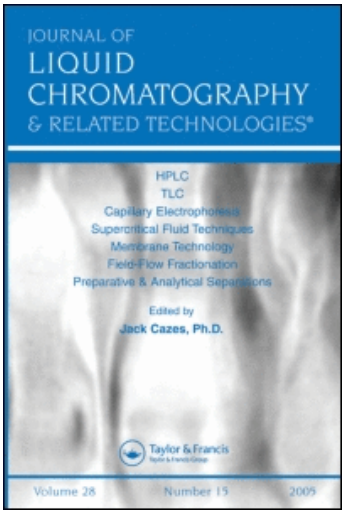

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A Thermodynamic and Fluorimetric Investigation of Micelle Chromatography: Effect of Temperature, Micelle Concentration and Polarity of Solutes

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A THERMODYNAMIC AND FLUORIMETRIC INVESTIGATION OF MICELLE CHROMATOGRAPHY: EFFECT OF TEMPERATURE, MICELLE CONCENTRATION AND POLARITY OF SOLUTES

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ABSTRACT

To understand the retention mechanisms involved in reversed-phase micelle chromatography, two models were examined to study the temperature and micelle concentration contribution to the retention process. The first model described the temperature contribution, using the van's Hoff equation, for each of the three equilibria: micelle-to-extra-micelle-mobile-phase, extra-micellar-mobile-phase-to-stationary phase and micelle-to-stationary phase. The second model described the temperature contribution on k' irrespective of the various equilibria involved. It was found that the enthalpy of retention obtained with the second model decreased with increasing micelle concentration. This contradicted a primary assumption in the first model that the equilibrium constants were independent of micelle concentration. No relationship was found between the enthalpy of retention evaluated with either model and the polarity of the partitioned solute molecules. A correlation was found between the entropy of retention and the polarity of these molecules. Fluorescence studies indicated that the micelle solvated molecules were probably located in the core of the micelles which contained other mobile phase components such as 1-propanol and water.

INTRODUCTION

The addition of micelles to reversed-phase liquid chromatography mobile phases solubilizes hydrophobic compounds that otherwise would not be soluble in the mobile phase. The theoretical framework that described micelle chromatography has been discussed by Armstrong and Nome (1), and by Arunyanart and Cline Love (2). Both models describe the retention controlling equilibria as the partitioning of the hydrophobic compound between three phases: the stationary phase, the mobile phase and the micellar phase.

To our knowledge there has been no formal investigation and discussion in the literature about the effect of temperature on the retention processes in micelle chromatography. This study will discuss theoretically and test experimentally two proposed models. The first model will describe the temperature contribution in terms of the van't Hoff equation for the three equilibria. The second model will use the van't Hoff equation to describe the contribution of temperature to the capacity ratio (k'), for each surfactant concentration without making any specific assumptions about the various equilibria involved.

Theory

For the following discussion, "probes" are defined as the solute molecules used to study the partitioning in a chromatographic process.

Micelle chromatography involves the partitioning of the chromatographed molecule (the probe) between the stationary phase and both the micelle and the extra-micellar phase:

$$k' = \frac{\phi[PL]}{[P] + [PM]} \qquad (1)$$

where k', θ, [P], [PL] and [PM] represent the capacity ratio, the phase ratio, the probe concentration associated with the extra micellar mobile phase,

the probe concentration associated with the stationary phase and the probe concentration associated with the mobile phase micelle assembly, respectively.

Model I

The first model describes the three reversible equilibria where the probe is associated with the different phases. The corresponding association constants are defined as follows:

$$k_1 = \frac{[PL]}{[P][L]} \tag{2}$$

$$k_2 = \frac{[PM]}{[P][M]} \tag{3}$$

$$k_3 = \frac{[PM][L]}{[PL][M]} \tag{4}$$

In the above equations, [L] and [M] represent the stationary phase ligand concentration and the mobile phase surfactant concentration self-associated in the micelle, respectively. This latter value is the surfactant concentration in excess of the critical micelle concentration (CMC). The first equilibrium describes the retention process in the absence of micelle in the mobile phase. Upon addition of micelle to the mobile phase, a second equilibrium is established which corresponds to a complex formed between the micelle assembly and the probe. The third equilibrium involves the reversible exchange of the probe between the stationary and micellar phases. This third equilibrium represents the sum of the first two, and as such, is a function of the first two. The rearrangement of the above four equations yields:

$$1/k' = \frac{[M]K_2}{\phi[L]K_1} + \frac{1}{\phi[L]K_1} \tag{5}$$

This equation describes the linear relationship between the reciprocal of the capacity ratio and the mobile phase surfactant concentration self-associated in micelles. If the retention in the absence of micelles is large, the intercept term becomes very small and is difficult to measure accurately. The derivation of equation 5 is that proposed by Arunyanart and Cline Love (2). The equilibrium constants, K_1, and K_2 obtained from equation 5 assume that the three equilibria are independent of the surfactant concentration.

