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Quantitative structure–retention relationships in comparative studies of behavior of stationary phases under high-performance liquid chromatography and capillary electrochromatography conditions

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Abstract

Quantitative structure–retention relationships (QSRR) have been employed in studying the molecular mechanism of chromatographic separations under pressure- (HPLC) and electro-driven (CEC) conditions. Logarithms of retention factors corresponding to zero percent of organic modifier in aqueous eluent, log k_w, were determined on eight reversed-phase stationary phases under both HPLC and CEC conditions at similar eluent flow velocities. QSRR equations describing log k_w in terms of linear solvation energy relationship (LSER) parameters of analytes, in terms of simple structural descriptors acquired by calculation chemistry, and in terms of logarithms of *n*-octanol–water partition coefficients, were derived. Parameters of corresponding QSRR equations for individual stationary phases were compared for both HPLC and CEC modes and the resulting similarities and differences in retention mechanisms were discussed. It has been concluded that at least in the case of regular neutral analytes the specific inputs to separation mechanism due to the electric field in CEC are of secondary importance.

Keywords: Quantitative structure–retention relationship; Linear solvation energy relationship; Stationary phases, LC; Stationary phases, CEC; Retention mechanisms

1. Introduction

Over two decades of development of capillary electrochromatography (CEC) many articles have been published on the underlaying theory [1–10]. As a highly efficient separation technique CEC was proposed mainly for analysis of steroids, peptides and proteins [11–16]. Much effort was devoted to column technology, development of stationary phases and to specific column design.

CEC, combining the theory and practice of capillary electrophoresis (CE) and capillary high-performance liquid chromatography (HPLC), attracted special attention from the point of view of the molecular mechanism of retention. The retention

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behavior of test analytes has been studied by a number of authors, who usually compared retention factors or plate numbers of neutral compounds under both HPLC and CEC conditions. Recently, model-based methods have been employed, in these comparative studies. Among them the Galushko model [17,18] or models based on structural descriptors of analytes from molecular modeling or from linear solvation energy relationships (LSER) [19–25] are probably best known. The latter models have been successfully used in HPLC to differentiate reversed-phase (RP) stationary phases and to predict retention of analytes.

The simplest quantitative structure–retention relationship (QSRR) model used in comparative studies of stationary phases relates log k_w to logarithm of *n*-octanol–water partition coefficient, log *P*:

$$\log k_w = k_1 + k_2 \log P \quad (1)$$

where log k_w is retention factor extrapolated to a pure water (buffer) mobile phase, the coefficients k_1 and k_2 are characteristics of the systems representing differences in the individual properties between the mobile and the stationary phase.

The following model relates log k_w to structural descriptors of analytes provided by molecular modeling:

$$\log k_w = k_1' + k_2'\delta_{min} + k_3'\mu^2 + k_4'\mathrm{SAS} \quad (2)$$

where $\delta_{min}$is the largest atomic excess of electrons, μ^2 is square of total dipole moment and SAS is van der Waals surface area of a molecule that is accessible to a molecule of water, the coefficients k_1', k_2', k_3' and k_4' are characteristics of the systems representing differences in the individual properties between the mobile and the stationary phase.

The LSER model of QSRR is characterized by the following general equation:

$$\log k_w = k_1'' + k_2''R_2 + k_3''\pi_2^H + k_4''\alpha_2^H + k_5''\beta_2^H + k_6''V_x \quad (3)$$

where R_2 is excess molar refraction, π_2^H is dipolarity/polarizability, α_2^H is hydrogen-bond acidity, β_2^H is hydrogen-bond basicity and V_x is characteristic volume of McGowan, the coefficients k_1'', k_2'', k_3'', k_4'', k_5'' and k_6'' are characteristics of the systems representing differences in the individual properties between the mobile and the stationary phase.

Wei et al. [26] applied LSER in a comparative study of 3 μm ODS particles. They used 70% ACN:30% aqueous buffer (2 m*M* Tris–HCl) as a mobile phase and found out that QSRR parameters referring to CEC conditions differed from those obtained at RP-HPLC conditions. Unlike in RP-HPLC, hydrogen-bond basicity of a solute (β_2^H) was statistically significant in CEC. The parameters V_x, π_2^H, α_2^H and β_2^H were all of similar significance in CEC. On the other hand, in HPLC the most significant were V_x and β_2^H parameters; actually, π_2^H was statistically significant at 95% significance level but it was of lesser importance for retention description.

Employing LSER Liu et al. [27] discussed the role of organic modifier in RP-HPLC and in CEC. The same group [28] also studied the behavior of Spherisorb ODS II stationary phase in CEC, pressurized electrochromatography (PEC) and HPLC. For acetonitrile as an organic modifier, the reported LSER equations obtained under CEC, PEC and HPLC conditions were closely similar. In the view of limited and rather fragmental actual knowledge it appeared worthwhile to systematically study the molecular mechanism of separations in analogous chromatographic systems operated at CEC and HPLC conditions employing the QSRR approach. For that purpose eight RP stationary phases were subjected to a study under both HPLC and CEC conditions using acetonitrile as organic modifier.

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 and the total length was 25 and 33.5 cm, respectively.

Prior to use in the CEC mode, 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 for 10 min. Next, 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

Table 1
List of investigated columns

Column	Column diameter [μm]	Average particle size [μm]	Pore size [Å]	Pore volume [cm^3/g]	Surface area [m^2/g]	Carbon load [%]
CEC Hypersil C_{18}	100	3	130	0.65	170	8.5
Hypersil C_8	100	3	120	0.65	170	6.5
Hypersil Phenyl	100	3	120	0.65	170	5
Spherisorb ODS	100	3	80	0.50	200	6.2
Spherisorb C_8	100	3	80	0.50	200	5.8
Unimicro C_{18}	100	3	N/A	N/A	N/A	N/A
Unimicro C_8	100	3	N/A	N/A	N/A	N/A
Unimicro Phenyl	100	3	N/A	N/A	N/A	N/A

N/A means that data are not available.

conditioned until the column pressure was stabilized (about 1 h).

The columns were tested under pressure- and electro-driven conditions using the same batches of eluents. All the columns were supplied in duplicate (the same batch with maximum 2% RSD in retention factor under HPLC conditions). The requirement was met for analysis of up to 1% RSD in retention factor under CEC conditions and of up to 0.5% RSD under HPLC conditions; each for six consecutive injections. HPLC conditions were adjusted to similar flow velocities as obtained in CEC. As a consequence, the HPLC experiments were not optimized with respect to the plate height.

2.2. Instrumentation

All the CEC chromatograms were obtained on a ^{3D}CE instrument (Agilent Technologies GmbH, Waldbronn, Germany) 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} electric field strength) was applied with 10 bar pressure at both ends of a capillary. The detection wavelength was 210 nm. High voltage was applied as a 6-s time ramp to avoid column stress. The column cassette temperature was maintained at 20 °C.

