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Chromatographic properties of reversed-phase stationary phases under pressure- and electro-driven conditions

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Abstract

Seven different reversed-phase (RP) stationary phases were examined under high-performance liquid chromatographic (pressure-driven, HPLC), and capillary electrochromatographic (electro-driven, CEC) conditions. Characterization of the stationary phases was performed following well-established test procedures providing a number of distinct column descriptors: hydrophobicity, hydrophobic selectivity and silanol activity. These parameters were used to describe the behavior of the RP-columns under both HPLC and CEC conditions. It is shown that chromatographic characteristics of porous RP-phases greatly depend on the mode of operation. By contrast, column descriptors of a non-porous viz. solid RP-phase material hardly differed for HPLC and CEC conditions. © 1999 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Capillary electrochromatography (CEC) is a recently developed separation technique combining the excellent efficiency usually achieved in electrophoretic separation techniques and the high selectivity that is characteristic for high-performance liquid chromatography (HPLC) [1,2]. At present CEC is being considered as a potential alternative technique for micro-HPLC, which is an established technique [3–7]. Many papers have been published recently on the theory and practical aspects in electrochromatography [8–22]. A number of these reports start from the theoretical concepts originating from capillary electrophoresis (CE) explaining the high efficiencies from the plug-like velocity profiles obtained in these techniques [20–22]. Yan et al. [23] compared the efficiencies that can be achieved in CEC and in micro-HPLC of 50 μm I.D. columns packed with 3 μm Hypersil ODS. They found significantly higher efficiency for the column under CEC conditions. As discussed in [24], in CEC the flow velocity and the plug-like velocity profile do not depend on the particle size down to approximately 0.5 μm or even lower as long as no double-layer overlapping occurs [24–26]. Opposite to HPLC where the use of smaller particles is seriously limited by pressure drop limitations, such small particles can

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easily be used in CEC. This potentially makes CEC a highly efficient technique compared to HPLC. Furthermore, based on detection developments in the field of micro-HPLC, on-column fluorescence detection was recently introduced into CEC too, providing an additional increase in efficiency [27,28]. At present the introduction of CEC is hampered by two major problems. First, the technical difficulties encountered in the manufacturing of suitable and reliable CEC columns still are substantial. In addition the achievement of a suitable and stable electroosmotic flow (EOF) and the mechanical stability of the packed bed in CEC-columns still are problematic. Second, there are a number of important questions unanswered the backgrounds of retention and selectivity in CEC. Concerning that framework the eventual changes in physico-chemical properties of HPLC stationary phases under CEC conditions is an issue of great interest. For instance Vissers et al. [29] has showed that while using the same stationary phase the retention for neutral compounds in CEC is about 20% higher than under micro-HPLC mode. In contrast T. Eimer et al. [30] found that for more hydrophobic analytes the retention factors were 36–40% lower in pressurized electrochromatography (PEC) compared to capillary LC. They concluded that this might be attributed to the higher polarity of the stationary phase under electric field conditions. Using RP columns Wei et al. [31] used solvatochromic parameters to study retention in CEC and found that the retention behavior under HPLC and CEC conditions is very dissimilar for these columns. It appeared that the hydrogen bond acidity and dipolarity/polarizibity of solutes play a more dominant role in CEC than in HPLC. Furthermore, they conclude that the effects of solute size and hydrogen bond basicity on retention are similar in both separation modes. Djordjevic et al. [32] compared the retention mechanism of neutral solutes under CEC and HPLC conditions and observed lower retention factors for the former mode on a column packed with CEC Hypersil C_{18} 3 μm. They attributed the differences to the heat generation in CEC, which causes significant differences between the set and the actual column temperature. Opposite to the findings reported above, Zhang et al. [33] found the retention behavior in CEC comparable to that in HPLC and obtained similar linear energy equations in CEC, PEC and HPLC using linear solvatation energy relationship analysis. In another report it has also been shown that HPLC methods for neutral compounds can be easily transferred to CEC [34]. Keeping other conditions identical the comparison of stationary phases in HPLC and CEC mode may reveal an answer to these partly contradictory findings.

With the above approach this paper seeks to characterize and to compare a number of reversed-phase stationary phases under HPLC versus CEC conditions. The characterization of the columns in both modes was performed using a well-defined standard test mixture and test procedure described by Galushko [35]. In addition other tests were performed for column evaluation too.

2. Experimental

2.1. Columns

The columns used in this study are listed in Table 1 together with relevant data provided by the manufacturer. The column packed bed was 25 cm, and 33.5 cm total length. Prior to use in CEC, the columns were conditioned. This was accomplished by applying 10 bar pressure on both sides of the column and increasing the voltage from 0 to 25 kV in 5 kV steps every 10 min. After that the pressure was increased to 12 bar and a 30 kV voltage was applied for 10 min. For the micro-HPLC experiments, the columns were conditioned until the column pressure was stabilized (requiring approx. 1 h). Note that in these experiments the same columns were tested under pressure and electro-driven con-

Table 1
List of investigated columns.

Column/stationary phase	Column diameter	Average particle size	Nominal pore size Å
Hypersil ODS	75 μm	3 μm	120
CEC Hypersil C_{18}	75 μm	3 μm	120
CEC Hypersil C_{18} [a]	100 μm	2.5 μm	120
CEC Hypersil C_{18} [a]	100 μm	2.5 μm	120
Unimicro C_{18}	75 μm	3 μm	300
Unimicro C_8	75 μm	3 μm	300
Unimicro Phenyl	75 μm	3 μm	300
NPS ODS	75 μm	3 μm	Non-porous

[a] Home-packed columns.

ditions using the same batches of eluents. All columns were preferably tested in the order CEC–HPLC to ensure the same flow velocity size.

2.2. Instrumentation

All CEC chromatograms were obtained via a Hewlett-Packard HP ^{3D}CE (Hewlett-Packard GmbH, Waldbronn, Germany) instrument equipped with a pressure facility of up to 12 bar at the outlet and/or inlet vial. This pressurization option of the instrument was used to prevent bubble formation in the capillaries. Samples were injected electrokinetically (5 kV for 2–15 s). For each run a voltage of 20 kV (600 V cm^{-1} electrical field strength) was applied with 10 bar pressure on both ends of a capillary. The detection wavelengths were 210 and/or 254 nm. High voltage was applied using a six-second time ramp to avoid column stress. The column cassette temperature was fixed at 20°C.

Micro-HPLC separations were carried out on a system consisting of a Phoenix 20 CU syringe pump (Carlo Erba Instruments, Milan, Italy), a microUVIS20 ultraviolet–visible absorbance detector (Carlo Erba Instruments, Milan, Italy) operating at 210 or 254 nm, and an injector with a 200 nl loop (VICI-AG Valco Europe, Schenkon, Switzerland). The flow-rate was approx. 0.2–0.3 μl/min with a 1/100-flow splitter. The experiments were performed at air-conditioned laboratory temperature (about 21°C) without additional thermostating.

