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Review

Retention modeling in micellar liquid chromatography

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Abstract

The aim of this review is to present the most relevant work on retention modeling in micellar liquid chromatography. First, physico-chemical models explaining the variation of capacity factors with one or more experimental variables (such as micellar concentration, organic modifier concentration, and pH) will be shown. Secondly, studies carried out to model the solute retention in micellar liquid chromatography by means of empirical equations will be presented, and finally new trends in this area will be introduced. © 1997 Elsevier Science B.V.

Keywords: Micellar liquid chromatography; Retention models; Reviews; Mobile phase composition; Artificial neural networks; Benzenes; Polynuclear aromatic hydrocarbons; Pyrazoles; Dihydropyridines

Contents

1. Introduction

As it is well known, the use of micellar solutions below their critical micellar concentration (CMC) as

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mobile phases in RP-HPLC leads to some advantages of this separation technique (micellar liquid chromatography, MLC) such as the low cost [1,2] and nontoxicity of surfactant [3–12] versus expensive and flammable solvents of chromatographic grade, unique selectivity [1,3,13–16], compatibility of mobile phases with salts and water-insoluble compounds [7,17], shorter equilibration times for gradient elution [5–7,9,11,18,19], and the possibility of direct injection of biological fluids due to the capability of some micellar solutions (anionic or nonionic surfactant solutions) to solubilize the proteic matrix of such samples [4,8–10,12,14,16,18–30].

Although MLC is a very interesting separation technique, the poor efficiency observed when pure aqueous micellar solutions are used as mobile phases [1,12,23] can seriously limit its practical applications. This problem is probably due to the poor wetting of the stationary phase and the restricted mass transfer, which can be solved, at least partially, by introducing a small percentage of organic modifiers in the mobile phase (usually alcohols of short or medium chain), increasing the temperature or reducing the flow-rate of the mobile phase. Therefore, the use of hybrid mobile phases has become a common practice to alleviate the poor efficiency of this chromatographic technique.

Thus, there are many variables that affect solute retention in MLC, such as nature and concentration of surfactant and organic modifier, solute nature, pH, temperature, etc., and it would be desirable to have models that may help to explain the relation of the solute retention with them and then to find the best conditions of the separation of a solute mixture. It is not an easy task, but some attempts have been made, especially in recent years, although more research will be required to exploit completely, the potential advantages of this separation technique more wisely.

The aim of the above studies is to find the optimum conditions to perform a solute mixture separation with minimum experimental effort. From a practical viewpoint, only partial answers have been obtained to this date; just the simultaneous evaluation of retention and chromatographic efficiency with all experimental variables can provide a general view of the problem. However, only retention models have been reported. The published work on this subject can be classified into physico-chemical models explaining the variation between capacity factors and one or two experimental variables (among micellar concentration, organic modifier concentration, and pH) and empirical models, without a chemical sense, but enabling to foresee the solute retention behavior in different experimental conditions. When physico-chemical models are not available, empirical models are often used.

2. Physico-chemical models

Some physicochemical models relating the variation of capacity factors with (i) micellized surfactant concentration, (ii) micellized surfactant concentration and pH, and (iii) micellized surfactant concentration and organic modifier concentration in MLC have been reported since Armstrong and Henry introduced MLC as a separation technique in 1980 [31]. It is important to note that no more than two variables are considered simultaneously.

2.1. Models relating retention to micellized surfactant concentration

The first studies on solute retention modeling in MLC were performed by Armstrong and Nome [32] and Arunyanart and Cline Love [33]. Armstrong and Nome [32] considered the retention of a solute by means of a three equilibria model as those presented in Fig. 1. The solute in this model can partitioning between the modified stationary phase (the stationary phase is modified by the adsorption of surfactant monomers) and the aqueous phase, characterized by the partition coefficient P_{sw}, between micelles and aqueous phase, P_{mw}, and the stationary phase and the micellar pseudophase, P_{sm}. The equation proposed by Armstrong and Nome [32] is the following

$$\frac{V_s}{V_e - V_m} = \frac{1}{P_{sw}} + \frac{P_{mw} - 1}{P_{sw}} v[M_m] \quad (1)$$

where V_s is the stationary phase volume, V_e the elution volume of the solute, V_m the mobile phase volume, v the surfactant molar volume, $[M_m]$ the micellized surfactant concentration (total surfactant concentration minus the CMC) and P_{sw} and P_{mw} the

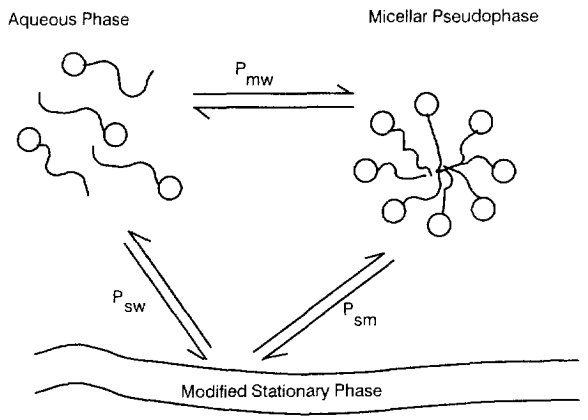

Fig. 1. Three equilibria model for solute partitioning among the different phases or pseudo-phases in MLC.

solute distribution coefficients between the stationary and aqueous phases and between the micellar pseudophase and the aqueous phase respectively.

The equation proposed by Arunyanart and Cline Love [33] relates the capacity factor with the micellized surfactant concentration as

$$\frac{1}{k'} = \frac{1}{\phi[L_s]k_1} + \frac{k_2}{\phi[L_s]k_1}[M_m] \quad (2)$$

$E_m + L_s \rightleftharpoons EL_s$; k_1 (equilibrium 1)

$E_m + M_m \rightleftharpoons EM_m$; k_2 (equilibrium 2)

where ϕ is the phase ratio (V_s/V_m), $[L_s]$ the concentration of stationary phase sites and k_1 and k_2 the constants of the two equilibria, E_m is the solute in the mobile phase and EL_s and EM_m are the complexes formed between the solute in the mobile phase and the stationary phase sites in the stationary phase and the micelles in the mobile phase, respectively.

