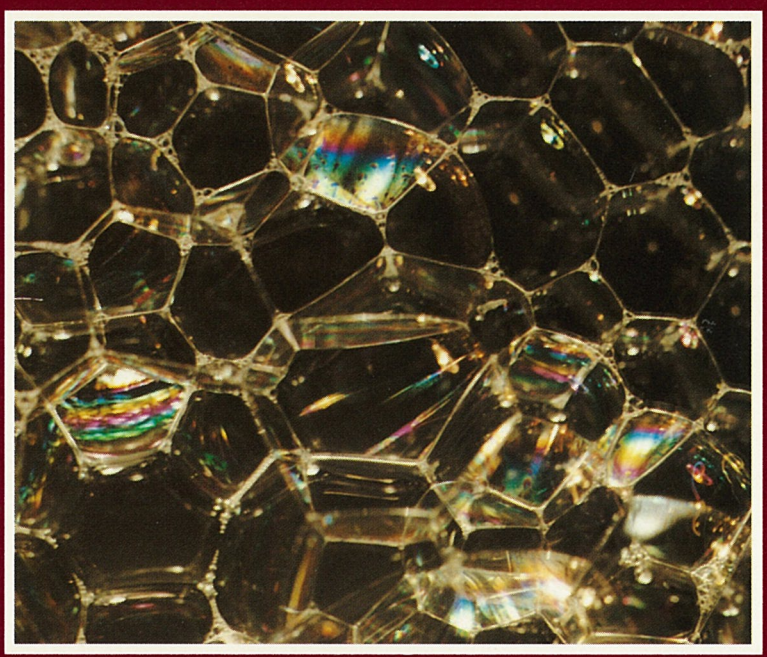
Micellar electrokinetic chromatography : fundamentals and applications

MICELLAR ELECTROKINETIC CHROMATOGRAPHY

Fundamentals and Applications



Pim Muijselaar

MICELLAR ELECTROKINETIC CHROMATOGRAPHY

Fundamentals and Applications

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de
Technische Universiteit Eindhoven, op gezag van
de Rector Magnificus, prof.dr. M. Rem, voor
een commissie aangewezen door het College
van Dekanen in het openbaar te verdedigen op
dinsdag 10 september 1996 om 16.00 uur

door

WILHELMUS GERARDUS HENDRIKUS MARIA MUIJSELAAR

geboren te Geertruidenberg

Dit proefschrift is goedgekeurd door de promotoren:

prof.dr.ir. C.A.M.G. Cramers
en
prof.dr.ir. F.M. Everaerts

Kikite ga kashikoi naraba
hanashite wa oroka de attemoyoi

De spreker mag dan een dwaas zijn
als de luisteraar maar wijs is

Japanse spreuk

Aan mijn ouders

Het in dit proefschrift beschreven onderzoek is financieel ondersteund door de Nederlandse organisatie voor Wetenschappelijk Onderzoek.

Muijselaar, Wilhelmus Gerardus Hendrikus Maria

Micellar electrokinetic chromatography; fundamentals and applications / Wilhelmus Gerardus Hendrikus Maria Muijselaar. - Eindhoven: Eindhoven University of Technology
Thesis Eindhoven University of Technology. - with ref. -
With summary in Dutch.
ISBN 90-386-0358-4
Subject headings: micellar electrokinetic chromatography / capillary electrophoresis

CONTENTS

INTRODUCTION AND SCOPE

CHROMATOGRAPHY

In modern analytical chemistry, chromatography has become the most widely used separation technique. In the beginning of this century, the first chromatographic experiments were carried out by Tswett [1], who separated plant pigments. Since these first experiments, many scientists have made substantial contributions to theoretical as well as practical aspects of this analytical technique, and, especially during the last decades, chromatography reached its present mature state. This development does not only stem from improvements in technology, but certainly also from the growing need of many scientists for better methods to separate complex mixtures. At present, chromatography is an essential analytical tool for the determination of various substances in many fields of science and industry [2]. The separation process in chromatographic analyses is based on differences in distribution equilibria of individual analytes between a mobile phase and a stationary phase. Generally, the movement of the mobile phase is induced by a pressure gradient across the column.

ELECTROPHORESIS

In electrophoresis, charged particles can be separated based on their movement under the influence of an external electric field. The first electrophoretic experiments were already performed by Von Reuss [3] in the beginning of the last century. In 1937, Tiselius demonstrated the possibilities of electrophoresis as analytical method by the separation of biological macromolecules [4]. In order to reduce thermal convection and diffusion, electrophoresis was initially performed in various stabilizing anti-convective media, such as polyacrylamide or agarose gels. Although efficient separations are obtained, slab-gel electrophoresis generally suffers from long analysis times and difficulties in detection and automation. Alternatively, narrow-bore tubes can be applied as separation compartment, thus

facilitating the performance of free solution electrophoresis. Moreover, in these tubes thermal gradients are reduced more effectively due to higher surface/volume ratios. The first free zone electrophoresis experiments were described by Hjertén in 1967, applying a rotating glass tube of 3 mm inner diameter (I.D.) [5]. In the 1970s, smaller I.D. capillaries were successfully applied, allowing the use of higher electric fields [6,7]. It was shown by Mikkers *et al.* [8] that high efficiencies could be achieved, as theoretically described by Giddings [9]. In 1981, Jorgenson and Lukacs demonstrated the potential of capillary electrophoresis as analytical technique, using 75 μm I.D. capillaries and on-column fluorescence detection [10]. Since that time, capillary electrophoresis has been applied for the separation of substances from many different origins, ranging from small inorganic ions to large proteins and oligonucleotides. The results of fundamental studies as well as numerous applications have been reported during the last decade and several reviews [11-13] and textbooks [14-17] are available.

MICELLAR ELECTROKINETIC CHROMATOGRAPHY

In 1984 Terabe *et al.* developed Micellar Electrokinetic Chromatography (MEKC), an analytical technique at the crossroads of chromatography and electrophoresis [18,19]. With this separation method the application area of capillary electrophoresis was extended from ionic species to neutrals. In MEKC a charged surfactant is added to the electrolyte solution, forming a micellar phase. The separation mechanism of neutral species is based on differences in the partitioning between the aqueous phase and the micellar phase (*chromatographic principle*). These two phases are moving with different velocities, according to electrokinetic transport phenomena (*electrophoretic principle*). The aqueous phase is transported by electroosmosis, whereas the micellar phase is transported by a combination of electroosmotic and electrophoretic migration. The movement of the 'pseudo-stationary' micellar phase is a fundamental difference with other chromatographic techniques. Besides neutral species, also mixtures of charged and neutral compounds can be separated by MEKC. In this case differences in electrophoretic mobility as well as differences in phase distribution can be exploited simultaneously to obtain separation. Primarily due to the characteristic flat velocity profile of the electroosmotic flow [20], high efficiencies are generally obtained. Since the introduction of MEKC, several fundamental characteristics as well as practical aspects have been described. A comprehensive textbook was published by Vindevogel and Sandra [21]. At present, major applications of MEKC are found in pharmaceutical and biotechnological sciences.

An important feature of MEKC is its flexibility. The composition of the electrolyte system

can easily be changed in order to control migration behaviour and optimize selectivity. In this respect the pseudo-stationary phase plays a key role, since its chemical nature has a major influence on the separation process. Various surfactant systems can be used as well as mixed micelles, possessing different solubilization characteristics. Besides micelles, also other macromolecular structures such as oligomers, micro-emulsions or dendrimers can be applied as pseudo-stationary phases. The application of chiral surfactants or cyclodextrines enables enantiomeric separations.

On the other hand, several limitations in MEKC can be recognized as well. Due to the movement of the pseudo-stationary phase, a finite elution window is observed, which makes MEKC less applicable for the separation of extremely complex mixtures or hydrophobic compounds. In addition, since commonly used micellar systems are not stable in organic media, the application of organic modifiers is limited.

Despite the ease of varying the experimental conditions, proper selection of a suitable electrolyte system in MEKC is still a difficult task. In gas chromatography stationary phases can be chosen on the basis of specific selective chemical interactions using the Rohrschneider-McReynolds scale [22], whereas in liquid chromatography mobile phases can be selected based on Snyder's selectivity triangle [23]. As stated by Khaledi [24], similar approaches in MEKC would greatly facilitate method development procedures. At present, however, the influence of the chemical nature of the pseudo-stationary phase and the structural properties of solutes on solute-micelle interactions is still not well understood. Moreover, the migration behaviour of charged as well as neutral compounds depends on many experimental parameters, which cannot be controlled independently in many cases [21,25]. Although these problems can be circumvented by statistical resolution optimization strategies [26], a better knowledge of the interaction phenomena during micellar solubilization and the influence of experimental conditions on migration behaviour would provide a theoretical background from which MEKC separation methods can be developed.

SCOPE OF THIS THESIS

In this thesis several fundamental aspects of the separation process during micellar electrokinetic chromatography are studied. The primary aim of these investigations was to get a better insight in the migration and selectivity mechanisms in MEKC, and, in addition, how these mechanisms can be characterized and quantified.

Chapter 1 gives a theoretical description of the basic principles in MEKC. Electrophoretic and electroosmotic transport phenomena are discussed and the migration behaviour of neutral compounds is described in terms of chromatographic as well as electrophoretic

parameters. A summary is given about the theoretical aspects of band-broadening in MEKC and the instrumentation, applied in this study, is described.

Chapter 2 deals with the separation performance. The resolution in MEKC is treated theoretically and the influence of various experimental conditions on efficiency, elution window and retention factors is evaluated.

Chapter 3 discusses the migration behaviour of charged compounds. A retention model and a mobility model are described and a comparison is made between these models, applying monovalent weak acids and an anionic surfactant system.

Chapter 4 discusses the application of the retention index concept in MEKC. It is demonstrated that retention indexes are independent of surfactant concentrations and that the relationship between retention indexes and *n*-octanol-water partition coefficients can provide information about micellar solubilization.

Chapter 5 describes two methods for the characterization of micellar pseudo-stationary phases. The influence of different solute-micelle interactions on micellar solubilization is studied by linear solvation energy relationships. It is shown that retention indexes can be applied for the classification of micellar pseudo-stationary phases in MEKC, in a similar way as the Rohrschneider-McReynolds scale in gas chromatography.

Chapter 6 deals with the electrokinetic properties of dendrimers and their potential as pseudo-stationary phases. In addition, the use of tetraalkylammonium ions for the separation of highly hydrophobic compounds in aqueous/organic media is investigated.

Chapter 7 discusses several physico-chemical properties of surfactant solutions, relevant for MEKC separations. Diffusion coefficients in micellar media are determined by the stopped migration method, and the migration behaviour of micelle counterions is evaluated.

Chapter 8 illustrates the potential of MEKC in pharmaceutical analyses by the qualitative as well as quantitative determination of various drugs in pharmaceutical formulations and biological samples.

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1

BASIC CONCEPTS OF MICELLAR ELECTROKINETIC CHROMATOGRAPHY

ABSTRACT

In this chapter the basic principles of the separation process during Micellar Electrokinetic Chromatography (MEKC) are described. The separation mechanism in MEKC is based on differences in distribution equilibria between an aqueous phase and a micellar phase. These phases are moving with different velocities, due to a combination of electrophoresis and electroosmosis. Both these electrokinetic transport phenomena are discussed. Equations for retention factor and mobility, two possible migration parameters, are given and various band-broadening mechanisms during MEKC analyses are evaluated. Finally, the instrumentation for performing MEKC experiments is described.

1.1 INTRODUCTION

Micellar Electrokinetic Chromatography (MEKC) is a highly efficient separation method, primarily suitable for the analysis of small neutral compounds [1-3]. The separation mechanism of this analytical technique is based on differences in distribution equilibria of sample compounds between an aqueous mobile phase and a pseudo-stationary micellar phase. A unique advantage of MEKC is the possibility to easily change the composition of the electrolyte system and, in particular, the chemical nature of the pseudo-stationary micellar phase. In this way differences in selectivity can be introduced rapidly in order to control migration behaviour and optimize resolution. Besides neutral compounds, also charged compounds with almost identical electrophoretic mobilities or mixtures of charged and uncharged compounds can be separated [4]. In this situation electrophoretic mobility as well as micellar solubilization determine the migration behaviour of the sample compounds. In MEKC both the aqueous phase and the pseudo-stationary micellar phase are transported by electrokinetic phenomena, including electroosmosis and electrophoresis. Consequently, MEKC separations are performed using capillary electrophoresis instrumentation.
In this chapter several basic principles of capillary electrophoresis are discussed with emphasis on micellar electrokinetic chromatography.

1.2 ELECTROPHORESIS

Electrophoresis is a process for separating charged particles based on their movement through a solution under the influence of an external electric field. Under given experimental conditions the linear velocity, v, of each particle is proportional to the applied electric field, E, according to:

$$v = m_{eff} E \tag{1.1}$$

The proportionality constant, m_{eff}, is called the effective mobility and is a characteristic property of each particle in a specific separation medium. To obtain an expression of the mobility in terms of different physical parameters, the forces acting on the particle at infinite solution can be considered.
When a charged particle is placed in an external electric field, it experiences an electric force, F_e, equal to the product of its net charge q and the electric field strength, E:

$$F_e = q\ E \tag{1.2}$$

Due to its motion the particle also experiences a friction force, F_f, which can be expressed according to Stokes' law as:

$$F_f = 6\ \pi\ \eta\ r\ v \tag{1.3}$$

where η is the viscosity of the surrounding medium, r is the effective hydrodynamic radius of the particle and v is the linear velocity. Practically immediately a steady-state will be reached, where the electric force, F_e, is balanced by the frictional force, F_f. Combination of eqns. (1.2) and (1.3) yields an expression for the mobility at infinite dilution:

$$m^0 = \frac{q}{6\ \pi\ \eta\ r} \tag{1.4}$$

The mobility at infinite dilution is related to the molar ionic conductivity at infinite dilution, λ^0, which, for 1:1 electrolytes, is expressed by:

$$m^0 = \frac{\lambda^0}{F} \tag{1.5}$$

where F is the Faraday constant. At finite dilution also relaxation and retardation effects, originating from the counter-ion atmosphere, should be taken into account. These effects have been quantitatively described by Debye, Hückel and Onsager [5]. For weak acids and bases the net charge and, consequently, the mobility, will strongly depend on the degree of dissociation. The effective mobility for weak ionic species, m_{eff}, is the sum of all products of the degree of dissociation and the mobilities at finite dilution of the subspecies, according to:

$$m_{eff} = \sum_i\ \alpha_i\ m_i \tag{1.6}$$

where α_i is the degree of dissociation and m_i is the mobility at finite dilution of the fully dissociated subspecies [6]. Clearly, differences in electrophoretic mobilities can arise as a result of differences in frictional properties, *i.e.* size or shape, or as a result of differences in the net charge of the species, *i.e.* degree of dissociation. Several experimental conditions can change these solute properties and, consequently, their migration behaviour. The

influence of different experimental variables on the migration mechanism and the way to control and optimize these variables play a key role in electrophoretic separation techniques.

1.3 ELECTROOSMOSIS

An important phenomenon in capillary electrophoresis is electroosmotic transport of ionic species during the analysis. Electroosmosis originates from the electrophoretic movement of the hydrated part of the electric double layer at the capillary wall [7]. In uncoated fused silica capillaries, which are most frequently used in capillary electrophoretic techniques, the capillary wall is negatively charged due to the ionization of surface silanol groups. This negative charge is balanced by cations from the solution, resulting in the formation of an electric double layer adjacent to the capillary surface. In Fig. 1.1 the resulting ionic distribution is illustrated. Cations close to the wall are tightly bound and immobile. This rigid inner layer is called the Stern layer. Further from the wall, the ionic atmosphere becomes more diffuse in a double layer with a net positive charge. This outer region is known as the Gouy-Chapman layer. Under the influence of an electric field this layer moves toward the cathode, and due to viscous drag the bulk liquid inside the capillary is transported along. This results in a continuous flow of the electrolyte solution which is referred to as the electroosmotic flow (EOF).
The velocity of the EOF, v_{EOF}, is given by the Helmholtz-Smoluchowski equation [8]:

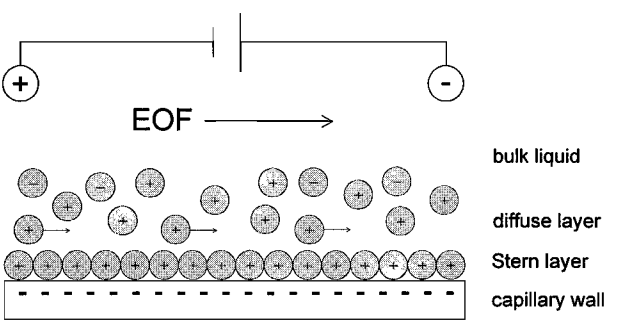

FIGURE 1.1 Schematic representation of the ionic distribution on a fused silica capillary wall and the resulting electroosmotic flow (EOF).

$$v_{EOF} = \frac{\varepsilon \zeta}{4 \pi \eta} E \tag{1.7}$$

where ε is the dielectric constant, ζ the zeta-potential on the capillary wall and η the viscosity at the capillary wall. Analogous to the effective mobility for ionic species, an electroosmotic mobility can be defined as:

$$m_{EOF} = \frac{\varepsilon \zeta}{4 \pi \eta} \tag{1.8}$$

All species in the bulk solution are affected to the same extent by the EOF, irrespective their radial position. Since the EOF shows a flat velocity profile, it does not significantly contribute to the dispersion of sample zones during the separation process. This in contrast with the parabolic flow profile, caused by a pressure drop, as observed in liquid chromatography. On applying an electrolyte system containing a cationic surfactant, a charge reversal of the capillary wall can be obtained, resulting in a reversal of the EOF [9]. This mechanism is illustrated in Fig. 1.2. Surfactant monomers adhere to the capillary surface through ionic interactions of the cationic head groups and anionic surface silanol groups. Due to hydrophobic interactions between apolar alkyl chains, an admicellar bilayer is formed. As a result the capillary wall obtains a cationic character and the EOF is directed towards the anode. This phenomenon occurs during micellar electrokinetic chromatography experiments with cationic surfactant systems. It can also be applied in order to reduce solute-wall interactions based on electrostatic repulsion in, *e.g.*, protein separations [10,11].

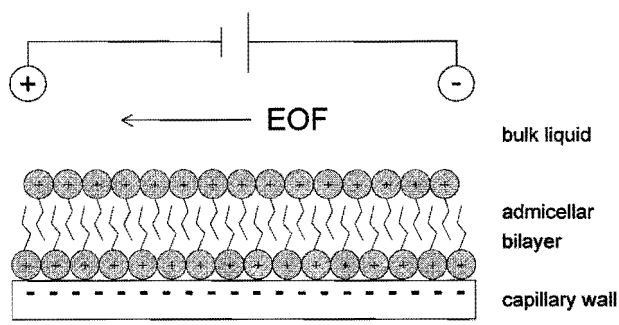

FIGURE 1.2 Schematic representation of the reversal of the electroosmotic flow (EOF) with a cationic surfactant.

1.4 SEPARATION MODES

In capillary electrophoresis, several separation modes can be distinguished, all possessing their own characteristic separation mechanism:

1.	Capillary Zone Electrophoresis	CZE
2.	Micellar ElectroKinetic Chromatography	MEKC
3.	Capillary Gel Electrophoresis	CGE
4.	Capillary IsoElectric Focusing	CIEF
5.	Capillary IsoTachoPhoresis	CITP

All these separation modes can be performed using the same equipment, which makes capillary electrophoresis a very versatile analytical technique. Since the different modes are mainly specified by the composition of the electrolyte system, they are easily accessed by a proper selection of the applied electrolyte solutions. In CZE separations are mainly based on differences in effective mobility, in MEKC on differences in micellar solubilization, in CGE on differences in molecular size, in CIEF on differences in isoelectric point and in CITP on differences in effective mobility. In the following section the separation mechanisms of capillary zone electrophoresis and micellar electrokinetic chromatography are discussed in more detail.

1.4.1 CAPILLARY ZONE ELECTROPHORESIS

Separations by Capillary Zone Electrophoresis (CZE) are performed in a homogeneous background electrolyte. After injection of a small band of a sample solution a high voltage is applied, resulting in a constant electric field across the capillary. All sample compounds migrate in discrete zones with a linear velocity, v, proportional to the vector sum of the electroosmotic and electrophoretic mobility, $m_{EOF} + m_{eff}$, according to:

$$v = (m_{EOF} + m_{eff})\, E \tag{1.9}$$

where E is the applied field strength. Notice that the electroosmotic mobility is superimposed on the electrophoretic mobility for all sample compounds. Due to the electroosmotic flow, in principle both cationic and anionic species can be separated during the same experiment. Cations migrate in the downstream mode with a high velocity. All neutral compounds migrate with the velocity of the EOF. Anions migrate in the upstream mode with a low velocity. The effective mobility, m_{eff}, can be calculated from the observed migration time, t_S, according to:

$$m_{eff} = \frac{l_c l_d}{t_S V} - \frac{l_c l_d}{t_{EOF} V} \tag{1.10}$$

where l_c is the total length of the capillary, l_d is the length from injection to detection, t_{EOF} is the migration time of the EOF and V is the applied voltage [12]. The migration time of the EOF can be determined with a UV absorbing neutral marker such as mesityl oxide or formamide.

1.4.2 MICELLAR ELECTROKINETIC CHROMATOGRAPHY

In Micellar Electrokinetic Chromatography (MEKC) a surfactant is added to the electrolyte system at a concentration above the critical micelle concentration (CMC). Surfactants are amphiphilic species, comprising both hydrophobic and hydrophylic regions [13,14]. They can be anionic, cationic, zwitterionic or nonionic. In most cases the surfactant contains a polar headgroup attached to a nonpolar hydrocarbon tail. Above the CMC the surfactant molecules associate to form colloidal-sized aggregates, resulting in a micellar phase. These micelles form dynamic entities that are constantly exchanging with monomer surfactant molecules from the bulk aqueous solution. In its most simple form micelles are generally viewed as being spherical. The hydrophobic tails of the surfactant molecules are oriented towards the centre forming a nonpolar core region and the hydrophobic head groups are directed towards the bulk aqueous solvent.

TABLE 1.1 Critical micelle concentration, CMC (mM), and aggregation number, n, for several surfactants used in MEKC.

Surfactant	abbreviation	CMC[a]	n[a]
anionic			
Sodium dodecylsulphate	SDS	8.1	62
Sodium dodecylsulphonate	SDSo	9.8	54
cationic			
Dodecyltrimethylammonium bromide	DTAB	15.0	50
Cetyltrimethylammonium bromide	CTAB	0.9	61
nonionic			
Polyoxyethylene-23-lauryl ether	Brij 35®	0.1	40
bile salts			
Sodium cholate	SC	12.5	3
Sodium deoxycholate	SDC	6.4	14

[a]From ref. [15].

Typically, micelles are composed of 40-100 monomeric surfactant molecules, which is referred to as the aggregation number. In Table 1.1 several surfactant systems, applied in MEKC are listed. The anionic surfactant sodium dodecylsulphate (SDS) is most commonly used in MEKC. Besides micelles also other organised media or macromolecular structures are applied as pseudo-stationary phases. In this case the term electrokinetic chromatography is (EKC) used.

The separation of neutral compounds in MEKC is based on differences in their partitioning between the aqueous phase and the micellar phase. In Fig. 1.3 the mechanism of MEKC is schematically presented for an anionic surfactant. The separation system consists of two moving phases, *viz.* an electroosmotically moving aqueous phase and an electrophoretically moving pseudo-stationary micellar phase. In contrast to conventional chromatography, the stationary phase, *i.e.* the micellar phase, is actually moving. Therefore it is referred to as a 'pseudo-stationary' phase. The micelles in this example have a negatively charged surface and possess an effective mobility in the direction of the anode. Using fused silica capillaries, the EOF will be directed to the cathode. If the electroosmotic mobility exceeds the absolute value of the effective mobility of the micelles, both the aqueous phase and the micellar phase will move in the direction of the cathode, however, the latter with a lower velocity. Totally insolubilized compounds migrate with the electroosmotic velocity. Totally solubilized compounds migrate with the velocity of the micelles. All other compounds migrate with a linear velocity between these two limiting values, determined by their partitioning with the pseudo-stationary micellar phase.

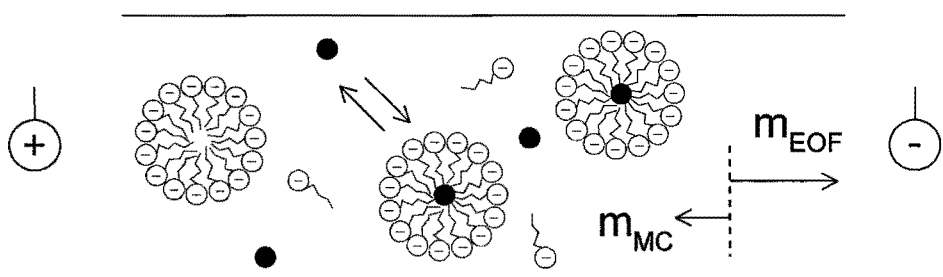

FIGURE 1.3 Schematic representation of the separation mechanism for neutral compounds in Micellar Electrokinetic Chromatography with an anionic surfactant system.

The time interval between the electroosmotic and micellar migration time is called the elution window. In chapter 2 the influence of various experimental parameters on the elution window will be discussed. For charged compounds the migration behaviour will be more complicated, due to electrophoretic migration in the aqueous phase and additional chemical equilibria. These phenomena will be discussed in more detail in chapter 3.
The separation mechanism of neutral compounds in MEKC is essentially based on chromatographic principles, *i.e.* differences in partitioning between two phases. However, the movement of these two phases is based on electrokinetic phenomena, *i.e.* electroosmosis and electrophoresis. In fact, MEKC is a hybrid separation technique, combining both chromatographic and electrophoretic principles. Therefore, the migration behaviour of sample compounds during MEKC analyses can be described both from a chromatographic and an electrophoretic point of view.

1.5 MIGRATION PARAMETERS IN MEKC

1.5.1 RETENTION FACTOR

The distribution between the aqueous phase and the micellar phase can, analogous to other chromatographic techniques, be expressed by the retention factor, k, according to:

$$k = \frac{n_{MC}}{n_{AQ}} \tag{1.11}$$

where n_{MC} and n_{AQ} are the numbers of moles of the solute incorporated in the micelles and in the aqueous phase, respectively. The migration velocity of the solute, v_S, is the weighted average of the electroosmotic velocity, v_{EOF}, and the velocity of the micelles, v_{MC}, according to:

$$v_S = \frac{n_{AQ}}{n_{AQ} + n_{MC}} v_{EOF} + \frac{n_{MC}}{n_{AQ} + n_{MC}} v_{MC} \tag{1.12}$$

or after combination with eqn. (1.11):

$$v_S = \frac{1}{1 + k} v_{EOF} + \frac{k}{1 + k} v_{MC} \tag{1.13}$$

Rearranging eqn. (1.13) and substituting inverse migration times for velocities yields the following expression for the retention factor:

$$k = \frac{t_S - t_{EOF}}{t_{EOF}\,(1 - \frac{t_S}{t_{MC}})} \tag{1.14}$$

where t_S, t_{EOF} and t_{MC} are the migration times of the solute, the electroosmotic flow and the micelles, respectively. The migration time of the micelles can be determined with a completely solubilized compound such as Sudan III [2] or timepidium bromide [16]. Eqn. (1.14) is modified from the normal chromatographic description of k to account for movement of the pseudo-stationary phase. Notice that if t_{MC} reaches infinity, *i.e.* the micelles form a true stationary phase, eqn. (1.14) reduces to the corresponding form of conventional chromatography.

1.5.2 MOBILITY

The migration velocity of the solute can also be expressed in terms of mobilities, by:

$$v_S = (m_{EOF} + \frac{k}{k+1}\, m_{MC})\, E \tag{1.15}$$

where m_{MC} is the effective mobility of the pseudo-stationary micellar phase [17,18]. In accordance with eqn. (1.9) a pseudo-effective mobility, m_{eff}^{ps}, can be defined by:

$$m_{eff}^{ps} = \frac{k}{k+1}\, m_{MC} \tag{1.16}$$

The pseudo-effective mobility can be calculated from the measured migration time of the solute, t_S, according to:

$$m_{eff}^{ps} = \frac{l_c l_d}{t_S V} - \frac{l_c l_d}{t_{EOF} V} \tag{1.17}$$

which is a similar equation as eqn. (1.10). An advantage of the use of pseudo-effective mobilities is that for their calculation the migration time of the micelles is not required.

1.6 BAND-BROADENING IN MEKC

During an MEKC separation there are several dispersive processes that contribute to the broadening of sample zones while they are transported through the capillary. The extra-capillary contributions caused by injection and detection are common to all capillary electrophoresis separation methods. They put specific demands on the experimental setup such as accurate injection of a short sample plug and a narrow detector bandwidth. The on-capillary factors that contribute to band-broadening in MEKC are more complex than in CZE due to the presence of a pseudo-stationary phase. Contributions to band-broadening stem not only from longitudinal diffusion but also from various mass-transfer phenomena which are intrinsic to the separation process.

TABLE 1.2 Expressions for the different contributions on the capillary plate height in MEKC [19].

Contribution	Expression
Longitudinal diffusion	$H_D = \frac{2(D_{AQ}+kD_{MC})}{1+(t_{EOF}/t_{MC})k}\frac{1}{v_{EOF}}$
Sorption/desorption kinetics	$H_{MC} = \frac{2(1-t_{EOF}/t_{MC})^2 k}{(1+(t_{EOF}/t_{MC})k)(1+k)^2}\frac{v_{EOF}}{k_d}$
Intermicelle diffusion	$H_{AQ} = (\frac{k}{1+k})^2 \frac{(1-t_{EOF}/t_{MC})^2}{1+(t_{EOF}/t_{MC})k}\frac{d^2 v_{EOF}}{4D_{AQ}}$
Temperature gradients	$H_T = \frac{(1-t_{EOF}/t_{MC})k}{24(D_{AQ}+kD_{MC})}\frac{B^2 I^4}{64\kappa^2\pi^4 r_c^2 \lambda_T^2 T^4} v_{EOF}$
Micelle microheterogeneity	$H_{EP} = \frac{(0.0053)^2(1-t_{EOF}/t_{MC})^2 k}{1+(t_{EOF}/t_{MC})k}\frac{v_{EOF}}{k_d}$

The different sources of band-broadening in MEKC have been evaluated in detail by Terabe *et al.* in terms of capillary plate heights [19]. The total capillary plate height, H_C, can be expressed by several additive factors as:

$$H_C = H_D + H_{MC} + H_{AQ} + H_T + H_{EP} \tag{1.18}$$

where H_D, H_{MC}, H_{AQ}, H_T and H_{EP} are plate height contributions due to longitudinal diffusion, sorption/desorption kinetics, intermicelle mass transfer, temperature gradients and micelle microheterogeneity. In Table 1.2 expressions for the different sources of band broadening are presented [19]. In order to estimate the contribution of each factor, the plate heights for the different band-broadening mechanisms were calculated as a function of the electroosmotic velocity. In Table 1.3 all numerical values for these calculations are presented [20]. The results, shown in Fig. 1.4, illustrate that longitudinal diffusion and sorption/desorption kinetics are the main band-broadening mechanisms during MEKC analyses. In open tubular liquid chromatography the contribution of diffusion in the stationary phase is considered negligible. However, in MEKC sample compounds will be subjected to micellar diffusion when they are solubilized in the pseudo-stationary micellar phase. In chapter 7 this phenomenon is discussed in more detail.

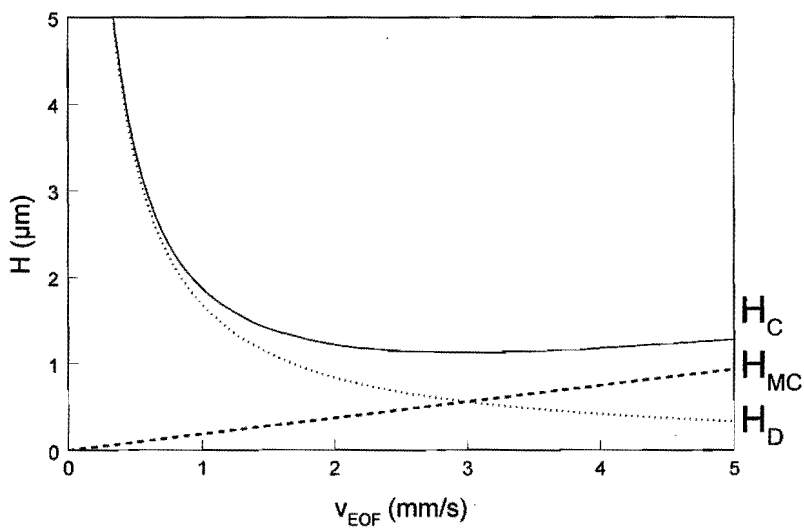

FIGURE 1.4 Plate height contributions of longitudinal diffusion, H_D, and sorption/desorption kinetics, H_{MC}, to the total capillary plate height, H_C. All other contributions are smaller than 0.001 μm.

TABLE 1.3 Numerical values for the parameters in TABLE 1.2 [20].

Variable	Definition	Typical value	Dimension
D_{AQ}	Diffusion coefficient in the aqueous phase	10^{-9}	$m^2.s^{-1}$
D_{MC}	Diffusion coefficient in the micellar phase	10^{-10}	$m^2.s^{-1}$
k_d	Desorption rate constant from the micelle	10^3	s^{-1}
d	Intermicelle distance	10^{-8}	m
B	Viscosity dependence on temperature	2400	K
I	Current	4.10^{-5}	A
κ	Specific conductivity	0.571	$S.m^{-1}$
r_c	Capillary radius	25.10^{-6}	m
λ_T	Thermal conductivity	0.573	$W.m^{-1}.K^{-1}$
T_0	Temperature electrolyte system	325	K
t_{MC}/t_{EOF}	Elution window	5	-
k	Retention factor	2.24[a]	-

[a] $2.24 = k_{opt}$ for $t_{MC}/t_{EOF} = 5$ (for explanation, see eqn. 2.4).

At a low electroosmotic velocity the contribution of sorption/desorption kinetics is of minor importance, due to the dynamic character of the micelles and the rapid solubilization equilibria. Only at higher electrical field strengths and consequently higher electroosmotic velocities the contribution of sorption/desorption kinetics becomes more important.

1.7 INSTRUMENTATION

The basic instrumentation for all modes of capillary electrophoresis is essentially the same. In Fig. 1.5 a schematic representation of the equipment is shown. The separation capillary is placed between two electrolyte reservoirs, filled with the electrolyte solution. These reservoirs also contain platinum electrodes which serve to connect the high voltage power supply. Sample injection is carried out either electrokinetically or hydrodynamically. Optical detection is carried out at the opposite end of the capillary. Two different instruments were applied for the MEKC and CZE experiments, described in this thesis, *viz.* a BioFocus 3000 capillary electrophoresis system (BioRad, Hercules, CA, USA) and an HP ^{3D}CE capillary electrophoresis system (Hewlett Packard, Waldbronn, Germany). Both instruments are fully automated computer controlled systems comprising a thermoregulated capillary cartridge, a high voltage power supply, a UV absorbance detector and one or two vial carousels containing randomly accessible sample and electrolyte reservoirs.

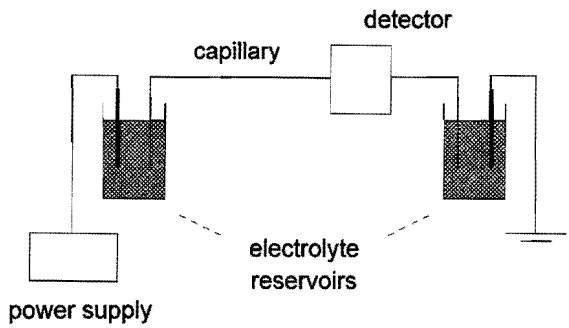

FIGURE 1.5 Schematic representation of capillary electrophoresis instrumentation.

INJECTION

Samples can be introduced by applying a relative low voltage for a short time interval (electrokinetic injection) or by applying a carefully controlled pressure for a short time interval (hydrodynamic injection). In order to maintain high efficiency, only minute volumes of sample are introduced, usually in the range of 0.1 - 50 nl. To improve the reproducibility of hydrodynamic injection, an integrated pressure-time profile with active feedback control is used to compensate for system risetime effects and variations in the applied pressure.

SEPARATION

Separations are carried out in polyimide coated fused silica capillaries, ranging from 20 to 100 cm in length and from 25 to 100 μm in internal diameter. The capillary is situated in a cartridge and is thermostated, using either a circulating liquid coolant (BioFocus 3000 system) or a forced air stream (HP ^{3D}CE system). Both systems are equipped with a high voltage power supply that can deliver up to about 30 kV with a current level of *ca.* 300 μA. The inlet electrode is connected to the high voltage whereas the outlet electrode is grounded. The polarity of the inlet electrode is software controlled and therefore easily reversible. All sample and electrolyte reservoirs are capped to reduce evaporation.

DETECTION

Sample zones are monitored by a programmable multi-wavelength UV absorbance detector at the outlet side of the capillary. A small section of the capillary serves as detection volume. To allow UV light pass through the capillary, a small part of the polyimide coating of the fused silica is removed, thus creating a UV transparent detection window. The optical beam is focused on the capillary detection volume automatically upon installation of the capillary cartridge in the instrument. The HP ^{3D}CE system is equipped with a diode array detector (DAD) which can be used for the validation of peak purity or the conformation of peak identities with spectral analyses.

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2

SEPARATION PERFORMANCE

ABSTRACT

The resolution of neutral compounds in MEKC is influenced by the efficiency, the elution window and the retention factors of the compounds. The influence of micellar diffusion on the efficiency is treated theoretically, showing that efficiency in MEKC strongly depends on the retention factor. Under practical MEKC conditions, however, the contribution of micellar diffusion to band-broadening is of minor importance. In addition, the influence of the electroosmotic mobility and the effective mobility of the micelles on the elution window is discussed and the effect of different experimental conditions on the elution window and the retention factors is determined. Although these variables cannot be controlled independently in many cases, it is demonstrated that the resolution in MEKC can be improved by adjusting the composition of the applied electrolyte system. Furthermore, the application of the separation number as parameter for the separation performance is discussed.

2.1 INTRODUCTION

Since the introduction of MEKC by Terabe *et al.* [1,2], several authors have paid attention to the fundamental characteristics of this separation method [3,4] and to the effect of different separation parameters on the migration behaviour [5-11]. Also the theoretical [12,13] and practical [14,15] aspects of resolution optimization have been well described. Assuming that selectivity is determined by the chemical nature of the applied surfactant system, resolution in MEKC is influenced by the efficiency, the elution window and the retention factors of the sample compounds. Therefore a good understanding of the effects of different experimental conditions on these variables is important for the development of MEKC analyses and for resolution optimization strategies.
The aim of this chapter is to evaluate the contribution of various parameters to the resolution of neutral species in MEKC experiments. The influence of different experimental conditions such as the applied field strength, buffer pH, ionic strength, capillary surface modifications, hydrocarbon chain length of the surfactant, surfactant concentration and organic modifier content on the elution window and the retention factors are investigated.

2.2 RESOLUTION EQUATION

Generally in chromatographic techniques, the degree of separation between two peaks is quantitatively described by the resolution, R_S, according to [16]:

$$R_S = \frac{t_2 - t_1}{2(\sigma_1 + \sigma_2)} \tag{2.1}$$

where t is the migration time, σ the temporal variance of the concentration distribution and the subscripts 1 and 2 denote the first and the last eluting peak, respectively. From eqns. (1.14) and (2.1) follows for the resolution between two closely eluting peaks in MEKC (assuming $k \approx k_1 \approx k_2$):

$$R_S = \frac{\sqrt{N}}{4} \frac{\alpha - 1}{\alpha} \frac{k}{1 + k} \frac{1 - (t_{EOF}/t_{MC})}{1 + (t_{EOF}/t_{MC})k} \tag{2.2}$$

where N and α are the number of theoretical plates and the separation factor k_2/k_1, respectively. In analogy with other chromatographic techniques, an efficiency term, a selectivity term and a retention term can be distinguished in this resolution equation. The

last term in eqn. (2.2) reflects the specific resolution characteristics in MEKC. If t_{MC} reaches infinity, *i.e.* the micelles form a true stationary phase, this term equals unity and eqn. (2.2) reduces to the well known resolution equation in conventional chromatography. The influence of the retention factor on the resolution is described by the retention term $f(k)$, formed by the product of the last two terms in eqn. (2.2):

$$f(k) = \frac{k}{1+k} \frac{1-(t_{EOF}/t_{MC})}{1+(t_{EOF}/t_{MC})k} \tag{2.3}$$

In Fig. 2.1 the dependence of $f(k)$ on k is shown for the different elution modes with various values for the elution window. From this figure it can be seen that the contribution of $f(k)$ to the resolution is less than in conventional chromatography. Notice that this situation is represented by $t_{MC}/t_{EOF}=\infty$. However, this difference is easily compensated by the high efficiency obtained by MEKC, which also contributes to the resolution according to eqn. (2.2). Fig. 2.1 also illustrates that increasing the elution window with a given retention factor leads to higher values of $f(k)$ and consequently to a higher resolution. For each value of the elution window the curve shows a maximum.

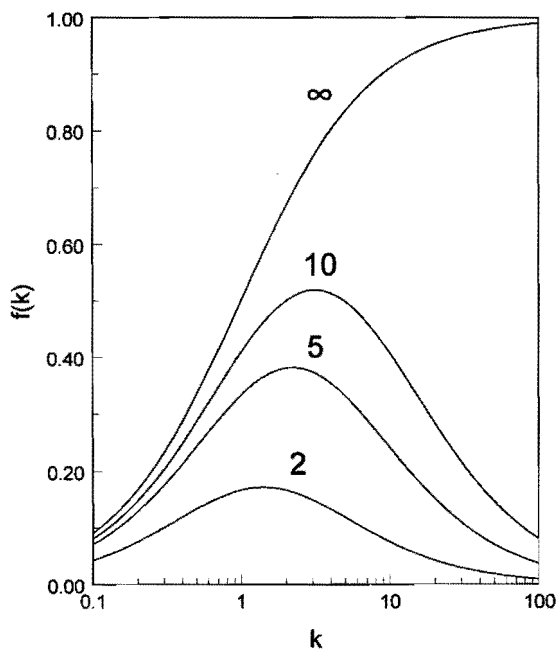

FIGURE 2.1 Calculated relationship between $f(k)$ and k for different values of the elution window.

The optimum retention factor, k_{opt}, for which the maximum resolution is obtained can be calculated by differentiating eqn. (2.5) with respect to k [12,16]:

$$k_{opt} = \sqrt{t_{MC}/t_{EOF}} \tag{2.4}$$

In the restricted elution mode and in the reversed direction mode (see section 2.2.2 for the definition of these terms) the contribution of $f(k)$ to the resolution can reach much higher values than in the normal mode. However, this will lead to long analysis times, especially for compounds with a retention factor close to $-(t_{MC}/t_{EOF})$ [6].

2.2.1 EFFICIENCY

In section 1.6 it was pointed out that under practical experimental conditions, *i.e.* electroosmotic velocities below 3 mm/s, longitudinal diffusion is the main band broadening mechanism during MEKC analyses. Since diffusion in micellar media is governed by aqueous as well as micellar diffusion, represented by D_{AQ} and D_{MC} respectively, both these factors have to be taken into account for the calculation of overall diffusion coefficients in MEKC electrolyte systems. This is described in more detail in chapter 7.

In order to investigate the influence of longitudinal diffusion on the efficiency in MEKC, the theoretical plate number, N, was calculated as a function of the retention factor, k, for different values of the elution window, assuming that longitudinal diffusion is the only band broadening mechanism. Following the same approach as Jorgenson and Lukacs [17] for capillary zone electrophoresis, the theoretical plate number in MEKC is given by:

$$N = \frac{(m_{eff}^{ps} + m_{EOF})\ V}{2\ D_{OV}} \tag{2.5}$$

where m_{eff}^{ps} is the pseudo-effective mobility, m_{EOF} is the electroosmotic mobility, V is the applied voltage and D_{OV} is the overall diffusion coefficient. In Fig. 2.2 all calculated curves are shown. For low retention factors ($k<1$) D_{MC} is negligible and consequently only small differences in N are obtained. For higher retention factors ($k>10$) the contribution of D_{MC} to efficiency is not negligible. Due to a decrease in overall diffusion coefficient N increases with increasing k. In practice, however, one works with t_{MC}/t_{EOF} values between 2-5 and $k<10$. Under these conditions the contribution of micellar diffusion on efficiency will be of minor importance.

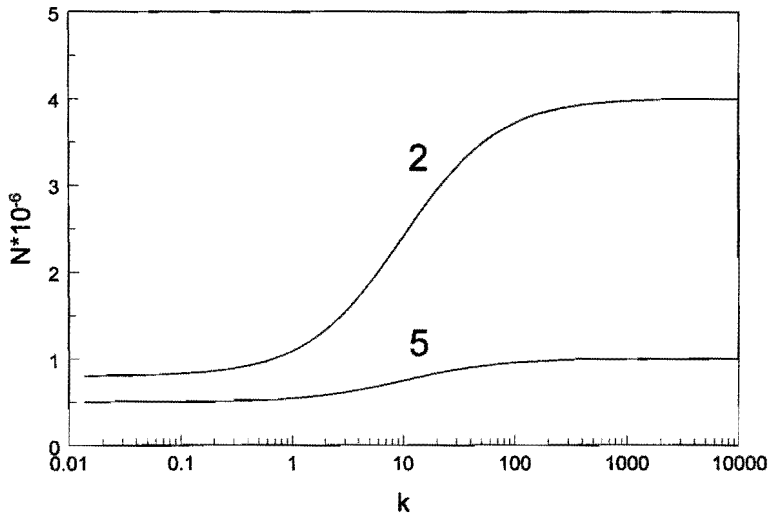

FIGURE 2.2 Calculated theoretical plate number, N, versus retention factor, k, for different values of the elution window, t_{MC}/t_{EOF}, assuming that longitudinal diffusion is the only band broadening mechanism. Model values: $D_{AQ}=10\cdot10^{-6}$ cm^2/s, $D_{MC}=10^{-6}$ cm^2/s, $m_{EOF}=$ $80\cdot10^{-5}$ or $50\cdot10^{-5}$ cm^2/Vs, $m_{MC}=$ $-40\cdot10^{-5}$ cm^2/Vs, $V=20$ kV.

2.2.2 ELUTION WINDOW

As pointed out in section 1.4.2, all neutral compounds will migrate within the elution window, the limited time interval between the electroosmotic and the micellar migration time, t_{EOF} and t_{MC}. These compounds will migrate with an overall linear velocity, v_S, given by:

$$v_S = \frac{1 + (t_{EOF}/t_{MC})k}{1 + k} m_{EOF} E \qquad (2.6)$$

where m_{EOF} and E are the electroosmotic mobility and the applied field strength, respectively. Under conditions of a constant field strength, the elution window, t_{MC}/t_{EOF}, is determined by the electroosmotic mobility, m_{EOF}, and the effective mobility of the micelles, m_{MC}, according to:

$$\frac{t_{MC}}{t_{EOF}} = \frac{m_{EOF}}{m_{EOF} + m_{MC}} \qquad (2.7)$$

The elution window can be increased by increasing $|m_{MC}|$ or by decreasing m_{EOF}. If the

absolute value of the effective mobility of the micelles exceeds the electroosmotic mobility, the elution window becomes negative.

As described by Vindevogel and Sandra [4], three different migration modes can be distinguished. If $t_{MC}/t_{EOF}>0$, all compounds will migrate to the detector, which is called the normal mode. If $t_{MC}/t_{EOF}<0$, only compounds for which holds $k<-(t_{MC}/t_{EOF})$ will migrate to the detector. This mode is called the restricted elution mode. Compounds for which holds $k>-(t_{MC}/t_{EOF})$ will migrate in the opposite direction. In order to detect these compounds a reversed polarity will have to be applied, which is called the reversed direction mode.

