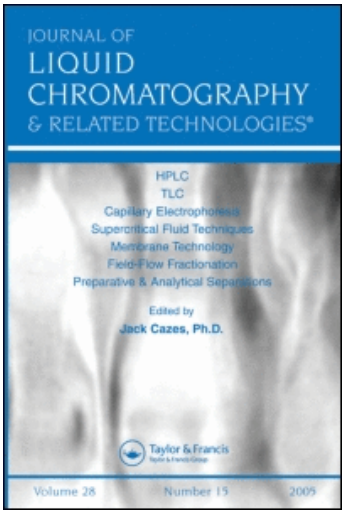

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SELECTIVITY IN MICELLAR LIQUID CHROMATOGRAPHY: SURFACTANT BONDED PHASE INTERACTIONS. I. C-18

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ABSTRACT

Micellar liquid chromatography and solid state ^{13}C NMR spectroscopy have been used to study the interactions of three ionic surfactants with the C_{18} alkyl bonded phase. The three surfactants, sodium dodecylsulfate (SDS), cetyltrimethyl ammonium bromide (CTAB), and dodecyltrimethylammonium bromide (DTAB), are commonly used in micellar RPLC. Surfactant adsorption is found to produce distinct changes in the selectivity of the stationary phase. Specifically, the differing nature of the surfactant monomer-bonded phase association is largely responsible for the observed differences in selectivity between SDS, CTAB, and DTAB micellar RPLC. For SDS, the association leads to the formation of an anionic hydrophilic surface layer on C_{18} which would explain the superior resolution

achieved by SDS for hydrophilic compounds. For CTAB or DTAB adsorbed on C_{18}, the nitrogen head group is probably incorporated (at least partially) in the bonded phase due to hydrophobic interactions between the methyl nitrogens and the C_{18} alkyl bonded phase. Chemical models depicting the structure of the surfactant coated C_{18} stationary phase are proposed from the NMR data, and these models are in good agreement with retention data obtained for these micellar RPLC systems.

INTRODUCTION

In reversed phase liquid chromatography (RPLC), a hydro-organic solvent mixture is commonly used as the mobile phase. However, aqueous micellar solutions, i.e., solutions containing surfactant at a concentration above the critical micelle concentration, have been shown to possess properties analogous to those of conventional mobile phases in RPLC. This unusual variation of the RPLC experiment is known as micellar liquid chromatography (MLC).

Armstrong and Henry[1] first demonstrated that micelles can be used in place of traditional organic modifiers, such as methanol or acetonitrile, in RPLC. Micelles, which are dynamic assemblies of surfactant molecules, can organize and compartmentalize solutes at various sites within the surfactant assembly. The actual location of the solute in the assembly is dictated by the nature of the solute and the surfactant system employed.[2-3] Each solubilization site, i.e. microenvironment, in the micelle is unique, and its properties (e.g., polarity, fluidity, and acidity) are distinctly different from those of the bulk solvent.

Retention in MLC has been shown to be correlated to surfactant type and to the concentration of surfactant in the mobile phase.[4-6] Solute retention in MLC generally decreases with increasing surfactant (i.e., micelle) concentration, but the rate of decrease can vary considerably from one organic solute to the next. Equations relating the capacity factor (k') to the concentration of micelles in the mobile phase have been formulated by Armstrong and Nome[7] and Cline-Love and Arunyanart.[8] These equations which are based on a three-way partition model have been verified experimentally[9-11] for a large number of organic compounds.

Many of the advantages offered by micellar mobile phases, e.g., enhanced luminescence detection, simultaneous separation of charged and neutral compounds, and the ability to directly inject biologicals onto the column without prior sample work-up, is due to the unique ability of micelles to

organize and compartmentalize solutes at the molecular level. The ability of micelles to selectively solubilize and interact with solute molecules is believed to be the basis of separation in MLC.[12] However, surfactant molecules are readily adsorbed on hydrocarbonaceous stationary phases. The architecture assumed by adsorbed surfactant molecules on conventional RPLC stationary phases has been postulated to vary from hemi-micellar or admicellar to mono-, bi-. Since many properties of HPLC stationary phases are altered by the process of surfactant adsorption, the modification of the bonded stationary phase by adsorbed surfactant molecules can have profound implications with regard to retention and selectivity in MLC.

Micellar liquid chromatography and solid state ^{13}C NMR spectroscopy have been used to study the interactions of three ionic surfactants with the C_{18} alkyl bonded phase. The three surfactants, sodium dodecylsulfate (SDS), cetyltrimethylammonium bromide (CTAB), and dodecyltrimethylammonium bromide (DTAB), are commonly used in micellar RPLC. Surfactant adsorption is found to produce distinct changes in the selectivity of the stationary phase. Specifically, the differing nature of the surfactant monomer-bonded phase association is largely responsible for the observed differences in selectivity between SDS, CTAB, and DTAB micellar RPLC. For SDS, the association leads to the formation of an anionic hydrophilic surface layer on C_{18} which would explain the superior resolution achieved by SDS for hydrophilic compounds. For CTAB or DTAB adsorbed on C_{18}, the nitrogen head group is probably incorporated (at least partially) in the bonded phase because of hydrophobic interactions between the methyl nitrogens and the alkyl bonded phase. Chemical models depicting the structure of the surfactant coated C_{18} stationary phase are proposed from the NMR data, and these models are in good agreement with retention data obtained for these different micellar RPLC systems.

EXPERIMENTAL

Chemicals

The six vanillin compounds (see Figure 1) which constituted the hydrophilic test mixture used to characterize the surfactant coated stationary phases were obtained from Aldrich and were used as received. Stock solutions of the various test solutes were prepared in methanol and then diluted to the appropriate working concentration (550 μg/mL) using 50% methanol in water. The surfactants, SDS, CTAB, and DTAB, were obtained from BDH Chemicals (99% purity) and were purified prior to use by first dissolving them in ethanol

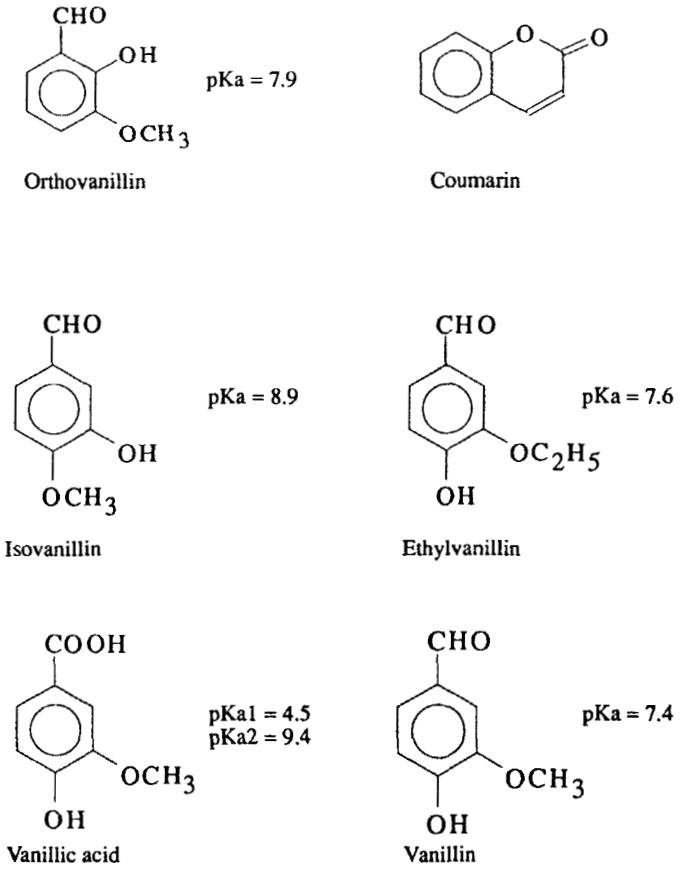

Figure 1. The vanillin compounds. The pKa values are from reference 13.