The contribution of temperature to the three equilibria can be studied from the regression parameters of equation 5 evaluated for each temperature:

$$\ln(1/k'_o) = -lnK_1 - \ln(\phi[L]) = \frac{\Delta H_1^o}{RT} - \frac{\Delta S_1^o}{R} - \ln A \quad (6)$$

$$\ln(m/k'_o) - lnK_2 = -\frac{\Delta H_2^o}{RT} + \frac{\Delta S_2^o}{R} \quad (7)$$

$$\ln(m) = lnK_3 + \ln(\phi[L]) = -\frac{\Delta H_3^o}{RT} + \frac{\Delta S_3^o}{R} + \ln(\phi[L]) \quad (8)$$

where ΔH^o and ΔS^o represent the standard enthalpies and entropies of binding, respectively, for the first, second and third equilibria. R is the universal gas constant, k'_o the intercept at [M] = o and m the slope at $k'_o = o$. This method of evaluating the equilibrium standard enthalpies of binding is valid assuming that both the phase ratio and the stationary phase ligand concentration are independent of temperature. This is often a reasonable assumption with small changes in temperature.

Model II

The second model describes the effect of temperature on retention without any specific reference to the various equilibria involved. The temperature contribution to the capacity ratio (k') can be described simply with the van't Hoff equation:

$$lnK' = -\frac{\Delta H_R^o(1/T)}{RT} + \frac{\Delta S_R^o}{R} + \ln\phi \quad (9)$$

where k', ΔH_R^o and ΔS_R^o represent the capacity factor, standard enthalpy of retention and standard entropy of retention, respectively and the intercept of the plot is a combination of both the entropy of retention and the phase ratio. A linear fit to equation 9 describes the usual retention behavior. A non-linear plot, on the other hand, has been shown by Horvath and co-workers to involve at least two independent enthalpic centers (3-6). The non-linearity of the van't Hoff plot also has been shown to arise from a temperature dependent enthalpy of retention (7). In this case, the non-linear van't Hoff plots have been studied using a second order polynomial:

$$T \ln k' = a + bT + cT^2 \qquad (10)$$

where T ln k' is related to the Gibb's free energy of the binding process. Using the Maxwell relationships, it has been shown (7) that the enthalpy of retention and entropy of retention can be computed from equation 10. Dividing both sides of equation 10 by temperature illustrates its similarity to the van't Hoff equation, where the extra "c" term can be thought of as the temperature dependent enthalpy or heat capacity contribution (7):

$$\ln k' = \frac{a}{T} + b + cT \qquad (11)$$

In this equation the "b" term combines the entropy contribution with the phase ratio constant. A linear regression of equation 11 where the "c" term is approximately zero would clearly indicate a linear van't Hoff plot.

MATERIALS AND METHODS

The high performance liquid chromatograph employed consisted of a HP1090 equipped with an automatic injector, a column oven, a diode array UV detector, a DPU board, a 10MB hard disk and a HP85B computer (Hewlett Packard Avondale, PA 19311).

The fluorescence equipment consisted of a Perkin Elmer luminescence spectrometer LS5 installed with a Perkin Elmer 3600 data station and a

thermostatically controlled turret cell holder (Perkin Elmer, Norwalk, CT 06856) . The fluorescence of the probes was measured in heptane, methanol and micelle-free mobile phase solutions at various concentrations of sodium dodecyl sulfate (SDS). All solvents used, unless otherwise stated, were of HPLC grade. The water used was collected from a Millipore Milli-Q reagent water system (Millipore Corp., Bedford, MA).