2.3 EXPERIMENTAL

INSTRUMENTATION AND SEPARATION CONDITIONS

All experiments were carried out on a BioFocus 3000 Capillary Electrophoresis System (BioRad, Hercules, CA, USA), unless otherwise noted. The wavelength of the detector was set at 200 nm and the temperature was kept constant at 25 °C. Pressure injection was carried out with an injection constant of 2 psi.s. All experiments were carried out with a constant voltage, with the anode placed at the inlet side and the cathode at the outlet side of the capillary, respectively. Two different fused silica capillaries of 50 μm I.D. were applied; an original BioRad standard capillary, total length 50.0 cm, distance between injection and detection 45.5 cm, and a capillary from Supelco (Bellefonte, PA, USA), total length 70.0 cm, distance between injection and detection 65.4 cm.

TABLE 2.1 Composition of background electrolytes at different pHs.

cation	buffering counter species	pH
0.01 M TRIS	acetic acid	4.9
0.01 M TRIS	MES	6.2
0.01 M TRIS	*o*-phosphoric acid	7.0
0.01 M TEA	MOPS	7.0
0.01 M TEA	HEPES	7.5
0.01 M TRIS	MOPS	7.9
0.01 M TRIS	MOPS	8.2
0.01 M TRIS	acetic acid	8.2
0.01 M TRIS	boric acid	8.5

For some of the experiments several different coated fused silica capillaries of 50 μm I.D. were applied; a C_{18} coated capillary from Supelco (CElect-H250, Bellefonte, PA, USA), a methyl silicone coated capillary and a polyethylene glycol coated capillary, both from Chrompack (Middelburg, The Netherlands), all with total length 70.0 cm, distance between injection and detection 65.4 cm. All experiments with the BioRad standard capillary were carried out three times, except for the experiments with the organic modifiers, which were carried out two times. The reported values are the average values.

SAMPLES AND SOLUTIONS

All chemicals were of analytical-reagent grade. In Table 2.1 the compositions of the background electrolytes at different pH values are listed. To these buffer solutions 50 mM sodium dodecylsulphate (SDS) was added, unless otherwise noted. Eight aromatic compounds were selected as sample compounds, covering a wide range of hydrophobicity. All sample compounds were dissolved at a final concentration of about 0.0005 M in a 50 mM SDS solution. Formamide was used as a neutral EOF marker and Sudan III as a micelle marker to measure t_{EOF} and t_{MC}, respectively.

2.4 PARAMETERS CONTROLLING THE ELUTION WINDOW AND RETENTION FACTORS

APPLIED FIELD STRENGTH

According to eqn. (2.1) the velocity of the sample compounds is linearly related to the applied field strength. Although a linear relationship was obtained at low field strengths, a positive deviation was observed at field strengths above *ca.* 200 V/cm. At a higher field strength the generated electric power will increase. Due to Joule heating, the mean temperature in the capillary will increase, which in turn will result in a decrease of the viscosity of the electrolyte solution. Both the electroosmotic mobility and the effective mobility of the micelles are inversely proportional to the viscosity.

The specific conductivity of the electrolyte solution, κ, can be calculated by Ohm's law:

$$V = \frac{l_c}{\pi r_c^2 \kappa} I \tag{2.8}$$

where V is the applied voltage, l_c and r_c are the length and the radius of the capillary, respectively, and I is the measured electric current. Since the specific conductivity is

inversely proportional to the viscosity, the electric current will proportionally increase if the viscosity decreases at a higher field strength. Therefore, a linear relationship was obtained for field strengths up to 400 V/cm between the velocity of the sample compounds and the measured electric current with regression correlation coefficients larger than 0.999. In Table 2.2 the measured electric current, the calculated specific conductivity, the electroosmotic mobility, the effective mobility of the micelles and the corresponding values for the elution window are listed for the different applied voltages.

TABLE 2.2 Applied voltage, V (kV), measured electric current, I (μA), specific conductivity, κ (S/m), electroosmotic mobility, m_{EOF} (10^{-5} cm^2/Vs), effective mobility of the micelles, m_{MC} (10^{-5} cm^2/V), and values for the elution window, t_{MC}/t_{EOF}, for different applied field strengths. BioRad standard capillary. Background electrolyte, 10 mM TRIS/acetic acid at pH 8.2.

V	I	κ	m_{EOF}	m_{MC}	t_{MC}/t_{EOF}
3	3.0	0.255	61.96	-40.71	2.92
6	6.0	0.255	62.20	-40.54	2.87
9	9.2	0.260	63.48	-41.21	2.85
12	12.7	0.270	65.69	-42.47	2.83
15	16.7	0.284	68.50	-44.21	2.82
18	21.3	0.301	71.25	-45.88	2.81
20	24.3	0.309	73.39	-47.18	2.80

BUFFER pH AND IONIC STRENGTH

In fused silica capillaries, the EOF originates from the dissociation of the surface silanol groups of the capillary wall. Therefore the electroosmotic mobility is dependent on the pH of the background electrolyte and, according to the double layer theory, also on the ionic strength. In Fig. 2.3 the pH dependence of the EOF is illustrated for phosphate buffers. As expected, a sigmoidal curve was obtained for m_{EOF} *versus* pH, also for the phosphate buffers containing 50 mM SDS. The lower m_{EOF} obtained with the buffers containing 50 mM SDS at high and intermediate pas is due to an increase in viscosity and ionic strength of the electrolyte systems. The more slightly decrease observed at lower pas is due to adsorption of SDS on the inner wall of the capillary. For a given surfactant, the effective mobility of the micelles can be expected to be almost independent of pH [6].

In order to examine the influence of buffer pH and ionic strength on the elution window and the retention factors in MEKC, experiments were carried out with the electrolyte systems given in Table 2.1, containing 50 mM SDS.

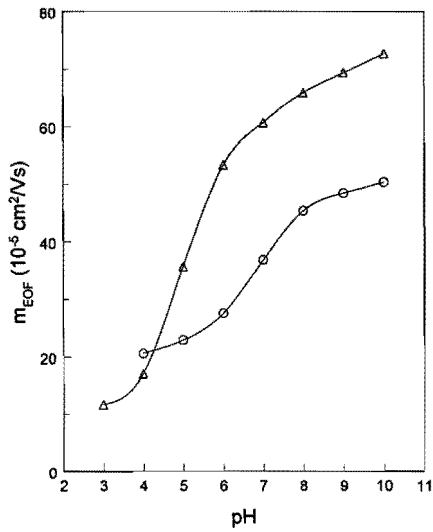

FIGURE 2.3 Dependence of the electroosmotic mobility, m_{EOF}, on the pH for phosphate buffers, containing (△) 0 and (○) 50 mM SDS. The phosphate buffers were prepared by adding *o*-phosphoric acid to a 10 mM KOH solution until the desired pH was reached. Capillary from Chrompack. Applied voltage, 20 kV.

In addition, experiments were carried out with electrolyte systems containing 0.02 M of the cation and 100 mM SDS. In Table 2.3 all calculated electroosmotic mobilities, effective mobilities of the micelles, corresponding values of the elution window and retention factors of the eight sample compounds are listed. As can be seen from these results the electroosmotic mobility is dependent on pH and ionic strength, whereas the effective mobility of the micelles and the retention factors of the compounds are virtually constant for a given surfactant concentration. Of course this only applies for uncharged compounds in MEKC. For charged compounds, the degree of dissociation and hence the retention factor may be strongly influenced by the pH of the electrolyte system [18]. The retention factors obtained with 100 mM SDS are less than twice those obtained with 50 mM SDS for all compounds, which is inconsistent with theory. Due to a higher electric current with the 100 mM SDS electrolyte system, the mean temperature in the capillary will increase. This will cause a decrease of distribution constants and retention factors of the sample compounds [19,20]. In Fig. 2.4 the relationship between t_{MC}/t_{EOF} and m_{EOF} for the experiments, listed in Table 2.3, is illustrated.

TABLE 2.3 Electroosmotic mobility, m_{EOF} (10^{-5} cm^2/Vs), effective mobility of the micelles, m_{MC} (10^{-5} cm^2/Vs), values for the elution window, t_{MC}/t_{EOF}, and retention factors, k, for (1) resorcinol, (2) phenol, (3) *p*-nitroaniline, (4) *p*-cresol, (5) 2,6-xylenol, (6) toluene, (7) 1,2-xylol and (8) propylbenzene, in several background electrolytes at different pHs; (I) Cation concentration 10 mM, containing 50 mM SDS; (II) Cation concentration 20 mM, containing 100 mM SDS. BioRad standard capillary. Applied voltage, 15 kV.

	pH	m_{EOF}	m_{MC}	t_{MC}/t_{EOF}	k							
					1	2	3	4	5	6	7	8
I	6.2	58.07	-44.31	4.22	0.22	0.52	1.14	1.47	2.87	3.33	8.24	24.79
	7.0[a]	56.43	-43.97	4.53	0.25	0.56	1.22	1.55	2.82	3.22	7.94	24.27
	7.5	61.26	-44.34	3.62	0.25	0.56	1.23	1.56	2.82	3.20	7.84	23.67
	7.9	64.59	-44.68	3.24	0.27	0.56	1.19	1.51	2.60	3.10	7.66	23.32
	8.2[b]	66.46	-44.95	3.09	0.28	0.56	1.19	1.51	2.74	3.12	7.62	23.06
	8.2[c]	65.94	-44.00	3.00	0.24	0.51	1.11	1.42	2.63	3.02	7.46	22.48
	8.5	66.75	-44.18	2.96	0.27	0.53	1.14	1.45	2.68	3.23	7.49	22.92
	Average		-44.35		0.25	0.54	1.17	1.50	2.74	3.17	7.75	23.50
	Standard Deviation		0.33		0.02	0.02	0.04	0.05	0.10	0.09	0.26	0.75
II	7.5	52.88	-44.29	6.02	0.36	0.95	1.90	2.61	4.70	5.92	14.29	43.40
	7.9	56.42	-44.45	4.72	0.35	0.91	1.81	2.49	4.54	5.76	13.95	42.40
	8.2[d]	57.98	-44.32	4.24	0.37	0.93	1.87	2.56	4.64	5.83	14.02	42.83
	8.2[e]	59.90	-45.09	4.05	0.38	0.93	1.86	2.55	4.66	5.84	14.20	43.02
	8.5	60.14	-44.90	3.95	0.41	0.97	1.94	2.65	4.83	6.01	14.48	43.99
	Average		-44.61		0.37	0.94	1.88	2.57	4.67	5.87	14.20	43.13
	Standard Deviation		0.32		0.02	0.02	0.04	0.05	0.09	0.09	0.18	0.54

[a]0.01 M TEA/MOPS; [b]0.01 M TRIS/MOPS; [c]0.01 M TRIS/acetic acid; [d]0.02 M TRIS/MOPS; [e]0.02 M TRIS/acetic acid

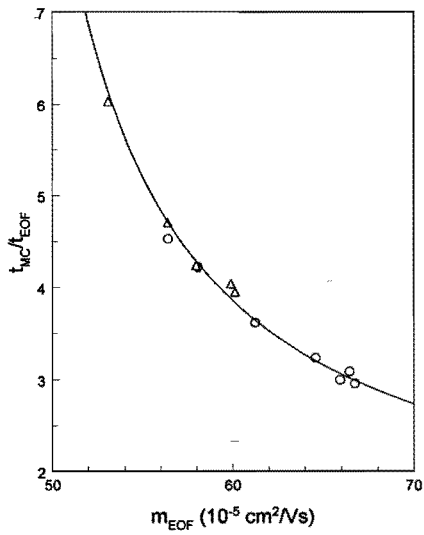

FIGURE 2.4 Relationship between elution window and electroosmotic mobility for the electrolyte systems at different pHs with (○) a cation concentration of 0.01 M, containing 50 mM SDS and (△) a cation concentration of 0.02 M, containing 100 mM SDS. The drawn line represents the theoretical curve for an m_{MC} of -44.46·10^{-5} cm^2/Vs. BioRad standard capillary. Applied voltage, 15 kV.

From the foregoing it can be concluded that for a given separation of neutral species with a specific surfactant, the pH of the electrolyte system can be used to optimize the elution window in MEKC. This is demonstrated in Fig. 2.5, where the electrokinetic chromatograms are shown for the separation of the sample mixture in an electrolyte system of 0.01 M TRIS/boric acid at pH 8.5 and 0.01 M TRIS/acetic acid at pH 4.9, respectively. In order to determine the effective mobility of the micelles and to be able to calculate retention factors with the low m_{EOF} at pH 4.9, Sudan III was injected at the outlet side of the capillary by electrokinetic injection with 10 kV for 10 s, after the hydrodynamic injection of the sample mixture at the inlet side of the capillary. In Table 2.4 all measured migration times and calculated retention factors are listed. From these results it can be calculated that a change in t_{MC}/t_{EOF} is obtained from 3.43 to -2.08 if the pH of the electrolyte system is lowered from 8.5 to 4.9. Consequently a higher resolution is obtained, however, at the cost of a longer analysis time. Notice that in Fig. 2.5A all compounds are migrating in the normal mode. In Fig. 2.5B resorcinol and phenol are migrating in the restricted elution mode, whereas Sudan III is migrating in the reversed direction mode.

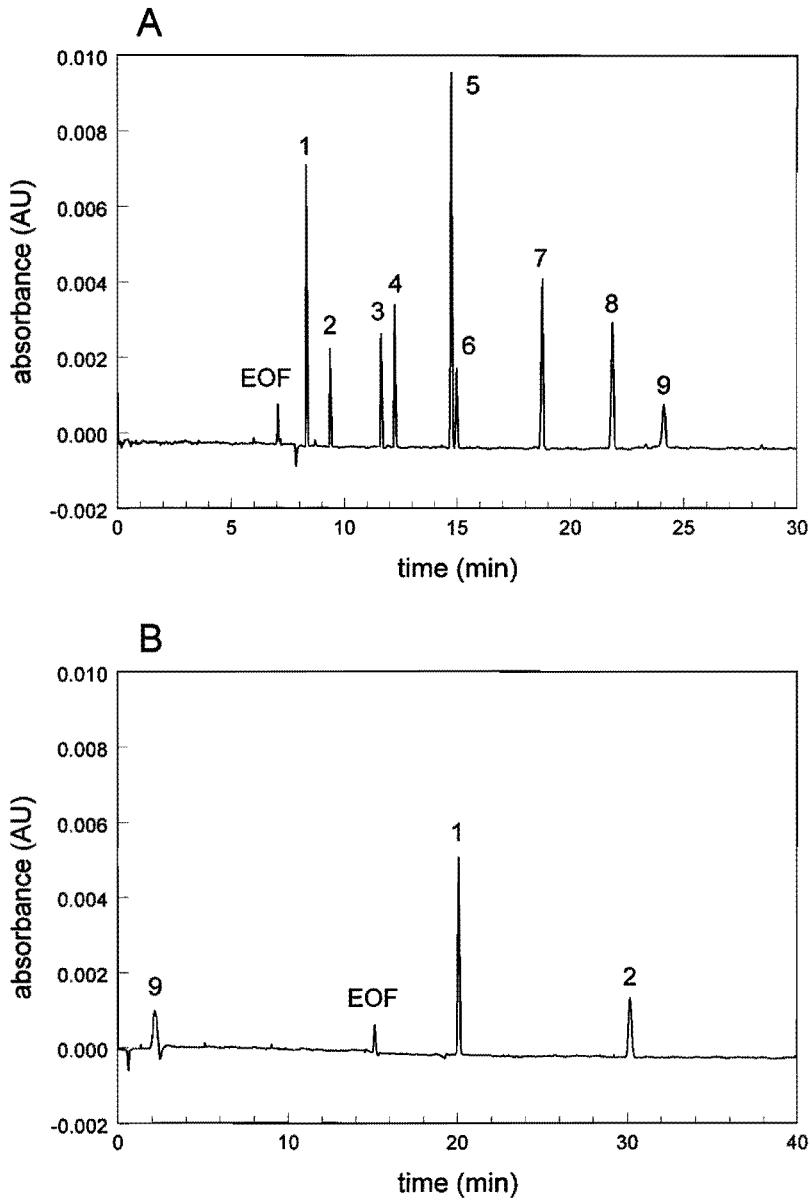

FIGURE 2.5 Electrokinetic chromatograms of the separation of (1) resorcinol, (2) phenol, (3) *p*-nitroaniline, (4) *p*-cresol, (5) 2,6-xylenol, (6) toluene, (7) 1,2-xylol, (8) propylbenzene and (9) Sudan III in a background electrolyte of (A) 10 mM TRIS/boric Acid at pH 8.5 and (B) 10 mM TRIS/acetic acid at pH 4.9. Capillary from Supelco. Applied voltage, 20 kV. For further explanation, see text.

TABLE 2.4 Migration times, t (min), and retention factors, k, with standard deviations (in parentheses), and theoretical plate numbers, N, in a background electrolyte of (I) 10 mM TRIS/boric Acid at pH 8.5 and (II) 10 mM TRIS/acetic acid at pH 4.9. Capillary from Supelco. Applied voltage, 20 kV. *(n=5)*.

	compound	t	k	$N \cdot 10^{-5}$
I	EOF	7.01 (0.04)	0	-
	resorcinol	8.26 (0.05)	0.272 (0.001)	1.35
	phenol	9.35 (0.03)	0.547 (0.005)	1.52
	p-nitroaniline	11.64 (0.03)	1.283 (0.024)	1.69
	p-cresol	12.27 (0.02)	1.538 (0.029)	1.87
	2,6-xylenol	14.78 (0.02)	2.889 (0.053)	2.24
	toluene	15.03 (0.02)	3.061 (0.059)	2.32
	1,2-xylol	18.77 (0.03)	7.702 (0.142)	2.24
	propylbenzene	21.82 (0.07)	23.172 (0.449)	1.75
	Sudan III	24.01 (0.11)	∞	1.73
II	EOF	15.11 (0.07)	0	-
	resorcinol	19.98 (0.13)	0.197 (0.001)	1.64
	phenol	29.77 (0.31)	0.499 (0.007)	1.31
	Sudan III[a]	2.21 (0.04)	∞	-

[a]Notice that the length from injection to detection is only 4.6 cm for Sudan III. For further explanation, see text.

CAPILLARY SURFACE MODIFICATIONS

Besides the composition of the electrolyte system, the electroosmotic mobility can be controlled by modification of the capillary surface [21-23]. To investigate the influence of several surface modifications in MEKC, the sample mixture was analyzed with one uncoated and three different coated fused silica capillaries in a 0.01 M TRIS/phosphoric acid electrolyte system at pH 7.0. In Fig. 2.6 all electrokinetic chromatograms are shown. The C_{18} and the polyethylene glycol coated capillaries showed a decrease in m_{EOF}, resulting in a larger elution window. However, also a decrease in efficiency was observed, probably due to solute-wall interactions. The methyl silicone coated capillary showed an increase in m_{EOF}, resulting in a smaller elution window. Toluene, 1,2-xylol, propylbenzene and Sudan III could not be detected, owing to adsorption of these compounds on the polymer coating. These results suggest that only for a limited number of compounds the application of coated capillaries in MEKC may be advantageous.

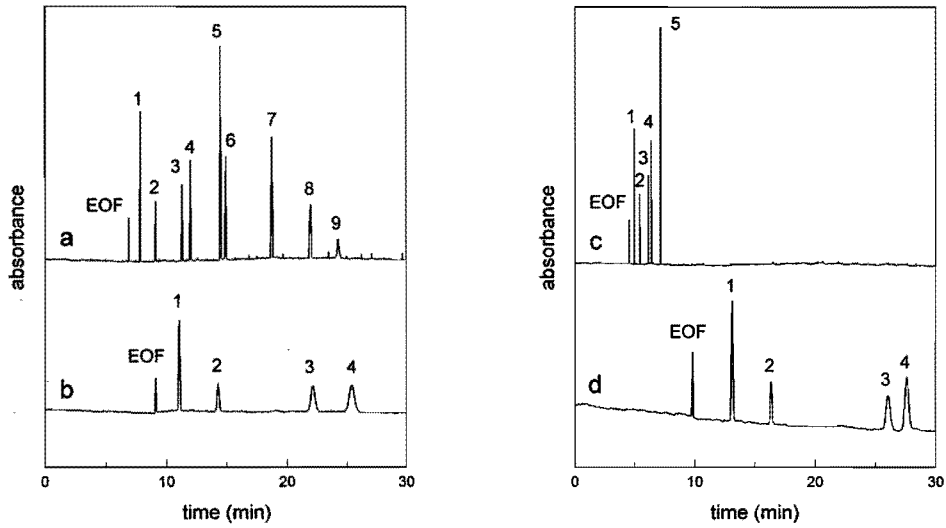

FIGURE 2.6 Electrokinetic chromatograms of the sample mixture obtained with capillaries with different surface modifications: (a) untreated fused silica capillary from Supelco, (b) C_{18} coated capillary from Supelco, (c) methyl silicone coated capillary from Chrompack and (d) polyethylene glycol coated capillary from Chrompack. Background electrolyte, 10 mM TRIS/phosphoric acid at pH 7.0. Applied voltage, 20 kV. See the legend of FIGURE 2.5 for the names of the compounds.

HYDROCARBON CHAIN LENGTH OF THE SURFACTANT

For micelles, the effective mobility will be strongly dependent on the nature of the surfactant. A shorter hydrocarbon chain will lead to a reduced aggregation number and consequently to a reduced effective charge, but also to a reduced micelle size [24]. Both these factors influence the m_{MC} in an opposite way. Moreover, the m_{EOF} is also influenced by the nature of the surfactant. At equal surfactant concentrations, the phase ratio will decrease if a surfactant with a shorter hydrocarbon chain is applied, due to an increase in the critical micelle concentration and a decrease in the partial molar volume of the micelles, as can be seen from eqn. (2.11). This is illustrated in Fig. 2.7. Consequently, a decrease in retention factors will be obtained. Therefore the elution window and the retention factors cannot independently being optimized by changing the hydrocarbon chain length of the surfactant.

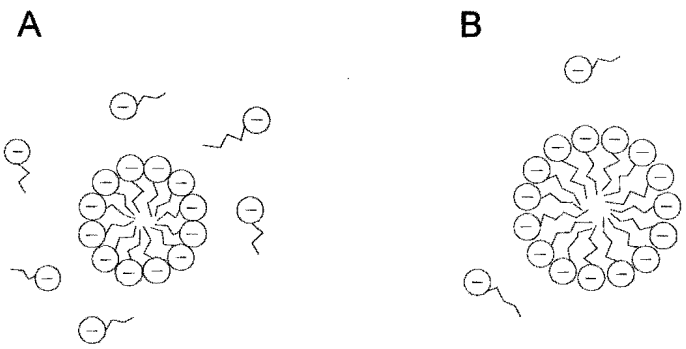

FIGURE 2.7 Schematic representation of two surfactant systems with (A) a short and (B) a long hydrocarbon chain.

SURFACTANT CONCENTRATION

The retention factor is related to the distribution constant, K, and the phase ratio, β, according to:

$$k = K\,\beta \tag{2.9}$$

The phase ratio can be calculated according to:

$$\beta = \frac{V_{MC}}{V_{AQ}} = \frac{\bar{v}\,(C_{SF} - CMC)}{1 - \bar{v}\,(C_{SF} - CMC)} \tag{2.10}$$

where V_{MC} and V_{AQ} are the volume of the micellar phase and the aqueous phase, respectively, $\bar{v}$ is the partial molar volume of the micelles, C_{SF} is the surfactant concentration and CMC is the critical micelle concentration. Under normal MEKC conditions the denominator of eqn. (2.10) is approximately equal to 1, leading to:

$$k = K\,\bar{v}\,(C_{SF} - CMC) \tag{2.11}$$

Thus the retention factor is linearly related to the surfactant concentration [2]. To investigate the influence of the phase ratio on the retention factors and the elution window, experiments were carried out with an electrolyte system of 0.01 M TRIS/MOPS at pH 8.2, containing different concentrations SDS, ranging from 30 mM to 100 mM. For the retention factors *versus* concentration SDS linear graphs were obtained with regression correlation coefficients larger than 0.997. As can be seen from the results, listed in Table 2.5, both

m_{EOF} and $|m_{MC}|$ decrease with increasing surfactant concentration, due to changes in viscosity and ionic strength. The increase in viscosity will be partly compensated by Joule heating with a higher electric current. Here it should be noted that in capillary electrophoretic techniques a distinction can be made between bulk viscosity (important for m_{MC}) and wall-surface viscosity (important for m_{EOF}) [25]. Probably bulk viscosity will be more influenced by Joule heating than wall surface viscosity. As a result, a small increase of the elution window is observed at higher surfactant concentrations as also reported by others [4,26]. Terabe *et al.* [21], however, reported a small decrease of the elution window, which may be due to differences in thermoregulating the capillary.

In Fig. 2.8 the effect of the surfactant concentration on the function *f(k)* is demonstrated. For weakly hydrophobic compounds with $k<k_{opt}$ like resorcinol and phenol, an increase in *f(k)* is observed with increasing surfactant concentration, whereas for strongly hydrophobic compounds with $k>k_{opt}$ like 1,2-xylol a decrease in *f(k)* is observed. For moderately hydrophobic compounds like *p*-cresol only a small influence of the surfactant concentration on *f(k)* is observed.

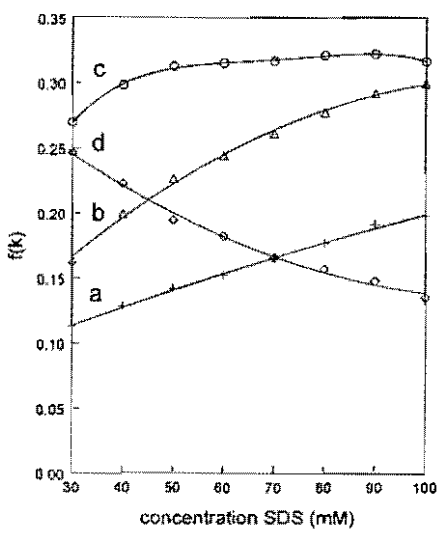

FIGURE 2.8 Relationship between *f(k)* and concentration SDS for (a) resorcinol, (b) phenol, (c) *p*-cresol and (d) 1,2-xylol. BioRad standard capillary. Background electrolyte, 10 mM TRIS/MOPS at pH 8.2. Applied voltage, 15 kV.

TABLE 2.5 Electroosmotic mobility, m_{EOF} (10^{-5} cm^2/Vs), effective mobility of the micelles, m_{MC} (10^{-5} cm^2/Vs), values for the elution window, t_{MC}/t_{EOF}, and measured electric current, I (μA), at different SDS concentrations, C_{SDS} (mM). BioRad standard capillary. Background electrolyte, 10 mM TRIS/MOPS at pH 8.2. Applied voltage, 15 kV.

C_{SDS}	m_{EOF}	m_{MC}	t_{MC}/t_{EOF}	I
30	60.62	-43.18	3.47	11.1
40	59.48	-43.93	3.60	13.7
50	58.38	-42.53	3.69	16.7
60	58.29	-42.50	3.69	19.7
70	57.93	-42.34	3.72	22.7
80	57.49	-42.49	3.83	25.8
90	57.49	-42.76	3.91	29.6
100	57.15	-42.47	3.89	33.4

ORGANIC MODIFIERS

Generally in MEKC hydrophobic compounds show retention factors much larger than k_{opt}. The separation of these compounds can be improved by the addition of an organic modifier to the background electrolyte in order to decrease their retention factors to more favourable values. Moreover, the elution window will be extended by the addition of organic modifiers [10,11]. To study the influence of different organic modifiers in MEKC, experiments were carried out with an electrolyte system of 0.01 M TRIS/boric acid at pH 8.5, containing different amounts of methanol, acetonitrile or urea. The migration times of the micelles for these experiments were calculated by an iteration procedure, applying the migration data of a homologous series of alkylbenzenes [20,27], as described in chapter 4. In Table 2.6 all calculated values for the electroosmotic mobility, the effective mobility of the micelles and the corresponding elution windows are summarized. At a methanol concentration above 20% (v/v) the restricted elution mode was obtained and t_{MC} could no longer be determined. A decrease in m_{EOF} and $|m_{MC}|$ is observed at increasing modifier concentrations, due to changes in the viscosity and the dielectric constant of the electrolyte systems. Moreover, the micelle structure and hence m_{MC} will be influenced by the addition of an organic modifier. As a result, an increase in the elution window is observed with increasing concentrations of methanol, acetonitrile or urea.

In reversed-phase liquid chromatography it has been shown that the variation of the retention factor, k, with the volume fraction of organic solvent in the aqueous-organic mobile phase, ϕ, is reasonably well described by [28]:

$$\ln k = A + B\phi + C\phi^2 \qquad (2.12)$$

where A, B and C are constants for a specific solute and eluent combination. For a small range of solvent compositions eqn. (2.12) can be approximated by

$$\ln k = A + B\phi \qquad (2.13)$$

Although in MEKC small changes in the phase ratio may occur, due to the influence of organic modifiers on micelle structures, for methanol and urea a linear relationship was obtained between the logarithm of the retention factor and the concentration modifier in the background electrolyte, as shown in Fig. 2.9. For acetonitrile a 2nd order relationship was obtained. From Fig. 2.9 and Table 2.6 it can be concluded that with an increase in modifier concentration a decrease in retention factors as well as an increase in elution window is obtained, resulting in a better resolution for hydrophobic compounds. As an example in Fig. 2.10 parts of the electrokinetic chromatograms are shown of the separation of alkylbenzenes with different amounts of methanol. As can be clearly seen a better resolution is obtained for strongly hydrophobic compounds migrating near t_{MC} at higher concentrations methanol, due to both a decrease in retention factor and an increase in elution window.

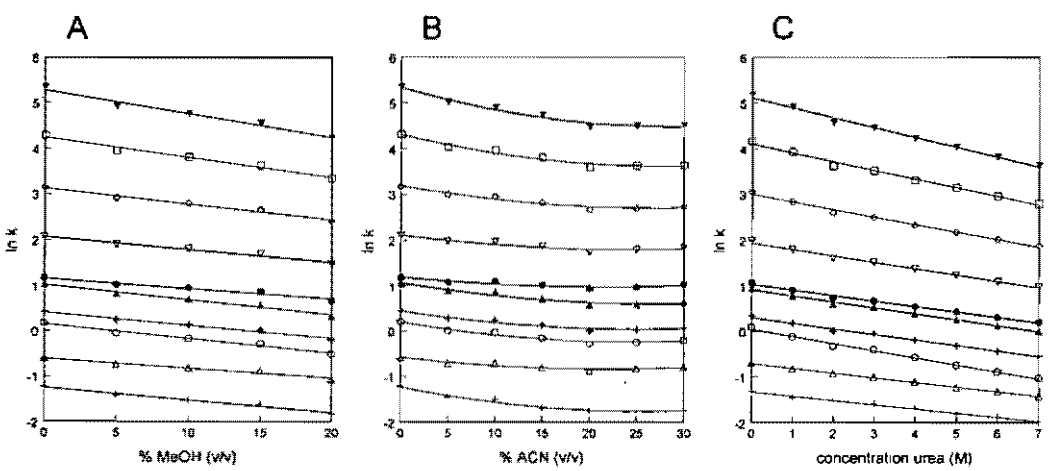

FIGURE 2.9 Logarithm of retention factor *versus* concentration modifier for (A) methanol (*v/v*), (B) acetonitrile (*v/v*) and (C) urea (M). BioRad standard capillary. Background electrolyte, 10 mM TRIS/boric acid at pH 8.5, containing different amounts of organic modifier. Applied voltage, 15 kV.

TABLE 2.6 Electroosmotic mobility, m_{EOF} (10^{-5} cm^2/Vs), effective mobility of the micelles, m_{MC} (10^{-5} cm^2/Vs), and values for the elution window, t_{MC}/t_{EOF}, at different concentrations of methanol (%(v/v)), acetonitrile (%(v/v)) and urea (M). Background electrolyte, 10 mM TRIS/boric acid at pH 8.5. BioRad standard capillary. Applied voltage, 15 kV.

methanol				acetonitrile				urea			
%	m_{EOF}	m_{MC}	t_{MC}/t_{EOF}	%	m_{EOF}	m_{MC}	t_{MC}/t_{EOF}	M	m_{EOF}	m_{MC}	t_{MC}/t_{EOF}
0	67.14	-44.21	2.93	0	63.75	-44.11	3.25	0	58.40	-43.12	3.82
5	56.68	-39.18	3.24	5	58.86	-42.58	3.62	1	56.19	-41.99	3.96
10	48.90	-35.62	3.68	10	56.74	-42.41	3.96	2	54.97	-40.49	3.80
15	41.72	-31.96	4.28	15	52.78	-40.96	4.46	3	53.73	-41.03	4.23
20	36.64	-29.41	5.06	20	50.72	-42.08	5.87	4	52.88	-40.26	4.19
25	32.84	-	-	25	49.04	-41.47	6.48	5	51.43	-40.11	4.55
30	29.76	-	-	30	47.21	-41.45	8.22	6	50.71	-39.26	4.43
								7	49.04	-38.65	4.73

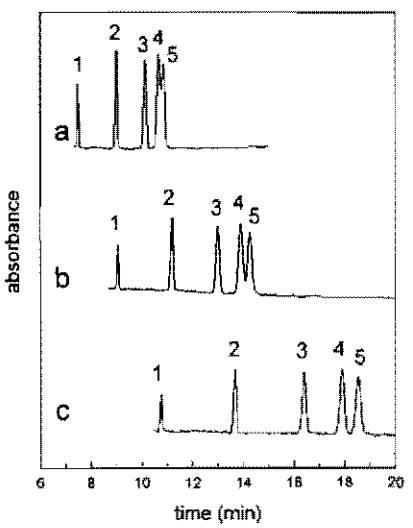

FIGURE 2.10 Electrokinetic chromatograms of the separation of the alkylbenzenes (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene and (5) butylbenzene in a background electrolyte of 10 mM TRIS/boric acid at pH 8.5, containing (a) 0%, (b) 5% and (c) 10% (*v/v*) methanol. BioRad standard capillary. Applied voltage, 15 kV.

2.5 SEPARATION NUMBER

Analogous to other chromatographic techniques, the separation number, *SN*, can be used in MEKC to describe the separation performance. The separation number is defined as the number of component peaks that can be placed between the peaks of two consecutive homologous standards with z and $z+1$ carbon atoms and separated with a resolution of R_S=1.177 [28]. Thus

$$SN = \frac{t_s^{z+1} - t_s^z}{w_{0.5}^{z+1} + w_{0.5}^z} - 1 \qquad (2.14)$$

where t_s^z and t_s^{z+1} are the migration times of the two consecutive homologues and $w_{0.5}^z$ and $w_{0.5}^{z+1}$ are their peak widths at half height on a temporal basis. In Fig. 2.11 *SN* is shown as a function of k for a homologous series of alkylaryl ketones, assuming that longitudinal diffusion is the only band broadening mechanism. In other chromatographic techniques, *SN* reaches a limiting value if k reaches infinity [29]. However, in MEKC a maximum is obtained for all values of the elution window as can be seen from Fig. 2.11. There are two reasons for this phenomenon. First, by analogy with the retention term *f(k)* (see Fig. 2.1), a maximum is observed in the curves representing the normal elution mode (t_{MC}/t_{EOF} = 2 or 5), due to the characteristic limited elution window. Second, due to micellar diffusion, *i.e.* diffusion of the pseudo-stationary phase, *SN* becomes 0 if k reaches infinity in the infinite elution mode (t_{MC}/t_{EOF} = ∞). Here it should be noted that the retention factors for the *SN*-maxima in Fig. 2.11 are not identical to those for the maxima in Fig. 2.1, calculated by eqn. (2.4), as in the latter case efficiency is not taken into account.

In Fig. 2.12 theoretical calculated and experimental determined separation numbers, *SN*, are shown for a homologous series of alkylaryl ketones in an electrolyte systems of 20 mM TRIS, adjusted to pH 8.2 by adding acetic acid, containing 50 mM SDS. The decrease in *SN* for more hydrophobic species clearly demonstrates the influence of the limited elution window in MEKC experiments. Lower separation numbers were obtained than the theoretical curve where only longitudinal diffusion was taken into account, indicating that other contributions to band broadening such as injection and micelle microheterogeneity also play a significant role in these MEKC analyses.

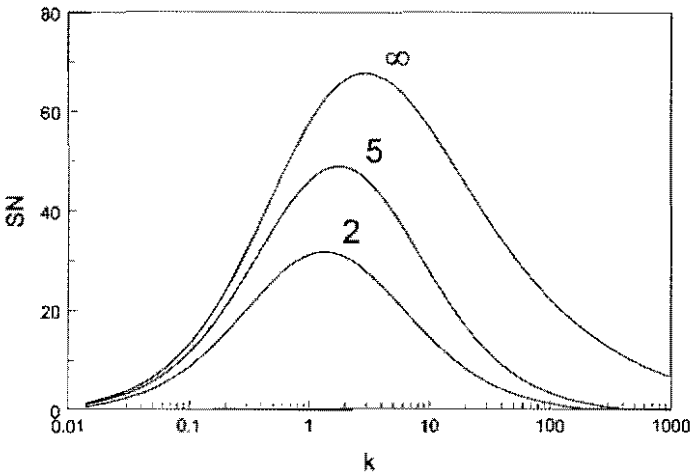

FIGURE 2.11 Calculated separation number, *SN*, versus retention factor, *k*, for a homologous series of alkylaryl ketones for different values of the elution window, t_{MC}/t_{EOF}, assuming that longitudinal diffusion is the only band broadening mechanism. Model values: $D_{AQ}=10\cdot10^{-6}$ cm²/s, $D_{MC}=10^{-6}$ cm²/s, $l_c=l_d=100.0$ cm, $V=20$ kV, $m_{EOF}=$ 80, 50 or $40\cdot10^{-5}$ cm²/Vs, $m_{MC}=-40\cdot10^{-5}$ cm²/Vs, log k = 0.35*z*-2.56 [20].

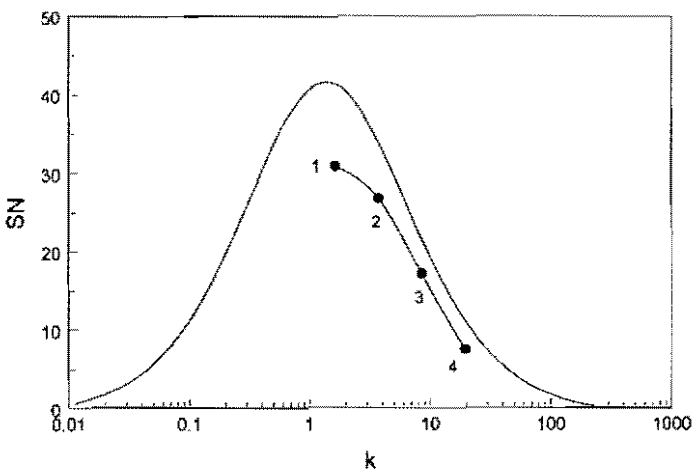

FIGURE 2.12 Theoretical calculated and experimental measured separation number, *SN*, versus retention factor, *k*, for a homologous series of alkylaryl ketones; (1) acetophenone, (2) propiophenone, (3) butyrophenone and (4) valerophenone. Electrolyte system, 0.02 M TRIS/acetic acid at pH 8.2, containing 50 mM SDS. For other conditions, see section 7.1.3. Calculated curves: $D_{AQ}=8.09\cdot10^{-6}$ cm²/s, $D_{MC}=1.17\cdot10^{-6}$ cm²/s (average values from TABLE 7.2), log k = 0.36*z*-2.67.

2.6 CONCLUSIONS

The overall linear velocity of neutral sample compounds was shown to be linearly related to the measured electric current. According to the resolution equation both the elution window and the retention factor influence the resolution in micellar electrokinetic capillary chromatography. These variables are often simultaneously affected by the experimental conditions and hence they cannot be controlled independently in many cases. The results demonstrate that the resolution of uncharged compounds can be improved by adjusting different separation parameters. The elution window can be increased by decreasing the pH of the electrolyte system, whereas the retention factors of the compounds remain fairly constant. With a low electroosmotic flow at low pH values, the restricted elution mode can be obtained, resulting in a better resolution for weakly hydrophobic compounds. The use of coated capillaries was shown to be of limited value in MEKC. The retention factor can be optimized in order to increase the function *f(k)* by changing the surfactant concentration. An increase in elution window as well as a decrease in retention factors can be obtained by the addition of an organic modifier to the background electrolyte, resulting in a better resolution for strongly hydrophobic compounds. For methanol and urea a linear relationship is obtained between the logarithm of the retention factor and the modifier concentration whereas for acetonitrile a 2nd order relationship is obtained.

It was demonstrated that for low retention factors micellar diffusion during MEKC analyses is negligible. However, for higher retention factors the contribution of micellar diffusion is not negligible. Although efficiency in MEKC strongly depends on the retention factor for small elution windows, the contribution of micellar diffusion to band-broadening is of minor importance under practical MEKC conditions.

The separation number was found to be a good parameter to describe the separation performance in MEKC experiments, and may be used in resolution optimization strategies.

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3

MIGRATION BEHAVIOUR OF MONOVALENT WEAK ACIDS MOBILITY MODEL *versus* RETENTION MODEL

ABSTRACT

Migration of charged compounds in MEKC is based on micellar solubilization as well as electrophoretic migration. Consequently, the migration behaviour of these compounds can be described with a retention model or a mobility model. Both models are discussed and evaluated for the migration behaviour of monovalent weak acids in an SDS micellar system. It is demonstrated that for hydrophobic compounds the calculation of retention factors may be troublesome, due to interaction with surfactant molecules in the aqueous phase. Therefore the mobility model is to be preferred. The simultaneous determination of dissociation constants by spectroscopic and electrophoretic methods is discussed and apparent dissociation constants in micellar media are determined, showing that pK_a-shifts may occur in MEKC analyses, due to micellar solubilization. Furthermore, a sample stacking procedure is described, based on differences in overall effective mobilities between aqueous and micellar electrolyte systems.

3.1 INTRODUCTION

Although micellar electrokinetic chromatography (MEKC) was primarily developed for the separation of neutral compounds [1,2], also mixtures of charged and uncharged compounds [3,4] and charged compounds with almost identical electrophoretic mobilities [5,6] can be efficiently separated using this analytical technique. The migration behaviour of charged compounds in MEKC is based on partitioning with the pseudo-stationary micellar phase as well as electrophoretic migration in the aqueous phase. A good understanding of the influence of experimental conditions on these partitioning and migration processes would greatly facilitate the development of optimization strategies for complex sample mixtures. Recently, the migration behaviour has been studied extensively and several models have been suggested which describe migration in terms of physico-chemical constants such as micelle binding constants and apparent dissociation constants in micellar media [7-11].
In this chapter mobility and retention models for monovalent weak acids in an anionic micellar system are evaluated and compared. Two substituted phenols and four alkylparabens were selected as test compounds, based on their intermediate pK_a-values and different hydrophobic properties. In addition to that, the determination of apparent dissociation constants in aqueous and micellar media by electrophoretic and spectroscopic methods simultaneously is examined. Furthermore a sample stacking procedure, based on mobility differences due to micellar solubilization, is discussed.

3.2 THEORY

The separation mechanism of charged compounds in MEKC is based on both chromatographic and electrophoretic principles. Therefore the migration of these compounds can be described in terms of the retention factor, *i.e.* a chromatographic parameter, as well as in terms of mobility, *i.e.* an electrophoretic parameter. Khaledi *et al.* [7] reported in detail a phenomenological approach to describe the migration behaviour of acidic compounds as a function of buffer pH and micelle concentration. In this approach the net migration parameter of an acidic compounds is assumed to be the weighted average of the migration parameter of the acid in the undissociated and the dissociated form, in the aqueous and the micellar phase, respectively. Thus the overall effective mobility, m_{eff}^{ov}, in a micellar electrolyte system is given by:

$$m_{eff}^{ov} = \frac{1}{1+k} m_{eff.AQ} + \frac{k}{1+k} m_{MC} \tag{3.1}$$

where k is the retention factor, $m_{eff.AQ}$ is the effective mobility of the acid in the aqueous phase and m_{MC} is the effective mobility of the micellar phase. The overall effective mobility can be calculated directly from the migration times of the electrokinetic chromatogram according to:

$$m_{eff}^{ov} = \frac{l_c l_d}{t_S V} - \frac{l_c l_d}{t_{EOF} V} \tag{3.2}$$

where l_c is the length of the capillary, l_d is the length from injection to detection, t_S is the migration time of the solute, t_{EOF} is the migration time of the electroosmotic flow (EOF), and V is the applied voltage. Notice that this equation is a similar expression as eqns. (1.10) and (1.17).

3.2.1 RETENTION MODEL

From eqn. (3.1) the following expression for the retention factor can be derived:

$$k = \frac{m_{eff}^{ov} - m_{eff.AQ}}{m_{MC} - m_{eff}^{ov}} \tag{3.3}$$

The effective mobility in the aqueous phase, $m_{eff.AQ}$, is a difficult accessible parameter from MEKC experiments. Therefore often effective mobilities, obtained with capillary zone electrophoresis (CZE) experiments are used in this equation. However, it should be noted that several assumptions are made in this case, *e.g.*, the influence of the micelles on ionic strength, dielectric constant and viscosity and consequently on the effective mobility are assumed negligible and interactions of sample compounds with surfactant monomers are assumed not to occur.

The retention factor for a monovalent acid with thermodynamic equilibrium

$$HA \rightleftharpoons H^+ + A^- \tag{3.4}$$

is a weighted average of the retention factor in the undissociated form, k_{HA}, and the dissociated form, k_{A^-}, according to:

$$k = (1 - \alpha)\, k_{HA} + \alpha\, k_{A^-} \tag{3.5}$$

where α is the degree of dissociation, given by:

$$\alpha = \frac{K_a}{K_a + [H^+]} \tag{3.6}$$

Combination of eqns. (3.5) and (3.6) leads to the following expression for the retention factor as a function of pH:

$$k = \frac{k_{HA} + k_{A^-}\,(K_a / [H^+])}{1 + K_a / [H^+]} \tag{3.7}$$

Eqn. (3.7) predicts a sigmoidal behaviour of k *versus* pH, identical to liquid chromatographic techniques, with k_{HA} and k_{A^-} as the limiting retention factors of the acid in the undissociated and the dissociated form, respectively.

3.2.2 MOBILITY MODEL

The effective mobility of an acid in the aqueous phase, $m_{eff,AQ}$, is given by:

$$m_{eff,AQ} = \frac{K_a}{K_a + [H^+]}\, m_{A^-,AQ} \tag{3.8}$$

where $m_{A^-,AQ}$ is the effective mobility of the fully dissociated acid in the aqueous phase. The apparent dissociation constant in micellar media is defined by [7,12]:

$$K_{a,app} = K_a \frac{K_{A^-}^m [M] + 1}{K_{HA}^m [M] + 1} \tag{3.9}$$

where $K_{HA}{}^m$ and $K_{A^-}{}^m$ are the binding constants to the micelles of the undissociated and the dissociated form of the acid, respectively, and [M] is the concentration of surfactant, present as micelles.

Combination of eqns. (3.1), (3.7), (3.8), and (3.9) leads to the following expression for the overall effective mobility as a function of pH:

$$m_{eff}^{ov} = \frac{m_{HA} + m_{A^-} (K_{a,app} / [H^+])}{1 + K_{a,app} / [H^+]} \tag{3.10}$$

with

$$m_{HA} = \frac{K_{HA}^m [M] \; m_{MC}}{1 + K_{HA}^m [M]} \tag{3.11}$$

and

$$m_{A^-} = \frac{m_{A^-,AQ} + K_{A^-}^m [M] \; m_{MC}}{1 + K_{A^-}^m [M]} \tag{3.12}$$

Eqn. (3.10) predicts a sigmoidal behaviour of m_{eff}^{ov} *versus* pH, with m_{HA} and m_{A^-} as the limiting mobilities of the acid in the undissociated and the dissociated form in micellar media, respectively.

3.2.3 SPECTROSCOPIC pK_a DETERMINATION

According to Beer's law, the absorbance, A, for a UV detector equals:

$$A = \varepsilon \; c \; l \tag{3.13}$$

where ε is the molar absorptivity, c is the concentration of the UV absorbing species and l is the effective path length. For a monovalent acid at concentration c_{HA}, the absorbance at a specific wavelength will be:

$$A = [(1 - \alpha) \; \varepsilon_{HA} + \alpha \; \varepsilon_{A^-}] \; c_{HA} \; l \tag{3.14}$$

where ε_{HA} and ε_{A^-} are the molar absorptivities of the acid in the undissociated and the dissociated form, respectively.