followed by addition of charcoal to the solution. After the charcoal was separated from the mother liquor by filtration, the surfactant was recrystallized from the ethanol and dried in an oven at 65° C. Micellar solutions were prepared from the recrystallized surfactants using HPLC grade distilled water. (Methanol-water mobile phases were also prepared using HPLC grade solvents.) All mobile phase solutions were filtered twice with a 0.45 μm Nylon membrane filter (Rainin Instruments, Woburn, MA) to remove particulate matter. Prior to use, the solutions were degassed and their pH adjusted to 3 with hydrochloric acid to prevent ionization of polar solutes in the mobile phase solutions.[13]

High Performance Liquid Chromatographic (HPLC) Measurements

All HPLC measurements were made using either a Perkin Elmer TriDet HPLC or a Rainin 81-20 M analytical HPLC system. The analytical column was Apex I C-18 (5-μm, 10cm x 4.6 mm i.d.). The columns were purchased from Jones Chromatography (Golden, CO). The analytical column was water-jacketed and temperature controlled. Separate columns were used for each surfactant because of strong and irreversible adsorption of ionic surfactants on the stationary phase of the C_{18} bonded phase. The dead volume of each column which was determined by injecting different solutions such as methanol-water, or water onto the Apex I column was approximately 1.0 mL and was used for all k' calculations. The k' values determined in this study were averages of at least triplicate determinations, and deviations in individual k' values were never greater than 5%. All k' measurements were made at a flow rate of 1.0 mL/min and were measured at 25°C for SDS and DTAB and 30°C for CTAB. (Since the Kraft point of CTAB is 23°C, it was necessary to perform the CTAB studies at a higher temperature.)

Estimation of Critical Partitioning Parameters in Micellar RPLC

Solute-stationary phase and solute-micelle binding constants were determined for the vanillin compounds using an equation developed by Cline-Love and Arunyanart[8]

$$1/k' = [M]K_2/\theta[L_s]K_1 + 1/\theta[L_s]K_1 \qquad (1)$$

where [M] is the concentration of surfactant, K_2 is the solute-micelle binding constant per monomer of surfactant, θ is the chromatographic phase ratio, $[L_s]$ is the concentration of ligate on the stationary phase, and K_1 is the solute-stationary phase binding constant. A plot of 1/k' vs [M] should yield a straight line, and in fact excellent linearity was observed for all six compounds using SDS, CTAB or DTAB.

Solid State NMR Measurements

Adsorption of SDS, DTAB, and CTAB on C_{18} chemically derivitized silica was investigated using cross polarization/magic angle spinning ^{13}C NMR with high-power proton decoupling (CP/MAS ^{13}C NMR). All NMR experiments were performed at 50 MHz on a Bruker/IBM WP-200 SY Spectrometer equipped with an IBM solids control accessory and a Doty-type solid-state probe that was software controlled which permitted automatic

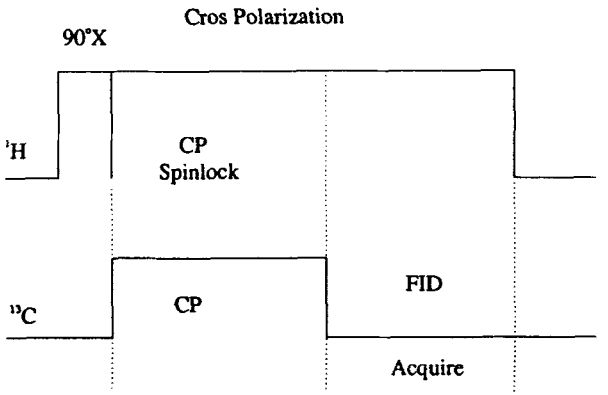

Figure 2. Pulse sequence for the determination of T_{CH}.

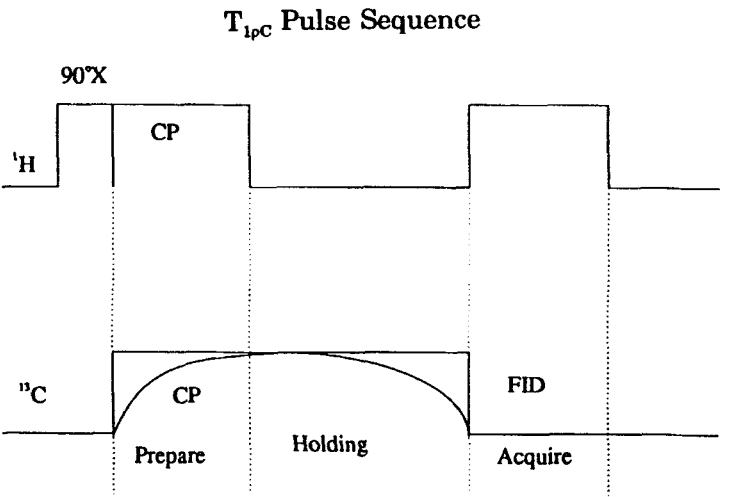

Figure 3. Pulse sequence for the determination of T_{1YC}.

variation of all pulse parameters. The magic angle spinning probe used was a double-tuned, single-coil design with a bullet type rotor which held a sample volume of 0.75 cm^3. Two different pulse sequences were used in these NMR experiments (see Figures 2 and 3). However, each pulse sequence was performed with a constant 3-s recycle time. The ^{13}C spectra collected were

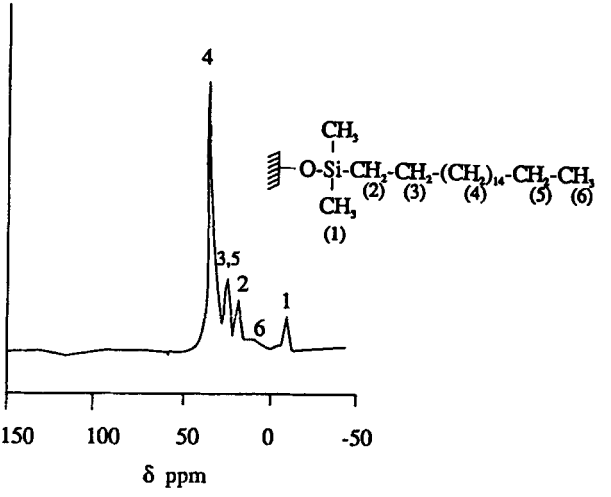

Figure 4. ^{13}C CP/MAS NMR spectrum and chemical shift assignments for C_{18}. A thousand pulses were used to generate the spectrum.

externally referenced to para-di-t-butyl benzene. All chemical shift values were expressed as parts per million down-field from tetramethylsilane. The ^{13}C data were collected in 2 Kbytes of memory, exponentially multiplied prior to Fourier transformation, and zero-filled to 8 kilobytes.

