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Journal of Chromatography A, 778 (1997) 67–75

JOURNAL OF CHROMATOGRAPHY A

Reliability of the capacity factor at zero micellar concentration and the solute–micelle association constant estimates by micellar liquid chromatography

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Abstract

In micellar liquid chromatography, MLC, the hydrophobicity of a compound is the predominant effect on its retention and interaction with micelles. The capacity factors at zero micellar concentration, k_m, and the solute–micelle association constants, K_{AM}, have recently been used as the hydrophobicity index of compounds and are important in QSAR studies. These parameters could be estimated (by regression) from the (k,[M]) data, where k is the capacity factor and [M] the surfactant concentration minus the critical micelle concentration. k_m and K_{AM} are usually obtained from the intercept and slope, respectively, of the plot $1/k$ vs. [M]. In spite of the general use of this equation, the reliability of the estimates has not received special attention. Two new regression models have been proposed: a nonlinear model (plot k vs. [M]) and a new linear model (k vs. k·[M]). The reliability of the k_m and K_{AM} estimates is studied with the aid of simulated data, and the conclusions obtained from the theoretical study are contrasted with experimental data. The influence of variables like the hydrophobicity of compounds, the K_{AM}/k_m ratio, the uncertainty of experimental k data and the experimental design have been considered. Some critical aspects of the models and recommendations for their use are provided. © 1997 Elsevier Science B.V.

Keywords: Retention factor; Hydrophobicity; Solute–micelle association constants; Association constants; Computer simulation; Regression analysis; Micellar liquid chromatography

1. Introduction

The biological activities of solutes are in most cases related to their physico–chemical properties. Compounds' hydrophobicity, expressed as the partition coefficients in the biphasic solvent system octanol–water, log P, is of great importance in quantitative–structure activity relationship (QSAR) studies, in drug design and toxicology [1–4]. The determination of log P using the traditional shake-flask method has several drawbacks, and different approaches have been proposed for estimating it [5]. Since the retention of a compound on reversed-phase liquid chromatography (RPLC) is governed by hydrophobic interactions, a linear relationship between the logarithm of the capacity factor and log P could be expected. This method has received much attention, but certain problems have been reported [6,7]. For instance, hydrophobicity estimations depend on the mobile phase composition and nature of the stationary phase, and for alkyl-bonded stationary phases, distinct relationships are observed for different groups of congeneric compounds, mainly due to

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PII S0021-9673(97)00545-1

the presence of free silanol groups on the stationary phase.

The use of micellar mobile phases instead of the traditional hydro-organic mobile phases to quantify hydrophobicity has several advantages. The first is that the retention behaviour of compounds (apolar, polar or ionic) chromatographed with anionic, cationic and non ionic surfactants has been accurately modelled [8,9]. Thus, the retention of a nonionizable compound as a function of micellar concentrations can be deduced by:

$$k = \frac{k_m}{1 + K_{AM}[M]} \quad (1)$$

where k is the capacity factor, [M] is the total concentration of surfactant in the mobile phase minus critical micellar concentration (CMC). K_{AM} is the solute–micelle association constant and k_m is the capacity factor at zero micellar concentration. k_m represents the partition coefficient of solute between the modified stationary phase and water multiplied by the phase ratio, $k_m = \phi P_{SW}$. Other advantages are that the adsorption of surfactant monomers on the alkyl-bonded stationary phase reduces silanophilic interactions and increases the hydrophobicity of stationary phase [10], and that the stationary phase environment in micellar liquid chromatography (MLC) is independent of the micelle concentration in the mobile phase and is quite similar to a purely aqueous eluent system. Finally, micelles are amphiphilic aggregates with anisotropic microenvironments that provide both hydrophobic and electrostatic sites of interaction. Structurally they are more similar to biomembranes than 1-octanol or RPLC stationary phases [11].

