

Micellar electrokinetic capillary chromatography theory based on conventional chromatography

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ABSTRACT

From the definitions of retention time (t_R) and resolution (R_s) in conventional chromatography, two fundamental equations for the retention behaviour and resolution of neutral solutes are derived and proved to be valid in all cases of micellar electrokinetic capillary chromatography (MECC). Two parameters, phase velocity ratio (P_r) and column availability (A_{co}), are introduced to reveal clearly the relationships and differences between MECC and conventional chromatography. The t_R and R_s values may be either positive or negative in MECC. A negative t_R indicates that the solute migrates toward the positive electrode and a positive t_R toward the negative electrode. $R_s > 0$ means that the solute with a smaller value of the capacity factor (k') in the pair of solutes reaches the detector first, while $R_s < 0$ means that the elution order is the opposite. MECC can be classified into eight cases depending on the values of P_r for convenience of discussion. So far, MECC was usually performed in case IV and the resolution was poorer than that in conventional chromatography for given values of theoretical plate number, selectivity and k' . However, a better resolution can be obtained in cases II, VI and VIII when $P_r < (1 - k')/2$. Cases VI, VIII and II are preferable to case IV for high resolution and should be more frequently employed in the future.

INTRODUCTION

Since micellar electrokinetic capillary chromatography (MECC) was first introduced to extend the power of capillary electrophoresis to the separation of neutral solutes by Terabe *et al.* in 1984 [1], it has exhibited great potential as an effective liquid separation technique [2,3]. With the rapid increase in applications, the basic theory to summarize and predict experimental results is desired, and is also necessary for this new technique to be widely accepted. In a

theoretical approach, the distribution mechanism and two fundamental equations to describe the retention behaviour and resolution of neutral solutes have been put forward by Terabe and co-workers [1,4]:

$$t_R = \frac{1 + k'}{1 + (t_0/t_{mc})k'} \cdot t_0 \quad (1)$$

$$R_s = \frac{N^{1/2}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'_2}{1 + k'_2} \cdot \frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})k'_1} \quad (2)$$

where t_R is the retention time of a solute, k' is the capacity factor (moles of the solute in the micellar phase/moles the aqueous phase), t_0 and t_{mc} are the retention times of the aqueous and micellar phases, respectively, R_s is the resolution

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of a pair of solutes, subscripts 1 and 2 refer to the two solutes with $k'_1 < k'_2$, α is the selectivity (k'_2/k'_1) and N is the theoretical plate number.

Based on the eqns. 1 and 2, the retention behaviour and resolution in MECC has been widely investigated [4–9]. However, because eqns. 1 and 2 were derived under the assumption $0 < t_0/t_{mc} < 1$ [1,4], most discussions have been restricted within these narrow limits. In fact, eqns. 1 and 2 can be applied for all values of t_0/t_{mc} from $-\infty$ to ∞ , which will be expounded in this paper. It can be seen from eqns. 1 and 2 that t_0/t_{mc} can affect the separation more significantly than k' . In some MECC analyses, a negative t_0/t_{mc} was demonstrated to be preferable to positive values for high resolution [10–13]. However, the theoretical explanation of the retention and resolution in terms of t_0/t_{mc} was inadequate. Gareil [8] developed three sets of retention and resolution equations for the three cases of MECC classified according to the value of t_0/t_{mc} from $-\infty$ to ∞ . However, there was a lack of mathematical evidence and continuity [8]. It still remains ambiguous over what range of values t_0/t_{mc} should be employed for higher resolution.

In this paper, eqns. 1 and 2 are mathematically derived from the definitions of t_R and R_s in conventional chromatography without any assumptions. To reveal clearly the relationships and differences between MECC and conventional chromatography, the parameters phase velocity ratio, column availability and virtual column length are introduced, and then concise forms of eqns. 1 and 2 are presented. The retention behaviour and resolution in all cases of MECC are systematically discussed in comparison with those in conventional chromatography, and some conditions for MECC are suggested for improving the resolution.

