THEORETICAL INTERPRETATION OF RETENTION DATA OF POLYCHLORINATED BIPHENYLS IN LIQUID CHROMATOGRAPHY

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The new approach to the theoretical evaluation of Gibbs free energy of solvation is applied to the estimation of retention data in RP-HPLC. Simple models of stationary and mobile phases in RP-HPLC are used. The correlation between retention data of a series of 15 polychlorinated biphenyls (measured on chemically bonded stationary phase with C_{18} alkyl chains) and the contribution of the Gibbs solvation energy is investigated in order to determine the dominant factor controlling retention. The retention in RP-HPLC can be predicted on the basis of our thermodynamic retention model. This approach is suitable especially when no standards are available for some derivatives (e.g. metabolites of biologically active substances) and when the interpretation of experimental elution is not clear.

Recent development of chromatography is accompanied by systematic research of the relation between the structure and retention data of separate compounds. Several reports have dealt with this topic, the so-called Quantitative Structure–Retention Relationships (QSRR). A brief survey of these methods can be found in refs[1-4]. The fundamental aim of the QSRR study is to find suitable molecular and submolecular descriptors of solutes which determine their retention behaviour. Correct understanding of these generalized relations is necessary for good interpretation of the results obtained by chromatographic methods, especially gas liquid chromatography (GLC) and high-performance liquid chromatography on normal or reversed phases (HPLC, RP-HPLC). Martin in his fundamental work[5] on QSRR expressed the relation of the partition coefficient to the character of the substituent and to the properties of the mobile and stationary phases. Since then, many different forms of linear energy relationships have been applied in chromatography.

In the fragment approach, the Gibbs free energy of molecular retention is calculated by linear combination of retention energy contributions of a molecule fragment[6-9], but it is not possible to consider actual changes of the electron density redistribution in the molecule which may cause significant deviations in some cases. Moreover, the fragment constants are not always unambiguous.

A number of methods have been suggested to account for the relation between the chromatographic retention and various physico-chemical parameters[10–23] such as boiling point[10,11], surface tension[11], number of carbon atoms[12], molecular weight[13], dipole moment[17], molar parachor[18] and molecular polarizability[20], further topological parameters[11,24–30] and quantum chemical indices[11,31,32]. These methods are purely empirical approaches.

In other approaches, the Gibbs free energy of retention is not fragmented but calculated as a sum of separate contributions corresponding to the Gibbs free energy of retention. Many works have appeared in this area recently. For GLC chromatography Lamparczyk et al.[33] tried to express the partition energy approximately. Starting from the basic equation for the interaction potential energy, the authors expressed the dependence of the interaction energy on the atomic and molecular parameters. Similarly Jinno et al.[34] formulated a simplified method expressing the logarithm of the capacity factor $\log k'$ suitable for RP-HPLC. A solvatochromic comparison method was used to study the interaction between solutes and solvents in GLC and later in RP-HPLC[35–43].

Melander and Horváth[44] calculated the cavitation and interaction energies in accordance with the solvophobic theory[45,46] and compared them with the retention parameters. According to their detailed model, the solute is partitioned by "solvophobic interactions", where the role of the mobile phase is emphasized. The weak points of this method consist in the approximate evaluation of electrostatic and dispersion contributions and in neglecting the role of the stationary phase. Moreover, many physicochemical constants are required which are not easily found in the literature or determined experimentally.

Our aim was to develop a thermodynamic model allowing to express theoretically the solvation Gibbs free energy in terms of the interactions of a particular compound with both the stationary and mobile phases. Our calculation of the Gibbs free energy of solvation is based on a sophisticated expression for the electrostatic, dispersion, repulsion, and cavitation contributions.

As an example of the application of our method, the Gibbs free energy of solvation is calculated for a series of 15 polychlorinated biphenyls (PCB) with regard to interactions in the stationary and mobile phases in RP-HPLC. Further the possibilities of modelling the stationary chemically bonded C_{18} phase and the mobile phase represented by the water–methanol mixture are evaluated. In this way, the correctness of our theoretical model for the studied compounds is checked. Finally, the calculated contributions of the Gibbs free energy of solvation are correlated with the capacity factors and the predictivity of the proposed method is evaluated.

THEORETICAL

The capacity factor k', used for quantitative description of the chromatographic process, is defined as

$$k' = \frac{C_s}{C_m}\frac{V_s}{V_m} = \frac{t_R - t_M}{t_M}\,, \qquad (1)$$

where C_s/C_m is the ratio of the concentration in the stationary phase to that in the mobile phase, V_m and V_s are the volumes of the mobile and stationary phases, t_R is the retention time and t_M the dead time.

