetic capillary chromatography (MECC) have been derived, which are analogous to the corresponding capillary zone electrophoresis (CZE) equations for the resolution and the migration time. The components in the theoretical plate height expression for MECC are compared with the appropriate parameters of the Van Deemter equation. MECC optimum resolution has been found for neutral solutes in three cases, where the migration mobility of the micelle is negative, zero, and positive. For the first case, which is similar to the CZE one, the condition has been found that makes the resolution approach infinity. For the two cases of positive and zero migration mobility of micelle, the optimal ranges of the capacity factors for good resolution and resolution per unit time have been found to be between 2 and 5. These optimal ranges approximate to that for conventional column chromatography, even though the physical causes of flow are different.

INTRODUCTION

Capillary electrophoresis has been growing very rapidly during this decade because it offers speed and highly efficient separations, especially for macromolecules in the important area of analytical biotechnology. Electrophoretic separation in capillaries was originally introduced in 1974 by Virtanen (*1*). After a short period in which no publications appeared, Mikkers et al. (*2*), in 1979, reported plate heights less than 10 μm for a zone electrophoresis separation. There are several important theoretical works that contribute to the foundation of capillary electrophoresis theory; one is the 1965 work of Rice and Whitehead (*3*), which is a theoretical study of electrokinetic flow in narrow cylindrical capillaries; the other, published in 1969, is due to Giddings (*4*), whose theory permits an evaluation of the ultimate capabilities of zone electrophoresis. Then, in 1981, Jorgenson and Lukas (*5*) provided a theory for capillary zone electrophoresis in which they proposed two fundamental equations for resolution and the migration time. This latter work followed the approach of Giddings for the plate number and borrowed the concept of theoretical plates from chromatography theory, assuming diffusion is the only cause of zone broadening. In CZE, the migration time of a solute is given by

$$t_s = \frac{L^2}{(\mu_E + \mu_{EO})V} \quad (1)$$

where t_s is the migration time, L is the capillary length, μ_E is the solute's electrophoretic mobility, μ_{EO} is the electroosmotic mobility, and V is the applied voltage. The plate number, N, for a particular solute is

$$N = \frac{(\mu_E + \mu_{EO})V}{2D} \quad (2)$$

where D is the diffusion coefficient of the solute. The resolution, R_S, is given by

$$R_s = \frac{(\mu_{E,A} - \mu_{E,B})}{(\bar{\mu}_E + \mu_{EO})} \frac{N^{1/2}}{4} \quad (3)$$

where $\bar{\mu}_E$ is the average of the electrophoretic mobilities of solute A and B. Substituting eq 2 into eq 3, the resolution can be expressed as

$$R_s = \frac{(\mu_{E,A} - \mu_{E,B})}{(\bar{\mu}_E + \mu_{EO})^{1/2}} \frac{V^{1/2}}{4(2)^{1/2}D^{1/2}} \quad (4)$$

Although CZE is a highly efficient separation technique, only ionic or charged compounds can be separated by this method because the separation principle is based on the

* Corresponding author.

0003-2700/90/0362-2714$02.50/0

difference in electrophoretic mobilities. In 1984, Terabe et al. (6, 7) introduced micellar electrokinetic capillary chromatography (MECC). This technique involves the addition of surfactant ions, at concentrations above their critical micelle concentration, to the mobile phase in a capillary. Neutral compounds are separated based on their differential partitioning between an electroosmotically pumped aqueous mobile phase and the hydrophobic interior of the micelles, which are moving at a velocity generally different from that of the mobile phase due to electrophoretic effects. This technique enables neutral components to be separated in addition to ionic species, while retaining the advantages of the capillary electrophoresis format. The two fundamental equations for the retention time and resolution in MECC (7) are

$$t_R = \left(\frac{1 + \tilde{k}'}{1 + (t_o/t_{mc})\tilde{k}'}\right)t_o \tag{5}$$

$$R_s = \frac{N^{1/2}}{4}\,\frac{\alpha - 1}{\alpha}\,\frac{\tilde{k}_B}{1 + \tilde{k}'_B}\,\frac{1 - t_o/t_{mc}}{1 + (t_o/t_{mc})\tilde{k}'_A} \tag{6}$$

where $\tilde{k}'$ is the capacity factor, N is the number of theoretical plates, α is the selectivity, t_o and t_{mc} are the retention time of the aqueous and micellar phases, respectively, and $\tilde{k}'_A$ and $\tilde{k}'_B$ and the capacity factors of solute A and solute B.

MECC combines both electrophoresis and chromatography and many papers that contribute in this area classify CZE and MECC in the same family (8). However, although according to Terabe (9) electrokinetic chromatography is an interface between electrophoresis and chromatography, fundamental equations for MECC (eqs 5 and 6) have been derived on a chromatographic basis, not an electrokinetic one. Since MECC is a combination of chromatography and electrophoresis, it is possible to develop MECC theory incorporating electrokinetic principles. In this paper, we have derived the electrokinetic equations for the migration time and the resolution for MECC as a function of the mobilities of the components. These expressions provide better representation for putting CZE and MECC in the same family than the alternative, chromatographic approach to a theoretical analysis. Also, it will be shown how the electroosmotic mobility has a key role in MECC and how by manipulating it, the optimum resolution can be obtained. In general, MECC can be divided into two categories, MECC with neutral solutes and MECC with charged solutes, but the fundamental equations for the migration time and resolution are derived only for neutral solutes.

THEORY

Effective Electrophoretic Mobility and Effective Migration Mobility for the Neutral Solute. The relation between the applied electric field, E, and the velocities v_{EO}, v_{mc}, and v_{EP} are given as

$$v_{EO} = \mu_{EO}E \tag{7}$$

$$v_{EP} = \mu_{EP}E \tag{8}$$

$$v_{mc} = \mu_{mc}E \tag{9}$$

where μ_{EO} is the electroosmotic mobility, μ_{EP} is the electrophoretic mobility of the micelle, and μ_{mc} is the net micelle mobility. The μ_{mc} and μ_{EP} values are related by the relation

$$\mu_{mc} = \mu_{EO} + \mu_{EP} \tag{10}$$

We define two new mobilities: the effective electrophoretic mobility and the effective migration mobility for the neutral solute. Although the solute moelcules are neutral, they spend a fraction of time in the micelles and a fraction of time in the electroosmotic flow. In other words, the solute molecules are mobile and carried at each instance by either the micelles or the electroosmotic flow. Correspondingly, for the neutral solute, there is an effective electrophoretic mobility and an effective migration mobility. The effective electrophoretic mobility of the neutral solute can be defined as

$$\mu^*_{EP} = \left(\frac{n_{mc}}{n_{aq} + n_{mc}}\right)\mu_{EP} \tag{11}$$

where n_{mc} and n_{aq} are the total moles of the solutes in the micelle and in the aqueous phase, respectively. By use of the capacity factor definition $k = n_{mc}/n_{aq}$ the effective electrophoretic mobility in eq 8 can be shown in terms of the capacity factor and the electrophoretic mobility of the micelle