Sample Preparation

To prepare a sample for solid-state NMR, 0.5 g of 5μm C_{18} reversed phase material was equilibrated with 10 mL of 0.05 M aqueous CTAB, DTAB, or SDS solution. The equilibration period for the stationary phase material and surfactant was at least 24 h.

During equilibration, a wrist action shaker was periodically used to agitate the samples. After equilibration, each sample was vacuum filtered onto a 0.45 μm Nylon 66 membrane filter and vacuum dried at 35°C for 2 days prior to being packed into the rotor of the solid-state probe.

X-ray Diffraction Studies

Low angle X-ray diffraction spectra were obtained for pure SDS, CTAB, and DTAB with a Siemens Crystalloflex 4, with a Tennelec detector system, PSD 100. The path length was 50 cm, and the sample was suspended in a 0.5 mm capillary with 0.01 mm wall thickness. The spectra were run at room temperature at 40 kV and 30 mA.

RESULTS & DISCUSSION

Solid state NMR was employed in this study because surfactant molecules not in contact with the bonded phase can be readily differentiated from surfactant molecules that are intercalated or in direct contact with the bonded phase. However, solid state NMR cannot sample the stationary phase under chromatographic conditions. Even though solid state NMR measurements can provide information about the structural environment of the surfactant coated stationary phase, no direct information on how the stationary phase interacts with the solute is provided by this NMR technique. One approach for the direct measurement of these interactions is the use of retention probes which can be selected to emphasize specific physical or chemical interactions of the solute with the mobile or stationary phase. Because our objective was to study hydrophilic interactions in MLC, not hydrophobic interactions (which is the usual practice), we chose a set of six vanillin compounds to serve as retention probes. NMR and micellar RPLC retention data obtained for a C_{18} bonded phase are summarized and discussed below for three ionic surfactants: SDS, CTAB, and DTAB.

NMR

Solid state ^{13}C NMR spectra are shown in Figures 4, 5, 6, and 7 for the following materials: (1) C_{18}, (2) pure SDS, (3) SDS adsorbed on C_{18}, (4) pure CTAB, (5) CTAB adsorbed on C_{18}, (6) DTAB, and (7) DTAB adsorbed on C_{18}. Chemical shift assignments were made on the basis of previously published literature reports[14-16] on related materials and the observed CP/MAS spectra of pure SDS, CTAB, and DTAB. For pure SDS (see Figure 5a) in order of increasing frequency from left to right, the lowest field line at 68 ppm is assigned to the methylene carbon nuclei alpha to the sulfate head group (i.e., the alpha carbon), the highest field line at 12 ppm is assigned to the terminal methyl group. There is a second peak for the alpha carbon at 50 ppm which only appears after adsorption of SDS on C_{18} (see Figure 5b). This peak represents surfactant monomer in contact with the bonded phase. We attribute

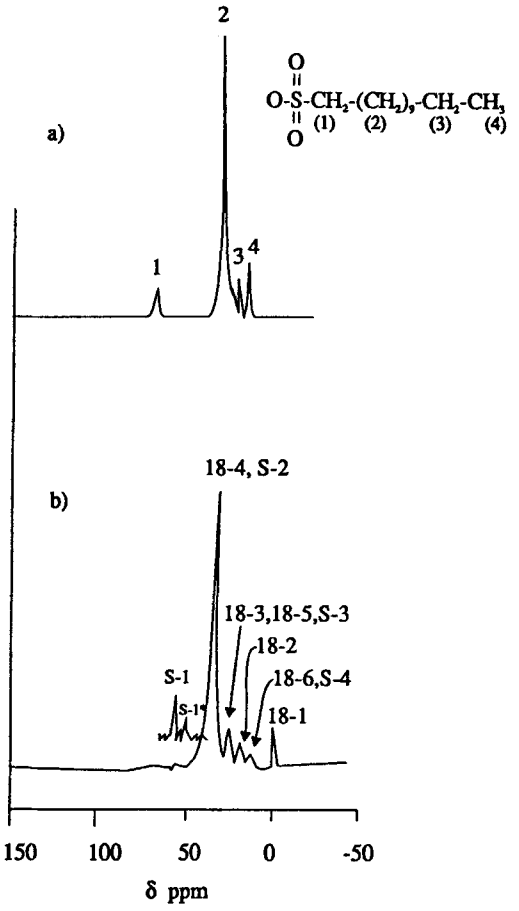

Figure 5. ^{13}C CP/MAS NMR spectrum and chemical shift assignments for (a) pure SDS, and (b) SDS adsorbed on C_{18}. Bonded phase resonances are indicated by labels starting with numerals (i.e., 18-), while surfactant resonances are indicated by labels beginning with letters (S=SDS).

the change in the chemical shift value of the alpha carbon to a change in the chemical environment of this nuclei. This conclusion is reinforced by X-ray diffraction studies performed in our laboratory which show that pure SDS has an ordered structure with an interlayer spacing of 37.3 A which is in good agreement with the calculated length of a close-packed SDS structure,[17] whereas a less ordered arrangement is implied by the accepted model[18-19] for

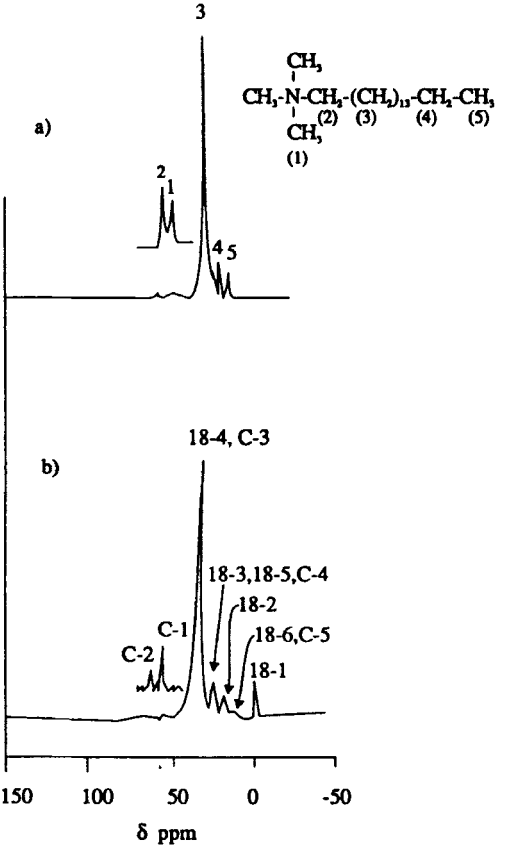

Figure 6. ^{13}C CP/MAS NMR spectrum and chemical shift assignments for (a) pure CTAB, and (b) CTAB adsorbed on C_{18}. Bonded phase resonances are indicated by labels starting with numerals (i.e., 18-), while surfactant resonances are indicated by labels beginning with letters (C=CTAB).

surfactant adsorption at a buried interface. Because of differences in ordering between these two phases (solid vs adsorbed SDS), a change in the chemical shift value of the alpha carbon is not unexpected and constitutes direct evidence for wetting of the C_{18} bonded phase by the surfactant monomer.