The usefulness of MLC in estimating a compound's hydrophobicity has been reported by several authors [11–17]. In a previous paper an empirical model that describes the dependence between retention in MLC and log P was proposed. It was also demonstrated that k_m and K_{AM} are adequate indexes for chromatographically quantifying the hydrophobicity of solutes using micellar mobile phases, i.e. log k_m was the parameter showing the best correlation with log P [18].

The k_m and K_{AM} values are usually obtained by the intercept and slope, respectively, of a plot $1/k$ vs. [M]. This approach has been used for many different types of solutes, surfactants and stationary mobile phases [13,16,19–21], but in spite of this widespread use, the statistics on the estimates have not received special attention.

In this paper we have examined the reliability of k_m and K_{AM} estimations of compounds in MLC. Three different models were assayed, and a statistical study of the accuracy and the uncertainty of the parameters was carried out. The simulation study includes the influence of variables like the hydrophobicity of the compounds (in terms of k_m values), the K_{AM}/k_m ratio, precision in experimental k measurements and the experimental design. The conclusions derived from the theoretical study were tested using some experimental results.

2. Experimental

2.1. Models for estimating k_m and k_{am} values

For estimating k_m and K_{AM} parameters, simulated (k') or experimental (k) capacity factors, corresponding to different [M] values were used. The data were fitted to three different regression models: a non linear regression model (model NL), which uses Eq. (1), and two lineal regression models (models L and I), derived from this equation. Model L is the habitual way to estimate the parameters k_m and K_{AM}:

$$1/k = 1/k_m + K_{AM}/k_m[M] \quad (2)$$

where a plot of $1/k$ vs. [M] yields an intercept ($1/k_m$) and a slope (K_{AM}/k_m) from which k_m and K_{AM} may be an estimate. Finally, a new linear model (model I) is proposed as an alternative way to find the parameters:

$$k = k_m - K_{AM}k[M] \quad (3)$$

In this model the plot k vs. $k \cdot [M]$ gives the estimations of k_m (the intercept) and K_{AM} (the slope) directly.

2.2. Simulation study

The simulation study was performed following various steps:

1. Fix k'_m values in the range 10–100 000.
2. Fix K'_{AM}/k'_m ratio values in the range 0.25–4.
3. Calculate K'_{AM}.
4. Fix the surfactant concentrations to 0.05, 0.075, 0.1, 0.15 and 0.2 *M*. Note: The CMC value should be substracted from those values to obtain [M] (i.e. 0.0082 *M* is the CMC of an aqueous solution of sodium dodecyl sulphate (SDS) at 25°C).
5. Use k'_m and K'_{AM} in Eq. (1) to calculate the theoretical k' values at the five [M] values.
6. Fix the relative standard deviation for k', R.S.D(k), in the 1–10% range.
7. Calculate the standard deviation (s_k) for k' values from R.S.D.(k).
8. Round off the k' values using the number of significant figures according to s_k . For instance, Fig. 1 (upper left part) shows the k' values obtained with the following simulation variables: log k' in the 1–5 range (each 0.2); $K'_{AM}/k'_m=1$; R.S.D.(k)=3%, and five [M] values (0.0418, 0.0668, 0.0918, 0.1418 and 0.1918).
9. Fit k' and [M] data in Eqs. (1)–(3) (models NL, L and I, respectively). For instance, Fig. 1 shows the 21 series of five pairs of k',[M] data plotted according to model NL (upper right part), model L (lower left part), and model I (lower right part).
10. Obtain the fitting parameters, k_m and K_{AM}, and the regression statistics. Calculate the relative differences, %E, between the theoretical (k'_m, K'_{AM}) and calculated parameters (k_m, K_{AM}). Calculate the standard deviation (s) and R.S.D. for k_m and K_{AM}.

