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Journal of Chromatography A, 911 (2001) 177–190

JOURNAL OF CHROMATOGRAPHY A

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Selectivity of stationary phases in reversed-phase liquid chromatography based on the dispersion interactions

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Received 21 March 2000; received in revised form 13 October 2000; accepted 24 November 2000

Abstract

Selectivity of 15 stationary phases was examined, either commercially available or synthesized in-house. The highest selectivity factors were observed for solute molecules having different polarizability on the 3-(pentabromobenzyloxy)propyl phase (PBB), followed by the 2-(1-pyrenyl)ethyl phase (PYE). Selectivity of fluoroalkane 4,4-di(trifluoromethyl)-5,5,6,6,7,7,7-heptafluoroheptyl ($F_{13}C_9$) phase is lowest among all phases for all compounds except for fluorinated ones. Aliphatic octyl (C_8) and octadecyl (C_{18}) phases demonstrated considerable selectivity, especially for alkyl compounds. While PBB showed much greater preference for compounds with high polarizability containing heavy atoms than C_{18} phase, $F_{13}C_9$ phase showed the exactly opposite tendency. These three stationary phases can offer widely different selectivity that can be utilized when one stationary phase fails to provide separation for certain mixtures. The retention and selectivity of solutes in reversed-phase liquid chromatography is related to the mobile phase and the stationary phase effects. The mobile phase effect, related to the hydrophobic cavity formation around non-polar solutes, is assumed to have a dominant effect on retention upon aliphatic stationary phases such as C_8, C_{18}. In a common mobile phase significant stationary phase effect can be attributed to dispersion interaction. Highly dispersive stationary phases such as PBB and PYE retain solutes to a significant extent by (attractive) dispersion interaction with the stationary phase ligands, especially for highly dispersive solutes containing aromatic functionality and/or heavy atoms. The contribution of dispersion interaction is shown to be much less on C_{18} or C_8 phases and was even disadvantageous on $F_{13}C_9$ phase. Structural properties of stationary phases are analyzed and confirmed by means of quantitative structure-chromatographic retention (QSRR) study. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Stationary phases, LC; Dispersion interactions; Selectivity; Retention mechanisms; Quantitative structure–retention relationships

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PII: S0021-9673(00)01193-6

1. Introduction

Understanding of intermolecular interactions in high-performance liquid chromatography (HPLC) will help choosing a right stationary phase and help to delevop new ones suitable for the target analysis. This is a complicated task, since there are multiple variables that influence retention and separation process. Several authors attempted to explain the retention mechanisms in normal phase mode (NPLC) [1,2] as well as reversed-phase chromatography (RPLC) [3–5]. Based upon experimental observations and theoretical interpretations, these papers present fundamental concepts of chromatographic retention.

Retention in RPLC is described in terms of free energy change, $\Delta G°$, upon transfer of a solute from an aqueous mobile to a non-polar stationary phase, and expressed as the retention factor k:

$$\Delta G^0 = -RT(\ln k - \ln \phi)$$

$$\ln k = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \phi \quad (1)$$

where T is absolute temperature, R is the gas constant, ϕ is the phase ratio (the ratio of stationary and mobile phase volumes), $\Delta H°$ and $\Delta S°$ are enthalpy and entropy, respectively, associated with the transfer of a solute from the mobile phase to the stationary phase.

In RPLC a mobile phase is aqueous (dipolar) and a stationary phase is hydrophobic (non-polar). Retention of solutes will follow their respective affinity to two phases. Following Giddings [6], one may state that usually in two phase-partitioning processes of solutes the enthalpy values determine the equilibrium, driven by the intermolecular interactions between a solute and the two phases. These interactions were systematically described by Kaliszan [7] and they are divided into several types. For non-ionized components of a chromatographic system one may distinguish a Van der Waals interaction class including the following: dipole–dipole interaction (Keesom's), dipole-induced dipole interaction (Debye's) and instantaneous dipole-induced dipole interaction (London's or dispersive).

For solute retention in RPLC system one should, therefore, take into account two processes.

1. Attractive interaction: (a) dispersive between the solutes and stationary phase and, to some extent, mobile phase components (more likely an organic modifier); (b) dipolar in the case of species posessing considerable dipole moments. Both attractive interaction types can be referred to the so-called stationary phase effect [8].
2. Hydrophobic interaction or a mobile phase effect sometimes called *solvophobic* effect [9]. Although studied for many years, the hydrophobic effect is not entirely understood and there are various models created to investigate it [10,11]. Introduction of a non-polar molecule into water causes an increase in the free energy of an entire system. One possible explanation is that water molecules become more organized in order to create cavity which may adopt the introduced molecule [12].

We report in this work that some stationary phases show the selectivities that can be attributed to the dispersive interactions. General statements relating selectivity phenomena to dispersive interactions were previously reported by Zhao and Carr [13], where the stationary phase effects were analyzed quantitatively. The applicability of dispersion-driven retention mechanism was also described in our previous work on highly dispersive properties of heavy atom-containing aromatic stationary phases [14] and extremely weak dispersive properties of fluoroalkane stationary phase [15], where we described a critical influence of van der Waals (in general) and London (in particular) interactions. The isotope effect in HPLC or the separation of hydrogen/deuterium isotopic compounds (H/D isotopologues) may also be related to this type of interaction [16,17].

2. Experimental

Complete LaChrom™ HPLC equipment with HPLC data manager from Merck-Hitachi was employed (Hitachi, Tokyo, Japan; http://www.hitachi.co.jp/) and, additionaly, a differential refractive index detector RI-8 was from ToSoh (Tokyo, Japan). All chromatographic data were collected at 30°C using a thermostated water bath. Mobile phase was prepared by mixing methanol and water at the volume ratios 60:40, 70:30 and 80:20. Flow rate was

1.0 ml/min. Aromatic compounds were detected by UV absorption at 254 nm, and aliphatic compounds by refractive index (RI) detector. Dead-volume markers were uracil for UV and D_2O for RI detection. Following stationary phases (packed in a column 4.6 mm ID, 10–25 cm long) were from the commercial source: octyl (C_8), octadecyl (C_{18}), 3-(pentabromobenzyloxy)propyl (PBB), 2-(1-pyrenyl)ethyl (PYE) and 3-(*p*-nitrophenyloxy)-propyl (NPO) from Nacalai Tesque (Kyoto, Japan; http://www.nacalai.co.jp/), 4,4-di(trifluoromethyl)-5,5,6,6,7,7,7-heptafluoroheptyl ($F_{13}C_9$ or Fluofix™) from NEOS Corporation (Shiga, Japan; http://www.neos.co.jp/). Preparation of other stationary phase materials by standard procedures was described elsewhere [14,18]. Structures of the stationary phases are presented in Fig. 1. Test solutes were either from Nacalai Tesque, Tokyo Chemical Industry (Tokyo, Japan), Wako Pure Chemical Industries (Osaka, Japan) or Aldrich (Milwaukee, WI, USA; http://www.sigma-aldrich.com/). Chromatographic data for the selected test solutes are presented in Table 1 (other data will be available on request). Injection amount was kept as low as possible in order to provide infinite dilution-like conditions, i.e. ca. 0.5 μg was injected in the case of aromatic compounds and ca. 5 μg in the case of aliphatic compounds.

