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New Insights and Recent Developments in Micellar Liquid Chromatography

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Abstract: Micellar liquid chromatography (MLC) is an efficient alternative to conventional reversed–phase liquid chromatography with hydro-organic mobile phases. Almost three decades of experience have resulted in an increasing production of analytical applications. Current concern about the environment also reveals MLC as an interesting technique for ''green'' chemistry because it uses mobile phases containing 90% or more water. These micellar mobile phases have a low toxicity and are not producing hazardous wastes. After a rapid overview of the two first decades of the technique, this review focuses on the recent advances on fundamental aspects and analytical applications. Traditional and new surfactants, search of new organic solvents as mobile phase modifiers, and the use of new columns are addressed. Surfactant-bonded phase association, combination of diverse surfactant effects, interaction between organic solvents and micelles, and resolution performance are also considered. A special attention has been paid to the limited efficiency and weak elution strength, which are the main limitations usually pointed out in MLC. An effort has been made to clarify some wrong and sometimes unjustified ideas about MLC. The potential of this chromatographic mode is also shown for routine analytical procedures.

Keywords: Micellar liquid chromatography, critical micelle concentration, surfactants, organic solvents, elution strength, resolution, efficiency, analytical procedures

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INTRODUCTION

Ionic surfactants (amphilic molecules that comprise a hydrophobic moiety and an ionic head group) below the critical micelle concentration (CMC, i.e., the concentration at which the surfactant monomers form dynamic aggregates called micelles) have been added to the mobile phase in reversed–phase liquid chromatography (RPLC) since long in the so-called ion–pair chromatography. In this chromatographic mode, surfactant monomers in the mobile phase adsorb on the stationary phase and act as hydrophobic counter-ions, leading to increased retention of charged solutes. Micellar mobile phases were originally suggested by Armstrong for size exclusion chromatography in 1977 (1) and thin layer chromatography in 1979 (2) and HPLC was just a logical extension of this. In 1980, Armstrong and Henry suggested the use of aqueous solutions of surfactants of different nature (with either ionic and non-ionic head groups) at concentrations exceeding the CMC as mobile phases (3). That is, an RPLC mode with mobile phases containing micelles in addition to surfactant monomers. Armstrong called this new chromatographic mode ''micellar liquid chromatography.'' He later introduced the term ''pseudophase'' to include non-micellar entities such as cyclodextrins (4). Later the term ''micellar'' was preferred to ''pseudophase'' and the denomination ''micellar liquid chromatography'' (MLC) became the established name of the technique (5).

The unique capability of micellar mobile phases is attributed to the ability of micelles to selectively compartmentalize and organize solutes at the molecular level. However, the associations between the surfactant monomers and the bonded phase, forming a surface similar to the exterior of micelles, have profound implications with regard to retention and selectivity. In fact, solutes are separated on the basis of their differential partitioning between the bulk aqueous phase and the micellar aggregates in the mobile phase, and between the bulk aqueous phase and the surfactant-coated stationary phase. For water-insoluble species, partitioning can also occur via direct transfer of the solute in the micellar pseudophase to the surfactant-modified stationary phase.

In the very first reports on MLC, only pure micellar solutions were suggested as mobile phases. The idea is attractive, but suffers two main problems compared to RPLC with hydro-organic mobile phases: the excessive retention of apolar compounds and the reduced efficiency observed for solutes of various polarities. Dorsey et al. (6) recommended small additions of a short-chain alcohol to the micellar eluent to enhance efficiency. Later, the potential use of organic solvents added to micellar mobile phases to decrease the analysis time to acceptable values was also

proved. Indeed, most of the procedures published on MLC use hybrid micellar mobile phases (solutions containing surfactants above the CMC and an organic solvent).

In spite of the problems found in its initial development, after almost three decades of experience, MLC appears to be a possible alternative to conventional RPLC with hydro-organic mobile phases, and with increasing interest in ''green'' chemistry. The capabilities and advantages derived from the use of micellar mobile phases in RPLC are several:

(i) The variety of interactions between solutes, stationary phase, aqueous phase and micelles yield unique selectivity for many compounds.
(ii) Both charged and neutral solutes can be separated with the same mobile phase.
(iii) The analysis of samples containing compounds in a wide range of polarities is possible using isocratic elution. However, in case gradient elution is needed, equilibration times are shorter than those with hydro-organic mixtures.
(iv) The high solubilization capability of micelles facilitates dissolution of most matrices, which saves time in sample preparation. Thus, for example, the direct on–column injection of physiological fluids is possible.
(v) The compartmentalization of organic compounds by micelles produce enhanced luminescence detection.
(vi) The concentration of organic solvent needed in the preparation of hybrid micellar mobile phases is appreciably smaller than in conventional RPLC with hydro-organic mixtures (even for highly hydrophobic steroids with log $P_{o/w} = 7–8$). This is translated in a lower cost and toxicity, and the reduction of the environmental impact of hazardous wastes.
(vii) Organic solvents are highly retained in the micellar medium, which decreases the risk of evaporation. This makes micellar phases stable for a long time. Consequently, retention is highly reproducible and can be modeled accurately to predict changes in retention times with mobile phase composition (concentration of surfactant and volume fraction of organic modifier). This facilitates the optimization of the separation conditions.
(viii) MLC uses the same hardware (pumps, injectors, tubing, detectors, etc.) and columns as conventional RPLC. The solid nature of the surfactants usually employed in MLC should be considered to avoid conditions of precipitation inside the column. In spite of this, the long life of hardware and columns used in MLC routines has been repeatedly demonstrated.

Despite the advantages derived from the use of MLC, the applicability of the technique in the analytical laboratories is scarce, which should be explained by the hard comments in the literature about the limitations described in the first reports on this technique: the weak eluting power and reduced efficiency of pure micellar mobile phases, the non-reversible adsorption of some surfactants, and some questionable ideas about column damage and complexity of the experimental work, among others. In our opinion, these comments are far too superficial. Along the years, several valid solutions to these limitations have been proposed. The only real limitation up-to-date is related to the use of mass spectrometry (MS) detection, since direct on-line coupling to MLC is hindered by the presence of high concentrations of surfactant in the mobile phase. The present literature survey on MLC coupled with other detectors reveals that MLC is a highly competitive technique that should be considered more frequently.

MLC REPORTS

Since the first report on MLC (1), several research groups have been interested in the study of fundamental studies and development of applications. The seminal articles came from North-American research teams (7–12). The total production amounted to more than five hundred scientific reports at the end of 2007. Figure 1 shows the growth in the number of reports on MLC since 1980 to present days. A steady growth is observed over the first 15 years (1980−1995), as a result of the rapid and increasing interest that this technique arose at that moment. From the maximum (40 articles) of 1995 to the present time, the number of chemical reports remained approximately constant, averaging 30 articles per year. This indicates that the potential of MLC has not declined, but also has not risen any further. It must be pointed out that MLC makes less than 1% of the works produced in HPLC (between 3000 and 4000 per year).

The first reviews on MLC were published in the early 1980s coming exclusively from North-American research teams (3, 13). Successive advances were extensively reviewed by other authors throughout the decade (14–20). The second period characterizes on the publication of reports dealing with more specific topics and authors coming from the United States, Europe and Asia. Our research group in Valencia introduced MLC in Spain in the early 1990s. We graduated several Spanish students that found the topic so interesting that they started successful academic careers developing and using MLC in Spain. Today, we are proud to point out that Spanish groups are authoring

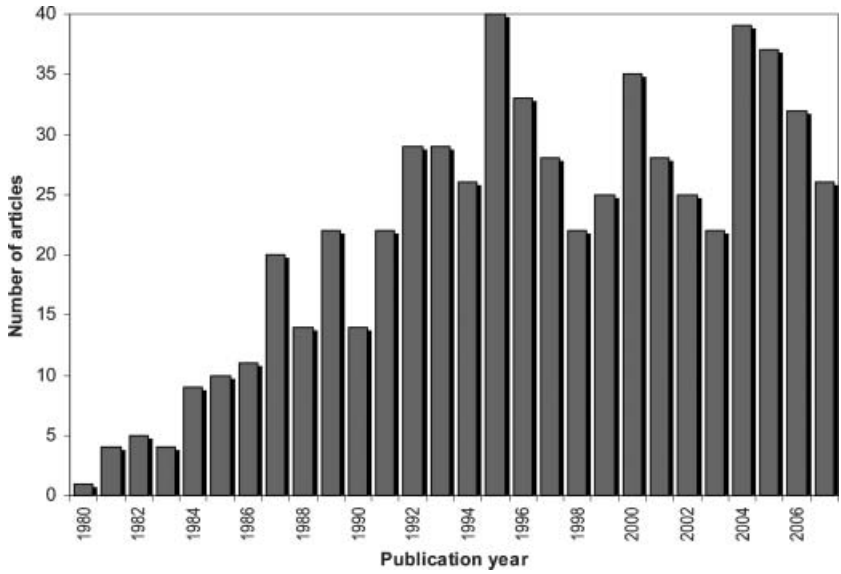

Figure 1. Publications dealing with MLC published over the 1980–2007 time period (612 articles found in the Elsevier, CAS and MedLine databases, Scopus® and SciFinder® search engines on December 2007).

close to half of all scientific articles dealing with MLC produced annually (21–40).

Many reviews on MLC were published in the 1992–2005 period. They focused on retention modeling (21–23), efficiency (24), hybrid mobile phases (25, 26), determination of distribution coefficients (27), and quantitative structure-retention relationships (QSRR) or quantitative retention–activity relationships (QRAR) (28, 29). Several general reviews have been also reported in this period (30–34). Finally, since the early years of MLC, numerous analytical applications involving this chromatographic mode have been reviewed (35–40).

In 2000, we prepared a reference work on MLC for experts and new researchers about to venture in this chromatographic mode (41). This monograph includes a detailed and extensive revision of the MLC literature published up to 1998. Ten years later, our interest for this chromatographic mode has not decayed, and we think as necessary the critical analysis of some recent advances on the knowledge of MLC fundamental aspects. This is the reason for this additional review article. We compiled in several tables the most relevant details on post-1998 publications on analytical applications of MLC.

EXPLORING THE CAPABILITIES OF MICELLAR MOBILE PHASES

Suitable Surfactants

For practical purposes, a suitable surfactant for MLC should have low CMC, aggregation number and, for ionic surfactants, a low Kraft point, which is defined as the temperature at which the ionic surfactant solubility equals its CMC. This should be preferably much smaller than the ambient temperature. A high CMC would imply operating at high surfactant concentration, which would result in viscous solutions and cause undesirable high pressure in a column and background noise in UV detectors. Since these detectors are the most commonly used in MLC, a suitable surfactant must have small molar absorptivity at the operating wavelength. Being the size of micelles a few nanometers, no problem caused by light scattering by micelles is expected.

Several surfactants of diverse nature fulfill these conditions, but the number of surfactants that have been used in MLC is reduced. Figure 2a indicates the frequency of use of surfactants in this chromatographic mode in the MLC reports that we found over the 1997–2007 time period. The anionic sodium dodecyl sulfate (SDS, also known as sodium lauryl sulfate) is, by far, the most common surfactant in MLC used in two thirds of the selected works. SDS is followed by the cationic cetyltrimethylammonium bromide (CTAB) and the non-ionic polyoxyethylene-(23)-dodecyl ether (Brij–35). Other surfactants include ionic, non-ionic and even zwitterionic surfactants.

SDS is usually selected due to its commercial availability in high purity with a relatively low cost. SDS is highly used in the manufacturing of detergents and cosmetics, and in other scientific fields. It gives rise to more economical procedures with respect to RPLC with hydro-organic mixtures. Also, when dealing with physiological matrices (urine, plasma, serum, etc.), SDS efficiently dissolves proteins allowing for direct injection of the samples in the chromatograph without any other treatment than filtration (13, 38). This is not possible with cationic surfactants. Conventional SDS-modified octadecyl (C_{18}) columns can accommodate hundreds of injections of biological matrices without any increase in back pressure or decrease in column performance. However, despite these reasons, SDS is often chosen simply because it has been used in hundreds of MLC studies, or because the dynamics of SDS micelles is better known than that of other micellar systems.

