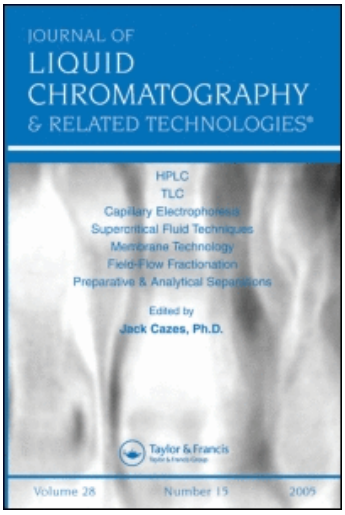

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LINEAR SOLVATION ENERGY RELATIONSHIP STUDY OF RETENTION IN MICELLAR LIQUID CHROMATOGRAPHY ON A C18 COLUMN USING SODIUM DODECYL SULFATE AND CETYLTRIMETHYLAMMONIUM BROMIDE MOBILE PHASES WITH ALCOHOL MODIFIERS

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ABSTRACT

The fundamental chemical interactions governing the retention of 15 solutes in 40 micellar reversed-phase liquid chromatographic systems using sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), methanol, n-propanol, and n-butanol as mobile phase additives are studied using linear solvation energy relationships (LSERs). The influence of solute properties on retention in MLC and the trends in the coefficients as a function of SDS and CTAB concentrations are investigated. The ability of the LSERs to account for the chemical interactions underlying solute retention is shown.

A comparison of predicted and experimental retention factors suggests that LSER formalism may not completely model the energetics of retention in MLC, but that the discrepancies, although systematic, are generally small. Finally, the effects of the addition of 0.035 M SDS to 10% methanol/90% water mobile phases on solute retention are discussed.

INTRODUCTION

Surfactant micelles, when added to reversed-phase liquid chromatographic (RPLC) mobile phases, alter the retention and selectivity of charged and neutral solutes.[1,2] Cationic, anionic, and neutral surfactants have been used in separations of a wide range of solute classes including pharmaceutical, biological, and industrial samples.[3-8] Additionally, micellar liquid chromatography (MLC) represents a technique for measuring solute-micelle binding constants through the use of the Armstrong-Nome equation[9] and Arunyanart and Cline Love's extension of it to charged solutes.[10] MLC is also used to quantify solute hydrophobicity via correlations of retention factors in micellar mobile phases with octanol-water partition coefficients.[11-14]

In some situations, MLC can offer many advantages over conventional RPLC. For instance, cationic, anionic, and neutral species can be separated simultaneously in MLC.[2,15] Also, gradient elution analyses can be very fast since the concentration of free surfactant monomers in the mobile phase remains essentially constant in the post-critical micelle concentrations (CMC) region.[16]

Thus, the amount of sorbed surfactant in the stationary phase remains constant, and little or no column re-equilibration time is required before a new separation is started.[16]

Finally, MLC offers enhanced selectivity in some separations through increased luminescence intensity for some solutes[17,18] when they are incorporated in micelles, but more typically because of the complex phase-transfer phenomenon occurring within the column.[1,2,19-22]

The major disadvantage of MLC, however, is the poor chromatographic efficiencies observed,[1,16,22] especially in mobile phases comprised solely of water and the surfactant being used. This limitation can be overcome, however, by the addition of small amounts of organic modifiers to the mobile phase.[1,16,23,24] Landy and Dorsey found that adding 3% n-propanol to sodium dodecyl sulfate (SDS) mobile phases and working at 40°C produced efficiencies similar to those obtained using hydro-organic mobile phases.[23]

Similar results were found with cetyltrimethylammonium bromide (CTAB) and polyoxyethylene(23) lauryl ether (Brij-35) mobile phases.[16]

Additionally, Khaledi *et al*. investigated the effect of methanol, 2-propanol, and n-butanol in MLC on solvent strength and selectivity.[2] Finally, Berthod and Roussel studied the effects of methanol, n-propanol, n-pentanol, and tetrahydrofuran and concluded that n-propanol and tetrahydrofuran produced the largest increases in efficiency in MLC.[24]

Given that the addition of alcohol modifiers to micellar mobile phases has become standard practice due to the above mentioned studies, we have studied the effects of methanol, n-propanol, and n-butanol as modifiers in MLC with the aim of understanding retention in these systems. Specifically, our interest lies in understanding the fundamental chemical interactions responsible for retention in MLC and variations of these interactions as a function of the nature and concentration of the surfactant and organic modifier. In that regard, we have used linear solvation energy relationships (LSERs)[25-28] to explain retention in MLC systems using aqueous mobile phases containing SDS or CTAB and 3 to 10% (v/v) methanol, n-propanol, or n-butanol.

The data in this study cover 40 different micellar mobile phases using 15 aromatic solutes with different functional groups to probe a variety of chemical interactions.

In this study we use the same LSER methodology that has been used by several authors to correlate solute retention factors in conventional RPLC[25,29-33] with parameters describing the solute's size/polarizability (V_X), dipolarity/polarizability (π_2^H), hydrogen bond (HB) donating ability ($\Sigma\alpha_2^H$), HB accepting ability ($\Sigma\beta_2^H$), and excess molar refraction (R_2).[34] The general LSER equation used is:

$$\log k = \log k_o + m(V_X/100) + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + rR_2 \qquad (1)$$

where the coefficients **m**, **s**, **a**, **b**, and **r** are obtained through a multiparameter linear regression and reflect differences in the two bulk phases between which the solute is transferring.[35] The log k_o term is simply the intercept of the regression and is comprised of constant contributions from the solutes and the chromatographic system. We note that V_X and π_2^H are blends of two different interactions.

