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Determination of diffusion coefficients and separation numbers in micellar electrokinetic chromatography

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Abstract

Diffusion coefficients in micellar electrokinetic chromatography (MEKC) were determined for a homologous series of alkylaryl ketones. Overall diffusion coefficients in micellar electrolyte systems were calculated from measured spatial peak variances, using the stopped migration method. From overall diffusion coefficients at two different surfactant concentrations, diffusion coefficients in the aqueous phase and in the micellar phase, respectively, were determined. Diffusion in the micellar phase was found to be approximately one order of magnitude lower than diffusion in the aqueous phase for the alkylaryl ketones. The influence of micellar diffusion on efficiency is treated theoretically. It is demonstrated that efficiency in MEKC strongly depends on the retention factor, especially for small values of the elution window. Under practical MEKC conditions, however, the influence of micellar diffusion is of minor importance. The determination of separation numbers in MEKC is evaluated and their use as parameter for the separation performance is discussed.

Keywords: Diffusion coefficients; Separation number; Micellar diffusion; Alkyl aryl ketones; Ketones

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 the literature, including quasi-elastic light scattering [6], nuclear magnetic resonance [7] and the Taylor dispersion method [8,9].

In micellar electrokinetic chromatography (MEKC) [10,11], surfactant solutions are applied for the separation of neutral species, based on differences in micellar solubilization. The different band broadening mechanisms in MEKC have been described in detail by Terabe et al. [12]. They concluded 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. Therefore a

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better knowledge of diffusional processes in surfactant solutions is also interesting from a chromatographic point of view.

In this work we describe the determination of diffusion coefficients in micellar media by MEKC. The contribution of micellar diffusion to the separation efficiency during MEKC analyses is evaluated. In addition to that, the application of the separation number as parameter for the separation performance is discussed.

2. Theory

Capillary electrophoresis (CE) offers some unique advantages for the determination of diffusion coefficients such as small sample size, suitability for solute mixtures and substances containing impurities and feasibility for automation. Several methods for the determination of diffusion coefficients by CE have been described in the literature [13–16]. In the low field method [13,14] 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 [14], 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 the measured peak variance, depend on the applied field strength. Moreover, these effects are more pronounced for sample compounds possessing high retention factors. Therefore this method cannot be applied for the determination of diffusion coefficients in MEKC.

In the dual-detector method [15], peak variances are measured at two positions on the capillary at a specific distance. This approach requires an experimental set-up with two on-column detectors.

In the stopped migration method [13,14,16], 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 [17]:

$$\Delta\sigma_s^2 = 2D_{OV}t_i \tag{1}$$

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:

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

where v is the migration velocity of the sample compound. In contrast to conventional liquid chromatography, where the 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 weighed average of the diffusion coefficients in the aqueous phase, D_{AQ}, and the pseudo-stationary micellar phase, D_{MC}, according to [12]:

$$D_{OV} = \frac{1}{1+k} D_{AQ} + \frac{k}{1+k} D_{MC} \tag{3}$$

where k is the retention factor. For the relatively small sample compounds, generally analyzed by MEKC, D_{MC} is approximately one order of magnitude smaller than D_{AQ} [18]. If overall diffusion coefficients are determined in two electrolyte systems, containing different surfactant concentrations, D_{AQ} and D_{MC} can be calculated using Eq. (3).

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 $R_S = 1.177$ [19]. Thus

$$SN = \frac{t_s^{z+1} - t_s^z}{w_{0.5}^{z+1} + w_{0.5}^z} - 1 \quad (4)$$

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. 1 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, diffusion in the stationary phase is considered negligible. Consequently SN reaches a limiting value if k reaches infinity [21]. This situation is represented by the dotted line in Fig. 1 ($D_{MC}=0$). However, due to diffusion of the pseudo-stationary phase in MEKC a maximum is obtained for all values of the elution window. There are two reasons for this phenomenon. First, analogous with the retention term *f(k)* from the MEKC resolution equation [11], a maximum is observed in the curves representing the normal elution mode ($t_{MC}/t_{EOF}=2$–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 ($t_{MC}/t_{EOF}=\infty$). Here it should be noted that the retention factors for the SN-maxima in Fig. 1 for the normal elution mode are not identical to those obtained by differentiating the retention term *f(k)*, as in the latter case efficiency is not taken into account.

