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Research Article

Thermodynamic studies of the interaction of molecular micelles and copolymerized molecular micelles with benzodiazepines and alkyl phenyl ketones using MEKC

In this study, polymers of sodium 10-undecenoyl L-leucinate (SUL) and sodium undecenyl sulfate (SUS) as well as their copolymerized molecular micelles (CoPMMs) were applied in MEKC as pseudostationary phases to separate benzodiazepines and alkyl phenyl ketones. SDS, a common pseudostationary phase used in MEKC, was also used for comparison. The van't Hoff relationship was applied to compute the temperature dependence of the MEKC retention factors of the test solutes to estimate the enthalpy, entropy, and the Gibbs free energy. Nonlinear van't Hoff plots were obtained with the majority of benzodiazepines indicating that the thermodynamic parameters were temperature-dependent in all surfactant systems for these solutes. In contrast, all alkyl phenyl ketones resulted in linear van't Hoff plots.

Keywords:

Benzodiazepines / Compensation temperature / Copolymerized molecular micelles / Enthalpy / Entropy
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1 Introduction

MEKC is a mode of CE, which was first introduced by Terabe *et al.* [1]. The separation principle is based on the differential distribution of molecules between an aqueous buffer solution and the moving charged pseudostationary phase. A commonly used pseudostationary phase in MEKC is SDS. As alternatives to conventional micelles (*e.g.*, SDS), molecular micelles have been used as pseudostationary phases in MEKC because of their distinct advantages over conventional micelles. First, molecular micelles have zero CMC [2, 3]. Thus, they can be used at concentrations well below the CMC of the nonpolymerized surfactants [4]. Secondly, molecular micelles are stable in the presence of organic solvents due to the presence of covalent linkage between the individual surfactant molecules [3]. Finally, due to their high molecular weights, molecular micelles can be conveniently used

in MEKC-MS applications without background interference from surfactant monomers of low molecular weights [5, 6].

It should be noted that there are some disadvantages associated with the use of molecular micelles in MEKC. First, molecular micelles are not commercially available. Thus, they need to be synthesized and polymerized to be used as pseudostationary phases. The synthesis of the molecular micelles may be time consuming and requires some expertise. Secondly, in general, gamma radiation or chemical means are used for polymerization. The former is not always easily accessible and may have harmful effects on human health. For chemical polymerization, initiators and other side products are sometimes difficult to eliminate and these impurities may reduce the performance of the pseudostationary phases. Finally, batch-to-batch variations are common and may have some effect on the precision of the separation. Despite these possible disadvantages, the use of molecular micelles in MEKC has been shown to have many distinct advantages [3–8].

Although poly(sodium 10-undecylenate) (poly-SUA), the first successful anionic molecular micelle used as a potential pseudostationary phase in MEKC, has separated a wide range of neutral compounds, its electrophoretic mobility was influenced drastically by the ionization of the carboxylated

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Abbreviations: CoPMM, copolymerized molecular micelle; SUL, sodium 10-undecenoyl L-leucinate; SUS, sodium undecenyl sulfate

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head groups, thus resulting in poor reproducibility in analysis times [7]. Moreover, poly-SUA has limited solubility, *i.e.*, not soluble below pH 7.0. To overcome these problems, Shamsi *et al.* [8] and Palmer and Terabe [9] polymerized a surfactant with a sulfate head group, *i.e.*, poly(sodium 10-undecenyl sulfate) (poly-SUS). In addition, chiral molecular micelles have also been introduced as pseudostationary phases in MEKC [10, 11]. A major advantage of chiral molecular micelles is that manipulation of selectivities is feasible through the integration of a variety of chiral head groups into the molecular micelles.

Temperature has a major impact on retention, selectivity, and resolution in chromatographic separations [12]. In chromatographic systems, the distribution coefficient of a solute between the mobile phase and stationary phase is a function of the difference in free energy of the solute in the two phases [13]. Thus, a better understanding of the partition mechanism between the pseudostationary phase and the solute is important to control selectivity. Terabe *et al.* [14] have studied the micellar solubilization of a group of analytes by MEKC using SDS as a pseudostationary phase. Peterson and Foley [15] have investigated the micellar solubilization of chiral compounds using a monomeric chiral surfactant. Their results showed that structural differences of solutes had a significant effect on the enthalpy and entropy of solubilization of the solutes in pseudostationary phases. These studies showed also that small differences in the enthalpy and entropy of solute transfer play an important role in selectivity. In addition, the temperature dependence of the retention factor of PTH DL-amino acids in MEKC using two chiral molecular micelles has been recently reported [16]. Both surfactant systems with different head groups were found to provide different thermodynamic quantities using the same set of solutes.

In the study reported here, polymers of sodium 10-undecenyl L-leucinate (SUL) and SUS as well as their copolymerized molecular micelles (CoPMs) were applied in MEKC as pseudostationary phases for the separation of chiral and achiral molecules. Furthermore, the separations obtained with the new molecular micelles (CoPMs, poly-SUS, and poly-SUL) were compared to the results obtained with SDS as a pseudostationary phase. Using the van't Hoff relationship, the enthalpy and entropy of transfer of seven benzodiazepines (flunitrazepam, nitrazepam, clonazepam, temazepam, diazepam, oxazepam, lorazepam) and seven alkyl phenyl ketones (acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, and octanophenone) were measured from the aqueous phase to the pseudostationary phases. In addition, distribution coefficients, Gibbs free energies, and compensation temperatures of the pseudostationary phase-solute complexes were determined. To investigate the partition mechanism, which enables pseudostationary phases to selectively separate many compounds, the distribution coefficients, free energy, enthalpy, and entropy of transfer were determined and are reported in this study.