As the separation is also based on the distribution between two phases, MECC naturally belongs to chromatographic systems according to the definition of chromatography [14]. The fundamental difference between MECC and conventional chromatography is only that both of the phases in MECC are moving. Hence the theory of MECC can be deduced on the basis of conventional chromatography.

THEORY AND DISCUSSION

Retention behaviour

One of the two phases in MECC is the bulk aqueous solution, migrating at a velocity strictly determined by the electroosmotic flow. The other phase is the micelles, migrating at a velocity (V_{mc}) determined by the sum of the electroosmotic velocity (V_{eo}) of the bulk solution and the electrophoretic velocity of the micelles (V_{ep}):

$$V_{mc} = V_{eo} + V_{ep} \quad (3)$$

The velocity is positive when the migration is towards the negative electrode and negative when it is towards the positive electrode [4,5,10]. In this instance, V_{eo} , V_{mc} and V_{ep} may be either positive or negative.

The definition of retention time in MECC is identical with that in conventional chromatography, that is, the time when just half amount of a solute has been eluted from the column or migrated away from the detector. At this moment the following equation is true:

$$V_{R,aq} C_{aq} + V_{R,mc} C_{mc} = V_{c,aq} C_{aq} + V_{c,mc} C_{mc} \quad (4)$$

where $V_{c,aq}$ and $V_{c,mc}$ are the volumes of the aqueous and micellar phases in the column, respectively, $V_{R,aq}$ and $V_{R,mc}$ are the retention volumes of the aqueous and micellar phases, respectively, and C_{aq} and C_{mc} are the equilibrium concentrations of the solute in the aqueous and micellar phases, respectively. The right-hand side of eqn. 4 represents the amount of the solute still remaining in the column and the left-hand side represents that having been eluted from the column. In contrast with conventional chromatography, the left-hand side of eqn. 4 has one more term, because the two phases both flow out the column. Eqn. 4 can be rewritten as

$$\frac{V_{R,aq}}{V_{c,aq}} + \frac{V_{R,mc}}{V_{c,aq}} \cdot \frac{C_{mc}}{C_{aq}} = 1 + \frac{V_{c,mc}}{V_{c,aq}} \cdot \frac{C_{mc}}{C_{aq}} \quad (5)$$

It is evident that $V_{R,aq}/V_{c,aq} = t_R/t_0$ and the right-hand of eqn. 5 is equal to $1 + k'$, as in conventional chromatography. The second term on the left-hand side can be expressed as

$$\begin{aligned} \frac{V_{R,mc}}{V_{c,aq}} \cdot \frac{C_{mc}}{C_{aq}} &= \frac{t_R F_{mc}}{V_{c,aq}} \cdot \frac{C_{mc}}{C_{aq}} \\ &= \frac{t_R}{V_{c,aq}} \cdot \frac{V_{c,mc}}{t_{mc}} \cdot \frac{C_{mc}}{C_{aq}} = \frac{t_R}{t_{mc}} \cdot k' \end{aligned} \quad (6)$$

where F_{mc} is the volume flow velocity of the micellar phase. By combination with eqn. 5, the fundamental retention equation is derived:

$$t_R = t_0(k' + 1) \cdot \frac{P_r}{(k' + P_r)} \quad (7)$$

where P_r is the phase velocity ratio and defined as

$$P_r = \frac{V_{eo}}{V_{mc}} = \frac{t_{mc}}{t_0} \quad (8)$$

It is worth noting that there are no assumptions in the above derivation and eqn. 7 is valid in all cases of MECC. Eqn. 7 is equivalent to eqn. 1 but more concise by using P_r instead of t_{mc}/t_0 . The phase velocity ratio (P_r) highlights the relationships and difference in retention behaviour between MECC and conventional chromatography. P_r is the decisive parameter that determines the retention characteristics of

MECC. Hence MECC can be classified into eight cases according to the value of P_r for convenience of discussion, as shown in Table I. This classification is made so that the cases of MECC change in turn with the continuously changing experimental conditions.

