Causes and Remediation of Reduced Efficiency in Micellar Liquid Chromatography

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Review

Causes and remediation of reduced efficiency in micellar liquid chromatography

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Abstract

Broad peaks are obtained when purely aqueous micellar phases are used in micellar liquid chromatography (MLC). The causes of reduced efficiency in MLC are investigated. Slow solute mass-transfer kinetics between micelles, the aqueous phase and the surfactant covered stationary phase are the origins of the efficiency loss. Knox plots show that the reduced efficiency comes from A term increase and, for lipophilic solutes, A and C terms increases. Surfactant adsorption reduces the pore volume and surface area of the stationary phase changing the flow anisotropy (A term). The surfactant adsorbed layer slows down the mass transfer (C term). Three ways for efficiency loss remediation are known: flow-rate reduction, temperature increase and alcohol addition. Alcohols are known to change the micelle structure and to increase the kinetics of micelle formation–destruction. It is shown that the ratio of the alcohol chain length to surfactant alkyl chain length, $C_{n,OH}/C_{n,surf}$, should be equal or higher than 1/3 to produce the best efficiency enhancements in MLC. Also, the volume of alcohol to be added is not absolute but relative to the surfactant concentration. The alcohol to surfactant concentration ratio should be kept constant. Temperature increases and especially alcohol additions reduce the retention factors. Thermodynamic and kinetics of the micellar exchanges in MLC cannot be dissociated. © 1997 Elsevier Science B.V.

Keywords: Micellar liquid chromatography; Reviews; Efficiency; Thermodynamic parameters

Contents

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1. Introduction

Micellar liquid chromatography (MLC) uses micellar phases as the mobile phase with classical stationary phases. Micellar phases are aqueous solutions containing micelles. Micelles are associations of surfactant molecules that are formed due to a delicate equilibrium between the attractive hydrophobic forces and repulsive electrostatic forces. In the case of non-ionic surfactants, the micelles form due to a balance between the attractive hydrophobic forces of the hydrocarbon surfactant tails and the hydrophobic forces of the polar part of the surfactant molecules.

The interest of MLC is extensively developed in other articles of this issue [1]. The particular interest for the practising chromatographer is that MLC uses exactly the same hardware, pumps, connecting tubing, detectors, injectors and columns as the classical HPLC technique. However, it was noted early that broad peaks, i.e., a poor efficiency, were obtained when micellar mobile phases were used instead of classical hydro–organic mobile phases [2,3]. Dorsey attributed the reduced micellar efficiency to a poor wetting of the apolar stationary phase by the aqueous surfactant phase [3]. He proposed the addition of 3% 1-propanol to remedy the problem [3,4]. Yarmchuck et al. thought that a slow mass transfer between the micelles and the stationary phase was responsible for reduced efficiency [5]. Armstrong [6], Berthod [7] and Hinze [8] concluded that poor mass transfer within the stationary phase itself was mainly responsible for the observed low efficiency. Two main approaches were proposed to enhance the efficiency in MLC: (1) addition of low amounts of *n*-propanol [3] and (2) increases of temperature [4,5].

This work tries to describe the causes of the reduced efficiency in MLC. The chromatographic process is rapidly exposed and the differences between a classical hydro–organic reversed mobile phase and a micellar phases are pointed out. The chromatographic process can be modelled using the Knox equation that relates the reduced plate height to the reduced mobile phase velocity. Knox plots with classical hydro–organic mobile phases and micellar mobile phases are compared and discussed. Surfactant adsorption is the main difference between classical and micellar phases. It is probably responsible for part of the efficiency loss. The effect of the addition of various organic solvents on the micelle physicochemical structure and surfactant adsorption is exposed. The efficiency enhancements obtained with temperature changes and/or organic modifier additions are described.

2. Chromatographic process and efficiency

2.1. Thermodynamics

The retention volume, V_R, of a solute is related to the solute affinity constant for the stationary phase, K, by

$$V_R = V_0 + KV_S \quad (1)$$

in which V_0 and V_S refer to the mobile and stationary phase volumes inside the column. The retention factor, k', is expressed by

$$k' = (V_R - V_0)/V_0 = K\varphi \quad (2)$$

with $\varphi(=V_S/V_0)$, the phase volume ratio.

The peak retention volume and factor are due to thermodynamic phenomena which are temperature dependent. The classical Gibbs free energy equation can be applied:

$$\Delta G^0 = -RT\ln K = \Delta H^0 - T\Delta S^0 \quad (3)$$

Combining Eqs. (2) and (3) yields:

$$\ln k' = -\Delta H^0/RT + \Delta S^0/R + \ln\varphi \quad (4)$$

The plots of ln k' versus $1/T$, the Van't Hoff plots, will be linear if ΔH^0 and ΔS^0 are constant over the temperature range studied. Variations of these parameters would indicate a change of the chromatographic mechanism with temperature.

2.2. Kinetics

The kinetics of the chromatographic process is linked to efficiency. The number of theoretical plates, N, that measures column efficiencies, is best estimated using the Foley–Dorsey equation [9]:

$$N = \frac{41.7(t_R/W_{0.1H})^2}{B'/A' + 1.25} \quad (5)$$

in which $W_{0.1\,H}$ is the peak width at 10% peak height, B'/A' is the peak asymmetry factor measured by the ratio of the back (B') to front (A') half portion of the $W_{0.1\,H}$ peak width referring to its t_R retention time. This equation takes in account the peak asymmetry. In MLC, the efficiency decreases are always associated with peak tailing, i.e., asymmetry. All plate counts given were obtained using the Foley–Dorsey equation, then, the asymmetry B'/A' factor will not be listed. If efficiency increases, the B'/A' factor becomes closer to unity.

According to Snyder and Kirkland [10], the contributions to band broadening in a column can be represented by

$$H = C_e d_p + \frac{C_m d_p^2 u}{D_m} + \frac{C_d D_m}{u} + \frac{C_{sm} d_p^2 u}{D_m} + \frac{C_s \phi_f^2 u}{D_s} \quad (6)$$

in which the C values are constant plate height coefficients related to eddy diffusion (e), mobile phase mass transfer (m), longitudinal diffusion (d), stagnant mobile phase mass transfer (sm) and stationary phase mass transfer (s), ϕ_f is the thickness of the stationary phase layer, D_m the solute diffusion coefficient in the mobile phase, D_s the solute diffusion coefficient in the stationary phase layer and u is the mobile phase velocity.