The equilibrium of direct transfer between the micellar phase and the stationary phase has been neglected because it depends on the formers.

Both equations predict a retention decrease when the micellized surfactant concentration increases. Although this is the most common situation experimentally found, sometimes when CN and C_1 stationary phases have been used, some solutes can be found for which retention increases or does not vary when increasing in the micellized surfactant concentration in the mobile phase. Thus, Armstrong and Stine [34] proposed three categories for solutes classification: (i) binding solutes, those that can bind with micelles ($k_2>0$) in the mobile phase and, consequently, retention decreases when the micellized surfactant concentration increases; (ii) non-binding solutes, those that cannot bind with micelles ($k_2=0$) in the mobile phase, therefore, retention does not vary with the micellized surfactant concentration; (iii) anti-binding solutes, those that are excluded by micelles ($k_2<0$) and their retention increases when the micellized surfactant concentration is greater. Although it has no chemical sense to obtain equilibrium constants with negative values, this phenomenon is quite real and reproducible and might be due to electrostatic repulsion [34].

The equations proposed by Armstrong and Nome [32] and Arunyanart and Cline Love [33] (Eqs. (1) and (2)) have been widely used with several types of compounds, surfactants, and stationary phases [4,14,28,33,35–47] and have enabled to check the validity of these models constituting a simple way to calculate solute–micelle association constants. However, some problems have been found when these models are used. First, errors in the determination of solute–micelle association constants increase as the hydrophobicity of the solute is greater. This problem can be minimized by using more polar stationary phases [12,48,49], since retention diminishes when these phases are employed. Also, negative intercepts in the plots of the inverse of capacity factors versus micellized surfactant concentration have been often reported. This fact prevents the calculation of association constants. Some studies have been carried out to study this phenomenon [28,39,50] and several explanations have been reported. Borgerding et al. [39,50] think that these intercepts are zero more than negative and they are due to the high values of the solute partition coefficient between the aqueous and the modified stationary phase. In this regard, when very hydrophobic solutes are studied, their solubility in the aqueous phase is very limited so the retention implies a direct transfer from the micelles to the stationary phase. Therefore, these solutes' retention can be expressed as:

$$k' = P_{sm}\frac{\phi}{V[M_m]} \quad (3)$$

that is, capacity factors are inversely related to the

micellized surfactant concentration, the slope depends on the solute partition coefficient between micelles and stationary phase and the intercept is zero. Other authors [28,43] have reported the variation of the CMC, the aggregation number, and micelle geometry as possible causes for obtaining negative intercepts, as the surfactant concentrations increases; recently, Torres-Lapasió et al. [51], have reported problems in the calculation of dead time when micellar mobile phases are used.

These models, although extensively used, have a limited applicability due to the low efficiency of the chromatographic peaks when pure aqueous micellar mobile phases are employed. However, they are also valid when hybrid mobile phases in which constant percentages of organic modifiers are used [9]. This fact has been verified for different organic modifiers such as methanol [14,38,47,52], propanol [4,6,47,52–55], butanol [15,47,52,56] and acetonitrile [57], in which case solute micelle association constants lower than those calculated in water are obtained because the organic modifier can compete to a different extent with the solutes for interaction with micelles and the stationary phase.

As an example of the validity of Arunyanart and Cline Love's model [33] (Eq. (2)) when hybrid mobile phases [containing hexadecyltrimethylammonium bromide (CTAB) as the surfactant and *n*-propanol as the organic modifier] are used, (Table 1) the equations and r^2 values obtained for some solutes (*p*-nitrophenylpyrazoles and dinitrophenylpyrazoles) studied by our research team [58] are shown. It can be observed that r^2 values are very close to unity, indicating the validity of Arunyanart and Cline Love's [33] equation. It is also interesting to note that intercepts are close to zero or negative due to the high hydrophobicity of the compounds under study (the logarithm of the octanol–water partition coefficient, log P_0, for these compounds ranges between 2.16 and 3.15).

The models of Armstrong and Nome [32] and Arunyanart and Cline Love [33] can only solve partially the optimization of the conditions of a real separation problem because many important vari-

Table 1
Equations for the plots of $1/k'$ versus micellized surfactant concentrations for mobile phases containing CTAB as the surfactant and constant concentrations of *n*-propanol as the organic modifier for a group of nitrophenylpyrazoles and dinitrophenylpyrazoles [58] (substituting groups in the pyrazole molecule are shown as R1, R2 and R3)

