Development and Validation of a Procedure for Estimating the Hydrophobicity of Structurally Unrelated Compounds by Micellar Liquid Chromatography

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Abstract

Reversed-phase liquid chromatography has been used most often to estimate values of log *P*, but despite years of study, there is no universally accepted method of performing these estimations. The main problem has to do with the fact that the hydrophobic parameter, log k_w, depends on the hydrogen bond acceptor–donor character of the compounds. The use of micellar mobile phases to perform these estimations is evaluated here, and the influence of the nature of the surfactant (anionic, cationic, and nonionic) on the log *k*–log *P* relationships is studied. The use of a nonionic surfactant, such as Brij35, to prepare the mobile phases provided adequate results regardeless of the hydrogen bond acceptor–donor character of the compounds, whereas noncongener behaviors are found with anionic and cationic surfactants. This enabled the establishment of a calibration set consisting of 7 compounds with variable hydrophobicity in order to calibrate new columns and predict log *P* values. In these conditions, the hydrophobicity of structurally unrelated compounds in the –0.1 < log *P* < 4.5 range can be measured. To evaluate the accuracy of predictions, the hydrophobicity of 27 compounds is determined and compared with the log *P* values calculated using the ACD–log *P* program. Nonsignificant differences between the predicted and the theoretical log *P* values were achieved at 95% probability level.

Introduction

Predictions of toxicity, reactivity, and transport parameters are in great demand in pharmaceutical and environmental areas. Quantitative structure–activity relationships (QSARs) represent the entire area of such estimations (1–4). Among the descriptors used in QSAR correlations, the hydrophobic parameter, log *P*, usually expressed as the partition coefficient in the octanol–water system, is the most popular. However, the shake-flask technique that is used to measure the log *P* values is prone to errors and experimental problems, and alternative methods for estimation have been reported (5).

The retention of a compound on reversed-phase liquid chromatography (RPLC) has been used as a hydrophobic parameter. Although the RPLC method is convenient and simple to use, however, a universal procedure including the RPLC conditions for simulating log *P* has not yet been established (6–8). It has been reported that in conventional RPLC, compounds with similar log *P* values and different hydrogen bond acceptor–donor character show different retention behavior. It was demonstrated that the log k_w value gives accurate estimates of log *P* for compounds free from strong hydrogen bounders, whereas it tends to overestimate the log *P* value of hydrogen acceptors (HBA compounds) and underestimate that of hydrogen donors (HBD compounds) (9,10).

The use of surfactants at concentrations above the critical micellar concentration (cmc) as mobile phases in micellar liquid chromatography (MLC) provides several advantages for estimating the hydrophobicity of compounds. The adsorption of surfactant monomers on the stationary phase reduces silanophilic interactions and increases the hydrophobicity of the stationary phase. The usefulness of MLC has been reported (11–19), but thorough studies of the influence of the nature of surfactants on hydrophobicity estimations have not been carried out.

Yang et al. (20) studied the relationships between the logarithm of retention factors, log *k*, in micellar electrokinetic chromatography (MECK) and the log *P* values for 60 aromatic compounds using cetyltrimethylamonium (CTAB) as a cationic surfactant and 3 different anionic surfactants: sodium dodecylsulphate (SDS), sodium cholate, and lithium perfluorooctane sulphonate. In the SDS system, 3 different lines were recognized for the congener subgroups of compounds. This behavior was explained as being caused by the hydrogen bond donor characteristic of SDS micelles that selectively differentiate between the solutes with different hydrogen bond acceptor strength, and they concluded that retention in this system is not based solely on hydrophobicity. A similar result

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was observed for a CTAB–MEKC system, but the hydrogen bond acceptor characteristic of CTAB selectively differentiates between the solutes with different hydrogen bond donor strength.

In the present report, the influence of the nature of the surfactant (anionic, cationic, and nonionic) on the chromatographic estimation of the hydrophobicity of aromatic compounds with different hydrogen bond acceptor–donor characters is studied. For each surfactant, structural similarities between compounds are evaluated, and relationships between log *P* and log *k* are established. Finally, a calibration set of compounds is proposed and checked in order to established a general procedure for estimating the hydrophobicity of any compound.

Experimental

Instrumental and measurement

A Hewlett-Packard (Palo Alto, CA) HP 1100 chromatograph with an isocratic pump, an ultraviolet (UV)–visible detector, and an HP Vectra computer was used. Data acquisition was done with the HP ChemStation software (1996 version, Hewlett-Packard). The solutions were injected into the chromatograph through a Rheodyne (Rohnert Park, CA) valve with a 20-µL loop. For each surfactant, a Spherisorb octadecylsilane ODS-2 C_{18} column (120 × 4 mm, 5-µm particle size) and the corresponding guard columns of similar characteristics (35 × 4 mm) were used. The mobile phase flow rate was 1 mL/min. The detection was performed in UV at 254 nm. All the assays were carried out at room temperature (20 ± 2°C). The retention factors (*k* values) determined in this study were averages of at least triplicate determinations. The dead time value, t_m, was determined for each injection as the first perturbation in the chromatogram.