2 Materials and methods

2.1 Instrumentation

A Beckman P/ACE model 5510 CE instrument (Fullerton, CA, USA) was employed for MEKC separations. System Gold software was used for system control and data handling. The MEKC separations were performed in a 57 cm total length (50 cm effective length) \times 50 μ m id (367 μ m od) fused-silica capillary (Polymicro Technologies, Tucson, AZ, USA). The temperature of the capillary in the CE instrument was controlled by use of a fluoroorganic fluid. New capillaries were prepared by rinsing each with 1 M NaOH (1 h) and triply deionized water (30 min). After each separation with the same pseudostationary phase, the capillary was rinsed sequentially 5 min with triply deionized water, 0.1 M NaOH (3 min), and separation buffer (3 min). The capillary was reactivated each day by sequentially rinsing with 1 M NaOH (15 min), triply deionized water (2 min), and the running buffer (5 min). When the pseudostationary phase was changed, the capillary was reconditioned with triply deionized water (15 min), 0.1 M NaOH (10 min), and with the separation buffer (5 min). Unless otherwise noted, the time used for pressure injection (0.5 psi) was 2 s. Enantiomeric mixtures were used for temazepam, oxazepam, and lorazepam, and the elution order for the specific enantiomers was not determined. Thus, the standard notation throughout the text will be based upon the solutes elution order *i.e.* oxazepam 1 elutes before oxazepam 2. Each solute/pseudostationary phase was run at least in triplicate.

2.2 Materials

The benzodiazepines (flunitrazepam, nitrazepam, clonazepam, temazepam, diazepam, oxazepam, lorazepam) and L-leucine were obtained from Sigma (St. Louis, MO, USA). The compounds *N*-hydroxysuccinimide, undecylenic acid, dicyclohexylcarbodiimide (DCC), ethyl acetate, disodium hydrogen phosphate, sodium bicarbonate, and sodium carbonate were all obtained from Aldrich (Milwaukee, WI, USA) in reagent-grade form. The undecylenyl alcohol, alkyl aryl ketone homologs (acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, and octanophenone), chlorosulfonic acid, SDS, and pyridine (PY) were of analytical reagent grade and were also purchased from Aldrich. All chemicals were used as received.

The details of the synthesis of monomeric achiral SUS have been reported elsewhere [3]. Monomeric chiral SUL was synthesized according to a procedure reported by Lapidot *et al.* [17]. The details of the modified synthetic procedure are reported in a study by Wang and Warner [10]. The synthesis of CoMMs has been previously published [18].

2.3 Preparation of separation buffers and standard solute solutions

A 100 mM stock solution of phosphate buffer (pH 8.0) was prepared by dissolving the appropriate amount of sodium dihydrogenphosphate in triply deionized water. The solution of each pseudostationary phase was prepared by first dissolving 0.1 g of the surfactant in 5.0 mL of triply deionized water. Two milliliters of the 100 mM phosphate stock buffer were then added to this solution. The pH of the surfactant solution was adjusted to 8.0 using either dilute phosphoric acid or dilute NaOH. The final volume was then adjusted to 10.0 mL with triply deionized water. Finally, the buffers were sonicated (10 min), filtered (0.45 μm ; Nalgene, Rochester, NY, USA), and then resonicated (3 min). Stock solutions of the benzodiazepines, acetophenone, propiophenone, butyrophenone, and valerophenone were prepared in a 1:1 methanol/triply deionized water mixture while hexanophenone, heptanophenone, and octanophenone were prepared in pure methanol. The concentrations of the solutes ranged from 0.15 to 0.30 mM.

2.4 Calculations

The capacity factor, k' , of neutral solutes was measured by use of the following equation [19]:

$$k' = \frac{t_R - t_{eo}}{t_{eo} \left[1 - \left(\frac{t_R}{t_{psp}} \right) \right]} \quad (1)$$

where t_R , t_{eo} , and t_{psp} are the respective migration times of the retained solute, the EOF, and the pseudostationary phase. Methanol was used as the t_{eo} marker and was measured from the time of injection to the first deviation from the baseline. Decanophenone was used as a tracer for t_{psp} [20]. Also, t_R can be measured for the first and last eluted solutes, and the separation windows $t_{psp} - t_{eo}$ and $t_1 - t_f$ are then defined. The capacity factor is related to the distribution coefficient, K , by the following equation:

$$k = K \left(\frac{V_{psp}}{V_{aq}} \right) \quad (2)$$

where V_{psp} is the volume of the pseudostationary phase and V_{aq} is the volume of the aqueous phase. The ratio of V_{psp}/V_{aq} is known as the phase ratio, β , which can be determined using the following relationship [14]:

$$\beta = \frac{\bar{v}(C_{psp} - \text{CMC})}{1 - \bar{v}(C_{psp} - \text{CMC})} \quad (3)$$

where \bar{v} is the partial specific volume of the pseudostationary phase, C_{psp} is the concentration of the pseudostationary phase, and CMC is the critical micelle concentration of the pseudostationary phase. The \bar{v} values were determined in 5°C increments from 15 to 40°C and reported elsewhere [18].

