Quantitative Structure–Retention Relationships for Polychlorinated Biphenyls and Chlorobenzenes on Selected Normal-Phase Liquid Chromatographic Stationary Phases

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Abstract

Linear regression between capacity factors and solute properties is performed to evaluate quantitative structure–retention relationships. A general increase in retention on porous graphitic carbon (PGC) and 2-(1-pyrenyl)ethyldimethyl silica (PYE) stationary phases operated in normal-phase liquid chromatography is shown to correlate with increasing polarizability and decreasing energy of the lowest unoccupied molecular orbital of the solutes. The best prediction of capacity factors is achieved when the retention model is restricted to chlorinated benzenes or non-ortho-substituted chlorobiphenyls (i.e., solutes with a low degree of steric hindrance). The retention of chlorinated benzenes on PGC and PYE is successfully described by only one parameter, the third order valence path molecular connectivity index ($^3\chi_{vp}$). The coefficients of determination between measured and predicted log k' values are 0.994 and 0.992 for PGC and PYE, respectively. A 2,4-dinitroanilinopropyl silica column demonstrated other retention characteristics clearly different from PGC and PYE.

Introduction

High-performance liquid chromatography (HPLC) is frequently applied in the cleanup of samples prior to analysis for halogenated environmental pollutants such as polychlorinated biphenyls (PCBs) and hexachlorobenzene. The aim of HPLC fractionation is to isolate the solutes from other material that can interfere in the final analysis using gas chromatography (GC). HPLC is also used for isolation of selected non-ortho chloro-substituted toxic PCB congeners (i.e., 3,3',4,4'-tetrachlorobiphenyl [CB-77], 3,3',4,4',5-pentachlorobiphenyl [CB-126], and 3,3',4,4',5,5'-hexachlorobiphenyl [CB-169]) from the bulk of PCBs. The most applied HPLC stationary phases for fractionation of these non-ortho PCB congeners are porous graphitic carbon (PGC) and 2-(1-pyrenyl) ethyldimethyl silica

(PYE). The authors have previously used 2,4-dinitroanilino-propyl silica (DNAP) for isolation of CB-77, CB-126, and CB-169 in human blood plasma (1).

However, there is a lack of knowledge regarding the mechanisms that govern the separation. In this work, a quantitative structure–retention relationships (QSRR) approach is used to study the retention characteristics of individual PCB congeners and chlorinated benzenes on PGC, PYE, and DNAP stationary phases. The subject of QSRR has been thoroughly described by Kaliszan (2,3). QSRR can be used for the prediction of retention data, which can be further applied for identification of solutes. QSRR is a tool in studies of retention mechanisms, and it has also been applied for the determination of physical data from retention (e.g., octanol–water partition coefficients from retention in reversed-phase HPLC systems) (4). However, most published QSRR studies apply to GC. Good correlations between predicted and measured retention in GC have been demonstrated for PCBs (5–7). A QSRR approach has been used for identification of individual PCB congeners in sediment samples analyzed by dual-column capillary GC with electron-capture detection (8).

There are also a number of QSRR studies in reversed-phase HPLC. Hasan and Jurs (6) modeled the retention of halogenated biphenyls on an octadecyl silica stationary phase and achieved a good correlation between measured and predicted retention with an equation containing five different parameters. The same chromatographic data were used by Seybold and Bertrand (9), who demonstrated good correlations taking into account only the type and number of halogens in the entire molecule and in ortho- position specifically. A similar approach to quantitatively describe retention on silica and alumina LC columns by use of the substitution pattern of halogenated benzenes and biphenyls has been reported (10). In both GC and LC, it has shown to be more difficult to describe the separation of polar solutes than nonpolar solutes, and it is more difficult with polar stationary phases than with nonpolar stationary phases (3). Hence, quantitatively describing retention in normal-phase HPLC systems through the use of physical and chemical

parameters is a difficult application of QSRR.

In this study, special attention was paid to the correlation between solute retention and parameters that are often regarded as important in chromatography. Van der Waals-type interactions (i.e., dispersive [London], dipole-induced dipole [Debye], and dipole–dipole [Keesom]) have been described in terms of polarizability, ionization potential, and dipole moment (2,11). Electron donor–acceptor interaction has been correlated to the lowest unoccupied molecular orbital (LUMO), highest occupied molecular orbital (HOMO), and ionization potential (12). In this study, regression analysis was performed to find the best description of retention from any parameters selected among the 38 available parameters.

Experimental

Chemicals

All individual chlorobenzenes and chlorobiphenyls were obtained from Ultra Scientific (Kingston, RI) except 2,3,3',4,4',5,5'-pentachlorobiphenyl, which had been synthesized at the Department of Environmental Chemistry at Stockholm University (Stockholm, Sweden) (13). All chlorobiphenyls included in the study are listed in Table I. In addition, all 12 chlorinated benzenes, benzene, and biphenyl were used.