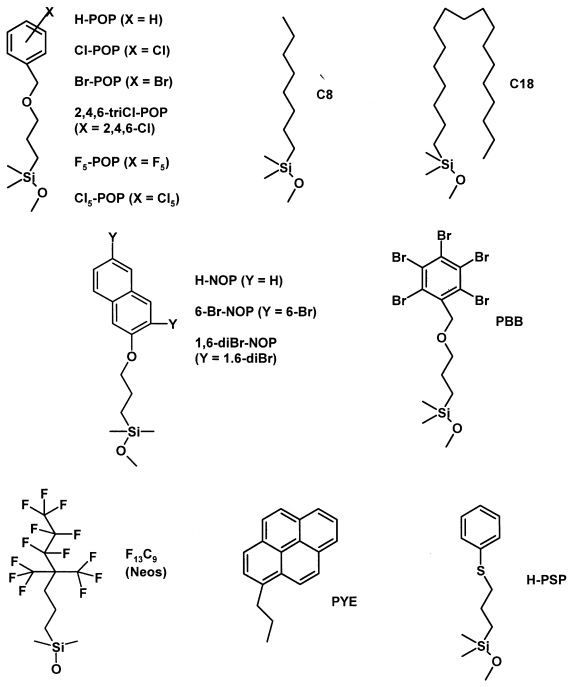

Fig. 1. Structures of the stationary phases studied.

The following 34 compounds were used for QSRR column characterization: benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, fluorobenzene, chlorobenzene, bromobenzene, nitrobenzene, acetophenone, ethyl phenyl ketone, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,2-dibromobenzene, 1,3-dibromobenzene, 1,4-dibromobenzene, *o*-xylene, *m*-xylene, *p*-xylene, 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene, methyl benzoate, ethyl benzoate, propyl benzoate, butyl benzoate, phenol, 4-fluorophenol, 4-chlorophenol, 4-bromophenol, 4-iodophenol, 4-methylphenol and 4-methoxyphenol.

Chemometric multiple regression analysis calculations were performed with Essential Regression program, freely available for scientific community [19], working together with MS-Excel™ (http://www.microsoft.com/). Molecular modelling and structural properties were calculated with HyperChem™ 5.11 and ChemPlus™ 1.6 package (Hypercube, Gainesville, FL, USA; http://www.hyper.com/) on PentiumII™ class Windows NT™ workstation. In order to obtain structural electronic descriptors, the structures of solutes were primarily optimized in vacuum by classical approach (MM+ force field) followed by semi-empirical quantum approach (Restricted Hartree–Fock PM3 method). Geometry optimization procedures employed Polak-Ribiere algorithm. QSAR descriptors were computed by ChemPlus™ package default procedures. Molecular visualization was done using WebLab Viewer™ (Molecular Simulations; http://www.msi.com/). The results of molecular modelling for test solutes are available on request.

3. Results and discussion

3.1. Comparative analysis of chromatographic data

3.1.1. Comparison of C_{18}, $F_{13}C_9$ and PBB phases

The chromatograms demonstrating elution order of substituted benzenes in mobile phase of MeOH/

Table 1
Retention factors for the test compounds upon all stationary phases. Mobile phase: MeOH/water 70:30

Samples	CAS number	log P^b	C_8	C_{18}	$F_{13}C_9$	H-NOP	6-Br-NOP	1,6-diBr-NOP	H-POP	F_5POP	Cl-POP	2,4,6-triCl-POP	Cl_5POP	Br-POP	H-PSP	PBB	PYE
Benzene	71-43-2	2.13	1.14	1.68	0.42	0.50	0.53	0.59	0.48	0.74	0.52	0.61	0.86	0.60	0.58	1.22	0.88
Toluene	108-88-3	2.73	1.83	2.94	0.56	0.76	0.85	0.96	0.69	1.16	0.79	0.97	1.41	0.93	0.81	2.16	1.45
Ethylbenzene	100-41-4	3.15	2.79	4.63	0.72	1.10	1.23	1.40	0.95	1.52	1.12	1.36	1.94	1.33	1.11	3.04	2.21
Propylbenzene	103-65-1	3.69	4.43	7.83	0.96	1.61	1.85	2.16	1.33	2.09	1.63	2.00	2.90	1.96	1.51	4.66	3.42
Butylbenzene	104-51-8	4.38	7.04	13.34	1.28	2.39	2.83	3.37	1.87	2.97	2.39	3.03	4.43	2.91	2.08	7.51	5.49
Amylbenzene	538-68-1	4.90	11.1	22.6	1.69	3.49	4.29	5.21	2.60	4.11	3.44	4.53	6.68	4.27	2.85	11.9	8.59
Hexylbenzene	1077-16-3	5.52	17.5	38.5	2.23	5.05	6.48	8.08	3.60	5.72	4.94	6.86	10.2	6.24	3.87	19.2	13.6
Naphthalene	91-20-3	3.30	2.39	4.20	0.46	1.46	1.74	2.12	1.15	1.89	1.36	1.59	2.81	1.65	1.34	6.61	3.06
Fluorobenzene	462-06-6	2.27	1.12	1.54	0.50	0.49	0.53	0.58	0.48	0.80	0.52	0.63	0.87	0.59	0.57	1.16	0.91
Chlorobenzene	108-90-7	2.84	1.75	2.68	0.52	0.81	0.90	1.02	0.73	1.11	0.83	0.98	1.42	0.97	0.86	2.29	1.64
Bromobenzene	108-86-1	2.99	1.98	3.14	0.49	0.99	1.11	1.27	0.85	1.20	0.98	1.15	1.66	1.18	1.01	2.99	2.09
Iodobenzene	591-50-4	3.25	2.42	4.09	0.44	1.35	1.42	1.70	1.09	1.38	1.22	1.39	2.02	1.56	1.25	4.38	3.00
1,2-Difluorobenzene	367-11-3	2.37	1.2	1.53	0.64	0.53	0.56	0.63	0.50	0.94	0.58	0.67	0.94	0.67	0.59	1.16	1.03
1,3-Difluorobenzene	372-18-9	2.21	1.26	1.68	0.66	0.51	0.55	0.61	0.50	0.92	0.56	0.68	0.95	0.61	0.57	1.16	0.95
1,4-Difluorobenzene	540-36-3	2.13	1.10	1.43	0.57	0.52	0.52	0.58	0.49	0.81	0.52	0.63	0.86	0.57	0.54	1.08	0.98
1,2-Dichlorobenzene	95-50-1	3.43	2.60	4.11	0.64	1.28	1.51	1.74	1.04	1.67	1.31	1.51	2.30	1.55	1.22	4.19	3.07
1,3-Dichlorobenzene	541-73-1	3.53	3.15	5.39	0.74	1.44	1.68	1.96	1.15	1.75	1.43	1.77	2.58	1.70	1.32	4.63	3.51
1,4-Dichlorobenzene	106-46-7	3.44	2.72	4.61	0.65	1.50	1.63	1.90	1.13	1.52	1.31	1.61	2.35	1.58	1.25	4.44	3.65
1,2-Dibromobenzene	583-53-9	3.64	3.09	5.27	0.52	1.79	2.14	2.51	1.34	1.81	1.68	1.92	2.95	2.12	1.57	6.77	4.62
1,3-Dibromobenzene	108-36-1	3.75	4.01	7.45	0.64	2.10	2.41	2.92	1.54	2.00	1.94	2.36	3.42	2.46	1.77	7.77	5.66
1,4-Dibromobenzene	106-37-6	3.79	3.48	6.48	0.58	2.25	2.51	2.97	1.52	1.76	1.81	2.18	3.21	2.32	1.66	7.67	6.10
o-Xylene	95-47-6	3.12	2.69	4.62	0.69	1.11	1.31	1.55	0.95	1.75	1.17	1.45	2.28	1.37	1.11	3.9	2.34
m-Xylene	108-38-3	3.20	2.91	5.08	0.74	1.14	1.34	1.60	0.97	1.86	1.19	1.52	2.32	1.40	1.12	3.68	2.39
p-Xylene	106-42-3	3.15	2.90	5.12	0.73	1.11	1.34	1.63	0.95	1.85	1.16	1.54	2.44	1.37	1.11	3.86	2.29
Trifluoromethylbenzene	98-08-8	3.01	–	2.59	1.04	–	–	–	–	1.43	–	–	–	–	–	1.81	1.37
1,3-Bis(trifluoromethyl)benzene	402-31-3	3.83	–	4.83	3.24	–	–	–	–	3.13	–	–	–	–	–	2.01	2.06
1,4-Bis(trifluoromethyl)benzene	433-19-2	3.83	–	4.62	2.69	–	–	–	–	2.78	–	–	–	–	–	2.07	2.99
Anisole	100-66-3	2.11	1.04	1.46	0.36	0.66	0.68	0.78	0.56	0.86	0.63	0.72	1.05	0.75	0.65	1.66	1.30
Thioanisole	100-68-5	2.74	1.70	2.61	0.41	1.18	1.23	1.43	0.95	1.22	1.07	1.13	1.67	1.33	1.06	3.49	2.49
Pentane	109-66-0	3.39	4.21	8.35	1.49	0.88	0.97	1.20	0.85	1.57	0.91	1.40	2.00	1.20	0.99	2.30	1.79
Hexane	110-54-3	3.90	6.65	15.1	1.97	1.35	1.52	1.90	1.21	2.28	1.31	2.17	3.08	1.81	1.39	3.70	2.89
Heptane	142-82-5	4.66	10.7	25.4	2.58	2.01	2.35	3.02	1.70	3.29	1.91	3.26	4.72	2.70	1.93	5.88	4.59
1,3-Dichloropropane	142-28-9	2.00	0.85	1.23	0.32	0.57	0.54	0.59	0.51	0.63	0.47	0.58	0.84	0.69	0.65	1.14	1.18
1,3-Dibromopropane	109-64-8	2.37	1.22	1.90	0.32	0.93	0.89	0.98	0.77	0.79	0.71	0.86	1.24	1.10	0.96	2.12	2.02
1,3-Diiodopropane	627-31-6	3.02	2.25	4.03	0.34	2.03	1.98	2.19	1.42	1.19	1.36	1.58	2.33	2.28	1.78	5.88	4.95
1-Fluoropentane	592-50-7	2.33	1.53	2.47	0.79	0.64	0.65	0.75	0.58	0.97	0.58	0.79	1.08	0.82	0.71	1.20	1.36
1-Chloropentane	543-59-9	–	2.59	4.62	0.89	1.20	1.23	1.38	0.98	1.44	1.02	1.33	1.86	1.47	1.15	2.46	2.69
1-bromopentane	110-53-2	3.37	3.10	5.79	0.89	1.52	1.56	1.77	1.17	1.63	1.25	1.60	2.26	1.83	1.40	3.36	3.53
1-Iodopentane	628-17-1	–	4.34	8.80	0.93	2.17	2.30	2.64	1.59	2.03	1.69	2.19	3.14	2.62	1.87	5.60	5.35
Triphenylamine[a]	603-34-9	5.74	–	7.87	0.29	–	–	–	–	–	–	–	–	–	–	10.2	7.30
Triphenylphosphine[a]	603-35-0	5.69	–	6.36	0.24	–	–	–	–	–	–	–	–	–	–	11.7	6.64
Triphenylarsine[a]	603-32-7	–	–	8.86	0.26	–	–	–	–	–	–	–	–	–	–	15.4	8.17
Triphenylantimony[a]	603-36-1	–	–	11.2	0.26	–	–	–	–	–	–	–	–	–	–	22.7	11.2
Triphenylbismuth[a]	603-33-8	–	–	10.1	0.22	–	–	–	–	–	–	–	–	–	–	24.8	13.9
Triphenylmethane[a]	519-73-3	–	–	6.42	0.27	–	–	–	–	–	–	–	–	–	–	7.90	6.35