Reagents and standard

Mobile phases were prepared by aqueous solutions of polyoxyethylene(23) lauryl ether (Brij35) (Acros Chimica, Geel, Begium), SDS (Merck, Dietikon, Switzerland), and CTAB (Acros). The pH of the micellar eluent was adjusted to 7.4 and 3.5 with 0.05M phosphate buffer. In order to adjust the ionic strength to 0.2M, NaCl was added to the micellar mobile phase.

Nineteen aromatic compounds with different acceptor–donor hydrogen bond properties were selected. Acetanilide, acetophenone, aniline, *p*-nitroaniline (Scharlau, Barcelona, Spain) and caffeine (Fluka, Buchs, Switzerland) were selected as the aromatic compounds with a hydrogen bond acceptor character. Acetylsalicylic acid, salicylic acid (Panreac, Barcelona, Spain), benzoic acid, 2-iodobenzoic acid, pyrogallic acid (Scharlau), phenol (Probus, Badalona, Spain), and resorcinol (Doesder, Barcelona, Spain) were selected as the aromatic compounds with a hydrogen bond donor character. Anthracene, biphenyl, bromobenzene, naphthalene, pyrene (Scharlau), benzene, and toluene (Probus) were selected as the aromatic compounds with nonhydrogen-bond properties. Table I shows the logarithm of protonation constants, log *k*, and the log *P* values for the nonionic forms of the aromatic compounds studied (21,22).

Stock standard solutions of aromatic compounds were prepared by dissolving 10 mg of the compound in 10 mL of phosphate buffer (or in acetonitrile for highly hydrophobic compounds). Working solutions were prepared by diluting the stock standard solutions using phosphate buffer solution or acetonitrile. The solutions were stored in the refrigerator at 4°C.

Barnstead (Dubuque, IA) E-pure deionized water was used throughout. The mobile phase and the solutions injected into the chromatograph were vacuum filtered through 0.45-µm and 0.22-µm nylon membranes, respectively.

Software and data processing

The log *P* values for the nonionic forms of the aromatic compounds and the protonation constants of the compounds were taken from the literature (21) or calculated by means of the ACD–log *P* program (22). For compounds partially ionized at the working pH, the apparent log *P* values were calculated according to the following equation:

$$\log P_{app} = \log P + \log (\beta_i h^i/(1 + \beta_1 h + \beta_2 h^2 + ... + \beta_n h^n) \qquad \text{Eq. 1}$$

where β_i is the accumulated protonation constant of the neutral form of the compound for the *n*-protic system and $b_n = K_1 K_2 K_3 ... K_n$.

Excel 7.0 Microsoft Office (Microsoft, Redmond, WA) software was used to perform the statistical analysis of the linear regressions.

Results and Discussion

Retention behavior of aromatic compounds

The retention of the selected aromatic compounds was measured using mobile phases containing different concentrations of a nonionic surfactant, (0.02, 0.04, and 0.06M Brij35), an anionic surfactant (0.075, 0.10, and 0.15M SDS), and a cationic surfactant (0.02, 0.04, and 0.05M CTAB). The mobile phase pH was adjusted to 7.4, except for the acidic compounds; for those compounds, the mobile phase pH was adjusted to 3.5 in order to increase their retention. As can be expected, for the highly hydrophobic compounds studied, large changes in the retention were obtained upon increasing the surfactant concentration in the mobile phase, whereas the retention of the slightly hydrophobic compounds was scarcely modified. This behavior indicates, as expected, that the eluent strength of the surfactant increases as the compound hydrophobicity increases.

The experimental values (log *k* and surfactant molar concentration C_s) were adjusted to the equation $\log k = \log k_w - S C_s$, where $\log k_w$ is the logarithm of the retention factor in the absence of surfactant and C_s is the total concentration of surfactant. Table II shows the intercept and slope values, as well as the regression statistics. In all cases, appropriate correlation coefficients *r* were obtained ($r > 0.99$). As can be expected, the

log k_w and S values increased as the hydrophobic character of the compounds increased.

The log k_w values obtained for the neutral compounds for each surfactant were similar. However, for compounds with a hydrogen bond donor character, the log k_w values obtained for CTAB were larger than those obtained with Brij35 and SDS. This behavior could indicate that the retention of compounds not only depends on hydrophobic interactions but also on the electronic interactions between compounds and surfactant modified stationary phase.