HPLC

The HPLC system consisted of a Waters 590 pump (Milford, MA), a Marathon autosampler (Spark Holland BV, Emmen, The Netherlands) equipped with a 20-µL injection loop and an LKB model 2152 ultraviolet (UV) detector (Stockholm, Sweden) operated at 254 or 225 nm. HPLC-grade hexane (Rathburn, Walkerburn, Scotland) was used as the mobile phase. Three different HPLC stationary phases were used: PYE (150-mm × 4.6-mm column, 5-µm particle size) (Cosmosil, Naclai Tesque, Japan), DNAP (250-mm × 4.6-mm column, 5-µm particle size) (ES Industries, Berlin, NJ), and Hypercarb PGC (20-mm × 4.6-mm column, 7-µm particle size) (Shandon Scientific, Cheshire, England; packed by HPLC-teknik, Robertsfors, Sweden). The HPLC columns were regulated at 25°C by a Crococil (Sainte Foy La Grande, France) thermostat. The injected solute amount varied between 10 and 200 ng, and the system dead time was determined as the start of the elution of pentane that was added to all samples. Both biphenyl and CB-77 were added to all samples analyzed on the PYE and DNAP columns to check for drift in retention times. On the PGC column, however, only pentane was added in addition to the test solute. CB-77 was omitted because its retention time on this stationary phase was extremely long, and the biphenyl peak complicated the determination of the capacity factor for compounds eluting close to the dead volume. Hence, biphenyl was analyzed separately between the runs of the other solutes on the PGC column.

Molecular modeling

The molecules were drawn using the MacMimic (InStar Software, Lund, Sweden) computer program, and the figures were exported to be geometrically energy-minimized using the MM2 molecular mechanics software (N.L. Allinger, University of Georgia, Athens, GA). Subsequently, the energy-optimized molecular structures were used for generating a number of molecule parameters utilizing the semi-empirical molecular orbital program Mopac (version 6.00, F.J. Seiler, U.S. Air Force Academy, Colorado Springs, CO; available from the Quantum Chemistry Program Exchange, Indiana University, Bloomington, IN). Multipole moment, kappa and connectivity indices, length, breadth, and height were calculated by a computer program constructed by one of the authors (14). A total of 38 parameters were determined for all solutes. However, many of the parameters were highly correlated to one another. Furthermore, correlation between parameters depended on which set of solutes was modeled. For example, more correlated parameters were found in data sets that contained only chlorinated benzenes than in the entire set that contained chlorinated benzenes and chlorinated biphenyls.

Regression analysis

The solute retention, calculated as the logarithm of the capacity factor, was correlated to the parameters by partial least-squares regression (PLS) (15) and multiple linear regression (MLR). These calculations were performed by a computer program constructed by one of the authors (16). In the models presented here, the optimum number of PLS factors were, in all cases, the same as the total number of parameters in the model, which is equivalent to using MLR. No models presented by us contain parameters that had a coefficient of determination higher than 0.75 when regressed against each other. Outlier analysis was performed on the residuals with a 5% significance level to test whether benzene and biphenyl could be regarded as belonging to the groups of chlorinated benzenes and chlorinated biphenyls, respectively. Evaluation of all regression equations were performed by the use of predicted residual sum of squares (PRESS) statistics, in which each capacity factor is predicted by a model constructed from all other solute capacity factors in the data set (17). The resulting root mean square errors of prediction (RMSP) and the root mean square errors (RMS) from the modeling are given as a percentage of the total span of capacity factors for the solutes in the model. For all models presented, the F-tests for goodness of fit (18) indicated that the parameter effects were significantly different from zero with a confidence level higher than 99%.

Results and Discussion

The retention times were found to be very stable when utilizing the PYE and DNAP columns, indicated by small variations in the capacity factors of the CB-77 and the biphenyl added to all samples. The relative standard deviation of the retention times of biphenyl and CB-77 were less than 0.4 and 1.4% on the PYE and DNAP columns, respectively. The variation in retention on the PGC column was somewhat higher; the relative standard deviation was 6.2%. However, the retention of the test compounds spanned a much wider interval on

the PGC than on the two other columns; capacity factors were between 0.2 and 147. On the PYE column, the capacity factors were in the range of 0.2–3.2, whereas the capacity factors were in the range of 0.2–2.3 for the DNAP stationary phase. Capacity factors for all PCB congeners included in the study are listed in Table I, and capacity factors for the chlorobenzenes are listed in Table II.

In trying to find the correlation between retention and the calculated parameters, it was soon realized that there were

differences between subgroups of the solutes. In most cases, a better prediction of retention was obtained if the chloro-benzenes and the non-ortho-substituted chlorobiphenyls were treated separately. This indicated differences in the retention mechanisms, probably caused by a higher degree of steric hindrance of ortho-substituted chlorobiphenyls compared with chlorobenzenes and non-ortho PCB congeners. A difference in chromatographic behavior between PCB congeners according to the degree of ortho-substitution was recognized by Brodsky and Ballschmiter (4). When predicting octanol–water partition coefficients from retention data in reversed-phase HPLC, they divided the chlorobiphenyls into three groups: non-ortho-, mono-ortho-, and ditetra-ortho-substituted chlorobiphenyls.

