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MICELLES IN SEPARATIONS: A PRACTICAL AND THEORETICAL REVIEW

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I. INTRODUCTION

Surface active agents (a.k.a. surfactants, amphiphiles, detergents, etc.) have been utilized for years in a variety of separation processes (Table I). Most of the earlier techniques can be classified as nonmicellar methods. That is, the separation process did not require the surfactant to be in an aggregated or micellar form. These methods have been expanded and improved and remain widely useful today. The focus of this work, however, is on the more recent micellar techniques (Table I). Readers interested in the nonmicellar methods are referred to the many fine reviews and articles that have appeared on these subjects.[1-6] In making a division between micellar and nonmicellar separation techniques, one should understand that there are some cases where this distinction is not clear-cut.

The deliberate use of micelles and their unique properties for separations probably began in the mid to late 1970's.[7-9] Also at this time micelles were being used to improve a variety of

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TABLE I.

Examples of Separation Techniques Which Utilize Surfactants

Micellar Techniques	Nonmicellar Techniques
a. pseudophase or micellar liquid chromatography	a. ion interaction chromatography
b. liquid-liquid extractions	b. foam flotation
c. cloud point extractions	c. floc foam floation
d. capillary electrokinetic separations	d. polyacrylamide gel electrophoresis
e. membrane techniques	e. precipitation and flocculation

other titrimetric,[10] spectroscopic[11-17] and electrochemical techniques.[18] The existence of the micellar pseudophase was a fundamental requirement for all of these techniques. In some instances, it is possible for micelles to be present but not contribute to a separation process or that the desired effect is caused by monomer surfactant. These should not be confused with true micellar methods (Table I).

An overview of the structure and properties of micelles will be given prior to the discussions of various micellar separation techniques. A thorough knowledge of micelles is useful for those who want to understand, utilize and optimize these methods. It is hoped that this review will help stimulate further work and innovation in the area of micellar separations.

II. STRUCTURE AND PROPERTIES OF MICELLES

A. Normal Micelles

Structure. Micelles are dynamic species consisting of aggregated surfactants in an aqueous continuum.[19-27] Surfactants are amphiphilic molecules that contain both hydrophilic (water-loving) and hydrophobic (water-hating) parts.[28,29] They are generally classified by the nature of their hydrophilic "head-groups" as cationic, anionic, nonionic or zwitterionic surfactants. The hydrophobic "tail" of the surfactant is generally a linear or branched hydrocarbon containing between seven and twenty one carbons. Occasionally aromatic ring systems will be present as well. Surfactants usually exist as discrete monomers in very dilute aqueous solution (generally less than 10^{-4} M), although there is some evidence of premicellar aggregation or oligomerization.[27] If one increases the concentration of surfactant in solution, a point will eventually be reached where extensive aggregation occurs and many of the bulk physical solution properties change. The aggregate is called a micelle and its shape varies from that of a prolate ellipsoid to a rough sphere depending on the surfactant and environment. The point at which aggregation occurs is referred to as the critical micelle concentration (i.e., CMC, c.m.c., or Cm) and can be detected by a variety of physico-chemical techniques as illustrated in Figure 1. Aggregation actually occurs over a narrow range of concentrations and seems to be a highly cooperative process.[25] It is not unusual to obtain slightly different CMC values depending on one's method of measurement.

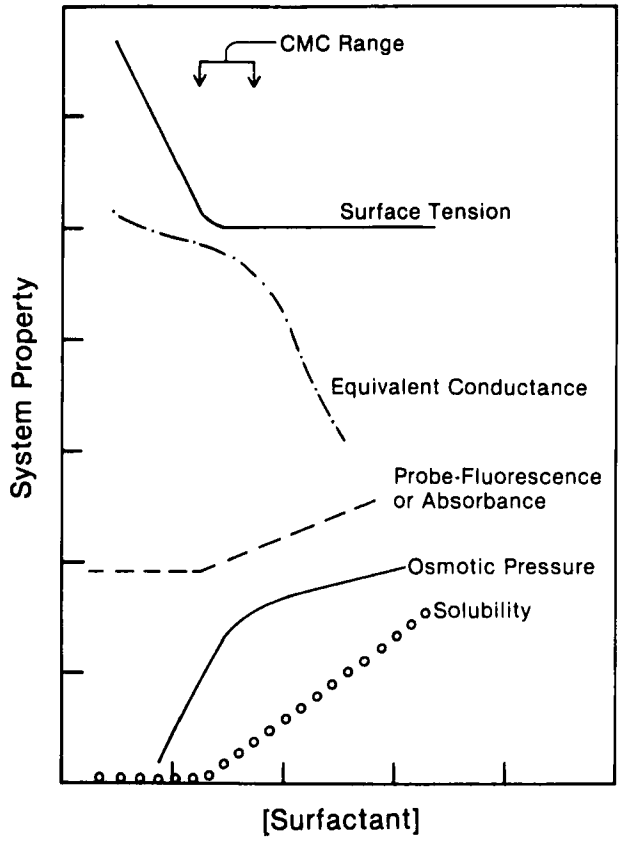

Figure 1

Typical changes in an aqueous surfactant system's properties which reflect the onset of aggregation.

The structure of aqueous micelles continues to be a subject of some controversy. A variety of models have been proposed, based on a variety of sometimes conflicting experimental evidence. Four basic models will be considered, in roughly the chronological order of their appearance in the literature. It should be noted

that in a brief treatment such as this, some oversimplification in the interest of space and clarity is unavoidable.

A cross-sectional representation of the classical micelle is given in Figure 2. This is often referred to as the Hartley, oil-drop, reef and/or radial model.[27,30-32] This model assumes the micelle has approximately spherical geometry, a somewhat rough surface with all hydrocarbon chains in extended anti configurations, and little or no water penetration into the core. Indeed, it resembles a small hydrocarbon "pool" or oil droplet surrounded by polar headgroups, counterions and water.[27,30-32]

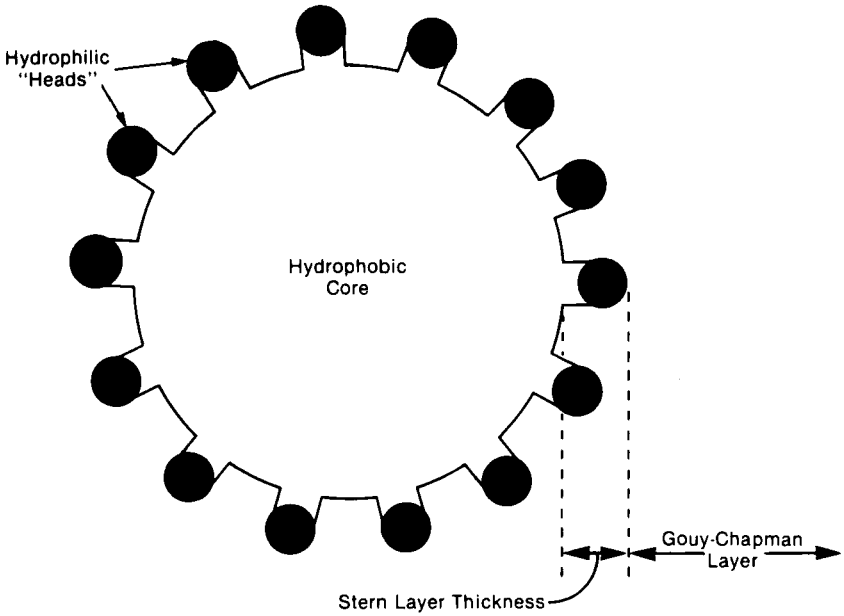

Figure 2

Typical cross-sectional schematic representing the classical view of an aqueous micelle. Counterions are not shown.[23,30,31]

There are a number of contradictions in this model which have induced other researchers to propose alternatives. For example, how can radially aligned, extended, anti hydrocarbons be like a disordered oil droplet, or how can the ends of the hydrocarbon chains occupy the same volume at the center of the micelle core? Yet, for years this simple, esthetically pleasing, but uncritical view of the micelle has been used to adequately explain a number of experimental observations which require a hydrophobic pseudophase. Increasingly, theoretical and experimental evidence indicate a number of shortcomings with the classical model. Use of probe molecules, direct analysis, and model building all indicate that there is significant contact between water and the hydrocarbon "tails" of the surfactants which constitute the micelle (as well as with hydrophobic solutes associated with the micelle).[27] This can occur in at least three different ways: (a) if there is significant water penetration past the head groups and into the hydrophobic core of the micelle, (b) if there is no penetration of water into the micelle core but significant portions of the hydrocarbon "tails" are exposed at the micelle-water interface, (c) some combination of (a) and (b). A cross-sectional representation of the "Menger-micelle" is shown in Figure 3.[33] There are two conformational extremes for the Menger-model, one in which the hydrocarbon chains are fully extended and the other where the hydrocarbon chains are folded or compacted.[33-37] In both cases water has access to the hydrocarbon interior of the micelle. The surface of the "Menger micelle" is

rougher and more poorly defined than that of the classical micelle and the surfactants that comprise the micelle are oriented in a more random manner (Figure 3). In addition to being more sterically acceptable, this model explains a number of experimental results (e.g., viscosity, polarity, kinetics) better than the classical model.[33-37]

Two of the more recent micelle models have a hydrophobic core largely devoid of water (as in the classical model) and yet have considerable contact between water and the hydrophobic "tail" portion of the surfactants (as in the Menger-model). This is

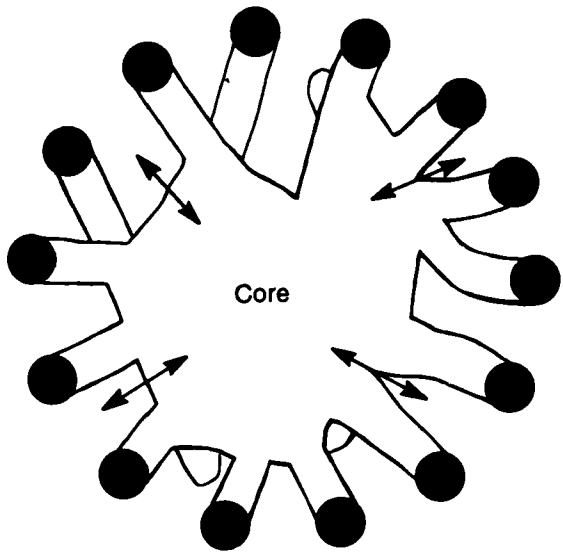

Figure 3

A possible representation of the Menger micelle in which the hydrocarbon "tails" are extended. In this model the size of the core can vary, the surface is relatively rough and water is able to penetrate past the Stern layer.[33-36] Counterions are not shown.

accomplished by having hydrocarbon portions of the surfactant as well as "head-groups" exposed at the surface. Fromherz developed a spherical structure for the micelle using space filling block models.[38] The surfactant blocks are assembled in a parallel, strainfree, water-free packing with the head groups separated as far as possible. While the hydrocarbon tails are considered to be in an extended-anti conformation, gauche conformations near the head group of the surfactant are allowed to minimize electrostatic repulsion.[38] The result, shown in Figure 4, is a highly structured hybrid of a bilayer and classical micelle. As can be

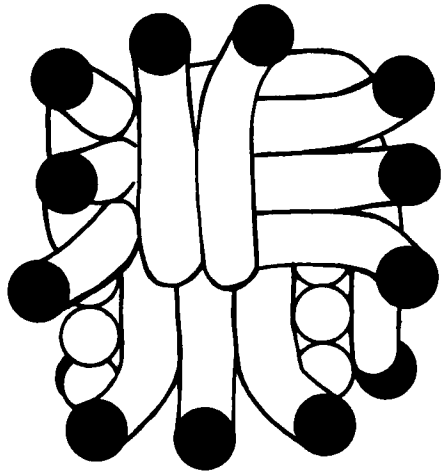

Figure 4

A possible cross-sectional view of the Fromherz micelle. This model is constructed using cylindrical sticks to represent the surfactant molecules. The "molecules" are arranged in a square lattice. The models can be bent by one gauche conformation near the headgroup. These and additional constraints result in a model with a more traditional hydrophobic core, however, the average amount of hydrocarbon subject to surface wetting is high.[38] Counterions are not shown.

seen in Figure 4, there is considerable hydrocarbon exposure to water at the micelle surface. More extensive details of this model have been published.[38]

In the Dill model of the micelle, the hydrocarbon "tails" are considerably less structured (Figure 5).[39-41] Statistical theory in conjunction with a lattice model allows a random distribution of chains on which certain steric constraints are imposed. Thus, the structure of the roughly spherical micelle is thought to be the result of: (a) Langmuir's principle of differential solubility in which hydrocarbon tails are packed into a waterless

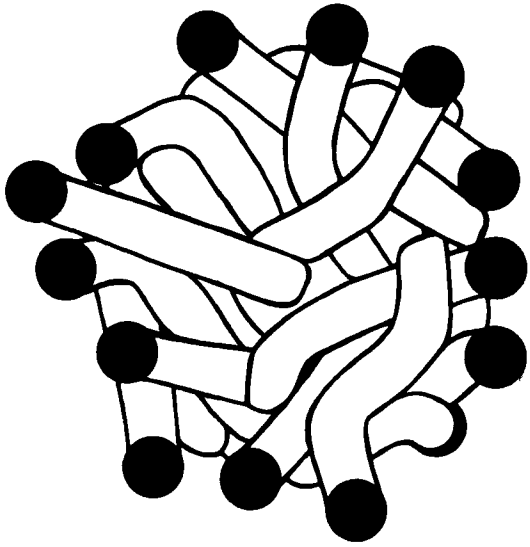

Figure 5

A cross-sectional schematic of the Dill model for the micelle. The hydrocarbon tails are more randomly distributed as dictated by statistical considerations. There is a definitive hydrophobic core with little water penetration. There is also an appreciable amount of hydrocarbon exposed at the surface. Counterions are not shown.[39-41]

core (b) steric forces which determine the structure of the core, and (c) molecular chain configurations of equal energy are of equal likelihood.[39-41] Again, there is a considerable amount of hydrocarbon exposed at the surface of the micelle. This, as in the previous model, allows hydrophobic solutes to be solubilized near the surface, and explains how these solutes (as well as portions of the surfactant hydrocarbon "tail") can be in contact with water when associated with the micelle (Figure 5).

