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Separation parameters via virtual migration distances in high-performance liquid chromatography, capillary zone electrophoresis and electrokinetic chromatography

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Abstract

Among the various differential migration processes of separation, high-performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) have emerged as the two major high-performance analytical techniques for separation of complex biological substances. In both HPLC and CZE with electroendoosmotic flow, the differential migration process can be divided into a separative component, which involves selective interactions with the stationary phase or differences in the electrophoretic migration velocities, and a non-separative component representing migration by convection that does not contribute directly to separation. The introduction of virtual migration distances leads to an additivity relationship for the two components that is applicable to both of the above techniques and facilitates the recognition of the underlying similarities as well as the expression and comparison of the various separation parameters. Examination of the key migration parameters led to the characterization and the classification of the various modes of CZE with electroendoosmotic flow. The treatment was extended to the analysis of capillary electrochromatography and micellar electrokinetic chromatography; two hybrid processes which exhibit features borrowed from HPLC and CZE. The use of virtual migration distances also led to a consistent and unified description of the characteristic parameters of these separation systems.

Keywords: Migration distances, virtual; Electrochromatography; Selectivity; Electrokinetic chromatography; Separation parameters; Retention factor; Electroendoosmotic flow

1. Introduction

The concept of differential migration as a separation and analytical technique dates back to the introduction of chromatography by Tswett [1] who was the first to recognize its major features and significance. In a differential migration process, the components of a mixture move in a suitable conduit with different velocities either by convection coupled with their distribution between a mobile and stationary phase or under the influence of a field. Separation occurs when their partitioning or interaction with the field is of different magnitudes. Among the differential migration processes, chromatography, electrophoresis, ultracentrifugation, field-flow fractionation and time of flight mass spectrometry are most prominent at present. A general treatment of this subject and a classification of differential migration processes have been given earlier by Strain et al. [2,3] and Giddings [4–7].

Recently, high-performance liquid chromatography (HPLC) and capillary zone electrophoresis

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(CZE), both based on the concept of differential migration, have emerged as the two major instrumental analytical techniques for separation of complex biological substances [8–11]. The term chromatography encompasses all processes which involve two phases, one stationary and one moving, and the migration velocity of the sample components is determined by their distribution between the two phases and the flow velocity [12]. In electrophoresis, charged molecules migrate in electric field with or without bulk flow [13]. Both HPLC and CZE employ highly sophisticated instruments that follow the paradigm set by the gas chromatograph and the separation of the sample components is brought about with high speed and resolution by using high pressure and high voltage gradient across the column, respectively.

Mobile phase flow is a characteristic feature of any chromatographic system and thus an integral part of the migration process in HPLC. On the other hand, in electrophoresis, bulk flow due to electroendoosmosis had long been considered an undesirable secondary feature [14], and only in CZE with fused-silica capillaries has the electroendoosmotic flow (EOF) been intentionally exploited to control the time of separation and even the direction in which the sample components migrate. For capillary electrophoresis in free solution, EOF is usually a part of the migration process which is then appropriately called "electrorheophoresis" [15], to distinguish it from classical electrophoresis that is carried out under essentially arheic conditions, i.e., in the absence of bulk flow.

Both in HPLC and CZE, the migration of the separands can be readily divided into two parts: one representing their selective interactions with the stationary phase or the electric field that is directly responsible for the separation and the other representing migration that does not contribute directly to the separation. The latter non-separative component of the migration process can be measured with an inert tracer, which is not subject to the fundamental interactions underlying the separation process, i.e., partitioning between the mobile and stationary phase in HPLC or electrophoretic migration in CZE. As we shall see in capillary electrochromatography (CEC) and micellar electrokinetic chromatography (MEKC), the complexity of the process, which combines features of HPLC and CZE, does not allow for a simple distinction between separative and non-separative migration.

Our goal is to treat linear chromatography and electrophoresis exemplified by HPLC and CZE in a unified fashion by using the concept of virtual migration lengths that leads to a simple additivity relationship of the two components of the migration process in both the techniques. Thereafter, the approach is extended to the more complex processes of CEC and MEKC in order to compare the prominent features of these analytical processes and of the expressions employed for the measurement of their efficiencies.

2. Separative and non-separative migration

In HPLC and CZE, separation occurs due to differences in the retention volumes, that are at constant flow conveniently represented by retention times, and the electrophoretic migration velocities of the separands, respectively. Yet, the time they spend in the mobile phase or the distance they move with EOF does not contribute directly to the separation because they are the same for all sample components. This distinction between separative and non-separative migration leads to expressions for the various separation parameters that are based on the additivity of the mobile and stationary phase holdup times in HPLC and the additivity of the electrophoretic and electroendoosmotic velocities in CZE. In the following, these additivity relationships are reviewed for HPLC and CZE to show that the natural framework for their expressions lies in the time and velocity domains, respectively.

2.1. High-performance liquid chromatography

In HPLC, the mobile phase flow and retention by the stationary phase take place in series so that the total migration time, t_R, is the sum of the times the eluite spends in the stationary and mobile phases

$$t_R = t_o + t'_R = t_o + k't_o \quad (1)$$

where t_o is the retention time of an unretained tracer, i.e., the holdup time in the mobile phase, t'_R is the adjusted retention time, i.e., the holdup time in the

stationary phase and k' is the chromatographic retention factor that, among others, expresses the mass distribution of the eluite between the two phases.

Dividing Eq. (1) by the migration distance, i.e., the length of the column, L_m, we have a relationship in terms of velocities

$$\frac{1}{u_b} = \frac{1}{u_o} + \frac{1}{u_o/k'} \tag{2a}$$

Rearranging Eq. (2a) we obtain that

$$u_b = u_o - k'u_b \tag{2b}$$

In the above equations u_b is the migration velocity of the eluite band, u_o is the velocity of an inert tracer, whereas, u_o/k' and $k'u_b$ are virtual velocities which account for the retention by the stationary phase. Thus, in HPLC, the use of the velocity frame does not allow for the expression of the separative and non-separative components of the migration process by a simple additivity relationship because it leads to the introduction of fictitious velocities that are coupled terms. In view of Eq. (1), the time domain offers the natural framework for such an additivity relationship in chromatography.