Under linear chromatographic conditions, the temperature dependence of the retention of a given solute can be expressed with the following van't Hoff equation [21]:

$$\ln K = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (4)$$

where ΔH^0 is the change in enthalpy associated with the transfer of the solute from the aqueous buffer solution to the pseudostationary phase, ΔS^0 is the corresponding change in entropy, R is the ideal gas constant, and T is the absolute temperature. A plot of $\ln K$ versus $1/T$ is expected to be linear if these thermodynamic properties (*i.e.*, ΔH^0 and ΔS^0) are temperature-independent and the phase ratio, β , is independent of temperature over the temperature range studied [22]. Graphical analysis of $\ln K$ versus $1/T$ plot would yield the ΔH^0 and ΔS^0 values from the slope ($-\Delta H^0/R$) and y -intercept ($\Delta S^0/R$), respectively. Once ΔH^0 and ΔS^0 are known, Gibbs free energy, ΔG^0 , associated with the transfer of the solute from the aqueous solution to the pseudostationary phase can be calculated using the following equation:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (5)$$

3 Results and discussion

3.1 Effect of temperature on separation of benzodiazepines

The retention times of the benzodiazepines decreased as the temperature was gradually increased from 15 to 40°C. The separation window also decreased as the temperature was elevated from 15 to 40°C (data not shown). However, the effect was less pronounced in poly-SUS and SDS. As the temperature was increased from 20 to 40°C, the migration order and resolution of the majority of the benzodiazepines were significantly changed. The electropherograms of benzodiazepines at 40°C are shown in Fig. 1 and the corresponding electropherograms for 20°C are presented in our earlier publication [18]. For example, at 40°C, the migration order of diazepam (peak 5) and oxazepam (peak 6) was reversed using poly-SUL (Fig. 1A). The resolution between diazepam and oxazepam (peaks 5 and 6) increased and the resolution between lorazepam and diazepam (peaks 7 and 5) decreased with increasing temperature (Fig. 1B) with poly-L₈S₂. Using poly-L₆S₄, lorazepam and diazepam (peaks 7 and 5) are baseline-resolved at 20°C, but coeluted at 40°C (Fig. 1C). In addition, the migration order of flunitrazepam and nitrazepam was reversed at 40°C as compared to the migration order at 20°C using poly-L₂S₈ (Fig. 1E). In contrast, for SDS the migration order of the benzodiazepines was unaffected at both temperatures; but, the resolution between the adjacent peaks improved at 40°C. Unlike benzodiazepines, no change in retention order of alkyl phenyl ketones is expected due to the known properties of homolog series; that is, their beha-

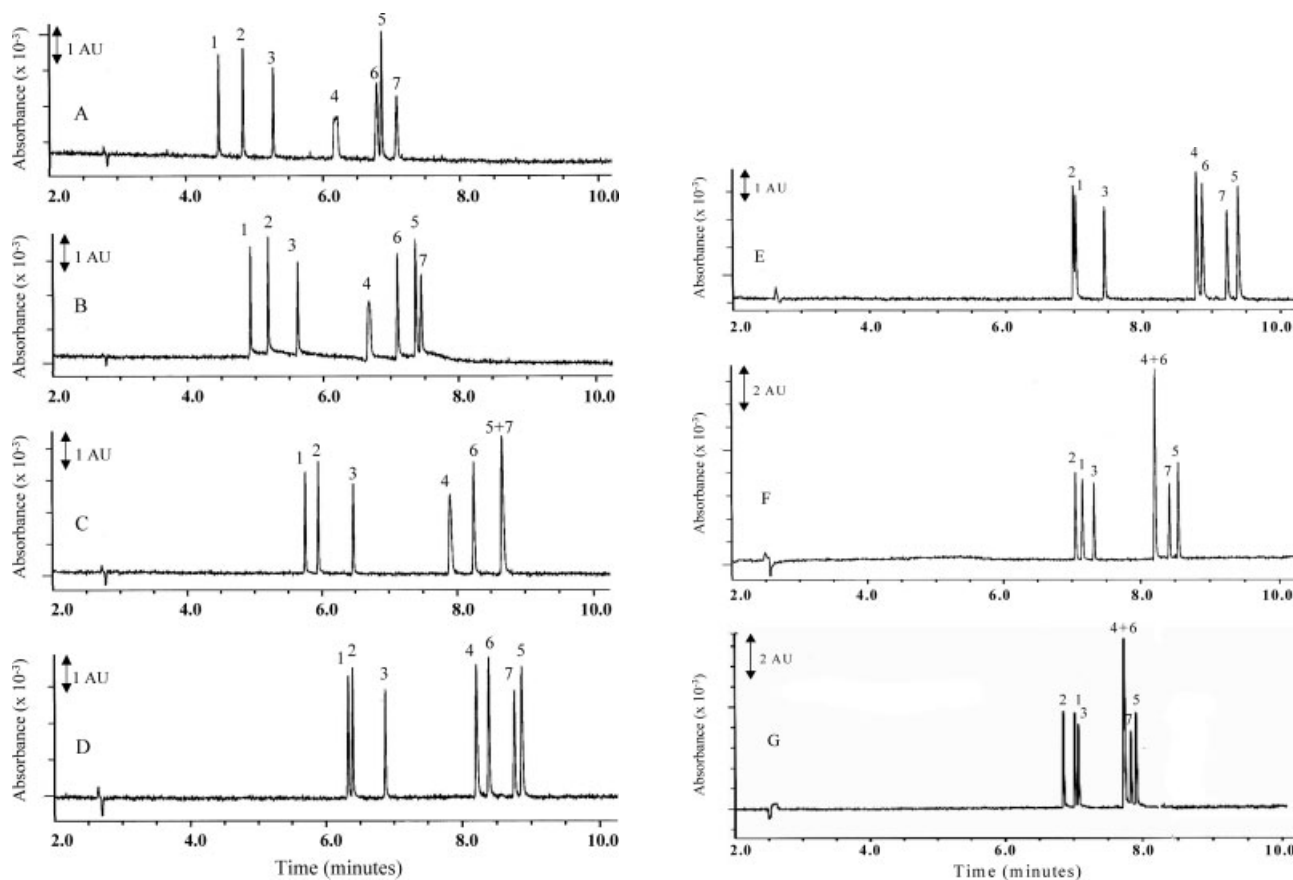


Figure 1. Comparison of (A) poly-SUL, (B) poly-L₈S₂, (C) poly-L₆S₄, (D) poly-L₈S₂, (E) poly-L₂S₈, (F) poly-SUS, and (G) SDS for separation of seven benzodiazepines. MEKC conditions: mobile phase, 1.0% w/v each surfactant in 20 mM phosphate buffer (pH 8.0); pressure injection, for 2 s; applied voltage, +25 kV; temperature, 40°C, UV detection: 254 nm. Peak identifications, 1, flunitrazepam; 2, nitrazepam; 3, clonazepam; 4, temazepam; 5, diazepam; 6, oxazepam; and 7, lorazepam. Each sample was run at least in triplicate.