[a] Mobile phase: MeOH/water 80:20.

[b] Experimental Log P (logarithms of *n*-octanol/water partition coefficient) values (mainly after Hansch and Leo, 1995) were obtained from LogKow database [for details see W.M. Meylan and P.H. Howard, J. Pharm. Sci., 84 (1995) 83]. Trial version is freely available on-line at http://esc_plaza.syrres.com/interkow/logkow.htm.

water 60:40 upon three selected, commercialy available stationary phases are presented in Fig. 2. Elution orders generally do not differ from 70:30 mobile phase (as listed in Table 1). It has to be emphasized that absolute retention values cannot serve to compare the columns, because of the different phase ratios (ϕ). Therefore, we mainly deal in this section with the relative comparison of stationary phases with respect to their differentiation of structural units of compounds from the same chemical groups (arene and alkane derivatives). In other words, the differences between elution orders are discussed.

Fig. 2a shows the comparison among the molecules Ph–X–CH_3 (where X=CH_2, O, S) and toluene. PBB differentiated –O– and –S– very clearly while $F_{13}C_9$ did not. In addition, PBB showed the greatest preference toward –S–, while C_{18} and $F_{13}C_9$ showed the preference for alkyl groups. We emphasize that a sulphur atom is highly polarizable. Calculated partial molecular polarizability of S in thioanisole molecule is 3.00 $Å^3$, of O in anisole it is only 0.64 $Å^3$, for aliphatic CH_2 group it is 1.79 $Å^3$. However, calculated partial vdW surface of thioanisole sulphur (21.17 $Å^2$) is almost equal to CH_2 group (21.12 $Å^2$), i.e. size of both units is similar resulting in similar hydrophobic cavity size. Thus, the preference shown by the highly dispersive PBB phase toward thioanisole can be explained in terms of London forces. It is in agreement with the previously published observations, where PBB demonstrated highest retentive properties for C_{60} fullerene (as retention of this compound is a good indication of dispersive potential) [14].

The chromatograms in Fig. 2b are for monohalogenated benzenes. On all stationary phases, except $F_{13}C_9$, they were eluted in the following order: –F<–Cl<–Br<–I. Such selectivity may be expected in the reversed-phase systems, especially for C_{18} phase, based on the results in *n*-octanol/water partition process (log *P*). Here again, highly dispersive PBB phase showed the preference (α values) according to increasing polarizability of halogen substituents (–F<–Cl<–Br<–I). However, the exception was $F_{13}C_9$ phase, where we observed the elution order: –I<–Br<–F<–Cl. The $F_{13}C_9$ phase showed tendency opposite from the other phases, i.e. the larger and more polarizable halogen substituents are (–Cl<–Br<–I), the lower overall retention. In addition, attractive interactions between fluorinated alkane phase and fluorobenzene were observed. Because one can understand RPLC retention as being divided into two parts-mobile phase effect and stationary phase effect, and because the mobile phase effect is common to all the systems, one can think of this reversed elution order for monohalogenated benzenes in terms of the differences in stationary phase effect. Some factor must be responsible for very small preference of fluoroalkane phase towards the –I and –Br atoms. In fact, plot of the retention factors against refractive index of monosubstituted benzenes in Fig. 3 shows a slightly negative trend in selectivity for fluoroalkane phase. One should take into account that in this particular chromatographic system, the 70% MeOH/water mobile phase has even higher dispersive properties than the stationary phase (''attractive'' mobile phase effect visible), which may explain the negativity of the slope for halogen substituents. The interaction between –F and –I compounds is not energetically favourable. Actually, much smaller hydrophobic selectivity [$\alpha(CH_2)$] can be observed on $F_{13}C_9$ phase than on the other phases.