Solute	R1	R2	R3	3% propanol		5% propanol		10% propanol	
				Equation	r^2	Equation	r^2	Equation	r^2
1[a]	-Me	-H	-Me	$y=0.565x+4.1\cdot10^{-3}$	0.9998	$y=0.576x+7.3\cdot10^{-3}$	0.9975	$y=0.778x+0.0163$	0.9997
2[a]	-H	-Br	-Me	$y=0.481x+6\cdot10^{-4}$	0.9998	$y=0.502x+1.4\cdot10^{-3}$	0.9996	$y=0.684x+6.3\cdot10^{-3}$	0.9991
3[a]	-Me	-Br	-Me	$y=0.408x-1\cdot10^{-4}$	0.9998	$y=0.425x+3\cdot10^{-4}$	0.9994	$y=0.593x+3.3\cdot10^{-3}$	0.9993
4[a]	-Ph	-H	-H	$y=0.461x-2.9\cdot10^{-3}$	0.9992	$y=0.497x-1.7\cdot10^{-3}$	0.9994	$y=0.728x-2.1\cdot10^{-3}$	0.9997
5[a]	-t-Bu	-Me	-t-Bu	$y=0.191x-9\cdot10^{-4}$	0.9996	$y=0.183x-6\cdot10^{-4}$	0.9999	$y=0.263x+1\cdot10^{-4}$	0.9991
6[a]	-Ph	-H	-Ph	$y=0.271x-1.9\cdot10^{-3}$	0.9988	$y=0.274x-1.4\cdot10^{-3}$	0.9998	$y=0.400x-1.4\cdot10^{-3}$	0.9995
7[b]	-H	-Me	-Me	$y=0.673x+6.4\cdot10^{-3}$	0.9999	$y=0.279x+9.1\cdot10^{-3}$	0.9993	$y=0.986x+0.0232$	0.9991
8[b]	-H	-Br	-Me	$y=0.249x-9\cdot10^{-4}$	0.9996	$y=0.242x-1.1\cdot10^{-3}$	0.9999	$y=0.306x+2\cdot10^{-4}$	0.9996
9[b]	-Me	$-NO_2$	-Cl	$y=0.656x+2.1\cdot10^{-3}$	0.9999	$y=0.742x+2.4\cdot10^{-3}$	0.9996	$y=1.03x+8.8\cdot10^{-3}$	0.9992
10[b]	-Br	-Me	-H	$y=0.667x-4\cdot10^{-4}$	0.9997	$y=0.718x-9\cdot10^{-4}$	0.9997	$y=0.959x+3.3\cdot10^{-3}$	0.9997
11[b]	-Me	-Br	-Me	$y=0.477x+8\cdot10^{-4}$	0.9999	$y=0.514x+1.0\cdot10^{-3}$	0.9994	$y=0.726x+4.7\cdot10^{-3}$	0.9994
12[b]	-Br	-Br	-Me	$y=0.508x-8\cdot10^{-4}$	0.9998	$y=0.545x-4\cdot10^{-4}$	0.9996	$y=0.768x+1.6\cdot10^{-3}$	0.9996
13[b]	-Me	-H	-t-Bu	$y=0.429x+1.5\cdot10^{-3}$	0.9999	$y=0.455x+2.0\cdot10^{-3}$	0.9989	$y=0.636x+6.6\cdot10^{-3}$	0.9994
14[b]	-H	-Me	-Ph	$y=0.438x-7\cdot10^{-4}$	0.9995	$y=0.447x+4\cdot10^{-4}$	0.9996	$y=0.620x+2.4\cdot10^{-3}$	0.9997
15[b]	-H	-Br	-Ph	$y=0.386x-9\cdot10^{-4}$	0.9998	$y=0.407x-7\cdot10^{-4}$	0.9994	$y=0.586x+6\cdot10^{-4}$	0.9995
16[b]	-Ph	-Me	-Me	$y=0.398x-7\cdot10^{-4}$	0.9996	$y=0.421x-5\cdot10^{-4}$	0.9996	$y=0.600x+6\cdot10^{-4}$	0.9995
17[b]	-Ph	-Br	-Me	$y=0.374x-1.9\cdot10^{-3}$	0.9990	$y=0.387x-9\cdot10^{-4}$	0.9998	$y=0.563x-4\cdot10^{-4}$	0.9997
18[b]	-t-Bu	-H	-t-Bu	$y=0.202x-3\cdot10^{-4}$	0.9996	$y=0.204x-8\cdot10^{-5}$	0.9999	$y=0.318x+9\cdot10^{-4}$	0.9993
19[b]	-t-Bu	-Me	-t-Bu	$y=0.181x-8\cdot10^{-4}$	0.9996	$y=0.180x-5\cdot10^{-4}$	0.9984	$y=0.281x+4\cdot10^{-6}$	0.9996

Me, methyl; Ph, phenyl; *t*=Bu, *tert.*-butyl.
[a] Basic structure: *p*-nitrophenylpyrazole.
[b] Basic structure: dinitrophenylpyrazole (nitro groups: *o*- and *p*- positions).

ables are not considered. Thus, when ionic solutes are studied, the mobile phase pH and the micellized surfactant concentration should be optimized simultaneously.

2.2. Models relating retention to micellized surfactant concentration and pH

Examples of these models are those proposed for weak acids and bases [59] and for zwitterionic solutes [26]. When these are considered, the equilibria that can be established between the different species and phases are shown in Fig. 2 (Fig. 2a weak acids and Fig. 2b zwitterionic compounds) and equations relating to the variation of capacity factors with the micellized surfactant concentration and pH are the following:

weak acids

$$k' = \frac{k'_0(1 + k_2[M_m]) + k'_1(1 + k_4[M_m])k_{am}/[H^+]}{1 + k_2[M_m] + (1 + k_4[M_m])k_{am}/[H^+]} \quad (4)$$

where k'_0 and k'_1 the limiting capacity factors for the neutral and the dissociated form, respectively.

zwitterionic solutes

$$k' = \frac{k'_{sc} + k'_{sz}k_{a1}/[H^+] + k'_{sa}k_{a1}k_{a2}/[H^+]^2}{[1 + k_{mc}[M_m] + (1 + k_{mz}[M_m])k_{a1}/[H^+] + (1 + k_{ma}[M_m]k_{a1}k_{a2}/[H^+]]^2} \quad (5)$$

where $k'_{sc} = k_{sc}\phi[L_s]$, $k'_{sz} = k_{sz}\phi[L_s]$, and $k'_{sa} = k_{sa}\phi[L_s]$.

In these cases the variation of the capacity factor with pH, when the micellized surfactant concentration remains constant, is of a sigmoidal type.

These studies suggest that a shift in the ionization constants is obtained when the micellized surfactant concentration is modified, so optimization of the separation conditions must be attained considering both variables simultaneously.

The results obtained when Eq. (5) is used to predict the retention behavior of phenylalanine, tryptophan, methionine, and phenylpropionic acid range between 4.1% and 10.5%. Although these values can be considered too high, it should be noted that a small change in the pH can reduce the error significantly due to the strong dependence of retention on this variable, that is, the retention prediction can be poor when the pH is within (±) 1 pH unit of the solute apparent ionization constant.

Only recently, some models relating simultaneously retention to micellized surfactant and the organic modifier concentrations, have been reported.

