Journal of Chromatography, 316 (1984) 183–191
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 443

GRADIENT ELUTION MICELLAR LIQUID CHROMATOGRAPHY

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SUMMARY

The usefulness of micellar mobile phases for gradient elution reversed-phase chromatography is demonstrated. Adsorption isotherms are shown to prove that no further stationary phase modification occurs above the critical micelle concentration. Micelle concentration gradients are then possible with no column re-equilibration necessary between injections. The strength of common micellar solutions is calculated and compared to methanol. Micellar concentration gradients are also shown to be compatible with electrochemical detection with minimal background current shifts.

INTRODUCTION

Gradient elution techniques are the most versatile and popular techniques for solving the general elution problem in liquid chromatography. Snyder and co-workers[1–3] have thoroughly addressed both theoretical and experimental aspects of gradient elution. Besides the overall reduction in separation time, gradient elution also has the advantages of peak compression, yielding lower limits of detection for strongly retained compounds, and increased column load capacity. The major disadvantage of gradient elution is that the composition of the stationary phase changes during the course of the separation in both normal- and reversed-phase techniques. This means that after a gradient separation, many column volumes of the initial mobile phase must be flushed through the column to re-equilibrate the stationary phase to its initial conditions. Therefore, despite the faster gradient separation, re-equilibration time spent can be so prohibitive that even a very long isocratic analysis will often be tolerated. The changing of the stationary phase composition during a gradient also introduces two non-ideal processes into theoretical considerations[3], *viz.* solvent demixing due to preferential uptake of one mobile phase component by the stationary phase, and changes in column deadtime due to changes in stationary phase composition.

It is this problem of changing stationary phase composition that we have recently solved[4].

Surfactants have been used for many years as a component of reversed-phase eluents, their principal role being that of ion pairing with oppositely charged solutes. While several reports mentioned possible micelle formation at high surfactant concentrations, Armstrong and Henry[5] were the first to utilize aqueous micellar solutions

0021-9673/84/$03.00

for reversed-phase separations. They showed that the micelles can provide a hydrophobic site for interaction with solutes in the mobile phase and can be used in place of traditional organic modifiers such as methanol or acetonitrile. The concentration of micelles can then be used to control the strength and selectivity of reversed-phase eluents. One of the early problems of micellar chromatography was a serious loss of efficiency when compared to hydro-organic mobile phases. This problem has been overcome by the demonstration that efficiencies comparable to hydro-organic mobile phases can be achieved with the addition of 3% propanol to the micellar mobile phase and an increase in column temperature to 40°C[6]. For C_1 alkyl phases and weakly polar phases, only elevated temperatures may be necessary[7].

Since the first report of micellar chromatography, the advantages of selectivity, low cost, and low toxicity have been emphasized. While certainly worthwhile, these are not compelling reasons for a major shift to the use of such mobile phases. Other, more dramatic advantages are beginning to appear however, that should shift the role of micellar mobile phases from laboratory curiosity to practical utility. Landy and Dorsey[4] have recently demonstrated that micellar concentration gradients are possible which speed the elution of strongly retained compounds without altering the composition of the stationary phase. This means that no column re-equilibration is necessary between samples, which will allow gradient techniques to be useful for repetitive, routine analyses with dramatic savings of both time and solvent. Arunyanart and Cline-Love[8] have developed a new three phase equilibrium model, relating capacity factor to micellar mobile phase concentration, and derived equations which allow calculation of the equilibrium constant for the solute between the bulk aqueous phase and micellar aggregate. More significantly, if the equilibrium constant is available from independent methods, the equations can accurately predict the chromatographic capacity factor at zero or higher mobile phase micelle concentration. This means that for the first time spectroscopic measurements can accurately predict chromatographic retention, a goal not yet realized for hydro-organic mobile phases.

Here we report additional evidence, in the form of adsorption isotherms, that there is no further stationary phase modification at surfactant concentrations above the critical micelle concentration (CMC). The strength of micellar mobile phases, necessary for proper utilization of gradient theory, is calculated and compared to methanol. We further demonstrate that micelle concentration gradients are compatible with the use of electrochemical detectors, which heretofore have not been generally amenable to gradient techniques.