Generally in capillary electrophoretic techniques peak areas are expressed on a temporal basis [13]. On applying a non-UV absorbing electrolyte system, the measured temporal peak area, A_T, will be proportional to:

$$A_T \propto Q_{inj} [(1 - \alpha) \varepsilon_{HA} + \alpha \varepsilon_{A^-}] t_S \quad (3.15)$$

where Q_{inj} is the injected sample amount. From eqns. (3.6) and (3.15) it can be concluded that for a constant sample amount the spatial peak area A_S, *i.e.* the temporal peak area multiplied by the migration velocity, is proportional to:

$$A_S \propto \frac{\varepsilon_{HA} + \varepsilon_{A^-} (K_a / [H^+])}{1 + K_a / [H^+]} \quad (3.16)$$

Eqn. (3.16) predicts a sigmoidal behaviour of A_S *versus* pH. This expression can be applied for the spectroscopic determination of dissociation constants in aqueous media, pK_a, by CZE experiments [14] or apparent dissociation constants in micellar media, p$K_{a,app}$, by MEKC experiments, provided that the molar absorptivities ε_{HA} and ε_{A^-} differ sufficiently at the applied wavelength. Notice that for micellar media K_a should be replaced by $K_{a,app}$.

3.3 EXPERIMENTAL

INSTRUMENTATION AND METHODS

All CZE and MEKC experiments were carried out on a BioFocus 3000 Capillary Electrophoresis System (BioRad, Hercules, CA, USA) at a constant temperature of 25 °C. Pressure injection was carried out with an injection constant of 2 psi.s, unless noted otherwise. Two fused silica capillaries from Chrompack (Middelburg, The Netherlands) were applied; 50 μm I.D., total length 70.0 cm, distance between injection and detection 65.4 cm or 75 μm I.D., total length 50.0 cm, distance between injection and detection 45.5 cm. UV absorbance spectra were measured with a Perkin Elmer UV/VIS Lambda 3B spectrophotometer (Perkin Elmer, Cupertino, CA, USA), using 1-cm cuvets at 25 °C. Non-linear regression of eqns. (3.7), (3.8), (3.10) and (3.16) was carried out with SlideWrite Plus (Advanced Graphics Software, Carlsbad, CA, USA), using the Levenberg-Marquardt algorithm.

SAMPLES AND SOLUTIONS

Methylparaben, ethylparaben, propylparaben and butylparaben were obtained from Sigma (St. Louis, MO, USA), *2,4,5*-trichlorophenol, *o*-nitrophenol, *p*-nitrophenol and polyoxyethylene(23)lauryl ether (Brij 35) were obtained from Merck (Darmstadt, Germany), sodium dodecylsulphate (SDS) was obtained from Aldrich (Steinheim, Germany). All other

chemicals were of analytical-reagent grade. Samples were dissolved in water at a final concentration of 0.25 mM, unless noted otherwise. Water was purified by a Milli-Q water purification system (Waters Millipore, Milford, MA, USA). In Table 3.I the compositions of the electrolyte systems at different pH values are listed. All buffer solutions were filtered through 0.45-μm filters prior to use.

TABLE 3.1 Composition of electrolyte systems at different pHs.

cation	buffering counter species	pH
0.02 M TRIS	formic acid	4.0
0.02 M TRIS	acetic acid	5.0
0.02 M TRIS	MES	6.0
0.02 M TRIS	*o*-phosphoric acid	7.0
0.02 M TRIS	acetic acid	8.0
0.02 M TRIS	boric acid	9.0
0.01 M NaOH	CAPS	10.0
0.01 M NaOH	CAPS	11.0

3.4 RESULTS AND DISCUSSION

3.4.1 MIGRATION BEHAVIOUR IN CZE

In order to study the migration behaviour of monovalent weak acids in CZE, experiments were carried out applying the electrolyte systems listed in Table 3.1. From the measured migration times effective mobilities, $m_{eff,AQ}$, were calculated and $m_{A^-,AQ}$ and pK_a values were determined, using eqn. (3.8). In Fig. 3.1 and Table 3.2 these results are presented. For comparative purposes with the MEKC data, the subscript AQ is also applied in the CZE data. The calculated pK_a values are in good agreement with those reported in literature [15]. As expected from theory, a decrease in $m_{A^-,AQ}$ is observed for the alkylparabens with an increase in alkyl chain length.

TABLE 3.2 Effective mobility of the fully dissociated acid, $m_{A^-,AQ}$ (10^{-5} cm^2/Vs), and pK_a-values with standard deviations (in parentheses), determined with eqn. (3.8) by CZE and pK_a-values from literature. *(n=8)*.

compound	$m_{A^-,AQ}$	pK_a	pK_a lit.[15]
methylparaben	-25.04 (0.53)	8.27 (0.06)	
ethylparaben	-23.47 (0.48)	8.29 (0.06)	
propylparaben	-22.43 (0.51)	8.30 (0.06)	
butylparaben	-21.49 (0.48)	8.32 (0.06)	
o-nitrophenol	-30.78 (0.33)	7.31 (0.03)	7.23
2,4,5-trichlorophenol	-25.99 (0.41)	6.91 (0.05)	6.72

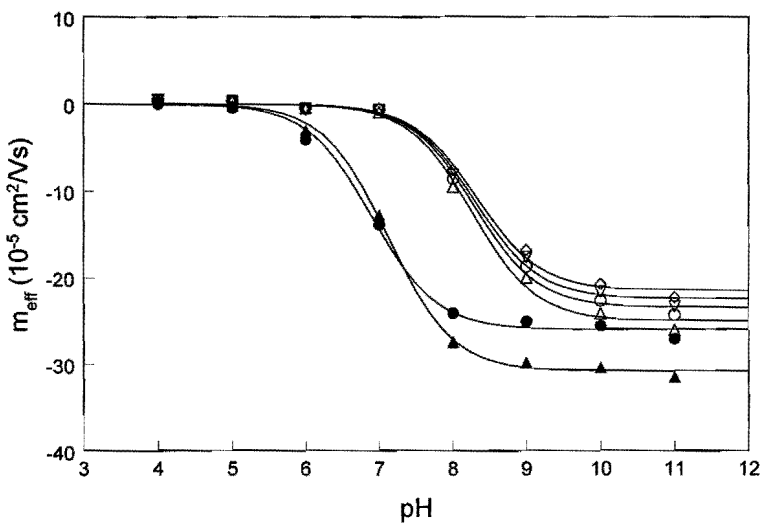

FIGURE 3.1 Effective mobility, m_{eff}, *versus* pH by CZE for (△) methylparaben, (○) ethylparaben, (▽) propylparaben, (◇) butylparaben, (▲) *o*-nitrophenol and (●) *2,4,5*-trichlorophenol. Capillary 50 μm I.D., l_c=70.0 cm, l_d=65.4 cm. Voltage, 20 kV. Lines represent calculated curves according eqn. (3.8).

3.4.2 MIGRATION BEHAVIOUR IN MEKC

The effective mobilities, shown in Fig. 3.1, indicate that alkylparabens can be separated by CZE at high pH. However, the differences in effective mobility are rather small. Much better separations are obtained by MEKC due to the additional separation mechanism based on micellar solubilization. This is illustrated in Fig. 3.2 for an electrolyte system of 0.02 M TRIS/boric acid at pH 9.0. In CZE (Fig. 3.2a) the migration behaviour of the

alkylparabens is mainly determined by their effective hydrated radii. In MEKC (Fig. 3.2b) a reversal of the migration order is obtained, owing to stronger interactions of the larger, more hydrophobic alkylparabens with the pseudo-stationary micellar phase.

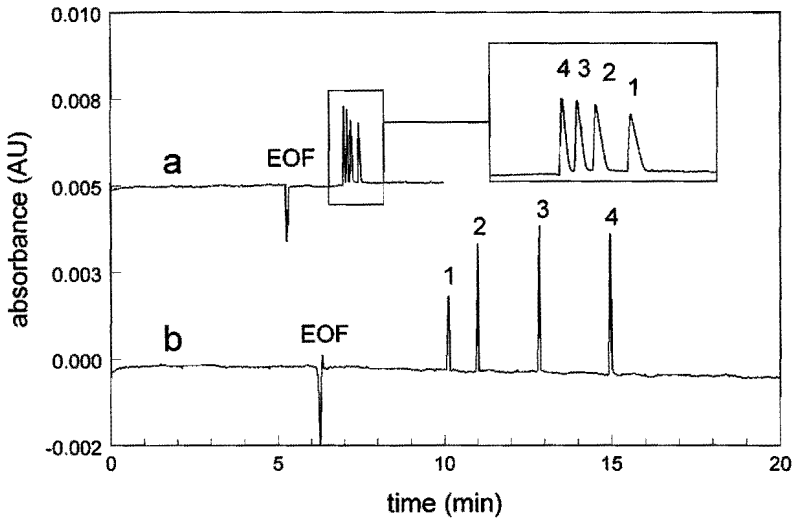

FIGURE 3.2 Electrokinetic chromatograms for the separation of (1) methylparaben, (2) ethylparaben, (3) propylparaben, (4) butylparaben in an electrolyte system of 0.02 M TRIS/boric acid at pH 9.0, containing (a) 0 mM and (b) 50 mM SDS. Capillary 50 μm I.D., l_c=70.0 cm, l_d=65.4 cm. Voltage, 20 kV. Detection wavelength, 200 nm.

MOBILITY MODEL

To study the migration behaviour of monovalent weak acids in MEKC, experiments were carried out applying electrolyte systems from Table 3.1 in the pH range 6-11, containing 50 mM SDS. With the electrolyte systems at pH 4.0 and 5.0 the restricted elution mode was obtained where the micelle migration time could not be determined [16]. From the measured migration times overall effective mobilities, m_{eff}^{ov}, were calculated and m_{HA}, m_{A^-} and $pK_{a,app}$ values were determined, using eqn. (3.10). In Table 3.3 the results are listed. The relatively small differences between m_{A^-} and $m_{A^-,AQ}$ (see Table 3.2) indicate that the dissociated forms of the sample compounds do not interact with the micellar phase due to electrostatic repulsion. The small decrease of m_{A^-} compared to $m_{A^-,AQ}$ may be attributed to an increase in ionic strength and viscosity of the micellar electrolyte system. Here it should be noted that with a decrease in mobility we mean a decrease in the absolute value of the mobility. The low value for butylparaben is due to a less accurate fit. As expected from eqn. (3.11) an

increase in m_{HA} is observed for the alkylparabens with an increase in hydrophobicity. The ΔpK_a values demonstrate that higher pK_a-shifts are obtained for more hydrophobic species, owing to a stronger interaction with the micellar phase. For methylparaben the variation in overall effective mobility as a function of pH is small, which makes the determination of $pK_{a,app}$ more difficult. In Fig. 3.3 the overall effective mobility is shown as a function of pH for *o*-nitrophenol and *2,4,5*-trichlorophenol. At low pH (undissociated form) the migration is mainly determined by micellar solubilization, whereas at high pH (dissociated form) the migration is mainly determined by the effective mobility in the aqueous phase.

TABLE 3.3 Values of m_{HA} (10^{-5} cm^2/Vs), m_{A^-} (10^{-5} cm^2/Vs) and $pK_{a,app}$ with standard deviations (in parentheses), determined with eqn. (3.10) by MEKC and ΔpK_a-values, compared to CZE values from TABLE 3.2. *(n=6)*.

compound	m_{HA}	m_{A^-}	$pK_{a,app}$	ΔpK_a
methylparaben	-26.58 (0.44)	-23.87 (0.33)	7.94 (0.40)	-0.33
ethylparaben	-32.38 (0.51)	-22.44 (0.72)	9.17 (0.18)	0.88
propylparaben	-35.77 (0.33)	-20.54 (0.79)	9.90 (0.09)	1.60
butylparaben	-37.64 (0.35)	-17.43 (2.23)	10.57 (0.15)	2.25
o-nitrophenol	-20.31 (0.35)	-28.01 (0.19)	7.45 (0.10)	0.14
2,4,5-trichlorophenol	-35.11 (0.72)	-24.92 (0.62)	8.24 (0.19)	1.33

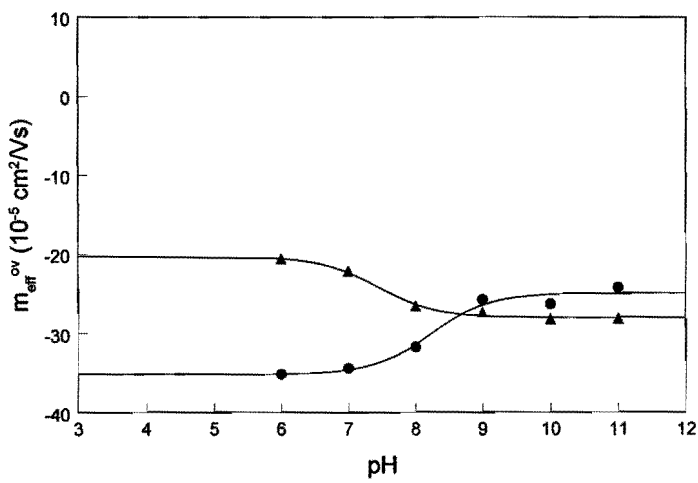

FIGURE 3.3 Overall effective mobility, m_{eff}^{ov}, *versus* pH by MEKC for (▲) *o*-nitrophenol and (●) *2,4,5*-trichlorophenol. Capillary 50 μm I.D., l_c=70.0 cm, l_d=65.4 cm. Voltage, 20 kV. Lines represent calculated curves according eqn. (3.10).

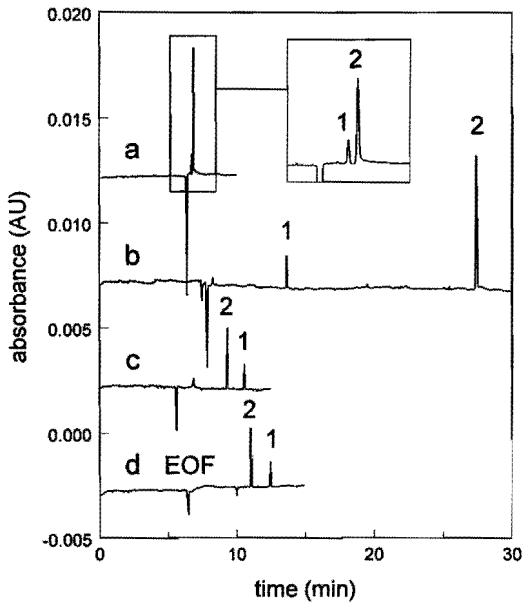

FIGURE 3.4 Electrokinetic chromatograms for the separation of (1) *o*-nitrophenol and (2) *2,4,5*-trichlorophenol in electrolyte systems of (a and b) 0.02 M TRIS/MES at pH 6.0 and (c and d) 0.02 M TRIS/CAPS at pH 11.0, containing (a and c) 0 mM and (b and d) 50 mM SDS. Capillary 50 μm I.D., l_c=70.0 cm, l_d=65.4 cm. Voltage, 20 kV. Detection wavelength, 200 nm.

The foregoing results indicate that MEKC offers the possibility to control the migration behaviour in order to improve the separation of charged compounds with almost identical effective mobilities, provided that the degree of interaction with the micellar phase is sufficiently different. In Fig. 3.4 the influence of an SDS pseudo-stationary phase on the migration of *o*-nitrophenol and *2,4,5*-trichlorophenol is illustrated for electrolyte systems at pH 6.0 and 11.0, respectively. At pH=6.0 (Fig. 3.4a and 3.4b, strong interaction) large mobility differences are obtained, whereas at pH=11.0 (Fig. 3.4c and 3.4d, weak interaction) only small differences are observed.

RETENTION MODEL

As was pointed out in the theoretical part, the migration behaviour of charged compounds in MEKC can also be described in terms of a retention factor [7]. For all sample compounds retention factors, k, were calculated according eqn. (3.3), using the effective mobilities obtained with CZE experiments (see Fig. 3.1) for $m_{eff,AQ}$. Sigmoidal relationships were obtained for all sample compounds which is illustrated in Fig. 3.5 for four alkylparabens.

From these data k_{HA}, k_{A^-} and pK_a values were determined, using eqn. (3.7). In Table 3.4 the results are presented. The low values for k_{A^-} support the suggestion that the dissociated forms of the sample compounds do not interact with the micellar phase. An increase in k_{HA} is observed with an increase in hydrophobicity of the compounds. The calculated pK_a values are comparable with those obtained with CZE experiments, listed in Table 3.2. For butylparaben a higher value was obtained due to a less accurate fit.

TABLE 3.4 Values of k_{HA}, k_{A^-} and pK_a with standard deviations (in parentheses), determined with eqn. (3.7) by MEKC. *(n=6)*.

compound	k_{HA}	k_{A^-}	pK_a
methylparaben	2.19 (0.07)	-0.07 (0.06)	8.12 (0.08)
ethylparaben	5.64 (0.14)	0.01 (0.11)	8.15 (0.06)
propylparaben	15.32 (0.44)	0.24 (0.38)	8.24 (0.08)
butylparaben	39.54 (3.41)	-0.84 (4.45)	9.01 (0.27)
o-nitrophenol	1.13 (0.07)	-0.24 (0.03)	7.14 (0.10)
2,4,5-trichlorophenol	14.60 (0.70)	-0.08 (0.17)	6.64 (0.07)

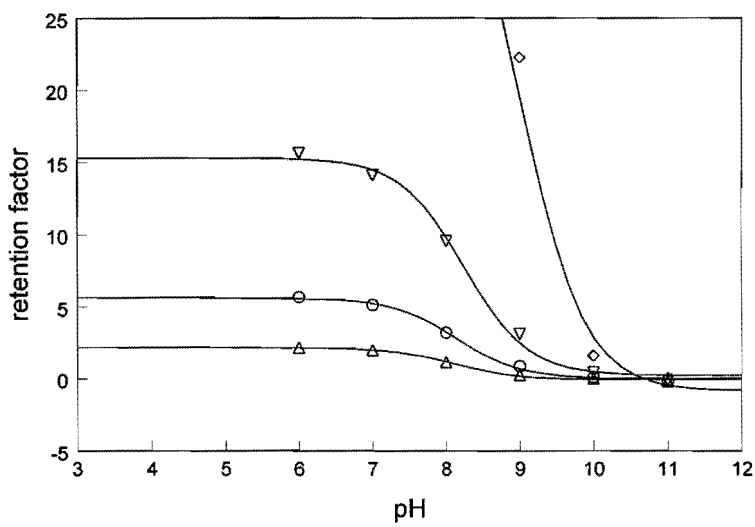

FIGURE 3.5 Retention factor *versus* pH by MEKC for (△) methylparaben, (○) ethylparaben, (▽) propylparaben and (◊) butylparaben. Capillary 50 μm I.D., l_c=70.0 cm, l_d=65.4 cm. Voltage, 20 kV. Lines represent calculated curves according eqn. (3.7).

3.4.3 MOBILITY MODEL *versus* RETENTION MODEL

From the foregoing it can be concluded that both the mobility model and the retention model describe well the migration behaviour of monovalent weak acids during MEKC analyses. For the correct calculation of retention factors according eqn. (7), effective mobilities in the aqueous phase are required. However, $m_{eff,AQ}$ is a difficult accessible quantity which makes the determination of retention factors troublesome. This difficulty has also been addressed by others [6,17]. As mentioned in the theoretical part, several assumptions are made if mobility data from CZE experiments are used for retention factor calculations. A fundamental difference between the composition of the aqueous phase in MEKC and the electrolyte system in CZE is the presence of surfactant monomer at a concentration, equal to the critical micelle concentration (CMC).

In order to study the influence of the surfactant concentration on $m_{eff,AQ}$, experiments were carried out in electrolyte systems of pH 7.0 and 9.0 containing different SDS concentrations, including concentrations below the CMC. These pHs were selected to ensure partly dissociation of the sample compounds. In Fig. 3.6 the experimental overall effective mobilities are shown for (A) *o*-nitrophenol and *2,4,5*-trichlorophenol at pH=7.0 and for (B) four alkylparabens at pH=9.0, respectively.

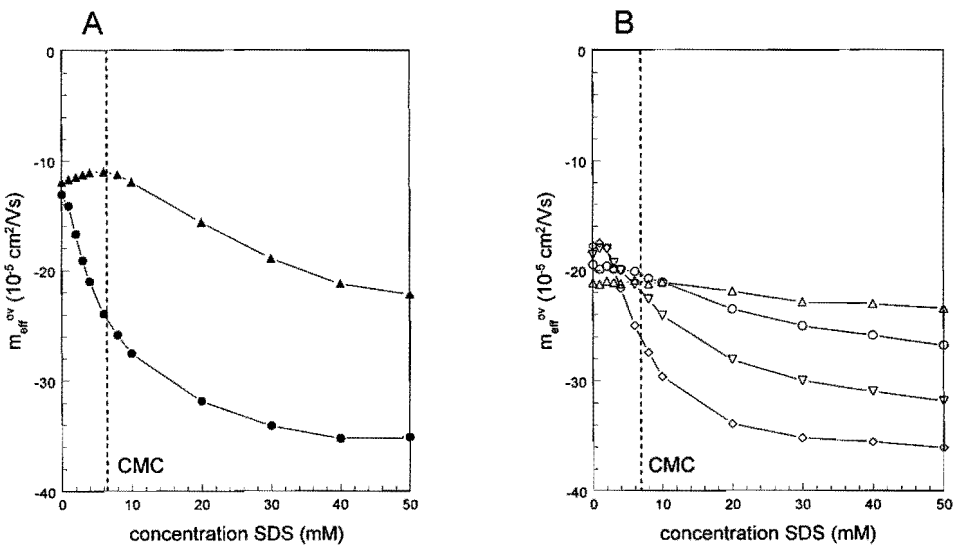

FIGURE 3.6 Overall effective mobility, m_{eff}^{ov}, as a function of concentration SDS for (△) methylparaben, (○) ethylparaben, (▽) propylparaben, (◇) butylparaben, (▲) *o*-nitrophenol and (●) *2,4,5*-trichlorophenol in electrolyte systems of (A) 0.02 M TRIS/*o*-phosphoric acid at pH 7.0 and (B) 0.02 M TRIS/boric acid at pH 9.0. Capillary 50 μm I.D., l_c=70.0 cm, l_d=65.4 cm. V=20 kV.

In these figures also the CMC's are indicated which were determined from the inflection points of the measured current *versus* concentration SDS. Above the CMC an increase in m_{eff}^{ov}, is obtained for all sample compounds due to micellar solubilization. Below the CMC, for *o*-nitrophenol a small decrease is observed in $m_{eff,AQ}$, owing to an increase in ionic strength. For *2,4,5*-trichlorophenol, however, a large increase in $m_{eff,AQ}$ is obtained below the CMC with an increase in SDS concentration (see Fig. 3.6A). These experiments were repeated three times with freshly prepared electrolyte systems and sample solutions and identical results were obtained in each case. The same behaviour was observed, although to a smaller extent, for propylparaben and butylparaben (see Fig. 3.6B). We assume that this phenomenon is due to interaction of the undissociated forms of the sample compounds with surfactant monomers. Several authors reported the separation of neutral compounds, based on hydrophobic interaction with surfactant monomers [18-20]. Probably this type of interaction also occurs in the aqueous phase during MEKC experiments, resulting in an increase of $m_{eff,AQ}$ for hydrophobic compounds.

To investigate the influence of the degree of dissociation on the interaction with surfactant monomers in more detail, additional experiments were carried out in electrolyte systems at pH 5.0 and 9.0, respectively, with surfactant concentrations in the range 0 - 10 mM. A decrease in interaction was observed for *2,4,5*-trichlorophenol with an increase in degree of ionization (i.e. higher pH), as shown in Fig. 3.7. These results support the suggestion that the undissociated forms of hydrophobic compounds may show interaction with surfactant monomers. Vindevogel and Sandra reported different results in borate and TRIS buffers [6], indicating that also the chemical nature of the buffer may affect the interaction mechanism. In Table 3.5 $m_{eff,AQ}$ is listed for all sample compounds for a CZE electrolyte system (concentration surfactant, C_{SF}=0) and for an MEKC electrolyte system (C_{SF}=CMC), respectively. Large differences are observed for hydrophobic compounds, which will have a pronounced effect on the calculation of retention factors according eqn. (3.7). This is illustrated in Fig. 3.8 for *o*-nitrophenol and *2,4,5*-trichlorophenol, where k is shown as a function of the concentration SDS. Although linear graphs are obtained with both $m_{eff,AQ}$ values, large differences in k are observed for *2,4,5*-trichlorophenol.

These results demonstrate that mobility data obtained with CZE experiments should be used with caution to calculate retention factors of charged compounds in MEKC experiments. Therefore the mobility model is to be preferred to describe their migration behaviour. Moreover, mobilities are more directly related to migration times than retention factors, which makes the overall effective mobility a more representative migration parameter.

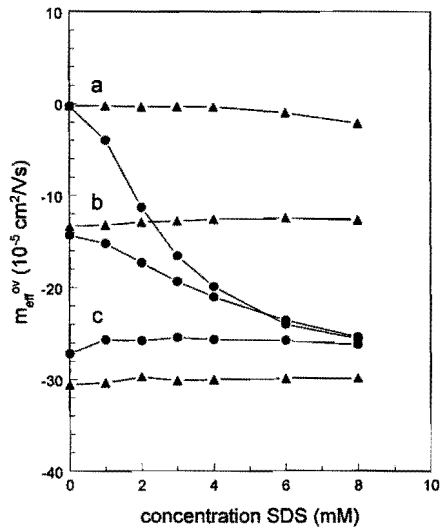

FIGURE 3.7 Overall effective mobility, m_{eff}^{ov}, as a function of concentration SDS for (▲) *o*-nitrophenol and (•) *2,4,5*-trichlorophenol in electrolyte systems of (a) 0.02 M TRIS/acetic acid at pH 5.0, (b) 0.02 M TRIS/*o*-phosphoric acid at pH 7.0 and (c) 0.02 M TRIS/boric acid at pH 9.0. Capillary 50 μm I.D., l_c=70.0 cm, l_d=65.4 cm. Voltage, 20 kV.

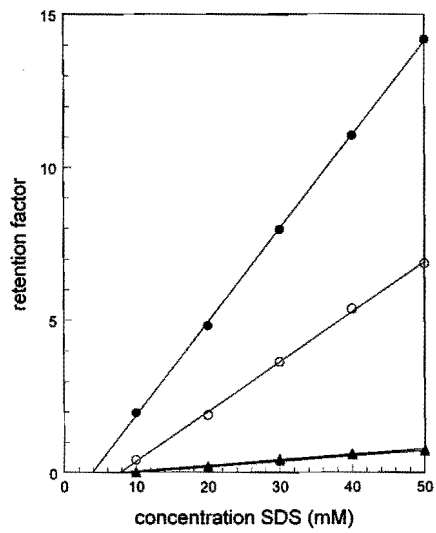

FIGURE 3.8 Calculated retention factor according eqn. (3.3) *versus* concentration SDS for (▲) *o*-nitrophenol and (•) *2,4,5*-trichlorophenol. Calculations with (closed symbols) $m_{eff,AQ}$ at C_{SF}=0 and (open symbols) $m_{eff,AQ}$ at C_{SF}=CMC, respectively. Capillary 50 μm I.D., l_c=70.0 cm, l_d=65.4 cm Voltage, 20 kV.

TABLE 3.5 Effective mobility in the aqueous phase, $m_{eff,AQ}$ (10^{-5} cm^2/Vs), determined from FIGURE 3.6, at SDS concentrations C_{SF}=0 and CMC, respectively. For experimental conditions, see FIGURE 3.6.

compound	$m_{eff,AQ}$ (C_{SF}=0)	$m_{eff,AQ}$ (C_{SF}=CMC)
methylparaben	-21.14	-21.05
ethylparaben	-19.47	-20.36
propylparaben	-18.58	-21.85
butylparaben	-17.75	-26.01
o-nitrophenol	-11.93	-11.06
2,4,5-trichlorophenol	-13.09	-24.45

3.4.4 SPECTROSCOPIC pK_a DETERMINATION

Due to partitioning with the micellar pseudo-stationary phase, shifts in dissociation constants of weak acids can be observed in MEKC experiments [7,21]. The magnitude of these pK_a-shifts depend on the structural properties of the solute and the micelles, and, as demonstrated in Table 3.3, can be more than 2 pH units in an SDS micellar system. The migration models described above demonstrate that dissociation constants play an important role in the migration behaviour. Therefore a good determination of pK_a-values in micellar media will be beneficial for a better understanding of the separation mechanism and to develop optimization strategies. Moreover, dissociation constants play a key role in several chemical and biological phenomena such as reaction kinetics and biological uptake and transport. Since most capillary electrophoresis instruments are equipped with a UV detector, this technique enables the simultaneous determination of pK_a-values by electrophoretic and spectroscopic methods. To investigate this possibility, CZE and MEKC experiments were carried out with *p*-nitrophenol in electrolyte systems of 0.02 M TRIS/acetate in the pH range 5-10 with steps of 1 pH unit. For the MEKC experiments 50 mM SDS and 50 mM Brij 35 (neutral surfactant) were applied as pseudo-stationary phases. For the spectrophotometric method the best results are obtained with a large difference between the molar absorptivities of the undissociated form, ε_{HA}, and the dissociated form, ε_{A^-}, respectively. In order to select proper wavelengths, the UV absorbance spectra of *p*-nitrophenol were measured in an aqueous solution, a 50 mM SDS solution and a 50 mM Brij 35 solution, containing 0.01 M NaOH or 0.01 M HCl, respectively. The local maxima of the undissociated form (λ=317 nm) and the dissociated form (λ=407 nm) were found to be unaffected by the presence of the surfactants. These wavelengths were applied for detection. From the measured migration times overall effective mobilities were calculated and apparent dissociation constants were

determined, using eqn. (3.10), and from the measured temporal peak areas spatial peak areas were calculated and apparent dissociation constants were determined, using eqn. (3.16). In Table 3.6 the results are presented, showing that 50 mM Brij 35 causes a larger pK_a-shift for *p*-nitrophenol than 50 mM SDS. For the CZE and 50 mM SDS electrolyte system considerable differences are observed between the two methods. Better results were reported for CZE experiments by Cleveland *et al.* [14] with an equation including activity correction for buffer ionic strength, applying more migration data obtained with different buffers and UV detection at 237 nm.

TABLE 3.6 Dissociation constants of *p*-nitrophenol in CZE and MEKC electrolyte systems, determined with mobility data, m_{eff}^{ov}, according eqn. (3.10) and spatial peak areas, A_S, at 317 and 407 nm according eqn. (3.16) with standard deviations (in parentheses) and ΔpK_a-values. *(n=6)*.

	CZE	50 mM SDS	ΔpK_a	50 mM Brij 35	ΔpK_a
m_{eff}^{ov}	7.29 (0.11)	7.40 (0.12)	0.11	7.97 (0.06)	0.68
A_S^{317}	7.27 (0.12)	7.66 (0.18)	0.39	7.94 (0.08)	0.67
A_S^{407}	7.44 (0.17)	7.96 (0.10)	0.52	7.96 (0.01)	0.52

3.4.5 m_{eff}^{ov}-MEDIATED SAMPLE STACKING

Differences in overall effective mobilities caused by a neutral surfactant can be used for a sample stacking procedure of weak acids. This procedure is schematically illustrated in Fig. 3.9 and works as follows. The acid is dissolved in an electrolyte system at a pH around its pK_a. The analysis is carried out with the same electrolyte system, containing a neutral surfactant. The presence of the neutral surfactant will lead to a decrease in overall effective mobility of the acid, due to (i) a micelle-induced pK_a-shift resulting in a lower degree of dissociation, (ii) partitioning with neutral micelles and (iii) an increase in viscosity of the electrolyte system. The higher effective mobility in the sample solution will result in a higher linear velocity and consequently in stacking of the acid at the rear side of the sample plug. In this way sample stacking is possible, even if the sample is dissolved in a solution with the same ionic strength as the electrolyte system. This procedure was demonstrated for *p*-nitrophenol, applying an electrolyte system of 0.02 M TRIS/MOPS (morpholino-propanesulphonic acid) at pH 7.0 and 50 mM Brij 35 as neutral micellar phase. For comparative purposes also a field amplified sample stacking procedure was carried out, *i.e.* compounds dissolved in deionized water [22]. Notice that in this situation the higher linear velocity in the sample plug is primarily based on a higher field strength and not on a higher effective mobility.

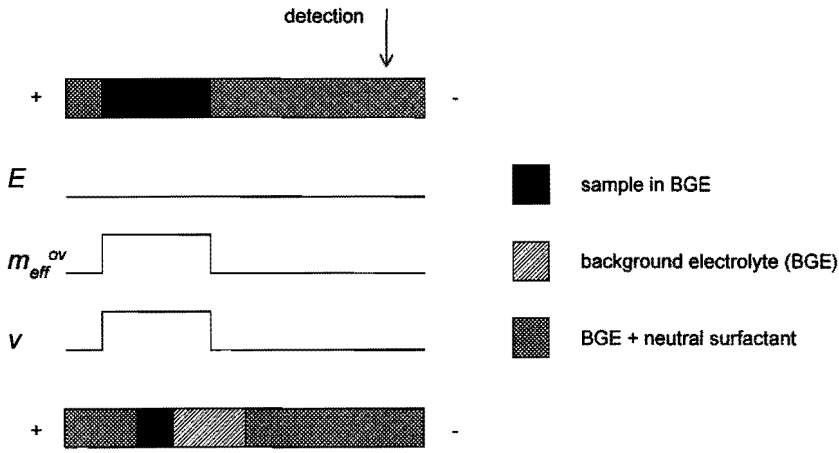

FIGURE 3.9 Schematic representation of m_{eff}^{ov}-mediated sample stacking. Sample dissolved in background electrolyte and capillary filled with background electrolyte containing a neutral surfactant. E=field strength, m_{eff}^{ov}=overall effective mobility and v=linear velocity. For further explanation, see text.

In Fig. 3.10 the results are shown for (a) no sample stacking, (b) field amplified sample stacking and (c) m_{eff}^{ov}-mediated sample stacking, applying injection constants of 2 and 20 psi.s, respectively. Without sample stacking a broad sample zone is obtained for the long injection time. The gradual shape at the front side of the plug is due to the parabolic flow profile during hydrodynamic injection. The migration times for the two injection constants (front side of the peak) are almost identical. With field amplified sample stacking good peak shapes are obtained for both injection times. Due to differences in the electroosmotic mobilities of water and the electrolyte system a higher EOF is obtained at longer injection times. For longer sample plugs it takes more time for the compound to migrate out of the sample plug and therefore it is detected closer to the EOF. With m_{eff}^{ov}-mediated sample stacking also good peak shapes are obtained for both injection times. The injected amount is smaller and the migration times are longer, due to a higher viscosity of the electrolyte system. For the longer injection time a higher EOF is obtained owing to the higher electroosmotic mobility of the sample plug. Here it takes also more time for the compound to migrate out of the long sample plug.

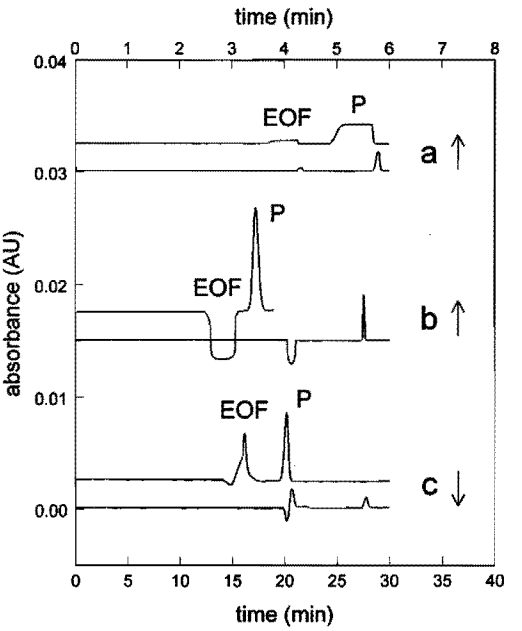

FIGURE 3.10 Electrokinetic chromatograms for (P) *p*-nitrophenol dissolved in (a and c) background electrolyte and (b) water. Electrolyte system, 0.02 M TRIS/MOPS at pH 7.0 containing (a and b) 0 mM and (c) 50 mM Brij 35. Injection constant, (upper curve) 20 and (lower curve) 2 psi.s. Capillary 75 μm I.D., l_c=50.0 cm, l_d=45.4 cm. Voltage, 15 kV. Detection wavelength, 215 nm.

In order to investigate the contribution of the increase in viscosity to the sample stacking procedure, the viscosity ratio of electrolyte systems with and without 50 mM Brij 35 was determined. A solution of the electrolyte system, containing 1 μl/ml mesityl oxide, was injected for a long period and the time to reach the detector was measured. The ratio of the times for both electrolyte systems equals their viscosity ratio, which was 1.76. The ratio of overall effective mobilities of *p*-nitrophenol in these electrolyte systems was 5.91, illustrating that the contribution of differences in viscosity is small and that the decrease in m_{eff}^{ov} is mainly based on micellar partitioning. In Fig. 3.11 peak heights are shown as a function of the injection constant. Without sample stacking the maximum peak height is obtained with 2 psi.s. With field amplified sample stacking peak shapes start to deteriorate with longer sample plugs due to a mismatch in the EOF of the sample plug and the electrolyte system [23]. For the m_{eff}^{ov}-mediated sample stacking procedure the curve starts to level of at higher injection constants, also due to an EOF mismatch.

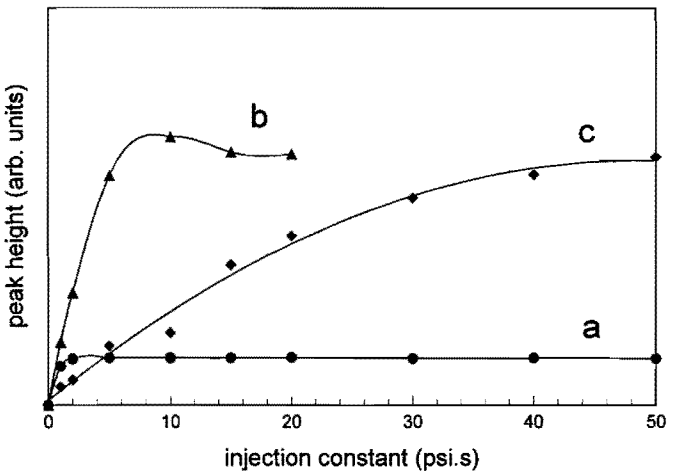

Figure 3.11 Peak height *versus* injected amount under (a) non-stacking, (b) field amplified sample stacking and (c) m_{eff}^{ov}-mediated sample stacking conditions. Capillary 75 μm I.D., l_c=50.0 cm, l_d=45.4 cm. Voltage, 15 kV. Detection wavelength, 215 nm.

3.5 CONCLUSIONS

Both the retention model and the mobility model can be applied to describe the migration behaviour of monovalent weak acids during MEKC experiments. However, the determination of effective mobilities in the aqueous phase may be troublesome, due to interaction of the undissociated form of the acid with surfactant monomers. This phenomenon may have a markedly influence on the calculation of retention factors for hydrophobic species. The results demonstrate that retention factors, calculated with mobility data obtained with CZE experiments, should be used cautiously. Moreover, overall effective mobilities are more directly related to the migration behaviour than retention factors. Therefore the mobility model is to be preferred to describe the migration behaviour of charged compounds in MEKC.

Shifts in pK_a-values may occur during MEKC analyses, due to micellar solubilization. CZE and MEKC were shown to be suitable techniques to determine apparent dissociation constants in aqueous and micellar media by electrophoretic and spectroscopic methods simultaneously. It was demonstrated that differences in overall effective mobilities, based on interaction with a neutral surfactant, can be applied for a sample stacking procedure, even if the sample compounds is dissolved in a solution with the same ionic strength as the applied electrolyte system.

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4

APPLICATION OF THE RETENTION INDEX CONCEPT

ABSTRACT

The application of the retention index concept in MEKC was evaluated for the identification of neutral species. Homologous series of alkylbenzenes and alkylaryl ketones were applied as retention index standards and also for the calculation of the micelle migration time by an iteration procedure. The relationship between retention indices, I, and n-octanol-water partition coefficients is discussed and ΔI values were calculated from retention indices, obtained with both anionic and cationic surfactant systems, to study the separation mechanism for solutes with different functionalities in MEKC. The influence of the phase ratio is treated theoretically and it is demonstrated that the capacity factor is linearly related to the surfactant concentration, whereas the retention index is independent of the surfactant concentration. The temperature dependence of the retention index was investigated and the standard enthalpy, entropy and Gibbs free energy for micellar solubilization of the sample compounds were determined, showing that the hydrophobic interaction plays a significant role in MEKC analysis.

4.1 INTRODUCTION

In chapter 1 it was pointed out that both retention factors and pseudo-effective mobilities can be used for peak identification in MEKC. Besides these two parameters, also retention indexes can be used for identification purposes. In gas chromatography (GC) retention indexes have found widespread application for the identification of substances in complex matrices, because they are considered to express the retention with the best reproducibility and precision [1-3]. In 1958 Kovats described the fundamental concept and derived the basic equations for the retention index system. Retention indexes in GC are not only applied for peak identification but also as an aid in structure analysis and for the evaluation of stationary phases. In reversed-phase liquid chromatography (LC) retention indexes have found use in peak identification, the investigation of structure-activity relationships and the characterization of different eluents and reversed-phase stationary phases [4-8]. In this chapter the applicability of the retention index system for neutral species in MEKC is described.

4.2 RETENTION INDEXES IN MEKC

In contrast to most other chromatographic techniques, in MEKC two moving phases can be distinguished, *viz.* an electroosmotically pumped aqueous phase and a pseudo-stationary micellar phase. Hence both the velocity of the EOF and the velocity of the micelles are characteristic for an MEKC analysis. These two velocities determine the elution window t_{MC}/t_{EOF}, which influences the resolution. The retention factor of a neutral solute can be regarded as the migration time of the solute, related to both t_{EOF} and t_{MC}, according to:

$$k = \frac{\left(\frac{t_S - t_{EOF}}{t_{EOF}}\right)}{\left(\frac{t_{MC} - t_S}{t_{MC}}\right)} = \frac{t_S - t_{EOF}}{t_{MC} - t_S} \cdot \frac{t_{MC}}{t_{EOF}} \qquad (4.1)$$

This is illustrated in Fig. 4.1. Analogous to the Kovats retention index scale in GC [1], a retention index scale in MEKC can be derived, based on a homologous series with an increasing number of methylene groups [9]. The members of the homologous series are assigned retention index values equal to one hundred times the number of their carbon atoms, z, *i.e.* benzene has a retention index equal to 600, toluene 700, ethylbenzene 800,

etc. The retention index of a solute can be calculated by the logarithmic interpolation between the two neighbouring members of the homologous series, according to the equation:

$$I = 100z + 100 \cdot \frac{\log k_S - \log k_z}{\log k_{z+1} - \log k_z} \tag{4.2}$$

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 is the retention factor of the solute. From eqn. (4.2) it can be seen that the retention index, just as the retention factor, is independent of the elution window. Combination of eqns. (4.1) and (4.2) leads to an expression for the retention index as a function of the migration times:

$$I = 100z + 100 \cdot \frac{\log\left(\frac{t_S - t_{EOF}}{t_{MC} - t_S}\right) - \log\left(\frac{t_z - t_{EOF}}{t_{MC} - t_z}\right)}{\log\left(\frac{t_{z+1} - t_{EOF}}{t_{MC} - t_{z+1}}\right) - \log\left(\frac{t_z - t_{EOF}}{t_{MC} - t_z}\right)} \tag{4.3}$$

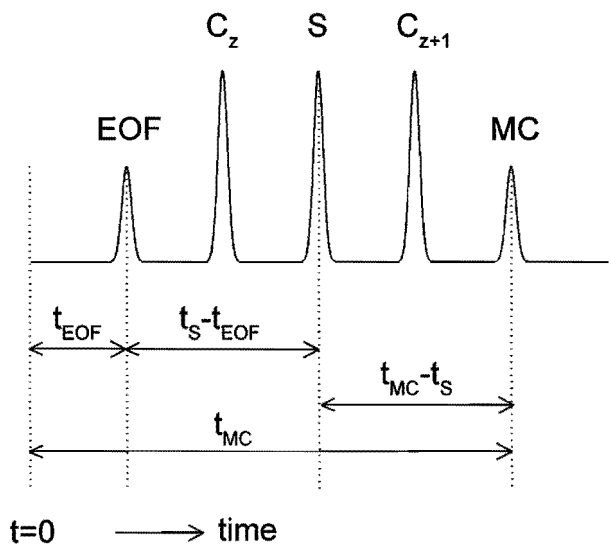

FIGURE 4.1 Schematic illustration of an electrokinetic chromatogram of an EOF marker (EOF), a neutral solute (S) and a micelle marker (MC) with the corresponding ratios of the migration times. C_z and C_{z+1} are the two neighbouring members of a homologous series for solute S with z and $z+1$ carbon atoms, respectively.

Compared to retention factors, retention indexes of hydrophylic components are less sensitive to uncertainties in the determination of t_{EOF} and retention indexes of hydrophobic components are less sensitive to uncertainties in the determination of t_{MC}. Moreover, the retention index is not influenced by the phase ratio, *i.e.* is independent of the surfactant concentration. Therefore retention indexes provide more accurate qualitative data in MEKC. Calculation of ΔI values from retention indexes obtained with different surfactant systems can also provide information about the interaction phenomena between sample molecules with different functionalities and micelles in MEKC experiments. This is illustrated in more detail in chapter 5. For these reasons retention indexes are a valuable way of expressing migration data in MEKC.

4.3 EXPERIMENTAL

CHEMICALS

Benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, sodium dodecylsulphate (SDS) and dodecyltrimethylammonium bromide (DTAB) were obtained from Aldrich (Steinheim, Germany); acetophenone, propiophenone, butyrophenone, valerophenone and hexanophenone from Pierce (Rockford, Ill, USA) and cetyltrimethylammonium bromide (CTAB) and tris(hydroxymethyl)amminomethane (TRIS) from Merck (Darmstadt, Germany). All other chemicals were of analytical-reagent grade.

INSTRUMENTATION

For all MEKC experiments a BioFocus 3000 Capillary Electrophoresis System (BioRad, Hercules, CA, USA) was used. All separations were carried out in 50 μm I.D. fused-silica capillaries from Chrompack (Middelburg, The Netherlands). For the experiments with the anionic surfactant SDS the total length was 50.05 cm and the distance between injection and detection was 45.45 cm. For the experiments with the cationic surfactants CTAB and DTAB the total length was 50.55 cm and the distance between injection and detection was 45.90 cm. The wavelength of the UV detector was set at 200 nm or 225 nm.

Conductivity measurements were carried out with a CDM 83 conductivity meter (Radiometer, Copenhagen, Denmark), equipped with a type CDC 314 conductivity cell. The SDS solutions for conductivity measurements were thermostated with a water bath (Ultra Thermostat, Colora, Germany).

SEPARATION CONDITIONS

All analysis were carried out at 25 °C in an electrolyte system of 0.02 M TRIS adjusted to pH 8.5 by adding boric acid, with 50 mM of the appropriate surfactant, unless otherwise noted. Samples were introduced by pressure injection with an injection constant of 2 psi.s. For all experiments a constant voltage of 20 kV was applied, except for the experiments with different temperatures where a constant current of 10 μA was applied. With the anionic surfactant SDS the anode was placed at the inlet side and the cathode at the outlet side of the capillary, and *vice versa* with the cationic surfactants CTAB and DTAB.

SAMPLES AND SOLUTIONS

Besides the reference compounds nine benzene derivatives were selected with different functionalities as the sample compounds. Resorcinol, phenol and naphtalene were dissolved at a concentration of 0.0005 M and all other sample compounds at a concentration of 0.15 μl/ml in a 50 mM solution of the appropriate surfactant, unless otherwise noted. Formamide was used as a neutral EOF marker. All CTAB solutions were stored at 30 °C. Water was purified by a Milli-Q water purification system (Waters Millipore, Milford, MA, USA). All buffer solutions were filtered through a 0.45 μm filter prior to use.

