Electrokinetic Chromatography

Theory, Instrumentation and Applications

Edited by UTE PYELL

University of Marburg, Germany

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Preface

In their pioneering papers in 1984 and 1985 Terabe *et al*. [1,2] discovered that separations based on electrokinetic phenomena (electrophoresis and electroosmosis) can be described as chromatographic processes provided that an additive (also called separation carrier or pseudostationary phase) having a velocity different from that of the analytes is present in the separation electrolyte, and this is able to interact with the analytes to be separated. In the first papers on this topic this additive was a micelle-forming ionic surfactant at a concentration above the critical micelle concentration. However, it was also realized very early by Terabe [3] that the presence of micelles is not a prerequisite of electrokinetic chromatography (EKC). Since the presentation of the general separation carrier concept many variants of EKC have been developed that employ 'polymeric micelles', microdroplets, other types of colloidal phases, dissolved linear polymers and dendrimers or oligomeric units as separation carriers.

In the last two decades EKC has been mainly regarded as a special form of capillary electrophoresis (CE), which has matured into a powerful analytical separation technique that brings speed, reproducibility and automation to the labour intensive methods of classical electrophoresis. However, the IUPAC recommendations concerning the terminology for analytical capillary electromigration techniques published in 2004 [4] clearly regard EKC and CE (also known as capillary zone electrophoresis, CZE) as two equal members of the large family of capillary electromigration techniques. Of course, regarding the unique position of EKC as an interface between electrophoresis and chromatography there inherently remain fuzzy borders between EKC and CE.

This book is designed to be a guide to the large, rapidly growing and diverse field of EKC for a broad audience: those new to EKC, those more experienced, those interested in method development including instrumental developments, and those involved with applications research in various fields. The book aims to bring together a thorough theoretical description of methodological aspects, an overview of the current status of the various forms of EKC, and current and emerging applications, as well as looking forward to future developments. This book should be of interest for all those who need a high-efficiency separation technique with easily adaptable selectivity and having the additional features of low sample volume requirements, short run times and high versatility.

The task of compiling this book required the cooperation of internationally recognized experts with special competence in their respective fields. The volume is composed of 21 chapters organized into three major parts: I Separation Principles, II Instrumentation, and III Applications. Part I includes an introduction to the terminology used to describe the separation process in EKC (Chapter 1), a review of electrokinetic methods to investigate

the micelle-formation process (Chapter 2), an introduction to the fundamentals of the solvation parameter model for the (selectivity) characterization of separation carriers (Chapter 3), a general guide to method development and resolution optimization with micellar pseudostationary phases (Chapter 4), an introduction to concepts for computer-based rapid method optimization (Chapter 5), and reviews of various forms of EKC including microemulsion electrokinetic chromatography (MEEKC), EKC with polymeric pseudostationary phases and dendrimers, EKC with pseudostationary ion-exchange phases and enantioselective EKC (Chapters 6–9). The part is completed by a detailed discussion on techniques employed for on-line sample enrichment in combination with EKC (Chapter 10).

In the Part II general aspects of instrumentation are treated including the use of coated capillaries (Chapter 11). This part also presents reviews of different detection methods (laser-induced fluorescence detection, amperometric detection, photothermal detection and mass spectrometric detection) having high potential as sensitive and/or selective detection techniques for EKC (Chapters 12–15). The part is completed by a review of the implementation of EKC on microfluidic devices (Chapter 16). The final part is concerned with applications of EKC in the fields of pharmaceutical analysis, the analysis of body fluids, food analysis, chiral analysis and environmental analysis (Chapters 17–21). With this structure the book intends to illuminate many facets of this relatively young member in the family of separation methods, bringing together expert knowledge from various directions that will not only help the novice to estimate how EKC might help in solving analytical tasks, but will also assist the more experienced user in broadening and deepening their knowledge of this technique, which has already found its way into routine laboratories.

I would like to thank all of the contributors and further scientists for their support and cooperation. My personal hope is that bringing together a comprehensive review of the state of the art of this fascinating technique will become a factor in its further dissemination in various fields of application.

Ute Pyell

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Part I
Separation Principles

1

Theory of Electrokinetic Chromatography

Ute Pyell

1.1 Introduction

Electrokinetic chromatography (EKC) is a term that was coined by Terabe and coworkers in 1985 [1,2]. EKC belongs to a family of electromigration separation techniques that employ electrokinetic phenomena (electrophoresis and electroosmosis) for the separation of constituents in a sample. EKC invariably also involves chemical equilibria, e.g. distribution, ion exchange and/or complex formation. According to Terabe and coworkers [1,2] EKC is defined as a capillary electromigration separation technique employing a separation carrier. The separation carrier, also called pseudostationary phase, is a unity (e.g. a microdroplet, a micelle, a dendrimer, or a dissolved polymer) that interacts with the solutes to be separated while its migration velocity is, in general, virtually unaffected by this interaction. The property of the migration velocity of the separation carrier being virtually unaffected by the interaction with dissolved solutes will be taken here to define the difference between a pseudostationary phase and a simple complex-forming agent, which is used in capillary electrophoresis to modify the effective electrophoretic mobility of the solutes to be separated. If the solutes to be separated do not posess an effective electrophoretic mobility without the presence of the separation carrier, the separation carrier must have an electrophoretic mobility.

According to the IUPAC recommendations [3], EKC is a separation technique 'based on a combination of electrophoresis and interactions of the analytes with additives (e.g. surfactants), which form a dispersed phase moving at a different velocity [...than the analytes (*editorial note*)]. In order to achieve separation either the analytes or this

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secondary phase should be charged'. Micellar EKC (MEKC) is 'a special case of EKC, in which the secondary phase is a micellar dispersed phase in the capillary' and microemulsion EKC (MEEKC) 'is a special case of EKC, where a microemulsion is employed as the dispersed phase.'

In chromatography the observed velocity of a solute zone is the weighted mean of two velocities (velocity of the mobile phase and 'velocity' of the stationary phase) resulting from the partitioning of the solute between these two phases. In EKC, as defined above, a noncharged solute will migrate either with the velocity of the electroosmotic flow or with the velocity of the separation carrier. Consequently, the separation of neutral solutes differing in their partitioning coefficients (between the separation carrier and the surrounding phase) is possible and the separation process in EKC can be described in chromatographic terms. In fact, conventional chromatography can be regarded as a special case of EKC, where the observed velocity of the separation carrier is zero.

In their first papers on EKC, Terabe and coworkers [1,4] had already emphasized the chromatographic nature of the underlying separation process (re-)defining parameters known from chromatographic theory. Their treatment is the basis of further considerations on rational resolution optimization [5], method development [6] or experimental determination of physicochemical parameters from EKC data [7,8]. One of the peculiarities of EKC is the nonexistence of a stationary phase, hence the solute zone is also transported (in the direction of the detector or in the opposite direction) when incorporated into the pseudostationary phase. Another peculiarity is the possibility of combining electrophoretic and chromatographic phenomena.

The instrumentation used in EKC is identical to that employed in capillary electrophoresis (CE) (see Part II 'Instrumentation'). Separation takes place in a (fused silica) capillary with an inner diameter less than 100 μm and a length mostly varying between 20 and 100 cm. The capillary is filled with the separation electrolyte containing the separation carrier and is immersed at both ends in vessels filled with the same electrolyte. The sample is injected directly into the first segment of the capillary. A high voltage (up to 35 kV) is applied between two electrodes (incorporated into the vessels) producing a very high field strength within the capillary. The migration of analyte zones during the separation process is then caused by electrokinetic effects. In order to avoid instrumental band broadening, detection is mainly done over a short segment of the capillary (e.g. photometric or fluorimetric detection). The resulting trace, detector signal versus time, can be called an electropherogram or chromatogram, which reflects the position of EKC as being between electrophoresis and chromatography.

In this chapter chromatographic and electrophoretic terms that are needed to describe and to optimize the separation process will be introduced.

1.2 Electrokinetic Phenomena

Two electrokinetic phenomena are of interest in EKC: electrophoresis and electroosmosis. Electrophoresis is the migration of a charged unity (e.g. an ion), surrounded by a medium, due to the presence of an electric field. The charged unity will experience acceleration due to electrostatic forces and friction due to the surrounding medium.

Under steady-state conditions the two opposite forces balance each other. A final electrophoretic velocity v_{ep} is reached which remains constant at constant electric field strength E.

$$v_{ep} = \frac{\varepsilon\zeta E}{6\pi\eta} = \mu_{ep}E \tag{1.1}$$

where ε is the electric permittivity of the surrounding medium, ζ is the electrokinetic potential (zeta potential) at the surface of the charged unity, η is the viscosity of the surrounding medium, and μ_{ep} is the electrophoretic mobility of the charged unit in the specific medium. (In a capillary filled with a homogeneous buffer, E is given by voltage U divided by the total length L_T of the capillary.)

