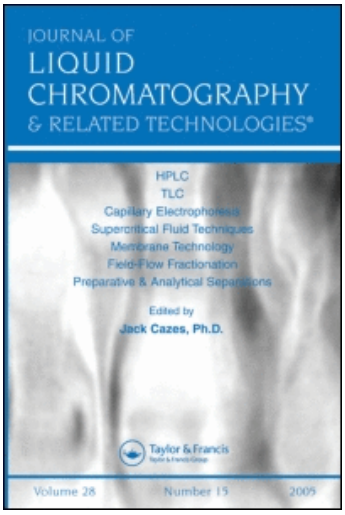

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Micellar Liquid Chromatography with Hybrid Eluents

M. L. Marina[a]; M. A. García[a]
[a] Departamento de Química Analítica, Facultad de Ciencias, Universidad de Alcalá de Henares, Madrid, Spain

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MICELLAR LIQUID CHROMATOGRAPHY WITH HYBRID ELUENTS

M. L. MARINA* AND M. A. GARCÍA
Departamento de Química Analítica
Facultad de Ciencias
Universidad de Alcalá de Henares
28871 Alcalá de Henares, Madrid, Spain

ABSTRACT

The characteristics of Micellar Liquid Chromatography with hybrid eluents are described. The influence of the addition of an organic modifier to the mobile phase on the retention, eluent strength, efficiency and selectivity is discussed. The application of MLC with hybrid eluents for predicting solutes hydrophobicity is studied.

I. INTRODUCTION

The combination of hydrophobic and hydrophilic properties in the molecules of surfactants confers to micellar systems some special characteristics in aqueous solution and this has made these systems applicable in different areas (**1-8**). The ability of micellar systems to solubilize hydrophobic compounds in aqueous solution (**9**) or improve

different analytical methodologies (**7,10**) should be emphasized. The use of surfactant solutions above their critical micellar concentration or c.m.c. as mobile phases in Reversed Phase High Performance Liquid Chromatography (RPLC) originates Micellar Liquid Chromatography (MLC) which is an interesting alternative to the use of hydro-organic mobile phases in chromatography (**11-13**).

The great number of interactions that are possible in the separations by MLC techniques, as electrostatic, hydrophobic and esteric (**14-16**) and the modification of the stationary phase by adsorption of monomeric surfactants (**17,18**) make these systems more complicated than conventional RPLC with hydro-organic mobile phases. In fact in MLC, three different equilibria can be considered (**19**): the distribution equilibrium of the solute (1) between the micellar mobile phase and the aqueous mobile phase, (2) between the micellar mobile phase and the stationary phase and (3) between the stationary phase and the aqueous mobile phase. From these equilibria, different equations have been developed to describe the chromatographic behavior of eluted solutes (**19-21**). These equations have allowed the determination of the solute-micelle association constants of several solutes with different micellar systems (**15,19,20,22-27**). This method can be applied to a great variety of solutes without the limitations or pre-requisites needed when other methods are employed (**28**).

MLC techniques also present other advantages such as: a) micellar mobile phases have low cost and low toxicity as compared with hydro-organic mobile phases since they are mainly composed of water (**11,19**). b) It is possible to separate ionic and nonionic solutes due to some special characteristics of micelles (**11**). c) Luminiscence detection can be improved in MLC because many solutes show enhanced fluorescence (**10,29-33**) and in some cases, room temperature liquid phosphorescence (**29,33,34**)

when associated with micelles. Furthermore, many metal-dye complexes show increased absorbance in the presence of micelles (**10,35**). d) It is possible to inject biological fluids directly into the chromatographic system because of the solubilization of the proteins by anionic surfactants as sodium dodecylsulphate (SDS) or nonionic surfactants such as polioxietilen[23]dodecanol (Brij-35) (**36-44**). e) Rapid elution gradients can be achieved in MLC because micellar gradients do not require reequilibration time (**45**). This is due to the amount of surfactant adsorbed on the stationary phase which remains practically constant after the equilibrium is reached and the surfactant concentration in mobile phase is above the c.m.c. (**46-48**). f) The control of separation selectivity is exerted through a great number of parameters such as nature (type and charge) and concentration of the surfactant in the mobile phase, the presence of additives as organic modifiers and salts and the pH (**16,23,49-52**). g) The correlation between chromatographic retention of several organic compounds in MLC and their logarithm of octanol-water partition coefficient (**53,54**) or their bioactivity (**55**) has been shown so this technique can be considered interesting in the evaluation of solute hydrophobicity.

