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Characterization of Pseudostationary Phases in Micellar Electrokinetic Chromatography by Applying Linear Solvation Energy Relationships and Retention Indexes

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The influence of different solute–micelle interactions on micellar solubilization is studied by using linear solvation energy relationship (LSER) modeling. The LSER results for six anionic micellar systems demonstrate that the migration behavior of neutral species in micellar electrokinetic chromatography (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 sodium dodecyl sulfate (SDS) and mixed SDS/Brij 35 micellar systems, primarily due to different hydrogen bonding characteristics. It is demonstrated that retention indexes can be applied for quantitative characterization of pseudostationary phases in MEKC in a way similar to the use of the Rohrschneider–McReynolds scale in GC. This method facilitates classification of pseudostationary phases according to several solvatochromic quantities with a limited number of experiments. Using this approach, six anionic and two cationic micellar systems were classified according to their hydrogen bond donor and hydrogen bond acceptor strengths, respectively, giving results comparable to those obtained with 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.

Micellar electrokinetic chromatography (MEKC) is a highly efficient separation method, especially suitable for the analysis of neutral compounds. The separation mechanism of this technique is based on differences in the distribution equilibria of sample compounds between an aqueous mobile phase and a pseudostationary 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, in particular, the pseudostationary phase can easily be changed by rinsing the capillary. Different surfactant systems and mixed micelles have been applied in order to control migration behavior and optimize selectivity.5-10 Selectivity in MEKC is mainly determined by the hydrophilic moieties of the applied micellar system. However, the influence of the chemical nature of the pseudostationary phase and the structural properties of the solute molecules on solute–micelle interactions is still not well understood. Despite the ease of varying the experimental conditions, proper selection of a suitable surfactant system may be a difficult task.

Yang and Khaledi applied linear solvation energy relationship (LSER) modeling 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. Previously it was demonstrated that the retention index is a good migration parameter for comparing different surfactant systems since it is a relative quantity, independent of the concentration, molar volume, and critical micelle concentration of the applied surfactant.13,14 Moreover, retention indexes facilitate the classification of sample compounds in terms of functional group selectivity.

In this work, we describe the application of the retention index concept for the quantitative classification of pseudostationary phases in MEKC. First, LSER methodology was applied for the characterization of micellar solubilization in six anionic surfactant systems. Based on these results, six anionic and two cationic micellar systems were classified according to their hydrogen bonding characteristics by applying retention indexes. 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 behavior in MEKC experiments.

THEORY

In LSER modeling, solute properties that depend on solute–solvent interactions are correlated to several solvatochromatic

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parameters of the solute according to \(^{15}\)

\[
SP = SP_0 + mV/100 + s\pi + b\beta + a\alpha
\]  

(1)

where \(SP\) is the solvent-related solute property, \(SP_0\) is a regression constant which depends among others on the phase ratio, \(V\) is the molar volume of the solute, \(\pi\) is a measure of the solute dipolarity/polarizability, \(\beta\) is the hydrogen bond acceptor (HBA) ability, and \(\alpha\) is the hydrogen bond donor (HBD) ability of the solute. The coefficients \(m, s, b, a\) 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. \(^{15}\)

In chromatographic techniques, the logarithm of the retention factor, \(\log k\), is generally used as a solute property. \(^{11}\) Accordingly, for M E K C, eq 1 can be written as

\[
\log k = \log k_0 + mV/100 + s\pi + b\beta + a\alpha
\]  

(2)

where \(\log k_0\) is a regression constant and coefficients \(m, s, b, a\) 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 (disfavorable) or an exoergic (favorable) 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 M E K C analyses. \(^{11,12}\)

EXPERIMENTAL SECTION

Chemicals. Benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, sodium dodecyl sulfate (SDS), cortisone, hydrocortisone, and corticosterone were obtained from Aldrich (Steinheim, Germany). Tris(hydroxymethyl)aminomethane (TRIS), sodium dodecane sulfonate (SDSo), and poly[(oxyethylene)(23)]-dodecanol (Brij 35) were obtained from Merck (Darmstadt, Germany). Tris(hydroxymethyl)aminomethane (TRIS) was obtained from Sigma (St. Louis, MO). All other chemicals were analytical-reagent grade.