Structural similarities between compounds were investigated using a micellar mobile phase in a way similar to the conventional RPLC system (23). For this purpose, the experimental S and log k_w values (Table II) were adjusted using the following equation:

$$S = a + b \log k_w \qquad \text{Eq. 2}$$

Table III shows the intercept and slope values obtained by adjusting the pairs of data S, log k_w of all compounds (global model), and those obtained by adjusting the pairs of data corresponding to the different groups of compounds. As can be observed, when SDS was used, adequate correlations were obtained for each group of compounds (r = 0.89–0.97). However, the correlation obtained for the global model was poor (r = 0.35), indicating that the hydrogen bond interactions have a great influence on the retention behavior in MLC using this surfactant. Similar results were observed for CTAB.

In contrast, when Brij35 was used as the micellar mobile phase, similar and adequate correlations were obtained for the global model and for the models obtained with the different groups of compounds (r = 0.83–0.97). This indicates the existence of structural similarities between the compounds.

Table I. Aromatic Compounds, log *P* Values, and Logarithm of Protonation Constant (log *k*)

Compound	Symbol	Group	log *P*	log *k*
Acetanilide	A1	acceptor	1.21	0.50
Acetophenone	A2	acceptor	1.66	–
Aniline	A3	acceptor	0.90	4.63
Caffeine	A4	acceptor	0.07	14.0, 0.6
p-Nitroaniline	A5	acceptor	1.33	0.991
Acetylsalicylic Acid	D1	donor	1.23	3.5
Benzoic Acid	D2	donor	1.89	4.20
Phenol	D3	donor	1.48	9.89
2-Iodobenzoic Acid	D4	donor	2.40	2.85
Pyrogallic acid	D5	donor	0.29	9.85
Resorcinol	D6	donor	0.80	11.32, 9.15
Salicylic Acid	D7	donor	2.06	13.4, 2.97
Anthracene	N1	neutral	4.54	–
Benzene	N2	neutral	2.13	–
Biphenyl	N3	neutral	4.06	–
Bromobenzene	N4	neutral	2.99	–
Naphthalene	N5	neutral	3.35	–
Pyrene	N6	neutral	4.88	–
Toluene	N7	neutral	2.74	–

Table II. logk_w and *S* Values for the Aromatic Compounds Eluted with Different Surfactants

	SDS		Brij35		CTAB	
Compound	$S \pm s$	log $k_w \pm s$	$S \pm s$	log $k_w \pm s$	$S \pm s$	log $k_w \pm s$
Acetanilide	4 ± 2	1.4 ± 0.3	6.4 ± 0.5	1.07 ± 0.02	7.5 ± 0.3	1.367 ± 0.010
Acetophenone	4.4 ± 0.3	1.83 ± 0.03	6.6 ± 0.3	1.593 ± 0.014	3.5 ± 0.9	1.39 ± 0.03
Aniline	1.51 ± 0.07	1.026 ± 0.008	5.7 ± 1.7	1.09 ± 0.07	4.8 ± 0.5	1.206 ± 0.016
Caffeine	5.82 ± 0.17	1.807 ± 0.018	2.4 ± 0.3	0.438 ± 0.016	7.7 ± 1.2	0.61 ± 0.04
p-Nitroaniline	2.76 ± 0.08	1.083 ± 0.008	11 ± 3	1.56 ± 0.11	11.7 ± 1.1	1.71 ± 0.04
Anthracene	4.5 ± 0.6	2.22 ± 0.06	12.1 ± 0.9	2.26 ± 0.04	15 ± 4	2.21 ± 0.15
Benzene	1.718 ± 0.009	1.4371 ± 0.0009	7.7 ± 1.0	1.74 ± 0.04	6 ± 2	1.55 ± 0.08
Biphenyl	4.0 ± 0.7	2.10 ± 0.08	13 ± 2	2.35 ± 0.09	12 ± 5	2.12 ± 0.16
Bromobenzene	3.24 ± 0.17	1.988 ± 0.018	8.4 ± 0.6	2.08 ± 0.03	12 ± 3	2.05 ± 0.11
Naphthalene	3.9 ± 0.6	1.99 ± 0.07	11.6 ± 1.5	2.12 ± 0.06	12 ± 4	2.03 ± 0.14
Pyrene	5.6 ± 0.4	2.33 ± 0.04	14 ± 2	2.36 ± 0.09	15 ±6	2.2 ± 0.2
Toluene	2.72 ± 0.15	1.719 ± 0.017	12 ± 5	2.1 ± 0.2	10 ± 4	1.87 ± 0.12
Acetylsalicylic acid	11.8 ± 0.5	1.55 ± 0.06	6.3 ± 0.9	1.07 ± 0.04	39 ± 19	2.7 ± 0.7
Benzoic acid	8.19 ± 0.12	1.624 ± 0,013	8 ± 2	1.38 ± 0.09	17 ± 4	2.10 ± 0.14
Phenol	2.7 ± 0.3	1.06 ± 0.04	9.6 ± 1.3	1.45 ± 0.06	12 ± 2	1.82 ± 0.08
2-Iodobenzoic acid	14.5 ± 0.5	1.94 ± 0.05	10 ± 3	1.52 ± 0.12	11 ± 3	2.24 ± 0.10
Pyrogallic acid	—	—	5.6 ± 0.4	0.884 ± 0.018	16 ± 3	1.55 ± 0.11
Resorcinol	—	—	9 ± 2	1.26 ± 0.10	13 ± 2	1.84 ± 0.07
Salicylic acid	3 ± 2	1.0 ± 0.2	10 ± 2	1.45 ± 0.09	18.56 ± 0.1	2.364 ± 0.004

