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Fluorinated bonded stationary phases in micellar liquid chromatography

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Abstract

The usefulness of fluorinated bonded stationary phases in micellar liquid chromatography (MLC) is examined. Different selectivities and higher efficiencies were observed using a flurooctyl (FO) column as compared to that of a C_{18} column for both non-ionic and ionic compounds in MLC. Shortened analysis times for amino acids and small peptides and increased retention for the early eluting sulfonamides were observed on the FO column. The unique phenomenon of the simultaneous enhancement of solvent strength and selectivity that often occurs in the MLC systems with the alkyl-bonded stationary phases was also observed for the FO column. This is due to the existence of the competing partitioning equilibria in MLC and because of the interactive nature of the two eluent parameters, micelle concentration and the volume fraction of organic modifiers, both of which influence solvent strength and selectivity. Consequently, simultaneous optimization of these two parameters is the most effective strategy for the MLC systems with the fluorinated bonded stationary phases. Subsequently, the iterative regression strategy was utilized to optimize these two mobile phase parameters for a group of amino acids and small peptides. Excellent agreement was obtained between the observed optimum chromatogram and the one predicted by the iterative regression strategy using only five initial experiments. The successful application of the iterative regression optimization procedure indicates that the retention pattern in MLC with fluorinated bonded stationary phases is easily predictable. This is a similar behavior to that using hydrocarbonaceous phases and can be attributed to the linear variations in retention with the two mobile phase parameters and to the highly reproducible retention behavior in MLC.

1. Introduction

Micellar liquid chromatography (MLC) is a powerful alternative to ion-pair chromatography (IPC) for the separation of charged compounds [1–3]. This is because MLC offers a combination of several capabilities such as capability of simultaneous separation of ionic and non-ionic compounds, reproducible and predictable retention behavior, simultaneous enhancement of solvent strength and separation selectivity, and rapid gradient capability. Other unique advantages like possibility of on-column injection of physiological fluids, enhanced luminescence detection, applications in quantitative structure–biological activity relationships, low cost and low toxicity have also been reported [4–10].

The most serious drawback of MLC is the additional band broadening as compared to that in conventional reversed-phase LC with hydro–organic eluents. However, the typical MLC column efficiency is about the same as that in

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IPC at similar elution strengths [3]. Several workers have concluded that adsorption of monomer surfactants on the alkyl-bonded stationary phases in MLC contributes significantly to the band-broadening process. In general, slow kinetics of mass transfer in stationary phase and in mobile phase have been identified as the reasons behind the poor chromatographic efficiency [11–14]. In general, incorporation of additional chemical equilibria in an LC system (such as ion pairing or micelle partitioning) provides enhanced selectivity at the expense of additional band broadening.

Alkyl-bonded stationary phases have been used in nearly all of the MLC separations that have so far been reported. Fluorinated bonded stationary phases in MLC may offer several advantages over hydrocarbonaceous bonded stationary phases. First, due to the reduced interaction between hydrocarbon and fluorocarbon functional groups (as compared to the hydrocarbon–hydrocarbon interactions), it is expected that the extent of adsorption of hydrocarbon surfactants on the fluorinated bonded stationary phases be less than that on the alkyl-bonded stationary phases. Reduction in surfactant adsorption may lead to an improvement in the kinetics of mass transfer across the stationary phase in MLC and subsequently to higher column efficiencies. Second, hydrocarbon compounds are generally less retentive on fluorinated bonded stationary phases than on alkyl-bonded stationary phases. Therefore, for the separation of hydrophobic hydrocarbon compounds either lower micelle concentration is required which leads to better efficiency in MLC [13], or faster separations are achieved at a given solvent strength. Third, different chromatographic selectivity can be observed on a fluorinated bonded stationary phase especially for compounds with polar functional groups. In addition, the differences in polarities and types of interactions that exist in MLC systems with hydrocarbon ionic micelles in the mobile phase and fluorinated bonded stationary phases are larger than those in traditional MLC systems with hydrocarbon micelles and alkyl-bonded stationary phases. This might lead to a better control on retention and higher degree of selectivities. Generally, larger differences between the mobile phase and stationary phase polarities provide better selectivities [15,16].

The usefulness of fluorinated bonded stationary phases in RPLC system was first reported by Berendsen *et al.* [17]. As compared to the hydrocarbonaceous bonded stationary phases (such as C_{18}) in RPLC, fluorocarbon columns have different chemistry and offer less retention for hydrocarbon compounds and specific interactions between fluorine and some polar functional groups (such as–CHO,–$COCH_3$,–OH,–NO_2, –CN,–OCH_3,–F and–COOH) [15,18–20]. They are potentially useful for the separation of very hydrophobic hydrocarbon compounds [21], proteins [22], and some polar compounds [20].