Thus, the coefficients of these parameters are also blends of the corresponding properties. Specifically, **m** is the difference in the cohesivity/dispersive ability of the two bulk phases, and **s** is the difference in the ability of the two phases to interact through dipole-dipole and dipole-induced dipole interactions. Numerous examples of LSERs pertaining to liquid chromatography and their interpretations have been published and the interested reader is referred to them for a more detailed discussion of equation 1 and the meaning of the parameters.[29-33]

Relative to non-micellar RPLC systems, the interpretation of MLC LSERs is complicated by the fact that the mobile phase contains discrete aggregates which provide microenvironments into which the solutes can partition.[9,10] The system is therefore commonly described using a three-phase model (mobile, stationary, and micellar phases) with three accompanying partition coefficients (mobile to stationary phase, mobile to micelle phase, and stationary to micelle phase transfers).[9,10] We note that we performed regressions on retention factor data only, which, as Hinze and Weber emphasize, relies solely on the distribution of the solutes between the mobile and stationary phases.[36] Thus, thermodynamically, this is the only distribution upon which our mathematical results depend; we invoke the three-phase model only as a means of interpreting the LSER results. Specifically, we view the mobile phase as a concentration-weighted sum of the micellar and bulk aqueous phases.

Thus, changes in the LSER coefficients with increasing surfactant concentration are interpreted by asserting that the contribution of the micellar phase to the overall effective chemical nature of the mobile phase has increased. In other words, based on mass action principles, the influence of the micellar phase on the behavior of solutes increases as the amount of micellar phase present in the mobile phase increases.

Regarding the interpretations of the role of the stationary phase in determining changes in the LSER coefficients as a function of surfactant concentration, we point out that Dorsey, Khaledi, Landy, and Lin,[37] Berthod, Girard, and Gonnet[38] and Jandera and Fischer[39] have shown that the amount of surfactant sorbed by the stationary phase on a C-18 column remains constant above the CMC for SDS- and CTAB- containing mobile phases in pure water and 5% methanol/95% water mixtures.[40] Since we are working at concentrations well above the CMC and using C-18 bonded stationary phases, we feel it is reasonable to assume that the stationary phases in our studies do not change as the surfactant concentration is varied.

We, at times, rely on this assumption when interpreting changes in the LSER coefficients as a function of surfactant concentration. Finally, we note that different modifiers and different modifier concentrations within the same surfactant system have been shown to cause changes in the total amount of sorbed surfactant, but that the amount will not vary as a function of the surfactant concentration.[24]

EXPERIMENTAL

The benzene and naphthalene derivatives used were: (1) benzene, (2) benzyl alcohol, (3) benzamide, (4) toluene, (5) benzonitrile, (6) nitrobenzene, (7) phenol, (8) 2-phenylethanol, (9) chlorobenzene, (10) phenylacetonitrile, (11)

3,5-dimethylphenol, (12) naphthalene, (13) 1-naphthol, (14) 2-naphthol, (15) 1-naphthylamine.

Sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), n-propanol, and n-butanol were from Merck and methanol was from Scharlau. All were of the highest purity available and used as received.

Experimental micellar liquid chromatography data used in this work were determined as previously described.[22,41] The chromatographic system consisted of a Model 510 pump, a Model U6K injector, a Model 440 fixed-wavelength (254 nm) detector, and a Model 740 data module (all from Waters). Retention data (three replicates, RSD from 0 to 4.5 %) were obtained with a 15 cm x 3.9 mm I.D. Spherisorb ODS 2 (dp = 5 μm) column (Teknokroma) and a 15 cm x 3.9 mm I.D. Nova-Pak C-18 (dp = 4 μm) column (Waters).

The columns and mobile phases were water-jacketed and maintained at 25°C with a circulating water bath. LSERs were determined using the regression algorithm in Excel and solute parameters from Abraham et al.[34]

RESULTS AND DISCUSSION

The LSER equations describing each subset of data are presented in Table 1 and Table 2. The correlation coefficients are lower than we normally obtain for RPLC, ranging from as low as 0.920 to a quite acceptable 0.992. The average correlation coefficient of all the regressions is 0.976 (0.983 if the 3% n-propanol/CTAB systems are omitted) and the average standard error of the regressions is 0.080 (0.074 if the 3% n-propanol/CTAB is omitted). While these correlation coefficients are poorer than those typically obtained in RPLC LSERs (approximately 0.99), the average standard errors are similar to those encountered in RPLC LSERs (0.078).[33] The poorer correlations may be the result of inadequate modeling of the complex transfer phenomena occurring in MLC. Further evidence of inadequate modeling is presented below.

In Table 3 the variance-covariance matrix for the solute parameters is presented. We note that all of the parameters covary to some extent, with a few covarying to a very great extent. Correlations such as these can lead to inaccurate determinations and large standard deviations of the LSER coefficients. Although the uncertainties in the coefficients that we observe are indeed quite large, the coefficients and trends in the data are chemically reasonable and consistent with known properties of micelles and liquid chromatography, lending confidence to their reliability and chemical significance. Therefore, we will interpret the trends with the acknowledged possible influence of parameter covariance and apparent statistical equivalence of most of the coefficients.

Table 1

LSER Equations for 20 Different SDS Mobile Phases with C-18 Stationary Phases[a]