In capillary electrophoresis (CE) solutes can be separated into zones if they differ sufficiently in their effective electrophoretic mobilities μ_{eff}. In the case of fast equilibria (e.g. protonation equilibria, complexation equilibria) being involved, the efficient electrophoretic mobility is the weighted mean (respecting the degree of dissociation/protonation or complexation) of the electrophoretic mobilities of all solute species being present in equilibrium. Hence fast equilibria are exploited in CE for resolution optimization, e.g. via the adjustment of the pH (separation of solutes differing in their degree of dissociation/protonation) or the addition of a complex forming agent to the separation buffer (separation of solutes differing in their degree of complexation).

The second electrokinetic phenomenon that is important in EKC is electroosmosis. Regarding the typical instrumentation used for EKC, electroosmosis is the bulk flow of liquid inside the capillary at constant velocity due to the effect of the electric field on the counterion layer adjacent to the charged capillary wall. In bare fused silica capillaries the surface is negatively charged under most pH conditions. There will be an excess of positive counterions in the zone forming the boundary layer. This zone of surplus charge adjacent to the capillary wall will be accelerated in the electric field and will also experience friction by the medium next to this layer. In consequence, a steady-state constant velocity, the electroosmotic velocity v_{eo}, is reached in the liquid outside the electrical double layer (Helmholtz Smoluchowski equation) [9].

$$v_{eo} = \frac{\varepsilon\zeta E}{4\pi\eta} = \mu_{eo}E \tag{1.2}$$

where ζ is the electrokinetic potential (zeta potential) at the surface of the charged wall and μ_{eo} is the electroosmotic mobility.

Equation (1.2) is only valid if the capillary inner diameter is large compared with the thickness of the electric double layer. However, this restriction is fulfilled in practice. It is important to note that v_{eo} is independent of the capillary inner diameter and (outside the electric double layer) the velocity of a liquid segment is not a function of the radial position (in contrast to pressure-induced laminar flow). Consequently, neither electrophoresis nor electroosmosis contribute to zone broadening in EKC. The observed (apparent) velocity v_s of a solute zone corresponds to the sum of the effective electrophoretic velocity v_{ep} of a solute and the electroosmotic velocity v_{eo} ($v_s = v_{ep} + v_{eo}$).

1.3 The Separation Carrier

It is the application of a separation carrier that transforms capillary electrophoresis (electrokinetic separation in a homogeneous electric field [10]) into EKC. The term 'separation carrier' was coined by Terabe [2,11] generalizing the concept of micellar electrokinetic chromatography (MEKC) [1,3], in which a micellar pseudophase is employed. The term pseudostationary phase, which is more often used in the literature [12], has an identical meaning. The separation carrier is a unit that is added to (or dissolved/dispersed in) the separation electrolyte. In general (see Section 1.1) the separation carrier has an effective electrophoretic mobility and is able to interact with the solutes of interest. In Figure 1.1 the separation mechanism in EKC for a neutral solute and a micelle forming anionic surfactant as separation carrier is depicted.

In chromatography, solutes are separated in a system consisting of a stationary and a mobile phase. The velocity v_s of a solute zone in the chromatographic bed corresponds to:

$$v_s = \frac{t_{mob}}{t_{mob} + t_{stat}} v_{mob} = \frac{1}{k+1} v_{mob} \tag{1.3}$$

where t_{mob} is the residence time in the mobile phase, t_{stat} is the residence time in the stationary phase, and k is the retention factor (t_{stat}/t_{mob}).

In EKC the separation carrier replaces the stationary phase. However, the separation carrier is not immobilized, and hence can have an observed velocity different from zero. Consequently, the observed velocity of a solute zone (neutral solute) is the weighted mean of the velocity of the mobile phase and of the observed velocity of the separation carrier:

$$v_s = \frac{t_{mob}}{t_{mob} + t_{rsc}} v_{mob} + \frac{t_{rsc}}{t_{mob} + t_{rsc}} v_{sc} = \frac{1}{k+1} v_{mob} + \frac{k}{k+1} v_{sc} \tag{1.4}$$

where t_{rsc} is the residence time associated with the separation carrier, v_{sc} is the observed velocity of the separation carrier ($v_{sc} = v_{epsc} - v_{eo}$), and v_{epsc} is the electrophoretic velocity of the separation carrier.

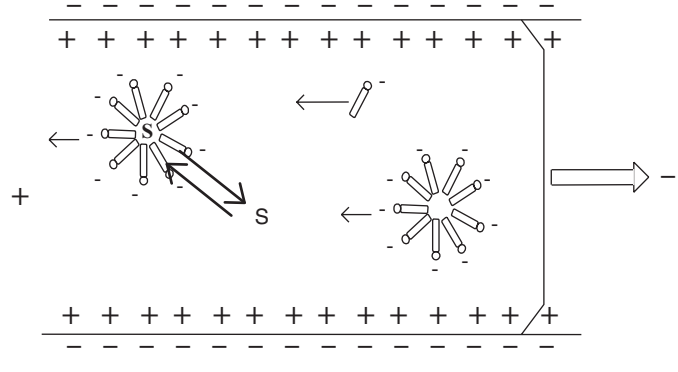

Figure 1.1 *Scheme illustrating the separation mechanism in micellar EKC (anionic surfactant, normal elution mode)*

Table 1.1 *Modes in EKC*

Mode	Separation carrier
Micellar EKC (MEKC)	Micellar pseudophase: anionic, cationic, or nonionic surfactant (or mixture of nonionic surfactant with ionic surfactant) in a concentration above the CMC
Microemulsion EKC (MEEKC)	Microdroplets present in oil-in-water or water-in-oil microemulsions
Polymeric EKC	Polymerized micelles or amphiphilic (charged) linear copolymers or cocondensates
Dendrimeric EKC	Charged dendrimers
Ion-exchange EKC (IE-EKC)	Soluble linear polymers with ion-exchange sites or other moieties with ion-exchange sites (interaction analyte/separation carrier via electrostatic forces)
Secondary-equilibrium modified MEKC, e.g. cyclodextrin-modified MEKC (CD-MEKC) or ligand-exchange MEKC (LE-MEKC)	Micellar pseudophase plus dissolved complex ligand or metal complex

Here the retention factor k is redefined to be the ratio t_{rsc}/t_{mob}. Equation (1.4) shows why EKC is considered to be a chromatographic process if the observed velocity v_{sc} of the separation carrier is vitually unaffected by the interaction with the solute. When associated with the separation carrier the solute is transported with the velocity of the free separation carrier.

EKC was introduced first in 1984 by Terabe and coworkers [1,2,4], who employed charged micelles formed by anionic or cationic surfactants as separation carriers. In 1991 Watarai [13] introduced microemulsion EKC (MEEKC) employing charged microdroplets as the separation carrier. Later, polymeric micelles, charged dissolved (amphiphilic) polymers and charged dendrimers were used as separation carriers in EKC [14,15]. Table 1.1 gives an overview of developed modes in EKC. Generally, all modes of interaction of the solute with the (pseudo)stationary phase known in chromatography should also be applicable in EKC: hydrophobic interaction, Van-der-Waals interaction, electrostatic interaction, coordinative interaction, etc.

In contrast to EKC, a capillary electromigration separation technique employing a true stationary phase is called capillary electrochromatography (CEC) [16]. According to the IUPAC recommendations [3] CEC is 'a special case of capillary liquid chromatography, where the movement of the mobile phase through a capillary, filled, packed or coated with a stationary phase, is achieved by electroosmotic flow'.

EKC was first developed to make it possible to separate noncharged compounds by using an electromigration separation technique without the participation of a stationary phase. Figure 1.2 shows the separation of positional isomers of neutral nitrotoluenes by employing micelles of the anionic surfactant sodium dodecylsulfate (SDS) as the separation carrier. However, EKC also proved to be a versatile tool for mixtures of charged and uncharged compounds as well as for charged compounds with similar effective electrophoretic mobilities. As EKC is invariably performed in a chamber of high electric field strength, electrophoresis will contribute to the separation if the solutes are permanently or partially charged (weak electrolytes). Consequently, measures taken