The solutions for the chromatographic mobile phases and the spectroscopic measurements consisted of a 1:10 (v:v) mixture of 1-propanol (Burdick & Jackson, Laboratories Inc.) and a 0.02 M phosphate (sodium phosphate and phosphoric acid, American Chemicals Ltd., Montreal, Canada) pH 2.1 aqueous solution. The various SDS solutions were made by the addition of the appropriate amounts of powdered SDS. All of the mobile phases used in this study, were made from the same batch of surfactant free solution to control the mobile phase characteristics. The 10 cm HPLC column contained C18 Spherisorb packing material (CSC Inc, Montreal, Canada).

The probes used were: anthracene, A; 9-anthraldehyde, A(CHO); anthracene-9-carboxylic acid, A(COOH); 9-anthracenemethanol, A(CH2OH) (Aldrich Chemical Co. Milwaukee, WI 53201). To avoid obtaining retention times under column overload conditions, the probe concentrations injected were kept to a minimum but were sufficient to obtain undistorted peaks of high plate count.

All of the statistical computations were performed with RS/1 (release 2.2 or 3.0, BBN Research System, Cambridge MA 02238), operated on a VAX-11/780 superminicomputer (Digital Equipment Corp). To improve the residuals distribution, most data sets required a weighting factor that was computed for each datum as the reciprocal of the independent variable (8).

Measurement of the void time

The definition of the best void time marker for chromatography has been described elsewhere (9-11). For micelle chromatography, a probe molecule may experience different volumes depending on whether it resides within the micelle or remains in the bulk solution. Hence the true void volume is dependent on the weighted average of the residence times in either phase. Such a measurement is not practical. However, we have found, like others (2), that methanol gave reproducible results over the range of conditions used in the present study.

The capacity ratio for each test probe and each condition was computed using the void time value described above with retention times measured in triplicate.

RESULTS AND DISCUSSION

Determination of the CMC values

To evaluate the effect of micelle concentration on retention, as described by equation 5, the surfactant CMC must be known at each temperature investigated.

The CMC was determined with turbidimetry and fluorescence techniques. The turbidimetry measurements were close to the noise level of the UV spectrometer used and thus lacked the required sensitivity for very low CMC values. A(COOH) was used for the fluorescence probe CMC determination. This method of CMC evaluation also proved to be difficult due to a non-linear relationship between the surfactant concentration and the emission. Considering these limitations, experiments in our laboratory indicated that the CMC of SDS in 0.02M phosphate (pH 2.1)and 10% 1-propanol was less than 0.0005M. This value was considered to be insignificant in the context of equation 5 when compared with the SDS concentration used in the present study.

TABLE 1

Critical Micelle Concentration of Sodium Dodecyl Sulphate in Aqeuous Solutions with Sodium Chloride or 1-Propanol as Additives (12).

Additive	Concentration (% v/v)	Temperature (C)	CMC (M)
1-propanol	5.0	25	.0038
1-propanol	5.0	50	.0051
1-propanol	9.2%	25	.0013
1-propanol	13.3%	25	.0006
sodium chloride	.02M	25	.0038
sodium chloride	.03M	70	.0032
sodium chloride	.03M	25	.0031

Literature values (12) for the SDS critical micelle concentration in solutions containing either NaCl or 1-propanol at various temperatures are shown in Table 1. This table shows that the CMC is significantly reduced in the presence of individual additives to less than 0.003 M for NaCl and 0.001M for 1-propanol. The combination of both additives probably results in a further decrease of the CMC values. Our experimentally estimated CMC values appear in reasonable agreement with these literature data.

The effect of micelle concentration on retention (Model I)

Since the CMC, for the present set of conditions, is much smaller than the lowest surfactant concentration used in the present study, the value of M in equation 5 is equivalent to the SDS concentration.

The effect of surfactant concentrations on retention times at different temperatures is shown in Fig. 1. Fitting the data to equation 5 yields correlation coefficients greater than 0.9999 for each curve and residuals less than 1%. The slope and intercept parameters are significant ($p<0.001$) in all cases (see Fig. 1 and Table 2).