The similarity or divergence in retention mechanisms within groups of compounds can be studied using a thermodynamic approach in which $\log k'$ is determined at different temperatures (19). In practice, however, this is very difficult to perform successfully in LC because of the limited temperature range that can be applied and the difficulties in accurately controlling the temperature of the HPLC system.

Two examples of the differences between subgroups of the solutes in this study are illustrated in Figure 1 and Figure 2; $\log k'$ on the PYE phase is plotted against solute LUMO and polarizability, respectively. For the whole set of solutes, a trend of increased retention with decreasing LUMO and increasing polarizability was noted. However, it was clearly demonstrated that a better correlation between $\log k'$ and the parameter is achieved if regression is performed on selected subgroups of solutes. This is illustrated by the black dots in Figure 2, which represent the chlorobenzenes.

All three stationary phases studied here are often regarded to interact by electron donor–acceptor (EDA) mechanisms. LC of EDA stationary phases has been reviewed by Nondek (12) and by Sander and Wise (20). In addition to EDA interaction, the PGC stationary phase has been associated with dispersive and polar interactions as the main retention mechanisms (21,22). There are several studies that emphasize the importance of solute planarity for retention on PYE and PGC (23–25), and accordingly, the best models of retention in this study were obtained for the chlorinated benzenes, which are all planar.

Table I. Capacity Factors of Biphenyl and the Chlorinated Biphenyls Included in the Study*

Structure	IUPAC no.	PGC	PYE	DNAP
Biphenyl		2.22	0.27	0.90
3	2	3.20	0.37	0.94
4	3	7.40	0.42	1.03
3,3'	11	7.19	0.53	1.20
3,4	12	12.89	0.56	1.35
3,4'	13	10.72	0.66	1.19
3,5	14	4.44	0.41	0.84
4,4'	15	47.10	0.81	1.34
2,3,5	23	1.30	0.40	0.63
2,4,4'	28	1.33	0.56	0.83
3,3',4	35	33.92	0.92	1.63
3,3',5	36	10.67	0.62	1.01
3,4,4'	37	95.48	1.22	1.80
3,4,5	38	23.29	0.67	1.34
2,2',4,4'	47	0.43	0.49	0.66
2,2',5,5'	52	0.76	0.55	0.68
2,3,3',5'	58	1.70	0.65	0.87
2,3,4,5	61	2.30	0.56	0.83
2,3,5,6	65	1.44	0.48	0.57
3,3',4,4'	77	105.0	1.83	2.30
3,3',4,5	78	32.86	1.13	1.62
3,3',4,5'	79	30.24	1.25	1.42
3,3',5,5'	80	12.40	0.76	0.90
3,4,4',5	81	53.9	1.73	1.82
2,2',4,5,5'	101	0.67	0.61	0.67
2,2',4,5',6	103	0.37	0.42	0.50
2,3,3',4,4'	105	3.03	1.21	1.54
2,3,4,4',5'	114	3.00	1.05	1.04
2,3,4,5,6	116	2.23	0.60	0.62
2,3',4,4',5	118	2.10	1.04	1.07
2',3,3',4,5	122	2.60	0.94	1.30
3,3',4,4',5	126	102	2.47	2.28
3,3',4,5,5'	127	34.7	1.53	1.40
2,2',3,4,4',5'	138	0.70	0.87	1.01
2,2',4,4',5,5'	153	0.58	0.72	0.67
2,2',4,4',5,6'	154	0.37	0.43	0.47
2,3,3',4,4',5	156	2.84	1.44	1.28
2,3,3',4,4',5'	157	4.25	1.57	1.49
2,3',4,4',5,5'	167	2.00	1.24	1.05
3,3',4,4',5,5'	169	147	3.21	2.27
2,2',3,3',4,4',5	170	1.10	1.13	1.07
2,2',3,4,4',5,5'	180	0.77	0.92	0.78
2,3,3',4,4',5,5'	189	2.58	1.70	1.25
2,2',3,3',4,5,5',6	198	0.80	0.65	0.55
2,2',3,4,4',5,6,6'	204	0.23	0.32	0.37

* The individual polychlorinated biphenyls are identified by numbers according to the International Union of Pure and Applied Chemistry (IUPAC) (35).