Instrumentation and Separation Conditions. All experiments were carried out on a BioFocus 3000 capillary electrophoresis system (Bio-Rad, Hercules, CA) at a constant voltage of 20 kV. A 50- \(\mu\)m id fused silica capillary (Polymicro Technologies, Phoenix, AZ) 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. A homologous series of alkylbenzenes was applied as retention index standards. The migration time of the micelles was calculated by an iteration procedure as described previously. \(^{13,16}\)

Samples and Solutions. Stock solutions of the sample compounds and the retention index standards (alkylbenzenes) were prepared in methanol at concentrations of about 1 mg/mL and 2 \(\mu\)L/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 phosphoric acid, was used and for the electrolyte system containing TDS, a buffer of 20 mM TRIS adjusted to pH 7.0 with phosphoric acid was used. All SDSo solutions were stored at 40 °C. Water was purified by using a Milli-Q water purification system (Waters Milipore, Milford, MA). All buffer solutions were filtered through 0.45-\(\mu\)m filters prior to use.

RESULTS AND DISCUSSION

Linear Solvation Energy Relationships. To elucidate the contribution of different solute—micelle interactions to M E K C migration behavior, LSER modeling was applied for six different anionic surfactant systems, viz., the sodium and the TRIS salts of dodecyl sulfate (SDS and TDS, respectively), sodium dodecane-sulfonate (SDSo), and various mixed micellar systems of SDS and the neutral surfactant Brij 35. As micelles are surrounded by a considerable number of counterions, \(^{17}\) special attention was paid to the composition of the electrolyte systems, i.e., the cation of the background electrolyte was identical to the micelle counterion. The LSER studies were carried out for a group of 18 aromatic compounds possessing different functionalities and hydrophobicity. In Table 1, all solvatochromatic parameters and n-octanol–water partition coefficients, \(\log P_{ow}\), of these compounds are listed. \(^{15,18}\)

Table 1. n-Octanol–Water Partition Coefficient, a (log \(P_{ow}\)) and Solvatochromic Parameters, b V/100, \(\pi, \beta,\) and \(\alpha,\) of 18 Sample Compounds

<table>
<thead>
<tr>
<th>no.</th>
<th>compound</th>
<th>(\log P_{ow})</th>
<th>V/100</th>
<th>(\pi)</th>
<th>(\beta)</th>
<th>(\alpha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>phenol</td>
<td>1.46</td>
<td>0.536</td>
<td>0.72</td>
<td>0.33</td>
<td>0.61</td>
</tr>
<tr>
<td>2</td>
<td>benzaldehyde</td>
<td>1.48</td>
<td>0.606</td>
<td>0.92</td>
<td>0.44</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>nitrobenzene</td>
<td>1.85</td>
<td>0.631</td>
<td>1.01</td>
<td>0.30</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>benzyl cyanide</td>
<td>1.56</td>
<td>0.590</td>
<td>0.90</td>
<td>0.37</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td>acetophenone</td>
<td>1.58</td>
<td>0.690</td>
<td>0.90</td>
<td>0.49</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>chlorobenzene</td>
<td>2.84</td>
<td>0.581</td>
<td>0.71</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>7</td>
<td>naphthalene</td>
<td>3.37</td>
<td>0.753</td>
<td>0.70</td>
<td>0.15</td>
<td>0.00</td>
</tr>
<tr>
<td>8</td>
<td>anisol</td>
<td>2.11</td>
<td>0.638</td>
<td>0.73</td>
<td>0.32</td>
<td>0.00</td>
</tr>
<tr>
<td>9</td>
<td>bromobenzene</td>
<td>2.99</td>
<td>0.624</td>
<td>0.79</td>
<td>0.06</td>
<td>0.00</td>
</tr>
<tr>
<td>10</td>
<td>p-cresol</td>
<td>1.94</td>
<td>0.634</td>
<td>0.68</td>
<td>0.34</td>
<td>0.58</td>
</tr>
<tr>
<td>11</td>
<td>1,2-xylol</td>
<td>3.12</td>
<td>0.668</td>
<td>0.51</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>12</td>
<td>benzyl alcohol</td>
<td>1.08</td>
<td>0.634</td>
<td>0.92</td>
<td>0.52</td>
<td>0.39</td>
</tr>
<tr>
<td>13</td>
<td>p-nitrotoluene</td>
<td>2.45</td>
<td>0.729</td>
<td>0.97</td>
<td>0.31</td>
<td>0.00</td>
</tr>
<tr>
<td>14</td>
<td>benzene</td>
<td>2.13</td>
<td>0.491</td>
<td>0.59</td>
<td>0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>toluene</td>
<td>2.65</td>
<td>0.592</td>
<td>0.55</td>
<td>0.11</td>
<td>0.00</td>
</tr>
<tr>
<td>16</td>
<td>ethylbenzene</td>
<td>3.13</td>
<td>0.668</td>
<td>0.53</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>17</td>
<td>propylbenzene</td>
<td>3.69</td>
<td>0.769</td>
<td>0.51</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>18</td>
<td>butylbenzene</td>
<td>4.28</td>
<td>0.867</td>
<td>0.49</td>
<td>0.12</td>
<td>0.00</td>
</tr>
</tbody>
</table>