2.3. Models relating retention to micellized surfactant and organic modifier concentrations

The first model proposed to relate capacity factors with the micellized surfactant and alcohol concentrations [60] can be considered an extension of Arunyanart and Cline Love's [33]. This model considers the modification of stationary phase sites and micelles concentration due to the presence of an alcohol, that is, the alcohol can compete with the solute for interaction with the stationary phase and

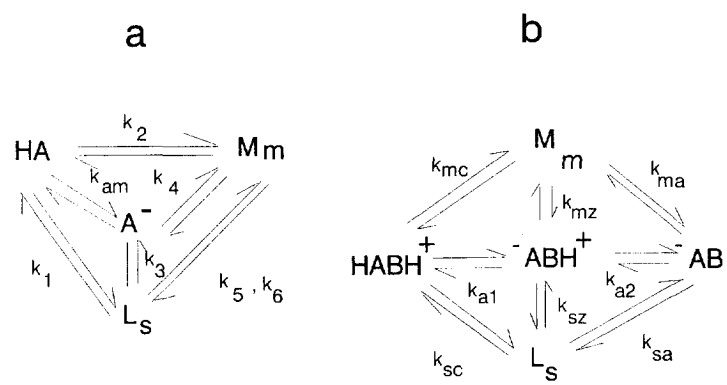

Fig. 2. Equilibria established among the different species and phases for (a) weak acids and (b) zwitterionic compounds.

micelles. Therefore, the equilibria proposed are those considering solutes interaction in the mobile phase and stationary phase sites and micelles in the mobile phase (equilibria 1 and 2, introduced earlier) and the following:

$A_m + L_s \rightleftharpoons AL_s$; k_3 (equilibrium 3)

$A_m + M_m \rightleftharpoons AM_m$; k_4 (equilibrium 4)

being A_m the alcohol concentration in the mobile phase (in molarity) and AL_s and AM_m the complexes formed between the alcohol in the mobile phase and stationary phase sites and micelles respectively.

If these four equilibria are considered, the resulting equation for the capacity factor is:

$$k' = \frac{\phi k_1[L_s](1 + k_4[A_m])}{1 + (k_3 + k_4)[A_m] + k_2[M_m](1 + k_3[A_m]) + k_3k_4[A_m]^2} \quad (6)$$

It should be noted that depending on the constant values and alcohol concentration, this model can be simplified and some linear equations relating the inverse of the capacity factor with the alcohol concentration are obtained. Some of these expressions are shown at Table 2. Linear or quadratic variations of capacity factors with the alcohol concentration can be obtained for low alcohol concentrations, when the interaction of alcohol and micelles is of little importance, or both. Moreover, for very hydrophobic solutes (high k_1 values), interacting strongly with micelles (high k_2 values) and using alcohols interacting weakly with the stationary phase, the model predicts a direct transfer of the solutes from the micelles to the stationary phase (Eq. 9 in Table 2) which slope (plot of $1/k'$ versus $[M_m]$) depends on the alcohol concentration and with zero intercept (when this variable remains constant).

So, this model can predict a nonlinear, linear or quadratic variation of capacity factors with the alcohol concentration in the mobile phase (when micellized surfactant concentration is constant) and a linear variation of the inverse of capacity factors with the micellized surfactant concentration (when the alcohol concentration remains constant).

These variations concur with the experimental behavior as shown in Fig. 3 (plots obtained for two different pyrazole derivatives, a and b, representing respectively those possessing the least and the highest hydrophobicity of the compounds studied) and with some empirical equations previously reported [61] and tested with solutes of a different nature, surfactants and alcohols [61–63] which will be covered in the next section, and it is able to explain why depending on the solute, the surfactant, and the

Table 2
Simplified expressions derived from the general Eq. (6) and the approximations made to obtain them (reproduced from J. Chromatogr. A, 719 (1996) 15–26 with permission from the authors)

Equation	Simplified expressions[a]	Approximation
7	$\frac{1}{k'} = \frac{1}{a} + \frac{k_3}{a}[A_m] + \frac{k_2}{a}[M_m] + \frac{k_2k_3}{a}[M_m][A_m]$	$k_4[A_m] \ll 1$ $k_3 \gg k_4$ $k_3k_4[A_m]^2 \ll 1$
8	$\frac{1}{k'} = \frac{1}{a} + \frac{k_3 + k_4}{a}[A_m] + \frac{k_2}{a}[M_m] + \frac{k_2k_3}{a}[M_m][A_m] + \frac{k_3k_4}{a}[A_m]^2$	$k_4[A_m] \ll 1$
9	$\frac{1}{k'} = \frac{k_2}{a}[M_m] + \frac{k_2k_3}{a}[M_m][A_m]$	$k_4[A_m] \ll 1$ $k_2 \uparrow\uparrow$ $k_3 \downarrow$
10	$\frac{1}{k'} = \frac{1}{a} + \frac{k_3}{a}[A_m] + \frac{k_2}{a}[M_m] + \frac{k_2k_3}{a}[M_m][A_m] + \frac{k_3k_4}{a}[A_m]^2$	$k_4[A_m] \ll 1$ $k_3 \gg k_4$
11	$\frac{1}{k'} = \frac{1}{ak_4}\frac{1}{[A_m]} + \frac{k_3 + k_4}{ak_4} + \frac{k_2}{ak_4}\frac{[M_m]}{[A_m]} + \frac{k_2k_3}{ak_4}[M_m] + \frac{k_3}{a}[A_m]$	$k_4[A_m] \gg 1$

[a] $a = k_1\phi[L_s]$.

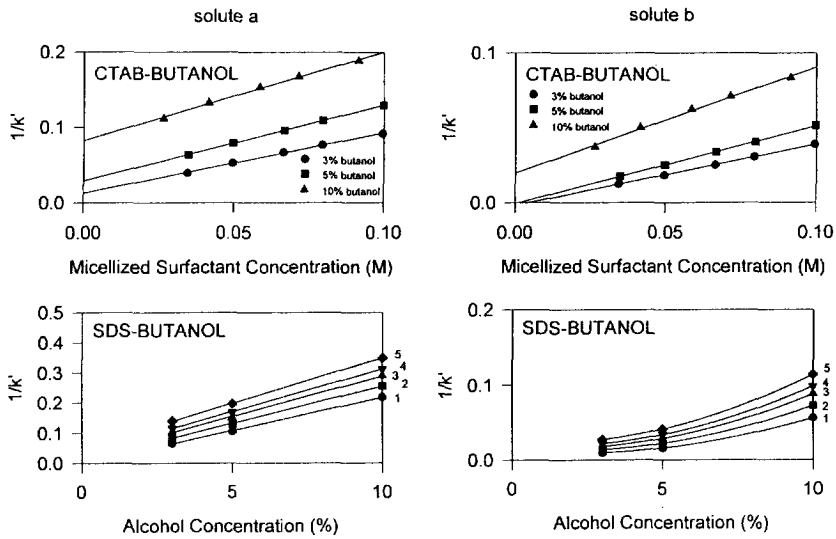

Fig. 3. Experimental variation found for the inverse of capacity factors as a function of micellized surfactant concentration (for constant concentrations of butanol) (up), and alcohol concentration (total surfactant concentration: (1) 0.035 *M*, (2) 0.050 *M*, (3) 0.067 *M*, (4) 0.080 *M*, and (5) 0.1 *M*) (down) for solutes a (left), and b (right) being the least and the highest hydrophobic compounds of the pyrazole derivatives studied in Ref. [58].

alcohol nature and concentrations, different equation showing the retention behavior can be found.