2.3. Chemicals

The buffer consisted of di-sodium tetraborate decahydrate (Merck, Darmstadt, Germany), dissolved in deionized water and adjusted to pH=8.0 using concentrated hydrochloric acid (Merck, Darmstadt, Germany). Acetonitrile (ACN) and methanol (MeOH) were used as organic modifiers and were of HPLC supra-gradient-grade purity (both from Biosolve, Valkenswaard, Netherlands). The eluents were prepared by mixing the tetraborate buffer with an appropriate amount of the organic modifier and degassed (5 min) with helium prior to use. The same batch of eluent was used to test a specific column in both separation modes. Ionic strength in the eluent was kept constant at a tetraborate concentration of 1.5 mmol/l^{-1}. The test sample contained the following compounds: thiourea (t_0), phenol, aniline, benzene, toluene, *p*-ethylaniline, *N*,*N*-dimethylaniline, ethylbenzoate, ethylbenzene, biphenyl, naphthalene, fluorene, anthracene (all from Merck, Darmstadt, Germany). Samples were prepared by dissolving these compounds in the mobile phase or in the pure organic modifier and then diluted with the tetraborate buffer.

2.4. Test procedure

For the characterization of the RPLC stationary phases under CEC and HPLC conditions a test procedure as described by Galushko was applied [35]. Contrary to the aqueous methanol eluents used in the original test, we applied a tetraborate buffer pH=8.0 in our experiments (to guarantee a sufficiently electroosmotic flow velocity for all tested columns together with minimal packing degradation [50]) instead of water. Furthermore, acetonitrile was used next to methanol as another organic modifier in these column tests. Both modifiers were used at various concentrations in the eluents. Unless otherwise noted the standard test conditions were the following:

Eluent: methanol–aqueous tetraborate buffer pH=8.0 60:40 v/v

Temperature: 20°C

Test compounds: thiourea (t_0), aniline, phenol, benzene, and toluene.

Column descriptors were obtained using ChromLife software (Merck, Darmstadt, Germany) with the following parameters:

1. Hydrophobicity $(H) = (k_{\text{benzene}} + k_{\text{toluene}})/2$
2. Hydrophobic (methylene) selectivity (HS); retention data of benzene, toluene and phenol are used to calculate capacity factors of ethylbenzene and propylbenzene as described by Galushko [35].

   $$(HS) = k_{\text{propylbenzene}}/k_{\text{ethylbenzene}}$$

3. Silanol activity $(NI) = 1 + 3 \times [k_{\text{aniline}}/k_{\text{phenol}} - 1]$

   k = retention factor

To preserve a sufficiently wetted state of the stationary phase ligands, column tests were not

Table 2
Retention times of thiourea in CEC as a t_0 marker for all columns measured at several percentages of methanol and acetonitrile as organic modifier; test compound: thiourea (t_0); eluent: mixtures of methanol or acetonitrile and tetraborate buffer (1.5 m*M* in total); for other experimental conditions see text

Column	ACN (%)						MeOH (%)						
	30	40	50	60	70	80	50	60	65	70	75	80	90
Hypersil ODS 3 µm	4.049 ±0.024	3.186 ±0.013	3.504 ±0.010	3.010 ±0.010	2.714 ±0.022	–	5.676 ±0.029	5.965 ±0.023	5.699 ±0.014	5.714 ±0.010	5.023 ±0.017	–	–
CEC Hypersil C_{18} 3 µm	–[a]	2.048 ±0.021	2.085 ±0.011	2.340 ±0.017	2.318 ±0.020	2.400 ±0.004	–	–	–	–	–	–	–
CEC Hypersil C_{18} 2.5 µm (1)	4.467 ±0.025	4.118 ±0.024	3.915 ±0.015	3.875 ±0.013	3.579 ±0.021	3.510 ±0.015	7.721 ±0.027	8.475 ±0.039	8.396 ±0.033	8.249 ±0.036	8.043 ±0.035	–	–
CEC Hypersil C_{18} 2.5 µm(2)	4.065 ±0.027	3.582 ±0.020	3.604 ±0.009	3.603±0.001	3.444 ±0.010	3.285 ±0.003	–	–	–	–	–	–	–
Unimicro C_{18} 3 µm	–	5.379 ±0.013	5.183 ±0.006	4.955 ±0.018	4.744 ±0.024	4.616 ±0.012	–	13.014 ±0.009	12.880 ±0.008	13.078 ±0.012	15.588 ±0.011	12.106 ±0.010	10.626 ±0.015
Unimicro C_8 3 µm	–	3.374 ±0.004	2.269 ±0.002	3.149±0.007	3.119 ±0.007	3.047 ±0.005	–	15.601 ±0.007	15.371 ±0.012	15.125 ±0.019	14.086 ±0.016	13.694 ±0.025	12.907 ±0.023
Unimicro Phenyl 3 µm	–	4.029 ±0.014	4.058 ±0.018	3.871 ±0.009	3.758 ±0.015	3.410 ±0.012	9.292 ±0.013	9.308 ±0.012	9.098 ±0.009	8.899 ±0.023	8.633 ±0.019	8.472 ±0.022	–
NPS ODS 3 µm	5.310 ±0.001	5.162 ±0.003	5.133 ±0.002	–	–	–	11.075 ±0.009	13.004 ±0.006	14.149 ±0.008	–	–	–	

[a] (–) = data not available.

performed below 20% organic modifier. Table 2 further summarizes retention times of thiourea in CEC as a t_0 marker for all columns and all mobile phase compositions. The flow velocity in HPLC mode for a given condition was adjusted to that in CEC mode.

For details about this test method the reader is referred to paper [35] and references therein. The other test compounds (PAH's, *p*-ethylaniline, *N,N*-dimethylaniline) were used for additional tests of the RP stationary phases under study. Under all conditions all solutes are supposed to behave as neutral compounds. None of them was subject to electrophoretic mobility, which has been proved by capillary zone electrophoresis experiments.

3. Results and discussion

3.1. Column hydrophobicity and hydrophobic selectivity

As an example in Fig. 1a and b hydrophobic selectivities (HS) obtained on the Unimicro C_{18} 3 μm and Unimicro Phenyl 3 μm columns under pressure-driven (HPLC) and electro-driven (CEC) conditions are plotted. Under HPLC conditions for RP-phases of similar ligand length, the selectivity of specific increments (e.g. CH_2-group) generally is fairly constant under constant experimental conditions and decreases with increasing portions of organic modifier in the eluent [36–38]. In addition, ideally under further similar conditions, stationary phases behaving identically under both HPLC and CEC eluent-drive conditions show equal to one ratios of specific chromatographic properties like hydrophobicity or hydrophobic selectivity. The HS-values on the Unimicro C_{18} 3 μm column (Fig. 1a) are in good agreement with data usually obtained under HPLC conditions on RP-columns. Furthermore, the HS-values obtained under HPLC and CEC conditions on this column do differ not much if at all, suggesting similar behavior for this stationary phase for both separation modes. In contrast the HS-values obtained at 70 and 80% organic modifier on the Unimicro Phenyl 3 μm column differ significantly up to 23%.