One must understand that models by their very nature are oversimplifications of the real situation. The debate on micelle structure will undoubtedly continue. Much of the current controversy over micelle structure stems from Menger's summary of conflicting experimental results and his alternative micelle structure (vide supra) which attempted to resolve these conflicts.[33] Regardless of which view of the micelle is eventually accepted, Menger must be given credit for pointing out the shortcomings of the classical micelle, which resulted in a considerable amount of discussion and modeling by others.

Properties. The properties of aqueous micelles have been extensively reviewed by a number of researchers.[19-29] A summary of this material will be given with particular emphasis on those characteristics that are most pertinent to separations.

Several selected micelle forming surfactants are listed in Table II including their CMC's, Krafft points, aggregation numbers and fraction of charges. As will be seen, all of these properties are important in micellar separations. The aggregation number is

the average number of monomer surfactant molecules per micelle. The Krafft point is the temperature at which the solubility of a surfactant is equal to its CMC. The fraction of charge for micelles composed of ionic surfactants is the ratio of the number of counterions in the Stern Layer of the micelle to the aggregation number subtracted from 1. Most of these properties are dependent on the temperature of the system and are also strongly affected by the presence of organic and/or inorganic impurities. For example, slightly increasing the ionic strength of the solution will decrease the CMC and increase the aggregation number of most ionic micelles.[22,42] Some organic impurities seem to induce micelle formation at lower surfactant concentrations while others, such as methanol or ethanol, tend to disrupt micelle formation.[21] Figure 6 is a phase diagram for typical pure ionic surfactants. It illustrates the regions in which precipitate, monomers and micelles exist as a function of temperature and concentration. At still higher surfactant concentrations, it is possible for some surfactants to undergo sphere to rod transitions and formation of lyotropic liquid crystals.[19,20,37]

It is important to understand that there are some distinct differences between ionic and nonionic micelles. In general, micelles composed of nonionic surfactant tend to have lower CMC's and higher aggregation numbers than analogous ionic micelles.[19,20] This is thought to be due, in part, to the lack of electrostatic repulsion between the "head groups" of nonionic surfactants. In ionic micelles, this repulsion tends to limit the size and CMC of

TABLE II

Selected Micelle Forming Surfactants and Associated Aggregational Parameters.[a]

Surfactant	CMC, M (25°C)	N[b]	α[c]	KP[d]
Cationic				
Hexadecyltrimethyl ammonium bromide	9.2×10^{-4}	61	0.10	22
Tetradecyltrimethyl-ammonium bromide	3.5×10^{-3}	75	0.1	-
Dodecyltrimethyl-ammonium bromide	1.5×10^{-2}	50	0.19-0.12	-
Dodecylammonium chloride	1.5×10^{-2}	55.5	0.64-0.11	-
Hexadecylpyridinium chloride	9×10^{-4}	95[e]	0.31	-
Anionic				
Sodium tetradecylsulfate	2.1×10^{-3}	80[f]	-	25
Sodium dodecylsulfate	8.1×10^{-3}	62	0.13-0.21	16
Lithium dodecylsulfate	8.8×10^{-3}	63	0.23	-
Sodium 4-dodecylbenzene-sulfonate	1.6×10^{-3}	24	-	-
Sodium dodecyl-sulfonate	9.8×10^{-3} [g]	54	-	32
Sodium dodecanoate	2.4×10^{-2}	56[h]	0.36[i]	-
Potassium perfluoro-heptanoate	2.8×10^{-2}	-	-	25.6
Sodium perfluoro-heptanoate	3.0×10^{-2}	-	-	8.0
Lithium perfluoro-octylsulfonate	6.3×10^{-3}	-	-	<0
Sodium perfluoro-octylsulfonate	-	-	-	56.5
Zwitterionic				
n-Octyl-N,N-dimethyl ammonium-3-propionate	2.5×10^{-1}	24	0	<0
n-Dodecyl-N,N-dimethyl-ammonium-3-propionate	5.3×10^{-3} [j]	-	0	-

(continued)

TABLE II, Continued

Surfactant	CMC, M (25°C)	N[b]	α[c]	KP[d]
n-Dodecyl-N,N-dimethyl-ammonium-3 propane-1-sulfonate	3×10^{-3}	55	0	<0
n-Dodecyl-N,N-dimethyl-glycine	1.8×10^{-3}	-	0	-
Nonionic				
Polyoxyethylene (6) dodecyl ether	8.7×10^{-5}	400	0	-
Polyoxyethylene (12) dodecyl ether	5×10^{-5}	81	0	-
Polyoxyethylene (23) dodecyl ether	5.5×10^{-5}	40	0	-
Polyoxyethylene (7) hexadecyl ether	1.7×10^{-6}	594	0	-
Polyoxyethylene (12) hexadecyl ether	7.5×10^{-5}	152	0	-
Polyoxyethylene (10) nonylphenyl ether	7.5×10^{-5}	276[k]	0	-
n-Octyl glucoside	2.5×10^{-2}	52[k]	0	-
n-Dodecyl glucoside	1.9×10^{-4}	-	0	-

[a]All listed parameters were determined in pure water at 25°C unless otherwise indicated. All data is summerized from references 19-29.

[b]Aggregation number (25°C).

[c]Fraction of charge.

[d]Krafft Point, °C.

[e]Measured in 0.0175 M NaCl.

[f]Measured at 60°C.

[g]Measured at 38°C.

[h]Measured in 0.013 M KBr.

[i]Measured in 0.21 M NaCl.

[j]Measured at 30°C.

[k]Taken from: R. Roxby and B. P. Mills, Fed. Proc., 39, 6, 1985 (1980).

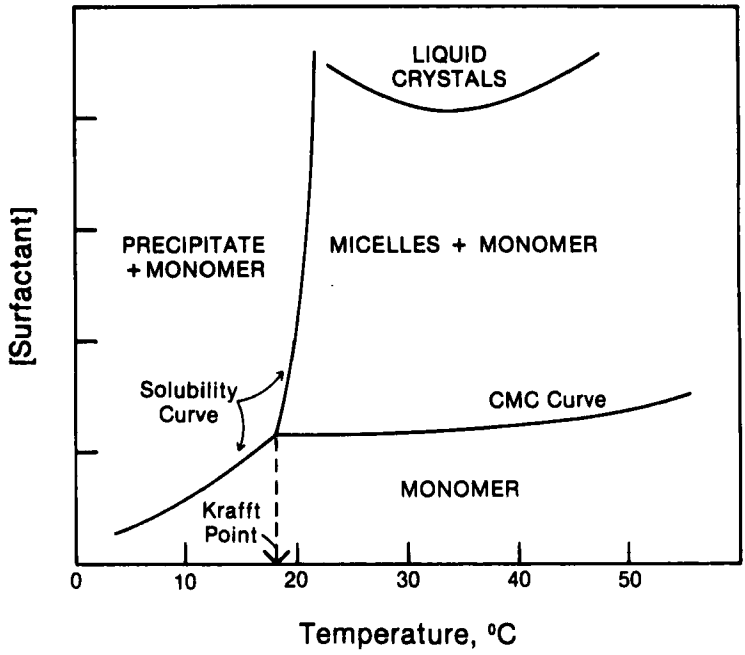

Figure 6

An example of a phase diagram for an aqueous ionic surfactant system.

the aggregate. Favorable entropic and van der Waal's forces are thought to provide the driving force for aggregation of all normal micelles.[19-28] The two remaining factors that also contribute to a micelle's size and shape are surface energy minimization and steric exclusion (particularly of the "tails").[41] In solutions of high ionic strength, where electrostatic repulsions are minimized, the CMC's and aggregation numbers of ionic micelles tend to approach those of analogous nonionic micelles. Pure nonionic micelles obviously have no fraction of charge. Their "head groups" do have significant dipole moments, however, and they can

TABLE III

Cloud Points of Several Nonionic Surfactants[a]

Surfactant[b]	Concentration	Cloud Point, °C
Polyoxyethylene (9.5) isooctylphenyl ether (Triton X-120)	0.25%	64
" "	7.0%	65
" "	33.0%	76
Polyoxyethylene (7.5) nonylphenyl ether	0.125%	1
" "	5.0%	6
" "	20.0%	25
Polyoxyethylene (6) dodecyl ether	1%	48
" "	10%	50.5
Polyoxyethylene (20) nonylphenyl ether	10%	>100
Polyoxyethylene (23) dodecyl ether	1-6%	100

[a]Summarized data from references 19, 20, 123 and 160.

[b]The number in parentheses after polyoxyethylene refers to the average number of oxyethylene groups.

also form weak complexes with some metal ion impurities that may be present in solution. Most unusual is the fact that nonionic surfactants (unlike ionic surfactants) can undergo a phase separation (referred to as the cloud point) with increasing temperature in aqueous solution. Table III lists cloud points of

TABLE IV

A Comparison of Solute Solubility in Aqueous and Micellar Solutions

Compound	Molar Solubility in Water	Surfactant Concentration[a]	Molar Solubility in Micellar Solution	Solubility Enhancement Factor
Pyrene[b]	6×10^{-7}	0.07 M SDS	7×10^{-2}	120,000
		0.04 M HTAB	4.1×10^{-1}	680,000
Perylene[b]	1.6×10^{-9}	0.01 M SDS	1.9×10^{-3}	1,190,000
		0.01 M HTAB	3.3×10^{-2}	1,500,000
Anthracene[b]	2.2×10^{-7}	0.02 M SDS	6.3×10^{-3}	28,700
		0.02 M HTAB	3.3×10^{-2}	150,000
Naphthalene[b]	2.2×10^{-4}	0.04 M SDS	3.8×10^{-1}	1,700
		0.02 M HTAB	1.11	5,000
1-Bromo-naphthalene[b]	4.5×10^{-5}	0.05 M SDS	6.6×10^{-1}	15,000
		0.05 M HTAB	4.3	96,000
Biphenyl[b]	4.1×10^{-5}	0.05 M SDS	2.1×10^{-1}	5,100
		0.05 M HTAB	1.0	24,000
Benzene[b]	2.3×10^{-2}	0.05 M SDS	2.5	109
		0.05 M HTAB	12.3	530
N-methyl-acridone[c]	3.45×10^{-5}	0.01 M HTAC	3.2×10^{-4}	9
		0.4 M HTAC	1.0×10^{-2}	290
		0.01 M SDS	6.4×10^{-3}	2
		0.4 M SDS	3.5×10^{-3}	101
		0.1 M SB-12	7.8×10^{-4}	23
		0.01 M Brij-35	1.4×10^{-4}	4
		0.2 M Brij-35	3.4×10^{-3}	99
Lucigenin[c]	1.28×10^{-2}	0.4 M HTAC	5.95×10^{-4}	4.6
		0.04 M SDS	8.92×10^{-2}	1.7
Progesteone[d]	1×10^{-4}	0.05 M TTAB	8.7×10^{-3}	87
Methyl-parabene[b]	1.45×10^{-2}	0.04 M SDS	3.39×10^{-2}	2.3
Ethyl-parabene[d]	5.4×10^{-3}	0.04 M SDS	2.27×10^{-2}	4.2

(continued)

TABLE IV., Continued

Compound	Molar Solubility in Water	Surfactant Concentration[a]	Molar Solubility in Micellar Solution	Solubility Enhancement Factor
Butyl-parabene[d]	1.1×10^{-3}	0.04 M SDS	2.43×10^{-2}	22
Sulphaethyl-thiadiazole[d]	0.4 mg/ml	30% P-80	11.3 mg/ml	28
Sulphis-oxazole[d]	0.3 mg/ml	30% P-80	80 mg/ml	27
Iodine[d]	0.2 mg/ml	200 g/L CMG	27 mg/ml	135

[a]Surfactant abbreviations are as follows: sodium dodecylsulfate (SDS), hexadecyltrimethylammonium bromide (HTAB), 3-(N-dodecyl-N,N-dimethylammino)propane-1-sulfonate (SB-12), tetradecyltrimethylammonium bromide (TTAB), polysorbate 80 (P-80), and cetomacrogeol (CMG).

[b]M. Almgren, F. Grieser and J. K. Thomas, J. Am. Chem. Soc., 101, 279 (1979).

[c]W. L. Hinze, T. E. Riehl, H. N. Singh and Y. Baba, Anal. Chem., 56, 2180 (1984).

[d]D. Attwood and A. T. Florence, " Surfactant Systems" Chapman and Hall, New York, 1983.

several nonionic surfactants. Note that the cloud point can vary with surfactant concentration and with any impurities present in solution.

The association or solubilization of solutes by aqueous micelles is responsible for most of the useful applications of these aggregates. Solutes can interact electrostatically (e.g., inorganic ions), hydrophobically or more likely by a combination of these effects (both factors are involved for most organic solutes).[43,44] Table IV shows that association with a micelle can

increase the solubility of a variety of sparingly water soluble compounds, sometimes by several orders of magnitude. It was originally believed that hydrophobic solutes were dissolved in the hydrocarbon "core" of the micelle in much the same manner as they dissolve in an organic solvent. This analogy may be sufficient for oil and water microemulsions,[45] but is probably an oversimplification for the normal micellar system. Given the structure of the Menger, Fromherz or Dill micelle it is likely that the interaction between many hydrophobic solutes and the micelle may be somewhat akin to a surface adsorption phenomenum where both hydrophobic and electrostatic interactions are important. This would not only explain the apparent presence of relatively nonpolar solutes (such as benzene) near the surface of the micelle,[43] but also account for the fact that some polar solutes have a greater solubility in micellar solutions than in either water or hydrocarbon solvents.[27] Some solutes, such as aliphatic hydrocarbons, may still have access to or be solubilized by the hydrophobic core of the micelle. Certain solutes that can comicellize (such as dodecanol) obviously will have a portion of their structure integrated into the micelle core. Consequently, the micelle can be thought of as having at least two type of interaction sites.[32,46,47] One which is analogous to a "hydrophobic dissolved state" (i.e., the core) and another which is a more polar "adsorbed state" near the micelle surface.[32,46]

Micelles are sometimes mistakenly thought of as static species. In reality they, and any solutes associated with them,

are in dynamic equilibrium with their surrounding. Surfactant monomers leave and enter the micelle on a microsecond time scale.[23-27] These monomers are freely exchanged with monomers in the bulk solution, other micelles, as well as with surfactants adsorbed on any solid surfaces. Complete dissolution and redistribution of a micelle can occur in a millisecond time scale.[23-27] Solutes associated with a micelle can also be freely exchanged with the bulk solution, other micelles or any other surface present.