2.3. Experimental data

Replicate experimental capacity factors of phenol, aniline, acetanilide and *o*-tolidine ($n=9$; 10 ppm concentration; 0.1 *M* SDS mobile phase; ODS2 C_{18} 120×4.6-mm I.D., 5-μm column; room temperature; intra-day assay) and two independent series of theophylline, theobromine and caffeine ($n=20$; 1–10 or 2–20 ppm range, 0075 or 0.05 *M* SDS mobile phase, respectively, ODS2 C_{18} 120×4.6 mm I.D., 5-μm column; room temperature; inter-day assay) were obtained. The standard deviation of the experimental k values (s_k) was used to fix the R.S.D(k) range for the simulation study.

Some experimental data were collected from our laboratory to check the predictions made with the

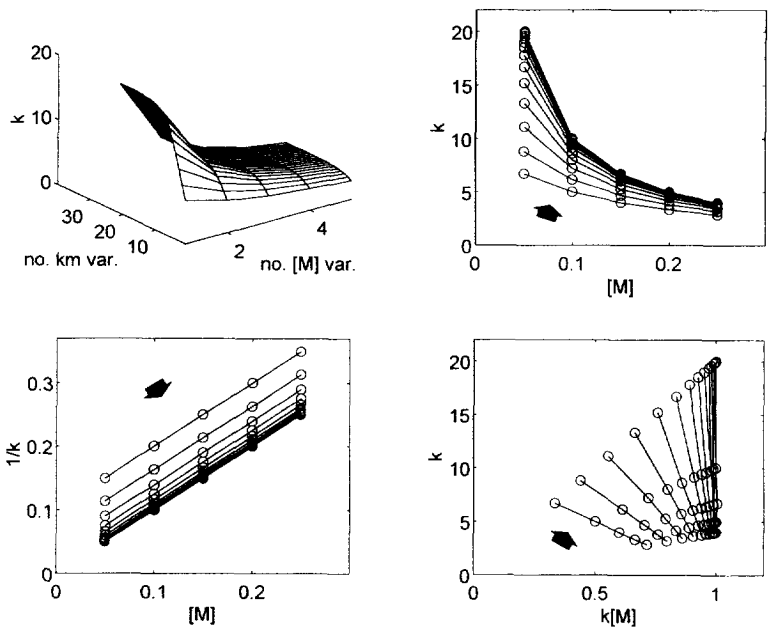

Fig. 1. Simulation steps. Simulated k' values (upper left part). Series of five pairs of k',[M] data plotted according to model NL (upper right part), model L (lower left part), and model I (lower right part). See Section 2.2 for simulation variables.

Table 1
k_m and K_{AM} estimations for some diuretics. k,[M] data obtained from [22]

Compound	Mobile phase	Model L		Model I		Model NL	
		$k_m \pm s$	$K_{AM} \pm s$	$k_m \pm s$	$K_{AM} \pm s$	$k_m \pm s$	$K_{AM} \pm s$
Acetazolamide	1	1.56±0.14	1.60±0.94	1.56±0.13	1.60±1.0	1.58±0.13	1.8±1.1
	2	1.18±0.08	2.70±1.60	1.18±0.08	2.5±1.8	1.18±0.08	2.5±1.7
Hydrochlorothiazide	1	1.7±0.4	4.0±2.5	1.7±0.3	4.0±2.9	1.8±0.3	5.2±3.2
	2	1.8±0.1	13.0±0.7	1.8±0.1	13±2	1.8±0.1	12±2
Chlorthalidone	1	25±9	40±15	27±7	44±16	32±7	56±17
	2	15±8	36±20	15±7	30±30	21±10	63±46
Bendroflumethiazide	1	46±17	60±20	51±14	60±20	61±13	81±23
	2	45±2	81±3	45±1	82±3	46±1	83±3
Amiloride	1	140±110	110±80	150±100	120±80	280±180	240±160
	2	158±4	175±4	160±3	177±4	161±2	178±3
Spironolactone	1	1000±2000	300±400	600±300	140±80	630±190	150±50
	2	130±70	60±30	130±60	60±40	190±80	100±60
Triamterene	1	500±600	200±200	400±500	100±200	2000±4000	1000±1700
	2	310±100	190±60	260±50	150±40	250±40	150±30