In Fig. 2 the CH_2-selectivity ratios HS_{HPLC}/HS_{CEC} of all columns are presented together with the ideal line $HS_{HPLC}/HS_{CEC}=1$; HS_{HPLC} and HS_{CEC} represent hydrophobic selectivity obtained in the HPLC and CEC modes, respectively, under further similar experimental conditions. Hypersil ODS 3 μm (ACN), more polar phases such as the CEC Hypersil C_{18} 2.5 μm – No. 2 (ACN), Unimicro Phenyl 3 μm (MeOH), Unimicro C_8 3 μm (ACN and MeOH), and one of the self-packed CEC Hypersil C_{18} 2.5 μm – No. 2 (ACN), showed deviations in HS_{HPLC}/HS_{CEC}-ratios of up to ±10%. In addition somewhat lower deviations in HS-ratios were obtained of up to −8% for the Unimicro C_8 3 μm column for both acetonitrile and methanol as organic modifier, and ±6% for the Hypersil ODS 3 μm (ACN) columns.

An unexplained exception is the 5% and the 23% deviation found for the Unimicro Phenyl 3 μm column using 70 and 80% of acetonitrile, respectively. For all other modifier concentrations of this column the HS-ratio deviation has maximally 5%. All other columns showed only moderate deviations of a few percent in their HS_{HPLC}/HS_{CEC} ratios. More specifically the changes were up to 1.7%, 4% and −2% for the CEC Hypersil C_{18} 3 μm under ACN-conditions, the NPS ODS 3 μm and the Hypersil ODS 3 μm, respectively, with methanol as the organic modifier. Hydrophobicity and hydrophobic selectivity are related to length, ordering and orientation of the ligands on a substrate's surface [39,40]. More particularly, orientation and ordering also depends on ligand coverage density and the nature and concentration of the organic modifier [41]. Since the column tests only differed in the mode of application (CEC vs. HPLC), we speculate that the observed deviations in HS_{HPLC}/HS_{CEC} ratios must be attributed to stationary phase changes under electrical field conditions. This is in agreement with findings of others such as Eimer et al. [30], Wei et al. [31], and Angus et al. [42], who also observed dissimilarities in stationary phase properties under HPLC and CEC conditions. Obviously, not all nominally identical stationary phases respond in a similar way to the application of an electrical field. For instance, the HS-values of the CEC Hypersil C_{18} 2.5 μm and the CEC Hypersil C_{18} 2.5 μm (No. 2), both under acetonitrile conditions, are 10 and 2%, respectively.

In contrast to these relatively small deviations in

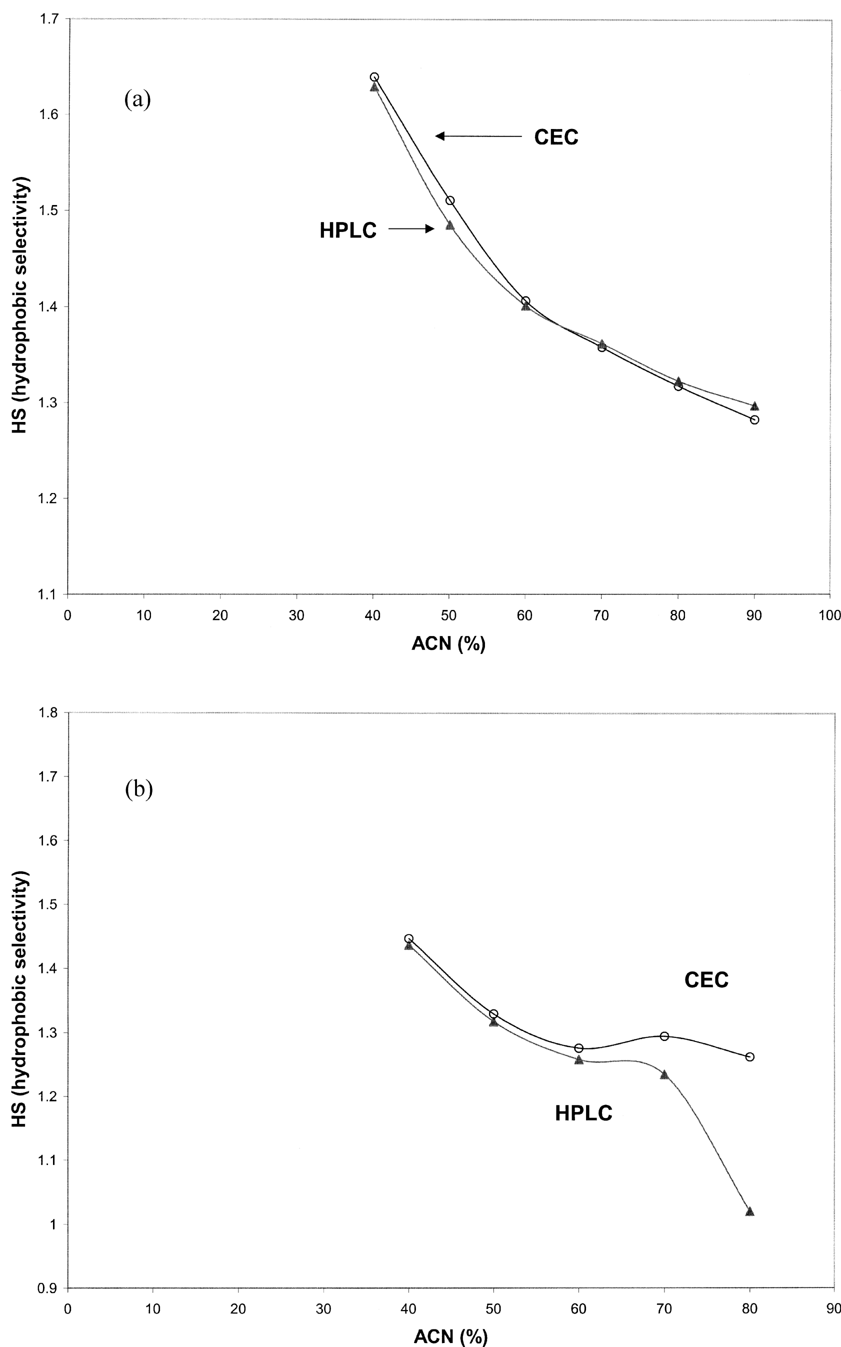

Fig. 1. (a) Hydrophobic selectivity (HS) values for the Unimicro C_{18} 3 μm column under pressure (HPLC) and electro-driven (CEC) conditions; eluent: tetraborate buffer (1.5 m*M* in total), pH=8.0 and acetonitrile, detection: 254 nm; test compounds: thiourea (t_0), phenol, benzene, toluene; for other experimental conditions see Section 2.2 Instrumentation. (b) Hydrophobic selectivity (HS) values for the Unimicro Phenyl 3 μm column under pressure (HPLC) and electro-driven (CEC) conditions; eluent: tetraborate buffer (1.5 m*M* in total), pH=8.0 and acetonitrile, detection: 254 nm; test compounds: thiourea (t_0), phenol, benzene, toluene; for other experimental conditions see Section 2.2 Instrumentation.

