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EFFECTS OF RESTRICTED MASS TRANSFER ON THE EFFICIENCY OF MICELLAR CHROMATOGRAPHY

PAUL YARMCHUK, ROBERT WEINBERGER, ROLAND F. HIRSCH and L. J. CLINE LOVE*
Seton Hall University, Department of Chemistry, South Orange, NJ 07079 (U.S.A.)
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SUMMARY

A model treating restricted mass transfer in micellar chromatography based on the kinetics of adsorption–desorption of the solute on the stationary phase and entrance–exit rates from micelles in the mobile phase is proposed. This model provides an explanation for the lower chromatographic efficiency of micellar mobile phases relative to conventional mobile phases. Based on our studies, the efficiency of micellar chromatography can be optimized by use of elevated temperatures, low flow-rates and minimal micelle concentrations. All of these experimental variables are targeted at maximizing the kinetic rate parameters.

INTRODUCTION

Surfactants are valuable mobile phase additives in liquid chromatography (LC). Their most notable uses are as ion interaction reagents which employ secondary equilibria to increase retention and enhance selectivity for appropriate solutes[1–3]. Virtually all of these and similar examples utilize the surfactant below the critical micelle concentration (CMC), or the mobile phase contains sufficient organic modifiers that alter or disrupt the micellar assembly. Accordingly, micelles are not implicated in these separation mechanisms.

Early work with surfactants in paper chromatography by Farulla *et al.*[4] showed that double spots were formed which were attributed to micelles at the solvent front. In this case the surfactant was not a true component of the mobile phase, but was present either in the sample itself or was impregnated into the paper prior to the chromatography. In the first work in "soap chromatography" by Knox and Laird[5], the concentration of cetyltrimethylammonium bromide in the mobile phase was sufficient to form micelles, and good efficiencies were obtained. However, as discussed in that work, the high concentration of propanol in the mobile phase would tend to form smaller micelle-like clusters which would differ from true micelles in their degree of interaction with the solutes.

More recent examples in high-performance liquid chromatography (HPLC) have described the use of surfactants as the sole organic modifier at concentrations above the CMC in reversed-phase systems[6,7]. A micellar model that treats the mobile

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phase as being microscopically heterogeneous provides a theoretical basis for surfactant mobile phase behavior[7]. The role of electrostatic and hydrophobic interactions on retention was studied to allow the contribution of each effect to be defined[8]. Other studies took advantage of the profound effects micelles can have on spectroscopic properties by combining micellar chromatography with micelle stabilized room temperature phosphorescence to develop a new LC detection technique[9].

While all of the preceding examples illustrate the novelty and uniqueness of micellar mobile phases, they offer separations that yield poor LC efficiency compared with the state-of-the-art methods. This paper describes how restricted mass transfer of a solute between the micelle, bulk water and stationary phase accounts for this behavior. The effects of solute entrance–exit rate constants for the micelle and stationary phase are treated both theoretically and experimentally in terms of a random walk model. Optimization of experimental variables to maximize efficiency is described.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Constametric III pump and UV III monitor set at 254 nm (Laboratory Data Control, Riviera Beach, FL, U.S.A.), and a Model 7120 sample injector with a 20-μl loop (Rheodyne, Berkeley, CA, U.S.A.). The column (15 cm × 4.6 mm I.D.) was packed with 5-μm Supelcosil LC-1 (Supelco, Bellefonte, PA, U.S.A.). Separate columns were used for anionic and cationic surfactants. A pre-column (12.5 cm × 4.6 mm I.D.) packed with silica gel (37–53 μm) (Whatman, Clifton, NJ, U.S.A.) was located between the pump and sample injector in order to saturate the mobile phase with silica and minimize dissolution of the column packing. A Fisher Recordall, Model 5000, strip chart recorder (Fisher Scientific, Springfield, NJ, U.S.A.) was used to record the chromatograms. Column temperature was controlled by immersing the precolumn and analytical column in a water bath where the temperature was maintained by a Model 73 circulating heater (Fisher Scientific).

Reagents

The sodium dodecyl sulfate (SDS) was electrophoresis grade obtained from Bio-Rad Laboratories and was used as received. The dodecyltrimethylammonium bromide (DTAB) was from Fisher Scientific and was recrystallized twice from acetone–chloroform. The solutes were obtained from various companies and were used as received. The methanol, *n*-propanol, and *n*-butanol were from Fisher Scientific.