The Knox equation [11] is used in LC to determine the contributions to the final solute band width. It can be expressed as

$$h = A\nu^{1/3} + \frac{B}{\nu} + C\nu \quad (7)$$

where A, B and C are the constants of the Knox equation, h is the reduced plate height calculated as $h = H/d_p$, where H is the column plate height ($H = L/N$, L being the column length and N the number of theoretical plates), d_p is the stationary phase particle diameter, ν is the reduced mobile phase velocity, i.e., $\nu = ud_p/D_m$, with the mobile phase velocity, u, in cm s^{-1} and D_m in $cm^2\ s^{-1}$.

The A, B and C terms are related to flow anisotropy, molecular longitudinal diffusion and mass transfer processes, respectively. The theoretical support for the Knox equation was derived by Horvath [12]. The A term cannot be expressed simply. The theoretical treatment links A to structural parameters of the column packing, porosity, pore volume, pore diameter and tortuosity [12]. A is related to the flow pattern and the general band spreading due to 'eddy' diffusion [13]. The B term is linked to longitudinal molecular diffusion. It was written as [13]:

$$B = 2[\gamma_m + \gamma_s(D_s/D_m)k'] \quad (8)$$

where γ_m and γ_s are obstruction factors for diffusion through granular and/or porous material. The C term of the Knox equation represents the mass transfer contribution to solute band broadening. It was written as [13]

$$C = q\left(\frac{k' + \psi}{1 + k'}\right)^2\left(\frac{D_m}{\gamma_{sm}\psi D_m + k'\gamma_s D_s}\right) \quad (9)$$

where the γ terms are obstruction factors with the subscripts defined as in Eq. (6), ψ is the stagnant mobile phase fraction and q is a geometrical factor depending on porosity [12–14].

Band broadening and/or unusual retention are also known to occur when specific interactions take place between particular solutes and the stationary phase. The most common cause of such band broadening is stationary phase overload. It can be also exclusion phenomena due to the small size of the stationary phase pores and the large size of the solute. Acid–base interactions between the surface silanols and

basic compounds, specially the amine compounds, dramatically broaden the corresponding peaks. Peak deformations either frontings or tailings are observed.

2.3. External band broadening

The plate number, i.e., the chromatographic efficiency, is a parameter difficult to estimate correctly [15]. The use of methods assuming Gaussian peaks may produce grossly overestimated peak efficiency. The asymmetry based method [9] (Eq. (5)) was designated as the manual method giving plate numbers closest to the exact plate numbers obtained with the moment method [15,16]. The second central reduced moment of a peak corresponds to its variance [17]. It is known, but too often overlooked, that the overall peak variance, σ^2, is a combination of the column variance plus all the other extra-column variances

$$\sigma^2 = \sigma^2_{col} + \sigma^2_{ct} + \sigma^2_{inj} + \sigma^2_{det} + \sigma^2_{other} \qquad (10)$$

in which the subscripts refer to the column, connecting tubing, injector, detector and other variance contributions (electronic time constants, frits, unions). It is possible and desirable to reduce all extra-column variances. However, external band broadenings cannot be fully eliminated. The measured variance is always the sum of the actual column variance, σ^2_{col}, plus a constant term. If the column efficiency is constant for a variety of solutes, the square root of the column variance, σ_{col}, increases linearly with time or elution volume. The other variance contributions are rather constant with time. As the retention time increases, the external band broadening contribution to the global variance becomes less significant. The measured efficiencies of highly retained peaks appear higher than the efficiencies of low k' peaks. Fig. 1 (bottom) shows that a plateau of constant efficiency, the true column efficiency, is rapidly reached. Most methods for improving the efficiency reduce the retention factor at the same time that they decrease the σ^2_{col} parameter. Then, the experimental observed efficiency may show a maximum value [12] for a given k' value as illustrated by Fig. 1 (top). In MLC the efficiency enhancement is always associated with a retention

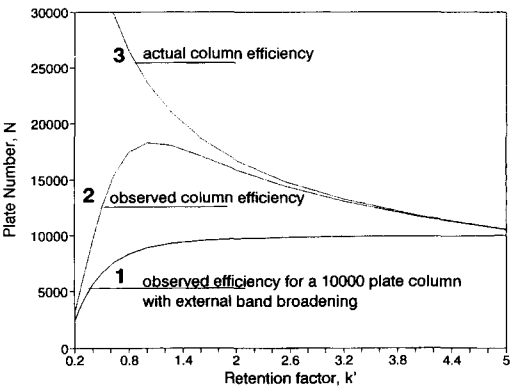

Fig. 1. Efficiency experimental measurement. (1) Observed efficiency for a 10 000 plate column, the extra-column band broadening is heavy in decreasing the column efficiency for low k' solutes, (2) observed efficiency with extracolumn band broadening and efficiency enhancement combined with k' reduction, a maximum N value seems to exist, (3) true column efficiency when enhancements are used in MLC.

factor decrease. This enhancement can be annihilated by the extra-column effects. This is specially true when non-optimized chromatographic systems are used.

3. Chromatographic process and micellar phases

3.1. The three phase model

The chromatographic process with micellar phase was first described by the three phase model of Armstrong [18]. A secondary chemical equilibrium is added to the classical mobile–stationary phase equilibrium. The solute can interact with the stationary phase, the exchange is modelled using a constant K_{SW}. The solute can also interact with the micelles and a K_{MW} constant is defined. The micelle can transfer directly the solute to the stationary phase (constant K_{SM}). The letters M, S and W stand for micelle, stationary and water phases, respectively. The solute retention equation is

$$\frac{1}{k'} = \frac{1}{K_{SW}\varphi}[(K_{MW} - 1)\bar{V}C_{mic} + 1] \qquad (11)$$

in which C_{mic} is the surfactant concentration in the

micelles, i.e., the total surfactant concentration minus the critical micelle concentration (CMC). The K_{SM} constant is derived from the two others using [18]

$$K_{SM} = K_{SW}/K_{MW} \quad (12)$$

Other workers derived similar linear relationships between $1/k'$ and C_m [19] through the solute binding micellar constant which is directly related to the K_{MW} constant through the surfactant molar volume. The drawback of the binding constant is that it has a dimension. Its unit is $1\,mol^{-1}$ or $ml\,g^{-1}$ or other units. The binding constant value depends on the unit selected. The K_{MW} constant is dimensionless, there is no ambiguity on its numerical value. However, it should be noted that it is a constant per surfactant molecule. The micellar distribution constant corresponds to the K_{MW} constant multiplied by the micelle aggregation number.

The equilibrium of the three exchanges, MW, SW and SM, of the model are not reached instantaneously. There are kinetic parameters. These kinetic parameters intervene in the global observed efficiency.

