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Review

Modelling of retention behaviour of solutes in micellar liquid chromatography

M.C. García-Alvarez-Coque*, J.R. Torres-Lapasió, J.J. Baeza-Baeza

Departamento de Química Analítica, Facultad de Química, Universidad de Valencia, 46100-Burjassot, Valencia, Spain

Abstract

In micellar liquid chromatography (MLC), the resolution for a given multi-component mixture can be optimized by changing several variables, such as the concentrations of surfactant and organic modifier, the pH and temperature. However, this advantage can only be fully exploited with the development of mathematical models that describe the retention and the separation mechanisms. Several reports have appeared recently on the possibilities of accurately predicting the solute retention in MLC. Although the retention and selectivity may strongly change with varying concentrations of surfactant, organic modifier and/or pH, the observed changes are very regular, and are well described by simple models. This characteristic enables a successful prediction of retention times and compensates the negative effect of the broad and tailed chromatographic peaks obtained for some solutes when micellar eluents are used. An overview of the models proposed in the literature to describe the retention behaviour in pure micellar eluents and micellar eluents containing an organic modifier, at a fixed pH or at varying pH, is given. The equations derived permit the evaluation of the strength of micelle–solute and stationary phase–solute interactions. The prediction of the retention based on molecular properties and the use of neural networks, together with the factors affecting the prediction capability of the models (linearization of the equations, dead time, critical micellar concentration, ionic strength and temperature) are commented on. The strategies used for the optimization of resolution are also given.

Keywords: Reviews; Retention models; Micellar liquid chromatography; Mobile phase composition

Contents

*Corresponding author.

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1. Introduction

In reversed-phase liquid chromatography, the presence of micelles in the mobile phase provides a great variety of interactions (Fig. 1): the solutes can remain outside the micelle associated with the polar head of the surfactant, can penetrate into the micelle core, or can form a part of the outer palisade layer. The monomers of ionic surfactants are adsorbed on the alkyl-bonded stationary phases mainly through hydrophobic interaction between the tail of the surfactant and the alkyl chains of the stationary phase. The charged head of the surfactant will then remain in contact with the polar solution. Solutes can experience hydrophobic interactions with either the non-polar tail of the adsorbed surfactant and the bonded non-modified stationary phase, and polar interactions with the ionic head of the adsorbed surfactant and with the free silanol groups on the stationary phase. Non-polar solutes should only be affected by hydrophobic interactions with both micelle and stationary phase, but for solutes that are charged, two distinct additional situations will exist, according to the sign of the charged solute, which can be the same or opposite to the sign of the head of the surfactant and, therefore, can be attracted or repelled by the surfactant.

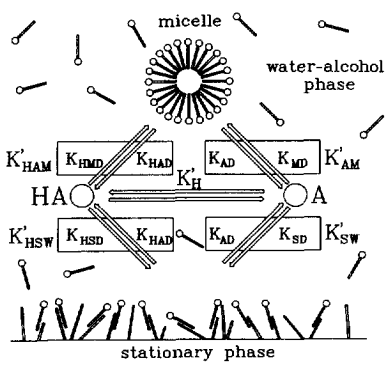

Fig. 1. Solute–micelle and solute–stationary phase interactions in micellar mobile phases of surfactant and organic modifier.

Most of the reported procedures for the determination of compounds in micellar liquid chromatography (MLC) make use of micellar mobile phases containing an organic modifier, generally, a short-chain alcohol (hybrid micellar mobile phases), owing to the increased eluent strength, especially important for the most non-polar solutes, and often improved shape of the chromatographic peaks. The modifiers vary the critical micellar concentration (CMC), the aggregation number of the surfactant and the polarity of the bulk water phase. The most hydrophillic alcohols do not penetrate the micelles, but butanol and pentanol can be inserted into the micelle with their hydroxyl group orientated towards the Stern layer, and their hydrocarbon chain remaining inside the non-polar micelle core. The incorporation of the alcohol in the micelle can result in an additional interaction with the solutes. On the other hand, alcohols solvate the bonded stationary phase and reduce the amount of surfactant adsorbed, the effect being larger with increasing concentration and hydrophobicity of the alcohol. The rigidity of the surfactant-alkyl-bonded ligand structure may also be affected.

In such systems, the retention of solutes will be governed by three different equilibria (Fig. 1). The first one is the distribution of the solutes between the micelle and bulk water, the second equilibrium is the

partition of the solutes between the stationary phase and bulk water, and the last one implies the direct transfer between the surfactant-modified stationary phase and the micelle. The latter equilibrium can be neglected in most situations, but it is significant for highly non-polar (water-insoluble) solutes, which have a great affinity for both stationary phase and micelle. A decrease in retention time is usually observed when either the micellar concentration or the concentration of organic modifier is increased. However, different components in a mixture respond in different ways to changes in concentration of surfactant and/or organic modifier, resulting in a change in resolution. Selection of the pH in the mobile phase is also often extremely important owing to the side protonation reactions of many solutes. Indeed, the manipulation of the pH can yield the resolution of complex mixtures.

In MLC, the chromatographer is, therefore, supplied with several tools to refine a separation and optimize the resolution for a given multi-component mixture. However, this advantage can only be exploited to the fullest when all variables are simultaneously taken into account. By only using the proton, surfactant or the organic modifier concentration, or by varying one after optimizing the other, a better separation can be easily missed. The use of an interpretive optimization strategy, which needs specific information on the retention of the individual components in a mixture, may be much more efficient and reliable, and requires fewer experiments to derive an acceptable separation. However, the model used in the prediction of the retention behaviour should be sufficiently accurate.

The separation process in a micellar chromatographic system requires a structured approach in the development of practical applications. Several reports have appeared recently on the possibilities of accurately predicting the solute retention in MLC. A regular change in the retention behaviour is observed with the variation of pH, and the concentrations of surfactant and organic modifier. This characteristic enables a successful prediction of retention times and compensates the negative effect of the broadening and distortion of chromatographic peaks obtained for some solutes when micellar eluents are used. Simultaneous optimization of the resolution and solvent strength is also possible.

2. Retention behaviour in pure micellar eluents

2.1. Micellar concentration as unique experimental variable

The capacity factor, k', in micellar media at a given pH, is related to the concentration of monomers of surfactant-forming micelles ([M]=total concentration of surfactant minus CMC), through a very simple equation. Three theoretical approaches have been proposed to describe the retention of solutes for liquid chromatographic systems at various micellar concentrations: the models of Armstrong and Nome [1], Arunyanart and Cline-Love [2] and Foley [3]. The equations derived are similar and allow the evaluation of the strength of micelle–solute and stationary phase–solute interactions.

In 1981, Armstrong and Nome [1] extended to micellar media the classic model of Martin and Synge [4] and Herries et al. [5]. The model describes the situation that a given solute experiences in an ideal system formed by a number of identical plates, where partition equilibria take place. The authors considered three partition coefficients corresponding to the three transitions that can occur in the three environments that exist in a micellar chromatographic system, the aqueous phase, the micelles and the stationary phase: the partition coefficients of the solute between the stationary phase and water, P_{AS}, between the micelle and water, P_{AM}, and between the stationary phase and the micelle, P_{MS}. The latter coefficient was, however, not included in the model, since it can be obtained by combination of the two former. The mass fraction of solute in each environment and each theoretical plate, was obtained from P_{AS} and P_{AM}. Based on the expression giving the maximally occupied theoretical plate, the following equation was obtained:

$$\frac{V_e - V_0}{V_s} = \frac{k'}{\phi} = \frac{P_{AS}}{1 + \upsilon(P_{AM} - 1)[M]} \quad (1)$$

where V_e represents the total volume of eluent needed to elute a given solute from the column, V_s is the volume of the active surface of the stationary phase, V_0 is the column dead volume, $\phi = V_s/V_0$ is the phase ratio, and υ is the partial specific volume of the monomers of surfactant in the micelle. The

calculation of P_{AM} requires knowledge of v. The model assumes that, above the CMC, the stationary phase is saturated with the adsorbed surfactant. When micelles are not present in the mobile phase, Eq. (1) reduces to the partition equation of conventional reversed-phase liquid chromatography:

$$V_e = V_0 + V_s P_{AS} \quad (2)$$

Unlike Armstrong and Nome, Arunyanart and Cline-Love [2] explained the retention in terms of the following chemical equilibria:

(i) Association of the solute in the bulk water, A, with the stationary phase binding sites, S:

$$A + S \overset{P_{AS}}{\rightleftarrows} AS \quad (3)$$

(ii) Association of the solute in the bulk water with a monomer of surfactant in the micelle:

$$A + M \overset{K_{AM}}{\rightleftarrows} AM \quad (4)$$

(iii) Direct transfer of the solute in the micelle to the stationary phase:

$$AM + S \overset{P_{MS}}{\rightleftarrows} AS + M \quad (5)$$

The coefficient P_{MS} may be calculated as: $P_{MS} = P_{AS}/K_{AM}$. Substitution of the equilibrium constants P_{AS} and K_{AM} in the expression giving the capacity factor leads to:

$$k' = \phi \frac{[AS]}{[A] + [AM]} = \frac{\phi P_{AS}[S]}{1 + K_{AM}[M]} \quad (6)$$

However, since usually [S] is constant, this quantity can be included in the partition constant P_{AS} . The calculation of P_{AS} requires knowledge of ϕ. The difficulties in the determination of the volume of the bonded surface of the stationary phase compel the use of the entire volume of the silica solid support particles, which gives an underestimation of P_{AS}.