Procedure

Micellar mobile phases were prepared by dissolving the appropriate amount of surfactant in water and filtering through a 0.5-μm cellulosic membrane filter (Rainin Instrument, Woburn, MA, U.S.A.). Stock solutions of the test solutes were prepared in methanol and then diluted to the appropriate working concentration with 0.10 *M* SDS or DTAB for the micellar systems and with the mobile phase for the conventional reversed-phase systems. The working concentrations were phenol (42

μg/ml), benzene (380 μg/ml), 2-naphthol (42 μg/ml), naphthalene (52 μg/ml), anthracene (6.1 μg/ml), and *p*-nitroaniline (25 μg/ml). Retention times and peak widths were measured manually. Plate counts were determined using the formula $N = 5.54(t_R/W_{\frac{1}{2}})^2$ where t_R is the retention time and $W_{\frac{1}{2}}$ is the peak width at half height.

The effect of temperature on efficiency was performed with 0.10 *M* DTAB at a flow-rate of 2.0 ml/min. The column was equilibrated at each temperature until constant retention times were obtained.

The flow-rate study was conducted at 25°C using three mobile phases: (i) 0.10 *M* SDS; (ii) 30% methanol in water; and (iii) 45% methanol in water (used exclusively for anthracene). These concentrations of methanol and surfactant produced approximately the same k' for each solute with both micellar and conventional mobile phases. Flow-rates were measured by collecting the effluent in a 10-ml graduated cylinder for a sufficient length of time to collect at least 5 ml.

The effects of surfactant concentration and added organic modifiers on the chromatographic efficiency were studied at 25°C with a flow-rate of 2.0 ml/min. The column was allowed to equilibrate until constant retention times were obtained. The mobile phases containing added organic modifiers were prepared by adding the appropriate volume of alcohol to a 0.20 *M* SDS solution and diluting with water to an SDS concentration of 0.10 *M*.

Determination of adsorption–desorption rate constants

Adsorption and desorption rate constants to and from the stationary phase were obtained by chromatographing the test solutes at 25°C using 0.005 *M* SDS at a flow-rate of 4.5 mm/sec (2.0 ml/min). At this surfactant concentration, which is just below the CMC, no micelles should be present, but the surface of the stationary phase should have approximately the same amount of adsorbed surfactant as is present above the CMC, as shown by Hung and Taylor[10]. The values for the desorption rate constant, k_d, were obtained from the measured chromatographic parameters substituted into eqn. 1 from the random walk theory[11].

$$H = \frac{2k'}{(1 + k')^2} \frac{v}{k_d} \tag{1}$$

where H is the height equivalent of a theoretical plate, k' is the capacity factor, and v is the linear velocity. The adsorption rate constant, k_a, was calculated using a basic chromatographic expression, $k' = k_a/k_d$.

RESULTS AND DISCUSSION

In order to determine the reasons for the generally low efficiencies observed with micellar chromatography as compared with conventional reversed-phase chromatography, the factors characterized by the Van Deemter equation

$$H = A + \frac{B}{v} + Cv \tag{2}$$

were considered, where A, B, and C are constants for a given column and solute and

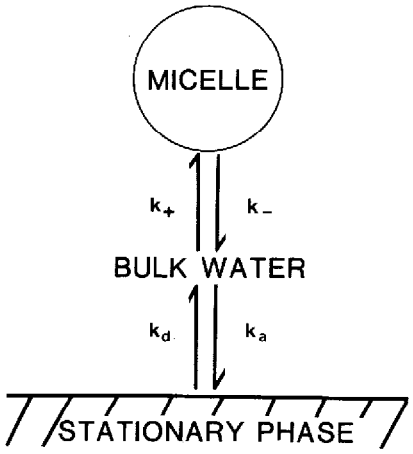

Fig. 1. A diagram illustrating the two primary equilibria in a micellar system when a stationary phase is present. The rate symbols are indicated.

v is the linear velocity. The first term, which describes eddy diffusion, is determined primarily by the packing structure of the column bed and the particle diameter of the packing material. Since these are column parameters, conventional and micellar mobile phases should have approximately the same eddy diffusion term for a given column.