[SDS] (M) 10% Methanol	$Logk_0$	m	s	A	b	r	R	s.d.
0.035	0.24 (0.24)	1.93 (0.35)	-0.57 (0.15)	-0.47 (0.11)	-1.50 (0.24)	0.35 (0.14)	0.984	0.092
0.050	0.29 (0.22)	1.76 (0.32)	-0.52 (0.14)	-0.45 (0.10)	-1.37 (0.22)	0.28 (0.13)	0.984	0.083
0.067	0.36 (0.23)	1.48 (0.33)	-0.48 (0.15)	-0.46 (0.11)	-1.21 (0.23)	0.30 (0.14)	0.979	0.087
0.080	0.44 (0.18)	1.31 (0.27)	-0.47 (0.12)	-0.47 (0.09)	-1.14 (0.18)	0.29 (0.11)	0.985	0.070
0.100	0.41 (0.17)	1.30 (0.25)	-0.45 (0.11)	-0.41 (0.08)	-1.11 (0.17)	0.22 (0.10)	0.985	0.066
10% n-Propanol								
0.035	0.30 (0.25)	1.99 (0.37)	-0.44 (j0.16)	-0.58 (0.11)	-1.90 (0.23)	0.23 (0.16)	0.989	0.088
0.050	0.45 (0.21)	1.65 (0.30)	-0.43 (0.13)	-0.56 (0.09)	-1.65 (0.19)	0.25 (0.13)	0.991	0.072
0.067	0.39 (0.19)	1.61 (0.28)	-0.38 (0.12)	-0.55 (0.08)	-1.59 (0.18)	0.18 (0.12)	0.991	0.067
0.080	0.46 (0.19)	1.44 (0.28)	-0.36 (0.12)	-0.56 (0.08)	-1.49 (0.17)	0.19 (0.12)	0.992	0.066
0.100	0.45 (0.19)	1.32 (0.28)	-0.35 (0.12)	-0.55 (0.08)	-1.41 (0.18)	0.20 (0.12)	0.990	0.066
10% n-Butanol								
0.020	0.33 (0.22)	1.62 (0.32)	-0.56 (0.14)	-0.33 (0.10)	-1.89 (0.21)	0.28 (0.13)	0.989	0.082
0.035	0.36 (0.21)	1.38 (0.30)	-0.50 (0.13)	-0.38 (0.10)	-1.67 (0.20)	0.25 (0.12)	0.987	0.078
0.050	0.39 (0.20)	1.20 (0.29)	-0.49 (0.13)	-0.39 (0.09)	-1.52 (0.20)	0.26 (0.12)	0.986	0.077
0.067	0.41 (0.21)	1.06 (0.30)	-0.46 (0.13)	-0.39 (0.10)	-1.41 (0.20)	0.26 (0.12)	0.984	0.078
0.080	0.41 (0.20)	0.98 (0.29)	-0.45 (0.13)	-0.39 (0.10)	-1.32 (0.20)	0.25 (0.12)	0.984	0.075

Table 1 (Continued)

[SDS] (M) 5% n-Butanol	Logk$_a$	m	s	A	b	r	R	s.d.
0.020	0.32	1.86	-0.65	-0.41	-1.94	0.36	0.977	0.129
	(0.34)	(0.49)	(0.22)	(0.16)	(0.34)	(0.20)		
0.035	0.35	1.65	-0.59	-0.47	-1.77	0.33	0.981	0.107
	(0.28)	(0.41)	(0.18)	(0.13)	(0.28)	(0.17)		
0.050	0.39	1.45	-0.56	-0.46	-1.60	0.32	0.981	0.099
	(0.26)	(0.38)	(0.17)	(0.12)	(0.26)	(0.16)		
0.067	0.42	1.33	-0.53	-0.45	-1.51	0.27	0.981	0.093
	(0.25)	(0.36)	(0.16)	(0.11)	(0.24)	(0.15)		
0.080	0.46	1.18	-0.49	-0.44	-1.38	0.27	0.981	0.086
	(0.23)	(0.33)	(0.14)	(0.11)	(01.26)	(0.14)		

[a] Standard deviations for each coefficient are shown in parentheses.

SDS Systems

Influence of Solute Properties on Retention in SDS Systems

The magnitudes of LSER coefficients as a function of SDS concentration with 10% methanol (v/v) in the eluent (Table 1) indicate that solute size (V_X) and HB basicity ($\Sigma\beta_2^H$) are the two most important solute parameters determining retention in this system. This is completely consistent with studies of RPLC using non-micellar aqueous mobile phases.[29-33] The relative magnitudes of the coefficients in MLC and non-micellar RPLC are discussed below.

As in non-micellar RPLC, larger solutes are more retained than are smaller solutes, presumably a manifestation of the hydrophobic effect. Compounds with higher HB basicities are less retained than compounds with lower HB basicities.

This arises from the HB interactions between the strong HB donating ability of water in the mobile phase and the HB accepting ability of HB basic solutes. Solutes with high dipolarity/polarizability and HB acidity are less retained than are solutes with lower ability to interact through these forces. Again, this is presumably due to favorable interactions of the solute with the water in the aqueous phase. Finally, solutes with higher excess molar refractivities are more retained than are those with lower excess molar refractivities.

Table 2

LSER Equations for 20 Different CTAB Mobile Phases With C-18 Stationary Phases

[CTAB] (M) 3% n-Propanol	$Logk_a$	m	s	a	b	r	R	s.d.
0.035	0.71	0.99	-0.34	-0.02	-1.07	0.44	0.930	0.139
	(0.36)	(0.53)	(0.23)	(0.17)	(0.17)	(0.22)		
0.050	0.74	0.81	-0.30	-0.00	-0.93	0.39	0.927	0.121
	(0.32)	(0.47)	(0.20)	(0.15)	(0.32)	(0.19)		
0.067	0.71	0.70	-0.32	-0.03	-0.86	0.41	0.930	0.116
	(0.30)	(0.45)	(0.19)	(0.14)	(0.30)	(0.18)		
0.080	0.70	0.59	-0.28	-0.06	-0.74	0.34	0.923	0.106
	(0.23)	(0.41)	(0.18)	(0.13)	(0.28)	(0.15)		
0.100	0.66	0.51	-0.25	-0.08	-0.66	0.34	0.920	0.098
	(0.26)	(0.38)	(0.16)	(0.12)	(0.26)	(0.15)		
5% n-Propanol								
0.035	0.64	1.27	-0.27	0.09	-1.45	0.22	0.978	0.076
	(0.20)	(0.29)	(0.13)	(0.09)	(0.20)	(0.12)		
0.050	0.78	0.95	-0.32	0.02	-1.08	0.21	0.978	0.063
	(0.17)	(0.24)	(0.11)	(0.08)	(0.17)	(0.10)		
0.067	0.70	0.88	-0.23	0.01	-1.07	0.15	0.975	0.062
	(0.16)	(0.23)	(0.10)	(0.08)	(0.16)	(0.10)		
0.080	0.66	0.84	-0.20	0.00	-1.02	0.13	0.975	0.058
	(0.15)	(0.22)	(0.09)	(0.07)	(0.15)	(0.09)		
0.100	0.65	0.74	-0.17	-0.01	-0.93	(0.11)	0.975	0.051
	(0.13)	(0.20)	(0.08)	(0.06)	(0.13)	(0.08)		
10% n-Propanol								
0.035	0.54	1.34	-0.35	-0.07	-1.55	0.28	0.987	0.065
	(0.17)	(0.25)	(0.11)	(0.08)	(0.17)	(0.10)		
0.050	0.57	1.15	-0.31	-0.08	-1.36	0.23	0.985	0.062
	(0.16)	(0.24)	(0.10)	(0.08)	(0.16)	(0.10)		
0.067	0.58	0.94	-0.30	-0.10	-1.18	0.26	0.980	0.065
	(0.17)	(0.25)	(0.11)	(0.08)	(0.17)	(0.10)		
0.80	0.57	0.90	-0.26	-0.08	-1.14	0.20	0.986	0.050
	(0.13)	(0.19)	(0.08)	(0.06)	(0.13)	(0.08)		
0.100	0.54	0.82	-0.24	-0.09	-1.04	0.17	0.987	0.045
	(0.12)	(0.17)	(0.08)	(0.06)	(0.12)	(0.07)		