The non-ionic Brij-35 has been also used in clinical analysis (38), and in QSAR studies (29). It was stated that RPLC with solutions of Brij–35 micelles as mobile phases could emulate in vitro the partitioning process in biomembranes. For this reason, the use of MLC with Brij-35 micellar mobile phases has been dubbed "biopartitioning MLC" (29). However,

this surfactant has the practical disadvantage of being strongly adsorbed on C_{18}–bonded stationary phases.

Surfactant–Bonded Phase Association

Numerous studies have demonstrated that the stationary phase is modified when the mobile phase contains a surfactant, producing stronger solute–modified stationary phase interactions. This explains the interest in ascertain the modification of alkyl and cyanopropyl bonded phase columns when SDS or CTAB (the most common surfactants in MLC) are incorporated into the mobile phase. For this

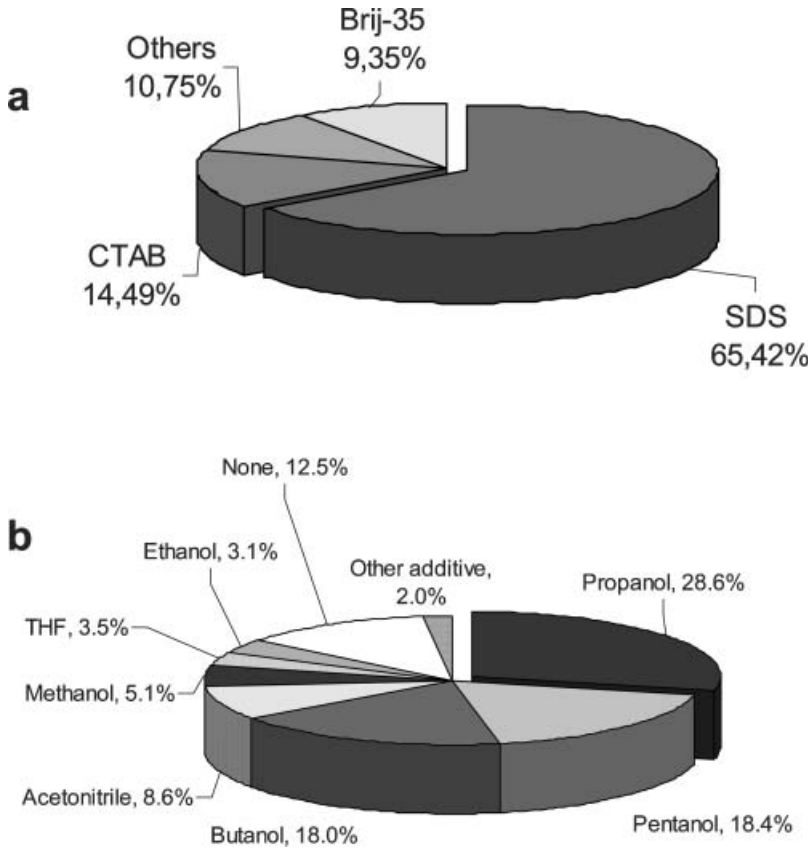

Figure 2. Statistical study on scientific articles dealing with MLC on the time period 1997–2007; (a) surfactants and (b) organic modifiers employed in the hybrid micellar mobile phases. Alcohol additives make a total of 73.2%. Non-ionic surfactants are most often used without additives.

purpose, solid state nuclear magnetic resonance (NMR, cross- polarisation/ magic angle spinning ^{13}C NMR with high-power proton decoupling) was used (42). This technique has the ability to differentiate surfactant molecules intercalated or in direct contact with the bonded phase. However, although information about the nature of the association between the surfactant and the bonded phase was provided, no direct knowledge on how the surfactant-modified stationary phase interacts with solutes in the mobile phase was obtained.

Differences in selectivity between MLC with SDS and CTAB on alkyl bonded phases for several vanillin compounds used as retention probes were attributed to the differing nature of the SDS- and CTAB-bonded phase association (43) (Figure 3). For SDS, the hydrophobic tail was found to be associated with the C_{18} alkyl chain bonded to the silica stationary phase, the sulfate head group oriented away from the surface (Figure 3a). This creates a negatively charged hydrophilic surface layer on the C_{18} surface. This charged layer affects the penetration depth of vanillin compounds in the bonded phase due to strong hydrogen-bonding interactions. The expected result is a decrease in hydrophobic interactions between the vanillin compounds and the C_{18} stationary phase. It would explain the superior resolution achieved by SDS micellar mobile phases. In the case of CTAB mobile phases, the surfactant association leads to a more hydrophobic bulk stationary phase because the positive nitrogen head group is partially incorporated into the C_{18} phase (Figure 3b).

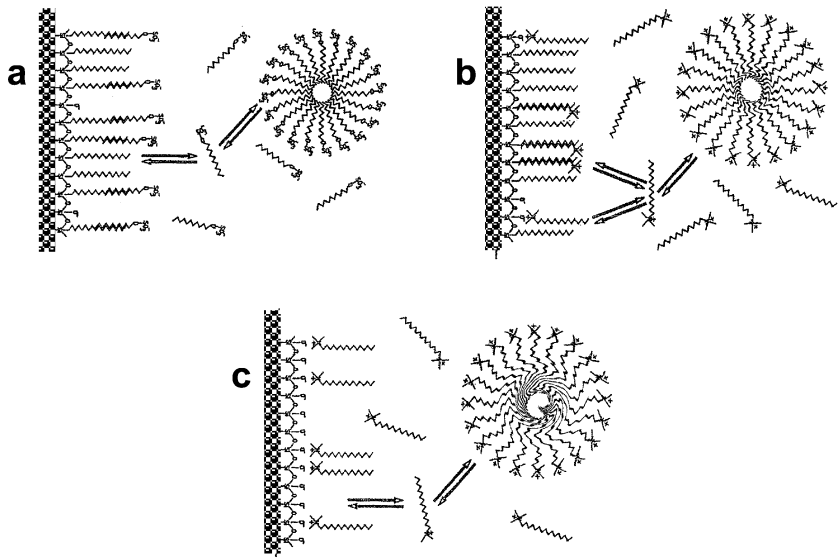

Figure 3. Three–phase systems in MLC: (a) C_{18}-bonded silica–SDS; (b) C_{18}-bonded silica-CTAB; (c) bare silica-CTAB (adapted from Ref. 42).

Solid state NMR explained also the so-called antibinding behavior observed with cyanopropyl bonded phase columns and ionic surfactants. The increased retention time of compounds possessing the same charge as the surfactant with increasing micelle concentration is a behavior opposite to what is usually observed (15). The negative sulfate head group of SDS and the positive *N*-alkyl head group of CTAB probably interact with the cyano group of the polar bonded phase through some type of electrostatic interaction. Consequently, both charged surfactant ions adsorb head down with their tails projected upward, creating a pseudo-alkyl bonded phase (Figure 3c). Hence, there is little free electrostatic charge to prevent migration of the ionized solute into the polar bonded phase. The lower surface charge of the SDS- and CTAB-modified cyanopropyl bonded phases is responsible for solute-micelle interactions playing a more important role in the retention of weak bases and weak acids, respectively, on cyanopropyl bonded phase columns (42).

Investigating Other Surfactants

The capabilities of SDS, CTAB and Brij-35 may explain the scarce interest in searching new surfactants adequate for MLC analysis. In one of such reports, it was shown that not only the surfactant type (neutral, anionic or cationic), but also the structure of the surfactant can affect the chromatographic behavior of solutes. For this purpose, the behavior of three surfactants (CTAB, cetyltrimethylammonium chloride CTAC, SDS, and dodecyltrimethylammonium chloride DTAC) was studied in the MLC separations of polycyclic aromatic hydrocarbons (PAHs), with pentanol as organic modifier (43). A difference in the separation was observed between CTAC and SDS/DTAC. At the same surfactant concentration, DTAC and SDS showed weaker elution strength compared to CTAC. Also, under each optimized separation conditions, CTAC-modified mobile phase provided the poorest separation. In addition to the four-carbon difference in their carbon tails (C_{16} versus C_{12}), the head groups of SDS and CTAC are also different, with SDS having a sulfate and CTAC a trimethylammonium head groups. DTAC has the same head group as CTAC and the same tail as SDS, consequently, the apolar carbon tail length appeared to be the main cause of the observed differences.

Other interesting reports refer to the zwitterionic surfactant *n*-dodecyl-*N*,*N*-dimethylamino-3-propane-1-sulfonate (C_{12}DAPS) (44), and the non-ionic surfactants Triton X-114 (45), and Genapol X-080 (46). C_{12}DAPS is one of the common surfactants that maintain its zwitterionic character over all the pH range used in RPLC. Compared to anionic and cationic surfactants, it gives the highest partitioning constants

with the stationary phase and micelle, which results in larger retention. Also, the dipolar environment is greater. This affects the MLC partition considerably, and allows an easier binding to acidic solutes than SDS.

Diverse Surfactant Effects Combined with MLC

The new interest in non-ionic surfactants in MLC is mainly related to the combined use of micellar cloud-point extraction and surfactant-assisted RPLC separation (45–47). Many aqueous solutions of non-ionic surfactants separate in two phases on heating above a particular temperature: the clouding temperature. The homogeneous solution becomes turbid (cloudy) and gives rise to a lower water-rich phase (containing a small portion of the surfactant equal to the CMC) and an upper organic surfactant-rich phase. The two phases can easily be separated by centrifugation and collection of the aqueous phase. A solute dissolved in the homogeneous solution at low temperature may partition preferentially in one phase or the other, but cloud-point extraction is commonly used to extract and/or concentrate apolar solutes in the surfactant-rich phase. The alternative methodology to cloud-point extraction implies extraction in a suitable organic solvent, evaporation to dryness and re-dissolution before injection into the RPLC system, implying a risk of sample loss and contamination along with extensive analysis time. Surfactant solutions are easier and safer to handle in extraction processes, producing less toxic wastes and saving the environment.

Several biogenic amines (putrescine, cadaverine, agmatine, tyramine, tryptamine, phenylethylamine, spermine, spermidine, and histamine) were analyzed in fish samples, after entrapping within the micelles of the non-ionic surfactant Triton X-114 (45). The surfactant–rich phase was re-dissolved in methanol and separated by MLC with gradient elution, where the mobile phase consisted of a 0.40 M SDS solution and increased acetonitrile. The use of the same surfactant in both cloud-point extraction and MLC separation may be advantageous. Both non-ionic surfactants Brij-35 and Genapol X-080 allowed the on-line combination of sample pre-treatment with MLC analysis of cholesterol in egg yolk (47), and six herbicides (chlortoluron, netoxuron, chloridazon, simazine, propazine and atrazine) (46), respectively. For cholesterol, the MLC separation was carried out with a cyano-bonded column and Brij-35 mobile phases modified with propanol, and for the herbicides, with a C_{18}-bonded column and Genapol X-080 mobile phases modified with methanol.

Other attractive example of the use of micellar mobile phases is related to the post-column use of immobilized enzyme reactors to modify

the detectability of some analytes (47, 48). The activity of enzymes (natural polypeptidic molecules) decreases rapidly and irreversibly due to denaturation when mixed with organic solvents. This denaturation problem renders the on-line use of enzyme reactors in RPLC expensive due to the frequent replacement of the reactor damaged by the mobile phase organic modifier. The use of a minimum amount of organic solvent in the mobile phase or dilution of the mobile phase before it enters the post-column enzyme reactor have been proposed to reduce this problem. However, these solutions result in either prohibitively long retention times or loss in sensitivity. The non-ionic surfactant Brij-35 was found to be far more gentle to the enzyme denaturation than any organic solvent (ionic surfactants are not adequate since they bind to charged groups on proteins and/or enzymes blocking them). Two examples have been reported where immobilized reactors are combined with MLC using Brij–35: the detection of amino acids by fluorescence of the homovanilic dimer, and cholesterol and several metabolites by UV absorption (47, 48). The non-ionic surfactant allowed the reactor activity to remain at a higher level and for much longer time (at least one week without losing activity) than with classical RPLC with hydro-organic solvents, and the limits of detection (LODs) were lower.