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

2.3. Chemicals

Acetonitrile (ACN) of HPLC supra gradient-grade purity (Biosolve, Valkenswaard, Netherlands) was used as the organic modifier in various concentrations. The eluents were prepared by mixing phosphate buffer (pH 7.0, final concentration 1 m*M*) with an appropriate amount of the organic modifier and degassed ultrasonically for 15 min prior to use. The same batch of eluent was used to test a given column at both separation modes. The set of test analytes is listed in Table 2 together with their structural descriptors. The series of analytes was taken as previously designed [29] with the well-defined hy-

Table 2
Structural descriptors of test analytes used in QSRR equations

No.	Solute	log P	R_2	π_2^H	α_2^H	β_2^H	V_x	δ_{min}	μ^2	SAS
1	*n*-Hexylbenzene	5.52	0.591	0.50	0.00	0.15	1.562	−0.2104	0.03880	415.40
2	1,3,5-Triisopropylbenzene	6.36	0.627	0.40	0.00	0.22	1.985	−0.2057	0.00624	478.27
3	1,4-Dinitrobenzene	1.47	1.130	1.63	0.00	0.41	1.065	−0.3418	0.00012	312.07
4	3-Trifluoromethylphenol	2.95	0.425	0.87	0.72	0.09	0.969	0.2454	4.39321	302.54
5	3,5-Dichlorophenol	3.62	1.020	1.10	0.83	0.00	1.020	0.2434	1.98246	306.77
6	4-Cyanophenol	1.60	0.940	1.63	0.79	0.29	0.930	−0.2440	10.9693	290.61
7	4-Iodophenol	2.91	1.380	1.22	0.68	0.20	1.033	−0.3021	2.51856	301.47
8	Anisole	2.11	0.708	0.75	0.00	0.29	0.916	−0.2116	1.56000	288.13
9	Benzamide	0.64	0.990	1.50	0.49	0.67	0.973	−0.4334	12.8450	293.30
10	Benzene	2.13	0.610	0.52	0.00	0.14	0.716	−0.1301	0.00000	244.95
11	Chlorobenzene	2.89	0.718	0.65	0.00	0.07	0.839	−0.1295	1.70824	269.49
12	Cyclohexanone	0.81	0.403	0.86	0.00	0.56	0.861	−0.2944	8.83278	269.31
13	Dibenzothiophene	4.38	1.959	1.31	0.00	0.18	1.379	−0.2709	0.27457	364.54
14	Phenol	1.47	0.805	0.89	0.60	0.30	0.775	−0.2526	1.52028	256.72
15	Hexachlorobutadiene	4.78	1.019	0.85	0.00	0.00	1.321	−0.0750	0.06708	352.14
16	Indazole	1.77	1.180	1.25	0.54	0.34	0.905	−0.2034	2.39011	285.46
17	Caffeine	−0.07	1.500	1.60	0.00	1.35	1.363	−0.3620	13.3298	367.02
18	4-Nitrobenzoic acid	1.89	0.990	1.07	0.62	0.54	1.106	−0.3495	11.7786	321.77
19	*N*-Methyl-2-pyrrolidinone	−0.38	0.491	1.50	0.00	0.95	0.820	-0.3532	12.9168	270.53
20	Naphthalene	3.30	1.340	0.92	0.00	0.20	1.085	−0.1277	0.00000	313.25
21	4-Chlorophenol	2.39	0.915	1.08	0.67	0.20	0.898	−0.2482	2.18448	280.38
22	Toluene	2.73	0.601	0.52	0.00	0.14	0.716	−0.1792	0.06916	274.50
23	Benzonitrile	1.56	0.742	1.11	0.00	0.33	0.871	−0.1349	11.1222	277.91
24	Benzoic acid	1.87	0.730	0.90	0.59	0.40	0.932	−0.3651	5.85156	288.00
25	1,3-Diisopropylbenzene	4.90	0.605	0.46	0.00	0.20	1.562	−0.2055	0.08820	399.79

log P = logarithm of *n*-octanol–water partition coefficient; R_2 = excess molar refraction; π_2^H = dipolarity/polarizability; α_2^H = hydrogen-bond acidity; β_2^H = hydrogen-bond basicity; V_x = characteristic volume of McGowan; δ_{min} = highest electron excess charge on an atom in the analyte molecule (in electrons); μ^2 = square of total dipole moment (in Debyes); SAS = solvent (water)-accessible molecular surface area (in Å^2).

drogen-bond capacity descriptors derived from the complexation scale of Abraham [25,26]. Samples were prepared by dissolving the analytes in the mobile phase or in the pure organic modifier and then diluting with phosphate buffer.

2.4. Test procedure

Analytes were chromatographed with mobile phases being mixtures of organic modifier with an aqueous buffer of composition ranging from 90/10 (v/v) to 40/60 (v/v), hold-up time (t_0) was measured using thiourea added to the solutions of the analytes and varied between 3 and 7 min depending on column and percentage of organic modifier. t_0 time in HPLC was adjusted to the obtained t_0 time in CEC for particular column and particular mobile phase composition. Based on the linear relationship between the logarithm of retention factor (log k) and the percentage of organic modifier in the mobile phase, the values of log k_w corresponding to 100% aqueous eluent were obtained by extrapolation. The data are summarized in Table 3.

3. Results and discussion

Table 3 summarizes values of log k_w (retention factor extrapolated to 100% aqueous mobile phase) for all the columns under both HPLC and CEC conditions. It is evident that both Spherisorb stationary phases (C_{18} and C_8) show retention patterns different than the remaining phases. Values of log k_w of polar compounds, such as 1,4-dinitrobenzene, 3,5-dichlorophenol, 4-cyanophenol or cyclohexanone, are much higher than the corresponding data determined on Hypersil or Unimicro stationary phases. Principal component analysis (PCA) clearly distin-

Table 3
Logarithms of retention factors extrapolated to 100% aqueous eluent in individual chromatographic systems