4.4 RESULTS AND DISCUSSION

4.4.1 SELECTION OF A HOMOLOGOUS SERIES

Standard reference compounds for the determination of retention indexes in MEKC should satisfy a number of requirements:

1. The relationship between log k and the number of carbon atoms in the molecules of the homologues must be linear;
2. The lowest homologue should be reasonably polar, in order to obtain a wide scale of retention indexes, covering the greater part of the elution window;
3. Containing a strong chromophore to detect them spectrophotometrically, as most capillary electrophoresis instruments apply on-column UV detection;
4. Not possessing an electrophoretic mobility, *i.e.* they should be uncharged;
5. Readily available at reasonable price;
6. Chemically stable in common electrolyte systems;
7. Not interacting with the fused-silica capillary wall.

According to the Martin equation [10], a homologous series with an increasing number of methylene groups shows a linear relationship between log k and the number of carbon atoms:

$$\log k = az + b \tag{4.4}$$

where z is the number of carbon atoms in the molecules of the homologues. The constant b is characteristic for the functional group of the homologues and depends on the phase ratio. Both the constants a and b depend on the nature of the aqueous phase and the micellar phase [11]. The retention index, I, can be calculated by interpolation and for components migrating faster than the first homologue by extrapolation of the equation:

$$\log k = a\frac{I}{100} + b \tag{4.5}$$

In first instance a number of alkan-2-ones were applied, but these compounds have only a weak chromophore and no reasonable UV signal was obtained. Therefore homologous series of alkylbenzenes and of alkylaryl ketones were tested as possible retention index standards. Five members of each series were analyzed, applying three different surfactants, *viz.* SDS, CTAB and DTAB. From the observed migration times the retention factors, k, were calculated and linear graphs were constructed for log k *versus* carbon number of the homologues. From these graphs the retention indexes were calculated, according to eqn. (4.5). In Table 4.1 all observed migration times and calculated retention factors and retention indexes are listed. The small deviations in the retention index values of the homologues from their nominal values give an indication of the precision of this method. For the last homologue the retention index equals the nominal value exactly, due to the iteration procedure for the determination of t_{MC} (*vide infra*). From these results it can be seen that for SDS benzene is the first homologue with the lowest retention factor, whereas for CTAB and DTAB acetophenone is the first homologue with the lowest retention factor. Moreover, for all components the calculated retention factors obtained with DTAB are smaller than those obtained with CTAB, due to the smaller interior of the DTAB micelles and a higher amount of free surfactant molecules in the aqueous phase; the critical micelle concentration (CMC) for DTAB and CTAB in water at 25 °C are 15.64 mM and 0.92 mM, respectively [12]. The linear graphs for log k *versus* carbon number of the homologues are shown in Fig. 4.2.

TABLE 4.1 Average values of the retention factors, k, and retention indexes, I, with standard deviations (in parentheses) for the alkylbenzenes and the alkylaryl ketones with the three different surfactants *(n=5)*.

	SDS		CTAB		DTAB	
Compound	k	I	k	I	k	I
Benzene	1.081 (0.005)	598.2 (0.1)	2.294 (0.018)	601.1 (1.0)	1.061 (0.009)	598.3 (0.2)
Toluene	3.108 (0.025)	704.6 (0.2)	6.252 (0.038)	701.7 (0.9)	2.749 (0.019)	705.1 (0.2)
Ethylbenzene	7.656 (0.054)	795.5 (0.2)	15.333 (0.232)	791.8 (1.3)	6.087 (0.030)	794.3 (0.3)
Propylbenzene	21.685 (0.185)	900.4 (0.2)	46.328 (2.972)	902.5 (1.3)	15.784 (0.060)	901.2 (0.2)
Butylbenzene	58.223 (0.550)	1000.0 (0.0)	122.415 (9.011)	1000.0 (0.0)	38.075 (0.265)	1000.0 (0.0)
Acetophenone	1.844 (0.011)	802.6 (0.1)	1.656 (0.012)	800.2 (0.5)	0.837 (0.009)	798.9 (0.2)
Propiophenone	3.957 (0.023)	896.7 (0.2)	4.048 (0.019)	900.6 (0.4)	1.894 (0.028)	902.0 (0.2)
Butyrophenone	8.970 (0.081)	997.6 (0.3)	9.529 (0.066)	996.7 (0.9)	4.049 (0.067)	998.0 (0.2)
Valerophenone	20.846 (0.240)	1101.5 (0.2)	23.957 (0.707)	1100.2 (0.8)	9.054 (0.155)	1099.6 (0.2)
Hexanophenone	46.376 (0.711)	1200.0 (0.0)	58.286 (1.892)	1200.0 (0.0)	20.050 (0.417)	1200.0 (0.0)

TABLE 4.2 Slope (-), intercept (-) and correlation coefficient for the graphs shown in FIGURE 4.2, electroosmotic mobility, m_{EOF} (10^{-5} cm^2/Vs), and effective mobility of the micelles, m_{MC} (10^{-5} cm^2/Vs), with standard deviations (in parentheses) for the three different surfactants and measured electric current (μA) *(n=5)*. Homologous series of (I) alkylbenzenes and (II) alkylaryl ketones.

Surfactant		Slope	Intercept	r	m_{EOF}	m_{MC}	I
SDS	I	0.43	-2.54	0.99976	53.40 (0.13)	-38.82 (0.13)	13.1
	II	0.35	-2.56	0.99983	55.46 (0.07)	-39.25 (0.06)	13.2
CTAB	I	0.43	-2.24	0.99911	-72.26 (0.36)	41.22 (0.28)	11.7
	II	0.39	-2.87	0.99981	-72.20 (0.40)	40.75 (0.24)	11.5
DTAB	I	0.39	-2.29	0.99967	-54.96 (0.25)	35.19 (0.24)	20.0
	II	0.34	-2.82	0.99985	-57.08 (0.56)	35.41 (0.07)	20.0

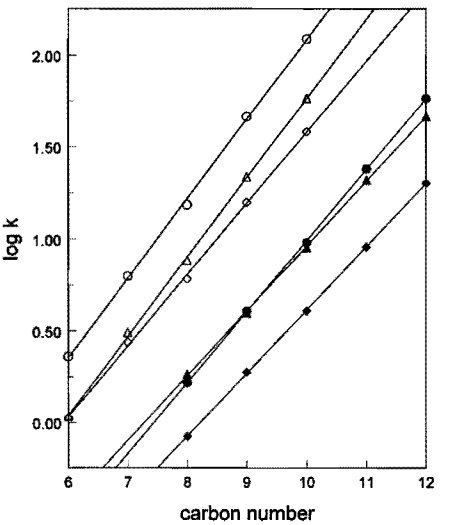

FIGURE 4.2 Relationship between log k and carbon number for the alkylbenzenes (open symbols) and the alkylaryl ketones (closed symbols) applying a background electrolyte containing (△) 50 mM SDS, (○) 50 mM CTAB or (◇) 50 mM DTAB.

As can be seen from Fig. 4.2, with all surfactants higher retention factors are obtained for the alkylbenzenes compared to the alkylaryl ketones with an equivalent carbon number. In Table 4.2 the electroosmotic mobility, m_{EOF}, the effective mobility of the micelles, m_{MC}, and the regression data for the graphs in Fig. 4.2 are given. From these results it can be concluded that both the alkylbenzenes and the alkylaryl ketones show a linear relationship between log k and the carbon number of the homologues. Hence both homologous series can be used as retention index standards in MEKC. For the anionic surfactant SDS the alkylbenzenes are to be preferred with respect to the scale of the retention factors, whereas for the cationic surfactants CTAB and DTAB the alkylaryl ketones are more favourable.

4.4.2 DETERMINATION OF THE MICELLE MIGRATION TIME

According to eqns. (4.1) and (4.3), both t_{EOF} and t_{MC} must be known in order to calculate the retention factor and the retention index. Formamide proved to be a suitable EOF marker in MEKC because it is a neutral compound that absorbs UV radiation and is not solubilized by the micelles. The micelle migration time can be determined by a compound which is completely solubilized by the micelles, such as Sudan III, anthracene or timepidium bromide [13]. In an alternative method, described by Bushey *et al.* [14,15], the migration data of a homologous series are used to determine t_{MC} by an iteration procedure. The migration time

of the last homologue is used as an estimation for t_{MC}. With this t_{MC} the retention factors of the other homologues are calculated and a linear graph of log k *versus* carbon number is constructed for these components. From this graph the retention factor for the last homologue is determined and with the migration time a new t_{MC} is calculated, using eqn. (1.14). With this new t_{MC} the retention factors of the other homologues are recalculated. This iteration procedure is repeated until the difference in consecutive calculated t_{MC} values is considered negligible. It is obvious that the linear graph of log k *versus* carbon number of the homologous series, constructed for the determination of t_{MC}, can be applied for the calculation of the retention indexes, according to eqn. (4.5).

In order to compare these two methods for the determination of t_{MC}, experiments were carried out with the homologous series of alkylbenzenes and alkylaryl ketones with Sudan III as a micelle marker. In Fig. 4.3 the electrokinetic chromatograms are shown of the separation of the alkylbenzenes and the alkylaryl ketones, applying a background electrolyte containing 50 mM SDS. The difference in the migration time of Sudan III in these electrokinetic chromatograms is a result of a small difference in the EOF.

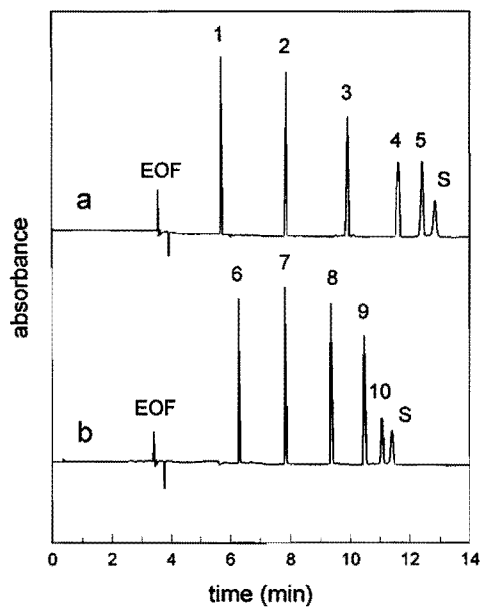

FIGURE 4.3 Electrokinetic chromatograms of the separation of (a) the alkylbenzenes (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene and (5) butylbenzene and (b) the alkylaryl ketones (6) acetophenone, (7) propiophenone, (8) butyrophenone, (9) valerophenone and (10) hexanophenone with Sudan III (S) as t_{MC} marker in a background electrolyte containing 50 mM SDS. Concentration of alkylbenzenes and alkylaryl ketones, 0.50 and 0.06 μl/ml, respectively. λ=200 nm.

TABLE 4.3 Average migration times, t (min), and calculated retention factors, k, using Sudan III as the micelle marker with standard deviations (in parentheses) for the alkylbenzenes and the alkylaryl ketones in a background electrolyte containing 50 mM SDS. MC is the calculated value for t_{MC} according to the iteration procedure. *(n=5)*.

Compound	t	k
Formamide (EOF)	3.55 (0.01)	0
Benzene	5.70 (0.01)	1.091 (0.005)
Toluene	7.89 (0.02)	3.163 (0.026)
Ethylbenzene	9.94 (0.03)	7.948 (0.058)
Propylbenzene	11.63 (0.03)	23.994 (0.210)
Butylbenzene	12.44 (0.03)	77.766 (1.199)
Sudan III (MC)	12.85 (0.03)	∞
MC (calculated)	13.00 (0.03)	-
Formamide (EOF)	3.42 (0.00)	0
Acetophenone	6.32 (0.02)	1.890 (0.011)
Propiophenone	7.87 (0.03)	4.157 (0.044)
Butyrophenone	9.41 (0.04)	9.803 (0.076)
Valerophenone	10.53 (0.04)	25.618 (0.195)
Hexanophenone	11.12 (0.05)	77.804 (0.908)
Sudan III (MC)	11.46 (0.05)	∞
MC (calculated)	11.69 (0.05)	-

From the observed migration times of the homologues the t_{MC} was calculated, using a laboratory-written iteration program. The iteration procedure was continued until the difference between consecutive calculated t_{MC} values was smaller than 0.1 %. In Table 4.3 all measured migration times and the calculated values for t_{MC} are listed. In this table also the calculated retention factors, using the migration time of Sudan III as t_{MC}, are included. As can be seen from these results, the values for t_{MC}, calculated with the iteration procedure, are higher than the migration time of Sudan III in both cases. Probably the micellization of Sudan III causes a small decrease in the effective mobility of the micelles. As a consequence, the calculated retention factors, using the migration time of Sudan III as t_{MC}, are higher than the calculated retention factors, using the iterative obtained t_{MC} (see Table 4.1), especially for components migrating near t_{MC}. Although these differences are observed, both methods provide reproducible results and the differences between the calculated effective mobilities of the micelles are rather small; $0.16 \cdot 10^{-5}$ and $0.33 \cdot 10^{-5}$ cm^2/Vs for the alkylbenzenes and the alkylaryl ketones, respectively. Hence both methods can be used for

the determination of the micelle migration time in MEKC with aqueous electrolyte systems. In all further experiments the laboratory-written iteration program was used for the calculation of t_{MC}.

4.4.3 DETERMINATION OF RETENTION FACTORS AND RETENTION INDEXES

In order to determine the retention factors and retention indexes of the sample compounds, experiments were carried out with a sample mixture consisting of resorcinol, aniline, phenol, benzaldehyde, nitrobenzene, acetophenone, chlorobenzene, bromobenzene, naphtalene and the five alkylbenzenes as the retention index standards with the anionic surfactant SDS. In Fig. 4.4 the electrokinetic chromatogram of this sample mixture in a background electrolyte containing 50 mM SDS is shown.

To investigate the influence of a rinsing procedure, the experiments were performed with and without rinsing the capillary between the analysis for two minutes with background electrolyte, respectively. The experiments were carried out ten times and the retention factors, k, and the retention indexes, I, were calculated from the observed migration times, t, using eqns. (1.14) and (4.5).

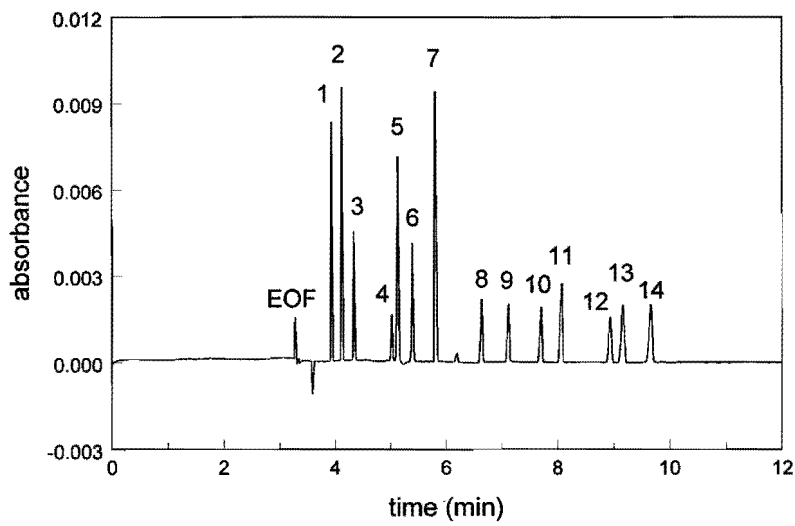

FIGURE 4.4 Electrokinetic chromatogram of the separation of (1) resorcinol, (2) aniline, (3) phenol, (4) benzene, (5) benzaldehyde, (6) nitrobenzene, (7) acetophenone, (8) toluene, (9) chlorobenzene, (10) bromobenzene, (11) ethylbenzene, (12) naphtalene, (13) propylbenzene and (14) butylbenzene applying a background electrolyte containing 50 mM SDS. Detection wavelength 200 nm.

TABLE 4.4 Average migration times, t (min), retention factors, k, and retention indexes, I, with standard deviations (in parentheses) for the different sample compounds in a background electrolyte containing 50 mM SDS *(n=10)*; standards, alkylbenzenes. I: rinsing of the capillary between the analyses with background electrolyte for two minutes. II: no rinsing between the analyses.

Compound	I			II		
	t	k	I	t	k	I
EOF	3.29 (0.00)	0 -	- -	3.30 (0.01)	0 -	- -
Resorcinol	3.93 (0.01)	0.325 (0.004)	477.7 (1.6)	3.95 (0.01)	0.324 (0.002)	475.6 (1.6)
Aniline	4.13 (0.01)	0.436 (0.003)	507.3 (0.6)	4.17 (0.01)	0.450 (0.004)	508.7 (0.7)
Phenol	4.34 (0.01)	0.570 (0.004)	534.3 (0.5)	4.38 (0.01)	0.583 (0.004)	534.6 (0.5)
Benzene	5.04 (0.01)	1.076 (0.010)	598.4 (0.2)	5.09 (0.02)	1.101 (0.011)	598.6 (0.1)
Benzaldehyde	5.15 (0.01)	1.169 (0.010)	606.6 (0.3)	5.20 (0.02)	1.195 (0.011)	606.9 (0.2)
Nitrobenzene	5.41 (0.02)	1.412 (0.013)	625.7 (0.2)	5.47 (0.02)	1.443 (0.013)	625.9 (0.3)
Acetophenone	5.81 (0.02)	1.846 (0.018)	652.7 (0.2)	5.88 (0.02)	1.883 (0.016)	652.7 (0.3)
Toluene	6.65 (0.02)	3.080 (0.028)	704.3 (0.1)	6.72 (0.02)	3.138 (0.027)	704.1 (0.2)
Chlorobenzene	7.12 (0.02)	4.097 (0.039)	733.0 (0.3)	7.20 (0.03)	4.182 (0.036)	733.0 (0.3)
Bromobenzene	7.70 (0.02)	5.910 (0.055)	769.9 (0.2)	7.78 (0.03)	6.035 (0.048)	769.9 (0.3)
Ethylbenzene	8.06 (0.03)	7.620 (0.076)	795.5 (0.3)	8.14 (0.03)	7.762 (0.070)	795.2 (0.2)
Naphtalene	8.92 (0.03)	16.377 (0.189)	872.6 (0.3)	9.00 (0.03)	16.766 (0.098)	872.8 (0.4)
Propylbenzene	9.14 (0.03)	21.607 (0.270)	900.5 (0.2)	9.22 (0.03)	22.149 (0.212)	900.8 (0.2)
Butylbenzene	9.63 (0.03)	57.992 (0.743)	1000.0 (0.0)	9.71 (0.04)	59.353 (0.617)	1000.0 (0.0)
MC (calculated)	9.96 (0.03)	∞ -	- -	10.04 (0.04)	∞ -	- -

As can be seen from the results, listed in Table 4.4, both the retention factor and the retention index can be used for peak identification in MEKC. However, the retention index shows a better repeatability. This is mainly due to the fact that it is a relative quantity which is less sensitive to small fluctuations in the experimental conditions than the retention factor [3]. Rinsing of the capillary with background electrolyte between the analysis had no significant influence on the repeatability of the migration times.

4.4.4 CORRELATION BETWEEN RETENTION INDEXES AND *n*-OCTANOL-WATER PARTITION COEFFICIENTS

In HPLC the correlation between retention indexes and *n*-octanol-water partition coefficients has been used for the examination of structures and physical properties of the analytes and for the prediction of retention characteristics. Also the *n*-octanol-water partition coefficients have proved to be useful for the quantitative correlation of biological activity with chemical structure. Baker [4] and Veith *et al.* [16] observed a linear relationship between the retention index and the logarithm of the *n*-octanol-water partition coefficient (log P_{OW}) for several organic compounds. The *n*-octanol-water partition coefficient is usually measured by UV spectroscopy. In Table 4.5 log P_{OW} values, taken from the literature, for the alkylbenzenes and the nine benzene derivatives with different functionalities are listed.

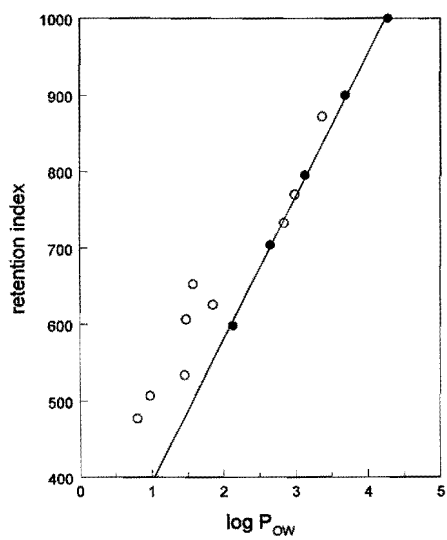

FIGURE 4.5 Relationship between retention index and log P_{OW} for (•) the alkylbenzenes and (○) the sample compounds with different functionalities obtained with a background electrolyte containing 50 mM SDS.

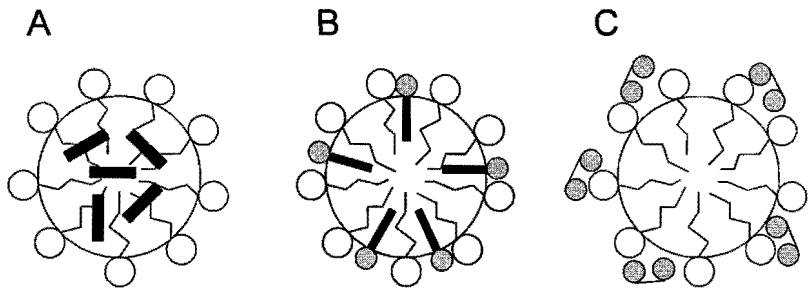

FIGURE 4.6 Schematic representation of different ways of micellar solubilization. (A) non polar compound, (B) compound with non polar and polar moieties, (C) polar compound.

If the distribution mechanism of the analytes in MEKC follows the same free energy relationship as the distribution in the *n*-octanol-water system, both log k and I will be linearly related to log P_{OW}. In order to determine the correlation between the retention index and the *n*-octanol-water partition coefficient, a graph was constructed of the retention indexes, listed in Table 4.4 (I), *versus* the log P_{OW} values, listed in Table 4.5. As can be seen from this graph, shown in Fig. 4.5 a linear relationship is obtained between I and log P_{OW} for the homologous series of the alkylbenzenes. The non polar compounds chlorobenzene, bromobenzene and naphtalene also follow this relationship. The polar compounds, however, show a deviation from the curve. These results indicate that non polar and polar compounds are solubilized in different ways. In aqueous solutions it is generally accepted that non polar compounds are solubilized in the hydrophobic interior of the micelle and polar compounds are solubilized by adsorption on the micelle surface, oriented with their hydrophobic moieties inside the micelle and their polar groups toward the aqueous phase [19,20]. This is illustrated in Fig. 4.6 Therefore distribution coefficients of non polar compounds are mainly influenced by the hydrocarbon chain of the surfactant whereas distribution coefficients of polar compounds are mainly influenced by the hydrophylic group of the surfactant. In chapter 5 the relation between I and log P_{OW} is studied in more detail.

TABLE 4.5 Functionalities, *n*-octanol-water partition coefficients, log P_{OW}, and calculated ΔI values obtained with the three surfactants for the different sample compounds; standards, alkylbenzenes.

Compound	Functionality	log P_{OW}[a]	I^{SDS}	I^{CTAB}	I^{DTAB}	ΔI_1[d]	ΔI_2	ΔI_3
Resorcinol	-OH, -OH	0.80	477.7	670.4	658.0	192.7	180.4	12.3
Aniline	$-NH_2$	0.98	507.3	540.2	531.2	32.9	23.9	9.0
Phenol	-OH	1.46	534.3	656.0	652.2	121.7	117.9	3.8
Benzene	-	2.13	598.4	604.4	598.4	6.0	0.1	5.9
Benzaldehyde	-COH	1.48	606.6	552.7	546.3	-54.0	-60.3	6.4
Nitrobenzene	$-NO_2$	1.86	625.7	616.9	615.7	-8.8	-10.0	1.2
Acetophenone	$-COCH_3$	3.18	652.7	574.0	573.7	-78.8	-79.0	0.3
Toluene	$-CH_3$	2.65[b]	704.3	698.9	704.7	-5.3	0.4	-5.7
Chlorobenzene	-Cl	2.84[c]	733.0	740.3	748.4	7.3	15.4	-8.1
Bromobenzene	-Br	2.99[c]	769.9	774.3	792.9	4.4	22.9	-18.5
Ethylbenzene	$-C_2H_5$	3.13[b]	795.5	786.6	794.1	-9.0	-1.5	-7.5
Naphtalene	$-C_4H_4$	3.37	872.6	914.3	925.8	41.7	53.2	-11.6
Propylbenzene	$-C_3H_7$	3.69[b]	900.5	904.9	901.0	4.4	0.4	3.9
Butylbenzene	$-C_4H_9$	4.28[b]	1000.0	1000.0	1000.0	0.0	0.0	0.0

[a]from ref. [16]. Marked values are from [b]ref. [17] and [c]ref. [18].
[d]$\Delta I_1 = I^{CTAB} - I^{SDS}$, $\Delta I_2 = I^{DTAB} - I^{SDS}$, $\Delta I_3 = I^{CTAB} - I^{DTAB}$.

4.4.5 CALCULATION OF ΔI-VALUES

In order to get more insight in this phenomenon, ΔI values were determined from the retention indexes obtained with the three different surfactants for all sample compounds, with the alkylbenzenes as the retention index standards. In Table 4.5 the calculated retention indexes and ΔI values for all sample compounds are listed. Since the cationic surfactants CTAB and DTAB differ only in the hydrocarbon chain length and the retention index is a relative quantity, the values for ΔI_3 are small for all sample compounds. For resorcinol, aniline and phenol positive values were obtained for ΔI_1 and ΔI_2. These compounds showed to have more interaction with the cationic micelles than with the anionic micelles. Here it should be noted, however, that at the pH of the electrolyte systems the acidic compounds resorcinol and phenol (pK_a = 9.81 and pK_a = 9.89, respectively) are partly ionized. This probably accounts for the high values of ΔI_1 and ΔI_2, obtained for resorcinol and phenol. Although these compounds are ionized to some extend, their migration behaviour is mainly based on micellar solubilization. For benzaldehyde, acetophenone, and nitrobenzene negative values were obtained for ΔI_1 and ΔI_2. These polar compounds showed to have more interaction with the anionic micelles than with the cationic micelles. The positive values of

ΔI_1 and ΔI_2, obtained for naphtalene are probably due to the fact that with the CTAB and DTAB surfactant systems naphtalene comigrates with propylbenzene. Therefore the calculation of I for naphtalene is less accurate in these surfactant systems.
In chapter 5 the influence of various interaction phenomena on micellar solubilization and, consequently, on selectivity, is described in more detail. Besides the approach described above, it is obvious that also by the calculation of ΔI values within one surfactant system information can be obtained about the interaction of different molecular functionalities with this specific micellar phase.

4.4.6 INFLUENCE OF THE PHASE RATIO

In section 2.4 it was pointed out that the retention factor is linearly related to the phase ratio (see eqn. (2.9)). The retention index, however, is independent of the phase ratio as it is a relative quantity for expressing migration data. Hence the retention index will be independent of the surfactant concentration. This can be clearly seen after combination of eqns. (4.2) and (2.9), leading to:

$$I = 100z + 100 \cdot \frac{\log K_S - \log K_z}{\log K_{z+1} - \log K_z} \qquad (4.6)$$

Of course the relative retention, which relates the retention factor of a neutral solute to that of a standard substance, will also be independent of the phase ratio [3].
In order to demonstrate the influence of the surfactant concentration on both the retention factor and the retention index, experiments were carried out with electrolyte systems containing different amounts of SDS, ranging from 25 mM to 100 mM. All experiments were carried out five times and from the observed migration times the average retention factors and retention indexes were calculated, applying the alkylbenzenes as retention index standards. For the retention factor *versus* surfactant concentration linear graphs were obtained, as shown in Fig. 4.7 The retention index showed to be independent of the surfactant concentration, which is illustrated in Fig. 4.8 This independence is advantageous for the comparison of experimental results obtained with different batches of electrolyte systems or in different laboratories. The decrease of the retention indexes of resorcinol with increasing SDS concentration is probably due to the fact that this compound is partly ionized, or that these retention index values are obtained by extrapolation, which may be less accurate than interpolation. For ionic species not only the distribution between the micellar phase and the aqueous phase, but also the effective mobility of the species determine the separation mechanism in MEKC [21,22], as described in chapter 3.

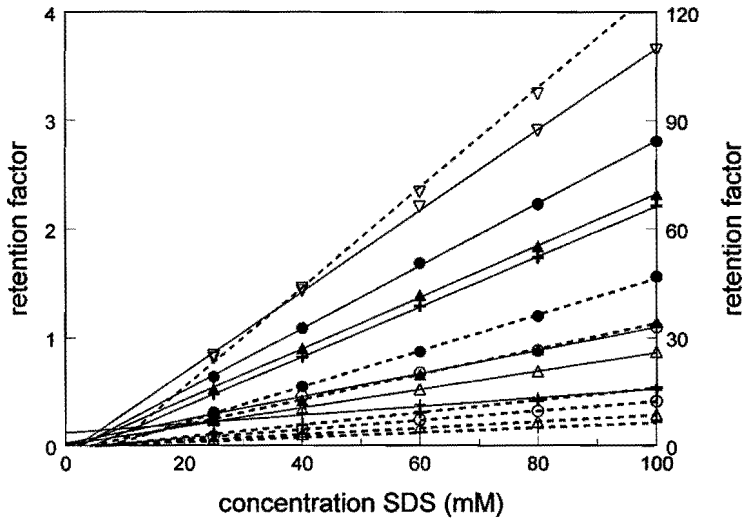

FIGURE 4.7 Graphs of the retention factor *versus* the concentration SDS for (drawn lines) (+) 1, (△) 2, (○) 3, (+) 4, (▲) 5, (●) 6, (▽) 7 and (dotted lines) (+) 8, (△) 9, (○) 10, (+) 11, (▲) 12, (●) 13 and (▽) 14. Drawn lines for the left and dotted lines for the right ordinate, respectively. See the legend of FIGURE 4.4 for the names of the components.

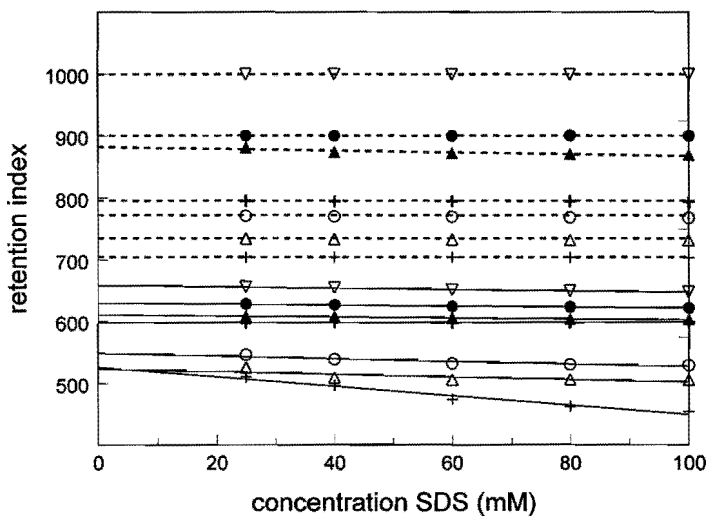

FIGURE 4.8 Graphs of the retention index *versus* the concentration SDS for (drawn lines) (+) 1, (△) 2, (○) 3, (+) 4, (▲) 5, (●) 6, (▽) 7 and (dotted lines) (+) 8, (△) 9, (○) 10, (+) 11, (▲) 12, (●) 13 and (▽) 14. See the legend of FIGURE 4.4 for the names of the components.

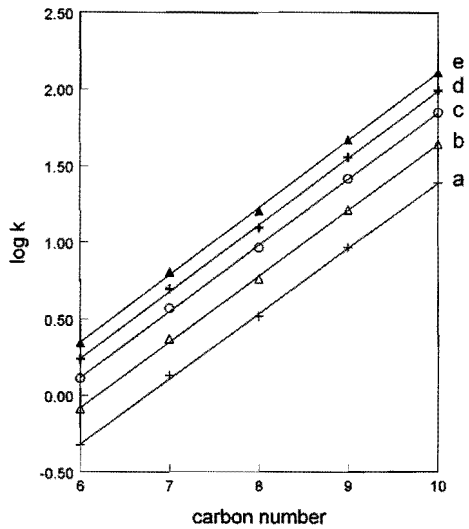

FIGURE 4.9 Relationship between log k and carbon number for the alkylbenzenes applying a background electrolyte containing (a) 25, (b) 40, (c) 60, (d) 80 and (e) 100 mM SDS.

The contribution of the effective mobility, however, decreases with increasing surfactant concentration. For all surfactant concentrations linear graphs were obtained for log k *versus* carbon number of the alkylbenzenes, according to eqn. (4.4), which are shown in Fig. 4.9. rom Fig. 4.9 it can be seen that the intercept (b) increases if the surfactant concentration increases, whereas the slope (a) remains fairly constant.

4.4.7 INFLUENCE OF TEMPERATURE AND CALCULATION OF THERMODYNAMIC QUANTITIES

To investigate the dependence of the retention index on the temperature in MEKC, experiments were carried out at different temperatures between 15 °C and 40 °C with a background electrolyte containing 50 mM SDS. At each temperature a constant current of 10 μA was applied. All experiments were carried out five times and from the observed migration times the average retention factors and retention indexes were calculated, applying the alkylbenzenes as the retention index standards. For all sample compounds the temperature dependence of the retention index, expressed as dI/dT, is listed in Table 4.6. As can be seen from these values the retention index, based on the alkylbenzenes, decreases with increasing temperature for all analytes. Moreover, the temperature dependence of the retention index is rather small, especially if the temperature working range for MEKC experiments is taken into account (*e.g.* with the BioFocus 3000 apparatus the capillary temperature can be varied between 15 °C and 40 °C).

TABLE 4.6 Temperature dependence of the retention index, dI/dT, and the distribution coefficients, K, of the sample compounds and measured voltage drop (kV) at different temperatures, T (°C).

Compound	dI/dT	Distribution coefficient					
		15	20	25	30	35	40
Resorcinol	-0.52	32.1	30.9	29.1	27.8	27.0	26.4
Aniline	-0.47	44.8	43.2	40.6	39.6	38.3	36.7
Phenol	-0.43	57.5	55.7	52.6	50.9	49.4	47.5
Benzene	0.01	103	102	98.7	97.4	96.0	93.7
Benzaldehyde	-0.42	119	114	108	104	101	97.0
Nitrobenzene	-0.25	141	136	130	126	124	119
Acetophenone	-0.44	190	180	170	164	158	152
Toluene	-0.03	300	292	282	276	271	264
Chlorobenzene	-0.13	407	392	377	366	358	347
Bromobenzene	-0.21	595	569	545	526	512	494
Ethylbenzene	0.01	747	722	699	682	670	652
Naphtalene	-0.57	1760	1630	1530	1450	1380	1300
Propylbenzene	-0.04	2160	2070	2000	1940	1890	1820
Butylbenzene	0.00	5850	5480	5370	5180	5040	4850
Voltage		17.8	16.8	15.8	14.8	14.2	14.2

The migration data of the sample compounds at different temperatures can be used for the determination of several thermodynamic quantities of micellar solubilization [23]. From the determined retention factors the distribution coefficient can be calculated with eqns. (2.9) and (2.10), provided that the partial molar volume and the critical micelle concentration at the different temperatures are known. In Table 4.7 literature data for $\bar{v}$ and CMC at different temperatures in pure water are listed. The partial molar volume of SDS showed to be almost independent of the constituents of the buffer solution [23]. Since the CMC generally decreases with increasing ionic strength of the surfactant solution, the CMC for SDS in the applied electrolyte system was determined by conductivity experiments as described by Saitoh *et al.* [25]. In Fig. 4.10 he graphs of the specific conductivity *versus* the concentration SDS in the TRIS/borate electrolyte system at different temperatures are shown. From the inflection points of these graphs the CMC's were determined, which are listed in Table 4.7. As can be seen from these results, at all temperatures the CMC in the electrolyte systems is lower than the CMC in pure water. From the determined retention

factors and the experimentally obtained CMC's the distribution coefficients of the sample compounds at the different temperatures were calculated using eqns. (2.9) and (2.10). These values are listed in Table 4.8.

TABLE 4.7 Partial molar volume, $\bar{v}$ (ml.mol^{-1}), and critical micelle concentration (CMC) (mmol.l^{-1}) for SDS at different temperatures, T (°C). I: in pure water. II: in a 0.02 M TRIS/borate electrolyte system at pH 8.5, from own measurements.

T	$\bar{v}$ [a]	*CMC*	
		I[b]	II
15	243.5	8.43	6.28
20	245.6	8.25	6.39
25	247.8	8.16	5.34
30	250.0	8.23	5.28
35	252.1	8.39	5.19
40	254.3	8.60	5.38

[a]from ref. [24]; [b]from ref. [12].

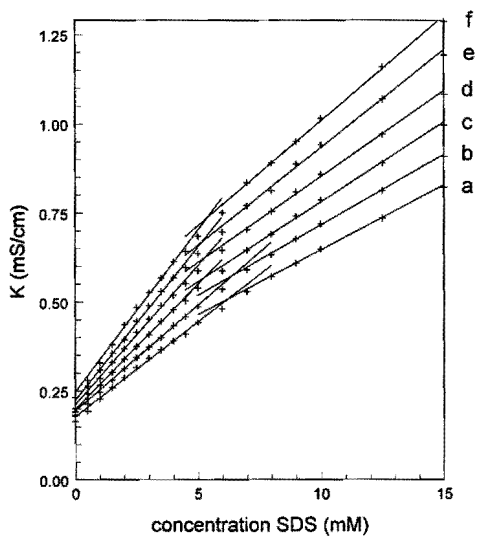

FIGURE 4.10 Graphs of the specific conductivity, κ, *versus* the concentration SDS in a TRIS/borate electrolyte system at pH 8.5 at (a) 15, (b) 20, (c) 25, (d) 30, (e) 35 and (f) 40 °C.

TABLE 4.8 Correlation coefficients for the graphs shown in FIGURE 4.11 and the standard enthalpy, ΔH^0 (kJ.mol^{-1}), standard entropy, ΔS^0 (J.mol^{-1}.K^{-1}) and standard Gibbs free energy, ΔG^0 (kJ.mol^{-1}) at 25 °C with standard deviations (in parentheses) for the micellar solubilization of the different sample compounds by the SDS micelles.

Compound	r	ΔH^0	ΔS^0	ΔG^0
Resorcinol	0.985	-6.1 (0.2)	7.5 (0.7)	-8.4 (0.3)
Aniline	0.968	-6.0 (0.3)	10.9 (1.0)	-9.2 (0.4)
Phenol	0.982	-5.8 (0.2)	13.6 (0.7)	-9.8 (0.3)
Benzene	0.896	-2.9 (0.3)	28.7 (0.9)	-11.4 (0.4)
Benzaldehyde	0.976	-6.2 (0.3)	18.3 (0.9)	-11.6 (0.4)
Nitrobenzene	0.964	-5.0 (0.3)	23.8 (0.9)	-12.1 (0.4)
Acetophenone	0.977	-6.5 (0.3)	20.8 (0.9)	-12.8 (0.4)
Toluene	0.936	-3.8 (0.3)	34.2 (0.9)	-14.0 (0.4)
Chlorobenzene	0.958	-4.7 (0.3)	33.6 (0.9)	-14.7 (0.4)
Bromobenzene	0.971	-5.5 (0.3)	33.9 (0.9)	-15.6 (0.4)
Ethylbenzene	0.941	-4.0 (0.3)	41.1 (0.9)	-16.3 (0.4)
Naphtalene	0.988	-8.8 (0.3)	31.5 (0.9)	-18.2 (0.4)
Propylbenzene	0.946	-5.0 (0.3)	46.6 (1.1)	-18.8 (0.5)
Butylbenzene	0.882	-5.3 (0.5)	53.7 (1.8)	-21.3 (0.7)

The standard enthalpy, ΔH^0, and the standard entropy, ΔS^0, for the micellar solubilization of the sample compounds can be calculated according to the van 't Hoff equation:

$$\ln K = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} \tag{4.7}$$

where R and T are the gas constant and the absolute temperature, respectively. The standard Gibbs free energy, ΔG^0, for the micellar solubilization can be calculated according to:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{4.8}$$

In Fig. 4.11 the van 't Hoff plots for all sample compounds are shown. From the slope and the intercept of these plots ΔH^0 and ΔS^0 and with eqn. (4.8) ΔG^0 at 25 °C were calculated. These values are listed in Table 4.8. The differences between these results and the results reported by Terabe *et al.* [23] may probably be due to differences in the applied electrolyte systems, or to the fact that they use retention factor values, extrapolated to zero electroosmotic mobility.

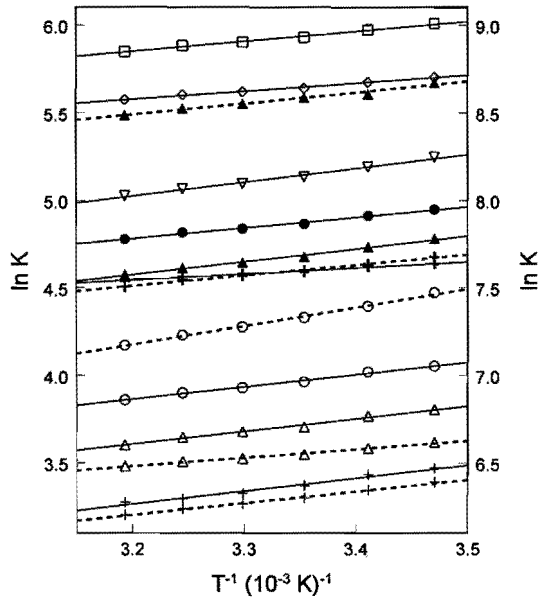

FIGURE 4.11 Van 't Hoff plots for (drawn lines) (+) 1, (△) 2, (○) 3, (+) 4, (▲) 5, (●) 6, (▽) 7, (◇) 8, (□) 9, and (dotted lines) (+) 10, (△) 11, (○) 12, (+) 13 and (▲) 14. Drawn lines for the left and dotted lines for the right ordinate, respectively. See the legend of FIGURE 4.4 for the names of the components.

As can be seen from Table 4.8, the enthalpy change of the alkylbenzenes decrease with an increase in the hydrocarbon chain length. This indicates that the affinity for the micelles is stronger for alkylbenzenes with a longer alkyl chain, *i.e.* for more hydrophobic species. For the entropy change, however, an increase was observed with an increase of the alkyl chain length. Moreover, for all sample compounds a positive value for the entropy change was obtained, which seems rather contradictory to the apparent lower degree of freedom of the solutes incorporated in the micelles. This phenomenon can be explained by a strong contribution of the hydrophobic interaction, as described by Terabe *et al.* [23]. From the entropy changes, listed in Table 4.8, it can be concluded that the hydrophobic interaction plays a significant role in the micellar solubilization of sample compounds in MEKC. The Gibbs free energy for micellar solubilization showed to be consistent with the migration order of the sample compounds (see Fig. 4.4).

4.5 CONCLUSIONS

Both the retention factor and the retention index can be used as a parameter for peak identification in MEKC. The retention factor provides fundamental information concerning the distribution coefficient of the analytes between the aqueous phase and the micellar phase and is linearly related to the phase ratio. However, the retention index shows a better repeatability and as it is a relative quantity the retention index is independent of the phase ratio, *i.e.* independent of the surfactant concentration.

Both the homologous series of alkylbenzenes and of alkylaryl ketones show a linear relationship between the logarithm of the retention factor and the carbon number of the homologues. Hence both series can be applied as retention index standards in MEKC. Moreover, this linear relationship can be used to calculate the micelle migration time by an iteration procedure. The alkylbenzenes are to be preferred with the anionic surfactant SDS with respect to the scale of the retention factors, whereas the alkylaryl ketones are to be preferred with the cationic surfactants CTAB and DTAB.

The correlation between retention indexes and *n*-octanol-water partition coefficients and the calculation of ΔI values from retention indexes obtained with different surfactant systems can provide information about the interaction phenomena and the separation mechanism in MEKC.

The temperature dependence of the retention index was found to be rather small. From the calculated thermodynamic quantities it can be concluded that the hydrophobic interaction contributes significantly to the micellar solubilization of sample compounds in MEKC.

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5

CHARACTERIZATION AND CLASSIFICATION OF MICELLAR PSEUDO-STATIONARY PHASES

ABSTRACT

The influence of different solute-micelle interactions on micellar solubilization is studied by Linear Solvation Energy Relationships (LSER) and retention indexes. The results of LSER modelling for six anionic micellar systems demonstrate that the migration behaviour of neutral species in MEKC is mainly determined by their molar volume and their hydrogen bond acceptor ability. Their polarity and hydrogen bond donor ability are shown to be of minor importance with these anionic surfactants. Large differences in selectivity were observed for SDS and mixed SDS/Brij 35 micellar systems, due to different hydrogen bonding characteristics. It is demonstrated that retention indexes can be applied for a quantitative characterization of pseudo-stationary phases in MEKC in a similar way as the Rohrschneider-McReynolds scale in GC. This method facilitates a classification of pseudo-stationary phases according to several solvatochromic quantities, with a limited number of experiments. Three anionic and two cationic micellar systems were classified according to their hydrogen bond donor and hydrogen bond acceptor strength, respectively, giving the same results as the LSER models. The relationship between retention indexes and *n*-octanol-water partition coefficients is treated theoretically and is applied for the prediction of retention indexes in a mixed SDS/Brij 35 micellar system.

5.1 INTRODUCTION

The separation mechanism of MEKC is based on differences in the distribution equilibria of sample compounds between an aqueous mobile phase and a pseudo-stationary phase [1,2]. During the last decade, MEKC has proved to be a powerful analytical tool in many fields of chemical analyses [3,4]. A main advantage of this separation technique is its flexibility. The composition of the electrolyte system and the pseudo-stationary phase can easily be changed in order to optimize selectivity. Different surfactant systems and mixed micelles have been applied to control the migration behaviour and selectivity [5-10]. Selectivity in MEKC is mainly determined by the hydrophylic moieties of the applied micellar system. However, the influence of the chemical nature of the pseudo-stationary phase and the structural properties of the solute molecules on solute-micelle interactions is still not well understood.

Recently, Khaledi *et al.* applied Linear Solvation Energy Relationship (LSER) modelling for the characterization of solute-micelle interactions [11,12]. They demonstrated that LSER studies provide quantitative information about different interaction phenomena and can be used to elucidate which mechanisms play a dominant role in MEKC selectivity. In chapter 4 it was demonstrated that the retention index is a good migration parameter to compare different surfactant systems and facilitates the classification of sample compounds in terms of functional group selectivity [13].

The aim of this chapter is to investigate the application of LSER methodology and the retention index concept for the characterization of solute-micelle interactions. Moreover, both methods are used for the classification of several pseudo-stationary phases in MEKC according to their hydrogen bonding characteristics. In addition to that, the relationship between retention indexes and *n*-octanol-water partition coefficients is discussed in view of the prediction and explanation of migration behaviour in MEKC experiments.

5.2 LINEAR SOLVATION ENERGY RELATIONSHIPS

In LSER modelling, solute properties that depend on solute-solvent interactions are correlated to several solvatochromic parameters of the solute according to [14]:

$$SP = SP_0 + mV/100 + s\pi + b\beta + a\alpha \qquad (5.1)$$

where SP is the solvent-related solute property, SP_0 is a regression constant which depends

amongst others on the phase ratio, V is the molar volume of the solute, π is a measure of the solute dipolarity/polarizability, β is the hydrogen bond acceptor (HBA) ability and α is the hydrogen bond donor (HBD) ability of the solute. The coefficients m, s, b and a are related to the chemical nature of the solvent system. The cavity term ($mV/100$) measures the endoergic process of separating the solvent molecules to provide a suitably sized cavity for the solute. The dipolarity/polarizability term ($s\pi$) measures the exoergic effect of solute-solvent dipole-dipole and dipole-induced dipole interactions. The hydrogen bonding terms ($b\beta$ and $a\alpha$) measure the exoergic effects of hydrogen bonding interactions involving the solute as HBA and the solvent as HBD and/or the solute as HBD and the solvent as HBA, respectively [14].