3.2. Micelle–aqueous phase exchanges

The micellar reduced efficiency was early on attributed to a diminished rate constant for solute exit from the micellar aggregates [5]. If the solute stays a long time inside the micelles that move with the mobile phase, it interacts with less stationary phase. It 'sees' a shorter column. Assuming the solute entrance in the micelle is diffusion controlled and equivalent for all solutes, the exit rate constant is inversely proportional to the K_{MW} constant. The solutes with the highest affinity for the micellar phase are the most retained and indeed the ones with the poorer efficiency [5]. The reduced solute exit rate from the micelle produces a decreased solute diffusion coefficient in the mobile phase, D_m.

It was shown that the D_m diffusion coefficient was decreased by micelle inclusion related to the K_{MW} constant [6]. The overall solute diffusion coefficient, D_m, in a micellar solution depends on the micelle diffusion coefficient, D_M, and the solute diffusion coefficient in the aqueous phase, D_W,

$$D_m = \frac{D_M}{1+\Psi} + \frac{D_W}{1+\frac{1}{\Psi}} \quad (13)$$

in which Ψ is related to the solute affinity constant, K_{MW}, measured by MLC:

$$\Psi = \frac{1 - C_m\bar{V}}{K_{MW}C_m\bar{V}} \quad (14)$$

The product $C_m\bar{V}$ is the volume percent of micellar phase and $1-C_m\bar{V}$ is the volume percent of the aqueous phase [6].

Fig. 2 illustrates the decrease of the benzene diffusion coefficient in various micellar solutions. Table 1 lists the diffusion coefficients of various solutes in different micellar media. Depending on the solute affinity for the micellar phase, the D_m diffusion coefficient can be decreased by a factor varying from 2–10 (Fig. 2). Such a D_m decrease may highly increase the B and C terms of the Knox equation as shown by Eqs. (8) and (9), respectively. The corresponding reduced velocity, ν, is also increased by the D_m decrease which should reduce the observed negative effect at low flow-rates and magnify it at high flow-rates. An important surfactant concentration seems not to be favorable for efficiency.

3.3. Knox plots and the causes of micellar reduced efficiency

As mentioned earlier, the three constants of the

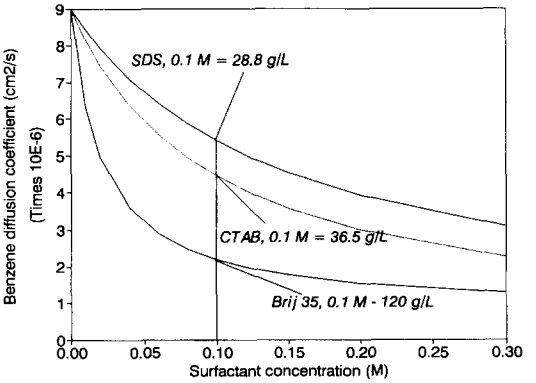

Fig. 2. Decreases of the benzene diffusion coefficient due to its inclusion in micelles of different surfactants.

Table 1
Diffusion coefficients in various micellar phases at 24°C

Solute	K_{MW}	P	D_m ($\times 10^{-6}$ cm^2/s)
Brij 22, 5% w/v; 0.08 *M*			
Benzyl alcohol	16.5	1600	5.1
Benzaldehyde	21.6	2100	4.7
Benzene	72.2	7000	2.9
Micelle	–	–	0.87
Brij 35, 5% w/v; 0.042 *M*			
Benzyl alcohol	10	400	6.3
Benzaldehyde	16	640	5.5
Benzene	45	1800	4.4
Micelle	–	–	0.94
SDS, 1.4% w/v; 0.05 *M*			
Benzene	77.4	4800	6.8
Toluene	242	15 000	3.4
Ethylbenzene	694	43 000	1.7
Propylbenzene	1940	120 000	0.94
Micelle	–	–	0.57

P is the micellar partition coefficient ($=K_{MW}N_{aggr.}$).
Brij 22=C12E10, M_r=610, $\bar{V}$=0.9542 ml g^{-1} or 0.582 l mol^{-1}, $N_{aggr.}$=97, CMC=0.09 m*M*.
Brij 35=C12E23, M_r=1200, $\bar{V}$=1.064 l mol^{-1} or 0.867 ml g^{-1}, $N_{aggr.}$=40, CMC=0.1 m*M*.
SDS, M_r=288, $\bar{V}$=0.246 l mol^{-1} or 0.854 ml g^{-1}, $N_{aggr.}$=62, CMC=8.2 m*M*. Data from [7].

Knox equation (Eq. (7)) are related to the kinetics of the chromatographic process. To be meaningful, the Knox plots should be obtained on the same column and different mobile phases. Table 2 lists the *A*, *B* and *C* values taken from different references [5,8,20,21]. On column 1, with the benzene solute, the efficiency loss is due to *A* increases (+300%) and *B* increases (+700%) compared to the values obtained with the methanol–water mobile phase. The *C* term is little changed. With the ethylbenzene solute, the *A* and *B* values are similar to the benzene ones but the *C* term is increased by 350%. On column 2, the efficiency reduction compared to the acetonitrile–water phase is due to moderate increases of both the *A* (+54%) and *C* (+16%) terms; the decrease of the *B* term is not significant considering the raw data set [21,22]. On column 3, the hydro–organic reference values were not given. Assuming the column 1 values can be used, the efficiency loss is again due to the *A* and *B* term increases (+300% and +80%, respectively). When the temperature was raised from 25°C to 45°C, this decreased the *A* and *C* terms by −21% and −70%, respectively. The effect of alcohols on the Knox parameters is a decrease of the *A* terms by −26% and −45% and a dramatic decrease of the *C* parameters by −93% and −97% when only 2% (v/v) propanol and 1% (v/v) pentanol, respectively, were added [23].