Table II. Capacity Factors of Benzene and the Chlorinated Benzenes

Structures	PGC	PYE	DNAP
Benzene	0.20	0.13	0.25
1	0.24	0.17	0.34
1,2	0.46	0.27	0.50
1,3	0.41	0.22	0.33
1,4	0.45	0.24	0.33
1,2,3	1.06	0.38	0.56
1,2,4	0.83	0.31	0.36
1,3,5	0.61	0.23	0.23
1,2,3,4	2.69	0.61	0.52
1,2,3,5	1.81	0.45	0.31
1,2,4,5	1.93	0.52	0.32
1,2,3,4,5	6.03	0.94	0.36
1,2,3,4,5,6	19.4	1.92	0.36

The most successful modeling for the entire set of compounds (i.e., 12 chlorobenzenes, 44 chlorinated biphenyls, benzene, and biphenyl) was obtained for the DNAP column. The retention was described by multiple linear regression with two parameters: stretch energy and steric energy (Figure 3). For the other two columns, it was not possible to satisfactorily model the retention of the entire set or a solute set restricted to the chlorinated biphenyls. These difficulties probably originated from a varying degree of steric hindrance that could not be fully accounted for by a parameter containing the torsion angle between the two phenyl rings. Consequently, this study concentrated on modeling retention for subgroups of compounds with a low degree of steric hindrance (i.e., the chlorinated benzenes and the non-ortho-substituted chlorobiphenyls).
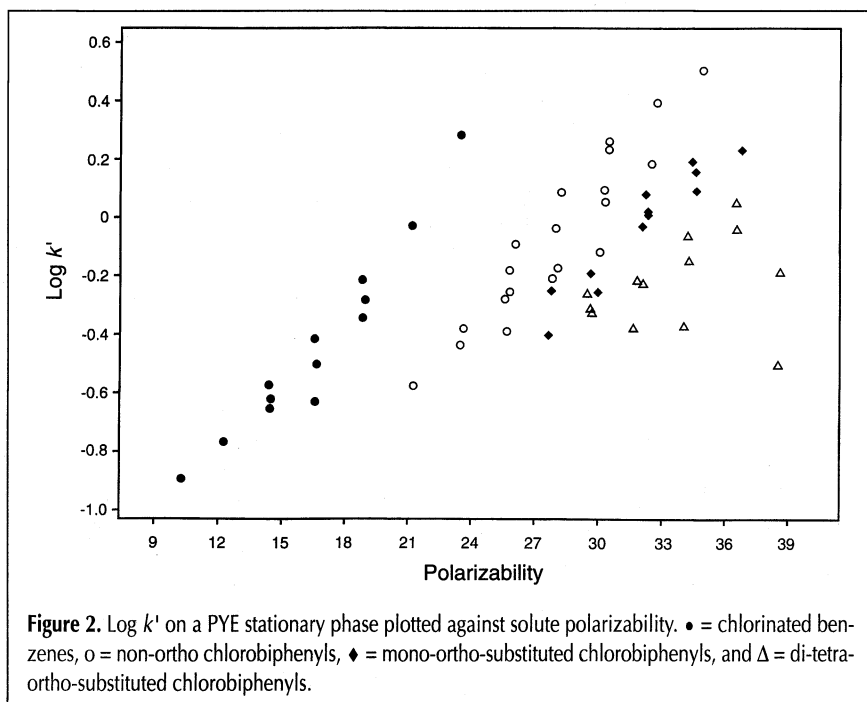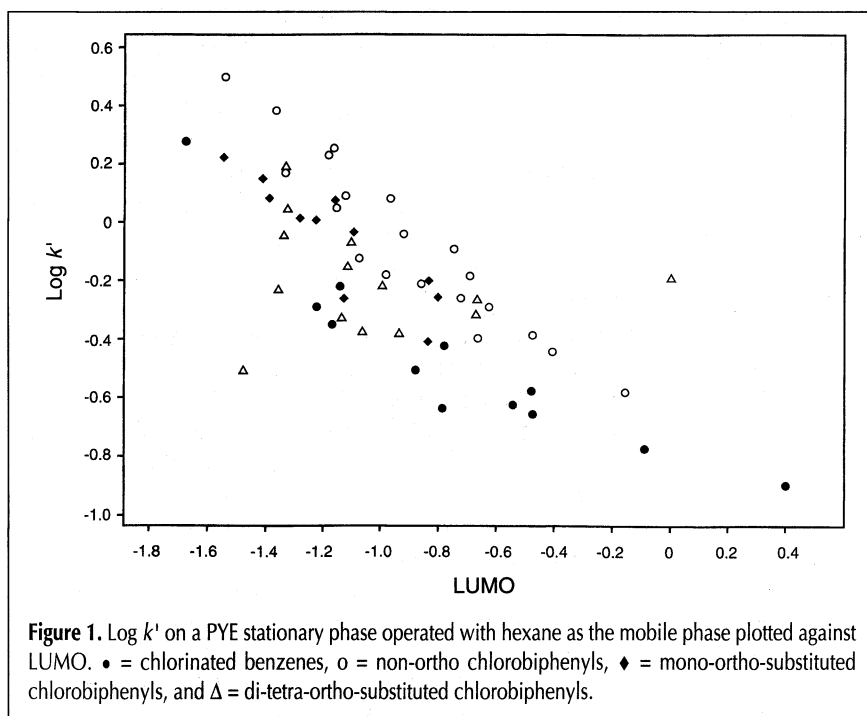
Chlorinated benzenes

With a solute set restricted to only the chlorinated benzenes, the parameter correlations to retention can be studied without any confounding effects caused by a lack of planarity.