2.2. Capillary zone electrophoresis

In electrorheophoresis, the overall migration velocity, u_m, is the sum of the electrophoretic velocity of the charged separand, u_{ep}, and the electroendoosmotic velocity measured by a neutral tracer, u_{eo}, as both migration processes take place simultaneously. Thus

$$u_m = u_{ep} + u_{eo} \tag{3a}$$

where each velocity is a signed quantity and can be either positive or negative. The velocity of a migrant in electrophoresis is the product of its mobility and the applied electric field, so that Eq. (3a) can be expressed as

$$\mu_m = \mu_{ep} + \mu_{eo} \tag{3b}$$

where μ stands for the mobility of a migrant and the subscripts have the same meanings as in Eq. (3a). In terms of the migration times, Eq. (3a) can be written as

$$\frac{1}{t_m} = \frac{1}{t_{eo}} + \frac{1}{t_{ep}} \tag{4}$$

where t_m is the total migration time, t_{eo} is the migration time of a neutral tracer that traverses the capillary by EOF and t_{ep} is the migration time that would be observed if the charged separand traversed the capillary under arheic conditions, i.e., in the absence of EOF. The additivity of the reciprocal migration times has the same drawback in CZE as the additivity of the reciprocal velocities in HPLC, i.e., it does not allow for an uncoupling of the separative and non-separative migration components. Consequently, in CZE, the velocity is considered as natural frame for the additivity relationship and this is the reason for the sparse use of migration times, although they can be read off in most cases directly from the electropherogram.

3. Virtual migration distances

An additivity relationship for the separative and non-separative parts of the overall migration process that is equally applicable to HPLC and CZE arises naturally upon introduction of "virtual migration distances", that represent the product of the migration time and velocity. They are obtained by dividing the real migration distance, i.e., the appropriate length of the column, into two virtual distances: l_s and l_o. The first, l_s, represents the separative component of the overall migration process and thus it reflects the magnitude of the adjusted retention time, t'_R, in HPLC and that of the electrophoretic velocity in CZE. The other virtual migration distance, l_o, represents that part of the overall migration process that does not lead directly to separation and thus reflects the retention time of an inert tracer, i.e., the mobile phase holdup time, t_o, in HPLC and the velocity of an uncharged tracer, i.e., that of EOF, in CZE. By definition, the sum of the two virtual lengths, l_s and l_o, always equals the actual migration distance.

3.1. High-performance liquid chromatography

In HPLC, the virtual distance, l_s, representing separative migration is defined as

$$l_s = u_b t'_R = L_m\left(\frac{k'}{1+k'}\right) \quad (5)$$

where L_m is the actual migration distance, i.e., the column length. On the other hand, l_o, the virtual migration distance accounting for the holdup time of an inert tracer is given by

$$l_o = u_b t_o = L_m\left(\frac{1}{1+k'}\right) \quad (6)$$

In HPLC, both virtual lengths, l_s and l_o, are positive quantities and therefore, neither of them can be greater than the total migration distance.

The meaning of the virtual distances can be interpreted in a number of ways. For instance, Eqs. (5,6) can be written in dimensionless form as

$$\frac{l_s}{L_m} = \frac{t'_R}{t_R} = \frac{k'}{1+k'}$$

and

$$\frac{l_o}{L_m} = \frac{t_o}{t_R} = \frac{1}{1+k'}$$

The above two expressions show that the virtual length components represent the probabilities of the migrant being either in the stationary or in the mobile phase, respectively [12]. Fig. 1 illustrates the dependence of the two dimensionless virtual distance components on the retention factor in HPLC. Whereas, l_s increases first steeply then only slowly with k' and approaches unity when $k'>10$, l_o decreases with k' and becomes negligibly small when $k'>10$. The significance of the so called chromatographic retardation factor, $1/(1+k')$, and of the chromatographic migration factor, $k'/(1+k')$, and their effect on the separation has received great attention in the early literature of gas chromatography mainly with regard to the comparison of the separation efficiencies of packed and capillary columns [16].

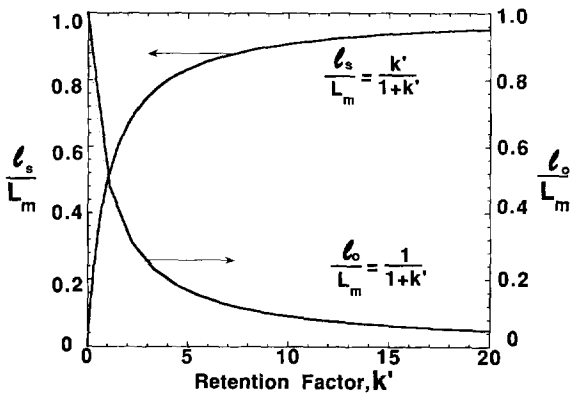

Fig. 1. Graph illustrating the dependence of the dimensionless virtual migration distances accounting for the separative, l_s/L_m, and non-separative, l_o/L_m, parts of the migration process against the retention factor, k', in HPLC.

3.2. Capillary zone electrophoresis

In CZE, the virtual distance associated with separative migration, l_s, is

$$l_s = u_{ep} t_m \quad (7)$$

whereas, the virtual migration distance, l_o, arising from EOF is

$$l_o = u_{eo} t_m \quad (8)$$

It is recalled that u_{eo} is the velocity of a neutral tracer that migrates with the EOF velocity.

Both l_s and l_o are signed quantities and their sign depends on the direction of the migration. When electrorheophoresis is codirectional, i.e., the electrophoretic velocity of the separands and the EOF have the same direction, both l_s and l_o have the same sign. In counterdirectional modes of CZE the signs of the two are opposite. Since the sum of l_s and l_o has to be equal to the total migration distance, L_m, if one of them is negative the other has to be positive and larger than L_m. Thus, the magnitude and sign of either of the two virtual distances reveal whether the electrorheophoretic process is co- or counterdirectional as will be discussed later.

Evidently, the virtual distances, l_s, have to be different for the sample components separated by HPLC or CZE and as a consequence the corresponding l_o values have also to be different. Such an interdependence of l_o on l_s may be counterintuitive since it arises from the fictitiousness of the virtual length domain used here to unify the natural additivity relationships of times and velocities in HPLC and CZE, respectively.

4. Fundamental migration parameters in HPLC and CZE

4.1. Chromatographic retention factor

In view of Eq. (1), the retention factor in HPLC can be expressed in terms of the two virtual length components as

$$k' = \frac{t_R - t_o}{t_o} = \frac{l_s}{l_o} \tag{9}$$

As mentioned before, the retention factors are always positive in chromatography, therefore, both l_s and l_o have to be positive.

4.2. Electrophoretic velocity factor

In CZE, the ratio of the two virtual lengths defines a migration parameter that is analogous to the chromatographic retention factor and is conveniently called the electrophoretic velocity factor, k'_e. It is given by

$$k'_e = \frac{l_s}{l_o} = \frac{u_{ep}}{u_{eo}} = \frac{t_{eo} - t_m}{t_m} \tag{10}$$

Since the direction of EOF can be the opposite of the electrophoretic migration of the separand, the velocity factor k'_e can be negative. Eq. (10) shows that the velocity factor is the ratio of the migration velocities of the charged and neutral sample components in CZE and can also be considered as the dimensionless electrophoretic velocity of a charged migrant normalized to the velocity of EOF.