$$\mu^*_{EP} = \left(\frac{\tilde{k}'}{1 + \tilde{k}'}\right)\mu_{EP} \tag{12}$$

We define the other mobility, the effective migration mobility of the solute, μ^*_s, as the summation of the effective electrophoretic mobility of the solute and the electroosmotic mobility

$$\mu^*_s = \mu^*_{EP} + \mu_{EO} \tag{13}$$

The relations between the applied electric field, E, and the net solute velocity, v_s, and the effective electrophoretic velocity of the solute, v^*_{EP}, are given as

$$v_s = \mu^*_s E \tag{14}$$

$$v^*_{EP} = \mu^*_{EP}E \tag{15}$$

From eqs 12 and 13, it can be seen that if the micelle was not present in the solution or if the solute is insolubilized in the micelle, then $\tilde{k}' = 0$ and so $\mu^*_{EP} = 0$, and therefore the effective migration mobility of the neutral solute becomes $\mu^*_s = \mu_{EO}$. By adding the micelle to the solution, we create an effective electrophoretic mobility for the neutral solute.

MIGRATION TIME

The migration time, t_s, for the solute to eluate can be found by combining eq 14 and the migration time $t_s = L/v_s$

$$t_s = L/\mu^*_s E \tag{16}$$

$$\text{Since } E = V/L, \text{ then } t_s = L^2/\mu^*_s V \tag{17}$$

By substituting μ^*_s from eq 13 into 17, we find the migration time t_s

$$t_s = \frac{L^2}{[\mu^*_{EP} + \mu_{EO}]V} \tag{18}$$

where V is the voltage applied across the capillary. It can be shown with proper substitution and algebraic manipulation that eq 18 is equivalent to its counterpart chromatography equation of the migration time (eq 5). The advantage of eq 18 over eq 5 is that it is in terms of the physical parameters of the system and illustrates, for example, that the time can be increased or decreased by manipulation of the electroosmotic mobility or voltage.

Resolution. The resolution R_s is defined as (10, 11) $R_s = ((t_s)_B - (t_s)_A)/W_{AVG}$, and $N = 16(t_s/W)^2$. Then

$$R_s = \frac{[(t_s)_B - (t_s)_A]}{\frac{1}{2}[(t_s)_B + (t_s)_A]}\,\frac{N^{1/2}}{4} \tag{19}$$

where N is the number of theoretical plates. By insertion of $t_s = L/\mu_s E$ in eq 19 and with some algebraic manipulation, we obtain

$$R_s = \frac{(\mu^*_s)_A - (\mu^*_s)_B}{\frac{1}{2}[(\mu^*_s)_B + (\mu^*_s)_A]}\,\frac{N^{1/2}}{4} \tag{20}$$

μ_s^* given by eq 13 is substituted in eq 20, from which we derive the expression

$$R_s = \frac{\mu^*_{EP,A} - \mu^*_{EP,B}}{\frac{1}{2}[(\mu^*_s)_B + (\mu^*_s)_A]} \frac{N^{1/2}}{4} \quad (21)$$

Since these equations are applied to a pair of solutes whose effective electrophoretic mobilities are similar enough to make their separation difficult, we can make the approximation

$$\mu^*_{EP,A} \simeq \mu^*_{EP,B} = \bar{\mu}^*_{EP} \quad (22)$$

and

$$\bar{\mu}^*_{EP} = \left(\frac{\bar{k}'}{1 + \bar{k}'}\right)\mu_{EP} \quad (23)$$

Now by inserting $^1/_2[(\mu^*_s)_B + (\mu^*_s)_A] = \bar{\mu}^*_s = \bar{\mu}^*_{EP} + \mu_{EO}$ in eq 21, the resolution in new form is

$$R_s = \frac{\mu^*_{EP,A} - \mu^*_{EP,B}}{\bar{\mu}^*_{EP} + \mu_{EO}} \frac{N^{1/2}}{4} \quad (24)$$

Equation 24 describes the resolution in terms of mobilities. This equation is equivalent to its counterpart, the chromatography equation of resolution (eq 6). However, this is not the whole story because N also is a function of the mobilities. Sepaniak and Cole have studied experimentally the factors that influence column efficiency in MECC (*12*). Their work showed Van Deemter-like behavior of plate height vs applied voltage. Recently, Terabe et al. have reported their theoretical and experimental results on band broadening phenomena that occur in MECC separations (*13*). In the Appendix, we compare the theoretical plate height based on the work of Terabe et al. with the Van Deemter equation. Proper coefficients have been used to summarize the column plate height, H. The term N from eq A21 can be replaced in eq 24 to give

$$R_s = \frac{1}{4} \frac{\mu^*_{EP,A} - \mu^*_{EP,B}}{\bar{\mu}^*_{EP} + \mu_{EO}} \times \left(\frac{L^2\mu_{EO}V}{(B'_M + B'_S)L^2 + (C'_S + C'_M + D' + E')\mu_{EO}^2V^2}\right)^{1/2} \quad (25)$$

Equation 25 is the resolution in terms of mobilities, capillary length, and the applied voltage. It needs to be remembered that the coefficients in the denominator of the second term in eq 25 are implicitly a function of μ_{mc}/μ_{EO}. This is because, as shown in the Appendix, they are functions of t_o/t_{mc} and $t_o/t_{mc} = \mu_{mc}/\mu_{EO}$.

If we want to compare the resolution equation R_s for MECC (eq 25) with eq 4 for the resolution of CZE, we need to simplify eq 25 further. Since for the CZE resolution, eq 4, the thermal band broadening has not been included, we can eliminate the H_t term in eq 25, especially because its contribution is small (*13*). By referring to definitions of B', C'_S, C'_M, and E', we see that they all have the term $(1 + (t_o/t_{mc})\tilde{k}')$ in their denominators. Then B'', C''_S, C''_M, and E'' are defined as follows:

$$B'' = B'[1 + (t_o/t_{mc})\tilde{k}'] \quad (26)$$

or

$$B''_M = B'_M\left[1 + \left(\frac{t_o}{t_{mc}}\right)\tilde{k}'\right] \quad \text{and}$$

$$B''_S = B'_S\left[1 + \left(\frac{t_o}{t_{mc}}\right)\tilde{k}'\right] \quad (27)$$

$$C''_S = C'_S[1 + (t_o/t_{mc})\tilde{k}'] \quad (28)$$

$$C''_M = C'_M[1 + (t_o/t_{mc})\tilde{k}'] \quad (29)$$

$$E'' = E'[1 + (t_o/t_{mc})\tilde{k}'] \quad (30)$$

Substituting $t_o/t_{mc} = \mu_{mc}/\mu_{EO}$ and combining eqs 25–30, we get

$$R_s = \frac{1}{4}\left[\frac{\mu^*_{EP,A} - \mu^*_{EP,B}}{\bar{\mu}^*_{EP} + \mu_{EO}}\right] \times \left[\frac{L^2\mu_{EO}[1 + (\mu_{mc}/\mu_{EO})\tilde{k}']V}{(B''_M + B''_S)L^2 + (C''_S + C''_M + E')\mu_{EO}^2V^2}\right]^{1/2} \quad (31)$$

and by substituting $\mu_{mc} = \mu_{EO} + \mu_{EP}$ in $[1 + (\mu_{mc}/\mu_{EO})\tilde{k}']$ and some rearrangements, we obtain

$$\mu_{EO}\left[1 + \left(\frac{\mu_{mc}}{\mu_{EO}}\right)\tilde{k}'\right] = (1 + \tilde{k}')\left[\mu_{EP}\left(\frac{\tilde{k}'}{1 + \tilde{k}'}\right) + \mu_{EO}\right] = (1 + \tilde{k}')(\bar{\mu}^*_{EP} + \mu_{EO}) \quad (32)$$