This model enables the calculation of different equilibrium constants, being very helpful to study the solute retention mechanism when a hybrid mobile phase is considered, the normal conditions due to the efficiency enhancement in such media. The different equilibrium constants calculated for a group of benzene derivatives, polycyclic aromatic hydrocarbons and dihydropyridines are reported in Refs. [60,63]. From the results presented in these references some conclusions can be drawn. The equations that best fit the retention behavior of the solutes under study depends on the solute nature, and the surfactant and alcohol nature and concentrations. For solutes of low hydrophobicity all the equilibrium constants can influence the retention so in this case the general model (Eq. (6)) should be applied. In contrast, when very hydrophobic solutes are considered, Eqs. (7) and (9) in Table 2 are usually found to explain their retention.

The method used to calculate physico-chemical constants is simple and only requires the use of nonlinear fitting software, but care must be taken due to the inherent risk of obtaining local minimum of the response surface instead of a global minimum. Also, because of the equations parameters equilibrium constants are, a restriction has been included for the values, that is, they can only be zero or positive. The mean relative errors of fitting reported for benzene derivatives and polycyclic aromatic hydrocarbons ranged between 2.5% and 10.3% and 1.06% and 15.70% for dihydropyridines. The highest errors obtained for benzene derivatives and polycyclic aromatic hydrocarbons in sodium dodecyl sulfate (SDS)–butanol mobile phases and for dihydropyridines in CTAB–butanol mobile phases could be explained by the change in the retention mechanism due to the solubility enhancement of the most hydrophobic compounds at high alcohol concentrations.

Recently, García-Alvarez-Coque et al. [64] have proposed a similar model that takes into account the shift of the different equilibria in these hybrid systems. Their study is about the impact of some fitting procedures (non-linear regression, and non-weighted and weighted regression) on the constants values and associated error. They conclude that the non-linear fitting and the weighted linear fittings provide the best results (weight being $1/k'^4$) but what it is not clear is the model that must be chosen to perform a separation. They reported that errors

obtained for the most hydrophobic solutes, such as pyrene, get better if the shift of the solute partition coefficient between the hydro–organic and the stationary phase due to the presence of alcohol is considered.

The physico-chemical models presented herein comprise state-of-the-art procedures on the subject although much remains to be done concerning the potential of this chromatographic technique. In our opinion, models that can help to explain retention as a function of micellized surfactant concentration, alcohol concentration, pH and temperature should be developed. It would also be desirable to model the chromatographic efficiency which concomitantly would facilitate optimization of separation conditions.

Some progress have been made but research must continue, both, in order to succeed in developing some physico-chemical models and, in their absence, some empirical methods, such as empirical equations, are being proposed.

3. Empirical equations

These models, as mentioned above, have no chemical sense but are very valuable tools for predicting retention solutes as a function of different variables. Sometimes, they lead to better results than physico-chemical models since their parameters have no physico-chemical meaning.

Among the different empirical equations reported in literature, some models can be found relating retention to (i) the organic modifier concentration, (ii) organic modifier and surfactant concentrations, and (iii) organic modifier and surfactant concentrations and pH.

3.1. Empirical equations relating retention to organic modifier concentration

The simplest model is that proposed by Khaledi et al. [21] and Kord et al. [8] relating the capacity factor to the organic modifier concentration when surfactant concentration is constant. The equation is the following:

$$\log k' = -S\varphi + \log k'_0 \quad (12)$$

where φ is the volume fraction of the organic modifier, S the parameter of elution strength, and k'_0 the capacity factor in absence of the organic modifier. Although this model can explain the decrease observed in the capacity factors in presence of organic modifiers, deviation of linearity and some significant differences are obtained between the intercept and the experimental capacity factor in the absence of an organic modifier. From an experimental viewpoint, its applicability is limited because the variation of the surfactant concentration is not considered. Borgerding et al. [39] and Torres-Lapasió et al. [61] have shown that this equation is only valid when methanol is used as the organic modifier and that only the variation of the inverse of the capacity factor with the volume fraction of the organic modifier is linear.

With the purpose of optimizing the separation conditions for different types of solutes, some models relating capacity factors to the surfactant concentration and volume fraction of the organic modifier have been proposed.

3.2. Empirical equations relating retention to surfactant and organic modifier concentrations

Among these, the models of Strasters et al. [65], Torres-Lapasió et al. [61,66] and Yang and Khaledi [67] can be cited. Strasters et al. [65] and Yang and Khaledi [67] use equations relating the logarithm of the capacity factor to the volume fraction of the organic modifier and the surfactant concentration; in our opinion its applicability is very limited because the variation of the capacity factor logarithm with the volume fraction of the organic modifier is only linear when methanol is considered. Moreover, predicting capacity factors as a function of the two variables is very complex. Thus, they use a dimensional space design determined for the capacity factors for five mobile phases (four at the corners and one at the center) and then an equation like

$$\log k' = A\mu + B\varphi + C \quad (13)$$

is fitted in each of the four triangle subspaces with three measurements, two at the corners and the central point (μ being the total surfactant concentration and φ the volume fraction of the organic modifier). With the parameters calculated the predic-

tion of capacity factors for mobile phases pertaining to these four subspaces is achieved, calculating an optimum for the variables conditions. If the values obtained do not concur experimentally with that calculated by the equation, more data is introduced in the model and the process is repeated.