For modelling the chromatographic retention in a RP-HPLC system the equation

$$\log k' = -\frac{\Delta G^{s/m}}{2{\cdot}3\boldsymbol{R}T} + \log V_s/V_m \qquad (2)$$

was used, where $\Delta G^{s/m}$ represents the Gibbs free energy of retention between the stationary and mobile phases, $\boldsymbol{R}$ is gas constant and T is the absolute temperature. The quantity $\Delta G^{s/m}$ can be evaluated as the difference between Gibbs free energies of solvation in these phases:

$$\Delta G^{s/m} = \Delta G^{s}_{solv} - \Delta G^{m}_{solv}\,. \qquad (3)$$

In accord with our model, we express Eq. (2) by the components of the solvation Gibbs free energies:

$$\Delta G^{s/m} = (\Delta G^{s}_{el} - \Delta G^{m}_{el}) + (\Delta G^{s}_{dr} - \Delta G^{m}_{dr}) + (\Delta G^{s}_{cav} - \Delta G^{m}_{cav})\,, \qquad (4)$$

where ΔG^{x}_{el} represents the electrostatic, ΔG^{x}_{dr} the dispersion-repulsion and ΔG^{x}_{cav} the cavitation contribution of Gibbs free energy of solvation in the stationary ($x = s$) and mobile ($x = m$) phases.

Equation for the Electrostatic Contribution

Two approximations are introduced: (i) simplified model of the coulombic contribution developed by Jano[44], (ii) model of polarizable continuum developed in our laboratory[46–48]. According to Jano[44], the coulombic contribution to the Gibbs free energy of solvation can be approximated by the extended Born equation:

$$\Delta G_{el} = \Delta G_{coul} = -(1/2)(1 - 1/\varepsilon)\sum_{A,B} Q_A Q_B / 4\pi\varepsilon_0 r'_{AB}\,, \qquad (5)$$

where ε is the relative permittivity of the solvent, ε_0 is the permittivity of vacuum, Q_A and Q_B are charges on the atoms A and B of the solute obtained by a quantum-chemical calculation, and r'_{AB} is the solvent–solute atom distance approximated as[45]:

$$r'_{AB} = r_{AB} + r_A^{vdW} ,$$
$$r'_{AA} = r_A^{vdW} , \qquad (6)$$

where r_A^{vdW} is the van der Waals radius of atom A and r_{AB} is the distance between the solute atoms A and B.

The inclusion of the Coulomb energy only is often insufficient. The model of polarizable continuum, proposed by Miertuš et al.[46–48] offers a more exact way to express the electrostatic component: the interaction of a molecule with the solvent is approximated by an infinite polarizable continuum with a relative permittivity ε. The solute molecule is situated in the cavity formed in the dielectric by penetrating spheres with their centers in the atom cores which form the molecule with a radius equal to the van der Waals radius of the atoms. On the surface of the cavity the point charges induced by the charge distribution of the solvated molecule are calculated. Their size is calculated from a numerical solution of the Laplace equation by an iterative process considering solute-solvent polarization and the selfpolarization of the solvent.

The final expression derived[46–48] for charges, $q_{K_i}^m$, on the individual surface elements of the cavity, S_{K_i}, is

$$\begin{aligned} q_{K_i}^m = q_{K_i}^o - [(\varepsilon - 1)/(4\pi\varepsilon)] \{(\Delta S_{K_i}/\delta_{K_i}|) \sum_{L_j K_i} q_{L_j}^{m-1} \,. \\ . [|(r_{K_i} + \delta_{K_i}) - r_{L_j}|^{-1} - |r_{K_i} - r_{L_j}|^{-1} - 2\pi q_{K_i}^{m-1} \,. \\ . \{1 - [\Delta S_{K_i}(4\pi R_K^2)^{-1}]^{1/2}\}\} , \end{aligned} \qquad (7)$$

where K and L denote atoms, *i* and *j* surface elements on the spheres, *m* is the number of selfpolarization cycles, ε is the relative permittivity of the solvent, *r* is the positional vector of the element, δ is the vector of numerical differentiation of the potential, and R_K is the radius of the *K*-th sphere. The second right-hand term expresses the mutual polarization of elements $L_j \neq K_i$, whereas the third term is the contribution from the selfpolarization of element K_i.