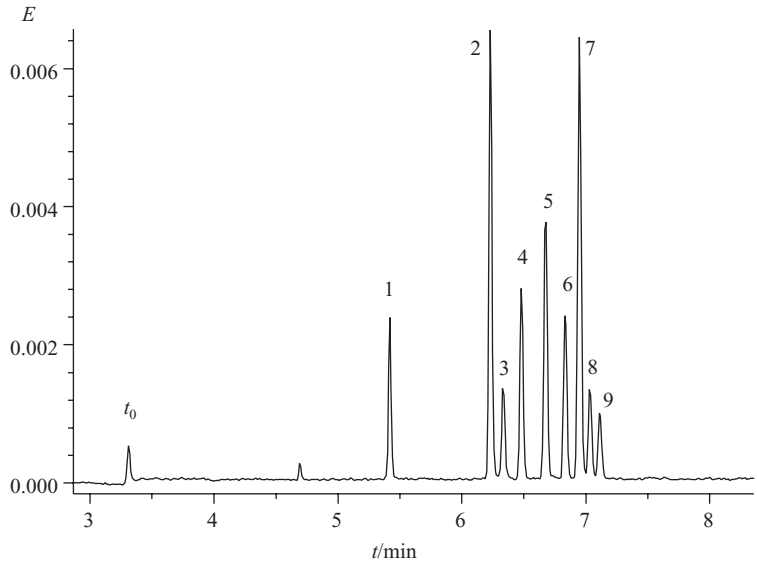

Figure 1.2 *Separation of neutral nitrotoluenes by micellar EKC. Electrolyte: c(SDS) = 55 mmol L^{-1}, c(urea) = 1.8 mol L^{-1}; c($Na_2B_4O_7$) = 10 mmol L^{-1}. Solutes: 1 = 2,4,6,-trinitrotoluene; 2 = 2,4-dinitrotoluene; 3 = 2,5-dinitrotoluene; 4 = 2,6-dinitrotoluene; 5 = 3,4-dinitrotoluene; 6 = 2-nitrotoluene; 7 = 2,3-dinitrotoluene; 8 = 4-nitrotoluene; 9 = 3-nitrotoluene; capillary: 75 μm i.d., 50 cm effective length, 56.5 cm total length; voltage: 25 kV; 25 °C; pressure injection: 1.5 s; detection: photometric, λ = 254 nm. (Reprinted from U. Pyell, U. Bütehorn, J. Chromatogr. A.,* ***716****, 81–95 (1995), copyright 1995, with permission from Elsevier)*

to modify the effective electrophoretic mobility in CE (e.g. addition of a complex forming agent, variation of pH) can be also applied in EKC to optimize the selectivity of the separation system.

1.4 Separation of Neutral Solutes

1.4.1 Retention Factor

Corresponding to theory in chromatography the retention factor k (older term: capacity factor k' or $\tilde{k}'$, sometimes referred to as migration factor k' [17]) in EKC is defined as residence time in the separation carrier devided by residence time in the surrounding liquid phase. If we assume the separation carrier to be a homogeneous (pseudo)phase, the separation process can be understood to be due to distribution between two distinct phases having two different observed mobilities:

$$k = \varphi P = \frac{V_{sc}}{V_{mob}} P \tag{1.5}$$

(φ = phase ratio, V_{sc} = volume of separation carrier, V_{mob} = volume of surrounding (mobile) phase, P = partition coefficient).

If the molar (or mass) concentration and the partial molar (or specific) volume of the separation carrier are known, the distribution coefficient can be calculated from the retention factor [1]. This makes EKC a valuable tool for the determination of liquid–liquid partition coefficients [18]. Replacing the velocities in Equation (1.4) with the respective distance-over-time and rearranging results in Equation (1.6) [1,4]:

$$k = \frac{t_s - t_0}{t_0 (1 - t_s/t_{sc})} \tag{1.6}$$

(t_0 = migration time of the front of the surrounding (mobile) phase, t_s = migration time of the solute zone, t_{sc} = migration time of the front of the separation carrier).

It should be noted that micelles (molecular aggregates of surfactants), which are mainly used as the separation carrier, have too small an aggregation number to be regarded as a phase in the usual sense and, on the other hand, also contain too many surfactant molecules to be considered as a chemical species [19]. In contrast to bulk phases, whose properties are invariant with position, the properties of small aggregates are expected to vary with distance from the interface (spatial heterogeneity). However, those solutes that enter the micelle, can diffuse rapidly within the micelle and experience a wide range of microenvironments, so that an averaging effect would prevail. Consequently, micelles are complex solvents and can only be treated in an approximate sense as bulk solvents. In spite of these restrictions, the retention data for a large number of solutes are homogeneous with respect to the construction of solvation parameter models, suggesting a uniform average solvation environment for all solutes [19].

In addition to these considerations, the interaction of the solute with the dispersed separation carrier can also be described by the binding model, where solute–separation carrier 'binding' is defined as occurring whenever the solute interacts with the separation carrier unity (e.g. a micelle) [20]. An equilibrium binding constant K_b can be defined for the equilibrium $S + SC \rightleftharpoons [SSC]$. On one hand, the binding model is more universal, as it is also possible to describe equilibria where the observed velocity of [SSC] is not identical to the observed velocity of the separation carrier. On the other hand, only one-to-one associates are taken into consideration. In general, the phase model is preferred to the binding model.

Equation (1.6) is valid in the case of so-called normal elution mode according to Vindevogel and Sandra [12]. In this elution mode, v_{eo} and v_{sc} have identical direction and $|v_{eo}| > |v_{sc}|$. It is important to state that in other elution modes v_{eo} and v_{sc} can have opposite directions.

Gareil [21] has shown that in the case that the observed velocity of the solute zone being opposite to v_{eo} (reversed direction mode according to Vindevogel and Sandra [12]), k has to be determined using Equation (1.7):

$$k = \frac{t_s + t_0}{t_0 (t_s/t_{sc} - 1)} \tag{1.7}$$

In that case t_s and t_{sc} can be determined simultaneously in one run whereas the determination of t_{eo} is only possible after the reversal of polarity or the injection of a marker solution at the opposite end of the capillary. Equation (1.4) has to be rewritten,

provided that only absolute velocities ($v = |\vec{v}|$) are given:

$$v_s = -\frac{1}{k+1} v_{eo} + \frac{k}{k+1} v_{sc} \tag{1.8}$$

In case of observed velocity of the solute zone being opposite to v_{sc} (restricted elution mode according to Vindevogel and Sandra [12]) Equation (1.9) and (1.10) are valid [21].

$$v_s = \frac{1}{k+1} v_{eo} - \frac{k}{k+1} v_{sc} \tag{1.9}$$

$$k = \frac{t_s - t_0}{t_0 (t_s/t_{sc} + 1)} \tag{1.10}$$

A scheme of the three elution modes compared here is given in Figure 1.3. Figure 1.4 shows the development of solute zones during the separation process for EKC in the normal elution mode. Generally, the effective electrophoretic mobility of the separation carrier is opposite to the electroosmotic mobility of the mobile phase, because a

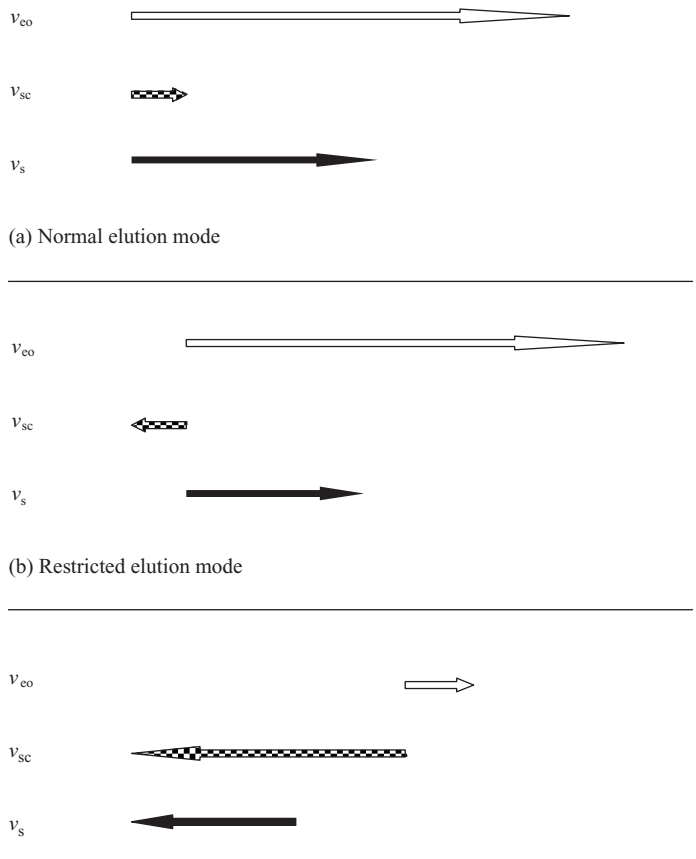

Figure 1.3 *Elution modes in electrokinetic chromatography*

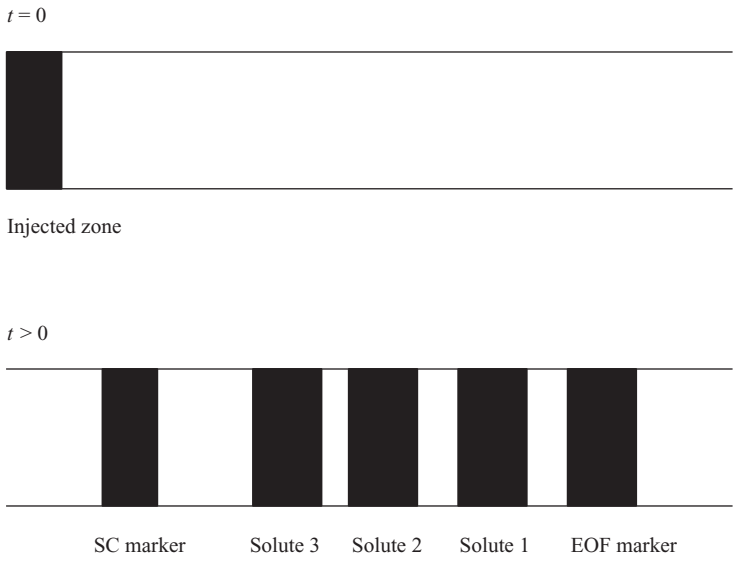

Figure 1.4 *Development of solute zones during the chromatographic run, normal elution mode, neutral solutes, P(Solute 1) < P(Solute 2) < P(Solute 3) [see Equation (1.5)]*

separation carrier of opposite charge to the surface of the capillary wall (e.g. a cationic surfactant or a cationic polymer in a negatively charged fused-silica capillary) will be adsorbed onto the surface of the capillary wall reversing the direction of the electro-osmotic flow. There are two special cases: (i) v_{sc} equals zero, (ii) v_{eo} equals zero. The first case corresponds to conventional chromatography, the second also corresponds to conventional chromatography if we rename the phases.