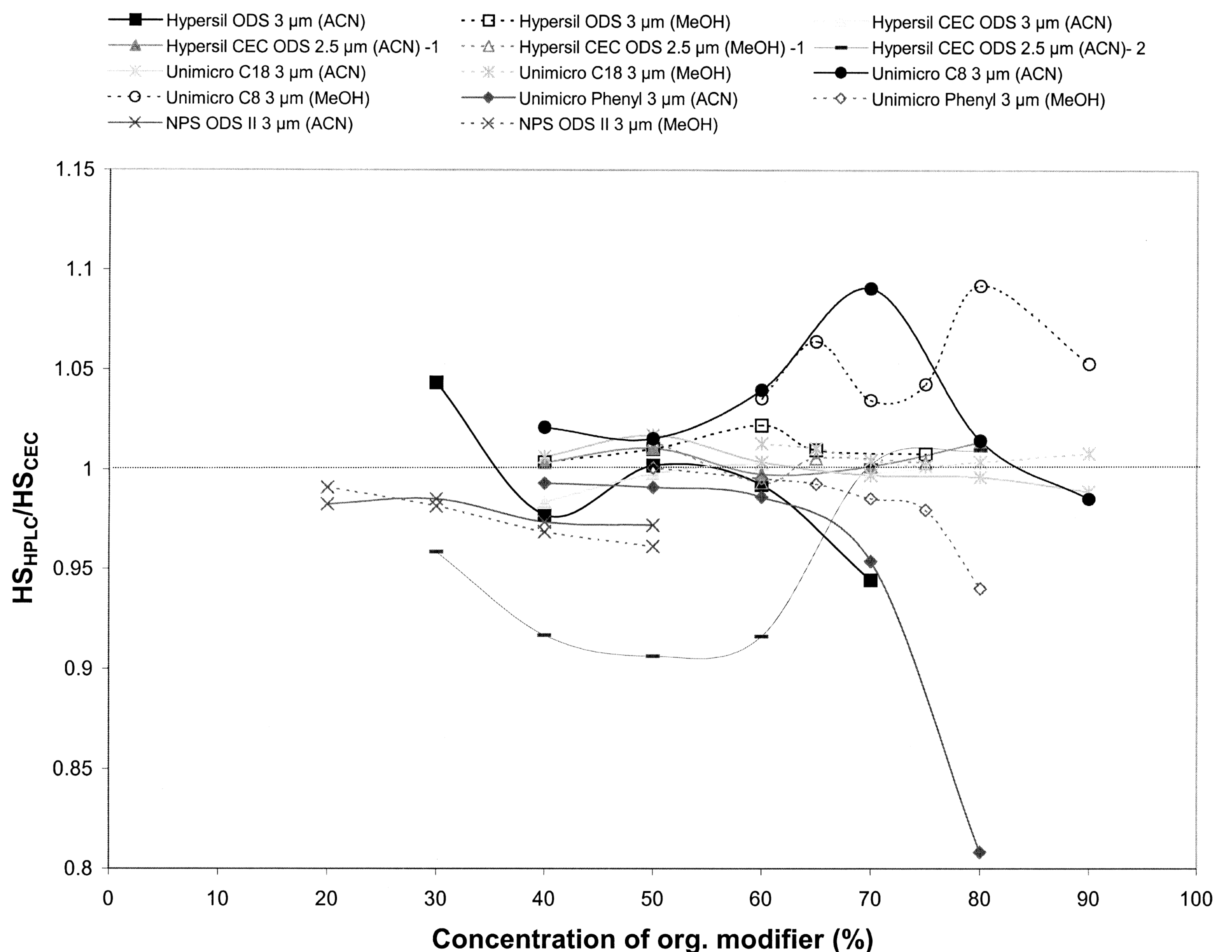

Fig. 2. Ratios of CH_2 selectivities under pressure (HPLC) and electro-driven (CEC) conditions HS_{HPLC}/HS_{CEC} for all columns for methanol and acetonitrile as organic modifier; eluent: tetraborate buffer (1.5 m*M* in total) pH=8.0 and organic modifier (for each column indicated in brackets); detection: 254 nm; test compounds: thiourea (t_0), phenol, benzene, toluene; for other experimental conditions see Section 2.2 Instrumentation. (. . .)=$HS_{HPLC}/HS_{CEC}=1$.

HS-ratios, much larger differences in hydrophobicity (H) properties were found. As an example the hydrophobicity (H) values obtained under HPLC and CEC conditions for CEC Hypersil C_{18} 3 µm for acetonitrile as organic modifier are presented in Fig. 3. Obviously, for this column hydrophobicity differs significantly under both these conditions. In addition, column hydrophobicity in the CEC-mode is smaller than in the HPLC mode under further similar experimental conditions for all cases. This finding is in accordance with earlier results of Eimer et al. [30] who reported an average decrease of approx. up to 40% in hydrophobicity, when applying an RP-column under PEC-conditions. Furthermore, for this column hydrophobicity under HPLC conditions increases relatively more compared to CEC conditions at decreasing fraction of acetonitrile in the eluent.

To compare stationary phase behavior, log *k* values of benzene vs. percentage of organic modifier (ACN and MeOH) were plotted for the Unimicro C_8 3 µm column in Fig. 4a and b respectively. For both separation modes and both modifiers a linear relationship was found with regression coefficients *r* from 0.9988 to 0.9999. As statistically determined (on the level of significance of 0.05) slopes log *k* vs. percentage of acetonitrile are not identical. Fig. 4b