The induced charges on the individual surface elements of the cavity are calculated and their sum, forming the potential V_σ, is included in the Hamiltonian of the solute molecule. The quantum chemical solution of the problem rests in expressing the electrostatic energy of solvation ΔE_{el} as the SCF energy difference for solute with and without the V_σ potential included:

$$\Delta E_{el} = \langle \Psi | H_0 + V_\sigma | \Psi \rangle - \langle \Psi | H_0 | \Psi \rangle . \qquad (8)$$

According to this theory, the electrostatic contribution to the Gibbs energy of solvation can be written as

$$\Delta G_{el} = -(1/2)\int_{\Sigma} \sigma V_{\sigma}\, dS \approx -(1/2)\, V_i q_i\,, \tag{9}$$

where σ is the surface charge density of the solute molecule, V_{σ} is the potential arising from the solute charge distribution, q_i is the i-th charge component on the cavity surface, and V_i is the potential in the centre of the i-th element of the cavity wall. Detailed information about the method can be found elsewhere[46-48].

Equation for the Dispersion and Repulsion Contributions

The dispersion and repulsion contribution to the interaction energy can be expressed by means of the London relation for the dispersion term[49] and the Born relation for the repulsion term[50]:

$$\Delta G_{dr} = \Delta G_d + \Delta G_r = -C/r^6 + B/r^{12}\,. \tag{10}$$

Considering the solute molecule in a spherical cavity with radius r_{so} surrounded by N spherical solvent molecules with radius r_{sv} which form the first solvation layer situated in the balanced distances $r_{so\text{-}sv}$, it is possible to obtain for both contributions a unified expression[51]:

$$\Delta G_{dr} = -3/4N\alpha_{so}\alpha_{sv}\left[\frac{\langle E\rangle_{so}\langle E\rangle_{sv}}{\langle E\rangle_{so} + \langle E\rangle_{sv}}\right]\frac{1}{(r_{so} + r_{sv})^6}\,, \tag{11}$$

where α is the polarizability, $\langle E\rangle$ represents the mean excitation energy approximated by ionization potentials[52], and so/sv designates the solute/solvent.

This relation can be expanded by considering the interactions between all solvent molecules surrounding the solute molecule in all solvation spheres[47]:

$$\Delta G_{dr} = B\sum_{i=1}^{n} \alpha_{i,so}[(r_{i,so}/r_{sv}) + 1]^{-3.91}\, S_i/S\,, \tag{12}$$

where

$$B = -3/4\alpha_{sv}\left[\frac{\langle E\rangle_{so}\langle E\rangle_{sv}}{\langle E\rangle_{so} + \langle E\rangle_{sv}}\right](1{\cdot}01\,\pi/r_{sv}^6)\,, \tag{13}$$

n is the number of atoms in the solute molecule, $r_{i,so}$ is the van der Waals radius of the i-th atom of solute, α_i is the atomic polarizability (which can be found in refs[53,54]), S_i is the area of the corresponding atomic element of the cavity and S is the total cavity surface area.

Expression for the Cavitation Contribution

Cavitation energy is the energy required for the formation of a cavity (with a defined volume) in the solvent, in which a molecule of the substance is subsequently placed. This is a destabilization of the system, which in the model corresponds to a redistribution of the solvent molecules. To express the cavitation contribution, it is possible to apply the Sinanoglu[55] or the Pierotti[56] approximation.

Sinanoglu expressed the cavitation energy $\Delta S_{\mathrm{cav,S}}$ (the subscript S refers to the Sinanoglu approach) based on the microscopic surface tension theory as

$$\Delta G_{\mathrm{cav,S}} = \gamma_{\mathrm{sv}} k_{\mathrm{sv}}(r_{\mathrm{sv}}/r_{\mathrm{so}})\, S\left(1 - \frac{\partial \ln \gamma}{\partial \ln T} - 2/3 A_{\mathrm{b}} T\right), \qquad (14)$$

where γ_{sv} is the macroscopic surface tension of the solvent, S is the cavity surface of the solute molecule, A_{b} is the thermal expansion coefficient, T is the absolute temperature and $k_{\mathrm{sv}}(r_{\mathrm{sv}}/r_{\mathrm{so}})$ is the microscopic cavitation factor for different radii of the solvent, r_{sv}, and solute, r_{so}. The microscopic cavitation factor can be evaluated as follows:

$$k_{\mathrm{sv}}(r_{\mathrm{sv}}/r_{\mathrm{so}}) = 1 + (r_{\mathrm{sv}}/r_{\mathrm{so}})^2\,[k_{\mathrm{sv}}(1) - 1]\,, \qquad (15)$$

where $k_{\mathrm{sv}}(1)$ is the microscopic cavitation factor for the pure liquid (the solvent).