Table III. Intercept (*a*) and Slope (*b*) Values Obtained by Adjusting the Pairs of Data *S* and log k_w to the Equation $S = a + b \log k_w$

Surfactant	Group	$b \pm S_b$	$a \pm S_a$	*r*
SDS (donor)	H-Acceptors	4.0 ± 1.0	–2.0 ± 1.4	0.89
	H-Donors	12 ± 2	–9 ± 3	0.96
	Neutral	4.0 ± 0.5	–4.2 ± 0.9	0.97
	Global	2.7 ± 1.7	0 ± 3	0.35
CTAB (acceptor)	H-Acceptors	7 ± 4	1 ± 5	0.67
	H-Donors	12 ± 4	–6 ± 8	0.78
	Neutral	12.8 ± 1.3	–14 ± 3	0.97
	Global	11 ± 3	–8 ± 6	0.73
Brij35	H-Acceptors	5.3 ± 1.8	0 ± 2	0.83
	H-Donors	9.0 ± 0.9	–3.3 ± 1.1	0.97
	Neutral	10 ± 2	–10 ± 5	0.88
	Global	5.2 ± 0.8	0.8 ± 1.4	0.88

Table IV. Explained Variance Corresponding to Each Principal Component

nºr PC	Eigen value	% Explained variance	% Accumulated explained variance
1	8.43	49.57	49.57
2	4.43	25.98	75.55
3	2.45	14.39	89.95
4	7.75E-1	4.56	94.51
5	6.43E-1	3.78	98.29
6	1.46E-1	0.86	99.15
7	5.52E-2	0.32	99.47
8	4.39E-2	0.26	99.73
9	2.63E-2	0.15	99.89
10	7.45E-3	0.04	99.93

Retention–structure relationships

The possibility of predicting the retention behavior of compounds from the physico-chemical properties and experimental conditions was evaluated. Prior to the study of the regression models, an exploratory data analysis was carried out. Principal component analysis (PCA) was applied to the retention data of the aromatic compounds with different concentrations of SDS (variables 01–03), Brij35 (variables 04–06), CTAB (variables 07–09), and several molecular descriptors (22) in order to establish the relationships between variables. The molecular descriptors used were log *P* (variable 10) as hydrophobic parameter; molecular weight (variable 11), molar refractivity (variable 12), molar volume (variable 13), and parachor (variable 14) as steric descriptors; the polarizability (variable 18) as an electronic parameter; and other physical properties, such as refraction index (variable 15), surface tension (variable 16), and density (variable 17). Because the variables are in different scales, the data were autoscaled before applying the PCA model. Table IV shows the explained variance corresponding to each principal component. Three principal components explain more than 89% of the variance. The use of the first four latent variables accounts more than 94% of variance in the data. Figure 1 shows the loading plot corresponding to the first two principal components (upper part) and that corresponding to the third and first principal components (lower part). The retention of compounds obtained using SDS, Brij35, and CTAB mobile phases (variables 01–09) correlate well with log *P* (variable 10); the highest correlation degree was for Brij35, as can be observed in the PC3–PC1 plot.