An important drawback of MLC techniques is the decrease in chromatographic efficiency (**56**) as compared to that obtained in conventional RPLC with hydro-organic mobile phases. This efficiency loss can be precluded by adding an organic modifier to the mobile phase and increasing the working temperature.

Because the addition of alcohols to micellar mobile phases can increase the efficiency and selectivity (**57**) and reduce the analysis time, the use of micellar mobile phases modified by organic modifiers has acquired importance in last years. The term hybrid is used for the ternary eluents of water-organic solvent-micelles throughout the text.

Some of the most significant features of MLC techniques with hybrid eluents and their applications are described in this work.

II. EFFICIENCY

One of the main drawbacks of MLC techniques is the loss observed in the chromatographic efficiency as compared with that obtained in RPLC with hydro-organic mobile phases. This efficiency loss is attributed to the increase in the resistence of mass transfer of the solute from the mobile phase to the stationary phase (**58**).

However, the addition of small quantities of organic modifier to the mobile phase (3% propanol) and the increase in working temperature (40ºC) have shown to allow the obtainment of efficiencies similar to those obtained in RPLC with hydro-organic mobile phases (**59,60**). Other authors suggest working with low flow rates, high work temperatures, and low surfactant concentration in mobile phase (**58**). In fact, it has been shown that the use of an elevated surfactant concentration in mobile phase can cause a chromatographic efficiency loss (**61**).

Surfactant adsorption on the stationary phase seems to have a great influence on the efficiency (**62-65**). The addition of a short or medium chain alcohol causes surfactant desorption out of the stationary phase and improves efficiency (**66**). This effect increases with increasing concentration and hydrophobicity of the modifier (**27,57,64**).

Alcohols may also improve the efficiency obtained in MLC with micelles of ionic surfactants because their presence can reduce the net electrical charge density of the ionic micellar surface decreasing the repulsive barrier (**61**). In fact, the addition of alkanes does not affect the

surface charge density and does not improve the efficiency obtained for very hydrophobic solutes. This explains why an efficiency enhancement is not observed with alcohols for nonionic surfactants as Brij-35 that are not charged. In fact, the efficiency observed for very hydrophobic solutes with a Brij-35 micellar mobile phase was better than that obtained with ionic micelles.

III. INFLUENCE OF MICELLE CONCENTRATION ON RETENTION

As previously stated, three different equilibria can be considered in MLC: the distribution of the solute between the micelle and bulk water, with the corresponding P_{MW} partition coefficient; the partitioning of the solute between the stationary phase and the micelle, with P_{SM} as partition coefficient, and the distribution of the solute between the stationary phase and water, with P_{SW} as partition coefficient.

According to these equilibria, several equations have been developed relating chromatographic retention in MLC and micelle concentration in mobile phase. Armstrong and Nome (**19**) reported the following equation:

$$V_s/(V_e - V_m) = \{v(P_{MW} - 1)/P_{SW}\}\, C_M + 1/P_{SW} \qquad [1]$$

where V_s, V_e, and V_m are the stationary phase volume, elution volume of the solute and the void volume of the column, respectively; v is the molar volume of the surfactant and C_M is the micellized surfactant concentration in the mobile phase (C_M = C - c.m.c., C being the total surfactant concentration in solution). A plot of $V_s/(V_e - V_m)$ vs C_M is linear and the term

$v(P_{MW} -1)$ can be obtained from the slope: intercept ratio. Since from the Berezín treatment (**67**), $v(P_{MW} -1)$ is equal to the solute-micelle association constant, this parameter can be obtained from this treatment and also the partition coefficient of solute between bulk water and micelle, P_{MW}, if the surfactant molar volume,v, is known.

Arunyanart and Cline-Love (**20**) have derived a similar equation that correlates the capacity factor, k', to micellized surfactant concentration, C_M, in the form:

$$1/k' = \{K_2/\Phi\ [L_s]K_1\}\ C_M + 1/\Phi\ [L_s]K_1 \qquad [2]$$

where K_2 is the solute-micelle association constant, Φ is the phase ratio (the ratio of the stationary phase volume, V_s, to the volume of the mobile phase, V_m, in the column), $[L_s]$ is the stationary phase concentration, and K_1 is the binding constant for the solute between the bulk solvent and the stationary phase. Again, a plot of 1/k' vs C_M should result in a straight line and the value of the solute-micelle binding constant K_2, can be obtained from the slope: intercept ratio.

