

# Systematic Studies of the Retention of Organic Compounds on an Octadecylsiloxane Column Using Sodium Dodecyl Sulfate as a Mobile Phase

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## ABSTRACT

The retention of ionizable, polar and nonpolar solutes on an octadecylsiloxane column (ODS) when sodium dodecyl sulfate (SDS) is used as a mobile phase was investigated. Studies were conducted at different surfactant concentrations, mobile phase acidities, concentrations of 2-propanol and ionic strengths of the mobile phase. The elution time of the ionizable solutes, aniline, pyridine and benzoic acid, is dependent on the electrostatic interaction (repulsion or attraction) with the negatively charged sites of the surfactant molecules bonded to the stationary phase. The micellar system resembles the reverse-phase chromatographic system in the retention of polar compounds (nitrobenzene, acetophenone, phenol, and benzaldehyde) and nonpolar compounds (toluene and benzene). The retention time of these solutes is determined by their partition between the nonpolar portions of the stationary phase and the lipophilic core of the micelle.

## INTRODUCTION

At surfactant concentrations above the critical micellar concentration (CMC), micelles are formed in the aqueous solution, i.e., aggregation of surfactant molecules. Therefore, the aqueous solution consists of two phases, the micelle and the bulk water. These, together with the stationary phase, form a three-phase system. Armstrong and Nome (1981) demonstrated the use of micellar surfactants, at concentrations above the critical micellar concentration, as mobile phase components in liquid chromatography. They proposed a model for the partition of the solute between the stationary phase and the aqueous phase (Figure 1). Accordingly, the solute is distributed between the micelle and the bulk water, the bulk water and the stationary phase, and the stationary phase and the micelle. The elution behavior of the solute is based on the combined effects of the three partition coefficients.

Since the introduction of micellar chromatography, other investigations have been reported on the use of surfactants as a mobile phase. Yarmchuk and co-workers (1982) studied the selectivity of methylsiloxane type column when sodium laurylsulfate and

dodecyltrimethylammonium bromide were used as mobile phases. Dorsey and co-workers (1983) enhanced column efficiency with sodium dodecyl sulfate as a mobile phase by the use of up to 3% n-propanol and temperatures of about 40°C. Landy and Dorsey (1985) extended the previous study to include the cationic surfactant, cetyltrimethylammonium bromide (CTAB), and the nonpolar surfactant, Brij-35. Arunyanart and Cline Love (1985) examined the retention behavior of the cyano type stationary phase when SDS was used as a mobile phase. Berthod and co-workers (1986) compared retention behavior of silica gel, C<sub>1</sub>, C<sub>8</sub>, C<sub>18</sub>, and CN bonded stationary phases at room temperature and pH 6.0. Their work was extended to the additive effects of 0.10 M NaCl and 5% methanol on the adsorption of SDS and CTAB (1986). Berthod and Roussel (1988) studied the effect of several organic modifiers (methanol, propanol, pentanol and tetrahydrofuran) on column efficiency. Kord and Khaledi (1992) used amino acids to compare elution strength and selectivity between micellar liquid chromatography and ion pair chromatography. Rodgers and Khaledi (1994) extended the above work with amino acids to include the effect of the acidity of the mobile phase.

Previously reported investigations have not provided a comprehensive examination of the retention behavior of organic compounds ranging from polar to ionizable to nonpolar on the octadecylsiloxane column when sodium dodecyl sulfate is used as a mobile phase. This work provides a study of this type.

## MATERIALS AND METHODS

### Instrumentation

The HPLC system consisted of a Perkin Elmer Series 3 Liquid Chromatograph equipped with a variable wavelength LC-65T UV detector/oven, an LC1-100 integrator and a 20  $\mu$ L Rheodyne sample injector (Model 7125, Berkeley, CA). The column (25 cm long and 4.6 mm i.d.) was packed with 5  $\mu$ m Supelcosil LC-18 (Supelco, In., Bellefonte, PA). The void volume of the system was calculated by using the peak of injected water. All pH measurements were performed with ALTEX Model 3560 Digital pH meter and Corning combination glass electrode. The flow rate was fixed at 1.0 mL min<sup>-1</sup>, the wavelength was set to 254 nm and the column temperature was maintained at 40°C.