Now we can substitute eq 32 into 31 and simplify the denominator of the first term with the numerator of the second term with the assumption of $\tilde{k} \simeq \bar{k}'$; it follows that

$$R_s = \frac{1}{4}\left(\frac{(\mu^*_{EP,A} - \mu^*_{EP,B})\mu_{EP}}{[\bar{\mu}^*_{EP} + \mu_{EO}]^{1/2}}\right) \times \left(\frac{(\bar{k}' + 1)L^2V}{(B''_M + B''_S)L^2 + (C''_S + C''_M + E')\mu_{EO}^2V^2}\right)^{1/2} \quad (33)$$

Resolution is thus expressed as a function of basic electrochemical parameters and this will enable optimal conditions to be predicted and tested. R_s also can be transformed to its chromatography form by multiplying the numerator and the denominator by $(\bar{k}' + 1)^{1/2}$, and setting $\tilde{k}'_B/\tilde{k}'_A = \alpha$, $\tilde{k}'_A \simeq \tilde{k}'$, and $\mu_{EP} = \mu_{mc} - \mu_{EO}$, we obtain this intermediate expression

$$R_s = \left[\frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{\tilde{k}'_B}{1 + k'_B}\right) \times \left(\frac{(1 - (\mu_{mc}/\mu_{EO}))}{[1 + ((\mu_{mc}/\mu_{EO})k'_A)]^{1/2}}\right)\right] \times \left(\frac{\mu_{EO}L^2V}{(B'_M + B''_S)L^2 + (C''_S + C''_M + E')\mu_{EO}^2V^2}\right)^{1/2} \quad (34)$$

Equation 34 can be transformed further to yield the chromatography form by substituting $\mu_{mc}/\mu_{EO} = t_o/t_{mc}$, and $v_{EO} = \mu_{EO}V/L$, and making some algebraic manipulations

$$R_s = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{\tilde{k}'_B}{\tilde{k}'_B + 1}\right)\left(\frac{(1 - (t_o/t_{mc}))}{(1 + (t_o/t_{mc})\tilde{k}'_A)^{1/2}}\right) \times \left(\frac{L}{\frac{(B''_M + B''_S)}{v_{EO}} + (C''_S + C''_M + E')v_{EO}}\right)^{1/2} \quad (35)$$

In addition, eq 35 can be derived by substituting N

$$N = \frac{L}{H} = \frac{L}{H_1 + H_{mc} + H_{aq} + H_t + H_{ep}} \quad (36)$$

in the chromatography resolution (eq 6), setting $H_t = 0$ and using eqs A2, A6, A12, and A21, and simplifying $(1 + t_o/t_{mc})\tilde{k}')$ components. The chromatography resolution equations are provided in Table I. The electrokinetic equations for migration time and resolution for both CZE and MECC are given in Table II. The similarities can be seen much better in these tables by using the concept of effective migration mobility, μ^*_S, of the solute for MECC. Below, we apply these equations, especially the electrokinetic equations, to find the optimal

Table I

$$R_s = \left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{\tilde{k}'_B}{\tilde{k}'_B + 1}\right)\frac{(1 - (t_o/t_{mc}))}{(1 + (t_o/t_{mc})\tilde{k}'_A)} \times \frac{1}{4}\left(\frac{L}{(B'_M + B'_S)/v_{EO} + (C'_S + C'_M + D' + E')v_{EO}}\right)^{1/2}$$

where $B' = B'_M + B'_S$ and

$$B'_M = \frac{2D_{aq}}{1 + (t_o/t_{mc})\tilde{k}'}, \qquad B'_S = \frac{2\tilde{k}'_m D_{mc}}{(1 + (t_o/t_{mc})\tilde{k}')}$$

$$C'_S = \frac{2(1 - t_o/t_{mc})^2}{(1 + (t_o/t_{mc})\tilde{k}')}$$

$$C'_M = \left(\frac{\tilde{k}'}{1 + \tilde{k}'}\right)^2 \frac{1 - (t_o/t_{mc})^2}{1 + (t_o/t_{mc})\tilde{k}'} \frac{d^2}{4D_{aq}}$$

$$D' = \frac{(1 - t_o/t_{mc})\tilde{k}'}{24(D_{aq} + \tilde{k}'D_{mc})} \frac{B^2 I^4}{64 k_o^2 \pi^4 r_c^2 \lambda^2 T_o^4}$$

$$E' = \frac{0.026(1 - t_o/t_{mc})^2 \tilde{k}'}{(1 + (t_o/t_{mc})\tilde{k}')k_d}$$

$$R_s = \left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{\tilde{k}'}{\tilde{k}' + 1}\right)\frac{(1 - t_o/t_{mc})}{(1 + (t_o/t_{mc})\tilde{k}')^{1/2}} \times \frac{1}{4}\left(\frac{L}{B''/v_{EO} + (C''_S + C''_M + E')v_{EO}}\right)^{1/2}$$

where $\tilde{k}'_A \simeq \tilde{k}'_B \simeq \bar{k}' \simeq \tilde{k}'$, $\quad B'' = B'(1 + (t_o/t_{mc})\tilde{k}')$,

$$B''_M = B'_M(1 + (t_o/t_{mc})\tilde{k}'), \qquad B''_S = B'_S(1 + (t_o/t_{mc})\tilde{k}')$$

$$C''_S = C'_S(1 + (t_o/t_{mc})\tilde{k}'), \qquad C''_M = C'_M(1 + (t_o/t_{mc})\tilde{k}')$$

$$H_t = 0, \quad \text{and} \quad E'' = E'(1 + (t_o/t_{mc})\tilde{k}')$$

resolution attainable in MECC and compare it with that in CZE.

In summary, two new, fundamental equations for MECC have been derived for the migration time and the resolution, which are analogous to the fundamental equations for capillary zone electrophoresis. The electrokinetic characteristics of MECC can be described by defining two important quantities called effective migration mobility, μ^*_S, and effective electrophoresis mobility of the neutral solute. This theoretical approach provides for a more general and accurate physical model to predict or simulate the roles of micelle transport in MECC. As for the chromatographic approach, local equilibration of the solute and micelle is assumed to be rapid relative to the velocities. Further, the components of the theoretical plate height, based on the work of Terabe et al. (*13*), are compared via the components of the Van Deemter equation in the Appendix and it is shown that for optimization, in contrast to stationary phase column chromatography, the number of theoretical plates N cannot be considered independent, nor a weak function of the capacity factor $\tilde{k}'$, unless the important term $(1 + (t_o/t_{mc})\tilde{k}')$ is removed from the N expression. It is noteworthy that this term exists in all components of H except for H_t.