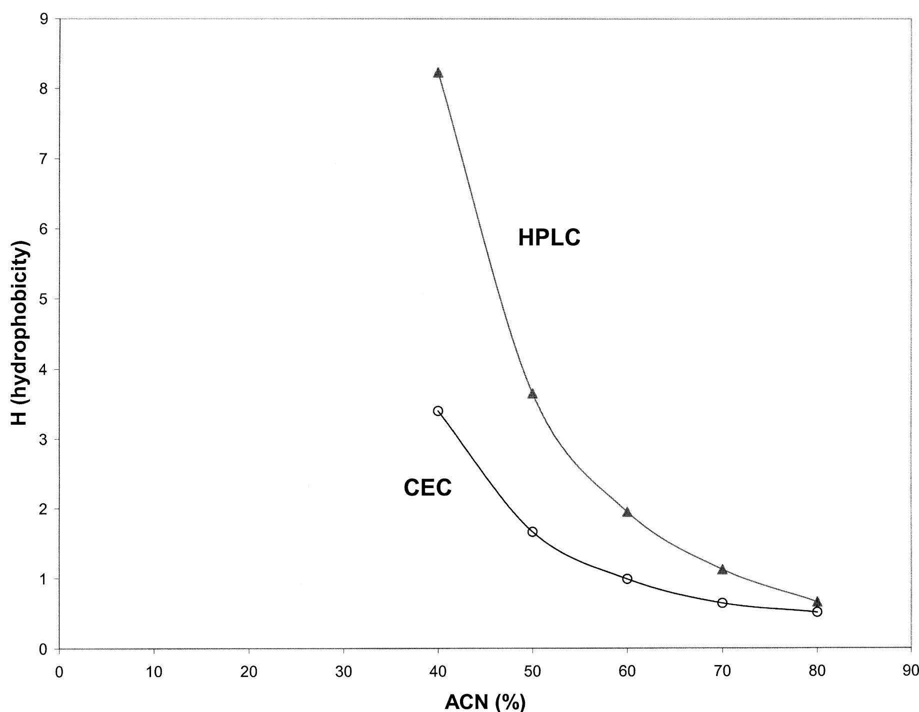

Fig. 3. Hydrophobicity (H) values for the CEC Hypersil C_{18} 3 μm column under pressure (HPLC) and electro-driven (CEC) conditions; eluent: tetraborate buffer (1.5 m*M* in total) pH=8.0 and acetonitrile; test compounds: thiourea (t_0), benzene, toluene; further experimental conditions see Section 2.2 Instrumentation.

further shows that log k_w values of benzene (retention factor of benzene extrapolated to pure water as the mobile phase) differ in methanol as organic modifier. This implies differences between the separation modes and modifiers used.

In Fig. 5 the hydrophobicity ratios H_{HPLC}/H_{CEC} for all columns are presented together with the ideal line $H_{HPLC}/H_{CEC}=1$. From these results it is obvious that significant differences in hydrophobicity occur depending on whether a column is used under HPLC or CEC conditions. Besides the specific properties of the stationary phase, this also appears to depend on the nature and concentration of the applied organic modifier. For instance, deviations in column H_{HPLC}/H_{CEC}-ratios are up to 47% for Hypersil ODS 3 μm and −59% for CEC Hypersil C_{18} 3 μm for acetonitrile as the organic modifier in the buffer. In addition, deviations in H_{HPLC}/H_{CEC}-ratios of up to −25% in ACN and also in MeOH for the Unimicro C_{18} 3 μm, 31% for the Unimicro C_8 3 μm column and up to 22% for CEC Hypersil C_{18} 2.5 μm (No. 2) were observed, both for acetonitrile. Note that these Hypersil and Unimicro packings have nominal pore sizes of 120 Å and 300 Å, respectively. Within this limited number of packing materials no clear influence of pore size on H_{HPLC}/H_{CEC}-ratios could be observed. The smallest deviations were found for the CEC Hypersil C_{18} 2.5 μm column (No. 1) (up to 5%) and NPS ODS 3 μm (± 5%for both ACN and MeOH).

With an exception of CEC Hypersil ODS 2.5 μm (No. 2) H_{HPLC}/H_{CEC}-ratios of the porous packing materials generally are much closer to one for methanol than acetonitrile as the organic modifier. These modifiers differ significantly in their hydrogen bond donor capacity (MeOH; 0.43 vs. 0.15 for ACN) and polarity/polarizability (0.60 for ACN and 0.28 for methanol) [43]; (normalized values).

These differences in the physico-chemical properties between both these modifiers are responsible for different states of wettability and interphase layers of the ligands under normal HPLC-conditions

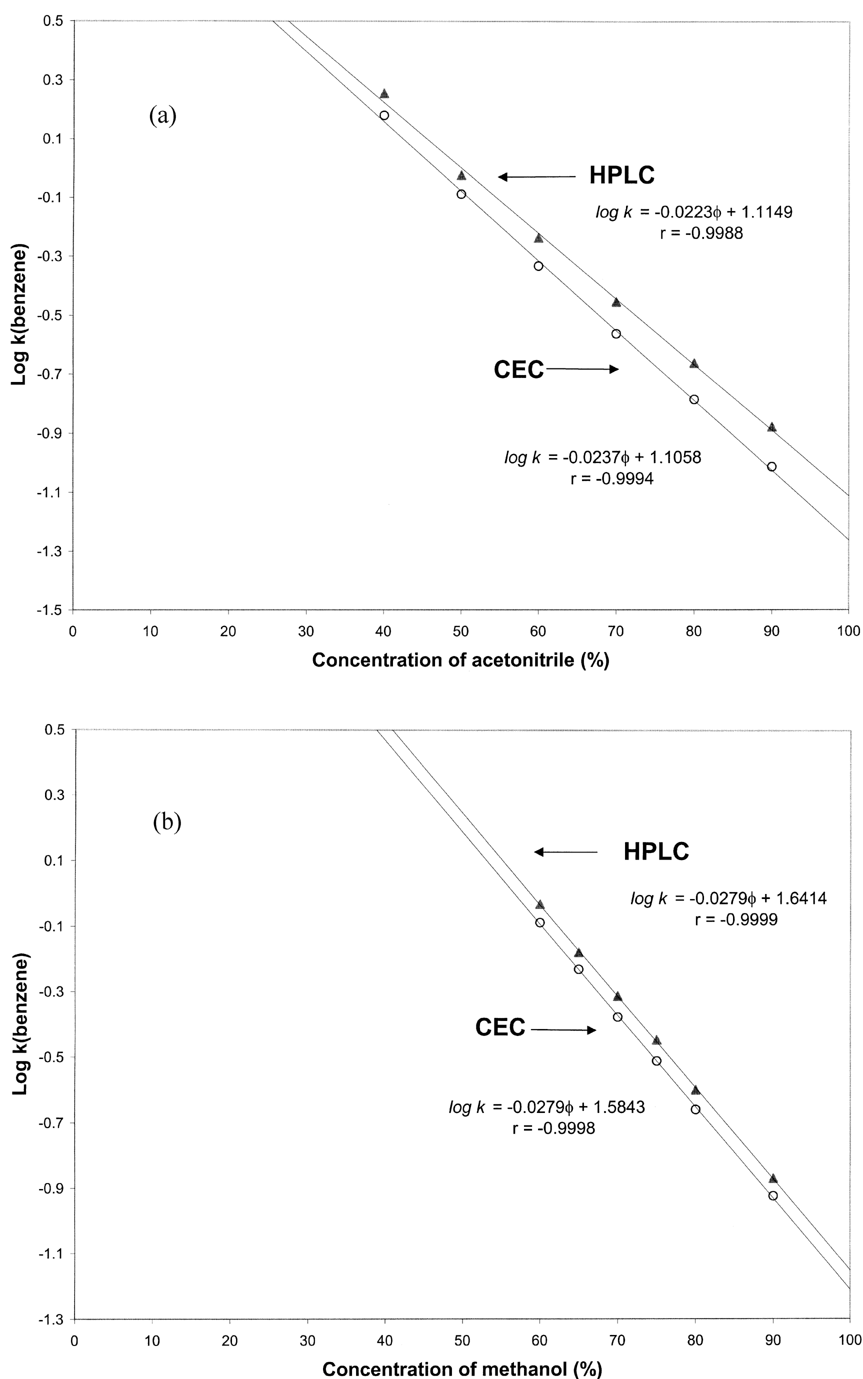

Fig. 4. (a) Log k values of benzene for the Unimicro C_8 3 μm column under pressure (HPLC) and electro-driven (CEC) conditions; eluent: tetraborate buffer (1.5 mM in total) pH=8.0 and acetonitrile; test compound: benzene; further experimental conditions see Section 2.2 Instrumentation. (b) Log k values of benzene for the Unimicro C_8 3 μm column under pressure (HPLC) and electro-driven (CEC) conditions; eluent: tetraborate buffer (1.5 mM in total) pH=8.0 and methanol; test compound: benzene; further experimental conditions see Section 2.2 Instrumentation.