With EKC it is possible easily to vary the volume ratio φ (volume of separation carrier/volume of surrounding (mobile) phase) by increasing the concentration of the separation carrier in the separation buffer. Assuming that the volume of the mobile phase is not significantly reduced by addition of the separation carrier, we would expect a linear increase in the retention factor with concentration of separation carrier [refer to Equation (1.5)]. This expectation has been confirmed by many authors [22]. We would also expect a retention factor of zero at a separation carrier concentration of zero. In the case of a micellar separation carrier it has to be taken into account that the concentration of the separation carier is zero at or below the critical micelle concentration (CMC). Consequently, in micellar EKC (if there is no interaction of the solute with the capillary wall and with surfactant monomers) by plotting the retention factor versus the surfactant concentration, straight lines are obtained that pass through the identical x-axis intercept, which corresponds to the CMC (see Figure 1.5).

1.4.2 Secondary Complex Equilibria

If a compound that forms complexes with the solutes to be separated is added to the separation electrolyte containing a separation carrier, then the apparent retention factor will be decreased due to the coupled equilibria. This effect is used in cyclodextrin-modified

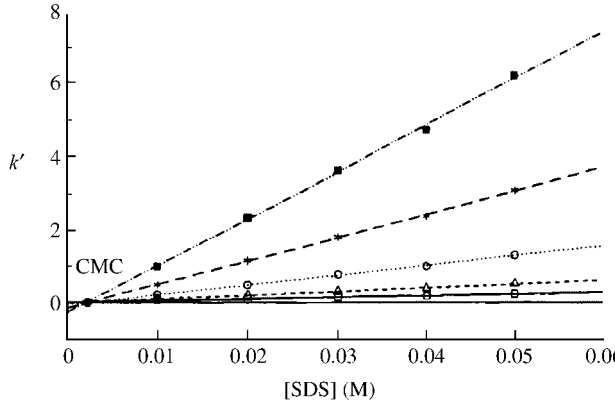

Figure 1.5 *Dependence of the retention factor* k *for several neutral solutes on the molar concentration of the anionic surfactant SDS in the separation buffer; solutes:* ■ *2-naphthol,* ∗ *toluene,* ◯ *nitrobenzene,* △ *phenol,* □ *resorcinol. (Reprinted with permission from M.G. Khaledi, S.C. Smith, J.K. Strasters, Anal. Chem.,* **63***, 1820–1830 (1991), copyright 1991 American Chemical Society)*

micellar electrokinetic chromatography for chiral separations and for the separation of hydrophobic compounds [23]. The partitioning coefficient P [see Equation (1.5)] corresponds to the ratio of the concentration of solute in the separation carrier to the concentration of uncomplexed solute in the surrounding phase. The concentration of the uncomplexed solute in the surrounding phase is dependent on the concentration of the complex forming additive and the complex formation equilibrium constant K_C. When assuming a 1:1 complex, the degree of complexation β of the solute in the surrounding phase is given by:

$$\beta = \frac{K_C \times c(A)}{1 + [K_C \times c(A)]} \tag{1.11}$$

where $c(A)$ is the molar concentration of the free complex forming additive. β remains constant if $c(A)$ remains constant. In general, $c(A)$ can be approximated by the total concentration of the complex forming additive. β can only take values between 0 and 1. It is evident that the situation will become more complicated if several separation carriers or several complex forming additives are used for selectivity optimization.

1.4.3 Resolution

The resolution of two adjacent zones in the normal elution mode is given by [1]:

$$R_s = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha}\right) \left(\frac{\bar{k}}{\bar{k}+1}\right) \left(\frac{1 - t_0/t_{sc}}{1 + (t_0/t_{sc})\,\bar{k}}\right) \tag{1.12}$$

(R_s = resolution, N = plate number, α = selectivity factor, $\bar{k}$ = mean retention factor = arithmetic mean of the two retention factors for the two solutes investigated).

Comparing Equation (1.12) with the equation for the resolution of two solute zones in conventional chromatography reveals that the dependence of R_s on the mean retention

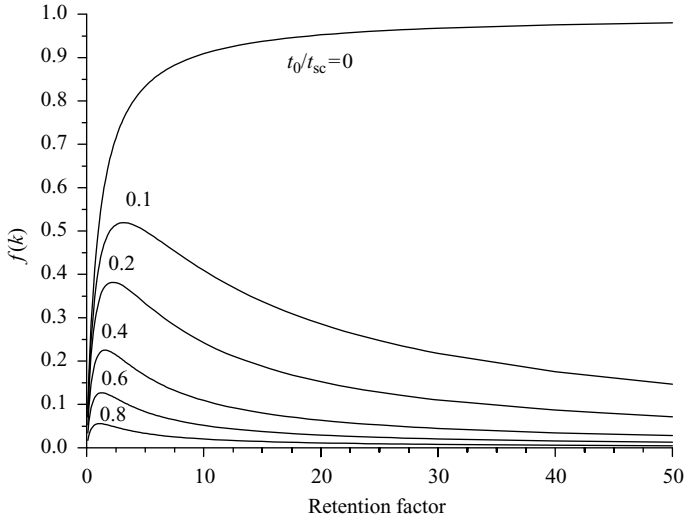

Figure 1.6 *Dependence of f(k) on the retention factor in EKC in the normal elution mode [see Equation (1.13)] for several ratios t_0/t_{sc}. The ratio $t_0/t_{sc} = 0$ corresponds to conventional chromatography with an immobilized stationary phase (according to [12])*

factor is more complex in EKC and that the time ratio t_0/t_{sc} has a major impact on the achievable resolution. In normal elution mode, the time span (window) in which a neutral compound can be eluted is restricted to values between t_0 and t_{sc}. Consequently, t_0/t_{sc} or its reciprocal value t_{sc}/t_0 have mainly been used in the literature to characterize the ratio of the observed velocities of the two 'phases' in EKC. One widely accepted term for this time ratio is migration (time) window.

Plotting the last two factors of Equation (1.12) [$f(k)$, see Equation (1.13)] against $\bar{k}$ reveals that $f(k)$ reaches a maximum and that this maximum is smaller than 1 in all instances, with 1 as the limiting value if t_{sc} approaches infinity (see Figure 1.6).

$$f(\bar{k}) = \left(\frac{\bar{k}}{\bar{k}+1}\right)\left(\frac{1 - t_0/t_{sc}}{1 + (t_0/t_{sc})\,\bar{k}}\right) \tag{1.13}$$

In their pioneering paper on micellar EKC Terabe *et al.* [1] recognized that the lower resolution obtained in the normal elution mode with identical N, α, and $\bar{k}$ is a disadvantage of EKC as compared with conventional chromatography that can be, however, compensated for by the large plate numbers achievable under routine conditions in EKC (200 000–300 000). It has to be emphasized that the equation to determine the resolution of two solute zones is dependent on the elution mode [see Equations (1.7) to (1.10)] [21].

In the reversed direction mode, Equation (1.14) is valid.

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{\bar{k}}{\bar{k}+1}\right)\left(\frac{1 + t_0/t_{sc}}{(t_0/t_{sc})\,\bar{k} - 1}\right) \tag{1.14}$$

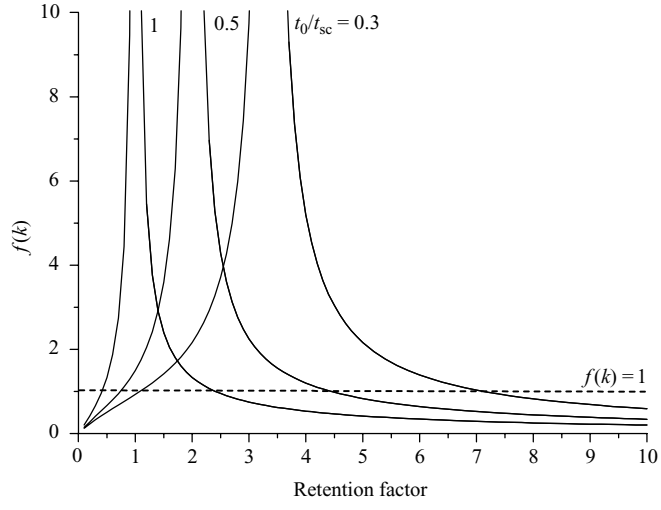

Figure 1.7 *Dependence of f(k) on the retention factor in EKC in the restricted elution mode [left side of the graph, see Equation (1.15)] and the reversed migration mode [right side of the graph, see Equation (1.14)] for several ratios t_0/t_{sc}. The dashed line indicates f(k) = 1 (according to [21])*

In the restricted elution mode, Equation (1.15) is valid.

