Journal of Chromatography, 388 (1987) 51–64
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 113

DIFFERENCES BETWEEN RETENTIONS OF VARIOUS CLASSES OF AROMATIC HYDROCARBONS IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

IMPLICATIONS OF USING RETENTION DATA FOR CHARACTERIZING HYDROPHOBICITY

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(First received April 23rd, 1986; revised manuscript received September 22nd, 1986)

SUMMARY

Capacity factors of a series of alkylbenzenes (C_1–C_{10}), 12 chlorobenzenes, 9 chlorotoluenes, 17 chloronaphthalenes and 65 chlorobiphenyls have been measured on an octadecylsilica column. Aqueous methanol of four different compositions (80–95% methanol) was used as eluent. Logarithms of capacity factors of all eluites are linearly related to the amount of organic modifier in the eluent.

In addition, linear relationships between the solvent strength and the logarithms of capacity factors extrapolated to zero methanol have been revealed. The proportionality factors are dependent on the structures of the eluites.

Thermodynamic consideration of the retention processes shows that, within each type of eluite, enthalpy–entropy compensation is found. The compensation temperatures are not significantly different for the various types of eluite. Furthermore, it is shown that the compensation temperatures increase with increasing water content of the eluent.

Since the intercepts of the ΔG^0–ΔH^0 plots are not equal for the various types of eluite, it was concluded that the distribution processes causing retention of benzene, naphthalene and biphenyl are different.

When only the free energies of retention (*i.e.* the capacity factors) of different types of eluite are compared, no accurate information on the hydrophobicity of the eluites can be obtained if aqueous methanol is used as eluent. Therefore the possibilities for relating or predicting other physico-chemical parameters of the test compounds, such as octan-1-ol–water partition coefficients with isocratic retention data, will be limited.

INTRODUCTION

During the past decade many papers have reported on the mechanism causing retention of hydrophobic non-electrolytes in reversed-phase high-performance liquid chromatography (RP-HPLC)[1–3].

In RP chromatography aqueous eluents are usually employed. It is often suggested that hydrophobic interactions in the mobile phase dominate the retention process. If so, the retention times or capacity factors should be related to aqueous solubility[4–7] and may be used as representative of the eluite's hydrophobicity. This implies, however, a simplification of the retention mechanism because the rôles of the eluent's organic modifier and the stationary phase are neglected.

In the debate concerning the influence of the stationary phase at least three aspects can be distinguished:

(i) What is the nature of the stationary phase, *i.e.* is it a two-dimensional surface onto which eluite and eluent molecules can adsorb competetively[8,9] or a three-dimensional phase into which molecules can be "dissolved"[9,10], or is it a layer of adsorbed eluent molecules into which eluites can be "dissolved"[11]?

(ii) What is the nature of the interactions between stationary phase and eluites, and what is the influence of these interactions on retention[9,12–15].

(iii) What is the size of the adsorption surface or the phase volume of the stationary phase, and what is the relationship between this size of stationary phase and the eluent composition[16–18]?

A priori, little is known about these aspects. However, in both the "solvophobic theory"[18,19] and in the "solubility parameter concept"[20,21] it is usually assumed that the influence of the stationary phase on the relative retentions of different eluites is limited. Hence most studies are concerned only with the influence of the mobile phase composition on retention[18,22,23].

For different types of organic modifier in aqueous eluents various types of relationship between capacity factors and organic modifier content (φ) have been proposed. For aqueous methanol eluents with octadecyl-modified silica as stationary phase, however, proportionality between the organic modifier content and the logarithm of the capacity factors of the eluites (log k'_φ) has usually been reported. Recently it has been shown that the proportionality factor between log k'_φ and φ is dependent not only on the nature of the organic modifier, but also on the nature of the eluite[18,24].

In the present study the influence of the organic modifier on the retention of structurally related non-electrolytes is investigated. Alkylbenzenes and chlorinated aromatic hydrocarbons, *i.e.* benzenes, toluenes, naphthalenes and biphenyls are used to study the rôle of interactions between eluent components, *i.e.* water and methanol, and the different parent compounds. To investigate whether or not the distribution mechanisms of the various eluites are similar, the existence of enthalpy–entropy compensation for the retentions of the various types of eluite is tested for.

In addition, the possibilities of using retention data (capacity factors) to characterize the hydrophobic nature of eluites are considered. This is discussed together with the implications of relating or predicting other physico-chemical properties, such as octan-1-ol–water partition coefficients or aqueous solubilities with capacity factors.

MATERIALS AND METHODS

Chemicals

n-Alkylbenzenes were obtained from PolyScience Corporation. Chlorinated benzenes and most of the chlorinated biphenyls were obtained from Analabs. Other polychlorinated biphenyls as well as chlorotoluenes and lower chlorinated naphthalenes were available from previous studies or were synthesized. All chemicals were of more than 95% purity.