Pierotti derived a relation for the Gibbs energy of cavitation $\Delta G_{\mathrm{cav,P}}$ (the subscript P refers to Pierotti's approach) by means of the hard sphere approximation. The spherical approximation of the shape of molecules is, however, not much exact. Therefore, we modified the Pierotti's procedure[47] so that the individual atomic spheres are considered in the calculation and the total Gibbs energy of cavitation is calculated as the sum of the atomic contributions[57],

$$\Delta G_{\mathrm{cav,P}} = \sum_{\mathrm{i}=1}^{\mathrm{n}} \left[A(r^{\mathrm{i}}_{\mathrm{so-sv}})^2 - B r^{\mathrm{i}}_{\mathrm{so-sv}} + C\right] S_{\mathrm{i}}/S\,, \qquad (16)$$

where S_{i} is the protruding part of the surface of the i-th atom, $r^{\mathrm{i}}_{\mathrm{so-sv}} = r_{\mathrm{i,so}} + r_{\mathrm{sv}}$ is the sum of radii of the i-th atom of solute and solvent, and A, B and C are constants characteristic of the solvent.

The Gibbs energy of cavitation (kJ/mol) for water has thus the form

$$G^{\mathrm{w}}_{\mathrm{cav,P}} = \sum_{\mathrm{i}=1}^{\mathrm{n}} \left[0{\cdot}239\,(r^{\mathrm{i}}_{\mathrm{so-sv}})^2 - 0{\cdot}480 r^{\mathrm{i}}_{\mathrm{so-sv}} + 0{\cdot}272\right] S_{\mathrm{i}}/S \qquad (17)$$

and for octanol

$$G^{\mathrm{w}}_{\mathrm{cav,P}} = \sum_{\mathrm{i}=1}^{\mathrm{n}} \left[0{\cdot}001\,(r^{\mathrm{i}}_{\mathrm{so-sv}})^3 + 0{\cdot}167\,(r^{\mathrm{i}}_{\mathrm{so-sv}})^2 - 0{\cdot}961 r^{\mathrm{i}}_{\mathrm{so-sv}} + 1{\cdot}442\right] S_{\mathrm{i}}/S\,. \qquad (18)$$

The contributions to the Gibbs energy of solvation expressed in this way are applied in Eq. (*3*) so that, finally, the solvation Gibbs free energy is expressed in the stationary phase and the same quantity in the mobile phase. Under the assumption that Eq. (*2*) is valid, the relation between log k' and the calculated Gibbs free energy of retention $\Delta G^{s/m}$ (or the individual contributions) can be correlated with the equation

$$\log k' = a\,\Delta G^{s/m} + b\,, \tag{19}$$

where a and b are constants. We used the correlation coefficient r and tested the linearity or the correlation function by means of the F-criterion[58], viz. by comparison with the critical F-distribution values F (S.R.) for the degrees of freedom $\nu_1 = k$ and $\nu_2 = n - k - 1$, where n is the number of substances in the series studied, k is the number of independent variables in the correlation function. In this way it is possible to evaluate the correctness of the Gibbs free energy and also its individual contributions. Moreover, it is possible to use the obtained correlation equations for the prediction of retention of other compounds.

The experimental values of the logarithm of the capacity factor log k' were taken from ref.[59]. The log k' values referr to RP-HPLC experiments on a commercial column with Separon Six C18 5 μm packing using the methanol–water (90 : 10) mobile phase. The values were determined by methods whose error generally does not exceed 5%. Such an error in the experimental values does not affect the statistical parameters (r, S.R.) so as to bring about a change in qualitative conclusions of this work.

Modelling of Stationary and Mobile Phases

RP-HPLC is generally characterized by application of a relatively non-polar stationary phase, usually a chemically bonded phase with n-alkyl chains and a mixed mobile phase, mostly a water methanol mixture. We modelled the mobile phase as a continuum, either as pure water, or as a mixed eluent water–methanol. This model is altogether justified for the mobile phase. The modelling of the stationary phase is problematical. In spite of the popularity of RP-HPLC, there is still a lack of information about the contribution of the stationary phase to the retention of the solute and to the selectivity. It is probable that the chemically bonded stationary phase with n-alkyl chains plays more the role of the active than of the passive solute receptor. Basically, two mechanisms of separation can be considered:

a) partition mechanism and *b*) adsorption mechanism.