The model proposed by Foley [3] is also based on chemical equilibria, the assumptions made being not very different from those of Arunyanart and Cline-Love [2]. The association between the solute in the bulk water and the micelles (Eq. (4)) is considered as a secondary equilibrium, which affects the retention of a solute in the absence of micelles, given by k'_A.

$$k' = \frac{1}{1 + K_{AM}[M]} k'_A \quad (7)$$

The equation is again similar to the equation of Armstrong and Nome (Eq. (1)). The capacity factor of free solute, k'_A, coincides with ϕP_{AS} in Eq. (1), and $\phi P_{AS}[S]$ in Eq. (6), whereas K_{AM} in Eq. (7) corresponds to the same constant in Eq. (6) and to the product $v(P_{AM} - 1)$ in Eq. (1), when the volume of the aqueous phase is taken as the total volume of the mobile phase. The three models lead to similar linear equations $1/k'$ vs. [M], and can be rewritten as:

$$\frac{1}{k'} = \frac{1}{K_{AS}} + \frac{K_{AM}}{K_{AS}}[M] \quad (8)$$

Eq. (8) has been verified experimentally for a large number of solutes (ionic, neutral, polar and non-polar), surfactants (anionic, cationic and non-ionic) and column materials (C_8, C_{18} and cyano). This equation is also valid for mobile phases with the same organic modifier concentration. Deviations from the model are, however, observed for highly and lowly retained solutes. In the former case, irreversible adsorption of the solute on the stationary phase may occur, whereas in the latter the elution of the solute will take place with the dead volume.

The constant K_{AM} is referred to the association of the solute with a surfactant monomer in the micelle, and should be multiplied by the aggregation number to get the constant referred to the whole micelle. If the value of K_{AM} is available from independent sources, the model can be used to predict the chromatographic behaviour at any micellar concentration. Alternatively, the MLC technique provides a convenient mode of estimating K_{AM}, since the concentration of the solute need not be known, the impurities in the sample are chromatographically separated, and simultaneous determination of the constants of several solutes is possible.

Eq. (8) describes the retention of solutes that can form associates or inclusion complexes both with the micelles and the surfactant adsorbed on the stationary phase. This is the case for neutral compounds and compounds with an opposite charge to the surfactant. However, compounds having the same charge as the surfactant will be excluded from the

micelles, and repelled by the modified stationary phase, unless other interactions exist that neutralize the electrostatic repulsion. For the solutes repelled from the micelles (called antibinding solutes), the retention increases with the concentration of surfactant. In this way, fitting of the experimental data to Eq. (8) gives negative solute–micelle association constants, physically meaningless. Since K_{AM} is the relationship of the equilibrium concentrations of solutes between bulk water and the micelles, it should be positive.

Jandera and Fischer [6] reported that this limitation suggests a severe inconsistency in the retention model of Eq. (8). To include the repulsion effect, these authors proposed that a part of the active stationary phase is inaccessible to the solutes. The relative reduction in the accessible volume will be proportional to the amount of adsorbed surfactant, Q_{CMC}, which is constant above the CMC:

$$\frac{\Delta V_s}{V_{s0}} = f_s Q_{CMC} \tag{9}$$

Similarly, a fraction of the volume of the mobile phase could not participate in the interactions with the solute:

$$\frac{\Delta V_m}{V_{m0}} = f_m[M] \tag{10}$$

where f_s and f_m are the fractions of the stationary and mobile phase inaccessible to the solute, respectively. According to this approach, four equations were formulated corresponding to different situations. Eq. (8) is valid for solutes that are not excluded from both the stationary phase and the micelles. For solutes repelled by the adsorbed monomers of surfactant on the stationary phase, but associated to the micelles:

$$k' = \frac{\phi P_{AS}}{1 + K_{AM}[M]}(1 - f_S Q_{CMC}) \tag{11}$$

whereas for solutes repelled by both the stationary phase and micelles:

$$k' = \phi P_{AS}\frac{1 - f_s Q_{CMC}}{1 - f_m[M]} \tag{12}$$

A similar equation can be obtained for solutes non-excluded from the stationary phase but repelled by the micelles ($f_s = 0$ in Eq. (12)). The two latter situations correspond to antibinding solutes. All these equations can be rewritten as:

$$\frac{1}{k'} = c_0 + c_1[M] \tag{13}$$

where the coefficients c_0 and c_1 have different signs according to the nature of the interactions in the stationary phase and micelles.

On the other hand, the separation of mixtures of compounds showing a wide range of polarities can be advantageously made using gradient elution. This is favoured in MLC because at moderate concentrations of ionic surfactant, the composition of the stationary phase remains constant during the micellar concentration gradient. Therefore, the only re-equilibration process necessary before the next gradient run is flushing the chromatography system with the initial mobile phase. The prediction of the retention in gradient conditions in MLC was reported, based on the gradient elution theory developed by Snyder [7], and assuming the linear model given by Eq. (8) and a linear change in the micellar concentration [8]:

$$[M] = a + bV \tag{14}$$

where V is the volume of the mobile phase delivered. The equation finally derived was:

$$t_g = \frac{t_0 K_{AS}}{K_{AM} b V_0}\left(-\frac{1}{k'_0} + \sqrt{\left(\frac{1}{k'_0}\right)^2 + 2\frac{K_{AM} b V_0}{K_{AS}}\left(1 - \frac{t_D}{t_0 k'_0}\right)}\right) + t_0 + t_D \tag{15}$$

where t_g is the gradient retention time, t_0 is the dead time, V_0/t_0 is the volume flow-rate, k'_0 is the capacity factor of the solute at the initial mobile phase, and t_D is the delay time (the time before the gradient actually reaches the top of the column). The model showed excellent results for a variety of solutes and sodium dodecyl sulphate (SDS) gradients in the 0.10–0.50 M range.

2.2. Simultaneous effect of pH and micelle concentration

Arunyanart and Cline-Love [9] and Rodgers and

Khaledi [10,11] studied the effect of pH on the MLC retention of weak acids and bases. These authors derived an equation that describes the observed behaviour in terms of pH and micellar concentration. For a monoprotic system showing a protonation equilibrium in water:

$$A + H \rightleftarrows HA, \quad K_H = \frac{[HA]}{[A]h} \tag{16}$$

where h is the proton concentration and K_H is the protonation constant; the capacity factor in a micellar chromatographic system will be given as follows:

$$k' = \phi \frac{[AS] + [HAS]}{[A] + [HA] + [AM] + [HAM]} \tag{17}$$

where [AS], [AM] and [A] refer to the non-protonated species, and [HAS], [HAM] and [HA] refer to the acidic species. Substituting Eq. (16) and the equilibrium constants relating all these species in Eq. (17), and making $K_{AS} = \phi P_{AS}$ and $K_{HAS} = \phi P_{HAS}$:

$$k' = \frac{K_{AS} + K_{HAS}K_H h}{1 + K_H h + K_{AM}[M] + K_{HAM}K_H h[M]} \tag{18}$$

Rewriting this expression, an equation similar to the reciprocal of Eq. (8) is obtained:

$$k' = \frac{\dfrac{K_{AS} + K_{HAS}K_H h}{1 + K_H h}}{1 + \dfrac{K_{AM} + K_{HAM}K_H h}{1 + K_H h}[M]} = \frac{K^H_{AS}}{1 + K^H_{AM}[M]} \tag{19}$$

where K^H_{AS} and K^H_{AM} are apparent constants with respect to proton concentration. Eq. (18) may also be rewritten as follows:

$$k' = \frac{\dfrac{K_{AS}}{1 + K_{AM}[M]} + \dfrac{K_{HAS}}{1 + K_{HAM}[M]} \dfrac{1 + K_{HAM}[M]}{1 + K_{AM}[M]} K_H h}{1 + \dfrac{1 + K_{HAM}[M]}{1 + K_{AM}[M]} K_H h} = \frac{k'_A + k'_{HA}K^M_H h}{1 + K^M_H h} \tag{20}$$

where k'_A and k'_{HA} are the capacity factors of the basic and acidic species, respectively. This equation indicates that the retention varies with pH, following a sigmoidal behaviour between the retention of the acidic species and the retention of the basic species (Fig. 2). The effect of the concentration of surfactant on the apparent protonation constant is also shown in this equation. Eq. (20) may be easily extended to solutes exhibiting several protonation equilibria in the pH working range (between 2.5 and 7.5 for the C_{18} columns) [10,11].