It can be seen from eqn. 7 that the retention time may be negative in MECC. A negative t_R has been accepted in the literature [5,10,12,13], although it is unnatural in conventional chromatography. A negative t_R indicates that the solute migrates towards the positive electrode and a positive t_R towards the negative electrode [5]. The migration direction of a solute can be seen straightforward from the signs of t_R and the polarity of power supply does not have to be specified. The absolute value of t_R indicates only the time that a solute takes to elute out of the column, if the power polarity is suitable. Hence adding signs to t_R in MECC is not only rational in theory but also meaningful in practice.

Almost all the discussions on t_R and R_s have been in terms of capacity factor [4–9]. In fact, the effect of the phase velocity ratio is more significant. Hence plots of t_R versus P_r are shown in Fig. 1, according to eqn. 7. The outline of the retention behaviour of MECC can be seen from

TABLE I

CHARACTERISTICS OF THE RETENTION BEHAVIOUR AND COLUMN AVAILABILITY (A_{co}) IN MECC

Subscripts 1 and 2 refer to a pair of solutes with $k'_1 < k'_2$. MECC is classified into eight cases depending on the value of the phase velocity ratio (P_r).

Case	P_r	Retention behaviour			A_{co}
		Elution order ($t_0 > 0$)	Elution order ($t_0 < 0$)	Absolute value of t_R	
I	0	$0 < t_{mc} < t_{R,2} < t_{R,1} < t_0$	$t_0 < t_{R,1} < t_{R,2} < t_{mc} < 0$	$ t_R < t_0 $	$-1/k'_1$
II	0–1	$0 < t_{mc} < t_{R,2} < t_{R,1} < t_0$	$t_0 < t_{R,1} < t_{R,2} < t_{mc} < 0$	$ t_R < t_0 $	$-1/k'_1 - 0$
III	1	$t_{mc} = t_{R,2} = t_{R,1} = t_0$	$t_0 = t_{R,1} = t_{R,2} = t_{mc}$	$ t_R = t_0 $	0
IV	1–∞	$0 < t_0 < t_{R,1} < t_{R,2} < t_{mc}$	$t_{mc} < t_{R,2} < t_{R,1} < t_0 < 0$	$ t_R < t_0 (1 + k')$	0–1
V	–∞, ∞	$0 < t_0 < t_{R,1} < t_{R,2} < \infty$	$-\infty < t_{R,2} < t_{R,1} < t_0 < 0$	$ t_R = t_0 (1 + k')$	1
VI	–∞ to $-k'_2$	$0 < t_0 < t_{R,1} < t_{R,2} < \infty$	$-\infty < t_{R,2} < t_{R,1} < t_0 < 0$	$ t_R > t_0 (1 + k')$	1–∞
VII	$-k'_2$ to $-k'_1$	$t_{R,2} < 0 < t_{R,1}$	$t_{R,1} < 0 < t_{R,2}$	$ t_R > t_0 (1 + k')$	–∞–0
VIII	$-k'_1 - 0$	$-\infty < t_{R,1} < t_{R,2} < t_{mc} < 0$	$0 < t_{mc} < t_{R,2} < t_{R,1} < \infty$	$ t_R > t_0 < -k'/2$	$A_{co} < -1$ when $P_r < (1 - k'_1)/2$