Pyridinium chloride (PC) has been reported as a selective fluorescence quencher of alternant PAHs, with little effect on the non-alternants, simplifying fluorescence-detected chromatograms of complex PAHs samples (49). Interestingly, unlike the parent moiety of PC, cetylpyridinium chloride (CPC) can play a dual role as micellar mobile phase modifier and selective fluorescence quenching agent of PAHs (49). However, because of the high quencher concentration that would exist in the micellar mobile phase, micelles only composed of pure CPC would quench fluorescence signal of all PAHs. Therefore, CTAC, which is not a fluorescence quencher and has similar properties to CPC (a C_{16} tail and positively charged head groups), was proposed as a co-surfactant to lower the concentration of CPC in the mobile phase (49).

Micellar media may also give a solution to lower the LODs of some analytes. There are two recent interesting examples of coupling this possibility with an MLC separation. In one of these procedures, solid phase extraction (SPE) was combined with on-line derivatization to determine biogenic amines at very low levels. SPE was done using an octadecylsilane (ODS) guard column installed instead of the filling loop on the HPLC apparatus (50). The on-line derivatization was accelerated by the presence of the non-ionic surfactant Triton X−114. It was performed simultaneously with the extraction by sequential injection of the derivatization reagent (benzoyl chloride) adsorbed on the SPE column followed by the solution containing the biogenic amines. Resolution of the peaks and quantification was further enhanced by

MLC with SDS and acetonitrile, and sensitization of the benzene ring absorption at 254 nm. LODs of the benzoyl derivatives of biogenic amines were in the hundredth ppt range (~0.1 μg/L). Such LODs were lower than those obtained with simple fluorescence detection and unparalleled to any other UV approach (50).

Finally, fluorimetric detection is a good option to achieve low LODs for the analysis of steroids in physiological fluids, but most steroids are non-fluorescent and must be derivatized to be detected, making the procedures too laborious. One option for steroids showing an α,β-unsaturated carbonyl group in the A-ring, such as cortisone, progesterone, and several hormones derived from testosterone, is the measurement of the sensitized fluorescence of a lanthanide ion, such as terbium(III) (51). This is achieved when a molecule (the donor) transfers energy to the low absorbing lanthanide ion (the receptor), which enhances greatly its fluorescence. The fluorescence yield of terbium(III) depends on the spatial proximity to the donor. Micellar media favor this proximity since both donor and acceptor can be accommodated simultaneously in the micelles, and therefore, be found in a small volume. Coupling of sensitized terbium fluorescence in micellar media with MLC using SDS-acetonitrile and SDS-pentanol was shown as a good alternative for steroid analysis, although the concentration of organic solvent should be limited to avoid quenching of the signal. In the recommended procedure, terbium(III) was added to the micellar mobile phase. LODs were in the 0.07–0.2 ppm or μg/mL range (51).

ORGANIC SOLVENTS AS MODIFIERS OF THE ELUTION STRENGTH AND RESOLUTION

Addition of Organic Solvents to Enhance Efficiency and Elution Strength

In the first reports on MLC, the mobile phase contained only a surfactant and, occasionally, a buffer compound. As soon as 1983, Dorsey et al. (6) recommended the addition of a short-chain alcohol, 1-propanol or butanol, to the micellar eluent to enhance the efficiency. It was later that the potential use of organic solvents to increase the elution strength of micellar mobile phases was appraised. Figure 2b shows that propanol is the most usual alcohol additive. More recently, acetonitrile, a common solvent in hydro-organic RPLC, has called attention.

The organic solvent (i) lowers the polarity of the aqueous solution, (ii) alters the micelle structure, and (iii) acts on the stationary phase changing the amount of adsorbed surfactant. Also, organic solvent molecules wet the bonded phase, changing its physicochemical structure (rigidity) and hydrophobicity.

Hybrid micellar mobile phases were found so useful that most applications in MLC are done with them today. Table 1 lists the MLC separations of pharmaceutical compounds in physiological fluids found in References 52 to 85. Most separations used hybrid micellar mobile phases (52–85). Table 2 lists also the MLC separations of pharmaceutical compounds but in the case of the control of shelf formulations such as pills, syrups, tablets, powders, injectable preparations, ointments, sprays or other forms (86–116). Here again, most Table 2 mobile phases are hybrid micellar mobile phases containing several volume percents of various organic modifiers. Table 3 gathers similarly the MLC separations of bioactive compounds found in food and water samples, environmental, cosmetic and samples of various origins (117–130). All micellar mobile phases in Table 3 were hybrid containing a surfactant and an organic modifier mostly added to enhance the elution strength.

Although the separation mode is still predominantly micellar in nature, the micelle itself is perturbed by the organic solvent, which can cause changes in micellar parameters, such as the CMC and the surfactant aggregation number. Indeed, a high percentage of organic solvent is not desirable, because the role of the micelle as a modifier would be diminished or annulled. Organic solvent rich mobile phases can also sweep out completely the adsorbed surfactant molecules from the bonded phase surface. The concentration of organic solvent that preserves the integrity of micelles is approximately 15% for propanol and acetonitrile, 10% for butanol, and 6% for pentanol (131).

Search of New Organic Modifiers

As commented, the number of organic solvents employed routinely in MLC is small. There has been, however, an interest in investigating the effect of other organic solvents on the chromatographic behavior in MLC. The solubilizing power of micellar media allows for the potential use of a wide variety of organic additives for the control of retention. Additives that would normally not be considered in conventional hydro-organic RPLC due to insufficient solubility can form stable mobile phases with micelles. Thus, for instance, in a 0.285 M SDS medium, the molar concentrations of 2-methyl-1-butanol, 1-pentanol, 1-hexanol and pentane were found to be 0.46, 0.92, 0.79 and 0.095, respectively, whereas their molar solubilities in water at 25°C are only 6.1×10^{-3}, 4.5×10^{-3}, 1.2×10^{-3}, and 9.5×10^{-6}, respectively (132).

In a comprehensive study, 21 additives (alkanols, alkane diols, dipolar aprotic solvents, and alkanes) were added to an SDS micellar mobile phase to determine the behavior of two solutes of widely differing hydrophobicity (benzene and 2–ethylanthraquinone) in a C_{18} column

Table 1. Experimental characteristics of MLC procedures for the analysis of physiological fluids.

Compounds	Sample (number of compounds)/Mobile phase/Stationary phase/ Elution mode/Temperature	Ref.
Analgesics: acetaminophen	Serum and urine (1)/0.02M SDS, pH 7/C_{18}/isocratic	52
Anesthetics: Procaine, tetracaine	Plasma (2)/0.15M SDS–10% propanol–0.5% triethylamine, pH 2.5/ Spherisorb ODS–2 (125 × 4 mm i.d.)/isocratic	53
Antibacterial agents: Ciprofloxacin, enoxacin	Serum (2)/0.075M SDS–3% propanol, pH 3/Licrospher 100RP18 (125 × 4 mm i.d.)/isocratic	54
Anticonvulsant agents: Bromazepam, carbamazepine, diazepam, flunitrazepam,	Serum (8)/0.06M SDS–5% butanol, pH 7/Kromasil C_{18} (120 × 4.6 mm i.d.)/ isocratic/25°C	55
halazepam, medazepam, nitrazepam, oxazepam, phenobarbital, phenytoin, tetrazepam	Serum (3)/0.05M SDS–butanol (70 ml/l), pH 7/C_{18} (250 × 4 mm i.d.)/isocratic	56
Barbiturates: Amobarbital, barbital, butabarbital,	Plasma and urine (1)/0.02M CTAB–15% propanol, pH 7.5/isocratic	57
diallybarbituric acid, hexobarbital, pentobarbital, phenobarbital, secobarbital	Urine (5)/0.07M SDS–0.3% propanol, pH 7.4/Spherisorb ODS–2 (120 × 4 mm i.d.)/isocratic	58
	Plasma (1)/0.03M CTAB–3% propanol, pH 7/Spherisorb ODS–2 (125 × 4 mm i.d.)/isocratic	59
	Plasma (2)/0.04M CTAB–3% propanol, pH 7.5/Spherisorb ODS–2 (125 × 4 mm i.d.) isocratic	60
	Serum (4)/0.10M SDS–4% butanol, pH 7/C_{18} (120 × 4 mm i.d.)/isocratic/25°C	61
β–Blockers: Acebutolol, atenolol, celiprolol, labetalol, metoprolol, nadolol, propranolol	Urine (7)/0.1M SDS–15% propanol, pH 3/Spherisorb ODS–2 (120 × 4.6 mm i.d.)/isocratic	62
β–Blockers and diuretics: Acebutolol, atenolol, labetalol, metoprolol, nadolol, propranolol, and amiloride, bendroflumethiazide, piretanide, triamterene	Urine (10)/0.11M SDS–15% propanol, pH 3/ODS–2 (120 × 4.6 mm i.d.)/ isocratic	63

β–Blockers and metabolites: Desisopropylpropanolol, α–naphtoxylactic acid, α–naphtoxyacetic, propranolol, propanolol glycol	Urine (5)/0.15M SDS–10% propanol, pH 3/ODS–2 (125 × 4.6 mm i.d.)/isocratic	64
Biogenic amines and metabolites: Dopamine, homovanilic acid, hydroxyindoleacetic acid, serotonin, tyramine	Serum (5)/0.15M SDS, pH 3/Kromasil C_{18} (120 × 4.6 mm i.d.)/isocratic	65
Bronchodilators: Caffeine, theophyline	Serum (2)/0.05M SDS–2.5% propanol, pH 7/Kromasil C_{18} (250 × 4.6 mm i.d.)/isocratic	66
Calcium channel blockers: Nifedipine, verapamil	Urine and serum (1)/0.125M SDS–3% pentanol, pH 3/Kromasil C_{18} (150 × 4.6 mm i.d.)/isocratic	67
	Urine and serum (1)/0.15M SDS–5% pentanol, pH 7/Kromasil C_{18} (250 × 4.6 mm i.d.)/isocratic	68
Cathecolamines and metabolites: Epinephrine, nor–metanephrine, norepinephrine, metanephrine	Serum (4)/0.075M SDS–1.6% butanol, pH 7/C_{18}/isocratic/25°C	69
Desferroxiamine and chelates with Al and Fe	Serum (3)/0.2M SDS–5% acetonitrile or 0.5% Brij–35, pH 7.4/Spherisorb ODS–2 (300 × 4.6 mm i.d.)/isocratic	70
Diuretics: Althiazide, amiloride,	Urine (6)/0.055M SDS–propanol/ODS–2 (120 × 4.6 mm i.d.)/isocratic	71
bendroflumethiazide, benzthiazide, bumetanide, canrenoic acid, canrenone, chlortalidone,	Urine (15)/0.055M SDS–6% propanol, pH 3/ODS–2 (125 × 4.6 mm i.d.)/isocratic	72
chlorthiazide, clopamide, cyclothiazide, dichlorphenamide, ethacrynic acid, furosemide,	Urine (19)/0.040M SDS–4% tetrahydrofuran, pH 3.2/Hypersil C_{18} (150 × 3 mm i.d.)/isocratic/50°C	73
hydrochlorthiazide, hydroflumethiazide, indapamide, piretanide, polythiazide, probenecid,	Urine (1)/0.05M SDS–6% propanol, pH 3/ODS–2 (125 × 4.6 mm i.d.)/isocratic	74
spironolactone, torasemide, triamterene, trichlormethiazide, xipamide	Urine (1)/0.05 M SDS/C_{18} (125 × 4.6 mm i.d.)/isocratic	75
Heroin and metabolites: Benzoylecgonine, heroin, 6−monoacetylmorphine, morphine	Serum (4)/0.1M SDS–4% butanol, pH 7/Kromasil C_{18} (250 × 4.6 mm i.d.)/isocratic	76
Nicotinic acid and nicotinamide	Serum and urine (2)/0.15M SDS–6% pentanol, pH 3/Kromasil C_{18} (250 × 4.6 mm i.d.)/isocratic	77

(*continued*)

Table 1. Continued.