Generally in RP-HPLC the partition mechanism is considered to be dominating, although it is never possible to eliminate entirely the simultaneous adsorption of some molecules on the stationary phase surface. Modelling of adsorption on chemically bonded stationary phases is discussed in our another work[60]. In the present paper we presume the partition mechanism, we model the stationary phase as a continuum, and neglect its anisotropy. For the C_{18} stationary phase it is necessary to define the "effective" continuum parameters. In view of the fact that C_{18} stationary phase contains long hydrophobic (alkyl) chains and a certain number of free OH groups, we decided to represent it by octanol or by a mixture of octanol with a less polar solvent (heptane). Our choice of octanol is a consequence of its structural similarity to C_{18} phases, because it contains one long C_8 hydrophobic chain and one OH group. In comparison with pure octanol, heptane has generally to model a lower polarity of the C_{18} phase.

RESULTS AND DISCUSSION

Retention Data for PCB *in a Model System Water–Octanol*

At first we calculated the individual contributions to the Gibbs energy of solvation for the series of 15 PCB. Following values of parameters were used for water (w) and octanol (o): relative permittivity $\varepsilon_w = 80{\cdot}0$, $\varepsilon_{oct} = 10{\cdot}3$, molecular polarizability $\alpha_w = 1{\cdot}49 \,.\, 10^{-30}\ m^3$, $\alpha_{oct} = 16{\cdot}2 \,.\, 10^{-30}\ m^3$. The standard geometries of PCB molecules were considered; bond lengths and bond angles were taken from refs[61,62].

TABLE I

Electrostatic contributions of Gibbs free energies of solvation (kJ/mol) in water and octanol calculated for 15 PCB derivatives

PCB derivative	ΔG^{w}_{el}	ΔG^{o}_{el}	$\Delta G^{w}_{el,G}$	$\Delta G^{o}_{el,G}$
2-Cl	−10·55	−9·64	−129·14	−109·88
2,2′-Cl	−19·72	−18·04	−158·61	−134·69
2,3′-Cl	−19·89	−18·19	−165·82	−140·97
2,6-Cl	−18·55	−16·96	−156·29	−131·63
2,5,4′-Cl	−27·43	−25·08	−189·62	−161·46
2,4,4′-Cl	−27·61	−25·25	−190·34	−162·06
2,5,3′-Cl	−27·08	−24·76	−196·53	−166·97
2,3,2′,3′-Cl	−32·61	−29·82	−231·99	−195·13
2,3,2′,5′-Cl	−33·76	−30·87	−229·31	−193·14
3,4,3′,4′-Cl	−32·59	−29·80	−257·99	−219·13
2,5,2′,5′-Cl	−34·96	−31·96	−226·67	−191·56
2,5,3′,4′-Cl	−31·65	−28·94	−231·87	−196·45
2,4,2′,5′-Cl	−35·21	−32·19	−228·11	−192·27
2,4,2′,4′-Cl	−35·28	−32·26	−223·53	−188·90
2,3,4,2′,5′-Cl	−39·63	−36·23	−272·77	−227·97

We have chosen (i) a constant torsion angle $\Theta = 60°$, (ii) torsion angle optimized by the method of empirical potentials. The results of statistical analysis do not differ significantly, thus we further give only the results for $\Theta = 60°$. The pure charges applied in the calculation of the electrostatic contribution were obtained from the CNDO/2 semiempirical quantum-chemical method[63].

The electrostatic contributions calculated according to Eq. (*5*) are given in Table I along with the electrostatic contribution values $\Delta G_{el,G}$ calculated from the polarizable continuum model according to Eqs (*7*), (*8*) and (*9*) for water and octanol. In Table II, the dispersion-repulsion contributions ΔG_{dr} and cavitation contributions $\Delta G_{cav,X}$ calculated according to Sinanoglu (index S) and Pierotti (index P) are given. As it can be seen, the individual components are fairly different. The values of electrostatic and dispersion-repulsion contributions in water and in octanol (Tables I and II) seem to be correct. The electrostatic contributions, $\Delta G_{el,G}$, are larger (in absolute values) in comparison with those, ΔG_{el}, involving only the coulombic contributions. Also, the values of ΔG_{el} are much smaller than those of the dispersion-repulsion contribution. This is caused by the low polarity of PCB. The dispersion-repulsion for PCB with the serial number from 1 to 5 is larger in water than in octanol (in absolute value). On the contrary, for PCB with the serial number from 6 to 15 it is