The retention selectivities for the chlorinated benzenes were very similar for the PYE and PGC columns. For both stationary phases, retention increased with the number of chlorine substituents. As has previously been concluded for the separation of chlorinated biphenyls on PYE by Haglund et al. (23), it was also observed for the chlorinated benzenes that congeners with adjacent chlorine substituents were more retained than solutes with the chloro-substituents spread out. The enhanced retention of compounds with the chlorines close together can imply a polarity influence. However, that would not explain the high capacity factor of hexachlorobenzene. In contrast, the retention of chlorinated dioxins on a PYE column operated in reversed-phase mode was found to be governed by symmetrical distribution of the chlorine atoms (26).

For the chlorinated benzenes, the parameters of polarizability, number of chloro-substituents, and LUMO are highly related to one another. These three parameters are all roughly related to retention of the chlorinated benzenes on PGC and PYE, though the coefficient of determination for polarizability and number of chloro-substituents are higher than those for LUMO. Furthermore, a slightly convex-shaped distribution was obtained when LUMO was plotted versus log k'. However, none of these three parameters explain the stronger retention of compounds with adjacent chloro-substituents that is shown within each isomeric group. For the entire set of chlorinated benzenes, there was no relation between dipole moment and retention. However, within each isomeric group, the congener with the highest dipole moment had the longest retention. The retention mechanisms of some substituted benzenes (e.g., phenol, nitrobenzene, and 1-chlorobenzene) separated on porous graphitic carbon with heptane as the mobile phase were studied by Bassler et al. (21). The main correlation was attributed to a submolecular polarity parameter, and furthermore, the PGC was supposed to act as an acceptor of n-electrons.

In regression analysis of the chlorinated benzenes, the best model of retention was obtained by a model that involved only one

Figure 1. Log k' on a PYE stationary phase operated with hexane as the mobile phase plotted against LUMO. • = chlorinated benzenes, o = non-ortho chlorobiphenyls, ♦ = mono-ortho-substituted chlorobiphenyls, and △ = di-tetra-ortho-substituted chlorobiphenyls.

Figure 2. Log k' on a PYE stationary phase plotted against solute polarizability. • = chlorinated benzenes, o = non-ortho chlorobiphenyls, ♦ = mono-ortho-substituted chlorobiphenyls, and △ = di-tetra-ortho-substituted chlorobiphenyls.

parameter, third order valence path molecular connectivity index ($^3\chi_{vp}$). This was demonstrated by a good correlation between log k' and $^3\chi_{vp}$ for both the PGC and PYE columns (Figure 4).

The connectivity indices were introduced by Randic (27), further developed by Kier and Hall (28), and also illustrated by Doherty et al. (29). Molecular connectivities are topological shape-specific indices that take into account the nature of the substituents (i.e., the chlorine atoms). The indices are constructed from the connective relations between atoms in a hydrogen-suppressed molecule.

There are many examples in which connectivity indices have been included in QSRR equations (6,30–33). With respect to the studied chlorinated benzenes and biphenyls, the first and second order valence path connectivity indices are the same for all isomeric compounds (e.g., tetra-chlorinated benzenes). Concerning $^3\chi_{vp}$ and the chlorobenzenes, the index generally increased with a higher degree of chloro-substitution. In addition, compounds in which the chloro-substituents were close together had higher indices than isomers in which the chlorine atoms were farther apart.

However, within the subgroup of chlorinated benzenes, there are a number of parameters that are highly correlated to $^3\chi_{vp}$. Of 38 different parameters, 18 had a correlation coefficient to $^3\chi_{vp}$ higher than 0.9. As expected, the retention can then be described by a number of single parameters with good correlation. Some examples of this are presented in Table III.