Apparatus

A Beckman 110 A liquid chromatograph was used, which was equipped with an Altex 254 nm mercury lamp, and a Rheodyne 7010 20-μl injection loop. A Hypersil ODS (5 μm) column (200 mm × 4.6 mm I.D.) was used with aqueous methanol as eluent at a flow-rate of 1.0 ml/min.

A mixture of methanol (Merck) and distilled water was used after de-aeration and filtration, using PTFE (0.25 μm) and cellulose (0.22 μm) Millipore filters, respectively.

RESULTS AND DISCUSSION

The capacity factor k' of an eluite in liquid chromatography can be related to its partition coefficient K between the stationary phase and the mobile phase by:

$$k'_\varphi = K_\varphi \psi \tag{1}$$

Here ψ denotes the stationary phase–mobile phase ratio and φ refers to the percentage of organic modifier in the aqueous eluent. In addition, the partition coefficient K can be expressed by

$$K_\varphi = e^{-\Delta G^0_\varphi/RT} \tag{2}$$

in which R denotes the gas constant (J $\mathrm{mol^{-1}\,K^{-1}}$), T the experimental temperature (K) and ΔG^0_φ the Gibbs free energy of the partition process.

In the present paper the logarithm of k' will be used, which by combining eqn. 1 and 2 can be expressed as:

$$\log k' = -\Delta G^0_\varphi/2.3\,RT + \log \psi \tag{3}$$

Determination of the hold-up time

To allow investigation of the retention behaviour of structurally related compounds, very precise measurement of the hold-up time or the void volume of the RP-HPLC system under study is of paramount importance. In the literature several methods for the determination of void volumes have been discussed[25–29], and it has been concluded that the method proposed by Berendsen *et al.*[27] usually provides good results.

In the present study this method is employed with a series of alkylbenzenes (C_2–C_{10}) for the determination of the column hold-up times at various temperatures and with different organic modifier concentrations (Table I).

TABLE I

HOLD-UP TIME IN SECONDS AS DETERMINED WITH THE METHOD PROPOSED BY BERENDSEN *et al.*[27]

Eluent composition (methanol–water)	*Temperature (°C)*			
	22	*32*	*48*	*56*
80:20	108.5 ± 0.9 (n = 11)	108.4 ± 0.8 (n = 4)	108.7 ± 0.6 (n = 4)	108.3 ± 0.8 (n = 4)
85:15	106.1 ± 0.7 (n = 18)	106.7 ± 0.5 (n = 5)	106.2 ± 0.3 (n = 4)	106.6 ± 0.7 (n = 4)
90:10	103.7 ± 0.4 (n = 17)	103.9 ± 0.7 (n = 5)	103.6 ± 0.6 (n = 5)	103.6 ± 0.5 (n = 5)
95:5	101. ± 0.2 (n = 11)	101.9 ± 0.6 (n = 5)	101.6 ± 0.2 (n = 4)	101.7 ± 0.4 (n = 4)

Additionally, convergence testing of the t_0 values by the method proposed by Krstulovic *et al.*[28], to check the measured hold-up times, showed convergence values (t_0^c) that fell within the 95% confidence limits of the mean values of the data of Table I, for all experimental conditions.

As is shown in Table I, the hold-up time was not influenced by the experimental temperature, which is in agreement with the observation of Neidhart *et al.*[29]. It is also consistent with the assumption that unretained compounds do not interact with the stationary phase, *i.e.* $\Delta G^0 = 0$ (eqn. 3). In addition, this observation indicates that, over a temperature range of 34°C, no change of the stationary phase–mobile phase ratio (ψ) was found for all eluent compositions.

Furthermore, Table I shows that an increase of the methanol content of the eluent results in a decrease of the hold-up time. This cannot be explained simply by a decreasing viscosity of the eluent with increased methanol content, which according to Darcy's law should result in increased flow-rates. This because the RP-HPLC system was flow-controlled (1.0 ml/min). In addition, temperature increases will also reduce the eluent's viscosity. However, while this did not influence the hold-up time, it may be suggested that the altered hold-up time with varying organic modifier content is not a consequence of Darcy's law, but a manifestation of a change in the column's phase ratio, ψ. As is shown in Table I, however, the change in the hold-up times using methanol contents between 80 and 95% is limited. Hence it can be assumed that, for comparing the measured capacity factors of single eluites, with various eluent compositions, use of uncorrected capacity factors may provide accurate information on the stationary phase–mobile phase partition coefficients (K_ϕ in eqn. 2).

Capacity factors and solvent strength

Since the test compounds under study have low aqueous solubilities, it is usually assumed that solvophobic expulsion forces in the aqueous mobile phase dominate the retention process, and that the manifestation of these forces is dependent on the type and concentration of the organic modifier.