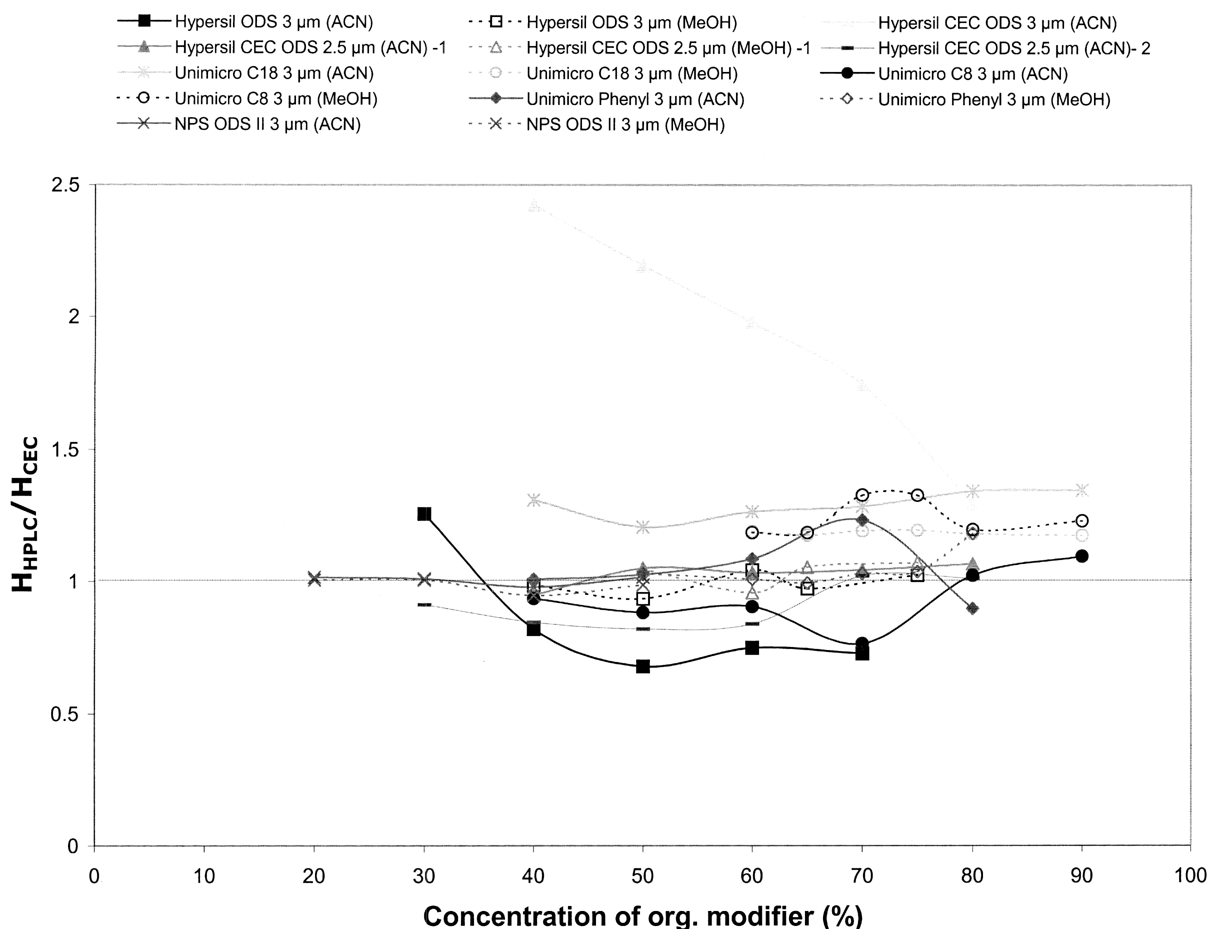

Fig. 5. Ratios of hydrophobicities H_{HPLC}/H_{CEC} of the columns under pressure (HPLC) and electro-driven (CEC) conditions using methanol and acetonitrile as organic modifier (for each column indicated in brackets); (. . .) = $H_{HPLC}/H_{CEC} = 1$; further experimental conditions see Section 2.2 Instrumentation.

[41,44,45]. We assume that in addition to these effects the application of an electrical field may cause different ligand orientations in the interphase resulting in the observed differences in H_{HPLC}/H_{CEC}-ratios. An alternative explanation might be found in the phase ratios of CEC columns under HPLC and CEC conditions. As discussed by Antle and Ying et al. [39,40] the various phase ratios of RP-columns often count significantly for the major part of the observed differences in hydrophobicities between columns rather than differences in distribution coefficients. Rathore and Horváth discussed the phenomenon of "electroosmotic whirlwind" around particles and pores [46] of packings under CEC-conditions.

These effects may cause limited access of test probes to the stationary phase internal pore volume contributing to virtually different phase ratios.

It seems safe to assume that this effect will depend on the mean pore size and more particularly on the pore size distribution (fraction micropores versus macropores) in a specific packing material among other things. Further support for this assumption is found in the close to one H_{HPLC}/H_{CEC}-values observed for the non-porous (NPS) stationary phase in this study. For this latter type of packing and for

both organic modifiers these ratios are 0.95–1.02 over the entire range of the modifier concentration. We assume that the nearly complete absence of pores of this NPS phase prevents the occurrence of different phase ratio values, in turn causing the constant H_{HPLC}/H_{CEC}-ratios found.

3.2. Silanol activity

Silanol activity of RP-columns is a rather empirical term and may include a number of Van der Waals and ion-exchange solute to stationary phase interactions [47]. In the Galushko test applied here, silanol activity is based on the measurement of the ratio of retention factors of aniline and phenol.