The chromatograms shown in Fig. 2c demonstrate separation of the mixture of mono and di-substituted benzenes. PBB phase showed the greatest preference for xylene, followed by bis(trifluoromethyl)benzene, toluene and trifluoromethylbenzene. The same elution order appeared for *meta*- and *para*-disubstituted benzenes and monosubstituted benzenes. The retention of bis(trifluoromethyl)benzene (molecule larger than xylene) is similar to that for much smaller toluene on the same column. Fluorine atoms have smaller polarizability than hydrogens, thus the selectivity of PBB should be interpreted in terms of attractive London interactions.

The elution order on $F_{13}C_9$ indicates much higher selectivity of this phase for the fluorinated species: bis(trifluoromethyl)benzene and trifluoromethyl-benzene are preferred than xylene and toluene. C_{18} phase shows the greater preference for fluorinated benzenes, but to much smaller extent than fluoroalkane phase.

General elution order of *ortho*, *meta* and *para* difluoro, -dichloro, -dibromobenzenes and xylenes varies systematically with the change of substituents (–diF<–diCH_3<–diCl<–diBr) indicating similar

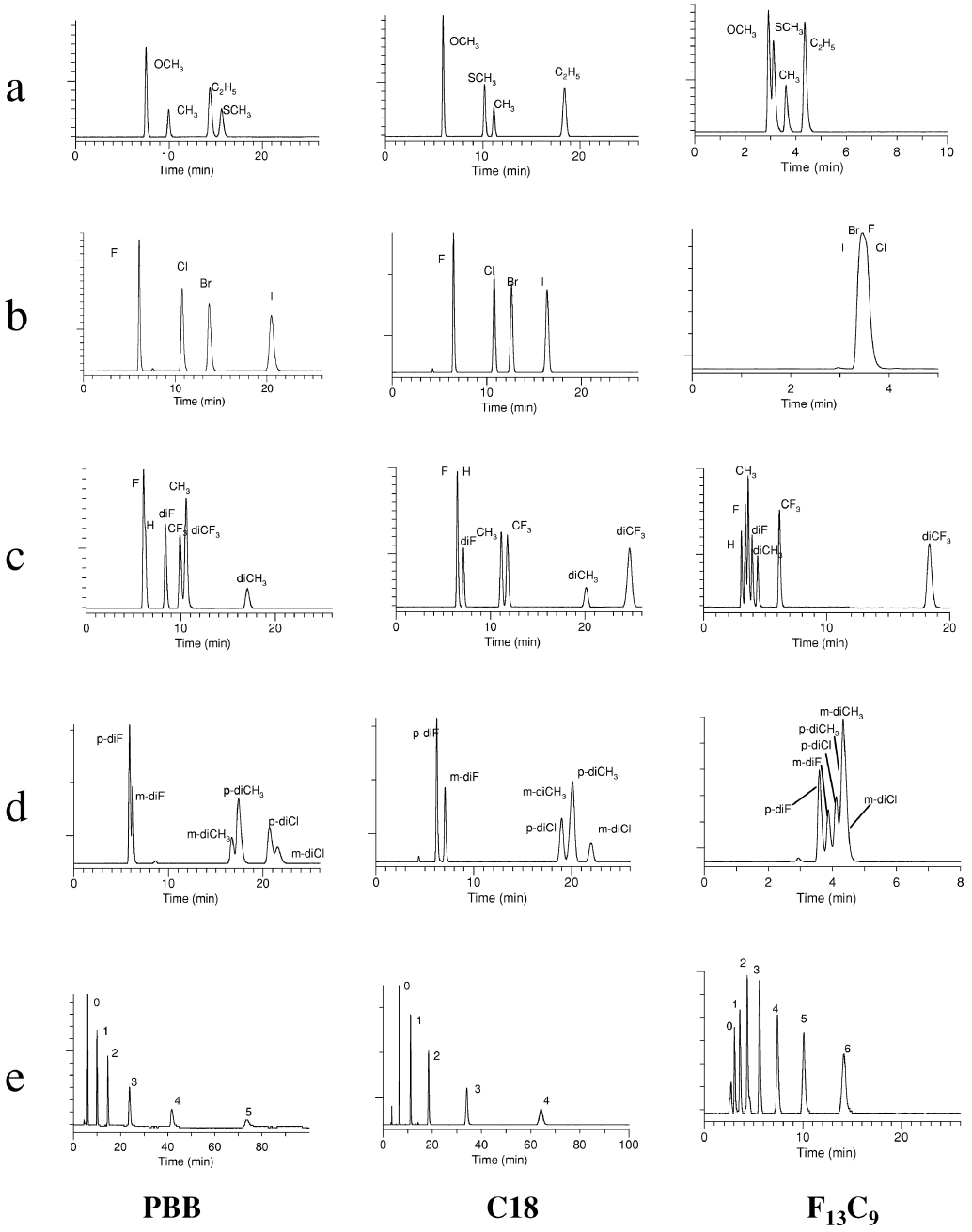

Fig. 2. Chromatograms collected on PBB, C_{18} and $F_{13}C_9$ stationary phases in 60:40 MeOH/water mobile phase for (a) anisole ($-OCH_3$), thioanisole ($-SCH_3$), toluene ($-CH_3$) and ethylbenzene ($-C_2H_5$); (b) fluorobenzene (–F), chlorobenzene (–Cl), bromobenzene (–Br) and iodobenzene (–I); (c) benzene (–H), fluorobenzene (–F), 1,3–difluorobenzene (–diF), toluene ($-CH_3$), m-xylene ($-diCH_3$), trifluoromethylbenzene ($-CF_3$) and 1,3-bis(trifluoromethyl)benzene ($-diCF_3$); (d) 1,3-difluorobenzene (m-diF), 1,4-difluorobenzene (p-diF), *m*-xylene (m-$diCH_3$), *p*-xylene (p-$diCH_3$), 1,3-dichlorobenzene (m-diCl) and 1,4-dichlorobenzene (p-diCl); e) alkylbenzenes (numbers stand for number of alkyl carbons).

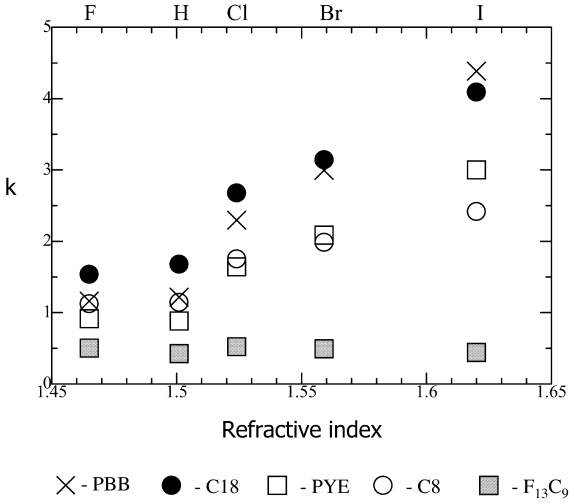

Fig. 3. Plot of refractive indices (n_D^{20}) of monosubstituted benzenes against their retention factors on five selected stationary phases in 70% MeOH/water mobile phase. The values of n_D^{20} (taken from Aldrich® catalogue) were 1.465 for fluorobenzene (F), 1.501 for benzene (H), 1.524 for chlorobenzene (Cl), 1.559 for bromobenzene (Br) and 1.620 for iodobenzene (I).

tendencies as in the case of monosubstituted benzenes, but it is not systematic within these groups (see Fig. 2d). Although we cannot explain the selecitivty mechanism here, we want to mention that there are considerably different dipole moments between all *ortho*, *meta* and *para* substituted benzenes studied, thus one may think of contribution of dipolar interactions to selectivity in each case. The shape recognition effects may also be important [20,21].