According to Hildebrand's solubility parameter concept for the solution of chemicals in binary solvent mixtures, a relationship between a solute's capacity factor and the organic modifier's partial molar volume (φ) has been derived, which can be expressed by [18,24,30,31]

$$\log k'_{\phi,i} = \log k'_{w,i} + A\varphi^2 - B\varphi \qquad (4)$$

Here $\log k'_{w,i}$ represents the logarithm of the capacity factor of solute i, for pure water as eluent. It has been proposed that the magnitudes of A and B are dependent on the components of the binary solvent mixture, and on the solute's molar volume. However, it has often been shown, using methanol–water as eluent, that the parameter A is close to zero, so the influence of φ^2 is negligible. Hence a simplified form of eqn. 4 showing proportionality between $\log k'_{\phi,i}$ and φ seems to be appropriate. When this type of relationship was initially proposed[32] the variable B was replaced by S, which was called the solvent strength of the organic modifier:

$$\log k'_{\phi,i} = \log k'_{w,i} - S\varphi \qquad (5)$$

As shown in Table II, the logarithms of the measured capacity factors of all test chemicals fit excellently with the eluent composition as described by eqn. 5. The high correlation coefficients of the linear relationship between φ and $\log k'_{\phi,i}$ support the assumed lack of influence of the φ^2 term[24].

Although it has sometimes been suggested that S is a constant, characterizing the solvent's properties only, it is obvious from the data listed in Table II that S is a variable. Recently a relationship between S and $\log k'_{w,i}$ has been proposed, which shows[33–35]:

$$S = p \cdot \log k'_{w,i} + q \qquad (6)$$

TABLE II

EXTRAPOLATED LOGARITHMS OF CAPACITY FACTORS ($\log k'_w$) AND SOLVENT STRENGTHS (S) OF TEST COMPOUNDS

Compound	*log k'_w*	*S*	*r*
Alkylbenzenes			
Benzene	0.993	1.77	0.9999
Toluene	1.536	2.27	0.9998
Ethylbenzene	2.093	2.76	0.9997
Propylbenzene	2.655	3.26	0.9998
Butylbenzene	3.212	3.74	0.9998
Pentylbenzene	3.767	4.23	0.9998
Hexylbenzene	4.320	4.72	0.9999
Heptylbenzene	4.862	4.72	0.9999
Octylbenzene	5.423	5.69	0.9999
Nonylbenzene	5.972	6.17	0.9998
Decylbenzene	6.522	6.65	0.9999

(*Continued on page 56*)

TABLE II (*continued*)

Compound	*log k'_w*	*S*	*r*
Chlorobenzenes			
Monochlorobenzene	1.463	2.21	0.9998
1,2-Dichlorobenzene	1.912	2.59	0.9999
1,3-Dichlorobenzene	2.170	2.80	0.9998
1,4-Dichlorobenzene	2.078	2.77	0.9997
1,2,3-Trichlorobenzene	2.494	3.06	0.9998
1,2,4-Trichlorobenzene	2.647	3.19	0.9999
1,3,5-Trichlorobenzene	2.922	3.36	0.9998
1,2,3,4-Tetrachlorobenzene	3.060	3.49	0.9999
1,2,3,5-Tetrachlorobenzene	3.303	3.67	0.9998
1,2,4,5-Tetrachlorobenzene	3.234	3.63	0.9999
Pentachlorobenzene	3.743	3.99	0.9998
Hexachlorobenzene	4.346	4.43	0.9999
Chlorotoluenes			
2-Monochlorotoluene	2.016	2.65	0.9998
3-Monochlorotoluene	2.057	2.71	0.9999
4-Monochlorotoluene	2.031	2.70	0.9998
2,4-Dichlorotoluene	2.711	3.23	0.9997
2,5-Dichlorotoluene	2.633	3.19	0.9998
2,6-Dichlorotoluene	2.702	3.21	0.9999
3,4-Dichlorotoluene	2.488	3.08	0.9997
3,5-Dichlorotoluene	2.761	3.28	0.9998
2,4,5-Trichlorotoluene	3.369	3.81	0.9998
Chloronaphthalenes			
1-Monochloronaphthalene	1.863	2.53	0.9994
2-Monochloronaphthalene	2.366	2.88	0.9995
1,2-Dichloronaphthalene	2.466	3.04	0.9995
1,3-Dichloronaphthalene	2.974	3.41	0.9996
1,4-Dichloronaphthalene	3.161	3.55	0.9998
1,5-Dichloronaphthalene	3.117	3.50	0.9998
1,8-Dichloronaphthalene	2.690	3.18	0.9994
2,3-Dichloronaphthalene	2.988	3.47	0.9996
2,7-Dichloronaphthalene	3.102	3.61	0.9996
1,3,7-Trichloronaphthalene	3.868	4.14	0.9999
2,3,6-Trichloronaphthalene	3.599	4.02	0.9998
1,2,3,4-Tetrachloronaphthalene	4.222	4.28	0.9997
1,2,3,5-Tetrachloronaphthalene	4.267	4.34	0.9998
1,3,5,7-Tetrachloronaphthalene	4.664	4.67	0.9997
1,3,5,8-Tetrachloronaphthalene	4.238	4.33	0.9996
Octachloronaphthalene	6.253	5.58	0.9995
Chlorobiphenyls			
Biphenyl	2.121	2.84	0.9994
2-Monochlorobiphenyl	2.775	3.39	0.9994
3-Monochlorobiphenyl	2.934	3.44	0.9993
4-Monochlorobiphenyl	2.947	3.48	0.9996
2,2'-Dichlorobiphenyl	2.906	3.59	0.9996
2,4-Dichlorobiphenyl	3.479	3.94	0.9998
2,4'-Dichlorobiphenyl	3.555	3.97	0.9995
2,5-Dichlorobiphenyl	3.361	4.09	0.9994
2,6-Dichlorobiphenyl	3.151	3.69	0.9995
2,2',5-Trichlorobiphenyl	3.567	4.13	0.9997
2,3,4-Trichlorobiphenyl	4.042	4.46	0.9995