Optimization. MECC with neutral solutes can be classified into three modes, depending on the net mobility of the micelle, μ_{mc}. Case I is when $\mu_{mc} < 0$ or $-\mu_{EP} > \mu_{EO}$. For this case, which has been referred to by Terabe et al. (*14*) as negative t_o/t_{mc}, the micelles migrate in a direction opposite to the electroosmotic flow. *In this work, we have assumed that any mobility is positive if its direction is the same as the electroosmotic flow vector and it is negative if its direction is opposite.* In case II, if we can modify the electroosmotic mobility such that $\mu_{EO} = -\mu_{EP}$, then $\mu_{mc} = 0$. Since $\mu_{mc} = 0$, then t_{mc} goes to infinity and $t_o/t_{mc} = 0$. Case III is when $\mu_{mc} > 0$ or $-\mu_{EP} < \mu_{EO}$. This mode of operation is the conventional one, which occurs usually without manipulation of the electroosmotic mobility. Without any extra coatings of the capillary, or other types of modification of the electroosmotic mobility, its magnitude is usually greater than the electrophoretic mobility of the micelle. The optimizations for each of these modes of operation follow below.

Case I: $\mu_{mc} < 0$ or $-\mu_{EP} > \mu_{EO}$. In this case, the micelles are migrating oppositely to the electroosmotic mobility. However, for separation the solute should migrate in the same direction as the electroosmotic flow. In order to maintain this condition, the effective migration mobility of the solute, μ^*_S must be greater than zero

$$\mu^*_S = \mu^*_{EP} + \mu_{EO} > 0 \tag{37}$$

By substituting eq 13 in the above inequality, we can obtain the following condition for maintaining the inequality:

$$\frac{\tilde{k}'}{1 + \tilde{k}'} < \frac{\mu_{EO}}{\mu_{EP}} \tag{38a}$$

We can solve the above inequality for the acceptable range of k

$$\tilde{k}' < \frac{-\mu_{EO}}{\mu_{EO} + \mu_{EP}} \quad \text{or} \quad \tilde{k}' \leq \frac{-\mu_{EO}}{\mu_{mc}} \tag{38b}$$

Setting the denominator of the first term in eq 33 equal to zero, the resolution goes to infinity

$$(\bar{\mu}^*_{EP} + \mu_{EO})^{1/2} = 0 \quad \text{or} \quad \mu_{EO} = -\bar{\mu}^*_{EP} \tag{39}$$

Table II. Electrophoresis Equations

CZE $\quad t_S = \dfrac{L^2}{\bar{\mu}^*_s V} = \dfrac{L^2}{(\bar{\mu}^*_{EP} + \mu_{EO})V}$ $\quad$ If $\mu_{EO} = -\bar{\mu}_E$ then R_s and t_S go to infinity

$$R_s = \frac{\mu_{s,A} - \mu_{s,B}}{\bar{\mu}_s}\frac{N^{1/2}}{4} = \left(\frac{\mu_{E,A} - \mu_{E,B}}{(\bar{\mu}_E + \mu_{EO})^{1/2}}\right)\left(\frac{V^{1/2}}{4(2^{1/2})D^{1/2}}\right)$$

MECC $\quad t_S = \dfrac{L^2}{\bar{\mu}^*_s V} = \dfrac{L^2}{(\bar{\mu}^*_{EP} + \mu_{EO})V}$ $\quad$ If $\mu_{EO} = -\bar{\mu}^*_{EP}$ then R_s and t_S go to infinity

$$R_s = \frac{\mu^*_{s,A} - \mu^*_{s,B}}{\bar{\mu}^*_s}\frac{N^{1/2}}{4} = \left(\frac{\mu^*_{EP,A} - \mu^*_{EP,B}}{(\bar{\mu}^*_{EP} + \mu_{EO})^{1/2}}\right)\frac{1}{4}\left(\frac{(\tilde{k}' + 1)L^2 V}{B''L^2 + (C''_S + C''_M + E)\mu_{EO}{}^2 V^2}\right)^{1/2}$$

where $\mu^*_{EP,A} = \dfrac{\tilde{k}'_A}{1 + \tilde{k}'_A}\mu_{EP}$, $\quad \mu^*_{EP,B} = \dfrac{\tilde{k}'_B}{1 + \tilde{k}'_B}\mu_{EP}$, $\quad$ and $\quad \bar{\mu}^*_{EP} = \dfrac{\bar{k}'}{1 + \bar{k}'}\mu_{EP}$

At this latter condition, the electroosmotic flow just balances the effect electrophoretic mobility of the neutral solute. In other words, the neutral solute is carried by the micelles in one direction as much as the solute is carried by the electroosmotic flow in the opposite direction, at which point substances with extremely small differences in their effective electrophoretic mobilities (extremely small differences in capacity factors) may be resolved. According to eq 33, the maximum resolution will be obtained, however, at a large expense in time, as may be seen by referring to eq 18 and imagining μ_{EO} and $\bar{\mu}^*_{EP}$ as being nearly equal but opposite. This argument is very similar to that for resolution optimization in capillary zone electrophoresis; namely, when $\mu_{EO} = -\bar{\mu}_E$, the resolution and migration time (eqs 1 and 4) mathematically approach infinity.

Case II: $\mu_{EO} = -\mu_{EP}$ or $\mu_{mc} = 0$. In this case, the micelle is stationary and its net velocity is zero. This case is equivalent to $t_{mc}/t_o = \mu_{EO}/\mu_{mc} = \infty$, or $t_o/t_{mc} = \mu_{mc}/\mu_o = 0$. Now the chromatographic situation pertains and by using eq 34 or 35 and substituting $\mu_{mc}/\mu_o = 0$, or $t_o/t_{mc} = 0$, one obtains

$$R_s = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{\tilde{k}'}{\tilde{k}' + 1}\right) \times \left(\frac{\upsilon_{EO}}{(B_M + B_S)L^2 + (C_S + C_M + E)\upsilon_{EO}}\right)^{1/2} \quad (40)$$

Since the dominant contributions to theoretical height dispersion are terms H_1 and H_{ep} (*13*), we keep B and E, which are the coefficients in H_1 and H_{ep}, and eliminate C_S and C_M. By substituting B and E, which are given by eqs A3a and A22, into eq 40, we obtain

$$R_s \approx \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{\tilde{k}'}{\tilde{k}' + 1}\right) \times \left(\frac{\upsilon_{EO}}{2(D_{aq} + \tilde{k}'D_{mc}) + (0.026/kd)\tilde{k}'\upsilon_{EO}^2}\right)^{1/2} \quad (41)$$