For both pure and adsorbed CTAB (see Figure 6) and DTAB (see Figure 7), the peak at the most down-field position (62 ppm) is due to the methylene carbon alpha to the ammonium head group (i.e., the α-carbon), while the peak

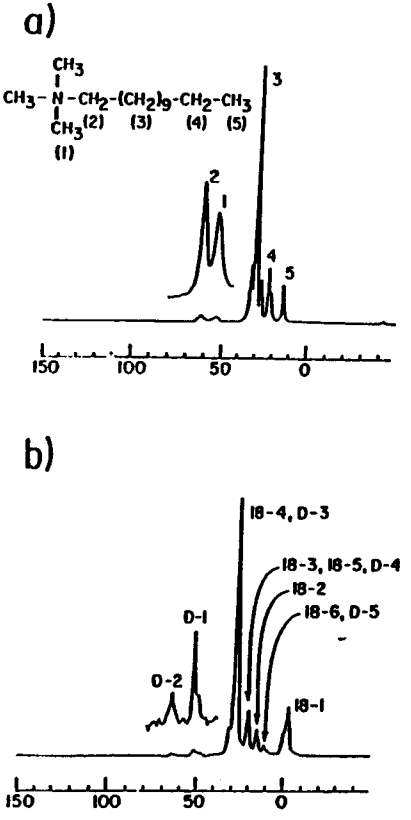

Figure 7. ^{13}C CP/MAS NMR spectrum and chemical shift assignments for (a) pure DTAB, and (b) DTAB adsorbed on C_{18}. Bonded phase resonances are indicated by labels starting with numerals (i.e., 18-), while surfactant resonances are indicated by labels beginning with letters (D=DTAB).

at 54 ppm is due to the N-methyl carbon. There is no change in the chemical shift value of the α-carbon after adsorption of CTAB or DTAB onto the bonded phase, and this is consistent with low angle X-ray diffraction data which shows both pure CTAB and DTAB to be amorphous solids. Hence, we should not observe any change in the chemical shift value of the CTAB or DTAB α-carbon because of the similarity in ordering of these two phases (solid vs adsorbed surfactant).

Table 1

Relaxation Parameters of the α-Carbon Nuclei*

Surfactant	T_{CH} (ms)	$T_{1\rho C}$ (ms)
CTAB (62 ppm)	0.03 (± 0.003)	14.5 (± 0.58)
CTAB on C_{18} (62 ppm)	0.54 (± 0.22)	24.2 (± 1.58)
DTAB (62 ppm)	0.03 (± 0.002)	25.7 (± 0.5)
DTAB on C_{18} (62 ppm)	0.30 (± 0.11)	20.8 (± 5.2)**
SDS (68 ppm)	0.13 (±0.03)	203.4 (± 0.24)
SDS on C_{18} (68 ppm)	0.12 (± 0.03)	21.3 (± 0.68)
SDS on C_{18} (50 ppm)	1.23 (± 0.00)	Dispersion Pattern

* The uncertainty in T_{CH} and $T_{1\rho C}$ was determined from the statistical parameters of the least squares fitting.
**At short holding times, i.e., less than 2 milliseconds.

From an examination of the NMR spectra, it is evident that the methylene carbon nucleus alpha to the head group of the surfactant can serve as a probe to study the sorptive behavior of SDS, CTAB, and DTAB on C_{18}. Resonances from the other surfactant nuclei are obscured by resonances from the bonded phase, preventing their use as probes. In other words, there is significant peak overlap in the 0 to 50 ppm region - one simply cannot distinguish aliphatic surfactant resonances from other resonances due to the bonded alkyl phase. Although the ^{13}C nucleus of the N-methyl group of CTAB and DTAB is not obscured by other surfactant or bonded phase resonances, the N-methyl group is not a good probe of molecular motion because of the rapid rotation of the methyl groups which can partially decouple the carbon and hydrogen nuclei.

It is also important to note that in Figures 5, 6, and 7, the relatively small peaks available to us as probes for this work (e.g., S-1, C-2, and D-2) appear to be inconsequential. In reality, this is not true. There is plenty of signal available to accurately measure peak intensity, with signal to noise (S/N) ratios of 100 or greater. The favorable S/N ratio of these peaks is simply obscured by the intensity of the "larger" peaks.

Table 1 lists cross polarization time constants (T_{CH}) for the alpha carbon of SDS, CTAB, and DTAB before and after surfactant adsorption onto the bonded phase. Since cross polarization is most efficient for static and near static C-H dipolar interactions, it can be related to the mobility of the nuclei

under investigation. For CTAB and DTAB, a significant increase in T_{CH} is observed after surfactant adsorption onto the bonded phase which indicates that the polar head group of the surfactant is more mobile after adsorption than in the pure solid form. This observation is consistent with a model of the CTAB or DTAB head group in intimate contact with the semi-rigid fluid like alkyl bonded phase. Hence, the decrease in the polarization transfer rate (i.e., the increase in T_{CH}) is significant because it constitutes direct evidence for wetting of the bonded phase by the surfactant monomer.

For adsorbed SDS, we observe two resonances for the α-carbon - one at 68 ppm and the other at 50 ppm. The 68 ppm resonance is very similar to the α-carbon peak of pure SDS as evidenced by the similar T_{CH} values (see Table 1), which suggests this peak represents SDS not in direct contact with the C_{18} bonded phase. However, the 50 ppm resonance behaves differently - the carbon magnetization build-up is not as rapid, and the T_{CH} value of the 50 ppm peak is substantively larger, suggesting that an increase in the mobility of the α-carbon nuclei has occurred. Hence, the 50 ppm peak probably corresponds to SDS in direct contact with the bonded hydrocarbon chains. During the cross-polarization experiments, $T_{1\rho H}$ (which represents ^{13}C magnetization relaxation through ^{1}H magnetization) was also determined for both pure and adsorbed surfactant. However, we could not relate this relaxational parameter to changes in motional behavior of the samples under investigation due to the problem of maintaining the Hartman-Hahn match at long contact times which is a concern since $T_{1\rho H}$ is determined from the falling portion of the variable contact time plot. Furthermore, the observed $T_{1\rho H}$ is an average over all the protons in the sample as a result of spin diffusion. Hence, a simple and direct interpretation of $T_{1\rho H}$ in terms of the various types of motion of carbon nuclei is not possible.