(1) 0.05 *M* SDS mobile phases with 0.01 *M* phosphate buffer (pH 4.5). (2) 0.05 *M* SDS +2% propanol mobile phases with 0.01 phosphate buffer (pH 4.5). Other conditions: ODS-2 C_{18} column (120×46-mm I.D.; 5 μm). Room temperature.

simulated data. Triplicate capacity factors at four different SDS concentrations in the mobile phase were obtained for a series of seven diuretics [22]. Two SDS mobile phases were used, one of which contained 2% 1-propanol. Other experimental conditions are depicted in Table 1.

2.4. Software

The Statgraphics 7.0 statistical package was used to perform the estimation of the parameters and statistical analysis of the linear and non linear regression models. This software uses the Least Squares method and the Marquardt iterative search algorithm for the linear regression (models L and I) and non linear regression (model NL) models, respectively.

3. Results and discussion

3.1. Simulation parameters and statistical considerations

The simulation study focused on the comparison of the quality of the three regression models in order to adjust the k,[M] data. The conclusions should reveal whether the current method (model L) for estimating the k_m and K_{AM} parameters is reliable enough, and if other approaches (models I and NL) incorporate more information and quality than model L. In order to be realistic, the ranges of the simulation variables were adjusted to values normally present in the molecules' features and experimental situations. For instance, k_m values in the 10–10 000 range should represent compounds with log P values in the 1–6 range [18]. When experimental k data were obtained in our laboratory (see Section 2), the R.S.D.(k) values found, ranged between 1.5–3.6% and 4.3–8.4%, for the intra-day and inter-day assay, respectively. Therefore, the R.S.D.(k) range for the simulation study was fixed at 1–10%. On the other hand, five surfactant concentrations were used to estimate the k_m and K_{AM} parameters by regression, when three, four, or five experimental mobile phases are usually used in the bibliography for this purpose.

On the other hand, the estimation of the uncertainty of k_m and K_{AM} parameters, deserves some comments. Strictly speaking, the retention data t_R and t_0 follow a normal distribution, while k does not. However, t_0 could be considered practically a constant. In this assumption, k follows a normal distribution and models I and NL, which uses k as a dependent variable and may be used to obtain the standard deviation of the parameters. Therefore, for models NL and I, the standard deviation of the

parameters, $s(k_m)$ and $s(K_{AM})$ were directly obtained from the statistical results.

In contrast, model L uses $1/k$ as a dependent variable. Since this variable is not subject to a normal distribution, it should not be applied to the parametric statistics to obtain the uncertainty of the parameters k_m and K_{AM}. From this point of view, the two proposed regression models (I and NL) possess an added advantage over the L model. Only for comparative purposes, the standard deviation of the parameters was also calculated, using the propagation of uncertainty theory, from the intercept (s_I) and slope (s_S) standard deviation values from Eq. (2):

$$s(k_m) = s_I \cdot k_m^2 \quad (4)$$

$$s(K_{AM}) = K_{AM}[(s_S k_m / K_{AM})^2 + (s_I k_m)^2]^{1/2} \quad (5)$$

Therefore, the uncertainty associated with k_m not only depends on the quality of the linear regression model but also on the value of k_m. Larger k_m values imply greater uncertainty in k_m estimations. For instance, the coefficient of variation is also dependent on the k_m value:

$$\text{R.S.D.}(k_m) = s_I \cdot k_m \cdot 100 \quad (6)$$

On the other hand, the uncertainty associated with K_{AM} depends on the quality of the linear regression model (standard deviation of intercept and slope) and on k_m and K_{AM} values.