It can be seen that N, which is the last expression, is a strong function of $\tilde{k}'$. The first term is independent of $\tilde{k}'$, so we need for R_s optimization to optimize the remainder of the expression

$$h(\tilde{k}') = \left(\frac{\tilde{k}'}{\tilde{k}' + 1}\right)\left(\frac{\upsilon_{EO}}{2(D_{aq} + \tilde{k}'D_{mc}\tilde{k}') + (0.026/k_d)\upsilon_{EO}^2\tilde{k}'}\right)^{1/2} \quad (42)$$

For algebraic simplification, we define the following two constants:

$$\beta_1 = 2D_{aq} \quad \text{and} \quad \beta_2 = 2D_{mc} + \frac{0.026}{k_d}\upsilon_{EO}^2 \quad (43)$$

By substituting the two constants in eq 42, we obtain

$$h(\tilde{k}') = \left(\frac{\tilde{k}'}{\tilde{k}' + 1}\right)\frac{\upsilon_{EO}^{1/2}}{(\beta_1 + \beta_2\tilde{k}')^{1/2}} \quad (44)$$

To find the optimal value of $\tilde{k}'$, it is necessary to take the derivative of eq 42 and to set it equal to zero. The following quadratic equation results:

$$\beta_2\tilde{k}'^2 - \beta_2\tilde{k}' - 2\beta_1 = 0 \quad (45)$$

The physical acceptable solution is

$$\tilde{k}'_{opt}(\text{maximum } R_s) = \frac{\beta_2 + (\beta_2^2 + 8\beta_2\beta_1)^{1/2}}{2\beta_2} \quad (46)$$

To obtain a quantitative estimate, the following values have been taken from the paper of Terabe et al. (*13*): $D_{mc} = 5 \times 10^5$ (mm^2·s^{-1}); $\upsilon_{EO} = 2$ (mm·s^{-1}); $D_{aq} = 8 \times 10^{-4}$ (mm^2·s^{-1}); $\beta_1 = 1.6 \times 10^{-3}$; $\beta_2 = 2.04 \times 10^{-4}$. By use of β_1 and β_2, $\tilde{k}'_{opt}$(maximum R_s) is calculated to be

$$\tilde{k}'_{opt}(\text{maximum } R_s) \approx 4.5 \quad (47)$$

and for $\upsilon_{EO} = 1$ (mm·s^{-1});k'_{opt}(maximum R_s) $\simeq 5.6$.

Case III: $\mu_{mc} > 0$ or $-\mu_{EP} < \mu_{EO}$. This mode is the conventional mode of operation for MECC. To find the optimum resolution for this case, it can be seen that the denominator of the first term in eq 33 cannot be zero as for case I because it is always positive. Since $-\mu_{EP} < \mu_{EO}$, it follows that $[-\tilde{k}'/(\tilde{k}' + 1)]\mu_{EP} = -\bar{\mu}^*_{EP} < \mu_{EO}$, and $\mu_{EO} + \bar{\mu}^*_{EP} = \bar{\mu}^*_s > 0$. The selectivity factor has been found to be independent of the micelle concentration for neutral solutes (*15–17*), which means that it is independent of $\tilde{k}'$, too. The last term in eq 34 is

$$\left(\frac{\mu_{EO}L^2V}{(B''_M + B''_S)L^2 + (C''_S + C''_M + E'')\mu_{EO}^2V^2}\right)^{1/2} \quad (48)$$

For lower values of $\tilde{k}'$, $B''_M = D_{aq}$ is the dominant component in the denominator of expression (*48*), because D_{mc} is 1 order of magnitude smaller than D_{aq} and $2D_{aq} = B_M$ is dominant in comparison to $2D_{mc}\tilde{k}' = B_S$ for lower values of $\tilde{k}'$. According to Terabe et al. (*13*), H_{mc} and H_{aq} contribute very little to the theoretical plate height, so C''_S and C''_M, which are the coefficients of H_{mc} and H_{aq}, are not dominant in expression 48. E'' is 1 to 2 orders of magnitude smaller than B''_M for lower values of $\tilde{k}'$. Therefore, at low values of $\tilde{k}'$, the dominant term in the denominator of expression 48 is B''_M, which is independent of $\tilde{k}'$. Now we can optimize the remainder of the resolution equation (34), which is a strong function of $\tilde{k}'$

$$f(\tilde{k}') = \left(\frac{\tilde{k}'}{\tilde{k}' + 1}\right)\frac{(1 - \mu_{mc}/\mu_{EO})}{[1 + (\mu_{mc}/\mu_{EO})\tilde{k}']^{1/2}} \quad (49)$$

where $\tilde{k}'_B \simeq \tilde{k}'_A \simeq \tilde{k}'$. Setting the derivative of eq 49 equal to zero, we obtain the following quadratic equation:

$$\tilde{k}'^2 + \left(2\frac{\mu_{mc}}{\mu_{EO}} - 1\right)\tilde{k}' + \left(2 - 2\frac{\mu_{EO}}{\mu_{mc}}\right) = 0 \quad (50)$$

Only the positive of the two roots is acceptable

$$\tilde{k}'_{opt}(\text{maximum } R_s) = \frac{-\mu_{mc}}{\mu_{EO}} + \frac{1}{2} + \frac{1}{2}\left(4\left(\frac{\mu_{mc}}{\mu_{EO}}\right)^2 - 4\frac{\mu_{mc}}{\mu_{EO}} + 8\frac{\mu_{EO}}{\mu_{mc}} - 7\right)^{1/2} \quad (51)$$

The values of $\tilde{k}'_{opt}$(maximum R_s) have been calculated from eq 51 for different values of μ_{EO}/μ_{mc} and are presented in Table III. As shown, by increasing μ_{EO}/μ_{mc}, $\tilde{k}'_{opt}$(maximum R_s) increases. The best resolution is obtained if μ_{EO}/μ_{mc} goes to infinity. This happens if μ_{mc} becomes zero. However, we cannot apply eq 51 to find $\tilde{k}'_{opt}$ when μ_{EO}/μ_{mc} goes to infinity because this violates the assumption that eq 51 is applicable for lower values of $\tilde{k}'$. In fact, when μ_{EO}/μ_{mc} goes to infinity or μ_{mc} goes to zero, we obtain case II, for which the optimum capacity factor for the best resolution is ~5. Therefore eq 51 is applicable for $\tilde{k}' \leq \sim 5$ with SDS micelles or other surfactant systems in which the terms that are independent of $\tilde{k}'$ can be assumed to be dominant. Another important parameter for optimization is the relation of the migration time with a given resolution. The ratio of t_R/R^2_S is obtained by using eqs 18 and 34

$$\frac{t_R}{R_S^2} = L^2(\tilde{k}' + 1)/[1 + (\mu_{mc}/\mu_{EO})\tilde{k}']\mu_{EO}V \Big/ \frac{1}{16}\left(\frac{\alpha - 1}{\alpha}\right)^2\left(\frac{\tilde{k}'}{\tilde{k}' + 1}\right)^2\frac{(1 - \mu_{mc}/\mu_{EO})^2}{[1 + (\mu_{mc}/\mu_{EO})\tilde{k}']} \times \left(\frac{\mu_{EO}L^2V}{(B''_M + B''_S)L^2 + (C''_S + C''_M + E')\mu_{EO}^2V^2}\right) \quad (52)$$