4.3. Electromigration factor

Recently the electromigration factor, f_m, has been introduced by Reijenga and Kenndler [17] to express the relative contribution of electrophoretic mobility to the total mobility, i.e., the sum of the electrophoretic mobility of a separand and the mobility of a neutral tracer, as

$$f_m = \frac{\mu_{ep}}{\mu_{ep} + \mu_{eo}} \tag{11}$$

The electromigration factor can be expressed in terms of the virtual migration distance and the velocity factors as

$$f_m = \frac{l_s}{L_m} = \frac{k'_e}{1 + k'_e} \tag{12}$$

In our terminology f_m is the dimensionless virtual distance of separative migration that measures the separative fraction of the total column length.

Per analogy with the virtual length components in HPLC, defined in Eqs. (5,6), we find f_m in terms of the retention factor as

$$f_m = \frac{l_s}{L_m} = \frac{k'}{1 + k'} \tag{13}$$

Eq. (13) implies that the chromatographic equivalent of f_m is the migration factor, $k'/(1+k')$, that has wide currency in chromatography.

The results obtained in this section are summarized in Table 1.

Table 1
Characteristic migration parameters of a single component in HPLC and CZE

Parameter	HPLC	VMD	CZE
Chromatographic retention factor, k'	$(t_R - t_o)/t_o$	l_s/l_o	–
Electrophoretic velocity factor, k'_e	–	l_s/l_o	μ_{ep}/μ_{eo}
Electromigration factor, f_m	–	l_s/L_m	$\mu_{ep}/(\mu_{ep}+\mu_{eo})$
Chromatographic migration factor	$k'/(1+k')$	l_s/L_m	–

The middle column, VMD, shows the expressions in terms of the virtual migration distances that are applicable to both HPLC and CZE with EOF.

5. The four main operational modes of CZE

Unlike in HPLC, where the sample components and the mobile phase move in the same direction, in CZE we encounter four fundamentally different operational modes as far as the relative directions and magnitudes of the electrophoretic and electroendoosmotic migration velocities are concerned. The four modes and their characteristics, in terms of the sign and the magnitude of the corresponding key

Table 2
The four main modes of capillary zone electrophoresis characterized by the signs and limiting values of the key migration parameters

Mode	Velocity factor, k'_c		Electromigration factor, f_m		Virtual migration distances				Typical electropherogram shown in
					l_s (separative)		l_o (non-separative)		
	Sign	Magnitude	Sign	Magnitude	Sign	Magnitude	Sign	Magnitude	
A	+	∞	+	1	+	L_m	+	0	Fig. 3a
B	+	[0,∞]	+	[0,1]	+	[0,L_m]	+	[L_m,0]	Fig. 3b
C	−	[1,∞]	+	[∞,1]	+	[∞,L_m]	−	[∞,0]	Fig. 3c
D	−	[0,1]	−	[0,∞]	−	[0,∞]	+	[L_m,∞]	Fig. 3d

Mode A, arheic CZE; Mode B, codirectional CZE; Mode C, counterdirectional CZE with the mobility of the neutral tracer being the smallest; Mode D, counterdirectional CZE with the mobility of the neutral tracer being the greatest. The notation [a,b] means: from a to b, including both a and b.

migration parameters, including the virtual migration distances are given in Table 2. Figs. 2–4 are complementary to Table 2 and provide graphical illustration of the various features associated with the four main modes, A–D, of capillary zone electrorheophoresis.

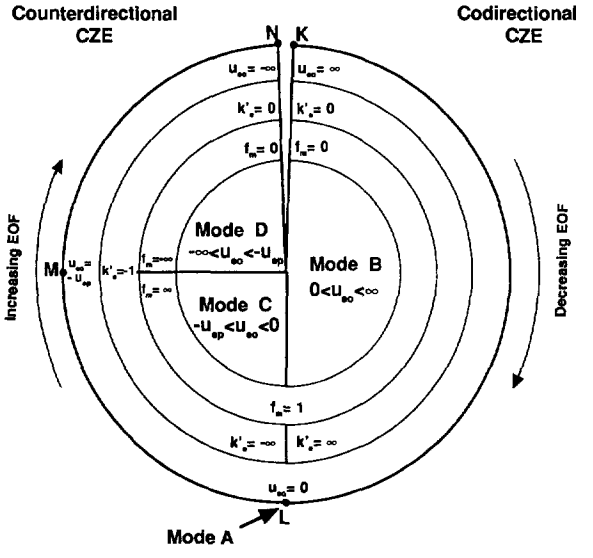

Fig. 2. Schematic illustration of the four main operational modes of CZE and the pertinent values of some key migration parameters. The right hand side of the chart represents the codirectional Mode B and the left hand side shows the two counterdirectional Modes C and D. Mode A lies at point L at the bottom. At a fixed electrophoretic velocity of the separand, the EOF decreases as we go clockwise from K to L. At point L, a transition occurs from the codirectional to counterdirectional CZE and as we move further clockwise the magnitude of the EOF increases in the opposite direction in Mode C. Point M where both EOF and electrophoretic migration have the same velocity is the transition point to Mode D, the other counterdirectional mode of CZE. Finally, at point N, the relative magnitude of EOF becomes very large in the direction opposite to that of electrophoretic migration.

The interrelationship between the various operational modes is revealed by Fig. 2, that shows the effect of changing EOF at a fixed value and sign of the electrophoretic velocity of the sample components. Our starting point is K, where the EOF velocity is codirectional and much higher than that of the electrophoretic migration ($u_{eo} \approx \infty$). As we move clockwise from point K along the chart that encompasses the four operational modes, the EOF diminishes and becomes zero at point L. Thus, the right hand side of the chart is the domain of Mode B, which encompasses codirectional CZE with the velocity factor increasing from zero to infinity and f_m reaching unity at point L. This point represents Mode A when there is no EOF, i.e., arheic conditions prevail. It is also the point of transition from codirectional to counterdirectional CZE where the EOF velocity and hence the velocity factor change sign. Moving further in clockwise direction, on the left side of the graph, we encounter sectors of Mode C and D, the two counterdirectional modes of CZE. The magnitude of EOF, the direction of which is now opposite to that of the electrophoretic velocity, increases in Mode C as we move from point L to point M, where the magnitude of the EOF becomes the same as that of the electrophoretic velocity and the velocity factor equals −1. Thus at M, which is the point of transition between Modes C and D, there is no migration due to the countervailing effect of the two velocities. The EOF velocity increases as we move along the sector of Mode D in clockwise