EXPERIMENTAL

Apparatus

The liquid chromatograph was composed of two Altex (Altex Scientific, Berkeley, CA, U.S.A.) 100A pumps and an Altex 210 sample injection valve with 5- and 20-μl loops. Detectors employed were an Altex 153 UV detector (254 nm) with an 8-μl flow cell, a Waters (Waters Assoc., Milford, MA, U.S.A.) R-401 refractive index detector, a Wescan (Wescan Instruments, Santa Clara, CA, U.S.A.) 213 conductivity detector, and a Bioanalytical Systems (Bioanalytical Systems, West Lafayette, IN, U.S.A.) LC-4 electrochemical detector with a TL-5 glassy carbon working electrode and an Ag/AgCl reference electrode.

Columns used were Altex Ultrasphere ODS 250 × 4.6 mm and 150 × 4.6 mm. A silica saturator precolumn was prepared from bulk silica and was placed before the injector. Both the precolumn and analytical column were thermostated by means of water jackets and a Haake D1 circulator.

Reagents

The surfactants were hexadecyltrimethylammonium bromide (CTAB) (purum grade) and sodium dodecyl sulfate (SDS) (puriss. grade), both from Fluka (Hauppauge, NY, U.S.A.) and were used as received. Surfactant solutions were made in deionized, distilled water and were filtered through a 0.45-μm Nylon-66 membrane filter (Rainin Instruments, Woburn, MA, U.S.A.). All mobile phase solutions also contained 1-propanol (3%, v/v)[6]. Solutes were from various manufacturers and were used as received. Solutes were dissolved in either methanol or aqueous micellar solution.

Procedures

Adsorption isotherms were measured by determining the amount of surfactant adsorbed onto the stationary phase from frontal chromatography experiments. The breakthrough of the surfactant was monitored by both refractive index and conductivity detectors. Since Knox and Hartwick[9] have shown that it is extremely difficult to remove all adsorbed SDS from C_{18} phases, a stepwise increase in concentration was used, each previous concentration being used as the new baseline. At each new concentration the column was first disconnected and the precolumn volumes flushed with the new concentration of surfactant. The breakthrough volume (V_R) was measured to the top of the plateau, and the void volume of the column (V_0) was subtracted. The amount of surfactant adsorbed was then calculated as

$$q_{ads} = (V_R - V_0)\, C_m = AC_s \tag{1}$$

where C_m is the concentration of surfactant in the mobile phase, C_s is the surface concentration of adsorbed surfactant, and A is the surface area of the stationary phase. A was reported to be 235 m^2/g or 752 m^2/column[10].

RESULTS AND DISCUSSION

Because of their extensive use as ion-pairing reagents, the adsorption of surfactants by reversed-phase sorbents has received much attention[9,11–14]. It is generally recognized that equilibration times of surfactant containing mobile phases are quite long, and this leads to an intuitive belief that micellar concentration gradients would be futile. However, a very unique property of micellar solutions is that there is always a constant amount of free surfactant present in solution, and any change in total surfactant concentration serves only to change the concentration of micelles[15]. Therefore after an initial column equilibration with any surfactant concentration above the CMC, it is possible with micellar concentration gradients to speed the elution of strongly retained compounds without altering the composition of the stationary phase[4]. This allows a step gradient back to the initial conditions and the only re-equilibration necessary before the next sample is that amount of mobile phase needed