Silanol activity data were calculated as a function of the nature and percentage of organic modifier; the results are summarized in Table 3. In Fig. 6 the ratios of silanol activities NI(HPLC)/NI(CEC) measured on each column are plotted together with the ideal line NI(HPLC)/NI(CEC)=1. From these results it can immediately be seen that for some columns the silanol activity ratios vary substantially as a function of the nature and fraction of the organic modifier in the buffer. For instance, the NI_{HPLC}/NI_{CEC}-ratios of Hypersil ODS 3 μm and Unimicro C_{18} and C_8 3 μm with methanol vary substantially over the investigated concentration range. Under acetonitrile conditions these ratios are smoother and less pronounced for these columns. Note that from these columns (under acetonitrile conditions) the Hypersil ODS 3 μm shows an NI_{HPLC}/NI_{CEC}-ratio$>$1. In all other cases depending on the nature and concentration of the modifier, NI-ratios larger or smaller than one were found. In contrast to the findings mentioned above other columns in this set showed for more smooth and much less pronounced NI_{HPLC}/NI_{CEC}-ratios over the investigated modifier concentration range for both modifiers. For Unimicro phenyl 3 μm and Hypersil CEC ODS 2.5 μm (1) for example rather smooth and constant ratios were found for both modifiers. There was an exception for Unimicro Phenyl 3 μm in the 60–80% acetonitrile range. Note that the NI_{HPLC}/NI_{CEC}-ratio is <1 under all conditions for the latter column.

For NPS ODS no difference in retention of aniline and phenol was found for either modifier. Consequently, NI_{HPLC}/NI_{CEC}-ratios were one over the entire modifier concentration range. Obviously the silanol activity of this latter column type is rather independent of the modifier's nature (acetonitrile vs. methanol) and operating conditions (HPLC vs. CEC).

From the results reported in Table 3 and Fig. 6 it is clear that for porous RP-packing materials silanol activity generally is not independent of the mode of operation and the applied modifier. For example, for the Unimicro Phenyl column under 50% methanol conditions, silanol activity is 1.51 and 5.81 under HPLC and CEC-conditions, respectively. In addition, for the same column substantially different silanol activities of 1.81 and 1.51 for 50% acetonitrile and methanol, respectively, under the same HPLC-mode can be observed.

Earlier studies have shown that under HPLC conditions silanol activity of porous RP columns may depend substantially on the nature of the modifier independent of whether methanol or acetonitrile is used in the eluent ([48,49] and refs. therein). Our results from the present study are in agreement with these earlier findings. In addition, the same appears to be true for porous packing materials under CEC-conditions.

Similar to hydrophobicity in the Galushko-test, silanol activity is determined from retention factors of two different compounds (see Section 2.4). Referring to the previous section on column hydrophobicity, we believe that the observed differences in silanol activity for porous packings must also be attributed to the following:

(i) Apparent differences in phase ratios under HPLC and CEC-conditions caused by electroosmotic whirlwind effects.

(ii) Different ligand orientations and thus silanol accessibility under both HPLC and CEC modes.

It should further be note that for porous packings from the results in Fig. 6 and Table 3 it can be concluded that the differences and changes in silanol activity under CEC and HPLC conditions are much more pronounced for methanol instead of acetonitrile.

In contrast to the observations for porous stationary phases the non-porous NPS ODS 3 μm packing showed remarkably different silanol activity behavior. Irrespective of the modifier (methanol or

Table 3
Silanol activity results for all columns measured at several percentages of methanol and acetonitrile as organic modifier; test compounds: thiourea (t_0), aniline, phenol; eluent: mixtures of methanol or acetonitrile and tetraborate buffer (1.5 m*M* in total); for other experimental conditions see text

Column	Mode	ACN (%)						MeOH (%)						
		30	40	50	60	70	80	50	60	65	70	75	80	90
Hypersil ODS 3 μm	HPLC	0.6389	1.0203	1.2970	1.3295	1.6266	–[a]	0.0382	0.0053	0.0725	0.2443	–	–	–
	CEC	0.6499	0.7464	0.9655	1.0000	1.0000	–	0.0192	0.0827	0.0733	0.2618	–	–	–
CEC Hypersil C_{18} 3 μm	HPLC	–	1.4705	1.5236	1.9696	2.1921	2.4776	–	–	–	–	–	–	–
	CEC	–	2.0528	2.4648	1.5936	2.2676	2.8684	–	–	–	–	–	–	–
CEC Hypersil C_{18} 2.5 μm (1)	HPLC	–	1.5074	1.7563	1.9602	2.2542	3.0038	–	1.2591	1.2425	–	1.5077	–	–
	CEC	–	1.7159	1.8734	2.1197	4.2826	3.0571	–	2.2358	1.9579	1.8254	1.8741	–	–
CEC Hypersil C_{18} 2.5 μm (2)	HPLC	1.3773	1.6008	1.8248	2.0891	2.4127	2.9429	–	–	–		–	–	–
	CEC	1.1623	1.7141	1.7349	1.6689	2.7333	2.4103	–	–	–	–	–	–	–
Unimicro C_{18} 3 Cm	HPLC	–	1.0000	1.1924	1.5264	1.6849	1.9813	2.1811	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	CEC	–	1.0528	1.3255	1.5227	1.8250	2.3043	2.0714	0.6747	0.8250	0.3407	1.0000	−0.8235	0.8000
Unimicro C_8 3 μm	HPLC	–	1.2281	1.5389	2.0893	2.2712	2.7333	–	1.0000	1.0000	−0.0588	1.0000	1.0000	−0.2973
	CEC	–	1.0000	1.3726	1.6606	2.1739	6.8696	–	0.5257	0.7107	1.0000	1.0000	1.0000	1.0000
Unimicro Phenyl 3 μm	HPLC	–	1.5042	1.8116	2.5644	5.3548	1.0000	1.5106	1.9345	2.5370	2.9412	3.3939	4.4160	–
	CEC	–	1.5878	1.9310	2.7869	10.269	10.390	5.8146	6.8984	7.9805	15.697	16.181	19.570	–
NPS ODS 3 μm	HPLC	1.0000	1.0000	1.0000	–	–	–	1.0000	1.0000	1.0000	–	–	–	
	CEC	1.0000	1.0000	1.0000	–	–	–	1.0000	1.0000	1.0000	–	–	–	

[a] (–) = data not available.