The solute micelle association constant obtained in this way is called the association constant per monomer. If this constant is multiplied by the aggregation number of the micelle, the association constant per micelle is obtained. Likewise, the P_{MW} and K_2 values only depend on the solute and the micellar system employed but not on the stationary phase (**15**).

Equations [1] and [2] show how the retention of a solute in MLC decreases when micelle concentration in mobile phase increases. This is in contrast to reversed-phase ion-interaction chromatography where the surfactant concentration is below the c.m.c., that is, no micelles exist, and the addition of an ionic surfactant will increase retention for compounds which interact electrostatically with it (**23**).

Equations [1] and [2] have frequently been employed with the aim of determining solute-micelle association constants in purely micellar systems (**15,19,20,22-27**). However, its validity for hybrid eluents has been shown (**15,27,68**). This has allowed the determination of the solute-micelle association constants in micellar media modified by alcohols. The addition of an organic modifier to a micellar solution can modify the characteristics of the micellar system (c.m.c. and the aggregation number) and this can originate a variation of the solute-micelle interactions (**69-71**) which, in turn, can change the chromatographic retention.

On the other hand, the error obtained during the determination of K_2 increases with solute hydrophobicity since P_{sw} values for these compounds are elevated (intercept very small, see equation [1]). With hybrid eluents, the value of P_{sw} decreases and the error in the determination of the solute-micelle association constants for very hydrophobic compounds also decreases (the intercept in equation [1] increases).

Although the validity of equations [1] and [2] has been shown for octylsilica and octadecylsilica stationary phases, cyano bonded columns have also been employed. In these columns, the retention for hydrophobic compounds considerably decreases especially when anionic surfactants as sodium dodecylsulphate (SDS) are used. This has allowed the determination of the solute-micelle association constants with similar or lower errors than those obtained for octadecylsilica columns but in considerably less time (**72**).

Solute-micelle interactions generally decrease in media modified by alcohols. In fact, solute-micelle association constants for a group of benzene and naphthalene derivatives with SDS and hexadecyltrimethylammonium bromide (CTAB) are greater in purely

micellar media than in solutions modified by a 5% or 10% n-butanol (**68**). This result has been attributed to the existence of a competing effect between the solute and the alcohol to interact with the micelle. However, the addition of a salt as NaCl can increase the interactions between the above-mentioned solute and SDS micelles. This is shown by obtaining of similar or higher association constants in the NaCl modified solution than in a purely micellar medium (**68**).

IV. INFLUENCE OF THE ORGANIC MODIFIER PERCENTAGE ON RETENTION

Khaledy et al. (**57**) proposed the following equation to relate solute retention (lnk') in MLC and volume fraction of organic modifier (Φ_{org}):

$$\ln k' = -S_{hyb}\,\Phi_{org} + \ln k'_{o} \qquad [3]$$

where S_{hyb} is the solvent strength parameter and $\ln k'_{o}$ is the retention of the solute in a purely micellar mobile phase.

This equation is simillar to that used to describe the retention variation with fraction volume of modifier in RPLC where lnk' linearly varies with Φ_{org} over a limited range. The slope of this straight line is called solvent strength parameter, S, and is generally proportional to the retention and molecular weight of the solute (**73,74**).

Equation [3] shows how solute retention in MLC decreases when Φ_{org} increases. However, in the same article where equation [3] is proposed, it was observed that the variation of lnk' with Φ_{org} for some amino acids and alkylbenzenes in SDS and CTAB mobile phases was not

linear. In other articles, a deviation from linearity was also observed as is the case of a group of benzene and naphthalene derivatives in a MLC system with SDS - n-butanol mobile phases (**75**). For other groups of solutes, the linear variation of lnk' with Φ_{org} was only found when methanol was used as organic modifier (**76**).

Recently, Torres-Lapasió et al. (**76**) have proposed a new model to describe the variation of solute retention in MLC with Φ_{org}. In this model, retention can be expressed by the following equation:

$$1/k' = A\mu + B\Phi + C\mu\Phi + D \qquad [4]$$

where μ and Φ are the surfactant and alcohol concentrations in mobile phase, respectively. The validity of this model has been shown for several solutes as catecholamines, amino acids, peptides, and other aromatic compounds with organic modifiers different from methanol (**76**).

