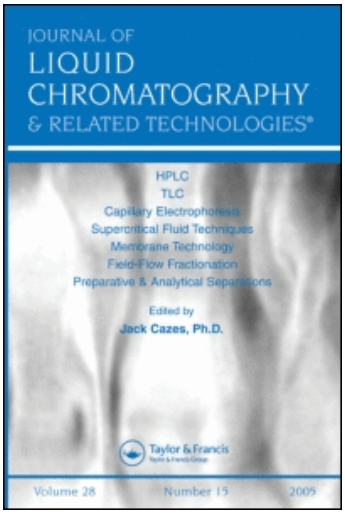

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THE EFFECT OF THE CHAIN LENGTH OF BONDED PHASE ON RETENTION OF AROMATIC SOLUTES IN RP LC WITH PURE WATER AS MOBILE PHASE

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THE EFFECT OF THE CHAIN LENGTH OF BONDED PHASE ON RETENTION OF AROMATIC SOLUTES IN RP LC WITH PURE WATER AS MOBILE PHASE

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□ *Retention factors (log k_w) in pure water were calculated for a homologous series of alkylbenzenes, PAHs, and aromatic monosubstituted derivatives for column C18, C8, and C4, using the numerical method based on the linear form of the Ościk's equation, which were correlated with the log k_w values determined by the linear extrapolation method from the log k dependence on methanol volume fraction in binary eluent. The chromatographic parameters (log k_w), being the hydrophobicity measure, were compared with the experimental partition coefficients in n-octanol/water system (log P). The author examined the contribution of the substituents (–CH_2–, –OH, –Cl, –NO_2, and –CHO) in the substance retention in pure water for columns of different hydrocarbon chain length of the bonded phase. Moreover, the transfer thermodynamics of the particular functional groups from water to the stationary phase, as well as the influence of the kind of the stationary phase on the thermodynamics of this process were studied. For this purpose the standard free enthalpy (ΔG^o) of the functional groups transfer from water to the stationary phase and from the gas to stationary one was calculated.*

Keywords aromatic solutes, log k_w, log P, reversed-phase liquid chromatography, substituent effect, thermodynamics of retention

INTRODUCTION

The retention factor in pure water in RP LC (log k_w) is mainly of a double usage. It is a commonly accepted chromatographic parameter of hydrophobicity used in lieu of the partition coefficient in n-octanol/water system (log P), including quantitative structure-activity relationship (QSAR) studies.[1–9] It is also used for the theoretical description of retention in RPLC in quantitative structure-retention relationship (QSRR) studies.[10,11] Unfortunately, experimental measuring of the log k_w parameter is

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impossible for most substances because of a very strong retention in the case of pure water as the mobile phase; log k_w values exceed not infrequently the order 10^2, which results in very high retention times and strong chromatographic peak broadening. For direct measuring of this parameter, the use of shorter columns, a modified detector, and a sampling valve are necessary.[12] In practice, log k_w values are most frequently determined from a series of chromatographic measurements by using a binary mobile phase of different contents of the organic modifier. Methanol or acetonitrile is most often used as the organic modifier. The obtained log k dependence on the volume fraction (φ) or molar fraction (x) of the organic modifier is extrapolated to zero concentration of the organic modifier by a linear or parabolic function.[12–16] However, the log k_w values determined by these methods differ in dependence on the type of extrapolation function, the concentration range of the organic modifier for which chromatographic measurements were made, and the type of organic modifier. One of the alternative calculation methods of this parameter is the numerical method[17–20] based on the linear form of the Ościk's equation,[21] which was also used in this study.

In the theoretical description of retention, quantitative structure–retention relationship (QSRR) analysis is often used, which states a basic assumption of the additivity of free standard enthalpy of the solute distribution between the stationary and mobile phase (ΔG_r^0).[22] This means that it can be expressed as the sum of free enthalpies of the individual structural elements "i" of molecule (ΔG_i^0). The logarithm of the retention factor (log k), frequently designated as κ, can be connected with the change of retention standard free enthalpy by the known relation:

$$\kappa = -\frac{\Delta G_r^0}{2.303RT} + \log \Phi \tag{1}$$

where R, T, and Φ are the gas constant, absolute temperature, and phase ratio of the column, respectively. The consequence of additivity of standard free enthalpy of retention is additivity of the retention factor logarithm:

$$\kappa = \sum \tau_i + \log \Phi \tag{2}$$

where τ_i is the logarithmic retention factor for a structural element "i" of the solute. The τ_i for a given substituent or functional group "X" can be easily calculated as difference of the logarithmic retention factor of substance, which contains the given substituent (κ_{RX}) and that of an identical substance without this substituent (κ_{RH}).

$$\tau_X = \kappa_{RX} - \kappa_{RH} \tag{3}$$

This parameter is the logarithm of so called group selectivity (α_X), i.e., relative retention of the solute containing substituent X with respect to an identical one without this substituent.

$$\tau_X = \log \alpha_X = \log \frac{k_{RX}}{k_{RH}} \tag{4}$$

In the use of pure water as the mobile phase, the parameter $\log k_w$ is to be denoted by the symbol κ_w. Similarly to Eq. (3), it can be the definite hydrophobic substituent value denoted as π^*.[23] For a given substituent "X," it is calculated by the equation:

$$\pi^*_X = \kappa_{w,RX} - \kappa_{w,RH} \tag{5}$$

Using this parameter, we can calculate the contribution of the functional group to the change of standard free enthalpy of a transfer of 1 mole of solute from water to the stationary phase as the difference of appropriate changes of free enthalpy for the substituted (RX) and not the substituted compound (HX):

$$\Delta G^0_{wX} = \Delta G^0_{wRX} - \Delta G^0_{wRH} \tag{6}$$

The results of the deductions from Eqs. (1) and (5) are as follows:

$$\Delta G^0_{wX} = 2.303RT(\kappa_{wRH} - \kappa_{wRX}) = 2.303RT\pi^*_X \tag{7}$$

where $\Delta G^0_{w(X)}$ is the standard free enthalpy of transfer of 1 mole of substituents X from water to the stationary phase.