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{\bar{k}}{\bar{k}+1}\right)\left(\frac{1 + t_0/t_{sc}}{1 - (t_0/t_{sc})\,\bar{k}}\right) \tag{1.15}$$

In these modes there is no restricted time span (window) during which a neutral compound can be eluted and $f(k)$ can exceed unity. Foley [5] and Gareil [21] have shown that in normal elution mode $f(k)$ is maximum for $\bar{k} = \sqrt{t_{sc}/t_0}$. However, for the reversed direction mode and for the restricted elution mode $f(k)$ increases dramatically, when $\bar{k}$ approaches t_{sc}/t_0 (see Figure 1.7) [12,21]. Here, if $\bar{k} = t_{sc}/t_0$, then the velocity of the solute zone is zero. Consequently, in these modes very high resolution can be obtained even for very low selectivity, but at the expense of migration time of the solutes to be separated. For example, Bushey and Jorgenson [24] succeeded in separating isotopically substituted compounds (dansylated methylamine and dansylated methyl-d_3-amine) by micellar EKC with migration times of more than 90 minutes.

In 1993 Zhang *et al*. [25] published a paper describing phenomena in EKC based on conventional chromatography theory. They defined three new parameters. One is the phase velocity ratio P_r, which is identical to t_{sc}/t_0. They define a negative time as that where the direction of migration is towards the positive electrode (the anode), and a positive time if the direction of migration is towards the negative electrode (the cathode).

The second new parameter is the column availability A_{co}, which corresponds to the last term in Equation (1.13):

$$A_{co} = \frac{P_r - 1}{P_r + \bar{k}} \tag{1.16}$$

The third parameter is the virtual column length L', which corresponds to the actual length of the capillary to the detector multiplied by the column availability A_{co}. In the case of conventional chromatography, $A_{co} = 1$ and the solute zone is only transported by the mobile phase to the detector. Zhang *et al.* [25] showed that in normal elution mode, $A_{co} < 1$, whereas in restricted elution mode and in reversed direction mode A_{co} can exceed unity.

1.4.4 Peak Capacity

According to Giddings [26], the peak capacity n corresponds to the maximum number of components resolvable in one chromatographic run. It is obvious that for the analysis of a complex sample with a high number of constituents a high peak capacity is mandatory in order to avoid comigration of solutes. In deriving an equation approximating the number of components resolvable in one chromatographic run, Giddings assumed a constant plate number independent of the solute and the retention factor:

$$n = 1 + \frac{\sqrt{N}}{4} \ln\left(\frac{t_2}{t_1}\right) \tag{1.17}$$

t_1 is the migration (or elution) time of the first solute zone, while t_2 is the migration time of the last solute zone. In normal elution mode in EKC, t_1 is identical to t_0 and t_2 is identical to t_{sc}, because all neutral solutes have to be eluted within this time span. Consequently, the migration window has a direct impact on the peak capacity in normal elution mode. In other elution modes of EKC there is no fundamental restriction of migration time.

It is, however, important to note that the requirement for Equation (1.17), constant plate numbers independent of the retention factor, is not fulfilled in practice. Some authors have therefore preferred to use the separation number SN instead of the peak capacity [27,28]. The separation number is defined as the number of component peaks that can be placed between the peaks of two consecutive homologous standards (e.g. the homologous series of *n*-alkyl phenyl ketones) with z and $z + 1$ carbon chain atoms, separated by a resolution of 1.177. Also the separation number is dependent on the migration window. The separation number takes into account the varying band broadening with increasing migration time. Kolb *et al.* [28] have therefore suggested calculating the overall peak capacity from the sum of separation numbers within a given z range.

1.4.5 Determination of the Velocities of the Mobile Phase and the Separation Carrier

Generally, for the determination of the electroosmotic velocity, which is identical to the velocity of the mobile phase, a sample that contains a neutral compound is injected (the marker of the velocity of the mobile phase), which is not retained by the separation carrier or the capillary wall and which has an effective electrophoretic mobility of zero. Several polar substances have been used to this end: acetone, formamide, and thiourea [29]. The marker must be detected by the detector in use. In the case of a UV detector the baseline disturbance caused by a zone of different refractive index to the separation buffer can be used as a signal [30].

If a substance (the marker of the velocity of the separation carrier) is available that is exclusively transported by the separation carrier and not transported by the mobile phase ($k \rightarrow \infty$) and this substance can be detected by the detector in use, then the velocity of the separation carrier can also be determined with a sample containing a marker. It is important to note that the marker of the velocity of the separation carrier does not have to be a neutral substance, and mainly nonpolar azo dyes (Sudan III, Sudan IV) [1,31], dodecanophenone [32] or polycyclic aromatic hydrocarbons [33] have been employed as marker substances. When using negatively charged micellar phases as separation carriers, Terabe *et al*. suggested using positively charged compounds with a nonpolar structure unit [34]. One of these substances is timepidium bromide [35]. Another compound employed to this end is quinine hydrochloride [8,36]. However, determinations of the velocity of the separation carrier with a simple marker should be treated with caution. Careful investigations have shown that these data can be misleading, especially if mobile phases are used that contain a considerable volume fraction of an organic solvent [37].

Bushey and Jorgenson [24,38] therefore suggested an iteration procedure to determine the separation carrier migration time. This iteration procedure is now used by many scientists working in the field. For chromatographic separations based on solvophobic interaction the Martin equation holds true: there is a linear relationship between the logarithm of the retention factor and the carbon number of the members of a homologous series. Muijseaar *et al*. [39] verified that this linear relationship is also valid in EKC with a micellar separation carrier (for the homologous series of n-alkyl benzenes and n-alkyl phenyl ketones as solutes and buffers containing sodium dodecylsulfate, decyltrimethylammonium bromide, or hexadecyltrimethylammonium bromide). According to the iteration procedure suggested by Bushey and Jorgenson, the migration time of the longest chain homologue is taken as approximation of the separation carrier migration time. Then the retention factors of the shorter chain homologues are calculated using this approximated separation carrier migration time. With the retention factors of the shorter chain homologues (plotting logarithm of k versus carbon number) a regression line is calculated. A new value for the retention factor of the longest chain member of the series is obtained by extrapolation of the regression line to the corresponding carbon number. Then a new value for the separation carrier migration time is calculated from the extrapolated value of the retention factor for the longest chain member of the series, and this procedure is continued until the difference between a new value for t_{sc} and the value calculated in the last iteration step is below a threshold value. This general procedure has been used by many groups in estimating the migration time of the separation carrier with different mobile phases and different pseudostationary phases: micelles [40–43], microdroplets [44], dendrimers [45] and polymeric pseudostationary phases [46].

Certainly, the marker method is more convenient than the iterative procedure. Several researchers have shown that in the case of purely aqueous mobile phases, the results for t_{sc} obtained from the migration time of a suitable marker can be equivalent to that obtained by the iterative procedure [30,39]. However, Bailey and Dorsey [47] emphasize that small errors in determining the migration time of the marker can lead to drastic errors in the calculated retention factor. From several potential markers of the velocity of the separation carrier, dodecanophenone has been selected by several authors as the most suitable due to its solubility properties and its high absorbance coefficient [30,47].

Methods to regulate the migration window have been reviewed recently [37]. The migration window reflects the velocity ratio of the two 'phases' involved. While it is difficult to modify the effective electrophoretic mobility of the pseudostationary phase, the electroosmotic mobility generated using a native fused-silica capillary can easily be modified by changing the pH of the separation electrolyte. For neutral solutes, the pH has no impact on the retention factor. Rasmussen and McNair [48] showed for micellar EKC with SDS that the elution order for n-alkyl parabenes can easily be reversed by decreasing the separation buffer pH from 7.0 to 3.37. This corresponds to a change in the elution mode (normal elution to reversed direction mode). Also Otsuka and Terabe [49] investigated the effect of the pH of the separation buffer on the velocities of the mobile and the micellar phase (SDS as surfactant). The pH range investigated was from 7.0 to 3.0. The electroosmotic velocity decreased dramatically with a decrease in pH below 5.5, while the electrophoretic velocity of the micellar phase was almost constant throughout the pH-range investigated. At a pH of 5.0 the absolute velocity of the micellar phase was identical to the absolute electroosmotic velocity.

Another approach to modifying the velocity of the mobile phase is to use coated capillaries. Janini *et al.* [50,51] showed that in micellar EKC with SDS and polyacrylamide-coated capillaries, hydrophobic solutes can be separated with high efficiency in a short run time. With these capillaries the electroosmotic flow is almost completely suppressed [52], so that in this case the separation carrier takes over the role of the mobile phase and the surrounding medium can be regarded as equivalent to the stationary phase of conventional chromatography. Consequently, here the column availability A_{co} is 1 and there is no restricted migration window.