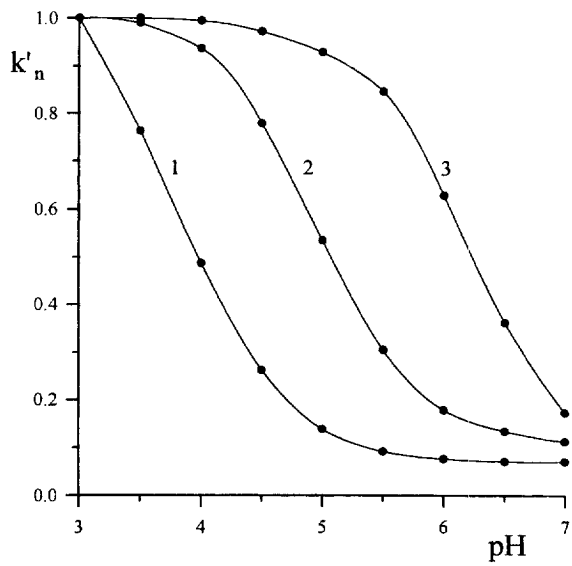

Fig. 2. Normalized capacity factors as a function of pH for: (1) tyrosine, (2) benzocaine, and (3) xipamide, eluted with a 0.10 *M* SDS mobile phase.

3. Retention behaviour in hybrid micellar eluents

The addition of an organic modifier allows a micellar mobile phase to improve the efficiency and to increase its solvent strength, thus overcoming the drawback of being a weak eluent [12]. Solute association constants to micelles and their partitioning into the stationary phase both decrease as a result of the addition of a modifier, especially for highly hydrophobic solutes. However, the K_{AM}/K_{AS} ratio increases and, therefore, the elution power of the mobile phase.

Khaledi et al. [13] assumed that the linear relation-

ship between $\log k'$ and the volume fraction of organic modifier, φ, followed in conventional reversed-phase liquid chromatography over a small range of values of φ, was also valid in hybrid MLC at a constant micellar concentration:

$$\log k' = -S\varphi + \log k'_0 \quad (21)$$

According to these authors, $\log k'_0$ in Eq. (21) was the logarithm of the capacity factor at a given micellar concentration. However, linear relationships are only actually obtained with methanol, probably owing to the weak eluent strength of this modifier. It was found for a set of catecholamines that the difference between the experimental and calculated k'_0 values was larger when an alcohol of longer alkyl chain length was used [14].

Eq. (21) was used to describe the change in retention during a gradient of organic modifier in MLC, keeping constant the concentration of surfactant [15]. The agreement between the experimental and calculated data indicated that the integrity of the micelles was maintained during the gradient runs. In addition, it was shown that little re-equilibration time after each organic solvent gradient run is required, due to the limited range of modifier concentrations used.

3.1. Use of an iterative regression strategy

Strasters et al. [16] were the first authors which intended to model the retention of solutes in hybrid micellar systems. They proposed a procedure that used the retention data of only five mobile phases: four measurements at the corners of the selected two-dimensional variable space defined by the concentrations of surfactant and modifier, and the fifth in the centre (design VI in Fig. 3). The boundaries of the variables included in the model should be set by the operator on the basis of previous experience. The extreme values are imposed by the practical limitations of the chromatographic system: the lower surfactant concentration must be well above the CMC and strong enough to cause elution of all components. The upper surfactant concentration is determined by a combination of the solubility of the surfactant, the viscosity of the resulting mobile phase and the degradation of the efficiency at higher concentrations. The organic modifier concentration is

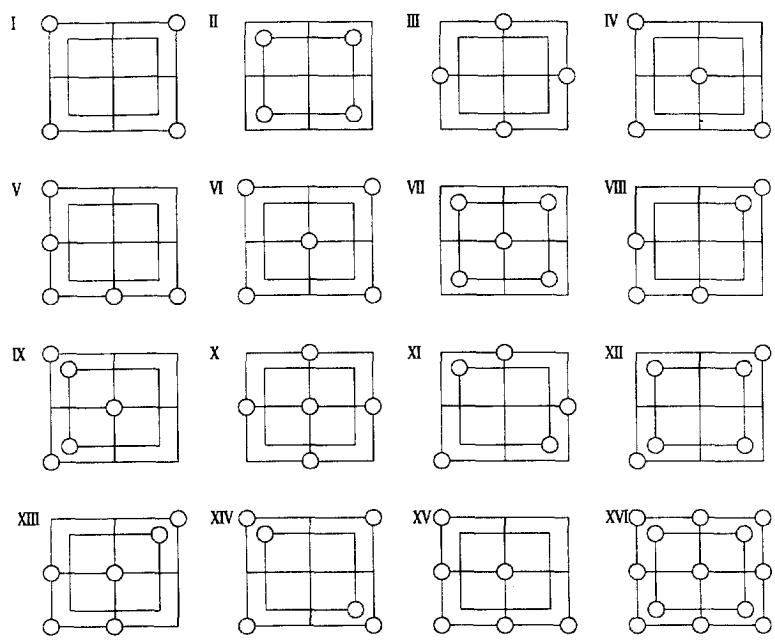

Fig. 3. Some experimental designs used in hybrid MLC. The abscissas represent surfactant concentration, and the ordinates modifier concentration.

limited to a maximum to ensure the integrity of the micelles. The maximum concentrations of modifier reported for modelling purposes have been 15% propanol, 6% butanol, 3% pentanol and 20% acetonitrile. However, solutions of surfactant at concentrations above their CMC in aqueous solution, where the micelles were broken by the presence of very high amounts of organic solvents, have shown interesting characteristics, such as shorter retention times and sharper peaks. Li and Fritz demonstrated that Eq. (8) is also followed for these mobile phases [17].

The rectangular variable space described above was divided into four triangular subspaces defined by three of the five measurements: two neighbour corner points and the central point. The method will be called here, for this reason, the method of triangles. It was assumed that the retention of solutes was linearly related to the mobile phase variables within a selected portion of the space. Strasters et al. [16] fitted a separate logarithmic linear function:

$$\ln k' = c_0 + c_1[\mathrm{M}] + c_2\varphi \qquad (22)$$

in each triangular subspace, and optimized the separation of a set of fifteen phenols eluted with hexadecyltrimethylammonium bromide (CTAB) and 2-propanol at pH 7, and thirteen amino acids and small peptides eluted with SDS and 2-propanol at pH 2.5. Torres et al. [18] used instead of a logarithmic function, a hyperbolic dependence. The retention in other mobile phases was calculated by linear interpolation inside each triangle where the coordinates belonged. Further, a chromatogram was simulated and compared with experimental data to verify the quality of the prediction.

When the experimental and predicted chromatograms coincided, a confirmation of the assumed linearity was obtained. When strong deviations of the linear model were observed, additional data points were included, without leaving the region of the variable space initially chosen, to refine the prediction by a further subdivision of the response surface into smaller triangles. This, obviously, can result in an undesirable large number of experiments and can cause the elimination of significant maxima in the first steps of an optimization process. If the optimum is found near one of the experimental points of the design, the prediction will be reliable. On the contrary, if it is at the centre of one triangle subspace, serious errors may result.

The success or failure of the method of triangles depends on the correctness of the linearity assumption of the retention model. The use of the logarithm of a function instead of the function, to make a linear interpolation, is a common practice when the range of variation of the function must be reduced. The use of a hyperbolic function makes less necessary the addition of new mobile phases to refine the predictions due to its better fitting accuracy. On the other hand, the division of the variable space into four triangles reduces even more the variations of the function, i.e., a function that does not fit in the whole variable space may be acceptable over a smaller range. The method of triangles can be considered as intermediate between a true sequential method and an interpretive method.