The use of the Knox plots to study the causes of micellar reduced efficiencies leads to the following conclusions. The micellar phase flow anisotropy seems to be much higher than the flow anisotropy obtained with a hydro–organic phase of comparable

Table 2
Parameters of the Knox equation

Solute	Mobile phase	Column	Optimal flow (ml min^{-1})	*A*	*B*	*C*	*k'*
Benzene	MeOH–water (70:30, v/v)	1	0.2	1.1	3.1	0.2	1.1
	0.05 *M* SDS (1.4%, w/v)	1	0.3	3.2	20	0.2	15.6
	25°C 0.05 *M* SDS (1.4%, w/v)	2	0.2	3.4	5.6	0.16	17.8
	45°C 0.05 *M* SDS (1.4%, w/v)	2	0.7	2.7	11.4	0.05	16.3
	ACN–water (30:70)	3	1.2	0.65	16.4	0.026	17.3
	0.042 *M* Brij 35 (5%, w/v)	3	<0.1	1.0	6	0.03	26.1
Ethylbenzene	MeOH–water (70:30 v/v)	1	0.2	1.1	3.8	0.15	3.5
	0.05 *M* SDS (1.4%, w/v)	1	0.3	3.1	23	0.5	45
Coumarin	0.01 *M* SDS (0.3%, w/v)	2	0.1	5.4	5.8	0.26	–
	0.01 *M* SDS+2% C_3OH	2	0.2	4.0	8.5	0.018	–
	0.01 *M* SDS+1% C_5OH	2	0.3	3.0	8.8	0.007	

Column 1: 10 cm×4.6 mm I.D., Radial-Pack ODS, 10 μm (Waters), data from [21].
Column 2: 10 cm×4.6 mm I.D., Apex ODS, 5 μm (Jones), data from [23].
Column 3: 10 cm×4.6 mm I.D., C_{14} bonded phase, 5 μm, 3.5 μmol/m^2, data from [21].
Room temperature, unless otherwise indicated.

viscosity (increased A term). This is only very partly due to the micellar viscosity. The main reason of such difference in flow patterns is the partial clogging of the stationary phase pores by adsorbed surfactant molecules [23]. A temperature raise decreases the mobile phase viscosity and the amount of adsorbed surfactant [23]. Both effects decrease the flow anisotropy and the A term. It will be revealed later that alcohol additions to a micellar phase reduce dramatically the amount of adsorbed surfactant.

The B term is linked to solute diffusion coefficient which is reduced by micelle inclusion (Eq. (8) Fig. 2). The increase of the B term in micellar phases is due to the D_m change and also to the k' increase (Table 2). Considering the C term equation (Eq. (9)), one would expect that micellar phases decrease C because k' values are higher and D_m coefficients are lower. This is not obtained. It means that the surfactant adsorption causes dramatic decreases of the D_s parameter. The solute diffusion coefficient in the surfactant covered stationary phase is very difficult [6,7,21,24]. It slows down the solute mass-transfer. This effect becomes dominant for lipophilic solutes that have a high affinity for the stationary phase (ethylbenzene, Table 2). The peaks corresponding to lipophilic solutes become very broad with micellar mobile phases. Both a temperature raise and an alcohol addition decrease the amount of adsorbed surfactant [23–25]. Both actions reduce the C term (Table 6) and improve the observed efficiency.

Clearly, the Knox plot study points out the surfactant adsorption on the stationary phase as responsible for the chief part of the efficiency loss observed using micellar phases.

3.4. Surfactant adsorption on the stationary phases

If the main property of surfactant solution is the micelle formation, the second important property is the adsorption of surfactant on any interface. Specially, the surfactant molecules adsorb on the large solid surface of the porous stationary phases [26]. The surfactant adsorbed layer changes the solute–stationary phase and micelle–stationary phase interaction and kinetics. Figs. 3 and 4 show the amount of sodium dodecyl sulfate (SDS) and cetyl trimethylammonium bromide (CTAB) respectively adsorbed

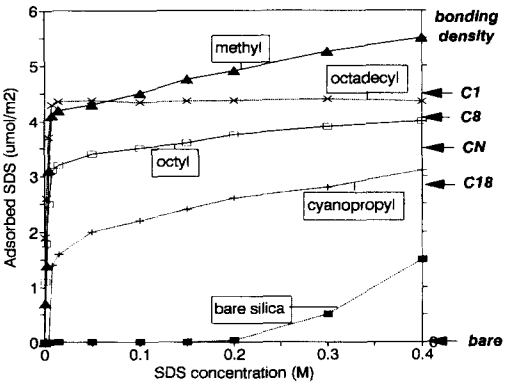

Fig. 3. Amount of adsorbed anionic SDS on 5 different Hypersil silica phases. The arrows on the right point the bonding density of the organic layer. Full characteristics of the phases are listed in Table 3. Data from [25].

on different stationary phases whose physicochemical characteristics are listed in Table 3. The amount of adsorbed surfactant increases rapidly up to the critical micelle concentration (CMC) value. For surfactant concentrations above the CMC, there is a plateau with constant adsorbed amount for SDS on ODS (C_{18}) phase and for CTAB on all phases but the cyanopropyl phase. A small additional amount of SDS adsorbs on the Fig. 3 phases, but ODS. This amount is about 0.1 μmol/m^2 (~3%) per 0.1 M SDS concentration step. The amount of adsorbed SDS on

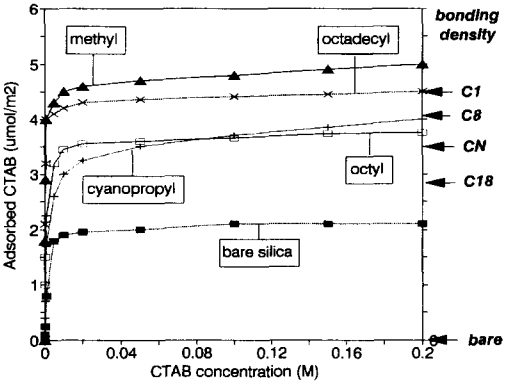

Fig. 4. Amount of adsorbed cationic CTAB on 5 different Hypersil silica phases. The arrows on the right point the bonding density of the organic layer. Full characteristics of the phases are listed in Table 3. It should be noted that bare silica is able to adsorb significant amounts of CTAB (silanol interactions). Data from [25].

Table 3
Physicochemical properties of stationary phases

Trade name	Bonded moiety (dimethylsilyl (group)	S ($m^2 g^{-1}$)	Pore diameter (nm)	Pore volume (ml g^{-1})	% C (w/w)	Bonding density ($\mu mol/m^2$)
Hypersil	Bare	150	12	0.6	0	0
CPS CN	Cyanopropyl	115	12	0.5	4.2	3.5
SAS C_1	Trimethyl	104	12	0.5	2.6	4.5
MOS C_8	Octyl	129	12	0.5	7.0	4.1
ODS C_{18}	Octadecyl	105	12	0.5	8.5	2.8

Monomeric bonding, S = surface area of the bonded phase, data from [26,42].

the plateau is close to one SDS molecule per bonded moiety for the C_1 and C_8 grafted phases. It is close to two SDS molecules per C_{18} chain for the ODS phase and only 1 SDS molecule for two cyanopropyl chains. Similar amounts were obtained for the cationic CTAB surfactant. These results were corroborated by other works [27]. Corresponding results were obtained for non-ionic surfactants [8,20]. The adsorbed surfactant molecules fill up part of the silica pore volume and doing so, they reduce the stationary phase surface area [20,24]. The surfactant adsorbed layer increases the thickness of the stationary phase organic layer, ϕ_f. This parameter intervenes quadratically in the last term of Eq. (6). Also, as pointed out in the Knox plots study, the surfactant layer decreases the kinetics of the exchanges with the stationary phase.