TABLE II

Dispersion-repulsion, ΔG_{dr}, and cavitation contributions, $\Delta G_{cav,X}$ (calculated by the method of Sinanoglu[55], X = S, and Pierotti[56], X = P) to the Gibbs free energy of solvation (kJ/mol) in water and octanol calculated for 15 PCB derivatives

PCB derivatives	ΔG^{w}_{dr}	ΔG^{o}_{dr}	$\Delta G^{w}_{cav,S}$	$\Delta G^{o}_{cav,S}$	$\Delta G^{w}_{cav,P}$	$\Delta G^{o}_{cav,P}$
2-Cl	−116·71	−113·84	94·90	35·11	183·97	106·18
2,2′-Cl	−135·86	−135·00	100·56	36·83	191·94	111·00
2,3′-Cl	−140·65	−140·38	102·03	37·28	193·86	112·22
2,6-Cl	−137·92	−137·53	100·65	36·86	191·33	110·64
2,5,4′-Cl	−162·57	−165·18	109·41	39·53	204·24	118·31
2,4,4′-Cl	−162·03	−164·69	109·41	39·53	204·24	118·31
2,5,3′-Cl	−163·72	−166·19	109·34	39·51	204·10	118·29
2,3,2′,3′-Cl	−187·71	−190·99	112·10	40·35	206·40	119·95
2,3,2′,5′-Cl	−178·86	−183·38	112·92	40·60	207·81	120·79
3,4,3′,4′-Cl	−184·55	−189·57	116·28	41·62	213·87	124·23
2,5,2′,5′-Cl	−181·46	−186·15	113·87	40·89	209·45	121·66
2,5,3′,4′-Cl	−184·18	−189·25	115·69	41·44	212·67	123·49
2,4,2′,5′-Cl	−180·64	−185·40	113·87	40·89	209·45	121·66
2,4,2′,4′-Cl	−179·88	−184·82	113·94	40·91	209·60	121·68
2,3,4,2′,5′-Cl	−200·12	−207·17	119·29	42·54	216·41	125·99

larger in octanol than in water. This means that with increasing content of chlorine the "hydrophobicity" of molecules increases and thus the dispersion-repulsion contribution in octanol increases (see Tables I and II).

It is seen from Table II that the two different approaches of the evaluation of cavitation contribution give very different results. This problem has already been mentioned by other authors[64]. The difference between the two methods is still more evident if we compare the calculated values for water and octanol as a solvent. However, with the increasing number of chlorine atoms the cavitation contribution both in water and in octanol increases in both methods. As a consequence, this leads to better PCB solvation in octanol. At present, it is difficult to decide which of the two methods is more accurate. The decrease in the $\Delta G_{cav,P}$ values according to Pierotti when passing from water to octanol seems to be too great. Moreover, this cause that the ΔG_{solv} values are negative both in water and in octanol and are greater in the absolute value in octanol. Therefore, it is more important to evaluate the relative trends in the change of both the Gibbs energy of solvation and its individual contributions. The differences in the Gibbs energies of solvation, $\Delta G_S^{o/w}$ and $\Delta G_P^{o/w}$, (Table III) are both negative. With increasing number of chlorine atoms in PCB the stabilization in the nonpolar phase increases, which corresponds with the increase in the log k' values taken from ref.[59] (Table III).

TABLE III

Theoretically calculated differences between the total Gibbs free energies of solvation (kJ/mol) in octanol and water and measured values of capacity factors k' (ref.[59]) for 15 PCB derivatives

PCB derivative	$\Delta G_S^{o/w}$	$\Delta G_P^{o/w}$	k'_{exp}
2-Cl	−56·01	−74·01	1·01
2,2′-Cl	−61·18	−78·38	1·18
2,3′-Cl	−62·78	−79·67	1·36
2,6-Cl	−61·82	−78·72	1·30
2,5,4′-Cl	−70·14	−84·90	2·80
2,4,4′-Cl	−70·17	−86·23	2·90
2,5,3′-Cl	−69·99	−85·97	2·91
2,3,2′,3′-Cl	−72·24	−86·94	1·91
2,3,2′,5′-Cl	−73·95	−88·64	2·20
3,4,3′,4′-Cl	−76·90	−91·88	4·14
2,5,2′,5′-Cl	−74·68	−89·49	2·58
2,5,3′,4′-Cl	−76·60	−91·53	3·92
2,4,2′,5′-Cl	−74·73	−89·54	2·92
2,4,2′,4′-Cl	−74·85	−89·73	3·14
2,3,4,2′,5′-Cl	−80·40	−94·08	3·36

The relative trends may be assessed best by using linear regression. Therefore, correlations of the logarithm of capacity factors, log k', with calculated Gibbs energies of solvation or its contributions were evaluated for fifteen PCB derivatives by the least squares method. In the correlation equations, r is the correlation coefficient, S.R. is the regression significance, n is the number of points considered in the correlation, and $1 - \alpha$ is the level of significance. The correlation coefficient is a measure of relation between the experimental and the theoretical values. The regression significance makes it possible to find out the quality of correlation, i.e. $100\,(1 - \alpha)\,\%$ intervals of reliability in which the regression coefficients of the correlation equations can be found.