\(a\) Log \(P_{ow}\) from ref 18. \(b\) From ref 19. \(c\) Solvatochromic parameters from ref 15.
Table 2. Regression Constant (log $k_0$) and System Coefficients $m$, $s$, $b$, and $a$ with Standard Deviations (in Parentheses), Correlation Coefficient ($r$) and Standard Deviation (SD), of LSER Models for Six Different Surfactant Systems and log $P_{OW}$ ($n = 18$)

<table>
<thead>
<tr>
<th>surfactant system</th>
<th>log $k_0$</th>
<th>$m$</th>
<th>$s$</th>
<th>$b$</th>
<th>$a$</th>
<th>$r$</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mM SDS</td>
<td>-1.56 (0.23)</td>
<td>4.19 (0.26)</td>
<td>-0.35 (0.20)</td>
<td>-1.56 (0.26)</td>
<td>-0.31 (0.13)</td>
<td>0.989</td>
<td>0.09</td>
</tr>
<tr>
<td>50 mM TDS</td>
<td>-1.54 (0.20)</td>
<td>4.12 (0.28)</td>
<td>-0.31 (0.21)</td>
<td>-1.59 (0.27)</td>
<td>-0.31 (0.14)</td>
<td>0.988</td>
<td>0.09</td>
</tr>
<tr>
<td>50 mM SDS\textsubscript{50}</td>
<td>-1.50 (0.20)</td>
<td>3.94 (0.23)</td>
<td>-0.40 (0.17)</td>
<td>-1.58 (0.22)</td>
<td>-0.17 (0.11)</td>
<td>0.991</td>
<td>0.08</td>
</tr>
<tr>
<td>50 mM SDS + 2 mM Brij 35</td>
<td>-1.61 (0.23)</td>
<td>4.30 (0.27)</td>
<td>-0.27 (0.20)</td>
<td>-2.15 (0.26)</td>
<td>-0.16 (0.13)</td>
<td>0.991</td>
<td>0.09</td>
</tr>
<tr>
<td>50 mM SDS + 5 mM Brij 35</td>
<td>-1.50 (0.23)</td>
<td>4.24 (0.26)</td>
<td>-0.17 (0.20)</td>
<td>-2.56 (0.26)</td>
<td>0.03 (0.13)</td>
<td>0.991</td>
<td>0.09</td>
</tr>
<tr>
<td>50 mM SDS + 10 mM Brij 35</td>
<td>-1.45 (0.23)</td>
<td>4.40 (0.27)</td>
<td>-0.18 (0.20)</td>
<td>-2.77 (0.26)</td>
<td>0.14 (0.13)</td>
<td>0.992</td>
<td>0.09</td>
</tr>
<tr>
<td>log $P_{OW}$</td>
<td>0.24 (0.18)</td>
<td>5.49 (0.21)</td>
<td>-0.61 (0.16)</td>
<td>-3.77 (0.20)</td>
<td>-0.12 (0.10)</td>
<td>0.998</td>
<td>0.07</td>
</tr>
</tbody>
</table>

$^a$ Log $P_{OW,0}$

Retention factors were determined for all sample compounds, and the system coefficients $m$, $s$, $b$, and $a$ from eq 2 were calculated by multiple linear regression. These values are listed in Table 2. In addition, the coefficients for the LSER model of log $P_{OW}$ were calculated and are included in Table 2 for comparative purposes. The high correlation coefficients ($r > 0.98$) suggest that migration behavior in MEKC can be well described by LSER models. The large absolute values of the system coefficients $m$ and $b$ indicate that the migration behavior of solutes in MEKC with these surfactant systems is mainly influenced by their size ($V/100$) and their HBA ability ($\beta$). Their dipolarity/polarizability ($\pi$) and HBD ability ($\alpha$) 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 marked importance in MEKC; consequently, retention 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 $\pi$, $\beta$ and $\alpha$, higher retention factors are obtained for larger, more hydrophilic 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. These LSER results are consistent with those reported earlier by Yang and Khaledi.