4. Remediation of reduced efficiency

The serious waste of efficiency in MLC is a major drawback hindering the development of the technique. To be useful for the practising chromatographer, the efficiency observed in MLC must at least approach that of conventional HPLC. As early as 1983, Dorsey et al. suspected a slow mass transfer coming from a poor wetting of the stationary phase by the aqueous micellar phase. They proposed the addition of 3% (v/v) 1-propanol to the micellar phase and heating the chromatographic system to 40°C [3]. The dramatic enhancement of the MLC efficiency gave rise to numerous studies of the effect of organic additives and temperature in MLC.

4.1. Effect of alcohols on micellar solutions

It is know in colloid science that the addition of medium chain alcohols to micellar solutions changes the micelle structure [28]. Microemulsion formation is obtained by associating ionic surfactants with medium chain alcohols used as co-surfactants [29]. The change of micelle structure can be monitored by CMC shifts. Table 4 lists the CMC and structure of the SDS micelles when various alcohols are added to the micellar phase. Small amounts (<5%, v/v) of methanol and ethanol do not change the micelle structure. Propanol and the following alcohols decrease the CMC and are incorporated into the micelle changing its size [30–32] and decreasing its internal viscosity [33].

4.1.1. Dynamic structure of micelles

It was demonstrated that there are two relaxation times in the kinetics of micelle formation–destruction: (1) a fast relaxation time, in the low nanosecond range, corresponding to the exchange rate of a surfactant molecule with the micelle and (2) a slower relaxation time, in the microsecond range, was linked to the average lifetime of a micelle [34]. Methanol and ethanol at low concentrations do not partition significantly with the SDS micelles [35]. Neither alcohol affects the two micelle relaxation times noticeably. The C_3–C_7 alcohols were found to increase the kinetics of the exchange rate of an SDS surfactant or alcohol molecule and to decrease the two relaxation times [35]. The formation–destruction of the SDS micelles is highly accelerated by these alcohols. The alcohol molecules incorporate in the micelles with their polar hydroxyl groups in the

Table 4
Organic additive effects on ionic surfactant micellization

Additive	Concentration		CMC (m*M*)	Aggregation number	*R* (nm)	K^{a}_{MW}
	(*M*)	% (v/v)				
Sodium dodecyl sulfate (SDS) +						
None	–	–	8.2	63	1.8	
Methanol	0.1	0.4	7.9	63	1.8	<1
	1.5	6	8.0	63	1.8	
Ethanol	0.1	0.58	7.7	63	1.8	2
	0.9	5.3	7.5	63	1.8	
1-Propanol	0.1	0.75	7.4	60	1.8	7
	0.5	3.7	5.6	51	1.8	
	1	7.5	4.7	44	1.8	
1-Butanol	0.05	0.46	7.1	63	1.8	25
	0.1	0.91	5.4	63	1.8	
	0.3	2.7	3.0	–	–	
1-Pentanol	0.02	0.22	5.6	68	1.8	85
	0.1	1.1	3.0	54	1.8	
	0.2	2.2	1.0	57	1.9	
1-Hexanol	0.01	0.13	5.8	45	–	400
	0.02	0.25	3.0	48	–	
	0.064	0.80	4.9	40	1.7	
1-Heptanol	0.028	0.40	5.8	53	1.9	1400
	0.043	0.61	5.3	43	1.9	
1-Octanol	0.012	0.19	6.5	64	1.9	5000
	0.025	0.39	5.7	59	2	
Decyltrimethylammonium bromide (DTAB) +						
None	–	–	64	39		
Methanol	1.24	5.0	64	39		
Ethanol	0.85	5.0	64	39		
1-Propanol	0.20	1.5	60	33		
	0.47	3.5	55	29		
	0.93	7.0	45	23		
	1.91	14.3	22	20		
1-Butanol	0.1	0.91	57	38		
	0.24	2.2	48	38		
	0.48	4.4	34	37		
	0.70	6.4	25	19		
1-Pentanol	0.025	0.27	58	39		
	0.1	1.1	44	39		
	0.225	2.4	24	27		

[a] K_{MW} value of the corresponding alcohol in a SDS micellar solution with trace amount of the alcohol added (K_{MW} is the partition coefficient of the alcohol per SDS molecule [41]). Data compiled from [8,20,26,29–32,41,49].

Stern layer and their alkyl chain in the micelle cores. This moves away the ionic sulfate groups [36]. The charge density of the micellar interface decreases which may facilitate the intermicellar migration of solutes. Similar results were obtained with the cationic CTAB surfactant [37]. The intermicellar migration of a dye solute was orders of magnitude increased by low concentrations of C_3–C_6 alcohols [38].

The opposite effect was observed with shorter chain surfactant (decyltrimethylammonium bromide, DTAB) and C_3–C_5 alcohols [31,32]. High concentrations of both SDS and medium chain alcohols (C_4 and up) produced a micelle size polydispersity and

anisotropy [39]. In such conditions, disorganization of the micelle structure with increased mobility and decreased lifetime was observed [39,40].

For MLC, it is necessary to summarize the vast amount of work done on the effects of alcohols on the ionic micelle structure distinguishing two points: (1) low amounts (≤5%, v/v) of methanol and/or ethanol have minimum effect and (2) the ratio of the alkyl chain length of the alcohol over that of the surfactant, $C_{n,OH}/C_{n,surf}$, can be used as a reference parameter. If the ratio $C_{n,OH}/C_{n,surf}$ is lower than 1/3, e.g. propanol and butanol with SDS micelles, or propanol, butanol and pentanol with CTAB micelles, the alcohol increases the kinetics of both the formation–destruction of the micelle and the exchange rate molecule– micelle. If $C_{n,OH}/C_{n,surf}$ is higher than 1/3, the concentration of the alcohol should be compared to the surfactant concentration. Low amounts of alcohol increase the kinetics of the micellar exchanges which is good for MLC efficiency. Significant alcohol concentrations disorganize the micelle structure which is unfavorable for MLC efficiency. At a constant surfactant concentration, the maximum beneficial alcohol concentration decreases as the $C_{n,OH}/C_{n,surf}$ ratio increases.