TABLE II (*continued*)

Compound	*log* k'_w	*S*	*r*
2,3′,4′-Trichlorobiphenyl	3.858	4.28	0.9996
2,3′,5-Trichlorobiphenyl	4.022	4.44	0.9994
2,3,6-Trichlorobiphenyl	4.432	3.92	0.9995
2,4,5-Trichlorobiphenyl	4.149	4.47	0.9998
2,4′,5-Trichlorobiphenyl	3.990	4.42	0.9999
2,4,6-Trichlorobiphenyl	4.110	4.52	0.9996
2,2′,3,3′-Tetrachlorobiphenyl	3.647	4.17	0.9995
2,2′,3,5′-Tetrachlorobiphenyl	3.908	4.39	0.9999
2,2′,4,4′-Tetrachlorobiphenyl	4.251	4.64	0.9998
2,2′,4,5′-Tetrachlorobiphenyl	4.395	4.83	0.9995
2,2′,4,6-Tetrachlorobiphenyl	4.170	4.63	0.9998
2,2′,5,5′-Tetrachlorobiphenyl	4.144	4.59	0.9998
2,2′,5,6′-Tetrachlorobiphenyl	3.690	4.24	0.9992
2,2′,6,6′-Tetrachlorobiphenyl	3.095	3.76	0.9993
2,3′,4,4′-Tetrachlorobiphenyl	4.431	4.74	0.9996
2,3,4,5-Tetrachlorobiphenyl	4.575	4.78	0.9998
2,3′,4′,5-Tetrachlorobiphenyl	4.463	4.79	0.9998
2,3′,4,6-Tetrachlorobiphenyl	4.548	4.90	0.9999
2,3′,5,5′-Tetrachlorobiphenyl	4.859	5.12	0.9998
2,3,5,6-Tetrachlorobiphenyl	4.281	4.60	0.9998
2,4,4′,6-Tetrachlorobiphenyl	4.598	4.95	0.9999
3,3′,4,4′-Tetrachlorobiphenyl	4.572	4.88	0.9997
2,2′,3,4,5′-Pentachlorobiphenyl	5.546	5.91	0.9999
2,2′,3′,4,5-Pentachlorobiphenyl	5.235	5.35	0.9994
2,2′,3,5,6-Pentachlorobiphenyl	4.227	4.59	0.9990
2,2′,4,5,5′-Pentachlorobiphenyl	4.861	5.16	0.9996
2,2′,4,5,6-Pentachlorobiphenyl	5.287	5.78	0.9991
2,3,4,5,6-Pentachlorobiphenyl	4.902	5.05	0.9999
2,2′,3,3′,4,4′-Hexachlorobiphenyl	4.834	5.11	0.9998
2,2′,3,3′,5,5′-Hexachlorobiphenyl	5.463	5.67	0.9997
2,2′,3,3′,6,6′-Hexachlorobiphenyl	4.218	4.71	0.9999
2,2′,3,4,4′,5′-Hexachlorobiphenyl	6.606	6.94	0.9997
2,2′,3,4,5,5′-Hexachlorobiphenyl	5.280	5.47	0.9998
2,2′,3,5,5′,6-Hexachlorobiphenyl	4.954	5.27	0.9996
2,2′,4,4′,5,5′-Hexachlorobiphenyl	5.480	5.66	0.9998
2,2′,4,5,5′,6′-Hexachlorobiphenyl	5.413	5.64	0.9996
2,2′,4,4′,6,6′-Hexachlorobiphenyl	5.080	5.32	0.9999
2,3,3′,4,4′,5-Hexachlorobiphenyl	4.784	4.84	0.9996
2,3,3′,4,5,6-Hexachlorobiphenyl	4.952	5.12	0.9997
3,3′,4,4′,5,5′-Hexachlorobiphenyl	5.703	6.05	0.9994
2,2′,3,3′,4,4′,6-Heptachlorobiphenyl	5.363	5.55	0.9998
2,2′,3,4,4′,5,6-Heptachlorobiphenyl	5.841	5.96	0.9993
2,2′,3,4,4′,5′,6-Heptachlorobiphenyl	5.827	5.98	0.9999
2,2′,3,4,5,5′,6,6′-Heptachlorobiphenyl	5.612	5.77	0.9998
2,2′,3,3′,4,4′,5,5′-Octachlorobiphenyl	6.382	6.37	0.9997
2,2′,3,3′,4,4′,5,6-Octachlorobiphenyl	6.164	6.24	0.9997
2,2′,3,3′,4,4′,6,6′-Octachlorobiphenyl	5.841	5.90	0.9996
2,2′,3,3′,4,5,5′,6-Octachlorobiphenyl	6.415	6.47	0.9999
2,2′,3,3′,5,5′,6,6′-Octachlorobiphenyl	5.625	5.77	0.9998
2,2′,3,4,4′,5,6,6′-Octachlorobiphenyl	6.220	6.23	0.9995
2,2′,3,3′,4,4′,5,5′,6-Nonachlorobiphenyl	6.549	6.46	0.9997
2,2′,3,3′,4,4′,5,6,6′-Nonachlorobiphenyl	6.755	6.69	0.9998
2,2′,3,3′,4,5,5′,6,6′-Nonachlorobiphenyl	6.573	6.54	0.9998
Decachlorobiphenyl	6.977	6.74	0.9997