Torres-Lapasió et al. [61,66] have studied the retention prediction errors when using equations relating the inverse or the logarithm of the capacity factor with the total surfactant concentration and the volume fraction of organic modifier for different types of solutes, such as catecholamines, aminoacids, and aromatic compounds. The process implies the calculation of the parameter equations with a factorial design such as that mentioned earlier, but the difference is that only one equation is used to predict the retention behavior of solutes in the space. The following are some of the equations they have evaluated:

$$z = A\mu + B\varphi + C \quad (14)$$

$$z = A\mu + B\varphi + C\mu\varphi + D \quad (15)$$

$$z = A\mu + B\varphi^2 + C\varphi + D \quad (16)$$

$$z = A\mu + B\varphi^2 + C\varphi + D\mu\varphi + E \quad (17)$$

$$z = A\mu^2 + B\mu + C\varphi^2 + D\varphi + E \quad (18)$$

z being the reciprocal or the logarithm of the capacity factor, μ the total surfactant concentration, and φ the volume fraction of the organic modifier. A, B, C, D, and E are the parameters of the equations. These authors conclude that the equation that best predicts the capacity factors in the conditions studied is:

$$1/k' = A\mu + B\varphi + C\mu\varphi + D \quad (19)$$

although in our experience we think that perhaps it is not clearly justified, because the errors obtained with it are of the same order than with the following

$$1/k' = A\mu + B\varphi^2 + C\varphi + D\mu\varphi + E \quad (20)$$

and even worse. In order to clarify this fact, our research team have performed some studies. Thus, Eqs. (19), (20), (15), in which z is log k', have been used to predict the retention behavior of twenty-three benzene derivatives and polycyclic aromatic hydrocarbons [62], twenty-seven dihydropyridines [63] and nineteen pyrazole derivatives [58] when hybrid mobile phases containing CTAB or SDS as surfactants and *n*-propanol or *n*-butanol as organic modifiers have been considered. As an example, in Fig. 4, the mean relative errors obtained when these equations are applied to the prediction of capacity factors of a group of pyrazoles derivatives are plotted [58]. Our results show that, generally, the worst results are obtained when the equation that relates the logarithm of the capacity factor with the total surfactant concentration and the volume fraction of the organic modifier is used. With respect to the other two equations we have found that depending on the solutes and the mobile phase composition both can provide similar errors (phases containing SDS–propanol in Fig. 4) or not (hybrid mobile phases containing CTAB–propanol, CTAB–butanol and SDS–butanol). When the most hydrophobic solutes

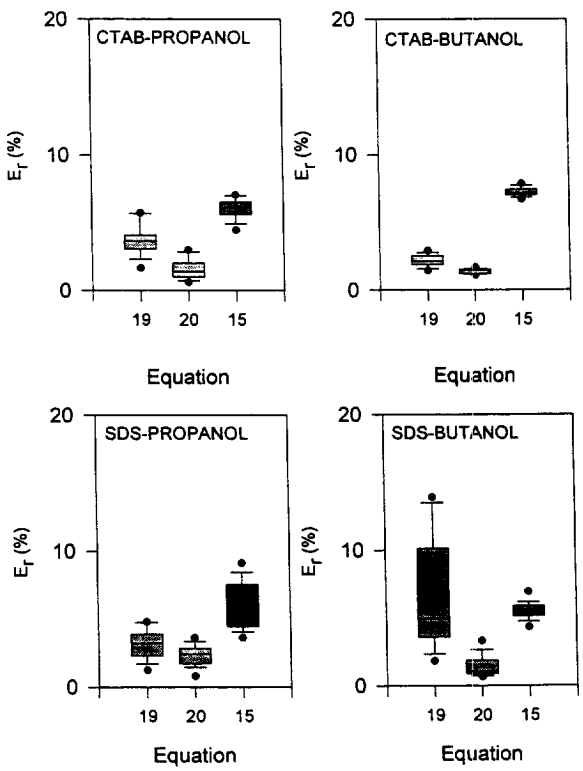

Fig. 4. Mean relative errors (in absolute value) obtained in the retention prediction of a group of pyrazoles by means of empirical equations. See Ref. [58].

with mobile phases containing *n*-butanol as organic modifier, the variation of the inverse of the capacity factor with the volume fraction of the organic modifier is clearly quadratic (Fig. 3) so in these cases the best results are obtained with Eq. (20). This quadratic variation has been reported earlier in conventional RP-HPLC by Schoenmakers et al. [68]. Therefore, in our opinion, Eq. (20) is of more general applicability. These discrepancies found with respect to the empirical equation that best explain the retention behavior of solutes in MLC with hybrid mobile phases can be understood if the physico-chemical model mentioned earlier is considered (Eq. (6)). The retention of a solute in this type of separation technique depends on the balance among the different possible interactions so depending on the nature of such compounds, the surfactant, and the alcohol different approaches can be made which means that different equations can be found.

In order to compare the modeling errors obtained with the physico-chemical model (Eq. (6) and related) and those achieved with the empirical equation that best explain the retention behavior, in Fig. 5, the errors corresponding to a group of benzene derivatives and polycyclic aromatic hydrocarbons are shown. It can be observed that the best results are obtained when the empirical Eq. (20) is used and that important differences are encountered when solute hydrophobicity increases. In our opinion, best results are obtained with empirical equations because the parameters, as it was mentioned, are not restricted while with the physico-chemical models the constants can only be zero or positive. In addition, when the most hydrophobic compounds are considered and at high alcohol concentrations, the variation of solute solubility must play an important role in the retention mechanism because a shift in the partition equilibria from the micelles towards the hydro–organic phase can be possible.

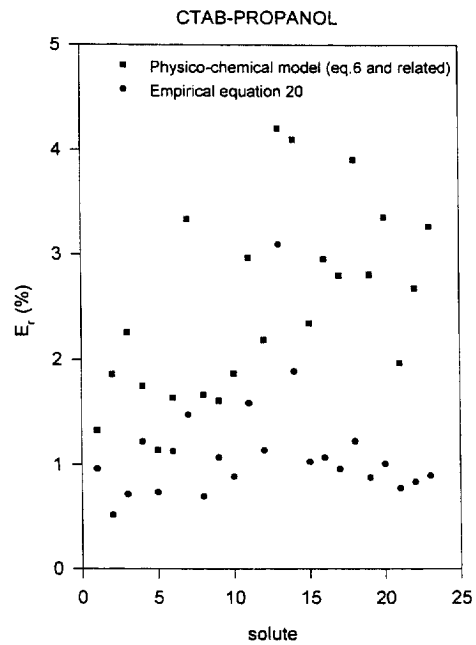

Fig. 5. Mean relative errors (in absolute value) obtained in the retention modeling of a group of benzene derivatives and polycyclic aromatic hydrocarbons by means of the physico-chemical model (Eq. (6) and related) and empirical Eq. (20), see Ref. [58].