3.2. Simulation study. Reliability of the k_m and k_{AM} estimations

The usual way to estimate the parameters k_m and K_{AM} is by means of Eq. (2) (model L). In general, appropriate correlation coefficient values are obtained (i.e. $r>0.999$) and they provide apparent, but not proven, estimation of reliability. On the other hand, the influence of the uncertainty associated with k values on estimation quality is not considered in the bibliography and deserves more attention. As an exemple we can take a compound with known parameter values, i.e. $k'_m = 1000$ and $K'_{AM} = 1000$. Using the simulation scheme described in the experimental section (steps 2 to 5 for $K'_{AM}/k'_m = 1$), the theoretical k' values are calculated. In a real situation, the number of decimal figures to be used in k depends on the precision of k measurements.

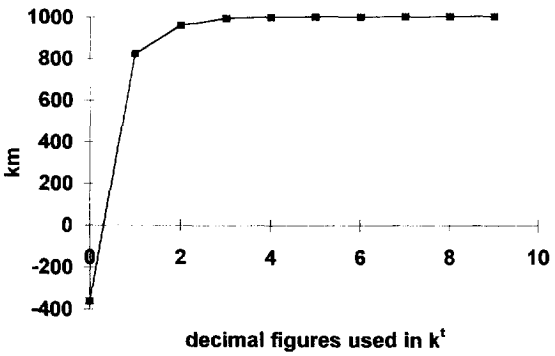

Fig. 2. Influence of capacity factor decimal figures number on the k_m estimation using model L. Theoretical k'_m value = 1000.

Fig. 2 shows the effect of the number of decimal figures used in k' on the k_m estimation using model L. With more than three decimal figures, accurate estimations were found (relative difference $<1\%$). In contrast, when 0, 1, 2, or 3 decimal figures were used, large errors in k_m estimates were observed. However, the correlation coefficient values found by applying model L to these k',[M] data were adequate in all instances (i.e. $r = 0.999_2$ and 0.99999_4 for zero and one decimal figures, respectively). This means that this statistical parameter does not assure the quality of estimations. Similar behaviour was observed for K_{AM} estimations (not shown). These results suggest that certain precautions could be necessary when dealing with some of the k_m and K_{AM} values published in the bibliography.

Model L is not the only way to linearize Eq. (1). We propose model I (Eq. (3)), which gives an alternative plot (k vs. $k\cdot$[M]) that makes it possible to find the parameters. Fig. 3 shows this plot for the same k',[M] data as in Fig. 1, when 1, 2, 3 and 9 decimal figures were used in k'. As can be observed, as fewer decimal figures were used, worse data fits were obtained with respect to the theoretical model (i.e. the solid line in Fig. 2 corresponding to nine decimal figures). This behaviour was not observed when data were plotted using the models L or NL, which suggest that model I is an 'indicator' of the experimental errors in the data. Therefore, it is useful for detecting random but also systematic errors in the experimental data sets and may also reveal the

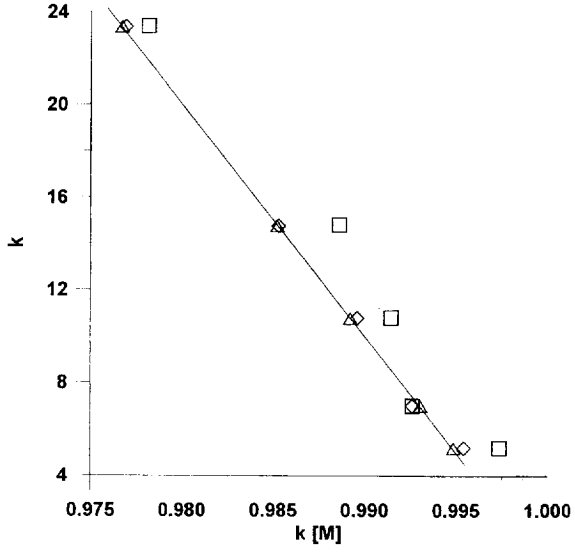

Fig. 3. k vs. k·[M] relationships (model I) obtained using 1(□); 2 (◇); 3 (△) and 9 (solid line) decimal figures in k.