In this paper, the usefulness of a flurooctyl (FO)-bonded stationary phase in MLC is examined with the emphasis on retention and selectivity behaviors. Retention behavior of ionic and non-ionic compounds were investigated in MLC with the FO column and sodium dodecyl sulfate (SDS) micellar eluents. The influence of the stationary phase on retention and selectivity for alkylphenone homologous series, amino acids, small peptides and sulfonamides is discussed. As expected, higher efficiencies were observed on the FO column than those on a C_{18} column for different test solutes. It was found that the simultaneous enhancement of solvent strength and selectivity, which was previously observed for the C_{18}-based MLC system [1–3], may also occur in the FO-based MLC system. The iterative regression optimization strategy [23,24] was successfully used for optimizing the separation of ten amino acids and small peptides. The effects of stationary phase on retention and selectivity for amino acids, small peptides and sulfonamides is also discussed.

2. Experimental

2.1. Chromatographic system

The chromatographic apparatus consisted of an HPLC pump (Model 400; Applied Biosys-

tems, Foster City, CA, USA) and a variable-wavelength absorbance detector (Model 783A, Applied Biosystems) set at 210 nm for amino acids and small peptides and at 254 nm for other test solutes. The HPLC system was controlled by the Chemresearch chromatographic data management system controller software (ISCO, Lincoln, NE, USA) running on a PC-88 Turbo personal computer (IDS, Paramount, CA, USA). A 5-μm particle size FO column (E.S. Industries, Berlin, NJ, USA), 150 × 4.6 mm, and a 5-μm particle size C_{18} column (Rainin Instruments, Woburn, MA, USA), 150 × 4.6 mm, were used. The columns were thermostated at 40°C by a water circulator bath (Lauda Model MT-6; Brinkmann Instruments, Westbury, NY, USA). A silica precolumn was used to saturate the mobile phase with silicates and protect the analytical column. Two 1.5-μm precolumn filters (Rainin Instruments) were placed between the silica precolumn and a VIGI injector (Valco, Houston, TX, USA) and between the injector and the analytical column. The column dead times were measured from the injection point of water samples and the first deviation of the baseline. The iterative regression program [23,24] was used to optimize and reconstruct the experimental chromatograms. The simulated chromatograms in this paper are based on a Gaussian peak shape, using the theoretical plates and the dead times experimentally observed.

2.2. Reagents

The stock solution of SDS (Sigma), was prepared by dissolving the required amount of surfactant in doubly distilled, deionized water and was filtered through a 0.45-μm nylon-66 membrane filter (Rainin Instruments). All the test solutes were obtained from Sigma. The sample solutions were prepared by diluting the stock solutions (5 mg/ml in methanol) with the mobile phase. The ionic strength was adjusted by adding phosphate buffer so that the total buffer concentration of the final solution was 0.020 *M*. After adding the required amount of organic solvents (such as 1-propanol) the pH was adjusted to 3.0. All other chemicals were obtained from Sigma, Aldrich or Fisher.

3. Results and discussion

Several mobile phase parameters such as the type/concentration of surfactant and organic solvent, pH, ionic strength and temperature can influence the MLC separations. In this study, the effects of surfactant concentration, type and volume fraction of organic solvent on the chromatographic behavior of the test solutes using a FO column and a C_{18} column were examined.

3.1. Retention behavior of homologous series

Alkyl homologous series are suitable test compounds for the investigation of retention mechanisms, especially in new RPLC systems [25,26]. The linear increase of retention (*i.e.* $\ln k'$) due to the addition of a methylene group is recognized as a measure of hydrophobic interaction in a given RPLC system.

The retention and selectivity of *n*-alkylphenones on the FO column were studied in the micellar eluents comprised of SDS micelles and an organic modifier (referred to as hybrid mobile phases [27,28]). Three different organic modifiers (1-propanol, 2-propanol and tetrafluoro-1-propanol) were investigated, and the results are listed in Table 1. It is clear from Table 1 that the retention factor (k'), instead of the logarithm of k', is linearly dependent on the carbon number (n_C) for all three hybrid eluents. For these systems, the plot of $\ln k'$ *vs.* n_C has a clear curvature and a quadratic equation provides a better correlation than the linear regression. However, a quadratic fit of k' *vs.* n_C plot does not improve the correlation. These results are consistent with those previously observed in the C_{18}-based MLC systems [27]. It has been concluded that the curvature in $\ln k'$ *vs.* n_C plots, which reflects the variation of methylene selectivity with n_C, is due to the fact that different compounds of a homologous series occupy various locations (with different microenvironment polarities) in micelles [27].