3. Experimental

3.1. Chemicals

Sodium dodecyl sulphate (SDS) was obtained from Aldrich (Steinheim, Germany); acetophenone, propiophenone, butyrophenone, valerophenone and hexanophenone from Pierce (Rockford, IL, 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).

3.2. Instrumentation and separation conditions

All experiments were carried out on an HP^{3D} CE 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

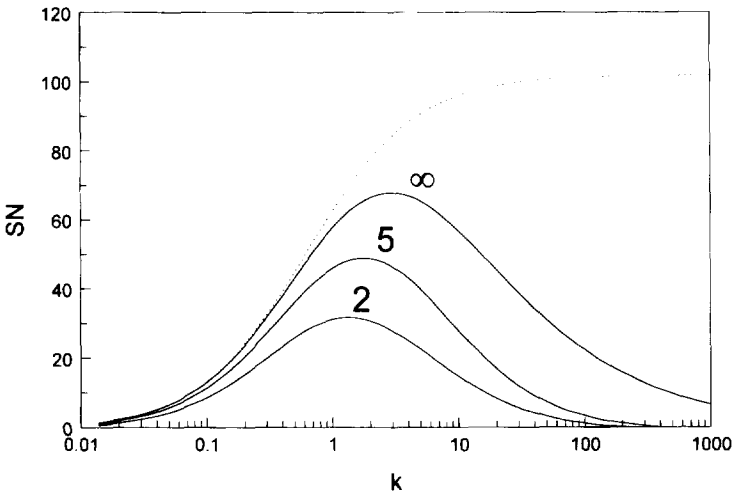

Fig. 1. Calculated separation number, SN, versus retention factor, k, for a homologous series of alkylaryl ketones for different values of the elution window, t_{MC}/t_{EOF}, assuming that longitudinal diffusion is the only band broadening mechanism. Model values: $L_C=L_D=100.0$ cm, $V=20$ kV, $\log k=0.35z-2.56$ [20]. Drawn lines: $D_{AQ}=10\cdot10^{-6}$ cm^2 s^{-1}, $D_{MC}=10^{-6}$ cm^2 s^{-1}, $m_{EOF}=80$, 50 or $40\cdot10^{-5}$ cm^2 V^{-1} s^{-1}, $m_{MC}=-40\cdot10^{-5}$ cm^2 V^{-1} s^{-1}. Dotted line: $D_{AQ}=10\cdot10^{-6}$ cm^2 s^{-1}, $D_{MC}=0$ cm^2 s^{-1}, $m_{EOF}=40\cdot10^{-5}$ cm^2 V^{-1} s^{-1}, $m_{MC}=-40\cdot10^{-5}$ cm^2 V^{-1} s^{-1}. For further explanation, see text.

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 m*M* Tris, adjusted to pH 8.2 by adding acetic acid, containing 25 m*M* or 50 m*M* SDS was used. All samples were dissolved at a concentration of approximately 0.02 $\mu l\ ml^{-1}$ in a 25 m*M* or 50 m*M* 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. Electrokinetic chromatograms were analysed by HP^{3D} CE CHEMSTATION software. Peak variances were determined from peak widths at half height.