The change of standard free enthalpy of retention (ΔG^0_r) can be expressed by means of free enthalpy of the solute transfer from the gas to mobile phase, i.e., salvation free enthalpy—or hydration free enthalpy if the mobile phase is pure water—(ΔG^0_m), and free enthalpy of the solute transfer from the gas phase to the stationary phase (ΔG^0_s):[24,25]

$$\Delta G^0_r = \Delta G^0_s - \Delta G^0_m \tag{8}$$

Determining the values of these free enthalpies, we can evaluate the contribution of the interactions of substances with the stationary and mobile phase in the retention process in an RPLC system.

In the present paper, the author determined the $\log k_w$ values by the numerical method and by linear extrapolation from $\log k$ dependence on methanol volume fraction in a binary eluent for a homologous series of alkylbenzenes, PAHs, and mono-substituted aromatic derivatives. Using these values, the parameter π^* was calculated for the methyl group and

functional groups −OH, −Cl, $-NO_2$, and −CHO. Also, the contribution of these substituents to the thermodynamics of retention in RPLC with pure water as an eluent was examined.

EXPERIMENTAL

HPLC Measurements

All chromatographic data were obtained using the Shimadzu Vp liquid chromatographic system equipped with LC 10AT pump, SPD 10A UV-VIS detector, SCL 10A system controller, CTO-10 AS chromatographic oven, and Rheodyne injector valve with a 20 μL loop. The Class-Vp computer program controlling hardware and registering and storing data to determine the retention time was used.

Three stainless-steel columnswere packed with LiChrospher RP-18e, LiChrosorb RP-8e (Merck) (12.5 cm × 4 mm, i.d.) and with Kromasil RP-4 (Phenomenex) (25 cm × 4.6 mm, i.d.); all particles of 5 μm in diameter were used in each experiment. Mixtures of methanol-water were used as effluents. The methanol molar fraction ranged from 0.1 to 0.9 at 0.1 steps. The mobile phase flow-rate was 1 mL min^{-1}. The flow-rate was 1.2 mL min^{-1} only for eluents rich in water (molar fraction of organic modifier ranging from 0.1 to 0.3). All measurements were made at 20°C. The test compounds were separately dissolved (10^{-3} mg mL^{-1}) in methanol and detected under UV light ($\lambda = 254$ nm). For calculation, average values of the retention factors from at least three experimental data were taken. The retention factor (k) was calculated according to:

$$k = (t_R - t_0)/(t_0 - t_{ec}) \tag{9}$$

where t_R, t_0, and t_{ec} were the gross retention time, the dead time, and the extra-column time, respectively. The dead time was evaluated from the uracil peak, whereas the extra-column time was determined experimentally using zero-volume connection in lieu of the chromatographic column. The extra-column volume was equal to 75 μL, and the extra-column time was calculated from this value.

Materials

HPLC-grade methanol was purchased from Merck. Alkylbenzenes: benzene, toluene, ethylbenzene, propylbenzene, and butylbenzene; PAHs: naphthalene, antracene, phenantrene, chrysene and fluorine; monosubstituted aromatics: phenol, nitrobenzene, chlorobenzene, benzaldehyde,

benzyl alcohol, and naphthols, all of analytical grade, were obtained from various sources.

RESULTS AND DISCUSSION

Determination of log k_w Values

The experimental log k values for column C8 and C4 are in Table 1; whereas, those for column C18 were given in a previous paper.[26] Using these values the author calculated the logarithms of the retention factors of the substances studied in pure water. For this purpose, the numerical method based on the linear form of the Ościk's equation[21] was used

$$G(x) = \frac{x_0(1 - x_0)}{\log k - x_0 \log k_0 - (1 - x_0) \log k_w} = ax_o + b \qquad (10)$$

where x_0 is the molar fraction of organic modifier in the mobile phase, k_o, k_w, and k are the solute retention factors in pure organic modifier, pure water, and mixed eluent, respectively. The left term of this equation, denoted as $G(x)$, is the linear function of the organic modifier molar fraction in the mobile phase. The values of log k_0 are determined by extrapolating the relation of log k *vs.* x_0 in the concentration range of organic modifier from 0.7 to 0.9. The numerical method consists in adjusting such a log k_w value to Eq. (10) to obtain the best linear relationship between $G(x)$ and x_0. For this purpose, the relation course of d r^2/d log k_w vs. log k_w was analyzed, where r was the linear correlation coefficient. The details of this method were given in previous publications.[17,20,26] For calculation of log k_w values by the numerical method, the log k values of the whole available concentration range of the given substance were used, because the range of the organic modifier concentrations at which measurements were made has not a significant influence on the results obtained by this method.

Also, the log k_w values were determined by the linear extrapolation method using the relationship of log k vs. φ (φ is the volume fraction of methanol in eluent). In this case, the concentration range (φ) from 0.35 to 0.8 was used,[12,13,16] except those substances for which measurements in this range could not be made because of a strong retention; they were: propylbenzene and naphthalene for column C18 and C8; and butylbenzene, antracene, phenantrene, fluorene, and chrysene for all columns studied.