3.2. Use of empirical equations describing the whole variable space

The scheme of interpolation followed in the iterative regression strategy is also not very simple from a practical point of view. The method of triangles requires at least four different equations, one for each established subspace. It is more convenient to use a single equation to describe the retention behaviour of a solute in the whole variable space. Torres et al. [14] obtained large errors in the description of the retention of a set of catecholamines with the method of triangles using the logarithmic equation. They also found a similar variation of the capacity factors of some solutes with respect to both concentrations of surfactant and modifier. The authors then decided to study the capability of a series of empirical equations to describe the retention behaviour at any surfactant and modifier concentration. Table 1 shows some of the models (equations) considered, where the logarithm (Eqs. (22)–(29)) or the reciprocal of the capacity factor (Eqs. (30)–(37)) are related to the micellar concentration and the volume fraction of organic modifier. The errors in the prediction of the retention of a set of five catecholamines were evaluated using more than one hundred experimental designs, some of them represented in Fig. 3.

The results given by the logarithmic equations

Table 1
Relative global errors (%) in the prediction of capacity factors for five catecholamines (noradrenaline, adrenaline, adrenalone, dopamine and isoprenaline) eluted with mobile phases of SDS (0.035–0.15 *M*) and 1-propanol (0–10%) at pH 6.8, using several equations and experimental designs [14,19]

Models		Experimental designs[a]															
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
Logarithmic equations																	
$\ln k' = c_0 + c_1[M] + c_2\varphi$	(22)	20.5	11.3	12.8	17.9	19.9	17.6	13.6	25.3	45.9	15.4	32.2	35.6	25.0	17.6	20.8	20.6
$\ln k' = c_0 + c_1[M] + c_2\varphi + c_3[M]^2$	(23)	25.0	12.2	9.7	9.9	11.7	10.1	8.0	25.4	>50	10.4	17.6	22.7	24.4	18.0	11.7	13.3
$\ln k' = c_0 + c_1[M] + c_2\varphi + c_3\varphi^2$	(24)	18.5	15.4	11.8	12.3	16.5	12.5	9.5	36.4	>50	14.6	18.0	21.8	34.7	13.8	16.7	29.6
$\ln k' = c_0 + c_1[M] + c_2\varphi + c_3[M]\varphi$	(25)	22.1	11.0	22.5	27.2	38.1	24.4	13.8	13.6	33.4	21.7	33.2	24.1	16.2	22.6	24.8	16.1
$\ln k' = c_0 + c_1[M] + c_2\varphi + c_3[M]\varphi + c_4[M]^2$	(26)	[b]	[b]	[b]	[b]	>50	8.4	7.9	11.8	>50	26.6	18.7	21.2	13.1	9.9	11.5	10.6
$\ln k' = c_0 + c_1[M] + c_2\varphi + c_3[M]\varphi + c_4\varphi^2$	(27)	[b]	[b]	[b]	[b]	103	10.6	9.3	16.6	>50	29.5	24.6	23.3	22.4	12.1	23.7	14.1
$\ln k' = c_0 + c_1[M] + c_2\varphi + c_3[M]\varphi + c_4[M]\varphi^{1/2}$	(28)	[b]	[b]	[b]	[b]	40.5	13.1	25.9	13.6	>50	23.3	>50	>50	14.7	13.8	29.4	14.9
$\ln k' = c_0 + c_1[M] + c_2\varphi + c_3[M]\varphi + c_4\varphi[M]^{1/2}$	(29)	[b]	[b]	[b]	[b]	37.9	17.4	11.5	13.4	>50	>50	>50	32.4	14.1	15.0	25.3	13.8
Hyperbolic equations																	
$1/k' = c_0 + c_1[M] + c_2\varphi$	(30)	25.3	17.4	>50	23.1	32.3	20.3	17.5	25.5	17.3	>50	17.3	17.1	22.7	21.2	28.2	20.4
$1/k' = c_0 + c_1[M] + c_2\varphi + c_3[M]^2$	(31)	29.1	16.4	>50	23.9	32.2	20.8	38.9	32.3	14.2	>50	15.8	15.3	27.3	20.6	29.2	20.3
$1/k' = c_0 + c_1[M] + c_2\varphi + c_3\varphi^2$	(32)	24.8	19.8	39.5	24.4	31.7	21.6	38.9	35.1	19.3	44.1	15.1	15.1	29.5	21.5	28.0	20.0
$1/k' = c_0 + c_1[M] + c_2\varphi + c_3[M]\varphi$	(33)	3.4	4.9	9.4	3.3	28.9	3.3	4.7	3.7	5.4	9.2	4.8	4.5	3.6	3.6	3.5	3.9
$1/k' = c_0 + c_1[M] + c_2\varphi + c_3[M]\varphi + c_4[M]^2$	(34)	[b]	[b]	[b]	[b]	>50	3.4	5.2	32.3	5.4	7.4	5.0	4.8	4.0	3.7	3.4	3.8
$1/k' = c_0 + c_1[M] + c_2\varphi + c_3[M]\varphi + c_4\varphi^2$	(35)	[b]	[b]	[b]	[b]	26.2	3.1	6.9	>50	5.3	25.0	4.0	3.8	3.9	3.4	3.1	3.5
$1/k' = c_0 + c_1[M] + c_2\varphi + c_3[M]\varphi + c_4[M]\varphi^{1/2}$	(36)	[b]	[b]	[b]	[b]	43.5	2.8	9.8	12.8	5.5	8.9	3.4	3.1	3.5	2.8	4.1	2.5
$1/k' = c_0 + c_1[M] + c_2\varphi + c_3[M]\varphi + c_4\varphi[M]^{1/2}$	(37)	[b]	[b]	[b]	[b]	29.2	3.4	5.2	6.2	5.7	32.3	5.7	8.0	3.7	3.9	3.5	3.9

[a] Experimental designs shown in Fig. 3.

[b] Insufficient experimental points for the fitting.

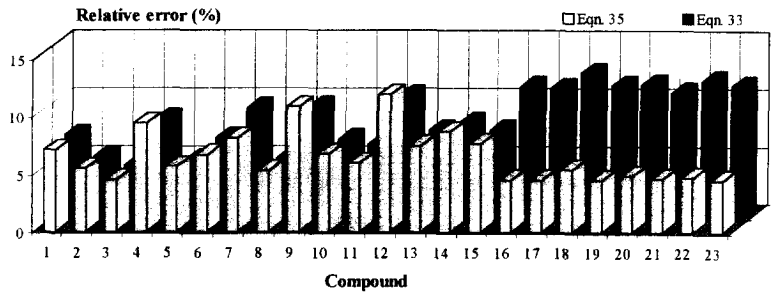

Fig. 4. Relative errors (%) obtained in SDS–butanol mobile phases, for the capacity factor prediction of: (1) benzene, (2) benzylic alcohol, (3) benzamide, (4) toluene, (5) benzonitrile, (6) nitrobenzene, (7) phenol, (8) 2-phenylethanol, (9) chlorobenzene, (10) phenylacetonitrile, (11) 3,5-dimethylphenol, (12) naphthalene, (13) 1-naphthol, (14) 2-naphthol, (15) 1-naphthylamine, (16) pyrene, (17) phenanthrene, (18) 2,3-benzofluorene, (19) fluorene, (20) fluoranthene, (21) acenaphthylene, (22) acenaphthene, and (23) anthracene. From [24].

were poorer. It was found that, frequently, these functions systematically gave larger k' values [18]. Eq. (30) gave a bad prediction of the retention when used to describe the whole variable space. However, this simplified equation yielded acceptable results in a small region of the variable space, as when applied in the method of triangles. It is evident that a term including both surfactant and modifier concentrations is needed in the retention equations. The smallest errors were obtained with Eqs. (33)–(37) [14,19].

The most simple equation giving acceptable results was Eq. (33). It has been checked that the prediction capability of this equation is similar to the other more complex (Eqs. (34)–(37)) ones, for polar or moderately non-polar compounds, such as amino acids [20], sulphamides [21], β-blockers [22] and diuretics [23] (C_{18} column and mobile phases of SDS and 1-propanol or pentanol), and some benzene and naphthalene derivatives (C_8 or C_{18} columns and mobile phases of SDS or CTAB, and 1-propanol or butanol) [14,24]. Thus, for these compounds, the plot of the reciprocal of k' vs. the concentration of modifier, at a fixed surfactant concentration, is linear.