Table 1
Retention and selectivity of alkylphenones in hybrid SDS System (FO column)

Compounds	0.10 *M* SDS, 15% 2-PrOH		0.10 *M* SDS, 15% 1-PrOH		0.20 *M* SDS, 3% tetrafluoro-1-PrOH	
	k'	α	k'	α	k'	α
Octanophenone	[a]		11.25	1.18	[a]	
Heptanophenone	12.19	1.14	9.53	1.12	12.25	1.12
Hexanophenone	10.70	1.19	8.49	1.19	10.94	1.13
Valerophenone	8.96	1.27	7.16	1.26	9.69	1.15
Butyrophenone	7.03	1.39	5.69	1.38	8.46	1.18
Propiophenone	5.04	1.44	4.12	1.44	7.14	1.18
Acetophenone	3.49		2.86		6.03	
Linear regression (R^2)						
k' *vs.* n_C	0.9980		0.9978		0.9996	
ln k' *vs.* n_C	0.9655		0.9586		0.9923	

[a] The peaks for octanophenone were not observed at these two mobile phase conditions.

The ratio of retention factors of two compounds differing only in a–CH_2 group, $\alpha(CH_2)$, is smaller on the FO column than that on the C_{18} and C_8 columns [28] at the same mobile phase conditions. This indicates that the interaction between a–CH_2 group and the fluorocarbon phase is less than that with the alkyl phases. This observation has also been reported for conventional hydro–organic mobile phases [19]. Note that $\alpha(CH_2)$ decreases as the homologues become more hydrophobic (with one exception), which was also observed with the alkyl-bonded stationary phases [28]. It has been concluded that the $\alpha(CH_2)$ differences between the compounds in a homologous series are due to the different locations (*i.e.* the different microenvironment polarities) of solubilization in/on micelles in MLC [28]. Also note that the $\alpha(CH_2)$ values for the tetrafluoro-1-propanol-containing eluent are smaller than those for the propanol-containing eluents. This may be due to smaller differences between the polarities of the mobile phase and the stationary phase in the former case despite the fact that the hybrid mobile phase with tetrafluoro-1-propanol is the weakest. Generally, $\alpha(CH_2)$ is inversely related to solvent strength in RPLC, however, this is not the case for MLC as was shown previously [1,3].

3.2. Efficiency

The typical numbers of theoretical plates for different compounds in MLC with the FO column and the C_{18} column are listed in Table 2.

The efficiencies for amino acids, small peptides and sulfonamides are higher on the FO column than those on the C_{18} column. The higher efficiencies obtained on the FO column in MLC are perhaps partly due to the smaller adsorption of surfactant (SDS) on the FO stationary phase surface than that on the C_{18} stationary phase surface. Smaller surfactant adsorption should accelerate the mass transfer and decrease the flow anisotropy [14]. However, this should be further confirmed by studying the adsorption isotherms of the surfactant on both FO column and C_{18} column. It is also clear from Table 2 that the micelle concentration has a great effect on efficiency. The efficiencies of amino acids and small peptides were reduced by about 10 000 theoretical plates per meter with an increase in the SDS concentration from 0.050 to

Table 2
Column efficiencies in MLC[a]

Compounds	Column	Mobile phase	N (plates/m, average)	h [b]	k' range
Amino acids and peptides	FO	0.10 M SDS, 15% 2-propanol	23 300	8.57	1.1–3.4
Amino acids and peptides	C_{18}	0.10 M SDS, 15% 2-propanol	16 000	12.5	3.0–9.9
Sulfonamides	FO	0.1075 M SDS, 8% 1-propanol	23 300	8.57	0.9–7.4
Sulfonamides	C_{18}	0.1075 M SDS, 8% 1-propanol	13 300	15.0	0.5–8.4
Amino acids and peptides	FO	0.05 M SDS, 3% 1-propanol	26 000	7.5	9.3–21.7
Amino acids andpeptides	FO	0.20 M SDS, 3% 1-propanol	16 000	12.0	2.8–5.4

[a] Calculated by using Foley and Dorsey's equation [29].
[b] Reduced plate height.

0.200 M. These results suggest that both stationary phase and mobile phase effects contribute to band broadening in MLC [11–14].

3.3. Solvent strength and selectivity

Simultaneous enhancement of solvent strength and separation selectivity was previously reported in the C_{18}-based MLC system [1–3]. A similar behavior was also observed in the FO-based MLC system for both amino acids and small peptides (ionic compounds) and sulfonamides (non-ionic compounds at pH 3.0) using the hybrid SDS micellar mobile phase conditions (pH 3.0).

In the C_{18}-based MLC system Eqs. 1 and 2 [1–3] describe the dependence of retention factor in MLC on the volume fraction of organic solvent and the micelle concentration, respectively.

$$\ln k' = -S\varphi_{\mathrm{org}} + \ln k'_0 \quad (1)$$

$$1/k' = (K_{\mathrm{mw}}[\mathrm{M}] + 1)/(P_{\mathrm{sw}}\phi) \quad (2)$$

where k' is the retention factor of a solute, φ_{org} is the volume fraction of the organic solvent, k'_0 is the retention factor in a purely aqueous micellar mobile phase, S is the solvent strength parameter, [M] is the micelle concentration, ϕ is the phase ratio, K_{mw} is the binding constant of solute to micelles, and P_{sw} is the partition coefficient of a compound from mobile phase into stationary phase [6].