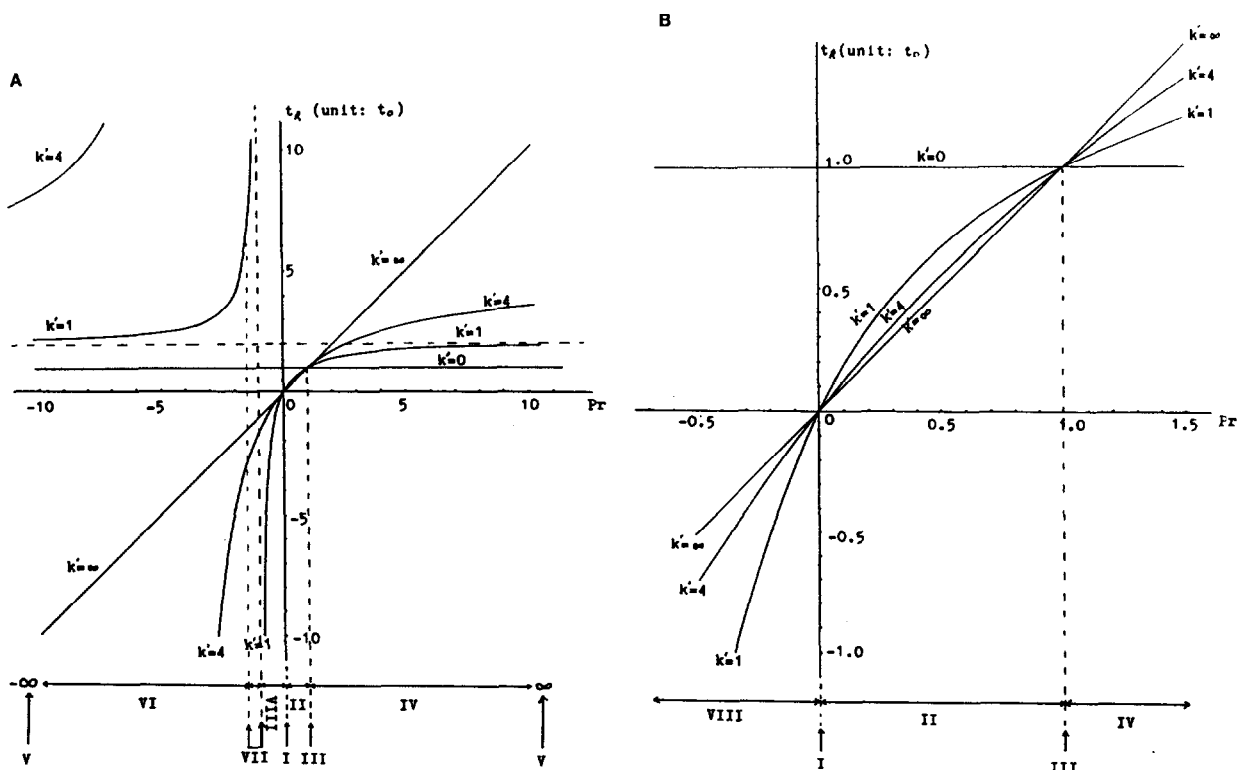


Fig. 1. Dependence of retention time on phase velocity ratio (P_r). The section for case II in (A) is expanded tenfold in (B). The values of the capacity factor are given on each line. The classifications of cases VI–VIII refer to a pair of solutes with $k'_1 = 1$ and $k'_2 = 1.5$. The units on the ordinate are relative to the retention time of the aqueous phase (t_0).

Fig. 1 and the details in each instance are discussed below. Some retention characteristics of the eight cases are summarized in Table I.

Case I: $P_r = 0$. In this case, the aqueous phase is immobile in contrast with the too fast moving micellar phase (see eqn. 8). As $P_r = 0$ and t_0 approaches infinity, the retention equation should be rewritten as $t_R = t_{mc}(1 + k')/k'$ by substituting $P_r t_0 = t_{mc}$ and $P_r = 0$ into eqn. 7. Because the unit of t_R is set as t_0 in Fig. 1, all the lines pass through the origin and the details of the retention behaviour cannot be seen.

Case II: $0 < P_r < 1$. It can be seen from Fig. 1B that the solutes with larger values of k' move faster than those with smaller values of k' , thus having shorter retention times in this case, similarly to case I. The elution order in cases I and II seemingly contradicts that in conventional chromatography, but actually it does not. In fact, the micellar phase should be considered as the

mobile phase and the aqueous phase as the stationary phase in cases I and II, because the micellar phase moves faster than the aqueous phase ($|V_{mc}| = |V_{co}|/P_r > |V_{co}|$). With this consideration, the dependence of t_R on k' would be similar to that in conventional chromatography if the capacity factor was defined as $V_{c,aq} C_{aq} / V_{c,mc} C_{mc}$.

Case III: $P_r = 1$. All the solutes migrate at the same velocity and there is no separation in this case, because the velocity of the micellar phase is equal to that of the aqueous phase and there is no relative movement of the two phases. Substitution of $P_r = 1$ into eqn. 7 yields $t_R = t_0$.