In MEKC the solute property can be the logarithm of the retention factor [11], log k, or the retention index, I. Accordingly eqn. (5.1) can be written as:

$$I = I_0 + mV/100 + s\pi + b\beta + a\alpha \qquad (5.2)$$

where I_0 is a regression constant and coefficients m, s, b and a represent the cohesiveness, dipolarity, HBD ability and HBA ability of the applied surfactant system, respectively. The sign of a coefficient denotes whether the term represents an endoergic (disfavourable) or an exoergic (favourable) process in the partitioning of the solutes between the aqueous phase and the micellar phase. These system coefficients can provide quantitative information about solute-micelle interaction phenomena and the influence of different surfactant systems on selectivity in MEKC analyses [11,12].

5.3 EXPERIMENTAL

CHEMICALS

Benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, sodium dodecylsulphate (SDS), cortisone, hydrocortisone and corticosterone were obtained from Aldrich (Steinheim, Germany). Tris(hydroxymethyl)aminomethane (TRIS), sodium dodecylsulphonate (SDSo) and polyoxyethylene(23)lauryl ether (Brij 35) were obtained from Merck (Darmstadt, Germany). The TRIS-salt of dodecylsulphate (TDS) was obtained from Sigma (St. Louis, MO, USA). All other chemicals were analytical-reagent grade.

INSTRUMENTATION AND SEPARATION CONDITIONS

All experiments were carried out on a BioFocus 3000 Capillary Electrophoresis System

(BioRad, Hercules, CA, USA) at a constant voltage of 20 kV. A 50 μm I.D. fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used, total length 50.0 cm, distance between injection and detection 45.4 cm. The temperature was kept constant at 25°C, except for the experiments with SDSo where the temperature was kept constant at 40°C. The wavelength of the detector was set at 200 or 240 nm. Samples were introduced by pressure injection with an injection constant of 1 psi.s. For the determination of retention indexes the sample injection was followed by a 1 psi.s pressure injection of a solution with the retention index standards prior to applying the voltage. The migration time of the micelles was calculated by an iteration procedure applying the migration data of the homologous series of alkylbenzenes [13,15].

SAMPLES AND SOLUTIONS

Stock solutions of the sample compounds and the retention index standards (alkylbenzenes) were prepared in methanol at a concentration of *ca.* 1 mg/ml and 2 μl/ml, respectively. These solutions were diluted 10 times with the appropriate electrolyte system. For the electrolyte systems containing SDS, SDSo and SDS/Brij 35 a buffer of 20 mM NaOH adjusted to pH 7.0 with *o*-phosphoric acid was used and for the electrolyte system containing TDS a buffer of 20 mM TRIS adjusted to pH 7.0 with *o*-phosphoric acid was used. All SDSo solutions were stored at 40°C. Water was purified by a Milli-Q water purification system (Waters Millipore, Milford, MA, USA). All buffer solutions were filtered through 0.45-μm filters prior to use.

5.4 RESULTS AND DISCUSSION

5.4.1 LINEAR SOLVATION ENERGY RELATIONSHIPS

In this work solute-micelle interactions in six different anionic surfactant systems were investigated, *viz.* the sodium- and the TRIS-salt of dodecylsulphate (SDS and TDS, respectively), sodium dodecylsulphonate (SDSo) and mixed micellar systems of SDS and the neutral surfactant Brij 35. As micelles are surrounded by a considerable amount of counter ions [16], special attention was paid to the composition of the electrolyte systems, *i.e.* the cation of the background electrolyte was identical to the micelle counter ion. LSER studies were carried out for a group of 18 aromatic compounds possessing different functionalities and hydrophobicity. In Table 5.1 all solvatochromic parameters and *n*-octanol-water partition coefficients, log P_{OW}, of these compounds are listed [14,17,18].

TABLE 5.1 *n*-Octanol-water partition coefficient[a], log P_{OW}, and solvatochromic parameters[c], $V/100$, π, β and α, of 18 sample compounds.

Nr.	compound	log P_{OW}	$V/100$	π	β	α
1	phenol	1.46	0.536	0.72	0.33	0.61
2	benzaldehyde	1.48	0.606	0.92	0.44	0.00
3	nitrobenzene	1.85	0.631	1.01	0.30	0.00
4	benzylcyanide	1.56	0.590	0.90	0.37	0.00
5	acetophenone	1.58	0.690	0.90	0.49	0.04
6	chlorobenzene	2.84	0.581	0.71	0.07	0.00
7	naphtalene	3.37	0.753	0.70	0.15	0.00
8	anisol	2.11	0.639	0.73	0.32	0.00
9	bromobenzene	2.99	0.624	0.79	0.06	0.00
10	*p*-cresol	1.94	0.634	0.68	0.34	0.58
11	1,2-xylol	3.12	0.668	0.51	0.12	0.00
12	benzylalcohol	1.08	0.634	0.99	0.52	0.39
13	*p*-nitrotoluene	2.45	0.729	0.97	0.31	0.00
14	benzene	2.13[b]	0.491	0.59	0.10	0.00
15	toluene	2.65[b]	0.592	0.55	0.11	0.00
16	ethylbenzene	3.13[b]	0.668	0.53	0.12	0.00
17	propylbenzene	3.69[b]	0.769	0.51	0.12	0.00
18	butylbenzene	4.28[b]	0.867	0.49	0.12	0.00

[a]log P_{OW} from ref. [17]; [b]from ref. [18].
[c]solvatochromic parameters from ref. [14].

In order to calculate the system coefficients *m*, *s*, *b* and *a* in eqn. (5.2), retention indexes were determined for all sample compounds, applying a homologous series of alkylbenzenes as retention index standards as described previously [13]. The system coefficients were calculated by multiple linear regression and are listed in Table 5.2. In addition, the coefficients for the LSER model of log P_{OW} were calculated and are included in Table 5.2 for comparative purposes. The high correlation coefficients ($r > 0.98$) suggest that migration behaviour in MEKC can be well described by LSER models. As can be seen from the results only minor differences are obtained for the regression constant I_0. This is due to the fact that the retention index is a relative quantity which is independent of the phase ratio. The large absolute values of the system coefficients *m* and *b* indicate that the migration behaviour of solutes in MEKC with these surfactant systems is mainly influenced by their size ($V/100$) and their HBA ability (β). Their dipolarity/polarizability (π) and HBD ability (α) are of minor importance. For all surfactant systems the cavity term is the most important

factor in the LSER models. The large positive values for *m* indicate that the cohesiveness term is of markedly importance in MEKC and consequently retention indexes will increase with increasing solute size. This is in accordance with the observation that for a homologous series of alkylbenzenes with almost identical values for coefficients π, β and α, higher retention indexes are obtained for larger, more hydrophobic members of the series. Moreover, the positive *m* values show that it is easier to create a suitably sized cavity in the micellar phase than in the aqueous phase because water is a more cohesive solvent than the organic interior of the micelles. This is in agreement with the observed positive entropy change for micellar solubilization of hydrophobic species [13,19].

Comparable system coefficients were obtained for the SDS and TDS micellar systems. Although the composition of the aqueous phase, surrounding the micelles (*i.e.* micelle counter ions and buffer ions) may influence the solubilization thermodynamics [19], almost no effect of the micelle counter ion on selectivity was observed. Only small differences in selectivity were observed between the SDS and SDSo micellar systems. However, large differences were observed for the system coefficients representing the hydrogen bonding characteristics (*b* and *a*) between the SDS and mixed SDS/Brij 35 micellar systems. Notice that the system coefficient *a*, representing the HBA ability of the pseudo-stationary micellar phase, changes from a negative to a positive value, *i.e.* from an endoergic to an exoergic process.

TABLE 5.2 Regression constant, I_0, and system coefficients, *m*, *s*, *b* and *a* with standard deviations (in parentheses), correlation coefficient, *r*, and standard deviation, S.D., of LSER models for six different surfactant systems and log P_{OW}. ($n=18$).

surfactant system	I_0	*m*	*s*	*b*	*a*	*r*	S.D.
50 mM SDS	220 (58)	994 (66)	-88 (50)	-367 (64)	-77 (33)	0.988	22
50 mM TDS	207 (57)	1008 (66)	-73 (50)	-388 (63)	-75 (33)	0.989	22
50 mM SDSo	223 (51)	1001 (59)	-97 (44)	-404 (57)	-40 (29)	0.991	20
50 mM SDS +2 mM Brij 35	214 (56)	1012 (65)	-63 (49)	-509 (63)	-36 (32)	0.990	22
50 mM SDS +5 mM Brij 35	206 (56)	1024 (65)	-38 (49)	-617 (62)	+7 (32)	0.991	22
50 mM SDS +10 mM Brij 35	211 (52)	1021 (60)	-38 (46)	-664 (58)	+32 (30)	0.992	20
log P_{OW}	0.24[a] (0.18)	5.49 (0.21)	-0.61 (0.16)	-3.77 (0.20)	-0.12 (0.10)	0.998	0.07

[a]log $P_{OW,o}$

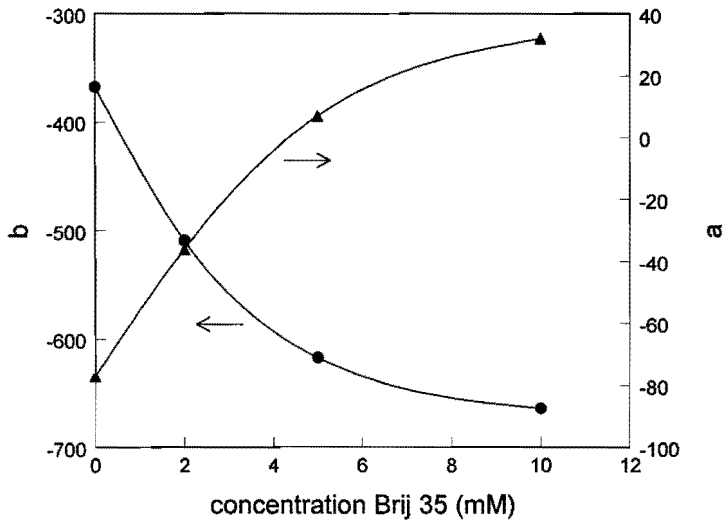

FIGURE 5.1 System coefficients *b* (•) and *a* (▲) as a function of the concentration Brij 35 (mM) added to an electrolyte system containing 50 mM SDS. For further explanation, see text.

In Fig. 5.1 coefficients *a* and *b* are shown as a function of the concentration Brij 35, illustrating that the *b* value decreases and the *a* value increases with increasing concentration Brij 35. This means that lower retention indexes are obtained for HBA solutes whereas higher retention indexes are obtained for HBD solutes in the mixed SDS/Brij 35 micellar systems compared to SDS. Probably the anionic surface of the SDS micelles is shielded by the polyoxyethylene chains of Brij 35 surfactant molecules when mixed micelles are formed [20]. This causes a decrease in interaction with HBA solutes. On the other hand, an increase in interaction with HBD solutes is observed. In the case of nonionic surfactants which possess polyoxyethylene groups, solutes may be incorporated into these groups as illustrated in Fig. 5.2. Due to this type of solubilization solute-micelle interactions with HBD solutes will increase because of hydrogen bond formation with the HBA ether oxygen of the oxyethylene groups [21].

From the foregoing results it can be concluded that LSER studies can provide useful information about migration behaviour and interaction phenomena in MEKC analysis. Therefore it is possible to use these models for the classification of pseudo-stationary phases in MEKC. In this respect it is interesting to mention the observed differences in selectivity between SDS and diaminobutane-based poly(propyleneimine) dendrimers, which were used recently as pseudo-stationary phases [22], as described in section 6.1. These dendrimers

which possess HBA amine groups showed to have strong interactions with HBD solutes ($\alpha > 0$) whereas for solutes without HBD abilities ($\alpha = 0$, *e.g.* alkylbenzenes) no interaction was observed.

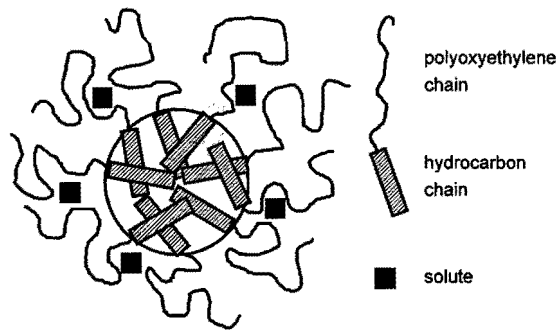

FIGURE 5.2 Schematic representation of solubilization in the polyoxyethylene chain of a Brij 35 micelle.

5.4.2 RETENTION INDEXES

Besides LSER modelling we also investigated the applicability of retention indexes to study solute-micelle interactions and for the classification of pseudo-stationary phases in MEKC. In chapter 4 the application of the retention index concept in MEKC for the identification of neutral species is described [13]. It was demonstrated that the retention index, I, is independent of the surfactant concentration and that ΔI values from retention indexes obtained with different micellar systems can provide information about interaction phenomena in MEKC analysis. ΔI values were calculated for retention indexes obtained with the anionic surfactant SDS and the cationic surfactants cetyltrimethylammonium bromide (CTAB) and dodecyltrimethylammonium bromide (DTAB), respectively. High positive ΔI values were obtained for the HBD compounds phenol and resorcinol whereas negative ΔI values were obtained for the HBA compounds benzaldehyde and acetophenone. These results suggest that the cationic micellar systems CTAB and DTAB provide a stronger HBA and a weaker HBD environment than the anionic micellar system SDS. This is consistent with LSER studies in Micellar Liquid Chromatography, reported by Yang and Khaledi [12].
For all sample compounds, listed in Table 5.1, and for 10 additional compounds (3 aromatic compounds, 4 xanthines and 3 corticosteroids) retention indexes were determined in the six anionic micellar systems and ΔI values were calculated according to:

$$\Delta I_{SF} = I_{SF} - I_{SDS} \tag{5.3}$$

where I_{SF} is the retention index obtained with a specific surfactant system and I_{SDS} is the retention index obtained with SDS. In this study SDS was chosen as reference system because this is the most widely used surfactant system in MEKC. Bearing in mind the results of the LSER calculations for SDS, TDS and SDSo (see Table 5.2), it is not surprising that for ΔI_{TDS} and ΔI_{SDSo} small values were obtained for all sample compounds. However, the $\Delta I_{SDS/Brij35}$ values for the mixed SDS/Brij 35 micellar system were more pronounced and are illustrated in Fig. 5.3. From these results it can be seen that four different subgroups can be distinguished; (A) HBD phenols and *p*-nitroaniline, (B) HBA aromatic compounds, (C) xanthines and (D) corticosteroids. Positive $\Delta I_{SDS/Brij35}$ values were obtained for HBD phenols and *p*-nitroaniline, due to hydrogen bond interaction with HBA polyoxyethylene chains as described before. For HBA aromatic compounds negative $\Delta I_{SDS/Brij35}$ values were obtained, due to shielding of the anionic surface of the SDS micelles by Brij 35 surfactant molecules. For all xanthines and corticosteroids large negative $\Delta I_{SDS/Brij35}$ values were obtained as these compounds possess strong HBA carbonyl and hydroxyl groups.

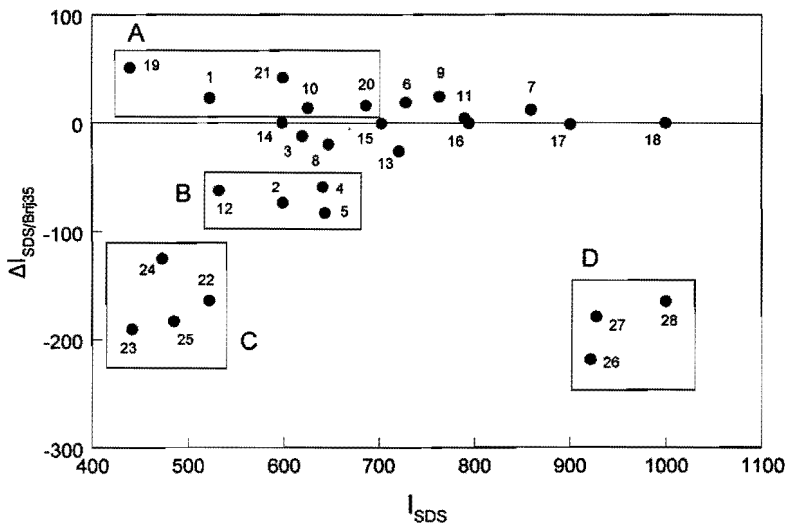

FIGURE 5.3 $\Delta I_{SDS/Brij35}$ (50 mM SDS + 10 mM Brij 35) *versus* I_{SDS} (50 mM SDS) for (1-18) all compounds listed in Table 5.1, (19) resorcinol, (20) 2,6-xylenol, (21) *p*-nitroaniline, (22) caffeine, (23) theobromine, (24) theophylline, (25) β-hydroxyethyl-theophylline, (26) cortisone, (27) hydrocortisone and (28) corticosterone. Four subgroups can be distinguished: (A) HBD phenols and *p*-nitroaniline, (B) HBA aromatic compounds, (C) xanthines and (D) corticosteroids.

From the foregoing it can be concluded that sample compounds can be divided in different subgroups according to their ΔI values. These ΔI values represent functional group selectivities and are mainly based on hydrogen bonding characteristics. These results demonstrate the possibility for a quantitative characterization and classification of pseudo-stationary phases in MEKC, analogous to the Rohrschneider-McReynolds scale in GC [23,24]. This classification would greatly facilitate the selection of an optimum composition of the micellar system for a given separation problem. Moreover, it would contribute to a better understanding of the separation process during MEKC analysis.
Based on the results of the LSER models, differences in retention indexes can be expressed in terms of two parameters, representing HBA and HBD strength of both solutes and micellar phases, according to:

$$\Delta I = \beta x + \alpha y \tag{5.4}$$

where β and α are physical quantities which represent the HBA and HBD strength of the solutes, and x and y are constants which represent the HBD and HBA strength of the micellar pseudo-stationary phase, respectively. Based on our LSER results, differences in cohesiveness and dipolarity between the pseudo-stationary phases were assumed to be of minor importance. Therefore these parameters were not taken into account in this simplified model. As standard compounds acetophenone (strong HBA) and phenol (strong HBD) were selected. Their solvatochromic parameters were used as the physical quantities β and α, respectively, in eqn. (5.4). These values can be found in Table 5.1. For five anionic systems (TDS, SDSo and SDS/Brij 35 at three different concentration ratios) and for two cationic systems (CTAB and DTAB) constants x and y were calculated and are listed in Table 5.3.

TABLE 5.3 System constants x and y for five anionic and two cationic micellar systems.

surfactant system	x	y
50 mM TDS	5	-3
50 mM SDSo	-39	36
50 mM SDS + 2 mM Brij 35	-87	48
50 mM SDS + 5 mM Brij 35	-154	105
50 mM SDS + 10 mM Brij 35	-180	135
50 mM CTAB	-185	300
50 mM DTAB	-185	293

From these results and from Fig. 5.1 it can be concluded that it is possible to create a gradually scale of pseudo-stationary phases in terms of HBD and HBA strength, applying surfactant systems with different SDS/Brij 35 concentration ratios. With the constants listed in Table 5.3 the applied micellar systems can be classified according to their HBD strength (x) as:

$$\text{TDS} \approx \text{SDS} > \text{SDSo} > \text{SDS/Brij 35} \approx \text{CTAB} \approx \text{DTAB}$$

and according to their HBA strength (y) as:

$$\text{CTAB} \approx \text{DTAB} > \text{SDS/Brij 35} > \text{SDSo} > \text{SDS} \approx \text{TDS}$$

In this classification the mixed micellar system of 50 mM SDS/10 mM Brij 35 was used. These results are identical to a classification based on the corresponding system coefficients b and a, calculated with the LSER models (Table 5.2 and LSERs from retention index data in Table 4.5). From the foregoing it can be concluded that besides LSER models also retention indexes can be used for the quantitative characterization and classification of pseudo-stationary phases in MEKC in a similar way as the Rohrschneider-McReynolds scale in GC. This method using retention indexes has several advantages. First, a relative small amount of experiments is required for the classification of pseudo-stationary phases in terms of solvatochromic quantities. Second, only for a limited number of reference compounds the solvatochromic parameters are needed. Third, this method also enables a classification of solutes in different micellar systems, according to functional group selectivities. Fourth, ΔI values are calculated directly from MEKC migration data. Therefore this method provides a good insight into the influence of a specific micellar phase on MEKC migration behaviour and the expected electrokinetic chromatogram. Of course this model can be made more accurate by taking into account more parameters in eqn.(5.4).

5.4.3 SELECTIVITY

To illustrate differences in selectivity, in Fig. 5.4 two electrokinetic chromatograms are shown obtained with an electrolyte system containing 50 mM SDS and a mixed micellar system containing 50 mM SDS and 10 mM Brij 35, respectively. In Table 5.4 all pseudo-effective mobilities and theoretical plate numbers for these experiments are listed. As a practical application three corticosteroids were separated applying a mixed SDS/Brij 35 micellar system. In Fig. 5.5 the electrokinetic chromatogram is shown. These compounds

can not be separated in an SDS system as they all migrate on or near the migration time of the micelles. By the addition of 10 mM Brij 35 to the electrolyte system a considerable decrease in retention indexes and pseudo-effective mobilities is obtained and a good separation is possible. In Table 5.5 all pseudo-effective mobilities in SDS and SDS/Brij 35 together with log P_{OW}-data are listed.

TABLE 5.4 Pseudo-effective mobility, m_{eff}^{ps}(10^{-5} cm^2/Vs), with standard deviations (in parentheses) and theoretical plate number, N, for the electrokinetic chromatograms shown in FIGURE 5.4. (n=5).

compound Nr.	50 mM SDS		50 mM SDS + 10 mM Brij 35	
	m_{eff}^{ps}	$N \cdot 10^{-5}$	m_{eff}^{ps}	$N \cdot 10^{-5}$
19	-7.07 (0.07)	1.47	-9.85 (0.07)	1.73
25	-9.81 (0.12)	1.65	-2.38 (0.02)	1.75
12	-14.32 (0.23)	1.53	-9.05 (0.07)	2.27
21	-21.68 (0.19)	1.77	-20.16 (0.05)	2.20
3	-23.23 (0.28)	1.91	-18.08 (0.07)	2.56
5	-25.41 (0.30)	1.77	-14.84 (0.08)	2.18
20	-29.51 (0.34)	1.91	-23.16 (0.07)	2.62

TABLE 5.5 n-Octanol-water partition coefficient, log P_{OW}, and pseudo-effective mobilities, m_{eff}^{ps}(10^{-5} cm^2/Vs), with standard deviations (in parentheses) for three corticosteroids in an SDS and a mixed SDS/Brij 35 micellar system. (n=5).

compound	log P_{OW}	50 mM SDS	50 mM SDS + 10 mM Brij 35
cortisone	1.42	-40.36 (0.02)	-23.38 (0.08)
hydrocortisone	1.55	-40.54 (0.02)	-24.98 (0.06)
corticosterone	1.94	-41.50 (0.01)	-26.78 (0.07)

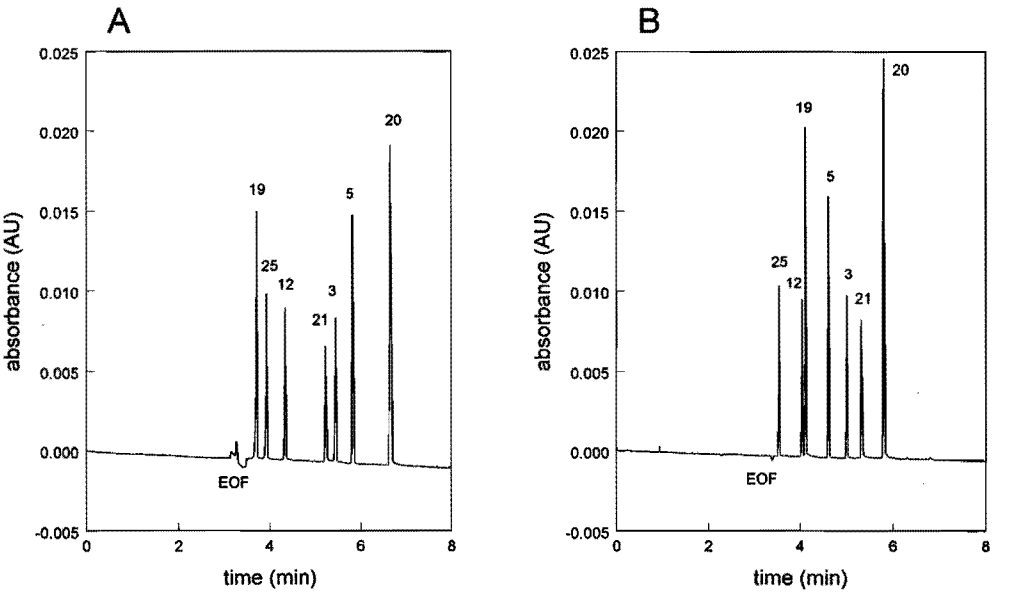

FIGURE 5.4 Electrokinetic chromatograms for the separation of (19) resorcinol, (25) β - hydroxyethyltheophylline, (12) benzylalcohol, (21) *p*-nitroaniline, (3) nitrobenzene, (5) acetophenone and (20) 2,6-xylenol in a micellar system of (A) 50 mM SDS and (B) 50 mM SDS + 10 mM Brij 35. λ=200 nm.

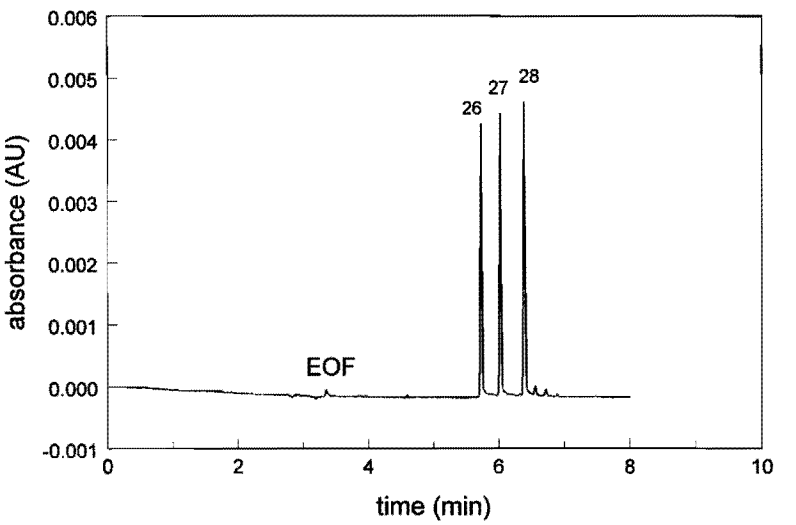

FIGURE 5.5 Electrokinetic chromatogram for the separation of (26) cortisone, (27) hydrocortisone and (28) corticosterone in a mixed micellar system of 50 mM SDS + 10 mM Brij 35. λ=240 nm.

5.4.4 EFFICIENCY AND ELUTION WINDOW

Besides the selectivity, also the efficiency and the elution window are affected by the mixed micellar system. As can be seen from the results, listed in Table 5.4, higher theoretical plate numbers are obtained for all sample compounds in the mixed SDS/Brij 35 micellar system. There are two possible explanations for this effect. First, sorption-desorption kinetics may be influenced when mixed micelles are formed, due to a different type of interaction or a decrease in the amount of micelle counter ions. Second, the viscosity of the electrolyte system will increase with the addition of Brij 35 and hence diffusion coefficients will decrease. In Table 5.6 electroosmotic mobility, m_{EOF}, and effective mobility of the micelles, m_{MC}, are listed as a function of the concentration Brij 35. At a higher concentration of the neutral surfactant the effective charge and therefore m_{MC} of the mixed micelles will decrease. Due to a higher viscosity both m_{EOF} and m_{MC} will decrease. As a result a decrease in elution window is observed [25] at higher Brij 35 concentrations.

TABLE 5.6 Electroosmotic mobility, m_{EOF} (10^{-5} cm^2/Vs), and effective mobility of the micelles, m_{MC} (10^{-5} cm^2/Vs), and resulting elution window as a function of the concentration Brij 35 (mM). All electrolyte systems contain 50 mM SDS.

concentration Brij 35	m_{EOF}	m_{MC}	$m_{EOF}/(m_{EOF}+m_{MC})$
0	62.64	-45.06	3.56
2	58.21	-39.93	3.19
5	52.26	-36.92	3.41
10	59.86	-29.06	1.94
25	57.50	-15.27	1.36

5.4.5 *n*-OCTANOL-WATER PARTITION COEFFICIENTS

n-Octanol-water partition coefficients, log P_{OW}, have been widely used as parameter for lipophilicity of substances and are applied in various disciplines such as drug design, toxicology and environmental monitoring of pollutants. In quantitative structure-activity relationships (QSAR), log P_{OW}-values are used to predict lipoidal transport and bioactivity [26]. The conventional shake-flask technique for the direct measurement of log P_{OW} is time consuming, requires highly pure compounds in reasonable quantities and has a limited dynamic range. Therefore alternative techniques have been developed for the indirect determination of log P_{OW}, such as reversed phase LC [27].

Recently, several authors paid attention to log P_{OW}-screening and QSAR studies using MEKC [28-32]. This microscale separation technique offers some unique advantages such

as speed, small sample size, suitability for mixtures and substances containing impurities and feasibility for automation. Moreover, micellar media provide a microenvironment which is much more similar to biological membranes than *n*-octanol or reversed phase stationary phases. For these reasons MEKC may be an attractive technique for the determination of biological activities by QSAR studies. In reverse, the existing large data bases of log P_{OW} can be applied to predict migration behaviour in MEKC. From the LSER results, listed in Table 5.2, it can be concluded that the *n*-octanol-water partition and micellar solubilization are distribution processes with comparable mechanisms. In both cases the cohesiveness and the HBD ability of the solvent (*m* and *b*) are the main system coefficients. Therefore a linear relationship can be expected between *I* and log P_{OW}, according to [32]:

$$I = p \log P_{OW} + q \tag{5.5}$$

where *p* and *q* are constants. In chapter 4 it was demonstrated that not all compounds follow this relationship with an SDS micellar system (see Fig. 4.5). Higher *I*-values were obtained for HBA compounds ($\beta > 0$). Recently Yang and Khaledi [33] pointed out that these differences are due to differences in the HBD strengths of SDS micelles and *n*-octanol. They recognized three different subgroups, based on the HBA abilities of the compounds; subgroup (A) with weak HBA strength ($\beta \leq 0.2$), subgroup (B) with intermediate HBA strength ($0.2 < \beta < 0.35$) and subgroup (C) with strong HBA strength ($\beta \geq 0.35$). This is illustrated in Fig. 5.6. In order to complete the data set with strong HBA solutes with a high log P_{OW}-value, additional experiments were carried out with propiophenone and butyrophenone. Since SDS micelles provide a stronger HBD environment than *n*-octanol, higher retention indexes are obtained for species with stronger HBA abilities. This also explains the high pseudo-effective mobilities for strong HBA corticosteroids in an SDS micellar system. These values are much higher than expected based on their moderate log P_{OW}-values (see Table 5.5). In Table 5.7 all constants *p* and *q* for eqn. 5 are listed for four anionic surfactant systems. For strong HBA compounds lower retention indexes are obtained in a mixed 50 mM SDS/10 mM Brij 35 micellar system. As a consequence the system coefficients of this micellar system correspond better to those of log P_{OW} than the system coefficients of the 50 mM SDS system. This is demonstrated by the quotient *-b/m* which is 0.37, 0.65 and 0.69 for 50 mM SDS, 50 mM SDS/10 mM Brij 35 and log P_{OW}, respectively. Hence a better correlation is obtained for the 50 mM SDS/10 mM Brij 35 system. In Fig. 5.7 the prediction of *I* from log P_{OW}-values is illustrated for this mixed micellar system. Of course less accurate predictions were obtained for the SDS system.

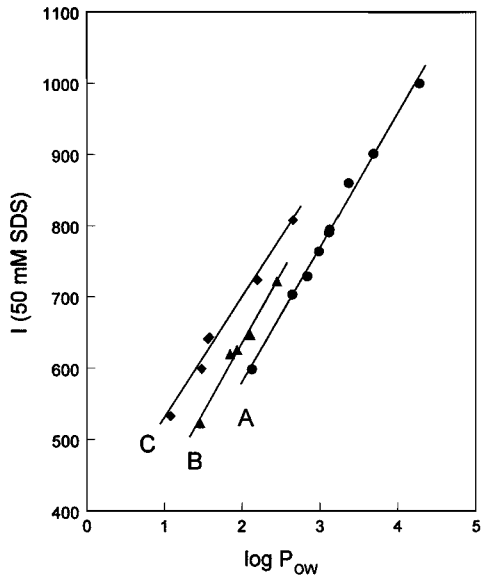

FIGURE 5.6 *I versus* log P_{ow} for the 18 sample compounds, listed in TABLE 5.1, propiophenone and butyrophenone. Three subgroups can be distinguished: (A) $\beta \leq 0.2$, (B) $0.2 < \beta < 0.35$ and (C) $\beta \geq 0.35$. For further explanation, see text.

TABLE 5.7 Regression constants p and q with standard deviations (in parentheses), correlation coefficient, r, and standard deviation, S.D., for the graphs of *I versus* log P_{ow} for four different surfactant systems.

surfactant system	p	q	r	S.D.
50 mM SDS	140 (10)	366 (25)	0.965	35
50 mM TDS	141 (10)	364 (25)	0.965	35
50 mM SDSo	143 (8)	354 (21)	0.975	29
50 mM SDS + 10 mM Brij 35	162 (5)	300 (14)	0.991	20

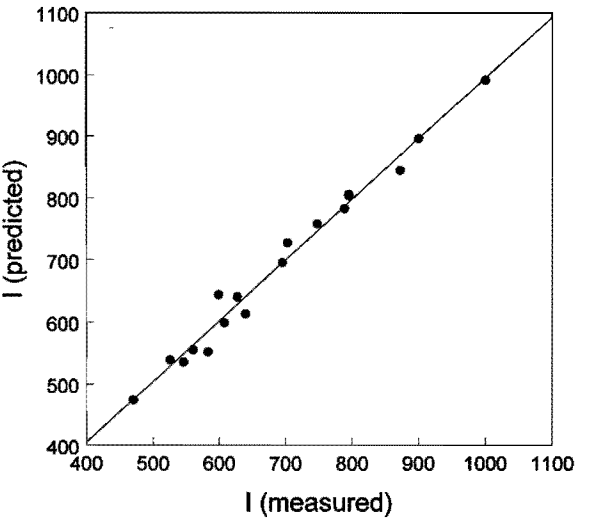

FIGURE 5.7 Predicted I values from log P_{OW}-data *versus* measured I values for the 18 sample compounds, listed in TABLE 5.1, applying a mixed micellar system of 50 mM SDS + 10 mM Brij 35.

5.5 CONCLUSIONS

The migration behaviour of neutral species in MEKC can be well described by LSER modelling. The LSER system coefficients provide quantitative information about which type of solute-micelle interactions play a dominant role in MEKC analyses. With the six anionic micellar systems applied in this study, the retention of the sample compounds is mainly determined by their molar volume and their HBA ability. Their dipolarity/polarizability and HBD ability were shown to be of minor importance.

The results demonstrate that ΔI values, calculated from retention indexes obtained with different micellar systems, can be used to elucidate the functional group selectivity of these specific micellar systems. Moreover, ΔI values can be applied for the classification of pseudo-stationary phases in MEKC, analogous to the Rohrschneider-McReynolds scale in GC. Using this method, four anionic and two cationic micellar systems were classified according to their HBD and HBA strength, respectively. The obtained results were similar to a classification based on LSER models.

Small differences in selectivity were observed between SDS, TDS and SDSo micellar systems. However, large selectivity changes were obtained between SDS and mixed

SDS/Brij 35 micellar systems, mainly due to differences in hydrogen bonding characteristics. Retention indexes of HBA compounds were found to decrease whereas retention indexes of HBD compounds were found to increase with increasing concentration of the neutral surfactant Brij 35.

The LSER models demonstrate that besides micellar solubilization also *n*-octanol-water partitioning is mainly determined by the cohesiveness and the HBD ability of the solvent. As SDS micelles possess a higher HBD strength than *n*-octanol, different subgroups can be distinguished in the correlation between I and log P_{OW}, based on the HBA ability of the compounds. However, a good linear relationship for I *versus* log P_{OW} was obtained in a mixed SDS/Brij 35 micellar system which could be applied for the prediction of retention indexes from log P_{OW} literature data.

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6

DENDRIMERS AND TETRAALKYLAMMONIUM IONS AS PSEUDO-STATIONARY PHASES

ABSTRACT

Dendrimers and tetraalkylammonium ions were evaluated as potential pseudo-stationary phases in electrokinetic chromatography (EKC). Effective mobilities were determined for five generations diaminobutane-based dendrimers (DAB-*dendr*-$(COOH)_x$) with capillary zone electrophoresis and indirect UV detection. DAB-*dendr*-$(COOH)_{64}$ was found to have a comparable effective mobility with SDS micelles at pHs in the range 7-9, thus providing a sufficient large elution window. Large differences in selectivity were obtained for hydrogen bond donating compounds between DAB-*dendr*-$(COOH)_{64}$ and SDS micelles, due to hydrogen bond accepting tertiary amines present in the dendrimer interior.
Tetraalkylammonium ions were applied for the separation of highly hydrophobic compounds in aqueous/organic media. The direction of the electroosmotic flow and, as a consequence, the migration behaviour of the hydrophobic compounds is shown to be strongly dependent on tetraalkylammonium concentrations and the organic modifier content of the electrolyte system. The potential of tetraalkylammonium pseudo-stationary phases in EKC is illustrated by the separation of several geometric isomers of polycyclic aromatic hydrocarbons.

6.1 DENDRIMERS

6.1.1 INTRODUCTION

Micellar Electrokinetic Chromatography (MEKC) [1,2] is the most commonly used mode of Electrokinetic Chromatography (EKC). In this mode a micellar pseudo-stationary phase is applied, generally created by the addition of a surfactant, *e.g.* sodium dodecylsulphate (SDS), to the electrolyte system at a concentration above the critical micelle concentration. Besides micelles, also micro emulsions [3] and oligomer [4] have been applied as pseudo-stationary phase in EKC. Recently, Tanaka *et. al.* reported about the application of dendrimers as pseudo-stationary phase in EKC with both aqueous and aqueous/organic electrolyte systems [5]. Dendrimers are three dimensional, highly structured macromolecules, formed by a controlled cascade of organic-synthetic reactions. Unlike micelles, dendrimers are macromolecules that are stable under a wide variety of experimental conditions. Moreover, the size and the chemical structure of the dendrimers can be well controlled during the synthesis. This enables the introduction of different functionalities, which may provide specific selectivities in EKC.

DENDRIMERS

The interest in dendritic macromolecules originates from the unique properties of these highly branched structures that have a defined number of generations and functional end groups [6]. The high degree of control over molecular weight and shape has led to the construction of spherical and cone-shape mesostructures, as well as stratified dendrimers possessing generations of different structure. Diameters of the spherical dendrimers generally range from 5-10 nanometer, making these molecules capable to be building blocks of a new field of chemistry. In some cases, dendrimers can be considered as a kind of unimolecular micelles [7]. However, a fundamental difference is the fact that the structure of dendritic macromolecules is static, with all end groups covalently bonded to a central core, whereas the structure of micelles is dynamic. Dendrimers may show some interesting features as pseudo-stationary phase in EKC. Moreover, EKC may be a convenient method to study the interaction of dendrimers with small organic molecules.

The dendrimers employed in this study are synthesized by the divergent approach [8]. A repetitive reaction sequence using the double Michael addition of a primary amine to acrylonitrile followed by the heterogeneously catalyzed hydrogenation of the nitriles into primary amines, yields diaminobutane-based poly(propyleneimine) dendrimers (DAB-*dendr*-$(CN)_x$) with 4, 8, 16, 32 and 64 nitrile end groups, respectively. These dendrimers which can be prepared on a large scale, are very flexible and possess glass transition temperatures

of approximately -40 °C. These nitrile terminated dendrimers are subsequently transformed into carboxylic acid terminated dendrimers (DAB-*dendr*-$(COOH)_X$) by treatment with aqueous HCl. In this section the results of an investigation on the electrophoretic properties of these dendritic macromolecules and their potential as pseudo-stationary phase in EKC are described. In Fig. 6.1 the structural formula of DAB-*dendr*-$(COOH)_{64}$ is illustrated.

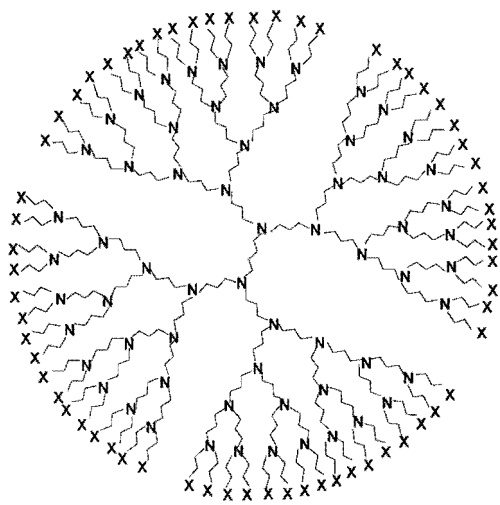

FIGURE 6.1 Structural formula of DAB-*dendr*-$(COOH)_{64}$, X=COOH.

6.1.2 EXPERIMENTAL

All experiments were carried out on a BioFocus 3000 capillary electrophoresis system (BioRad, Hercules, CA, USA) at a constant temperature of 25 °C. The wavelength of the detector was set at 200 nm. Pressure injection was carried out with an injection constant of 2 psi.s. For all experiments a 50 μm I.D. fused silica capillary was used (Polymicro Technologies, Phoenix, AZ, USA), total length 30.0 cm, length to detection 25.4 cm. The anode was placed at the inlet side and the cathode at the outlet side of the capillary, respectively. All chemicals were of analytical-reagent grade and all buffer solutions were filtered through a 0.45 μm filter prior to use.

6.1.3 DETERMINATION OF DENDRIMERS BY CZE WITH INDIRECT UV

An utmost requirement for dendrimers to be applicable as pseudo-stationary phase in EKC, is that they should have a high effective mobility, in order to obtain a sufficiently wide

elution window. For this reason, the effective mobility of the carboxylic acid terminated dendrimers was determined as a function of the pH of the background electrolyte by capillary zone electrophoresis (CZE) experiments. As these dendrimers show only little UV absorbance, these experiments were carried out in the indirect UV mode. In Table 6.1 the compositions of the background electrolytes at different pH values are listed. Notice that all background electrolytes contain the UV-absorbing negative ionic species benzoic acid. In Fig. 6.2 the electropherograms are shown for dendrimers DAB-*dendr*-$(COOH)_X$ having 4, 8, 16, 32 and 64 carboxylic acid end groups, respectively, in a background electrolyte at pH 8.2. Some minor impurities can be observed in the electropherograms of higher dendrimer generations. The broader sample zones, obtained for these generations, are probably caused by a high concentration of ammoniumchloride, present in these dendrimer samples due to the synthesis procedure. The non-UV-absorbing positive ammonium ions can be observed as a positive UV peak before the electroosmotic flow (EOF) in the electropherograms. According to the Kohlrausch regulation function, the total ionic concentration of a compound in a sample zone is higher than that of the co-ion of the background electrolyte, if the effective mobility of this compound is higher than that of the co-ion of the background electrolyte.

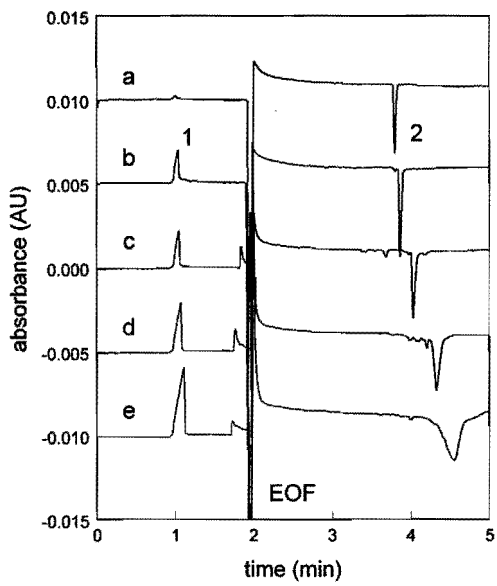

FIGURE 6.2 Electropherograms of (1) NH_4^+ and (2) DAB-*dendr*-$(COOH)_x$ having (a) 4, (b) 8, (c) 16, (d) 32 and (e) 64 end groups. The sample concentration of all dendrimers was 0.0002 M. Background electrolyte, 0.01 M benzoic acid/TRIS at pH 8.2.

TABLE 6.1 Compositions of background electrolytes at different pHs. All buffer solutions were prepared by adding the cation to 0.01 M benzoic acid until the selected pH was achieved.

cation	pH
DEA	9.0
TRIS	8.2
imidazole	7.0
histidine	6.0
creatinine	5.0
TRIS	4.0

According to the electroneutrality condition, the concentration of the UV-absorbing counter ion of the background electrolyte will also be higher, resulting in a positive UV peak for the non-UV-absorbing sample compound. In Table 6.2 all calculated effective mobilities of the dendrimers at different pHs are listed. From these results it can be concluded that DAB-*dendr*-$(COOH)_{64}$ migrates between pH 7.0 and 9.0 with an effective mobility, comparable to that of SDS micelles under identical experimental conditions (*ca.* $-38 \cdot 10^{-5}$ cm^2/Vs). As expected from the reduction of net charges, the effective mobility decreases with decreasing pH for all dendrimers. Besides net charges, also hydrodynamic radii of carboxylic acid terminated dendrimers will be influenced by the pH of the electrolyte system [9,10].

TABLE 6.2 Effective mobilities, m_{eff} (10^{-5} cm^2/Vs), for five generations of DAB-*dendr*-$(COOH)_x$ with different numbers of end groups, x, at different pHs.

x	pH					
	9.0	8.2	7.0	6.0	5.0	4.0
4	-35.14	-31.84	-31.65	-30.31	-28.85	-15.41
8	-37.74	-33.09	-27.50	-21.64	-18.64	-
16	-38.01	-33.86	-31.20	-23.10	-	-
32	-39.81	-36.20	-29.83	-	-	-
64	-39.65	-36.45	-35.11	-	-	-

6.1.4 DENDRIMERS AS PSEUDO-STATIONARY PHASES

In order to examine the applicability of these dendrimers as pseudo-stationary phase in EKC, experiments were carried out with a sample mixture containing hydroquinone, resorcinol, phenol, benzyl alcohol, *o*-cresol and 2,6-xylenol in a background electrolyte containing 0.002 M DAB-*dendr*-$(COOH)_{64}$ adjusted to pH 8.2 by adding TRIS. The same sample compounds were analyzed in an electrolyte system of 0.01 M TRIS adjusted to pH 8.2 by adding acetic acid, containing 0.05 M SDS. In Fig. 6.3 the electrokinetic chromatograms with these two pseudo-stationary phases are shown. For Fig. 6.3A benzyl alcohol and for Fig. 6.3B *o*-cresol were omitted in the sample mixture as these compounds comigrated with phenol.

Migration data of neutral species in EKC are generally expressed by means of the retention factor, k. However, for calculation of the retention factor, the migration time of the pseudo-stationary phase must be known, which may be troublesome to determine in case of dendrimers. Therefore pseudo-effective mobilities, m_{eff}^{ps}, were applied as parameter for peak identification, as described in section 1.5.2.