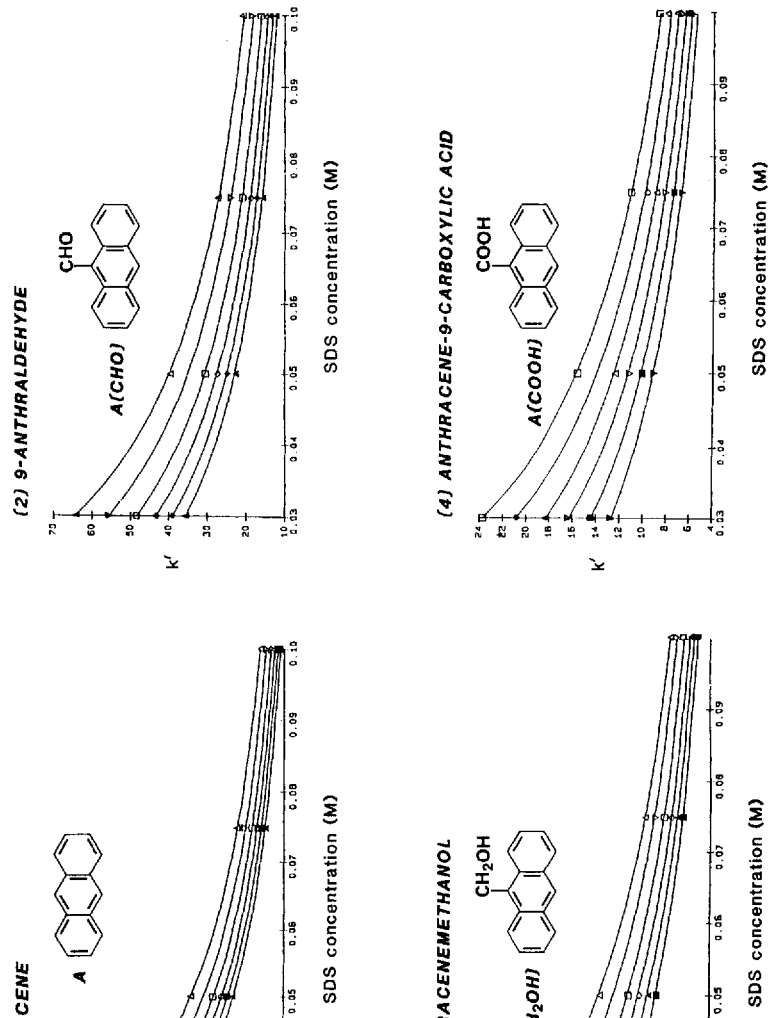

FIGURE 1. Effect of surfactant concentration on the retention of the probes at different temperatures. The curves represent the result of a linear regression with equation 5 replotted as a function of k′. The following range of temperatures used was 303-353 K corresponding to the largest and the smallest retention respectively.

TABLE 2

Regression Analysis of Equation 5 for Each Probe and Temperature Studied. The Data Shown in this Table are Plotted in Fig. 1 and Fitted to Equation 5 of in the Text. The Labels are: A, Anthracene; A(CHO), 9-Anthraldehyde; A(CH2OH), 9-Anthracenemethanol; A(COOH), Anthracene-9-Carboxylic Acid; (T) Temperature in Degrees Kelvin. The Significance Level is $p<0.03$. The Three Equilibrium Constants are Calculated with: $1/K_3$ = slope, $1/K_2$ = intercept and K_1 = slope/intercept. (n=4 for each of the curves except for T = 313.15 where n=3).

Probe	T(K)	Slope	Intercept
A	303.15	.323	-.0015
A	313.15	.355	-.0015
A	323.15	.388	-.0017
A	333.15	.415	-.0016
A	343.15	.444	-.0017
A	353.15	.478	-.0022
A(CHO)	303.15	.474	.0013
A(CHO)	313.15	.530	.0021
A(CHO)	323.15	.600	.0027
A(CHO)	333.15	.666	.0031
A(CHO)	343.15	.723	.0039
A(CHO)	353.15	.781	.0046
A(CH2OH)	303.15	.980	.0097
A(CH2OH)	313.15	1.03	.0129
A(CH2OH)	323.15	1.12	.0156
A(CH2OH)	333.15	1.22	.0174
A(CH2OH)	343.15	1.27	.0214
A(CH2OH)	353.15	1.32	.0262
A(COOH)	303.15	1.06	.0102
A(COOH)	313.15	1.18	.0126
A(COOH)	323.15	1.25	.0172
A(COOH)	333.15	1.36	.0203
A(COOH)	343.15	1.44	.0261
A(COOH)	353.15	1.51	.0333

The linear regression for anthracene resulted in a statistically significant negative y-intercept. This physically impossible result was also observed by Arunyanart and Cline Love (2) and can be related to the large value of K_1 and the steep curve which can produce instability during the fitting process. To approximate a large K_1 value, the intercept was set to zero. The resulting figures of merit were substantially different and indicative of a much poorer fit. Very hydrophobic compounds can induce

micelle formation at concentrations below the CMC, which could cause a departure from predicted values. In this context, the value of n and K_2 for surfactant concentration below the true CMC should be different from those above the CMC. This could cause a departure at low surfactant concentration and explain the observed negative intercept.