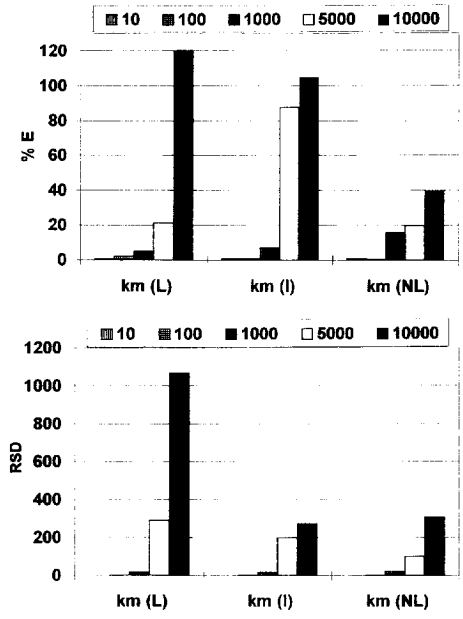

Fig. 4. Quality of k_m estimations as a function of theoretical k'_m values: $\%E$ (upper part) and R.S.D. (lower part). Simulation variables: $K'_{AM}/k'_m = 1$; R.S.D.(k) = 3%.

presence of outliers. However, this does not imply that the estimations obtained with this model are worse than those obtained with models L and NL.

From experimental k data R.S.D.(k) values in the 1–10% might be expected depending on the experimental conditions. These values give rise to 0–2 decimal figure k values (applying the significant figures criteria), which are in the critical range shown in Fig. 2. Figs. 2 and 3 illustrate the importance of uncertainty in k measurements on the k_m and K_{AM} estimations, but also indicate that the applicability and limitations of the regression models should be established. For this purpose, the influence of variables like the hydrophobicity of the compounds, the K_{AM}/k_m ratio and the R.S.D.(k) and the effect of the experimental design on the estimation reliability should be quantified. Extensive computer simulations were carried out in order to study the validity of the models over the fixed range of these variables. Some representative results are shown in the following paragraphs in relation to the quality of the k_m estimation (similar results, not shown, were found for K_{AM}).

The hydrophobicity of compounds is usually characterized by the parameter log P. As excellent correlations between log k_m and log P have been described [18], k_m has been used here as a hydrophobicity descriptor. Fig. 4 shows the quality of the k_m estimations in terms of accuracy ($\%E$) and precision (R.S.D.), corresponding to models L, I and NL, as a function of the theoretical k'_m values used in the simulation (10–10 000 range). Larger k_m values provided greater $\%E$ and R.S.D. values in the k_m estimations. For low k'_m values (up to 100), accurate and precise estimations were obtained in all cases (i.e. for $k'_m = 100$, the worst calculated k_m value, obtained with the model L, was 97.8 ± 1.3, with $\%E = 2.2$ and R.S.D. = 1.3%). A limited quality was found for $k'_m = 1000$ (i.e. model L offered the best result, $k_m = 950 \pm 170$). For large k'_m values (over 5000), the estimations were poor in all cases (i.e. the best results were obtained using NL model, 5992 ± 6048, which do not make sense from a statistical point of view). In such situations, the models should not be used to estimate k_m and K_{AM}.

The influence of the K_{AM}/k_m ratio and precision on k measurements was also studied. The quality of the k_m estimations, as a function of these parameters, also depended on the hydrophobicity level. For low k'_m values (10 or 100) the influence of the K'_{AM}/k'_m ratio and R.S.D.(k) was negligible for the three