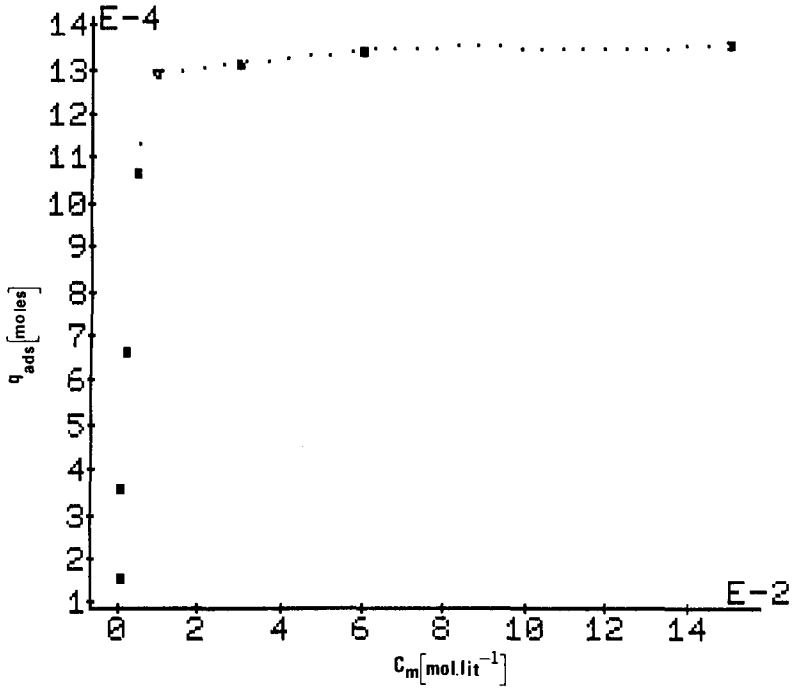

Fig. 1. Adsorption isotherm of SDS on an Altex Ultrasphere ODS column at 30°C. Mobile phase is 1-propanol–water (3:97). E-2 = 10^{-2}, E-4 = 10^{-4}.

to sweep the mixer and other precolumn volumes. We have recently shown this to be true by careful statistical evaluation of retention measurements of an early-eluted solute, following micellar gradients, and a step back to initial conditions. Failure to regenerate a column completely after a gradient will cause wide variability from one experiment to the next in the retention of early-eluted peaks[1]. We now address this argument from the perspective of the stationary phase.

Adsorption isotherms

Fig. 1 is the adsorption isotherm of SDS with a standard mobile phase of 1-propanol–water (3:97). The maximum concentration of surfactant adsorbed on the stationary phase occurs at a mobile phase concentration of *ca.* 10^{-2} *M* and gives a surface concentration of *ca.* 1.8 μmoles/m^2 of adsorbed SDS. Fig. 2 is a log–log plot of surface concentration *vs.* mobile phase concentration and, again, it is clear that there is apparent saturation of the stationary phase (*vide infra*).

Figs. 1 and 2, then, are supporting evidence for the conclusion that no column re-equilibration is necessary after a micelle concentration gradient. In fact, these plots should show a break at the CMC value of the surfactant, as that represents the maximum concentration of free surfactant that will exist in solution. Because of the nature of the curvature of these plots, they are not true Langmuir isotherms. That is, they do not show a break when the stationary phase becomes truly saturated, rather the break is a result of the micellization of the surfactant.

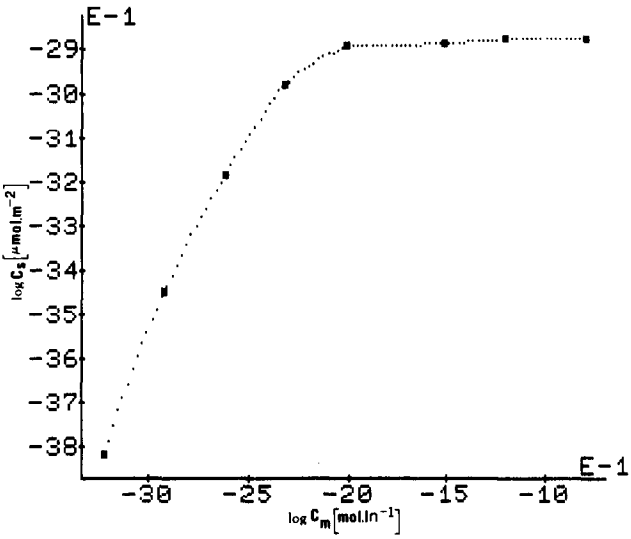

Fig. 2. Log–log representation of Fig. 1. Apparent saturation of stationary phase is obvious, but see text. E-1 = 10^{-1}.

These isotherms differ significantly from those of Knox and Hartwick[9], who reported Freundlich-type isotherms for SDS. While they reported mobile phase concentrations at or above the CMC value of SDS, it must be noted that their solvent was methanol–water (20:80), and it is highly doubtful that micellization occurred, even in their solution with the highest concentration. The CMC of SDS in pure water at 25°C is $8 \cdot 10^{-3}$ *M* and has been reported first to decrease and then to increase with small additions of methanol. In solutions of 0.27 mole fraction methanol, micellar aggregation of SDS molecules is precluded[16]. These facts both indicate that micelles did not exist in their mobile phase. They measured the surface concentration of SDS on ODS-Hypersil to be *ca.* 8 μmole/m^2, greater than that reported here by a factor of 4.