Clearly, spin diffusion can complicate the analysis of carbon relaxation behavior which is the reason why $T_{1\rho C}$ was used in the present study as an indicator of carbon relaxation behavior. Unlike $T_{1\rho H}$, spin diffusion is not a serious problem because the low natural abundance of ^{13}C ensures a physical separation within the solid and hence a slow spin diffusion rate.

Figures 8, 9, and 10 show the results from several variable holding time ($T_{1\rho C}$) experiments for SDS, CTAB, and DTAB. The holding time data were plotted in familiar semilog fashion. Information about the relaxation and motional behavior of the nuclei can be obtained from these plots. A linear decay plot suggests homogeneous relaxation behavior, whereas a nonlinear decay plot indicates a distribution of relaxation times for the nucleus. From the reciprocal of the slope of the semilog decay curve, $T_{1\rho C}$ can be obtained.

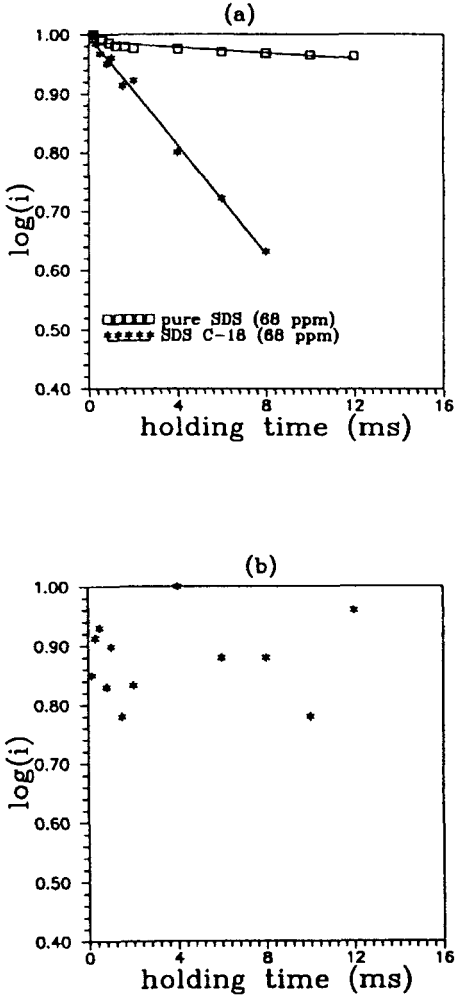

Figure 8. A plot of log intensity versus holding time for the alpha carbon of pure and adsorbed SDS: (a) 68 ppm resonance, and (b) 55 ppm resonance.

The semilog decay curves for the 68 ppm peak of solid and adsorbed SDS (see Figure 8) are linear, which suggests homogeneous relaxation behavior. Taken together, T_{CH} and $T_{1\rho C}$ data (see Table 1) suggest that the carbon atom associated with the 68 ppm peak in the NMR spectrum of SDS adsorbed on C_{18} is as rigid as the α-carbon nuclei of pure SDS. In other words, the 68 ppm peak represents solid SDS. On the other hand, the 50 ppm peak, which is

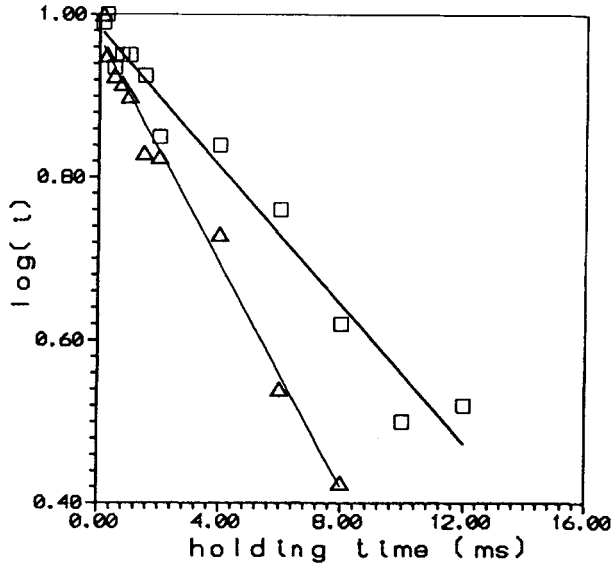

Figure 9. A plot of log intensity versus holding time for the alpha carbon of pure and adsorbed CTAB. (triangles = CTAB; squares = CTAB adsorbed on C_{18})

only observed after adsorption of SDS onto C_{18} and C_8, does not exhibit homogeneous relaxation behavior. We attribute the so-called dispersion pattern in the decay plot to the sulfate head group of SDS which is very mobile. The sulfate head group is not in direct contact with the bonded phase and will have many different orientations available to it. Because the α-carbon will possess a unique relaxation time for each orientation available to the sulfate head group, it is not surprising that a dispersion pattern is observed for the α-carbon nuclei which is in direct contact with the fluid-like bonded phase.

The decay curves shown for CTAB (see Figure 9) are also linear which indicates that the α-methylene carbon atom of both adsorbed and solid CTAB exhibits homogeneous relaxation behavior. The value of $T_{1\rho C}$ for the α-methylene carbon atom of adsorbed CTAB is greater than $T_{1\rho C}$ for pure CTAB, suggesting that an increase in the mobility of the carbon nuclei has occurred after adsorption of CTAB onto the C_{18} bonded phase (see Table 1). These conclusions are reinforced by the variable contact time data previously

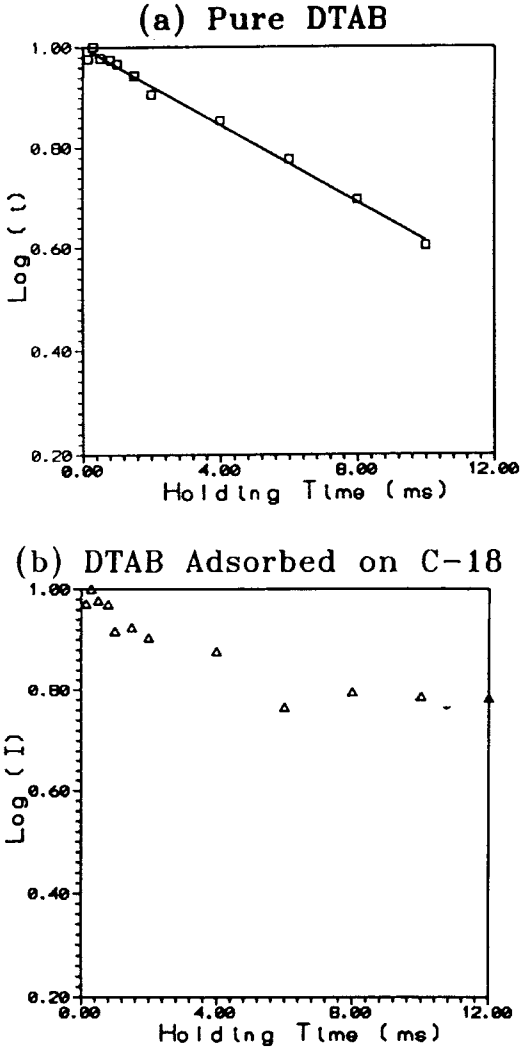

Figure 10. A plot of log intensity versus holding time for the alpha carbon of pure and adsorbed DTAB.

discussed, where T_{CH} of the CTAB α-carbon nuclei increased after adsorption of CTAB onto the bonded phase. Taken together, changes in T_{CH} and $T_{1\rho C}$ values of the α-carbon nuclei indicate that the polar head group of CTAB is in intimate contact with the fluid-like bonded phase.