Effect of temperature on K_1, K_2 and K_3

The association constants K_1, K_2 and K_3 describe the probe equilibria for the three micelle chromatography phases. The contribution of temperature to these equilibria can be explicitly described with the van't Hoff equations (see equations 6 to 8). Using these equations, the values obtained having the intercepts and slopes shown in Table 2, were used to determine values for the three equilibrium constants. The logarithmic values for the equilibrium constants were plotted against 1/T in Fig. 2 for each equilibrium constant. From the regression analysis of these plots the value for the equilibrium enthalpies and intercepts were obtained and are listed in Table 3. The correlation coefficients (see Table 3) and the distribution of the points around the regression line indicated that the van't Hoff relationships were valid. This suggested a temperature contribution to the aggregation number that was not significant. This is in agreement with the general knowledge about small micelles (13). No relationship between the polarity of the probes and the equilibrium enthalpies were found. On the other hand, a positive relationship between the probe polarity and the K_1 and K_2 intercept (the equilibrium entropies for K_1 and K_2) was found, which confirmed that the retention process was governed by entropic effects.

Effect of temperature on k' (Model II)

The capacity ratio, k', describes retention as an equilibrium between the stationary phase and the mobile phase, irrespective of the solvation process in the mobile phase (see equation 1). The temperature contribution to the retention process can be described with a van't Hoff equation (see equation 9) or, for non-linear cases, with equation 11.

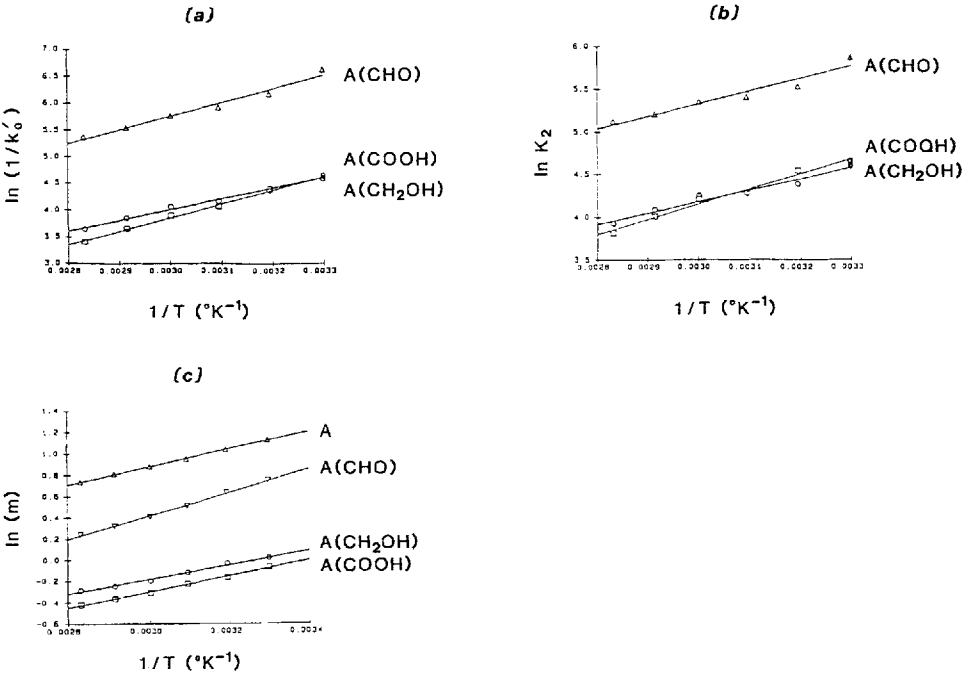

FIGURE 2. Van't Hoff plots to determine K_1, K_2 and K_3 equilibrium enthalpies (Model I) using a) equation 6 to evaluate the equilibrium enthalpies for K_1, b) equation 7 to evaluate the equilibrium enthalpies for K_2, and c) equation 8 to evaluate the equilibrium enthalpies for K_3.