On the other hand, Figure 1 indicates that a poor correlation exists between the retention of compounds and the other

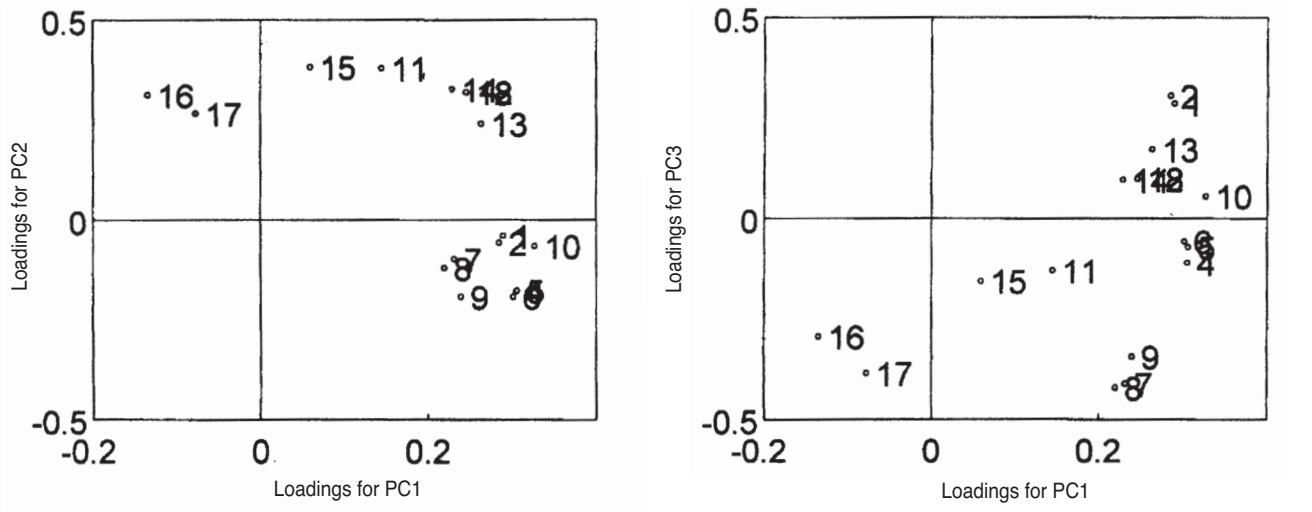

Figure 1. Loading plots corresponding to the PCA analysis (number corresponds to variables).

descriptors. These results could indicate that the retention of compounds can be explained by means of univariate models of log k = f(log P) type.

Figure 2 shows the log k–log P relationships obtained using the retention data of aromatic compounds in different micellar media: 0.02M CTAB, 0.075M SDS, and 0.02M Brij35. Several conclusions can be obtained. Using CTAB as micellar eluent (Figure 2A), the retention of compounds with hydrogen bond donor character was generally higher than can be expected from their log P values. This behavior could be due to the formation of hydrogen binding between CTAB (surfactant) and compounds with hydrogen bond donor character. For SDS, surfactant with hydrogen bond acidity character or hydrogen bond donor, the retention of the compounds with hydrogen bond acceptor character was slightly higher than that corresponding to donor or neutral compounds with similar log P values (Figure 2B). Similar behaviors were obtained for the other surfactant concentrations used.

These results are in agreement with the conclusions previously reported by Lavine et al. (13) and Yang et al. (20) for ionic surfactants. From the results, it can be concluded that the use of SDS and CTAB is not appropriate for establishing the general