Case IV: $1 < P_r < \infty$. This case is the most frequently encountered in MECC analyses. The retention behaviour in this case has been widely investigated [4–9], which can also be seen from Fig. 1 and Table I.

Case V: $P_r = -\infty$ and ∞ . The micellar phase

(pseudo-stationary phase) really becomes stationary in this case, as $V_{mc} = 0$. Hence the situation in this case is identical with that in conventional chromatography. Eqn. 7 turns into the well known equation in conventional chromatography by substituting $P_r = \infty$ into eqn. 7.

Case VI: $-\infty < P_r < -k'_2$. The micellar phase moves in the direction opposite to the aqueous phase in this and the following cases ($P_r < 0$), whereas in cases I–V the two phases move in the same direction ($P_r > 0$). It should be noted that the boundary conditions in this and the following cases are dependent on the capacity factors of the solutes. The discussion here refers to a pair of solutes with $k'_1 < k'_2$. Those solutes with $k' > -P_r$ cannot be detected in this case and their situations belong to case VIII, because they migrate in the opposite direction to the electroosmotic flow. By substituting the inequality $-\infty < P_r < -k'$ in eqn. 7, we obtain $|t_R| > |t_0|(1 + k')$, whereas in case IV $|t_R| < |t_0|(1 + k')$. This means that the retention time in this case is longer than that in case IV.

Case VII: $-k'_2 \leq P_r \leq -k'_1$. By consideration of the range of P_r in eqn. 7, one obtains that $t_{R,1}$ is of the same sign as t_0 but $t_{R,2}$ is not. The pair of solutes do not migrate in the same direction. When $P_r = -k'_2$ or $P_r = -k'_1$, $t_{R,2}$ or $t_{R,1}$ approaches infinity. Hence this case is not acceptable for common separations.

Case VIII: $-k'_1 < P_r < 0$. By substituting the inequality $-k' < P_r < 0$ in eqn. 7, we obtain that t_R has the opposite sign to t_0 . In this case, the migration of the solute forced by the electroosmotic flow cannot compensate for the opposite migration of the solute induced by the oppositely moving micellar phase. As a result, the solute migrates in the opposite direction to the electroosmotic flow. The detection end must be set opposite to the electroosmotic flow in this case, in contrast to the other cases. It can be performed simply by reversing the polarity of the power supply. The elution order in this case is similar to that in cases I and II, but contrary to that in the other cases and conventional chromatography, as Fig. 1 shows. In cases I, II and VIII the micellar phase will arrive first at the detector, so those solutes which are inclined to be solubilized by the micellar phase (larger value of k')

will elute early. Those solutes with $k' < -P_r$ migrate in the same direction as the electroosmotic flow and will not be detected in this case; their situations belong to case VI.

As an example, the elution order at $\text{pH} < 3.5$ was the reverse of that at $\text{pH} > 6$ in the literature [5,11], where the situation at the lower pH corresponded to case VIII and that at the higher pH to case IV. Another example is the reported separation of amines [12], where P_r was -2.5 according to our calculation. Thus, Dns-methylamine ($k' = 0.82$) and Dns-methyl- $^{2}\text{H}_3$ amine ($k' = 0.81$) migrated in the direction of the electroosmotic flow and were detected at the negative end, and the situation belonged to case VI ($P_r < -k'$), whereas the situation for Dns-hexylamine ($k' = 71.3$) and Dns-octylamine ($k' = 279$) belonged to case VIII ($P_r > -k'$) and the power polarity had to be reversed in order to detect them.