Compounds that associate with a micelle have characteristic binding constants (K's) or partition coefficients (P's). The binding constant of solute to a micelle is equivalent to the ratio of the entrance and exit rate constants. Typical entrance and exit rate constants for several solutes to micelles have been published.[48,49] It is important to note that a solute can interact with a micelle without binding or partitioning to it. For example, an ion of the same charge as an ionic micelle could be electrostatically repelled when its double layer interacts with that of the micelle.

The association behavior of normal aqueous micelles is frequently referred to as a "monomer:n-mer" association and is characterized by equation 1. Where S is the self-associating

$$n\ S_1 \longrightarrow S_n \qquad (1)$$

solute and n is the aggregation number (typically n = 20 to 100)

for ionic micelles and somewhat larger for nonionic micelles). In its ideal form, this model allows surfactant to be present as monomers and monodisperse aggregates. When additional surfactant is added to the system, more micelles of the same size and aggregation number are produced while the concentration of the monomer remains roughly constant and equivalent to the CMC.[19-28] In reality, micelles are thought to be narrowly dispers but not monodisperse. Near the CMC, addition of surfactant does indeed seem to produce additional, "replicate micelles." At higher concentrations, however, the size, shape, aggregation number, etc. of some micelles will change. For example, micelles composed of hexadecyltrimethylammonium bromide (HTAB) will undergo sphere to rod transitions with an accompanying increase in aggregation number at surficiently high surfactant concentrations. Lyotropic liquid crystal formation can eventually occur at even higher concentrations. On the other hand, micelles of hexadecyltrimethylammonium chloride (HTAC) tend to remain spherical in shape at equivalent concentrations. In addition, the monomer concentration tends to increase slightly with increasing surfactant and micelle concentration. However, at surfactant concentrations near the CMC, the amount of additional monomer is sufficiently small that it is often negligible.

B. Reversed Micelles

When surfactants are dissolved in nonpolar organic solvents rather than water, aggregation can also occur. Unlike

aqueous micelles, the hydrophilic "head groups" are located in the interior of the aggregate while the hydrophobic "tails" extend into the continuous nonpolar phase (Figure 7). These aggregates are also dynamic-equilibrium species. It has been argued that the term "micelle" should not be used for these aggregates because the forces that lead to their formation, their aggregational behavior, structure and properties are generally different from those of normal aqueous micelles. It has even been implied that the presence of trace impurities (particularly water, which is nearly impossible to completely remove from surfactants) may be necessary for surfactant aggregation in organic solvents.[50,51] Despite these facts the term "reversed micellar" has achieved a widespread

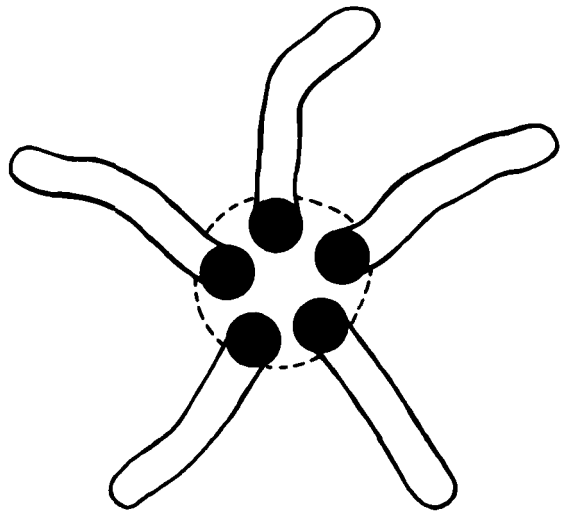

Figure 7

Model of a reversed micelle in a nonpolar organic solvent from which all possible water and impurities have been removed.

popular acceptance for these aggregates.

The association energy of reversed micelles seems to be mainly enthalpic in nature rather than entropic as in the case of aqueous micelles. Ion pairs and dipolar interactions between surfactants and their counterions predominate in nonpolar-organic media.[51-53] Consequently, one might expect the aggregational behavior of reversed micelles to be different from that of normal aqueous micelles. The association behavior of reversed micelles can be divided into at least two ideal types. The first type is sequential indefinite self-association as indicated in equation (2).[52-54] Where: S is a surfactant monomer, S_2 is a dimer, S_3 is a trimer and the equilibrium constants (K_1, K_2, etc) are equal. Surfactant systems such as dodecylammonium propionate (DAP) in cyclohexane or benzene and dodecylammonium pyrene-1-butyrate (DAPB) in benzene, exhibit this type of behavior. The aggregates are generally small in the absence of impurities (average n = 3-6)

$$S + S \rightleftharpoons S_2, \quad K_1 = \frac{[S_2]}{[S]^2} \qquad (2)$$

$$S + S_2 \rightleftharpoons S_3, \quad K_2 = \frac{[S_3]}{[S_2][S]}$$

$$\vdots \qquad\qquad \vdots$$

but increase in size with increasing surfactant concentration.[51,56] It is obviously difficult to define a CMC for such a system.[53] At best, one can define it as the concentration at which the onset of any aggregation occurs. Other surfactants

(e.g., dioctylsulfosuccinate and dodecylammonium benzoate) seem to form reversed micelles via monomer:n-mer type aggregation, as in equation (1).[58,59] This aggregational behavior is somewhat similar to that of aqueous micelles. The aggregation number also tends to be larger for this second type of reversed micelle. Unlike the first type of reversed micelle, the second type seems to have a reasonably distinct CMC. It is apparent that the reversed micelle is affected by a number of factors including the nature of the surfactant and/or surfactant counterion, solvent, concentration, temperature, and the presence of any solutes and/or impurities such as water.

Reversed micelles tend to adjust their size to accommodate or surround whatever impurity or solute is introduced into the solution. Various amounts of water (and other polar solutes can be solubilized in the hydrophilic core of a reversed micelle (Figure 8). The nature of the water in the core of a reversed micelle is dependent on its concentration.[53] At low concentrations (<0.4 M) there is little or no "free" water, as all of the molecules are occupied in hydrating the polar "head-groups" and counterions of the surfactant. This "bound" water exhibits high "microviscosities" and other unusual properties.[53] At higher concentrations of added water a "pool" of free or bulk water is also present (Figure 8).

The reversed micelle is a good model for the active site of an enzyme. Much larger rate enhancements for reactions have been observed in reversed micelles than in normal micelles for

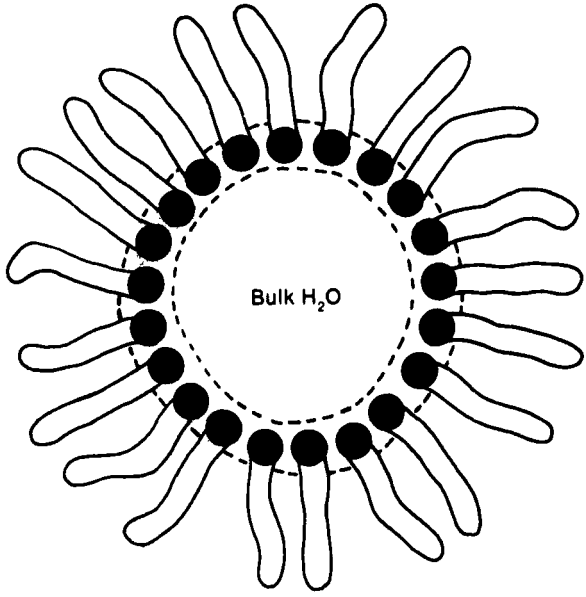

Figure 8

Model of an expanded-reversed micelle or water-in-oil microemulsion. The water between the dashed lines is tightly bound to the headgroups and associated counterions. Consequently its properties and behavior are somewhat different from that of the bulk water.

example.[53] The reasons for this are apparent. One can sequester a large number of reactant species in a very small reversed micellar core. These reactants can be oriented along an interface. One can functionalize the surfactant head groups and/or counterions in a reversed micelle. Consequently one can locate nucleophilic, hydrogen bonding and/or electron withdrawing groups in close proximity to one's reactants. Lastly, one can control the "type" and amount of water present in the reaction core.

Perhaps the most important feature of reversed micelles, from a separations point of view, is their ability to selectively solubilize a variety of polar solutes in a nonpolar media. Micellar effects on the physico-chemical properties of various solutes could also be useful in their detection as well as in stabilizing easily decomposed compounds. This is true for normal as well as reversed micelles.

III. LIQUID CHROMATOGRAPHY

A. Gel Permeation Chromatography

Aqueous micellar solutions have been successfully used as mobile phases in a variety of liquid chromatographic techniques. Although micellar mobile phases have been extensively utilized in "high performance liquid chromatography" (HPLC), their use originated with gel permeation chromatography (GPC) and thin layer chromatography (TLC). The first deliberate use of the unique properties of micelles in a chromatographic separation was probably in the GPC resolution of three transfer RNAs on a 109 x 1.8 cm Sephadex G-100 column (see Figure 9).[60] In order to accomplish this separation, the chromatographic conditions had to be carefully controlled. The ionic strength of the mobile phase was adjusted so that the hydrated tRNAs could just enter the gel while the tRNA-micelle complex was excluded from the interstitial volume of the gel.[60] Consequently, fractionation was the result of differential binding of tRNAs to the cationic hexadecyltrimethylammonium chloride (HTAC) micelle which was a component of the mobile phase.[60]

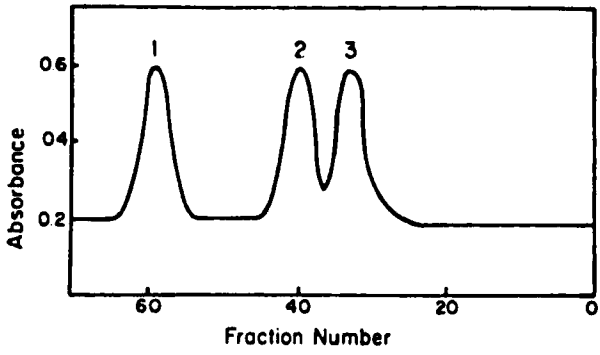

Figure 9

Separation of tRNAs of Sephadex G-100-120 in the presence of 1.0 M NaCl and 1.0 10^{-3} M HTAC. Glutamic acid-II tRNA (1), tyrosine tRNA (2) and phenylalanine tRNA (3). Each fraction contains 3.2 ml. Reprinted with permission from ref. 7.

A more straight-forward approach for micellar GPC separations involves the use of a small pore stationary phase (e.g., Sephadex G-25 or G-10). In this case the relatively large micelle can exist only in the excluded volume of the column (where it is rapidly eluted) while small solutes can also exist in the interstitial pore volume (resulting in longer elution times). However, if a small solute binds to the micellar component of the mobile phase, it will tend to elute more rapidly (with the micelle). Consequently, the GPC elution behavior of a small solute is regulated by its interaction with the micelle and not by the traditional exclusion mechanism. This behavior is illustrated in Figure 10.[61] The nucleosides adenosine and uridine are not appreciably separated by GPC with an aqueous or buffered mobile phase (Figure 10A). When sodium dodecanoate (SD) micelles are

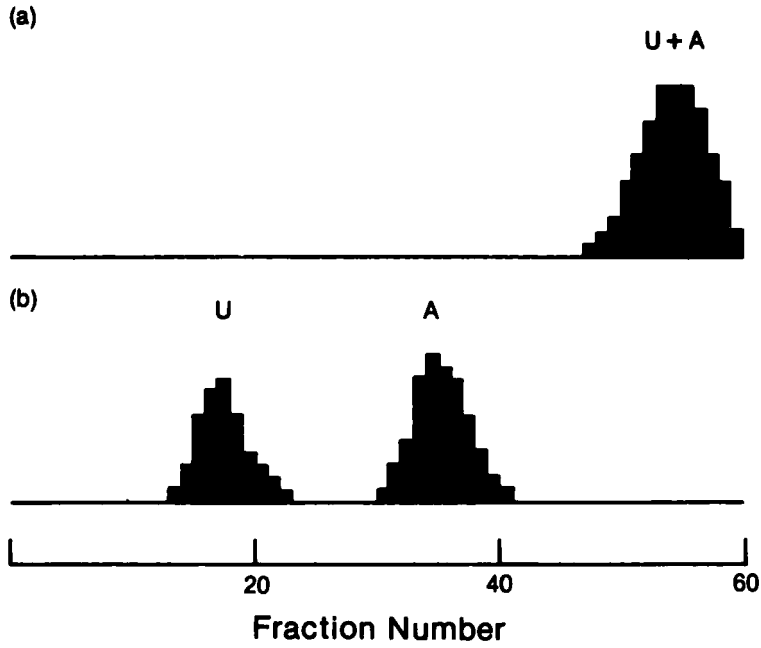

Figure 10

Histogram (a) shows the elution pattern of uridine (U) and adenosine (A) on a Sephadex G-25 column using an aqueous buffer solution. Histogram (b) shows the result of an analogous experiment when 0.02 g/ml sodium dodecanoate was added to the mobile phase.

added to the mobile phase, however, separation is achieved (Figure 10B). Uridine is eluted more quickly because it binds more strongly to SD micelles.[61,62]

Shortly after the micellar separation of tRNAs was reported,[60] Maley and Guarino found that basic and aromatic amino acids could be separated from acidic and neutral amino acids by chromatography on a 0.9 x 90 cm Sephadex G-25 column using an aqueous sodium dodecyl sulfate (SDS)-formic acid mobile phase.[63]

They speculated that different amino acids would bind different amounts of SDS therby forming aggregates of different size that could separate via classic size exclusion. Today, we know that this mechanism is improbable and that the separation was due to the partitioning of small solutes to large, roughly uniform micellar aggregates that existed only in the excluded volume of the column. Both the protonated-basic and hydrophobic amino acids eluted rapidly because they partitioned strongly to SDS micelles. Regardless, this remains one of the interesting early studies involving micellar mobile phases in chromatography.[63]

The effect of solute partitioning (between micellar and aqueous phases) during gel filtration on columns of Sephadex G-25 was first recognized by Herries et al. and used to calculate partition coefficients.[64] Their highly pertinent results will be considered in section III-D, on Theory. Unfortunately no consideration was given to the possible usefulness of micellar mobile phases for chromatography in general or GPC in particular. This was understandable at the time since the resolution one could obtain via the micellar-Sephadex G-25 technique was sufficiently poor as to make it of questionable use for most separations.