According to Eqs. 1 and 2, increasing the solvent strength in MLC through an increase in the volume fraction of organic solvent or an increase in the micelle concentration leads to a decrease in retention. Fig. 1 shows that Eqs. 1

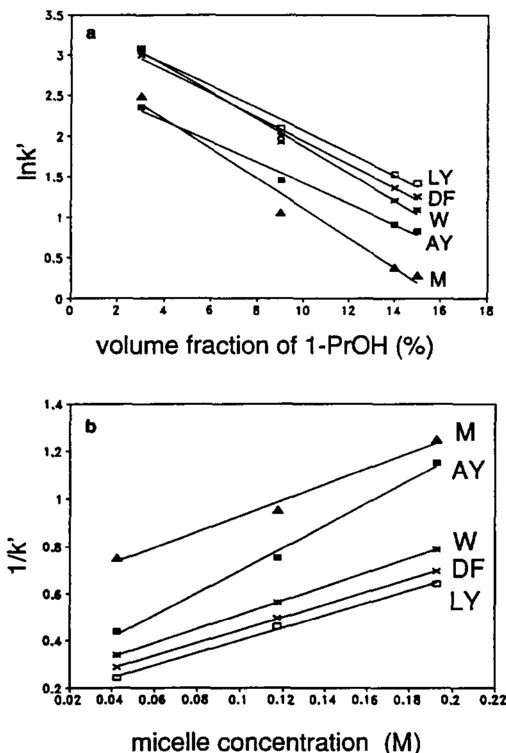


Fig. 1. The effect of (a) the volume fraction of 1-propanol (0.05 M SDS) and (b) the SDS concentration (15% 1-propanol) on the retention of amino acids and small peptides.

and 2 are also valid for an MLC system with fluorinated bonded stationary phase using a group of amino acids and small peptides as test compounds. Good linearity (r, the correlation coefficient, >0.99) for both $\ln k'$ *vs.* φ_{org} and $1/k'$ *vs.* [M] was obtained with the exception of Met ($r = 0.988$) in Fig. 1a, which may be due to the experimental errors. Similar results were also observed for sulfonamides.

Eqs. 3 and 4 show the dependence of selectivity in MLC systems on the volume fraction of organic solvent and micellar concentration [1].

$$\ln \alpha = -(S_2 - S_1)\varphi_{org} + (\ln k'_{0,1} - \ln k'_{0,2}) \quad (3)$$

$$\alpha = \frac{(\alpha_{sw})([M] + 1/K_{mw,1})}{(\alpha_{mw})([M] + 1/K_{mw,2})} \quad (4)$$

Eq. 3 describes the change in selectivity between compounds 1 and 2 ($\alpha = k'_2/k'_1$) when the volume fraction of organic solvent increases from φ_a to φ_b ($\varphi_b > \varphi_a$). In Eq. 4, $K_{mw,1}$ and $K_{mw,2}$ are the binding constants of solutes 1 and 2 to micelles. α_{sw} is the stationary phase partitioning selectivity ($P_{sw,2}/P_{sw,1}$) and α_{mw} is the selectivity of binding to (or partitioning into) micelles ($K_{mw,2}/K_{mw,1}$).

The experimental results for amino acids and small peptides are illustrated in Fig. 2.

Previously, it was shown that there is no direct relation between solvent strength and selectivity in MLC with alkyl-bonded stationary phases [1–3]. This is in contrast to many situations in RPLC and IPC where solvent strength and selectivity are inversely related because of the direct relationship between solvent strength parameter (S) and retention (intercept of Eq. 1, $\ln k'_0$) [1,3,30]. In the MLC system with alkyl-bonded stationary phases, the S values for different compounds would depend on the extent of their interactions with micelles; *i.e.* S values are no longer linearly related to $\ln k'_0$ [1–3]. This is also observed for the FO column as shown in Fig. 3. As a result, simultaneous selectivity enhancement with solvent strength can also occur in MLC systems with fluorinated bonded stationary phases.

The occurrence of simultaneous enhancement of solvent strength and separation selectivity in