### Chemicals and Reagents

Sodium dodecyl sulfate, benzene, toluene, pyridine, aniline, benzoic acid, benzaldehyde, acetophenone and nitrobenzene were obtained from Aldrich Chemical Company, Inc. These chemicals were used as received. The stock solution of 0.15 M SDS was prepared in deionized water and filtered through a 0.45- $\mu$ m membrane (Rainin Instrument Co., Inc., Woburn, MA). The mobile phase was adjusted to the desired pH with 0.01 M Na<sub>2</sub>HPO<sub>4</sub>/H<sub>3</sub>PO<sub>4</sub>. Stock solutions of the test solutes were prepared in 0.10 M SDS solution. The working concentrations were as follows: benzene (1198  $\mu$ g/mL), toluene (1254  $\mu$ g/mL), pyridine (100  $\mu$ g/mL), aniline (100  $\mu$ g/mL), benzoic acid (100  $\mu$ g/mL), benzaldehyde (5  $\mu$ g/mL), acetophenone (5  $\mu$ g/mL), phenol (100  $\mu$ g/mL) and nitrobenzene (100  $\mu$ g/mL).

### Column Preparation and Reliability

The ODS-bonded silica column was coated with SDS by passing an aqueous solution of 0.10 M SDS at a flow rate of 1.0 mL min<sup>-1</sup> through the column for 2 h. Following

multiple separations the column was regenerated by washing with deionized water for 10 min at 1 mL min<sup>-1</sup>, followed by pure methanol until all surfactant was removed (about 1 h), and then with deionized water until all methanol was removed (about 1 h).

The reliability of the method was tested by injecting quadruplicate samples. A relative standard deviation of less than 0.5% was found. Based on these results, all studies were performed using single injections.

The capacity factor ( $k'$ ) was calculated using:

$$k' = \frac{t_r - t_o}{t_o} \quad \text{where: } t_r = \text{total retention time of the solute}$$

$$t_o = \text{retention time of the solute in mobile phase}$$

## RESULTS AND DISCUSSION

### Effect of Surfactant Concentration

The behavior of each solute was studied at concentrations of SDS ranging from 0.05 M to 0.15 M. As the concentration of SDS increased, the capacity factor decreased for all solutes (Figure 2). This is due to an increase in the number of micelles in the mobile phase as the concentration of surfactant increases (Yarmchuk, et. al., 1982).

These results are in contrast to reverse-phase ion-pair chromatography where the concentration of ionic surfactant is below the CMC. Under those conditions, while an increase in the concentration of surfactant has no influence on the retention of polar and nonpolar solutes, the retention of those compounds which are attracted electrostatically to the surfactant is increased (Snyder and Kirkland, 1979).

When surfactant is above the CMC, polar and nonpolar solutes are partitioned between the polar bulk aqueous phase and the nonpolar portions of the micelle and the stationary phase (Figure 1). The capacity factors for the polar and nonpolar compounds studied decrease in the same order as their polarity increases (Figure 2). This indicates that for these types of compounds the micellar system resembles reverse-phase chromatography.

The results in Figure 2 show a sharper decrease in the retention of the ionizable solutes, aniline and pyridine, as compared to the polar and nonpolar solutes. This can be explained by assuming that such ionizable solutes can interact electrostatically as well as hydrophobically with the micelle and the stationary phase.

### Micelle Bulk pH Effect

When the pH was varied from 2.82 to 7.00, the capacity factor of the nonpolar and polar solutes was found to be independent of the acidity of the mobile phase (Figure 3). On the other hand, the capacity factor of ionizable benzoic acid decreased as the pH of the mobile phase increased. The  $pK_a$  of benzoic acid (4.21) indicates that the concentration of the benzoate anion increases as the pH of the solution increases. Consequently, electrostatic

repulsion between the benzoate anion and the surfactant-modified stationary phase may increase its concentration in the mobile phase and therefore decrease its elution time.

The capacity factors of the ionizable species, aniline and pyridine, increased as the pH of the mobile phase decreased. From the  $pK_a$  values of the anilinium cation (4.60) and the pyridinium cation (5.22), the two solutes are expected to be partially ionic in solutions at pH below their  $pK_a$  values. This implies that the increase in capacity factor at the lower pH values may be due to the net electrostatic interaction of the solute with the micelles and the stationary phase.