Equation [4] shows that for a constant surfactant concentration in mobile phase, the term 1/k' should linearly vary with Φ_{org}:

$$1/k' = (A\mu + D) + (B + C\mu)\,\Phi \qquad [5]$$

On other hand, in purely micellar mobile phases ($\Phi = 0$):

$$1/k' = A\mu + D \qquad [6]$$

and an equation similar to that obtained by Arunyanart and Cline-Love (equation [2]) is obtained.

More work is required for different solutes, different surfactants, and different organic modifiers to show the validity range of equations [3] and [4].

V. SOLVENT STRENGTH OF HYBRID MICELLAR ELUENTS

In MLC, purely micellar eluents can have a quite small eluent strength (**57**). Eluent strength of purely micellar eluents increases when micelle concentration in mobile phase also increases (**57**). However, an increase in micelle concentration in mobile phase generally causes an efficiency loss.

For these reasons, the addition of organic modifiers to micellar mobile phases is of great interest: it is possible to increase both eluent strength and efficiency.

Solvent strength (S_{hyb}) in MLC with hybrid eluents has been defined as the slope of straigth line resulting from the variation of lnk' as a function of Φ_{org}. The value for S_{hyb} has been calculated for fourteen alkylbenzenes in micellar phases of CTAB modified by methanol (MeOH), 2-propanol (PrOH) and butanol (BuOH) (**57**). S_{hyb} values can be ranked as $S_{BuOH} > S_{PrOH} > S_{MeOH}$ which is similar to conventional hydro-organic systems as BuOH is the strongest solvent and MeOH is the weakest. The larger S_{hyb} for BuOH and PrOH indicate that these solvents interact more with micelles and, consequently, can solvate more effectively and/or can better compete with micelles for solute interactions. However, all values obtained for S_{hyb} for the group of compounds studied are still smaller than for those in absence of micelles, as S_{hyb} for BuOH is even smaller than S values for MeOH in conventional hydro-organic eluents.

Another consideration which also demonstrates the impact of micelles is the fact that the ranking of S_{hyb} for different solutes is different for MeOH, PrOH and BuOH. On the contrary, in conventional hydro-organic systems the same ranking of S values can be anticipated for different solutes. This is because in MLC, solvents interact differently with micelles and, therefore, their own microenvironment in micelles is different.

Since S values reflect the extent of solvation of solutes by organic solvents, the location of solutes and/or organic solvents in micelles can greatly influence the sensitivity of retention to changes in the concentration of organic solvent. In a conventional hydro-organic system, S significantly varies with solute molecular weight and functional groups. As an example, anthracene has a large S value and its retention in conventional RPLC with hydro-organic mobile phases (methanol-water) is more sensitive to variations in the concentration of organic solvent than other compounds with a minor S value. However, in the presence of CTAB micelles, the S_{hyb} value for anthracene in methanol is small and, therefore, its retention is less affected by the addition of organic solvents. This is because this compound strongly interacts with micelles and is less accessible to a polar solvent such as methanol. However, the relationship between S_{hyb} and solutes' structural properties cannot be easily recognized and it cannot be concluded that S_{hyb} is inversely related to hydrophobicity of solute (**57**).

VI. SELECTIVITY

Solute retention in MLC generally decreases when micelle concentration increases, as indicated in section III. The rate of change in retention of different solutes varies with charge and hydrophobicity of solutes as well as the length of alkyl chain, charge, and concentration of micelles (**77**). This fact causes inversions of elution order that are the result of two competing equilibria: solute-micelle association characterized by K_2 and solute-stationary phase interaction characterized by P_{sw}. The parameters K_2 and P_{sw} have a different effect on retention. When P_{sw} increases, retention also increases but when K_2 increases, retention decreases. When the surfactant concentration in mobile phase increases,

the effect that K_2 has on retention also increases and reversals in elution order can be obtained if the difference in K_2 values for two solutes is quite different (**23**). Therefore, separation selectivity in MLC can be controlled by modifying surfactant nature and concentration. Furthermore, when organic modifiers are added to the mobile phase, the solvent strength parameter S_{hyb} for a group of compounds does not have the same ranking for different alcohols due to the different interaction of these modifiers with micelles. For these reasons, MLC techniques are very interesting for chromatographic separation.

Although the conditions to optimize separation selectivity in MLC can vary with solutes' nature, several works show an increase in separation selectivity for aromatic compounds in MLC with hybrid eluents when the micelle concentration in the mobile phase decreases (**57,75,77**). However, for a group of amino acids and peptides, an increase in micelle concentration can cause an increase or decrease in selectivity (**57**).