The second term is a measure of longitudinal or axial diffusion and is proportional to the diffusion rate of the solutes in the mobile phase. In LC the solute diffusion rates are very low, and this term is only significant at very low flow-rates. Therefore, we concentrated on the third term, which is concerned with mass transfer, both in the mobile and stationary phases.

Mass transfer effects

The generally low efficiencies often encountered in micellar chromatography appear due to restricted mass transfer. For good chromatographic efficiency, the equilibration of solute between the stationary phase and the mobile phase should be rapid as is normally observed with conventional LC. In reversed-phase LC, in the absence of side reactions, only one equilibrium must be considered, that of the solute

TABLE I

DESORPTION AND ADSORPTION RATE CONSTANTS

Mobile phase, 0.005 *M* SDS; column, LC-1 (15 cm × 4.6 mm I.D.) (Supelco); flow-rate, 2.0 ml/min; temperature, 25°C.

	k'	H (mm)	k_d (sec^{-1})	k_a (sec^{-1})
Phenol	11.8	0.0257	25.0	295
Benzene	31.2	0.0313	8.6	268
p-Nitroaniline	25.8	0.0273	11.7	302
2-Naphthol	140.0	0.0271	2.3	322

between the stationary and mobile phases. However, in micellar chromatography there are two solute equilibria (Fig. 1), one between the stationary phase and bulk water and a second between bulk water and the micelle. This two-fold solute equilibrium is a fundamental property of the system, and gives micellar chromatographic systems their uniqueness, but also imposes efficiency problems since mass transfer across an additional barrier is required.

Mass transfer in micellar chromatography is grossly affected by the entrance–exit rates of the solute in and out of the micellar aggregate. As shown in Fig. 1 there are four rate constants which must be considered: k_+ and k_-, the entrance and exit rate constants of a solute with the micelle, and k_a and k_d, the adsorption and desorption rate constants of a solute with the stationary phase. If all these rate constants were large, mass transfer would not limit efficiency, but the rate constants can vary greatly.

Almgren *et al.*[12] calculated k_- based on equilibrium data assuming k_+ to be diffusion controlled and equal to $7 \cdot 10^9\ M^{-1}\ \text{sec}^{-1}$ for all solutes. For a 0.10 *M* SDS solution, assuming an aggregation number of 62, the corresponding k_+ effectively becomes $1.0 \cdot 10^7\ \text{sec}^{-1}$. The values for k_- ranged from $4.4 \cdot 10^6\ \text{sec}^{-1}$ for benzene to $4.1 \cdot 10^3\ \text{sec}^{-1}$ for pyrene. This difference in k_- is very significant in terms of the amount of time these different solutes reside in the micelle where it is assumed they are unavailable to partition to the stationary phase. This factor will be considered later in this paper.

Values for k_a and k_d using a 0.005 *M* SDS mobile phase (below the CMC) were determined as described aand are listed in Table I. Horvath and Lin[13] have shown that *H* is due primarily to kinetics for a *well packed column*. The measured plate heights in Table I are two to three times above the optimum for a modern column of twice the particle diameter, but the system was not optimized for minimum plate height and some of the plate height is due to factors other than kinetics. The total plate heights were used to calculate k_d, however, since even an increase of a factor of two in k_a and k_d would have only a minimal effect compared with k_+ and k_-. As expected, the values for k_a are very similar since the rate of adsorption is diffusion controlled.

Even though they are both diffusion controlled, k_+ is much larger than k_a. We believe this is due to the distance which a solute molecule travels between stationary phase encounters as compared with the distance between micelle encounters. The number of steps, *n*, from random walk theory[11] can be calculated from the equation $n = (t_R - t_o)/t_d$ where t_R is the retention time of the solute of interest, t_o is the retention time of an unretained solute, and t_d is the desorption time for the solute of interest equal to $1/k_d$. With the chromatographic conditions used to determine k_d (0.005 *M* SDS), this results in 9100 steps for benzene and 11,000 steps for 2-naphthol. Assuming an average value of 10,000 steps, with a 15-cm long column, this results in a step length, *l*, of 15 μm, disregarding the tortuosity of the column packing which would increase *l*. The number of micelles which a solute could encounter over this step length can be calculated as follows. Using a spherical solute with a radius of 2 Å and SDS micelles with an approximate radius of 18.5 Å[12], an encounter would occur whenever the distance between the center of the solute and the center of the micelle was 20.5 Å or less. In other words, the solute would have an encounter cylinder with a radius of 20.5 Å and a length of 15 μm which results in a volume of