4.1.2. Alcohols and K_{MW} solute parameter

Table 4 lists the affinity of the alcohols for the micellar phase as K_{MW} parameters. A linear relationship was established between log K_{MW} and the alkyl chain length of the primary alcohol [41]. As the alkyl chain length increases, the alcohol has more affinity for the micellar phase and less affinity for the aqueous phase. This explains that the solute affinity for micelles, measured by the K_{MW} constant, is always depressed by alcohol additions (Table 5). As seen in Section 3.2, it means that the solute micelle exit rate is increased. The magnitude of the K_{MW} decrease is linked to the alcohol alkyl chain length. A given amount of hexanol has a much dramatic depressing effect on the solute K_{MW} constant than the same amount of propanol. Also, the same K_{MW} reduction can be obtained with far less hexanol than propanol. Methanol depresses the K_{MW} values (Table 5) because it enhances the solute solubility in the aqueous phase without changing significantly the micelle kinetics.

Table 5
Effect of alcohols on the solute affinity for the micellar and stationary phases

Solute	Medium	K_{MW}	ϕK_{SW}
Benzene	SDS	90	60
	SDS + 4% propanol	68	50
	SDS + 4% butanol	61	32
	SDS + 4% pentanol	48	23
	SDS + 3% hexanol	24	15
	Brij 35	50	85
	Brij 35 + 15% ethanol	39	40
Acetophenone	SDS	55	49
	SDS + 4% propanol	56	23
	SDS + 4% butanol	31	11
	SDS + 4% pentanol	22	8
	SDS + 3% hexanol	16	6
	Brij 35	29	20.4
	Brij 35 + 15% ethanol	14	15.4
Toluene	SDS	240	140
	SDS + 5% methanol	200	124
	SDS + 3% propanol	128	86
	SDS + 10% propanol	114	55
	CTAB	390	190
	CTAB + 5% methanol	240	125

The ϕK_{SW} value are only suggestive since different C_{18} columns were used.
Data compiled from [8,24,25,44,45].

4.1.3. Alcohols and stationary phases

Alcohols and surfactants molecules compete for adsorption on the stationary phase. Alcohol additions reduce the amount of adsorbed surfactant [20,24,25]. Table 6 lists the amounts of adsorbed surfactant when micellar mobile phases with or without alcohol was used [7]. The surfactant desorption is depending on the alcohol chain length [7,20]. Surfactant desorp-

Table 6
Alcohol effect on surfactant adsorption

Mobile phase	Adsorbed amount (μmol/m^2)
0.05 *M* SDS	4.6
+ 5% v/v methanol	4.4
+ 3% v/v propanol	3.0
+ 2% v/v pentanol	3.1
0.02 *M* CTAB	4.5
+ 5% v/v methanol	4.4
+ 3% v/v propanol	3.2
+ 2% v/v pentanol	2.2

Column ODS Hypersil, C_{18}, 5 μm.
Data from [7].

tion is linked to a ϕ_f reduction that should decrease the HETP (Eq. (6)). The kinetics of the surfactant adsorption–desorption process is enhanced by alcohol additions [5,7,21]. Spectroscopic studies have shown that short chain alcohols (C_1–C_3) just wet a monomeric C_{18} silica bonded layer without changing its organization. In contrast, long chain alcohols (C_7–C_{10}) interpenetrate with the C_{18} chains [43]. These alcohols decrease the surface tension and viscosity of the C_{18} bonded layer by minute amounts [43].

4.1.4. Which alcohol is best and how much to add?

The 1983 work of the Dorsey research group established the use of 3% (v/v) 1-propanol in micellar phases to reduce the MLC efficiency problem [3]. It is now confirmed that the addition of alcohol to micellar phases (i) increases the rate of the solute mass- transfer between the micelles and the aqueous phase by increasing the solute micelle exit rate constant, (ii) increases the solute mass transfer kinetics between the stationary phase and the aqueous phase by decreasing the stationary phase viscosity and the amount of adsorbed surfactant. The problem of alcohol additions to micellar phases is that kinetics enhancement cannot be dissociated from thermodynamics changes. The efficiencies increase and the retention times decrease [46]. A hybrid alcohol–micelle mobile phase has to have a higher solvent strength than a purely aqueous phase [47]. It was shown that alcohols were changing the micelles and the stationary phase in a comparable manner [48] as noted on the K_{SW} and K_{MW} parallel variations in Table 5.

Fig. 5 shows the efficiency enhancement for benzene and 2-ethylanthraquinone (EAQ) obtained with 5% (v/v) alcohol additions to a 0.285 *M* SDS micellar phase. The *x*-axis is the log K_{MW} value of the alcohol, a parameter almost proportional to the alcohol carbon number [41]. In pure SDS solution, the benzene and EAQ efficiencies were respectively 1400 and 49 plates (HETP, 14 d_p and 410 d_p). The maximum efficiencies, 3400 and 1100 plates (5.5 d_p and 18 d_p), respectively, were obtained with 5% (v/v) pentanol. The benzene efficiency was more than doubled and the EAQ lipophilic solute was multiplied by a factor 22 (Fig. 5). The EAQ k'

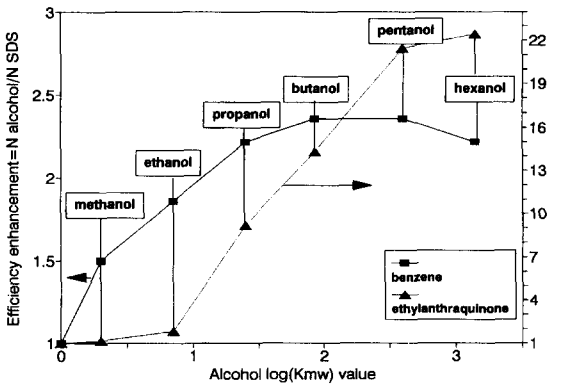

Fig. 5. Efficiency enhancement obtained with 5% (v/v) alcohol addition to a 0.285 *M* SDS phase. Column, 10 cm×4.6 mm I.D., 5 μm C_{18} Astec (Whippany, NJ, USA); flow-rate, 1 ml min^{-1} (0.5 ml min^{-1} for hexanol); 23°C. Reference efficiency: benzene, 1400 plates (left *Y*-axis); EAQ, 49 plates (right *Y*-axis) in pure SDS micellar phase. Data from [49].

retention factor was reduced by 82%, from 45 in pure SDS phase to 8 with 5% (v/v) pentanol [49]. The maximum efficiency increase for benzene was obtained for butanol and pentanol that have $C_{n,OH}/C_{n,surf}$ parameter of 1/3 and 0.42, respectively. 5% (v/v) hexanol seems to much with a $C_{n,OH}/C_{n,surf}$ parameter of 1/2, the benzene efficiency decreases, the SDS micelles may be disorganized. The EAQ efficiency seems identical with the pentanol and hexanol addition (Fig. 5), but the hexanol efficiency value was obtained at 0.5 ml min^{-1}. Pentanol seems the best alcohol with SDS solutions.