Compounds	Sample (number of compounds)/Mobile phase/Stationary phase/ Elution mode/Temperature	Ref.
Opiates: Codeine, morphine, thebaine	Serum (3)/0.15M SDS–7% butanol, pH 7/Kromasil C_{18} (250 × 4.0 mm i.d.)/ isocratic/25°C	78
Steroids: Acetonide, betamethasone, corticosterone, cortisol, cortisone, deflazacort,	Urine (6)/0.05M SDS–9% butanol/Spheri–5 RP_{18} (100 × 4.6 mm i.d.)/ isocratic	79
dehydrotestosterone, dexamethasone,	Urine (16)/0.036M SDS–1.9% butanol/Hypersil C_{18} (250 × 3.2 mm i.d.)/ isocratic/50°C	80
dydrogesterone, fludrocortisone, fludrocortisone acetate, hydroxycorticosterone,	Urine (13)/0.12M SDS–7% pentanol/Spherisorb ODS–2/(120 × 4.6 mm i.d.)/ isocratic	81
21−hydroxydeflazacort,	Urine (2)/0.018M SDS–8.3% tetrahydrofuran/Hypersil C_{18} (150 × 3.2 mm	82
11α−hydroxyprogesterone, medroxyprogesterone, medroxyprogesterone acetate, methandienone, methelonone enanthate, methylprednisolone, methyltestosterone, nandrolone, nandrolone decanoate, norethisterone, prednisolone, prednisone, progesterone, testosterone, testosterone enanthate, testosterone propionate triamcindone, triamcinolone	i.d.)/isocratic	
Stimulants: Amphetamine, ephedrine, methoxyphenamine, phenylephrine, phenylpropanolamine	Urine (5)/0.15M SDS–3% pentanol, pH 3/ODS–2 C_{18} (120 × 4.6 mm i.d.)/ isocratic	83
Tricyclic antidepressants: desipramine, imipramine	Serum (2)/0.15 M SDS–6% pentanol, pH 7/C_{18} (250 × 4.6 mm i.d.)/isocratic	84
Vitamins: B6 (pyridoxal, pyridoxine and pyridoxamine)	Serum (3)/0.15M SDS–2% pentanol, pH 3/Kromasil C_{18} (120 × 4.6 mm i.d.)/ isocratic	85

Table 2. Experimental characteristics of MLC procedures for the analysis of pharmaceutical formulations

Compounds	Sample (number of compounds)/Mobile phase/Stationary phase/Elution mode/Temperature	Ref.
Anesthetics: Bupivacaine, lidocaine, mepivacaine, procaine, propanocaine, tetracaine	Diverse formulations (6)/0.15M SDS–propanol (9:1), pH 3/Spherisorb ODS–2 (120 × 4 mm i.d.)/isocratic	86
Anesthetics and muscle relaxants: Lidocaine, tolperisone	Diverse formulations (2)/0.075M SDS–7.5% propanol/C_{18} (125 × 4.6 mm i.d.)/isocratic	87
Antianginals: Diltiazem, nadolol, nifedipine, propranolol, verapamilo	Diverse formulations (5)/0.05M SDS–5% pentanol, pH 7/Kromasil C_{18} (150 × 4.6 mm i.d.)/isocratic	88
Antibacterial agents: Azithromycin, cefuroxime, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfathiazole, trimethoprim	Diverse formulations (6)/0.04M SDS–2% 2–propanol/Nucleosil C_{18} (150 × 4.6 mm i.d.)/isocratic/40°C	89
	Medicine and veterinary formulations (7)/SDS–6% acetonitrile, pH 3/C_{18}/isocratic	90
	Tablets and capsules (1)/0.1M SDS–15% butanol, pH 7/Hypersil C_{18} (200 × 4.6 mm i.d.)/isocratic/60°C	91
	Tablets (1)/0.02M SDS–8% acetonitrile/XTerra C_{18} (150 × 4.6 mm i.d.)/isocratic/50°C	92
	Diverse formulations (2)/0.1M SDS–3% butanol/Hypersil ODS (125 × 4.6 mm i.d.)/isocratic/35°C	93
Anticonvulsant agents: Bentazepam, carbamazepine, clorazepate, chlordiazepoxide, diazepam, diltiazem, ethosumixine, halazepam, oxazepam, phenobarbital, phenytoin, pinazepam, tetrazepam, zoplicone	Diverse formulations (3)/0.1M SDS–3% butanol, pH 3/ODS–2/isocratic	94
	Pills and capsules (6)/0.1M SDS–3% butanol–0.1% triethylamine, pH 3/C_{18}/isocratic	95
	Capsules, pills, tablets, injections, drops and suppositories (7)/CTAB/C_{18}/isocratic	96
Antidiabetic drugs: metformin, glipizide, glicazide	Diverse formulations (3)/Zorbax XDB C_{18}/isocratic	97

Antihistamines: Azatadine, carbinoxamine, cyclizine, cyproheptadine, diphenhydramine, doxylamine, tripelennamine, brompheniramine, chlorcyclizine, chlorphenamine, flunarizine, hydroxyzine, promethazine, terfenadine, tripelennamine, tripolidine	Tablets, capsules, powders, solutions and syrups (7)/0.15M SDS–6% pentanol/C_{18}/isocratic	98
	Tablets, capsules, suppositories, syrups and ointments (11)/0.02M CTAB–3% propanol, pH 6; 0.02M CTAB–3% propanol, pH 7; 0.04M CTAB–3% butanol, pH 3; 0.04M CTAB–3% butanol, pH 5/Spherisorb C_{18} (250×4.6 mm i.d.)/isocratic	99
Antihistamines and phenethylamines: Carbinoxamine, chlorpheniramine, dexbrompheniramine, dexchlorpheniramine, diphenhydramine, doxylamine, pheniramine, phenyltoloxamine, tripolidine, azatadine and ephedrine, methoxyphenamine, phenylephrine, phenylpropanolamine, pseudoephedrine	Diverse formulations (15)/0.05M SDS–6% pentanol, pH 7/Eclipse XDB C_8 (150×4.6 mm i.d.)/isocratic	100
	Cough cold preparations (4)/0.15M SDS–6% pentanol, pH 7/ODS–2 C_{18} (120×4.6 mm i.d.)/isocratic	101
Calcium channel blockers: Flunarizine, nifedipine, verapamil, degradation products	Diverse formulations (6)/0.15M SDS–10% propanol–0.3% triethylamine, pH 4 or 6.8/cyanopropyl–bonded (250×4.6 mm i.d.)/isocratic	102
Diuretics: Althiazide, bendroflumethiazide, chlorthiazide, cyclothiazide, furosemide, hydrochlorthiazide, hydroflumethiazide, trichlormethiazide	Tablets and capsules (7)/0.02M SDS, pH 7 or 0.15M SDS, pH 7/Spherisorb ODS–2 (120×4.6 mm i.d.)/isocratic	103
	Tablets, capsules, injectables and drops (1)/0.06M SDS–8% propanol, pH 3/ ODS–2 (125×4.6 mm i.d)/isocratic	104
	Diverse formulations (1)/0.05M SDS/C_{18} (125×4.6 mm i.d.)/isocratic	75
Nicotinic acid and nicotinamide	Diverse formulations (2)/0.15M SDS–6% pentanol, pH 3/Kromasil C_{18} (250×4.6 mm i.d)/isocratic	77
Phenethylamines: Amphetamine, arterenol, ephedrine, phenylephrine, phenylpropanolamine, mephentermine, methoxyphenamine, pseudoephedrine, tyramine	Capsules, tablets, pills, powder, syrup and drops (9)/0.15M SDS–5% pentanol, pH 7/Spherisorb OD–2 (120×4.6 mm i.d.)/isocratic	105

(*continued*)

Table 2. Continued.

Compounds	Sample (number of compounds)/Mobile phase/Stationary phase/ Elution mode/Temperature	Ref.
Non-steroideal anti–inflammatory drugs: Acemetacin, diclofenac, indomethacin, ketoprofen, nabumetone, naproxen, tolmetin, piketoprofen	Diverse formulations (8)/0.06M CTAB–10% butanol, pH 7/Kromasil C_{18} (150 × 4.0 mm i.d.)/isocratic	106
	Diverse formulations (6)/0.15M SDS–10% propanol, pH 3/Spherisorb ODS–2 (250 × 4.6 mm i.d.)/isocratic	107
Steroids (anabolics and corticoids): Beclomethasone, bethamethasone, budesonide, danazol, dexamethasone, fludrocortisone, fluocinolone, hydrocortisone, methyltestosterone, triamcinolone	Creams, gels and ointments (7)/0.1M SDS–4% butanol, pH 7/C_{18} (12.5 × 4.6 mm i.d.)/isocratic	108
	Pills (1)/0.04M SDS–10% propanol/Hypersil ODS (150 × 4.6 mm i.d.)/ isocratic/60°C	109
	Capsules (1)/0.04M SDS–2% pentanol/Hypersil ODS (150 × 3 mm i.d.)/ isocratic/60°C	110
	Cocktails (2)/0.0325M CTAB–0.24% pentanol/Hypersil C_{18} (250 × 3 mm i.d.)/ isocratic/60°C	111
Tricyclic antidepressants: Amitryptiline, clomipramine, doxepin, imipramine, maprotiline, nortryptiline, trimipramine	Capsules, pills, tablets, injections (7)/0.04M CTAB–5 to 15% propanol, pH 3–4/Kromasil C_{18} (150 × 4.6 mm i.d.)/isocratic	112
	Tablets and capsules (7)/0.075M SDS–6% pentanol, pH 3/Eclipse XDB C_8 (150 × 4.6 mm i.d.)/isocratic	113
Vitamins: A, B3 (nicotinamide), B1 (thiamine), B2 (rivoflavin), B6 (pyridoxal, pyridoxine and pyridoxamine), B9 (folic acid), B12 (cyanocobalamin), C (ascorbic acid), E	Capsules, pills and syrups (5)/0.1M SDS–4% pentanol, pH 3/Kromasil C_{18} (120 × 4.6 mm i.d.)/isocratic	114
	Multivitamin tablets (7)/0.016M SDS–3.5 to 10% butanol, pH 3.6/Particil ODS–2 (250 × 4.6 mm i.d.)/gradient/35°C	115
	Multivitamin syrup (2)/0.077M SDS–12% butanol, pH 7/Spherisorb ODS–2 (100 × 3.9 mm i.d.)/isocratic/30°C	116

Table 3. Experimental characteristics of MLC procedures for the analysis of other samples.

Compounds	Sample (number of compounds)/Mobile phase/Stationary phase/ Elution mode/Temperature	Ref.
Antibacterial agents: Sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisoxazole	Honey and milk (11)/0.019M SDS–5.8% acetonitrile, pH 3/Hypersil ODS–2 (100 × 4.6 mm i.d.)/isocratic	117
Antioxidants: Butylated hydroxyanisole, butylated hydroxytoluene, docecyl gallate, nordihydroguaiaretic acid, octyl gallate, propyl gallate, tert–butylhydroquinone, 3–tert–buytl–4–hydroxyanisole, 2,4,5–trihydroxybutyrophenone	Edible oil (7)/0.1M SDS–2.5% propanol, pH 3/Spherisorb ODS–2 (125 × 4 mm i.d.)/isocratic	118
	Sunflower, corn and olive oils, margarine, lard and butter oil (4)/0.1M SDS–2.5% propanol, pH 3/Spherisorb ODS–2 (125 × 4 mm i.d.)/ isocratic	119
	Powered and liquid milk, cream of milk and dietetic supplements (7)/ 0.05–0.15M SDS–1–9% propanol, pH 3/Spherisorb ODS–2 (125 × 4 mm i.d.)/isocratic	120
	Olive oil (5)/0.01M SDS–30% propanol acid, pH 2/Lichrosorb RP–18 (150 × 3.9 mm i.d.)/isocratic	121
Biogenic amines and metabolites: Agmatine, cadaverine, dopamine, homovanilic acid, hydroxyindoleacetic acid, histamine, 2−phenylethylamine, putrescine, serotonin, spermidine, spermine, tryptamine, tyramine	Food substrates (9)/0.4M SDS–acetonitrile 70:30, 58:42, pH 3/ LiChrospher RP–18 (244 × 4.4 mm i.d.)/gradient	45
	Food substrates (9)/0.4M SDS–acetonitrile 70:30, 58:42, pH 3/ LiChrospher RP–18 (244 × 4.4 mm i.d.)/gradient	50
Carbamates: Carbaryl, carbofuran, desmedipham, methiocarb, propoxur	Commercial pesticide formulations and water (5)/0.07M Brij–35/ Kromasil C_{18} (25 × 4 mm i.d.)/isocratic	122
	Water samples (3)/0.15M SDS–6% pentanol, pH 3/C_{18} (250 × 4 mm i.d.)/isocratic	123
Cholesterol	Food (1)/0.03M Brij–35–10% propanol, pH 7.2/Zorbax CN (150 × 4.6 mm i.d.)/isocratic	124