The asymmetry factors calculated for all solutes except pyridine were less than 1.3. It is interesting to note that at pH 2.91 the pyridine peak showed extreme tailing (asymmetry factor = 9.8) which improved as the pH increased to 6.97 (asymmetry factor = 4.3). After washing the surfactant off the column with 300 mL of pure methanol and using methanol-water as a mobile phase, tailing was still observed at the higher pH values and again observed when a new column (never treated with surfactant) was used. These results may be explained by the electrostatic interaction of the pyridinium ion with the unprotonated hydroxyl group of the silica gel particles (Hux and Cantwell, 1984).

#### **Effect of 2-Propanol**

In reverse-phase chromatography, it is generally accepted that the addition of an organic modifier to the mobile phase decreases its polarity. This, in turn, causes the solvent strength to increase which ultimately leads to shorter retention of solutes. Similar results were observed in this study when the percentage of 2-propanol was increased from 3% to 15% in the presence of 0.10 M SDS in the mobile phase (Figure 4). In addition, there was a greater decrease in the retention of aniline and pyridine than in the other solutes studied. The retention of aniline and pyridine decreased by factors of 0.45 and 0.51, respectively, while the other solutes decreased by an average factor of  $0.65 \pm 0.04$ . The small decrease in capacity factor of the nonionic solutes may be related to the decrease in polarity of the mobile phase as the concentration of 2-propanol increases. The more significant decrease in capacity factors of aniline and pyridine may be a result of a decrease in the number of surfactant molecules bonded to the stationary phase when the polarity of the mobile phase is decreased as the amount of 2-propanol is increased. This decrease in the number of active sites for electrostatic interaction shifts the partition equilibrium of the solute to the mobile phase direction.

#### **Salt Effect**

In general, the solubility of an organic solute, in the ionic form, depends on the interaction of both its ionic and its non-ionic portions with the stationary phase. Thus, the retention behavior of such solutes as a function of salt concentration results from a combination of electrostatic and hydrophobic effects. The net effect of increasing the sodium chloride concentration up to 0.10 M was found to affect the slopes of aniline and pyridine more than that of the polar and nonpolar solutes (Figure 5). This may be attributed to the competition between the solute cations and the sodium ion for the active sites on the stationary phase.

## SUMMARY

Micellar chromatography can offer several mechanisms for manipulating retention times and controlling column selectivity. The effects of sodium dodecyl sulfate concentration, acidity of the mobile phase, percentage of 2-propanol and concentration of sodium chloride affected the retention times of the weak organic bases (aniline & pyridine) to a greater extent than the weak organic acid (benzoic acid) and the polar and nonpolar solutes studied.

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Figure 1. Diagram illustrating the partition of the solute between the stationary phase, micelle and the bulk aqueous phase.

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Figure 2. Effect of surfactant concentration on capacity factor. Conditions: eluent pH 3.10, flow rate 1.0 mL/min and 2-propanol (3%). Line identities: (t) = aniline, (●) = toluene, (□) = pyridine, (O) = benzene, (◇) = nitrobenzene, (Δ) = acetophenone, (‡) = benzoic acid, (s) = benzaldehyde, (n) = phenol.

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Figure 3. Effect of pH of the mobile phase on the capacity factor. Conditions: 0.10 M SDS concentration, flow rate 1.0 mL/min and 2-propanol (3%). Line identities: (●) = toluene, (t) = aniline, (O) = benzene, (□) = pyridine, (◇) = nitrobenzene, (Δ) = acetophenone, (s) = benzaldehyde, (t) = benzoic acid, (n) = phenol.

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Figure 4. Effect of percentage of 2-propanol on capacity factor. Conditions: 0.10 M SDS concentration, pH 3.06, flow rate 1.0 mL/min. Line identities: (●) = toluene, (t) = aniline, (O) = benzene, (□) = pyridine, (◇) = nitrobenzene, (Δ) = acetophenone, (s) = benzaldehyde, (t) = benzoic acid, (n) = phenol.

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Figure 5. Effect of concentration of sodium chloride on capacity factor. Conditions: 0.10 M SDS concentration, pH 3.10, flow rate 1.0 mL/min and 2-propanol (3%). Line identities: (●) = toluene, (t) = aniline, (O) = benzene, (□) = pyridine, (◇) = nitrobenzene, (Δ) = acetophenone, (s) = benzaldehyde, (t) = benzoic acid, (n) = phenol.

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