So, in our opinion, the physico-chemical models should be applied in retention mechanism studies. However, when the scope is the retention prediction, in order to optimize the best separation conditions, the use of the appropriate empirical equations, if available, should be considered.

3.3. Empirical equations relating retention to surfactant and organic modifier concentrations and pH

An empirical model relating the retention in MLC with hybrid eluents to the total surfactant concentration, volume fraction of modifier, and pH has been reported by Strasters et al. [69]. Equations used relate the variation of the logarithm of capacity factor to the variables linearly, and some important limitations can be cited. First, the variation of capacity factors with the pH is sigmoidal so the equation will only be valid in a very short range of pH. Thus, for example, in their study of amino acids separation with SDS as the surfactant and propanol as the organic modifier, the pH range is limited to values between 2.5 and 3.5. And, only with alcohols (such as methanol) the variation of the logarithm of the capacity factor with the volume fraction would be linear, therefore deviations of the model can be expected in other conditions.

3.4. Other empirical equations

From a different point of view, some empirical models have been reported by Rodríguez Delgado et

al. [70,71]. These models are intended to explain the retention as a function of molecular descriptors and the surfactant concentration or the organic modifier concentration. First of all, they study the molecular descriptors possessing the most relevant information and then the equation parameters relating the logarithm of the capacity factor to them and to the surfactant concentration or the organic modifier concentration are calculated. There is not question that valuable information is obtained from the molecular descriptors in order to understand the retention mechanism although equations applicability to optimize the best conditions for a mixture of solutes separation is limited as only one experimental variable (surfactant concentration or organic modifier concentration) is considered.

Now that the models reported in the literature have been described, some aspects must be highlighted. From a statistical viewpoint, it would be desirable that models be validated properly, not associating the success of a model with the number of nines in the correlation coefficients of plots relating calculated and experimental retention. This is a necessary condition but it is not the unique. In modeling studies, our goal must be clear to design the experiments in order to validate the model, and the experimental data must be established in three sets: training, to calculate the constants or parameters of the model, prediction, to check the prediction capability of our model, and validation. Of course, very often many experimental data are not available so in these circumstances the validation can be attained using the technique of omitting one sample. This design enables the detection of overparameterization and deviation problems in our models not easily detected by a simple inspection of the results obtained.

Until now, models presented herein required a mathematical equation, derived from chemical considerations or empirical in nature, but there are other methods that, although empirical too, need no such requirements, we mean artificial neural networks (ANNs).

4. New trends in micellar liquid chromatography modeling studies

Although ANNs were known for years, they have only been applied recently to model the retention in MLC with hybrid eluents.

Before studies on this subject are examined, a brief introduction to the theory of ANNs might be interesting (for more information, see Refs. [72,73]). ANNs are powerful tools for information processing. Initially, they were designed to simulate the human brain despite an important difference, the human brain works in parallel whereas conventional computers perform tasks sequentially.

Some important advantages of ANNs as opposed to other statistical techniques can be cited: the relationship among variables in a mathematical form is not required [72,74], they are capable of modeling nonlinear relationships [75,76]. Continuous and discrete variables can be used simultaneously [74]. By contrast, better results are not always obtained with ANNs [77] than with other techniques.

ANNs are constituted by simple processing units called neurons or nodes connected to form a net. The nodes are located in layers, one input layer, one or more hidden layers, and the output layer. The neurons are connected to those of the previous and the next layers but which are not interconnected. The information flows from the input layer to the output layer, when it gets to a neuron of the n layer, Net, is the sum of the signals of the nodes of the previous layer $(n-1)$ and it can be calculated as

$$\text{Net} = w_1 x_1 + w_2 x_2 + \cdots + w_m x_m \tag{21}$$

where w_1 and x_1 are the weight of the connexion of this node with the first node of the previous layer and the output signal of the first node of the previous layer, respectively. In every neuron, a mathematical transformation of the signal received occurs by means of a transfer function, being a sigmoidal function the most common. Thus, the output signal of the i node of the n layer can be calculated as

$$\text{Out}_i^n = \frac{1}{1 - e^{-\text{Net}_i^n}} \tag{22}$$

if the sigmoidal transfer function is used.

The net must learn the variables' relationships by means of a learning process. This process begins with an aleatory assignation of weight values for the different connexions of the net, then through a learning algorithm, the weights change until the minimum for the medium quadratic error is reached.

Although briefly covered, some ideas can be drawn for the fundamental aspects of the ANNs. As the net learns of the data without the need of a mathematical expression and since nonlinear fitting (choosing the appropriate transfer function) is available, it would be possible to model solute retention behavior in MLC with hybrid eluents as a function of different variables simultaneously. Perhaps it would also be possible to solve the problem of modeling retention and efficiency simultaneously as a function of these variables. However, care must be taken because overparameterization, overtraining and local minimum problems may arise.

To date, only one reference on the use of ANNs in modeling retention in MLC has been reported [78], one of our research works is in press [79] and one has been submitted for publication [80]. Xie et al. [78] have studied the capability of multilayer ANNs to model and predict the retention behavior (i) for a group of diuretics, phenols, aminoacids, catecholamines, and aromatic compounds as a function of the surfactant and alcohol concentration in the mobile phase, (ii) for several pharmaceutical products as a function of the pentanol concentration and temperature, and (iii) for some aminoacids as a function of SDS and propanol concentrations and pH. Good results have been reported but, in our opinion, comparisons with other available methods such as the use of empirical equations or physico-chemical models should have been included because only if results are better with ANNs, the difference in the computational time and data required can be justified.