Parabens: Benzoic acid, butyl esters of *p*–hydroxybenzoic acid, *p*–hydroxybenzoic acid, methyl– ethyl–, propyl–, isopropylparaben	Cosmetics (5)/0.1M SDS–2.5% propanol, pH 3/Spherisorb ODS–2/ isocratic	125
	Cosmetics and food samples (7)/2% Brij–35–20% propanol, pH 3/ Lichrosorb ODS (250 × 4.6 mm i.d.)/isocratic	126
Phenolic compounds: Caffeic acid, *p*–coumaric acid, oleuropeina, tyrosol	Virgin olive oil (6)/0.07M SDS–2.5% 2–propanol, pH 3/Nucleosil 120 C_{18} (200 × 4.6 mm i.d.)/isocratic	127
Phospholipids: Phosphatidylcholine, phosphatidylethanolamine		
Steroids (anabolics and corticoids): Androsterone, bolasterone, boldenone, corticosterone, cortisone, cortisol, dehydroepiandrosterone, deoxycorticosterone, epitestosterone, hydroxyprogesterone, 11β–hydroxyprogesterone, 11–ketotestosterone, nandrolone, nandrolone decanoate, medroxyprogesterone, medroxyprogesterone acetate, methyltestosterone, progesterone, testosterone, testosterone enanthate, testosterone propionate	Standard solutions (9)/0.075M SDS–12.4% acetonitrile or 0.13M SDS–4.5% pentanol with 0.01M Tb(III)/Hypersil C_{18} (150 × 3 mm i.d.)/ isocratic	51
	Standard solutions (13)/0.04M SDS–5% propanol/Hypersil C_{18} (150 × 3.0 mm i.d.)/isocratic/60°C	128
Sugars: Arabinose, glucose, lactose, maltose, xylose	Infant formula and syrups (5)/0.017M SDS–7.7% ethanol, pH 6.7/ Kromasil 100–10NH_2 (250 × 4.6 mm i.d.)/isocratic/35°C	129
Tetracyclines: Chlortetracycline, doxycycline, minocycline, oxytetracycline, tetracycline	Animal feeds (5)/0.05M SDS–5% butanol, pH 3/ODS–Hypersil (100 × 4.6 mm i.d.)/isocratic	130

(132). Several trends were drawn from the retention data: first, the presence of alkanols, alkanediols and dipolar aprotic solvents resulted in a diminution of the retention of the probe solutes, whereas alkane additives had relatively little effect. The advantage of the presence of micelles can be illustrated by the fact that the retention factors (k) for benzene and 2-ethylanthaquinone are $k = 6.1$ and 12.8, respectively, with a mobile phase of 0.285 M SDS containing 2% (v/v) pentanol, whereas they are as high as $k = 40.2$ and >200, respectively, with a 2.1:97.9 (v/v) pentanol-water mobile phase (in the absence of surfactant).

The elution strength was observed to parallel the octanol-water partition coefficients (i.e., hydrophobicity) of the additives or their ability to bind to SDS micelles (132). The retention decreased as the additive hydrophobicity increased, the effect being more intense for 2-ethylanthraquinone, the more hydrophobic probe solute, than for benzene. The correlation between log $P_{o/w}$ for the organic additives and the retention factor of benzene is shown in Figure 4. Similar plots were obtained when the micelle binding constants (K_{AM}) of the additives were plotted against the solute retention. Thus, the stronger the organic additive binds to the micelle in the mobile phase, the greater will be its ability to reduce the retention time of a given solute in MLC. Thus, if either $P_{o/w}$ or K_{AM} is

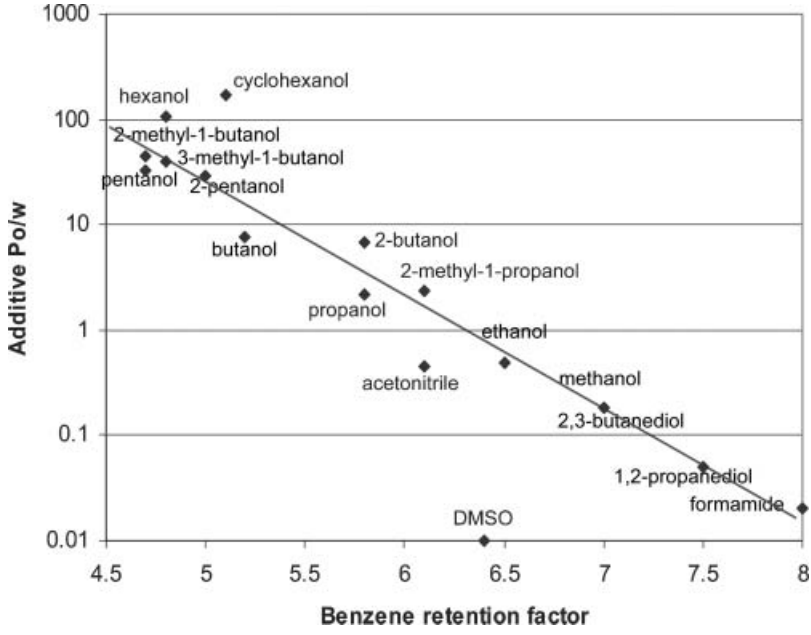

Figure 4. Correlation between the octanol-water partition coefficient of several organic additives and the retention factor of benzene eluted with a 0.285 M SDS mobile phase containing 5% (v/v) of the additives (adapted from Ref. 132).

available for a potential additive or can be estimated from the known solvatochromic relationships for these two parameters, then the qualitative relationships shown in Figure 4 can be used to predict the additive effect on solute retention for a particular mobile phase.

Interaction of Organic Solvents with Micelles and Effects on CMC and Retention

The addition of an additive to micellar solutions results in the partitioning of some or all of the additive to the micellar aggregates, the degree of binding increasing with the additive hydrophobicity. Such intercalation of additive molecules into the SDS micelle leads to an increase in the distance between adjacent anionic sulfate headgroups and a decrease in the micellar surface potential (charge density). In fact, the additive may produce a disordering of the micellar interface by ''opening-up'' the charged headgroup region, which in turn decreases the distinction between the hydrophilic and hydrophobic domains and fluidises the micelle interior (micelles become more labile). The magnitude of these effects depends on the concentration and nature (i.e., alkyl chain length and degree of branching) of the additive employed (133).

The analysis of CMC data, obtained after addition of methanol, ethanol, propanol, butanol, pentanol, acetonitrile and tetrahydrofuran to an SDS solution, allowed some understanding on the interactions between solute, SDS micelles and bulk liquid (134). Methanol, acetonitrile and tetrahydrofuran increased the CMC, whereas the other alcohols decreased it. For butanol and pentanol, the CMC barely changed for alcohol concentrations above 4% and 1.5%, respectively. The micelle is affected (i) by interaction with the polar alcohol groups located outside the micelles, (ii) by entry of alcohol into the micelle palisade and (iii) by dissolution of the alcohol in the micelle core. The first two effects might favor the formation of micelles, whereas the latter can substantially increase the amount of alcohol inside the micelle and produce a microemulsion.

Methanol, with the shortest carbon chain, is more polar and soluble than the other alcohols. Because for methanol the size of the chains is closer to that of the polar group, it can solvate the surfactant monomers more easily, increasing their dissolution in water. This hinders interaction of the monomers during formation of micelles and, consequently, the concentration of surfactant must be larger to stabilize the micellar aggregates. This could be one reason of the CMC increase with methanol concentration. Ethanol and propanol, which are miscible with water, remain mainly outside the micelles, dissolved in the bulk liquid, but they

interact with the micelle surface, reducing the repulsions among the ionic heads of the surfactant monomers. This benefits the formation of micelles and reduces the aggregation number and CMC.

In contrast, butanol and pentanol are inserted into the micellar assembly, due to their particular structure that combines a polar group with an apolar chain, similarly to the surfactant molecule. The alcohol molecules align with the surfactant molecules in the micelle palisade, the polar hydroxyl group of the alcohol orientated towards the Stern layer and the alkyl chain located in the apolar micelle core. This gives rise to swollen mixed micelle intermonomer spaces of the micelle structure, which makes entry of one surfactant monomer entities into the micelle more difficult (133).

Although acetonitrile, methanol and tetrahydrofuran have different polarities and structures, their effects on the variation of CMC values are very similar. The dipolar non-protic acetonitrile and polar and protic methanol are small molecules similar in structure, whereas tetrahydrofuran has a low dielectric constant. All three act on the water structure facilitating the solvation of the surfactant monomers. A greater amount of surfactant is required to form the micellar assemblies (134). The structure of tetrahydrofuran, cyclic rather than linear, is different. There will be serious steric problems to overcome when inserting a molecule of tetrahydrofuran into the micelle structure. Owing to the low polarity of this solvent, however, the formation of micelles is favored at low concentrations (0–5% concentration range), as shown by the slight reduction in the CMC values. At larger concentrations, the CMC increased at an increasing rate.

Interestingly, the dependence of the retention factors of several solutes on the concentration of modifier was found similar to that found for the CMC. This implies that the effects that change the CMC values upon addition of alcohol to a surfactant solution are, at least partially, those that induce the retention behavior in MLC (134). Thus, for pentanol, a strong CMC decrease was obtained for alcohol concentrations up to 1.5%, whereas the retention of the solutes decreased substantially for pentanol concentrations up to 1.5–2%. The change in retention upon addition of propanol and butanol was smaller, as occurred with the CMC.

Elution Strength of Surfactant and Organic Solvent

The more hydrophobic the probe solute, the greater is the additive effect on the apparent elution strength of the micellar mobile phase. The global ability of a hybrid micellar mobile phase to elute a solute is often measured without distinguishing between the elution strength of the

modifiers. A simple measurement of the elution strength is the slope (c_1 coefficient) of the classical linear retention model (131):

$$\text{at constant micellar concentration} : \log k = c_0 + c_1\varphi \quad (1)$$

$$\text{or at constant organic modifier content} : \log k = c_0 + c_1[\text{M}] \quad (2)$$

where φ is the volume fraction of organic modifier solvent, and [M] the molar concentration of the surfactant. Depending on the strength of the interactions, the surfactant or the organic modifier solvent has a preponderant effect on elution strength. Thus, for a group of sulfonamides, the elution strength in MLC with SDS/acetonitrile was observed to be determined mainly by the surfactant with c_1 values between 4 and 9 against acetonitrile c_1 values between 1 and 3.5. The elution strength of SDS was often greater than that of acetonitrile in the classical aqueous–organic RPLC mode with c_1 = 1.5–6 (131). For a group of β-blockers eluted with SDS/propanol, the changes in retention were also larger for the surfactant (c_1 = 4–5 against c_1 = 2–4 and 1−3.5 for acetonitrile in MLC and classical RPLC, respectively). It was hypothesized that the strong association of these compounds with the anionic micelles of SDS was responsible for the large changes observed in retention factor (62). In contrast, for a group of apolar steroids, eluted with a strong modifier (pentanol), the elution strength was dominated by the organic solvent (c_1 = 7–14), against c_1 = 2–5 for acetonitrile and methanol in the classical RPLC mode (131).

An algorithm was proposed to evaluate the elution strength of the modifiers in hybrid micellar mobile phases containing a surfactant and an organic solvent, based on a mechanistic retention model that takes into account the competing equilibriums of solutes among aqueous-organic phase, micelle, and stationary phase (135). The results were discussed according to the values of the partition constants of the solutes, and showed the complex behavior of the elution strength in the hybrid mobile phases, which depends on the relative concentration of the different modifiers.