Table 2 (Continued)

[CTAB] (M) 5% n-Butanol	$Logk_w$	m	s	a	b	r	R	s.d.
0.020	0.54	1.46	-0.53	-0.11	-1.85	0.36	0.985	0.090
	(0.24)	(0.35)	(0.15)	(0.11)	(0.23)	(0.14)		
0.035	0.60	1.16	-0.44	-0.14	-1.56	0.28	0.983	0.080
	(0.21)	(0.31)	(0.13)	(0.10)	(0.21)	(0.12)		
0.050	0.61	1.02	-0.40	-0.15	-1.38	0.23	0.984	0.069
	(0.18)	(0.27)	(0.12)	(0.08)	(0.18)	(0.11)		
0.067	0.62	0.87	-0.36	-0.15	-1.24	0.20	0.984	0.062
	(0.16)	(0.24)	(0.10)	(0.08)	(0.16)	(0.10)		
0.080	0.63	0.77	-0.34	-0.16	-1.15	0.18	0.984	0.058
	(0.15)	(0.22)	(0.10)	(0.07)	(0.15)	(0.09)		

[a] Standard deviations for each coefficient are shown in parentheses.

Table 3

Correlation Matrix of Solute Parameters

	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	V_x
R_2	1.000				
π_2^H	0.512	1.000			
$\Sigma\alpha_2^H$	0.398	0.304	1.000		
$\Sigma\beta_2^H$	0.304	0.716	0.460	1.000	
V_x	0.820	0.531	0.383	0.518	1.000

Trends in the Coefficients as a Function of SDS Concentration

The largest changes in the coefficients as a function of SDS concentration occur for **m** and **b,** whose magnitudes decrease as the SDS concentration increases. The **s** coefficient behaves similarly, but undergoes a much smaller change. Chemically, the **m** coefficient decreases because increasing the surfactant concentration increases the concentration of micelles. This allows more solutes to partition into the less cohesive/more dispersive micellar microenvironment in the mobile phase, resulting in an overall decrease in the effective mobile phase cohesivity and an increase in the mobile phase dispersive ability relative to the cohesivity/dispersion of the stationary phase. This also explains the behavior of **s** and **b** since, as more solutes partition out of the aqueous environment of the mobile phase into the micellar microenvironments, the average solute environment appears to be less polar and have less HB donating ability. In a sense, the micellar environment shields the solutes from interactions with water. The **a** and **r** coefficients do not exhibit distinct trends as a function of surfactant concentration.

The constant **a** coefficient implies that the overall effective basicity of the mobile phase does not change as a function of the SDS concentration. Since the cohesivity and the acidity change, the constancy of the mobile phase basicity relative to that of the stationary phase is somewhat surprising. However, it is possible that this behavior arises from interactions of the solute with the sulfate head groups, which offset the loss of interactions with water. The **r** coefficient shows that the stationary phase is slightly better able to interact with polarizable molecules (all other parameters being equal) than is the mobile phase and that this behavior remains relatively constant as a function of SDS concentration.

As explained in the introduction, the above discussions regarding variations of the LSER coefficients rely on the fact that the properties of the stationary phase remain essentially constant as the SDS concentration is increased. Thus, changes in the coefficients are interpreted entirely as changes in the mobile phase relative to the constant chemistry of the stationary phase.

The same trends in the LSER coefficients are present in the SDS systems containing 10% n-propanol, 10% n-butanol, and 5% n-butanol (see Figure 1). The interpretations of these systems are identical to that presented above for 10% methanol in SDS mobile phases. In general, these trends indicate that from the point of view of the solutes, as the concentration of surfactant increases, the mobile phase becomes more like the stationary phase in terms of its chemical interactions with the solutes.

Given the general structural similarity of surfactants and stationary phases (i.e. alkyl chains attached to polar heads groups), this is a chemically rea-