The studies carried out by our research team are only beginning and attempts are being made to establish ANNs' optimum architecture. Thus, the fitting error variation with the number of nodes in the hidden layer, the transfer function in those nodes, and the use of some transformations of the input and output variables are under study. The first study [79] comprises works on modeling for a group of benzene derivatives and polycyclic aromatic hydrocarbons when hybrid mobile phases containing CTAB as the surfactant and *n*-propanol as organic modifier are used. In the second, modeling was extended to a group of 27 dihydropyridines in mobiles phases containing CTAB or SDS as the surfactant and *n*-propanol or *n*-butanol as organic modifier. From these studies some considerations can be drawn: first, the election of the transfer function greatly affects ANNs' success. Of all transfer functions evaluated, linear and logarithmoid transfer functions provided the best results (two sigmoidal functions were also evaluated) but when the linear function was used the errors obtained were worse than those of empirical Eq. (20). On the other hand, when the logarithmoid function was used better results with ANN were obtained than when the same empirical equation was applied. In this case (using the logarithmoid function) the mean relative errors for every compound were better than 1%. Perhaps it can be argued that overtraining is the reason for such low error values but a different group of compounds was tested to see if this phenomenon was observed and this was not the reason. In order to compare the results obtained by means of ANNs and of empirical equations (Eq. (20) has been used), that have been not made in the published works, in Fig. 6, mean relative errors for a group of 27 dihydropyridines in different hybrid mobile phases obtained by means with each of these two techniques are shown as Box plots. It can be observed that errors obtained with ANNs are always lower than those of the empirical equations. So, the results reported clearly show ANNs as alternative statistical techniques with very good possibilities, although this case can be considered very simple (only two variables have been considered). In our opinion, ANNs will show a great expansion in modeling studies in coming years because they can afford the task of complex data treatment such as that required in MLC.

5. Conclusions

From the models presented some conclusions can be drawn:

It is only possible to model retention as a function of two or three experimental variables simultaneously depending on whether we consider physico-chemical models or empirical equations, and the efficiency modeling has been misleading. So, more work is required to introduce other important variables.

The experimental variation of capacity factors with the surfactant concentration and the organic modifier concentration have been adequately ex-

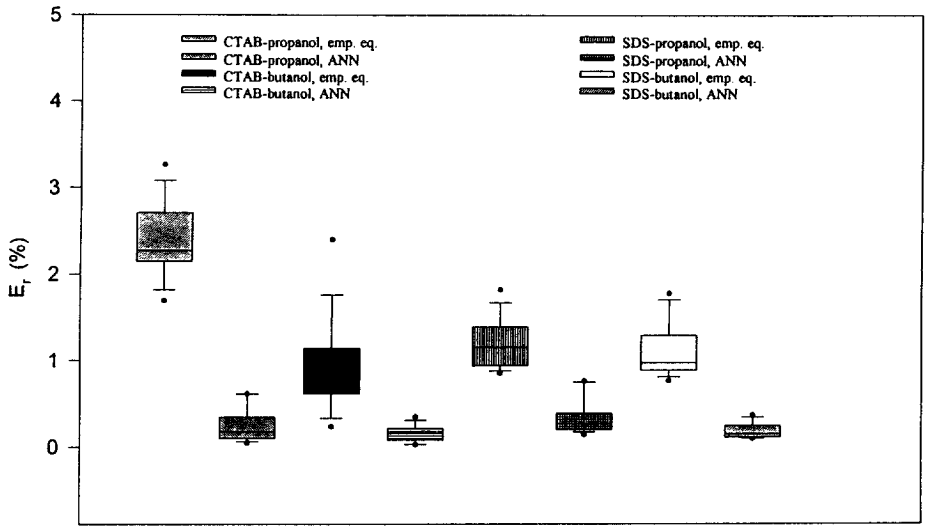

Fig. 6. Comparison of the errors obtained in retention modeling of a group of dihydropyridines by means of the empirical Eq. (20) and ANNs [58].

plained by means of physico-chemical and empirical methods. In contrast, when pH is introduced, as a variable, the empirical models proposed are only valid when some requirements are fulfilled, i.e., linear variations of the capacity factor logarithm with the experimental variables.

Physico-chemical models reported can explain the retention mechanism of organic compounds in MLC but some repulsive interactions between polar or ionic solutes with the surface charge of micelles and the modified stationary phases have not been considered, so deviations can occur in these cases.

Retention in MLC depends on the balance of different interactions among solutes and the stationary phase, micelles and the organic modifier. So, depending on the solute, the nature and concentrations of micelles and organic modifier, pH, etc., different equations can explain retention in such media. This fact pointed out, partially, by the physico-chemical models relating capacity factors to the surfactant and the alcohol concentrations, can explain the discrepancies found by different research teams in proposing a general empirical equation.

Our results indicate that the best errors in modeling studies of retention as a function of the surfactant and the alcohol concentrations are obtained when ANNs are used, so in our opinion, ANNs constitute a very promising alternative to classical statistical methods for retention modeling studies in MLC, although more work is needed and the inclusion of the efficiency is recommended.

6. Symbols

V_s	stationary phase volume
V_e	elution volume of the solute
V_m	mobile phase volume
k_i	constant of equilibrium i
v	surfactant molar volume
$[M_m]$	micellized surfactant concentration
P_{sw}	solute distribution coefficient between the stationary and aqueous phases
P_{mw}	solute distribution coefficient between the micellar pseudophase and the aqueous phase
k'	capacity factor
ϕ	phase ratio (V_s/V_m)
$[L_s]$	concentration of stationary phase sites
E_m	solute in the mobile phase
EL_s	complex formed between the solute in the mobile phase and the stationary phase sites in the stationary phase

EM_m complex formed between the solute in the mobile phase and the micelles in the mobile phase
P_{sm} solute distribution coefficient between micelles and stationary phase
P_0 octanol/water partition coefficient
A_m alcohol concentration in the mobile phase
AL_s complex formed between the alcohol in the mobile phase and the stationary phase, in the stationary phase
AM_m complex formed between the alcohol in the mobile phase and the micelles, in the mobile phase
φ volume fraction of the organic modifier
S elution strength parameter
μ total surfactant concentration
w_i connexion weight of node i
x_i output signal of the node i of the previous layer
E_r mean relative error, in absolute value

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