No.	Analyte	log k_w															
		Hypersil C_{18}		Hypersil C_8 MOS		Hypersil Phenyl		Spherisorb ODS		Spherisorb C_8		Unimicro C_{18}		Unimicro C_8		Unimicro Phenyl	
		HPLC	CEC	HPLC	CEC	HPLC	CEC	HPLC	CEC	HPLC	CEC	HPLC	CEC	HPLC	CEC	HPLC	CEC
1	*n*-Hexylbenzene	6.8896	6.7875	6.8952	6.7579	6.1838	6.2189	6.4547	6.4859	6.8524	6.4009	6.2938	6.1251	6.8634	6.8542	6.3947	6.2490
2	1,3,5-Triisopropylbenzene	7.5969	7.4694	7.8589	7.6340	7.0597	6.4122	7.0826	7.0962	7.9865	7.3278	7.0644	6.8385	7.8104	7.7546	7.2221	7.0719
3	1,4-Dinitrobenzene	3.3307	3.3280	3.5104	3.4699	3.1718	3.0677	6.3164	5.0714	4.6630	4.1411	2.6063	2.5997	3.8115	3.8364	3.6197	3.2227
4	3-Trifluoromethylphenol	3.3498	3.4113	3.8029	3.7493	3.5055	3.2564	6.3703	5.2672	4.1665	4.3150	2.9729	2.8641	4.0909	4.0652	3.8537	3.5613
5	3,5-Dichlorophenol	3.7453	3.8200	4.1998	4.1160	3.6170	3.0982	5.4174	4.3205	4.4597	3.7131	3.2747	3.2812	4.118	3.9484	3.9454	3.6621
6	4-Cyanophenol	2.4421	2.2699	1.9323	1.7582	2.1608	1.8683	8.0989	6.8314	2.1452	2.8712	1.3620	1.0909	2.4349	2.0986	2.8500	2.0727
7	4-Iodophenol	3.1399	3.1695	3.4818	3.3882	2.9769	2.9368	4.8794	4.1149	3.8838	3.5546	2.6673	2.5982	3.7421	3.6896	3.4974	3.1233
8	Anisole	2.9818	3.0310	3.3711	3.0874	2.8390	2.8745	3.8934	3.7837	3.3855	3.1648	2.5416	2.5052	3.5924	3.6088	3.1488	2.9127
9	Benzamide	−0.0636	0.0284	0.1706	0.1214	0.3093	0.0560	1.4421	0.3557	0.5670	0.2640	−0.5647	−0.6775	0.4340	0.5727	0.6348	0.4501
10	Benzene	2.9563	3.0568	3.3792	3.3502	2.6257	2.7196	3.4653	3.7403	3.2508	3.1517	2.5675	2.5478	3.6033	3.6499	3.1787	2.8514
11	Chlorobenzene	3.7446	3.8149	4.2018	4.1603	3.3607	3.3741	3.7416	4.0986	3.9358	3.8396	3.3984	3.3522	4.3773	4.3996	3.7288	3.4757
12	Cyclohexanone	0.5780	0.9771	0.9234	0.9817	0.6586	0.6516	3.2235	1.7929	1.5305	1.1457	0.3847	0.2806	1.4493	1.5365	1.3878	1.1013
13	Dibenzothiophene	5.2880	5.2275	6.0893	5.9210	4.8632	4.9205	5.1093	5.1538	5.6867	5.3776	4.9306	4.8239	5.5909	6.0155	5.0753	4.8745
14	Phenol	1.8713	1.9034	1.8566	1.8303	1.8956	1.6201	7.3360	4.5947	2.6005	2.2926	1.0660	1.0019	2.1819	2.2212	2.2888	1.8713
15	Hexachlorobutadiene	5.8905	5.8070	5.9595	5.8911	5.3608	5.4501	5.6157	5.6734	6.0137	5.6620	5.2663	5.0692	6.3909	6.3657	5.5756	5.4300
16	Indazole	1.4766	1.5621	1.5860	1.5356	1.4586	1.3001	3.3165	1.9024	1.9749	1.6479	0.8925	0.7694	1.8958	1.9067	2.0131	1.6564
17	Caffeine	−1.1170	−1.0608	−1.5547	−1.4163	−0.9153	−1.3497	−1.8807	−1.3489	−1.2510	−1.1745	−1.9988	−1.9089	−1.0928	−0.9130	−0.9496	−0.7478
18	4-Nitrobenzoic acid	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
19	*n*-Methyl-2-pyrrolidinone	−1.5731	−1.5032	−2.1933	−2.1923	−1.5955	−1.3497	−2.0614	−1.7704	−1.6867	−1.6597	−2.6003	−2.5132	−1.7852	−1.6161	−1.0976	−1.5471
20	Naphthalene	4.4222	4.4403	4.8830	4.7714	3.9157	3.9475	4.4095	4.4376	4.6420	4.3966	4.1020	4.0350	4.9962	4.9618	4.2294	4.0112
21	4-Chlorophenol	2.8017	2.8010	2.9211	2.8923	2.5838	2.5305	4.1911	4.2740	3.1546	3.3569	2.1210	2.0993	3.2379	3.2502	3.1150	2.7058
22	Toluene	3.6731	3.7153	4.1474	4.1025	3.2389	3.2980	4.0619	4.0697	4.0061	3.7965	3.3256	3.2924	4.2957	4.3017	3.6116	3.4061
23	Benzonitrile	2.4781	2.5528	2.7396	2.7173	2.2436	2.3027	3.2451	3.6883	2.7184	2.7694	1.9140	1.8747	3.0572	3.0946	2.8172	2.5092
24	Benzoic acid	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
25	1,3-Diisopropylbenzene	6.2297	6.1735	6.4277	6.3080	5.7691	5.7907	5.7980	6.0112	6.2177	6.2742	6.0303	5.4610	6.4494	6.4677	5.9979	5.8601

guishes the Spherisorb C_{18} stationary phase under both HPLC and CEC conditions as an outlier as regards the retention mechanism (Fig. 1a). PCA done for the columns remaining after excluding Spherisorb C_{18} evidences that behavior of the Spherisorb C8 stationary phase also differs from the other phases though the difference is not as pronounced as for the Spherisorb C_{18} stationary phase (Fig. 1b).

Comparing the data on the stationary phases given in Table 1 one may notice that, for example, Hypersil columns have a higher carbon load and a lower surface area than Spherisorb. The differences in log k_w on those phases are remarkable. Most probably, they are to some extent also due to the differences in column dipolarity/polarizibility. Spherisorb stationary phases are based on a type of silica substrate that is apparently different than in the case of other phases studied as the selectivity differences for the phases based on similar substrates are usually minor [30].

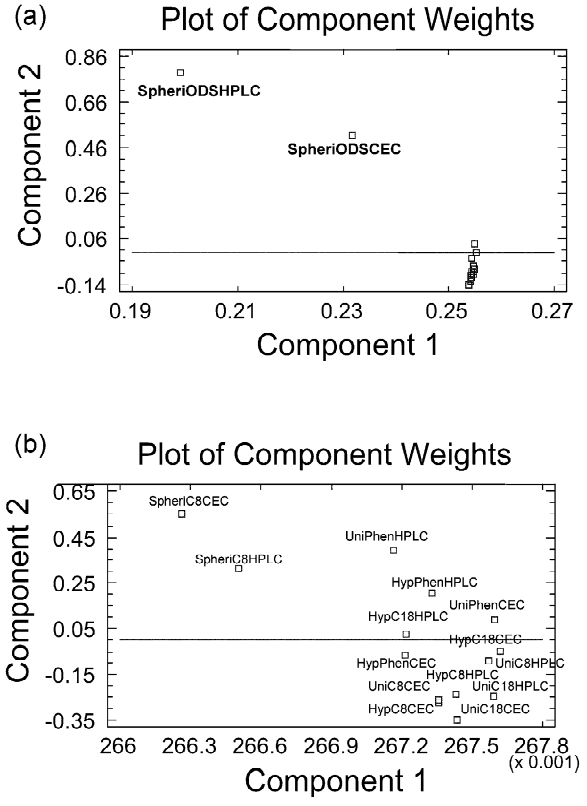

Fig. 1. Plot of first two component weights resulting from principal component analysis of log k_w data determined in (a) all the separation systems studied (b) with Spherisorb ODS stationary phases excluded. SpheriODS = Spherisorb ODS, SpheriC$_8$ = Spherisorb C$_8$, HypC$_{18}$ = Hypersil C$_{18}$, HypC$_8$ = Hypersil C$_8$, HypPhen = Hypersil Phenyl, UniC$_{18}$ = Unimicro C$_{18}$, UniC$_8$ = Unimicro C$_8$, UniPhen = Unimicro Phenyl; symbols CEC and HPLC after the name of the column indicate performed under CEC conditions or HPLC conditions, respectively.