The early micellar-GPC work could be considered more of a novelty than a viable analytical technique.[60-64] However, it was useful in that it provided an explanation for a number of chromatographic anomalies. It also illustrated the potential of using micellar mobile phases to alter selectivity and provided a mechanism that would serve as a basis for future related techniques.

The development of a high performance GPC packing that has an exclusion limit of about one to two thousand and is compatible with aqueous micellar mobile phases would allow micellar-GPC to develop into a viable technique. Initial attempts to use commercially available silica gel based, or cross-linked polymer packings in this mode indicated that there were considerable problems with packing stability, adsorption effects and lack of sufficient interstitial volume.[65]

B. Thin Layer Chromatography

Micellar solutions have been used to separate a wide variety of compounds in thin layer chromatography (TLC). Indeed, many of the advantages and characteristics of these moblie phases that are currently cited in HPLC were first developed in TLC. Micellar mobile phases were first used in TLC as a means to eliminate the use of organic solvents, and their associated problems from the chromatographic environment.[8] At the time this was considered somewhat unusual, given the accepted dogma that organic solvents were necessary for separating water insoluble organic species. Several possible advantages of micellar mobile phases were cited such as: (a) greater safety, because flamable and/or toxic organic solvents were not needed; (b) ease of disposal since most of the surfactants used were biodegradable; (c) greater versatility, because one can add a variety of salts to control ionic strength, pH, buffer capacity, etc. without running into solubility problems; (d) relatively low costs; and (e)

greater selectivity, because the highly specific interaction of a solute with a micelle (which utilizes hydrophobic, electrostatic and interfacial surface interactions) cannot be duplicated by any traditional pure or mixed solvent system.[8,9,66-68]

Several cationic, anionic and nonionic micelles in aqueous solution were tested on a variety of planar stationary phases to see if they produced viable separations. It was found that micellar mobile phases were not compatible with paper, microcrystalline cellulose and silica gel stationary phases.[8,9,66-68] Streaks with little or no separation were produced on these media rather than compact, discrete spots. Likewise, nonionic micelles tended to produce streaks, even on stationary phases that were compatible with ionic micelles. The best results were obtained with ionic micelles (e.g. SDS, HTAC, HTAB, etc.) and polyamide stationary phases (produced by Brinkman or Baker).[8,9,66-68] Alumina and reversed phase supports were also used with some success.[8,9,66-68]

It became apparent from this work that one could not only alter the selectivity (i.e., the α or separation factor) of separations by changing the micelle concentration but also by changing the nature of the aggregate (e.g., surface charge). This is illustrated in Figure 11, where analogous separations of benzamide, 2-naphthol and biphenyl on identical polyamide stationary phases produce chromatograms of very different elution order and Rf's. The difference in the selectivity of these two separations is mainly due to the different charge of the micelles

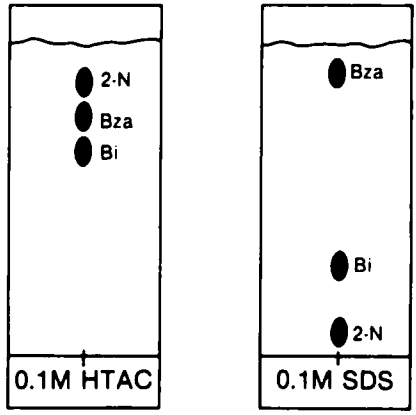

Figure 11

Two TLC separations which illustrate the effect of a micelle's charge on selectivity. Hexadecyltrimethylammonium chloride (HTAC) forms cationic micelles and sodium dodecyl sulfate (SDS) forms anionic micelles. The solutes 2-naphthol (2-N), benzamide (Bza) and biphenyl (Bi) were separated on identical polyamide plates.

in the mobile phase. While it is obvious that the charge of an ionic micelle will have a substantial effect on charged or ionizable solutes it also appears that micellar charge can have a profound effect on uncharged solutes.[44] Indeed, it has been suggested that while hydrophobic interactions are necessary to solubilize a variety of organic solutes, the selectivity is controlled by electrostatic and surface effects for any molecule with a dipole or dipole moment.[44] Spectroscopic studies tend to confirm this even for relatively hydrophobic, nonpolar solutes such as benzene.[43]

It was demonstrated that one could use micellar TLC to obtain partition coefficients and/or binding constants of solutes to

micelles.[44] The theory and mechanism were shown to be analogous to that for HPLC. Furthermore, one could substitute other compounds, such as cyclodextrins, for the micellar aggregate and achieve analogous results (see section VI).[44]

Sherma and co-workers separated amino acids on a variety of TLC plates with several different solvent systems.[69] They found that there was no reversal in the retention of most amino acids in going from normal to reversed phase TLC. Retention reversals were only observed when micellar mobile phases or surfactant impregnated stationary phases were used. This was thought to be due to an ion exchange mechanism.[69] These results indicated that simplified notions of "normal" and "reversed" phase chromatography were not always appropriate.[69]

Menger and Doll used micellar TLC to determine partition coefficients of fatty acids to SDS micelles.[70] The fatty acids could be visualized using a dilute $KMnO_4$ spray reagent after development on polyamide plates. This information was used in a more extensive report on the structure of the micelle.[70]

Stahr and Domoto used micellar solutions to separate a variety of mycotoxins.[71] Concentrated solutions of SDS were required for development but tended to deform the bands. Cyclodextrin mobile phases were used as well, and appeared to produce better separations for these compounds.[71]

The most extensive use of reversed micellar mobile phases has been in TLC (Table V). Several amino acids, nucleosides and quinones have been separated using hexane solutions of

dioctylsulfosuccinate (DOSS) and water.[8,9,72] Because of the viscosity of the solution, development times can take 12 to 48 hours. However, these separations often produce very small spot sizes and therefore high resolution.[8,9,67,72] Table V summarizes many of the separations that have been done via micellar TLC. Except in the case of slow developing reversed micellar systems, the efficiency of this technique (as indicated by spot size) tended to be less than that of more traditional TLC methods.

In a related technique, less viscous solutions of microemulsions can be used for more rapid separations.[73] This will be discussed in Section VI.

A series of TLC experiments commonly used in organic teaching laboratories have been altered so that micellar mobile phases could be used.[75] This allowed the elimination of many potentially hazardous organic solvents from the teaching laboratory. The net result was that students could run more separations safely and do them whenever the need arose.[75]

The possible advantages of micellar TLC have been discussed.[66-75] One should also be aware that there are some disadvantages to this method. First, there is a limited number of stationary phases (e.g., polyamide and alumina) that can be used with this technique. Visualization of the spots can sometimes be more difficult because of the presence of the surfactant. The capacity of a micellar mobile phase (to dissolve and carry a solute) is generally less than that of an organic solvent. Consequently, one must be careful not to spot too much material

TABLE V

Planar Separations Which Utilize Micellar Mobile Phases

Compound	Mobile Phase[a]	Stationary Phase	Ref
1. pesticides	(aq.) SDS or HTAB	PA or Al	1
2. polycyclic aromatic hydrocarbons	(aq.) SDS	PA	9
3. amino acids	(aq.) SDS or HTAC	RP	69
4. amino acids	(non aq. rev. micelle) DOSS	RP	9
5. nucleosides	(non aq. rev. micelle) DOSS	RP	8
6. substituted phenols, anilines and benzoic acids	(aq.) SDS or HTAC	PA, RP	67
7. substituted naphthalenes	(aq.) SDS	PA	74
8. food colors	(aq.) SDS	PA	75
9. indicators	(aq.) SDS	PA	75
10. caffeine and impurities	(aq.) SDS or HTAC	PA or Al	75
11. quinones	(aq.) SDS or HTAC	PA	72
12. thiols	(aq.) SDS or HTAC	PA	72
13. phthalimides	(aq.) SDS or HTAC	PA	72
14. mycotoxins	(aq.) SDS	PA, Al, RP	71
15. fatty acids	(aq.) SDS	PA	70

[a]The abbreviations in this column refer to type of surfactant which makes up the micelle in the mobil phase. Sodium dodecyl sulfate (SDS), hexadecyltrimethylammonium bromide (HTAB), hexadecyltrimethylammonium chloride (HTAC), and dioctylsulfosuccinate (DOSS).

[b]Polyamide (PA), alumina (Al), and reversed phase (RP).

which can cause streaking. Aqueous micellar mobile phases sometimes produce more diffuse spots than do traditional mobile phases.

C. Liquid Chromatography

To date, more micelle mediated separations have been reported by high performance liquid chromatography than by any other technique. A brief review of micellar or pseudophase liquid chromatography (in Japanese) was given by Saitoh.[76] The first example of a modern LC separation that utilized micellar mobile phases rather than organic solvents, demonstrated the effective resolution of nine phenols and two polynuclear aromatic hydrocarbons on a C_{18} reversed phase column (Figure 12).[77] It was

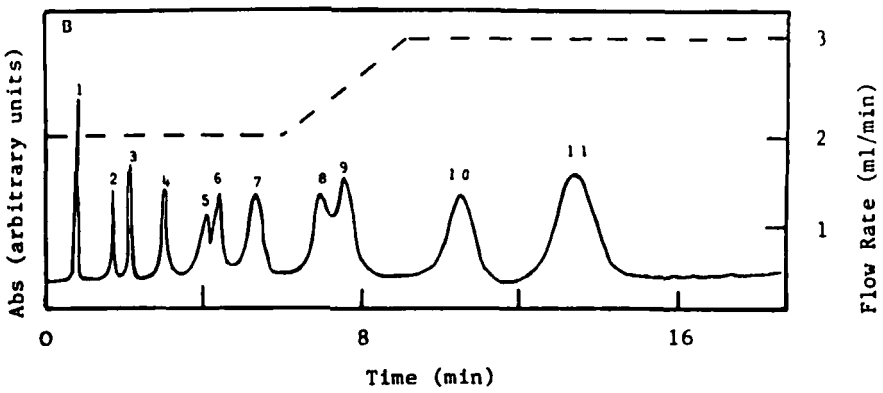

Figure 12

A C_{18} reversed phase LC separation of eleven compounds using a 0.2 M SDS (aq) mobile phase. A flow gradient was used and is indicated by the dotted line. The peaks are as follows: 1. picric acid, 2. hydroquinone, 3. resorcinol, 4. catechol, 5. phenol, 6. p-nitrophenol, 7. o-cresol, 8. o-isopropylphenol, 9. o-nitrophenol, 10. naphthalene, 11. anthracene. Reprinted with permission from ref. 77.

shown that hydrophobic, amphiphilic and hydrophilic molecules could be chromatographed at the same time with isocratic elution.[77] This was because the micelle could interact with a solute via both hydrophobic and electrostatic interactions. It was shown that varying the micelle concentration in the mobile phase affects the relative separation (α) of several solutes and that solute retention decreased when the micelle concentration was increased.[77] The possibility that micellar mobile phases might result in the improved detection of various compounds was noted for the first time.[77] Many of the advantages of micelles noted for planar chromatography (e.g., safety, versatility, etc.) also applied to LC. Likewise, many of the disadvantages of micellar mobile phases in TLC (section III-B) also applied to LC. Specifically the solution capacity of the micellar mobile phase is generally (but not always) less than that of equal volumes of many organic solvents. In preparative LC one must separate the final product from the surfactant by extraction, precipitation or some other technique.[75]

At the time the first reports concerning micellar chromatography appeared, a number of researchers were studying the mechanism of ion interaction chromatography in alcohol-water mixtures.[78-80] They noted that at higher concentrations of the ion interaction reagent (IIR), which was often a surfactant, a deviation from expected behavior occured. Plots of retention versus concentration of IIR produced plateaus or maxima. This was often attributed to the formation of micelles in the mobile

phase.[79,80] In some cases models were formulated to include micellar and ion interaction effects. While simple micelle formation was a convient way to explain the anomolous behavior of this highly useful technique, it is now apparent that this is a highly complicated hybrid system. Mobile phase modifiers such as methanol tend to alter and disrupt micelle formation (section II). Exactly what type of aggregational structures exist in surfactant-methanol-water mixtures is still open to question. Symmetrical ion interaction reagents such as tetrabutylammonium chloride will not form normal aqueous micelles but will tend to aggregate like reversed micelles in some organic solvents or solvent mixtures (section II-B). Added organic additives and modifiers can profoundly affect the partitioning and/or adsorption behavior of the stationary phase and bulk solvent through a variety of mechanisms.

The effect of small to moderate additions of organic modifier to a surfactant containing mobile phases was examined by Graham and Rogers.[79] The effect of surfactants below and above the CMC on the retention of nonionic solutes on a reversed phase column was discussed. They also noted the anomalous decrease in capacity factor at high surfactant concentrations. It was demonstarted that the surfactant adsorbed on the stationary phase did not significantly affect the retention of more hydrophobic solutes such as benzene or toluene, but did affect more hydrophilic, polar and/or charged compounds.[79] It was also shown that as the concentration of organic modifier was increased, the role of the

surfactant became less important. A simple model of adsorbed surfactant on the stationary phase was used to explain many of these observations.[79]

In 1981 several significant developments occurred in micellar LC. In addition to the separation of a greater variety of compounds, the three-phase model for micellar or pseudophase LC was proposed and supported by theory and experiment.[81] The use of micellar mobile phases for enhanced fluorescence and room temperature liquid phosphorescence detection was demonstrated.[82,83] It was shown that other additives could be added to water (such as cyclodextrins) and produce chromatographic behavior analogous to that of micellar mobile phases.[66,67] It was demonstrated that there were at least two effects which led to altered retention in micellar LC.[81] The first effect was due to adsorption of surfactant onto the stationary phase. This could affect retention by imparting on ion interaction capacity to the stationary phase (as discussed by other[1,2,78-80]) or by creating competing equilibria between the surfactant and solute for adsorption sites.[81] The second effect was a substantial decrease in solute retention in the presence of micelles as shown in Figure 13. Micellar LC theory was derived to explain and utilize the second, generally more dramatic of these effects.[81]

The three-phase model (Figure 14) allowed a theoretical description of micellar LC.[81] This and subsequent treatments are considered in section III-D. The useful thing about the three phase model is that one could substitute another pseudophase for the micelle (such as a cyclodextrin or crown ether) without

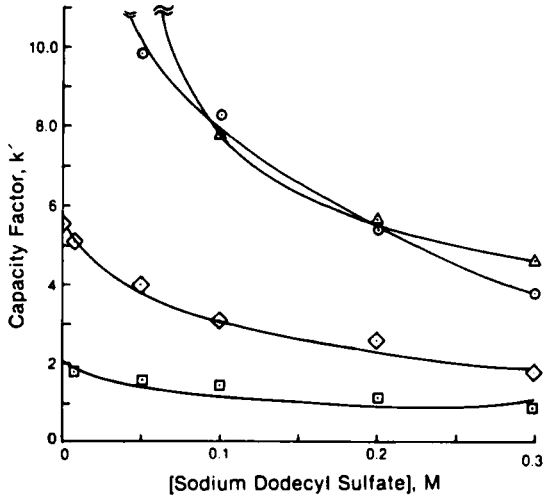

Figure 13

Plots of LC capacity factor k' vs surfactant concentration. Data taken from reference 81.