4. Results and discussion

4.1. Diffusion coefficients

Overall diffusion coefficients in MEKC were determined for a homologous series of alkylaryl ketones, using the stopped migration method. For the interrupted experiments the electromigration was stopped for 300 min, 10 min after injection. These experiments were performed with electrolyte systems containing 25 m*M* and 50 m*M* SDS, respectively. In Fig. 2 two resulting electrokinetic chromatograms are shown for an electrolyte system containing 50 m*M* SDS. All experiments were carried out four times and in Table 1 the average migration times and spatial peak variances are listed. The micelle migration times were calculated by an iteration procedure as described previously [20,22]. 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. In addition to that, D_{AQ} will be lower for larger species, resulting in a smaller sample zone. This is illustrated in more detail in Fig. 3 where the normalized peaks of acetophenone and hexanophenone from Fig. 2 are shown on a spatial basis. From Fig. 3 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.

From the results, listed in Table 1, retention factors and overall diffusion coefficients were calculated, using Eq. (1). Subsequently, diffusion coefficients in the aqueous phase, D_{AQ}, and in the micellar phase, D_{MC}, were calculated, using Eq. (3). In Table 2 all these values are listed. According to the Stokes–Einstein equation the diffusion coefficient is inversely proportional to the viscosity of the surrounding medium [13]. Therefore the difference in viscosity between the electrolyte systems containing 25 m*M* and 50 m*M* SDS was determined as follows. A solution of each electrolyte system, containing 10 $\mu l\ ml^{-1}$ formamide, was flushed through the capillary with a pressure of 50 mbar. From the time to reach the detector it could be calculated that the viscosity of the 50 m*M* SDS system is 4.4% higher than the viscosity of the 25 m*M* SDS system. Since this value is lower than all RSD values of the overall diffusion coefficients, listed in Table 2, we decided not to take viscosity changes into account.

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. In literature a value of $7.90 \cdot 10^{-6}\ cm^2\ s^{-1}$ is reported for D_{AQ} of acetophenone at infinite dilution [23]. The deviation with the results in Table 2 may be attributed to non-ideal behaviour of the electrolyte system and to the inaccuracy of the method. Diffusion coefficients of the alkylaryl ketones in the micellar phase were found to be approximately one order of magnitude lower than those in the aqueous phase, which is in agreement with values earlier reported [18]. From Eq. (3) it can be deduced that for compounds with low retention factors micellar diffusion is negligible. For compounds with high retention factors, however, micellar diffusion is not negligible, resulting in a lower overall diffusion coefficient. For comparative purposes, D_{MC} values from literature, determined with the Taylor dispersion method, are included in Table 2. These results show that the diffusion coefficients obtained with both methods agree well. However, more accurate data are obtained using the Taylor dispersion method [8,9].