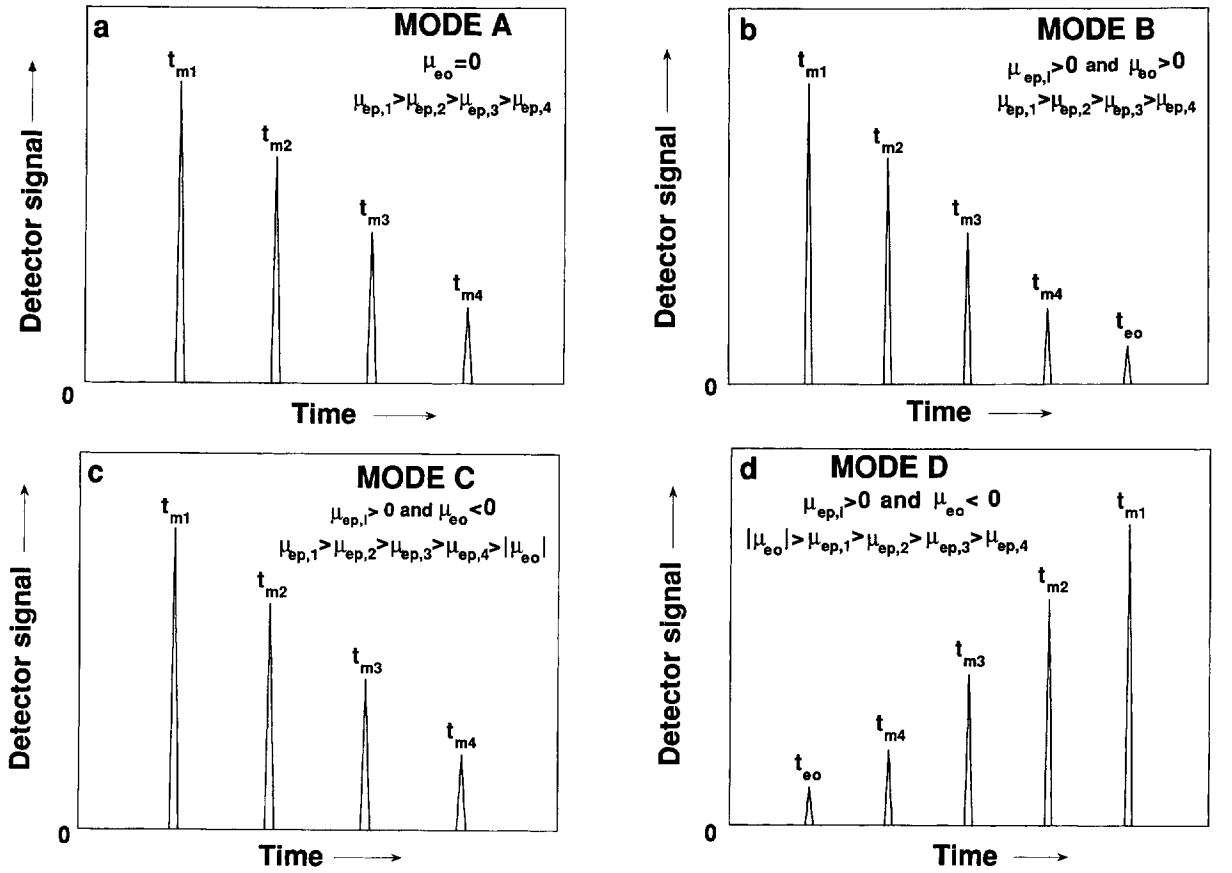

Fig. 3. Schematic illustration of electropherograms typical for the four main modes of CZE listed in Table 2. The conditions are stated on charts a to d for each mode: (a) arheic CZE; (b) codirectional CZE; (c) counterdirectional CZE when the neutral tracer migrates slower than any of the charged sample components; (d) counterdirectional CZE with the neutral tracer being the fastest migrating component.

direction and since it is higher than the electrophoretic velocity, the separands migrate in the direction of EOF, i.e., in the direction opposite to that of the electrophoretic velocity. Finally, we reach point N, where EOF becomes so high that both the velocity factor and electromigration factor diminish to zero. This corresponds to a situation where no separation occurs either because the separands are neutral or their electrophoretic velocity is negligibly small in comparison to the EOF velocity.

Another way to illustrate the four operational modes of CZE is by means of representative electropherograms as shown in Fig. 3. In Mode A, arheic conditions prevail and the sample components migrate solely by their electrophoretic velocity and appear in the order of their decreasing electrophoretic mobilities as depicted in Fig. 3a. In the absence of EOF, neutral sample components do not migrate, therefore, the velocity factor, k'_e, is infinitely large, f_m is unity and the non-separative component of the virtual length, l_o, is zero as shown in Table 2. The separative component of the virtual migration length is equal to the length of the column, i.e., l_o is zero, and this explains the absence of Mode A in Fig. 4.

Codirectional CZE is encompassed by Mode B, where the migration velocities of charged sample components are always greater than that of the neutral tracer for EOF. As seen in Fig. 3b the components appear on the electropherogram in the order of decreasing mobility as in Mode A. Both l_s and l_o are of the same sign in Mode B and each of them is smaller than the total capillary length and as

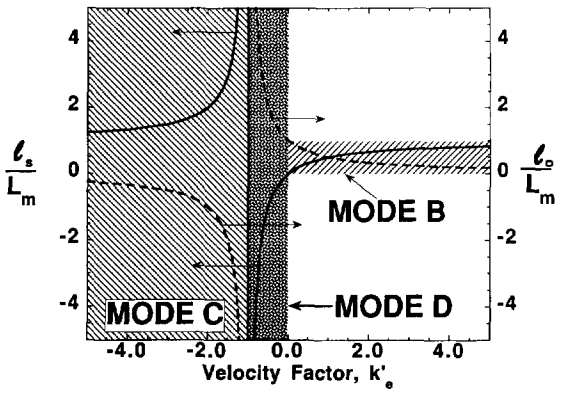

Fig. 4. Graph illustrating the dependence on the velocity factor, k'_e, of the dimensionless virtual migration distances for the (solid lines) separative, l_s/L_m, and the (broken lines) non-separative, l_o/L_m, parts of the migration process in CZE. Modes B, C and D are represented by differently shaded areas, each of them covers a particular range of operating conditions. Detailed information on the four operational modes of CZE is given in Fig. 2 and Table 2.

a result the k'_e is always positive and ranges between zero and infinity. It is seen in Fig. 4, which illustrates the dependence of the virtual migration distances on the velocity factor, that in Mode B, this dependence is the same as the dependence of l_s/L_m on k' depicted in Fig. 1. In Mode B, k'_e is always positive and f_m ranges between zero and unity and this behavior is analogous to that of the factors k' and $k'/(1+k')$ in HPLC. Thus Mode B of CZE, which is most widely used in free solution capillary zone electrophoresis, exhibits among the four modes the closest formal resemblance to chromatography.

Modes C and D both represent counterdirectional CZE with the restriction that all charged sample components exit at the same end of the capillary. As expected, the velocity factors are negative in both modes as shown in Table 2. In Mode C, the EOF velocity is lower than the electrophoretic velocity of any charged separand, therefore, the order of their appearance on the representative electropherogram, vide Fig. 3, is the same as in Modes A and B. However, no neutral tracer can traverse the column and, therefore, its peak is absent on the electropherogram in Fig. 3c. In Mode C as seen in Table 2, k'_e is greater than unity and negative, whereas, f_m is greater than unity and positive. Fig. 4 illustrates the dependence of the two virtual length components on the velocity factor, k'_e. When EOF is very small, the respective values of l_s and l_o approach L_m and zero. Both l_s and l_o increase with EOF but in the opposite direction, so that their sum remains the total migration length, L_m. Finally, when the EOF velocity approaches the electrophoretic velocity i.e. $k'_e = -1$ and $f_m = \infty$, l_s and l_o become $+\infty$ and $-\infty$, respectively. This is the case when a sample component forms a stagnant band in the capillary due to the countervailing effect of the two counterdirectional velocities.