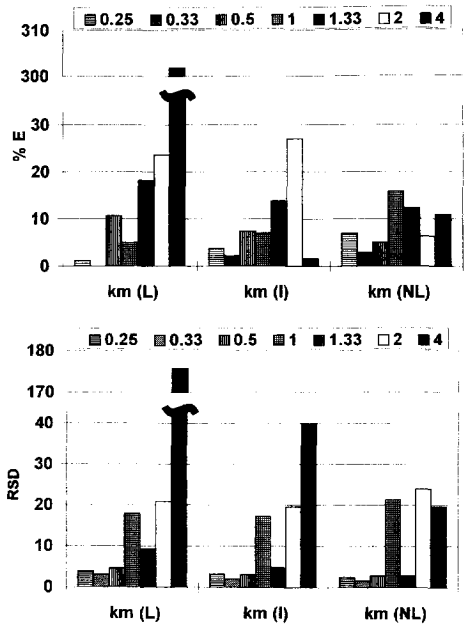

Fig. 5. Quality of k_m estimations as a function of K'_{AM}/k'_m ratio values: $\%E$ (upper part) and R.S.D. (lower part). Simulation variables: $k'_m = 1000$; R.S.D.(k) = 3%.

models. The differences between models became more evident for $k'_m = 1000$. For instance, Fig. 5 shows the quality of the k_m estimations corresponding to the models L, I and NL, as a function of the theoretical K'_{AM}/k'_m values used in the simulation (0.25–4 range). For model L, large $\%E$ and R.S.D. values were obtained as the ratio value increased. This behaviour was not observed for models I and NL. According Eq. (2), high ratio values imply high slope values in $1/k$ vs. [M] plots (model L). In such situations the intercept estimations are more influenced by experimental errors in k measurements, which could justify this particular behaviour.

As expected, the decrease in the precision of k measurements [R.S.D.(k) = 1, 3, 5 and 10%] revealed a decrease in the precision of k_m, with the following precision order (model L < model I < model NL). In contrast, accuracy was less dependant on this factor. Low errors ($\%E < 10\%$) were found in all cases for models I and NL, while large errors (>40%) were obtained with model L for R.S.D.(k) = 10%, which suggests that this model is less robust.

The experimental design i.e. the number and composition of the mobile phases also influenced the quality of k_m and K_{AM} estimations when models L, I and NL were applied. For instance, Table 2 shows the k_m and K_{AM} predictions and the regression statistics obtained using two different series of surfactant concentrations. In the second series, a low value of surfactant concentration was used (0.025 M) instead of 0.075 M in order to calculate [M]. As can be observed, a substantial change in the parameter estimation was obtained with the three models. For model L, no improvement in the absolute $\%E$ values was observed, which confirms that the good values on the regression statistics (r^2 and F) do not seem to relate to the estimation quality. In contrast, for model I, the new data set produced a drastic improvement

Table 2
Influence of the experimental design on the k_m and K_{AM} estimation quality with models L, I and NL. Simulation variables are: $k'_m = K'_{AM} = 1000$; $K'_{AM}/k'_m = 1$; R.S.D.(k) = 3%

Model	L		I		NL	
Plot	$1/k$ vs. [M][a]	$1/k$ vs. [M][b]	k vs. $k\cdot$[M][a]	k vs. $k\cdot$[M][b]	k vs. [M][a]	k vs. [M][b]
r^2	0.99999_2	0.99999_8	0.9_1	-0.99_8	0.9999_8	0.9999_9
F	404079	1497422	32	1298	99999	99999
k_m	950	1050	930	990	1200	974
(s)	(170)	(110)	(160)	(30)	(200)	(11)
K_{AM}	950	1050	930	990	1200	972
(s)	(170)	(110)	(160)	(30)	(300)	(11)
Intercept	0.00105	0.00095				
(s)	(0.00019)	(0.00009)				
Slope	0.9993	0.9998				
(s)	(0.0016)	(0.0008)				

[a] [M]: 0.0418, 0.0668, 0.0918, 0.1418, 0.1918 M.
[b] [M]: 0.0168, 0.0418, 0.0918, 0.1418, 0.1918 M.

in the estimation quality, together with an increase in the r^2 and F values. Finally, for model NL, an improvement in the estimation quality without changes in r^2 and F statistics was observed. Table 2 suggests that the estimation and quality of the intercept are the most critical aspect of model L, which may justify the high sensitivity of this model to the experimental variables. Model I seem to be more reliable than model L when an adequate correlation coefficient is obtained with this model.