Results of QSRR analysis of retention data for test series of solutes are collected in Tables 4–6. Table 4 summarizes coefficients k_1 and k_2 of the regression equations relating log k_w data to log P (Eq. (1)). There are no statistically significant differences in either k_1 or k_2 between HPLC and CEC modes (t-test, 95% confidence level) for none of the stationary phases tested. Therefore, in terms of analyte partition between mobile and stationary zone, the nature of the eluent driving force is not important. Also, the differences of k_1 and k_2 among individual phases studied are insignificant for either HPLC or CEC conditions. Perhaps that does not concern the Spherisorb ODS stationary phase. An evidently lower regression coefficient k_2 at log P term in Eq. (1), observed for Spherisorb ODS, indicates a lower lipophilicity of that phase which may arise from the specific properties of the silica substrate.

The observed similarity of partition properties of the stationary phases studied is not surprising because all of them are modern bonded-silica reversed-phase materials designed to maximally reduce specific, hard to control contributions to retention. It can be concluded that the log P parameter of analytes is not sensitive enough to clearly distinguish possible differences in retention properties of modern materials.

Lipophilicity (or hydrophobicity) parameters, like log P, are complex net measures of various intermolecular interactions between analyte, on one hand, and components of a given partition system, on the other hand. Two main types of intermolecular interactions are distinguished as governing both slow-equilibrium and chromatographic separations: non-specific ones, i.e., molecular-bulkiness-related, dispersive, London's interactions and structurally specific, polar interactions including dipole–dipole, dipole–induced dipole, hydrogen bonding and electron pair donor–electron pair acceptor interactions [19].

Unlike the rather crude analyte property descrip-

Table 4
Regression coefficients (±standard deviation), numbers of data points used to derive regression (n), correlation coefficient (R), standard errors of estimate (s) and F-test values (F) of regression equations $\log k_w = k_1 + k_2 \log P$

Column	Mode	k_1	k_2	n	R	s	F
CEC Hypersil	HPLC	−0.2886	1.3294	20	0.9601	0.6047	224
C_{18}		(±0.2436)	(±0.0888)				
	CEC	−0.1722	1.2961	20	0.9601	0.5892	224
		(±0.2374)	(±0.0866)				
Hypersil C8	HPLC	−0.3769	1.4502	20	0.9536	0.7150	191
		(±0.2881)	(±0.1050)				
	CEC	−0.3710	1.4206	20	0.9538	0.6988	192
		(±0.2816)	(±0.1027)				
Hypersil Phenyl	HPLC	−0.1667	1.1970	20	0.9618	0.5322	235
		(±0.2144)	(±0.0782)				
	CEC	−0.2934	1.2176	20	0.9558	0.5849	201
		(±0.2357)	(±0.0860)				
Spherisorb ODS	HPLC	1.7140	1.0461	20	0.6215	2.0596	12
		(±0.8298)	(±0.3025)				
	CEC	1.0111	1.1422	20	0.7630	1.1511	27
		(±0.6089)	(±0.2220)				
Spherisorb C8	HPLC	0.1181	1.3222	20	0.9387	0.7580	141
		(±0.3054)	(±0.1114)				
	CEC	0.154639	1.2404	20	0.9260	0.7895	114
		(±0.3181)	(±0.1160)				
Unimicro C_{18}	HPLC	−1.0248	1.4124	20	0.9618	0.6276	235
		(±0.2529)	(±0.0922				
	CEC	−1.0359	1.3894	20	0.9607	0.6272	227
		(±0.2527)	(±0.0921)				
Unimicro C8	HPLC	0.0782	1.3556	20	0.9464	0.7228	163
		(±0.2912)	(±0.1062)				
	CEC	0.1363	1.3421	20	0.9474	0.7080	167
		(±0.2853)	(±0.1040)				
Unimicro Phenyl	HPLC	0.3204	1.1605	20	0.9489	0.6029	172
		(±0.2429)	(±0.0886)				
	CEC	−0.0070	1.1772	20	0.9610	0.5289	230
		(±0.2131)	(±0.0777)				

The values are statistically significant on 99% confidence level.

tor, log P, in Eq. (1), in QSRR equations of the form of Eqs. (2) and (3), the terms are present which should account for differences in specific intermolecular interactions if such were to manifest themselves in CEC with respect to HPLC or among the individual stationary phases operated in a given separation mode. Table 5 summarizes parameters characterizing QSRR equations describing $\log k_w$ in terms of structural descriptors of analytes that are easily acquired by standard computational chemistry programs (Eq. (2)). Again, for none of the eight stationary phases under study any statistically significant difference (t-test, 95% confidence level) in regression coefficients $k_1'-k_4'$ was found between the HPLC and the CEC modes. On the other hand, when comparing respective QSRR equations for individual stationary phase materials one can distinguish Spherisorb ODS (both in HPLC and CEC mode). In QSRR equations in Table 5 for Spherisorb ODS the terms related to the highest electron excess on an atom in analyte molecule, δ_{min}, and to a water accessible van der Waals surface, SAS, are insig-

Table 5
Regression coefficients (±standard deviation), numbers of data points used to derive regression (n), correlation coefficient (R), standard errors of estimate (s) and F-test values (F) of regression equations $\log k_w = k'_1 + k'_2\ \delta_{min} + k'_3\ \mu^2 + k'_4$ SAS