In Mode D, as illustrated in Fig. 3d, the EOF velocity is higher than the electrophoretic velocity and the sample components appear at the detector in an order reversed, when the polarity of the electrical field is reversed with respect to those in all other operational modes. As seen, they appear at the other end of the capillary in the order of increasing electrophoretic mobilities, with the peak of the neutral tracer being the first. Fig. 4 illustrates that at $k'_e = -1$, the virtual length components, l_s and l_o, have infinitely large negative and positive values, respectively. This change in the signs of the two virtual migration length components in comparison to those in Mode C is due to the reversal in the direction of migration of the peaks as we enter Mode D from Mode C. The magnitude of both virtual distances decreases with that of the velocity factor and when k'_e reaches zero, l_s and l_o become zero and L_m, respectively. This corresponds to the case when the EOF velocity is infinitely large with respect to the electrophoretic velocity, and consequently, the migration is essentially of the non-separative kind. Table 2 shows that in Mode D, the k'_e is negative and less than unity and f_m has a negative value between infinity and zero.

In Eq. (10), the velocity factor was expressed also in terms of the migration times and this representation of k'_e as a dimensionless peak locator on the electropherogram is comparable to that of the retention factor in HPLC, vide Eq. (9). This notion of k'_e in CZE, however, applies only in Modes B and D. In Mode A, the migration velocity of a neutral tracer is zero and in Mode C, it is counterdirectional to that of the charged sample components and thus k'_e is infinity in the latter two modes.

The discussion above covers the four main modes of CZE. Special situations, e.g., modes with migrants carrying opposite charge and moving in opposite

directions, however, can be also treated as combinations of two or more of these modes.

6. Selectivity and other key separation parameters in HPLC and CZE

In HPLC, the inherent selectivity or the separation factor, α_{21}, of the chromatographic system for separands 1 and 2, in the absence of secondary equilibria is given by the ratio of the appropriate retention factors so that when separation occurs α_{21} is greater than unity [12]. In view of Eq. (9), in terms of the virtual length components α_{21} is expressed as follows

$$\alpha_{21} = \frac{k'_2}{k'_2} = \frac{l_{s2} l_{o1}}{l_{o2} l_{s1}} \tag{14}$$

where subscripts 1 and 2 refer to the faster and the slower migrating component, respectively. Thus it conforms to the convention that the selectivity in a separation process is never less than unity.

In CZE, by using the same definition of selectivity in terms of virtual migration distances as in Eq. (14), we obtain for the separation factor the following expression

$$\alpha_{21} = \frac{l_{s2} l_{o1}}{l_{o2} l_{s1}} = \frac{\mu_{ep,2}}{\mu_{ep,1}} \tag{15}$$

where $\mu_{ep,1}$ and $\mu_{ep,2}$ are the mobilities of the two components. Thus the inherent selectivity in the absence of secondary equilibria is given by the ratio of the electrophoretic mobilities of the two components.

In the literature of CZE, we often encounter another selectivity parameter [18,19], denoted here by δ_m. It is defined in terms of the mobilities and the virtual migration distances as follows

$$\delta_m = \frac{\Delta\mu_m}{\bar{\mu}_m} = \frac{l_{s2} - l_{s1}}{\bar{l}_o} = \frac{\Delta l_s}{\bar{l}_o} \tag{16}$$

where $\Delta\mu_m$ and $\bar{\mu}_m$ are the difference and the arithmetic mean of the electrophoretic mobilities of the two migrants and Δl_s and $\bar{l}_o$ are the corresponding values of the virtual migration distances. In order to distinguish the inherent selectivity, α_{21}, given in Eq. (15) from δ_m, defined by Eq. (16), the latter is referred to as the relative mobility difference.

Eq. (16) shows that δ_m depends not only on the separative but also on the non-separative virtual length component and hence on the magnitude of the EOF. Therefore, δ_m represents an operational selectivity that does not depend only on the properties of the separands. In contradistinction, the inherent selectivity as defined by Eq. (15) is unique for a given pair of migrants.

The chromatographic equivalent of δ_m is δ'_m that in view of the definition of the virtual migration distances in HPLC is given by

$$\delta'_m = \frac{\Delta l_s}{\bar{l}_o} = 2\left(\frac{\alpha - 1}{\alpha + 1}\right)\left(\frac{\bar{k}'}{1 + \bar{k}'}\right) \tag{17}$$

As shall be seen later in Tables 3 and 5, the operational selectivity parameter can be readily applied to electrokinetic chromatography as well. It should be noted that the factor $(\alpha - 1/\alpha + 1)$ $(\bar{k}'/1 + \bar{k}')$ with $\bar{k}'$ as the average retention factor of the two sample components, appears in several expressions for the efficiency of separation in chromatography, e.g., in one form of the resolution equation that is considered the fundamental equation of linear chromatography [20].

By using the virtual migration distances, the most important separation parameters, such as resolution, plate number and the required migration length, have been expressed for both HPLC and CZE in a unified fashion and are listed in Table 3. The results suggest that the use of the virtual migration distances offers a unifying approach for expression and comparison of the key separation parameters in various differential migration processes of separation.

7. Electrokinetic chromatography

The use of the virtual migration distances is extended to the two major representatives of electrokinetic chromatography: MEKC and CEC. Both techniques encompass a medley of features borrowed from HPLC and CZE and are carried out with instruments similar to those employed in CZE. Both use high electric field to generate the mobile phase flow by electroendoosmosis and to cause electro-

Table 3
Efficiency parameters for the separation of two bands in HPLC and CZE

Parameter	HPLC	VMD	CZE
Selectivity, α_{21}	$\frac{k'_2}{k'_1}$	$\frac{l_{s,2}l_{o,1}}{l_{s,1}l_{o,2}}$	$\frac{\mu_{ep,2}}{\mu_{ep,1}}$
Relative mobility difference, δ'_m or δ_m	$2\left(\frac{\alpha-1}{\alpha+1}\right)\left(\frac{\bar{k}'}{1+\bar{k}'}\right)$	$\frac{\Delta l_s}{\bar{l}_o}$	$\frac{\Delta\mu}{\bar{\mu}}$
Migration length required, L_m	$\frac{16HR^2}{\delta'^2_m}$	$16HR^2\left(\frac{\bar{l}_o}{\Delta l_s}\right)^2$	$\frac{16HR^2}{\delta^2_m}$
Number of plates, N	$\frac{16R^2}{\delta'^2_m}$	$16R^2\left(\frac{\bar{l}_o}{\Delta l_s}\right)^2$	$\frac{16R^2}{\delta^2_m}$
Resolution, R	$\frac{\sqrt{N}}{4}\delta'_m$	$\frac{\sqrt{N}}{4}\left(\frac{\Delta l_s}{\bar{l}_o}\right)$	$\frac{\sqrt{N}}{4}\delta_m$