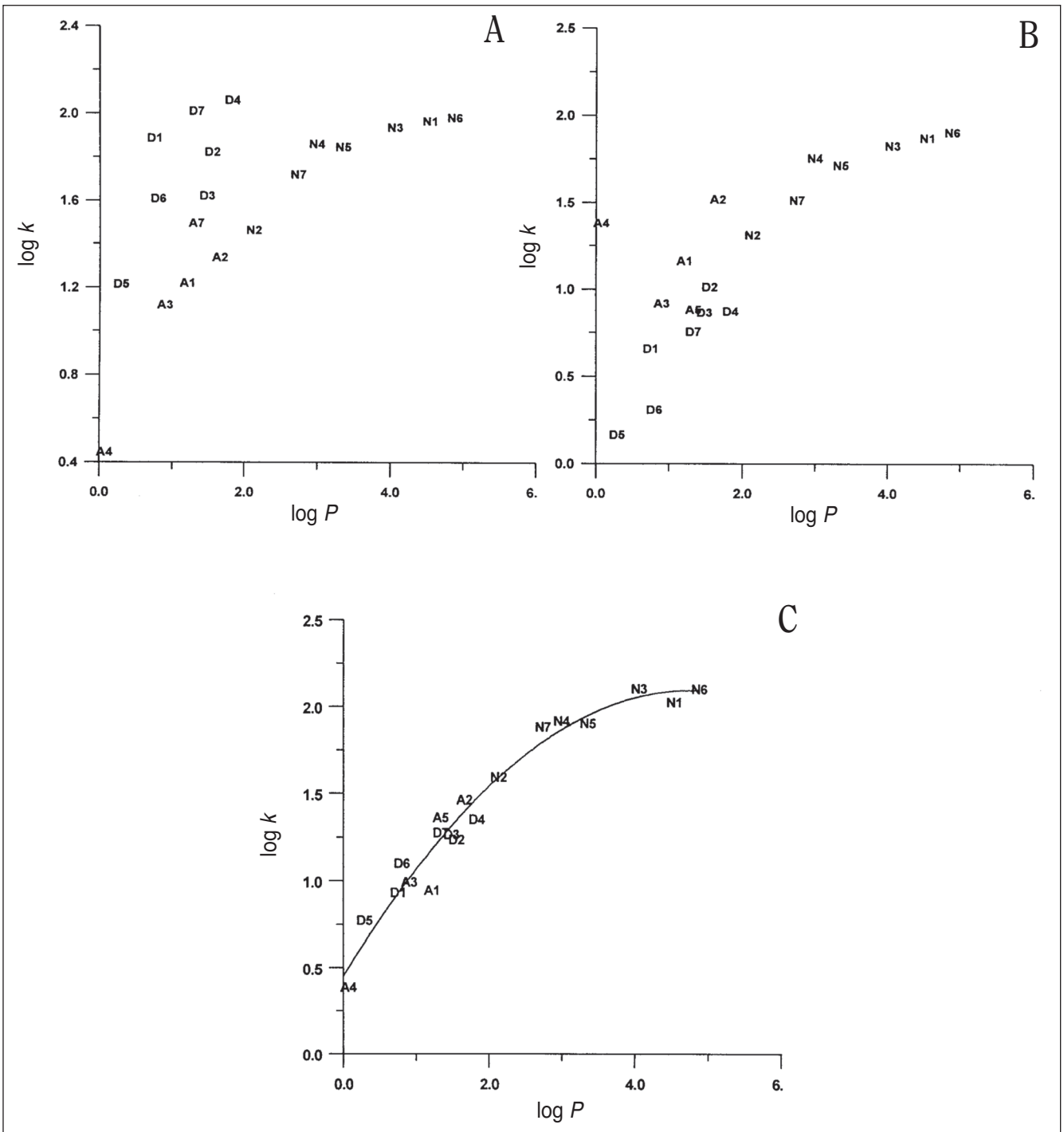

Figure 2. log k–log P relationships: 0.02M CTAB (A), 0.075M SDS (B), and 0.02M Brij35 (C) (see Table I).

Table V. Fitting Parameter Obtained by Adjusting the Retention Data of Compounds Obtained Using Different Concentrations of Brij35 in the Mobile Phase for Equation log $k = n + m_1 \log P + m_2 (\log P)^2$

Concentration	$m_1 \pm ts_{m1}$	$m_2 \pm ts_{m2}$	$n \pm ts_n$	r^2
0.02M	0.66 ± 0.19	–0.063 ± 0.004	0.47 ± 0.17	0.95
0.04M	0.53 ± 0.19	–0.05 ± 0.04	0.39 ± 0.16	0.94
0.06M	0.52 ± 0.18	–0.05 ± 0.04	0.31 ± 0.16	0.93

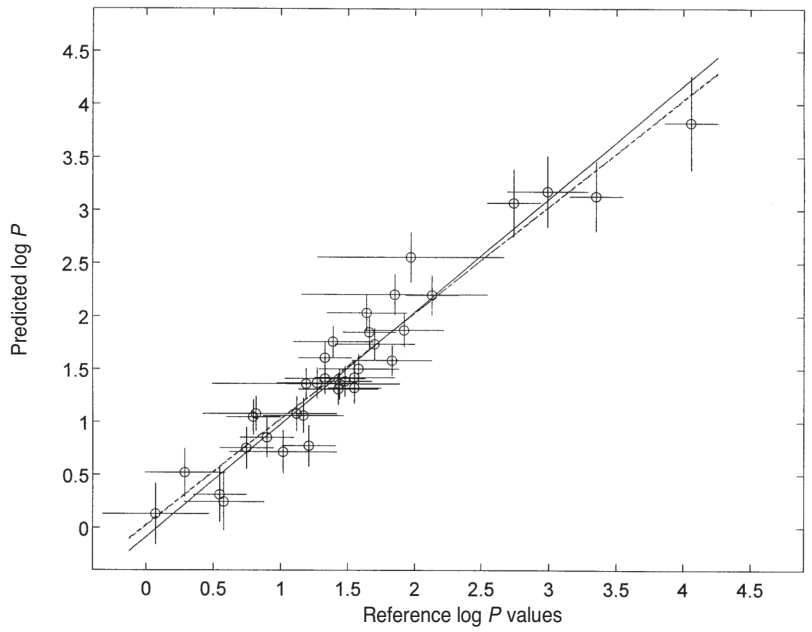

Figure 3. Predicted versus reference log *P* values of 27 compounds.