Gradient elution

The theory and advantages of gradient elution have been well documented[1–3]. There are some important distinctions, however, when considering reversed-phase micellar chromatography as compared to traditional hydroorganic mobile phases.

Hydroorganic mobile phases generally exhibit linear plots of log *k'* *vs.* percent organic component (at least over the range of *k'* values of interest in gradient elution). This then dictates that for a linear solvent strength gradient, a linear gradient from solvent A to B is the preferred shape. However, with micellar mobile phases, linearity occurs when log *k'* is plotted *vs.* log [surfactant][6,17]. This then dictates that a convex gradient would generally be the preferred shape for micellar mobile phases. However, the difference in the quality of separation between a linear and convex gradient is often slight[1].

A second difference in the two types of mobile phases is in selectivity. With hydroorganic mobile phases, plots of log *k'* *vs.* percent organic component for different solutes are (approximately) linear, but often not parallel. This change in se-

TABLE I

STRENGTH OF MICELLAR MOBILE PHASES

Reference state	*Surfactant*	*Concentration (M)*	ε^0
Methanol (100%)	SDS	$1 \cdot 10^{-2}$	−0.792
		$5 \cdot 10^{-2}$	−0.679
		$1 \cdot 10^{-1}$	−0.610
	CTAB	$1 \cdot 10^{-2}$	−0.801
		$5 \cdot 10^{-2}$	−0.664
Methanol–water (90:10)	SDS	$1 \cdot 10^{-2}$	−0.681
		$5 \cdot 10^{-2}$	−0.567
		$1 \cdot 10^{-1}$	−0.498
	CTAB	$1 \cdot 10^{-2}$	−0.690
		$5 \cdot 10^{-2}$	−0.553

lectivity can lead to changes in band position with changing gradient steepness[2]. This change in selectivity is even more common with micellar mobile phases. Yarmchuk *et al.*[17] have shown that retention order reversals can occur with changes in surfactant concentration. This implies that the elution order in an isocratic separation may be different from the elution order in a gradient separation.

Lastly, a question that must be considered is that of the strength of micellar mobile phases. Grushka *et al.*[18] have calculated ε^0 parameters of water–methanol mixtures as a function of temperature and mobile phase composition. The expression given by Snyder[19] for calculating ε^0 in normal-phase chromatography has also been used in reversed-phase liquid chromatography with carbon adsorbents[20]:

$$\ln (k'_1/k'_2) = a(\varepsilon_2^0 - \varepsilon_1^0) \quad (2)$$

where a is the molecular area of the solute and the subscripts 1 and 2 indicate solvents 1 and 2, respectively. By choosing one of the solvents as a reference state and setting $\varepsilon = 0$, eqn. 2 can be used for comparing solvent strengths. Table I shows strength values at 40°C for both SDS and CTAB at various concentrations when benzene is used as a solute and both 100% methanol and methanol–water (90:10) as the reference solvent. For comparison, Grushka *et al.*[18] reported a value of −0.0867 for methanol–water (4:1) with a reference of 100% methanol and decylbenzene as the solute. This indicates that 0.1 *M* SDS and CTAB are both considerably weaker reversed-phase eluents than methanol–water (4:1). This need not mean that micellar mobile phases must necessarily give longer separation times than hydroorganic mobile phases. We have successfully used SDS concentrations as high as 0.4 *M*, and it is also possible to reduce analysis time through the use of stationary phases with shorter chain length. Fig. 3 shows a gradient separation of a seven component test mixture.