García et al. [24] found that changes in surfactant and alcohol in the mobile phase influence the relative errors obtained with Eq. (33), which were lower for CTAB with respect to SDS, and propanol with respect to butanol. This indicated that this simple model failed to take into account some interactions of the solutes, which are more important in very hydrophobic systems and when solute–micelle interactions are diminished. One can expect that the amount of surfactant desorbed by butanol is greater than by propanol. Also butanol can compete to a greater extent than propanol with the micelle for the interaction of the solutes. These authors found that Eq. (35) (with a φ^2 term) provided a better description of the retention for highly hydrophobic polycyclic aromatic hydrocarbons (PAHs) than Eq. (33) (Fig. 4). For these compounds, the plot of $1/k'$ vs. φ was, thus, non-linear. Similar results were obtained for steroids eluted with SDS and acetonitrile [25]. Table 1 shows that the addition of a $[M]\varphi^{1/2}$ term improved the accuracy of the prediction for some experimental designs (Eq. (36)).

Although experimental designs of four, five or six points, such as designs I, VI and XIV in Fig. 3, were enough to achieve the fitting parameters of Eqs. (33), (35), (36), respectively, at least an additional measurement is recommended to allow checking of the accuracy of the fittings. The prediction of capacity factors for mobile phases showing the smallest eluent strength, such as a pure micellar with a concentration of surfactant close to the CMC is often very poor, especially for non-polar solutes. It must be considered that for these mobile phases, an extrapolation is performed in a region of a strong change in k', where small variations in the concentrations of surfactant and modifier highly affect the retention. In contrast, the extrapolation in a region of larger eluent strength is less problematic, because of the smooth variation in k'.

3.3. Physico-chemical meaning of the empirical models

The interpretation of the empirical equations based on physico-chemical properties will permit the im-

provement of the descriptive models, and the evaluation of parameters of interaction between the three environments involved in MLC. In this sense, a physico-chemical model was proposed where the organic modifier, O, was considered as a simple competitor of the solutes for the binding sites in the stationary phase and the micelles [26]. Two new equilibria were formulated:

$$O + S \overset{K_{OS}}{\rightleftarrows} OS \tag{38}$$

$$O + M \overset{K_{OM}}{\rightleftarrows} OM \tag{39}$$

The combination of equilibria, Eqs. (3), (4), (6), (38), (39), yields the following:

$$k' = \frac{K_{AS}[S](1 + K_{OM}\varphi)}{(1 + K_{OS}\varphi)(1 + K_{OM}\varphi) + K_{AM}[M](1 + K_{OS}\varphi)} \tag{40}$$

In this model, the possible interactions between the complexes *OS* or *OM*, and the solutes, are not considered. The effect of the modifier on the partition constants of the solute due to the change in the polarity of bulk water is also obviated. On the other hand, if the modifier takes part in the equilibria with the stationary phase and micelles (Eqs. (38) and (39)), the use of the total concentration of modifier instead of the free concentration can give significant errors in the evaluation of the physico-chemical parameters of the model.

In another approach, García-Alvarez-Coque et al. [27] related the coefficients in Eq. (33) with several parameters of retention. From the reciprocal:

$$k' = \frac{1}{c_0 + c_1[M] + c_2\varphi + c_3[M]\varphi} \tag{41}$$

an expression similar to the reciprocal of Eq. (8) can be obtained:

$$k' = \frac{K_{AS}\dfrac{1}{1 + K_{AD}\varphi}}{1 + K_{AM}\dfrac{1 + K_{MD}\varphi}{1 + K_{AD}\varphi}[M]} \tag{42}$$

where:

$$K_{AS} = \frac{1}{c_0};\ K_{AM} = \frac{c_1}{c_0};\ K_{AD} = \frac{c_2}{c_0};\ K_{MD} = \frac{c_3}{c_1}$$

This equation can be rewritten as:

$$k' = \frac{K^{\varphi}_{AS}}{1 + K^{\varphi}_{AM}[M]} \tag{43}$$

K^{φ}_{AS} and K^{φ}_{AM} being apparent constants with respect to the concentration of modifier. It was observed that the convergence of the iterative process in the non-linear regression was more rapid and stable when the experimental data were fitted to Eq. (41) instead of Eq. (42). The constants K_{AD} and K_{MD} measure the relative variation in the concentration of solute in bulk water and micelle, respectively, in the presence of modifier, taking the pure micellar solution (without modifier), as reference:

$$K^{\varphi}_{AS} = \frac{[AS]}{[A] + \Delta[A]} = K_{AS}\frac{1}{1 + K_{AD}\varphi} \tag{44}$$

$$K^{\varphi}_{AM} = \frac{[AM] + \Delta[AM]}{[M]([A] + \Delta[A])} = K_{AM}\frac{1 + K_{MD}\varphi}{1 + K_{AD}\varphi} \tag{45}$$

where [A] and [AM] are the concentrations of free solute in bulk water and solute associated to the micelle in a pure micellar solution, and Δ[A] and Δ[AM] are the changes in the concentrations produced by the modifier.

On the other hand, Eq. (35) can be rearranged as follows [27]:

$$k' = \frac{K_{AS}\dfrac{1}{1 + K_{AD}\varphi + K\varphi^2}}{1 + K_{AM}\dfrac{1 + K_{MD}\varphi}{1 + K_{AD}\varphi + K\varphi^2}[M]} \tag{46}$$

This equation introduces a new constant, K, that implies a quadratic hyperbolic variation in K^{φ}_{AS} and K^{φ}_{AM} with φ. An alternative model was proposed for highly hydrophobic solutes [27], which considers the additional change in the concentration of solute associated with the stationary phase, due to the presence of the modifier:

$$k' = \frac{K_{AS}\dfrac{1 + K_{SD}\varphi}{1 + K_{AD}\varphi}}{1 + K_{AM}\dfrac{1 + K_{MD}\varphi}{1 + K_{AD}\varphi}[M]} \tag{47}$$

This equation is an extension of Eq. (42) and was checked to give a significant improvement in the prediction of the retention of highly hydrophobic solutes, such as pyrene.

The constants K_{MD} and K_{AD} account for the displacement of the micelle–water equilibrium, whereas K_{SD} and K_{AD} describe the modification of the stationary phase–water equilibrium (see Fig. 1). These changes are due to the diminution in the polarity of the water and the modification of the interactions of the solute with the micelle and stationary phase.

3.4. Simultaneous effect of pH, micelle and organic modifier concentrations

Although two variables usually suffice for samples of moderate complexity to obtain a satisfactory separation, inclusion of a third variable will often further improve the quality of the obtainable optimum, with respect to both resolution and analysis time. In the optimization of the separation of weak organic acids and bases with micellar mobile phases, it is usual to fix the pH and only optimize the concentration of surfactant and modifier. The best pH for the separation is selected after examining the retention in a reduced number of mobile phases, at two or three pH values. However, the pH should be simultaneously optimized with the concentrations of surfactant and modifier, since the protonation constants suffer shifts depending on the composition of the mobile phase. This is caused by the different partitioning of the acidic and basic species of a solute in the micellar pseudophase, due to the electrostatic interactions (Fig. 1).

Strasters et al. [28] considered the simultaneous optimization of the concentrations of surfactant, modifier and pH, by using an approximate iterative procedure: the method of triangles adapted to three dimensions, and linear $\log k'$ vs. surfactant, modifier and pH functions. The procedure began with a design of fifteen points located in a three-dimensional space, which was divided into 24 tetrahedra. Therefore, 24 different equations of retention should be fitted. The retention in other mobile phases was calculated by a linear interpolation inside each tetrahedron. The range of pH values examined by the authors for the prediction of the retention of several amino acids and peptides, was intentionally reduced to pH 2.5–3.5 to prevent deviations from linearity in their retention behaviour. This was, obviously, a great limitation of the procedure.

A mathematical model was further developed [29], which extended the description made with Eq. (42), to take into account the influence of pH on retention. The effect of the modifier on the description of the retention can be considered by substitution of Eqs. (44) and (45), and others similar for the protonated species, in Eq. (20). The following was obtained:

$$k' = \frac{\dfrac{\dfrac{K_{AS}}{1+K_{AD}\varphi}}{1+K_{AM}\dfrac{1+K_{MD}\varphi}{1+K_{AD}\varphi}[M]} + \dfrac{\dfrac{K_{HAS}}{1+K_{HAD}\varphi}}{1+K_{HAM}\dfrac{1+K_{HMD}\varphi}{1+K_{HAD}\varphi}[M]}\kappa K_H h}{1+\kappa K_H h} \tag{48}$$

where:

$$\kappa = \frac{1+K_{HAM}\dfrac{1+K_{HMD}\varphi}{1+K_{HAD}\varphi}[M]}{1+K_{AM}\dfrac{1+K_{MD}\varphi}{1+K_{AD}\varphi}[M]}$$

Eq. (48) may be rewritten as:

$$k' = \frac{k'_A + k'_{HA} K_H^{M\varphi} h}{1 + K_H^{M\varphi} h} \tag{49}$$

where $K_H^{M\varphi}$ is the apparent protonation constant that depends on the concentration of both surfactant and modifier, and on the association capability of both acid–base species with the micelle. The modifier decreases $K_H^{M\varphi}$, whereas this constant slightly increases with the concentration of surfactant. Eq. (48) contains nine constants (K_{AS}, K_{AM}, K_{AD}, K_{MD}, K_{HAS}, K_{HAM}, K_{HAD}, K_{HMD} and K_H), and describes the change in the capacity factors of acid–base solutes at any concentration of surfactant, modifier and pH in the mobile phase.