After some algebraic simplifications

$$t_R = 16R_S^2\left(\frac{\alpha}{\alpha-1}\right)^2\left(\frac{(\tilde{k}'+1)^3}{\tilde{k}'^2}\right)\left(\frac{1}{(1-\mu_{mc}/\mu_{EO})^2}\right)\times \left(\frac{(B''_M+B''_S)L^2+(C''_S+C''_M+E')\mu_{EO}^2V^2}{\mu_{EO}^2V^2}\right) \quad (53)$$

The resolution could be maintained constant at a desired value by changing L while $\tilde{k}$ is changing. The term α is independent of $\tilde{k}'$, as mentioned earlier. The last term in eq 53 can be assumed to be independent of $\tilde{k}'$ for lower values of $\tilde{k}'$, based on the same argument we made for expression 48. The term $(1-\mu_{mc}/\mu_{EO})^2$ also is independent of $\tilde{k}'$. Therefore, to find the optimum response time with a constant R_S, in terms of the capacity factor, the following part of eq 53 is optimized:

$$g(\tilde{k}') = (\tilde{k}'+1)^3/\tilde{k}'^2 \quad (54)$$

$$dg(\tilde{k}')/d\tilde{k}' = 0$$

$$3(1+\tilde{k}')^2\tilde{k}'^2 - (1+\tilde{k}')^3 2\tilde{k}' = 0 \quad (55)$$

After the equation was simplified and the above equation solved, the answer is $\tilde{k}' = 2$. *Therefore, at $\tilde{k}' = 2$ the maximum resolution per unit time can be obtained.* This result is interesting because it is consistent with the optimum value, $k = 2$, for maximum resolution per unit time in the conventional column chromatographic method (*11*). The overall conclusion for cases II and III is that the optimal values of $\tilde{k}'$ are between 2 and ~5 for good resolution and resolution per unit time. It is very interesting that this optimum range is the same as that for column chromatography (*11*) even though the fundamental equations for t_R, R_S, and H are not the same in MECC as those for column chromatography. The summary of the results obtained for the three cases is shown in Figure 1.

COMPARISON WITH PREVIOUS OPTIMIZATION FOR MECC

There is not any previous work for optimization of case I. For this case, it is needed to optimize the product of the two new fundamental equations for t_S and R_S, which have been derived in this work based on an electrokinetic approach. For the other cases, II and III, Terabe et al. optimized more qualitatively the resolution in terms of the capacity factor (*7*). To evaluate the effect of $\tilde{k}'$ on resolution, they used the function $f(\tilde{k}')$, the product of the last two terms in the resolution equation (6)

$$f(\tilde{k}') = \left(\frac{\tilde{k}'}{\tilde{k}'+1}\right)\left(\frac{1-t_o/t_{mc}}{1+(t_o/t_{mc})\tilde{k}'}\right) \quad (56)$$

Foley (*18*) has used above equation to optimize the resolution more quantitatively.

The difference between the optimization procedures of previous works (*7*, *18*) and the present work is that in the previous work, N has been assumed to be independent of $\tilde{k}'$, but in this study N has been assumed to be a function of $\tilde{k}'$ and it has been included in the optimization process. The optimum capacity factor for resolution and resolution per unit time have been calculated for the present work and previous work (*18*) and are summarized in Table III. Even though the results are close over some ranges of the optimum capacity factor, the optimum values of the present work should be more accurate for $\mu_{EO}/\mu_{mc} = t_{mc}/t_o < 25$ because of the inclusion of the dependence of N on $\tilde{k}'$. The ranges over which the previous assumptions made to derive the optimum equations are valid or invalid are given in Table III.

In conclusion, MECC has been classified into three cases, depending on the migration mobility of the micelles. In case I, we have $\mu_{mc} < 0$. This case is the same as that which Terabe has referred to as the negative t_{mc}/t_o case (*14*). To meet this condition, it is required that the effective migration mobility of solute, μ^*_s, must be greater than zero. It has been shown that an asymptotic maximum exists, which makes the resolution and the migration time infinity. The condition for asymptotic maxima is $\mu_{EO} = -\bar{\mu}^*_{EP}$. Hence, here, MECC is very similar to capillary zone electrophoresis in which the ultimate resolution is obtained by setting $\mu_{EO} = -\bar{\mu}_E$. If this condition is exactly met, the resolution and the migration time approach infinity. For cases II and III, where $\mu_{mc} = 0$ and $\mu_{mc} > 0$, it is shown that a local maximum exists for each case to optimize the resolution. For practical purposes, the optimum range of $\tilde{k}'$ for good resolution and resolution per unit time is between 2 and 5 which is similar to that for column chromatography.

GLOSSARY

d	intermicelle distance
D	diffusion coefficient of a solute
D_{aq}	diffusion coefficient of a solute in an aqueous phase
D_{mc}	diffusion coefficient of a solute in a micellar phase
$\tilde{k}'$	capacity or retention factor = n_{sp}/n_{mp}
k_d	desorption rate constant of a solute from a micelle
L	length of capillary across which voltage is applied
N	separation efficiency; number of theoretical plates
n_{mp}	number of moles of solute in the mobile phase
n_{sp}	number of moles of solute in the stationary phase
R_S	resolution = $\Delta t/W_{AVG}$
t_o	migration time for an unretained solute
t_{mc}	migration time for a micelle
t_S	migration time for a solute in CZE
v	velocity of migration
V	applied voltage
α	selectivity; $\alpha = \tilde{k}'_B/\tilde{k}'_A$
μ_{EO}	electroosmotic mobility
μ_{EP}	electrophoretic mobility of a charged solute
$\mu_{EP,A}$	electrophoretic mobility of charged solute A
$\bar{\mu}_{EP}$	average electrophoretic mobility of two charged solutes A and B
μ_{MC}	net migration mobility of a micelle = $\mu_{EP,micelle} + \mu_{EO}$
$\mu^*_{EP,A}$	effective electrophoretic mobility of a neutral solute = $\mu_{EP}[\tilde{k}'/(1+\tilde{k}')]$
$\bar{\mu}^*_{EP}$	average effective electrophoretic mobility of two neutral solutes
μ^*_s	effective net migration mobility of a neutral solute = $\mu^*_{EP} + \mu_{EO}$