The log k_w values determined by the numerical method and linear extrapolation (log $k_{w(num)}$ and log $k_{w(lin)}$, respectively) for three columns: C18, C8, and C4 are presented in Table 2. It can be seen that they differ relatively significantly in the case of the substances of a very high hydrophobicity

TABLE 1 Values of the Logarithms of the Retention Factor (log k) Determined at Various Concentrations of Organic Modifier in the Mobile Phase on LiChrosorb RP-8e and Kromasil RP-4

	Molar fraction of methanol (x_o)																	
	C8									C4								
Solute	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
Benzene	1.42	1.09	0.75	0.51	0.22	−0.01	−0.21	−0.40	−0.61	1.16	0.85	0.55	0.22	−0.02	−0.25	−0.42	−0.52	−0.68
Toluene	1.82	1.49	1.07	0.75	0.42	0.15	−0.08	−0.34	−0.51	1.53	1.20	0.82	0.38	0.07	−0.12	−0.32	−0.44	−0.59
Ethylbenzene		1.62	1.36	1.02	0.60	0.30	0.03	−0.20	−0.43		1.36	0.85	0.43	0.15	−0.06	−0.21	−0.37	−0.52
Propylbenzene			1.70	1.29	0.80	0.46	0.16	−0.09	−0.35		1.49	1.10	0.64	0.32	0.10	−0.11	−0.29	−0.47
Butylbenzene				1.50	1.02	0.63	0.29	−0.01	−0.29			1.43	0.87	0.50	0.29	0.00	−0.20	−0.40
Naphthalene			1.38	1.00	0.57	0.24	−0.04	−0.25	−0.47		1.54	1.02	0.54	0.22	−0.07	−0.30	−0.46	−0.66
Antracene			1.81	1.39	0.93	0.53	0.19	−0.05	−0.31			1.44	0.86	0.46	0.10	−0.18	−0.38	−0.61
Phenantrene			1.91	1.36	0.87	0.51	0.20	−0.06	−0.32			1.45	0.83	0.41	0.07	−0.21	−0.40	−0.61
Fluorene			1.81	1.29	0.82	0.52	0.18	−0.07	−0.33			1.43	0.81	0.42	0.09	−0.17	−0.36	−0.57
Chryzene				1.87	1.24	0.78	0.42	0.11	−0.17			1.85	1.12	0.63	0.22	−0.10	−0.32	−0.51
Phenol	0.90	0.54	0.23	0.08	−0.17	−0.42	−0.59	−0.79	−1.01	0.83	0.45	0.12	−0.17	−0.37	−0.57	−0.75	−0.89	−1.02
1-Napthol	1.95	1.44	0.90	0.56	0.19	−0.10	−0.33	−0.52	−0.80		1.20	0.69	0.25	−0.07	−0.33	−0.54	−0.69	−0.91
2-Napthol	1.91	1.37	0.83	0.50	0.14	−0.16	−0.39	−0.57	−0.84		1.14	0.62	0.18	−0.12	−0.37	−0.59	−0.72	−0.95
Benzyl alcohol	0.91	0.51	0.19	−0.04	−0.25	−0.43	−0.59	−0.79	−1.01	1.02	0.57	0.20	−0.11	−0.32	−0.48	−0.66	−0.77	−0.92
Nitrobenzene	1.27	0.96	0.60	0.34	0.05	−0.14	−0.35	−0.52	−0.76	1.16	0.76	0.40	0.07	−0.17	−0.38	−0.56	−0.68	−0.88
Chlorobenzene	1.90	1.55	1.10	0.80	0.43	0.13	−0.12	−0.30	−0.53		1.25	0.84	0.44	0.15	−0.12	−0.32	−0.47	−0.66
Benzaldehyde	1.17	0.72	0.36	0.14	−0.13	−0.32	−0.47	−0.63	−0.84	1.02	0.57	0.20	−0.11	−0.32	−0.48	−0.66	−0.77	−0.92

TABLE 2 Retention Data for Aromatics in Pure Water for C18, C8, and C4 Bonded Phases. Logarithms of the Retention Factors (κ_w) Estimated by Extrapolation of log k vs. φ Relationships and Those Determined by Numerical Method from the Linear Form of Ościk's Equation using Methanol Organic Modifier

	Linear extrapolation					Numerical method				
Solute	C18	C8	C4	$\Delta\kappa_{w(\text{C8–C18})}$ [a]	$\Delta\kappa_{w(\text{C4–C8})}$ [b]	C18	C8	C4	$\Delta\kappa_{w(\text{C8–C18})}$ [a]	$\Delta\kappa_{w(\text{C4–C8})}$ [b]
Benzene	2.260	2.045	1.727	−0.215	−0.318	2.329	2.271	2.162	−0.058	−0.109
Toluene	2.893	2.735	2.199	−0.158	−0.536	2.943	2.842	2.678	−0.101	−0.164
Ethylbenzene	3.520	3.321	2.750	−0.199	−0.571	3.496	3.376	3.177	−0.120	−0.199
Propylbenzene	4.087	3.930	3.121	−0.157	−0.809	4.040	3.915	3.678	−0.125	−0.237
Butylbenzene	4.810	4.608	3.217	−0.202	−1.391	4.509	4.443	4.175	−0.133	−0.268
Naphthalene	3.516	3.403	2.815	−0.113	−0.589	3.710	3.640	3.283	−0.070	−0.357
Antracene	4.806	4.135	3.344	−0.671	−0.792	4.607	4.298	3.633	−0.309	−0.665
Phenantrene	4.557	4.245	3.502	−0.313	−0.743	4.462	4.385	4.187	−0.077	−0.198
Chryzene	5.462	5.230	4.175	−0.232	−1.055	5.721	5.525	4.874	−0.196	−0.651
Fluorene	4.454	4.043	3.414	−0.411	−0.630	4.295	4.186	4.004	−0.109	−0.186
Phenol	1.393	1.385	1.331	−0.008	−0.054	1.754	1.745	1.733	−0.009	−0.012
1-Naphthol		2.709	2.406		−0.303		3.048	2.880		−0.168
2-Naphthol		2.641	2.309		−0.331		3.068	2.782		−0.286
Benzyl alcohol	1.387	1.331	1.279	−0.056	−0.052	2.061	1.887	1.628	−0.073	−0.095
Nitrobenzene	2.085	1.896	1.705	−0.189	−0.191	2.391	2.280	2.168	−0.111	−0.112
Chlorobenzene	3.044	2.677	2.401	−0.365	−0.278	3.092	2.945	2.744	−0.147	−0.201
Benzaldehyde	1.714	1.668	1.478	−0.046	−0.191	2.090	1.945	1.832	−0.145	−0.113

[a] $\Delta\kappa_{w(\text{C8–C18})} = \kappa_{w(\text{C8})} - \kappa_{w(\text{C18})}$.
[b] $\Delta\kappa_{w(\text{C4–C8})} = \kappa_{w(\text{C4})} - \kappa_{w(\text{C8})}$.