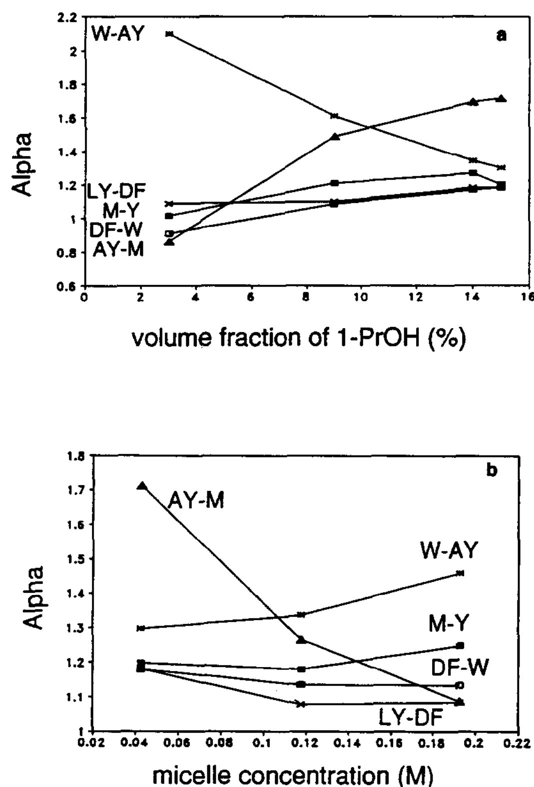


Fig. 2. The effect of (a) the volume fraction of 1-propanol (0.05 M SDS) and (b) the SDS concentration (15% 1-propanol) on the selectivity of amino acids and small peptides.

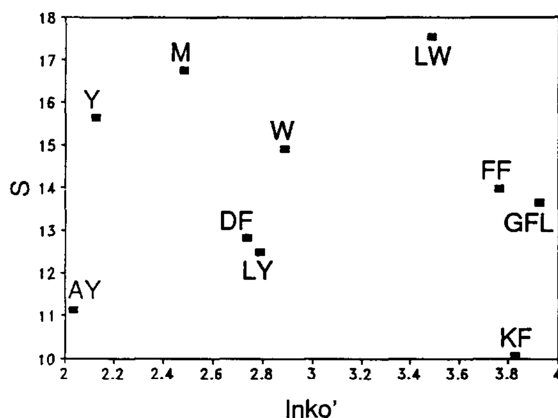


Fig. 3. The plot of solvent strength parameter (S) *vs.* $\ln k'_0$. FO column, 0.11 M SDS, 1-propanol, pH 3.0.

MLC has been attributed to the existence of the competing partitioning equilibria in MLC and to the influence of micelles on the role of the organic solvents [1–3].

Selectivity in MLC is a function of the types and concentrations of micelles and organic modifiers [1,28]. Due to the different types of interactions (such as electrostatic and hydrophobic) and the competing equilibria in MLC, one can expect any forms of the selectivity behavior with the changes in the micelle concentration and the volume fraction of solvent. This is a similar behavior to that in the C_{18}-based MLC system [1–3,28]. This means that the simultaneous optimization of the volume fraction of organic solvent and the micelle concentration is necessary in the C_{18}-based MLC system [3,23,24] as well as the fluorocarbon-based MLC system.

3.4. Type of organic solvents

Snyder's selectivity triangle is a widely accepted method for the characterization of LC solvents and has been employed for solvent selection in conventional HPLC [31–35]. In this paper, the influence of various organic modifiers on retention and selectivity in the FO based MLC system was studied for sulfonamides, amino acids and small peptides.

1-Propanol (from group II), methanol (from group II), tetrahydrofuran (from group III), acetonitrile (from group VIb) and tetrafluoro-1-propanol (from group VIII) were used as organic modifiers in the FO-based MLC of sulfonamides. The effect of organic modifiers on selectivity is illustrated in Fig. 4 for two different pairs of sulfonamides (IXZ-CPD and BZD-IXZ) (see Table 3 for abbreviations). The effect of organic solvents on the separation of eight sulfonamides is illustrated in Fig. 5. The volume fractions of the organic modifiers were adjusted so that the solvent strengths (retention factors) of all five mobile phases remain approximately the same for the last eluting solute (IMD, $k' = 20$). It is clear that the organic solvents have a different influence on the selectivity for a pair of compounds (Fig. 4). However, the differences and similarities in retention pattern and selectivity

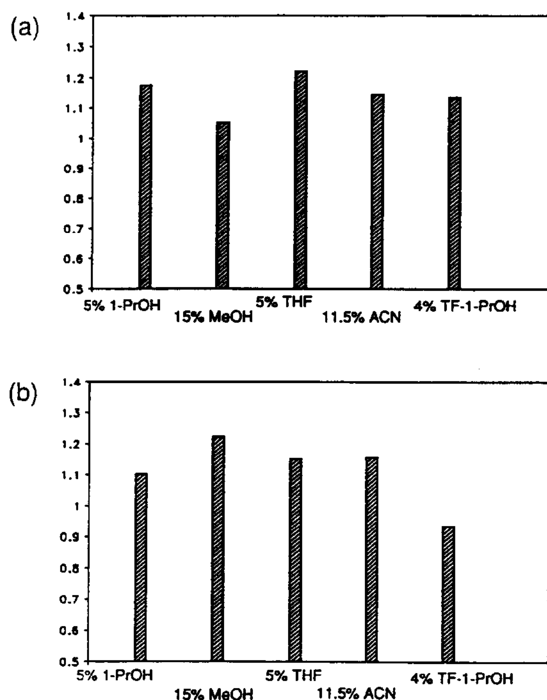


Fig. 4. The effect of organic modifiers on selectivity of sulfonamides at [SDS] = 0.02 *M*. (a) IXZ-CPD, (b) BZD-IXZ. ACN = Acetonitrile; TF = tetrafluoro.