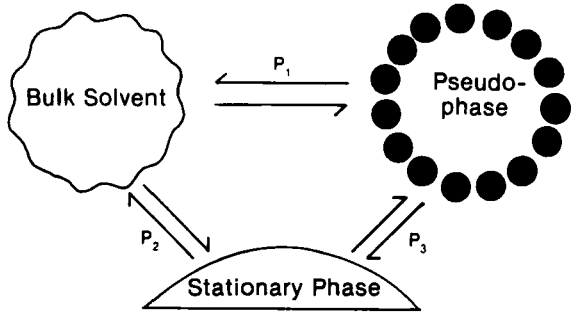

Figure 14

Representation of the origional three phase model which allowed a theoretical description of micellar or pseudophase LC.[81] "P" is the partition coefficient of a solute between the indicated phases.

appreciably changing the model or theoretical equations (section VI). In addition, one could use either a partitioning or binding approach to obtain related results, as was demonstrated in micellar kinetic studies (Figure 15).[84-86] One of the useful aspects of this work was that it allowed one to evaluate, chromatographically, partition coefficients and/or binding constants involving any or all phases.[81] It was also apparent that the separation factor changed with changing surfactant concentration, as it had in TLC.[81]

There is a significant amount of experimental support for the three phase or pseudophase model of micellar LC. First of all, plots of retention data fit the theoretical expressions (section III-D) for a variety of solutes which bind to micelles. Furthermore the experimental value of a solute partition coefficient or binding constant involving only micellar and

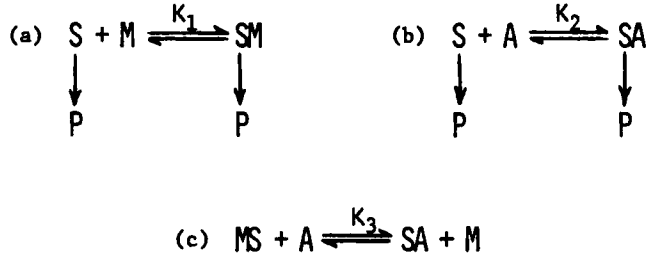

Figure 15

Typical kinetic reaction models which utilize binding constants (Ks) rather than related partition coefficients. Relationship (c) is adapted from classic adsorption chromatography. "S" represents a solute, "M" the Micelle, "A" a solid surface adsorption site, "MS" a micelle-solute complex, "SA" an adsorbed solute and "P" is the reaction product of "S".[21-27,84,148]

aqueous "phases" was shown to be independent of the stationary phase and/or chromatographic technique used.[44,81] Pelizzetti and co-workers later demonstrated that micellar binding constants or partition coefficients determined by LC (for over 20 different compounds) were in good agreement with those determined by other independent methods (e.g. spectral shift, kinetic studies, solubility studies, etc.).[87,88]

One factor that is frequently overlooked in micellar LC is that a number of assumptions have been made in deriving pseudophase retention equations.[81] For example, it is assumed that compounds that bind to a micelle will show decreased retention with increasing micelle concentration in the mobile phase, while compounds which do not bind to the micelle will show little change in retention.[81] It is further assumed that: the micelle-solute "complex" is of 1:1 stoichiometry; the stationary phase becomes saturated with surfactant at or below the CMC (or that different amounts of bound surfactant do not appreciably affect retention); the CMC, aggregation number and geometry of the micelle are not significantly altered by the presence of the chromatographed solutes; the aggregation number and geometry of the micelle does not change with increasing surfactant concentration; and so on.[81] In cases where these assumptions are not valid, one can find deviations from expected behavior. As will be shown at the end of this section, many of these "deviations" are not only interesting but also scientifically useful.

Shortly after the initial work on the three-phase model, Sybilska et. al. derived and experimentallly verified pseudophase retention equations for solutes that were weak to moderate strength acids and bases.[89,90] These equations (which will be discussed in section III-D) take into account the different binding of the ionized and unionized form of a solute to the pseudophase. By incorporating acid-base equilibria into the pseudophase retention equations Sybilska et al. could predict the dependence of retention, capacity factors and selectivity factors on both concentration of the pseudophase and pH.[89,90] Although these equations were originally used for a cyclodextrin pseudophase they are essentially identical for micellar mobile phases (section III-D).

Detection: It was originally thought that micellar mobile phases would have a beneficial effect on detection because these solutions were relatively incompressible compared to organic solutions.[77] It soon became apparent that there were additional benefits as well. It has been known for some time that micelles (as well as cyclodextrins) could alter the fluorescence behavior of a variety of compounds (e.g. by altering quantum yields, lifetimes, excitation and emission spectra, fluorescence depolarization and quenching effects).[14,17] It was also discovered by Turro et al.[91] and Thomas and co-workers[48,92] that the micelle could stabilize the triplet state of some molecules, therby allowing room temperature liquid phosphorescence (RTLP). The micelle also effectively separated solutes thereby preventing

triplet-triplet annihilation.[48,91-93] Hinze discussed the analytical possibilities of all of these results.[14,17] Shortly afterwards, the benefits of micellar enhanced fluorescence detection was demonstrated for a series of polynucleararomatic hydrocarbons (PAHs).[82,83] In the same report room temperature liquid phosphorescence (RTLP) detection was first demonstrated and several of the shortcomings and difficulties of the technique were mentioned.[82,83] Further work was done in RTLP detection by Weinberger et al., who studied the effects of surfactant concentration, addition of organic modifiers and postcolumn addition of micelles on the phosphorescence signal.[94] It was indicated that RTLP detection could improve one's selectivity. The detection limits of some PAHs were found to be as low as 5 ng, with linear dynamic ranges covering three orders of magnitude.[94] Vo-Dinh reviewed and evaluated much of the work concerning micellar effects on room temperature phosphorescence.[95]

A variety of nonchromatographic electroanalytical studies have been done in micellar solutions.[18,47,96-109] Micelles were shown to be useful in the voltammetry of a variety of water insoluble or weakly soluble compounds[96-109] and in stabilizing radical anions.[18,47] Recent studies have shown that LC-electrochemical detection (LCEC) is more compatible with gradient micellar LC than with traditional aqueous-organic gradients. Landy and Dorsey demonstrated the micellar mobile phases are uniquely well suited for repetitive gradient analyses.[110] Traditional gradient reversed phase LC (with

aqueous-organic solvent mixtures) requires a relatively long re-equilibration period (for the stationary phase) before the analysis can be repeated reproducibly. With ionic micellar mobile phases, the stationary phase often becomes quickly saturated with surfactant at relatively low mobile phase surfactant concentrations.[111,112] Above the CMC the surfactant exists as micelles and a small, relatively constant, concentration of monomers (section II-A). An increase in surfactant concentration (as in gradient elution) tends to increase the micelle concentration but does relatively little to the monomer or stationary phase concentrations. Consequently, little column re-equilibration is needed for repetitive analyses, resulting in a savings of time and solvent.[110,111] In addition the adsorption isotherm of SDS on reversed phase packing was measured and the role of organic modifier on the process was discussed.[111] Khaledi and Dorsey evaluated the parameters affecting baseline shifts in gradient elution LCEC.[113] They studied the shift in baseline caused by micellar concentration gradients at different potentials, pHs and ionic strengths. It was found that gradient induced shifts could be greatly reduced, particularly at high potentials, by balancing the pH and conductance of two micellar solutions.[113,114] It appears that the greater compatability of micellar gradients with electrochemical detectors is a significant advantage over traditional aqueous-organic mobile phases.

Micellar effects on UV-detection in LC have not been adequately considered. However, there are nonchromatographic

studies which indicate that micelles can affect the absorbance of certain compounds.[14,15,115,116] The interested reader is referred to these articles and the references therein.

Selectivity and efficiency are two parameters that have always been of paramount importance to the chromatographer. Micellar mobile phases have been known from the beginning to produce unusual selectivities for a variety of compounds as a result of their "multiple interaction ability" (section III-B). Conversely, micellar mobile phases have offered little or nothing for improved chromatographic efficiency. In fact, the efficiency of an LC separation is generally less when micellar mobile phases are used, as compared to traditional aqueous-organic solvent mixtures.

Selectivity in micellar LC is governed by the same principles that were outlined for planar methods (section III-B). Yarmchuk, et al. examined the retention behavior of several substituted aromatic compounds.[117] They also found that selectivity factors (α's) varied with surfactant concentration and charge. They confirmed (for HPLC) Maley and Guarino's report[63] that micellar mobile phases could effectively separate acidic (anionic), basic (cationic), and neutral compounds (section III-A); as well as reports by a variety of others on the ion interaction role of added surfactants.[78-81] It was also reported that repulsion from a micelle (of a similarly charged solute) should not affect retention and that nonpolar solutes such as benzene and toluene should be affected by hydrophobic but not by electrostatic

effects.[117] Previous and subsequent work has shown that electrostatic interactions can be as important for nonionizable compounds as for those that are able to ionize. Indeed, it has long been known that any molecule with a dipole moment can be profoundly affected by electrostatic interactions.[8,44] Even benzene has been shown to prefer interacting with the charge Stern layer of cationic micelles.[43] More recently it has been demonstrated that the repulsion of a solute from a micelle can result in very unusual selectivities.[118,119] In fact, the elution behavior is opposite to that of previously reported micellar separations in that retention increases with increasing surfactant concentration (Figure 16). This behavior is observed when one utilizes stationary phases which do not adsorb large quantities of surfactant (e.g., propyl nitrile or C_1 packings) and is thought to result from an excluded volume effect.[118,119] Essentially, a solute is forced onto the stationary phase when it is excluded from the mobile phase by the micelle and its double layer. It is apparent that in this situation, some of the simplifying assumptions used in the various pseudophase retention models[81,89,90] are violated. This becomes even more apparent when plotting retention data according to the model equations. Lines of negative slope are produced giving theoretically meaningless negative "coefficients."[118,119]. These "coefficients" may still be useful, however, since in some cases they can be correlated to the degree of electrostatic repulsion between a solute and micelle. This phenomena is very useful in terms of selectivity,

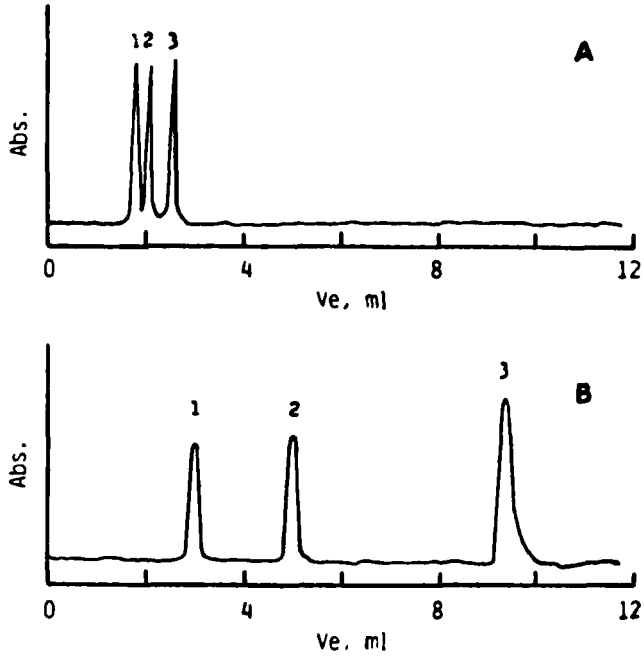

Figure 16

A comparison of the LC retention behavior of sodium nitroferricyanide (1), naphthol-6-sulfonic acid (2), and sodium naphthalenesulfonate (3), when eluted with a 0.025 M SDS mobile phase (A) and with a 0.4 M SDS mobile phase (B) on a 30 cm propylnitrile column. Note that the retention of all compounds increases when the micelle content of the mobile phase is increased. This is the opposite of normally reported chromatographic behavior. Reprinted with permission from ref. 118.

because increasing the micelle content of the mobile phase can cause decreased retention of some peaks and increased retention of others.[119]

Dorsey, et al. first studied efficiency enhancement in micellar LC.[120] Both the causes and remedies for lower efficiency

in micellar mediated separations were considered. It was thought that the lack of efficiency was due to poor mass transfer from the micelle to stationary phase.[120] Addition of low concentrations of organic modifiers and higher temperatures tended to enhance separation efficiency and return peak shapes to normal levels. This was thought to result from better wetting of the stationary phase by the modifier and a temperature induced lowering of the viscosity of the mobile phase.[120] It is apparent that this is a very complex hybrid system in which the organic modifier can increase partitioning to the bulk nonmicellar solvent, modify stationary phase surface interactions and alter the micelle structure. Yarmchuk, et al. also considered mass transfer effects on the efficiency of micellar LC.[121] They thought that the problem with mass transfer was a consequence of both slow micellar exit rates and slow desorption from the stationary phase.[121] The plate height, H, was shown to increase with increasing surfactant concentration. They also surmised, as had Graham and Rogers,[69] that the main effect of added organic modifier was to lessen the role of the added surfactant, thereby producing a system closer to traditional reversed phase chromatography. It was concluded that one should improve mass transfer by increasing temperature, reducing linear velocity and keeping the micelle concentration somewhat lower.[121] In a recent study, the diffusion coefficients and plate heights of several solutes were measured in the presence and absence of micelles.[122] Some of the solutes were known to bind strongly to the micelle while others were strongly repelled

from the micelle. The diffusion coefficients of strongly binding solutes approached that of the micelle, which is approximately ten times less than that of a small solute.[122] Solutes that did not bind to the micelle had much higher diffusion coefficients. Interestingly, both types of solutes (bound and repelled) showed decreased efficiency in micellar LC. This indicated that mass transfer from the stationary phase played a major role in the decreased efficiency of micellar LC.[122]