1.4.6 Retention Indices

In 1994 Muijselaar *et al.* [39] presented the application of the retention index concept in EKC. In chromatography, retention indices have been used for the identification of solutes because they are considered to express the retention with the best reproducibility and precision. They can also be used for structure–activity relationships and the characterization of stationary (and mobile) phases. Generally, the retention index I of a solute is calculated by the logarithmic interpolation between two neighbouring members of a homologous series according to:

$$I = 100z + 100\,\frac{\lg k_S - \lg k_z}{\lg k_{z+1} - \lg k_z} \tag{1.18}$$

where k_z and k_{z+1} are the retention factors of the homologues with z and $z+1$ carbon atoms, respectively, and k_S = retention factor of the solute.
Regarding the determination of k in EKC (normal elution mode) it follows:

$$I = 100\,z + 100\,\frac{\lg\left(\dfrac{t_s - t_0}{t_{sc} - t_s}\right) - \lg\left(\dfrac{t_z - t_0}{t_{sc} - t_z}\right)}{\lg\left(\dfrac{t_{z+1} - t_0}{t_{sc} - t_{z+1}}\right) - \lg\left(\dfrac{t_z - t_0}{t_{sc} - t_z}\right)} \tag{1.19}$$

where t_s is the migration time of solute, t_z and t_{z+1} are migration times of the homologues with z and $z+1$ carbon atoms respectively, t_0 is the migration time of the front of mobile

phase, and t_{sc} is the migration time of the front of the separation carrier. Consequently, for the determination of I the migration velocities of the separation carrier and of the surrounding phase also have to be determined exactly. In contrast to the retention factor, the retention index I (a relative quantity) is independent of the phase ratio and consequently, is also independent of the separation carrier concentration, which follows from theory and has been verified experimentally [39]. The dependence of retention indices on temperature was shown to be very small. A significant decrease in relative standard deviations was obtained by comparing retention indices to retention factors [53]. These features make the retention index the ideal parameter for the identification of peaks [54]. Muijselaar *et al.* [39] and Ahuja and Foley [55] demonstrated for micellar EKC that the series of alkylbenzenes, of *n*-alkyl phenyl ketone, and of 1-nitroalkanes show a linear relationship between the logarithm of the retention factor and the carbon number of the homologues. Hence, these series can be applied as retention index standards in MEKC.

The determination of I for different solutes and different micellar separation carriers indicated that polar and nonpolar compounds are solubilized in different ways. Hence, the shift in $I(\Delta I)$ for the same compound and a different separation carrier can serve as a valuable parameter for characterizing the selelectivity in EKC. ΔI values can be applied to the classification of separation carriers in EKC analogously to using the Rorschneider–McReynolds scale in gas chromatography [56]. Ishihama *et al.* [57] reported, for microemulsion EKC, a high correlation between I and the logarithmic octanol–water distribution coefficient ($\log P_{OW}$) for 53 aromatic sample compounds possessing different functionalities. They preferred I to k, because the reproducibility and the repeatability (batch-to-batch and run-to-run) were drastically improved, when using I as a parameter for correlation studies.

1.4.7 Efficiency

Efficiency is a measure of the band broadening occurring during separation. Terminology developed for chromatography has been transferred to capillary electromigration separation methods. The height equivalent to one theoretical plate, (or plate height) H corresponds to the peak variance (in length units) divided by the migrated distance. The plate number N is the migrated distance divided by the plate height. In EKC with standard experimental parameters plate numbers of 200 000 to 300 000 can be obtained under routine conditions. Yu *et al.* [58] have shown that plate numbers obtained in micellar EKC with neutral analytes having low to medium retention factors can be estimated by a simple model based on longitudinal diffusion and length of the injected sample plug. The efficiency for these neutral analytes was independent of the sufactant (SDS) concentration (15–100 mmol L^{-1}). This model has been refined by attributing the instrumental variance not only to the length of the analyte plug but by estimating it from the peaks of the micellar marker (regression method) [59]. In these investigations it was taken into consideration that the overall diffusion coefficient in a medium containing a separation carrier is the weighted average of the analyte diffusion coefficient in the mobile phase and the diffusion coefficient of the analyte-separation carrier adduct [60].

In the past it has been a subject of debate as to whether the efficiency in EKC is also influenced by nonequilibrium effects (separation carrier mass transfer, transchannel mass transfer) or separation carrier (micellar) polydispersity [61–63]. Recent investigations,

however, show that lower plate numbers than those that would be expected if only instrumental band broadening and diffusion are responsible mechanisms, can be explained by the radial variation of the effective separation carrier mobility resulting from Joule heat [59,64].

It was experimentally verified that peak asymmetries and efficiency loss found with samples of high analyte concentration (concentration overload) can be explained in terms of nonlinear chromatography [65]. The technique of 'vacancy injection' makes it possible to determine the distribution isotherms, which can be described by a Langmuir isotherm [66]. Generally, in capillary electromigration separation techniques, laminar flow due to pressure differences between the two ends of the capillary has to be avoided, as it can dramatically reduce efficiency. In the case of differences in the electroosmotic velocity in different segments of the capillary (e.g. partial filling technique) band broadening can result from intersegmental pressure [67]. A further source of band broadening can be analyte adsorption at the inner capillary wall, which should be suppressed by a suitable composition of the separation electrolyte or dynamic or static coating of the inner capillary wall.

1.5 Separation of Weak Electrolytes

1.5.1 Migration of Acids

The separation mechanism of charged compounds in EKC is based on both chromatographic and electrophoretic principles. In 1985, Otsuka *et al.* [68] studied the migration behaviour and separation of chlorinated phenols by micellar EKC with SDS as separation carrier. They describe the overall effective electrophoretic velocity of a (partially) ionized solute v_{eps} in a micellar medium as the weighted sum of the effective electrophoretic velocity v_{ep} of the solute and the electrophoretic velocity of the separation carrier v_{epsc}.

$$v_{eps} = \frac{1}{k+1} v_{ep} + \frac{k}{k+1} v_{epsc} \tag{1.20}$$

The true retention factor k [in contrast to the appararent retention factor k_{app} calculated according to Equation (1.6)] can only be calculated if the effective electrophoretic velocity v_{ep} of the solute zone in the separation electrolyte without separation carrier is known. This quantity is mainly determined by CE experiments. However, it should be noted that several assumptions are made in this case: the influence of the separation carrier on ionic strength, dielectric constant and viscosity are assumed to be negligible and, in the case of micellar or microemulsion EKC interactions of the solute with surfactant monomers, are assumed not to occur [69]. In that case, k can be calculated from:

$$k = \frac{v_{eps} - v_{ep}}{v_{epsc} - v_{eps}} = \frac{\mu_{eps} - \mu_{ep}}{\mu_{epsc} - \mu_{eps}} \tag{1.21}$$

With this equation, k can be calculated even for a fully ionized solute. Otsuka *et al.* determined v_{ep} for phenolic compounds with a buffer containing 5 mmol L^{-1} SDS, assuming that this concentration is below the CMC. Generally when using this approach, it has to be considered that the CMC of a surfactant in an aqueous separation electrolyte

can be substantially lower than that in pure water [70]. The phenomenological approach of Otsuka *et al*. [68] satisfactorily explains the dependence of the retention factor k on the pH of the separation buffer for the chlorinated phenols investigated. It is important to note that the retention factor for the negatively charged species was measurable and different from zero.

If the migration time t_{0sc} of a solute in absence of the separation carrier is known, then the true retention factor k can also be calculated from t_{0sc}, from the migration time t_s of the solute in presence of the separation carrier, and from the migration time of the separation carrier t_{sc} [71]:

$$k = \frac{t_s - t_{0sc}}{t_{0sc}\,(1 - t_s/t_{sc})} \tag{1.22}$$

Equation (1.22) is analogous to Equation (1.6). The problem here is the determination of t_{0sc}. If the electroosmotic velocity is not independent of the separation carrier concentration, then t_{0sc} cannot be directly determined and has to be calculated from the effective electrophoretic velocity of the solute v_{ep} and the electroosmotic velocity in the presence of the separation carrier [47]. In 1991 Khaledi *et al.* [72] investigated in detail the migration behaviour of acidic solutes in micellar EKC dependent on the pH and the concentration of an anionic surfactant. Their phenomenological approach confirms the observations made by Otsuka *et al.* [68].

The retention factor k of an acid is the weighted average of the retention factors of its undissociated (HA) and its dissociated (A) forms:

$$k = F_{HA}^{aq}\, k_{HA} + F_{A}^{aq}\, k_{A} \tag{1.23}$$

where F_{HA}^{aq} is the mole fraction of the undissociated acid in the aqueous phase, F_{A}^{aq} is the mole fraction of the dissociated acid in the aqueous phase, k_{AH} is the retention factor of the undissociated acid, and k_A is the retention factor of the dissociated acid, $F_{HA}^{aq} + F_{A}^{aq} = 1$.