In the middle column the parameters are given in terms of virtual migration distances, so that the expressions are equally applicable to both HPLC and CZE with EOF.

phoretic migration of charged sample components. The separation of uncharged components occurs due to their selective partitioning between the mobile and the stationary phases. In the case of MEKC the latter is a micellar pseudostationary phase [21]. In both kinds of electrokinetic chromatography in the limit with neutral separands (and a hypothetical stationary micellar phase in MEKC), all the expressions simplify to those derived earlier for HPLC and the expressions for MEKC of neutral species are widely available in the literature [22–25]. For charged separands when they are unretained, i.e., $k'=0$, the expressions reduce in both MEKC and CEC to those shown above for CZE. Therefore, we shall treat here the case when charged sample components, which are retained by the stationary phase, are subjected to electrokinetic chromatography. The dual characteristics of the separating system render the treatment of the techniques more involved than that of CZE or HPLC because of a coupling of the separative and non-separative parts of the overall migration processes. Consequently, neither in the time nor in the velocity domain can their relationship be expressed by a simple additivity rule. In the following, we will show that by using the concept of virtual migration distances, it is possible to disengage the two components and to formulate an additivity relationship that facilitates the appropriate definition of the key separation parameters. In the treatment below, with a few exceptions, the symbols used previously in HPLC and CZE will be employed. The results presented in Table 5 (below) illustrate the common features of the two techniques and shed light on their relationship to HPLC and CZE.

8. Capillary electrochromatography

CEC is a promising chromatographic technique which employs packed capillary columns with EOF for mobile phase flow [26–30] and is carried out by using a slightly altered version of the instruments presently used in CZE. Among the several advantages of CEC are its potential to offer column efficiencies higher than those obtained in HPLC with the same column and a loading capacity higher than that of CZE with a capillary of commensurate dimensions.

8.1. Migration velocities and times

In CEC the overall migration velocity of a charged sample component, u_c, is given by the sum of its velocities due to EOF and electrophoretic migration in the mobile phase multiplied by the retardation factor $1/(1+k')$ as follows

$$u_c = \left(\frac{1}{1+k'}\right)(u_{ep}+u_{eo}) = \frac{u_{ep}+u_{eo}}{1+k'} \qquad (18)$$

where k' is the chromatographic retention factor as

defined in Eq. (9) and all velocities are signed quantities. Dividing Eq. (18) by the total column length, L_m, we obtain the corresponding additivity relationship in the time domain as

$$\frac{1}{t_{Rc}} = \left(\frac{1}{1+k'}\right)\left(\frac{1}{t_{eo}} + \frac{1}{t_{ep}}\right) \tag{19}$$

where t_{Rc} and t_{eo} are the migration times of the sample component and an unretained neutral marker, respectively. The time, t_{ep}, is required by a charged sample component to traverse the column by virtue of electrophoretic migration alone. Eqs. (18,19) confirm that in CEC the separative and non-separative components of the migration are coupled in both the velocity and the time domains. In one limiting case, with a neutral migrant when $u_{ep}=0$ and $k'\neq 0$, Eqs. (18,19) reduce to Eqs. (1,2a), their respective counterparts in HPLC. In the other limit, with an unretained but charged migrant, when $k'=0$ and $u_{ep}\neq 0$, Eqs. (18,19) reduce to Eqs. (3a,4), the corresponding relationships in CZE.

8.2. Virtual migration distances

As in HPLC, the time spent by a sample component in or on the stationary phase is of the separative kind in CEC as well. However, a charged separand is subject to both electrophoretic migration and convection by EOF, just as in CZE, during the time it spends in the mobile phase. Therefore, in order to express the separative and non-separative virtual migration distances we shall combine features previously introduced for HPLC and CZE.

The virtual separative fraction of the column length due to retention by the stationary phase, l_s^r, can be expressed in the same way as in HPLC

$$l_s^r = \left(\frac{k'}{1+k'}\right)L_m \tag{20}$$

Thus, the other virtual distance resulting from migration in the mobile phase is given by

$$l_{os}^m = \left(\frac{1}{1+k'}\right)L_m \tag{21}$$

As indicated above, l_{os}^m has two components. One represents electrophoretic migration in the mobile phase, which contributes directly to separation, l_s^e, and a non-separative component due to EOF, l_o.

The two contributions can be expressed by using the chromatographic retention factor and the electrophoretic velocity factor as

$$l_s^e = \left(\frac{L_m}{1+k'}\right)\left(\frac{k'_e}{1+k'_e}\right) \tag{22}$$

and

$$l_o = \left(\frac{L_m}{1+k'}\right)\left(\frac{1}{1+k'_e}\right) \tag{23}$$

Since the overall separative virtual migration distance, l_s, is the sum of l_s^r and l_s^e we obtain that

$$l_s = l_s^r + l_s^e = L_m\left(\frac{1}{1+k'}\right)\left(k' + \frac{k'_e}{1+k'_e}\right) \tag{24}$$

Comparing Eqs. (5,24) we see that with respect to HPLC as the reference, the electrophoretic migration in the mobile phase may strongly affect the contribution of separative migration.

8.3. Electrochromatographic retention factor

By using the definition of the retention factor as the ratio of the separative and non-separative virtual migration lengths in Eq. (9) we arrive at the following expression of the electrochromatographic retention factor, k'_c,

$$k'_c = \frac{l_s}{l_o} = \frac{k' + k'_e/(1+k'_e)}{1/(1+k'_e)} = k' + k'k'_e + k'_e \tag{25}$$

The product $k'k'_e$ in Eq. (25) reflects the simultaneous occurrence of chromatography and electrophoresis. In the limit of $k'_e=0$ the electrochromatographic retention factor will be equal to k' and the separation process can be treated within the hermeneutics of HPLC alone. On the other hand when k'_e has non zero value, the electrochromatographic process assumes all features of CZE with the concomitant complexity of the process.

The potential complexity of CEC could also be illustrated in a similar fashion as Fig. 2, which depicts the main modes of CZE, since mutatis mutandis the same electrophoretic phenomena apply in both CZE and CEC to the migration of charged molecules. Thus, the classification of four operational modes of CZE on the basis of the magnitude and direction of the EOF in Fig. 2 and Table 2 applies, in

principle, to CEC as well, with the magnitude of the flow velocity being reduced by the retardation factor when retention by the stationary phase occurs. The signs and magnitudes of the electrochromatographic retention factor, k'_c, and the two virtual migration distances together with the velocity factors are presented in Table 4 for the four operational modes of CEC. Mode A, in the case of CEC, is mentioned only for the sake of completeness; the absence of bulk flow disqualifies this mode as chromatography.