At first, we correlated the differences between the total Gibbs free energies of solvation in octanol and in water for the two approaches to the calculation of the cavitation contribution $\Delta G_S^{o/w}$ and $\Delta G_P^{o/w}$.

For the series of 15 PCB derivatives ($n = 15$, $1 - \alpha > 99{\cdot}99\%$) the following correlation equations are obtained (in kJ/mol):

type Ia

$$\log k' = -0{\cdot}0259\,\Delta G_S^{o/w} - 1{\cdot}4456 \tag{20}$$

$$r = 0{\cdot}910 \quad \text{S.R.} = 62{\cdot}3$$

type Ib

$$\log k' = -0{\cdot}0312\,\Delta G_P^{o/w} - 2{\cdot}3264 \tag{21}$$

$$r = 0{\cdot}926 \quad \text{S.R.} = 78{\cdot}56\,.$$

In both equations, the negative sign of the linear term is consistent with the physical concept. The higher values of the r and S.R. parameters for Eq. (*21*) suggest that Pierotti's approach gives a better estimate of the cavitation contribution to the Gibbs free energy of retention for the selected compounds.

A question about the absolute term arises when comparing Eqs (*20*) and (*21*) with the corresponding Eq. (*2*). This term probably expresses the absolute error of the approximations used in modelling the retention with respect to the process actually taking place.

Further we correlated the differences of particular components of the Gibbs free energy of solvation:

type IIa

$$\log k' = -0{\cdot}3680\,\Delta G_{el}^{o/w} - 0{\cdot}0184\,\Delta G_{dr}^{o/w} - 0{\cdot}0770\,\Delta G_{cav,S}^{o/w} - 4{\cdot}1884 \tag{22}$$

$$r = 0{\cdot}952 \quad \text{S.R.} = 35{\cdot}31$$

type IIb

$$\log k' = -0{\cdot}0693\,\Delta G_{el}^{o/w} + 0{\cdot}0770\,\Delta G_{dr}^{o/w} - 0{\cdot}1192\,\Delta G_{cav,P}^{o/w} - 9{\cdot}4553$$

$$r = 0{\cdot}963 \quad \text{S.R.} = 47{\cdot}12\,. \tag{23}$$

Theoretically, it is feasible to set up six-parameter correlation equations involving the Gibbs free energies of solvation in the two phases as the independent variables. Such equations might enable us to assess the effect of the individual contributions and to provide a physical substantiation of the trends. We obtained the following correlation equations:

type IIIa

$$\log k' = -35{\cdot}480\,\Delta G^{w}_{el} - 0{\cdot}0643\,\Delta G^{w}_{dr} - 2{\cdot}292\,\Delta G^{w}_{cav,S} + $$

$$+ 38{\cdot}8103\,\Delta G^{o}_{el} + 0{\cdot}0803\,\Delta G^{o}_{dr} + 7{\cdot}9046\Delta G^{o}_{cav,S} - 58{\cdot}2857 \qquad (24)$$

$$r = 0{\cdot}981 \quad \text{S.R.} = 24{\cdot}94$$

type IIIb

$$\log k' = -25{\cdot}5868\,\Delta G^{w}_{el} + 0{\cdot}0255\,\Delta G^{w}_{dr} + 0{\cdot}4077\,\Delta G^{w}_{cav,P} + $$

$$+ 27{\cdot}9894\,\Delta G^{o}_{el} - 0{\cdot}0246\,\Delta G^{o}_{dr} - 0{\cdot}6415\,\Delta G^{o}_{cav,P} - 6{\cdot}68773 \qquad (25)$$

$$r = 0{\cdot}994 \quad \text{S.R.} = 113{\cdot}86\,.$$

Further statistical analysis of the correlation equations showed the dominant role of the cavitation and dispersion-repulsion contributions in the separation process. It is also possible to use the above equations to calculate the "theoretical capacity factor", k'_{calc}, in good accordance with the experimental one, k'_{exp}.