It is assumed that secondary equilibria with buffer constituents do not occur. The mole fractions of the undissociated and the dissociated acid in the aqueous phase are dependent on the pH and the acid constant K_a:

$$F_{HA}^{aq} = \frac{c(H^+)}{c(H^+) + K_a} \tag{1.24}$$

$$F_{A}^{aq} = \frac{K_a}{c(H^+) + K_a} \tag{1.25}$$

$$k = \frac{k_{HA} + k_A\,(K_a/c(H^+))}{1 + (K_a/c(H^+))} \tag{1.26}$$

According to Equation (1.26), the dependence of the retention factor for acidic solutes on the pH is a sigmoidal relationship with maximum slope for $pH = pK_a$. This also holds true for the partition coefficient P.

In EKC not only the retention factor k but also the observed (apparent) effective mobility μ can be taken as parameter to describe the migration behaviour of acidic solutes quantitatively:

$$\mu = F_{HA}^{SC}\, \mu_{SC} + F_{A}^{SC}\, \mu_{SC} + F_{A}^{aq}\, \mu_{A}^{aq} \tag{1.27}$$

where F is the molar fraction of the dissociated or undissociated species associated with the separation carrier or in the aqueous phase, μ_{SC} is the observed mobility of the separation carrier, and μ_A^{aq} is the observed mobility of the dissociated acid in the aqueous phase,

$$F_{HA}^{sc} + F_A^{sc} + F_{HA}^{aq} + F_A^{aq} = 1$$

If we define the apparent acid constant $K_{a,app}$ in a medium containing a separation carrier as:

$$K_{a,app} = \frac{(c_{SC}(A^-) + c_{aq}(A^-))\ c_{aq}(H^+)}{(c_{SC}(HA) + c_{aq}(HA))} \tag{1.28}$$

it can be shown that the observed overall effective mobility μ of a solute in a medium containing a separation carrier can be described as a function of μ_{HA} and μ_A (the apparent mobilities of the two species in the medium containing a separation carrier), the apparent acid constant $K_{a,app}$ and the molar proton concentration $c(H^+)$:

$$\mu = \frac{\mu_{HA} + \mu_A\ (K_{a,app}/c(H^+))}{1 + (K_{a,app}/c(H^+))} \tag{1.29}$$

with

$$\mu_{HA} = \frac{K_{HA}^{SC}\ c(SC)\ \mu_{SC}}{1 + K_{HA}^{SC}\ c(SC)} \tag{1.30}$$

$$\mu_A = \frac{\mu_A^{aq} + K_A^{SC}\ c(SC)\ \mu_{SC}}{1 + K_A^{SC}\ c(SC)} \tag{1.31}$$

On the basis of these equations, Smith and Khaledi [73] developed a model to predict the migration behaviour of organic acids in micellar EKC employing an anionic surfactant (SDS) in terms of the acid constant K_a, the separation carrier binding constants K_A^{SC} and K_{AH}^{SC}, the pH and the molar separation carrier concentration. In the case of micelles as separation carriers, K^{SC} is identical to the partition coefficient P multiplied by the molar volume of the micelles.

Quang *et al*. [74] generalized this phenomenological approach, also taking ion pair interactions into consideration. For an acidic solute this type of interaction would be expected for cationic surfactant micelles as separation carriers. In this case ion pair formation of the anionic species with the cationic surfactant monomers (reducing the effective electrophoretic mobility of the solute in the aqueous phase) can take place. In Figure 1.8 the possible interactions of an acidic or a basic solute in an anionic or in a cationic micellar phase are schematically depicted.

We then have to take into consideration the ion pair formation equilibrium $A + S_{mono} \rightleftarrows [AS_{mono}]$ (S_{mono} = surfactant monomer) with the ion pair equilibrium constant K_{IP}. The concentration of S_{mono} corresponds to the critical micelle concentration. The electrophoretic mobility of the ion pair $[AS_{mono}]$ is zero and following molar fractions have to be considered:

$$F_{HA}^{SC} + F_A^{SC} + F_{HA}^{aq} + F_A^{aq} + F_{ASmono}^{aq} = 1 \tag{1.32}$$

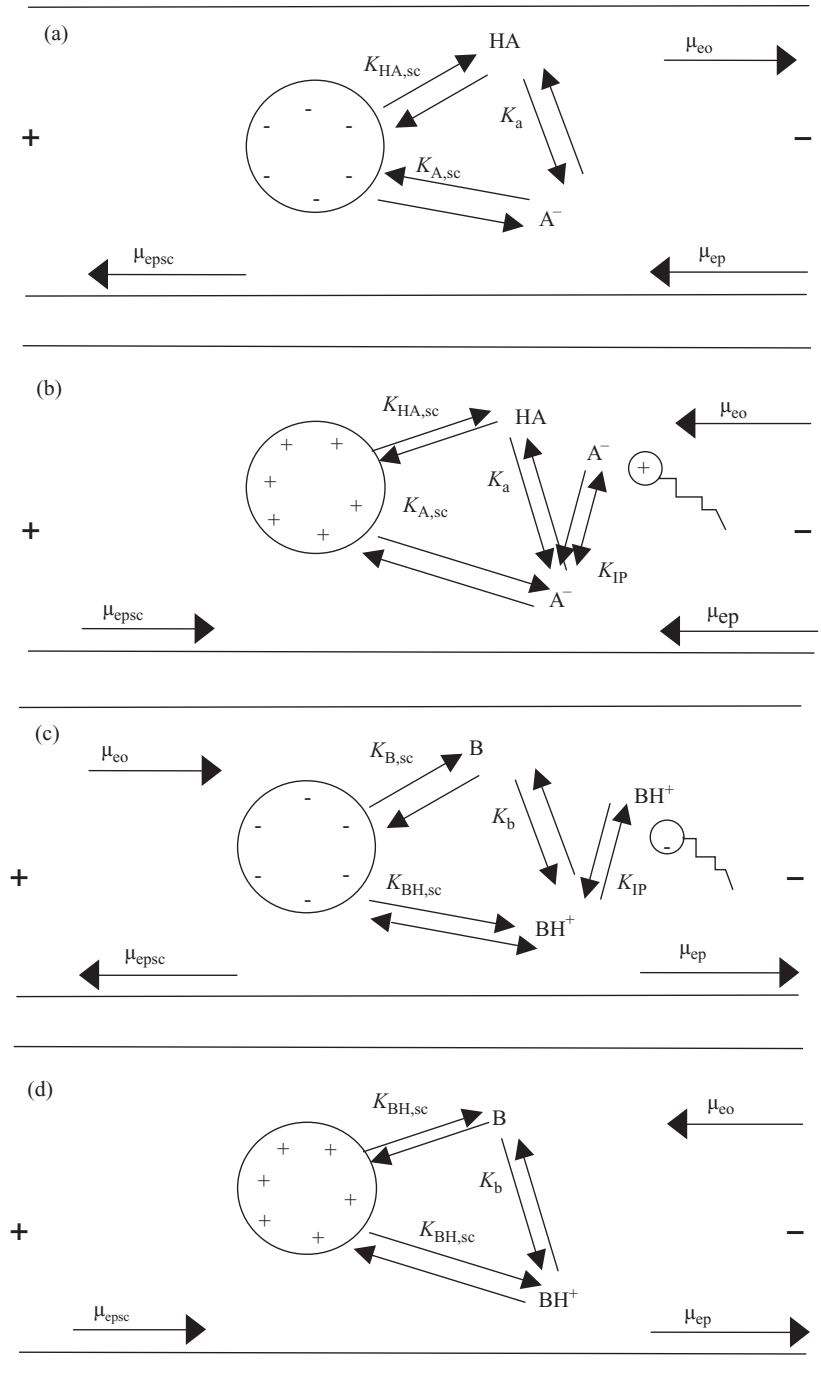

Figure 1.8 *Interaction of ionizable solutes with micellar separation carriers: (a) acidic solute (AH) with anionic SC; (b) acidic solute (AH) with cationic SC; (c) basic solute (B) with anionic SC; (d) basic solute (B) with cationic SC (according to [74])*

Table 1.2 *Influence of cationic surfactant monomers on electrophoretic mobilities of acidic solutes (electrolyte composition: (a) c(H_3BO_3) = 10 mmol L^{-1}, c($Na_2B_4O_7$) = 10 mmol L^{-1}, pH = 9.0; (b) c(H_3BO_3) = 10 mmol L^{-1}, c($Na_2B_4O_7$) = 10 mmol L^{-1}, c(DoTAB) = 5 mmol L^{-1}, pH = 9.0. capillary 75 μm i.d., 50 cm effective length, 57 cm total length; voltage 25 kV; temperature 25 °C; sample pressure injection: 2 s)*

Solute	$\mu_{ep}/(10^{-3}$ cm^2 V^{-1} s$^{-1})$ CE buffer	$\mu_{ep}/(10^{-3}$ cm^2 V^{-1} s$^{-1})$ CE buffer + DoTAB
4-Hydroxy-3-methoxybenzyl alcohol	−0.034	−0.034
4-Hydroxybenzyl alcohol	−0.036	−0.039
Ethylvanilline	−0.228	−0.223
Vanilline	−0.253	−0.246
4-Methoxybenzoic acid	−0.265	−0.256
4-Hydroxybenzaldehyde	−0.271	−0.269
Vanillic acid	−0.293	−0.297
4-Hydroxybenzoic acid	−0.311	−0.317
3,4-Dihydroxybenzoic acid	−0.394	−0.393