viors vary with respect to each other as the experimental conditions are changed. As discussed later, the differences in retention behavior are both solute- and pseudostationary phase-dependent. It is believed that the ionization of both analytes and the molecular micelle head groups improves with an increase in temperature. In addition, when the temperature rises, the hydrophilic head groups lose the water of hydration [23]. These changes, in turn, will manipulate the chemistry around the micellar head groups and thus influence analyte partitioning between the pseudostationary phase and the BGE. It should be mentioned that temperature has no significant effect on the methylene selectivity, which is correlated with the hydrophobicity [18].

3.2 Thermodynamic calculations

One of the objectives of this study is to evaluate the ΔH° and ΔS° values as the solutes transfer from the aqueous phase to the micellar phase as a function of temperature. The $\ln K$ values of benzodiazepines were plotted against $1/T$ (van't Hoff plot) (data not shown). At temperatures between 20 and

30°C, distinct deviation from linearity in van't Hoff plots were observed in all pseudostationary phases. The nonlinear van't Hoff plots indicate temperature dependence of the partitioning of benzodiazepines from the aqueous phase to the pseudostationary phase. It also indicates the entropy-controlled factors which are involved in complexation between the solutes and the pseudostationary phases [24]. Similar nonlinear trends of the van't Hoff plots have been noticed in HPLC [24–26], which were attributed to octadecyl stationary phase undergoing a phase transition of the octadecyl chains from a liquid-like state to a more ordered crystalline-like state upon cooling. In MEKC, however, this nonlinearity could be attributed to the conformation of both the solutes and the pseudostationary phases. Although the nonlinearity in van't Hoff plots was more pronounced for the longer retained benzodiazepines, which have more hydrogen bonding sites, (*i.e.*, temazepam, diazepam, oxazepam, and lorazepam), it was not noteworthy for less retained three benzodiazepines (*i.e.*, flunitrazepam, nitrazepam, and clonazepam). Thus, the thermodynamic properties of the first three benzodiazepines and that of seven alkyl phenyl ketones in seven pseudosta-

tionary phases were further examined. Enantiomers of the chiral benzodiazepines (temazepam, oxazepam, and lorazepam) were not studied due to the ineffective enantioseparation at or above 25°C.

The van't Hoff plots for three benzodiazepines are shown in Fig. 2. The K , ΔH° , ΔS° , and ΔG° values are listed in Table 1. The ΔG° values are negative for all three benzodiazepines in all pseudostationary phases, indicating that the partitioning of these solutes from the aqueous phase into pseudostationary phase is thermodynamically favored. The absolute values of ΔH° for all three benzodiazepines are larger in SDS than in poly-SUL, poly-SUS, and CoPMs. This observation demonstrates that these three solutes thermodynamically favor the SDS micelles more than the remaining micelles. In contrast to ΔH° , the ΔS° values are either negative or positive depending on the pseudostationary phases used. For example, the ΔS° value for flunitrazepam is positive in all pseudostationary phases except in SDS. Conversely, the ΔS° values of nitrazepam and clonazepam are negative in all pseudostationary phases except in poly-SUL (Table 1). The ΔS° value is negative for the hydrophilic analytes due to their weak or even nonexistence hydrophobic character, which results in a low degree of ordering in the

aqueous phase. In general, the negative ΔS° value indicates a more organized system, possibly due to competition between the hydrophobic interaction that tends to keep the solute in the hydrophobic core of the micelles and the hydrogen bonding and dipole–dipole interactions that tend to keep the solutes on the surface of the micelles. When these two different forces are balanced, the solute will exist in an “adsorbed” state between the hydrophilic head group and the hydrophobic core of the micelle. When the ΔS° values are positive, the solutes are in a “dissolved” state and freely migrate between the hydrophobic core and the polar head group of the micelles, which results in a more disorganized system. These variations in ΔS° are believed to be dependent on the chemical species (*i.e.*, water molecules, buffer ions, *etc.*) surrounding the micellar phase in the separation capillary.

Previous studies have demonstrated that a significant amount of water is present in palisade and Stern layers of the conventional micelles [27, 28]. The chemistry on and around the surface of the micelle is influenced through the water molecules residing in palisade and Stern layers of the micelle. It has been suggested that these water molecules can influence the polarity and the hydrogen bonding character of the micelle [28, 29]. Due to the hydrophobicity of the benzodiazepines (their $\log P$ values range from 2.31 to 3.86) and the pseudostationary phases, hydrophobic interaction plays a significant role in retention of these solutes. In addition, as a result of the hydrogen bond donating and/or hydrogen bond accepting functional groups on the benzodiazepine molecules, hydrogen bonding is believed to play a more significant role than the hydrophobic interaction [18]. Thus, the chemistry on and around the micellar surface can be altered by temperature. The ionization of water molecules as well as benzodiazepine molecules may increase as a factor of temperature. Thus, the interaction between the benzodiazepines and the pseudostationary phases can be significantly influenced by the temperature.