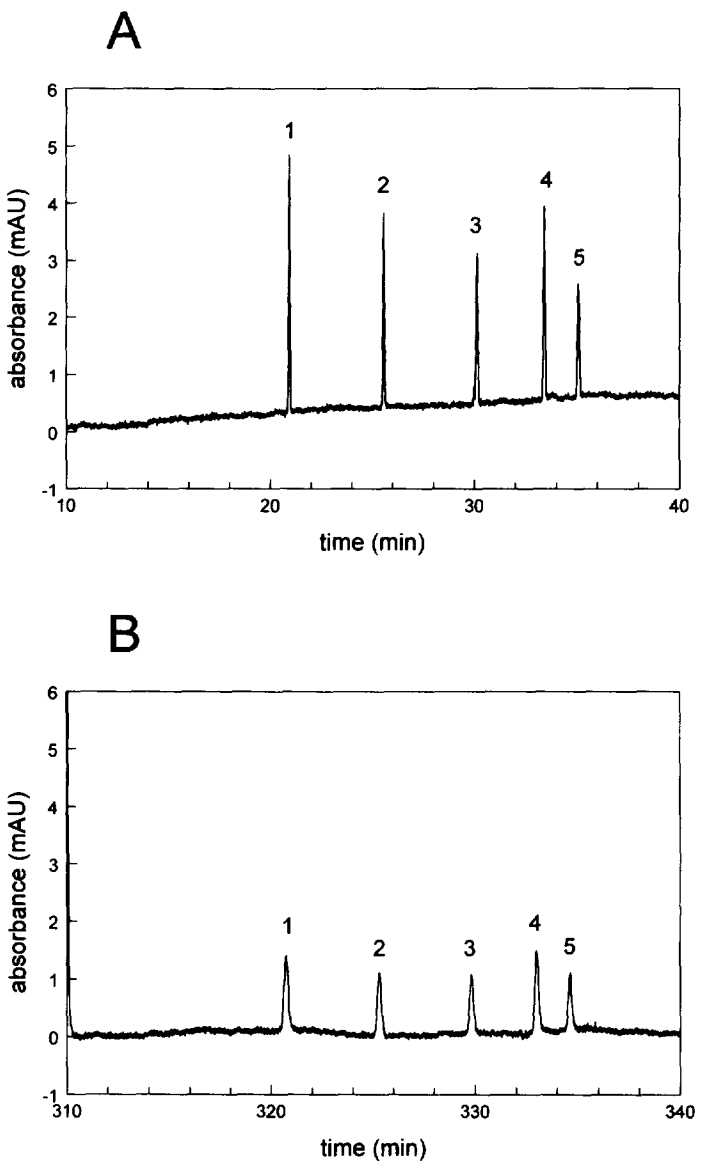

Fig. 2. Electrokinetic chromatograms of (1) acetophenone, (2) propiophenone, (3) butyrophenone, (4) valerophenone and (5) hexanophenone. Electrolyte system, 0.02 *M* Tris–acetic acid at pH 8.2, containing 50 m*M* SDS. Capillary, $L_C = 96.5$ cm and $L_D = 88.0$ cm. $V = 20$ kV (A) without and (B) with an interruption of 300 min, 10 min after injection. Detection wavelength, 240 nm.

4.2. Efficiency and separation numbers

From the foregoing it can be concluded that micellar diffusion plays an important role in the band broadening mechanism during MEKC experiments. In order to investigate the influence of longitudinal diffusion on the separation efficiency in more detail, 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 [24] for capillary zone electrophoresis, the theoretical plate number in MEKC is given by:

Table 1
Average migration times, t, and spatial peak variances, σ^2, 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	t (min)	RSD (%)	σ^2 (cm^2)	RSD (%)	t-300 (min)	RSD (%)	σ^2 (cm^2)	RSD(%)
		Experiment I				Experiment II			
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	–	–

RSD = relative standard deviation.