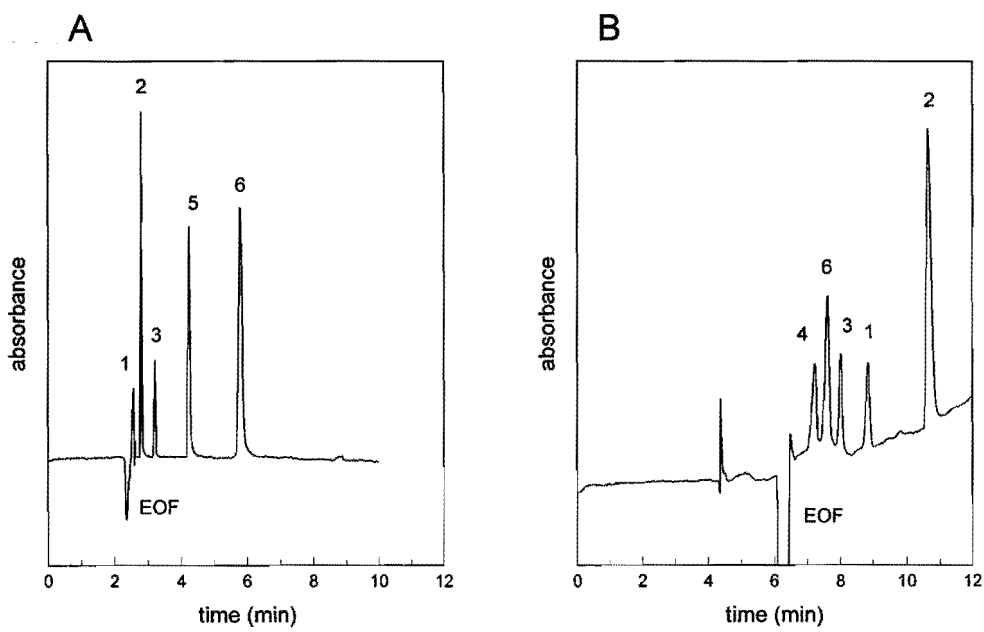

FIGURE 6.3 Electrokinetic chromatograms of the separation of (1) hydroquinone, (2) resorcinol, (3) phenol, (4) benzyl alcohol, (5) *o*-cresol and (6) 2,6-xylenol in an electrolyte system containing (A) 0.05 M SDS and (B) 0.002 M DAB-*dendr*-$(COOH)_{64}$.

TABLE 6.3 Pseudo-effective mobilities, m_{eff}^{ps} (10^{-5} cm^2/Vs), with standard deviations (in parentheses), of aromatic sample compounds for the two different pseudo-stationary phases.

compound	SDS	DAB-*dendr*-$(COOH)_{64}$
hydroquinone	-4.17 (0.07)	-8.17 (0.10)
resorcinol	-8.45 (0.06)	-9.62 (0.13)
phenol	-14.25 (0.18)	-5.69 (0.06)
benzyl alcohol	-14.95 (0.25)	-3.94 (0.04)
o-cresol	-23.98 (0.29)	-5.39 (0.11)
2,6-xylenol	-31.32 (0.41)	-5.13 (0.05)

In Table 6.3 all calculated pseudo-effective mobilities for the sample compounds in the two different electrolyte systems are listed. From these results it can be concluded that SDS-micelles and DAB-*dendr*-$(COOH)_{64}$ exhibit different interactions with the sample compounds, resulting in a different migration order. In chapter 5 it was argued that micellar solubilization of neutral compounds is mainly determined by their hydrophobic and hydrogen bonding characteristics. The interior of DAB-*dendr*-$(COOH)_{64}$ provides a different chemical environment than the interior of SDS-micelles, due to tertiary amines present. These tertiary amines possess strong hydrogen bond acceptor abilities and, consequently, these dendrimers exhibit a stronger interaction with, *e.g.*, hydroquinone and resorcinol, possessing hydrogen bond donor abilities. Moreover, local pH differences may influence distribution equilibria between acidic compounds and DAB-*dendr*-$(COOH)_{64}$. Spectrophotometric experiments of dendritic boxes with indicator dyes encapsulated [11], demonstrated that the pH inside diaminobutane-based dendrimers can be significantly higher than the pH of the surrounding aqueous phase.

6.1.5 CONCLUSIONS

Dendrimers were shown to be a potential pseudo-stationary phase in electrokinetic chromatography. Different selectivities were observed for aromatic sample compounds with SDS-micelles and DAB-*dendr*-$(COOH)_{64}$ as pseudo-stationary phase, respectively. These differences are due to the characteristic chemical structures of both the surface and the interior of micelles and dendrimers. These results indicate that electrokinetic chromatography may be an attractive technique to study interaction phenomena between dendrimers and small organic molecules.

6.2 TETRAALKYLAMMONIUM IONS

6.2.1 INTRODUCTION

In chapter 2 it was pointed out that for the determination of highly hydrophobic compounds with MEKC, the presence of a limited elution range forms a major limitation [12]. Due to high partition coefficients between the aqueous mobile phase and the micellar pseudo-stationary phase, these compounds possess retention factors, far too high to obtain optimum resolution [13]. They migrate close to or at the migration time of the micelles.

Different strategies have been described to overcome these problems. Terabe *et al.* [14] described the use of cyclodextrins in MEKC for the separation of polycyclic aromatic hydrocarbons (PAHs). Also, several organic modifiers such as methanol, acetonitrile and 2-propanol have been applied, in order to reduce the affinity of the sample compounds for the micellar phase [15,16]. However, the amount of organic solvents that can be used is limited as at modifier concentrations above *ca.* 20-30% (v/v) sodium dodecylsulphate (SDS) micelles are generally not stable. Separations at higher organic modifier concentrations have been reported [17,18], but the mechanism for these analyses is rather based on interactions with surfactant monomers than on micellar solubilization. Recently, several authors reported the application of highly branched macromolecular structures, such as unimolecular polymerized micelles [4,19], ionic polymers [20,21] and dendrimers [5,22,23]. These macromolecules form stable pseudo-stationary phases, allowing relatively high amounts of organic modifiers to be used. Walbroehl and Jorgenson [24] discussed the use of tetrabutylammonium ions in mixed water/acetonitrile solutions and demonstrated good separations of PAHs, based on solvophobic interaction phenomena.

In this section the application of two long-chain tetraalkylammonium surfactants, *viz.* tetraoctylammonium bromide and tetradecylammonium bromide, for the separation of highly hydrophobic compounds by electrokinetic chromatography in aqueous/organic media is described.

6.2.2 EXPERIMENTAL

CHEMICALS

Tetraoctylammonium bromide (TOAB), pentylbenzene and acetonitrile were obtained from Merck (Darmstadt, Germany), tetradecylammonium bromide (TDAB) from Sigma (St. Louis, MO, USA), propylbenzene and butylbenzene from Aldrich (Milwaukee, WI, USA) and hexylbenzene, octylbenzene, nonylbenzene, dodecylbenzene and all polycyclic aromatic hydrocarbons from Fluka (Frankfurt, Germany). Water was filtered by a Milli-Q purification system (Waters Millipore, Milford, MA, USA).

INSTRUMENTATION AND SEPARATION CONDITIONS

All experiments were carried out on a BioFocus 3000 Capillary Electrophoresis System (BioRad, Hercules, CA, USA) at a constant voltage of 20 kV. In the anionic mode the cathode was placed at the inlet side and the anode at the outlet side of the capillary, respectively, and *vice versa* in the cationic mode. A 75 μm I.D. fused silica capillary (Chrompack, Middelburg, The Netherlands) was used, total length 70.0 cm, distance between injection and detection 65.4 cm. From both ends of the capillary the polyimide coating was removed in order to prevent dissolving in the aqueous/organic electrolyte systems. The temperature was kept constant at 25 °C and the wavelength of the detector was set at 220 nm. Samples were introduced by pressure injection with an injection constant of 2 psi.s. Between each run the capillary was flushed subsequently for 2 min. with acetonitrile, 2 min. with 0.1 N NaOH, 2 min. with deionized water and 2 min. with the electrolyte solution. All samples were dissolved in the electrolyte system at a final concentration of at least 25 times smaller than the concentration of tetraalkylammonium ions.

6.2.3 ELECTROOSMOTIC FLOW AND MIGRATION MODES

Walbroehl and Jorgenson [24] applied electrolyte systems of tetrahexylamonium ions (THA^+) in mixed water/acetonitrile media for the separation of several PAHs. Due to solvophobic interactions of these hydrophobic compounds with the THA^+ ions, positively charged species are formed which possess an effective mobility and migrate in an electric field. This separation principle has much in common with MEKC and therefore this technique can be regarded as a form of electrokinetic chromatography (EKC) in aqueous/organic media with tetraalkylammonium ions as pseudo-stationary phase. Baseline separation was obtained for five PAHs with a different number of aromatic rings in a water/acetonitrile (50/50, v/v) electrolyte system containing 25 mM THA^+. However, lower resolutions were obtained for these PAHs with mixed water/acetonitrile electrolyte systems containing tetrabutylammonium ions [25], illustrating that the alkyl chain length plays an important role in the solvophobic interaction mechanism. Therefore we investigated the applicability of tetraoctylammonium (TOA^+) and tetradecylammonium (TDA^+) ions as pseudo-stationary phases for the separation of highly hydrophobic compounds with EKC in aqueous/organic media. In Fig. 6.4A an electrokinetic chromatogram is shown for the separation of a hydrophobic sample mixture, including several PAHs, in the cationic mode, applying a water/acetonitrile (50/50, v/v) electrolyte system containing 10 mM TOAB. In this experiment all compounds migrate in the downstream mode [26], *i.e.* they are detected before the electroosmotic flow (EOF).

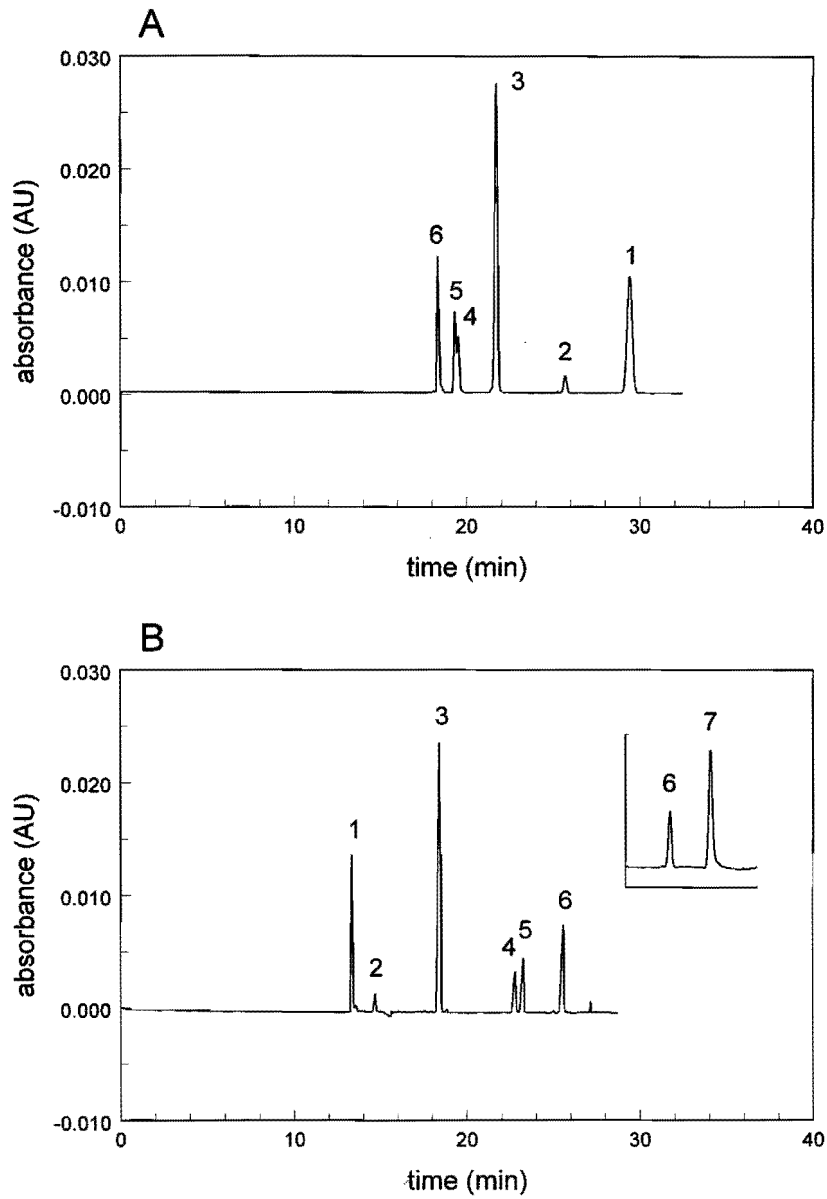

FIGURE 6.4 Electrokinetic chromatograms of the separation of (1) formamide (neutral EOF marker), (2) propylbenzene, (3) naphtalene, (4) anthracene, (5) phenantrene, (6) pyrene and (7) chrysene in electrolyte systems of (A) water/acetonitrile (50/50, v/v) and (B) water/acetonitrile (60/40, v/v), containing 10 mM TOAB.

The migration mechanism for this kind of analyses was proposed by Walbroehl and Jorgenson [24], involving an association of a positively charged surfactant monomer and a noncharged sample compound. This association will be stronger for larger, more hydrophobic compounds, resulting in a higher migration velocity (see Fig. 6.4A). They suggested that the association of a sample compound with more than one THA^+ ion is unlikely, due to electrostatic repulsion. However, TOAB and TDAB are known to show micellar aggregation in organic media [27]. Therefore a micellar pseudo-stationary phase can be formed by these surfactant systems in mixed water/acetonitrile electrolyte systems. Moreover, the formation of a dynamic coating of positively charged hemimicelles on the capillary wall may cause a change in the sign of the ζ-potential, resulting in a reversal of the direction of the EOF [28], as described in chapter 1. This is illustrated in Fig. 6.4B where the same sample mixture is separated in the anionic mode, applying a water/acetonitrile (60/40, v/v) electrolyte system containing 10 mM TOAB. Notice that the migration order is reversed compared to Fig. 6.4A. In this situation all compounds migrate in the upstream mode, *i.e.* they are detected after the EOF. Fig. 6.4B also illustrates that separation is obtained for several geometric PAH isomers, *e.g.* anthracene and phenantrene or pyrene and chrysene (see inset), using TOAB as pseudo-stationary phase. These results demonstrate that a difference of 10 % (v/v) acetonitrile in the electrolyte system strongly influences the condition on the capillary wall and the migration behaviour.

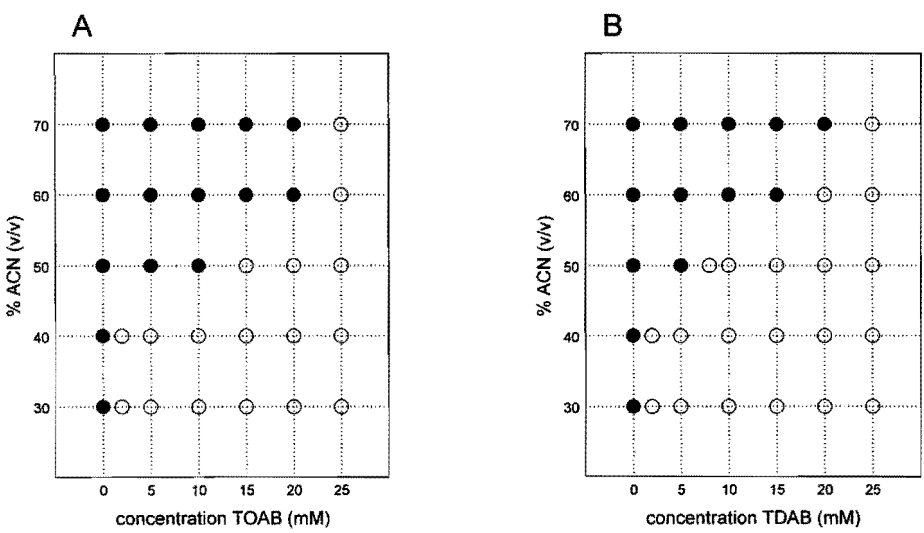

FIGURE 6.5 Influence of surfactant concentration an amount of acetonitrile on EOF direction and migration mode for (A) TOAB and (B) TDAB. (•) EOF directed to cathode, downstream mode, (○) EOF directed to anode, upstream mode.

In order to study the influence of the composition of the electrolyte system on the EOF and the migration mode of the sample compounds in more detail, experiments were carried out in water/acetonitrile electrolyte systems at various volume ratios, containing different concentrations TOAB and TDAB, respectively. The results, shown in Fig. 6.5, illustrate that for electrolyte systems containing more acetonitrile an EOF reversal is obtained at higher surfactant concentrations. Due to a lower critical micelle concentration, TDAB causes an EOF reversal at lower concentrations than TOAB at intermediate water/acetonitrile ratios.

6.2.4. TETRAALKYLAMMONIUM IONS AS PSEUDO-STATIONARY PHASES

INFLUENCE OF ELECTROLYTE SYSTEM ON MIGRATION BEHAVIOUR

To investigate the influence of the concentration TAA^+ ions on the migration behaviour of hydrophobic species, experiments were carried out with the sample mixture in water/acetonitrile (60/40, v/v) electrolyte systems containing different concentrations TOAB. In all these experiments the sample compounds migrate in the upstream mode. Pseudo-effective mobilities, m_{eff}^{ps}, were calculated according to eqn. (1.17). In Fig. 6.6 all pseudo-effective mobilities are shown as a function of the TOAB concentration, illustrating that m_{eff}^{ps} increases with increasing TOAB concentration. This effect is more pronounced for larger, more hydrophobic PAHs which possess stronger interactions with the pseudo-stationary phase. At higher TOAB concentrations the curves level off, as the limiting value of m_{eff}^{ps} is the effective mobility of the pseudo-stationary phase.

Besides the TAA^+ concentration also the water/acetonitrile volume ratio will influence the migration behaviour of the sample compounds [24]. This is illustrated in Fig. 6.7 for the separation of four PAHs in electrolyte systems containing 5 mM TOAB and 10 mM TDAB, respectively. All sample compounds migrate in the upstream mode in the electrolyte systems with 30% or 40% (v/v) acetonitrile containing 5 mM TOAB and with 40% or 50% (v/v) acetonitrile containing 10 mM TDAB , respectively, whereas in the other electrolyte systems all sample compounds migrate in the downstream mode. On increasing the amount of acetonitrile, solvophobic interactions will decrease, resulting in lower pseudo-effective mobilities. As would be expected, a stronger decrease is obtained for larger, more hydrophobic species. These results demonstrate that the resolution can be controlled by the composition of the electrolyte system, *i.e.* concentration TAA^+ and water/acetonitrile volume ratio.

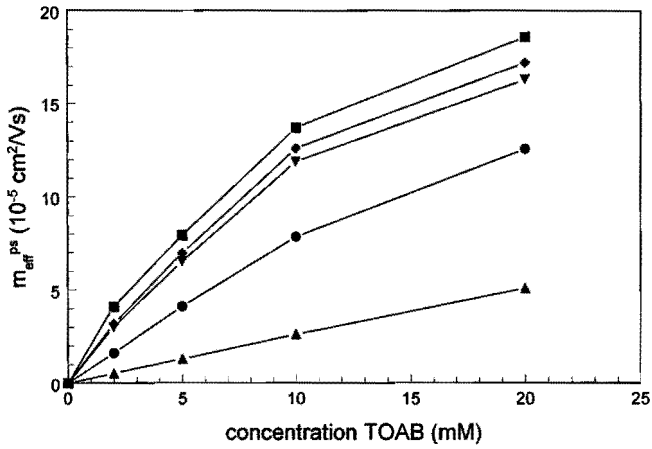

FIGURE 6.6 Pseudo-effective mobility, m_{eff}^{ps}, *versus* concentration TOAB in a water/acetonitrile (60/40, v/v) electrolyte system for (▲) propylbenzene, (●) naphtalene, (▼) anthracene, (◆) phenantrene and (■) pyrene.

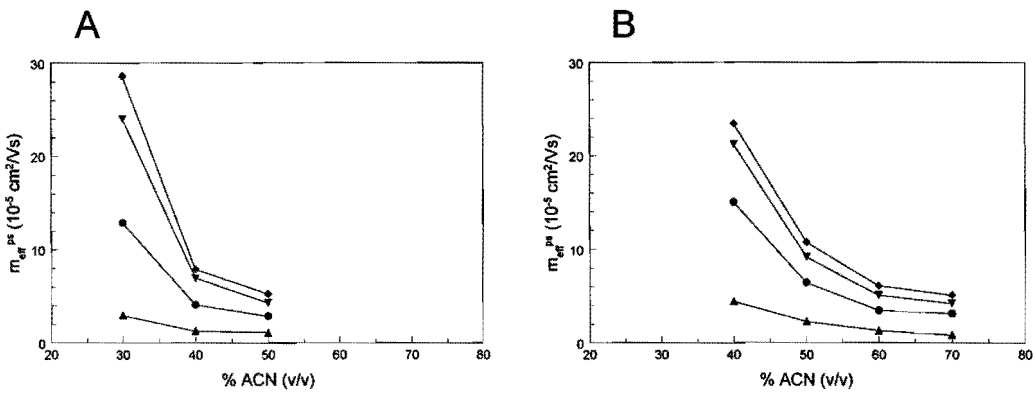

FIGURE 6.7 Pseudo-effective mobility, m_{eff}^{ps}, *versus* concentration acetonitrile in electrolyte systems containing (A) 5 mM TOAB and (B) 10 mM TDAB for (▲) propylbenzene, (●) naphtalene, (◆) phenantrene and (■) pyrene. For migration mode, see text.

MOBILITY OF THE PSEUDO-STATIONARY PHASE

The conformation of the pseudo-stationary phase in this form of EKC will depend strongly on the composition of the aqueous/organic electrolyte system. If the sample compounds are migrating in the downstream mode, the pseudo-stationary phase consists mainly of single TAA^+ ions. However, if the sample compounds are migrating in the upstream mode, the

pseudo-stationary phase consists of both single TAA^+ ions and micellar aggregates, formed by more TAA^+ ions. The aggregation number of TAA^+ ions and consequently micellar volume and shape, strongly depends on the amount of water in the aqueous/organic medium [27]. In order to study the migration behaviour of the tetraalkylammonium pseudo-stationary phase, experiments were carried out with a homologous series of alkylbenzenes. For a homologous series with an increasing number of methylene groups, log k will increase linearly with carbon number, according to [29]:

$$\log k = az + b \tag{6.1}$$

where k is the retention factor, representing the distribution equilibrium between the aqueous/organic phase and the pseudo-stationary phase, z is the carbon number of the homologues and a and b are constants. In Fig. 6.8 the electrokinetic chromatogram is shown for the separation of seven alkylbenzenes in the downstream mode, applying a water/acetonitrile (40/60, v/v) electrolyte system containing 20 mM TOAB.

Providing that the solvophobic interaction mechanism of the hydrophobic solutes is fast enough and that the migration behaviour of the TAA^+ ions is not markedly influenced by this interaction, the pseudo-effective mobility of the species, m_{eff}^{ps}, can be expressed by:

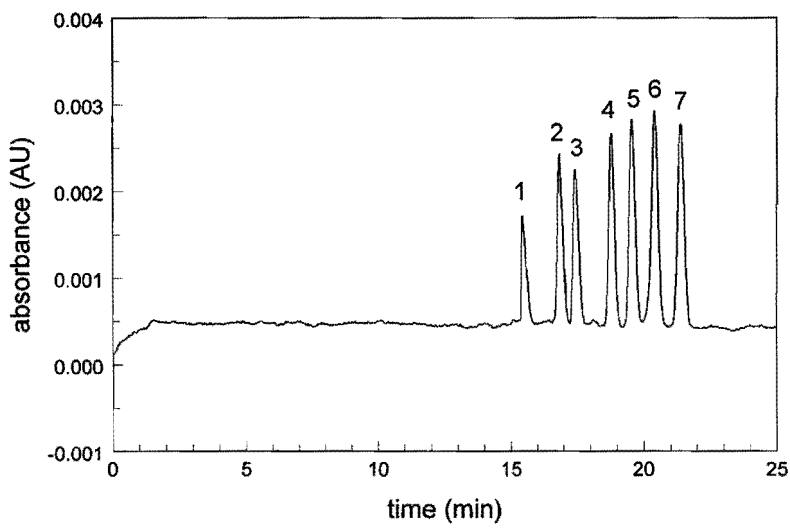

FIGURE 6.8 Electrokinetic chromatogram of the separation of (1) dodecylbenzene, (2) nonylbenzene, (3) octylbenzene, (4) hexylbenzene, (5) pentylbenzene, (6) butylbenzene and (7) propylbenzene in a water/acetonitrile (40/60, v/v) electrolyte system containing 20 mM TOAB.

$$m_{eff}^{ps} = \frac{k}{k+1} m_{eff,TAA} \tag{6.2}$$

where $m_{eff,TAA}$ is the effective mobility of the tetraalkylammonium pseudo-stationary phase. Combination of eqn. (2) and (3) leads to:

$$m_{eff}^{ps} = \frac{10^{az+b}}{1+10^{az+b}} m_{eff,TAA} \tag{6.3}$$

For the same homologous series of alkylbenzenes as in Fig. 6.8, m_{eff}^{ps} was determined in the upstream mode, applying electrolyte systems of water/acetonitrile (60/40, v/v), containing 20 mM TOAB and water/acetonitrile (50/50, v/v), containing 10 mM TDAB, respectively. From these migration data, $m_{eff,TAA}$ could be calculated by curve fitting of eqn. (6.3). This is illustrated in Fig. 6.9. For these calculations experimental data in the upstream mode were applied because in this mode the migration of the pseudo-stationary phase is probably less affected by the interaction with the homologues and better results for the curve fitting procedure were obtained. In Table 6.4 $m_{eff,TAA}$ is listed for both pseudo-stationary phases. In this table also the effective mobilities, calculated by an iteration procedure as described in chapter 4, are included, showing identical results.

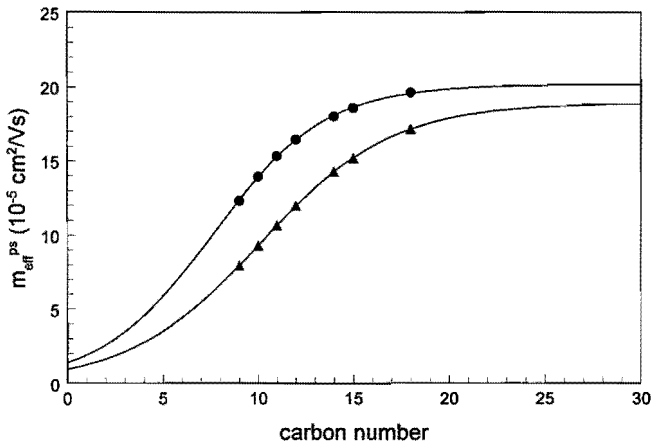

FIGURE 6.9 Pseudo-effective mobility, m_{eff}^{ps}, as a function of carbon number for a homologous series of alkylbenzenes in water/acetonitrile electrolyte systems of (•) (60/40, v/v) containing 20 mM TOAB and (▲) (50/50, v/v) containing 10 mM TDAB. Drawn lines represent the results of the curve fitting procedure.

6.2.5 CONCLUSIONS

Tetraalkylammonium ions were shown to be suitable pseudo-stationary phases for the separation of highly hydrophobic compounds by electrokinetic chromatography in aqueous /organic media. The direction of the EOF and the migration behaviour and consequently the resolution of the sample compounds strongly depend on the surfactant concentration and the organic modifier content of the electrolyte system.

A hydrophobic sample mixture, including several geometric PAH isomers could be separated in the upstream mode, applying a water/acetonitrile (60/40, v/v) electrolyte system containing 20 mM TOAB. The effective mobility of TOAB and TDAB pseudo-stationary phases was determined from the migration data of a homologous series of alkylbenzenes by curve fitting, giving identical results as an iteration procedure.

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7

PHYSICO-CHEMICAL PROPERTIES IN MICELLAR MEDIA

ABSTRACT

In this chapter several physico-chemical properties in micellar media, relevant for MEKC separations, are studied. Diffusion coefficients in surfactant solutions were determined by MEKC, using the stopped migration method. Initially, overall diffusion coefficients were measured at two different surfactant concentrations. From these values individual diffusion coefficients in the aqueous phase and in the micellar phase were calculated, showing that aqueous diffusion is about one order of magnitude higher than micellar diffusion.
The migration behaviour of micelle counterions is discussed and it is shown that, due to a high degree of counterion binding, micelle counterions in commonly used surfactant systems possess negative transport numbers under MEKC conditions. This phenomenon is illustrated for an SDS micellar system in which sodium ions possess a negative effective mobility. In addition, the influence of sodium and tris(hydroxymethyl)aminomethane as dodecylsulphate counterions on the effective mobility of micelles, efficiency and selectivity is evaluated.

7.1 DETERMINATION OF DIFFUSION COEFFICIENTS IN MEKC

7.1.1 INTRODUCTION

Surfactant solutions are frequently used as organized media in diverse industrial and pharmaceutical applications such as chemical reaction catalysis [1], cleaning and degreasing of surfaces [2] and drug solubilization [3,4]. Besides micellar solubilization, all these applications are dependent on molecular transport phenomena [5]. Therefore a good understanding of diffusional processes in these microheterogeneous systems is of great importance, *e.g.* to study reaction kinetics or to control the release rates of drugs from their formulations. Several techniques to measure diffusion coefficients in micellar systems have been described in literature, including quasi-elastic light scattering [6], nuclear magnetic resonance [7] and the Taylor dispersion method [8,9].

In micellar electrokinetic chromatography (MEKC) surfactant solutions are applied for the separation of neutral species, based on differences in micellar solubilization. In chapter 1 different band broadening mechanisms in MEKC have been discussed. It was suggested that if instrumental conditions are optimized, longitudinal diffusion is the main dispersive factor at low field strengths, whereas at higher field strengths sorption/desorption kinetics and micelle heterogeneity become more significant factors (see Fig. 1.5). Therefore a better knowledge of diffusional processes in surfactant solutions is also interesting from a chromatographic point of view. In this section the determination of diffusion coefficients in micellar media by MEKC experiments is evaluated.

7.1.2 THEORY

Capillary electrophoresis offers some unique advantages for the determination of diffusion coefficients such as small sample size necessity, suitability for solute mixtures and feasibility for automation. Several methods for the determination of diffusion coefficients by capillary electrophoresis have been described in literature [10-13].

In the *low field method* [10,11] a sufficiently low electric field is applied in order to minimize Joule heating effects. Diffusion coefficients are calculated directly from measured peak variances. A disadvantage of this approach is that contributions from injection and detection to band broadening must be calculated or estimated, or experimental conditions must be chosen in such a way that these contributions are negligible.

In the *graphical method* [11], peak variances obtained at various electric field strengths are plotted against migration time. From the slope of this linear graph the diffusion coefficient can be calculated. However, in MEKC the contributions of sorption/desorption kinetics and micelle heterogeneity to band broadening, and consequently to measured peak variances,

depend on the applied field strengths. Moreover, these effects are more pronounced for sample compounds possessing high retention factors. Therefore this method can not be applied for the determination of diffusion coefficients in MEKC.

In the *dual-detector method* [12], peak variances are measured at two positions on the capillary at a specific distance. This approach requires an experimental setup with two detectors.

In the *stopped migration method* [10,11,13], peak variances obtained after interrupting the electromigration for a specific time are compared with peak variances obtained with an uninterrupted experiment.

In this work we applied the stopped migration method for the determination of diffusion coefficients in MEKC. Assuming that other band broadening effects than longitudinal diffusion are the same in both experiments, overall diffusion coefficients in the applied electrolyte system, D_{OV}, can be calculated according to the Einstein equation [14]:

$$\Delta\sigma_s^2 = 2D_{OV}t_i \tag{7.1}$$

where $\Delta\sigma_s^2$ is the difference in spatial peak variances from both experiments and t_i is the period of interruption. Spatial peak variances , σ_s^2, can be calculated from temporal peak variances, σ_t^2, measured from the electrokinetic chromatogram, according to:

$$\sigma_s^2 = \sigma_t^2 v^2 \tag{7.2}$$

where v is the migration velocity of the sample compound. In contrast to open tubular liquid chromatography, where the contribution of diffusion in the stationary phase is considered negligible, in MEKC solubilized compounds will be subjected to micellar diffusion. Therefore, the overall diffusion coefficient in MEKC experiments, D_{OV}, is a weighted average of diffusion coefficients in the aqueous phase, D_{AQ}, and the pseudo-stationary micellar phase, D_{MC}, according to [15]:

$$D_{OV} = \frac{1}{1+k} D_{AQ} + \frac{k}{1+k} D_{MC} \tag{7.3}$$

For the relatively small sample compounds, generally analyzed by MEKC, D_{MC} is *ca.* one order of magnitude smaller than D_{AQ} [16]. If overall diffusion coefficients are determined in two electrolyte systems, containing different surfactant concentrations, D_{AQ} and D_{MC} can be calculated using eqn. (7.3).

7.1.3 EXPERIMENTAL

CHEMICALS

Sodium dodecylsulphate (SDS) was obtained from Aldrich (Steinheim, Germany); acetophenone, propiophenone, butyrophenone, valerophenone and hexanophenone from Pierce (Rockford, Ill, USA) and tris(hydroxymethyl)amminomethane (TRIS) and acetic acid from Merck (Darmstadt, Germany). Water was filtered by a Milli-Q water purification system (Waters Millipore, Milford, MA, USA).

INSTRUMENTATION AND SEPARATION CONDITIONS

All experiments were carried out on an HP^{3D} Capillary Electrophoresis instrument (Hewlett Packard, Waldbronn, Germany) with a 50 μm I.D. fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA), total length 96.5 cm, distance between injection and detection 88.0 cm. The temperature was kept constant at 25°C and the wavelength of the detector was set at 200 and 240 nm. For all experiments an electrolyte system of 20 mM TRIS, adjusted to pH 8.2 by adding acetic acid, containing 25 mM or 50 mM SDS was used. All samples were dissolved at a concentration of ca. 0.02 μl/ml in a 25 mM or 50 mM SDS solution. Samples were introduced by a 5 s. pressure injection with 50 mbar. A constant voltage of 20 kV was applied. In the interrupted experiments the voltage was switched off for 300 min., 10 min. after injection.

7.1.4 RESULTS AND DISCUSSION

Overall diffusion coefficients in MEKC were determined for a homologous series of alkylaryl ketones, using the stopped migration method. Experiments were performed with and without interruption of the electromigration for 300 min., 10 min. after injection, applying electrolyte systems containing 25 mM and 50 mM SDS, respectively. In Fig. 7.1 two resulting electrokinetic chromatograms are shown for an electrolyte system containing 50 mM SDS. All experiments were carried out four times and in Table 7.1 the average migration times and spatial peak variances are listed. The micelle migration times were calculated by an iteration procedure as described in chapter 4. For the experiments with an interruption of the electromigration, longitudinal diffusion is the main band broadening mechanism. Due to lower diffusion coefficients in the micellar phase than in the aqueous phase, lower spatial peak variances are obtained for more hydrophobic species. This is illustrated in more detail in Fig. 7.2 where the normalized peaks of acetophenone and hexanophenone from Fig. 7.1 are shown on a spatial basis. From Fig. 7.2 it can be clearly seen that a smaller sample zone is obtained for hexanophenone than for acetophenone in the interrupted experiment, although the migration time for hexanophenone is longer.

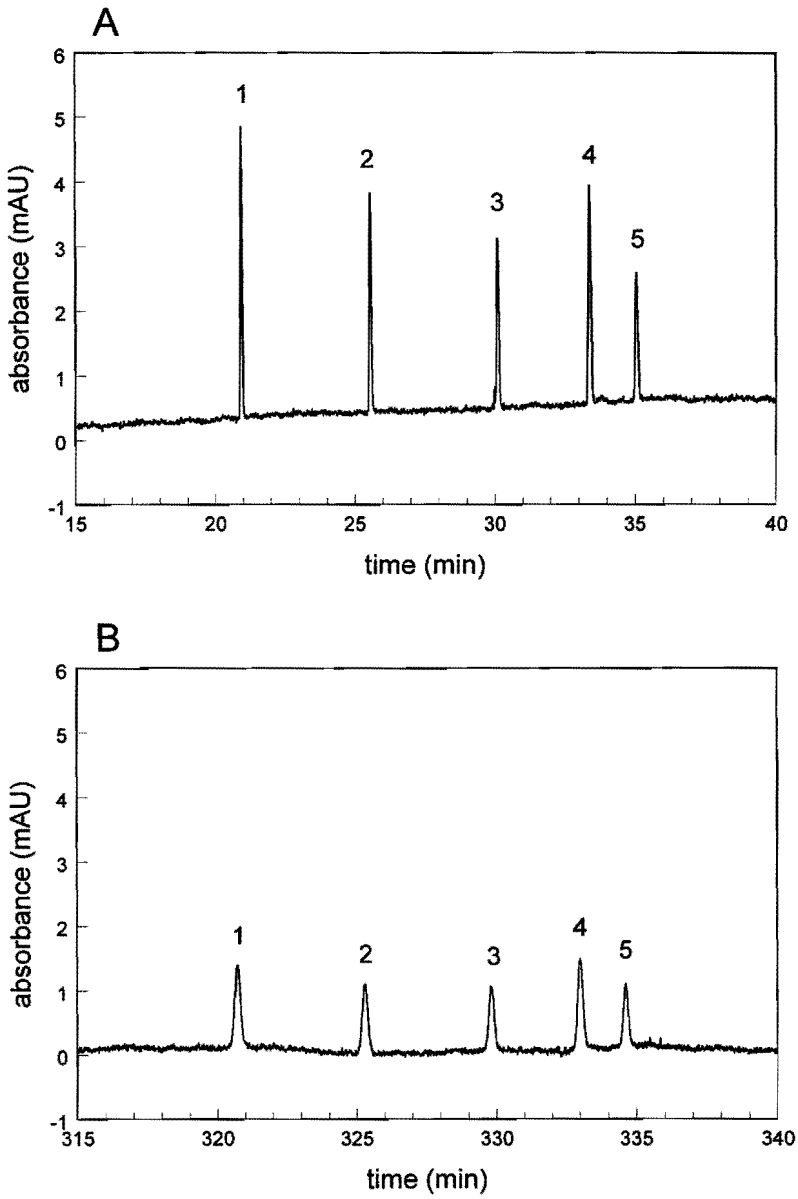

FIGURE 7.1 Electrokinetic chromatograms of (1) acetophenone, (2) propiophenone, (3) butyrophenone, (4) valerophenone and (5) hexanophenone. Electrolyte system, 0.02 M TRIS/acetic acid at pH 8.2, containing 50 mM SDS. Capillary, l_c=96.5 cm and l_d=88.0 cm. Voltage, 20 kV (A) without and (B) with an interruption of 300 min., 10 min. after injection. Detection wavelength, 240 nm.

TABLE 7.1 Average migration times, t (min), and spatial peak variances, σ^2 (cm^2), with relative standard deviations (RSD %) for experiments (I) without and (II) with an interruption of the electromigration for 300 min., 10 min. after injection, for electrolyte systems containing 25 mM or 50 mM SDS. ($n=4$).

compound	I				II			
	t	RSD	σ^2	RSD	t-300	RSD	σ^2	RSD
25 mM SDS								
EOF	11.09	(1.2)	-	-	11.09	(0.7)	-	-
acetophenone	15.30	(1.9)	0.0177	(5.1)	15.30	(1.0)	0.2241	(6.0)
propiophenone	18.37	(2.1)	0.0159	(6.2)	18.38	(1.1)	0.1513	(4.8)
butyrophenone	22.22	(2.1)	0.0168	(9.8)	22.21	(1.1)	0.0989	(6.0)
valerophenone	25.67	(2.0)	0.0188	(12.8)	25.69	(1.2)	0.0770	(5.1)
hexanophenone	27.81	(1.9)	0.0198	(9.6)	27.83	(1.2)	0.0610	(7.9)
MC	30.05	(1.5)	-	-	30.09	(1.1)	-	-
50 mM SDS								
EOF	12.16	(0.9)	-	-	12.16	(0.4)	-	-
acetophenone	20.83	(1.1)	0.0146	(4.0)	20.85	(0.4)	0.1784	(5.1)
propiophenone	25.42	(1.0)	0.0126	(4.3)	25.42	(0.3)	0.1062	(5.8)
butyrophenone	29.92	(1.0)	0.0113	(6.1)	29.88	(0.3)	0.0678	(7.0)
valerophenone	33.13	(0.9)	0.0107	(4.6)	33.07	(0.1)	0.0569	(6.0)
hexanophenone	34.76	(0.9)	0.0114	(9.4)	34.69	(0.2)	0.0458	(10.4)
MC	36.24	(0.8)	-	-	36.19	(0.3)	-	-

From the results, listed in Table 7.1, retention factors and overall diffusion coefficients were calculated, using eqn. 7.1. Subsequently, diffusion coefficients in the aqueous phase, D_{AQ}, and in the micellar phase, D_{MC}, were calculated, using eqn. 7.3. In Table 7.2 all these values are listed. As expected from theory, lower overall diffusion coefficients were obtained for sample compounds with higher retention factors and for electrolyte systems with higher surfactant concentrations. Diffusion coefficients of the alkylaryl ketones in the micellar phase were found to be *ca.* one order of magnitude lower than those in the aqueous phase, which is in agreement with values earlier reported [16]. From eqn. (7.3) it can be deduced that for compounds with low retention factors micellar diffusion is negligible, whereas for compounds with high retention factors micellar diffusion contributes significantly to band-broadening. However, as stated in chapter 2, under practical MEKC conditions, *i.e.* t_{MC}/t_{EOF} between 2-5 and $k<10$, the contribution of micellar diffusion to band-broadening is of minor importance.

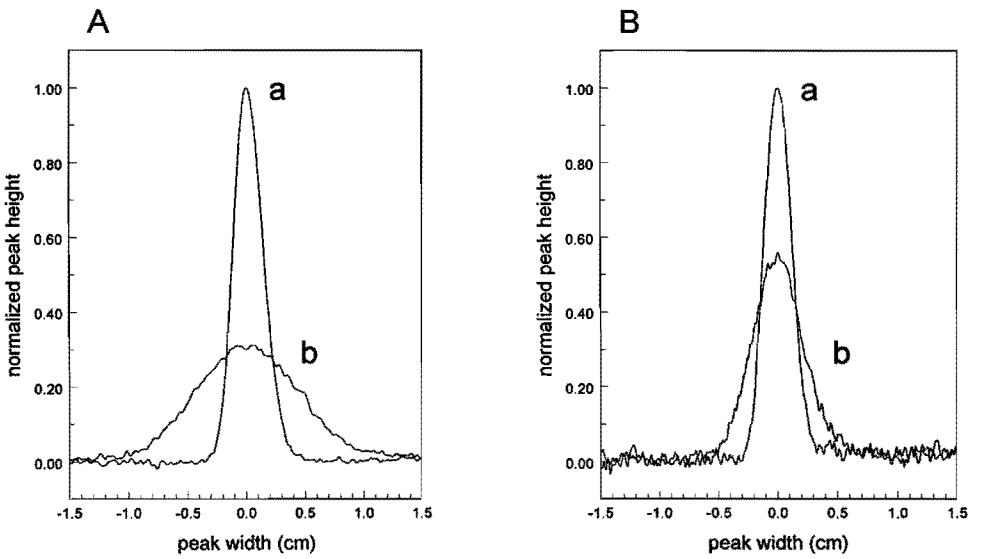

FIGURE 7.2 Normalized peaks of (A) acetophenone and (B) hexanophenone on a spatial basis. Electrolyte system, 0.02 M TRIS/acetic acid at pH 8.2, containing 50 mM SDS. Capillary, l_c=96.5 cm and l_d=88.0 cm. Voltage, 20 kV (a) without and (b) with an interruption of 300 min., 10 min. after injection. Peaks were normalized by putting the peak height for the uninterrupted experiment equal to 1.

TABLE 7.2. Average retention factors, k, and overall diffusion coefficients, D_{OV} (10^{-6} cm^2/s), with relative standard deviations (RSD %) for five alkylaryl ketones in electrolyte systems containing 25 mM or 50 mM SDS, and calculated diffusion coefficients in the aqueous phase, D_{AQ} (10^{-6} cm^2/s), and the micellar phase, D_{MC} (10^{-6} cm^2/s). (n=8).

compound	*25 mM SDS*				*50 mM SDS*					
	k	RSD	D_{OV}	RSD	k	RSD	D_{OV}	RSD	D_{AQ}	D_{MC}
acetophenone	0.77	(1.7)	5.7	(6.6)	1.69	(1.0)	4.6	(5.6)	8.4	2.3
propiophenone	1.69	(1.9)	3.8	(6.5)	3.67	(1.0)	2.6	(6.6)	8.4	1.0
butyrophenone	3.84	(2.1)	2.3	(7.5)	8.40	(2.2)	1.6	(8.5)	7.9	0.8
valerophenone	9.02	(2.2)	1.6	(8.0)	20.09	(2.2)	1.3	(7.5)	7.5	1.0
hexanophenone	20.21	(2.4)	1.2	(12.4)	45.26	(2.9)	1.0	(14.1)	8.3	0.8
										0.87[a]
										0.76[b]

[a]Determined with the Taylor dispersion method and methyl yellow as tracer in 20 mM SDS and [b]in 52.5 mM SDS, respectively [8].

For comparative purposes also D_{MC}-values from literature, determined with the Taylor dispersion method, are included in Table 7.2. These results show that the diffusion coefficients obtained with both methods agree well. However, here it should be mentioned that more accurate date are obtained using the Taylor dispersion method [8,9].

7.1.5 CONCLUSIONS

It was demonstrated that diffusivity in micellar media can be measured by micellar electrokinetic chromatography, using the stopped migration method. Overall diffusion coefficients were determined for a homologous series of alkylaryl ketones in electrolyte systems containing 25 and 50 mM SDS, respectively. From these values diffusion coefficients in the aqueous phase, D_{AQ}, and in the pseudo-stationary micellar phase, D_{MC}, were calculated, showing that D_{AQ} is about one order of magnitude larger than D_{MC}.

7.2 MIGRATION BEHAVIOUR OF MICELLE COUNTERIONS

7.2.1 INTRODUCTION

In chapter 5 the influence of different solute-micelle interactions on micellar solubilization have been discussed. Remarkable differences in selectivity were observed between SDS and mixed SDS/Brij 35 micellar systems, due to different hydrogen bonding characteristics of these micellar phases. Besides the hydrophylic moieties of the micellar phase, also the chemical nature of the counterions may affect solute-micelle interactions. Several authors paid attention to the influence of inorganic and organic counterions of dodecylsulphate micelles in MEKC [17-21]. Cohen *et al.* [17] reported an improved resolution for oligonucleotides due to complexation with divalent micelle counterions. Nishi *et al.* [18] and Nielsen and Foley [19] described the effect of tetraalkylammonium salts in an SDS micellar system. Ahuja and Foley [21] reported the influence of sodium, potassium and lithium counterions on efficiency, selectivity and elution window. Also, the high degree of counterion binding has been applied for the separation of anionic species applying MEKC with cationic surfactant systems [22,23].

Resolution in MEKC is influenced by the efficiency, selectivity, solute retention, and elution window, as described in chapter 2. All these terms depend on the structure and microenvironment of the pseudo-stationary micellar phase. The chemical nature of counterions may have a pronounced effect on micellar structure [16,24] and, consequently, on the resolution in MEKC experiments. Therefore a good understanding of the migration behaviour of micelle counterions and their influence on the separation process is important,

both from an electrophoretic and a chromatographic point of view. In this section the migration behaviour of sodium counterions during MEKC experiments with a sodium dodecylsulphate micellar system is studied. In addition, the influence of sodium and tris(hydroxymethyl)aminomethane counterions on micelle mobility, separation efficiency and selectivity is discussed.

7.2.2 THEORY

In an aqueous solution of an ionic surfactant three constituents can be distinguished, *viz.* the solvent water, an amphiphilic ion and a hydrophillic counterion. Above the critical micelle concentration (CMC) micelles will be formed which can be described by the following equilibrium [16]:

$$mM + nA \rightleftharpoons M_mA_n \tag{7.4}$$

where M denotes the counterions, A the amphiphilic ions and MA the micelles, respectively. Hence n is the aggregation number and m is the number of counterions bound to the micelle. The degree of counterion binding, θ, is defined as the ratio of counterions and amphiphilic ions in a micelle (m/n). For a large number of surfactant systems, θ lies in the range 0.5-0.8. The degree of dissociation of the micelles, α, equals 1-θ.