The van't Hoff plots of seven alkyl phenyl ketones are shown in Fig. 3, and the thermodynamic results are summarized in Table 2. All ΔH° values of alkyl phenyl ketones are negative, and in general their absolute values increase with an increase in alkyl chain length of the solute. Unlike benzodiazepines, the ΔS° values for all alkyl phenyl ketones in all surfactant systems are positive except for acetophenone which has a negative ΔS° value due to its hydrophilic character, as compared to the rest of the alkyl phenyl ketones, in SDS. The absolute value of ΔS° increases with the length of the alkyl chain of the ketones. This increase in ΔS° values, as a function of hydrophobicity of the alkyl phenyl ketones, can be attributed to the contribution from hydrophobic interactions between the solutes and the pseudostationary phases. It is reasoned that the presence of a hydrophobic solute forces the water molecules surrounding the solute to form a dense, ordered network of hydrogen bonds with each other to minimize their contact with the hydrophobic solute. This ordering lowers the total entropy of the aqueous system. This

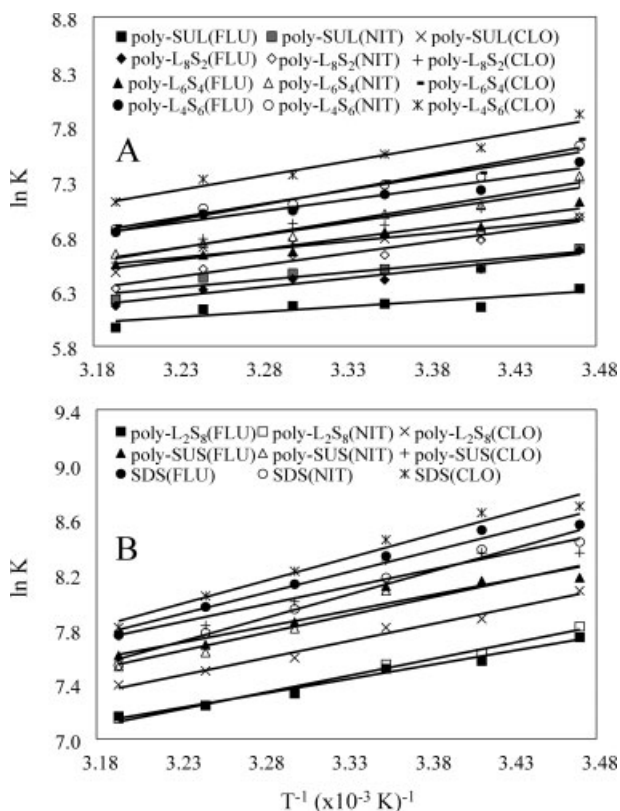


Figure 2. van't Hoff plots of three benzodiazepines (flunitrazepam, nitrazepam, and clonazepam) using (A) poly-SUL, poly- L_8S_2 , poly- L_6S_4 , and poly- L_4S_6 ; (B) poly- L_2S_8 , poly-SUS, and SDS. Legends are shown in the figure. Separation conditions are the same as in Fig. 1.

Table 1. Distribution coefficients (K), enthalpy (ΔH°), entropy (ΔS°), and Gibbs free energy (ΔG°) values for flunitrazepam, nitrazepam, and clonazepam in pseudostationary phases

Benzodiazepines		Pseudostationary phases						
		Poly-SUL	Poly-L ₈ S ₂	Poly-L ₆ S ₄	Poly-L ₄ S ₆	Poly-L ₂ S ₈	Poly-SUS	SDS
Flunitrazepam	K (at 20°C)	4.8×10^2	6.6×10^2	9.8×10^2	1.4×10^3	1.9×10^3	3.5×10^3	5.0×10^3
	ΔH° (kJ mol ⁻¹)	-7.8	-13.2	-16.5	-17.4	-17.3	-19.2	-20.9
	ΔS° (J·mol ⁻¹ ·K ⁻¹)	25.3	9.5	1.5	1.4	4.2	1.9	-1.4
	ΔG° (kJ·mol ⁻¹ at 20°C)	-15.2	-15.9	-16.9	-17.8	-18.5	-19.8	-20.5
Nitrazepam	K	6.7×10^2	8.7×10^2	1.2×10^3	1.5×10^3	2.0×10^3	3.5×10^3	4.4×10^3
	ΔH°	-11.4	-17.4	-20.8	-20.7	-20.1	-21.7	-23.9
	ΔS°	15.9	-2.8	-11.5	-9.0	-5.0	-6.4	-12.8
	ΔG°	-16.0	-16.6	-17.4	-18.1	-18.7	-19.8	-20.1
Clonazepam	K	9.0×10^2	1.2×10^3	1.6×10^3	2.0×10^3	2.6×10^3	4.3×10^3	5.7×10^3
	ΔH°	-12.1	-19.1	-22.7	-21.6	-20.6	-21.0	-23.2
	ΔS°	15.7	-5.9	-15.5	-9.8	-4.5	-2.6	-8.2
	ΔG°	-16.7	-17.3	-18.1	-18.8	-19.3	-20.3	-20.7

Percentage uncertainties in the calculated parameters were less than 4.3%.