(antracene, phenantrene, chrysene) and in regard to column C4. For polar substances, log $k_{w(lin)}$ values are always smaller than those determined by the numerical method. In columns C18 and C8 the differences are from about 0.28 for benzaldehyde on column C8 to about 0.38 nitrobenzene on the same column, while in C4 they are somewhat bigger and range from about 0.33 for benzyl alcohol to about 0.47 for naphthols. For aromatic hydrocarbons, the differences between log $k_{w(num)}$ and log $k_{w(lin)}$ values are the biggest in column C4, amounting to over 0.4 for substances of a lower hydrophobicity to about 0.7 for chrysene; the values determined by extrapolation are always smaller. The smallest differences of log k_w values in column C18 and C8 occur in the case of aromatic hydrocarbons of relatively lower hydrophobicity. They are of the order 0.025–0.07 for benzene, toluene, ethylobenzene, and propylobenzene on column C18 to about 0.25 for aromatics of bigger molar mass and for column C8.

The correlations between the log k_w values determined by the numerical method and linear extrapolations are shown in Figure 1 and the correlations parameters are listed in Table 3. In columns C18, C8, and C4, the correlations between log k_w determined by the two methods are relatively good for all substances studied ($r^2 = 0.9836$, SD = 0.0298; $r^2 = 0.9787$,

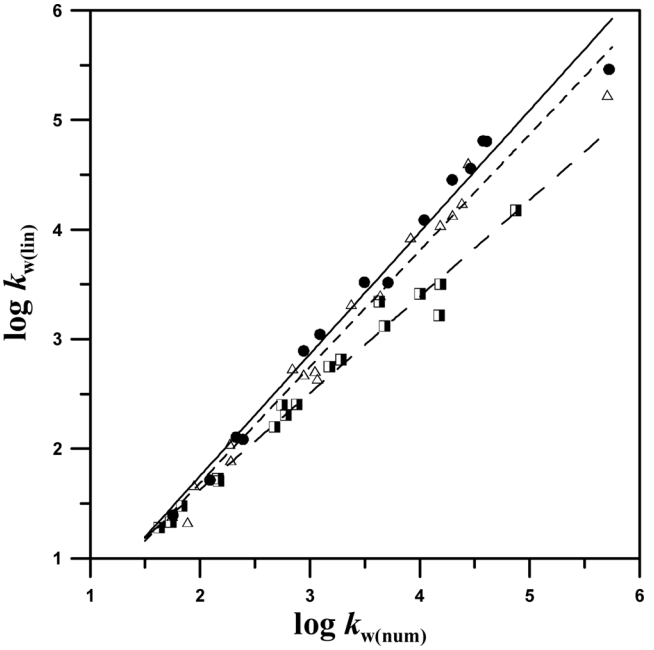

FIGURE 1 Correlation between the log k_w values calculated by the numerical method (log $k_{w(num)}$) and those obtained by linear extrapolation (log $k_{w(lin)}$) for C18 (circles), C8 (triangles), and C4 (squares) column.

TABLE 3 Regression Data for log $k_{w(num)}$ vs. $k_{w(lin)}$ Correlations for Column C18, C8, and C4

Column	Solutes	a	b	n	r^2	SD
C18	All studied	1.112	−0.473	15	0.9836	0.0298
	Alkylbenzenes	1.181	−0.624	5	0.9984	0.0023
C8	All studied	1.060	−0.433	17	0.9787	0.0316
	Alkylbenzenes	1.167	−0.603	5	0.9993	0.0009
C4	All studied	0.882	−0.137	17	0.9806	0.0149
	Alkylbenzenes	0.777	0.136	5	0.9550	0.0240

SD = 0.0316; and $r^2 = 0.9806$, SD = 0.0149 for column C18, C8, and C4, respectively) and are very good for the homologous series of alkylbenzenes except for C4 column ($r^2 = 0.9984$, SD = 0.0023; $r^2 = 0.9993$, SD = 0.0009; and $r^2 = 0.9550$, SD = 0.0240 for column C18, C8, and C4, respectively).

It can be seen that regardless of the method used for κ_w calculation, the values of the logarithmic retention factors in pure water decreased with the length of decrease in the hydrocarbon chain bonded on the packing surface (Table 2). This decrease of log k_w values for nonpolar or weakly polar substances can be caused by the attractive interactions of the solute with a stationary phase decrease as a result of short alkyl chains of the bonded phase, not with the whole solute surface interaction with ligands of the stationary phase. It is posited that an RP-8 phase already possesses sufficiently long chains which permits solute molecules to interact on practically their whole surface with the stationary phase.[27] Thus the partition mechanism of retention predominates for bonded phases with a C8 chain or a longer one; whereas, in the case of bonded phases with short chains, the adsorption mechanism dominates, as in Dill's theory.[28,29]

Another factor can be weaker and weaker screening of residual hydroxyl groups present on the silica surface by shorter hydrocarbon chains. These groups show repulsive interaction with non-polar molecules or their fragments and attractive interaction with polar substituents.