Column	Mode	k'_1	k'_2	k'_3	k'_4	n	R	s	F
CEC Hypersil	HPLC	−0.5592	8.3868	−0.2273	0.0209	22	0.9388	0.8691	47
C_{18}		(±1.1657)	(±2.5836)	(±0.0471)	(±0.0034)				
	CEC	−0.2529	8.1938	−0.2252	0.0198	22	0.9389	0.8497	47
		(±1.1329)	(±2.5108)	(±0.0458)	(±0.0033)				
Hypersil C8	HPLC	0.1180	8.3014	−0.2717	0.0198	22	0.9293	1.0045	40
		(±1.3471)	(±2.9857)	(±0.0544)	(±0.0039)				
	CEC	0.1451	8.2231	−0.2653	0.0194	22	0.9307	0.9705	41
		(±1.3015)	(±2.8846)	(±0.0526)	(±0.0038)				
Hypersil Phenyl	HPLC	−0.6564	6.6522	−0.2135	0.0190	22	0.9325	0.8252	42
		(±1.1067)	(±2.4528)	(±0.0447)	(±0.0032)				
	CEC	−0.1301	7.3085	−0.2163	0.0176	22	0.9298	0.8393	40
		(±1.1256)	(±2.4947)	(±0.0455)	(±0.0033)				
Spherisorb ODS	HPLC	5.5476	–	−0.3160	–	22	0.6144	2.0510	13
		(±0.5476)		(±0.0886)					
	CEC	5.1352	–	−0.3205	–	22	0.6779	1.7566	18
		(±0.4690)		(±0.0758)					
Spherisorb C8	HPLC	−0.5645	–	−0.3378	0.0172	22	0.9084	1.0316	47
		(±1.3180)		(±0.0460)	(±0.0040)				
	CEC	0.4576	6.6421	−0.2417	0.0172	22	0.9072	1.0115	29
		(±1.3565)	(±3.0065)	(±0.0548)	(±0.0039)				
Unimicro C_{18}	HPLC	−0.8682	9.0435	−0.2461	0.0209	22	0.9374	0.9262	46
		(±1,2422)	(±2.7532)	(±0.0502)	(±0.0036)				
	CEC	−0.6761	8.7647	−0.2456	0.0198	22	0.9403	0.8792	48
		(±1.1792)	(±2.6134)	(±0.0476)	(±0.0034)				
Unimicro C8	HPLC	0.7885	8.7656	−0.2459	0.0184	22	0.9284	0.9400	41
		(±1.2607)	(±2.7941)	(±0.0509)	(±0.0035)				
	CEC	0.6595	8.1854	−0.2485	0.0184	22	0.9325	0.9123	42
		(±1.2235)	(±2.7118)	(±0.0494)	(±0.0035)				
Unimicro Phenyl	HPLC	0.4209	6.8211	−0.2102	0.0169	22	0.9169	0.8869	33
		(±1.1894)	(±2.6361)	(±0.0481)	(±0.0034)				
	CEC	−0.3641	6.8911	−0.2076	0.0186	22	0.9332	0.8076	43
		(±1.0831)	(±2.4005)	(±0.0438)	(±0.0031)				

The values are significant on 99% confidence level, the values (–) are statistically not significant on 99% confidence level.

nificant. Instead, significant are the square of the total dipole moment, μ^2, and the free term k'_1 which is very large.

Table 6 summarizes statistical parameters of QSRR equations based on analyte descriptors from linear solvation energy relationship theory (Eq. (3)). With the series of test analytes employed, the LSER-based analyte descriptors R_2 and π_2 appeared insignificant in case of each stationary phase and the separation mode studied.

QSRR equations based on LSER descriptors also do not prove actual difference in molecular mechanism of separation between the two modes compared, i.e., between HPLC and CEC. There are no statistically significant differences (t-test, 95% confidence level) between the k''_4, k''_5 and k''_6 coefficients in HPLC and CEC. The lack of the significance of term corresponding to McGowan volume, V_x, in QSRR for Spherisorb ODS operated at HPLC conditions, whereas it is significant at CEC conditions, may not be conclusive. Furthermore, there has been no explanation found on low correlation coefficient

Table 6
Regression coefficients (±standard deviation), numbers of data points used to derive regression (n), correlation coefficient (R), standard errors of estimate (s) and F-test values (F) of regression equations $\log k_w = k_1'' + k_2''R_2 + k_3'' \pi_2^H + k_4'' \alpha_2^H + k_5'' \beta_2^H + k_6'' V_x$

Column	Mode	k_1''	k_4''	k_5''	k_6''	n	R	s	F
CEC	HPLC	0.9181	−1.2948	−5.8859	4.1017	22	0.9792	0.3640	298
Hypersil C_{18}		(±0.3237)	(±0.2504)	(±0.2536)	(±0.2243)				
	CEC	1.15623	−1.3581	−5.7990	3.89315	22	0.9831	0.3194	368
		(±0.2840)	(±0.2197)	(±0.2225)	(±0.2243)				
Hypersil C_8	HPLC	1.5178	−1.5598	−6.6188	3.9963	22	0.9799	0.3849	309
		(±0.3423)	(±0.2648)	(±0.2681)	(±0.2703)				
	CEC	1.5081	−1.5662	−6.4638	3.8934	22	0.9785	0.3889	289
		(±0.3458)	(±0.2675)	(±0.2709)	(±0.2731)				
Hypersil Phenyl	HPLC	0.6970	−0.9110	−5.2908	3.8519	22	0.9814	0.3114	335
		(±0.2769)	(±0.2142)	(±0.2169)	(±0.2187)				
	CEC	1.1717	−1.3617	−5.4672	3.4641	22	0.9799	0.3235	309
		(±0.2877)	(±0.2226)	(±0.2254)	(±0.2272)				
Spherisorb ODS	HPLC	6.3256		−6.3134		22	0.6037	1.6364	32
		(±0.4942)		(±1.1162)					
	CEC	2.9499		−6.0670	2.6854	22	0.8050	0.6920	41
		(±0.8434)		(±0.7376)	(±0.7248)				
Spherisorb C_8	HPLC	1.5344	−1.0892	−6.0795	3.8866	22	0.9678	0.4544	190
		(±0.4041)	(±0.3126)	(±0.3166)	(±0.3191)				
	CEC	1.5686	−0.9786	−5.8186	3.5986	22	0.9598	0.4821	151
		(±0.4287)	(±0.3316)	(±0.3358)	(±0.3385)				
Unimicro C_{18}	HPLC	0.6178	−1.5986	-6.3602	4.0938	22	0.9859	0.3156	444
		(±0.2806)	(±0.2171)	(±0.2198)	(±0.2216)				
	CEC	0.7257	−1.6406	−6.2226	3.8737	22	0.9843	0.3240	396
		(±0.2881)	(±0.2229)	(±0.2257)	(±0.2275)				
Unimicro C_8	HPLC	1.9426	−1.4767	−6.2899	3.6846	22	0.9804	0.3575	317
		(±0.3179)	(±0.2459)	(±0.2490)	(±0.2510)				
	CEC	1.9832	−1.6537	−6.1486	3.6645	22	0.9807	0.3515	321
		(±0.3126)	(±0.2418)	(±0.2449)	(±0.2468)				
Unimicro Phenyl	HPLC	1.4823	−0.8489	−5.3637	3.4852	22	0.9818	0.3001	341
		(±0.2669)	(±0.2065)	(±0.2091)	(±0.2107)				
	CEC	0.9686	−1.0362	−5.2434	3.7126	22	0.9828	0.2946	363
		(±0.2620)	(±0.2026)	(±0.2052)	(±0.2068)				

The values are significant on 99% confidence level, the values (–) are statistically not significant on 99% confidence level.

for this particular stationary phase for all three QSRR methods. On the other hand, there seems to be a systematic trend in coefficients collected in Table 6 when comparing analogous QSRR equations for HPLC and CEC. Namely, k_6' and k_5' (negative sign) tend to be higher whereas k_4'' (negative sign) tends to be lower in case of CEC. Physical meaning of that observation, if any, may better be checked if data given in Table 6 will be related to those in Table 5. That can be done because the QSRR equations of general form Eqs. (2) and (3) are mutually related. If one compares the ordering of separation systems on the plot of k_6' in Eq. (3) vs. k_4' in Eq. (2) (Fig. 2), i.e., according to the regression coefficients at the volume of the analyte (V_x) and at its van der Waals surface area that is accessible to water (SAS), one will notice a clear trend. Namely, the higher coefficients stand at the C_{18} compared to the C_8 stationary phases. It is rational because the C_{18} phases have a larger surface area of the hydrocarbon ligand that is accessible to the analyte. The same phases under CEC conditions (open symbols) have