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 counterions and buffer ions) may influence the solubilization thermodynamics, almost no effect of the micelle counterion on selectivity was observed. Only small differences in selectivity were observed between the SDS and SDS\textsubscript{50} 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 pseudostationary micellar phase, changes from a negative to a positive value, i.e., from an endoergic to an exoergic process. In Figure 1, coefficients $a$ and $b$ are shown as a function of the concentration of Brij 35, illustrating that the $b$ value decreases and the $a$ value increases with increasing concentration of Brij 35. This means that lower retention factors are obtained for HBA solutes whereas higher retention factors are obtained for HBD solutes in the mixed SDS/Brij 35 micellar systems compared to those obtained in SDS. Probably the anionic surface of the SDS micelles is shielded by the poly(oxyethylene) chains of Brij 35 surfactant molecules when mixed micelles are formed. 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 poly(oxyethylene) groups, solutes may be incorporated into these groups as illustrated in Figure 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. These results suggest that it is possible to create a gradual scale of pseudostationary phases in terms of HBD and HBA strength, applying surfactant systems with different SDS/Brij 35 concentration ratios. From the foregoing results, it can be concluded that LSER studies provide useful information about the contribution of different interaction phenomena to migration behavior in MEKC. The calculated system coefficients can be applied for quantitative classification of micellar pseudostationary phases according to solvatochromic parameters.

Retained Indexes. Besides LSER modeling, we also investigated the applicability of retention indexes for the classification

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of micellar pseudostationary phases in MEKC. Previously we reported the application of the retention index concept in MEKC for the identification of neutral species.\textsuperscript{13} It was demonstrated that the retention index, $I$, is independent of the surfactant concentration and that $\Delta I_{SF}$ values from retention indexes obtained with different micellar systems provide information about different ways of micellar solubilization in MEKC analysis. $\Delta I_{SF}$ values were calculated for retention indexes obtained with the anionic surfactant SDS and the cationic surfactants cetyltrimethylammonium bromide (CTAB) and dodecytrimethylammonium bromide (DTAB), respectively. High positive $\Delta I_{SF}$ values were obtained for the HBD compounds phenol and resorcinol, whereas negative $\Delta I_{SF}$ 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. These results are consistent with LSER studies in micellar liquid chromatography, reported by Yang and Khaledi.\textsuperscript{12}

For all sample compounds listed in Table 1, and for 10 additional compounds (three aromatic compounds, four xanthines, and three corticosteroids), retention indexes were determined in the six anionic micellar systems, and $\Delta I_{SF}$ values were calculated according to

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

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 the reference system because this is the surfactant system most widely used in MEKC. Bearing in mind the results of the LSER calculations for SDS, TDS, and SDSo (see Table 2), it is not surprising that, for $\Delta I_{TDS}$ and $\Delta I_{SDSo}$ 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 Figure 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 poly(oxyethylene) 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.

These results demonstrate that sample compounds can be divided in different subgroups according to their $\Delta I_{SF}$ values. These $\Delta I_{SF}$ values represent functional group selectivities. To investigate the contribution of different solute–micelle interactions to $\Delta I_{SDS/Brij35}$ in more detail, LSER studies were carried out for a 50 mM SDS system and a 50 mM SDS/10 mM Brij 35 mixed micellar system, applying the retention index $I$ as solute parameter in eq 1. In Figure 4, the contribution of the different terms in eq 1 (i.e., the products of the resulting system coefficients and the solvatochromic parameters) to $\Delta I_{SDS/Brij35}$ are illustrated for 13 sample compounds. These values clearly illustrate the contribution of different types of interaction to solute retention and selectivity. The observed differences in selectivity between 50 mM SDS and 50 mM SDS/10 mM Brij 35 are, for the greater part, due to the HBD strength of the micellar systems (negative term $\beta_I$) and, for compounds possessing a positive value for $\alpha$, also to the HBA strength of the micellar systems (positive term $\alpha_I$). The polarity term ($\sigma$) indicates a relatively small contribution to retention in the SDS/Brij 35 system compared to that in the SDS system.