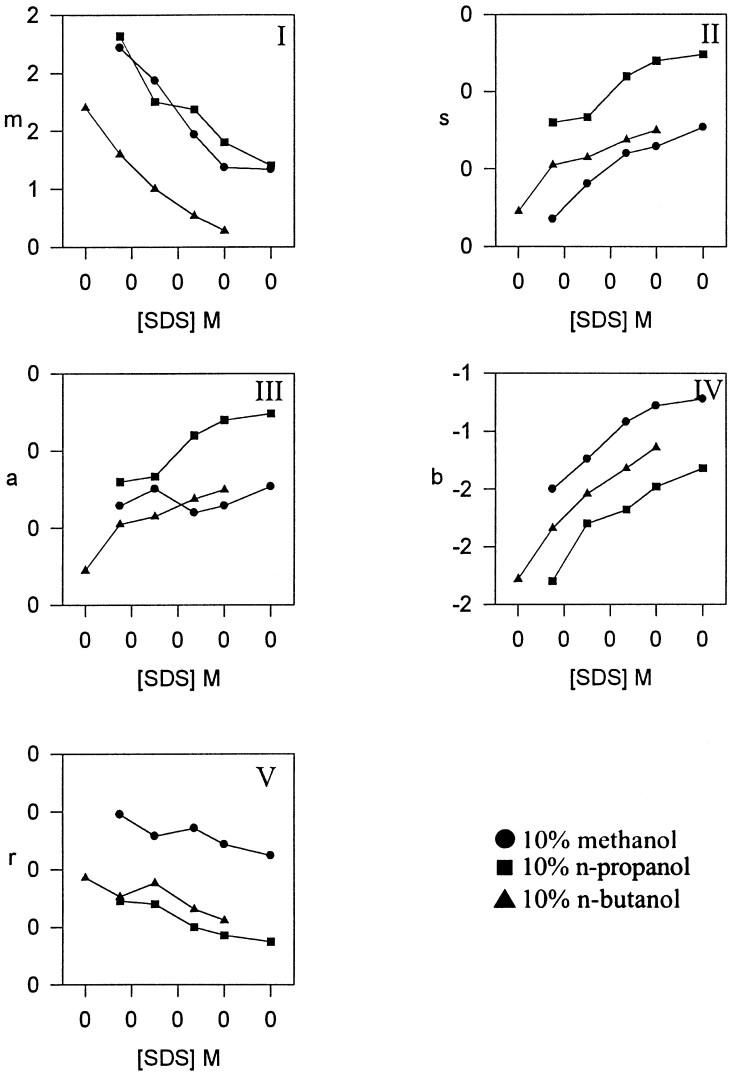


Figure 1. LSER coefficients as a function of modifier chain length and SDS concentration. Graphs I-V are as in Figure 1. Modifiers are 10% methanol, 10% n-propanol, and 10% n-butanol. Error bars have been omitted for clarity. Standard deviations for each coefficient are listed in Table 1.

sonable observation. This lends confidence to the assertion that the LSERs are properly reflecting changes in the system as a function of the SDS concentration.

Residuals of LSER Equations for SDS Systems

Figure 2a is a plot of the experimental log k values versus log k values calculated from the LSER equation for the 10% methanol/0.035 M SDS system. Figure 2b shows residual values (calculated minus experimental log k) for each solute in all five SDS concentrations in 10% methanol normalized to the average standard deviation for each fit. If the LSER model is entirely accurate, we expect to see a random distribution of residuals that is independent of SDS concentration. Instead, we see that the distribution of residuals remains nearly constant as the SDS concentration is varied.

We conclude from this that there are contributions to retention that are not properly accounted for by the LSER formalism — a conclusion which is further supported by the fact that the pattern of residuals is nearly the same in the other SDS systems with different alcohol modifiers (results not shown). In other words, we have not achieved the level of "exhaustive fitting".

One possible explanation for the systematic nature of the residuals is that the LSER equation is not appropriate for this system, given that LSERs were developed to model solute transfer in two-phase systems. MLC, however, is better represented by a three-phase model, as discussed in the introduction. Another explanation for systematic deviations is that the model as stated in equation 1 may be incomplete. It is reasonable to suggest that there are ion-dipole interactions that are not accounted for in the LSER formalism which arise from the solute interacting with the charged head groups and their counterions. The lack of such an ion-dipole interaction term in the LSER could then lead to systematic deviations. We note that the retention factor data are not questioned, as measurement errors in these values are random and would lead to random deviations from the LSER fits as a function of the surfactant concentration, not the systematic deviations that we observe.

Despite the systematic nature of these residuals, it is clear from Figure 2a that the LSER is doing a reasonable job of reproducing the log k values and can be used to predict, albeit only roughly, the log k value of a solute with known parameters.

This predictive power can aid in choosing a starting point for methods development when deciding which surfactant, modifier, and concentration of each of these mobile phase components to use to achieve a separation. Additionally, Figure 2b indicates that although the LSER model may be systematically inaccurate or incomplete, it does account for the large majority of