In all the chromatograms, obtained in the same mobile phase, we see clear trend that more polarizable solutes are preferred by PBB, and to the smaller extent by C_{18} phase. $F_{13}C_9$ stationary phase actually showed the opposite tendency. These three stationary phases, along with several others, can offer a selectivity spectrum for compounds with different polarizability. If we see overlapping peaks on C_{18} phase for compounds with different polarizability, we may expect separation in opposite elution order on PBB and $F_{13}C_9$ phase.

3.1.2. Analysis of selectivities (α)

There are systematic structural differences within the groups of derivatives (e.g. alkylbenzenes). In such case phase ratio ϕ cancels out according to the equations:

$$\alpha = \frac{k_2}{k_1}, \; -RT \ln \alpha = \Delta G_2^0 - \Delta G_1^0 = \Delta\Delta G_\alpha^0 \qquad (2)$$

where k_1 and k_2 denote retention factors of two subsequently separated compounds and $\Delta\Delta G_\alpha^0$ is the free energy difference associated with the transfer of these two compounds from a mobile to a stationary phase. When α is calculated between two subsequent homologous species one obtains the free energy of transfer of methylene unit CH_2. Other structural units can be investigated, e.g. C_4H_2 unit ($k_{naphthalene}/k_{benzene}$).

Plot of α values for aromatic unit, C_4H_2, which is highly polarizable, vs. α values for aliphatic CH_2 unit, which has lower polarizability, in 70% MeOH/water is presented in Fig. 4a. One can observe different trends for aromatic and aliphatic phases. Free energy of transfer of aliphatic CH_2 unit from the mobile to the stationary phase mainly depends on the mobile phase effect as it was found to be strongly dependent on the mobile phase composition (%B) [22], however, there must be a small contribution of the stationary phase effect (dispersion interaction), too. Free energy of transfer of CH_2 is higher than C_4H_2 on $F_{13}C_9$ phase indicating the weakest involvement of dispersion forces on this phase selectivity. Fluorinated hydrocarbons exhibit extremely low dispersive potential, as reflected by the lowest refractive indices among the organic compounds [23].

Temperature effect on selectivities was measured in our previous work [24], where thermodynamic parameters were calculated for CH_2 and C_4H_2 structural units. Fig. 4b demonstrates $\Delta\Delta G^0$, $\Delta\Delta H^0$ and $T*\Delta\Delta S^0$ values on four selected stationary phases. One can notice the exothermic character of the retention process in all cases studied. Thus, the enthalpy change ($\Delta\Delta H^0$) is the driving factor for the association with the stationary phase and, finally, the free energy change of binding. Upon $F_{13}C_9$ phase, however, $\Delta\Delta H^0$ is exceptionally smaller for C_4H_2 than for CH_2, again confirming very weak involvement of dispersion interaction on this phase selectivity.

Fig. 5 present logarithms of retention factors of

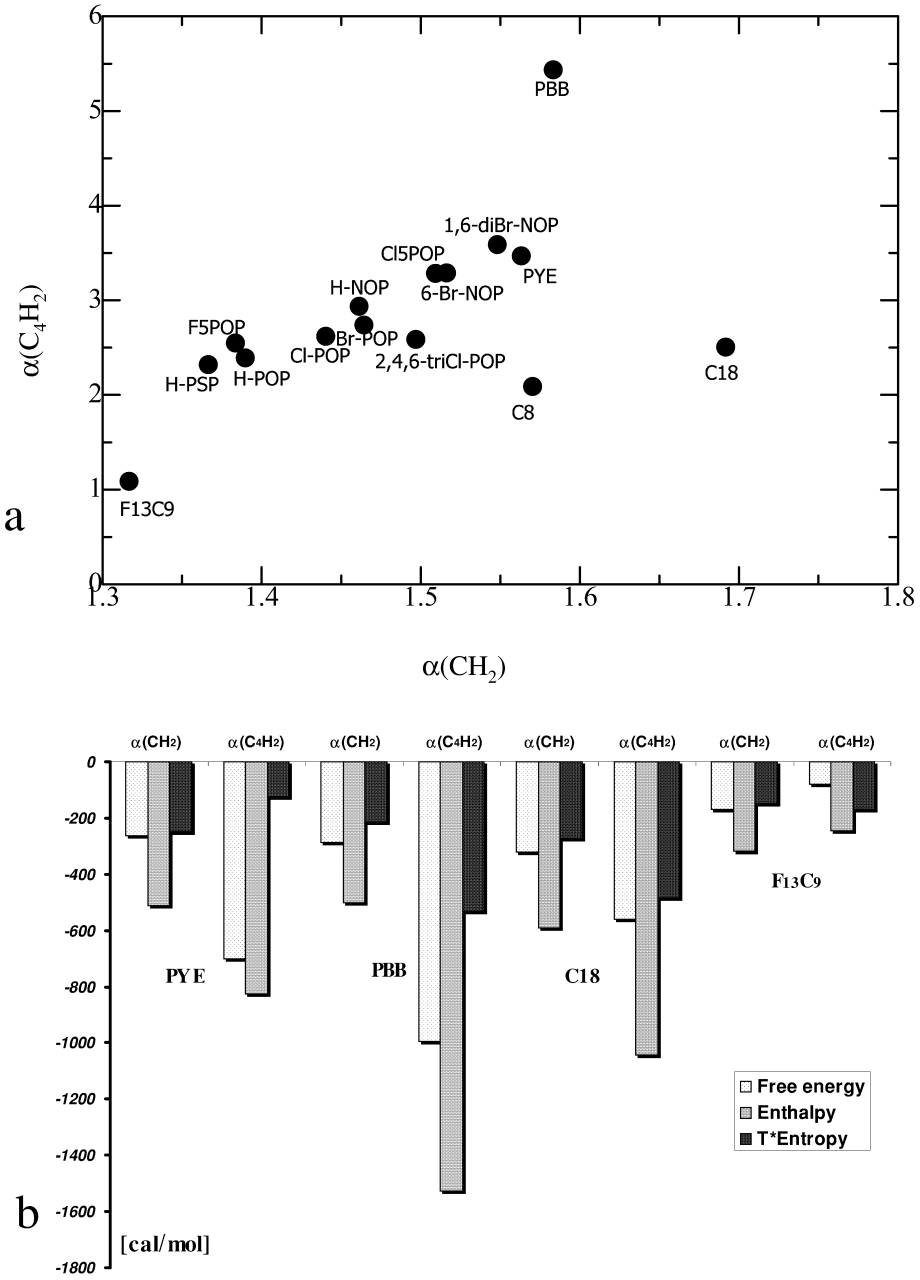

Fig. 4. (a) Comparison of selectivities of 10 selected stationary phases plotted for aromatic C_4H_2 ($k_{naphthalene}/k_{benzene}$) and aliphatic CH_2 ($k_{amylbenzene}/k_{butylbenzene}$) units. (b) Graph of the Gibbs free energy and its components for the aliphatic and aromatic retention unit on four stationary phases in 70% MeOH/water mobile phase at 30°C. Data are taken from the reference [24].