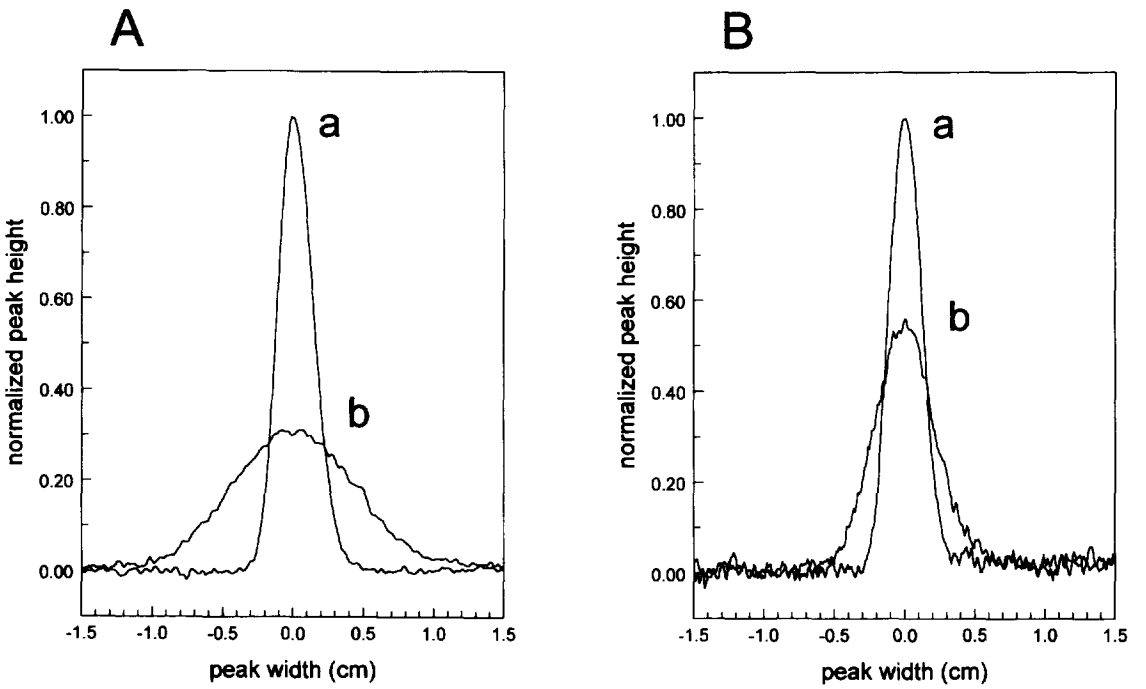

Fig. 3. Normalized peaks of (A) acetophenone and (B) hexanophenone on a spatial basis. Electrolyte system, 0.02 M Tris–acetic acid at pH 8.2, containing 50 mM SDS. Capillary, $L_C = 96.5$ cm and $L_D = 88.0$ cm. Voltage, 20 kV (a) without and (b) with an interruption of 300 min, 10 min after injection. Peaks were normalized by putting the peak height for the uninterrupted experiment equal to 1.

Table 2
Average retention factors, k, and overall diffusion coefficients, D_{ov} (10^{-6} cm^2 s^{-1}), with relative standard deviations (%) 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^{-1}), and the micellar phase, D_{MC} (10^{-6} cm^2 s^{-1}) ($n=8$)

Compound	k	RSD (%)	D_{ov}	RSD (%)	k	RSD (%)	D_{ov}	RSD (%)	D_{AQ}	D_{MC}
	25 mM SDS				50 mM SDS					
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.
[b] As [a] in 52.5 mM SDS [8].

$$N = \frac{(m_{eff}^{ps} + m_{eof})V}{2D_{ov}} \quad (5)$$

where m_{eof} is the electroosmotic mobility, V is the applied voltage and m_{eff}^{ps} is the pseudo-effective mobility of the solute, defined by [25]:

$$m_{eff}^{ps} = \frac{k}{k+1} m_{mc} \quad (6)$$

where m_{mc} is the effective mobility of the micelles. In Fig. 4 the 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 the efficiency is not negligible. Due to a decrease in overall diffusion coefficient N increases with increasing k. This phenomenon should be taken into account in theoretical resolution optimization strategies [26,27]. Under practical MEKC conditions, i.e., t_{MC}/t_{EOF} between 2–5 and $k<10$, however, the influence of micellar diffusion on the total solute band-broadening is of minor importance.

As was pointed out in Section 2, separation numbers can be used in MEKC to describe the separation performance. In Fig. 5 theoretically calculated and experimentally determined separation num-