The effect of the organic modifier content in mobile phase seems to be clearer. Generally, separation selectivity in MLC is improved in the presence of an organic modifier and increases with the volume fraction of the modifier in mobile phase (**57,75,77**). This result is opposed to that observed in conventional RPLC with hydro-organic mobile phases in which an increase in organic modifier content causes a decrease in solute retention and selectivity. Recently, a comparative study on the influence of organic modifier content and surfactant concentration on solvent strength and selectivity in Ion Pair Chromatography and in MLC has been completed (**78**). The selectivity enhancement observed in MLC when the solvent strength increases has been attributed to the competing partitioning equilibria in micellar systems and/or to the unique characteristics of micelles to compartmentalize solutes and organic solvents (**57**).

Although separation selectivity is generally improved when the volume fraction of organic modifier is increased, for some amino acids and peptides selectivity can decrease with the content of 2-propanol of a SDS micellar mobile phase (**77**). In this case, it has been shown that for pairs of peaks whose selectivities were reduced with increasing 2-propanol concentration, a selectivity enhancement was observed as a result of increasing micelle concentration and vice versa. These observations suggest that solvent strength increases with concentrations of both micelle and organic solvent, the effect of these two parameters on selectivity could be quite different, even opposite. Micelles and 2-propanol compete to interact with solutes and, as a result, they influence the role of one another in controlling retention and selectivity.

As a consequence of these results, a model has been developed which explains the dependence of the solvation ability of organic solvents in MLC (represented by solvent strength parameter, S_{hyb}, of solutes) and the degree of solute interactions with micelles. Whenever the difference in solvent strength parameter values of two solutes in micellar eluents, dS_{hyb}, was positive, maximum selectivity was observed at the weakest eluent strength. When dS_{hyb} was negative, an inverse relationship between retention and solvent strength parameter exists so that selectivity increases with volume fraction of organic solvent in micellar eluents (**77**).

The mutual effects of micelles and organic modifiers on one another would also require a simultaneous optimization of these two parameters. Like in the study of the separation selectivity of 15 benzene and naphthalene derivatives in MLC with SDS and CTAB mobile phases modified by methanol, n-propanol, and n-butanol, it was found that selectivity was better in SDS than in CTAB and that it increases when surfactant concentration in mobile phase decreased. Regarding organic

modifier content, selectivity was better in the presence of n-propanol or n-butanol at medium percentages, but the latter had the advantage of decreasing analysis time with respect to n-propanol (**75**). Obtaining maximum selectivities at medium alcohol percentages can be justified by the existence of pairs of compounds whose selectivity increases when eluent strength decreases.

VII. QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS (QSAR)

Another interesting possibility of MLC techniques is their application to the quantitation of physicochemical properties of biologically active compounds in QSAR studies, specifically for the prediction of hydrophobicity.

Hydrophobicity is commonly understood as a measure of the relative tendency of a solute to prefer a nonaqueous rather than an aqueous environment. Biological activity of many compounds, bioaccumulation of organic polutants, and soil sorption of environmental contaminants have all been correlated to the lipophilic character of molecules (**13**). The quantitation of hydrophobicity has both diagnostic and predictive value in various disciplines such as drug design, toxicology, and environmental monitoring (**79-82**). When comparing behavior of various solutes in the same environment, a quantitative scale can be used to demonstrate the abilities of individual solutes to participate in hydrophobic interactions. Octanol-water partitioning is a common reference system that provides the most recognized hydrophobicity measure: the logarithm of the partition coefficient, log P_{ow} (**83**). The standard "shake-flask" method for determining partition coefficients in liquid-liquid systems has several serious

disadvantages (**84**). Despite numerous efforts using a variety of methods, the measurement of P_{ow} is still difficult. In 1977 publications began to appear on what is now termed quantitative structure-retention relationships (QSRRs) (**84**). QSRRs result from applying the methodology used for quantitative structure-biological activity relationships (QSARs) (**83**) to the analysis of chromatographic data.