among these five organic solvents can not be explained according to the Snyder's classification. Tetrafluoro-1-propanol has the most significant overall difference on the separation of sulfonamides, which may be due to the similarity between stationary phase and mobile phase. Similar results were also obtained for amino acids and small peptides and in the C_{18}-based MLC systems. Apparently, the existence of the competing partitioning equilibria in MLC and the influence of micelles in the mobile phase alter the effects of organic modifiers such that their role can no longer be explained according to the Snyder's classification of solvent selectivity [36].

3.5. Optimization of separation

It has been previously shown that the effects of the micelle concentration and the volume fraction of organic solvent in MLC should be

Table 3
Abbreviation of solutes

Amino acids and small peptides		Sulfonamides	
Components	Abbreviation	Components	Abbreviation
Ala–Tyr	AY	Sulfisoxazole	IXZ
Asp–Phe	DF	Sulfachloropyridazine	CPD
Leu–Tyr	LY	Sulfabenzamide	BZD
Met	M	Sulfacetamide	CTM
Trp	W	Sulfadiazine	DIA
Tyr	Y	Sulfamerazine	MRZ
Leu–Trp	LW	Sulfadimethoxine	DMX
Lys–Phe	KF	Sulfisomidine	IMD
Gly–Phe–Leu	GFL	Sulfamethazine	MTZ
Phe–Phe	FF	Sulfinpyrazone	IPZ

evaluated simultaneously. Optimization of one mobile phase variable at a time is ineffective in MLC due to the interactive nature of the variables.

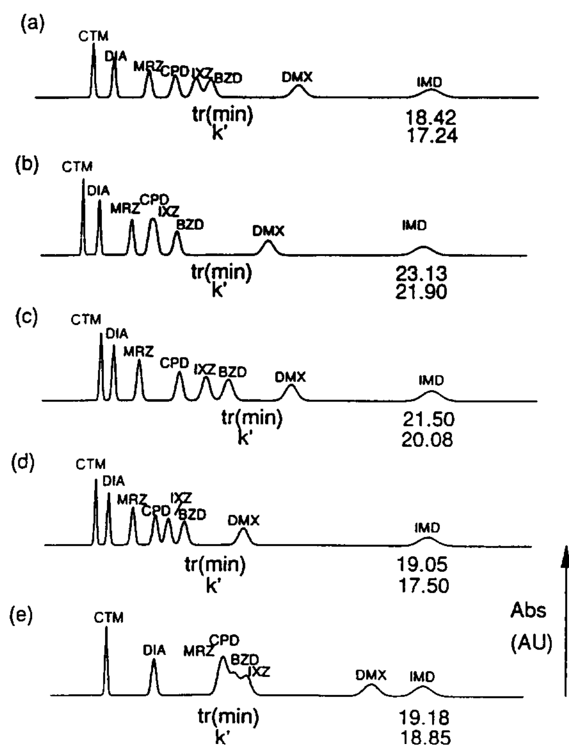


Fig. 5. The reconstructed chromatograms of a mixture of eight sulfonamides based on the experimental retention and efficiency data at 0.02 *M* SDS (a) 5% 1-propanol, (b) 15% methanol, (c) 5% tetrahydrofuran, (d) 11.5% acetonitrile, (e) 4% tetrafluoro-1-propanol.

Consequently, the iterative regression (IR) optimization design has been successfully applied for the multiparameter optimization in MLC [23,24]. This optimization strategy is based on the assumption that retention ($\ln k'$) is a linear function of the parameters within the parameter space. As shown in Eq. 1, it is certain that $\ln k'$ is linearly related to the volume fraction of organic modifier (1-propanol). According to Eq. 2, $1/k'$ is linearly proportional to the micelle concentration. However, the algorithm of the IR program that was used in this work is based on the assumption that retention ($\ln k'$) is a linear function of the two parameters (volume fraction of organic modifier and surfactant concentration) within the parameter space [23,24]. The results of the regressions between $1/k'$ *vs.* micelle concentration and $\ln k'$ *vs.* surfactant concentration are listed in Table 4 for the amino acids and small peptides. Acceptable linearity is observed for $\ln k'$ *vs.* surfactant concentration within the selected range (0.05–0.20 *M*). The experimental design for the optimization of the two important parameters, the concentration of surfactant (SDS) and the volume fraction of organic solvent (1-propanol), was based on only five initial experiments, four at the corners of a square and one at the center [23]. The corner values of the parameters are limited by the practical conditions of chromatographic systems. The lower surfactant concentration is chosen well above the critical micelle concentration of the surfactant (*ca.* 8 m*M* for SDS at ambient