This model does not consider the effect of the modification of K_H in the water–modifier bulk solvent, due to the change in the concentration of modifier. A more complete model would require the introduction of a new constant. It was checked, however, that the inclusion of this constant does not improve significantly the description of the retention,

since the large number of parameters of the model provides a high flexibility to the fitting, absorbing the deviations produced by this simplification.

A partial fitting of the data can be made to obtain initial values that facilitate the rapid and reliable convergence of Eq. (48) towards the correct solution, and to avoid local minima. Thus, the capacity factors in four mobile phases at pH 7 could be used to calculate the four constants of the non-protonated species (Eq. (42)). The parameters of the protonated species could be obtained similarly at a sufficiently acid medium, whereas the estimation of the protonation constant requires additional mobile phases at intermediate pH values. With the initial parameters, the global non-linear fitting of the complete set of experimental data can be made. Experimental data from at least nine mobile phases are needed, but extra data can be used to improve the reliability of the predictions. The model was successfully applied to the prediction of the retention behaviour of seven solutes inside the total pH range of a C_{18} column, and for the concentration ranges of SDS and propanol, 0.05–0.15 *M*, and 0–8% (v/v), respectively. The errors obtained were lower than 6% [29].

3.5. Prediction of the retention based on molecular properties

Several models have been published in the context of quantitative structure–retention relationships, in order to predict the retention behaviour in liquid chromatography [30]. These models correlate, through multiple linear regression, the retention with diverse molecular descriptors, such as the number of carbon atoms, number of π-electrons, molecular connectivity index, octanol–water partition coefficient, Van der Waals volume, length-to-breadth ratio of the molecule and dipole moment. One of the main problems in these studies is the number and nature of the descriptors. In principle, the more descriptors used, the better the correlation. However, using too many properties the physico-chemical meaning of the role of the different factors can be lost.

Rodriguez et al. studied the chromatographic behaviour of several PAHs eluted with SDS micellar mobile phases without alcohol [31], or with methanol, 2-propanol or 1-butanol, from C_{18} columns [32]. The behaviour of unsubstituted PAHs was well established using the model:

$$\log k' = aF + bL/B + c \tag{50}$$

where F is a correlation factor, calculated for each PAH as [(number of double bonds)+(number of primary and secondary carbon atoms)−0.5 for a non-aromatic ring], and L/B is the ratio of the maximalized length-to-breadth of the rectangle enclosing the molecules. The parameter F is directly related to the size of the molecule and non-directly to its hydrophobicity, whereas L/B refers to the shape of the molecule. The retention of the unsubstituted PAHs increased with both F and L/B. The regression coefficient decreased, however, for methyl-substituted PAHs, which was explained as due to the non-planarity of these compounds, produced by the methyl group. Two new descriptors (selected among thirteen starting descriptors subjected to factor analysis) were, therefore, added to the model: the fraction of non-polar unsaturated surface area (NUSA/TSA), and the dipole moment (DPMON). The retention increased when the NUSA/TSA ratio decreased, which gives information about the electron delocalization along the aromatic rings, and when the DPMON increased, although the relevance of this descriptor was lower and only acquired importance in describing the electronic properties of isomers. Finally, a more general equation was established where the coefficients in Eq. (50) were related, through second- and third-degree polynomia, with the concentration of surfactant or alcohol.

Yang and Khaledi [33] applied linear solvation energy relationships (LSERs) to the evaluation of the retention behaviour of uncharged substituted aromatic compounds of diverse hydrophobicity, using diphenyl and C_8 columns, and mobile phases of CTAB and SDS with 2-propanol and butanol as modifiers. In LSERs, solvent related properties of solutes, *SP* (such as k' or $\log k'$), can be described in terms of the solvatochromic parameters as follows:

$$SP = SP_0 + mV/100 + s\pi^* + b\beta + a\alpha \tag{51}$$

where SP_0 is the regression constant, V is the molar volume of solutes that is related to the solubility behaviour, π^* is a measure of the solutes' ability to engage in dipolarity/polarizability interactions with

the solvent, β is the solutes' basicity, and α is the solutes' acidity. The chemical interactions depended on the type of surfactant, modifier and stationary phase, which affected dramatically the retention behaviour. The values of the regression coefficients, which were between 0.949 and 0.975, showed the significance of the chemical interactions in explaining the retention. For SDS, the correlation was better using $\log k'$, whereas with CTAB, k' correlated better with the solvatochromic parameters. The $a\alpha$ term was positive for solutes binding to CTAB micelles, while it was negative for the SDS micellar phase. This suggested that CTAB provided more a basic environment for the binding of acidic solutes than SDS. On the other hand, CTAB micelles are less dipolar (they have more negative s values) for solutes than SDS micelles.

The statistical treatment required to find a correlation between retention and molecular properties must be performed with caution, since the high number of possible descriptors and the relations among them will, frequently, lead to good results. This will not indicate, however, necessarily that the studied properties influence the retention mechanisms that occur inside the chromatographic system.

3.6. Use of neural networks to predict the retention

In recent years, neural networks have widely been used for solving different chemical problems, including the field of liquid chromatography, such as modelling and prediction of the retention using structural descriptors, chromatographic peak classification and deconvolution of overlapped peaks. In MLC, Xie et al. [34] applied multi-layer feed-forward neural networks, trained with an error back-propagation algorithm, to model the retention behaviour as a function of the concentrations of surfactant and organic modifier, pH and temperature. Accurate retention predictions were achieved. The capacity factor was adopted as the output of the networks. Thus, the general relationship of k' with respect to the mobile phase parameters was considered to be non-linear.

The soft models defined by the weights of the neural networks are capable of accommodating all types of relationships, being especially useful when the dependence of the retention behaviour with the mobile phase parameters is unknown. However, neural networks learn the relationships from the data themselves, and, hence, more experimental points are needed with respect to hard-modelling methods. The use of networks is, therefore, only recommended for those cases where adequate theoretical or empirical models do not exist, such as retention modelling in MLC with four variables (surfactant, modifier, pH and temperature), as described above.

4. Factors affecting the prediction capability of the models

4.1. Linearization of the equations of retention

In the literature, linear regression is often applied to fit the experimental data to the model that describes the dependence of the capacity factor with the concentration of surfactant. However, the linearization process introduces a perturbation in the fitting procedure. A weighting strategy should, therefore, be employed to diminish the systematic errors produced with the linear transformation and to obtain output data similar to those given by non-linear regression. The least-squares method performs an implicit weighting of the experimental points that is proportional to the sensitivity of the signal with respect to the parameter being fitted. When an equation is linearized, the fitting data are modified and, consequently, the implicit weighting is perturbated. The perturbing effect of linearization can be eliminated by using sensitivity weights [35], such as:

$$W = \frac{\left(\frac{\delta f}{\delta P}\right)^n}{\left(\frac{\delta F}{\delta P}\right)^2} \tag{52}$$

where f and F are the non-linear and linearized equations, respectively, and P is a parameter of the model. Substitution in Eq. (52) of Eqs. (30)–(37), for $n=2$, results in:

$$W = \frac{1}{k'^4} \tag{53}$$

Fitting errors, in average 25% larger, were obtained

with the non-weighted fitting compared to the weighted fitting [27].

The evaluation of large retention constants is difficult. The constant K_{AS} is related to the retention in a mobile phase at the CMC; therefore, its calculation requires an extrapolation in a region of large variation of k'. When non-weighted linear fitting is performed, the errors can lead to negative intercepts in the $1/k'$ vs. [M] plots. The application of weights corrects these errors, at least partially.