The dependence of the electrochromatographic retention factor on the electrophoretic velocity factor, k'_e, is illustrated in Fig. 5 with the chromatographic retention factor, k', as the parameter. It is seen that k'_c is a linear function of k'_e with slope and intercept that depends on the value of k'. The domains of the major operational modes in CEC are also shown in Fig. 5. As k' value increases, the slope of the lines increases with k'_c attaining higher values over the same range of k'_e. Also we can see that when $k'_c = -1$, all lines intersect at $k'_c = -1$ where the electrophoretic and the EOF velocities are equal and in opposite direction, and due to their countervailing effect the migrant zone stagnates. In this case the chromatographic process comes to a halt.

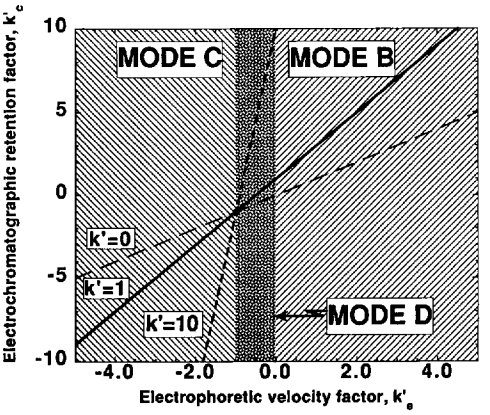

Fig. 5. Graph illustrating the dependence of the electrochromatographic retention factor, k'_c, in CEC on the electrophoretic velocity factor, k'_e, with the chromatographic retention factor, k', as the parameter. Detailed information on the various operational modes of CEC is given in Table 4. The classification of the modes is the same as shown for CZE in Figs. 2 and 4, and Table 2.

8.4. Separation parameters

The key separation parameters in CEC are expressed by following the scheme used earlier in HPLC. In essence, the chromatographic retention factor was replaced by the electrochromatographic retention factor. The final results are presented in Table 5, along with those in terms of the virtual migration distances and later derived for MEKC.

8.5. Micellar electrokinetic chromatography

In MEKC, migrants are separated on the basis of their differential partitioning into the micellar phase

Table 4
The signs and limiting values of the key migration parameters encountered in the four main operational modes of CEC that are essentially the same as those schematically illustrated in Fig. 2 and listed in Table 2 for CZE

Mode	Electrophoretic velocity factor, k'_e		Electrochromatographic retention factor, k'_c		Virtual migration distances			
					l_s (separative)		l_o (non-separative)	
	Sign	Magnitude	Sign	Magnitude	Sign	Magnitude	Sign	Magnitude
A	+	∞	+	∞	+	L_m	+	0
B	+	[0,∞]	+	[k',∞]	+	$\left[L_m\left(\frac{k'}{k'+1}\right),L_m\right]$	+	$\left[L_m\left(\frac{1}{k'+1}\right),0\right]$
C	−	[1,∞]	−	[1,∞]	+	[∞, L_m]	−	[∞,0]
D	−	[0,1]	±	[$+k'$,−1]	±	$\left[+L_m\left(\frac{k'}{k'+1}\right),-\infty\right]$	+	$\left[L_m\left(\frac{1}{k'+1}\right),\infty\right]$

The same capital letters are used throughout to denote the corresponding modes. The notation [a,b] means: from a to b, including both a and b.

Table 5
Separation parameters in MEKC and CEC

	CEC	VMD	MEKC
Overall retention factor, k'_c or k'_m	$k'+k'k'_e+k'_e$	$\frac{l_s}{l_o}$	$\frac{k'(t_{mc}-t'_m)}{t_{mc}+k't'_m}(k'_e+1)+k'_e$
Selectivity, α_{21}	$\frac{k'_{c,2}}{k'_{c,1}}$	$\frac{l_{s,2}l_{o,1}}{l_{s,1}l_{o,2}}$	$\frac{k'_{m,2}}{k'_{m,1}}$
Relative mobility difference, δ''_m or δ^*_m	$2\left(\frac{\alpha-1}{\alpha+1}\right)\left(\frac{\bar{k}'_c}{1+\bar{k}'_c}\right)$	$\left(\frac{\Delta l_s}{\bar{l}_o}\right)$	$2\left(\frac{\alpha-1}{\alpha+1}\right)\left(\frac{\bar{k}'_m}{1+\bar{k}'_m}\right)$
Resolution, R	$\frac{\sqrt{N}}{4}\delta''_m$	$\frac{\sqrt{N}}{4}\left(\frac{\Delta l_s}{\bar{l}_o}\right)$	$\frac{\sqrt{N}}{4}\delta^*_m$
Number of plates, N	$16\frac{R^2}{\delta''^2_m}$	$16R^2\left(\frac{\bar{l}_o}{\Delta l_s}\right)^2$	$\frac{16R^2}{\delta^{*2}_m}$
Migration length required, L_m	$\frac{16HR^2}{\delta''^2_m}$	$16HR^2\left(\frac{\bar{l}_o}{\Delta l_s}\right)^2$	$\frac{16HR^2}{\delta^{*2}_m}$

The corresponding expressions in terms of the virtual migration distances are listed in the middle column. They are applicable not only to MEKC and CEC but also to HPLC and CZE as shown in Table 3.

which itself is subject to migration and is, therefore, a pseudostationary phase. The technique employs a surfactant in the mobile phase above its critical micelle concentration to control the migration of the separands which need not be charged. In fact, MEKC is used mostly for the separation of neutral sample components and as such has received ample treatment in the literature [21–25].

Our interest is focused on the more complex case when the sample components are charged so that their overall migration rate depends not only on partitioning into the micellar pseudophase but also on their electrophoretic mobility. Under such conditions, the migration velocity of a charged migrant in MEKC, u_{me}, depends on the electroendoosmotic velocity, u_{eo}, the electrophoretic velocity of the migrant, u_{ep}, the retention factor of the migrant, k', with respect to the pseudostationary micellar phase, and the electrophoretic velocity of the micellar pseudophase, $u_{ep,mc}$.

8.6. Migration velocities and times

One way to evaluate the overall migration velocity of a charged migrant in MEKC is to calculate the velocities of the pseudostationary micellar phase and the mobile phase, multiply the velocities by the probabilities of the migrant being in the respective phases, and add the two terms together to obtain

$$u_{me}k_{m,2}=\left(\frac{k'}{1+k'}\right)(u_{ep,mc}+u_{eo})+\left(\frac{1}{1+k'}\right)(u_{ep}+u_{eo}) \quad (26)$$

where all velocities are signed quantities. A relationship similar to Eq. (26) was derived for the MEKC of anions [24].