Propanol is chosen often as the organic solvent modifier of micellar mobile phases. However, its elution strength is rather low and apolar compounds will elute at long retention times. The addition of more hydrophobic alcohols, such as butanol and pentanol, is the solution to decrease the retention of strongly retained solutes (105, 136). In many MLC reports, several organic solvents are assayed before selecting the most appropriate. Apparently, it was not easy to decide which modifier was the best and how much to add to obtain the desired separation, although it was evident that this depended on the analyte nature. In a comprehensive study, good correlation was observed between solute

polarity (measured as log $P_{o/w}$) and retention in MLC (131). The most suitable organic solvent in the micellar mobile phase is thus determined mainly by the polarity of the eluted compounds.

The following recommendations were given to assess in the selection of the modifier with SDS surfactant: a low volume fraction of propanol (≈1%) should be used to separate compounds with log $P_{o/w} < -1$, such as amino acids; a greater concentration of this solvent (≈5–7%) is needed for compounds in the range $-1 < \log P_{o/w} < 2$, such as diuretics and sulfonamides, and a high concentration of propanol (≈15%) or lower concentrations of butanol (<10%) are useful for less polar compounds with $1 < \log P_{o/w} < 3$, such as β–blockers. Pentanol (<6%) is more suitable for apolar compounds with log $P_{o/w} > 3$, such as steroids. For positively charged basic drugs such as phenethylamines with $0 < \log P_{o/w} < 1.7$, propanol is somewhat a too weak additive due probably to association of the protonated solutes with SDS adsorbed on the stationary phase (105).

In general, the behavior of charged analytes in a micellar mobile phase of an ionic surfactant changes drastically with respect to a conventional hydro-organic system. The elution of several phenethylamines with an SDS micellar mobile phase is a good example (105). These drugs elute at short retention times with conventional RPLC acetonitrile-water mobile phases, needing a weak eluent (<15% organic solvent). In contrast, in an anionic SDS micellar system they are strongly retained and need the addition of butanol or pentanol to be eluted from the column. This permits, however, a better control of the retention of the drugs, and the resolution of close compounds, such as ephedrine and pseudoephedrine, not separated in the conventional RPLC systems (105).

The pH of the Mobile Phase

Retention of ionizable compounds depends on the solute ionization state, which makes acidity a worthy factor to be taken into account in RPLC separations. However, changes in retention with pH are often associated with changes in selectivity, rendering pH control rather complex. The difficulties are increased since changes in organic solvent concentration affect solute acid–base constants. Often the pH is fixed at a convenient value and this worthy experimental factor is not used. Despite the theoretical and practical complexity of the pH problem, new efforts have been made to model the retention in MLC with hybrid eluents at variable pHs. Good models predicted optimal chromatograms that could be reproduced experimentally with great accuracy (137, 138).

In the recommended MLC procedures for weak acids, such as amino acids and phenols, using SDS as surfactant, the pH is often fixed at 2.5–3,

where the protonated species dominates (see Tables 1–3). In these conditions, the separation space is wider, which favors the resolution. Basic drugs, such as β–blockers and phenethylamines, do not change their retention in the working pH range of a conventional alkyl–bonded column, but a low pH is also selected to enhance the efficiencies (131). Moreover, a decrease in the strength of the acidic solutes (i.e. an increase in the acidity constant, pK_a) is generally observed in micellar solutions of anionic surfactants compared to aqueous solution (139). This fact expands the range where the acidic species is dominant to more basic pH, giving rise to two advantages: the column life is prolonged and as only one species dominates, the results are very reproducible.

It was recently suggested to replace the organic alcohol modifier by aliphatic carboxylic acids of two to six carbon atoms (139). Carboxylic acids play the double function of adjusting the pH and modifying the SDS medium, resulting in a simpler preparation of the mobile phase. The hydrophobicity of aliphatic alcohols and carboxylic acids with same number of carbon atoms is similar. Consequently, the characteristics of partition between bulk aqueous phase and micellar pseudophase and their properties as co-surfactants are also similar. However, the differences of properties of carboxylic and hydroxyl groups lead to different separation selectivity. The strength of aliphatic carboxylic acids as modifiers of SDS micellar mobile phases in the analysis of 2,4-dinitrophenyl derivatives of amino acids was found slightly smaller than that of the corresponding aliphatic alcohols, except for acetic acid and ethanol. This was explained by the slightly lower hydrophobicity of aliphatic acids in comparison with corresponding alcohols. However, in general, the resolution of peaks provided by mobile phases containing the aliphatic carboxylic acids was better. Thus, a new perspective for separation of acidic compounds by micellar eluents was opened, although the new modifiers are not appropriate for compounds which need a good control of pH and for columns which are unstable at pH < 3 (139).

Resolution Performance

Different organic solvents yield not only particular changes in the elution strength and selectivity of micellar mobile phases, but also in the chromatographic efficiency producing diverse resolution capabilities. In a comparative study of the effect of five organic modifiers (propanol, butanol, pentanol, acetonitrile and tetrahydrofuran) on the resolution of a mixture of five probe compounds with SDS micellar mobile phases, acetonitrile was observed to allow the most satisfactory separation in an extensive composition region (15–20% v/v) with very small overlapping and sufficiently low retention times (140). Maximal resolution was rather

low for the alcohol modifiers, with however much lower content (3–5% v/v). This report shows not only the differences in resolution with different modifiers, but also the good performance of acetonitrile. Acetonitrile improved selectivity, but also the efficiencies and symmetries of the chromatographic peaks. In other reports, the separation of steroids of low polarity (136) and sulfonamides (117) were also benefited from the greater resolution capability of acetonitrile added to an SDS micellar mobile phase at moderate (10–20% v/v) amounts.

Acetonitrile has the drawback of its moderate elution strength. Adding more than 20% in a micellar solution would greatly decrease its interest compared to classical RPLC mobile phases. The elution strength of 20% acetonitrile micellar mobile phases is not enough to elute highly apolar compounds in acceptable analysis times. However, this solvent can be used in lower amount and combined with a stronger solvent for these analyses (136). The challenge of the wide range of log $P_{o/w}$ (3–8) of steroidal hormones was solved using two SDS mobile phases sequentially. The first one contained acetonitrile and separated the steroids of lower polarity: dehydrotestosterone, dydrogesterone, medroxyprogesterone, medroxyprogesterone acetate, methandienone, methyltestosterone and testosterone. The second SDS mobile phase contained pentanol and was able to elute the apolar steroids of the sample: dehydrotestosterone, dydrogesterone, medroxyprogesterone acetate, methandienone, methyltestosterone, progesterone, testosterone enanthate and testosterone propionate (136).

Often MLC is a complementary technique to classical RPLC for the screening of compounds due to its peculiar behavior with regard to the selectivity and elution strength. Based on the chromatographic data of several reports, the screening capability of MLC was discussed for several sets of compounds (amino acids, β-blockers, diuretics, phenethylamines, phenols, PAHs, steroids and sulfonamides) (131). In all cases, compounds having a wide range of polarities were resolved isocratically by MLC in relatively short analysis times and using a low volume fraction of organic solvent. In several examples, with conventional C_{18} columns, micellar-organic and aqueous–organic mobile phases were compared; the resolution was similar or improved in the MLC mode. Figure 5 shows how the resolution attained for a set of 14 β-blockers was remarkably improved with respect to that found with aqueous–organic mobile phases (Figures 5a and b), even when special columns designed for basic compounds were used (Figure 5c) (141).

The monomers of surfactant modify the stationary phase, changing not only the peak shape, but also the retention capability of the column, an effect more pronounced for apolar compounds. The resulting effect can be compared to RPLC with organic solvent gradients, with a big saving in organic solvent volume. In several MLC reports, where a

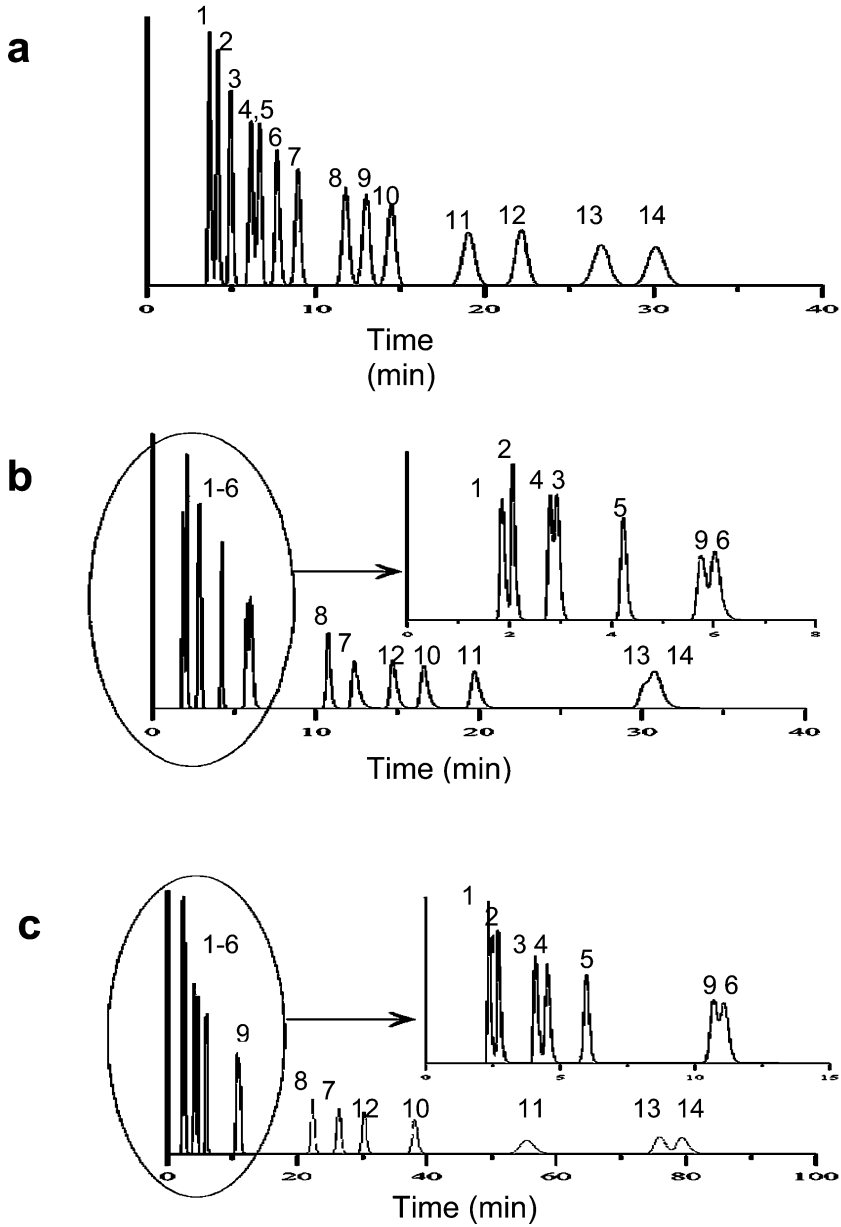

Figure 5. Chromatograms for a mixture of 14 β-blockers eluted with optimized mobile phases and different C_{18} columns (minimal resolution is given in parenthesis): (a) column Spherisorb and micellar mobile phase 0.10M SDS + 15% propanol ($Rs_{4,5}$ = 0.9); (b) column Spherisorb and hydro-organic RPLC mobile phase 21% acetonitrile + 0.1% triethylamine ($Rs_{13,14}$ = 0.2); (c) column

separation achieved in hydro-organic RPLC isocratic mode is compared with what is obtained with a micellar mobile phase, the compounds appear more evenly distributed in the MLC chromatogram. In hydro-organic RPLC, the total run time may be shortened using a gradient of organic solvent. In that case the total run time of the micellar mode may be higher than the gradient analysis time. When drugs in physiological fluids are analyzed, even after a sample cleanup, a protein band is obtained in the first minutes of the chromatograms. In this situation, MLC will be superior to gradient hydro-organic RPLC, being able to separate the drugs from the protein band (38).

MLC in the Framework of the Solvation Parameter Modeling

The linear solvation parameter approach based on the five descriptors proposed by Abraham:

$$\log k = c + eE + sS + aA + bB + vV \tag{3}$$

has been shown to be an interesting tool to study the particular behavior of micellar chromatographic systems (142–144). The solute descriptors in Eq. (3) are the excess molar refraction E modeling the solute polarizability, S for the solute dipolarity/polarizability, A and B the respective effective hydrogen-bond acidity and basicity, and V, the solute molecular McGowan volume calculated using the solute structure. These descriptors can be found in published tables. The six a-v system descriptors depend on the stationary phase and the mobile phase used to obtain the k retention factors. The regressed parameters refer to solute interaction differences between the selected stationary and mobile phase.