In the studied aromatic hydrocarbons, the change of log k_w values for column C18 and C8 was small, in the order of 0.1 (0.3 only for antracene). For column C8 and C4, it was approximately twice as large (Table 2), which confirmed the supposition that in the case of columns containing bonded hydrocarbon chains C8 and longer, the retention mechanism is the same; whereas, for columns of shorter bonded alkyls, it was changed. However, in the case of monosubstituted aromatic derivatives, the change of log k_w values for columns of a shorter and shorter boned hydrocarbon chain is effected by two opposing factors: on the one hand, a decreased attractive interaction of the hydrophobic molecule part with the stationary phase and increased repulsive interaction with residual hydroxyl groups diminishing retention occurred; and on the other hand, increased attractive interaction of

functional polar group with residual hydroxyls on the surface of packing that increases retention occurred. These effects are clearly visible, particularly in the case of phenol and benzyl alcohol, which belong to a hydroxyl group that is capable of a strong hydrogen interaction with the surface silanols of packing. This interaction essentially compensated for the decrease of the aromatic ring interaction with alkyls bonded on the packing surface, which, as a consequence, caused little change in the log k_w of phenol on the studied columns C18, C8, and C4 (Table 2). The influence of the chain length of bonded phase on naphthols log k_w values is more visible because attractive interaction of the solute hydroxyl group with surface silanols cannot compensate a stronger repulsive interaction of a large hydrophobic part of a naphthol molecule.

In Table 4 the author presented the π^* parameters calculated from Eq. (5) using log $k_{w(num)}$ values, which expresses the contribution of the given substituent to the total log k_w value of solute. For the methylene group ($-CH_2-$), it was calculated from the modified Eq. (5)

$$\pi^*_{CH_2} = \kappa_{w,n+1} - \kappa_{w,n} \tag{11}$$

where $\kappa_{w,n+1}$ and $\kappa_{w,n}$ are the log k_w values of alkylbenzene homologues differing by one carbon atom. Methylene group selectivities differ a small amount depending on the alkylbenzenes pair taken into the calculation, and it reaches the highest value for the benzene–toluene pair. This may be connected with the fact that the methyl group surface is somewhat larger than the methylene surface (34.89 and 22.67 ?^{2}, respectively)[30] that effects retention increase. The mean values of this parameter are 0.562,

TABLE 4 Hydrophobic Substituent Values (π^*) for Aromatic Hydrocarbons and Monosubstituted Aromatics for C18, C8, and C4 Bonded Phases

Substituent	C18	C8	C4
$-CH_2-$			
Toluene–benzene	0.614	0.571	0.516
Ethylbenzene–toluene	0.553	0.534	0.499
Propylbenzene—ethylbenzene	0.544	0.539	0.501
Butylbenzene–propylbenzene	0.536	0.528	0.497
Average	0.562	0.543	0.503
$-OH$			
Phenol	−0.575	−0.526	−0.429
1-Naphtol		−0.692	−0.403
1-Naphtol		−0.672	−0.501
Benzyl alcohol	−0.882	−0.955	−1.050
$-CHO$	−0.239	−0.326	−0.330
$-NO_2$	0.062	0.009	0.006
$-Cl$	0.763	0.674	0.528

0.543, and 0.503 for column C18, C8, and C4, respectively, and are comparable to the literature data.[22,23] Methylene group selectivity has a slightly lower value for column C8 compared to C18 and for column C4 compared to C8; in that, the difference is approximately twice as big in the latter case as in the case of the aforementioned discussed influence of the chain length of bonded phase on the κ_w values of alkylbenzenes. The results agree with the reports that methylene group selectivity increases as the bonded phase chain length increases.[31–34]

Among the studied substituents, the hydroxyl and aldehyde group causes κ_w value decrease, whereas chlorine and nitrate group causes its increase, which is very slight in the case of $-NO_2$ group (Table 4). the π^* values of the hydroxyl groups depend on whether the group belongs to phenols or aromatic alcohols. They are lower for phenol (-0.575, -0.526, and -0.429 for column C18, C8, and C4, respectively) than for benzyl alcohol (-0.882, -0.955, and -1.050 for column C18, C8, and C4, respectively). Thus, in the case of benzyl alcohol, the –OH group diminishes retention to a higher degree than for phenols and even more so when the chain of the bonded phase is shorter, which is different for hydroxyl groups of phenols and other functional groups in which the contribution to retention decreases for bonded phases with shorter chains.

Logarithms of retention factors in pure water are frequently used descriptors of hydrophobicity (or liophilicity) of substances instead of logarithms of partition coefficients in n-octanol/water system. The correlations between the log k_w values for column C18, C8, and C4 calculated by the numerical method and the experimental log P values[35] are presented in Figure 2, whereas the regression data are listed in Table 5. The best linear correlation was obtained for alkylbenzenes ($r^2 = 0.9921$, $r^2 = 0.9932$, and $r^2 = 0.9937$ for column C18, C8, and C4, respectively). In the case of monosubstituted aromatics, the correlations are not the best, which can be a result of the studied solutes belonging to different homologous series differing considerably in properties and showing differentiated intermolecular interactions, both in the mobile and stationary phase. We can see here a big influence of the chain length of the bonded phase on the quality of the obtained correlations, of which the best is for column C4 ($r^2 = 0.9791$, SD $= 0.0124$). In the case of polycyclic aromatic hydrocarbons (PAHs), the log k_w values for column C18 show a relatively good linear correlation with log P values ($r^2 = 0.9881$, SD $= 0.0128$). It is considerably weaker for column C8 ($r^2 = 0.9712$, SD $= 0.0312$) and very weak for column C4 ($r^2 = 0.8644$, SD $= 0.1467$). This could result from the fact that in the case of a bonded phase with a short alkyl chain (C4), particularly for large PAHs molecules, adsorption is the main retention mechanism, in contrast to bonded phase with long alkyl chain (C18 and C8), in which a partition mechanism predominates.