$2.0 \cdot 10^{-16}$ cm^3. A 0.10 *M* SDS solution contains $9.03 \cdot 10^{17}$ micelles/cm^3 which results in 180 micelles within the encounter cylinder. Therefore, a solute could encounter *ca.* 180 micelles before it encounters the stationary phase in a typical step so that the distance between micelle encounters is much less than between stationary phase encounters. Once a micelle is encountered the hydrophilic effect of the bulk water would tend to keep a hydrophobic solute in the vicinity of the micelle such that multiple encounters with the same micelle are very probable. This would further reduce the distance between micelle encounters. These factors, we believe, can account for the large difference between the two diffusion controlled rate constants.

Since k_+ is much larger than k_a, a solute molecule is more likely to enter a micelle than to enter the stationary phase. While in a micelle, the solute is isolated and probably cannot partition to the stationary phase without first exiting the micellar aggregate. However, small or readily water-soluble molecules with large micellar exit rates can rapidly move back into the bulk water where they become available to the stationary phase. Due to very large entrance–exit rate constants, small hydrophilic solute molecules can move in and out of a micelle many times before encountering the stationary phase. For these solutes, efficiencies are not limited by micellar equilibria. However, more hydrophobic molecules have k_- values which are several orders of magnitude smaller as shown in Table II, where k_- was determined by Almgren *et al.*[12]. The exit rate constant for anthracene is 260 times smaller and for pyrene 1000 times smaller than for benzene. As a result, the more hydrophobic compounds remain in the micelle much longer. Since they spend much less time in the bulk water, solute mass transfer between micelle and stationary phase is inhibited.

With bonded phase hydrocarbon columns the stationary phase becomes coated with surfactant monomers and acquires characteristics of the particular surfactant[3]. Thus, solutes with large micellar exit rates also have relatively large desorption rate constants compared with k_d for more hydrophobic solutes. This is illustrated in Table I, where k_d for phenol is ten times larger than for the more hydrophobic 2-naphthol. This means hydrophilic solutes can return to the bulk water frequently, and, thereby, are closer to equilibrium. Indeed, for hydrophilic solutes good efficiencies are obtained. However, the hydrophobic solutes with much smaller k_d values remain in the stationary phase much longer.

In addition, mass transfer in the mobile phase, and especially in stagnant mobile phase, depends upon the diffusion constants of the solutes. In water these are usually *ca.* 10^{-5} cm^2/sec. However, the diffusion constant for an SDS micelle is only *ca.* 10^{-6} cm^2/sec (ref. 14). Therefore, when a solute is within a micelle, its rate of diffusion decreases, further restricting mass transfer.

TABLE II

EXIT RATE CONSTANTS OF AROMATIC HYDROCARBONS FROM SDS MICELLES

Arene	k_- *(sec^{-1})**	*Arene*	k_- *(sec^{-1})**
Benzene	$4.4 \cdot 10^6$	Biphenyl	$9.6 \cdot 10^4$
Toluene	$1.3 \cdot 10^6$	Anthracene	$1.7 \cdot 10^4$
Naphthalene	$2.5 \cdot 10^5$	Pyrene	$4.1 \cdot 10^3$

* Calculated using $7 \cdot 10^9$ M^{-1} sec^{-1} as the entrance rate constant (from ref. 12).

The loss in efficiency can also be explained by the use of random walk theory[11] where the peak variance σ^2 equals nl^2. Since we have assumed that t_d is not affected by the addition of micelles, any decrease in retention with addition of micelles must be due to the time a solute resides in the micelles. This would cause a reduction in n and a corresponding increase in l. Since σ^2 depends upon the square of the step length, it will also increase signifying a loss in efficiency. As shown in our previous work[6], more hydrophobic solutes exhibit larger decreases in retention for a given increase in micelle concentration. Therefore, they would also exhibit a larger increase in l and a greater decrease in efficiency.