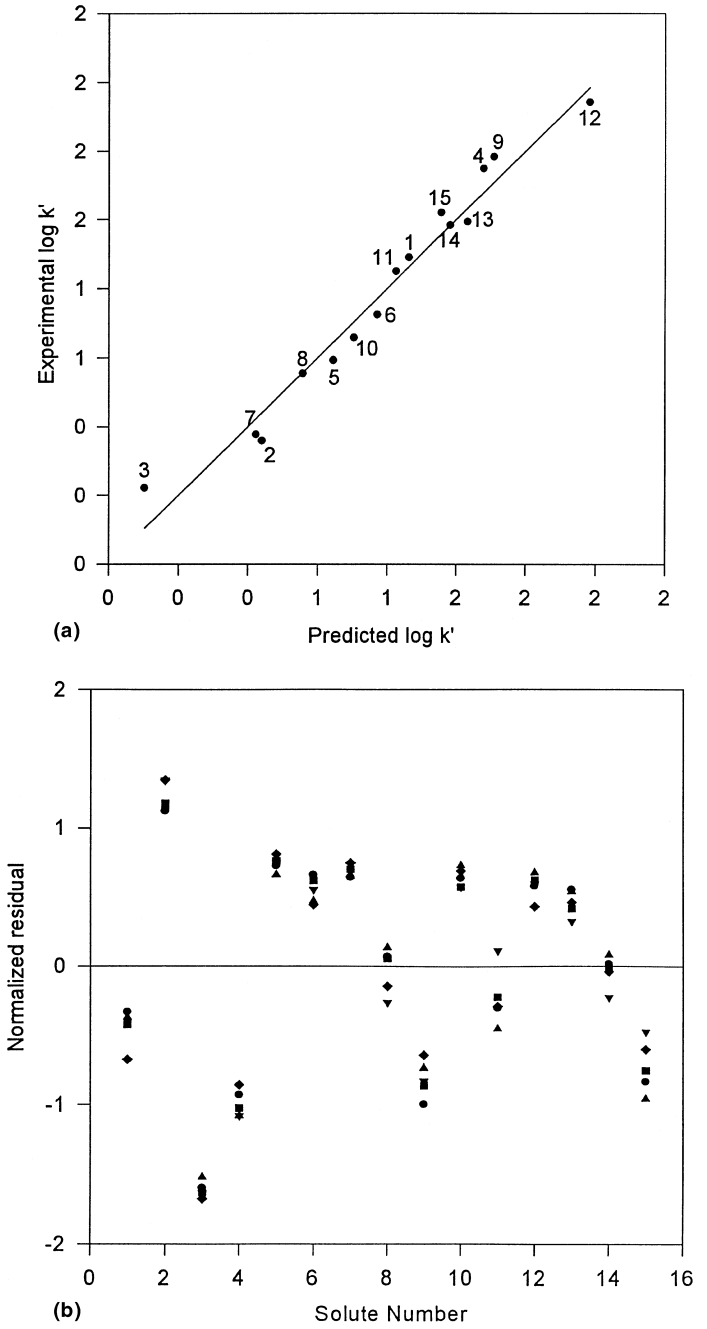


Figure 2. a) Experimental versus predicted log k values in 10% methanol/0.035 M SDS mobile phase. b) Normalized residuals (predicted minus experimental log k values) of the LSERs for 10% methanol/SDS mobile phases. Residuals are normalized to the average standard error of each LSER equation. SDS concentrations (M) are 0.035, 0.050, 0.067, 0.080, and 0.100. Solute numbers are as listed in the Experimental section.

the overall retention. Thus, the contribution of the systematic modeling errors to the overall retention is only a minor modification to the overall retention as predicted by the LSER model.

Effect of the Addition of Alcohols to SDS Systems

Shown in Figure 1 are the LSER coefficients obtained in 10% methanol, n-propanol, and n-butanol in SDS mobile phases. The **m** coefficients suggest that methanol and n-propanol offer greater selectivity for non-polar solutes of different sizes. Additionally, n-propanol offers the greatest selectivity based on solute HB basicities (all other parameters being equal). Since the other coefficients are small and similar in magnitude, there are essentially no differences in selectivity between the modifiers in term of their influence on solutes varying in their HB acidity, excess molar refractivity, and dipolarities/polarizabilities. Given this, it can be said that the selectivity for two solutes in these systems primarily depends on the relative differences in their sizes and HB basicities.

Again, we note that the standard errors of these coefficients overlap and that our conclusions are based entirely on the actual values of the coefficients. If statistical errors are considered, then all three modifiers are essentially equivalent with regards to selectivity based on specific interactions described by each LSER term. We further note that Khaledi et al.[2,42] and Cline Love et al.[43] have reported complex dependencies of selectivity on surfactant concentration, modifier type, and modifier concentration.

Their conclusions, however, are based on different solute sets which included ionic and zwitterionic compounds. Thus, the results presented here strictly pertain to only the solutes used in this study and may not apply to all possible solutes, especially solutes whose interactions with charged micelles could include ionic attractive and repulse interactions.

CTAB Systems

Effect of CTAB Concentration on the LSER Coefficients

LSER coefficients as a function of CTAB concentration with 3% n-propanol as the modifier (Table 2) show that as was the case for the SDS system, the volume and basicity of the solutes play the largest roles in determining their retention, with smaller contributions from their dipolarity/polarizability and excess molar refractivity.

The trends in the LSER coefficients as a function of CTAB concentration for the systems with 5% n-propanol, 10% n-propanol and 5% n-butanol are the same as those described for the 3% n-propanol/CTAB system and similar to those observed for SDS systems.

Residuals of LSER Equations for CTAB Systems

Figure 3a is a plot of the logarithm of experimental retention factors versus the logarithm of retention factors calculated using the LSER for the 3% n-propanol/CTAB system. Figure 3b shows the residuals (predicted minus experimental values) for each solute normalized to the average standard error of the fit. As was the case with the SDS mobile phases systematic deviations are present. This again illustrates that the LSER is not properly accounting for the energetics of the system, as discussed above.

The distribution of residuals is similar for all the CTAB systems studied (results not shown), with the absolute values of the residuals being smaller in the other systems than those shown in Figure 3b. Interestingly, the pattern of residuals in the CTAB system is different than that in the SDS systems (compare Figures 2b and 3b). This is a strong indication that the chemistry involved in the solute interactions is significantly different in the two surfactant systems. If the interactions governing retention were the same, we would expect to see the same residual pattern for each surfactant system due to the systematically incomplete modeling of retention expressed in the LSER equation.

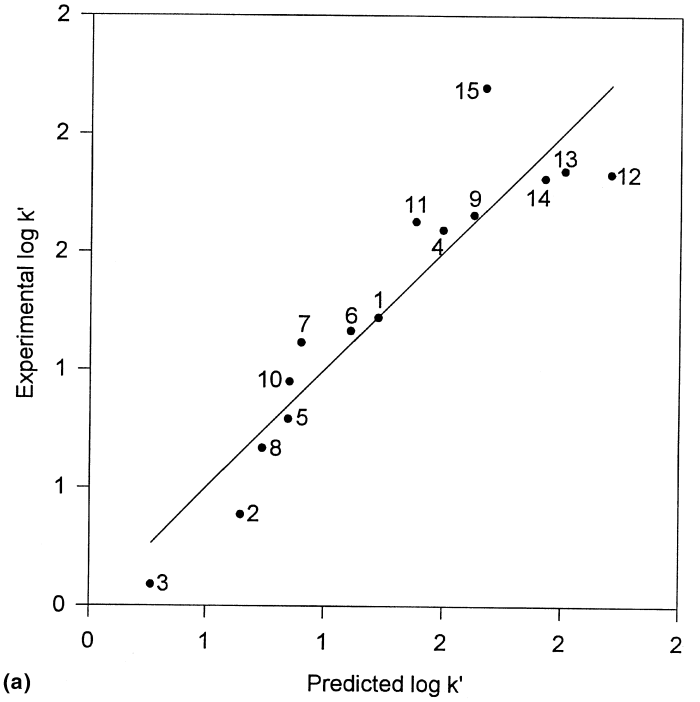
Effect of the Addition of Alcohols to CTAB Systems

Since the LSER coefficients are nearly equivalent with the two different alcohols, a great change in the selectivity would not be expected when changing n-propanol to n-butanol in the mobile phase. Again, relative retention for two solutes would depend on the relative differences in their properties and the global effect of micelle and alcohol concentrations on their retention.