TABLE III

VALUES FOR p AND q IN $S = p \log k'_{w,i} + q$ (EQN. 6)

Column type	*Eluate type*	p	q	n	*Ref.*
Hypersil ODS	Alkylbenzenes	0.88 ± 0.01	0.92 ± 0.02	9	This study
	Chlorobenzenes	0.76 ± 0.01	1.15 ± 0.04	12	This study
	Chlorotoluenes	0.83 ± 0.01	0.99 ± 0.05	9	This study
	Chloronaphthalenes	0.76 ± 0.03	1.18 ± 0.09	15	This study
	Chlorobiphenyls	0.85 ± 0.03	1.02 ± 0.08	64	This study
	Miscellaneous	0.74 ± 0.04	1.62 ± 0.09	30	33
	Miscellaneous	0.79 ± 0.02	1.57 ± 0.06	102	33
LiChrosorb RP-18	Alkylbenzenes	0.91 ± 0.02	0.81 ± 0.09	3	34
	Methylbenzenes	0.82 ± 0.01	1.02 ± 0.03	12	34
	Chlorobenzenes	0.77 ± 0.01	1.25 ± 0.05	12	34
	Chloroanilines	0.76 ± 0.02	1.47 ± 0.08	15	34
	Chlorophenols	0.76 ± 0.03	1.64 ± 0.08	12	34
	Fused arenes	0.73 ± 0.01	1.47 ± 0.10	5	34
	Polyphenyls	0.87	1.06	2	34
	Miscellaneous	0.77 ± 0.02	1.40 ± 0.06	79	34
Nucleosil RP-18	Miscellaneous	0.77	1.24	32	35
	Miscellaneous	0.75 ± 0.06	1.29 ± 0.14	30	35
	Miscellaneous	0.68 ± 0.04	1.54 ± 0.09	49	35

It was assumed that the parameters p and q were constants. The p and q values of the present study are listed in Table III together with previously reported data. It can be concluded that these do not support the assumption that both p and q have constant values, being independent of the system or eluites under study.

The variation of the p parameter is limited, *i.e.* ranging from 0.75 to 0.91, and seems to be mainly influenced by the eluites' structures. For instance, the values for the alkylbenzenes and chlorobenzenes of the present study (0.88 and 0.76, respectively) are in good agreement with those reported previously[34] (0.91 and 0.77, respectively).

The q parameter on the other hand is not only dependent on the eluites' structures, but also on the chromatographic system under investigation. This can be concluded from the data for alkyl benzenes and chlorobenzenes in the various systems. In addition, q values measured in one particular system seem to increase with increasing polarity of the eluites. These data support previous observations[24,36].

According to eqn. 2, the eluite's partition coefficient can be expressed by

$$K_{\mathrm{ODS/eluent}} = \gamma_{\mathrm{eluent}} \gamma_{\mathrm{ODS}}^{-1} \tag{7}$$

Substitution of eqn. 7 into eqn. 6 gives

$$S = p[\log (\gamma_{\mathrm{eluent}} \gamma_{\mathrm{ODS}}^{-1}) + \log \psi] + q \tag{8}$$

In addition, by rearranging eqn. 5 and using the two extreme eluent compositions, *i.e.* $\varphi = 0$ and $\varphi = 1$, the solvent strength can be expressed as:

$$S = \log\left(\frac{\gamma_{\mathrm{w}}}{\gamma_{\mathrm{ODS}}}\cdot\psi_{\mathrm{w}}\right) - \log\left(\frac{\gamma_{\mathrm{CH_3OH}}}{\gamma_{\mathrm{ODS}}}\cdot\psi_{\mathrm{CH_3OH}}\right) = (\log\gamma_{\mathrm{w}} - \log\gamma_{\mathrm{CH_3OH}})\psi \tag{9}$$

in which it is assumed that ψ_{w} and $\psi_{\mathrm{CH_3OH}}$ are equal. After combining eqns. 8 and 9 it can be concluded that p relates S to the properties of the eluite (*i.e.* γ_{w} and γ_{ODS}) as well as to properties of the RP-HPLC system (*i.e.* γ_{ODS} and ψ). In addition, q relates S to γ_{w}, γ_{CH_3OH}, γ_{ODS} and ψ. This means that, for a RP-HPLC system with a constant stationary phase–mobile phase ratio, two eluites having similar γ_{w} and γ_{ODS} values can have different log $k'_{\phi,i}$ values. This may be explained by different activity coefficients of these chemicals in pure methanol. So it can be concluded that the retention of various types of solute cannot uniquely be expressed by eqn. 5, because S is a parameter that can be described by log $k'_{\mathrm{w},i}$, p and q only. This conclusion is consistent with the interaction indices model used to describe RP chromatographic retentions[16,24,31].