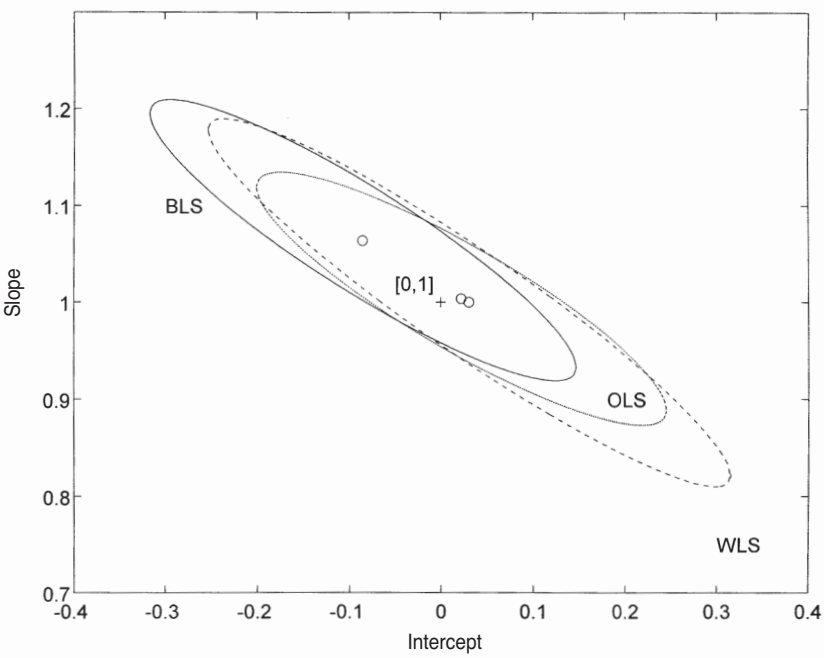

Figure 4. Covariance between the slope and the intercept.

conditions needed to determine the hydrophobicity of compounds.

In contrast, when Brij35 was used (Figure 2C), a single line described the relationship between retention in MLC and hydrophobicity for the group of aromatic compounds studied, regardless of their hydrogen bond acceptor–donor character. This result is in agreement with those indicated by Quina et al. (24) who, by comparing the solubilizing characteristics of SDS, CTAB, DTAB, and Brij35, concluded that Brij35 micelles should provide the best general solubilization medium for the widest variety of solutes.

As can be observed in Figure 2, the log *k*–log *P* relationship obtained for Brij35 was not linear in the log *P* range studied from –1 to 5. This has been reported previously (12,15,17), and it is due to the fact that the retention of a solute in MLC depends on two competitive equilibria (the interaction of the solute with micelles and with the stationary phase), and both processes depend on hydrophobicity.

In order to obtain predictive retention models, the retention of compounds obtained for different concentrations of Brij35 in mobile phase, log *k* values, and log *P* values were adjusted to different mathematical models. In a previous paper,it was demonstrated (17) that the log *k*–log *P* relationships in MLC can be described by the following equation:

$$\log k = n + m_1 \log P + \log (1 + m_2[M] + [M]10^{m_3 \log P}) \quad \text{Eq. 3}$$

where n, m_1, m_2, and m_3 are fitting parameters, and $[M]$ is the micellar concentration in the mobile phase. However, in spite of the fact that the experimental data fit the model well, the parameter estimations by means of iterative procedures did not produce stable results, because the model did not converge. A parabolic function of the type $\log k = n + m_1 \log P + m_2 (\log P)^2$ provided adequate results. Table V shows the regression statistics obtained by applying multiple linear regression. As can be observed, the correlation coefficients obtained were adequate ($r > 0.9$) for all the Brij35 concentration in the mobile phases assayed. On the other hand, the chromatographic system is less sensitive to hydrophobicity as the surfactant concentration in the mobile phase increases, as indicated by the variation in the fitting parameter m_1. The

applicability upper limit of the model is determined by the maximum of the parabola. The log *P* values corresponding to the function maximum were 4.68, 4.84, and 4.75 for 0.02, 0.04, and 0.06M Brij35 concentrations, respectively.

Hydrophobicity estimations of compounds using Brij35 mobile phases

One of the aims of this study was to find experimental conditions and a model able to predict the hydrophobicity of structurally unrelated compounds from the experimental retention data in MLC. The results shown above indicate the possibility of performing the hydrophobicity estimation of compounds from retention data in MLC using Brij35 as micellar mobile phase and C_{18} as stationary phase.