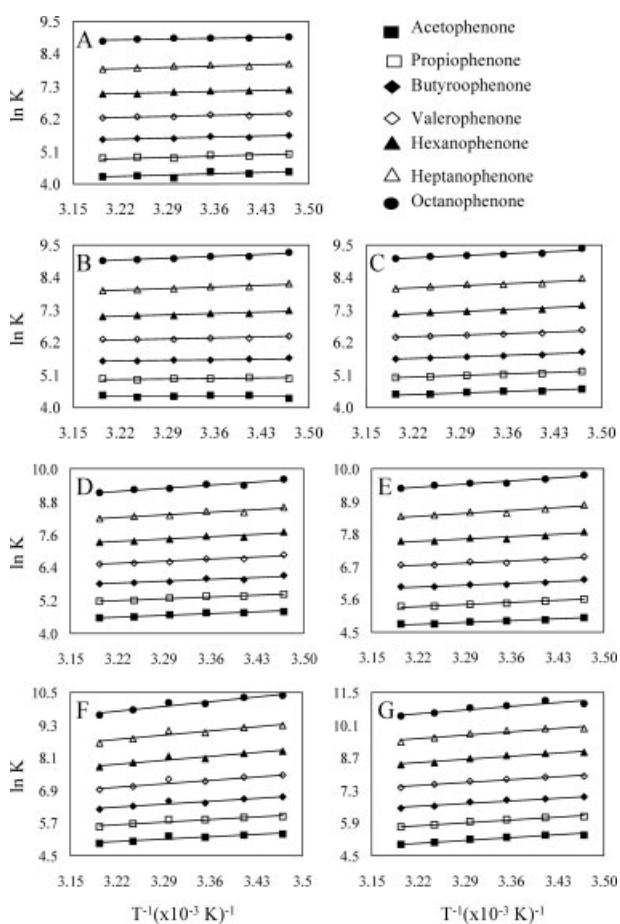


Figure 3. van't Hoff plots of alkyl phenyl ketones using (A) poly-SUL, (B) poly-L₈S₂, (C) poly-L₆S₄, (D) poly-L₈S₂, (E) poly-L₂S₈, (F) poly-SUS, and (G) SDS. Legends are shown in the figure. Separation conditions are the same as in Fig. 1.

ordered network of water molecules will disappear upon transfer of hydrophobic solutes from polar aqueous solution into a relatively hydrophobic pseudostationary phase. Consequently, the total entropy of the aqueous system increases significantly. This process can be explained using a two-state model [28]. The first state is the adsorbed state where the solute (in this case alkyl phenyl ketone) is attracted to the surface of the pseudostationary phase either through the dipole-dipole, dipole-induced dipole, or electrostatic interactions between the solutes and the pseudostationary phases. The second state is known as the dissolved state where the solutes will be solubilized into the core of the pseudostationary phase due to a match of the hydrophobicity of the solutes and the pseudostationary phase. The hydrophobicity of the alkyl aryl ketones increases as the length of the alkyl chain is increased. Thus, the K values increase due to stronger hydrophobic interaction between the solutes and the hydrophobic surfactant systems. As mentioned above, the ΔH° values of both benzodiazepines and alkyl phenyl ketones are negative in all surfactant systems, while the ΔS° values are generally negative for benzodiazepines but positive for alkyl phenyl ketones. The Gibbs free energy (ΔG°) values are negative for both groups of solutes, indicating a favorable or spontaneous partitioning of the solutes from relatively more hydrophilic aqueous phase to more hydrophobic micellar system.

The relative contributions of ΔH° and ΔS° to ΔG° for the benzodiazepines and the alkyl phenyl ketones in pseudostationary phases are shown in Tables 3 and 4, respectively. Since ΔG° values are always negative (Tables 1 and 2), the contribution of ΔH° and ΔS° to ΔG° is scientifically meaningful when the signs of both ΔH° and $-T\Delta S^\circ$ terms are negative. Therefore, the values for all benzodiazepines in poly-SUL and for flunitrazepam in all pseudostationary pha-

Table 2. The distribution coefficients (K), enthalpy (ΔH°), entropy (ΔS°), and Gibbs free energy (ΔG°) values for alkyl phenyl ketones in pseudostationary phases

Alkyl phenyl ketones		Pseudostationary phases						
		Poly-SUL	Poly-L ₈ S ₂	Poly-L ₆ S ₄	Poly-L ₄ S ₆	Poly-L ₂ S ₈	Poly-SUS	SDS
Acetophenone	$K^{a)}$	0.8×10^2	0.8×10^2	1.0×10^2	1.2×10^2	1.4×10^2	1.9×10^2	2.2×10^2
	$\Delta H^{b)}$	-5.1	0.8	-5.7	-7.1	-7.0	-10.4	-13.6
	$\Delta S^{c)}$	18.8	39.0	18.5	15.5	17.1	8.22	-1.86
	$\Delta G^{d)}$	-10.7	-10.7	-11.2	-11.6	-12.0	-12.8	-13.1
Propiophenone	K	1.4×10^2	1.5×10^2	1.7×10^2	2.1×10^2	2.5×10^2	3.7×10^2	4.6×10^2
	ΔH°	-4.5	-1.4	-6.0	-7.6	-7.6	-11.2	-13.8
	ΔS°	26.1	36.6	22.5	18.7	20.3	10.9	3.91
	ΔG°	-12.1	-12.2	-12.6	-13.1	-13.5	-14.4	-14.9
Butyrophenone	K	2.7×10^2	2.8×10^2	3.2×10^2	4.0×10^2	4.8×10^2	7.3×10^2	1.0×10^3
	ΔH°	-4.2	-2.9	-6.1	-8.1	-7.8	-12.6	-14.1
	ΔS°	32.2	37.1	27.7	22.6	25.1	11.7	9.54
	ΔG°	-13.7	-13.8	-14.2	-14.7	-15.1	-16.1	-16.9
Valerophenone	K	5.6×10^2	5.8×10^2	6.7×10^2	8.2×10^2	1.0×10^3	1.6×10^3	2.6×10^3
	ΔH°	-4.2	-3.8	-6.5	-9.2	-8.4	-14.5	-15.1
	ΔS°	38.6	40.1	32.2	24.9	29.0	11.7	13.9
	ΔG°	-15.5	-15.6	-16.0	-16.5	-16.9	-18.0	-19.2
Hexanophenone	K	1.3×10^3	1.3×10^3	1.5×10^3	1.9×10^3	2.3×10^3	3.9×10^3	7.1×10^3
	ΔH°	-5.0	-5.2	-7.6	-10.7	-9.6	-16.2	-16.7
	ΔS°	42.6	42.2	35.2	26.6	32.0	13.2	16.9
	ΔG°	-17.5	-17.6	-18.0	-18.5	-18.9	-20.1	-21.6
Heptanophenone	K	3.0×10^3	3.2×10^3	3.7×10^3	4.5×10^3	5.6×10^3	1.0×10^4	2.1×10^4
	ΔH°	-5.0	-6.3	-8.6	-11.6	-10.6	-18.3	-17.5
	ΔS°	49.9	46.0	39.4	31.0	36.0	14.0	22.7
	ΔG°	-19.6	-19.8	-20.1	-20.7	-21.1	-22.4	-24.1
Octanophenone	K	7.6×10^3	9.0×10^3	1.0×10^4	1.2×10^4	1.6×10^4	3.1×10^4	6.9×10^4
	ΔH°	-3.6	-7.6	-9.2	-12.8	-12.0	-21.3	-18.1
	ΔS°	62.3	50.3	45.8	35.1	39.2	12.9	30.0
	ΔG°	-21.8	-22.3	-22.6	-23.1	-23.5	-25.1	-26.8