In order to find whether or not the various types of structurally related eluite are retained by similar distribution processes, it became evident that the thermodynamics of the retention processes must be investigated. This is because application of eqn. 5 is essentially based on the assumption that one single mechanism is responsible for the retention of the various types of eluite. If so, enthalpy–entropy compensation must exist for the thermodynamics of retention of the various eluites.

Linear enthalpy–entropy compensation

Acording to Leffler and Grunwald[37], the availability of partial thermodynamic quantities (ΔH^0, ΔS^0) can provide information on the mechanisms of the physicochemical distribution of a series of eluites in aqueous solutions. It has been argued that one single mechanism may be responsible for the retention of various eluites, with ΔH^0 and ΔS^0 of this process being almost constant, if $\Delta\Delta H^0$ is proportional to $\Delta\Delta S^0$ (ref. 8). (Here $\Delta\Delta H^0$ and $\Delta\Delta S^0$ denote the alteration in the enthalpy and entropy changes of the distribution process caused by differences in the structures of the eluites.)

In addition, it has been argued that enthalpy–entropy compensation in RP chromatography can be expressed by[38,39]:

$$\log k'_{\phi,T} = \frac{\Delta H^0_\phi}{2.3R}\left(\frac{1}{T} - \frac{1}{\beta_\phi}\right) - \frac{\Delta G^0_{\beta,\phi}}{2.3\beta_\phi R} + \log\psi \tag{10}$$

Here log $k'_{\phi,T}$ denotes the logarithm of a capacity factor using eluent composition φ measured at temperature T, β_ϕ is the compensation temperature, and $\Delta G^0_{\beta,\phi}$ the Gibbs free energy of the retention process at $T = \beta_\phi$.

Enthalpies retention of the alkylbenzenes and some chlorinated benzenes, toluenes, naphthalenes and biphenyls with φ between 0.85 and 0.90 respectively, were obtained from the slopes of Van 't Hoff plots in the temperature range 20.0–56.0°C (Fig. 1).

It has been proposed that statistical variation in the log k'_ϕ–ΔH^0_ϕ plots is most limited if the log k'_ϕ values are measured at temperatures close to the harmonic mean of the experimental temperatures (T_{hm})[40]. Using capacity factors at T = 313 K,

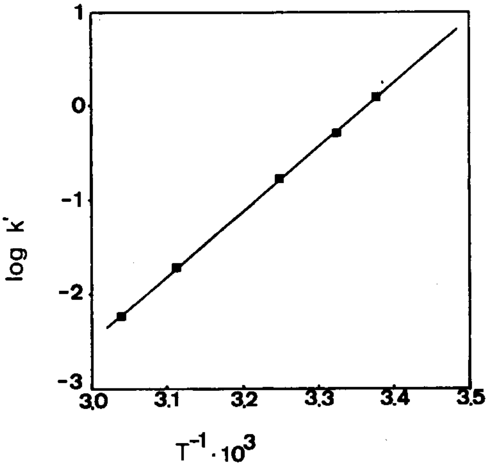


Fig. 1. Van't Hoff plot of the capacity factor of propylbenzene with $\varphi_{CH_3OH} = 0.85$.

TABLE IV

COMPENSATION TEMPERATURES AND INTERCEPTS OF EQN. 10 FOR RP-HPLC RETENTION WITH AQUEOUS METHANOL ELUENTS

Column type	*Eluate type*	φ	β *(K)*	*Exp. temp. (K)*	*Intercept*	*Ref.**	*r*	*n*
Hypersil ODS	Alkylbenzenes	85:15	609	313	−1.880	a	0.9999	9
	Alkylbenzenes	90:10	559	313	−1.444	a	0.9998	9
	Chlorobenzenes	85:15	603	313	−2.009	a	0.9996	6
	Chlorobenzenes	90:10	555	313	−1.412	a	0.9997	6
	Chlorotoluenes	85:15	603	313	−1.566	a	0.9998	5
	Chlorotoluenes	90:10	548	313	−1.181	a	0.9998	5
	Chloronaphthalenes	85:15	633	313	−2.144	a	0.9995	7
	Chloronaphthalenes	90:10	542	313	−1.333	a	0.9997	7
	Chlorobiphenyls	85:15	598	313	−1.430	a	0.9996	7
	Chlorobiphenyls	90:10	556	313	−1.065	a	0.9998	7
Nucleosil RP-18	*n*-Alkanal DNPH**	80:20	676	317	−0.963	39		
	2 *n*-Alkanal DNPH**	80:20	709	317	−0.984	39		
Permaphase ODS	Subst. benzenes	60:40	654	313	−1.35	41		
LiChrosorb RP-18	Subst. benzoates	80:20	794	308	−0.69	8		
Jasco FineSil C_{18}	Alkylbenzenes	76:24	682	293		42		
	Alkylbenzenes	80:20	639	293		42		
	Alkylbenzenes	90:10	517	293		42		
	Alkylbenzenes	99.9:0.1	682	293		42		

* a = Data from this study.