APPENDIX

According to Terabe et al., there are five causes of band broadening and the overall column plate height is given as

$$H = H_l + H_{mc} + H_{aq} + H_t + H_{ep} \quad (A1)$$

in which H_l, H_{mc}, H_a, H_t, and H_{ep} are plate heights generated by longitudinal diffusion, sorption–desorption kinetics of micellar solubilization, intermicelle mass transfer in the aqueous phase, radial temperature gradient effect on electrophoretic velocity, and electrophoretic dispersion of the micelles, respectively. Of course, it needs to be mentioned that these causes of band broadening are not exclusive according to Terabe et al. (*13*). An expression for the plate height contribution due to longitudinal diffusion (H_l) in conventional open capillary chromatography can be derived based on Einstein's law of diffusion. In that case, H_l is simply twice the diffusion coefficient (D) of the solute in the mobile phase divided by the mobile phase velocity (*4*). Since all solutes, regardless of their retention, spend the same length of time in the mobile phase, there is no dependence on a retention parameter. But in MECC all solutes do not spend the same length of time in the mobile phase (*12*). A more

Table III

$\mu_{EO}/\mu_{mc} = t_{mc}/t_o$	$\mu_{mc}/\mu_{EO} = t_o/t_{mc}$	ref 18 $\tilde{k}'_{opt}$(maximum $R_s = (t_{mc}/t_o)^{1/2}$	ref 18 $\tilde{k}'_{opt}$(maximum R_s/t_R)	present work $\tilde{k}'_{opt}$(maximum R_s)	present work $\tilde{k}'_{opt}$ (maximum R_s/t_R) = 2
1.5	0.67	1.22	1.2	0.84	2
2	0.5	1.41	1.19	1.24	2
3	0.33	1.73	1.31	2.19	2
4	0.25	2	1.39	3.14	2
5	0.2	2.24 approximate	1.45 approximate	3.53 valid	2
6	0.17	2.45 assumption,	1.50 assumption,	3.53 assumption	2
7	0.14	2.65 N independent of $\tilde{k}'$,	1.55 N independent of $\tilde{k}'$,	3.88	2 valid
8	0.13	2.83 close results	1.57 close results	4.08	2 assumption
9	0.11	3 to present work	1.60 to present work	4.43	2
10	0.1	3.16	1.63	4.66	2
15	0.07	3.87	1.72	5.74	2
25	0.04	5.00	1.81	7.40	2
100	0.01	10 invalid	1.94 valid	14.57 invalid	2
500	0.002	2.36 assumption,	1.99 assumption	32.09 assumption,	2
∞	0	∞ invalid results	2	∞ invalid results	2

complicated MECC expression for H_l, that includes the micelle solubilized solute, has been derived by Terabe et al. (*13*) to be

$$H_l = \frac{2(D_{aq} + \tilde{k}'D_{mc})}{1 + (t_o/t_{mc})\tilde{k}'} \frac{1}{v_{EO}} \quad \text{(A2)}$$

where D_{aq} and D_{mc} are the diffusion coefficients of the solute in the aqueous phase and in the micelle, respectively. From the above equation, if $D_{mc} = 0$ and t_{mc} goes to infinity, which is the case for column chromatography with stationary phse, the result is $H_l = 2D/v_{EO}$. This is simply the longitudinal diffusion component of the Van Deemter equation (*10*, *11*, *19*). Since $2D$ is usually represented with B (*11*), B' is chosen to represent the coefficient of H_l. If

$$B = B_M + B_S, \quad B_M = 2(D_{aq}), \quad \text{and} \quad B_S = 2(\tilde{k}'D_{mc}) \quad \text{(A3a)}$$

where

$$t_o/t_{mc} = 0$$

then

$$B' = \frac{2(D_{aq} + \tilde{k}'D_{mc})}{1 + (t_o/t_{mc})\tilde{k}'} = \frac{B}{1 + (t_o/t_{mc})\tilde{k}'} \quad \text{(A3b)}$$

B' also can be given based on two components, $B' = B'_M + B'_S$ where

$$B'_M + \frac{D_{aq}}{1 + (t_o/t_{mc})\tilde{k}'} \quad \text{and} \quad B'_S = \frac{2(\tilde{k}'D_{mc})}{1 + (t_o/t_{mc})\tilde{k}'}$$

$$H_l = \frac{B'}{v_{EO}} = \frac{B'_M + B'_S}{v_{EO}} \quad \text{(A4a)}$$

and

$$v_{EO} = \mu_{EO}V/L \quad \text{(A4b)}$$

The term v_{EO} can be substituted from eq A4b in eq A4a, so we obtain H_l in terms of the electroosmotic mobility and the voltage applied across the capillary

$$H_l = \frac{B'L}{\mu_{EO}V} = \frac{(B'_M + B'_S)L}{\mu_{EO}V} \quad \text{(A5)}$$

The term representing sorption–desorption kinetics of micellar solubilization (H_{mc}) also has been derived by Terabe et al. (*13*) and is given as

$$H_{mc} = \frac{2(1 - t_o/t_{mc})^2\tilde{k}'}{[1 + (t_o/t_{mc})\tilde{k}'](1 + \tilde{k}')^2} \frac{v_{EO}}{k_d} \quad \text{(A6)}$$

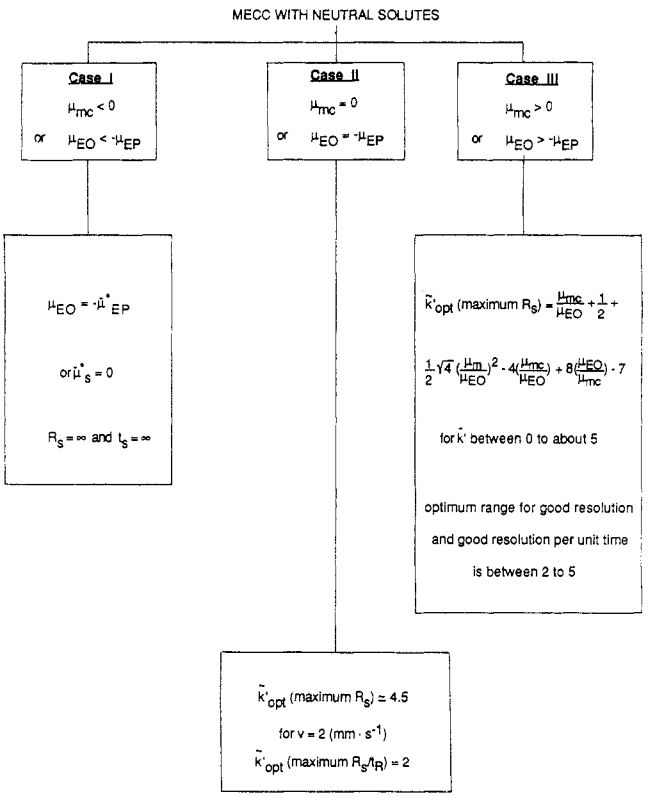

Figure 1. Summary of three modes of operation for MECC with their optimum ranges of operation.

where k_d is the desorption rate constant. It can be seen for stationary phase, t_{mc} again goes to infinity, and

$$H_{mc} = \frac{2\tilde{k}'}{(1 + \tilde{k}')^2} \frac{v_{EO}}{k_d} \quad \text{(A7)}$$