Mysels and Dulin [25] described a model for an anionic surfactant where three different ionic species will be present in solution, *viz.* M^+ ions, A^- ions and micelles composed of nA^- and θnM^+ ions with the empirical formula $M_\theta A$. If these three species are denoted by numerical subscripts *1*, *2* and *3* respectively, the following material balances can be derived for the concentration of the surfactant, C_{SF}, and the different species:

$$C_{SF} = C_M = C_A = C_2 + C_3 = C_1 + \theta C_3 \tag{7.5}$$

where C_M and C_A are the total concentrations of counterions and amphiphilic ions, respectively. The effective mobility of the amphiphilic ions, $m_{eff,A}$, is the weighted average of the mobility of the free A^- ions at the CMC, m_2, and the effective mobility of the micelles, m_3, according to:

$$m_{eff,A} = \frac{C_2}{C_{SF}} m_2 + \frac{C_3}{C_{SF}} m_3 \tag{7.6}$$

where C_2 is the CMC and $C_3 = C_{SF} - C_2$. The effective mobility of the counterions, $m_{eff,M}$, is the weighted average of the mobility of the free M^+ ions at the CMC, m_1, and the effective

mobility of the micelles, m_3, according to:

$$m_{eff,M} = \frac{C_1}{C_{SF}} m_1 + \frac{\theta C_3}{C_{SF}} m_3 \qquad (7.7)$$

where $C_1 = C_{SF} - \theta C_3$. The mobility of the free A^- ions and M^+ ions at the CMC, m_2 and m_1, can be calculated from their ionic mobilities at infinite dilution, $m^0_{A^-}$ and $m^0_{M^+}$, applying the Debye-Hückel-Onsager theory for the correction of relaxation and retardation effects [26]. An approximation for the degree of dissociation of the micelles, α, can be obtained from the differential conductivity, $\partial\kappa/\partial C_{SF}$, which above the CMC can be described by [27]:

$$\frac{\partial \kappa}{\partial C_{SF}} = \alpha(\lambda_{M^+} + Fm_3) \qquad (7.8)$$

where λ_{M^+} is the equivalent ionic conductivity of the counterions at the CMC and F is the Faraday constant. From literature data for the conductivity of sodium dodecylsulphate (SDS) [28] and assuming an effective mobility of -45.33 cm^2/Vs for SDS micelles, a value of 0.26 is calculated for α. This means that about 74 % of the counterions are bound to the micelles in an SDS micellar system. Here it should be noted that the degree of dissociation is actually not constant but increases slightly with increasing surfactant concentration [25].

In Fig. 7.3 $m_{eff,A}$ and $m_{eff,M}$ are shown as a function of surfactant concentration for a specific anionic micellar system. The reduction of viscous drag on micellization, *i.e.* a micelle formed by n surfactant molecules experiences less viscous drag than the total viscous drag experienced by n individual surfactant molecules, causes an increase in effective mobility of amphiphilic ions above the CMC. Due to the binding of counterions to micelles their effective mobility decreases above the CMC. The transport of bound counterions predominates over the migration of free counterions, due to a high degree of counterion binding and a high absolute value for the effective mobility of micelles. This results in a negative effective mobility for M^+ which means that the net migration of the positive counterions is towards the anode. In Fig. 7.3 also the transport numbers for A and M are shown. The transport number of an ionic species in solution is the fraction of the current carried by that species across a reference plane when an electric current is passed through the solution [29]. Thus, the transport number, t_i, is expressed by:

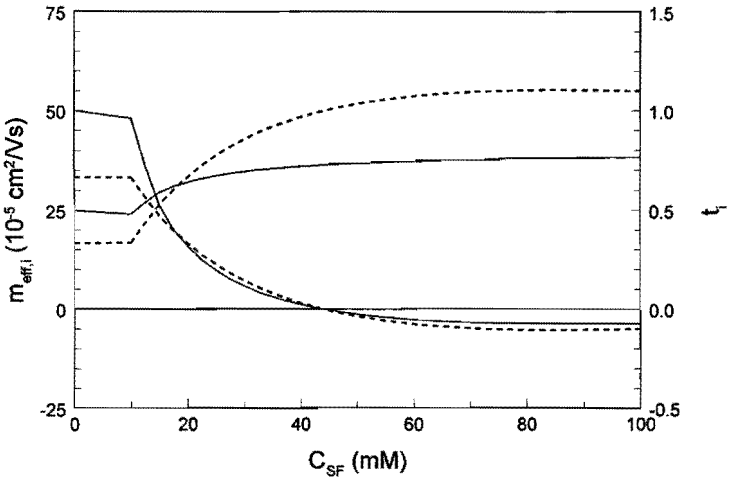

FIGURE 7.3 Effective mobility (drawn lines, left hand ordinate) and transport number (dotted lines, right hand ordinate) for amphiphilic ions, $-m_{eff,A}$ and t_A, and counterions, $m_{eff,M}$ and t_M, *versus* surfactant concentration, C_{SF}. Model values: CMC = 10 mM, $m^0_{A-} = -25 \cdot 10^{-5}$ cm²/Vs, $m^0_{M+} = +50 \cdot 10^{-5}$ cm²/Vs, $m_3 = -40 \cdot 10^{-5}$ cm²/Vs, $\alpha = 0.25 + 0.001 C_{SF}$.

$$t_i = \frac{i_i}{\sum_j i_j} = \frac{C_i z_i m_i}{\sum_j C_j z_j m_j} \tag{7.9}$$

where
i_i = current carried by the ion i,
C_i = concentration of ion i,
z_i = charge number of ion i,
m_i = effective mobility of ion i,
$\sum_j i_j$ = current carried by all the ions in solution.

The sum of transport numbers of all ionic species present in a solution equals unity:

$$\sum_i t_i = 1 \tag{7.10}$$

In the case of a single anionic surfactant solution with $C_M = C_A$ eqn. (7.9) reduces to:

$$t_i = \frac{i_i}{\sum_j i_j} = \frac{z_i m_i}{\sum_j z_j m_j} \tag{7.11}$$

Due to micellization t_A exceeds unity at higher surfactant concentrations, whereas t_M becomes negative due to counterion binding.

7.2.3 EXPERIMENTAL

CHEMICALS

Sodium dodecylsulphate (SDS), was obtained from Aldrich (Steinheim, Germany). Tris(hydroxymethyl)aminomethane (TRIS), was obtained from Merck (Darmstadt, Germany). The TRIS-salt of dodecylsulphate (TDS) was obtained from Sigma (St. Louis, MO, USA). All other chemicals were analytical-reagent grade.

INSTRUMENTATION AND SEPARATION CONDITIONS

All experiments were carried out on a BioFocus 3000 Capillary Electrophoresis System (BioRad, Hercules, CA, USA) at a constant voltage of 20 kV. 50 μm I.D. fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were used, total length 70.0 cm, distance between injection and detection 65.4 cm, or total length 50.0 cm, distance between injection and detection 45.4 cm. The temperature was kept constant at 25°C and the wavelength of the detector was set at 200 nm. Samples were introduced by pressure injection with an injection constant of 1 psi.s, unless otherwise noted.

7.2.4 RESULTS AND DISCUSSION

MIGRATION BEHAVIOUR MICELLE COUNTERIONS

In order to investigate the migration behaviour of sodium ions in an SDS micellar system, experiments were carried out with an electrolyte system comprising 20 mM imidazole adjusted to pH 8.5 by addition of boric acid, containing different concentrations SDS. A sample solution of SDS in deionized water was injected, containing the same concentration SDS as the electrolyte system. For the electrolyte system without SDS, a sample solution of 1 mM SDS was applied. Due to field amplified sample stacking a small increase in the sodium concentration is obtained in this sample plug, which can be visualized applying indirect UV detection. Besides the sodium peak and the electroosmotic flow (EOF) dip, also system peaks were observed as described by Beckers [30]. In Fig. 7.4 all electrokinetic chromatograms are shown.

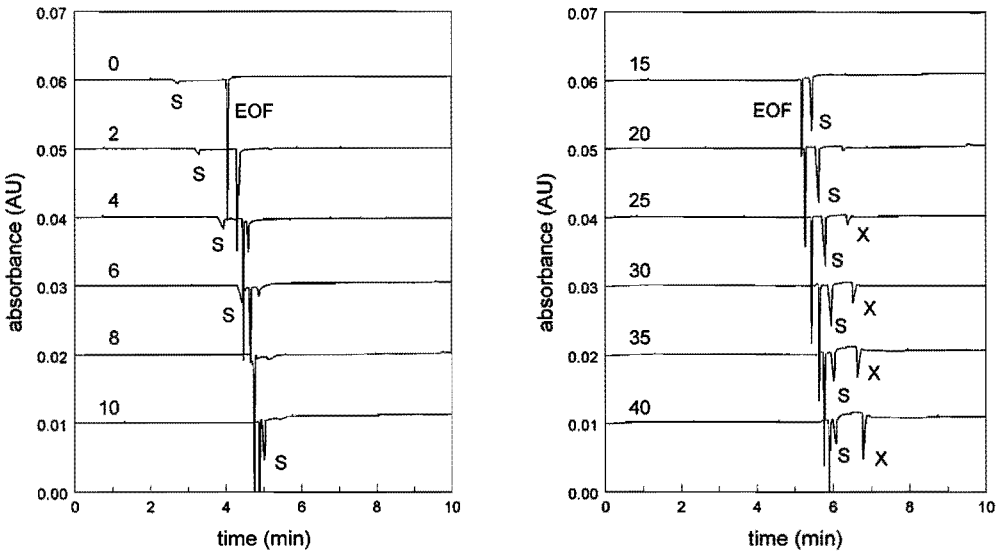

FIGURE 7.4 Electrokinetic chromatograms of sodium, S, in an electrolyte system of 20 mM imidazole adjusted to pH 8.5 by addition of boric acid, containing different concentrations SDS (mM). Sample solution, SDS in deionized water at the same concentration as the electrolyte system. Capillary length, 70.0 cm. X=system peak. For further explanation, see text.

Without SDS sodium migrates in the downstream mode [31] towards the cathode and is detected before the EOF. On increasing the SDS concentration the effective mobility decreases and becomes negative above *ca.* 10 mM SDS.In this situation sodium migrates in the upstream mode towards the anode and is detected after the EOF. Here it should be emphasized that it is difficult to distinguish between the two negative peaks after the EOF at higher SDS concentrations and to determine which peak should be attributed to sodium. Since the effect of field amplified sample stacking will decrease at higher SDS concentrations, we assume that the first peak represents sodium, as this peak decreases at higher SDS concentrations. In Fig. 7.5 all calculated effective mobilities are shown. These results illustrate that under normal MEKC conditions with an SDS micellar system the micelle counterions have a negative effective mobility and consequently a negative transport number.

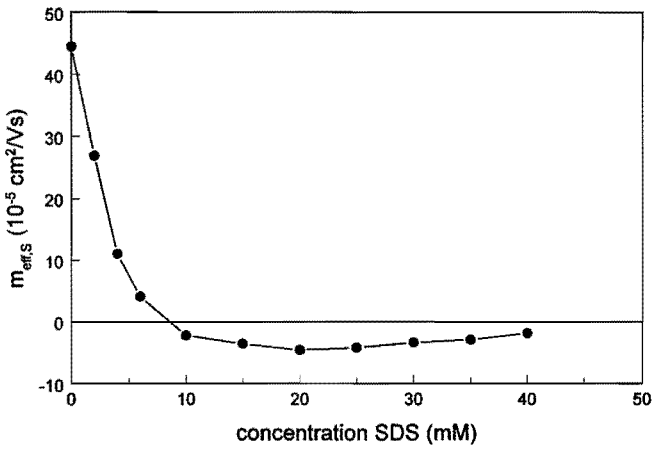

FIGURE 7.5 Calculated effective mobility for sodium, $m_{eff,S}$, *versus* concentration SDS in an electrolyte system of 20 mM imidazole adjusted to pH 8.5 by addition of boric acid.

MIGRATION BEHAVIOUR MICELLES

The size and valency of counterions associated with an ionic amphiphile may have a pronounced effect on micellar properties such as aggregation number and CMC. Also, the degree of ionization may be influenced by the effective hydrated radius of the counterion. In addition to that, the ionic strength of the applied background electrolyte may influence micellar size and shape [16,24]. Moreover, ionic constituents of the buffer may be bound to the micelles as counterions. For these reasons the effective mobility of micelles will not only be dependent on the applied surfactant system with a specific type of counterions, but also on the chemical nature of the background electrolyte.

TABLE 7.3 Effective mobility of DS micelles, m_{MC} (10^{-5} cm²/Vs), with standard deviations (in parentheses) with sodium and TRIS counterions in different electrolyte systems. *(n=5)*.

Cation	Buffering counter species	pH	surfactant system	m_{MC}
20 mM NaOH	*o*-Phosphoric acid	7.0	50 mM SDS	-45.33 (0.50)
20 mM TRIS	*o*-Phosphoric acid	7.0	50 mM TDS	-38.44 (0.11)
20 mM TRIS	*o*-Phosphoric acid	7.0	50 mM SDS	-39.16 (0.10)

In order to investigate the influence of counterions on the migration behaviour of micelles, effective mobilities of dodecylsulphate (DS) micelles were measured with sodium and TRIS as counterions, respectively. The migration time of the micelles was calculated by an iteration procedure applying the migration data of a homologous series of alkylbenzenes, as described in chapter 4. Special attention was paid to the composition of the background electrolytes so that the cation was identical to the DS counterion. As can be seen from the results, listed in Table 7.3, higher effective mobilities for DS micelles are obtained with sodium counterions (SDS) than with TRIS counterions (TDS). Due to a larger effective hydrated radius of TRIS compared to sodium, the degree of dissociation and consequently the net charge will be higher for TDS micelles, causing an increase in effective mobility. However, this effect is counteracted by a larger effective size for TDS micelles, causing a decrease in effective mobility. Obviously the larger micelle size predominates the higher degree of dissociation, resulting in a lower effective mobility of TDS micelles.
The results in Table 7.3 also illustrate that on applying an electrolyte system containing TRIS as buffering cationic species and SDS as micelle forming agent, the effective mobility of DS micelles is mainly determined by the presence of the TRIS cations. These results suggest that TRIS cations present in the background electrolyte are bound as counterions to DS micelles for a considerable extent in this electrolyte system.

EFFICIENCY

To study the influence of micelle counterions on efficiency in MEKC, experiments were carried out with a homologous series of alkylbenzenes, applying SDS and TDS as micellar system, respectively. As can be seen from the theoretical plate numbers, listed in Table 7.4, higher separation efficiencies are obtained with TDS for all compounds. Due to a smaller hydrated radius, sodium counterions are able to approach the charged sulphate groups of DS micelles more closely than TRIS counterions. As a consequence, the electrical repulsion of aggregated DS molecules will be reduced more effectively, allowing the sulphate groups to approach closer to each other [16,24]. Therefore, TDS micelles will possess a less rigid structure of the polar head groups and a less compact double layer than SDS micelles, resulting in improved sorption-desorption kinetics and higher separation efficiencies. This effect will be more pronounced for hydrophobic compounds, which explains the larger differences in theoretical plate numbers obtained for higher homologues.

TABLE 7.4 Theoretical plate numbers, $N \cdot 10^{-5}$, and methylene selectivity, $log\ \alpha_{CH_2}$, with standard deviations (in parentheses) for a homologous series of alkylbenzenes in SDS and TDS surfactant systems. Capillary length, 50.0 cm.

Compound	50 mM SDS	50 mM TDS
benzene	1.79	1.87
toluene	1.72	2.22
ethylbenzene	1.44	2.07
propylbenzene	0.91	2.39
butylbenzene	1.04	2.38
$log\ \alpha_{CH_2}$	0.418 (0.005)	0.410 (0.005)

SELECTIVITY

In chapter 5 it was demonstrated that SDS and TDS possess comparable solubilization characteristics and, consequently, almost no differences in functional group selectivity were observed between these micellar phases. However, differences in micellar structures, caused by different counterions, may influence hydrophobic selectivity [21]. Hydrophobic or methylene selectivity provides information about differences in polarity between the aqueous mobile phase and the pseudo-stationary micellar phase. The hydrophobic selectivity, $log\ \alpha_{CH_2}$, of a micellar system can be calculated from the migration data of a homologous series with an increasing number of methylene groups, according to [32]:

$$\log k = (\log \alpha_{CH_2})\ z + b \qquad (7.12)$$

where k is the retention factor, z is the number of carbon atoms in the homologues and b is a constant which depends amongst others on the phase ratio. A lower value of $log\ \alpha_{CH_2}$ indicates a more polar microenvironment in the micellar phase. In Table 7.4 $log\ \alpha_{CH_2}$ is listed for the homologous series of alkylbenzenes in an SDS and a TDS micellar system, respectively. As can be seen from these results, only a small difference is observed between the hydrophobic selectivity for these two pseudo-stationary phases. TDS micelles provide a slightly more polar microenvironment for the alkylbenzenes. The less rigid structure of the polar head groups allows for an increase of water penetration into the micellar surface and a higher extent of hydration of the sulphate groups. However, since nonpolar compounds are solubilized in the hydrophobic micelle interior, as described in chapter 4, only a minor influence is observed for the methylene selectivity of DS micelles applying alkylbenzene homologues.

7.2.5 CONCLUSIONS

Due to a high degree of counterion binding to micelles, negative transport numbers are obtained for micelle counterions in MEKC experiments. It was shown that sodium ions possess a negative effective mobility in commonly used SDS micellar systems. The mobility of micelles is influenced by the effective hydrated radius of counterions and the degree of counterion binding. Consequently, lower effective mobilities were obtained for TDS micelles than for SDS micelles. It was demonstrated that the effective mobility of DS micelles decreases if a background electrolyte is applied containing TRIS as buffering cationic species due to binding of TRIS to DS micelles as counterion. Higher efficiencies were obtained for TDS than for SDS, due to a less rigid structure of the micelle surface and a less compact double layer. The influence of micelle counterions on hydrophobic selectivity and functional group selectivity was shown to be of minor importance.

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8

DETERMINATION OF DRUGS IN PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL SAMPLES

ABSTRACT

In this chapter the application of micellar electrokinetic chromatography (MEKC) is evaluated for the qualitative and quantitative analyses of several drugs in various sample matrices. The quantitative abilities of MEKC are illustrated by the determination of phenothiazines, benzodiazepines and antiepileptic drugs in pharmaceutical formulations. In addition to that, the possibilities as well as the limitations of MEKC for the screening of drugs in biological samples are illustrated by the determination of benzodiazepines and antiepileptic drugs in serum at toxicologic and therapeutic concentration levels, respectively.

8.1 INTRODUCTION

In many aspects of pharmaceutical and biological industries, separations of complex mixtures are required, as well as qualitative and quantitative analyses of purified substances. In medical science, accurate determinations of drug levels in body fluids are important for the investigation of intoxications and to study pharmacokinetis. In the previous chapters of this thesis it is demonstrated that MEKC is an efficient separation method, suitable for the analysis of both charged and neutral compounds. The composition of the electrolyte system and the pseudo-stationary micellar phase can easily be changed in order to control the migration behaviour and optimize selectivity. Since pharmaceutical substances often possess different physical properties such as pK_a-values and hydrophobicity, MEKC may be an attractive analytical technique for the qualitative and quantitative determination of these compounds in different sample matrices. In the following sections the applicability of MEKC is evaluated for the determination of phenothiazines, benzodiazepines and antiepileptic drugs in pharmaceutical formulations and biological samples.

8.2 PHENOTHIAZINES

8.2.1 INTRODUCTION

Phenothiazine and its derivatives are a group of basic drugs used as antihistamines, antipsychotics and neuroleptics in the therapy of different diseases. So far, pharmaceutical preparations containing phenothiazine compounds have been analyzed by spectrophotometric methods [1], gas chromatography [2-4], isotachophoresis [5] and liquid chromatography [6]. Since their introduction, capillary zone electrophoresis (CZE) [7,8] and micellar electrokinetic chromatography (MEKC) [9,10] have developed into highly efficient separation methods with a great variety of applications. Amongst others, many pharmaceutical substances have been analyzed using these separation techniques. Although MEKC was initially developed for the determination of neutral compounds, it has proved to be also a powerful analytical tool for the separation of compounds with almost identical electrophoretic mobilities [11,12] and for mixtures containing both charged and uncharged compounds [13,14]. In this section the applicability of CZE and MEKC for the separation of fourteen structurally related phenothiazines is studied.

8.2.2 EXPERIMENTAL

INSTRUMENTATION

All experiments were carried out on a BioFocus 3000 Capillary Electrophoresis System (BioRad, Hercules, CA, USA). The temperature was kept constant at 25°C and the wavelength of the UV detector was set at 240 nm. Pressure injection was carried out with an injection constant of 2 psi.s. A 50 μm I.D. fused silica capillary from Polymicro Technologies (Phoenix, AZ, USA) was used, total length 70.0 cm, distance between injection and detection, 65.4 cm. All experiments were carried out with a constant voltage of 20 kV. In the anionic mode the cathode is placed at the inlet side and the anode at the outlet side of the capillary, respectively, and *vice versa* in the cationic mode.

CHEMICALS

Propiomazine maleate salt, ethopropazine hydrochloride, promethazine hydrochloride, acetopromazine maleate salt, triflupromazine hydrochloride, promazine hydrochloride, trimeprazine hemi-(+)-tartrate salt, chlorpromazine hydrochloride, trifluoperazine dihydrochloride, perphenazine, thioridazine hydrochloride, prochlorperazine dimaleate salt, fluphenazine dihydrochloride and methotrimeprazine maleate salt were obtained from Sigma (St. Louis, MO, USA), cetyltrimethylammonium bromide (CTAB) was obtained from Merck (Darmstadt, Germany) fluorinated alkyl quaternary ammonium iodide (FC 135) was obtained from Fluorad/3M (Leiden, The Netherlands). The critical micelle concentration (CMC) of FC 135 is *ca.*2 g/l, determined from surface tension data provided by the manufacturer. Phenergan (Pharma Chemie BV, Haarlem, The Netherlands) and Melleril (Sandoz Pharma AG, Basel, Switzerland) tablets were obtained at a local pharmacy.

STANDARD SOLUTIONS

Stock solutions of the phenothiazines were prepared by weighing accurately 10.0 mg of the compounds and dissolving them in 10.0 ml deionized water. Perphenazine and prochlorperazine dimaleate salt were dissolved in 10.0 ml methanol. All solutions were diluted 10 times with deionized water to give sample solutions with a final concentration of 0.1 mg/ml. For the calibration graphs, dilutions of the stock solutions were used at concentrations of 0.1, 0.08, 0.06, 0.04, 0.02, and 0.01 mg/ml.

SAMPLE PREPARATION

Phenergan and Melleril tablets, labelled to contain 25 mg promethazine and 100 mg thioridazine, respectively, were pulverized and dissolved in 100 ml deionized water by sonication for 30 min. These solutions were diluted 5 times and 20 times, respectively, so

that the concentration of the sample is near the middle of the linear range of the calibration graph. These dilutions were used for injection without further pretreatment.

8.2.3 RESULTS AND DISCUSSION

DETERMINATION OF PHENOTHIAZINES BY CZE WITH REVERSED EOF

Phenothiazines are basic compounds with pK_a-values between 8-10. They migrate in the downstream mode [15] at intermediate pH values in normal CZE experiments, carried out in the cationic mode. However, these analyses are hampered by adsorption of the basic compounds on the negatively charged capillary surface, resulting in very bad peak shapes and a low resolution. This is illustrated in Fig 8.1A, where an electropherogram is shown for the separation of six phenothiazines, applying a background electrolyte of 0.02 M TRIS/formic acid at pH 3.5. This adsorption can be efficiently reduced by a dynamical modification of the capillary surface with the fluorinated cationic surfactant FC 135, resulting in a charge reversal of the capillary wall and a reversed electroosmotic flow (EOF) [16-18], as described in section 1.3 (see Fig. 1.2). Consequently, these experiments are carried out in the anionic mode. Moreover, in these experiments the phenothiazines migrate in the upstream mode which makes a better separation possible [8,19].

The effective mobilities of the phenothiazines were determined as a function of pH. In Table 8.1 the compositions of the different background electrolytes are given and in Table 8.2 all effective mobilities are listed. In CZE the highest resolution is generally obtained at a pH near the pK_a-values of the analytes. However, for these solutes at higher pH-values deteriorating peak shapes were observed. This is due to a less effective shielding of the negatively charged surface silanol groups with increasing pH [18], resulting in adsorption of the basic phenothiazines on the capillary surface. To check the influence of FC 135 on the effective mobilities of the analytes, experiments were carried out in the cationic mode applying a background electrolyte of 0.02 M TRIS/formic acid at pH 3.5 without FC 135.

TABLE 8.1 Compositions of background electrolytes at different pHs. All background electrolytes contain the fluorinated cationic surfactant FC 135, unless otherwise noted.

Cation	Buffering counter species	pH
0.02 M TRIS	Formic acid	3.5
0.02 M TRIS	Acetic acid	5.0
0.02 M TRIS	MES	6.2
0.02 M TRIS	MOPS	7.2

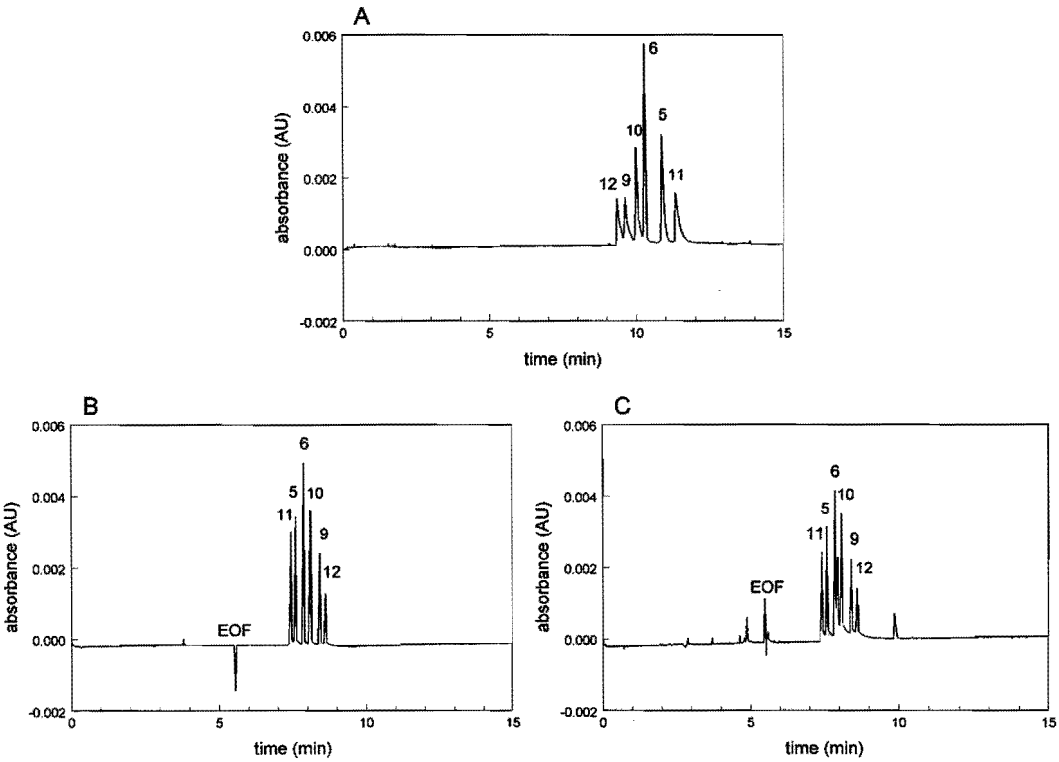

FIGURE 8.1 Electropherograms of the separation of (11) thioridazine, (5) triflupromazine, (6) promazine, (10) perphenazine, (9) trifluoperazine and (12) prochlorperazine (all 0.1 mg/ml) dissolved in (A and B) deionized water and (C) 10-fold diluted human urine. Background electrolyte, 20 mM TRIS/formic acid at pH 3.5, containing (A) 0 and (B and C) 50 mg/l FC 135.

As can be seen from the results, listed in Table 8.2, almost identical effective mobilities were obtained, showing that the migration of the analytes is not affected by the presence of the cationic surfactant FC 135 in the background electrolyte.

From the foregoing it can be concluded that phenothiazines can be determined by CZE with reversed EOF, applying an electrolyte system in the pH range 3.5-7.2 containing 50 mg/l FC 135. However, a complete separation of the fourteen drugs is not possible in this pH range, as the differences in effective mobilities are too small. In Fig. 8.1B an electropherogram is shown for the separation of six phenothiazines, applying a background electrolyte of 0.02 M TRIS/formic acid at pH 3.5 containing 50 mg/l FC 135. Notice that the migration order of the six phenothiazines is reversed compared to Fig. 8.1A.

TABLE 8.2 Effective mobilities, m_{eff} (10^{-5} cm^2/Vs), for fourteen phenothiazines as a function of pH applying electrolyte systems containing (I) 0 and (II) 50 mg/l FC 135.

Nr.	compound	I	II	II	II	II
		3.5	3.5	5.0	6.2	7.2
1	propiomazine	18.33	18.41	17.72	16.95	17.03
2	ethopropazine	19.17	19.10	18.73	17.74	17.86
3	promethazine	20.50	20.43	20.22	18.98	18.79
4	acetopromazine	19.02	19.16	19.01	17.83	17.59
5	triflupromazine	18.44	18.89	18.82	17.88	18.92
6	promazine	20.73	20.39	20.39	19.17	19.06
7	trimeprazine	19.84	19.60	19.57	18.37	18.36
8	chlorpromazine	19.51	19.64	19.49	18.32	18.23
9	trifluoperazine	22.92	23.59	17.12	15.88	16.69
10	perphenazine	21.53	21.76	16.28	14.56	12.22
11	thioridazine	17.39	17.58	17.61	16.30	16.65
12	prochlorperazine	24.27	24.57	17.54	15.71	14.61
13	fluphenazine	20.73	20.83	15.76	14.41	13.09
14	methotrimeprazine	18.78	18.58	18.64	17.33	17.07

TABLE 8.3 Migration time, t (min), and effective mobility, m_{eff} (10^{-5} cm^2/Vs), with standard deviations (in parentheses) for six phenothiazines dissolved in (I) water and (II) 10-fold diluted human urine. Background electrolyte, 20 mM TRIS/formic acid at pH 3.5, containing 50 mg/l FC 135. *(n=5)*.

compound	I		II	
	t	m_{eff}	t	m_{eff}
EOF	5.44 (0.06)	-70.11 (0.77)	5.54	-68.86
thioridazine	7.26 (0.10)	17.58 (0.09)	7.44	17.59
triflupromazine	7.42 (0.10)	18.69 (0.10)	7.60	18.67
promazine	7.67 (0.11)	20.39 (0.10)	7.87	20.39
perphenazine	7.88 (0.12)	21.71 (0.07)	8.10	21.76
trifluoperazine	8.19 (0.13)	23.51 (0.07)	8.43	23.61
prochlorperazine	8.37 (0.13)	24.51 (0.08)	8.62	24.61

In Table 8.3 all migration times and effective mobilities are listed for five experiments, showing a good repeatability. In order to study the influence of the composition of the sample matrix, experiments were carried out with 10-fold diluted human urine, spiked with the same sample compounds (all 0.1 mg/ml). In Fig. 8.1C the electropherogram for this separation is shown. As can be seen from the results, listed in Table 8.3, comparable migration times and effective mobilities were obtained for both sample mixtures.

DETERMINATION OF PHENOTHIAZINES BY MEKC

As phenothiazines possess hydrophobic properties, MEKC experiments were carried out with the cationic surfactant CTAB, thus introducing a second separation mechanism based on micellar solubilization. For these experiments the migration of the compounds can be described by the overall effective mobility, m_{eff}^{ov}, as described in chapter 3, which is a weighted average of the effective mobility in the aqueous phase, $m_{eff,AQ}$, and the effective mobility of the micelles, m_{MC}, according to eqn. (3.1). In Fig. 8.2 the influence of the CTAB concentration on the overall effective mobility, m_{eff}^{ov}, is illustrated. The electrolyte system consisted of 0.02 M TRIS/acetic acid at pH 5.0 containing 50 mg/l FC 135. As can be seen from Fig. 8.2, optimum resolution is obtained at a CTAB concentration of *ca.* 10 m*M*, although not all compounds can be separated with this electrolyte system.

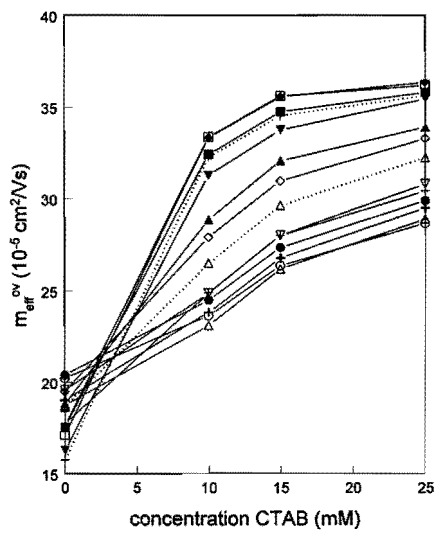

FIGURE 8.2 Overall effective mobility, m_{OV}, *versus* concentration CTAB for (drawn lines) (+) 1, (△) 2, (○) 3, (+) 4, (▲) 5, (●) 6, (▽) 7, (◊) 8, (□) 9, (▾) 10, (◆) 11, (■) 12 and (dashed lines) (+) 13 and (△) 14. See Table 8.2 for the names of the compounds. Background electrolyte, 20 mM TRIS/acetic acid at pH 5.0, containing 50 mg/l FC 135 and different concentrations CTAB.

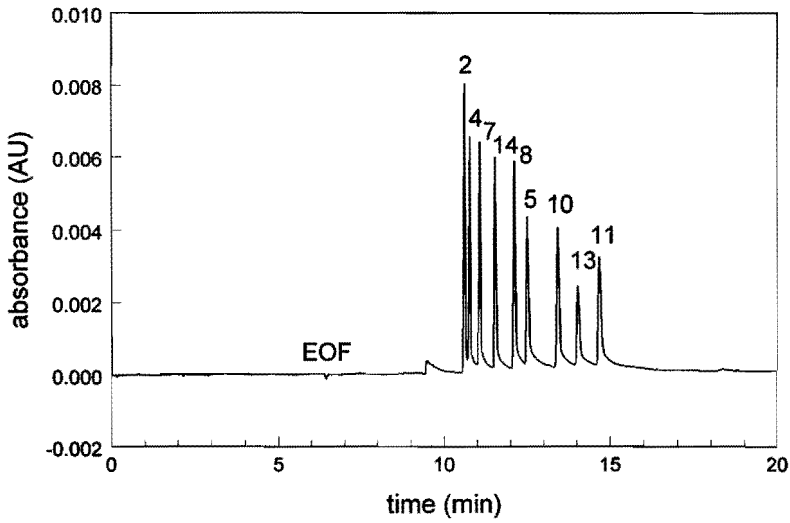

FIGURE 8.3 Electrokinetic chromatogram of the separation of (2) ethopropazine, (4) acetopromazine, (7) trimeprazine, (14) methotrimeprazine, (8) chlorpromazine, (5) triflupromazine, (10) perphenazine, (13) fluphenazine and (11) thioridazine (all 0.1 mg/ml). Background electrolyte, 20 mM TRIS/acetic acid at pH 5.0, containing 50 mg/l FC 135 and 10 mM CTAB.

In Fig. 8.3 an electrokinetic chromatogram is shown for the separation of nine phenothiazines, applying a background electrolyte of 0.02 M TRIS/acetic acid at pH 5.0 containing 50 mg/l FC 135 and 10 mM CTAB. The slightly tailing peaks are probably due to interactions between the sample compounds and a mixed micellar surfactant layer on the capillary wall. In Table 8.4 all migration times and effective mobilities are listed for five experiments, showing a good repeatability. For the MEKC system, also experiments were carried out with 10-fold diluted human urine, spiked with the same sample compounds (all 0.1 mg/ml). Comparable migration times and effective mobilities were obtained for both sample mixtures, which are also listed in Table 8.4.

QUANTITATIVE ASPECTS

In order to determine the quantitative abilities of CZE separations with reversed EOF, calibration graphs were set up for promethazine, triflupromazine, thioridazine and fluphenazine, applying a background electrolyte of 0.02 M TRIS/acetic acid at pH 5.0 containing 50 mg/l FC 135. In Table 8.5 the regression parameters for the calibration graphs are presented, showing a linear relationship between peak area and injected sample concentration.

As an application promethazine and thioridazine were determined in Phenergan and Melleril tablets, respectively. Both samples were measured three times. In Table 8.5 the determined concentrations and standard deviations for both compounds are listed. As can be seen from these results the labelled and measured values agree well.

TABLE 8.4 Migration time, t (min), and effective mobility, m_{eff} (10^{-5} cm^2/Vs), with standard deviations (in parentheses) for nine phenothiazines dissolved in (I) water and (II) 10-fold diluted human urine. Background electrolyte, 20 mM TRIS/acetic acid at pH 5.0, containing 50 mg/l FC 135 and 10 mM CTAB. *(n=5)*.

compound	I		II	
	t	m_{eff}	t	m_{eff}
EOF	6.41 (0.02)	-59.50 (0.20)	6.39	-59.70
ethopropazine	10.52 (0.05)	23.25 (0.05)	10.53	23.47
acetopromazine	10.68 (0.05)	23.78 (0.05)	10.69	24.02
trimeprazine	10.99 (0.05)	24.78 (0.06)	10.99	24.99
methotrimeprazine	11.45 (0.05)	26.18 (0.06)	11.45	26.38
chlorpromazine	12.02 (0.06)	27.75 (0.06)	12.02	27.96
triflupromazine	12.41 (0.06)	28.77 (0.08)	12.41	28.96
perphenazine	13.36 (0.07)	30.95 (0.10)	13.30	31.02
fluphenazine	13.96 (0.08)	32.17 (0.10)	13.88	32.22
thioridazine	14.55 (0.09)	33.27 (0.05)	14.50	33.39

TABLE 8.5 Regression correlation coefficients, r, limits of detection, LOD (μg/ml) for the calibration graphs of four phenothiazines and labelled and measured values with standard deviations (in parentheses) for Melleril and Phenergan tablets. *(n=6, m=3)*.

compound	r	LOD	labelled	measured
fluphenazine	0.99983	2.18		
thioridazine	0.99993	1.36	100	102.06 (0.64)
triflupromazine	0.99996	1.08		
promethazine	0.99999	0.63	25	28.86 (0.08)

8.2.4 CONCLUSIONS

Basic phenothiazine compounds can be successfully determined by capillary zone electrophoresis in the anionic mode with the addition of the cationic fluorinated surfactant FC 135 to the background electrolyte, resulting in a reversed wall charge and a reversed EOF. However, with micellar electrokinetic chromatography, combining both electrophoretic and chromatographic separation principles, a larger difference between the overall effective mobilities can be obtained, resulting in an improved resolution. Nine phenothiazines could be separated applying an electrolyte system of 0.02 M TRIS/acetic acid at pH 5.0 containing 50 mg/l FC 135 and 10 mM CTAB.

The quantitative abilities of CZE with reversed EOF were satisfactory and promethazine and thioridazine could easily be determined in pharmaceutical preparations without any sample pretreatment.

8.3 BENZODIAZEPINES

8.3.1 INTRODUCTION

Benzodiazepines are weak basic pharmaceuticals with hypnotic, tranquillizing and anticonvulsant properties. Quantitative determination of benzodiazepines and their methabolites in biological fluids is important for the investigation of their pharmacokinetics as well as intoxications. General analytical methods for therapeutical and toxicological screening of benzodiazepines include gas chromatography, liquid chromatography and gas chromatography/mass spectrometry [20]. Recently, Thormann *et al.* [21,22] reported the determination of benzodiazepines in human urine by micellar electrokinetic chromatography (MEKC). Bechet *et al.* [23] studied the influence of various experimental conditions on their migration behaviour. In this section the quantitative determination of benzodiazepines in pharmaceutical dosage forms and bovine serum by MEKC is investigated.

8.3.2 EXPERIMENTAL

CHEMICALS

Desmethylflunitrazepam, nitrazepam, flunitrazepam, clonazepam, temazepam, oxazepam, desalkylflurazepam, desmethyldiazepam and diazepam were kindly donated by the pharmacy of a local hospital (Diaconessenhuis, Eindhoven, The Netherlands), sodium dodecylsulphate (SDS) was obtained from Aldrich (Steinheim, Germany), methanol, acetonitrile, boric acid and tris(hydroxymethyl)amminomethane (TRIS) from Merck (Darmstadt, Germany), bovine serum from Sigma (St. Louis, MO, USA) and saline (154 mM NaCl) from NPBI BV

(Emmer Compascuum, The Netherlands). Water was filtered by a Milli-Q water purification system (Waters Millipore, Milford, MA, USA).

INSTRUMENTATION AND SEPARATION CONDITIONS

All experiments were carried out on an HP^{3D} Capillary Electrophoresis instrument (Hewlett Packard, Waldbronn, Germany) with a 75 μm I.D. fused silica capillary (Scientific Glass Engineering, Austin, Texas, USA), total length 64.5 cm, distance between injection and detection 56.0 cm. A constant voltage of 25 kV was applied. The temperature was kept constant at 25°C and the wavelength of the detector was set at 235 nm. For all experiments an electrolyte system of 20 mM TRIS, adjusted to pH 9.0 by adding boric acid was used, containing 25 mM SDS and either 20 % (v/v) methanol or 20 % (v/v) acetonitrile. Samples were introduced by a 2 sec. pressure injection with 50 mbar, unless otherwise noted.

STANDARD SOLUTIONS AND SAMPLE PREPARATION

Stock solutions of the benzodiazepines were prepared by weighing accurately 4.0 mg of the sample compounds and dissolving them in 4.0 ml methanol. These solutions were diluted 100 times with the appropriate electrolyte system to give sample solutions with a final concentration of 0.01 mg/ml.

Seresta and Valium tablets, labelled to contain 10 mg oxazepam and 10 mg diazepam, respectively, were pulverized and dissolved in 10 ml methanol by sonication for 15 min. These solutions were diluted 100 times with the electrolyte system and were used for injection without further pretreatment.

SAMPLE PRETREATMENT

Spiked serum samples were pretreated by a solid phase extraction (SPE) procedure, using C_{18} SPE cartridges (Alltech, Deerfield, IL, USA), containing 200 mg sorbent material with a reservoir volume of 3 ml and a Vac Elut equipment (Analytichem International, Harbor City, CA, USA). An extraction scheme described in detail by Evenson and Wiktorowicz [24] was applied with some minor modifications. The methanol fractions containing the benzodiazepines, collected after the final elution step, were evaporated to dryness under a gentle stream of nitrogen at room temperature and the residues were redissolved in 100 μl of the appropriate electrolyte system.

8.3.3 RESULTS AND DISCUSSION

INFLUENCE OF ORGANIC MODIFIER ON SELECTIVITY

For the determination of benzodiazepines by MEKC, an electrolyte system of 20 mM TRIS/boric acid at pH 9.0, containing 25 mM SDS was selected [23]. At this pH all benzodiazepines are neutral and, consequently, their migration behaviour is merely based on micellar solubilization. Initial experiments demonstrated that some of the benzodiazepines are rather hydrophobic compounds which co-migrate at the migration time of the micelles, even at the relatively low surfactant concentration of 25 mM SDS. Therefore an organic modifier had to be applied, in order to decrease their interaction with the pseudo-stationary micellar phase. To investigate the influence of the type of organic modifier, experiments were carried out in electrolyte systems containing 20 % (v/v) methanol and 20 % (v/v) acetonitrile, respectively. As can be seen from the electrokinetic chromatograms, shown in Fig. 8.4, a markedly difference in selectivity is obtained for (A) methanol and (B) acetonitrile. In chapter 5 it was demonstrated that solute-micelle interactions in SDS micellar systems are mainly determined by the molar volume and the hydrogen bond acceptor ability of the solute. Consequently, the observed differences in selectivity between electrolyte systems containing methanol and acetonitrile may be attributed to differences in hydrogen bonding characteristics of these organic modifiers [25]. In Table 8.6 all pseudo-effective mobilities, m^{ps}_{eff}, calculated according eqn. (1.17), for nine benzodiazepines are listed.

TABLE 8.6 Pseudo-effective mobilities, m^{ps}_{eff} (10^{-5} cm^2/Vs), for nine benzodiazepines in electrolyte systems of 20 mM TRIS/boric acid at pH 9.0, containing 25 mM SDS and 20 % (v/v) methanol or 20 % (v/v) acetonitrile, respectively.

compound	20 % (v/v) MeOH	20 % (v/v) ACN
desmethylflunitrazepam	-19.12 (0.16)	-13.12 (0.35)
nitrazepam	-20.08 (0.16)	-13.84 (0.38)
flunitrazepam	-20.45 (0.16)	-14.38 (0.39)
clonazepam	-21.58 (0.15)	-15.37 (0.41)
temazepam	-25.41 (0.10)	-17.77 (0.54)
oxazepam	-25.70 (0.08)	-18.51 (0.54)
desalkylflurazepam	-25.82 (0.09)	-19.62 (0.55)
desmethyldiazepam	-26.63 (0.08)	-21.09 (0.58)
diazepam	-26.63 (0.08)	-22.00 (0.57)

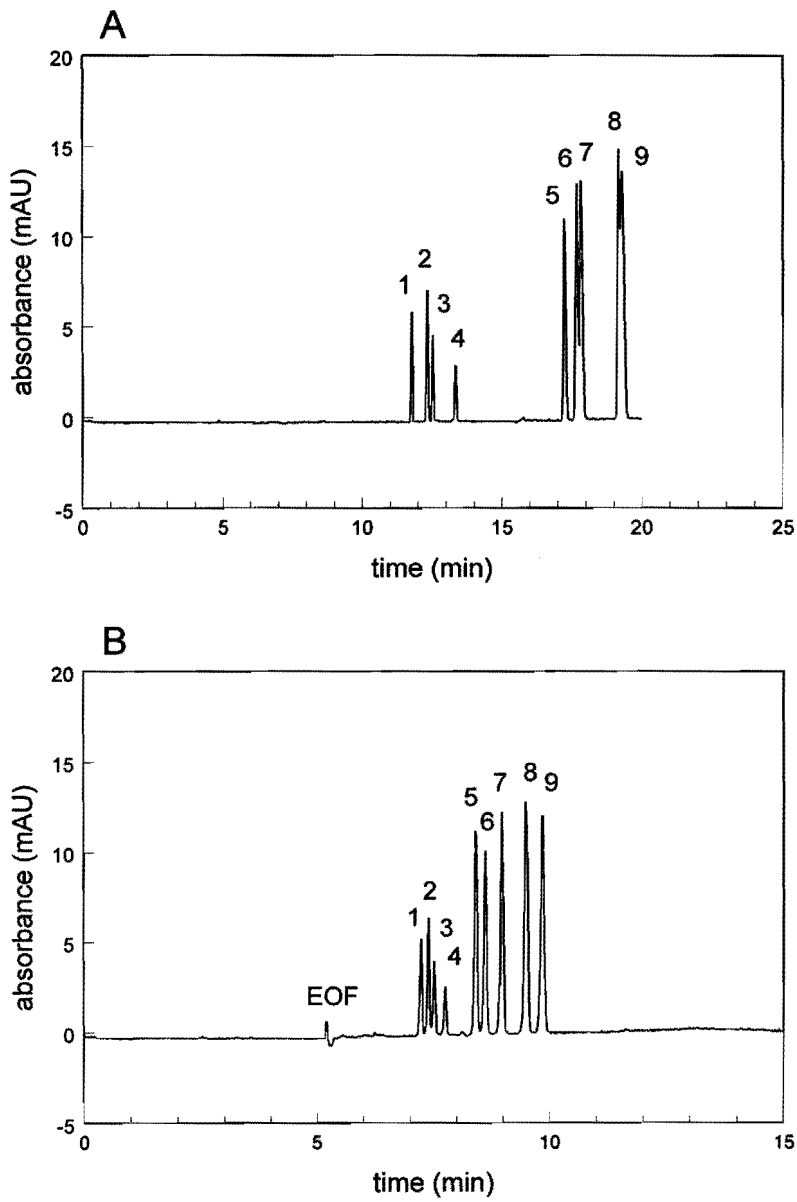

FIGURE 8.4 Electrokinetic chromatograms of the separation of (1) desmethylflunitrazepam, (2) nitrazepam, (3) flunitrazepam, (4) clonazepam, (5) temazepam, (6) oxazepam, (7) desalkylflurazepam, (8) desmethyldiazepam and (9) diazepam in electrolyte systems of 20 mM TRIS/boric acid at pH 9.0, containing 25 mM SDS and (A) 20 % (v/v) methanol or (B) 20 % (v/v) acetonitrile.