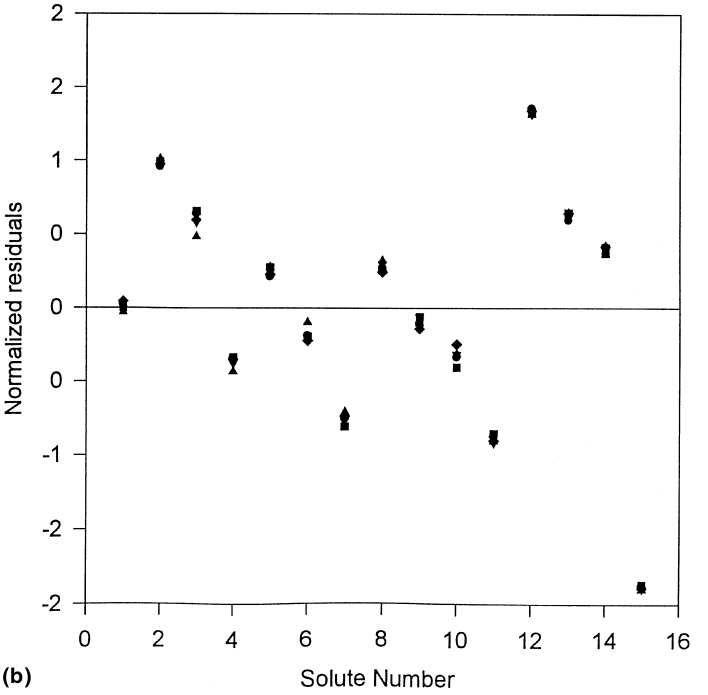
Comparison of SDS to CTAB

Comparison of the LSER coefficients in SDS and CTAB in 10% n-propanol and 5% n-butanol (see Tables 1 and 2) reveals very interesting differences in the fundamental chemical interactions governing retention in SDS and CTAB systems. The **m** coefficient shows that at equal surfactant concentrations the SDS mobile phases are more cohesive/less dispersive than CTAB mobile phases, assuming that the stationary phases are behaving identically in both systems. This may be simply a manifestation of the fact that at the same total surfactant concentration, more CTAB micelles than SDS micelles are present because of the lower CMC of CTAB (CMC = 0.9 mM for CTAB and 8.1 mM for SDS). Thus, more solutes may be in a micellar microenvironment in the mobile phase in CTAB than in SDS.

This has important implications for practical chromatography in that it reveals fairly significant differences between the effects on retention of SDS relative to CTAB as mobile phase modifiers. Specifically, at the same mobile



(a)



(b)

phase concentration, SDS is more selective based on the size of the molecule than is CTAB. That is, all else being equal, a change in the solute size will result in a larger difference in retention in the SDS system than in the CTAB system.

The **b** coefficients also differ for the SDS and CTAB systems, with the **b** coefficients of SDS having larger magnitudes than those of CTAB in both 10% n-propanol and 5% n-butanol. The most striking difference between the two systems, however, is seen in the **a** coefficients. In CTAB systems, the **a** coefficient is generally very close to zero while in SDS systems the **a** coefficients are considerably more negative. This means that the effective HB basicity of SDS mobile phases is greater than that of the stationary phase.

Chemically it is reasonable to relate the increased basicity of SDS mobile phases relative to CTAB mobile phases to differences in the nature of the head groups of the two surfactants. The sulfate head groups surely contribute to the effective basicity of the mobile phase while the ammonium head group cannot. This is an over-simplified view, however, as it totally neglects stationary phase effects and other potentially important mobile phase differences, such as the amount of water inside CTAB and SDS micelles and differences in the counterions associated with the micelles.

The differences in the **s** and **r** coefficients that are observed when comparing SDS to CTAB are smaller than the differences in the other coefficients. Thus, solute dipolarity and excess molar refractivity offer less significant differences in retention than the other coefficients. For solutes which are approximately of similar size and which are not HB acids or bases, however, the dipolarity and excess molar refractivity may be the only significant differences between the solutes to be separated, and thus the small differences in the **s** and **r** coefficients of SDS and CTAB may be quite important in separating non-HB solutes. Overall, the differences in the interaction abilities of SDS and CTAB, especially regarding the HB basicity, are important in that they can be used to estimate selectivity and thereby guide method development and optimization in separations of solutes similar to those used in this study.

Figure 3. a) Experimental versus predicted log k values in 3% n-propanol/0.035M CTAB mobile phase. b) Normalized residuals (predicted minus experimental log k values) of the LSERs for 10% n-propanol/CTAB mobile phases. Residuals are normalized to the average standard error of each LSER equation. CTAB concentrations (M) are 0.035, 0.050, 0.067, 0.080, and 0.100. Solute numbers are as listed in the Experimental section.

Comparison of SDS/Methanol Systems to Non-MLC

One of the most interesting aspects regarding MLC is the effect of micelles on retention and selectivity relative to non-micellar or sub-micellar mobile phases. The LSERs describing retention in RPLC with methanol/water mobile phases have been previously reported.[33] The percent methanol was varied from 20 to 50% in 10% increments, and the retention factors for at least 48 solutes were measured in each mobile phase on a Zorbax-C8 column. LSERs were then obtained for each mobile phase composition and from these, an LSER in 10% methanol was determined by extrapolation. This allows us to compare the extrapolated LSER to LSERs obtained in this study for 10% methanol/SDS systems.

Inherent in this comparison is the assumption that the C8 phase used in the RPLC study is chemically similar to the C18 phase used in the micellar studies. The extrapolated LSER is shown in equation 2.