** Dinitrophenylhydrazones.

values of β_Φ and $(\Delta G^0_{\beta,\Phi}/2.3R\ \beta_\Phi + \log \psi)$ were calculated from the slopes and intercepts of eqn. 10 (Fig. 2) and are listed in Table IV. The correlation coefficients of eqn. 10 were higher than 0.9995 for the experimental data under study. As shown, the compensation temperature for $\varphi = 0.85$ and $\varphi = 0.90$, of all the types of chemical under study are comparable with values reported previously[8,34,36,37] (Table IV). Table IV and Fig. 2 also show that the slopes of the equation are almost equal for the various chlorine derivatives of benzene, biphenyl and naphthalene. This means that the compensation temperatures of these compounds are comparable with fixed eluent compositions.

In addition, the data listed in Table IV support the previously observed increase of compensation temperatures with increasing water content of the eluent[42]. Since this holds true for all compounds, it may be clear that the influence of the structure of the parent compound on the compensation temperatures of a class is limited. In contrast the intercepts of eqn. 10 for the various types of eluite differ significantly. These different intercept values, with φ being constant, represent differences in $\Delta G^0_{\beta\Phi}$ only. This may be explained by the differences of the relative importance of interactions between eluent components, the stationary phase and the three structurally different parent aromatic hydrocarbons, *i.e.* benzene, naphthalene and biphenyl.

When using different eluent compositions, the intercepts of the log $k'_{\Phi,T}$–ΔH^0_Φ plots are not similar for the various types of eluite. This, however, is not surprising, since it cannot be assumed *a priori* that benzenes, naphthalenes and biphenyls have similar $(\gamma_{\text{eluent}}/\gamma_{\text{ODS}})$ ratios.

Moreover, it may be concluded that the relative influences of interactions between eluites, eluents and stationary phase compoonents causing retention of one particular eluite are not similar for different eluent compositions. Thus these different types of interaction will not simply change proportionally with changes of the eluent composition.

Contribution of chlorine and methylene to the retention

The extrathermodynamic contributions to the retention of parent eluites after substitution with chlorine or methylene groups can be investigated using[8]

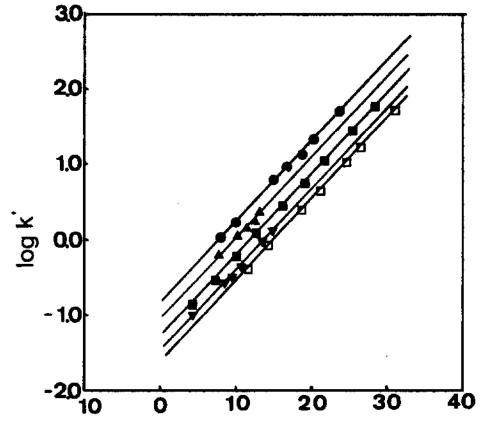
$$\tau_{\Phi,T} = \left(\frac{\Delta\Delta H^0_\Phi}{2.3R}\right)\left(\frac{1}{T_{\text{hm}}} - \frac{1}{\beta_\Phi}\right) + \frac{\Delta\Delta G^0_{\beta,\Phi}}{2.3R\beta_\Phi} \qquad (11)$$

Here τ_Φ, which is determined at temperature T, can be expressed by

$$\tau_\Phi = \log K_{\text{X},\Phi} - \log K_{\text{H},\Phi}$$

in which X denotes a substituent in the parent compound. Similarity in the slopes and intercepts of eqn. 11 was found for all types of eluite (Fig. 3).

Hence it can be concluded that substitution of chlorine atoms into the aromatic hydrocarbon parent compounds gives a contribution to the retention that is mechanistically comparable with the contribution of methylene addition. These extrathermodynamic group contributions are independent of the structure of the parent compound. If so, the full extent of the differences between the capacity factors of the



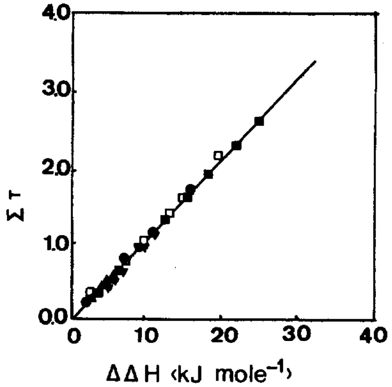


Fig. 2. Relationship between enthalpy and free energy changes (at T = 313 K) during the retention of alkylbenzenes (■) and some chlorobenzenes (▼), chlorotoluenes (▲), chloronaphthalenes (□) and chlorobiphenyls (●).