3.3. Experimental data

The simulation study reveals that a compound's hydrophobicity (in terms of k_m) is the most critical aspect affecting the accuracy and precision that can been expected in the k_m and K_{AM} estimation regardless of the regression model used. However, Figs. 4 and 5, suggest that model L is less reliable than models I and NL for high hydrophobic compounds. This agrees with Fig. 1, which shows that nearly zero or negative intercepts may be obtained from $1/k$ vs. [M] plots (model L) for large k_m values. This leads to false or nonsense k_m and K_{AM} estimations.

In such cases, the addition of an organic modifier, such as short-chain alcohols, to the micellar mobile phase provides an adequate reduction in k values and should improve the accuracy of the estimations. The presence of a constant amount of modifier in the micellar mobile phase produces modifications in k_m and K_{AM} values with respect to those obtained in a pure micellar mobile phase. However, it has been demonstrated that Eq. (1) is also valid [10]. On the other hand, the modified k_m and K_{AM} values may still be used as hydrophobicity descriptors [18].

Table 1 shows the parameter values estimated by the three models for a set of diuretics eluted with SDS and SDS–2%1-propanol mobile phases. In general, the results agree well with the predictions derived from the simulated data. When pure SDS mobile phases were used, the compound's hydrophobicity substantially influenced the estimation quality. For low hydrophobic diuretics (i.e. acetazolamide and hydrochlorotiazide) there is good agreement between the estimations obtained with the three models, especially models L and I. As the hydrophobicity increases (i.e. chlortalidone, bendroflumetiazine, amiloride) the results vary more from one model to another and large R.S.D. values were obtained. Finally, for the most hydrophobic compounds the estimations shows low reliability. For spironolactone, a nonsense value was obtained with model L while for trianterene a nonsense value was found in all cases.

The addition of 2% propanol to the SDS mobile phases produced an increase in the estimation quality, especially for the more hydrophobic compounds. Three beneficial effects may be observed: (1) k_m and K_{AM} can be estimated for all compounds using the three models; (2) in general, better coincidence between the estimations of the three models is obtained; (3) in general, lower R.S.D. values were found.

4. Conclusions

The three regression models assayed make it possible to estimate k_m and K_{AM} but, the estimation quality depends strongly on the compounds' hydrophobicity. Low hydrophobic compounds, which are weakly retained, lead to accurate and precise predictions. For such compounds, the models tolerate variations in the K_{AM}/k_m ratio and the R.S.D. of experimental k values. In contrast, medium and especially high hydrophobic compounds lead to poor predictions. In this case, modifiers should be added to the mobile phase.

In studies related to structure–retention (QSRR) and activity–retention (QRAR) quantitative relationships, it may be necessary to obtain k_m or K_{AM} values for a series of compounds with different hydrophobicities in the same chromatographic conditions. Therefore, it is common for compounds that are weakly retained to coincide with others that are strongly retained. In estimating these parameters, some particular features of the models should be considered that are more important, the more strongly retained the compounds are. (1) For model L, good r and F statistic parameters are insufficient to assure the reliability of estimations. In addition, it is less robust than models I and NL in relation to variables like K_{AM}/k_m ratio, R.S.D. of k values or the experimental design. (2) For model I, adequate correlation coefficients (i.e. 0.99) may be sufficient to guarantee reliable predictions. In addition, the k

vs. k[M] plots may help the data study and prevents the presence of outliers. (3) Model NL is the most robust model but the estimation quality is less predictable. On the other hand, it may be useful to obtain three models to estimate the parameters instead of one of them, since a good agreement of their results may increase the estimation reliability.

Acknowledgments

This work was supported by the CICYT Project SAF96/1709.

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