Of course, there is the possibility for direct transfer of a solute between the stationary phase and the micelle. For this discussion, it has been assumed that direct transfer is minimal. In order for a solute to transfer from a micelle to the stationary phase, the solute must be oriented towards the stationary phase so that contact can be made. No such orientation is necessary, of course, for transfer from the stationary phase to the micelle since the solute can enter the micelle at any point. Thus, if direct contact exchange is significant, it would tend to decrease the average t_d, but would probably have only a very small effect on k_-.

From these discussions, the mass transfer problem appears to be a consequence of slow micellar exit rates and slow desorption from the stationary phase, both of which are related to the hydrophobicity of the individual solute. If the favorable properties of micellar chromatography are to be employed, methods of improving mass transfer and, thus, efficiency must be realized.

Effect of temperature

Since the kinetics of the system are a major factor in the mass transfer, the efficiency should improve if the overall rate constants, notably k_- and k_d, can be increased. The easiest and most common method of providing that feature is to increase the temperature. A series of solutes were chromatographed at temperatures between 25°C and 70°C and the results of these experiments are shown in Table III.

Overall, the column efficiency increased with elevated temperature. For smaller solutes, such as phenol and benzene, only a 50–100% increase in plate count was seen, with the values leveling off at 50–60°C. This modest increase may be due in part

TABLE III

EFFECT OF TEMPERATURE ON EFFICIENCY

Chromatographic conditions: mobile phase, 0.10 *M* DTAB; column, LC-1 (15 cm × 4.6 mm I.D.) (Supelco); flow-rate, 2.0 ml/min.

Temperature (°C)	*N (per column)*				
	Phenol	*Benzene*	*2-Naphthol*	*Naphthalene*	*Anthracene*
25	2300	3000	750	1200	460
30	2800	3400	1000	1300	530
40	3200	3800	1300	1700	860
50	4100	4400	1900	2300	1200
60	4400	5000	2400	3000	1500
70	4100	4900	2700	3100	1700

to enhanced micellar kinetics but is also probably a consequence of lowering the mobile phase viscosity. This mode of increasing mass transfer is normally observed even in simple reverse phase separations. With the larger solutes such as naphthalene and anthracene, a 250–350% increase was seen. In these cases where the rates of k_- and k_d were particularly small, the increase in temperature resulted in very significant increases in efficiency. Similar results were observed with SDS mobile phases. These are shown in Fig. 2a and b where the peaks, especially for anthracene, are much broader at 25°C than at 60°C. The effect of temperature on retention, however, is small which indicates that mass transfer, not the partition coefficients, is primarily affected.

There is a disadvantage to working at elevated temperatures in terms of column life. Since there are no organic modifiers except the surfactant in the mobile phase, the solubility of the silica support in the mobile phase can be significant. This effect increases with temperature and at 60°C rapid deterioration of the column may be observed. To maximize column life, a saturator column packed with silica was placed between the pump and injector and immersed in the water bath. With this precaution, very little column dissolution was observed.

Effect of linear velocity

In conventional LC the typical Van Deemter plot shows a decrease in efficiency with increasing linear velocity above the optimum[15]. A similar but enhanced effect is seen with micellar mobile phases as shown in Figs. 3 and is further evidence of mass transfer effects. For small solutes such as benzene where mass transfer is satisfactory, the plot using a micellar mobile phase is quite similar to that of a conventional chromatographic system. However, for larger molecules such as naphthalene