A limiting parameter for the alcohol choice is viscosity. The hybrid micellar phase obtained has a higher viscosity that the micellar phase without alcohol. Viscosity increases with the alcohol chain length. The viscosity of the 0.285 *M* SDS phase was 1.65 cP producing a 95 kg/cm^2 (9.5 MPa or 1400 p.s.i.) pressure at 1 ml min^{-1} with a 10-cm column. The viscosity of the 5% (v/v) pentanol SDS phase was 2.32 cP producing a 127 kg/cm^2 (12.7 MPa or 1800 p.s.i.) pressure [49]. The viscosity of the 5% hexanol SDS phase was 11.8 cP which precluded its use at 1 ml min^{-1}. A flow-rate of 0.5 ml min^{-1} was used. This is likely to produce too high efficiencies for the hexanol points of Fig. 5.

Is 5% (v/v) the right amount for alcohol additions? Considering that large amounts of alcohols

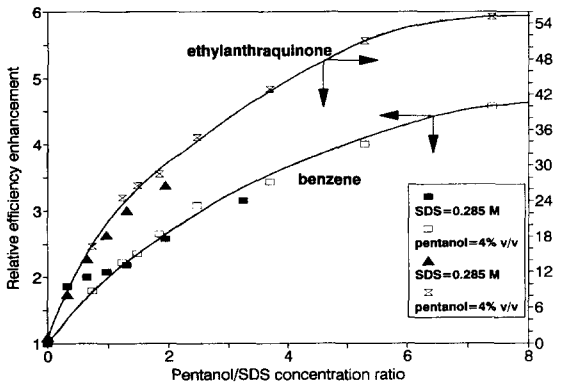

Fig. 6. Efficiency enhancement versus the pentanol to SDS concentration ratio. Same experimental conditions and reference efficiencies as in Fig. 5. Data from [49].

with a $C_{n,OH}/C_{n,surf}$ ratio higher than 1/3 disorganize the micelle structure [39], the alcohol to surfactant ratio is studied. Fig. 6 shows the efficiency enhancement obtained for benzene and EAQ plotted versus the pentanol over SDS concentration ratio. Two sets of experiments were done: (i) constant SDS concentration (0.285 *M*) and increasing pentanol additions and (ii) 4% (v/v) pentanol and increasing SDS concentrations [49]. The efficiency obtained are clearly linked to the pentanol/SDS ratio. The efficiency enhancement reaches a plateau for concentration ratios higher than 6. Similar efficiency enhancements were obtained with the cationic CTAC surfactant, an ODS (C_{18}) column and acetonitrile (ACN) as the organic additive [24]. A plateau at a three fold efficiency enhancement was obtained for ACN/CTAC concentration ratio higher than 12. Although these results were obtained for only two ionic surfactants and C_{18} columns, it seems possible to extend them to other micellar mobile phases and bonded silica stationary phases. The alcohol to surfactant ratio acts on both solute exchange rates: micelle–aqueous phase exchanges and stationary phase–mobile phase exchanges. It is directly related to the number of alcohol molecules per micelle. Also, this ratio dictates the total amount of surfactant surface coverage and fluidity of the composite organic layer of the stationary phase [7,26].

In conclusion, the best amount of alcohol to add depends on the surfactant concentration used. It means that efficiency enhancements cannot be dissociated from micellar solvent strength.

4.2. Effects of temperature

Table 7 lists the efficiencies obtained with temperature increases and pure micellar solutions. The efficiency of acetophenone on a C_{18} column was multiplied by 12 when the temperature was raised from 25°C to 73°C [23]. Two to three fold enhancements were obtained for the other solutes [5,23]. The retention factors were decreased by temperature. The magnitude of the k' decreases is far less important than what was observed with alcohol additions [47]. The k' decrease with temperature is predicted by the Van't Hoff equation (Eq. (4)). The equilibrium enthalpies and entropies of the retention process were measured in MLC for various surfactants [45,50]. These studies showed that the micellar retention process is essentially governed by entropic effect [50].

Enthalpy–entropy compensation studies are useful in the investigation of separation mechanisms [51]. They were used in the study of the retention mechanisms by C_{18} phases [52] and the study of the mechanism of chiral separations with cyclodextrin bonded phases in GC [53]. The enthalpy change, ΔH_{MW}, of the K_{MW} values of 6 solutes (the more lipophilic one was naphthalene) were determined in pure SDS micellar phase and 3% and 10% (v/v) propanol modified SDS phases [45]. The plot of the ln k' value of each solute versus the corresponding ΔH_{MW} value should produce straight lines with similar slopes if the separation mechanisms are similar [45,51–53]. Fig. 7 shows that the alcohol addition alters the micellar retention mechanism as is evident by the large change in slope and scattering of the data noted. Propanol has altered the stationary phase as well as the micellar phase [45]. It was noted that naphthalene could be separated by direct transfer from the micelles to the stationary phase because it was not soluble enough in the aqueous phase [54]. The naphthalene representative point is well off the regression line for the SDS micellar solution. The alcohol additions bring the naphthalene point closer to the other solute's points (Fig. 7). The hydro–alcoholic phase has a higher solvent strength.

The solubility of silica in hydro–organic phases is

Table 7
Effects of temperature on efficiency and retention parameters

Solute	Temperature (°C)	Efficiency (plates)	Relative enhancement (%)	Retention factor
Decyltrimethylammonium bromide 0.1 *M*, column LC1, 15 cm, 2 ml min^{-1} [5]				
Benzene	25	3000	100	7
	30	3400	113	
	40	3800	127	
	50	4400	147	
	60	5000	167	5
	70	4900	163	
Anthracene	25	460	100	25
	30	530	115	
	40	860	186	
	50	1200	260	
	60	1500	330	20
	70	1700	370	
Sodium dodecyl sulfate 0.05 *M*, column Apex C_{18}, 10 cm, 1 ml min^{-1} [23]				
Toluene	25	2000	100	33
	35	2500	125	31.6
	45	3100	155	30.6
	55	3300	165	28.4
	65	3900	195	26.1
	73	5900	295	25.5
Acetophenone	25	280	100	19.4
	35	400	143	16.0
	45	980	350	13.6
	55	1800	643	11.2
	65	2100	750	9.8
	73	3300	1180	9.5

enhanced by temperature increases. The rate of the silica solubilization depends on the pH and temperature of the mobile phase and also of the bonding quality of the stationary phase [42]. Silica solubility may become a serious problem when working at temperature higher than 50°C [5,23,45]. Column life is shortened.