the selected alkylbenzenes and halogenated benzenes upon five stationary phases, plotted against each other or vs. log *P* values. The analysis of trends (i.e. elution order differences) for the congeners is conducted. One can roughly predict retention for disubstituted halogenated benzenes looking at the position

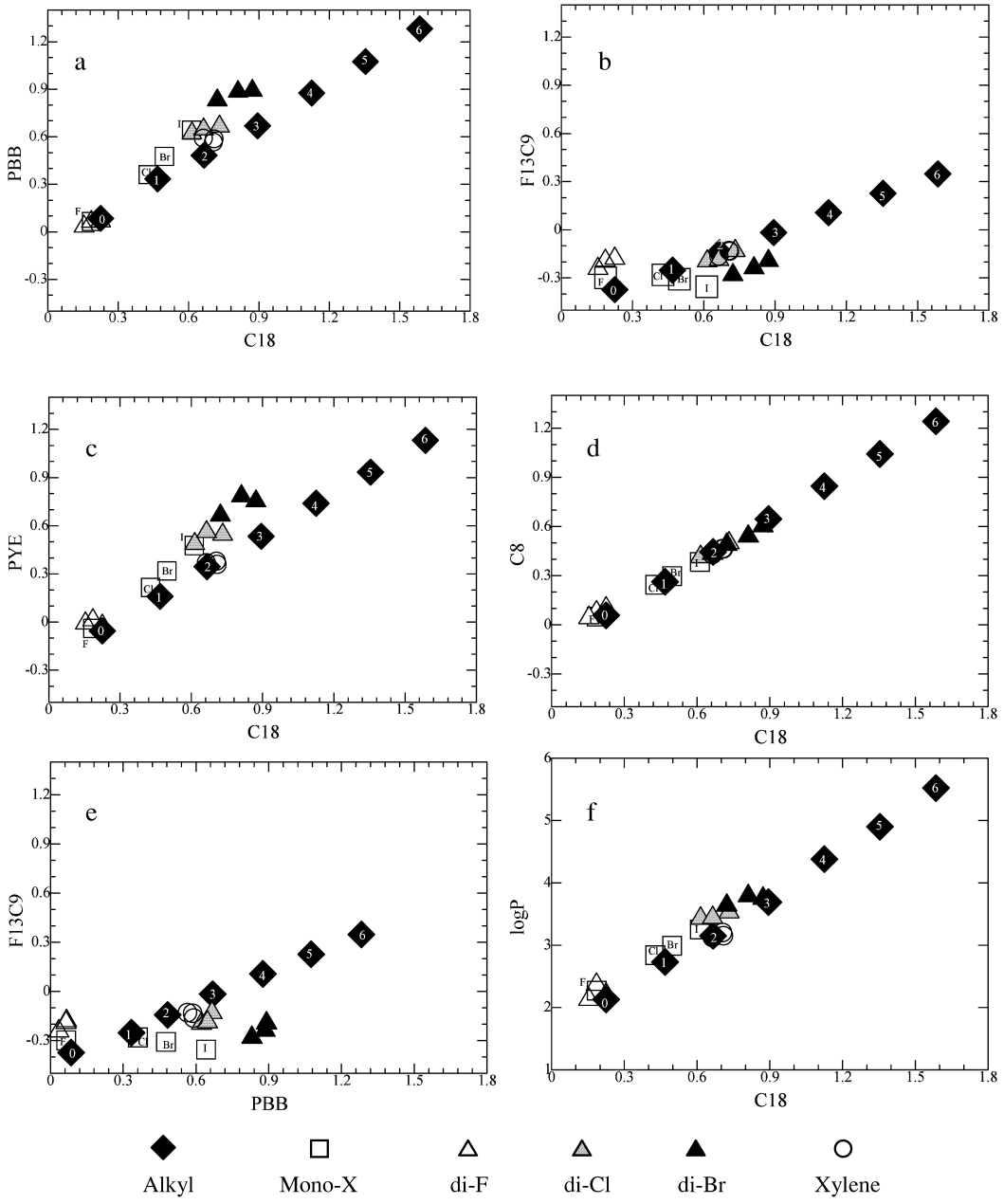

Fig. 5. (a) Mutual plots of logarithms of the retention factors for selected stationary phases and logarithm of *n*-octanol/water partition coefficient, log *P*, for alkylbenzenes (Alkyl; number stands for number of alkyl carbons), monohalogenated benzenes (Mono-X), dihalogenated benzenes (di-F, di-Cl and di-Cl) and xylenes.

of the monohalogenated derivatives. In all plots from Fig. 5 the trends for the aliphatic moieties (alkylbenzenes and xylenes) confirm typical behaviour of RP systems, i.e. there is a linear increase with the number of carbons in aliphatic chain [25]. Smaller slope than for all other phases is observed for $F_{13}C_9$ (Fig. 5b), although the relationship is positively proportional indicating presence of hydrophobic

properties of the fluoroalkane chains. The slopes for mono and dihalogenated benzenes are similar to the alkylbenzenes in Fig. 5d (C_8 vs. C_{18}), suggesting that the preference towards these two groups of compounds is caused by the similar mechanism on both phases. Fig. 5f relates the trends on C_{18} phase to *n*-octanol/water partition coefficients, indicating a similarity of these two systems. Trends of halogenated benzenes for PYE and PBB vs. C_{18} are different, however. Considerably greater slopes appearing in PBB and PYE phases (as compared to C_{18}) for halogenated benzenes indicate much greater involvement of the other interaction type in the retention of these compounds than it is apparent in the case of alkylbenzenes. Except fluorine, Cl, Br and I bound to organic carbon possess very high partial molecular polarizabilities (calculated values are 2.32 $Å^3$, 3.01 $Å^3$ and 5.41 $Å^3$, respectively, while for F it is 0.30 $Å^3$ and for H 0.36 $Å^3$). Especially, when thinking in terms of polarizability with respect to the size, e.g. described by vdW surface, the polarizabilities of halogen atoms appear to be considerably higher than the respective alkyl groups. Calculated partial vdW surfaces for –H, –F, –Cl, –Br and I are, respectively, 6.93 $Å^2$, 11.94 $Å^2$, 28.78 $Å^2$, 36.67 $Å^2$ and 43.74 $Å^2$. Calculated partial atomic polarizability and vdW surface for aliphatic CH_3 group of toluene are 2.15 $Å^2$ and 33.07 $Å^2$, respectively. Therefore, we conclude that the selectivity of Cl, Br and I substituted benzenes involves attractive London forces to a greater extent than in the case of alkyl groups, especially on aromatic stationary phases. The ratio of polarizability to vdW surface may help understanding two effects: attractive stationary phase interactions based on polarizability and repulsive mobile phase interaction based on cavity size.

It should be mentioned that between aromatic stationary phases and substituted benzenes one can also take into account possible charge-transfer interactions, which influence separation factors as well. However, stationary phases having various electron-withdrawing and electron-donating groups, including PYE and PBB, demonstrate similar tendencies for the selectivity of aromatic species, thus we assume that these stationary phases are showing highly dispersive properties.

The slopes for all halogenated benzenes are slightly negative on $F_{13}C_9$ phase when plotted against C_{18} or PBB phase (Figs. 5b and 5e, respectively). The trend is negative with increasing polarizability of substitutents. It confirms unfavourable interactions between non-polarizable stationary phase and compounds with high polarizability.

The tendencies for monosubstitued aliphatic solutes are presented in Fig. 6. Free energies of transfer of given structural units (calculated with respect to pentane according to Eq. (2)) are plotted for seven selected stationary phases vs. C_{18} phase. Only the aliphatic C_8 phase demonstrates similar tendency for all substitutents, indicating similar selectivity mechanism as C_{18}. Other phases of dispersive aromatic character and $F_{13}C_9$ phase with very low polarizability show different tendencies.