Non-micellar RPLC LSER (10% methanol/90% water):

$$\log k = 0.63 + 3.72V_x - 0.48\pi_2^H - 2.04\Sigma\beta_2^H - 0.35\Sigma\alpha_2^H \quad (2)$$

MLC LSER (10% methanol/0.035 M SDS):

$$\log k = 0.24 + 1.93V_x - 0.57\pi_2^H - 1.50\Sigma\beta_2^H - 0.47\Sigma\alpha_2^H + 0.35R_2 \quad (3)$$

Comparing equation 2 to the LSER for 10% methanol/0.035 M SDS (equation 3), we see that the micelles predominantly affect the **m** coefficient, changing it from 3.72 to 1.93 in the absence and presence of micelles, respectively. At 50% methanol the **m** coefficient in non-micellar RPLC is 2.38,[33] still larger than when just 0.035 M SDS is added to the mobile phase. The dramatic decrease in the **m** coefficient means that the addition of micelles significantly decreases the effective mobile phase cohesivity and/or increases its dispersive ability, presumably because the solutes are free to partition into the relatively disordered, more dispersive microenvironments of the micelles. As more surfactant is added and more micelles form, the decrease in the effective mobile phase cohesivity and the increase in its dispersive ability continue because more solutes are allowed to partition out of the relatively cohesive water into the micelles.

In terms of practical chromatography, this means that selectivity based on solute size is diminished when micelles are added to the mobile phase. This result is consistent with the finding of Khaledi[42] that the chromatographic selectivity of methylene units decreases as surfactant concentration increases.

The other noteworthy effect of adding micelles to the mobile phase relates to the **b** coefficient. We see that the **b** coefficients of the extrapolated LSER and the LSER of 10% methanol/0.035 M SDS are somewhat different (-2.04

compared to -1.50, respectively), but that this change is much less dramatic than that of the **m** coefficient.

This raises the question that if solutes are partitioning out of the water into the micelle phase and this causes a reduction in the effective cohesivity of the mobile phase, why then is there not a concomitant change in the effective HB acidity of the mobile phase? We suggest that the answer to this question lies in the fact that significant amounts of water are present in the solutes' micellar microenvironment.[44-46] Thus, the solutes inside the micelle are still able to participate in HB acid-base interactions.

The water in these microenvironments, however, almost certainly is not in as organized a network as it is in bulk water, since it is undoubtedly interacting with the polar, charged headgroups and may also be somewhat disrupted by the alkyl chains of the surfactants. Thus, the cohesivity of the microenvironments may be dramatically different than the cohesivity of bulk water, but the HB ability of the microenvironments may be close to the same strength as that of bulk water.

Another reason for the lack of change of the **b** coefficient relative to the **m** coefficient upon the addition of 0.035 M SDS relates to the extent of partitioning of the solutes being considered in each interaction. HB bases will not partition as strongly into the micellar phase as will non-HB solutes of the same size. Thus, at 0.035 M SDS, the basic compounds will tend to be in the water portion of the mobile phase. The effective acidity of the mobile phase (being a weighted sum of the acidity of the aqueous and micellar phases) reported by the HB-basic compounds will therefore be close to that of water.

As more micelles are formed, more HB-solutes will partition into them based on mass action principles and, thus, the overall HB acidity of the mobile phase reported by the solutes will better represent the acidity of the micelles, which, given the decrease in the **b** coefficient, is lower than that of the aqueous phase portion of the mobile phase.

To be more explicit regarding the above explanation, consider the solutes that are in the mobile phase and their distribution between the bulk aqueous and micellar phases which comprise the overall mobile phase. It is important to stress that we are considering only those molecules in the mobile phase and not the total number of solute molecules in the column. Using published partition coefficients,[47,48] we have estimated the fraction of mobile phase solutes that is in the aqueous phase and the fraction that is in the micellar phase.

Furthermore, we have done so as a function of surfactant concentration. For a solute such as benzamide that has a relatively small concentration-based partition coefficient ($K_{WM} \approx 54$), we find that in a 0.035 M SDS/10% methanol mobile phase, approximately 23% of the benzamide molecules present in the

mobile phase are associated with micelles, while the remaining 77% are in the bulk aqueous phase. At 0.100 M SDS this changes to 55% and 45%, respectively. This represents a greater than two-fold change in the number of benzamide molecules in the micellar phase.

Thus, as the SDS concentration is increased, the environment that is being sensed by these molecules shifts significantly toward that of the micellar phase and away from that of the aqueous phase. Contrast this with the behavior of naphthalene, which is 89% in the micelle phase at 0.035 M SDS and 97% in the micelle phase at 0.100 M SDS.

Overall, since nonpolar molecules such as naphthalene primarily determine the **m** coefficient, and HB bases such as benzamide primarily determine the **b** coefficient, we expect to see larger changes in the **m** coefficient with the addition of micelles to the mobile phase compared to changes in the **b** coefficient since the basic compounds are still primarily in the aqueous portion of the mobile phase. We also expect to see continued changes in both the **m** and **b** coefficients as the concentration of surfactant is increased, since the percentage of solutes associated with the micellar phase increases.

The **s**, **a**, and **r** coefficients vary somewhat with surfactant concentration, but these changes are quite small compared to the changes in the **m** and **b** coefficients. Additionally, the direction of change again seems to reflect increased solute partitioning into micelles as the surfactant concentration increases.

Overall, micelles added to conventional methanol/water RPLC mobile phases have their greatest effect on the effective cohesivity of the mobile phase. Secondarily, at higher surfactant concentrations, the **b** coefficient becomes smaller in magnitude, decreasing the influence of solute HB basicity on retention.

CONCLUSIONS

The relative importance of various chemical interactions such as dipolarity/polarizability, HB acidity, and HB basicity were determined in 40 different MLC systems using SDS, CTAB, methanol, n-propanol, and n-butanol as mobile phase modifiers. The same trends in the LSER coefficients were found regardless of the nature of the surfactant and the nature and concentration of the organic additives.

Solute size and basicity are the two most important solute parameters determining retention in the MLC systems studied. Analysis of residual values showed that there were contributions to retention that were not properly accounted for by the LSER formalism. Despite that, LSERs are able to reasonably reproduce log k values and to predict, albeit only roughly, the log k value of a solute with known parameters.

Thus, the contribution of the systematic modeling errors to the overall retention is only a minor modification to the overall retention as predicted by the LSER model. This has important implications for the practical application of MLC as it will influence the choice of surfactant when developing separation methods.

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