Resolution

The definition of the resolution (R_s) is identical with that in conventional chromatography [15]:

$$R_s = \frac{t_{R,2} - t_{R,1}}{2(\sigma_2 + \sigma_1)} \quad (9)$$

where subscripts 1 and 2 refer to the pair of solutes with $k'_1 < k'_2$ and σ is the standard deviation of the peak. By substituting eqn. 7 into eqn. 9 and using the same assumptions as in conventional chromatography [15], we obtain the approximate resolution equation of MECC:

$$R_s = \frac{N^{1/2}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'_2}{1 + k'_2} \cdot A_{co} \quad (10)$$

where A_{co} is the column availability, given by

$$A_{co} = \frac{P_r - 1}{P_r + k'_1} \quad (11)$$

Eqn. 10 is equivalent to eqn. 2 and valid in all cases of MECC. The column availability (A_{co}) is an important parameter that determines the characteristics of R_s in MECC, and its physical meaning will be discussed in the next section. Plots of A_{co} versus P_r are shown in Fig. 2 and

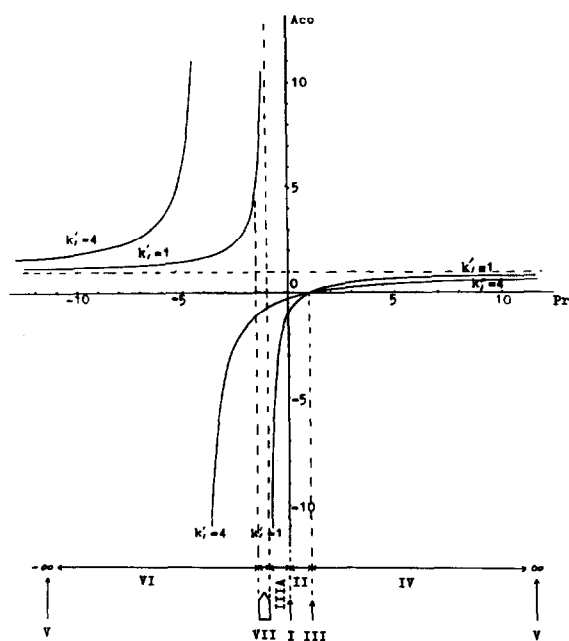


Fig. 2. Dependence of column availability (A_{co}) on phase velocity ratio (P_r). The values of the capacity factor are given on each line. Classifications of cases VI–VIII as in Fig. 1.

some characteristics of A_{co} in the eight cases are summarized in Table I. A_{co} indicates the relationships and differences in resolution between MECC and conventional chromatography, just as P_r does in retention behaviour.

In case I, the aqueous phase is stationary in contrast to the too fast moving micellar phase. The resolution is similar to that in conventional chromatography when the aqueous phase is considered as the stationary phase and the micellar phase as the mobile phase. Eqn. 10 would return to the style in conventional chromatography if the capacity factor was defined as $V_{c,aq}C_{aq}/V_{c,mc}C_{mc}$.

Substitution of $P_r = 1$ into eqn. 11 yields $A_{co} = 0$, and therefore there is no separation in case III. Case V corresponds to conventional chromatography, substitution of $P_r = \infty$ into eqn. 11 yielding $A_{co} = 1$. Case VII is useless for general separations because the pair of solutes will not elute at the same end.

MECC has usually been performed in case IV, where $0 < A_{co} < 1$, as Fig. 2 shows. The res-

olution resulting from the high plate number is partly offset by the column availability. Hence the resolution did not seem as good as expected when the plate number was as high as 40 000 [1]. Fortunately, better resolution can be obtained in cases VI, VIII and II.

By considering the range of P_r in case VI in eqn. 11, we have $A_{co} > 1$. This means that the resolution in case VI is always better than that in conventional chromatography for the given values of N , α and k' , which is the opposite of that in case IV. The improvement in resolution in case VI has been demonstrated experimentally in the literature [10,12]. The deuterated and non-deuterated compounds could be separated in case VI, whereas no separation was observed in the normal mode, case IV [12].

As Fig. 2 shows, R_s is negative in cases I, II and VIII. A negative R_s is unnatural in conventional chromatography, but is valid in MECC. A negative R_s means that the solute with the larger values of k' reaches the detector earlier than the other solute ($|t_{R,2}| < |t_{R,1}|$), whereas a positive R_s means $|t_{R,2}| > |t_{R,1}|$. The signs of R_s clearly indicate the elution order of the pair of solutes, while the absolute value of R_s indicates how good a separation is. It can also be seen from the definition of R_s (eqn. 9) that it will be negative when $t_{R,1} > t_{R,2}$.