4.2. Dead time measurement

The reliable determination of k' requires the accurate evaluation of the dead time of the chromatographic system. This is not an easy task when a micellar mobile phase is used. The shape, height and sign of the perturbations appearing at the head of the chromatograms are unpredictable, especially when the nature of the injected solution is very different to that of the mobile phase. In the MLC literature, the dead time is usually measured by injection of water, aqueous solutions of $NaNO_3$, NaI and KI, or organic solvents, such as methanol and acetonitrile. The criteria applied to locate the dead time is either the measurement of the position of the maximum of the first peak, or the measurement of the time from the injection to the first deviation from the baseline. The first criterion is very simple, but the variability in the shape and position of the first peak with mobile phases of diverse composition leads, frequently, to unprecise values. In contrast, the start of the first main peak in the chromatograms is fairly reproducible [36].

The values of dead time obtained by the procedures mentioned above depend on the nature of the injected solution. The dead time measured by injection of water is not very reproducible, especially when the data are taken at the maximum of the first perturbation of the baseline. This measurement is better performed by the injection of micellar solutions of solutes [36]. The experimental work can provide a great number of replicates which can be used for this purpose.

The dead time does not change appreciably with the composition of the eluent in hybrid mobile phases. Therefore, the same value can be used to predict the capacity factors of solutes eluted in a given chromatographic column, with mobile phases containing variable amounts of surfactant and alcohol [27,36]. Furthermore, an excellent prediction of retention times can be obtained using approximate values of dead time, since the capacity factor is only used as an intermediate variable in the prediction of the position of the chromatographic peaks. The evaluation of physico-chemical retention parameters requires, however, the use of an accurate value of dead time. Otherwise, ill-conditioned fittings giving non-sense negative parameters can be obtained.

4.3. Critical micellar concentration

An important factor that should be considered when physico-chemical retention constants are calculated from chromatographic data in MLC, is the correct subtraction of the CMC from the total concentration of surfactant. The CMC usually decreases at low concentrations of organic solvent. When the CMC is not subtracted from the total concentration of surfactant, the errors in the calculation of the retention constants can be larger than 100%. However, as occurs with the dead time, there is no significant difference between the use of total or micellar concentration of surfactant in the prediction of the retention behaviour (capacity factors) [19,27].

4.4. Ionic strength and temperature

The ionic strength of the mobile phase will influence the elution, again depending on the combination of hydrophobic and electrostatic interactions. Armstrong and Stine [37,38] observed that the negative c_1 coefficient in Eq. (13) for antibinding solutes, became more positive with increasing salt concentration in the mobile phase, and these solutes could even change to a binding type behaviour. This effect was explained as similar to the flocculation of colloidal systems: the counterion layer around the micelle is narrowed in a solution containing higher concentration of ions, which facilitates the approximation of the solute to the micelle assembly. Hydrophobic interactions between the solute and the nonpolar core of the micelle can then be established. For solutes that are electrostatically attracted to the micelle, the retention must also change because of

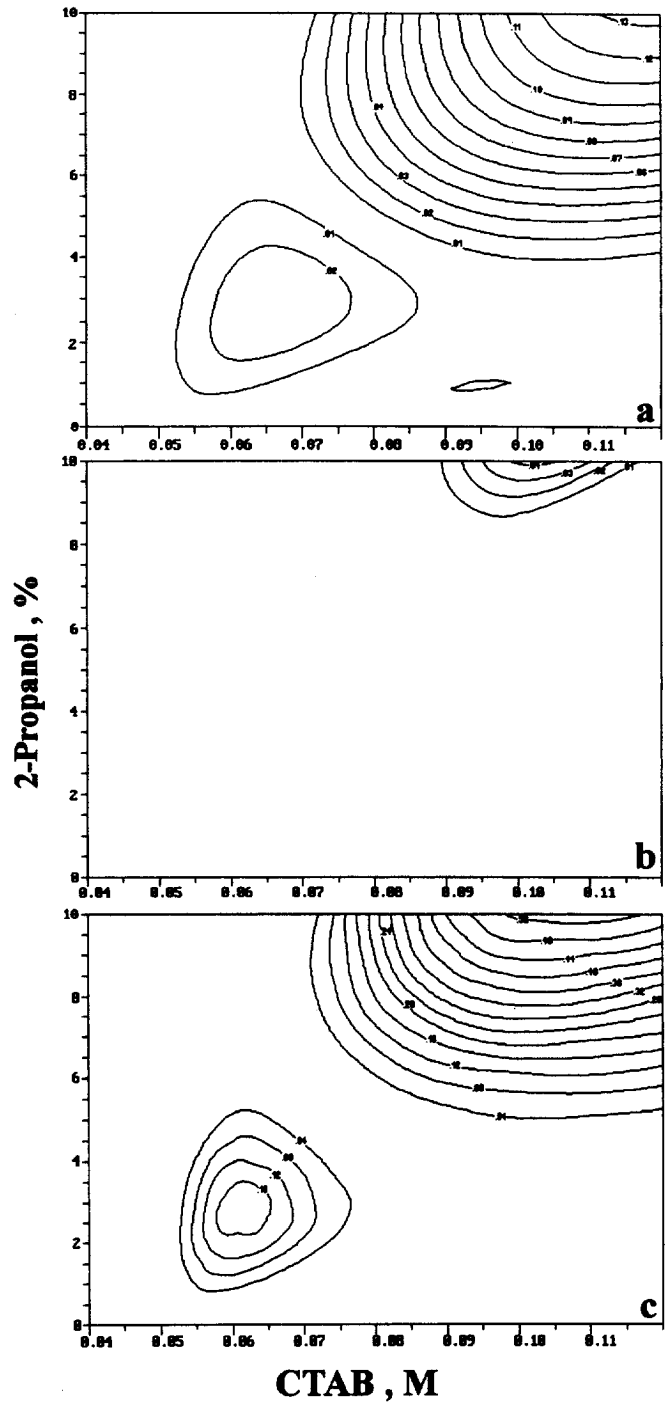

Fig. 5. Contour maps of global resolution for the mixture of fifteen phenols, and CTAB/2-propanol mobile phases (optima indicated), according to: (a) separation factor (0.12 *M*/10%); (b) valley-to-peak ratio (0.102 *M*/10%); (c) overlapping extent (0.107 *M*/10%); from [19].

the diminution of K_{AM} and K_{AS} in a medium of increasing ionic strength, since the presence of extra inert ions at moderate concentrations favours the displacement of the equilibrium towards dissociation. The retention will increase or decrease depending on the K_{AM}/K_{SW} ratio.

Dorsey et al. [12], in an effort to enhance the efficiency, proposed the use of elevated temperatures in MLC. Tomasella et al. [39,40] observed for several solutes that K_{AS} and K_{AM} decreased as the temperature increased, but K_{AS} decreased more rapidly, thus making the retention decrease. However, the effect of temperature on retention is less pronounced in MLC than in conventional liquid chromatography. Also, the change in temperature displays a minor change in the capacity factors when compared with the changes that are caused by the addition of organic modifier to the mobile phase.

5. Optimization of resolution

To optimize the resolution in the separation of a mixture of compounds, the following process should be applied: (i) achievement of the retention equations for each solute, (ii) search of the optimum mobile phase with the aid of contour maps of global functions of resolution of the mixture of solutes (Fig. 5), (iii) simulation of the chromatogram for the optimum mobile phase, and (iv) search of a new maximum when the selected optimum is not satisfactory. The four criteria of global resolution given below, based on the normalized product, r, of different properties, $X_{i,i+1}$, associated to pairs of consecutive peaks, were applied in MLC [16,19]:

$$r = \prod_{i=1}^{n-1} \frac{X_{i,i+1}}{\left(\sum \frac{X_{i,i+1}}{n-1}\right)^{n-1}} \tag{54}$$

The individual properties studied were the selectivity, separation factor, valley-to-peak ratio and overlapping extent. The three latter may vary from 0 to 1. The function of resolution, r, was maximized to obtain the optimum mobile phase. The proximity to unity indicated the good performance of the separation. The function r was chosen as the optimization criterion, since it originates an even distribution of all peaks in the chromatogram. Since the product of all observed resolutions is used, coelution will effectively cause the criterion to drop to zero. On the other hand, extremely long chromatograms with a number of unnecessarily large resolution values will also be represented by low criterion values.