This equation is the same as H_S in column chromatography with solid stationary phase (*10*), where $1/k_d = t_d$ and t_d is the average desorption time of the analyte from the surface

$$H_s = C_S v = \frac{2kt_d}{(1 + k)^2} v \quad \text{(A8)}$$

where C_S is the coefficient for mass transfer to and from the solid stationary phase in column chromatography. C'_S is chosen to show the coefficient of H_{mc} (eq A6) such that

$$H_{mc} = C'_S v_{EO} \quad \text{(A9)}$$

v_{EO} can be substituted from eq A4b in eq A9. So we obtain H_{mc} as

$$H_{mc} = C'_S \mu_{EO} V/L \quad \text{(A10)}$$

The expression for the plate height contribution of intermicelle diffusion in the aqueous phase, H_{aq}, also has been derived by Terabe et al. (*13*) and it is given as

$$H_{aq} = \left(\frac{\tilde{k}'}{1+\tilde{k}'}\right)^2 \frac{(1 - t_o/t_{mc})^2}{1 + (t_o/t_{mc})\tilde{k}'} \frac{d^2 v_{EO}}{4D_{aq}} \quad \text{(A11)}$$

where d is the intermicelle distance and D_{aq} is the diffusion coefficient of the solute in the aqueous phase. For the stationary phase when the migration velocity of the micelles is zero, t_{mc} becomes infinity and we get

$$H_{aq} = \left(\frac{\tilde{k}'}{1+\tilde{k}'}\right)^2 \frac{d^2 v_{EO}}{4D_{aq}} \quad \text{(A12)}$$

The parameter $[\tilde{k}'/(1+\tilde{k}')]^2(d^2 v_{EO}/4D_{aq})$ could be considered to be C_M, which is the coefficient in $H_M = C_M v_{EO}$ in column chromatography, where H_M is the contribution plate height due to mass transfer to the mobile phase with stationary phase in column chromatography. C'_M is chosen to show the coefficient of H_{aq} (eq A11) such that

$$H_{aq} = C'_M v_{EO} \quad \text{(A13)}$$

v_{EO} can be substituted from eq A4b in eq A13. So we get

$$H_{aq} = C'_M \mu_{EO} V/L \quad \text{(A14)}$$

The temperature gradient contribution to the plate height given by Terabe et al. (*13*) is as follows

$$H_t = \frac{(1 - t_o/t_{mc})\tilde{k}'}{24(D_{aq} + \tilde{k}'D_{mc})} \frac{B^2 I^4}{64 k_o^2 \pi^4 r_c^2 \lambda^2 T_o^4} v_{EO} \quad \text{(A15)}$$

where r_c is the radius of column, λ and k_o are electrical and thermal conductivities of the solution, respectively, I is current, and T_o is the temperature of the solution. The coefficient of v_{EO} in eq A15 is chosen to be D'. This term does not have a counterpart in the Van Deemter equation. The coefficient of eq A15 is chosen to be D' such that

$$H_t = D' v_{EO} \quad \text{(A16)}$$

Substituting eq A4b in eq A16 in previous cases, we obtain

$$H_t = D' \mu_{EO} V/L \quad \text{(A17)}$$

The electrophoretic dispersion contribution to the plate height (H_{ep}) has been obtained by Terabe et al. (*13*). This term is the dominant term in plate height for higher electroosmotic velocities

$$H_{ep} = \frac{0.026(1 - t_o/t_{mc})^2 \tilde{k}'}{1 + (t_o/t_{mc})\tilde{k}'} \frac{v_{EO}}{k_d} = E' v_{EO} \quad \text{(A18)}$$

(Equation A18 is valid only for SDS solutions, because the coefficient 0.026 is an estimated value for the SDS micelle (*13*).)

For t_{mc} equal to infinity for the stationary phase case, H_{ep} is given as

$$H_{ep} = 0.026(\tilde{k}'/k_d) v_{EO} = E' v_{EO} \quad \text{(A19)}$$

This term similarly does not have a counterpart in the Van Deemter equation. When $v_{EO} = \mu_{EO} V/L$ is substituted in eq A19, we get

$$H_{ep} = E' \mu_{EO} V/L \quad \text{(A20)}$$

The number of theoretical plates for all contributions is obtained by combining eqs A5, A8, A10, A14, and A20, with $N = L/H$, then

$$N = \frac{L^2 \mu_{EO} V}{(B'_M + B'_S)L^2 + (C'_S + C'_M + D' + E')\mu_{EO}^2 V^2} \quad \text{(A21)}$$

LITERATURE CITED

(1) Virtanen, R. *Acta Polytech. Scand.* **1974**, *123*, 1–67.
(2) Mikkers, F. E. P.; Everaerts, F. M.; Verheggen, Th. P.E.M. *J. Chromatogr.* **1979**, *169*, 11–20.
(3) Rice, C. L.; Whitehead, R. *J. Phys. Chem.* **1965**, *69*, 4017–4024.
(4) Giddings, J. C. *Sep. Sci.* **1969**, *4*, 181–189.
(5) Jorgenson, J.; Lukacs, K. D. *Anal. Chem.* **1981**, *53*, 1298–1302.
(6) Terabe, S.; Otsuka, K.; Ichikawa, K.; Tsuchiya, A.; Ando, T. *Anal. Chem.* **1984**, *56*, 111–113.
(7) Terabe, S.; Otsuka, K., Ando, T. *Anal. Chem.* **1985**, *57*, 834–841.
(8) Ewing, A. G.; Wallingford, R. A.; Olefirowicz, T. M. *Anal. Chem.* **1989**, *61*, 292A–303A.
(9) Terabe, S. *TrAC, Trends Anal. Chem.* **1989**, *8*, (4), 129–134.
(10) Skoog, D. A. *Principles of Instrumental Analysis*; Saunders, College Publishing: New York, 1985.
(11) Karger, B. L.; Snyder, L. R.; Horvath, C. *Introduction to Separation Science*; Wiley: New York, 1973.
(12) Sepaniak, M. J.; Cole, R. O. *Anal. Chem.* **1987**, *59*, 472–476.
(13) Terabe, S.; Otsuka, K.; Ando, T. *Anal. Chem.* **1989**, *61*, 251–260.
(14) Terabe, S.; Utsumi, H.; Otsuka, K.; Ando, T.; Inomata, T.; Kuze, S.; Hanaoka, Y. *HRC & CC, High Resolut. Chromatogr. Chromatogr. Commun.* **1986**, *9*, 666–670.
(15) Row, K. H.; Griest, W. H.; Maskarinec, M. P. *J. Chromatogr.* **1987**, *409*, 193–203.
(16) Fujiwara, S.; Honda, S. *Anal. Chem.* **1987**, *59*, 2778–2776.
(17) Fujiwara, S.; Iwase, S.; Honda, S. *J. Chromatogr.* **1988**, *447*, 133–140.
(18) Foley, J. P. *Anal. Chem.* **1990**, *62*, 1302–1308.
(19) Van Deemter, J. J.; Zuiderweg, F. J.; Klinkenberg, A. *Chem. Eng. Sci.* **1956**, *5*, 271–289.

RECEIVED for review February 20, 1990. Accepted September 5, 1990.