Electrochemical detection

As with any mobile phase or mobile phase additive, a question which must be addressed is the effect of the system on solute detectability. It has previously been

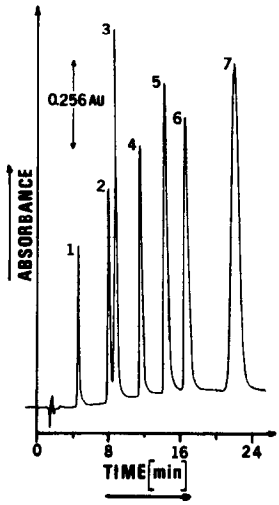

Fig. 3. Gradient separation of test mixture. Altex Ultrasphere ODS at 30°C, flow-rate 1.4 ml/min. Gradient program from 0.20 *M* to 0.40 *M* SDS both with 3% 1-propanol, 7.5 min linear ramp begun at injection. Peak identification: 1 = phenol, 2 = acetophenone, 3 = nitrobenzene, 4 = benzene, 5 = toluene, 6 = ethylbenzene, 7 = anthracene.

shown that micellar mobile phases lead to enhanced fluorescence and, even room temperature liquid phase phosphorescence, thus lowering the limits of detection by as much as an order of magnitude relative to conventional methanol–water mobile phases[21,22]. Here we report the compatibility of micellar mobile phases, including gradient techniques, with oxidative electrochemical detection.

Electroanalytical techniques have been applied often to fundamental studies of micelles, but there is a paucity of information about electroanalysis in micellar solution. The lack of attention may be attributed, in part, to a poor understanding of the effects of surfactants on the double-layer structure as well as on the kinetics and thermodynamics of the electron transfer process[23]. We have found no significant differences between micellar and hydroorganic mobile phases in limits of detection, sensitivity or noise for the small range of compound thus far studied by liquid chromatography with electrochemical detection[24].

Electrochemical detectors are generally considered to be incompatible with gradient elution techniques, particularly at high operating potentials. Changes in the conductance, viscosity, pH, etc., of the mobile phase during a gradient program yield steeply sloping baselines from the ever changing residual current. We have investigated the causes of residual current changes during gradient elution, and will present the results elsewhere[25].

Changing the micelle concentration will change the bulk properties of the mobile phase less than a change in concentration of an organic solvent. Furthermore, it is likely that only free surfactant interacts closely with the electrode surface, and this concentration does not change during a micelle gradient. Fig. 4 shows a separation of an eight-component test mixture with a micelle gradient and an applied potential of +1.2 V. By balancing the conductances with sodium perchlorate and buffering the two micelle solutions, gradients from 0.01 *M* to 0.40 *M* SDS show residual current shifts of only 8 nA at a potential of +1.20 V[25].

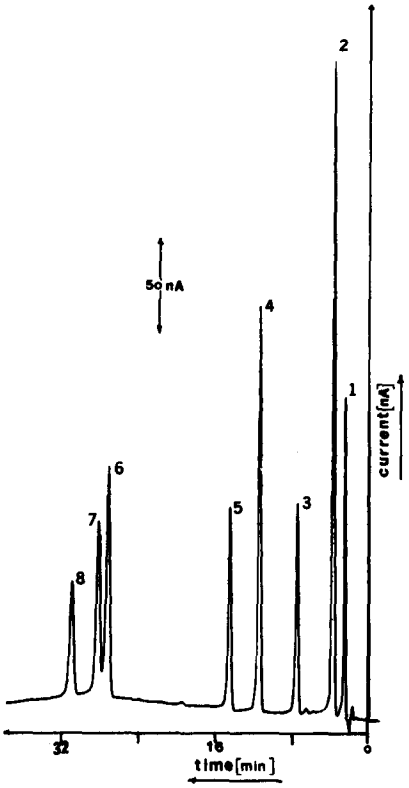

Fig. 4. Gradient separation with electrochemical detector at +1.2 V. Flow-rate 1.0 ml/min. Mobile phase A: 0.05 *M* SDS, 3% 1-propanol, pH 2.5 with phosphate buffer, sodium perchlorate added to balance conductivity with solvent B. Mobile phase B: 0.112 *M* SDS, 3% 1-propanol, pH 2.5 with phosphate buffer. Gradient program A to B in 12 min. Peak identification: 1 = hydroquinone, 2 = resorcinol, 3 = catechol, 4 = phenol, 5 = *p*-nitrophenol, 6 = *o*-nitrophenol, 7 = *p*-chlorophenol, 8 = *p*-bromophenol.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Michael McCown, Altex Scientific, for a gift of the Ultrasphere columns. J.G.D. acknowledges Eli Lilly and Company and the Alcoa Foundation for support of this work.

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