Better separations and shorter analysis times were obtained with the electrolyte system containing 20 % (v/v) acetonitrile. However, the electrolyte system containing 20 % methanol (v/v) showed much better repeatabilities. Therefore all other MEKC experiments were performed with an electrolyte system of 20 mM TRIS/boric acid at pH 9.0, containing 25 mM SDS and 20 % (v/v) methanol.

QUANTITATIVE ASPECTS

In order to investigate the quantitative abilities of this method, oxazepam and diazepam were determined in Seresta and Valium tablets, respectively. Since the available sample amount of the standard solutions was limited, calibration graphs were set up with one sample solution, containing 0.01 mg/ml oxazepam and 0.01 mg/ml diazepam. This solution was analyzed several times, applying pressure injection times of 0.5, 1, 2, 3, 4 and 5 sec., respectively. To construct calibration graphs, measured spatial peak areas were plotted against injection time multiplied by the sample concentration [26]. The Seresta and Valium samples were measured three times, applying a pressure injection time of 2 sec. In Table 8.7 the regression parameters for the calibration graphs and the labelled and determined concentrations in the tablets with standard deviations for both compounds are listed. As can be seen from these results, linear calibration graphs were obtained, and the labelled and measured values agree well.

TABLE 8.7 Regression correlation coefficients, r, limits of detection, LOD (μgs/ml) for the calibration graphs of oxazepam and diazepam and labelled and measured values with standard deviations (in parentheses) for Seresta and Valium tablets. *($n=6$, $m=3$).*

compound	r	LOD	labelled	measured
oxazepam	0.99971	1.10	10	10.08 (0.13)
diazepam	0.99988	0.76	10	9.88 (0.23)

DETERMINATION OF BENZODIAZEPINES IN SERUM

Thormann *et. al.* [27] described the direct injection of serum samples for therapeutic drug monitoring with MEKC. However, this method is only applicable for relatively hydrophylic compounds which migrate in front of the serum proteins. Moreover, serum proteins may have a dramatic effect on the electroosmotic flow and, consequently, on migration time repeatabilities [28]. In order to remove the serum proteins from the sample matrix, an SPE sample pretreatment was applied as described in the experimental section 8.3.2. As an

example in Fig. 8.5 an electrokinetic chromatogram is shown for a 200 μl serum sample, spiked with nitrazepam, clonazepam, oxazepam and diazepam at a concentration level of 0.01 mg/ml. The sample compounds were finally redissolved in 100 μl of the applied electrolyte system. To investigate the quantitative abilities of this SPE procedure, 100 μl of the electrolyte system, spiked with the same four benzodiazepines at 0.01 mg/ml, was extracted. As can be seen from the results, listed in Table 8.8, the recoveries and repeatabilities for this sample clean-up procedure are acceptable. The pseudo-effective mobilities are comparable to those listed in Table 8.6 and show a good repeatability. This method would be applicable for toxicological screening of, *e.g.*, oxazepam and diazepam, which may occur at high concentration levels in human serum. However, for therapeutic screening purposes the detection limits are far too high. Therapeutic concentration levels of benzodiazepines in human serum vary between *ca.* 20 and 500 μg/l. Hence for a quantitative determination of benzodiazepines in clinical samples, a high pre-concentration would be required and, consequently, a large sample amount. In practice, only small sample amounts are available in clinical pharmacology, which makes this method less applicable for clinical samples from a practical point of view.

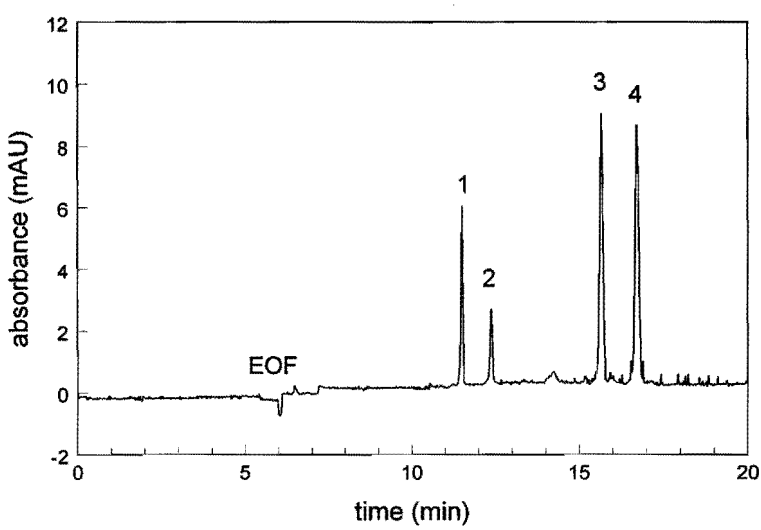

FIGURE 8.5 Electrokinetic chromatogram after SPE extraction of a 200 μl serum sample, spiked with (1) nitrazepam, (2) clonazepam, (3) oxazepam and (4) diazepam, all at a concentration level of 0.01 mg/ml.

TABLE 8.8 Pseudo-effective mobility, m_{eff}^{ps} (10^{-5} cm^2/Vs), with standard deviations (in parentheses) for four benzodiazepines in bovine serum, and recovery (%) with relative standard deviation (RSD %) of the SPE procedure. *(n=3)*.

compound	m_{eff}^{ps}	recovery	RSD
nitrazepam	-19.25 (0.03)	89	3.4
clonazepam	-20.74 (0.03)	87	5.8
oxazepam	-24.84 (0.02)	84	1.3
diazepam	-25.83 (0.02)	85	3.6

8.3.4 CONCLUSIONS

Micellar electrokinetic chromatography was shown to be a suitable analytical technique for the determination of benzodiazepines. Due to the hydrophobic character of some benzodiazepines, an electrolyte system with a relative low surfactant concentration and an organic modifier has to be applied. Remarkable differences in selectivity were observed between methanol and acetonitrile as organic modifiers, probably due to different hydrogen bonding characteristics of these solvents. Shorter analysis times and higher resolutions were obtained with an electrolyte system containing 20 % (v/v) acetonitrile. However, better repeatabilities were observed applying an electrolyte system containing 20 % (v/v) methanol. The quantitative abilities of MEKC were satisfactory, allowing a rapid determination of oxazepam and diazepam in pharmaceutical formulations. After an SPE sample pretreatment, four benzodiazepines were determined in bovine serum samples, thus illustrating the possibilities of MEKC as toxicological screening method. However, for the determination of benzodiazepines on a therapeutic concentration level the detection limits are far too high.

8.4 ANTIEPILEPTIC DRUGS

8.4.1 INTRODUCTION

Antiepileptic drugs cover a wide group of pharmaceuticals with different characteristics, used for the treatment of epilepsy in drug therapy. For the optimization of pharmacotherapy, accurate quantitative determinations of antiepileptic drugs in blood are required. Consequently, therapeutic drug monitoring plays an important role in fields like epilepsy, where life-long multiple-drug therapy is common. Generally, gas chromatography and liquid chromatography are utilized for the determination of antiepileptic drugs and their metabolites [29]. Also immunological methods are applied for routine screening purposes. Recently, Lee

et al. [30] reported the separation of six antiepileptic drugs by micellar electrokinetic chromatography (MEKC). Since the concentration levels of antiepileptic drugs in clinical samples are relatively high, MEKC could be utilized for the separation and identification of these drugs without preconcentration. In this section the applicabilaty of MEKC for the qualitative and quantitative determination of several antiepileptic drugs in pharmaceutical dosage forms and in serum at therapeutic concentration levels is studied.

8.4.2 EXPERIMENTAL

CHEMICALS

Ethosuximide, phenobarbital, phenytoin, carbamazepine, carbamazepine epoxide, valproic acid, Extrelut (Merck, Darmstadt, Germany) and reference serum (KKGT, 's-Gravenhage, The Netherlands) were kindly donated by the pharmacy of a local hospital (Centrale Ziekenhuisapotheek, 's-Hertogenbosch, The Netherlands), sodium dodecyl sulphate (SDS) was obtained from Aldrich (Steinheim, Germany), boric acid, tris(hydroxymethyl)-amminomethane (TRIS) and morpholinopropanesulphonic acid (MOPS) from Merck (Darmstadt, Germany). Water was filtered by a Milli-Q water purification system (Waters Millipore, Milford, MA, USA).

INSTRUMENTATION AND SEPARATION CONDITIONS

The instrumentation was identical as described in section 8.3.2. The detector was operated in the single wavelength mode at 200 nm or in the diode-array mode for spectral recognition. For all experiments an electrolyte system of 20 mM TRIS, adjusted to pH 8.1 by adding boric acid was used, containing 30 mM SDS. Samples were introduced by a 2 sec. pressure injection with 50 mbar, unless otherwise noted.

STANDARD SOLUTIONS AND SAMPLE PREPARATION

Stock solutions of the antiepileptic drugs were prepared by weighing accurately 4.0 mg of the drugs and dissolving them in 4.0 ml methanol. These solutions were diluted 100 times with the appropriate electrolyte system to give sample solutions with a final concentration of 0.01 mg/ml.

Carbamazepine and Diphantoine tablets, labelled to contain 200 mg carbamazepine and 46 mg phenytoin, respectively, were pulverized and dissolved in 200 ml and 50 ml methanol, respectively, by sonication for 15 min. These solutions were diluted 100 times with the applied electrolyte system and were used for injection without further pretreatment.

SAMPLE PRETREATMENT

Reference serum, containing a known relevant amount of antiepileptic drugs, was pretreated, applying a liquid-solid extraction with laboratory-made Extrelut columns. A 100 μl sample, spiked with an internal standard, was brought onto the column. The drugs were eluted with 5 ml ether. The ether fraction was evaporated to dryness under a gentle stream of nitrogen at room temperature and the residues were redissolved in 100 μl of the appropriate electrolyte system.

8.4.3 RESULTS AND DISCUSSION

SELECTION OF THE ELECTROLYTE SYSTEM

The antiepileptic drugs, investigated in this study, possess different physical properties such as solubility, pK_a-values and UV absorbance. Since MEKC is a suitable analytical method for the separation of mixtures of both charged and uncharged compounds, an MEKC electrolyte system was selected for the separation of these drugs. In Fig. 8.6 an electrokinetic chromatogram is shown of the separation of five antiepileptic drugs and aprobarbital in an electrolyte system of 20 mM TRIS/boric acid at pH 8.1, containing 30 mM SDS. Aprobarbital was applied as internal standard (I.S.) for the quantitative analyses in reference serum. Valproic acid could not be determined with this electrolyte system, due to the low UV absorbing properties of this compound. Therefore, valproic acid was determined separately by capillary zone electrophoresis (CZE) in the indirect UV mode, applying an electrolyte system of 20 mM TRIS/MOPS at pH 8.2. In Table 8.9 overall effective mobilities, m_{eff}^{ov}, calculated according eqn. (3.2) for all compounds are listed, showing good repeatabilities.

QUANTITATIVE ASPECTS

In order to study the quantitative abilities of this method, carbamazepine and phenytoin were determined in Carbamazepine and Diphantoine tablets, respectively. Calibration graphs were set up with one sample solution, containing 0.01 mg/ml carbamazepine and 0.01 mg/ml phenytoin, using the same procedure as described in section 8.3.3. The Carbamazepine and Diphantoine samples were measured three times, applying a pressure injection time of 2 sec. Linear calibration graphs were obtained for both compounds and the labelled and measured values agree well, as can be seen from the results, listed in Table 8.10.

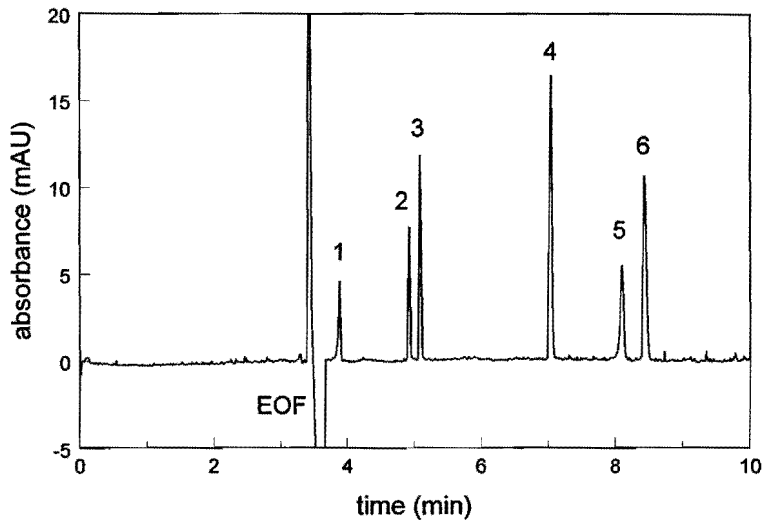

FIGURE 8.6 Electrokinetic chromatogram of the separation of (1) ethosuximide, (2) aprobarbital, (3) phenobarbital, (4) phenytoin, (5) carbamazepine and (6) carbamazepine epoxide in an electrolyte systems of 20 mM TRIS/boric acid at pH 8.1, containing 30 mM SDS.

TABLE 8.9 Overall effective mobilities, m_{eff}^{ov} (10^{-5} cm^2/Vs), with standard deviations (in parentheses) for six antiepileptic drugs and aprobarbital (I.S.) in an electrolyte system of 20 mM TRIS/boric acid at pH 8.1, containing 30 mM SDS.

compound	m_{eff}^{ov}
ethosuximide	-8.36 (0.04)
aprobarbital	-21.53 (0.15)
phenobarbital	-23.23 (0.19)
phenytoin	-36.55 (0.10)
carbamazepine	-40.80 (0.04)
carbamazepine epoxide	-41.98 (0.06)
valproic acid[a]	-23.65 (0.13)

[a]Determined by CZE with indirect UV detection, applying an electrolyte system of 20 mM TRIS/MOPS at pH 8.2.

TABLE 8.10 Regression correlation coefficients, r, limits of detection, LOD (μgs/ml) for the calibration graphs of carbamazepine and phenytoin and labelled and measured values with standard deviations (in parentheses) for Carbamazepine and Diphantoine tablets. *($n=6$, $m=3$).*

compound	r	LOD	labelled	measured
carbamazepine	0.99956	5.28	200	212 (1.72)
phenytoin	0.99980	3.78	46	48 (0.92)

TABLE 8.11 Labelled and measured concentrations (mg/l) with relative standard deviations (RSD %) of five antiepileptic drugs reference serum. Electrolyte system, 20 mM TRIS/boric acid at pH 8.1, containing 30 mM SDS. *($n=3$).*

compound	labelled	measured	RSD
ethosuximide	49.65	28	13
phenobarbital	27.76	31	10
phenytoin	13.14	13	6
carbamazepine	7.01	13	19
valproic acid[a]	74.60	4	10

[a]Determined by CZE with indirect UV detection, applying an electrolyte system of 20 mM TRIS/MOPS at pH 8.2.

DETERMINATION OF ANTIEPILEPTIC DRUGS IN SERUM

For the identification and quantification of antiepileptic drugs in reference serum, a liquid-solid extraction sample clean-up was carried out, in order to remove serum proteins from the sample matrix. As an example, in Fig. 8.7 an electrokinetic chromatogram of the reference serum is shown, illustrating that a sufficient clean sample is obtained with this relative simple extraction procedure. For the conformation of peak identities, these experiments were carried out with diode-array detection (DAD). The recorded spectra were compared with a spectral library, built up by the analyses of the separate compounds with a 5 sec. pressure injection. The drugs could easily be identified with match factors larger than 994. In Fig. 8.8 these spectra are shown for ethosuximide, phenobarbital, phenytoin and carbamazepine, respectively. Since carbamazepine epoxide co-migrates with an unknown compound, still present in the sample, it could not be identified (see Fig. 8.7).

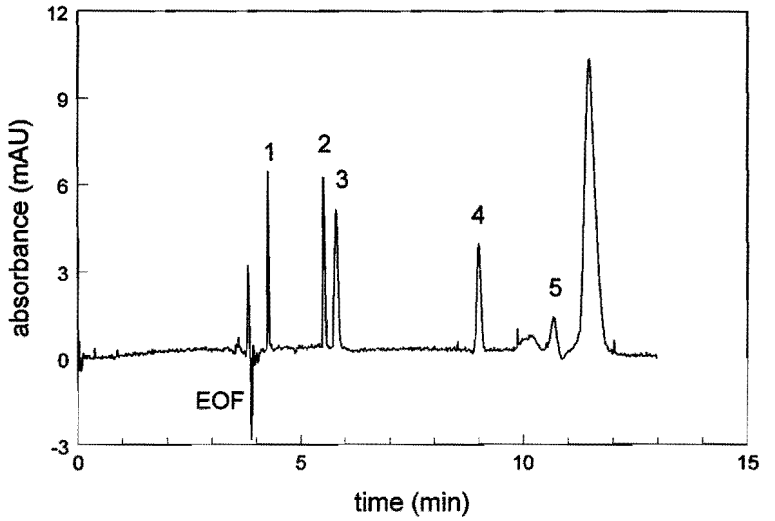

FIGURE 8.7 Electrokinetic chromatogram of (1) ethosuximide, (2) aprobarbital (I.S.), (3) phenobarbital, (4) phenytoin, and (5) carbamazepine in a reference serum sample. Electrolyte system, 20 mM TRIS/boric acid at pH 8.1, containing 30 mM SDS.

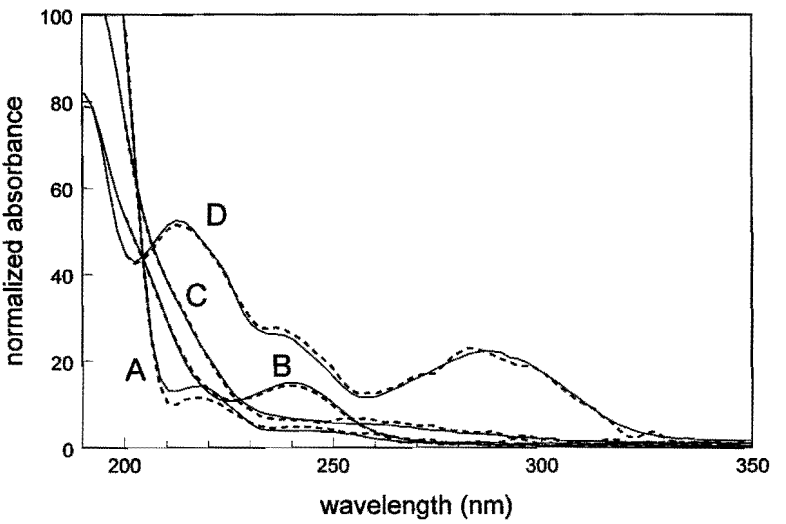

FIGURE 8.8 Normalized spectra of (A) ethosuximide, (B) phenobarbital, (C) phenytoin, and (D) carbamazepine. Drawn lines are from the spectral library and dotted lines from the electrokinetic chromatogram, shown in FIGURE 8.7.

In Table 8.11 the results for the quantitative determination of antiepileptic drugs in reference serum are presented. Three 100 μl samples were measured, applying a new prepared extraction column each time. The recovery for the extraction procedure, calculated from the spatial peak area of aprobarbital (I.S.) was acceptable (99 %), although the reproducibility (13 % RSD) indicates that an internal standard is required. The quantitative determination of phenobarbital and phenytoin is satisfactory. For carbamazepine the determined concentration is too high and less reproducible, probably due to difficulties in integrating small peaks. (see Fig. 8.7). For ethosuximide and valproic acid much lower concentrations were determined, which may be attributed to a low recovery and/or evaporative loss of these compounds during the extraction procedure [29,30].

8.4.4 CONCLUSIONS

It was demonstrated that micellar electrokinetic chromatography is an efficient method for the determination of antiepileptic drugs in pharmaceutical preparations and in serum. Five antiepileptic drugs could be rapidly separated, applying an electrolyte system of 20 mM TRIS/boric acid at pH 8.1, containing 30 mM SDS. The qualitative and quantitative abilities of this method were demonstrated by the analysis of reference serum, containing known amounts of several antiepileptic drugs. Proteins were removed from the sample matrix by an extraction procedure. Four antiepileptic drugs could be identified via spectral analysis, applying DAD detection. The quantitative determination of phenobarbital and phenytoin was satisfactory. For ethosuximide and valproic acid, however, quantification was hampered by loss of these compounds during the sample pretreatment procedure. These results clearly illustrate the possibilities of MEKC in therapeutic drug monitoring of antiepileptics. However, here it should be emphasized that in this respect a sufficient high concentration level of the sample compounds is a precondition.

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ABSTRACT

Micellar Electrokinetic Chromatography (MEKC) is an efficient analytical technique, developed at the crossroads of chromatography and electrophoresis. In MEKC neutral compounds can be separated in an electric field, based on differences in distribution equilibria of individual compounds between an aqueous phase and a pseudo-stationary micellar phase. These phases are moving with different velocities according to electroosmotic and electrophoretic transport phenomena. In addition, also charged compounds or mixtures of charged and uncharged compounds can be analyzed. In this case separations are based on differences in phase distribution as well as differences in electrophoretic migration. The composition of the applied electrolyte system and, in particular, the chemical nature of the pseudo-stationary phase, can easily be changed in order to control migration behaviour and optimize selectivity. Although MEKC continues to gain popularity in analytical chemistry, the exact role of many experimental parameters in migration and selectivity mechanisms still remains unexplained. It is the aim of this thesis to increase the knowledge about various fundamental aspects of the separation process in MEKC, and to study how migration and selectivity mechanisms can be described.

The separation mechanism of neutral compounds is essentially based on chromatographic principles. Since two moving phases can be distinguished, generally a limited elution window is observed and, as a consequence, modified chromatographic relationships have to be applied. The existence of a limited elution window has a major impact on the resolution equation in MEKC. In chapter 2 a detailed theoretical description is given about the influence of elution window and retention factors on efficiency as well as resolution. The effect of various experimental conditions on the elution window and retention factors is evaluated extensively and it is demonstrated that, although these variables often can not be controlled independently, resolution can be optimized by adjusting the composition of the applied electrolyte system.

The separation mechanism of charged compounds is based on chromatographic as well as electrophoretic principles. Consequently, the migration of charged compounds can be described in terms of retention or in terms of mobility. In chapter 3 a retention model and a mobility model are presented, which describe the migration behaviour of charged compounds in MEKC using micelle binding constants and apparent dissociation constants in micellar media. A comparison is made between both models by the investigation of the migration behaviour of monovalent weak acids in an SDS micellar system as a function of buffer pH and surfactant concentration. It is shown that effective mobilities in the aqueous phase may be influenced by interaction of the undissociated form of the acid with surfactant molecules. This phenomenon will have a marked influence on the calculation of retention factors. Therefore the mobility model is to be preferred. Moreover, mobilities are more directly related to migration velocities than retention factors, which makes the overall effective mobility a more representative migration parameter.

The results of a fundamental study on the application of the retention index concept in MEKC are presented in chapter 4. Since the retention index is a relative quantity, retention indexes are independent of the surfactant concentration, whereas retention factors are linearly related to the surfactant concentration. The relationship between retention indexes and *n*-octanol-water partition coefficients provides information about micellar solubilization. Observed differences in this relationship between polar and non polar compounds demonstrate that, in accordance with common belief, these compounds are solubilized in different ways. It is shown that retention indexes, obtained with different surfactant systems, can be applied to study solute-micelle interaction phenomena.

In chapter 5 the influence of different solute-micelle interactions on selectivity is studied by linear solvation energy relationships. For six anionic micellar systems it is shown that the migration behaviour of neutral compounds in MEKC is mainly determined by their molar volume and their hydrogen bond acceptor ability. Large differences in selectivity are observed between SDS and mixed SDS/Brij 35 micellar systems, due to their different hydrogen bonding abilities. Retention indexes can be applied for a quantitative characterization of micellar pseudo-stationary phases in MEKC in a similar way as the Rohrschneider-McReynolds scale in gas chromatography. For three anionic and two cationic micellar systems it is illustrated that this method facilitates a classification of micellar pseudo-stationary phases according to their hydrogen bonding characteristics with a limited number of experiments.

The use of two alternative types of pseudo-stationary phases in electrokinetic chromatography is evaluated in chapter 6. Diaminobutane-based dendrimers are shown to have specific selectivity properties compared to SDS micelles. Due to tertiary amines present in the dendrimer interior, differences in interaction and migration behaviour are observed for hydrogen bond donating compounds. Electrokinetic chromatography may be a suitable technique to study interaction phenomena of small organic molecules with macromolecular structures like dendrimers.
Tetraalkylammonium ions are shown to be suitable pseudo-stationary phases for the separation of highly hydrophobic compounds in aqueous/organic media. The direction of the electroosmotic flow and, as a consequence, the migration behaviour of the compounds strongly depend on the composition of the electrolyte system. The potential of tetraalkylammonium pseudo-stationary phases is illustrated by the separation of several geometric isomers of polycyclic aromatic hydrocarbons.

Several physico-chemical properties of surfactant solutions, relevant for MEKC, are studied in chapter 7. Individual diffusion coefficients in the aqueous as well as in the micellar phase were determined by MEKC experiments. Micellar diffusion was found to be one order of magnitude lower than diffusion in the aqueous phase. Under practical MEKC conditions the contribution of micellar diffusion to solute band broadening is of minor importance.
The migration behaviour of micelle counterions is discussed and it is shown that, due to a high degree of counterion binding to micelles, sodium ions possess a negative effective mobility in commonly applied SDS surfactant systems.

To illustrate the potential of MEKC in pharmaceutical analyses, several drugs were determined in various sample matrixes. Three groups of drugs were selected, possessing different physical properties. In chapter 8 the quantitative determination of phenothiazines, benzodiazepines and antiepileptics in pharmaceutical formulations is shown to be satisfactory.
Due to the relative high detection limits with on-column UV detection, the screening possibilities of MEKC for the determination of drugs in serum are limited. After an appropriate sample pretreatment procedure to remove serum proteins, benzodiazepines could be determined at toxicologic concentration levels whereas antiepileptic drugs could be determined at therapeutic concentration levels.

SAMENVATTING

Micellaire Electrokinetische Chromatografie (MEKC) is een efficiënte analysetechniek die ontwikkeld is op het kruispunt van chromatografie en electroforese. In MEKC kunnen neutrale componenten worden gescheiden in een electrisch veld, gebaseerd op verschillen in verdelingsevenwicht van de afzonderlijke componenten tussen een waterfase en een pseudo-stationaire micellaire fase. Deze fasen bewegen met verschillende snelheden volgens electroosmotische en electroforetische transportverschijnselen. Naast neutrale componenten kunnen ook geladen componenten of mengsels van geladen en ongeladen componenten worden geanalyseerd. In dit geval is de scheiding gebaseerd op zowel verschillen in verdelingsevenwicht als verschillen in electroforetische migratie. De samenstelling van het electroliet systeem en met name het type pseudo-stationaire fase kan eenvoudig worden gevarieerd teneinde het migratiegedrag te beïnvloeden en de selectiviteit te optimaliseren. Hoewel MEKC aan populariteit wint in de analytische chemie, is de rol van verschillende experimentele parameters in migratie- en selectiviteitsmechanismen nog onduidelijk. Het doel van dit proefschrift is om de kennis van verschillende fundamentele aspecten van het scheidingsproces in MEKC te vergroten en te bestuderen hoe migratie- en selectiviteits-mechanismen kunnen worden beschreven.

Het scheidingsmechanisme van neutrale componenten is gebaseerd op chromatografische principes. Aangezien beide fasen bewegen, is er in de meeste gevallen sprake van een gelimiteerd elutie window en dienen gemodificeerde chromatografische vergelijkingen te worden gebruikt. Dit gelimiteerde elutie window heeft een belangrijke invloed op de resolutievergelijking in MEKC. In hoofdstuk 2 wordt een uitgebreide theoretische beschrijving gegeven over de invloed van het elutie window en de retentie factoren op zowel de efficiency als de resolutie. Het effect van verschillende experimentele condities op het elutie window en de retentiefactoren wordt uitgebreid behandeld en er wordt aangetoond dat hoewel deze variabelen vaak niet onafhankelijk kunnen worden gevarieerd, de resolutie

geoptimaliseerd kan worden door het aanpassen van de samenstelling van het gebruikte electroliet systeem.

Het scheidingsmechanisme van geladen componenten is gebaseerd op zowel chromatografische als electroforetische principes. Derhalve kan de migratie van geladen componenten worden beschreven in de vorm van retentie of mobiliteit. In hoofdstuk 3 worden een retentiemodel en een mobiliteitsmodel gepresenteerd die het migratiegedrag van geladen componenten in MEKC beschrijven met behulp van micel bindingsconstanten en schijnbare dissociatieconstanten in micellaire media. Beide modellen worden vergeleken door het migratiegedrag van eenwaardig zwakke zuren in een SDS micellair systeem als functie van de pH en de surfactant concentratie te bestuderen. Uit het onderzoek blijkt dat de effectieve mobiliteit in de waterfase beïnvloed kan worden door interactie van de ongedissocieerde vorm van het zuur met surfactant moleculen. Aangezien dit verschijnsel een grote invloed heeft op de berekening van retentiefactoren verdient het mobiliteitsmodel de voorkeur. Bovendien zijn mobiliteiten, in tegenstelling tot retentiefactoren, direct gerelateerd aan migratiesnelheden, waardoor de overall effectieve mobiliteit een inzichtelijker migratieparameter is.

De resultaten van een fundamentele studie over de toepassing van het retentie index concept in MEKC worden beschreven in hoofdstuk 4. Aangezien de retentie index een relatieve grootheid is, zijn retentie indices onafhankelijk van de surfactant concentratie. Retentiefactoren daarentegen zijn lineair afhankelijk van de surfactant concentratie. Uit de relatie tussen retentie indices en *n*-octanol-water verdelingscoëfficiënten kan informatie worden verkregen over solute-micel interacties. Verschillen in deze relatie tussen polaire en apolaire componenten laten zien dat, in overeenstemming met wat algemeen wordt aangenomen, deze componenten zich op verschillende plaatsen in de micel bevinden. Daarnaast wordt uiteengezet dat retentie indices, verkregen met verschillende surfactant systemen, kunnen worden toegepast om solute-micel interacties te bestuderen.

In hoofdstuk 5 wordt de invloed van verschillende solute-micel interacties op de selectiviteit bestudeerd voor zes anionische micellaire systemen met behulp van 'linear solvation energy relationships'. Hieruit blijkt dat het migratiegedrag van neutrale componenten in MEKC voornamelijk bepaald wordt door hun molair volume en hun vermogen om op te treden als waterstofbrug acceptor. Grote verschillen in selectiviteit worden waargenomen tussen SDS en gemengde SDS/Brij 35 micellaire systemen, wat kan worden toegeschreven aan verschillen in waterstofbrugvorming. Retentie indices kunnen worden toegepast voor de

kwantitatieve karakterisering van micellaire pseudo-stationaire fasen in MEKC op eenzelfde manier als de Rohrschneider-McReynolds schaal in gas chromatografie. Voor drie anionische en twee cationische micellaire systemen wordt geïllustreerd dat deze methode het mogelijk maakt om micellaire pseudo-stationaire fasen te klassificeren naar hun vermogen tot waterstofbrugvorming met een gering aantal experimenten.

De toepassing van twee alternatieve typen pseudo-stationaire fasen in electrokinetische chromatografie wordt beschreven in hoofdstuk 6. Op diaminobutaan gebaseerde dendrimeren vertonen een specifieke selectiviteit ten opzichte van SDS micellen. Door de aanwezigheid van tertiare amines in het dendrimeer worden verschillen in interactie en migratiegedrag waargenomen voor waterstofbrug donerende componenten. Deze experimenten tonen aan dat electrokinetische chromatografie een bruikbare techniek is om interacties van kleine organische moleculen met macromoleculaire structuren zoals dendrimeren te bestuderen.
Daarnaast worden tetraalkylammonium ionen als pseudo-stationaire fasen gebruikt voor de scheiding van zeer hydrofobe componenten in waterige/organische media. De richting van de electroosmotische flow en het migratiegedrag van de componenten worden in sterke mate bepaald door de samenstelling van het electroliet systeem. De toepassingsmogelijkheden van tetraalkylammonium pseudo-stationaire fasen worden geïllustreerd door de scheiding van enkele geometrische isomeren van polycyclische aromatische koolwaterstoffen.

In hoofdstuk 7 worden een aantal voor MEKC relevante fysisch-chemische eigenschappen van surfactant oplossingen bestudeerd. Afzonderlijke diffusiecoëfficiënten in de waterfase en de micellaire fase zijn bepaald door middel van MEKC experimenten. De resultaten tonen aan dat diffusie in de micellaire fase een orde van grootte lager is dan diffusie in de waterfase. Onder praktische MEKC condities is de bijdrage van micellaire diffusie aan de totale bandverbreding zeer gering.
Daarnaast wordt het migratiegedrag van micel tegenionen gedetailleerd beschreven. Aangetoond wordt dat door een hoge mate van binding van tegenionen aan micellen natrium ionen een negatieve effectieve mobiliteit bezitten in algemeen toegepaste SDS surfactant systemen.

Om de toepasbaarheid van MEKC in pharmaceutische analyses te illustreren zijn een aantal geneesmiddelen bepaald in verschillende relevante matrices. Drie groepen geneesmiddelen werden geselecteerd met verschillende fysische eigenschappen. In hoofdstuk 8 wordt de kwantitatieve bepaling van phenothiazines, benzodiazepines en antiepileptica in diverse farmaceutische preparaten beschreven. Door de relatief hoge detectiegrens met UV detectie

zijn de mogelijkheden om MEKC als screeningsmethode toe te passen voor de bepaling van geneesmiddelen in serum beperkt. Na een geschikte monstervoorbewerkingsprocedure om serum eiwitten te verwijderen konden benzodiazepines op toxicologische concentratieniveaus en antiepileptica op therapeutische concentratieniveaus worden bepaald.

SYMBOLS

a	system coefficient LSER	[-]
	constant	[-]
A	absorbance	[AU]
	constant	[-]
	amphiphilic ion	[-]
A_S	spatial peak area	[AU]
A_T	temporal peak area	[AU.s]
b	system coefficient LSER	[-]
	constant	[-]
B	viscosity dependence on temperature	[K]
	constant	[-]
c	concentration	[mole.m^{-3}]
C	concentration	[mole.m^{-3}]
	constant	[-]
d	intermicelle distance	[m]
D	diffusion coefficient	[m^2.s^{-1}]
E	electric field strength	[V.m^{-1}]
F	Faraday constant	[C.mole^{-1}]
F_e	electric force	[N]
F_f	friction force	[N]
ΔG^0	Gibbs free energy	[J.mole^{-1}]
H	plate height	[m]
ΔH^0	standard enthalpy	[J.mole^{-1}]
i_i	current carried by ion i	[A]
I	retention index	[-]
	current	[A]
I_0	regression constant (eqn. 5.2)	[-]
k	retention factor	[-]
k_d	desorption rate constant	[s^{-1}]
k_{opt}	optimum retention factor	[-]
K	distribution constant	[-]
K^m	binding constant to micelles	[-]
K_a	dissociation constant	[-]
$K_{a,app}$	apparent dissociation constant in micellar media	[-]
l	effective pathlength	[m]
l_c	total capillary length	[m]
l_d	effective capillary length	[m]
P_{OW}	*n*-octanol-water partition coefficient	[-]
m	system coefficient LSER	[-]
	number of counterions bound to micelle	[-]
	number of readings	[-]
m^0	mobility at infinite dilution	[m^2.V^{-1}.s^{-1}]
m_{eff}	effective mobility	[m^2.V^{-1}.s^{-1}]

m_{EOF}	electroosmotic mobility	[$m^2.V^{-1}.s^{-1}$]
m_i	mobility at finite dilution of subspecies i	[$m^2.V^{-1}.s^{-1}$]
m_{MC}	effective mobility micelles	[$m^2.V^{-1}.s^{-1}$]
M	concentration surfactant present as micelles	[$mole.m^{-3}$]
	counterion	[-]
n	aggregation number	[-]
	number of measurements	[-]
	number of moles	[mole]
N	theoretical plate number	[-]
p	constant	[-]
q	net charge	[C]
	constant	[-]
Q_{inj}	injected sample amount	[mole]
r	effective hydrodynamic radius	[m]
	regression correlation coefficient	[-]
r_c	capillary radius	[m]
R	gas constant	[$J.mole^{-1}.K^{-1}$]
R_S	resolution	[-]
s	system coefficient LSER	[-]
ΔS^0	standard entropy	[$J.mole^{-1}.K^{-1}$]
SP	solute property	[-]
SP_0	regression constant (eqn. 5.1)	[-]
t	migration time	[s]
	transport number	[-]
T	absolute temperature	[K]
v	linear velocity	[$m.s^{-1}$]
$\overline{v}$	partial molar volume micelles	[$m^3.mole^{-1}$]
V	voltage	[V]
	volume	[m^3]
	molar volume solute	[$m^3.mole^{-1}$]
$w_{0.5}$	peak width at half height	[s]
x	constant	[-]
y	constant	[-]
z	number of carbon atoms	[-]
	charge number	[-]

Greek

α	degree of dissociation	[-]
	selectivity factor	[-]
	hydrogen bond donor ability solute	[-]
β	phase ratio	[-]
	hydrogen bond acceptor ability solute	[-]

ε	dielectric constant	$[C^2.J^{-1}.m^{-1}]$
	molar absorptivity	$[m^3.cm^{-1}.mole^{-1}]$
ζ	zeta potential	[V]
η	viscosity	$[N.s.m^{-2}]$
θ	degree of counterion binding	[-]
κ	specific conductivity	$[\Omega^{-1}.m^{-1}]$
λ_T	thermal conductivity	$[W.m^{-1}.K^{-1}]$
λ	molar ionic conductivity of an ionic species	$[m^2.\Omega^{-1}.mole^{-1}]$
λ^0	molar ionic conductivity of an ionic species at infinite dilution	$[m^2.\Omega^{-1}.mole^{-1}]$
π	dipolarity/polarizability solute	[-]
σ_s	spatial peak variance	[m]
σ_t	temporal peak variance	[s]
ϕ	volume fraction organic solvent	[-]

Superscript

ov	overall
ps	pseudo
z	number of carbon atoms

Subscript

A⁻	acid in dissociated form
AQ	aqueous phase
	due to intermicelle mass transfer
C	capillary
D	due to longitudinal diffusion
EP	due to micelle microheterogeneity
HA	acid in undissociated form
i	concerning subspecies i
MC	micellar phase
	due to sorption/desorption kinetics
OV	overall
S	solute
SF	surfactant
	surfactant system
T	due to temperature gradients
z	number of carbon atoms
1	first compound
2	second compound

ABBREVIATIONS

BGE	background electrolyte
Brij 35	polyoxyethylene-23-lauryl ether
CAPS	3-cyclohexylamino-1-propanesulphonic acid
CGE	capillary gel electrophoresis
CIEF	capillary isoelectric focussing
CITP	capillary isotachophoresis
CMC	critical micelle concentration
CTAB	cetyltrimethylammonium bromide
CZE	capillary zone electrophoresis
DAB	diaminobutane
DAD	diode array detection
DEA	diethanolamine
DS	dodecylsulphate
DTAB	dodecyltrimethylammonium bromide
EKC	electrokinetic chromatography
EOF	electroosmotic flow
FC 135	fluorinated alkyl quaternary ammonium iodide
GC	gas chromatography
HBA	hydrogen bond acceptor
HBD	hydrogen bond donor
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid
I.D.	internal diameter
I.S.	internal standard
LC	liquid chromatography
LOD	limit of detection
LSER	linear solvation energy relationship
MEKC	micellar electrokinetic chromatoraphy
MES	2-(N-morpholino)ethanesulphonic acid
MOPS	morpholinopropanesulphonic acid
PAH	polyaromatic hydrocarbon
psi	pounds per square inch
QSAR	quantitative structure-activity relationship
RSD	relative standard deviation
SC	sodium cholate
S.D.	standard deviation
SDC	sodium deoxy cholate
SDS	sodium dodecylsulphate
SDSo	sodium dodecylsulphonate
SN	separation number
SPE	solid phase extraction
TAA	tetraalkylammonium
TDS	TRIS dodecylsulphate
TEA	triethanolamine
THA	tetrahexylammonium

TDAB	tetradecylammonium bromide
TOAB	tetraoctylammonium bromide
TRIS	tris(hydroxymethyl)amminomethane
UV	ultra violet

DANKWOORD

Veel mensen hebben een bijdrage geleverd aan de totstandkoming van dit proefschrift. Graag wil ik vanaf deze plaats alle collega's van de vakgroep Instrumentele Analyse bedanken voor hun advies en hulpvaardigheid in de afgelopen jaren. Een aantal wil ik met name noemen.
Op de eerste plaats mijn beide promotoren, Carel Cramers en Frans Everaerts, bedankt voor de geboden mogelijkheden en het in mij gestelde vertrouwen. Mede door de grote mate van vrijheid in de opzet en uitvoering van het onderzoek heb ik veel van deze promotie geleerd.
Henk Claessens, ik bewaar goede herinneringen aan onze discussies met betrekking tot het onderzoek. Bedankt voor je adviezen en het nauwkeurig nakijken van mijn manuscripten.
Sjef Öllers, Harald Verhelst en Erica Basters, jullie hebben met veel inzet en enthousiasme een groot aantal experimenten uitgevoerd en een belangrijke bijdrage geleverd aan de inhoud van dit proefschrift. Ik kijk met veel plezier terug op onze samenwerking.
Marion van Straten, bedankt voor je prettige gezelschap gedurende de afgelopen jaren en voor de bijdrage aan een gedeelte van het experimentele werk.
Denise Tjallema dank ik voor al het secretariële werk en Anja Huygen en Hans van Rijsewijk voor het vervaardigen van vele prachtige dia's en posters.
Tevens wil ik een aantal mensen buiten de vakgroep bedanken voor hun bijdrage.
Hugo Billiet, Hugo Corstjens en Hans Frank van de Technische Universiteit Delft voor de waardevolle discussies en nuttige opmerkingen, vooral tijdens de beginfase van het onderzoek.
Johan Jansen ben ik zeer erkentelijk voor de synthese van de verschillende dendrimeren.
Cari Sänger, bedankt voor je interesse en de vele stimulerende lange-afstand conversaties.
Mijn familie en vrienden wil ik bedanken, in het bijzonder John en Annet, voor hun belangstelling en morele steun in de afgelopen jaren en de nodige (muzikale) afleiding.
Tot slot wil ik een speciaal woord van dank richten aan mijn ouders. Jullie zijn mij op velerlei terrein tot steun geweest en hebben mijn activiteiten altijd met zeer veel interesse gevolgd.

CURRICULUM VITAE

Pim Muijselaar werd geboren op 6 september 1967 te Geertruidenberg. In 1985 behaalde hij het VWO diploma aan het 'Dongemond College' te Raamsdonksveer. Aansluitend hierop begon hij met de studie Scheikundige Technologie aan de Technische Universiteit Eindhoven. Het afstudeeronderzoek werd verricht in de vakgroep Chemische Proceskunde onder leiding van dr.ir. C.W. van Oers en prof.dr.ir. P.J.A.M. Kerkhof, en betrof de transportverschijnselen bij de ultrafiltratie van multicomponentsystemen. In 1990 behaalde hij cum laude het ingenieursexamen. Van 1991 tot 1996 is hij werkzaam geweest in de vakgroep Instrumentele Analyse aan de Technische Universiteit Eindhoven. Hier werd het promotieonderzoek uitgevoerd onder leiding van prof.dr.ir. C.A.M.G. Cramers, waarvan de resultaten zijn beschreven in dit proefschrift.
Met ingang van november 1996 is hij werkzaam in de groep van prof.dr. S. Terabe aan het Himeji Institute of Technology in Japan.

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STELLINGEN

behorende bij het proefschrift

MICELLAR ELECTROKINETIC CHROMATOGRAPHY

Fundamentals and Applications

van

W.G.H.M. MUIJSELAAR

1. Het gebruik van retentie indices biedt een goede mogelijkheid voor het klassificeren van pseudo-stationaire fasen in micellaire electrokinetische chromatografie.
Dit proefschrift, hoofdstuk 4 en hoofdstuk 5.

2. In tegenstelling tot de bewering van Cai en El Rassi kan het elutie window in micellaire electrokinetische chromatografie met natrium dodecylsulfaat als pseudo-stationaire fase wel systematisch worden gevarieerd middels de pH van het gebruikte electroliet systeem.
J. Cai en Z. El Rassi, J. Chromatogr., 608(1992)31.
Dit proefschrift, hoofdstuk 2.

3. De door Ahuja en Foley waargenomen verkleining van het elutie window bij lithium dodecylsulfaat ten opzichte van natrium dodecylsulfaat wordt door hen ten onrechte toegeschreven aan een lagere mobiliteit van de micellen.
E.S. Ahuja and J.P Foley, Anal. Chem., 67(1995)2315.

4. Mede gezien de letterlijke betekenis van het woord 'chromatografie' kunnen de door Hoyer en Mysels beschreven 'tracer electrophoresis' experimenten beschouwd worden als de eerste aanzet tot micellaire electrokinetische chromatografie.
H.W. Hoyer en K.J. Mysels, J. Phys. Chem., 4(1950)966.

5. De impact van een publicatie over het spoelen van een capillair wordt niet vergroot door de vermelding van de wetenschappelijke output van het instituut waar de auteurs werkzaam zijn.
S. Abdel-Baky and R.W. Giese, J. Chromatogr., 608(1992)159.

6. Door het instationaire fluxgedrag bij ultrafiltratie experimenten te bestuderen kan een uitspraak worden gedaan over de vorming van een gellaag op het membraan.
C.W. van Oers, M.A.G. Vorstman, P.G. Muijselaar and P.J.A.M. Kerkhof, J. Membrane Sci., 73(1992)231.

7. Het verlengen van de referentielijst door het veelvuldig verwijzen naar 'unpublished results' levert geen wezenlijke bijdrage aan de inhoud van een publicatie en dient derhalve vermeden te worden.
E.S. Ahuja and J.P. Foley, Analyst, 119(1994)353.

8. Met het oog op een groeiend aantal industriële applicaties van micellaire electrokinetische chromatografie alsmede de validatie van deze methoden, dient de chromatografie nomenclatuur van de International Union of Pure and Applied Chemistry (IUPAC) herzien en uitgebreid te worden.
L.S. Ettre, Pure & Appl. Chem., 65(1993)819.

9. Bij de beschrijving van het retentiemechanisme in 'parallell-current open tubular liquid chromatography' verdient het aanbeveling om de snelheid van beide fasen in rekening te brengen.
K. Šlais, M. Horká and K. Klepárník, J. Chromatogr., 605(1992)167.

10. Na meer dan een eeuw wetenschappelijk onderzoek naar de invloed van diverse houtsoorten en metaallegeringen op de klankkleur is het nog steeds de fluitist en niet de wetenschapper die de toon zet bij de materiaalkeuze voor de bouw van dwarsfluiten.
Th. Boehm, "Ueber den Flötenbau und die neuesten verbesserungen desselben", B. Schott's Sœhnen, Mainz, 1847.
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N. Toff, "The Development of the Modern Flute", Taplinger, New York, 1979.

11. Het beleid van de Federatie van Nederlandse Rode Kruis Bloedbanken inzake de selectie van bloeddonoren staat op gespannen voet met de Algemene Wet Gelijke Behandeling.
"Bloeddruk", Donornieuws voor Zuid-Oost Brabant, 8(1996)6.
De Volkskrant, 20-2-1996, p.11.

12. De hoge mate van selectiviteit van verkeerslichten ten aanzien van stadsbussen en de daarmee gepaard gaande grote retentie van fietsers heeft een negatief effect op de verkeersveiligheid.

13. Vrijwilligerswerk is niet vrijblijvend.

14. In de hedendaagse telecommunicatiemaatschappij heb je pas echt iets bereikt als je niet bereikbaar hoeft te zijn.

Pim Muijselaar
Eindhoven, 10 september 1996