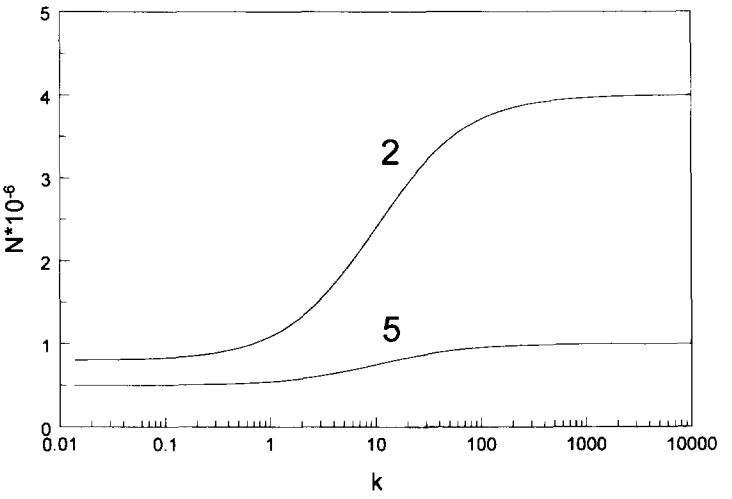

Fig. 4. Calculated theoretical plate number, N, versus retention factor, k, for different values of the elution window, t_{MC}/t_{EOF}, assuming that longitudinal diffusion is the only band broadening mechanism. Model values: $D_{AQ}=10\cdot10^{-6}$ cm^2 s^{-1}, $D_{MC}=10^{-6}$ cm^2 s^{-1}, $V=20$ kV, $m_{EOF}=80$ or $50\cdot10^{-5}$ cm^2 V^{-1} s^{-1}, $m_{MC}=-40\cdot10^{-5}$ cm^2 V^{-1} s^{-1}.

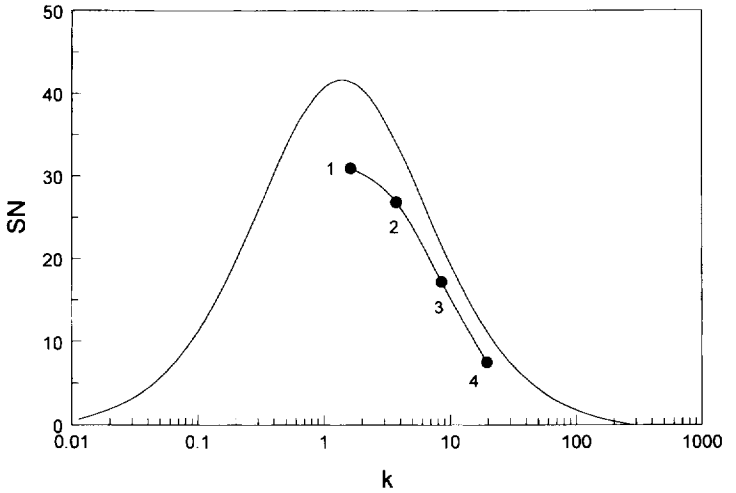

Fig. 5. Theoretical calculated and experimental measured separation number, SN, versus retention factor, k, for a homologous series of alkylaryl ketones. Electrolyte system, 0.02 M Tris–acetic acid at pH 8.2, containing 50 mM SDS. See Fig. 2 for the names of the compounds. Calculated curve: $D_{AQ}=8.09\cdot10^{-6}$ cm^2 s^{-1}, $D_{MC}=1.17\cdot10^{-6}$ cm^2 s^{-1} (average values from Table 2), log $k=0.36z-2.67$. Capillary, $L_C=96.5$ cm and $L_D=88.0$ cm. $V=20$ kV.

bers, SN, are shown for a homologous series of alkylaryl ketones in an electrolyte system containing 50 mM SDS. The decrease in SN for more hydrophobic species clearly demonstrates the influence of the limited elution window in MEKC experiments. Separation numbers lower than the theoretical curve were obtained where only longitudinal diffusion was taken into account, indicating that other contributions to band-broadening such as injection and micelle heterogeneity also play a significant role in these MEKC analyses.

5. Conclusions

MEKC was shown to be a suitable technique to study diffusivity in micellar media. Overall diffusion coefficients were determined, using the stopped migration method, for a homologous series of alkylaryl ketones in electrolyte systems containing different SDS concentrations. 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}. It was demonstrated that efficiency in MEKC strongly depends on the retention factor, especially for small values of the elution window. The separation number was found to be a good parameter to describe the separation performance in MEKC experiments, taking into account both efficiency and the limited elution window.

Acknowledgments

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