When analyzed separately from CH_2, all halogen substituents demonstrate linear tendency to increase the free energy of transfer with their increasing size and polarizability upon all phases, except $F_{13}C_9$. The steepest slope is observed for highly dispersive PBB phase indicating the highest involvment of attractive stationary phase effect. $F_{13}C_9$ phase demonstrates very flat trend for halogen atoms, similar to the case of halogenated benzenes confirming the weakest stationary phase effect among all phases studied.

A very condensed cluster for CH_2 unit is observed indicating that the free energy of transfer does not differ significantly between the stationary phases, although they demonstrate very distinctive properties for the other solutes. Except for C_8 phase, similar clustering is observed for weakly dispersive –F unit. On the other hand, a broad scattering is noticed for highly polarizable –Cl, –Br and, most intensively, –I units. In this case different free energies of transfer are observed for the same free energy of transfer on C_{18} phase — highest for PYE, H-NOP and PBB, lowest for weakly dispersive aliphatic $F_{13}C_9$ and C_8 phases. It indicates that the selectivity mechanism clearly differentiates between weakly polarizable aliphatic and highly polarizable aromatic stationary phases.

Fig. 7 shows selectivity comparison for four stationary phases with respect to the five group V elements (pnictogens) having triphenyl groups. Selectivity factor, α, was calculated as the ratio of retention factors to that of triphenylmethane, thus the influence of structural fragment (respective pnictogen

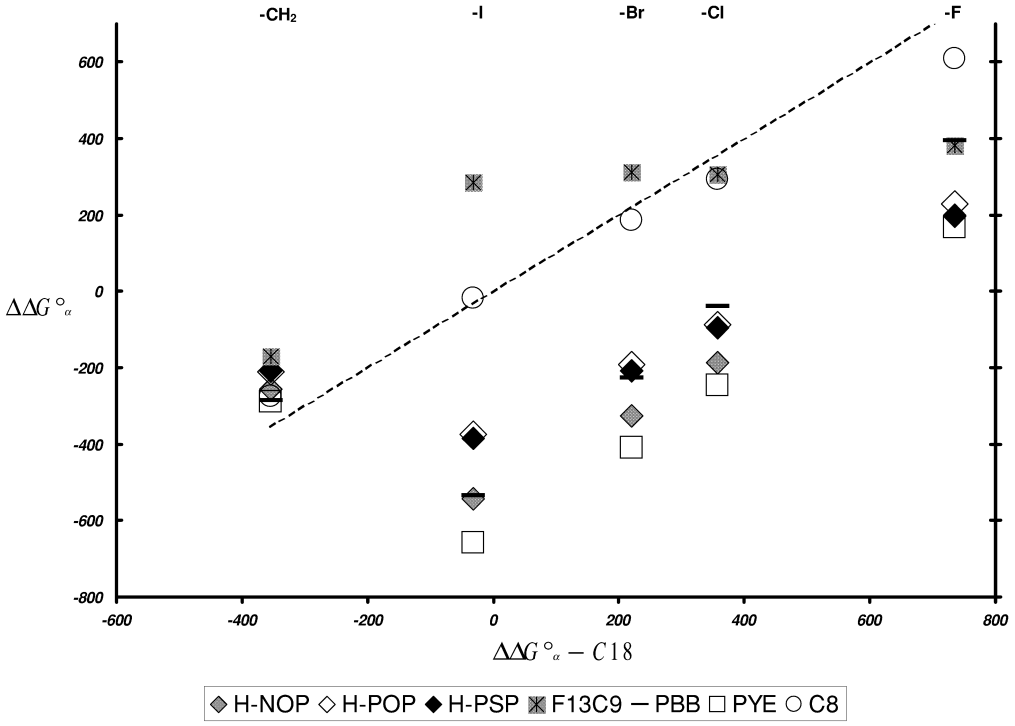

Fig. 6. Plot of free energies of transfer, $\Delta\Delta G^0_\alpha$, for structural units of substituted pentanes and hexane (all calculated with respect to pentane) on C_{18} phase (*X* axis) against the $\Delta\Delta G^0_\alpha$ values for these units on seven other stationary phases (*Y* axis). Dashed line, representing C_{18} phase itself, is added for comparison.

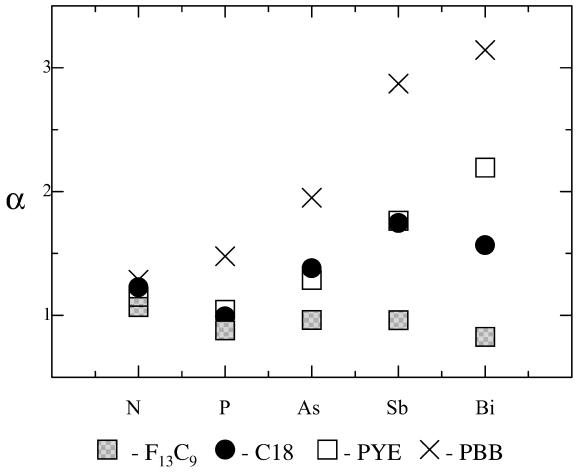

Fig. 7. Plot of selectivities of pnictogen-triphenyl derivatives (calculated with respect to triphenylmethane) for the four stationary phases analyzed.

atom) on selectivity of the stationary phases is demonstrated. One can note that with increasing the size of pnictogen atom (N<P<As<Sb<Bi) the selectivity of PBB phase increases significantly. Similar tendency, although to the smaller extent, is noticed for PYE and C_{18} phases. The behaviour of triphenylamine may be explained in terms of strong hydrogen bond basicity of nitrogen atom resulting in interactions with remaining silanols.

We attempt to explain the monotonous trends on PBB and PYE for triphenyl derivatives (excluding basic N) in terms of increasing molecular volume of pnictogen atoms, accompanyed by the increase in polarizability. It can be confirmed by the exceptional trend of the non-dispersive $F_{13}C_9$ phase, where no increase in separation factor was observed with the increase of size and polarizability of pnictogen atoms. Interestingly, C_{18} and PYE demonstrate similar selectivity to all pnictogen atoms except Bi,

which selectivity factor is much higher on PYE than on C_{18} phase.

3.2. Chemometric characterization of stationary phases properties

We employed quantitative structure–chromatographic retention relationships [7] (QSRR) analysis in order to characterize physicochemical properties of the stationary phases. QSRR becomes recently a useful tool for both column characterization [26–28] and investigation of the molecular mechanisms of retention via chemometric processing of retention data [29–31].

Retention of solutes is described in terms of their structural solvatochromic parameters (derived empirically, e.g. from the spectroscopic measurements) according to Abraham's formula [32]:

$$\log k = \log k_0 + s\pi^* + a\alpha_H + b\beta_H + rR_2 + mV_x \quad (3)$$

where log k denotes logarithm of retention factor of a test solute, log k_0 is the intercept, π^* is solute's dipolarity/polarizability parameter (reflecting the ability of interacting via both electrostatic and dispersion interactions), α_H is hydrogen bond acidity of solute, β_H is its hydrogen bond basicity, R_2 is solute's molar refraction coefficient (reflecting purely dispersive properties and interaction potential via London forces) and V_x stands for the solute's characteristic McGowan volume (that reflects the net hydrophobic properties and, to a certain extent, dispersive interaction potential as well). The coefficients s, a, b, r and m correspond to the properties of the mobile/stationary phase complex. Since the same mobile phase was used in all cases, the properties of the stationary phase can be extracted. Here, positive sign indicates that the stationary phase exhibits stronger properties than the mobile phase.