For the dinitroanilinopropyl silica column, a retention characteristic different from that of PGC and PYE was demonstrated. The DNAP stationary phase is often regarded as an acceptor phase in EDA chromatography, and, with this mechanism, aromatic compounds with an electron-rich, aromatic nucleus and a low ionization potential should preferably be retained. However, there was no correlation at all between log k' and ionization potential for the chlorinated benzenes (i.e., the coefficient of determination for linear regression was not significantly different from zero at any level of confidence). For polycyclic aromatic hydrocarbons separated on DNAP, 1/log k' values have been correlated to the ionization potentials (i.e., the longer the retention, the lower the ionization potential), which provides evidence for electron donor–acceptor interactions (34). It is noted that the log k' values for the chlorinated benzenes on DNAP were roughly distributed in three groups in which solutes that had two to four chlorine atoms grouped together in one part of the molecule (i.e., those with the highest dipole moment) had the highest retention. However, dipole moment does not explain the retention of symmetrically substituted congeners. Furthermore, there was no correlation between polarizability and log k'. Apparently, it was not possible to achieve an acceptable model of the retention on DNAP for a solute set that was restricted only to the chlorinated benzenes. Because the solutes were limited in number and also had log k' values distributed in three groups, the number of parameters that could have been used in a model was very limited. It was concluded that the available parameters did not sufficiently span the properties that affect retention.
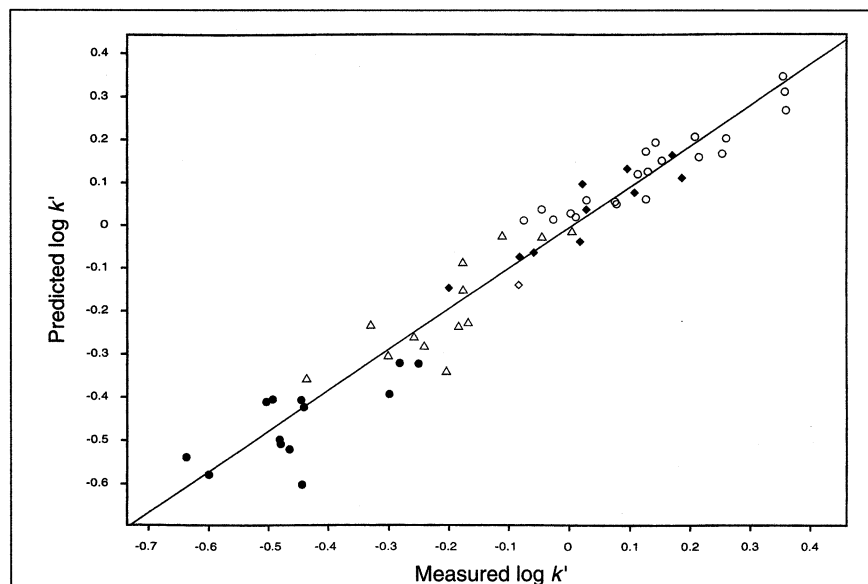

Figure 3. Plot showing predicted retention against measured retention on a DNAP column. Log k' = 10.27 × stretch energy – 0.077 × steric energy – 0.777. r^2 = 0.946, RMS = 6.0%, RMSP = 6.4%, ● = chlorinated benzenes, o = non-ortho chlorobiphenyls, ♦ = mono-ortho-substituted chlorobiphenyls, and Δ = di-tetra-ortho-substituted chlorobiphenyls.
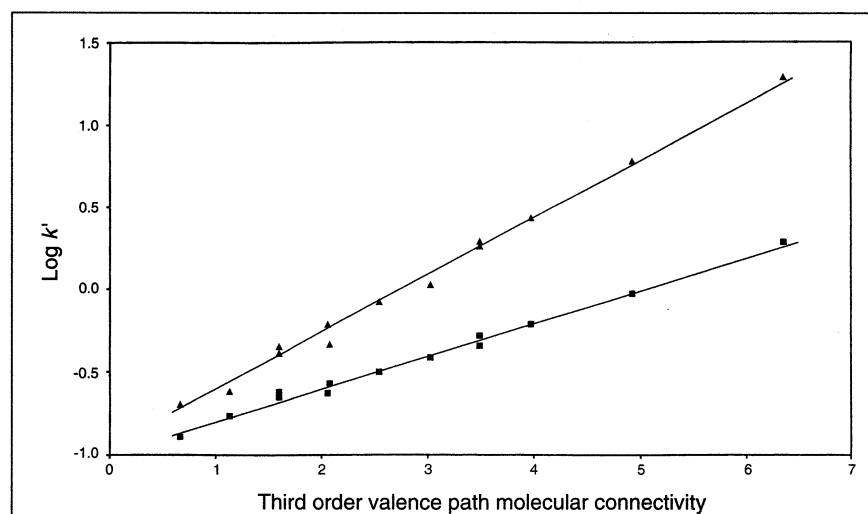

Figure 4. Log k' as a linear function of $^3\chi_{vp}$ for chlorinated benzenes and benzene on PGC (▲) and PYE (■) columns. PGC: log k' = 0.35 × $^3\chi_{vp}$ – 0.98, r^2 = 0.994, RMS = 2.2%, RMSP = 2.5%. PYE: log k' = 0.20 × $^3\chi_{vp}$ – 1.00, r^2 = 0.992, RMS = 2.4%, RMSP = 2.8%.

Non-ortho-substituted chlorinated biphenyls

The non-ortho chlorinated biphenyls were the PCB congeners with the smallest degree of steric hindrance. However, through molecular modeling, the torsional angle between the two phenyl rings was 40–42° for the non-ortho PCB congeners, so steric effects were expected to be of greater importance than for the chlorinated benzenes. When all the investigated chlorinated biphenyls were studied, a major difference between the PGC and PYE columns was observed. On PGC, all non-

ortho chlorobiphenyls except one (3-chlorobiphenyl) eluted after the other PCB congeners. On the PYE column, the non-ortho PCB congeners were spread over the whole range of capacity factors. Therefore, retention on PGC exhibited a more selective shape dependence than separation on a PYE stationary phase.