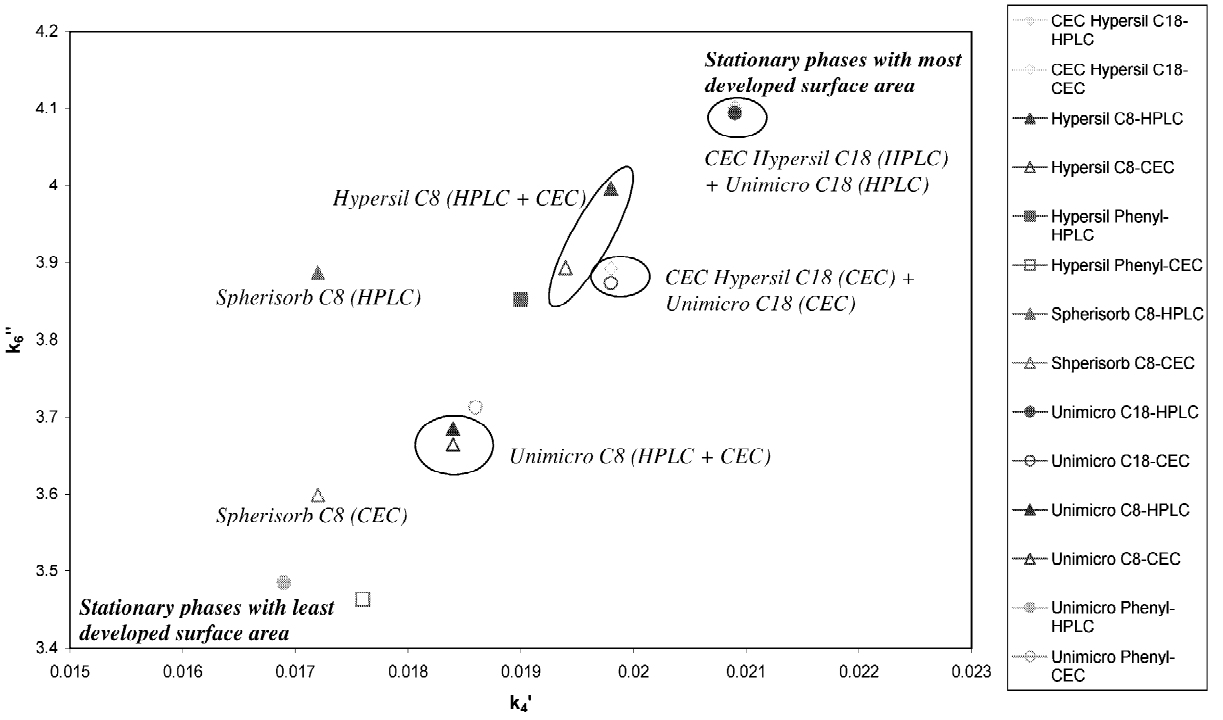

Fig. 2. Ordering of stationary phases according to their non-specific retentivity due to dispersion interaction characterized by the coefficient k_6'' for the V_x variable in Eq. (3) and the coefficient k_4' for the SAS variable in Eq. (2).

lower values of both k_6'' and k_4'' coefficients than under HPLC conditions. That finding seems to be reasonable in view of a previous study report by Jiskra et al. [31]. Those authors suggested that generating electroosmotic flow on the stationary phase under CEC conditions causes reordering of hydrocarbon chains of the ligand. That reordering may lead to a decrease of the overall contact of the solute with the hydrocarbonaceous stationary phase. Euerby et al. [34] used the CEC Hypersil C_{18}, Hypersil C_8 and Hypersil Phenyl for separation of barbiturates. The authors observed increase retention on the Hypersil C_8 stationary phase compared to the CEC Hypersil C_{18} stationary phase under CEC conditions while only minimum increase has been observed under HPLC conditions [35]. However, the separation order remained the same. This confirms further findings of this group, e.g. [36] and others, e.g. [36–38]. Wen et al. [39] found linear relationship between k_{HPLC} and k_{CEC} for neutral small molecules on the Spherisorb ODS (300 Å) and Zorbax ODS (80 and 300 Å). However, the slope of this relationship differed from one (namely 1.12). In this paper, the authors were further focused on the Van Deemter parameters *A* (Eddy diffusion term) and *C* (mass transfer resistance). It has been found that the value of both parameters was by a factor of 2–4 lower in HPLC compared to CEC due to the peculiarities of the EOF flow profile in the interstitial space and the generation of intraparticle EOF inside the porous particles of the column packing.

Coefficients k_5'' in Eq. (3) and k_3' in Eq. (2) (Tables 5 and 6), may both be related to the amount and activity of free silanol groups which are accessible to analytes. That would explain a correlation between the coefficients (Fig. 3). Similarly as in Fig. 2, the stationary phases having a less negative value are the phases with higher amount/activity of free silanols (therefore, these phases compete more effectively for analytes with strongly polar eluents). Typically, the phenyl stationary phases show higher silanol activity whereas the C_8 and C_{18} phases lower values. The exception is CEC Hypersil C_{18} stationary phase under both CEC and HPLC conditions.