5.1. Strategies strictly based on retention

The more simple criteria are based on the optimization of properties only depending on the retention, such as [16,41,42]:

$$\beta_{i,i+1} = 1 - \frac{1}{\alpha_{i,i+1}} = 1 - \frac{k'_{i+1}}{k'_i} \tag{55}$$

where $\alpha_{i,i+1}$ is the selectivity, and the separation factor [19]:

$$S_{i,i+1} = \frac{t_{i+1} - t_i}{t_{i+1} + t_i} = \frac{k'_{i+1} - k'_i}{k'_{i+1} + k'_i + 2} \tag{56}$$

The positional criteria can lead to a reliable optimum of resolution using the retention data from a few mobile phases. However, as the shape and width of the chromatographic peaks are not considered, achievement of an unacceptable optimum with peaks largely overlapped having a high positional resolution, could be obtained. If this is the case, only an increase in plate count will provide the desired

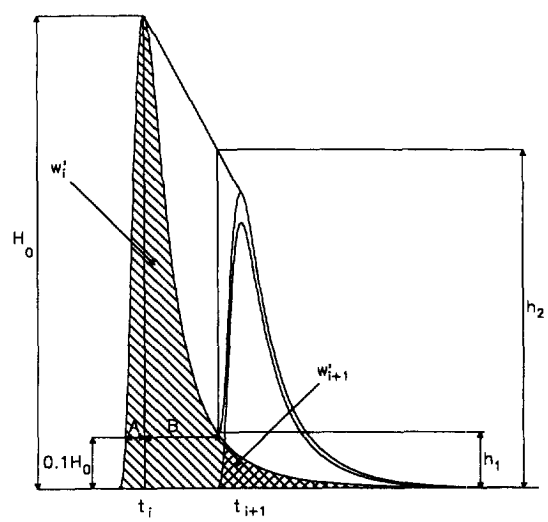

Fig. 6. Chromatographic peak properties. For details see text.

separation, for instance, using two identical columns in series [16]. Alternatively, a response surface related to a different criterion can be examined.

5.2. Strategies taking into account the position and peak profile

The major drawback of practical separations applying MLC is still the low chromatographic efficiency caused by the resistance to mass transfer in the processes involving the micelles, and the surfactant-modified stationary phase. This is especially important since the increased micellar concentrations causes a decrease in plate count, resulting in a varying efficiency over the variable space. In this respect, it is worthwhile to examine the inclusion of the expected peak profile in the expression of the chromatographic quality. The information given by the positional-shape criteria may be interesting, not only when the chromatographic peaks are asymmetrical or with peaks of low efficiencies, but also with mobile phases where symmetrical peaks are very close to each other. Thus, poorly defined optima obtained with the positional criterion often become clearer when the shape of the peaks is considered.

The valley-to-peak ratio:

$$P_{i,i+1} = 1 - \frac{h_1}{h_2} \tag{57}$$

and the overlapping extent

$$O_i = 1 - \frac{w'_{i+1}}{w'_i} \tag{58}$$

criteria consider not only the position, but also the shape (efficiency and asymmetry) of the peaks [19]. In Eqs. (57) and (58), h_1 is the height of the valley between two adjacent peaks; h_2 the interpolated height between the maxima of two adjacent peaks measured at the abscissa of the valley; w'_i is the total area of a given peak, and w'_{i+1} is the area of this peak overlapped by other peaks (Fig. 6). The overlapping criterion extends the global function of resolution to all the peaks in the chromatogram, thus $n-1$ should be substituted for n in Eq. (54).

The elution profile of each peak should be predicted from the values of efficiency and asymmetry factor, both interpolated using the values of these parameters obtained in the three experimental mobile phases closer to the simulated mobile phase. With this approach, accurate descriptions of peaks showing large asymmetry can be obtained.

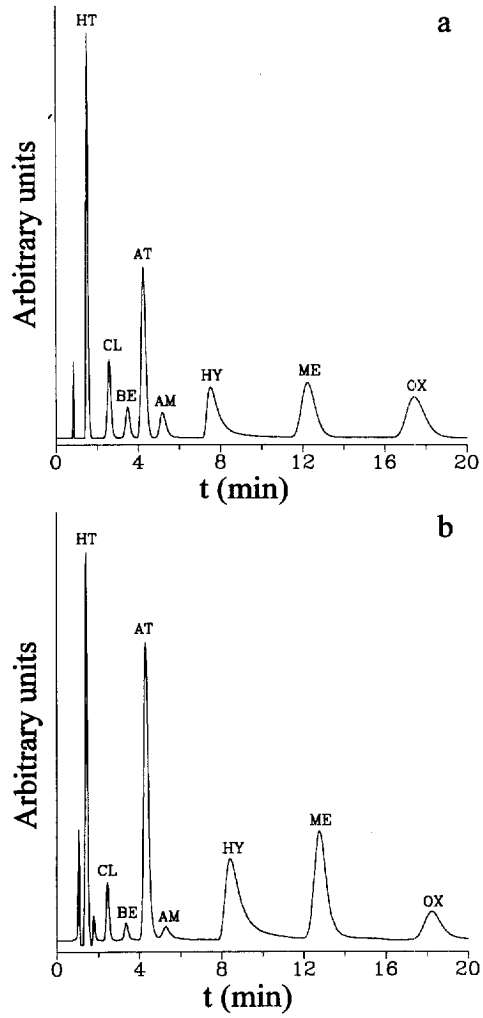

Fig. 7. Simulated (a) and experimental (b) chromatograms of a mixture of compounds, eluted with a 0.15 *M* SDS–7% 1-propanol mobile phase, buffered at pH 3.0. Symbols: HT, hydrochlorothiazide; CL, chlorthalidone; BE, bendroflumethiazide; AT, atenolol; AM, amiloride; HY, hydralazine; ME, metoprolol; and OX, oxprenolol; from [22].

The combined use of the optimization criteria may give complementary information for selecting the optimum mobile phase. The positional criterion gives a rough approximation of the region where the peaks

will be separated, but it does not indicate how well separated they will be. The valley-to-peak ratio criterion gives information about the region where the peaks will be apparent. Finally, the overlapping criterion will indicate the region where the peaks will be better quantified, because a larger surface of each peak will be exposed. As the application of the positional-shape criteria requires a good prediction of the position and shape of the chromatographic peaks, they are more susceptible to error. However, these criteria are always preferable, even when asymmetrical peaks are assumed to be symmetrical.

The selection of the optimum mobile phase should, however, not only consider the value of the resolution function, but also the suitability in the preparation of the mobile phase. Thus, an optimum found in a region of large variations in the resolution function will not be adequate in practice, as the errors in the prediction of the retention and in the preparation of the mobile phase may lead to results different from those expected. For complex response surfaces showing several maxima and minima, additional experimental mobile phases should be prepared in the region where the optimum appears. In other cases, the optimum will be very extensive, and mobile phases giving the shorter retention times will be more adequate.

As an example of the good performance of the models that permit the prediction of retentions and peak shapes, Fig. 7 shows the experimental and simulated chromatogram for a mixture of four diuretics, three β-blockers and a vasodilator, eluted with a mobile phase of 0.15 *M* SDS and 7% (v/v) 1-propanol at pH 3 [22]. The prediction of the position of the peaks was made using Eq. (33) and the optimum resolution was obtained according to the overlapping criterion.

A program has been developed for the general treatment of chromatographic data applied to MLC [42]. This program, called MICHROM, takes part in all the stages of the analytical process. It allows determination of the dead time, smoothing of chromatograms, measurement of peak parameters, modelling of the retention, and fitting of skewed peaks. Tools for the experimental design, optimization of the mobile phase composition to resolve a mixture of analytes, and simulation of chromatograms in several experimental conditions, are also implemented.

6. Conclusions

Modelling of the retention behaviour in MLC allows the understanding of the processes occurring inside the chromatographic system. Although the retention and selectivity strongly change with varying concentrations of surfactant, organic modifier and/or pH, the observed changes are very regular, and are well described by simple models. As a consequence, it is possible to predict the retention behaviour of solutes on the basis of a limited number of experiments, even though these experiments are relatively far apart in the variable space.

The many interactions that the solutes experience in a micellar chromatographic system enhances the differences among them. The possibility of using simultaneously the three most significant variables that affect the retention, e.g., surfactant and modifier concentrations and pH, will improve the capability of resolution of complex mixtures of ionic and non-ionic compounds. This will permit the reliable and relatively rapid optimization of the composition of the mobile phase, for the separation of a mixture of compounds, by using an interpretive method and a reduced number of mobile phases (five for two variables and nine for three variables).

The accurate prediction of the retention of each compound in a given mixture is more important with micellar mobile phases than with the conventional aqueous–organic eluents, due to the lower efficiencies of the former. The development of applications in MLC requires that the resolution of complex mixtures be made and optimized in a short time, with a low waste of reagents. The results shown in this review may contribute to this purpose.

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