Fig. 3. Relationship between extrathermodynamic group contributions to the enthalpies of retention and the substituent parameters of the retention of the test compounds at T = 313 K.

alkylated and chlorinated benzenes, chloronaphthalenes and chlorobiphenyls has to be attributed to the different interactions between parent compounds and stationary and mobile phases.

This enables us to conclude that the changes in RP chromatographic retention of non-electrolyte eluites may provide information on the solvophobic properties of these compounds if the derivatives of only one parent are compared. However, when comparing capacity factors of derivatives of different parent compounds, such as benzene, naphthalene and biphenyl, it must be noted that there are significant differences in the distribution processes causing retention of these parent compounds.

Moreover, the different $\Delta\Delta G^0_{\beta,\phi}$ values obtained for the three parent compounds indicate that neither naphthalene nor biphenyl can be considered as a derivative of benzene. This because otherwise all log k'_ϕ–ΔH_ϕ plots would have the same intercept. Thus, substitution of a phenyl group for a hydrogen in benzene will have an influence on eluite retention that is mechanistically different from that which re-

TABLE V

$\Delta\Delta S°$ AND $\Delta\Delta H°$ VALUES OF METHYLENE GROUPS IN ELUITES

Column	φ	$\Delta\Delta H°$ (kJ mol^{-1})	$\Delta\Delta S°$ (J mol^{-1} k^{-1})	*Ref.*
Hypersil ODS	85:15	−1.71	−2.81	This study
	90:10	−1.56	−2.79	This study
	80:20	−1.68	−2.18	44
	90:10	−1.12	−1.32	44
LiChrosorb	80:20	−1.94	−3.15	8
Nucleosil RP_{18}	80:20	−1.75		39
	80:20	−1.39		39

sults from chlorine and methylene substitution. Therefore it may be clear that in RP chromatography phenyl groups do not have their equivalent in a number of methylene groups, as has sometimes been suggested[43,44].

In Table V, the extrathermodynamic enthalpy and entropy and the contribution from methylene of the present study are listed, together with data obtained from the literature[8,39,44]. The extrathermodynamic enthalpy change seems to be increasingly exothermic with increasing water content of the eluent. This is consistent with expectations based on the rôle of hydrophobic interactions in RP chromatography.

While the retention of all parent compounds is exothermic, substitution of methylene or chlorine into the aromatic hydrocarbon under study makes the retention process more favourable.

Since the phase ratio of the mobile to the stationary phase is unknown, nothing can be concluded about the importance of the enthalpy of retention, relative to the entropy change of the system.

Concerning the extrathermodynamic group contribution, however, it can be concluded that the influence of $\Delta\Delta S^0$ on $\Delta\Delta G^0$ is limited. This is due to the high compensation temperatures listed in Table IV. As shown, the extrathermodynamic entropy changes are negative, *i.e.* unfavourable for the retention process, which is consistent with data reported previously. It has been argued that these negative values support the concept that adsorption rather than liquid–liquid exchange is the underlying physicochemical proces in RP HPLC retention[8].

Finally, the assumed entropy dominance of the dissolution and solvation of hydrophobic non-electrolytes in aqueous solvents[45] is not supported by the observed increasingly negative extrathermodynamic entropy contribution with increasing water content of the eluent.

CONCLUSIONS

By comparing only free energy changes or capacity factors measured with isocratic RP chromatography, little can be concluded about the solvophobic or hydrophobic properties of eluites, even when the study is limited to structurally related compounds. This is because the capacity factors of various types of eluite are not influenced to the same extent by the concentration of organic modifier in the eluent. In addition, it can be concluded that the relative importance of interactions between eluite–eluent and stationary phase, cause retention of structurally related eluites, is not necessarily constant.

The observed differences in retention of chlorine and alkyl derivatives of benzene, naphthalene and biphenyl could partly be explained by the differences between the structures of the parent compounds. This held true also for the different influence of the eluent composition on the retention of the various classes of aromatic hydrocarbons. The contributions of methylene and chlorine substitution on the retention were similar for all three parent compounds.

Since it was found that enthalpy–entropy plots for different eluent compositions yield different compensation temperatures, it can be concluded that employing measured capacity factors with aqueous methanol eluents does not provide suitable results for expressing the hydrophobicity of an eluite. The same holds true if the measured capacity factors are used to relate or predict other physico–chemical parameters, such as octan-1-ol–water partitioning or aqueous solubility.

Employing capacity factors extrapolated to 100% water as eluent may, however, provide better results, even though differences in interactions between the stationary phase and the various types of eluite mean that these parameters do not exclusively represent the hydrophobic properties of the chemicals

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* Note by the Editor: see also T. Braumann, *J. Chromatogr.*, 373 (1986) 191; this review was still in press when the present article was accepted.