4.3. Flow-rate

Considering the Knox plots, an obvious way to improve efficiency is to work at the optimum flow-rate. Table 2 lists the optimum flow-rates for different mobile phases. The strength of the pure micellar phases is much lower than the one of hydro–organic phases [47]. A flow-rates of 0.3 ml min^{-1} or lower will produce rebutting retention times. Table 2 shows that both alcohol addition and especially, a rise in temperature increase the optimum flow-rates. It was discovered that both remediation ways also increase the solvent strength, decreasing the retention times. If time is not a priority, it should be remembered that a decrease of the flow-rate can easily further enhance the micellar efficiency and consequently the chromatographic resolution.

4.4. Classical efficiency enhancements

Column overload and silanol interaction may broaden the chromatographic bands with or without micelles in the mobile phase. In case of a peak deformation due to a stationary phase overload, the decrease of the injected amount will enhance the peak shape. In the case of peak tailing due to silanol–amine interactions, it was shown that the peak shapes greatly improved when the mobile phase pH was decreased to below 5.5 [54]. The observed efficiency increase was attributed to protonation of

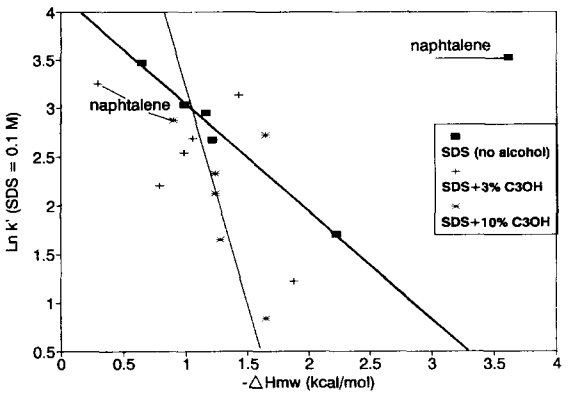

Fig. 7. Enthalpy–entropy compensation. Plot of the natural logarithms of the retention factor of phenol, naphthalene–methanol, anisole, benzene, toluene and naphthalene versus the corresponding ΔH_{MW} enthalpy obtained from Van't Hoff plots. The slope of the pure micellar phase does not correspond to the slope of the alcohol–micelle phases which indicates differing mechanisms. Column, 10 cm×4.6 mm I.D., 10 μm C_{18} Spheri-10 Brownlee Lab. (Santa Clara, CA, USA). Data from [45].

the silanol groups. Triethylamine is classically added to reversed-phase mobile phases to bind the silanol groups. This reduces the tailing of basic compounds which is not due to the micellar phase. The peak of propranolol in a Brij 35 (non-ionic surfactant) mobile phase was greatly improved and its asymmetry decreased by a small addition of triethylamine to the micellar mobile phase [55]. Higher additions of triethylamine produced a decrease of the propranolol retention factor with slight increase of peak shape and efficiency. This effect is probably due to an organic modifier effect similar to the effect described for alcohols.

5. Conclusion

The efficiency loss observed in MLC using purely aqueous micellar mobile phases is due to (i) a slow solute transfer from the aqueous to the micellar phase, (ii) to a slow transfer from the stationary phase to the aqueous phase and (iii) a change of the flow pattern in the column, change due to surfactant adsorption that changes the porosity and surface of the stationary phase.

MLC efficiency can be enhanced (i) by reducing the mobile phase flow-rate to work closer to the optimum of the Knox plot, (ii) by increasing the temperature which decreases the viscosities, increases the rate constants and decreases the amount of adsorbed surfactant, (iii) specially by adding an organic modifier such as an alcohol whose alkyl chain has a length such as the ratio $C_{n,OH}/C_{n,surf}$ close to 1/3. The amount of added alcohol should be increased if the surfactant concentration is increased to keep the alcohol to surfactant ratio constant.

Kinetics and thermodynamics are intimately linked. If flow rate reduction is excluded, all the other efficiency remediation methods produce thinner peaks eluting earlier. Then, the resolution factor may or may not be improved.

6. Abbreviations

A	Knox plot first parameter (flow anisotropy)
ACN	acetonitrile
B	Knox plot second parameter (solute diffusion)
B'/A'	peak asymmetry measured at 10% of the peak height
C	Knox plot third parameter (mass transfer)
C_m	part of the surfactant forming the micelles ($=C_T-$CMC) (M)
CMC	critical micelle concentration (M)
$C_{n,OH}$	alcohol chain length
$C_{n,surf}$	surfactant alkyl chain length
C_T	surfactant concentration (M)
CTAB	cetyl (C_{16}) trimethylammonium bromide
CTAC	cetyl (C_{16}) trimethylammonium chloride
d_p	silica particle diameter (μm)
D_m	global solute diffusion coefficient in the mobile phase ($cm^2\ s^{-1}$)
D_M	micelle diffusion coefficient in the mobile phase ($cm^2\ s^{-1}$)
D_S	solute diffusion coefficient in the stationary phase ($cm^2\ s^{-1}$)
D_W	solute diffusion coefficient in the aqueous part of the micellar phase ($cm^2\ s^{-1}$)
DTAB	decyltrimethylammonium bromide
EAQ	ethylanthraquinone
H	plate height (HETP, in μm or d_p number)
k'	solute retention factor
K	solute affinity constant
K_{MW}	solute affinity constant for the micelles

K_{SW} solute affinity constant for the stationary phase
K_{SM} solute affinity constant for a direct transfer micelle–stationary phase
L column length (cm)
MLC micellar liquid chromatography
N plate number
q geometrical porosity factor
SDS sodium dodecyl sulfate
t_R solute retention time (min)
T absolute temperature (K)
u mobile phase velocity (cm s^{-1})
$\bar{V}$ surfactant molar volume (l mol^{-1} or ml g^{-1})
V_0 column dead volume (ml)
V_R solute retention volume (ml)
V_S stationary phase volume (ml)
$W_{0.1\,H}$ peak width at 10% of the peak height ($A'+B'$) (min)

Greeks

ΔG^0 Gibbs free energy (kcal mol^{-1}; 1 cal = 4.184 J)
ΔH^0 transfer enthalpy (kcal mol^{-1})
ΔS^0 transfer entropy (kcal mol^{-1} K^{-1})
γ obstruction factor
ν reduced mobile phase velocity
σ^2 variance (band broadening in time or volume unit)
ϕ_f thickness of the stationary phase organic layer (μm)
Ψ used in Eqs. (13) and (14)
ψ stagnant mobile phase fraction
φ column phase ratio ($=V_S/V_0$)

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