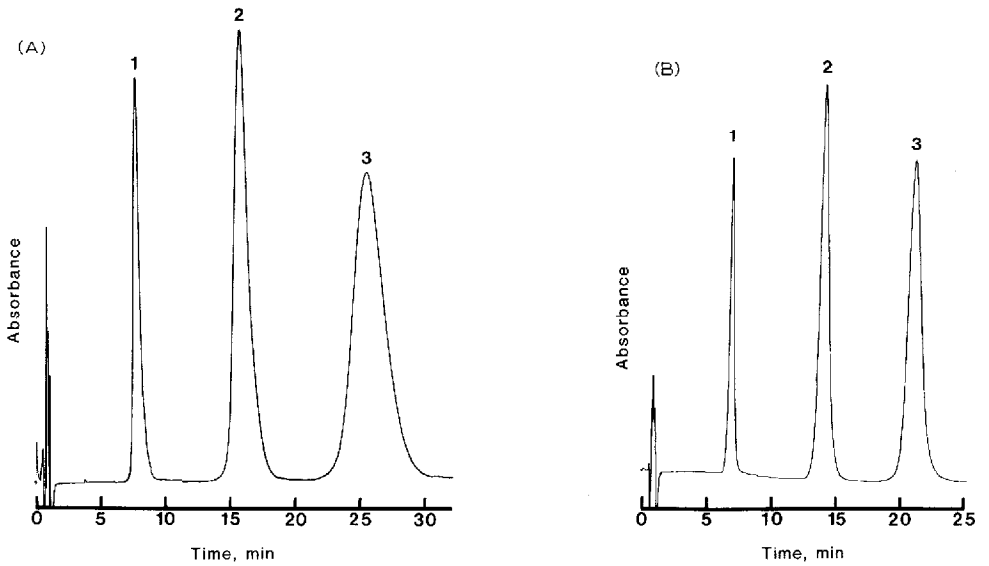

Fig. 2. Micellar chromatograms at (A) 25°C and (B) 60°C of (1) benzene, (2) naphthalene and (3) anthracene. Mobile phase, 0.10 *M* SDS; column, LC-8 (15 cm × 4.6 mm I.D.) (Supelco); flow-rate 2.0 ml/min; detector sensitivities, (A) 0.1 a.u.f.s., (B) 0.2 a.u.f.s.

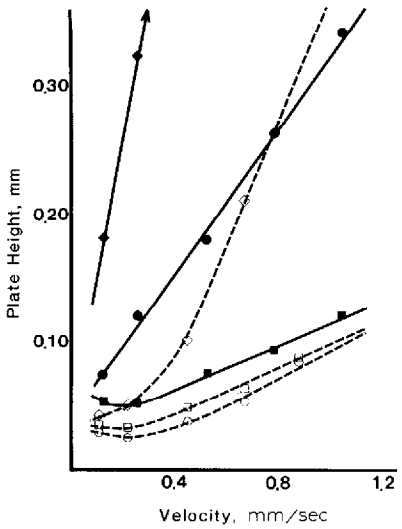

Fig. 3. Van Deemter plots for 0.10 *M* SDS (closed symbols) and water–methanol systems (open symbols). (■, □) Benzene, (●, ○) naphthalene, (◆, ◇) anthracene. Temperature, 25°C; column, LC-1.

or anthracene with poor mass transfer, the decrease in efficiency with increasing velocity is much greater with a micellar system and no minima appear in the plots. This is also shown in Table IV where the number of plates per column, N, drops to very low values for the larger molecules in the micellar system at high velocities.

The slow kinetics in micellar solutions are emphasized at high linear velocities. For small molecules where k_- and k_d are relatively large, mass transfer is fairly rapid, and flow is not as important. For large hydrophobic molecules where the rates are much slower, the mass transfer of the solute is inhibited. Therefore, at high linear velocities a micelle containing a hydrophobic solute molecule will be moved a relatively large distance along the column before it can exit the micelle and partition into the stationary phase.

Thus, at high flow-rates, the consequences of $\sigma^2 = nl^2$ are again highly emphasized, especially when compared with conventional chromatography. These results are analogous to conventional solvent systems except that the multiple equilibria demand more careful attention to flow-rates.

Effect of surfactant concentration

One of the first effects observed in working with micellar mobile phases was that the column efficiency decreased with increasing surfactant concentration as shown in Fig. 4. All four solutes showed an increase in plate height, H, as the surfactant concentration was increased from 0.02 to 0.20 *M* SDS. The increase was greatest for 2-naphthol which is the largest and most hydrophobic of the solutes studied.