Table 4
The comparison of regression

Components	$1/k'$ *vs.* [micelle]	r	$\ln k'$ *vs.* [surfactant]	r
AY	$1/k' = 4.739[M] + 0.223$	0.9977	$\ln k' = -6.423[SDS] + 1.126$	0.9976
DF	$1/k' = 2.719[M] + 0.172$	0.9999	$\ln k' = -5.902[SDS] + 1.510$	0.9909
LY	$1/k' = 2.655[M] + 0.135$	0.9988	$\ln k' = -6.474[SDS] + 1.689$	0.9843
M	$1/k' = 3.321[M] + 0.594$	0.9937	$\ln k' = -3.389[SDS] + 0.461$	0.9992
W	$1/k' = 2.997[M] + 0.210$	1.0000	$\ln k' = -5.641[SDS] + 1.339$	0.9933

Mobile phase compositions: SDS concentration range: 0.05–0.20 *M* and 15% 1-propanol. r = Correlation coefficient.

temperatures and without organic modifier) and can elute all the components. The upper surfactant concentration is controlled by a combination of some factors, such as the solubility of the surfactant in mobile phase, the viscosity of the resulting mobile phase and degradation of the efficiency at higher surfactant concentrations. The organic modifier concentration is limited to a maximum of *ca.* 15% to ensure the integrity of the micelles [23,24]. The retention of all the ten amino acids and small peptides were measured at these five mobile phase compositions. The optimum mobile phase composition of 14% 1-propanol and 0.05 *M* SDS was predicted by the IR program.

Excellent agreement exists between the predicted and the observed separation for these ten amino acids and small peptides as illustrated in Fig. 6. The plot of k' (observed) *vs.* k' (predicted) for these ten amino acids and small peptides is very good as shown in Fig. 7. Apparently, the assumed linear model of ln k' *vs.* the parameters is valid and retention behavior is reproducible. These results indicate that good separation of ionic solutes with a minimum experimental effort can be achieved in the FO-based MLC as well.

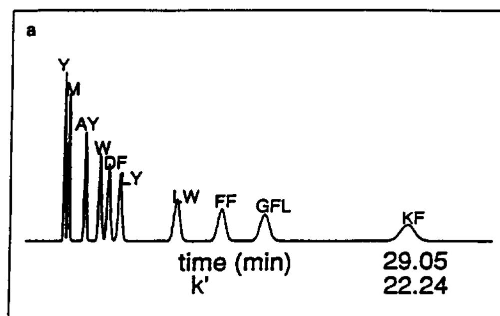
3.6. Stationary phase effect

Amino acids and small peptides (ionic compounds) and sulfonamides (non-ionic compounds at pH 3.0) were used as the test solutes to compare the stationary phase effects (FO *vs.* C_{18}) on retention, selectivity and the overall elution pattern.

The iterative regression optimization computer program [23,24] was used to optimize the separation of ten amino acids and small peptides on both the FO and C_{18} columns. The predicted optimized separations are illustrated in Fig. 8.

These ten amino acids and small peptides can be separated on a 15-cm FO column with the total analysis time of about 29 min. The same set of solutes could be separated on a 25-cm C_{18} column with the total analysis time of about 66.5

Predicted



Observed

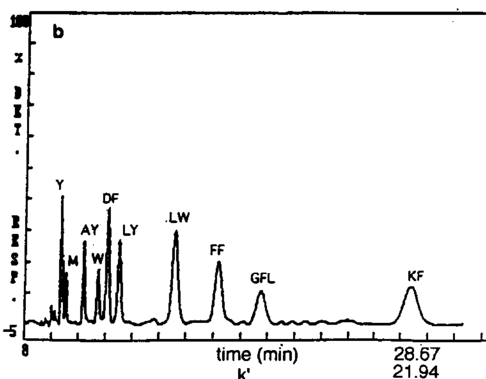


Fig. 6. The predicted (a) and observed (b) chromatograms of amino acids and peptides at the optimum mobile phase composition (0.05 *M* SDS + 14% 1-propanol) for ten amino acids and small peptides. FO column, 15 cm; pH 3.0.

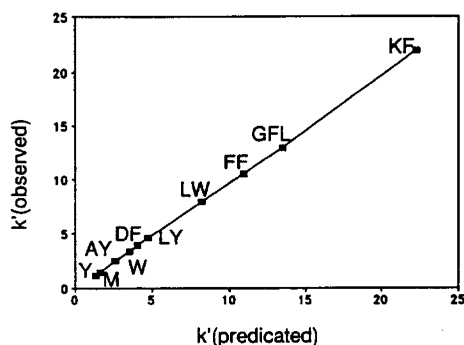


Fig. 7. The observed capacity factors *vs.* the predicted capacity factors for the ten amino acids and small peptides at the optimum mobile phase condition.

min. A shorter FO column (as compared to a C_{18} column) can be used to achieve the desired separation because of the higher efficiency gained from the faster mass transfer. Shortened analysis times are observed on the FO column due to both less interactions between fluorocarbon groups and the solutes and because of using a shorter column. Different elution orders (selectivity changes) of the solutes are observed on the FO column which indicates the different solute–stationary phase interactions. In addition, the differences in retention behavior could partly be attributed to the different mobile phase pH and organic modifiers.