In analogy to the Rohrschneider–M. Reynolds scale in GC,\textsuperscript{23,24} $\Delta I_{SF}$ values can be applied for the classification of pseudostationary phases in MEKC according to specific selective chemical interactions. This approach would greatly facilitate the fast 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. As a first approximation, differences in retention indexes can be expressed in terms of two parameters, representing the HBA and HBD strengths of both solutes and micellar phases, according to

\begin{align*}
\Delta I_{SDS/Brij35} &= I_{SDS/Brij35} - I_{SDS} \\
\Delta I_{SDS/Brij35} &= \Delta I_{SF} - \Delta I_{TDS} - \Delta I_{SDSo} - \Delta I_{SDS/Brij35}
\end{align*}

References:

where $\beta$ and $\alpha$ are physical quantities which represent the HBA and HBD strengths of the solutes, and $x$ and $y$ are constants which represent the HBD and HBA strengths of the micellar pseudostationary phase, respectively. Differences in cohesiveness and dipolarity between the pseudostationary 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 $\beta$ and $R$, respectively, in eq 4. These values can be found in Table 1. For six anionic systems (SDS, TDS, SDSo, and SDS/Brij 35 at three different concentration ratios) and for two cationic systems (CTAB and DTAB, retention index data from ref 13), constants $x$ and $y$ were calculated and are listed in Table 3. With these system constants, the applied micellar pseudostationary phases can be classified according to their HBD and HBA strengths, respectively, as shown in Figure 5. In this figure, classifications according to the corresponding system coefficients $b$ and $a$ from the LSER models are also included. Comparable results are obtained for both methods, except for the HBD strengths of the cationic surfactants CTAB and DTAB. Probably more parameters are necessary in eq 4, e.g., an additional polarity term, for an adequate characterization of these micellar systems.

From the foregoing, it can be concluded that retention indexes can be used for the quantitative characterization and classification of pseudostationary phases in MEKC in a way similar to the use of the Rohrschneider–McReynolds scale in GC. This approach has several advantages from a practical point of view. First, $\Delta_{ISF}$ values are calculated directly from MEKC migration data. Therefore, these values provide good insight into the influence of a specific micellar phase on MEKC migration behavior and the expected position of the peaks in the electrokinetic chromatogram. Second, only for the standards must retention indexes be determined. Consequently, a small number of experiments is required for the classification of pseudostationary phases, which enables the fast selection of a suitable electrolyte system. Third, only for the limited number of reference compounds are solvatochromic parameters needed. Of course, the described method can be made more accurate by taking into account more parameters in eq 4.

Selectivity. To illustrate differences in selectivity, in Figure 6, two electrokinetic chromatograms are shown that were obtained with an electrolyte system containing 50 mM SDS and a mixed micellar system containing 50 mM SDS and 10 mM Brij 35,

$$\Delta_{ISF} = \beta x + \alpha y$$  (4)
respectively. As a practical application, three corticosteroids were separated by applying a mixed SDS/Brij 35 micellar system. These compounds cannot be separated in an SDS system, as they all migrate at or near the migration time of the micelles. In Figure 7, both electrokinetic chromatograms are shown. With the addition of 10 mM Brij 35 to the electrolyte system, a considerable decrease in retention is obtained, and a good separation is possible.

**Efficiency and Elution Window.** Besides the selectivity, the efficiency and the elution window are also affected by the mixed micellar system. Higher theoretical plate numbers are obtained for all sample compounds in the mixed SDS/Brij 35 micellar system which may be attributed to improved sorption–desorption kinetics. In Table 4, electroosmotic mobility, $m_{EOF}$, and effective mobility of the micelles, $m_{MC}$, are listed as a function of the concentration of Brij 35. At a higher concentration of the neutral surfactant, the effective charge and, therefore, $m_{MC}$ of the mixed
micelles will decrease. As a result, a decrease in the elution window is observed at higher Brij 35 concentrations.

**n-Octanol—Water Partition Coefficients.** n-Octanol—water partition coefficients, log _P_ \text{OW}, have been widely used as a parameter for lipophilicity of substances and are applied in various disciplines such as drug design, toxicology, and environmental monitoring of pollutants. In quantitatively structure—activity relationships (QSAR), log _P_ \text{OW} values are used to predict lipoidal transport and bioactivity. The conventional shake-flask technique for the direct measurement of log _P_ \text{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_ \text{OW}, such as reversed phase LC.