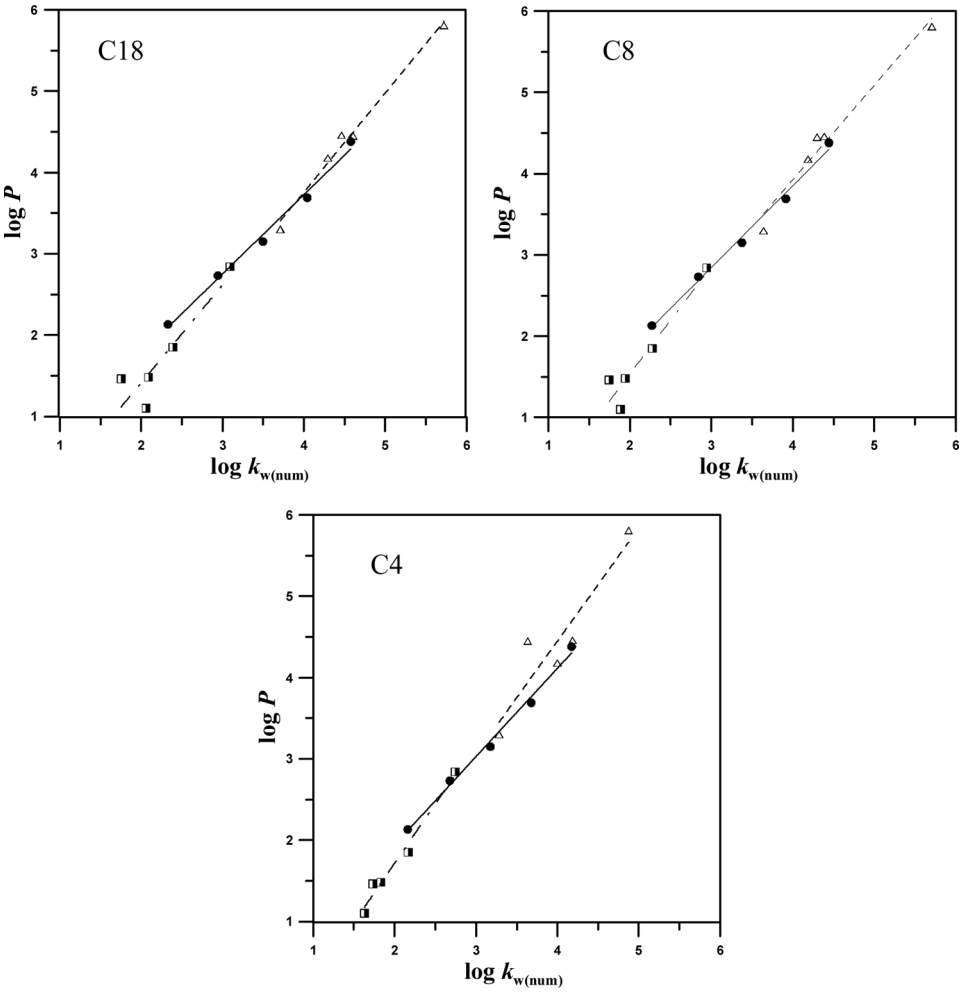

FIGURE 2 Correlations between log $k_{w(num)}$ and experimental log P values of alkylbenzenes (circles), PAHs (triangles), and monosubstituted aromatics (squares) for column C18, C8, and C4.

Free Enthalpy of Substituent Transfer to the Stationary Phase

The standard free enthalpy of substituent "X" (methylene group and functional groups: OH, −Cl, $-NO_2$, and –CHO) transfer from pure water to the stationary phase (C18, C8, and C4) ($\Delta G^0_{w(X)}$) was calculated from Eq. (7) using the log k_w values determined by the numerical method for alkylbenzenes and monosubstituted aromatic derivatives. Then, the contribution of the stationary phase to retention of the studied constituents was determined by calculating the standard free enthalpy of 1 mole of appropriate substituent "X" transfer from the gas to stationary phase ($\Delta G^0_{S(X)}$) using

TABLE 5 Regression Data for log $k_{w(num)}$ vs. log P Correlations for Column C18, C8, and C4

Column	Solutes	a	b	n	r^2	SD
C18	Alkylbenzenes	0.975	−0.175	5	0.9921	0.0079
	PAH-s	1.220	−1.124	5	0.9881	0.0128
	Monosubstituted aromatics	1.203	−0.995	5	0.8410	0.0942
	All studied	1.154	−0.832	15	0.9818	0.0366
C8	Alkylbenzenes	1.008	−0.179	5	0.9932	0.0068
	PAH-sMonosubstituted aromatics	1.162	−0.724	5	0.9712	0.0312
		1.325	−1.116	7	0.9118	0.0522
	All studied	1.159	−0.719	17	0.9857	0.0289
C4	Alkylbenzenes	1.086	−0.232	5	0.9937	0.0063
	PAH-s	1.392	−1.121	5	0.8644	0.1467
	Monosubstituted aromatics	1.459	−1.203	7	0.9791	0.0124
	All studied	1.321	−0.811	17	0.9730	0.0544

the modified Eq. (8):

$$\Delta G^0_{s(X)} = \Delta G^0_{r(X)} + \Delta G^0_{m(X)} \tag{12}$$

The value ($\Delta G^0_{m(X)}$) for the $-CH_2-$ group was assumed as 0.67 kJ/mol,[24,34] and for $-OH$ and $-Cl$ group, the assumption was 28.87 and 4.184 kJ/mol, respectively.[36] The calculated values are given in Table 6. The mean values of standard free enthalpy of methyl group transfer from water to stationary phase ($\Delta G^0_{w(CH_2)}$) became smaller as with the absolute value for packings with shorter chains of bonded phase (−3.151, −3.046, and −2.823 kJ/mol for C18, C8, and C4, respectively). Thus methylene groups showed a weaker and weaker tendency to transfer to the stationary phase with decreasing chain length of the bonded phase.