Following the first reports on reversed-phase TLC and HPLC methods of hydrophobicity parameterization, hundreds of reports on the application of chromatographically derived hydrophobicity descriptors in medicinal, agricultural, and environmental chemistry have appeared (**84**). In reversed-phase HPLC with hydro-organic mobile phases, a representative relationship has been obtained between the chromatographic measure of hydrophobicity (logk') determined on a deactivated phase for a noncongeneric series of nonionized basic, acidic, and neutral solutes, as well as their log P_{ow} values. Thus the advantages of the log P_{ow} hydrophobicity scale -its universality and continuity- are challenged by a more convenient, reproducible, fast, and inexpensive chromatographic approach. A systematic study could produce a large chromatographic hydrophobicity database similar to the one collected laboriously for log P_{ow} (**85**).

Another chromatographic approach used to evaluate octanol-water partition coefficients is countercurrent chromatography (CCC) with an octanol-water biphasic solvent system (**86,87**). The mobile phase is water saturated with octanol, and the stationary phase is octanol saturated with water. The measurable P_{ow} range was 0.003 to 300. A liquid stationary octanol phase permits the development of a dual-mode elution method using CCC which extends the measurable P_{ow} range to 5000. The co-current CCC method was developed to extend the range to P_{ow} = 20.000.

In co-current CCC, both the water and the octanol phase move in the same direction at different rates.

However, the use of a bulk solvent such as octanol as a model for complex systems such as biomembranes has been occasionally criticized. On the other hand, micelles have long been known as simple chemical models for biomembranes (**54**). Several workers have demostrated that the solubilization (or partitioning of solutes in micelles) closely resembles that of lipid bilayers and that both of these are different from the two-phase octanol-water system (**88-91**). Both micelles and biomembranes have amphiphilic properties and are anisotropic media. Molecular size and shape are significant factors in the partitioning of solutes in anisotropic environments while they are not determinant for the partition process in an isotropic solvent such as 1-octanol (**92**). These reports provide interesting examples confirming the suitability of micelles for representing biomembranes as far as hydrophobic interactions are concerned (**54**). A shake flask method has been presented for the determination of the partition coefficients involved in the distribution of polar solutes between octanol and aqueous micellar solutions (**93**).

Several studies have appeared in literature in which the correlation between retention in MLC and octanol-water partition coefficient or carbon number is studied. These works can be divided in two groups. In a first group are the studies in which a linear relationship is found between the logarithm of the capacity factor (logk') of compounds and log P_{ow} or number of carbom atoms (n_c) in the molecule. Like, a linear correlation logk' = f(log P_{ow}) has been found for a group of monosubstituted benzenes with mobile phases of sodium dodecylsulphate (SDS), hexadecyltrimethylammonium bromide (CTAB), and polyoxyethylene(23)dodecanol (Brij-35) (**53**). The same correlation has

been obtained for a group of phenols and other group of monosubstituted benzenes with mobile phases of SDS and CTAB that can be modified by alcohols (**94**), and for a series of aromatic polycyclic hydrocarbons with mobile phases of SDS, CTAB and polyoxyethylene(23)lauryl ether (Brij-35) (**95**). Also, this first group can include works in which a linear relationship is found for the variation of log P_{ow} with the logarithm of the solute-micelle association constants ($logK_z$) (**94,96**) or in which a linear variation is found for the transfer free energies from water to micelle as a function of the transfer free energy from octanol to water (**97,22,68**).

The second group includes studies in which a linear relationship between capacity factors (k') (and not logk') and log P_{ow} or n_c. For example, a linear relation has been found for k'-n_c in the case of groups of n-alkylbenzenes and n-alkylphenones with purely or hybrid SDS and CTAB mobile phases (**27**) and for a series of alkylbenzenes with mobile phases of SDS and Brij-35 (**98**). In the same way, a linear relationship has been found between k' and log P_{ow} for sixteen aromatic compounds in purely and hybrid SDS and CTAB mobile phases (**54**) and between k' and the bioactivity of 26 para-substituted phenols with tetradecyltrimethylammonium bromide (**55**). In this case, the addition of 10% 2-propanol to the micellar system (hybrid system) proved the best chromatographic system for the best estimation of the phenols bioactivity.

Regarding the nonlinearity observed for the variation of logk' as a function of carbon atoms (n_c), an equation has been derived recently which explains this apparently anomalous result (**99**). The equation is simply based on partitioning between moving and stationary phases. Experimental results on a variety of systems have displayed the nonlinearity seen previously. These data are adequately fit by the equation.

In summary, MLC appears as an interesting alternative to evaluate log P_{ow} and bioactivity of organic compounds, especially for hybrid

systems, utilized to extract the systematic information from diversified yet often highly intercorrelated sets of data, modern multivariate chemometric methods of data analysis must be used (**84**).

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