A comparison of the capacity factors calculated from Eqs (*20*), (*21*) and (*25*) with those obtained experimentally is given in Table IV. It can be seen that the correlation equation (*25*) offers the best accordance of the experimental and theoretical values of the capacity factor k'. The differences between them are probably due to additional effects occuring in the chromatographic column during separation, such as adsorption, steric effects, etc., which are not included in the model. The quantitative relationships between the experimental chromatographic retention data and the calculated Gibbs free energies of retention are more complex than could be predicted by Eq. (*2*).

More Accurate Models of the Stationary and Mobile Phases

For modelling the stationary and mobile phases, we have chosen the following approaches:

a) the stationary phase was modelled as octanol and the mobile phase as water (the results are mentioned above);

b) the stationary phase was modelled as octanol and the mobile phase as 10 : 90 water–methanol mixture;

c) the stationary phase was modelled as heptane and the mobile phase as water.

TABLE IV

Experimental and theoretical values of capacity factors k' for 15 PCB derivatives

PCB derivative	k'_{exp}	k'_{calc}		
		Eq. (*20*)	Eq. (*21*)	Eq. (*25*)
2-Cl	1·01	1·01	0·96	0·96
2,2'-Cl	1·18	1·37	1·32	1·31
2,3'-Cl	1·36	1·51	1·44	1·34
2,6-Cl	1·30	1·43	1·35	1·31
2,5,4'-Cl	2·80	2·35	2·10	2·75
2,4,4'-Cl	2·90	2·36	2·31	2·94
2,4,4'-Cl	2·90	2·36	2·31	2·94
2,5,3'-Cl	2·91	2·33	2·27	2·69
2,3,2',3'-Cl	1·91	2·66	2·43	1·90
2,3,2',5'-Cl	2·20	2·95	2·75	2·18
3,4,3',4'-Cl	4·14	3·51	3·47	4·12
2,5,2',5'-Cl	2·58	3·08	2·92	2·77
2,5,3',4'-Cl	3·92	3·45	3·38	4·10
2,4,2',5'-Cl	2·92	3·08	2·93	2·74
2,4,2',4'-Cl	3·14	3·11	2·97	3·16
2,3,4,2',5'-Cl	3·36	4·33	4·06	3·35
Max. rel. error, %		28·20	28·19	9·92
Mean rel. error, %		17·56	17·69	4·61

TABLE V

Prediction for PCB derivatives

PCB derivatives	$\Delta G_{\mathrm{P}}^{\mathrm{o/w}}$ kJ/mol	k'_{calc} (Eq. (*21*))
Biphenyl	−70·60	0·75
2,3',4-Cl	−85·97	2·27
2,4,5-Cl	−86·22	2·31
2,2',3,4-Cl	−88·69	2·76
2,2',3,5'-Cl	−89·44	2·91
2,3,3',4-Cl	−90·56	3·17
2,2',3,3',5-Cl	−94·69	4·25

Modelling the stationary phase as octanol and the mobile phase as 1 : 9 water–methanol mixture[65–68], we obtained a correlation equation of type I

$$\log k' = -0{\cdot}0185\,\Delta G_S^{o/wm} - 0{\cdot}6683 \qquad (26)$$

$$r = 0{\cdot}870 \quad \text{S.R.} = 41{\cdot}59\,.$$

By comparing with the previous models it follows that this model does not lead to an improvement of the correlation equations. In the type I and III equations the significance of regression decreases, in the type II equations correlation is slightly improved and the significance of regression increases.

By modelling the stationary phase as heptane, the following type I equation was obtained:

$$\log k' = -0{\cdot}0139\,\Delta G_S^{h/w} + 0{\cdot}0259 \qquad (27)$$

$$r = 0{\cdot}813 \quad \text{S.R.} = 25{\cdot}44\,.$$

In this case the correlation parameters of types II and III equations are higher than in case *b*), however, those of type I are lower.

Generally, these models of stationary and mobile phases (modelling in cases *b*) and *c*)) do not improve significantly the correlations between calculated and experimental data. Further development of more sophisticated models is therefore needed.

Prediction of Retention Data for Further PCB Derivatives

Due to high statistical significance mainly of Eqs (*21*) and (*25*) it is possible to use our approach for the prediction of retention data for those PCB derivatives for which experimental values of log *k'* are not known or which have not been synthesized.

We calculated the correlation Eq. (*21*) type I and the capacity factors *k'* for further derivatives for which the experimental data are not known. The calculated Gibbs free energies of retention and the predicted logarithms of capacity factors are in Table V. Further experiments are needed to substantiate these predictions.

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