For this purpose, the retention data corresponding to a single mobile phase was adjusted to a parabolic model in a way similar to that used to model the retention. Equation 4 shows the results obtained using the retention data for a 0.02M Brij35 concentration:

$$\log P = 0.2\ (\pm\ 0.8) - 0.2\ (\pm\ 1.3)\ \log k + 0.9\ (\pm\ 0.5)(\log k)^2,\ r^2 = 0.96 \qquad \text{Eq. 4}$$

where the numbers in brackets represent the confidence interval of estimates at a 95% probability level. As can be observed, the fitting parameters associated with the independent term and log *k* were not statistically significant. Therefore, the data were adjusted to Equation 5:

$$\log P = 0.86\ (\pm\ 0.02)(\log k)^2,\ r^2 = 0.96 \qquad \text{Eq. 5}$$

Equation 5 was obtained using the retention data of compounds with log *P* values lower than 4.1. Compounds with larger log *P* values deviated from the model, although the correlation coefficients obtained including these compounds (anthracene and pyrene) were adequate (r^2= 0.94). Using the retention data corresponding to 0.04 and 0.06M Brij35 micellar mobile phases, similar equations were obtained, but the correlation coefficients were lower (r^2 = 0.92 and 0.90, respectively).

On the basis of the adequate results obtained, the question of whether the use of the retention data corresponding to a reduced number of compounds could permit the hydrophobicity estimation of other compounds from their retention data was assessed. Seven compounds with different hydrogen bond acceptor–donor characters and log *P* were selected. The selected compounds were caffeine (log *P* = 0.07), pyrogallic acid (log *P* = 0.29), acetanilide (log *P* = 1.21), acetophenone (log *P* = 1.66), benzene (log *P* = 2.13), bromobenzene (log *P* = 2.99) and biphenyl (log *P* = 4.06). Using the retention data of these compounds for a 0.02M Brij35 mobile phase, an equation similar to Equation 5 was obtained:

$$\log P = 0.86(\pm\ 0.04)(\log k)^2,\ \ r^2 = 0.97 \qquad \text{Eq. 6}$$

The ability of the model to predict the hydrophobicity of new compounds was evaluated. For this purpose, from the log *k* values of 27 structurally unrelated compounds with different hydrogen bond character (13 barbiturates, 4 local anesthetics, and 10 aromatic compounds not included in the calibration set) obtained using a 0.02M Brij35 mobile phase, the log *P* values with their uncertainties were predicted. Figure 3 shows the predicted log *P* values versus the reference log *P* values provided by the ACD–log *P* program. For partially ionized compounds (i.e., some barbiturates, local anesthetics, and aromatic compounds with hydrogen donor character), the apparent log *P* values at the mobile phase pH were used. These values were calculated from the reference log *P* values using Equation 1.

Finally, in order to validate the accuracy of the procedure in the log *P* range studied ([0,4]), the predicted and the theoretical log *P* values were compared using the classic ordinary least-square (OLS) and the weighted least squares (WLS) methods and an alternative test suggested by Rius et al. (BLS method) (25).

The classic methods compare the intercept and slope values obtained by linear calibration with the theoretical values of zero and unity, respectively. However, both procedures have the drawback of considering the reference method (usually represented on the abscissa axis) as being free not only of systematic errors but of random errors, but these errors could be of the same order of magnitude as the new method to be validated. The BLS method is based on the joint confidence interval for the slope and the intercept of the regression line, which is calculated taking the uncertainties in both axes into account. The slope, intercept, and variances that are associated with the regression coefficients are calculated with bivariate least-squares regression.

Figure 4 shows the joint confidence intervals at 95% probability level obtained by applying the three regression techniques indicated. It can be seen that the joint confidence interval obtained by BLS, OLS, and WLS methods included the theoretical point ([0,1]) indicated the absence of significant differences between the estimated log *P* values obtained by proposed procedure and the theoretical values. The equation of the fitted line obtained by applying the BLS method was as follows:

$$\log P_{predicted} = -0.09\ (\pm\ 0.09) + 1.06\ (\pm\ 0.06)\ \log P_{reference} \qquad \text{Eq. 7}$$

Conclusion

A chromatographic procedure for estimating the hydrophobicity of structurally unrelated compounds with different hydrogen bond acceptor–donor characters is proposed. The procedure that uses Brij35 as a surfactant to prepare the micellar mobile phase and C_{18} as a stationary phase is accurate and provides results similar to those obtained with the ACD–log *P* program, with the advantage that is able to estimate the log P_{app} values at any pH. A reduced number of compounds can be used as a calibration set to predict the hydrophobicity of compounds with different structures and properties, and it could be used as a reference to calibrate new columns that would make it possible to compare the results obtained with different columns. The proposed procedure is superior to the

use of conventional mobile phases, because the behavior of structurally unrelated compounds is not homogeneous using hydroorganic mobile phases.

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