Recently, several authors reported on log _P_ \text{OW} screening and QSAR studies using MEKC. 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 much more similar to biological membranes than n-octanol or reversed phase stationary phases do. 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_ \text{OW} can be applied to predict migration behavior in MEKC.

From the LSER results, listed in Table 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_ \text{OW}, according to

\[ I = p \log P_\text{OW} + q \]  

where _p_ and _q_ are constants. Previously it was demonstrated that not all compounds follow this relationship with an SDS micellar system. Higher _I_ values were obtained for HBA compounds (\( \beta < 0.35 \)). Recently, Yang et al., 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 Figure 8. To complete the data set with strong HBA solutes with a high log _P_ \text{OW} value, additional experiments were carried out with propiophenone and butyrophone. 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 retention for strong HBA corticosteroids in an SDS micellar system. This retention is much higher than expected on the basis of their moderate log _P_ \text{OW} values. In Table 5, all constants _p_ and _q_ for eq 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_ \text{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_ \text{OW}, respectively. Hence, a better correlation is obtained.

### Table 4. Electroosmotic Mobility (\( m_{\text{EOF}}, 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1} \)), Effective Mobility of the Micelles (\( m_{\text{MC}}, 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1} \)), and Resulting Elution Window as a Function of the Brij 35 Concentration

<table>
<thead>
<tr>
<th>Conc Brij 35 (mM)</th>
<th>( m_{\text{EOF}} )</th>
<th>( m_{\text{MC}} )</th>
<th>( m_{\text{EOF}}/\left( m_{\text{EOF}} + m_{\text{MC}} \right) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>62.64</td>
<td>-45.06</td>
<td>3.56</td>
</tr>
<tr>
<td>2</td>
<td>58.21</td>
<td>-39.93</td>
<td>3.19</td>
</tr>
<tr>
<td>5</td>
<td>52.26</td>
<td>-36.92</td>
<td>3.41</td>
</tr>
<tr>
<td>10</td>
<td>59.86</td>
<td>-29.06</td>
<td>1.94</td>
</tr>
<tr>
<td>25</td>
<td>57.50</td>
<td>-15.27</td>
<td>1.36</td>
</tr>
</tbody>
</table>

\(^{a}\) All electrolyte systems contain 50 mM SDS.

### Figure 8. _I_ versus log _P_ \text{OW} for the 18 sample compounds, listed in Table 1, propiophenone and butyrophone. 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. Regression Constants _p_ and _q_ with Standard Deviations (in Parentheses), Correlation Coefficient (r), and Standard Deviation (SD), for the Graphs of _I_ versus log _P_ \text{OW} for Four Different Surfactant Systems

<table>
<thead>
<tr>
<th>Surfactant System</th>
<th><em>p</em></th>
<th><em>q</em></th>
<th>r</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mM SDS</td>
<td>140 (10)</td>
<td>366 (25)</td>
<td>0.965</td>
<td>35</td>
</tr>
<tr>
<td>50 mM TDS</td>
<td>141 (10)</td>
<td>364 (25)</td>
<td>0.965</td>
<td>35</td>
</tr>
<tr>
<td>50 mM SDSo</td>
<td>143 (8)</td>
<td>354 (21)</td>
<td>0.975</td>
<td>29</td>
</tr>
<tr>
<td>50 mM SDS + 10 mM Brij 35</td>
<td>162 (5)</td>
<td>300 (14)</td>
<td>0.991</td>
<td>20</td>
</tr>
</tbody>
</table>

< 0.2, (B) 0.2 < \beta < 0.35, and (C) \beta \geq 0.35. For further explanation, see text.
for the 50 mM SDS/10 mM Brij 35 system. In Figure 9, 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.

CONCLUSIONS

The migration behavior of neutral species in MEKC can be well described by LSER modeling. 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 $\Delta I_{SF}$ 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, $\Delta I_{SF}$ values can be applied for the classification of pseudostationary phases in MEKC, analogous to using the Rohrschneider-McReynolds scale in GC. Using this approach, six anionic and two cationic micellar systems were classified according to their HBD and HBA strengths, respectively. The obtained results were comparable to a classification based on LSER models.

Small differences in selectivity were observed between SDS, TDS, and SDO 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 of HBA compounds was found to decrease whereas retention of HBD compounds was found to increase with increasing concentration of the neutral surfactant Brij 35. With these mixed micellar systems, a gradual scale according to hydrogen bonding characteristics can be created.

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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