This can also be explained in terms of reduced mass transfer between the micelle and stationary phase. The entrance rate into the micelle is dependent upon the concentration of micelles in the mobile phase. With increasing micelle concentration, the probability of a solute encountering a micelle increases, thereby effectively in-

TABLE IV

EFFECT OF LINEAR VELOCITY ON PLATE COUNT *N* (PER COLUMN)

	0.10 M SDS					*30% Methanol*				
	Flow-rate (ml/min)					*Flow-rate (ml/min)*				
	0.54	*1.08*	*2.15*	*3.22*	*4.30*	*0.51*	*1.02*	*2.00*	*3.00*	*3.95*
	Velocity (cm/sec)					*Velocity (cm/sec)*				
	0.13	*0.26*	*0.53*	*0.79*	*1.05*	*0.11*	*0.23*	*0.45*	*0.67*	*0.88*
Phenol	3700	3300	2600	1700	1700	3700	3600	2400	1800	1300
Benzene	2900	2900	2000	1600	1300	4500	4700	3100	2400	1700
2-Naphthol	2100	1400	900	600	500	5800	5500	3500	2500	1600
Naphthalene	2000	1300	800	600	400	5200	6000	3900	2800	1800
Anthracene	800	500	300	200	100	3600*	3000*	1500*	700*	**

* 45% Methanol.

** Not obtained due to high column backpressure.

TABLE V

EFFICIENCY WITH ADDED ORGANIC MODIFIERS

Mobile phase, 0.10 *M* SDS containing appropriate modifier; column, LC-1 (15 cm × 4.6 mm I.D.) (Supelco); flow-rate, 2.0 ml/min; temperature, 25°C.

Modifier	*N (per column)*				
	Phenol	*Benzene*	*2-Naphthol*	*Naphthalene*	*Anthracene*
None	2500	2200	900	800	200
2% methanol	2000	2100	900	800	*
5% methanol	2400	2700	1100	900	200
10% methanol	2400	2900	1200	1000	300
2% *n*-propanol	2500	2800	1000	900	300
5% *n*-propanol	2100	2800	1200	1100	400
10% *n*-propanol	2100	2600	1400	1400	600
2% *n*-butanol	1600	2300	1000	1100	400
5% *n*-butanol	1700	3000	1300	1300	600
10% *n*-butanol	2000	2300	1400	1500	1100

* Not obtained.

creasing k_+. However, since the stationary phase and bulk water are not affected by the increase in surfactant concentration, and k_a is diffusion controlled, the adsorption rate from bulk water to stationary phase remains the same. This results in a shift of equilibrium and faster elution, but it also means that solute mass transfer between the micelle and stationary phase is reduced.

The effect of surfactant concentration on efficiency can be calculated starting with the theory proposed by Armstrong and Nome[7]. Their equation,

$$\frac{V_s}{V_R - V_m} = \frac{\bar{v}(K_{wm} - 1)}{K_{ws}} C_m + \frac{1}{K_{ws}} \tag{3}$$

where V_s is the volume of stationary phase, V_R is the elution volume of a solute, V_m is the volume of the mobile phase, $\bar{v}$ is the partial specific volume of the surfactant in the micelle, K_{wm} is the water to micelle partition coefficient, K_{ws} is the water to stationary phase partition coefficient, and C_m is the concentration of micelles, can be rearranged to yield

$$V_R = V_m + \frac{K_{ws} V_s}{\bar{v}(K_{wm} - 1)C_m + 1} \tag{4}$$

This is a variation of the fundamental chromatographic equation $V_R = V_m + KV_s$ where K is now the total partition coefficient as given in eqn. 5. Eqn. 4 reduces to the fundamental equation when $C_m = 0$.

$$K = \frac{K_{ws}}{\bar{v}(K_{wm} - 1)C_m + 1} \tag{5}$$

The capacity factor, k', is equal to $K(V_s/V_m)$. Using the expression for K from eqn. 5 and substituting for k' into the efficiency relationship (eqn. 1) yields:

$$H = \frac{2\nu}{K_d} \times \frac{K_{ws}(\bar{v}C_m(K_{wm} - 1) + 1)V_sV_m}{(V_m\bar{v}C_m(K_{wm} - 1) + 1 + K_{ws}V_s)^2} \quad (6)$$

If the values for k_d from Table I and data for K_{wm}, K_{ws}, V_m and V_s from previously reported work[8] performed under the same conditions are substituted into eqn. 6, and for $\bar{v}$ equal to 0.862 ml/g for SDS[16] the plate height can be calculated as a function of micelle concentration. These calculated values of H are shown in Fig. 4. For phenol the numbers are slightly low, while for benzene and *p*-nitroaniline they are high by *ca.* 50% and for 2-naphthol by a factor of two. However, as shown in Fig. 4, the plots of measured and calculated values *versus* micelle concentration for all the solutes are of the same shape indicating that eqn. 6, which shows a loss in efficiency with increasing surfactant concentration, predicts the correct trend.