The results of Abraham's multiple linear regression equation for the set of 34 test compounds analyzed on 15 columns are presented in Table 2. Only significant independent variables were taken into account (i.e. demonstrating significance level $P \leq 0.05$). All determination coefficients, R^2, were above 0.95.

The values of coefficient r reflect dispersive properties of stationary phases – highest is observed for PBB structure and the lowest negative r value is noticed for fluoroalkane stationary phase ($F_{13}C_9$). These results confirm the properties of these two phases found previously.

Direct interpretation of s coefficient as well as the entire $s\pi^*$ term is somehow ambiguous. It is, thereofre, more convenient to analyze s after combining with r, according to suggestion of Zhao and Carr [14], in order to cancel the influence of dispersive interaction in the s coefficient. In our study only for two columns, C_{18} and PBB, both variables (rR_2 and $s\pi^*$) were significant in one equation. For the PBB phase combination of r and s demonstrate a higher positive influence of dispersive properties over the smaller, negative influence of dipolarity on retention. In this case, however, a high standard error of estimate values should be notified. Aliphatic C_{18} demonstrates fairly higher negative influence of dipolar interactions (of mobile phase origin) on the retention of test compounds.

The values of m coefficient reflect the molecular size of stationary/mobile phase complex, thus mainly describe hydrophobicity of respective species in terms of repulsive cavity effect. The m coefficient has the greatest value upon the aliphatic C_{18}, followed by C_8 phase. The lowest m value is noticed for $F_{13}C_9$ phase. As it was recently pointed out by Reta et al., fluoroalkane phases do not exhibit similar hydrophobic (mobile phase effect related) properties to alkane phases, since the dispersive interactions on fluorocarbon phases are extremely low [33]. On the other hand, retention of homologues (of identical $s\pi^*$, $a\alpha_H$, $b\beta_H$ values) can be described by V_x, indicating a crucial importance of hydrophobic cavity formation in CH_2 retention, also on fluoroalkane phase [33].

High negative values of b signify that hydrogen-bond basicity of solutes works against retention. Direct physicochemical interpretation is that the mobile phase has stronger H-bond acidity than the stationary phase. The observed negative values for b in each stationary phase analyzed are in agreement with the character of MeOH molecules, which are more acidic than basic ($\alpha_H = 0.98$ and $\beta_H = 0.62$ for methanol [34]). Negative a values reflect (weaker) basic properties of methanol. However, it must be emphasized that since we deal mainly with the stationary phase properties arising from QSRR co-

Table 2
Results of QSRR characterization of the stationary phases. Independent variable coefficients are followed by standard error values (*italic*)[a]

Column	log k_0	r	s	a	b	m	R^2
C_8	−0.633	*n.s.*	−0.399	−0.194	−1.560	1.512	0.987
	0.056		*0.040*	*0.036*	*0.058*	*0.053*	
C_{18}	−0.528	0.243	−0.703	−0.301	−1.707	1.661	0.990
	0.061	*0.085*	*0.080*	*0.040*	*0.097*	*0.066*	
$F_{13}C_9$	−0.670	−0.410	*n.s.*	−0.449	−0.960	0.925	0.969
	0.063	*0.049*		*0.042*	*0.069*	*0.065*	
H-NOP	−1.195	*n.s.*	0.261	−0.651	−1.162	1.254	0.979
	0.063		*0.045*	*0.040*	*0.065*	*0.060*	
6-Br-NOP	−1.210	*n.s.*	0.280	−0.562	−1.271	1.327	0.969
	0.074		*0.053*	*0.048*	*0.077*	*0.071*	
1,6-diBr-NOP	−1.190	*n.s.*	0.232	−0.590	−1.303	1.408	0.975
	0.071		*0.051*	*0.046*	*0.074*	*0.068*	
H-POP	−1.023	0.079	*n.s.*	−0.445	−1.034	1.094	0.984
	0.045	*0.035*		*0.030*	*0.049*	*0.047*	
F_5POP	−0.714	−0.116	*n.s.*	−0.320	−1.055	1.131	0.954
	0.072	*0.056*		*0.048*	*0.079*	*0.075*	
Cl-POP	−1.102	*n.s.*	0.187	−0.470	−1.193	1.211	0.965
	0.072		*0.051*	*0.046*	*0.074*	*0.069*	
2,4,6-triCl-POP	−0.996	*n.s.*	*n.s.*	−0.424	−1.215	1.310	0.983
	0.048			*0.030*	*0.052*	*0.050*	
Cl_5-POP	−0.874	*n.s.*	*n.s.*	−0.383	−1.232	1.362	0.975
	0.058			*0.036*	*0.063*	*0.061*	
Br-POP	−1.101	*n.s.*	0.227	−0.553	−1.208	1.264	0.970
	0.072		*0.051*	*0.046*	*0.074*	*0.068*	
H-PSP	−0.919	0.099	n.s.	−0.340	−0.953	1.029	0.980
	0.044	*0.034*		*0.029*	*0.048*	*0.046*	
PBB	−0.872	0.595	−0.447	−0.419	−1.051	1.416	0.965
	0.092	*0.127*	*0.120*	*0.060*	*0.145*	*0.099*	
PYE	−1.171	*n.s.*	0.566	−0.933	−1.312	1.394	0.965
	0.097		*0.069*	*0.062*	*0.100*	*0.093*	

[a] R^2, determination coefficient (squared multiple linear regression coefficient). *n.s.*-not significant, significance level $P>0.05$ (independent variable rejected).

efficients, the bulk mobile phase influence is only reflected by negative or positive sign at *a* and *b*.

4. Conclusions

Comparative analysis of retention data confirms influence of the attractive stationary phase effect (van der Waals forces) and repulsive mobile phase effect (hydrophobic cavity) on the selectivity. Relative contribution of these two processes vary with respect to stationary phase structural properties. CH_2 selectivity, dominated by the mobile phase effect, is common to all cases but its magnitude varies upon transfer to different stationary phases. The differences arise from different stationary phase structure, including solvation with mobile phase organic modifier. Selectivity of aliphatic species on the weakly dispersive $F_{13}C_9$ phase proves the considerable role of the solvophobic mobile phase effect.

Aromatic stationary phases demonstrate higher selectivity for aliphatic species than $F_{13}C_9$ phase. Much higher selectivity is observed with respect to derivatives containing heavy atoms (Cl, Br, I, S, pnictogens) and C_4H_2 unit, especially on highly dispersive PBB and PYE phases. It indicates that the selectivity is mainly provided by the stationary phase effect (attractive interaction) on the aromatic stationary phases. These phases can be chosen for the analysis of compounds having differences in dispersive properties, if C_{18} phase failed to provide separation. The analysis of exceptional $F_{13}C_9$ phase

confirmed its negligible dispersive properties. It may also serve as a valuable tool for the separation of fluorinated organic compounds. By means of chemometric QSRR analysis we confirmed the difference in properties between highly dispersive aromatic phases and weakly dispersive aliphatic phases.

This study will help choosing an appropriate stationary phase for the separation, not possible with C_{18} phase, of compounds having different polarizability.

Acknowledgements

This work was supported by the Japan Society for the Promotion of Science (JSPS) through the postdoctoral fellowship (P97379) and the related Monbusho Grant-in-Aid. A support from Komitet Badan Naukowych (grant 4-PO5F-004-15) is also acknowledged. We would like to thank NEOS Corporation for providing Fluofix™ columns.

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