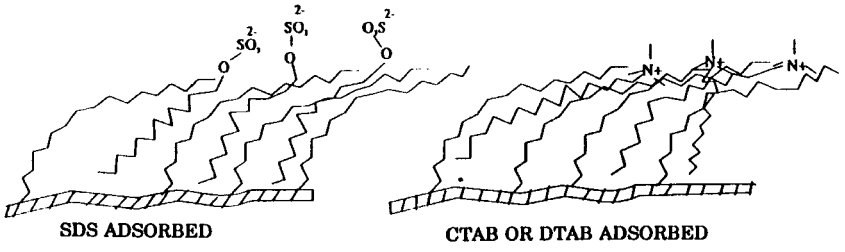

Figure 11. Model depicting the structure of SDS, DTAB, and CTAB-modified C_{18}.

Semilog decay curves for solid and adsorbed DTAB are shown in Figure 10. DTAB is similar to CTAB: T_{CH} and $T_{1\rho C}$ data follow the same trend. However, there are differences in the relaxation behavior of the α-methylene carbon atom of DTAB and CTAB. $T_{1\rho C}$ plots for adsorbed DTAB are not linear at long holding times, and the value of $T_{1\rho C}$ for the α-methylene carbon nuclei of adsorbed DTAB is not greater than $T_{1\rho C}$ for pure DTAB at short holding times. Since the only difference between DTAB and CTAB is hydrocarbon chain length, this factor is evidently important, influencing the adsorptive behavior of these amphiphiles on alkyl bonded phases.

We interpret the observed changes in α-carbon mobility upon adsorption of surfactant on the bonded phase as resulting from two entirely different forms of surfactant monomer association with the bonded phase. In the case of SDS, the hydrophobic alkyl tail and the alpha carbon of the adsorbed surfactant are associated with the C_{18} bonded phase, with the polar head group oriented away from the bonded phase surface. This orientation would prevent the establishment of a double layer structure at the stationary phase-mobile phase interface, i.e., the formation of hemi- or admicelles. On the other hand, the head group of CTAB and DTAB is oriented closer to the silica surface due to hydrophobic interactions between the N-methyl groups and the bonded phase. Evidently, CTAB is incorporated at least partly in the bonded phase giving rise to a modified bulk phase that is significantly denser. Figure 11 depicts the proposed model developed from the NMR data which summarizes SDS, CTAB, and DTAB adsorption on the C_{18} alkyl bonded phase at concentrations above the cmc of the surfactant.

Berthod and coworkers[20-21] have measured adsorption isotherms for SDS and CTAB on C_{18} bonded phase columns. The presence of large amounts of sodium chloride (ca. 0.20 M) in the micellar mobile phase increased markedly the amount of SDS adsorbed on C_{18} which is consistent with the proposed model for SDS adsorption since an increase in the ionic strength of the mobile

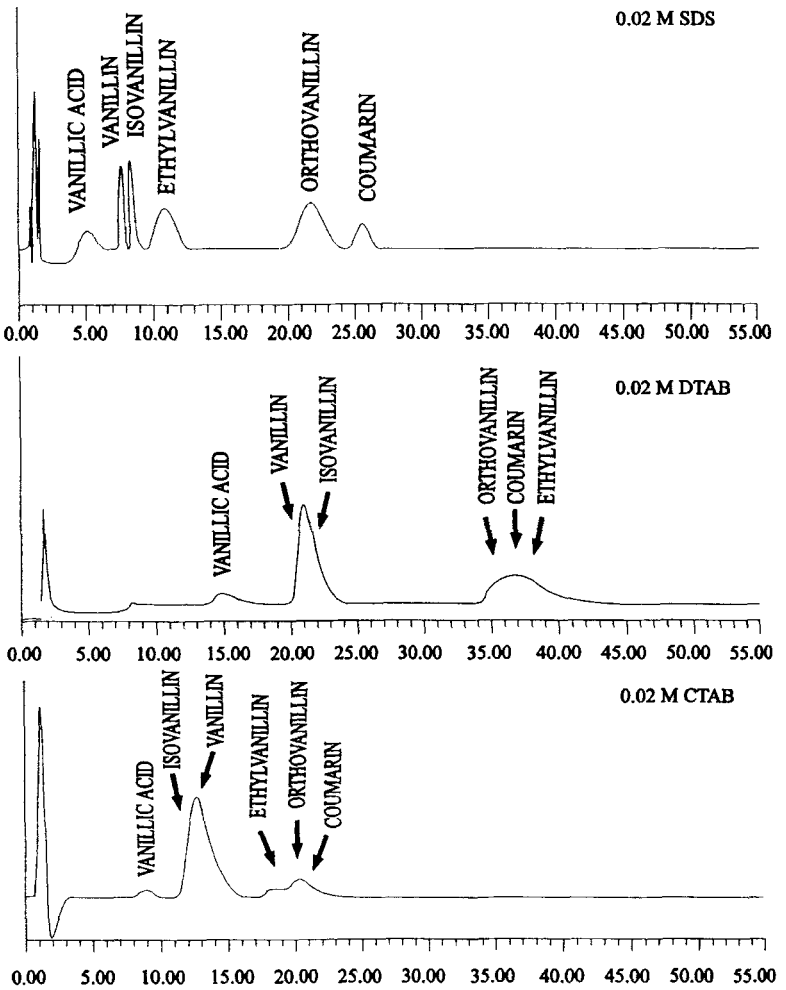

Figure 12. Separation of the vanillin test mixture on Apex I C-18 with a Perkin Elmer TriDet HPLC using the following mobile phases: 0.02 M SDS, 0.02 M DTAB, and 0.02 M CTAB. Flow rate was 1.0 mL/min, and the pH of each mobile phase was 3.0.

phase would diminish electrostatic repulsion between the sulfate head groups of the adsorbed surfactant molecules. However, the presence of a large amount of sodium chloride in the micellar mobile phase did not affect the total amount of CTAB adsorbed on C_{18} which is consistent with the proposed model for CTAB

adsorption since the N-alkyl head groups of adsorbed CTAB are already partly obstructed by the C_{18} alkyl bonded phase; hence, increasing the ionic strength of the mobile phase would not be expected to have much of an effect on reducing electrostatic repulsion between the N-alkyl head groups of adsorbed CTAB.