LSER approaches are often applied to individual mobile phase compositions. Recently, a solution was suggested to get a general model useful to predict retention at several mobile phase compositions, which include flag-variables to label each mobile phase (142). An interesting observation when this approach was applied to hydro-organic RPLC was that correlation plots of predicted and experimental log k values showed a curvature instead of the expected diagonal pattern for accurate predictions. Surprisingly, the curved pattern was absent in the micellar mode correlation plots, which was systematically confirmed with several sets of compounds (143, 144). This can be explained considering to the

XTerra MS® and hydro-organic RPLC mobile phase 15% acetonitrile ($Rs_{9,6}$ = 0.5). Peak identification: 1-atenolol, 2-sotalol, 3-carteolol, 4-nadolol, 5-pindolol, 6-acebutolol, 7-celiprolol, 8-esmolol, 9-timolol, 10-bisoprolol, 11-labetalol, 12-oxprenolol, 13-propranolol, and 14-alprenolol (adapted from Ref. 141).

different elution behaviors in conventional RPLC and MLC. As commented, when comparing the micellar mode with the isocratic conventional RPLC mode using the same sample and the same column, MLC retentions are more evenly distributed, with longer retention times for the least retained solutes (and often shorter times for the most retained ones) than hydro-organic RPLC retentions.

The study also revealed that, in the hydro-organic mode, the solvation parameters in Eq. (3) are smaller at higher solvent contents, indicating that the difference in solute interactions between the stationary and the mobile phases decrease. It means that the stationary and mobile phases become gradually similar (144). In MLC, a similar trend is observed with increasing surfactant concentration, but not with increasing concentration of organic modifier. It is speculated that increasing amounts of organic modifier reduce the thickness and/or amount of the surfactant layer adsorbed on the stationary phase. This surfactant depletion increases the structural differences between the stationary phase and micellar mobile phase. Also, in MLC the ranges of variation for the solvation parameters are appreciably narrower. The most outstanding was the range of variation observed for the hydrophobicity coefficient v, which for sets of phenols and β-blockers took values in the narrow range 1.1–1.4 in the micellar mode, whereas the range was 0.2–2 for phenols and 0.9–2.9 for β-blockers in the hydro-organic mode. This indicated that the hydrophobic interactions are scarcely affected by changes in the composition of micellar eluents, and explains the fact that these media are able to elute solutes of different hydrophobicity in retention time windows narrower than in conventional isocratic RPLC.

On the other hand, acidic and basic interactions (b and a terms in Eq. (3)) were observed to be affected by the charge of both the surfactant and the solute being eluted, but again the variability was significantly smaller than in hydro-organic RPLC (144). The micellar environment for both surfactants (SDS and CTAB) was similar in acidity to hydro-organic phases containing 30–40% acetonitrile. In the hydro-organic and micellar modes, respectively: $b \approx -0.3$ to -1.7 and $b \approx -1.4$ to -2.1 for phenols, and $b \approx -0.5$ to -1.8 and $b \approx -0.8$ to -0.9 for β-blockers.

The prediction capability with the traditional Abraham approach (Eq. (3)) was, however, not satisfactory in the micellar mode, showing larger error than in the hydro-organic mode. The Abraham model was not derived for charged entities, neither solutes nor phases. A sixth solute descriptor was thus proposed and added to the model to account for the ionic and steric interactions that take place inside a surfactant-modified column (144). Of course adding a parameter improved the model and the predictions became satisfactory. The new descriptors for different columns in the conventional RPLC mode were appreciably correlated with a slope close to unity. However, in MLC, significant differences in

behavior of the surfactant–modified column were evidenced using the extra parameter (144).

Use of a Second Organic Solvent

The potential use of a second organic solvent to the hybrid micellar eluent has also been considered. The selection of the most appropriate organic solvent/s in the analysis of a complex mixture of natural and synthetic anabolic steroids by MLC with SDS was carried out based on a partial version of the ''Glajch triangle.'' For this purpose, a pentagonal experimental design was applied using propanol, butanol, pentanol, acetonitrile and tetrahydrofuran as organic modifiers, allowing the inspection of mobile phases ranging from binary to quaternary (145). The best separation was obtained, however, with simple surfactant/organic modifier mobile phases.

Further, the addition of propanol as a second organic additive was checked to improve the MLC separation of PAHs with micellar mobile phases of CTAC, SDS or DTAC using pentanol as the main additive (125). Propanol was needed to decrease the retention without increasing the mobile phase viscosity. The beneficial effect of propanol on the separation was explained by the decrease in polarity of the aqueous phase, which increased the solubility of surfactant monomers and pentanol. This led to a more dynamic system with more pentanol available to displace the adsorbed surfactant molecules and modify the stationary phase. Also, the attraction of apolar solutes towards the less polar mobile phase was increased. Methanol and acetonitrile also improved the separation, but were less effective than propanol. The extent of the enhancement varied for the different surfactants, with the greatest improvements in resolution for CTAC (125).

CHANGING THE STATIONARY PHASE

Most analytical procedures in the MLC literature involve conventional C_{18} columns, and in a much lesser extent, octyl (C_8) columns. In some specific applications or studies, cyano-bonded columns are used. As commented, one of the limitations that have restricted the applicability of MLC is the weak eluting power of micellar mobile phases. The major difficulty with micellar mobile phases is the inability to effectively elute highly retained hydrophobic solutes. The use of shorter columns and shorter chain length stationary phases has been suggested to overcome this problem (146). However, although retention is effectively reduced, resolution often also decreases. Other

solutions, which have been suggested more recently is the use of alkyl-bonded stationary phases with large pore size (147, 148), and monolithic columns (149, 150).

Large-Pore Stationary Phases

The apparent lack of strength of micellar mobile phases has been suggested to be due to the exclusion of the micelles from the pores, within which nearly all (≥99%) of the stationary phase resides and the analytes spend most of their time. Since the excluded micelles do not have direct access to the analytes except when these have diffused out of the pores, even high concentrations of micelles are not sufficient to elute moderately to highly hydrophobic compounds (147). In the case of non-ionic surfactants that form large micelles, steric effects are most likely the cause of micellar exclusion from small pore materials. With ionic surfactants that form smaller but charged micelles, both electrostatic and steric effects are probably responsible for micellar exclusion. Monomers of common surfactants such as SDS and CTAB are known to adsorb quite strongly onto C_{18} stationary phases. The resulting charge buildup on the stationary phase surface within the pores gives rise to a Donnan-like potential that tends to repel like species from the pore. Large structures such as micelles, whose dimensions (typically 30–60 Å) are commensurate with pore diameters of typical (small pore) C_{18} columns, are repelled by such charge-charge interactions.

In order to determine whether large–pore stationary phases overcome this limitation in MLC, several C_8 and C_{18} stationary phases ranging from 100 to 4000 Å were investigated using test solutes of diverse nature and micellar solutions of non-ionic (Brij-22), anionic (SDS) and cationic (dodecyltrimethylammonium bromide, DTAB) surfactants as mobile phases (147). As the pore size of a porous material of a given particle diameter is increased, the specific surface area is reduced. As a consequence, the volume of the bonded stationary phase is also decreased, and thus the stationary phase-to-mobile phase ratio. Therefore, under equal mobile phase conditions, the retention of a solute in a large-pore column will necessarily be smaller than on an otherwise identical small-pore column. The authors compared the behavior of solutes of diverse nature eluted from columns of different pore sizes with the micellar solutions, compared to hydro-organic mixtures, and found that the large-pore columns allow better penetration of the micelles into the pores such that they can reach the solute at the internal surface of the stationary phase better, and elute the solutes in less time taking into account the unavoidable drop in retention factor with increasing pore size (147).

Under MLC conditions, 11 alkylphenones (acetophenone to dodecanophenone) were eluted with an SDS gradient and the 1000 Å column, whereas only the first four alkylphenones eluted from the 100 Å column. The retention time for acetophenone decreased by ≈80% going from the 100 to 1000 Å column, which exceeds what would be expected based on the decrease in the stationary phase surface alone (147). Thus, it appears that the wide pore stationary phases may be useful in reducing one of the important limitations in MLC.

It has been already commented that the usefulness of MLC in bioanalysis is related to the capability of injecting proteinaceous biofluids directly onto the chromatographic column. Typically, direct injection is conducted using conventional small-pore stationary phases, in which the plasma proteins are excluded from the stationary phase pores and eluted in the void volume. This has been thought to be important since plasma contains very high concentrations of proteins (≈60–80 mg/mL) relative to typical drug concentrations of $\leqslant 100\,\mu g/mL$. Having this in mind, the evaluation of large-pore (1000 Å) relative to conventional small-pore (100 Å) C_{18} stationary phases for the direct sample injection of drugs in plasma was found interesting (148). With large-pore stationary phases, plasma proteins may enter the pores and thus be retained and possibly interfere with the analytes. The analysis of plasma samples containing several drugs indicated, however, that the advantage of direct introduction of the sample was maintained with large–pore stationary phases (148).

On the other hand, generally, it is believed that 15–20 column volumes of mobile phase are required to re-equilibrate a column in hydro-organic gradient RPLC. Often, this re-equilibration time is as long as the sample elution time. Rapid re-equilibration in gradient MLC is believed to be produced by the restriction of micelles to the interparticle space. However, in large-pore columns the micelles penetrate into the stationary phase pores, and thus a slower column re-equilibration time might be expected. It was, however, checked that rapid column re-equilibration after gradient elution in MLC was maintained with large-pore stationary phases (148).

Monolithic Columns

Methods for hydro-organic RPLC on silica particle columns are easily transferred to silica monolithic columns. These columns are highly porous allowing for high mobile phase flow-rates associated with low column back-pressure. The working flow rates can be high giving fast analysis times without noticeable efficiency problems. Not surprisingly the performances of monolithic silica columns were tested in MLC (149,

150). The authors were interested in monoliths to speed up QSRR to estimate the membrane permeability of drugs. For this purpose, the elution behavior of diverse basic pharmaceutical drugs was determined on a classical particle-based column and compared with a monolithic column, both with hydro-organic and micellar mobile phases (149).

Micellar mobile phases and a monolithic column posed indeed no practical problems. Moreover, the transfer from particle-based columns to monolithic columns should be better than with hydro-organic RPLC, since with MLC the surfactant monomers cover the stationary phase surface screening possible differences in selectivity. However, since a pressure limit of 200 kg/cm^2 is recommended for this type of column, flow rates below or equal to 7 mL/min were advisable with the MLC method, whereas 9 mL/min can be reached in hydro-organic RPLC. The study comprised 25 drugs in a wide range of polarities (log $P_{o/w}$ = 0.03 to 6.45). For the isocratic hydro-organic RPLC method, the retention time window was very large, while the peaks of the least retained solutes were not completely separated from the solvent peak. Utilizing flow rates of 9 mL/min with this method did shorten the analysis times, but differentiation from the solvent peak of the least retained solutes and among them became almost impossible, resulting in a loss of information. With MLC, these problems did not occur: all 25 drugs could be clearly distinguished and were retained even at high flow–rate. Moreover, compared to isocratic hydro-organic RPLC at 1 mL/min, much shorter retention times for the most retained substances and, in general, longer retention times for the least retained substances were observed. Thus, for MLC, a smaller retention window was found with a remarkably lower analysis time both on a particle-based and monolithic columns. The results indicated that combining monolithic columns with micellar media leads to faster QSRR studies and possibly even better permeability predictions (150).