The retention data were analyzed using equations 9 thru 11. The natural logarithm of the capacity factors plotted against the inverse of temperature resulted in linear relationships for A(CHO), A(CH2OH) and A(COOH) at the SDS concentrations studied (Fig. 3). In all of these cases the "c" parameter in equation 11 was not significantly ($p<0.05$) different from zero. The slope parameter of the linear equation was significant ($p<0.05$) with a correlation coefficient larger than 0.999. For the anthracene probe, A, both equations 9 and 11 produced a statistically acceptable fit. However, an analysis of residuals from equation 9 showed a non-random distribution following a pattern indicative of curvature. As a result the data were fitted nonlinearly to the polynomial of equation 11 (Fig. 3).

TABLE 3

Equilibrium Enthalpies and Entropies of K_1, K_2 and K_3 Equilibria from Model I and Fig. 2. The Numbers in Parentheses Indicate the Percent Relative Standard Deviations and r Indicate the Correlation Coefficient (n=6). The Labels are: A, anthracene; (CHO), 9-anthraldehyde; A(CH_2COH) 9-anthracene-methanol; A(COOH, anthracene-9-carboxylic acid.

probe	K1 $-\Delta H_1^o$ (KH/Mole)		r	K2 $-\Delta H_2^o$ (KJ/Mole)		r	K3 $-\Delta H_3^o$ (KJ/Mole)		r
A	-	-	-	-	-	-	6.85	(.12)	.998
A(CHO)	21.1	(1.7)	.97	12.1	(1.6)	.93	9.00	(.22)	.997
A(CH_2OH)	16.7	(0.85)	.990	11.0	(1.1)	.96	5.68	(.28)	.990
COOH	21.0	(0.74)	.995	14.8	(0.96)	.98	6.25	(.24)	.994

	Intercept		$-\Delta S_2^o$ (KJ/KMole)		Intercept	
A	-	-	-	-	-1.59	(0.07)
A(CHO)	-3.73	(.28)	7.93	(5.0)	-2.83	(0.08)
A(CH_2OH)	-2.03	(.31)	1.64	(3.0)	-2.23	(0.11)
COOH	-1.88	(.62)	-9.80	(4.5)	-2.55	(0.08)

The enthalpies of retention evaluated from capacity factors for each surfactant concentration are shown in Table 4. Similar to Model I, there was no direct relationship between the polarity of the probes and the enthalpies of retention, while the entropies of retention (intercept) appeared to be proportional to polarity. However, an increase in surfactant concentration resulted in a reduction in the enthalpies of retention. This finding was in contradiction with an implicit assumption in Model I, where the equilibrium constant should be micelle concentration independent. A change in the enthalpies of retention with micelle concentration implies a change in the bonding strength between the micelle and the probe; this might arise from a physical change in the micelle itself as a function of the total surfactant concentration.

Contrary to the other more polar probes, the anthracene probe, A, showed no linear relationship between the surfactant concentration and