Borgerding and Hinze evaluated Brij-35 (a nonionic surfactant) micellar mobile phases for the reversed phase LC separation of aromatic alcohols, aldehydes, ketones and esters; as well as tobacco components.[123] Although elution seemed to follow the basic pseudophase retention expression,[81] there was some evidence that nonionic surfactants continue to adsorb onto the stationary phase in significant amounts at concentrations above the CMC. This is in contrast to what was reported for ionic surfactants by Hung and Taylor[112] and Dorsey, et al.,[111] and could possibly have an effect on a solute's partition coefficient or binding constant to the stationary phase. They also reported that addition of small amounts of ethanol or propanol (0-15%) to the mobile phase did not improve chromatographic efficiency.[123] Indeed, with 15% added ethanol, a decrease in efficiency was observed which seemed to result from the increased viscosity of the solution.[123] These and other experiments (involving the loading and stripping of Brij-35 from the stationary phase) seemed to indicate that the major cause of inefficiency is due to stationary phase mass transfer.[123]

Podcasy and Weber studied selectivity in micellar LC using the zwitterionic surfactant dodecyldimethylammonium propane sulfonate (sulfobetaine-12).[124] Micellar selectivity values (α's) were compared to those obtained with a traditional nonmicellar mobile phase consisting of 50:50, methanol:water. It was demonstrated that halogenated benzenes had a significantly greater selectivity for the micellar mobile phase. Furthermore, the order of selectivity was: iodobenzene > bromobenzene > chlorobenzene. However, the selectivity of the micellar mobile phase for substituted benzenes with polar substituents was not significantly greater than that of the methanol:water mobile phase. It was demonstrated that the selectivity of the micellar system tended to decrease as the concentration of surfactant increased. The reason for this was thought to be that the difference in polarity between the stationary phase and mobile phase tended to decrease as sulfobetaine-12 is added to the mobile phase.[124]

Kirkman[125] and Kirkman, et al.[126,127] studied the separation of ionic, neutral, chelated and organometallic metal species using a variety of micellar mobile phases and conditions. Because of the magnitude of this investigation only the highlights can be presented here. The interested reader is strongly referred to the origional references for further details of this impressive work. A variety of different surfactant micelles and bonded stationary phases were utilized. It was found that micellar LC could be used for separations that are commonly accomplished by ion exchange, normal phase and/or reversed phase LC, by simply selecting an

appropriate combination of surfactant type and bonded packing.[125] Micellar LC was used to separate both hydrophobic and hydrophilic compounds in a single determination. Kirkman and co-workers were the first to use plasma emission detection with micellar LC.[125-127] It was found that there was a surfactant induced enhancement of the emission signal in some cases and the mechanism of this enhancement was investigated. Both the separation and speciation of a variety of analytes were done via this technique. Micellar LC was used to fully resolve cis and facial trans isomers of cobalt(III) iminodiacetate anions.[127] In addition the separation of thermally labile isomers of aluminium trifluoroacetylacetonates was accomplished at 0°C.[125-127] Solute-micelle interactions were investigated using high resolution Fourier-Transform NMR.[125,126] Finally, the efficiency of micellar LC was investigated and compared to conventional reversed phase LC.[125]

Mullins and Kirkbright found that micellar solutions of HTAC could be useful in ion chromatography.[128] They successfully separated iodate, nitrite, bromide, nitrate and iodide. The effect of surfactant concentration and small amounts of organic modifier were also investigated.[128] It was found that the separation followed the three phase equilibrium theory proposed by Armstrong and Nome.[81] Detection limits (UV at 205 nm) ranged between 4 and 20 mg. An analysis of domestic tap water for nitrate and nitrite was also demonstrated.[128] There have been other reports on the use of surfactant solutions for the

separation of ions in which the surfactant concentration was sufficiently high for micellar aggregates to be present (although small amounts of organic modifiers were also present).[129,130] However, the possibility that an aggregational structure could be contributing to the separation of ions was not recognized or exploited.[129,130] Kirkbright and Mullins also reported the separation of five dithiocarbamates including N-methyldithiocarbamate (an insecticide) and disodium ethylenebisdithiocarbamate with HTAB micellar mobile phases.[131] The effect of ionic strength, surfactant concentration and added organic modifier were considered. Many of these separations were carried out with hybrid aqueous-organic-surfactant solutions in which the role and integrity of the micelle is not entirely clear.[131]

Barford and Sliwinski studied the LC separation of twelve proteins with buffered (pH 7) micellar mobile phases.[132] Unlike small solutes, proteins seemed to undergo an almost exponential change in retention for a given change in micelle concentration.[132] They found that proteins could be eluted near physiological pH using nonionic micellar solution but not with conventional alcoholic buffers. The proteins were classified by their elution behavior as low, intermediate and high retention species. Little or no correlation was found between protein retention and molecular weight, average hydrophobicity or surface charge.[132] Clearly, additional research is warranted on this interesting and potentially useful technique.

Arunyanart and Cline Love have modeled the pseudophase retention equation in the form of binding constants and capacity factors, rather than partition coefficients and elution volumes.[133] This work was analogous to Uekama, et al.[134] earlier treatment for cyclodextrin mobile phases. The relationship of these related approaches is discussed in the theory section, III-D. Other recent work (section III-D, Tables VI and VII) has demonstrated that the basic pseudophase retention equation can be similarly re-expressed in at least 39 related forms (which includes LC, TLC, and GPC). Although obtaining one form from another is mathematically trivial; there are certain statistical and other advantages in using different variations. This will be more fully discussed in section III-D on theory.

Mori studied the retention behavior of several catecholamines in cation exchange chromatography with micellar mobile phases.[135] He found it to be an effective technique which seemed to follow the traditional pseudophase retention equations.[135]

One aspect of micellar LC that hasn't been extensively explored is that of its "solvent strength". Dorsey et al.[111] briefly considered the "solvent strength" of 0.1 M SDS and CTAB. Both were found to be weaker reversed phase eluents than methanol:water (4:1). It was noted that shorter retention times could be achieved by using higher surfactant concentrations, gradients and shorter chain length bonded stationary phases.[111]

The applicability of micellar mobile phases is obviously growing. The advantages and limitations of this technique are

TABLE VI

Re-Expressions of the Pseudophase LC Retention Equation[a,b]

$$\frac{V_s}{V_e - V_m} = \frac{\nu(P_{mw} - 1)}{P_{sw} V_s} C + \frac{1}{P_{sw}} \quad (11)$$

$$\frac{V_s}{V_e - V_m} = \frac{V(P_{mw} - 1)}{P_{sw}} [M] \frac{1}{P_{sw}} \quad (12)$$

$$\frac{V_s}{V_e - V_m} = \frac{K_m}{K_s \phi [A]} [M] \frac{1}{K_s \phi [A]} \quad (13)$$

$$\frac{1}{V_e - V_m} = \frac{\nu(P_{mw} - 1)}{P_{sw} V_s} C + \frac{1}{P_{sw} V_s} \quad (14)$$

$$\frac{1}{V_e - V_m} = \frac{V(P_{mw} - 1)}{P_{sw} V_s} [M] + \frac{1}{P_{sw} V_s} \quad (15)$$

$$\frac{1}{V_e - V_m} = \frac{K_m}{V_s K_s \phi [A]} [M] + \frac{1}{V_s K_s \phi [A]} \quad (16)$$

$$\frac{1}{t_r - t_m} = \frac{\nu(P_{mw} - 1)}{P_{sw} V_s F} C + \frac{1}{P_{sw} V_s F} \quad (17)$$

$$\frac{1}{t_r - t_m} = \frac{V(P_{mw} - 1)}{P_{sw} V_s F} [M] + \frac{1}{P_{sw} V_s F} \quad (18)$$

$$\frac{1}{t_r - t_m} = \frac{K_m}{K_s V_s F \phi [A]} [M] + \frac{1}{K_s V_s F \phi [A]} \quad (19)$$

(continued)

TABLE VI., Continued

$$\frac{1}{k'} = \frac{\nu(P_{mw} - 1)}{P_{sw}\,\emptyset}\,C + \frac{1}{P_{sw}\,\emptyset} \qquad (20)$$

$$\frac{1}{k'} = \frac{V(P_{mw} - 1)}{P_{m}\,\emptyset}\,[M] + \frac{1}{P_{sw}\,\emptyset} \qquad (21)$$

$$\frac{1}{k'} = \frac{K_m}{K_s\,\emptyset[A]}\,[M] + \frac{1}{K_s\,\emptyset\,[A]} \qquad (22)$$

[a]Note that these equations can not only be used for micelle forming additives but for other species such as cyclodextrins, crown ethers, cryptands, etc. (11).

[b]V_s is the volume of the stationary phase, V_m is the volume of mobile phase, V_e is the elution volume of a solute, t_r is the retention time of a solute, t_m is the retention time of an unretained solute, k' is the capacity factor, P_{mw} is the partition coefficient of a solute between the micelle and water, P_{sw} is the partition coefficient of a solute between the stationary phase and water. K_m is the binding constant of a solute (aq) to a micelle (aq), K_s is the binding constant of a solute (aq) to the stationary phase (aq), ν is the partial specific volume of the surfactant in the micelle, V is the molar volume of the surfactant, $\emptyset$ is the phase ratio, F is the volume flow rate of the mobile phase, [A] is the concentration of stationary phase binding sites, C is the concentration of surfactant in the micelle in g/ml and [M] can either be the concentration of surfactant or micelles in the mobile phase in moles/liter.

becoming more apparent with time. The basic mechanism of separation is fairly well understood in some cases and there is a reasonable theoretical foundation (section III-D) on which to build. This is not to say that everything is clear cut. There is a healthy controversy over several aspects of micellar LC. The

TABLE VII

Re-Expressions of the Pseudophase TLC Retention Equation[a,b]

$$\frac{Rf}{1 - Rf} = \frac{\nu(P_{mw} - 1)}{P_{sw}\,\emptyset}\,C + \frac{1}{P_{sw}\,\emptyset} \qquad (23)$$

$$\frac{Rf}{1 - Rf} = \frac{V(P_{mw} - 1)}{P_{sw}\,\emptyset}\,[M] + \frac{1}{P_{sw}\,\emptyset} \qquad (24)$$

$$\frac{Rf}{1 - Rf} = \frac{K_m}{K_s\,[A]\,\emptyset^2}\,[M] + \frac{1}{K_s\,[A]\,\emptyset^2} \qquad (25)$$

[a]These equation can also be used for nonmicellar additives.

[b]All symbols are as defined in Table VI.

cause of lower efficiency seems to be mainly a stationary phase mass transfer problem, but the mobile phase may still be implicated in some cases. There is some question as to the role and usefulness of small amounts of organic modifier. The possible role of surface or interfacial tension needs to be examined in some cases.[136,137] Clearly, some of these differences result from the fact that different micellar systems and solutes are being examined. There can be significant differences between nonionic and ionic micelles as well as between different ionic aggregates (see section II-A). Furthermore, controversy on the structure and physicochemical properties of micelles is bound to affect the separations field as well. All of the theoretical models for pseudophase LC require several simplifying assumptions. In some

cases these assumptins are not justified and a deviation from "normal" elution behavior is seen. Typical examples of this include increased retention at higher micelle concentrations for repelled or excluded solutes,[118,119] variations in retention due to variable (e.g., non-Langmuir) adsorption of surfactant on the stationary phase,[123] and the unusual relationship between protein capacity factors and surfactant concentration.[132] As will be seen in the following section, there are reasonable explanations for some of these deviations.

D. Theory

Pseudophase or Micellar LC's theoretical origins extend back to the original work of Martin and Synge,[138] in which the driving force for separation was considered to be the equilibrium distribution of a solute between two phases. Even though this model allows one to draw reasonable conclusions about chromatographic retention and band broadening, it is now recognized that equilibrium (or quasi equilibrium) can be attained only at the band maximum and not at other points.[139] Most pseudophase theory attempts to explain retention (i.e., the location of the band maximum) in terms of the amount of a third phase present (e.g., the pseudophase which can be a micelle, cyclodextrin, etc.) plus the additional accompaning equilibria (Figure 14). The third phase is generally a well defined and controlled component of the mobile phase. Thus far, nonequilibrium or kinetic evaluations of micellar LC have

generally used the same approach proposed by Giddings[139] and van Deemter, et al.[140] for traditional chromatography.

In 1964, Herries, et al. devised a GPC method to determine the partition coefficients of compounds between micellar and aqueous phases.[64] These coefficients were needed to see if changes in reaction rates could be observed in systems where two reactants partition differently in a biphasic system. They defined a system in which the single partition coefficient of interest was that between aqueous and micellar phases and not between stationary and mobile phases.[64] Following the treatment of Martin and Synge,[138] they arrived at the following equation which describes the elution of a small solute on a Sephadex G-25 column with a micellar mobile phase:

$$\frac{V_i}{V_e - V_o} = \frac{\nu(P - 1)C}{k' K_d} + \frac{1}{k' K_d} \qquad (3)$$

Where V_i, V_o and V_e are imbibed (internal stationary phase), void and elution volumes respectively, ν is the partial specific volume of the detergent molecule in the micelle, K_d is the molecular sieving constant, k' is a proportionality constant, P is the partition coefficient of a solute between the micelle and water and C is the concentration of surfactant in the mobile phase.[64] More than a decade later, the first reports on micellar chromatography began to appear. One of these used a slightly modified version of equation 3 to describe the GPC separation and partitioning behavior of tRNA's (Section III-A):[60]

$$\frac{V_i}{V_e - V_o} = \frac{\nu Q(P - 1)C}{k' K_d} + \frac{1}{k' K_d} \qquad (4)$$

Where Q is the number of micelles in the external volume (V_o) divided by the total number of micelles in the column (in V_o + V_i). Both equations (3) and (4) reduce to the standard gel filtration equation in the absence of micelles as seen below.

$$V_e = V_o + P V_i \qquad (5)$$

The extension of partition theory to micellar HPLC required two additional steps. The first involved the formulation of the three-phase model with the three accompanying partition coefficients (Figure 14), and the second involved the derivation of an effective plate volume (V) term which takes into account all appropriate micellar parameters. The effective plate volume (V) is relatively simple and can be determined by inspection for traditional partition chromatography[138] and GPC[64] but is sufficiently complicated to require derivation in micellar LC.[81] Given the correct quantity for "V" one can easily complete the derivation and arrange the equation into the traditional format:

$$\frac{V_s}{V_e - V_m} = \frac{\nu(P_{mw} - 1)}{P_{sw}} C + \frac{1}{P_{sw}} \qquad (6)$$

where V_s, V_m and V_e are the volume of the stationary phase, mobile phase and elution respectively, P_{mw} is the partition coefficient

of a solute between micellar and aqueous phases and P_{sw} is that between stationary and aqueous phases.[81] The partition coefficient between micellar and stationary phases (P_{sm}) is given by the ratio of the other coefficients:

$$P_{sm} = \frac{P_{sw}}{P_{mw}} \tag{7}$$

Equation 6 can be derived so as to include any two of the three partition coefficients. When micelles are not present in the mobile phase, equation 6 reduces to the normal partition equation.[81] Berezin et al.,[84-86] Armstrong and Stine,[44,118,119] and Pelizzetti et al.[87,88] have indicated that obtaining binding constants (Ks) from partition coefficients (Ps) is a trivial process since:

$$K_{mw} = V(P_{mw} - 1) \tag{9}$$

where "V" is the molar volume.