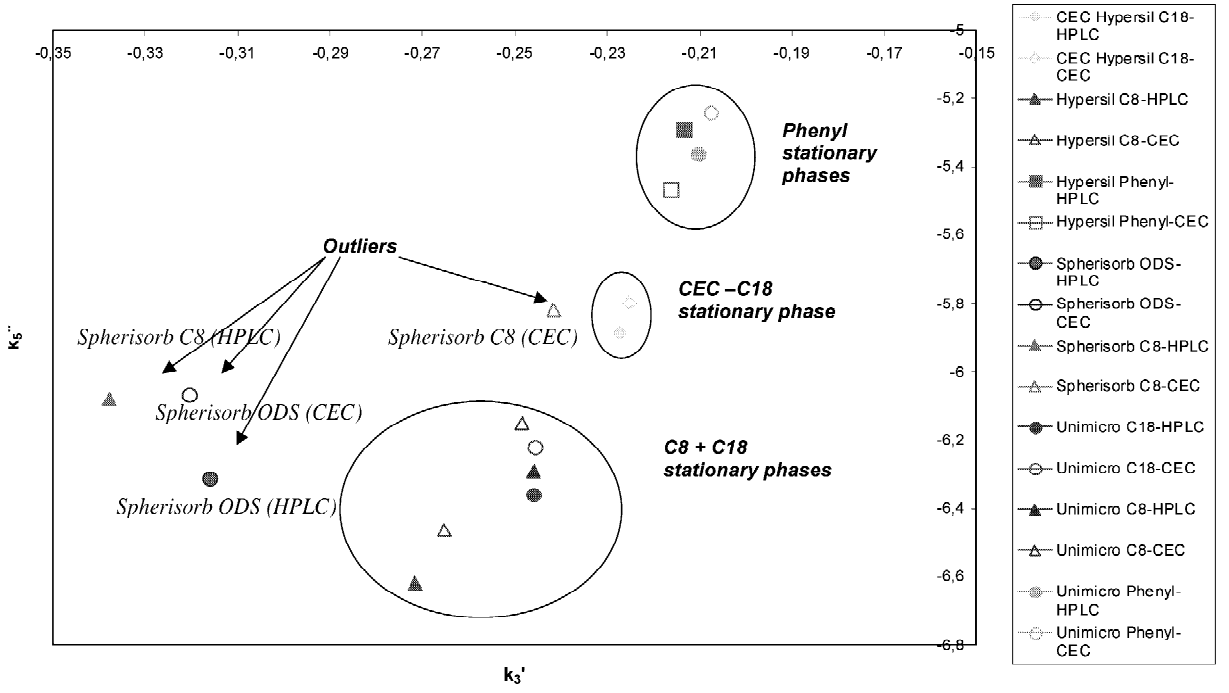

Fig. 3. Ordering of stationary phases according to their hydrogen-bond donor activity characterized by the coefficient k_5'' for the β_2^H variable in Eq. (3) and the coefficient k_3' for the μ^2 variable in Eq. (2).

This is not surprising as this particular stationary phase has been designed for use in CEC possessing higher amount of free silanols. The outliers are the Spherisorb ODS and Spherisorb C_8 stationary phases. In general, there is a trend that the stationary phases exhibit a higher silanol activity under CEC conditions (open symbols) than under HPLC conditions. That confirms previous reports [31–33].

Fig. 4 depicts a plot of stationary phase hydrogen-bond basicity, k_5'' in Table 6, under CEC conditions vs. that under HPLC conditions. Ideal line (tag $\alpha = 1$) and the regression line for all the stationary phases are given. Similarly as in Fig. 3 one can see separate clusters of the phenyl, the C_8 (except Hypersil C8) and the C_{18} stationary phases. Except the Hypersil Phenyl stationary phase, all the other stationary phases find themselves above the ideal line. In other words, under CEC conditions most of the tested stationary phases exhibit a higher activity of free silanols than at HPLC conditions. In the linear CEC-HPLC relationship the intercept is statistically different from zero according to the *t*-test value. That means that the activity of free silanol groups under CEC conditions is different from that under HPLC conditions.

In the same way as for hydrogen-bond basicity one can test regression coefficients at the hydrogen-bond acidity parameters of analytes, k_4'' in Table 6 (Fig. 5). That term describes the ability of an analyte to donate a proton to form a solute–solvent and/or solute–stationary phase hydrogen bond. In Fig. 5 the majority of stationary phases are below the ideal line of tag $\alpha = 1$. The exception is the Spherisorb C_8 stationary phase. The Spherisorb C_{18} stationary phase could not be included in the plot because for this phase the values of the coefficient of the solute hydrogen-bond acidity were not statistically significant. The clustering of stationary phases (especially C_8 and C_{18}) is not that evident as in the case of the coefficients of the solute hydrogen-bond basicity. The intercept in Fig. 5 differs significantly from zero implying differences in behavior under HPLC and CEC conditions.

Positive values of the coefficient of the McGowan parameter of analytes, k_6'' in Table 6, means that the dispersive interactions of the analyte with the hydro-

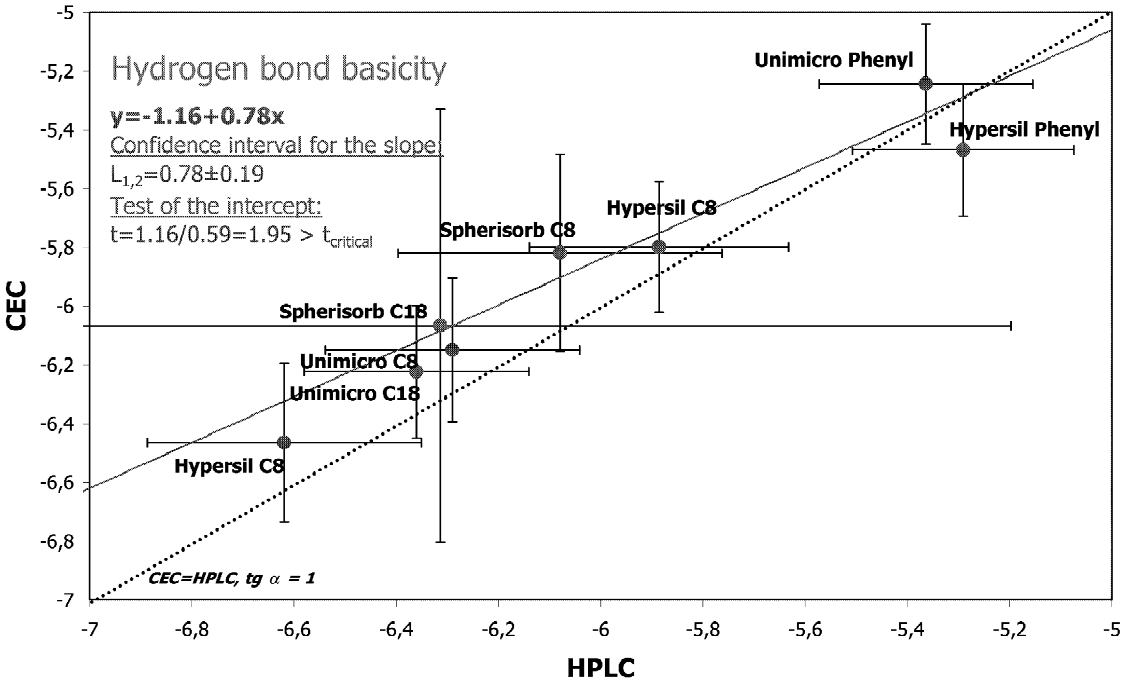

Fig. 4. Plot of regression coefficients at the hydrogen-bond basicity variable (β_2^H) in QSRR equations derived for HPLC and CEC modes. Error bars and the ideal line tag $\alpha = 1$ are given. The critical t-value is a value corresponding to 90% confidence level.