Comparing these values with standard free enthalpy of the $-CH_2-$ group transfer from the mobile phase (in this case water) to hexadecane ($\Delta G^0_{w(CH_2)C16}$), we can examine the contribution of the partition mechanism to retention in the studied RPLC system. According to the lattice model by Dill and Dorsey,[28,29] the ratio of $\Delta G^0_{w(CH_2)C16}$ to $\Delta G^0_{w(CH_2)}$), often denoted by F, is close to unity, if retention is of partition character, and it is bigger than unity when the adsorption contribution is the greater. Thus, calculated F parameters for $\Delta G^0_{w(CH_2)C16} = -3.200$ kJ/mol[24] are listed in Table 4. For column C18 and C8, they are very close to unity (mean values 1.015 and 1.051, respectively), whereas for column C4 an increase to 1.134 is visible. This means that the retention mechanism of the methylene group for column C4 becomes somewhat less partition-like compared with C8 and C18 phases. This is logical because short chains of bonded phase do not allow whole solutes to penetrate into interphase, though for mobile phases rich in water, the contribution of the adsorption mechanism is small, even

TABLE 6 Thermodynamics of Substituents Transfer from Pure Water and Gas Phase to Stationary Phase for Column C18, C8, and C4[a]

	C18		C8		C4		*F*		
Substituent	ΔG_w^0	ΔG_s^0	ΔG_w^0	ΔG_s^0	ΔG_w^0	ΔG_s^0	C18	C8	C4
$-CH_2-$									
Toluene–benzene	−3.444	−2.774	−3.203	−2.533	−2.894	−2.224	0.929	0.999	1.106
Ethylbenzene–toluene	−3.102	−2.432	−2.995	−2.325	−2.799	−2.129	1.032	1.068	1.143
Propylbenzene–ethylbenzene	−3.051	−2.381	−3.023	−2.353	−2.810	−2.140	1.049	1.059	1.139
Butylbenzene–propylbenzene	−3.006	−2.336	−2.961	−2.291	−2.788	−2.118	1.064	1.081	1.148
Average	−3.151	−2.481	−3.046	−2.376	−2.823	−2.151	1.015	1.051	1.134
–OH									
Phenol	3.225	−25.645	2.950	−25.920	2.406	−26.464			
1-Naphtol			3.881	−24.989	2.260	−26.610			
1-Naphtol			3.769	−25.101	2.810	−26.060			
Benzyl alcohol	4.947	−23.923	5.356	−23.514	5.889	−22.981			
–Cl	−4.279	−0.095	−3.780	0.404	−3.264	0.920			

[a]All ΔG^o data are in 20°C in kJ/mol.

for RP-1 and RP-3 bonded phases.[27] These results are in agreement with the π^* values discussed earlier.

Mean free enthalpies of methylene group transfer from gas to stationary phase ($\Delta G^0_{s(CH_2)}$) were −2.482, −2.376, and −2.151 kJ/mol for column C18, C8, and C4, respectively, and −2.53 kJ/mol for hexadecane,[24] whereas 0.67 kJ/mol from gas phase to water. Thus, attractive interactions of methylene groups with stationary phase were over three times higher than repulsive ones with water; thus, its interactions with bounded phase had the greatest contribution to the $-CH_2-$ group retention. Methylene group interaction with the stationary phase was weaker when the bounded phase chain was shorter, and it was the strongest in the case of solute partition between water and hexadecane.

As regards free enthalpy of phenol hydroxyl group transfer from gas to stationary phase ($\Delta G^0_{s(OH)}$), it was −25.645, −25.920, and −26.464 kJ/mol for C18, C8, and C4, respectively, while −28.87 kJ/mol from gas phase to water. Thus, the –OH group interactions were attractive both for stationary and mobile phase (water), but with water, they were somewhat stronger which caused a retention decrease. Because –OH group interacted stronger with the stationary phase when the chain of bonded phase was shorter, the absolute π^*_{OH} values decreased when the chain length of the bonded phase decreased. It looked similar to the case of the –OH group in naphthols and benzyl alcohol, in that in the latter hydroxyl group interactions with stationary phase were the weakest and decreased additionally with decreasing chain length of the bonded phase. Chlorine as a substitute showed minimal attractive interactions with stationary phase, which changed to repulsive with the decreasing chain length of the bonded phase ($\Delta G^0_{s(Cl)} = -0.095$, 0.404, and 0.920 for column C18, C8, and C4 respectively). These interactions practically have no influence on retention. Thus, retention of chlorine group was practically controlled by repulsive interaction with mobile phase, i.e., water.

CONCLUSIONS

The studies carried out showed that the chain length of the bonded phase effects the retention mechanism of the solutes in RPLC with pure water as an eluent. This has been testified both by the calculated logarithms of the retention factors (κ_w) and the hydrophobic substituent value (π^*) for $-CH_2-$, −OH, $-NO_2$, −Cl, and −CHO as well as thermodynamic analyses.

The shorter the chains of the bonded phase, the weaker are the attractive interactions of methylene group with the stationary phase. This is indicated by the calculated values of standard free enthalpy transfer of 1 mole of methylene groups from gas to stationary phase ($\Delta G^0_{s(CH_2)}$), the absolute

value of which decreases the shorter the chain of bounded phase. This in turn causes –CH_2– group selectivity in water eluent ($\pi^*_{CH_2}$) to decrease followed by weaker retention of alkylbenzenes with a chain length decrease of the bonded phase.