Retention Data

The conclusions regarding surfactant modification of C_{18} alkyl bonded phases can also be used to explain observed differences in selectivity between SDS, CTAB, and DTAB micellar RPLC. Adsorption of SDS on C_{18} in the manner described, with the sulfate head group projecting away from the bonded phase surface, would lead to the formation of a hydrophilic layer and would explain the superior resolution achieved by SDS for the vanillin compounds (see Figure 12) which probably undergo some form of selective hydrogen bonding interaction with this layer.

The hydrophilic layer formed on the stationary phase would also affect the penetration depth of the vanillin compounds into the bonded phase because of strong hydrogen bonding interactions between these compounds and the layer. The expected result would be a decrease in hydrophobic interactions between the vanillin compounds and the C_{18} stationary phase which would explain why the retention time of the vanillin compounds is greater for 0.02 M CTAB or 0.02 M DTAB than 0.02 M SDS. (The 0.02 M DTAB and 0.02 M SDS micellar solutions contain approximately the same number of micelles since DTAB and SDS have similar cmc's, whereas the CTAB solution contains significantly more micelles because its cmc is an order of magnitude lower than the cmc of SDS.) The proposed model for SDS adsorption can also explain the observation made by Yarmchuk and Cline-Love[6] that acidic solutes, such as phenols, have larger k' values when DTAB is used as the surfactant instead of SDS in micellar RPLC, whereas nonproton donor solutes, e.g., benzene, or nitrobenzene, possess similar k' values for DTAB and SDS.

The type of association between SDS and the bonded phase can also explain why the correlation coefficient for Log P and Log K_1 of SDS is so small (see Table 2), whereas incorporation of CTAB or DTAB in the manner described (see Figure 11) would ensure that much of the hydrophobic character of the modified bulk phase is retained which would explain why the correlation coefficient for Log P and Log K_1 of DTAB or CTAB (see Table 2) is so much larger.

Table 2

Solute Hydrophobicity as Represented by the [1,2]Log of the Octanol/Water Partition Coefficient (Log P) versus Log K_w or Log K_1 for the Vanillin Compounds on C_{18}

Compounds	Log P	[3]Log $K_{1(SDS)}$	[4] Log $K_{1(DTAB)}$	[5]Log $K_{1(CTAB)}$
Isovanillin	0.97	1.02	1.34	1.24
Vanillin	1.21	0.98	1.35	1.24
Orthovanillin	1.37	1.49	1.73	1.42
Coumarin	1.39	1.62	1.60	1.47
Vanillic Acid	1.43	0.62	1.34	1.23
Ethylvanillin	1.88	1.24	1.66	1.56

[1]Log P is a well known index of hydrophobicity.
[2]Log P values were obtained from the CLOGP Program, Medicinal Chemistry Project, Pomona College, Claremont, CA.
[3]The correlation coefficient for Log P and Log $K_{1(SDS)}$ is 0.188.
[4]The correlation Coefficient for Log P and Log $K_{1(DTAB)}$ is 0.600.
[5]The correlation coefficient for Log P and Log $K_{1(CTAB)}$ is 0.758.

Table 3

Sodium Dodecylsulfate

Compound*,**	$\Theta[L_s]K_1$	K_2
Vanillic Acid	4.2 ± 0.2	28.9 ± 2.1
Vanillin	9.6 ± 0.9	39.5 ± 6.1
Isovanillin	10.5 ± 1.0	37.6 ± 4.8
Ethylvanillin	17.5 ± 1.2	45.7 ± 3.7
Orthovanillin	31.2 ± 2.0	41.3 ± 3.0
Coumarin	41.7 ± 3.1	59.0 ± 4.6

* Compounds are listed in their order of elution from Apex I C-18. Concentration of SDS in the mobile phase varied from 0.01 to 0.10 M.
**Uncertainties in $\Theta[L_s]K_1$ and K_2 were determined from the statistical parameters of the least square fitting and from propagation of error.

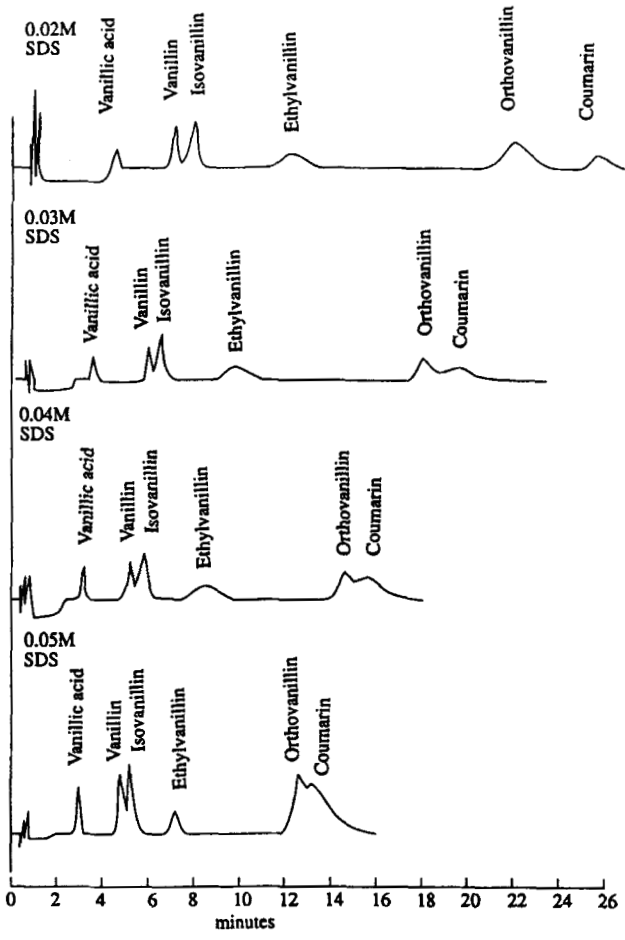

Figure 13. Separation of the vanillin test mixture on Apex I C-18 with SDS micellar solutions of differing surfactant concentration. Flow rate was 1.0 mL/min, and the pH of each mobile phase was 3.0.

Although the association between the surfactant and C_{18} bonded phase plays an important role in defining the selectivity of the separation process, micelle-solute interactions also play a role. For example, SDS micelles interact more strongly with vanillin than isovanillin, as evidenced by the larger K_2 value for vanillin (see Table 3), and this interaction is responsible, at least in part, for the 6 σ or baseline resolution achieved for these two isomers. Nevertheless, the separation of the hydrophilic test mixture is better at lower