Retention of the non-ortho chlorobiphenyls on PGC and PYE showed trends similar to the retention of chlorobenzenes. In general, higher retention corresponded to higher polarizability or lower LUMO.

On both PGC and PYE, the retention of 19 non-ortho chlorobiphenyls and biphenyl can be explained roughly by only two parameters: molecular length combined with one of some parameters highly correlated to one another (see Table IV). A high prediction ability for the model was demonstrated by the RMSP values, which are only slightly higher than the RMS values.

In all these equations for non-ortho PCB congeners on both PYE and PGC, MLR gave a better result than PLS with one component. However, in many cases, PLS modeling of retention using only one PLS factor or principal component regression (PCR) resulted in models that were nearly as good. An example is given in Figure 5, in which the retention on PYE is plotted against molecular length and polarizability. When the figure is viewed from a selected direction, a single PCR factor becomes visible. Retention was promoted by polarizability and the length of the molecule (i.e., chloro-substitution in para-position).

On PYE, the retention of non-ortho PCB congeners could also be very well-explained by an equation with two other parameters: quadrupole moment and bending energy (see Figure 6). Replacing bending energy with torsional energy also provides a good model because these parameters are correlated for this group of chlorinated biphenyls. However, modeling the retention of non-ortho chlorobiphenyls on PGC by these parameters did not give a satisfactory result. Interpretation of the model with the bending energy and quadrupole moment parameters was difficult. For the non-ortho PCB congeners, bending energy is correlated to the degree of adjacent chlorine-substitution with the highest bending energy for compounds that have many adjacent chloro-substituted carbons. The quadrupole moment itself had a coefficient of determination of 0.90 to log k' for the non-ortho CBs separated on PYE. The third order valence path molecular connectivity index, an excellent parameter for describing retention of chlorinated benzenes on both PGC and PYE, was less successful for the non-ortho chlorobiphenyls; the coefficient of determination was 0.89 and 0.71 for PYE and PGC, respectively.

On the third stationary phase studied, DNAP, the retention mechanism was much more complex. It was not possible to describe the retention by an appropriate number of the available parameters because of the grouping of capacity factors.

Table III. Regression Statistics from Modeling Retention, as Log k', for Chlorinated Benzenes on PGC and PYE Columns

Variable	PGC		PYE	
	RMS (%)/r^2	RMSP (%)	RMS (%)/r^2	RMSP (%)
$^3\chi_{vp}$	2.2 / 0.99	2.5	2.4 / 0.99	2.8
Polarizability	4.2* / 0.97*	5.1*	7.0 / 0.93	8.8
Sigma energy	5.0 / 0.97	7.1	3.2* / 0.98*	4.4*
No Cl atoms	5.1* / 0.96*	6.7*	8.6* / 0.92*	11.3*
Steric energy	3.8* / 0.98*	5.7*	3.4* / 0.98*	4.4*
Stretch energy	4.1 / 0.98	4.8	4.0 / 0.98	4.9

* Benzene removed as an outlier at the 5% level of significance.

Table IV. Multiple Linear Regression Modeling of the Retention of 19 Non-Ortho Chlorinated Biphenyls and Biphenyl on PGC and PYE Columns with Molecular Length and One Additional Parameter

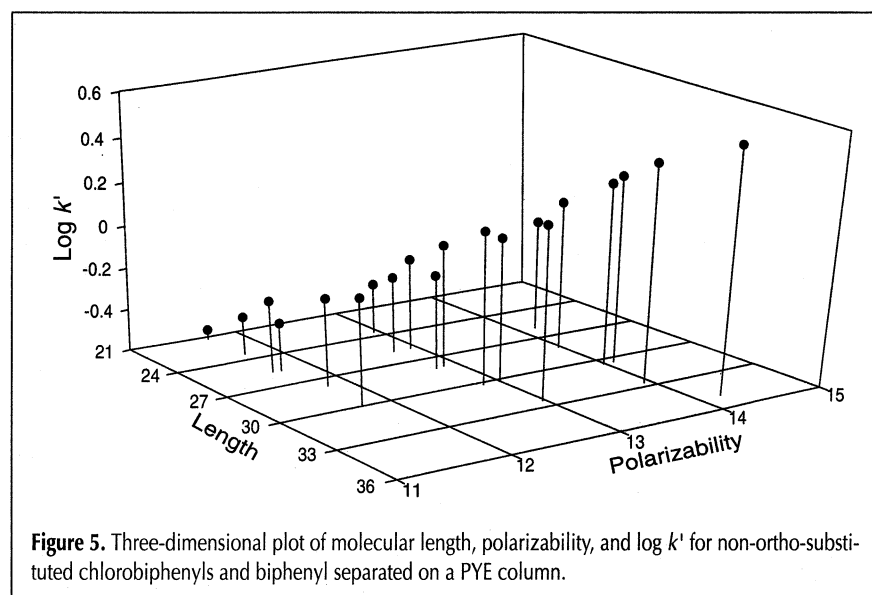| Additional parameter	PGC		PYE	
	RMS (%)/r^2	RMSP (%)	RMS (%)/r^2	RMSP (%)
Ionization potential	6.3 / 0.95	7.2	4.5 / 0.97	5.5
LUMO	6.3 / 0.95	7.2	5.2 / 0.96	6.1
Polarizability	6.6 / 0.95	7.7	4.1 / 0.98	4.8
Total energy	6.6 / 0.95	7.7	4.1 / 0.98	4.8
Heat of formation	6.4 / 0.96	6.9	5.2 / 0.96	6.8