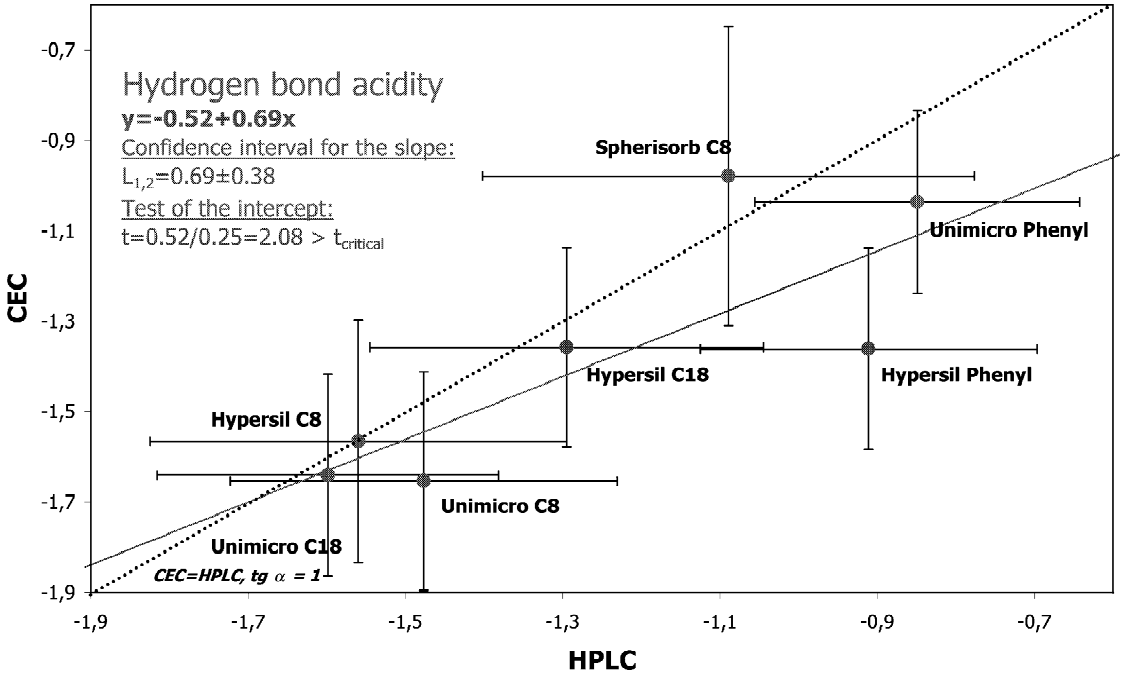

Fig. 5. Plot of regression coefficients at the hydrogen-bond acidity variable (α_2^H) in QSRR equations derived for HPLC and CEC modes. Error bars and the ideal line tag $\alpha = 1$ are given. The critical t-value is a value corresponding to 90% confidence level.

carbonaceous stationary phase are stronger than analogous interactions with the mobile phase. This explains the higher values of k_6'' observed for the C_8 and C_{18} stationary phases than for the phenyl stationary phases as the former phases contain more hydrocarbon ligand. When comparing k_6'' values for the same phase under both HPLC and CEC mode one notes that, with exception of the Unimicro Phenyl stationary phase, the values obtained in HPLC are generally higher than those found under CEC conditions. It means that either dispersive interactions between analyte and the hydrocarbonaceous phase are stronger under HPLC mode than under CEC mode or the interaction between the analyte and the mobile phase is stronger under the applied electric field (CEC), or a combination of both. As discussed in the previous paper [31], this may be due to the different orientation of hydrocarbonaceous chains under CEC conditions the interaction of an analyte with the stationary phase is weaker than under HPLC conditions. The *t*-test analysis demonstrated that *t*-value is lower than critical and the intercept in Fig. 6 is therefore statistically not significantly different from zero.

4. Conclusions

The QSRR models provide rational interpretation of differences and/or similarities in the molecular mechanism of chromatographic separations between HPLC and CEC reversed-phase systems. The models can be of help in objective comparison of separation properties of modern stationary phases.

Three models of QSRR relating standardized retention parameters as obtained on eight modern reversed-phase materials, demonstrated the lack of substantial differences in molecular mechanism of separation which would depend on the nature of the eluent driving force, i.e., high pressure in the HPLC mode or electroosmotic flow in the CEC mode. Neither the partition coefficient of analytes, nor their molecular size or polarity related structural descriptors from molecular modeling or from LSER theory clearly distinguished separation patterns on the same phase at HPLC and CEC conditions. Detailed comparative QSRR analysis supplied evidences of stronger nonspecific dispersive interactions attracting analytes to the hydrocarbonaceous stationary phase in the HPLC mode as related to the CEC mode and a

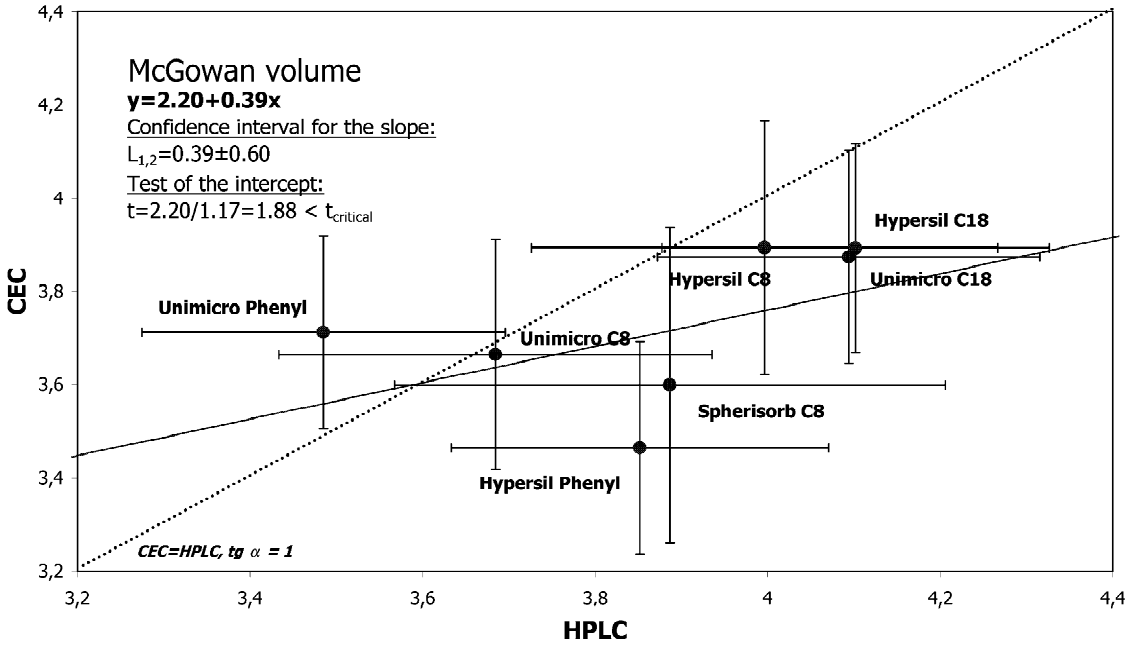

Fig. 6. Plot of regression coefficients at the McGowan volume variable (V_x) in QSRR equations derived for HPLC and CEC modes. Error bars and the ideal line tag $\alpha = 1$ are given. The critical *t*-value is a value corresponding to 90% confidence level.

higher activity of free silanols under CEC conditions with respect to HPLC. These differences do not manifest themselves strongly enough to substantially change the mechanism of retention in the two modes, however. On the other hand, these differences are significant enough to distinguish some reversed-phase materials from the other.

In view of this work there is a rather limited chance that replacing high pressure with electro-osmotic force will result in a dramatic improvement of separations. There are advantages of CEC over HPLC, like high peak capacity or different selectivity for complex analytes. On the other hand, CEC implies rather sophisticated technical solutions. Therefore, a question remains to be answered whether further development of CEC may result in a cost/effectiveness ratio that will be acceptable from the point of view of practical analytical applications.

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