The percent uncertainties in the calculated parameters were less than 5.2%.

Table 3. Contribution of enthalpy and entropy on Gibbs free energy for benzodiazepines in pseudostationary phases

Benzodiazepines		Pseudostationary phases						
		Poly-SUL	Poly-L ₈ S ₂	Poly-L ₆ S ₄	Poly-L ₄ S ₆	Poly-L ₂ S ₈	Poly-SUS	SDS
Flunitrazepam	$\Delta H^{a)}$ (%)	51.2	82.5	97.3	97.6	93.3	97.2	c)
	$\Delta S^{b)}$ (%)	48.8	17.5	2.7	2.4	6.7	2.8	c)
Nitrazepam	ΔH° (%)	71.0	c)	c)	c)	c)	c)	c)
	ΔS° (%)	29.0	c)	c)	c)	c)	c)	c)
Clonazepam	ΔH° (%)	72.6	c)	c)	c)	c)	c)	c)
	ΔS° (%)	27.4	c)	c)	c)	c)	c)	c)

a) Percent contribution of ΔH° .

b) Percent contribution of ΔS° on ΔG° .

c) Percent amount of hindered ΔH° by entropy effect was not determined due to ΔS° on ΔG° values.

Table 4. Contribution of enthalpy and entropy on Gibbs free energy for alkyl phenyl ketones in pseudostationary phases

Alkyl phenyl ketones		Pseudostationary phases						
		Poly-SUL	Poly-L ₈ S ₂	Poly-L ₆ S ₄	Poly-L ₄ S ₆	Poly-L ₂ S ₈	Poly-SUS	SDS
Acetophenone	ΔH° (%)	48.2	a)	51.3	61.0	58.4	81.2	a)
	ΔS° (%)	51.8	a)	48.7	39.0	41.6	18.8	a)
Propiophenone	ΔH° (%)	36.9	11.8	47.7	58.1	55.9	77.8	92.3
	ΔS° (%)	63.1	88.2	52.3	41.9	44.1	22.2	7.7
Butyrophenone	ΔH° (%)	31.0	21.0	42.8	54.9	51.4	78.7	83.5
	ΔS° (%)	69.0	79.0	57.2	45.1	48.6	21.3	16.5
Valerophenone	ΔH° (%)	27.0	24.4	40.9	55.7	49.7	81.0	78.7
	ΔS° (%)	73.0	75.6	59.1	44.3	50.3	19.0	21.3
Hexanophenone	ΔH° (%)	28.5	29.7	42.5	57.8	50.6	80.7	77.1
	ΔS° (%)	71.5	70.3	57.5	42.2	49.4	19.3	22.9
Heptanophenone	ΔH° (%)	25.3	32.0	42.7	56.1	50.2	81.7	72.4
	ΔS° (%)	74.7	68.0	57.3	43.9	49.8	18.3	27.6
Octanophenone	ΔH° (%)	16.3	34.0	40.6	55.5	51.2	84.9	67.2
	ΔS° (%)	83.7	66.0	59.4	44.5	48.8	15.1	32.8

a) Percent amount of hindered ΔH° by entropy effect was not determined due to ΔS° on ΔG° values.

ses (except SDS) are listed. Among the listed values, the ΔS° contribution to ΔG° is considerably higher in poly-SUL (Table 3) as compared to the remaining micellar systems. This can be attributed to the polar character of poly-SUL. As the mole fraction of sulfate head group in the molecular micelle is increased, the contribution of ΔH° to ΔG° becomes more significant. This trend becomes more noticeable with alkyl phenyl ketones. It should be mentioned that an increase in mole fraction of sulfate head group in the CoPMs improves the hydrophobicity of the molecular micelle [18]. For benzodiazepines the contribution of ΔH° to ΔG° is greater than that of ΔS° . For alkyl aryl ketones, however, the contribution is pseudostationary phase-dependent. That is, the contribution of ΔS° is dominant for the relatively polar surfactants (poly-SUL, poly-L₈S₂, and poly-L₆S₄) whereas the ΔH° contribution becomes significant for the sulfated rich molecular micelles (poly-L₄S₆, poly-L₂S₈, and poly-SUS) and SDS (Table 4). It is worth noting that the contribution of ΔH° decreases while that of ΔS° increases in almost all surfactant systems as the hydrophobicity of alkyl phenyl ketone increases. However, the trend is more noticeable with poly-SUL but less noticeable with poly-SUS.