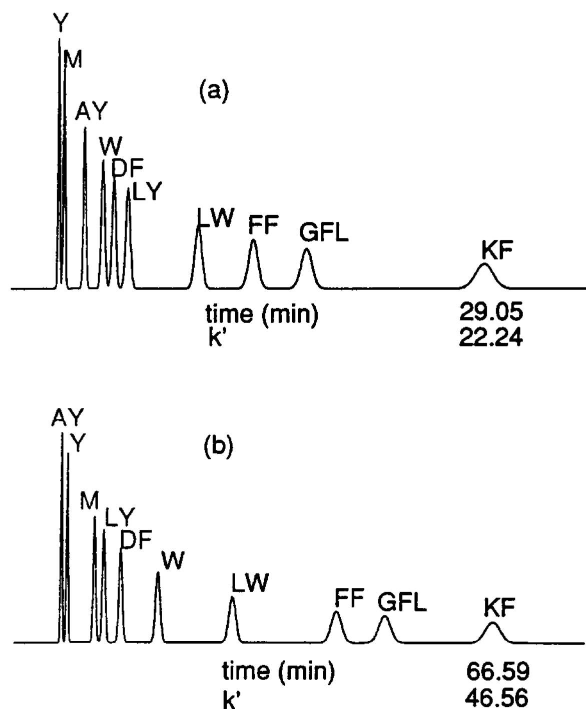


Fig. 8. The predicated optimized separation of ten amino acids and small peptides (a) 15-cm Flurooctyl column, 14% 1-propanol, 0.05 *M* SDS, pH 3.0; (b) 25-cm C_{18} column, 11% 2-propanol, 0.10 *M* SDS, pH 2.5.

To further confirm the stationary phase effect, the exact same mobile phase conditions were applied for the separation of sulfonamides on both FO and C_{18} columns. The reconstructed chromatograms of seven sulfonamides on a 15-cm FO column and a 15-cm C_{18} column are illustrated in Fig. 9 for both columns. Better separation was achieved on the FO column because of the increased retention and different selectivities for the early eluting peaks. The separations shown in Fig. 9 were obtained under non-optimized conditions using FO and C_{18} columns but were performed by using the same mobile phase composition. The same efficiency ($N = 3000$) was used to reconstruct the chromatograms for both FO and C_{18} columns; however, lower efficiency was observed on the C_{18}

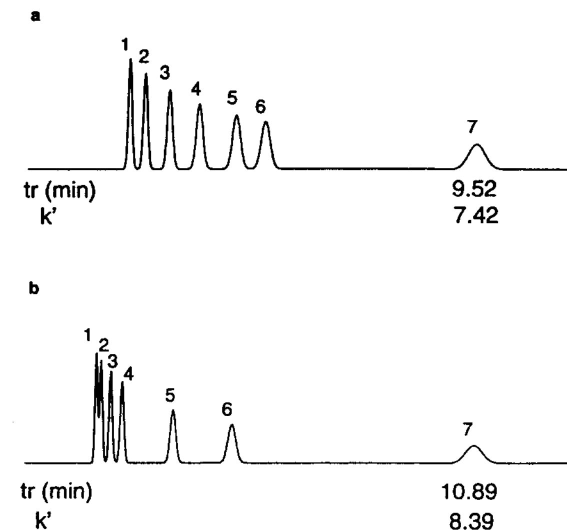


Fig. 9. The reconstructed chromatograms of seven sulfonamides based on the experimental retention data and $N = 3000$ at 0.1075 *M* SDS, 8% 1-propanol, pH 3.0 (a) 15-cm FO column, (b) 15-cm C_{18} column. Peaks: 1 = CTM; 2 = DIA; 3 = MRZ; 4 = MTZ; 5 = DMX; 6 = IMD; 7 = IPZ.

column (see Table 2). This means that a longer C_{18} column should be used to achieve the illustrated separation.

4. Conclusions

The possibility of using fluorinated bonded stationary phases in MLC was investigated. Higher efficiencies for both ionic and non-ionic compounds in MLC were obtained on the fluorinated bonded stationary phase probably because of the faster mass transfer. Shortened analysis times for hydrophobic compounds and increased retention for hydrophilic compounds were observed on a FO column. Better and faster separations can be obtained on the FO column for some compounds. In addition to the remedies that have already been suggested [11,13], the poor efficiency in MLC system might be improved by using stationary phases on which surfactant is less adsorbed. Stationary phase is a very important factor in MLC because it affects efficiency and overall separation. However, additional studies on the effect of stationary phase on efficiency and separation in MLC are needed.

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6. References

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