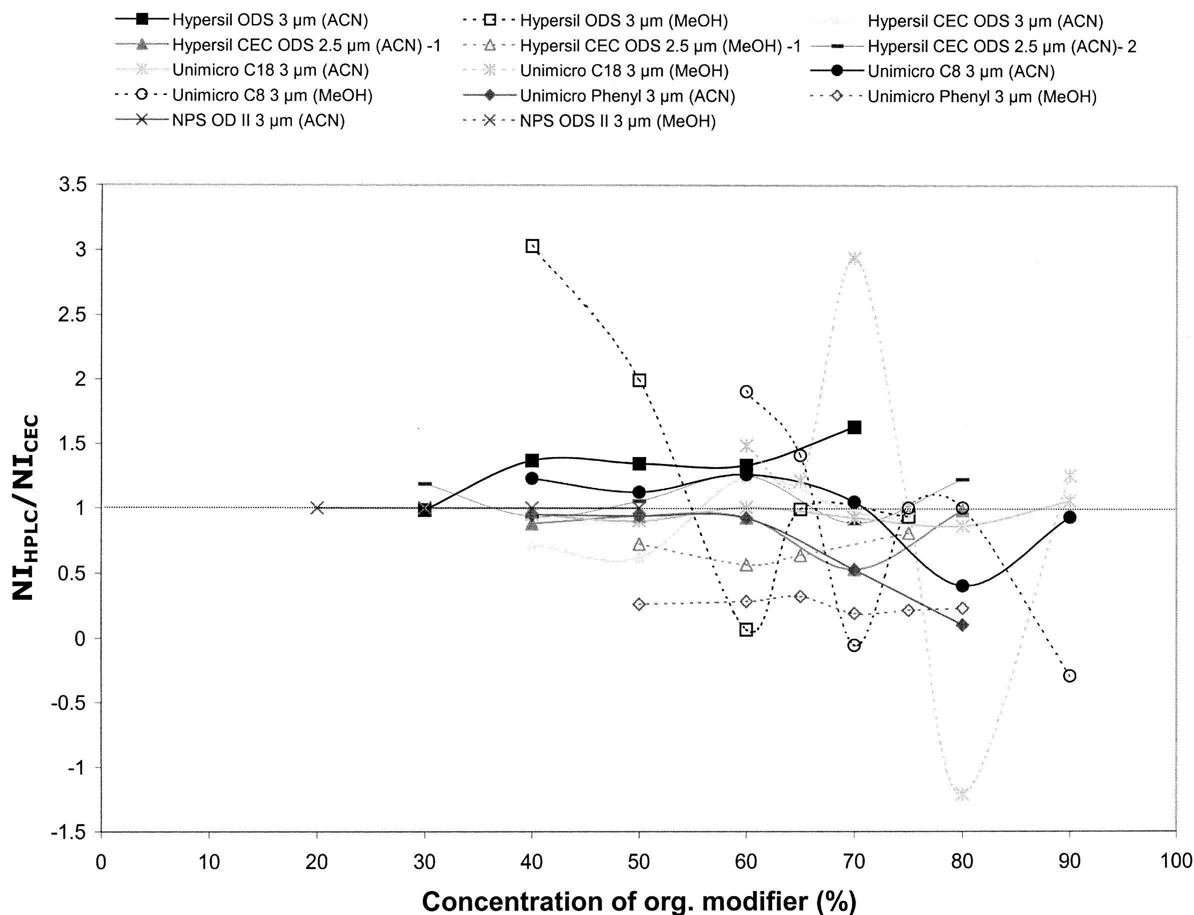

Fig. 6. Ratios of silanol activity values NI(HPLC)/NI(CEC) of the columns under pressure (HPLC) and electro-driven (CEC) conditions using methanol and acetonitrile as organic modifier (for each column indicated in brackets); (. . .) = NI(HPLC)/NI(CEC) = 1, further experimental conditions see Section 2.2 Instrumentation.

acetonitrile) or the applied mode (HPLC vs. CEC) silanol activity values of one were observed for all experiments. Again this might be taken as evidence that the absence of electroosmotic whirlwind effects in these solid packings are responsible for the more similar behavior under different conditions (modifier and mode of operation) compared to porous packing materials. In Table 4 two parameters, USP tailing factor and plate number of aniline, are both given separation modes and eluent containing 70% of acetonitrile as the organic modifier (40% of acetonitrile for the NPS ODS 3 μm column). Special attention was paid to the comparison of the electroosmotic flow (EOF) (Table 2) and the chromatographic behavior of the silanol sensitive compound (aniline) as long as the remaining silanol groups of stationary phase packing were responsible for the EOF. It can be concluded that columns under this particular condition generating lower EOF gave higher efficiencies (the Unimicro C_{18} 3 μm, the Unimicro Phenyl 3 μm and NPS ODS 3 μm columns). No direct relationship between EOF and USP tailing factor of aniline can be drawn, with the exception of the Unimicro C_{18} 3 μm and the Unimicro C_8 3 μm columns higher in CEC mode. Finally, no clear relationship between silanol activi-

Table 4
USP tailing factor and plate numbers of aniline in HPLC and CEC for all columns measured at 70% (40% for the NPS ODS 3 μm column) acetonitrile as organic modifier; test compound: aniline; eluent: 70% of acetonitrile and tetraborate buffer (1.5 m*M* in total); for other experimental conditions see text.

Column	Mode	USP Tailing factor	Plate number (half-width method)/column
Hypersil ODS 3 μm	HPLC	1.206	13115
	CEC	1.208	19800
CEC Hypersil C_{18} 3 μm	HPLC	1.225	14602
	CEC	1.257	18878
CEC Hypersil C_{18} 2.5 μm (1)	HPLC	1.155	12025
	CEC	1.329	22281
CEC Hypersil C_{18} 2.5 μm (2)	HPLC	1.198	12601
	CEC	1.311	22239
Unimicro C_{18} 3 μm	HPLC	1.258	16052
	CEC	1.205	36532
Unimicro C_8 3 μm	HPLC	1.229	10571
	CEC	1.075	26323
Unimicro Phenyl 3 μm	HPLC	1.092	17776
	CEC	1.390	30157
NPS ODS 3 μm	HPLC	1.067	23205
	CEC	1.087	28590

ty, average pore diameter and EOF [51] of the packing could be derived from the present results.

4. Conclusions

Under pH=8 condition and using methanol and acetonitrile as modifiers eight columns packed with seven different RP-phases were tested under pressure (HPLC) and electro-driven (CEC) conditions. With an exception for the Unimicro Phenyl column, methylene (hydrophobic) selectivity did not differ substantially between both modes: maximally 10% for the CEC Hypersil C_{18} 2.5 μm (No. 2) column. The limited ligand chain length can easily explain the strongly deviating results for the former column.

By contrast, for porous stationary phases, substantial differences in the major column descriptors hydrophobicity and silanol activity were found between the HPLC and CEC-modes. In addition, these differences were also a function of and strongly dependent on the nature and concentration of the applied modifier for some cases. These observations can probably be explained from different ligand orientations caused by the conditions of both eluent-driven modes. An alternative explanation of these findings may be found in the occurrence of electroosmotic whirlwind effects in porous packing causing different phase ratios.

For the non-porous stationary phase for the HPLC and CEC eluent-driven mode very similar hydrophobicity and silanol activity data were measured. This has also been taken as additional evidence for the electroosmotic whirlwind effect in porous packings. The results of the present study confirm the differences usually found in silanol activity between methanol and acetonitrile as the organic modifier under HPLC conditions. These effects, however, appear to be more manifest under CEC conditions. It was also found that the use of acetonitrile generally delivered smoother HPLC/CEC ratio curves versus percentage of modifier than methanol.

Finally, the results of this study clearly show that at least for porous stationary phases the transfer of existing HPLC methods to CEC analysis protocols is not straightforward.

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