CHROMATOGRAPHIC KINETICS IN MLC: THE EFFICIENCY PROBLEM

Understanding the Reduced Efficiency in MLC

The reduced efficiency in MLC worsens as the solute hydrophobicity increases. This problem has been attributed to several factors, including: (i) poor wetting of the hydrophobic stationary phase by the aqueous mobile phase (4), (ii) slow mass transfer between micelles, bulk aqueous phase and stationary phase (151), and (iii) modification of the stationary phase due to surfactant adsorption in amounts approximating that of the bonded hydrocarbon, which further reduces mass transfer within the

stationary phase (24, 152). Routinely, peak shape is improved by the addition of a short-chain alcohol, which desorbs some surfactant out of the stationary phase and reduces the viscosity of the surfactant-C_{18} structure (16). In parallel, the organic solvent changes the structure of the micelles and affects the retention, which complicates the interpretation of plate count data (152).

The interest in understanding and solving the problem of the reduced efficiency in MLC has not decayed. A mathematical model has been developed to determine the plate count as a function of the physical and chemical parameters governing the kinetics and transport in MLC separations (153). The equation incorporates solute mass–transfer between mobile and stationary phases, and kinetic limitations within the stationary phase, and assumes that only free surfactant is found in the pores of the alkyl-bonded phase. Model predictions were compared with experimental observations for a series of vanillin compounds injected onto a C_{18} column with mobile phases containing SDS. The proposed model provided a reasonable plate count prediction for the selected solute set. The set size was, however, too small to assess a general validity of the proposed method (153).

In hybrid MLC, the relationship between the peak shape parameters (efficiency and asymmetry) and the retention time depends on both organic solvent and surfactant. In a comparative study with hydro-organic RPLC, peak modeling was used to understand the factors that affect peak shape in MLC (154). The study revealed that the problem of achieving smaller efficiencies in MLC, compared to conventional RPLC, is not only related to the presence of surfactant covering the stationary phase, but also to the smaller concentration of organic solvent in the mobile phase. An extrapolation of the chromatographic behavior obtained for a group of diuretics eluted with SDS micellar mobile phases containing acetonitrile suggested that concentrations of acetonitrile above 20% (v/v) would produce efficiencies approaching those in the hydro-organic mode. However, such high concentrations would destroy the micelles (154).

Large-pore stationary phases were also investigated in MLC in relation to the problem of poor efficiency (155). Previous studies had suggested that hydrophobic compounds have a too low water solubility to participate significantly in the aqueous exchange of the three-way partitioning scheme (152). That is, they spend most of their time in the stationary phase or bound to the micelle. Consequently, with conventional small-pore size bonded phase silica in which the micelles are excluded from the pores, intraparticle mass transfer of solutes presumably should occur via diffusion of solutes in their free states, which will be relatively rapid. In contrast, when large-pore phases are employed, the micelles are able to penetrate the pores and interact with

solutes, reducing their effective diffusion coefficient and thus slowing the solutes' intraparticle mass transfer. Stationary phases of different nature were evaluated to determine the effect of pore size and chain length on efficiency in MLC. Improvements in the intraparticle mass transfer strictly due to pore size were, however, not observed (148, 155). The significant improvement in efficiency for different apolar stationary phases of large pore, such as C_4 and fluorooctyl columns, was again explained by the reduced amount of adsorbed surfactant. Also, alcohols should be used to enhance efficiency even in the case of wide-pore columns (155).

Is the Efficiency in MLC Really Lower?

It is known that the addition of suitable organic solvents to micellar mobile phases not only reduces retention times but also improves efficiencies. Peak asymmetries also decrease. If few tenth % v/v additions are accepted, acetonitrile is as good or even better an additive than short chain alcohols (117, 136). For polar compounds, the improvements in efficiency and symmetry can yield MLC peak shapes similar to those obtained in conventional RPLC (136). In this context, comments such as "In spite of the advantages of MLC and the fervor of its proponents, this separation technique has not seen widespread application because it tends to be less efficient than conventional RPLC" (153), "Despite the advantages, MLC has not found widespread use due to poor column efficiency" (147) or "The problem of reduced efficiency in MLC still remains, despite extensive study" (155) that question the validity of MLC, should be qualified as unjustified.

Several studies have been carried out to compare the performance of MLC and hydro-organic RPLC, using the same C_{18} column and solute set in both chromatographic modes (113, 131, 138, 141). For example, for a group of sulfonamides, the efficiencies and symmetries were observed to improve almost linearly when acetonitrile was added in the 0–6% v/v range to a mobile phase containing SDS micelles (131). The efficiency enhancement was associated to significant shorter retention times so possible extra-column effects cannot be eliminated. However, as expected, an increase in SDS concentration also diminished the retention times, but it conversely diminished efficiencies and deteriorated the peak symmetry. In that particular case, with isocratic RPLC acetonitrile-water mixtures, the behavior was that the efficiencies and the retention times decreased at increasing percentage of acetonitrile, confirming some extra-column effects. The peak asymmetries remained invariable or increased with more modifier (131). In another study, the efficiencies of diuretics eluted with micellar mobile phases of SDS-acetonitrile were found to be

smaller with respect to those in isocratic classical RPLC at similar retention times. However, the wider MLC peaks were more symmetrical than the corresponding thinner classical RPLC peaks (138).

Elution strength (thermodynamics) and efficiency (kinetics) are necessarily correlated in MLC (132). When different organic additives are considered, there is an initial steep increase in efficiency with increasing the additive log $P_{o/w}$ value. A plateau is soon reached. Since a more hydrophobic additive is needed to attain the maximum efficiency when working with very hydrophobic solutes, there is no single additive that will be the best in MLC. The optimal choice is dictated by the relative hydrophobicity of the test solutes. The study also showed that the addition of dipolar aprotic modifiers (e.g., acetonitrile and dimethyl sulfoxide) and weakly protic formamide resulted in plate counts greater than those predicted from their respective log $P_{o/w}$ values based upon the alkanol and alkanediol data (132). This observation was also made in a report on the separation of highly apolar compounds, as steroids, for which 16% v/v acetonitrile allowed higher efficiencies (N = 2000–4000) than 6% v/v pentanol (N = 500–2000). The elution strength of the pentanol containing micellar mobile phases were however clearly superior (136). A recent work has shown the advantage of using acetonitrile as an additive in MLC (144). The improved efficiencies result in better resolution for complex mixtures. Although evaluated in the early days of MLC, acetonitrile was not selected as a useful candidate to enhance efficiency because, at similar concentration, it was not improving the elution strength as well as short chain alcohols.

The Case of Basic Drugs

A particular case where MLC could have a great interest is the difficult analysis of basic compounds. Many drugs of interest contain basic amine groups. When these compounds are analyzed by classical RPLC, using C_{18} or C_8–modified silica stationary phases, several problems arise. Peak tailing is observed inducing low efficiencies and limited detection. Protonated basic compounds interact with the RPLC support through several mechanisms: beside the desired hydrophobic partitioning, ion exchange on silanol groups, salting–out and ion–pair formation occur depending on the nature of the basic solute, stationary phase and mobile phase (pH, temperature, and ionic strength) (156). Peak asymmetry is caused mostly by ionic interaction of the positively charged species with free silanols. Ion exchange can be avoided by reducing the pH of the mobile phase to suppress silanol ionization. A variety of base-deactivated packings from several manufacturers have also become widespread. However, when conventional RPLC packings are used, the interaction of

basic solutes with silanols is reduced by the addition of amine modifiers to the mobile phase. Triethylamine (TEA) is the most popular silanol screening agent (157).

Bare silica modified with long-chain quaternary ammonium ions added to the mobile phase (such as the cationic surfactant CTAB) has also been applied successfully to the analysis of basic drugs (158). At equilibrium, three phases are present in the chromatographic system: (i) a layer of electrostatically associated quaternary ammonium ions with their hydrophobic chains acting as stationary phase, (ii) the micelles, and (iii) the bulk solvent in which surfactant monomers are dissolved (Figure 3c). This system was able to separate a mixture of tricyclic antidepressants when no expensive commercial C_{18} deactivated column was able to give approaching results (158).

More recently, MLC with conventional C_{18} columns and hybrid SDS micellar mobile phases with propanol, butanol or pentanol have been reported to yield good performance in the analysis of basic drugs, such as β-blockers (141, 157), phenethylamines (105), tetracyclines (130), and tricyclic antidepressants (113). The silanol groups seem to play a less important role in the separation because they are screened by SDS adsorption. As shown by Figure 3a, the hydrophobic chain of SDS is inserted in the bonded organic layer with the sulfate group protruding outside. This adds negative charges to the silica surface. The positive basic drugs can interact by an ion-exchange mechanism with this layer (fast process) without penetrating too much the bonded alkyl layer to interact with the buried silanols (slow process) (157). The net effect is an experimentally improved efficiency.

Note that a cationic surfactant, such as CTAB, would not be effective in the analysis of basic drugs. Although ammonium groups of CTAB are buried inside the C_{18} layer, the stationary phase is positively charged and repels protonated basic drugs (Figure 3b). The kinetics is fast but the retention times are much reduced with compounds eluting at the void volume.

The benefits of covering the alkyl–bonded stationary phase with SDS are especially remarkable for β-blockers. MLC can really compete with hydro-organic isocratic RPLC in the separation of basic β-blockers. A comparative study of peak shape, elution behavior and resolution for β-blocker drugs separated by MLC and hydro-organic RPLC with classical C_{18} RPLC columns and a column especially designed for the separation of basic drugs (X-Terra MS® C_{18}) was proposed (141). Acetonitrile was the organic modifier selected for all classical RPLC mobile phases. Propanol was added to the SDS micellar mobile phases. MLC produced better β-blocker separations than conventional RPLC with isocratic elution even when an X–Terra MS® C_{18} special basic compound column was used (141, 157). Moreover, the chromatographic

peaks in the micellar system were almost symmetrical, whereas in conventional RPLC, even with addition of TEA, the peak asymmetries exceeded $B/A > 2$ (B and A being the left- and right-half widths of the chromatographic peak). It should be indicated here that the addition of TEA to micellar mobile phases also gives rise to further efficiency enhancements (62). Finally, with the X-Terra MS® column and acetonitrile–water mobile phases, high efficiencies were achieved for some β-blockers, with N = 900–7800, but the peaks were still slightly asymmetrical (B/A = 1.2–1.5). The combination of improved peak shapes, better selectivity, and smaller range in retention among compounds of extreme polarity leads to the logical observation that a greater number of solutes can be resolved in MLC in one run using isocratic elution (Figure 5). No study was done with gradient elution.

A large efficiency enhancement has also been observed for other basic compounds, such as phenethylamines (105). Using butanol or pentanol hybrid SDS mobile phases efficiencies between 3000 and 7000 plates were obtained. Conventional acetonitrile–water mobile phases and a classical C_{18} column could not give efficiencies higher than 4500 plates with these basic compounds. The significant phenethylamine peak tailings observed in the hydro-organic mode almost disappeared in MLC (105).

CONCLUSION

Ten years after our reference book in MLC (41) we observed that the interest for the technique remains steady. About 1% of the 3000–4000 reports dealing each year with liquid chromatography are MLC works which make an average stable number of about 30 articles per year. Half of this production comes from the European community and especially from Spain. Applications make the majority of the published works. They are concentrated in the separation of pharmaceutical compounds either to control the formulations or to follow the drug and/or its metabolites in the biological fluids during the medical treatment. In the latter case, the direct injection capability of MLC is especially appreciated. A very recent and original report using MLC presented the modeling of transport of environmental pollutants by groundwaters containing hydrogenated and perfluorinated surfactant (159).

The vast majority of reports uses hybrid micellar mobile phases in which an organic modifier is added to the surfactant. These additions enhance both the thermodynamics and the kinetics of the MLC process. The elution strength of the micellar mobile phase is greatly enhanced allowing for separation of hydrophobic compounds in a reasonable amount of time. Jointly, the peak efficiency is improved to values that can no longer be regarded as miserable when basic compounds are

separated. Acetonitrile was demonstrated to be an excellent organic modifier for micellar mobile phases. The only problem is that amounts in low tens of percent are needed to observe an acceptable enhancement of the elution strength. Short chain alcohols produce significant elution strength augmentation at much lower concentration. The efficiency (and resolution) enhancement is however inferior. Wide pore stationary phases were found to have interesting properties when associated with micellar mobile phases. To conclude this review, we think that it is now established that MLC can be a useful alternative in some particular occasions. There is no solution to a major drawback: MLC cannot be associated with an MS detector. However, we want to remind that a try of MLC would not need costly investment, just prepare the micellar mobile phase and let it run!

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