In the above fashion, the corresponding additivity relationship can be written in the time domain as

$$\frac{1}{t_m}=\left(\frac{k'}{1+k'}\right)\left(\frac{1}{t_{mc}}\right)+\left(\frac{1}{1+k'}\right)\left(\frac{1}{t_{eo}}+\frac{1}{t_{ep}}\right)=\left(\frac{k'}{1+k'}\right)\left(\frac{1}{t_{mc}}\right)+\left(\frac{1}{1+k'}\right)\left(\frac{1}{t'_m}\right) \quad (27)$$

where t_m, t_{eo} and t_{mc} are the respective migration times of the sample component, an unretained neutral tracer and the micelles. When it moves only by virtue of its electrophoretic velocity the migration time of a sample component is t_{ep}, and t'_m is the migration time of the separand when it moves both due to electrophoretic migration and EOF. In the case of a charged migrant that does not interact with the micellar phase, i.e. $k'=0$, Eqs. (26,27) reduce to the respective Eqs. (3a,4) for CZE. Similarly, when the micelles are stationary ($t_{mc}=\infty$) and the migrant is uncharged ($t_{ep}=\infty$) the above equations simplify

to Eqs. (2b,1) the corresponding expressions in HPLC.

As seen from Eqs. (26,27), the separative and non-separative components of the migration process are coupled and cannot be separated either in the velocity or the time domain as MEKC has characteristics of both HPLC and CZE. Again, we shall employ the concept of the virtual migration distances to separate the two components of the migration process and to formulate an appropriate additivity relationship.

8.7. Virtual migration distances

In MEKC, while the separation of charged species occurs by both partitioning and electrophoretic migration, EOF is the sole contributor to non-separative migration, in the same manner as in CEC, but the treatment of MEKC is more complicated because the micellar phase also migrates. We account for this effect by evaluating the adjusted retention time, t'_{Rm}, by using Eq. (27) as follows

$$t'_{Rm} = \frac{k' t'_m (t_{mc} - t'_m)}{t_{mc} + k' t'_m} \tag{28}$$

Using the above definition of the adjusted retention time, the virtual length component for the separative contribution arising from interaction of the sample component with the micellar phase, l_s^r, can be expressed as

$$l_s^r = u_{me} t'_{Rm} \tag{29}$$

In turn, the other virtual migration distance, l_{os}^m, is given by

$$l_{os}^m = u_{mc} t'_m \tag{30}$$

In view of the above discussion, l_{os}^m has two components: another separative contribution due to the electrophoretic migration in the mobile phase, l_s^e, and a non-separative component due to the EOF, l_o. The two contributions can be evaluated in the same fashion as in CEC upon multiplying l_{os}^m by the factors $k'_e/(1+k'_e)$ and $1/(1+k'_e)$ as follows

$$l_s^e = (u_{me} t'_m)\left(\frac{k'_e}{1+k'_e}\right) \tag{31}$$

and

$$l_o = (u_{me} t'_m)\left(\frac{1}{1+k'_e}\right) \tag{32}$$

The overall separative virtual migration distance is the sum of the l_s^r and l_s^e

$$l_s = l_s^r + l_s^e = u_{me}\left(t'_{Rm} + \frac{t'_m k'_e}{1+k'_e}\right) \tag{33}$$

The first term on the right hand side in Eq. (33), which is particular to MEKC, represents the chromatographic mechanism of the separation arising from the interaction of the separand with the micellar phase, whereas the second term stands for the electrophoretic separation of charged sample components. Thus they reflect the two limiting cases of MEKC. A similar approach was suggested by Zhang et al. [31] to treat the separation of neutral analytes in MEKC.

8.8. Overall retention factor and separation parameters

Again, using the definition of the retention factor as the ratio of the separative and non-separative virtual migration lengths, we obtain the following expression for k'_m, the overall retention factor for MEKC.

$$k'_m = \frac{l_s}{l_o} = \left(t'_{Rm} + \frac{t'_m k'_e}{1+k'_e}\right)\left(\frac{t_{m'}}{1+k'_e}\right)$$
$$= \frac{k'(t_{mc} - t'_m)}{t_{mc} + k' t'_m}(1+k'_e) + k'_e \tag{34}$$

The above definition of the overall retention factor allows us to express other key separation parameters by using the relationships given earlier for HPLC, and substituting the overall retention factor in MEKC for the chromatographic retention factor. The expressions so obtained are compiled in Table 5 together with those written in terms of the virtual migration distances and derived for CEC.

9. List of symbols and abbreviations

f_m	Electromigration factor
H	Plate height in HPLC and CZE
k'	Retention factor in HPLC

k'_c Electrochromatographic retention factor in CEC
k'_e Velocity factor in CZE
k'_m Overall retention factor in MEKC
l_s Separative virtual migration distance in HPLC, CZE, CEC and MEKC
l_s^e Separative virtual migration distance due to electrophoretic migration in CEC and MEKC
l_s^r Separative virtual migration distance due to retention in CEC and MEKC
l_o Non-separative virtual migration distance in HPLC, CZE, CEC and MEKC
l_{os}^m Virtual migration distance for migration in the mobile phase in CEC and MEKC
L_m Length of column
N Number of theoretical plates
R Resolution
t_{eo} Migration time of a neutral unretained migrant in CZE and CEC
t_{ep} Electrophoretic migration time of a charged but unretained migrant in CZE, CEC and MEKC without EOF
t_m Migration time of a charged migrant in CZE and MEKC
t'_m Electrophoretic migration time of a charged migrant in MEKC
t_{mc} Migration time of the micellar phase in MEKC
t_o Retention time of a neutral tracer in HPLC
t_R Retention time of a sample component in HPLC
t_{Rc} Migration time of a sample component in CEC
t'_R Adjusted retention time of a sample component in HPLC
t'_{Rm} Adjusted retention time of a sample component in MEKC
u_b Migration velocity of a sample component in HPLC
u_c Migration velocity of a sample component in CEC
u_{eo} Migration velocity of a neutral unretained migrant, i.e., the EOF velocity, in CZE, MEKC and CEC
u_{ep} Electrophoretic velocity of a migrant in CZE, MEKC and CEC
$u_{ep,mc}$ Electrophoretic velocity of the micellar phase in MEKC
u_m Migration velocity of a charged separand in CZE
u_{me} Migration velocity of a charged separand in MEKC
u_o Migration velocity of an inert tracer in HPLC

Greek letters

α_{21} Selectivity of component 2 with respect to component 1
δ_m Relative mobility difference in CZE
δ'_m Relative mobility difference in HPLC
δ''_m Relative mobility difference in CEC
δ^*_m Relative mobility difference in MEKC
μ_{eo} Mobility of a neutral migrant in CZE
μ_{ep} Electrophoretic mobility of a migrant in CZE
μ_m Mobility of a charged migrant in CZE

Acronyms

CEC Capillary electrochromatography
CZE Capillary zone electrophoresis
EOF Electroendoosmotic flow
HPLC High-performance liquid chromatography
MEKC Micellar electrokinetic chromatography

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