Armstrong and Stine showed that the chromatographic mechanism was fundamentally the same in micellar and cyclodextrin TLC as for LC and derived a related pseudophase TLC retention equation:

$$\frac{R_f}{1 - R_f} = \frac{v(P_{mw} - 1)}{\emptyset P_{sw}} C + \frac{1}{\emptyset P_{sw}} \tag{10}$$

where R_f is the TLC retardation factor and $\emptyset$ is the phase ratio (i.e., V_s/V_m).[44] In order to use eq. (10) in TLC one must first

verify that the concentration of the pseudophase remains constant during development or make appropriate corrections if it does not.[44]

Arunyanart and Cline Love utilized a pseudophase retention equation based on capacity factors (k') and binding constants.[132] In fact, one can rearrange pseudophase retention equations into at least 39 related forms for LC, TLC and GPC. Typical examples are shown in Table VI for LC and Table VII for TLC. For a given technique, obtaining one equation from another is conceptually and mathematically trivial. For example, one can obtain any equation in Table VI, from another, by making one or more of the following algebraic substitutions: $1/k' = V_m/(V_e-V_m)$, $1/\emptyset = V_m/V_s$, $V_e-V_m = F(tr-tm)$, $P_{sw} = K_s\,[A]$, and $K_m = (P_{mw}-1)$ and rearranging. All symbols are defined in Table VI and all of the above relationships are well known in the chromatographic literature. Proponents of this type of statistical re-expression claim that such excercises are worthwhile if the purpose is to put the equation into a form which is more convenient, yields more data, different data, more accurate data, etc.[141,142] In the case of the pseudophase retention equations for LC there are different advantages in plotting different equations. For example, values of P_{sw} are most easily obtained from plots of equations 11 and 12. One can avoid measuring ν or V by using equations 13, 16, 17, or 22. Equations 20-22 generally produce less accurate values of P or K. This is because the greatest relative error generally involves measurement of the void volume (V_m). Reported V_m values (which are technique

dependent) can vary 40% or more. In using capacity factors [$k'=(V_e-V_m)/V_m$], the relative error of V_m enters into the expression twice and in the same direction. Consequently the relative uncertainties in the left side of the various equations in Table VI can be different. For solutes with short retention times, the relative uncertainties in the various forms of the pseudophase retention equations are: $V_s/(V_e - V_m) < 1/(V_e - V_m) = 1/(tr - tm) < 1/k'$. For solutes with long retention times the relative uncertainties are: $1/(V_e - V_m) = 1/(tr - tm) < V_s/(V_e - V_m) < 1/k'$. In the former case, $V_s/(V_e - V_m)$ has the lowest relative uncertainty only if the volume of the stationary phase (V_s) is determined by subtracting V_m from the total column volume. This means that the relative uncertainity in V_s would be about the same and in the opposite direction to that of V_m, and therefore cancel. If V_s is determined independently, then plots of $1/(V_e - V_m)$ or $1/(tr - tm)$ would have the lowest relative uncertainty for both strongly and weakly retained solutes.

One of the useful aspects of the pseudophase retention equations is that they can be used to experimentally determine partition coefficients and/or binding constants. All forms of the equation are plotted in the same manner shown in Figure 17. Plotting the left hand side of any pseudophase retention equation versus surfactant concentration in the micelle (C) should give a straight line of positive slope (for solutes and pseudophases that adhere to the theoretical assumptions, section III-C). From the slope and intercept one can calculate any P or K value. Solutes

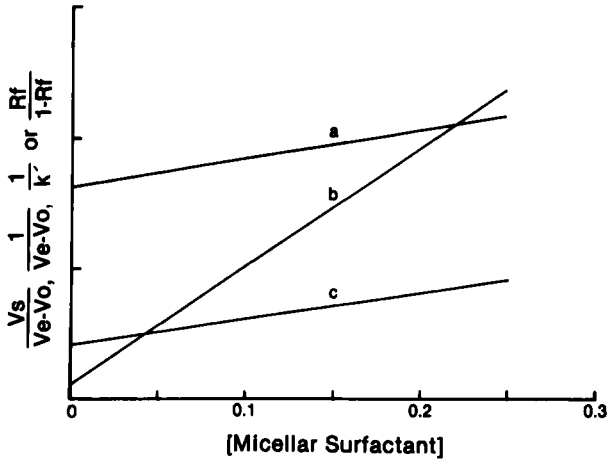

Figure 17

Typical plots of the pseudophase retention equations. One can calculate all pertinent binding constants and partition coefficients from the slopes and intercepts of plots such as these. Note, the crossing of one line by another (as in "b") indicates a change in retention order.

which deviate from this behavior will be discussed at the end of this section. If one plots the total amount of surfactant over a very wide range of concentration one obtains plots such as those shown in Figure 18. The first "break" in the curve at low surfactant concentrations occurs at the CMC. The leveling of the curve at high surfactant concentrations occurs when the solute is eluted near the void volume. If there are ion interaction effects below the CMC, the slope of this portion of the curve is negative.

The V_m-Intercept Paradox. An apparent way to minimize the effect of the large uncertainity of V_m is to adjust the chromatographic parameters so that large V_e values (which display significantly smaller bounds of uncertainty) are obtained. Indeed

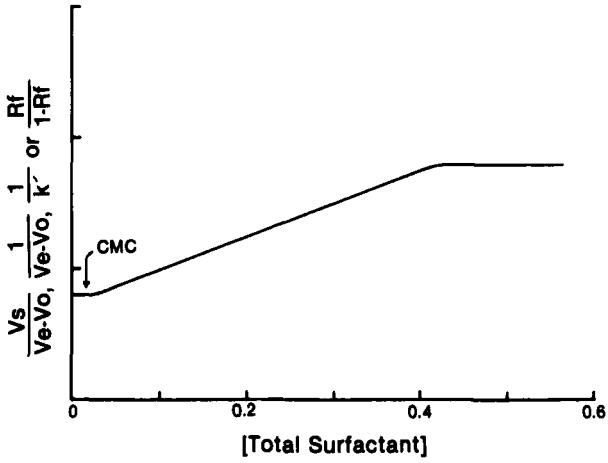

Figure 18

A plot of the pseudophase retention equation versus total surfactant concentration over a wider range of concentrations. Below the CMC there will be little change in retention unless there are ion interaction effects. At very high surfactant concentrations a solute can elute near the void volume and further increases in surfactant concentration will not lower retention.

as V_e increases the relative uncertainty in $V_e - V_m$ decreases. The only problem with this approach is that plots of any pseudophase equation for high retention systems generally produce lines with greater slopes as well as intercepts near zero. As was noted previously, this results in an increase in the relative error of the intercept.[81] Consequently, attempts to minimize the impact of the uncertainity of V_m by experimentally maximizing V_e will eventually result in a greater relative uncertainity in the intercept and vice versa.

Shortly after the first pseudophase retention equation for LC appeared,[81] Sybilska and co-workers derived expressions that

accounted for pH effects on the retention of weak acids and bases.[89,90] To accomplish this they had to include the binding constants of both the ionized and unionized forms of a solute as well as the K_a or K_b of the solute in the pseudophase retention expression. The result of their derivation was reported using both retention times (Eq. 26)[89] and capacity factors (Eq. 27):[90]

$$t_{obs} = \frac{t_1 + t_2Ka/[H^+] + t_3K_1[C\} + t_4K_2[C]Ka/[H^+]}{1 + Ka/[H^+] + K_1[C] + K_2[C]Ka/[H^+]} \quad (26)$$

$$k_{obs} = \frac{k_1 + k_2Ka/[H^+] + k_3K_1[C] + k_4K_2[C]Ka/[H^+]}{1 + Ka/[H^+] + K_1[C] + K_2[C]Ka/[H^+]} \quad (27)$$

where t_{obs} is the observed retention time; t_1, t_2, t_3, and t_4 are the retention times of the unionized ionized, complexed-unionized, and complexed-ionized solutes respectively; Ka is the acidity constant of the solute; K_1 and K_2 are the respective binding constants of unionized and ionized solutes to the pseudophase; $[H^+]$ is the hydrogen ion concentration; k'_{obs} is the observed capacity factor; and k'_1, k'_2, k'_3, and k'_4 are capacity factors which correspond to the above retention times. Sybilska et al. tested their equation using a cyclodextrin pseudophase.[89,90] They discussed the influence of added salt as well as pH. Three dimensional plots of α vs. pseudophase concentration and pH were made which illustrate the usefullness of equations (26) and (27) in optimizing separations. As in the case of micellar mobile phases, this method was found to be less efficient than conventional reversed phase LC.[89,90]

As previously mentioned, deviations from pseudophase retention theory can occur if any of the underlying assumptions are not valid. Two typical deviations from ideal theory are shown in Figure 19. In the first case (plot A) one obtains a line of negative slope. This occurs when the retention of a solute increases with micelle or pseudophase concentration (which is the opposite of expected behavior). Even more disconcerting is the fact that one obtains negative P and K values from such plots, which are theoretically impossible. Armstrong and Stine have discussed this phenomenum.[118,119] It seems to occur when a solute

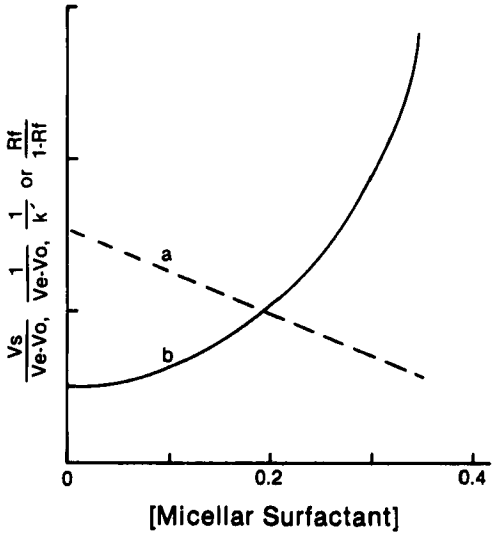

Figure 19

Plots of the pseudophase retention equation which illustrate two types of deviation from ideal behavior. Plots such as that in curve "a" can occur when a solute is strongly excluded from the pseudophase. Plots such as that in curve "b" can occur when the pseudophase-solute stoichiometry is not 1 to 1.

is strongly repelled from the micelle (as in a like charged solute) and when one is using a stationary phase that doesn't adsorb a large amount of surfactant. The solute is apparently excluded from the mobile phase by the micelle and forced onto the stationary phase.[118,119] This behavior, which is sometimes referred to as "antibinding", obviously violates a basic assumption of pseudophase theory (i.e., that the solute binds to, or is unaffected by the micelle). Menger and Dulaney found that "antibinding" behavior was not limited to aqueous micellar mobile phases.[143] The apparent excluded volume effect was observed with heptane solutions of derivatized β-cyclodextrin. TLC studies showed that 1,2-dimethylindole exhibited "antibinding" behavior while indole, 2,3-dimethylindole and p-nitrophenol exhibited binding behavior. Nonbinding was observed with o-nitrophenol in heptane and p-nitrophenol in acetonitrile. These results were used to study the nature of the host-guest interaction and demonstrated that binding could occur when the cyclodextrin cavity is more polar than the solvent.[143] Plot B of Figure 19 shows an entirely different type of deviation from ideal behavior. Instead of a straight line of positive slope, one obtains a curve of increasing slope. This behavior was observed by Barford and Sliwinski[132] for proteins and by Armstrong, et al. for both macromolecules (with micellar mobile phases) and small solutes (with cyclodextrin mobile phases).[144] Armstrong et al. have shown that upward curving plots (Figure 19B) can occur when the stoichiometry between the pseudophase and solute is 2:1 or

greater.[144] It is well known that some solutes can bind to two cyclodextrin molecules at the same time, for example. Analogous multiple equilibria can occur in micellar systems. For 2:1 complexes the correct pseudophase retention equation is:

$$\frac{1}{k'} \quad \text{or} \quad \frac{R_f}{1-R_f} = \frac{1}{K[A]\emptyset} + \frac{K_1 C}{K[A]\emptyset} + \frac{K_1 K_2 C^2}{K[A]\emptyset} \qquad (28)$$

where K, K_1 and K_2 are the binding constants to the stationary phase, first pseudophase, and second pseudophase respectively. When K_2 is zero, this equation reduces to the usual pseudophase retention equation (Tables VI and VII). This equation can be rearranged and plotted so that all binding constants can be calculated.[144]

Van Deemeter plots have been published by Yarmchuk et al.[121] and Borgerding and Hinze[123] for solutes separated with micellar mobile phases. Minima appear at linear velocities between 0.1 and 0.4 cm/sec. Borgerding and Hinze point out that according to previously derived rate equations,[145-148] the stationary phase mass transfer contribution is approximated by the slope of the van Deemter flow-efficiency curve at the higher linear velocities. They showed that increasing the coating of Brij-35 surfactant on the stationary phase dramatically decreased the efficiency of these separations.[123]

IV. EXTRACTION AND PARTITIONING

Micelles have been found to be useful in several different partitioning and extraction techniques all of which are akin to

liquid-liquid extractions. Four techniques will be discussed. The first involves the addition of micelles to the classic octanol-water partition system, the second method uses micellar solutions in liquid-solid extractions, the third involves the partitioning of solutes between nonionic micelles and water followed by a rapid concentration-isolation step, and the fourth technique involves the use of reversed micelles in a variety of organic solvent extractants.