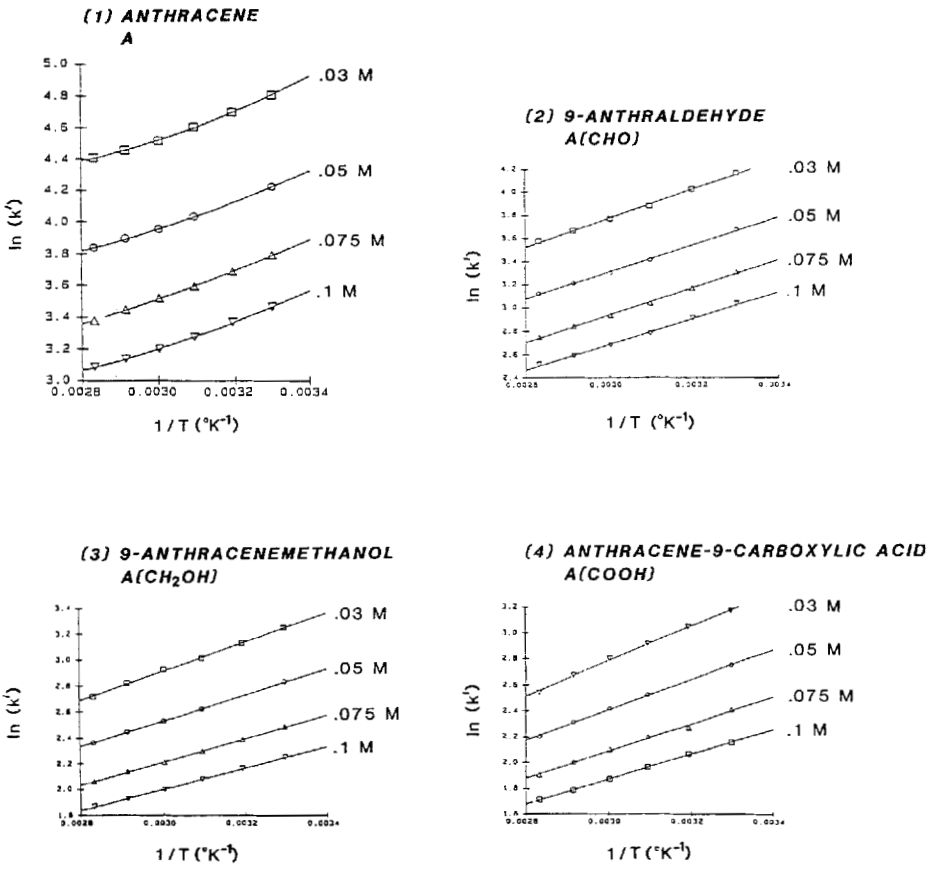

FIGURE 3. Van't Hoff plots of the chromatographic retention (ln k') for different micelle concentrations (Model II). Note the use of equation 11 for anthracene.

its enthalpy of retention (see Table 5). The minor non-linear nature of the van't Hoff plot probably resulted from a temperature dependent enthalpy of association between the probe and the micelle.

Fluorescence studies

To increase our understanding of the micelle solubilization process, an investigation of the interactions between the micelle and the probe was

TABLE 4

Enthalpies of Retention ($-\Delta H_R^o$) in KJ/K-Mole from Model II and Fig. 3 as a Function of Surfactant Concentration. Numbers in Parentheses Indicate the Percent Relative Standard Deviations. The Correlation Coefficient for Each Fit is at Least .995 and the Significance of the Slope is p<.001. The Labels are: A(CHO), 9-anthraldehyde; A(CH2OH), 9-anthracenemethanol; A(COOH), anthracene-9-carboxylic acid.

	SDS Concentration (M)			
	.03	.05	.075	.1
	Enthalpies of Retention ($-\Delta H_R^o$) (KJ/K-Mole)			
A(CHO)	10.49 (2.4)	9.88 (2.5)	9.83 (2.3)	9.21 (2.8)
A(CH2OH)	10.99 (2.0)	9.60 (1.5)	8.66 (3.0)	7.99 (0.83)
A(COOH)	9.17 (1.3)	7.47 (0.51)	7.47 (1.0)	6.85 (1.94)

TABLE 5

Results from the Non-linear van't Hoff Equation (Equation 11). The Enthalpies and the Entropies of Retention can be Calculated using: $-\Delta H_R = a - cT$ and $\Delta S_R = -b - 2cT$, respectively (see Equation 6 and 7 of ref 6). All of the Reported Parameters are Significantly (p<.01) Different from Zero. For Each Curve the Correlation Coefficient is greater than 0.999. The Percent Standard Deviations are Reported in Parentheses.

[SDS]	a/T	b	cT
.03	2616 (203)	8.819 (8.8)	0.01648 (0.0019)
.05	1932 (92)	5.294 (.56)	0.01036 (0.00086)
.075	1554 (137)	3.274 (.84)	.006884 (0.0013)
.1	2005 (106)	6.513 (.65)	0.01110 (0.0010)

undertaken. Specifically, it was of interest to determine whether the probe was located inside the micelle core or at the micelle surface. Fluorescence spectroscopy may provide more information concerning the solvation of the probe molecule. The shift of a fluorescence emission band toward lower or higher wavelengths generally indicates that the solvation environment is of lower or higher polarity, unless some specific interaction is involved (14). Such a specific interaction usually involves a change in the resonance structure of the fluorophore.