Figure 5. Three-dimensional plot of molecular length, polarizability, and log k' for non-ortho-substituted chlorobiphenyls and biphenyl separated on a PYE column.
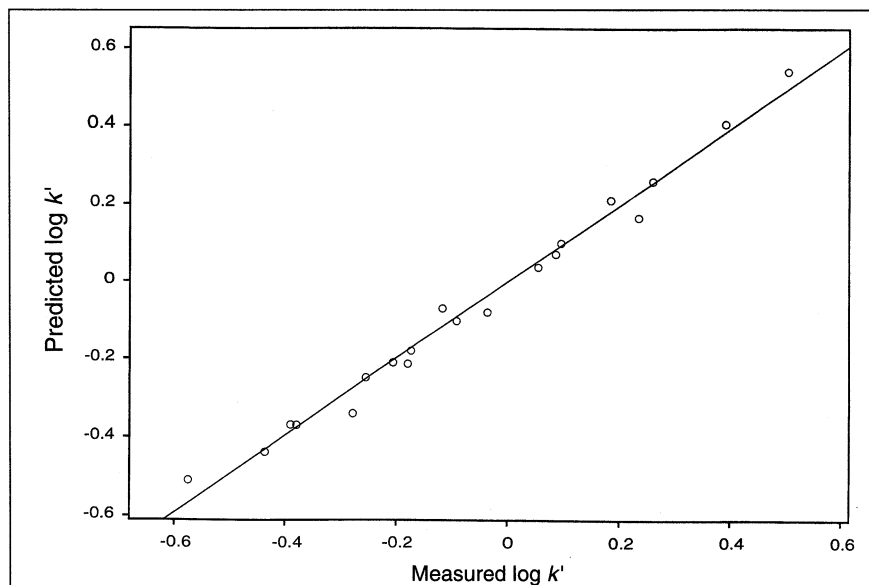
Figure 6. Plot showing the predicted log k' values versus measured log k' values for non-ortho chlorobiphenyls and biphenyl on PYE. Log k' = –0.005 quadrupole moment + 0.49 bending energy – 3.12. r^2 = 0.986, RMS = 3.1%, and RMSP = 3.7%.

With respect to parameters classically related to chromatographic retention, correlation to log k' was found when subgroups of solutes with a small degree of steric hindrance were studied separately. However, because of a high correlation between certain parameters (e.g., LUMO and polarizability), it could not be stated that a mechanism such as electron donor–acceptor interaction has a larger influence on retention than dispersive interactions or vice versa.

Applicability of QSRR to normal-phase HPLC

There is virtually no unambiguous way to compare the quality of QSRR equations between different modes of chromatography, but in most cases, the coefficient of determination between measured and predicted retention is reported. The most successful QSRR in this study were obtained for the chlorinated benzenes with the third order valence path molecular connectivity parameter, which yielded r^2 values of 0.992 and 0.994 for PYE and PGC, respectively. A high coefficient of determination (0.986) was also obtained for a two-parameter model of non-ortho chloro-substituted biphenyls separated on PYE. The best description of the entire set of chlorinated benzenes and chlorobiphenyls was obtained for DNAP. Modeling with two parameters resulted in a coefficient of determination of 0.946. However, for DNAP, the separation of solute subgroups that cover a smaller interval in both capacity factors and parameter values could not be modeled in a satisfactory way.

For GC analysis of chlorinated biphenyls, a coefficient of determination as high as 0.9991 (76 objects, 5 parameters) has been reported (8). In reversed-phase HPLC on an ODS column, the capacity factors of a mixture of halogenated biphenyls were modeled with an r^2 value of 0.968 (53 objects, 5 parameters) (6). In a QSRR study of substituted benzenes separated on PGC with heptane as the mobile phase, a coefficient of determination of 0.945 (11 objects, 2 parameters) between calculated and measured log k' was obtained (22).

In summary, the best results in these QSRR studies performed in normal-phase HPLC were obtained when a limited group of compounds with rather planar structure were separated on the PGC and PYE columns. Both PGC and PYE can be regarded as stationary phases with a low degree of complexity in retention mechanism. An interesting observation was the high correlation between log k' values for the chlorinated benzenes on PYE and PGC and the third order valence path molecular connectivity index.

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