Analysis of F parameters showed that retention for column C18 and C8 with pure water as eluent is controlled by the partition mechanism, while for column C4 a small participation of the adsorption mechanism is visible, which can result from the fact that C4 chains are not long enough for the solute to completely penetrate them. Because the retention mechanism is similar for column C18 and C8, the difference of the $\pi^*_{CH_2}$ and $\Delta G^0_{s(CH_2)}$ values for these columns is smaller in comparison to those for column C4.

Hydroxyl groups cause retention decrease. However, for –OH groups of phenols their attractive interaction with the stationary phase increases with chain length of the bonded phase decrease, while a reverse tendency exists for this group of benzyl alcohol. But, the chlorine group causes retention increase of aromatics because retention of this group is controlled largely by repulsive interaction with an aqueous eluent. Its contribution to retention is smaller when the chain of bonded phase is shorter, as interaction with stationary phase changes from weakly attractive for C18 to weakly repulsive for C8 and C4.

The values of κ_w are frequently been used to determine solute hydrophobicity instead of log P descriptor. These studies have shown that κ_w values for columns with long chains of bonded phases (C18 and C8) are more suitable to determine hydrophobicity of nonpolar substances (aromatic hydrocarbons), rather than for column C4, to determine that of monosubstituted aromatics.

REFERENCES

1. Sherblom, P. M.; Eganhouse, R. P. *J. Chromatogr.* **1988**, *454*, 37–50.
2. Siwek, A.; Śliwiok, J. *Chromatographia* **1988**, *25*, 797–800.
3. Bitteur, S.; Rosset, R. *J. Chromatogr.* **1987**, *394*, 279–293.
4. Braumann, T. *J. Chromatogr.* **1986**, *373*, 191–225.
5. Kaliszan, R. *J. Chromatogr.* **1981**, *220*, 71–84.
6. Thus, J. L. G.; Kraak, J. C. *J. Chromatogr.* **1985**, *320*, 271–279.
7. Valkó, K.; Bevan, Ch.; Reynolds, D. *Anal. Chem.* **1997**, *69*, 2022–2029.
8. El Tayar, N.; Van de Weterbeend, M.; Testa, B. *J. Chromatogr.* **1985**, *320*, 305–312.
9. Hansch, C.; Kurup, A.; Garg, R.; Gao, H. *Chem. Rev.* **2001**, *101*, 619–672.
10. Kaliszan, R. *Structure and Retention in Chromatography. A Chemometric Approach*, Harwood Academic Publishers: Amsterdam, 1997.
11. Kaliszan, R. *Quantitative Structure-Chromatographic Retention Relationships*, A Wiley-Interscience Publication: USA, 1987; 303.
12. Jandera, P.; Kubát, J. *J. Chromatogr.* **1990**, *500*, 281–299.
13. Hsieh, M.-M.; Dorsey, J. G. *J. Chromatogr.* **1993**, *631*, 63–78.
14. Schoenmakers, P. J.; Billiet, H. A. H.; De Galan, L. *J. Chromatogr.* **1983**, *282*, 107–121.
15. Jandera, P.; Churáček, J.; Čáslavský, J.; Vojáčová, M. *Chromatographia* **1980**, *13*, 734–740.

16. El Tayar, N.; Waterbeemd van de H.; Testa, B. *J. Chromatogr.* **1985**, *320*, 293–304.
17. Janicka, M.; Kwietniewski, L.; Matysiak, J. *J. Planar Chromatogr.* **2000**, *13*, 285–289.
18. Janicka, M. *Chromatographia* **2003**, *57*, 359–403.
19. Janicka, M.; Ościk-Mendyk, B.; Tarasiuk, B. *J. Planar Chromatogr.* **2004**, *17*, 186–191.
20. Janicka, M.; Kwietniewski, L.; Perišić-Janjić, N. U. *Chromatographia* **2006**, *63*, 87–93.
21. Jaroniec, M.; Ościk, J. *J. High Resol. Chromatogr. Chromatogr. Commun.* **1982**, *5*, 3–9.
22. Melander, W. R.; Horváth, Cs. *Chromatographia* **1982**, *15*, 86–90.
23. Tsantili-Kakoulidou, A.; El Tayar, N.; Van de Weterbeend, M.; Testa, B. J. *Chromatogr.* **1987**, *389*, 33–45.
24. Grushka, E.; Grinberg, N. *Advances in Chromatography*, CRC Press, Connecticut, 2006; Vol. 45, 285.
25. Ranatunga, R. P. J.; Carr, P. W. *Anal. Chem.* **2000**, *72*, 5679–5692.
26. Kwietniewski, L. *J. Liquid Chromatogr. & Rel. Technol.*, **2010**, *33*, 305.
27. Tan, L. Ch.; Carr, P. W. *J. Chromatogr. A* **1997**, *775*, 1–12.
28. Dorsey, J. G.; Dill, K. A. *Chem. Rev.* **1989**, *89*, 331–346.
29. Dill, K. A. *J. Phys. Chem.* **1987**, *91*, 1980–1988.
30. Möckel, H. J.; Welter, G.; Melzer H. *J. Chromatogr.* **1987**, *388*, 255–266.
31. Krstulović, A. M.; Colin, H.; Tchapla, A.; Guiochon, G. *Chromatographia* **1983**, *17*, 228–232.
32. Issaq, H. J. *J. Liq. Chromatogr.* **1981**, *4*, 1917.
33. Engelhardt, H.; Ahr, G. *Chromatographia* **1981**, *14*, 227–233.
34. Carr, P. W.; Li, J.; Dallas, A. J.; Eikens, D. I.; Tan, L. C. *J. Chromatogr. A* **1993**, *656*, 113–133.
35. http://146.107.217.178/lab/alogps/start.html
36. Xiang, T.-X.; Anderson, B. D. *Biophys. J.* **2002**, *82*, 2052–2066.