To have a better resolution than conventional chromatography, A_{co} should be smaller than -1 in cases II and VIII. By substituting $A_{co} < -1$ into eqn. 11, we have

$$P_r < \frac{1 - k'_1}{2} \quad (12)$$

The P_r range in case VI ($P_r < -k'_2$) is naturally sufficient for the demand of inequality 12, where $A_{co} > 1$. The values of P_r in case IV ($1 < P_r < \infty$) do not meet the requirement of inequality 12, thus $A_{co} < 1$. Hence inequality 12 is the necessary and sufficient condition to obtain a better resolution than in conventional chromatography for a given value of N , α and k' . The resolution in MECC can be greatly enhanced by adjusting the experimental parameters to meet inequality 12, which may be carried out by control of V_{co} [10,16–18], V_{cp} and k' [4,19–21].

The enhancement of resolution in case VIII has also been demonstrated in the separation of racemic dansylated amino acids [13]. At pH 3 the enantiomers of Dns-DL-methionine ($k' = 1.13$ and 1.15) were completely separated, where the P_r was -0.69 and the situation belonged to case VIII. In contrast, no separation was observed at pH 7 (case IV).

Column availability

As the micellar phase is moving in the MECC procedure, the movement of a solute can be divided into two components. One is the migration of the solute relative to the micellar phase at a velocity V'_s , which is ascribed to the distribution of the solute between the micellar and aqueous phases. The other is that the solute moves with the micellar phase at a velocity V_{mc} , which does not contribute to the separation. We call the former component the effective movement and the latter the ineffective movement. The total migration velocity of the solute (V_s) is the sum of V'_s and V_{mc} :

$$V_s = \frac{L}{t_R} = V'_s + V_{mc} \quad (13)$$

where L is the apparent column length from the injection end to the detection point, sometimes called the effective length [13]. Substitution of eqns. 3 and 7 into eqn. 13 yields

$$V'_s = \frac{-V_{ep}}{1 + k'} \quad (14)$$

We define the virtual column length (L') as the distance that a solute has migrated relative to the micellar phase within the time t_R , or the difference between the distance the micellar phase has moved and the apparent column length:

$$L' = V'_s t_R = L - V_{mc} t_R \quad (15)$$

By substituting eqns. 7 and 14 into eqn. 15 and using eqn. 11, we obtain

$$L' = LA_{co} \quad (16)$$

The virtual column length corresponds to the actual distribution length a solute undergoes. In essence, the physical meaning of L' is identical with that of column length in conventional chro-

matography. However, L' may be either positive or negative, because the solute may migrate in two ways. A negative L' indicates that the solute moves more slowly than the micellar phase in the same direction and a positive L' that it moves faster.

Eqn. 16 can be rewritten as $A_{co} = L'/L$, so that column availability essentially represents the ratio of the distribution length to the apparent column length. $|A_{co}| < 1$ means that the actual distribution length that is useful for separation is shorter than the apparent column length, whereas $|A_{co}| > 1$ means that the actual distribution length is longer than the apparent column length, hence the resolution can be enhanced. $A_{co} = 0$ means that there is no distribution and therefore no separation.

CONCLUSIONS

The fundamental retention and resolution equations (eqns. 7 and 10) are concise with the introduction of phase velocity ratio and column availability, which highlight the relationships and differences between MECC and conventional chromatography. As the micellar phase may move either in the direction of or opposite to the aqueous phase, t_R and R_s may be either positive or negative. MECC can be classified into eight cases for discussion. Case I, III, V and VII are the mathematical limits, which may not be approached in practical analysis but relate the actual modes in an overall understanding of the separation behaviour. Case IV is the most common mode in the literature but the resolution is not as good as expected. A better resolution can be obtained by adjusting the experimental parameters to meet $P_r < (1 - k'_1)/2$ in cases VI, VIII and II. It should be noted that all the solutes may not elute at the same end in cases VI and VIII.

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