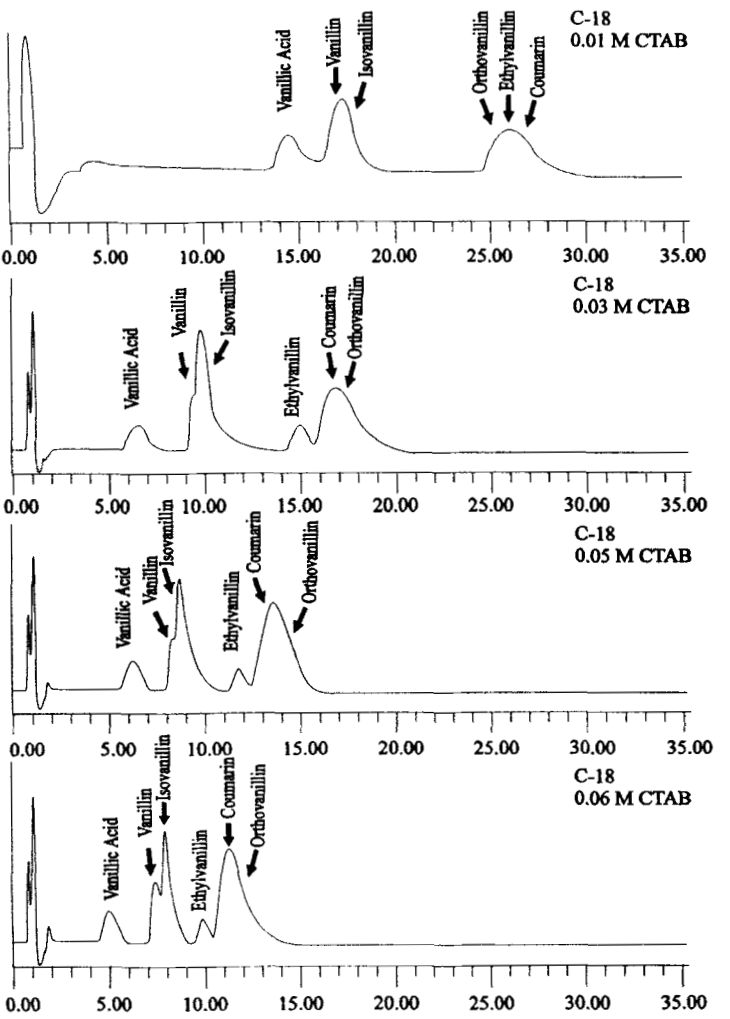

Figure 14. Separation of the vanillin test mixture on Apex I C-18 with CTAB micellar solutions of differing surfactant concentration. Flow rate was 1.0 mL/min, and the pH of each mobile phase was 3.0.

SDS concentrations (see Figure 13) which suggests that solute-stationary phase interactions are largely responsible for the separation. For CTAB and DTAB, the separation of the hydrophilic test mixture is better at higher surfactant

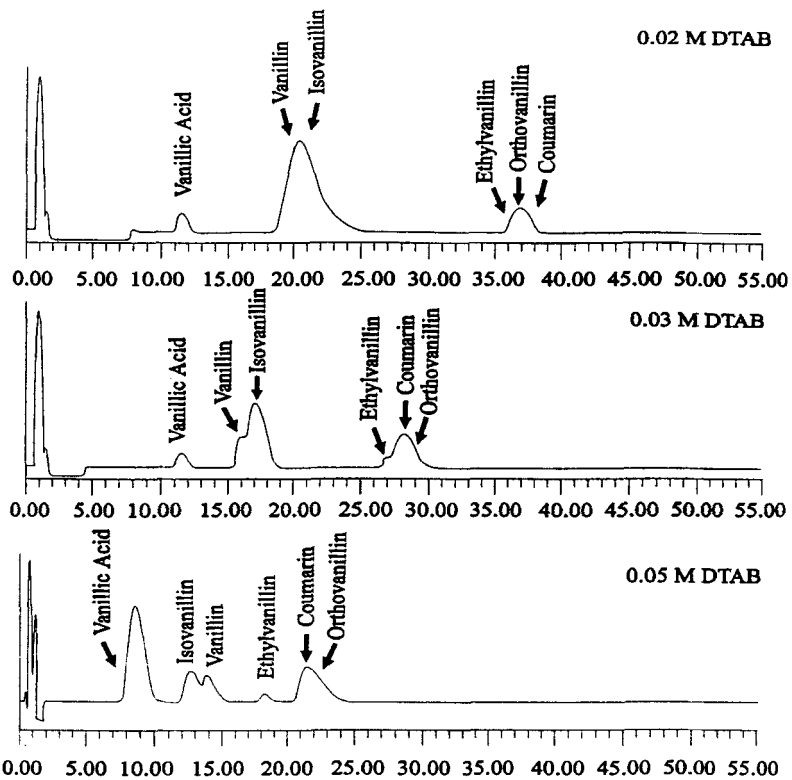

Figure 15. Separation of the vanillin test mixture on Apex I C-18 with DTAB micellar solutions of differing surfactant concentration. Flow rate was 1.0 mL/min, and the pH of each mobile phase was 3.0.

concentration (see Figures 14 and 15), which implies that micelle solute interactions are beneficial for the separation of the vanillin compounds on C_{18} when either CTAB or DTAB is used as the surfactant (see Tables 4 & 5).

This result is not surprising since the selectivity of CTAB and DTAB aggregates towards phenols is well known[22] and is probably the result of a secondary equilibrium process involving the transfer of a proton from the phenol to a water molecule in the Stern region of the micelle.

Table 4

Cetyltrimethylammonium Bromide

Compound*,**	$\Theta[L_s]K_1$	K_2
Vanillic Acid	16.9 ± 1.4	58.6 ± 5.2
Vanillin	17.2 ± 1.2	32.7 ± 2.7
Isovanillin	17.4 ± 1.2	29.7 ± 2.7
Ethylvanillin	36.0 ± 2.6	58.8 ± 4.5
Coumarin	29.5 ± 3.5	37.3 ± 5.3
Orthovanillin	26.5 ± 1.4	31.7 ± 2.3

* Compounds are listed in their order of elution from Apex I C_{18}.
Concentration of CTAB in the mobile phase varied from 0.006 to 0.15M.
**Uncertainties in $\Theta[L_s]K_1$ and K_2 were determined from the statistical parameters of the least squares fitting and from propogation of error.

Table 5

Dodecyltrimethylammonium Bromide

Compound*,**	$\Theta[L_s]K_1$	K_2
Vanillic Acid	21.9 ± 1.7	52.7 ± 5.8
Vanillin	22.8 ± 1.4	31.6 ± 2.7
Isovanillin	24.0 ± 1.6	29.7 ± 2.7
Ethylvanillin	46.1 ± 2.4	48.2 ± 3.1
Coumarin	40.7 ± 3.1	31.8 ± 3.4
Orthovanillin	53.8 ± 5.2	38.5 ± 6.3

* Compounds are listed in their order of elution from Apex I C-18.
Concentration of DTAB in the mobile phase varied from 0.01 to 0.14M.
**Uncertainties in $\Theta[L_s]K_1$ and K_2 were determined from the statistical parameters of the least squares fitting and from propogation of error.

CONCLUSION

Surfactant-bonded phase interactions in MLC are very important. A fundamental understanding of these interactions is crucial for developing separations with greater selectivity in MLC. Hence, finding the appropriate combination of surfactant and stationary phase is crucial in micelle mediated separations. Perhaps, some of the reported differences in selectivity between MLC and RPLC with hydro-organic mobile phases are due in some measure to the modification of the stationary phase by adsorbed surfactant.[23]

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