Plots of ΔH° versus ΔS° for the alkyl phenyl ketones are shown in Fig. 4. Due to insufficient data points, the plots of ΔH° versus ΔS° and the compensation temperature, β_T values for benzodiazepines are not reported. As seen in Fig. 4, the correlation coefficient values of the ΔH° versus ΔS° plots for the alkyl aryl ketones range from 0.27 to 0.95. It should be noted that the positive slope observed for the alkyl phenyl ketones in poly-SUL (Fig. 4A) is not statistically significant due to the observed low correlations coefficient ($R^2 = 0.27$). All other pseudostationary phases generated negative slopes

(Figs. 4B–G). The fact that not all solutes fall on the straight line indicates that these solutes may have different conformations when they are solubilized in the pseudostationary phases. The slope of the line gives the β_T , which is characteristic of the type of mechanism between the solute and the pseudostationary phase. As seen in Table 5, the β_T values obtained from alkyl phenyl ketones range from 20 to 1652 K. These values are not in agreement with previous results using MEKC [14, 15, 30] and RP-HPLC [31]. Peterson and Foley found β_T value of 287 to 298 K for hydrophilic solutes and 260 to 307 K for hydrophobic solutes using chiral micelles [30]. Terabe *et al.* [14] found a β_T value of 203 K for alkyl phenols using SDS. The β_T values found in this study as well as those of Peterson and Foley and Terabe *et al.* are much smaller than those typically found in RP-HPLC (*e.g.*, 625 K). The smaller β_T indicates that the contribution of the entropic term is very important in solubilization of the solutes in micellar phases. Different values of β_T for the solutes in the same type of pseudostationary phases suggest that these two groups of solutes are incorporated into the pseudostationary phase by different mechanisms, which is discussed in our earlier study [18].

4 Concluding remarks

This study is the continuation of our previous work on characterizing a novel class of pseudostationary phases (CoPMs) synthesized from varied mole fractions of two different types of surfactants, *i.e.*, SUS and SUL. To test the applicabilities of these surfactants as novel pseudostationary phases, seven benzodiazepines and seven alkyl aryl ketones

Table 5. Compensation temperatures for analytes in the seven pseudostationary phases and correlation coefficient values of ΔS° versus ΔH° plots

Solutes		Pseudostationary phases						
		Poly-SUL	Poly-L ₈ S ₂	Poly-L ₆ S ₄	Poly-L ₄ S ₆	Poly-L ₂ S ₈	Poly-SUS	SDS
Benzodiazepines	$\beta_T^{a)}$	420	370	360	350	340	290	270
	$R^{2b)}$	0.98	0.99	0.99	0.98	0.97	0.95	0.97
Alkyl phenyl ketones	β_T	20	485	137	313	217	1652	162
	R^2	0.27	0.70	0.91	0.95	0.92	0.65	0.91

a) Compensation temperature (K), obtained from the slope of ΔS° versus ΔH° plots.

b) Correlation coefficient of ΔS° versus ΔH° plots.

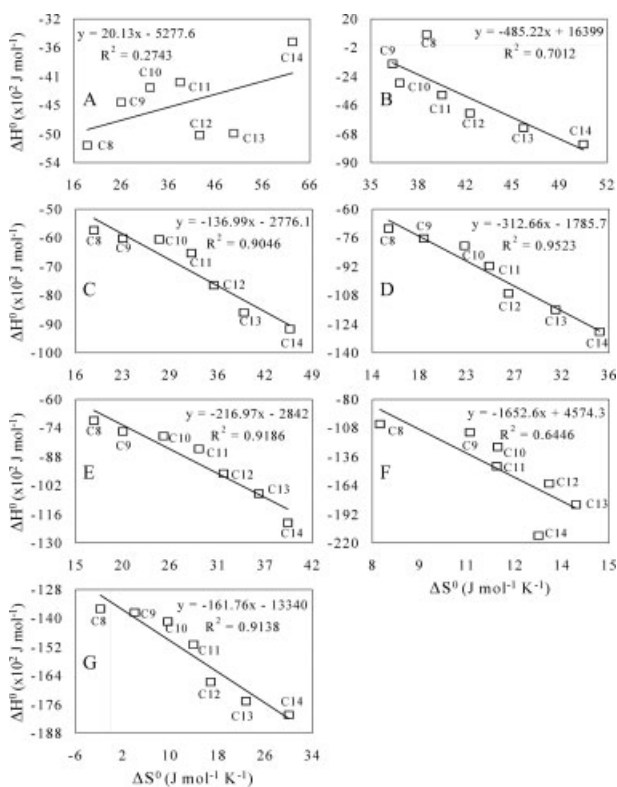


Figure 4. Enthalpy–entropy compensation in pseudostationary phases for alkyl phenyl ketones. Separation conditions are the same as in Fig. 1. Solutes: C8, acetophenone; C9, propiophenone; C10, butyrophenone; C11, valerophenone; C12, hexanophenone; C13, heptanophenone; and C14, octanophenone.

were separated at six different temperatures. Each pseudostationary phase was found to have different selectivities toward the benzodiazepines. These studies suggest the possibility of designing pseudostationary phases for the selected analytes. For each pseudostationary phase, differences in both ΔH° and ΔS° values were observed among the solutes. The van't Hoff plots for all alkyl phenyl ketones were linear, while these plots were nonlinear for the majority of benzodiazepines. This divergence suggests that the thermo-

dynamic parameters are mostly solute-dependent, rather than pseudostationary phase-dependent. The significant entropy differences and the wide range of entropy contribution to the Gibbs free energy change indicate the differences in selectivity of the pseudostationary phases. Our results for the thermodynamic parameters obtained in this study support our earlier deduction that hydrogen bonding between the solutes and the pseudostationary phases plays a major role in the retention of benzodiazepines, while hydrophobic interaction is a major factor in the retention of alkyl phenyl ketones.

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