While a plot of variation of H with micelle concentration, C_m, can be obtained since C_m is independent of the other terms in the equation, neither the partition coefficients nor k_d can be so plotted since they all depend upon the nature of the surfactant, and in order to change one of them, the others must change also.

Effect of added organic modifiers

Since mass transfer of the solute across the bulk water is one of the primary causes of band broadening, the addition of organic modifiers such as alcohols which render the bulk phase less polar should allow a nonpolar solute to exit the micelle more rapidly and therefore increase efficiency. Methanol should remain primarily in the bulk phase and has been shown to increase the self-diffusion of SDS in micellar solutions[17]. This should result in faster exit rates and increased mass transfer. Higher

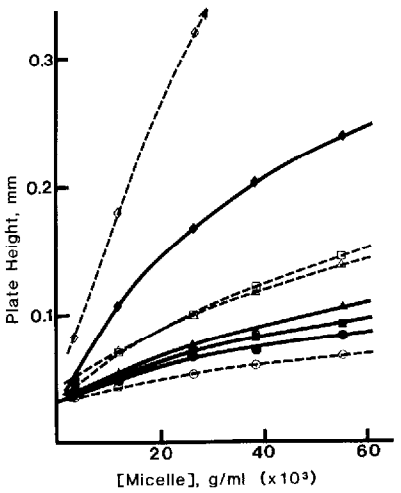

Fig. 4. Actual (closed symbols) and calculated (open symbols) plate heights as a function of micelle concentration. (●, ○) Phenol, (■, □) benzene, (▲, △) *p*-nitroaniline, (◆, ◇) 2-naphthol. Temperature, 25°C; column, LC-1.

alcohols, however, starting at about butanol tend to be incorporated into the micelle forming mixed micelles with a decrease in the CMC[17,18]. As expected, organic solvents enhance efficiency but at the cost of reducing k'. This means the role of the modifier serves to shift the mechanism of separation towards conventional reverse phase. The effects of different concentrations of three organic modifiers are shown in Table V. The small gains in efficiency are not worth the incorporation of organic solvents in this aqueous environment. The freedom from organic solvents is one of the features that make micellar chromatography attractive.

Dorsey *et al.*[19] have shown that the addition of small amounts of propanol to a micellar mobile phase can increase efficiency when using a C_{18} stationary phase due to wetting of the very hydrophobic stationary phase. In our work with the C_1 stationary phase, the major factor in retention is the adsorbed surfactant. The addition of alcohol to the mobile phase and its subsequent adsorption onto the stationary phase may result in a decrease in the amount of adsorbed surfactant and, thereby, have a much larger effect on retention. In addition, the compounds studied by Dorsey *et al.* were all single-ring compounds which have relatively high exit rate constants from micelles, thus lessening the effect of reduced mass transfer from the micelle.

CONCLUSIONS

Poor mass transfer between the micelle and stationary phase is a major cause of the reduced efficiency associated with micellar mobile phases. In order to improve mass transfer, it has been shown that (1) temperature should be increased; (2) linear velocity should be reduced; and (3) micelle concentration should be reduced. The use of additional organic modifiers such as alcohols appears to have minimal utility since they appear to affect the chromatography more than the efficiency.

Temperature has a large effect, and, as a result, micellar chromatography should be carried out at elevated temperatures, provided a means of preventing column deterioration, such as a saturator column, is used. Column packings which are not silica-based may be of great utility in this regard. When operating at elevated temperatures, the effects of flow-rate and surfactant concentration are minimized. However, for optimum efficiency, the flow-rate should be optimized while still maintaining a reasonable elution time. Likewise, a surfactant concentration close to but above the CMC should be used. When these factors are taken into account, reasonable efficiencies can be obtained for many larger, hydrophobic molecules, and the range of compounds for which micellar chromatography is practical will be expanded.

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