Where F_A^{aq} is the molar fraction of the free dissociated species in the surrounding phase, and F_{ASmono}^{aq} is the molar fraction of the ion-paired dissociated species in the surrounding phase. One possibility of studying the presence or absence of ion pair formation of charged solutes with oppositely charged surfactant monomers in the aqueous phase is afforded by capillary electrophoresis experiments with surfactants showing a high CMC. In Table 1.2 effective electrophoretic mobilities of several phenol and benzoic acid derivatives determined by CE in the presence and absence of 5 mmol L^{-1} dodecyltrimethylammonium bromide (DoTAB) are given. The CMC of DoTAB (21–23 °C) in these two buffers is 13–14 mmol L^{-1} (determined by conductometric titration [75]). Consequently, in these buffers DoTAB is only present as monomer. The comparison of effective electrophoretic mobilities shows that ion pair formation can be neglected in this case. There is no significant decrease in μ_{ep} due to the presence of surfactant monomers. It has to be emphasized that with 5 mmol L^{-1} DoTAB there is a reversal of the direction of the electroosmotic flow and consequently a reversal of the migration order. Consequently, DoTAB is also present in form of positively charged hemimicelles formed at the interface liquid–capillary. However, there is no band broadening due to solute–capillary wall (hemimicelle) interactions. It has to be emphasized that the solutes studied here are relatively hydrophilic.

Muijselaar *et al.* [69] verified experimentally that both the mobility model and the retention model describe the migration of monovalent acids in micellar EKC well. However, they observed (hydrophobic) interaction of the (hydrophobic) undissociated form of the acid with surfactant monomers. This interaction is a phenomenon that may have a marked influence on the determination of true retention factors for hydrophobic species.

1.5.2 Migration of Bases

The same concept outlined in the previous section was used by Strasters and Khaledi [76] in 1991 to describe the migration behaviour of cationic solutes in EKC with anionic

micellar separation carrier. They took into consideration the acid–base equilibrium, ion pair formation between the conjugated acid of the base and the surfactant monomer, and the distribution equilibria of both the base and its conjugated acid between the aqueous phase and the separation carrier (see Figure 1.8c).

In the case of an ion pair formation constant K_{IP} approaching infinity the solute will be present in the aqueous phase either as neutral species or as neutral ion pair. Consequently the effective electrophoretic mobility of this solute v_{ep} [refer to Equation (1.20)] in the aqueous medium is zero, and the true retention factor can be calculated from [refer to Equation (1.21)]:

$$k = \frac{\mu_{eps}}{\mu_{epsc} - \mu_{eps}} \tag{1.33}$$

In the absence of ion pair formation k can be calculated from Equation (1.21).

Employing these two equations, Strasters and Khaledi calculated the retention factors for several basic solutes dependent on the concentration of SDS in the separation electrolyte. They obtained linear relationships differing in the x-axis intersection. As the x-axis intersection should correspond to the CMC of SDS, negative values clearly indicate the invalidity of the approach. Their results suggest that ion pair formation must not be neglected, especially for the hydrophobic solutes.

On the basis of only five experiments, Quang *et al.* [74] succeeded in correctly modeling the migration behaviour of 17 aromatic amines separated by micellar EKC with an anionic surfactant (SDS) within a parameter range of $c(\text{SDS}) = 20$–85 mmol L^{-1} and pH = 7.0–12.0. In their approach it was assumed that the protonated base is present in the surrounding aqueous phase only as an ion pair formed with the surfactant monomer.

It would be interesting to model the migration behaviour of partly protonated bases or partly dissociated acids employing separation carriers that are not present as surfactant monomers (e.g. polymeric separation carriers) in order to verify the phenomenological approach outlined in the previous sections. Those equations concerning the resolution of charged solutes derived by Corstjens *et al.* [77] assume that the electrophoretic mobility of a charged solute in the surrounding medium is constant and not influenced by the separation carrier.

1.6 Separation of Ions

In 1992 Kaneta *et al*. [78] studied the migration behaviour of inorganic anions in micellar EKC using a cationic surfactant (cetyltrimethylammonium chloride). They employed the overall effective electrophoretic mobility μ_{eps} [which is not identical to the observed overall effective mobility μ of a solute in a medium containing a separation carrier in Equation (1.29)] as the parameter to describe the migration ($\mu = \mu_{eps} + \mu_{eo}$). In the presence of a cationic surfactant the electrophoretic mobility of an anion is influenced by interaction with the surfactant monomers (ion pair formation) and by interaction with the positively charged micellar pseudophase (‘distribution’). In fact, in this case the micellar pseudophase might be regarded as an ion-exchange separation carrier.

Below the CMC, the effective electrophoretic mobility μ_{ep} is given by:

$$\mu_{ep} = F_A^{aq} \times \mu_{ep,ion} \tag{1.34}$$

where F_A^{aq} is the molar fraction of free ion and $\mu_{ep,ion}$ is the electrophoretic mobility of free ion. Regarding that:

$$K_{IP} = \frac{c([AS_{mono}])}{c(A) \times c(S_{mono})} \tag{1.35}$$

and

$$F_A^{aq} = \frac{c(A)}{c(A) + c([AS_{mono}])} \tag{1.36}$$

it follows:

$$\frac{1}{\mu_{ep}} = \frac{c(S_{mono}) \times K_{IP}}{\mu_{ep,ion}} + \frac{1}{\mu_{ep,ion}} \tag{1.37}$$

Indeed, assuming that $c(S_{mono})$ is given by the total surfactant concentration and that plotting $1/\mu_{ep}$ versus $c(S_{mono})$ results in a straight line, this verifies the theoretical considerations. At the CMC there is a change in the slope, making it possible to determine the CMC and to determine the electrophoretic mobility of a solute at the CMC using a regression method. Above the CMC $c(S_{mono})$ remains constant, consequently also the electrophoretic mobility $\mu_{ep,CMC}$ of the anion in the surrounding aqueous phase remains constant independent of the total surfactant concentration. If the electrophoretic mobility of the separation carrier μ_{epsc} is known, the retention factor is then given by:

$$k = \frac{\mu_{eps} - \mu_{ep,CMC}}{\mu_{epsc} - \mu_{eps}} \tag{1.38}$$

Therefore, the method of Kaneta *et al.* [78] makes it possible to determine true retention factors in a micellar medium for charged and partially charged compounds without neglecting the effects of solute surfactant monomer association.

1.7 Application of Neutral Separation Carriers

In 1997 Collet and Gareil [79] reported the MEKC separation of long chain saturated and unsaturated free fatty acids in an alkaline medium with neutral micelles formed by polyoxyethylene-23-dodecyl ether (Brij 35). In this case, the two phases (the separation carrier and the surrounding phase) itself have the same velocity; however, this is not the case for the *solutes in the two phases*. Neutral separation carriers can only be used in EKC, if the solutes to be separated have an effective electrophoretic mobility in the surrounding phase. If the two solutes to be separated have identical effective electrophoretic mobilities in the surrounding phase, then all considerations made in Section 1.4 can be also applied, provided that the parameter t_0 (migration time of the front of the surrounding phase) is replaced by the parameter t_{0sc}, which is the migration time of a solute zone in the absence of the separation carrier.

1.8 Conclusions

As pointed out by Terabe [2] EKC can be regarded as an intermediate between electrophoresis and chromatography. Consequently, the separation process can be described either as a chromatographic or as an electrophoretic process. Both descriptions are valid and fully describe the migration behaviour for neutral solutes or for solutes being present in the mobile phase either in the uncharged form or in the form of an uncharged ion pair. In the case that the solute is also present in a charged form in the mobile phase, both the electrophoretic properties of the solutes and their interaction with the separation carrier have to be taken into consideration.

List of Symbols and Abbreviations

A_{co}	column availability
$c(A)$	molar concentration of the free complex forming additive
$c(H^+)$	molar concentration of protons
CMC	critical micellar concentration
E	electric field strength
F_A^{aq}	mole fraction of the dissociated acid in the aqueous phase
F_{HA}^{aq}	mole fraction of the undissociated acid in the aqueous phase
F_{ASmono}^{aq}	molar fraction of the ion-paired dissociated species in the aqueous phase
I	retention index
k	retention factor
$\bar{k}$	mean retention factor
k_A	retention factor of the dissociated acid
k_{AH}	retention factor of the undissociated acid
k_s	retention factor of the solute
k_z	retention factor of the homologue with z carbon atoms
k_{z+1}	retention factor of the homologue with z + 1 carbon atoms
K_a	acid constant
K_c	complex equilibrium constant
K_{IP}	ion pair equilibrium constant
L'	virtual column length
n	peak capacity
N	plate number
P	partition coefficient
P_{OW}	octanol/water partitioning coefficient
P_r	phase velocity ratio
R_s	resolution
t_0	migration time of the front of the surrounding (mobile) phase
t_{0sc}	migration time of a solute in absence of the separation carrier
t_{mob}	residence time in the mobile phase
t_s	migration time of the solute zone
t_{sc}	migration time of the front of the separation carrier
t_{rsc}	residence time associated with the separation carrier