All of the probes, except the A(COOH), behaved in the same manner. In these cases, the fluorescence spectra contained three peaks. The band at the lowest wavelength was most sensitive to polarity change. This band was used as a polarity indicator. The band position was shifted from low to high wavelength in the following order: heptane, methanol, mobile phase with surfactant and mobile phase without surfactant, respectively. These observations indicated that the probe, in the presence of micelles, is located in a hydrophobic medium of hydrophobicity between that of pure methanol and of the surfactant-free mobile phase. Since the micelle surface with the sulfonic acid present would be too polar, it is unlikely that the probe is located at the surface of the micelle. Although it was not possible to differentiate between a mixed micelle and the probe located in the micelle core, solvation environment of the probe may well include surfactant and aqueous 1-propanol.

A completely different picture was observed for the A(COOH). The fluorescence peak positions did not follow the same trend as for the others. The fluorescence band was shifted toward longer wavelengths and was severely distorted in the presence of surfactants or hydrophobic solvents like heptane (see Fig. 4). Increasing the probe concentration or adding sodium sulfate to methanol solutions resulted in a similar band distortion and shift toward longer wavelengths. These substantial differences from the other structurally related compounds were probably indicative of probe-to-ionic-species interactions in the micellar phase. The ionic species could be either another probe or the sulphonic moiety of the surfactant.

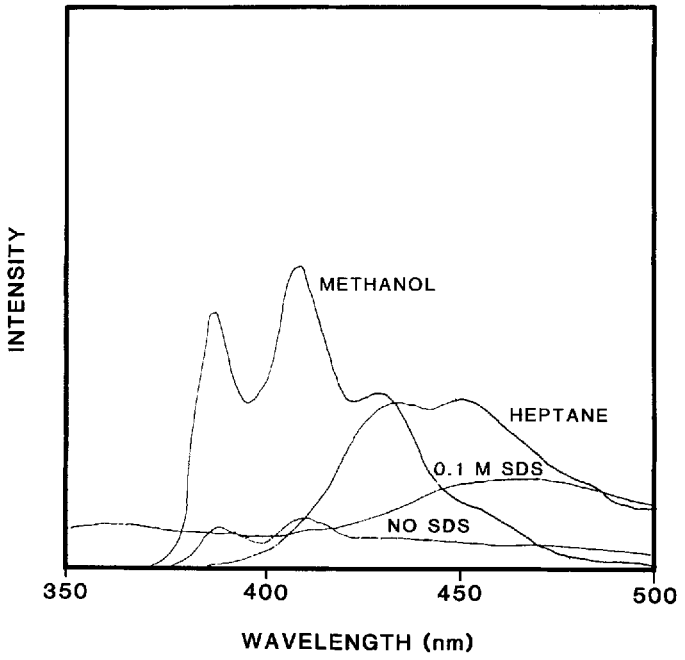

FIGURE 4. Emission spectra of anthracene carboxylic acid (excitation: 250 nm) as a function of solvent.

CONCLUSIONS

The present work has discussed two models to study micelle chromatography using temperature as a primary variable. The first model evaluated the retention enthalpies and retention entropies from van't Hoff plots of K_1, K_2 and K_3 equilibria. The second model investigated the retention of the probes without specific references to the various equilibria involved in the retention process. The strength of Model I is its ability to describe the effect of micelles on retention, while its ability to provide information about the energetics of the process is limited.

This second model indicated that the enthalpies of retention were reduced with an increase in surfactant concentration (see Table 4), contradicting the assumption in Model I that the equilibrium constants K_2

and K_3 are independent of surfactant concentration. However, it could be argued that this observation was the result of an ionic strength contribution.

An increase in surfactant concentration resulted in lower enthalpies of retention for the polar probes. This effect probably was the result of changes in the micelle physical characteristics as a function of the surfactant concentration. The hydrophobic probes were only marginally affected by temperature.

Both models indicated that the relative magnitude of the enthalpies of retention did not parallel their relative retention or polarities (see Tables 3-4). Both models also suggested (see Fig. 2 and 3) that the entropies of retention were inversely proportional to the probe polarity and to the micelle concentration. The fluorescence studies suggest that hydrophobic compounds associate with the micelle and reside in the micellar interior whereas more polar hydrophilic compounds may be located at the micelle-mobile phase interface.

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