Janini and Attari added SDS micelles to the aqueous phase of an octanol:water partitioning experiment.[149] They were particularly interested in the octanol:water system because of its widespread use as an empirical measure of hydrophobicity for biological and pharmacological compounds,[150,151] as well as in a number of other systems.[152-154] The addition of micelles to the aqueous layer allows one to treat this like a three phase system just as had been done in micellar LC.[81] A significant advantage of this system is that higher levels (which can be more accurately measured) of hydrophobic solutes can be found in the aqueous-micellar layer than can be found in a pure aqueous layer. The disadvantage is that one must be more careful of emulsion formation, and aggregates in the octanol layer can complicate the interpretation of data. Janini and Attari derived the following expression for the three phase partitioning system:

$$P_{app} = \frac{\nu(P_{mw} - 1)}{P_{ow}} C + \frac{1}{P_{ow}} \qquad (29)$$

where P_{app} is the apparent partition coefficient of a solute between the octanol and aqueous-micellar layer, P_{mw} and P_{ow} are the respective partition coefficients between the micelle and water, and octanol and water, ν is the partial specific volume of the surfactant and C is the concentration of surfactant in the micelles.[149] The distribution of resorcinol, catechol and hydroquinone was shown to follow eq. 29 and the relationship between this technique and micellar LC was discussed.[149]

Borgerding and Hinze used a 30% aqueous micellar soluton of Brij-35 to extract vanillin and ethylvanillin from tobacco leaves.[123] These solutes can then be easily quantitated by reversed phase LC with a 6% Brij-35 micellar mobile phase and UV detection. The precision of this technique, determined from replicate analyses, was excellent.[123]

Watanabe and co-workers developed a simple, efficient micellar extraction and concentration technique for a variety of metal chelates, nonpolar compounds and ion pairs.[155-162] This method requires the use of nonionic micellar solutions which are known to undergo phase separations when heated above their cloud point (see section II-A). In a typical experiment, 0.5 g of solution containing 20% of an appropriate nonionic surfactant plus chelating ligand is added to approximately 80 ml of a buffered sample containing the metal ion to be extracted.[160] The mixture is warmed until the cloud point is exceeded and centrifuged for one minute. The supernatant liquid is decanted leaving about one gram of material containing the chelated trace metal. This

material was adjusted to 2.00 ml of solution by addition of water plus a small amount of another nonionic surfactant which has a high cloud point. The concentration of metal ion (having been concentrated 40x) is then determined spectrophotometrically or by another appropriate technique.[160]

Watanabe, et al. did early experiments with Triton X-100 (cloud point > 64°C).[155-157] More recent experiments utilized polyoxyethylene nonyl phenyl ether with an average of 7.5 ethylene oxide units (PONPE-7.5).[158-162] PONPE-7.5 has a cloud point below room temperature at concentrations used for extraction.[160] The percent recovery of any extracted species is dependent on several factors including pH, foreign ions, type of surfactant additive, etc.[160,161] The partition theory including pH effects on ionizable solutes have been considered by Hoshino et al.[161] The distribution of a solute that can either gain or lose two protons is given by equation (30):

$$D = \frac{P_1 [H^+]/K_{a1} + P_2 + P_2P_3[H^+]}{1 + [H^+]/K_{a1} + K_{a2}/[H^+]} \qquad (30)$$

where D is the distribution coefficient; P_1, P_2 and P_3 are the partition coefficients of the postively charged doubly protonated species, neutral species and negatively charged species respectively; $[H^+]$ is the hydrogen ion concentration; and K_{a1} and K_{a2} are the acid dissociation constants of a diprotic solute.[161] This work and its experimental justification provide a quantitative explanation for the solubilization and equilibrium

behavior of ionizable solutes in nonionic micellar solutions. Watanabe and co-workers demonstarted the utility of this extraction method for several solutes including Mn, Zn, Cu and Ni chelates, I_2, $AsCl_2$, SnI_4, and a variety of thiocyanate complexes.[155-162] This technique was also used to determine Zn^{2+} in coastal seawater at the ppb level.[162]

Hinze and co-workers first used the nonionic micellar cloud point extraction technique to concentrate and quantitate polycyclic aromatic hydrocarbons (PAHs) and other organic solutes.[163] This method was particularly useful for dilute aqueous environmental samples because advantage can be taken of both the concentration factor and the enhanced luminescence of the species in micellar environments.[163]

A somewhat different liquid-liquid extraction technique involves the use of highly lipophilic surfactants dissolved in an organic solvent, to remove and concentrate a variety of ions from aqueous solution. This procedure was reviewed by Hinze[17] and is summarized below. Typical surfactants used were dinonylnaphthalene or didodecylnaphthalene sulfonates as well as alkylammonium surfactants. Both metal ions and complex ions can be extracted into organic solution. The main question in this system concerns the role the aggregate or reversed micelle plays in the separation. The majority of the evidence seems to indicate that the monomer surfactant is responsible for moving the aqueous ion across the interface, and into the organic layer.[17] Reversed micellar aggregates also exist in the organic layer (section

II-B). These aggregates can serve as a repository for the extracted ions. Undoubtedly the quantity and quality of ions residing in the reversed micelle will affect the selectivity and usefulness of the extraction.[17] This type of extraction is, mechanistically speaking, a good deal more complicated than other systems. There is also a considerable amount of controversy as to the exact role of the surfactants and/or surfactant aggregates. There are cases where aggregation does not seem to occur or when it occurs, seems to play little role in the separation process.[17] The mechanistic aspects of this technique remain one of the "gray areas" referred to in the introduction where the exact role and sometimes existence of the aggregate is not always clear. Despite the fact that the mechanism and theory of this method are not straightforward, there are numerous reports and reviews on practical applications.[17,164-166] The interested reader is referred to these for additional information.

V. ADDITIONAL MICELLAR METHODS

One of the more interesting separation techniques yet reported is a capillary electrokinetic method that utilizes micellar solutions. Terabe et al. described a number of chromatography-like separations that showed excellent selectivity and efficiency (see Figure 20).[167] Sixteen different phenols were separated in less than twenty minutes. Depending on the peak used for measurement, this technique generated between 300,000 and 500,000 plates per meter, with no back pressure problem or long

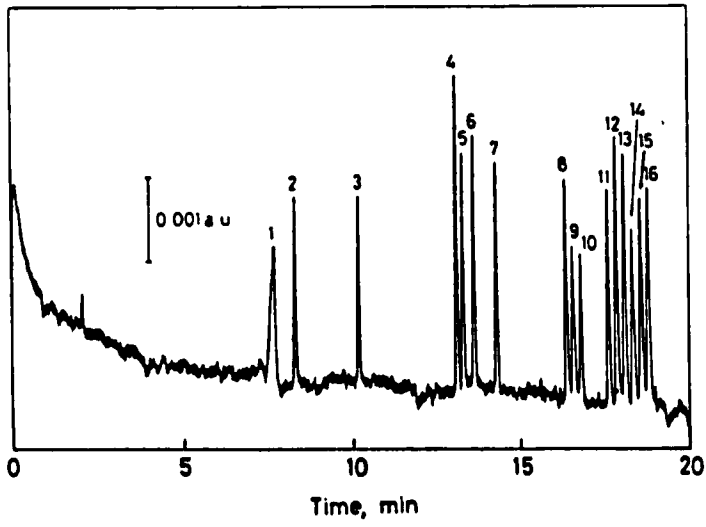

Figure 20

An electrokinetic separation of phenols with an SDS solution: (1) water, (2) acetylacetone, (3) phenol, (4) o-cresol, (5) m-cresol, (6) p-cresol, (7) o-chlorophenol, (8) m-chlorophenol, (9) p-chlorophenol, (10) 2,6-xylenol, (11) 2,3-xylenol, (12) 2,5-xylenol, (13) 3,4-xylenol, (14) 3,5-xylenol, (15) 2,4-xylenol, (16) p-ethylphenol; micellar solution, 1 mmol of SDS in 20 ml of borate-phosphate buffer, pH 7.0; current, 28 μA; detection wavelength, 270 nm; temperature, ca. 25°C. Reprinted with permission from ref. 167.

separation times. In a typical experiment, 900 mm X 0.05 mm i.d. microbore vitreous silica tubing was filled with a buffered SDS solution. Each end of the capillary was placed in a small beaker containing the surfactant solution and a platinum electrode. The sample to be separated is injected into the positive end of the tube and a voltage (25 kV) is applied across the tube. An on-line UV detector was placed 150 mm from the negative end of the tube. When anionic micelles were used the solutes migrated from the

positive to the negative end of the capillary. The opposite pattern was observed for cationic micelles.[167] The electrophoretic migration of the micelles is opposed by a more rapid electroosmotic flow of the solvent. Solutes that do not partition to the micelle would be expected to travel at the same velocity as the solvent and elute rapidly. Solutes which tend to bind to the micelles (which travel in the opposite direction of the solvent) will be eluted more slowly.[167] This is a beautiful example of a laminar "microscopic countercurrent" technique which results in high efficiencies. In subsequent work Otsuka et al.[168] used the electrokinetic-micellar method to separate a mixture of 22 phenylthiohydantoin (PTH)-amino acids. The separation behavior of these solutes was strongly dependent on the charge of the micelle.

Theoretical equations for "electrokinetic chromatography" were recently derived by Terabe, Otsuka and Ando.[169] They also observed that there was a linear relationship between current and migration velocities of water, micelle and any solute, but not between applied voltage and these velocities. This discrepancy was explained in terms of the temperature rise of the solution in the tube resulting from Joule heating.[169] The velocity of the micelle (v_m) in a capillary electrokinetic experiment is:

$$v_m = v_{eo} + v_{ep} \qquad (31)$$

where v_{eo} is the electroosmotic velocity of the micelle and v_{ep} is

the electrophoretic velocity of the micelle. Furthermore:

$$v_{eo} = \frac{6 \epsilon \zeta \ell}{r^2 eF} \sum_j \frac{a_j}{C_j Z_j^2} I \qquad (32)$$

and

$$v_{ep} = \frac{2 \epsilon \zeta}{3 \eta} f(\kappa a) E \qquad (33)$$

where ϵ is the permittivity of the liquid, ζ is the zeta potential, η is the viscosity of the liquid, ℓ is the length of the tube, r is the tube radius, e is the elementary charge, F is the Faraday constant, a_j is the radius of the solute ion "j", c_j is the number of moles per unit volume of solute "j", Z_j is the charge of solute "j", I is the current due to transport of charge by the fluid, κ is the Debye-Hu parameter where the function $f(\kappa a)$ depends on the particle shape and E is the electric field strength.[169]

The capacity factor for "electrokinetic chromatography" (k') is defined as the ratio of the amount of solute in the micellar phase over the amount in the bulk aqueous phase.[158] Note that this is different from the traditional chromatographic definition of capacity factor. The capacity factor, k', can be calculated from retention times by:

$$k' = \frac{t_r - t_o}{t_o(1 - t_r/t_{mc})} \qquad (34)$$

where t_r is the retention time of a solute, t_o is the retention time of a solute that does not associate with the micelle and t_m is the retention time of the micelle.[169] Given this capacity factor, all of the other traditional chromatographic parameters (e.g., resolution, plate number, partition coefficient, etc.) are easily determined.

Recently Terabe and co-workers have done "electrokinetic chromatography" using an ionizable β-cyclodextrin derivative.[170] The separation of several aromatic structural isomers was demonstrated. The retention parameters and distribution coefficients were discussed.[170]

Micellar solutions have been used by Patel and Foss to alter the partitioning behavior in equilibrium dialysis.[171] Ikeda and co-workers used micellar solutions in the dynamic dialysis of tetracycline antibiotics.[172,173] Using a Langmuir approach and appropriate acidity constants, they were able to relate the partition law to the amount of surfactant present and the pH of the system.[173] They found that the apparent partition coefficient (P_{app}) of a drug between the micelle and water was given by:

$$P_{app} = \frac{D_m/v}{D_w/(1 - v)} \qquad (35)$$

where v is the volume fraction of the micellar phase and D_m and D_w are the amount of the drug in the micelle and bulk water respectively.[173] The effect of pH on the apparent distribution of drug between micellar and aqueous phases is given by:

$$P_{app} = \frac{P_c[H^+] + K_a P_z}{[H^+] + K_a} \quad (36)$$

where K_a is the acid dissociation constant of the drug, $[H^+]$ is the hydrogen ion concentration and P_c and P_z are the partition coefficients of the protonated and zwitterionic species, respectively.[173] Dynamic dialysis experiments were done with cationic, anionic and nonionic micelles. The results were compared to octanol-water partition experiments. It was demonstrated that the ionized form of a drug associated more strongly with nonionic micelles than the neutral form. It was further noted that the pH dependency of the tetracycline-nonionic micelle interactions did not correlate with that of comparable octanol-water studies.[173]

IV. ANALOGOUS TECHNIQUES

Surfactant micelles are not the only thing one can add to a pure solvent, thereby creating an unusual mobile phase or solution for separtions. Cyclodextrins, for example, have already been mentioned because the same three phase model and theory used to describe micellar mobile phases can be used for solutions of these cycloamyloses. This was amply demonstrated by Vekama et al.,[134] Armstrong, Stine,[44] Sybilska et al.[89,90] (section III-D), Menger and Dulaney[144] and Terabe et al.[170] Cyclodextrin mobile phases tend to show greater selectivity towards structural and geometrical isomers than do micellar mobile phases.[66,174,175] This is because of their ability to form inclusion complexes with a

variety of solutes.[176-181] One advantage cyclodextrin mobile phase have over micellar mobile phases is that their UV cut off is less than 200 nm, which is as good or better than most HPLC-grade water. A disadvantage of cyclodextrins is that they are more expensive than most surfactants. Cyclodextrin mobile phases are but one aspect of the many uses of cyclodextrins in separations. Since it is beyond the scope of this work to consider all aspects of cyclodextrins mediated separations, the interested reader is referred to an excellent review on the subject by Hinze.[179]

Certain polymers can be treated as an additional phase when used as mobile phase or solution modifiers. Two examples will be given. Dasgupta and co-workers used polystyrenesulfonate to modify permitted and Donnan forbidden ion penetration rates through a variety of small diameter ion exchange membrane tubes.[182] Like micelles, ionic polymers behave as liquid ion exchangers, thereby reducing the concentrations of free counterions that can traverse a membrane.[182]

Hinze and co-workers have used a variety of linear dextran polymers as chromatographic mobile phase modifiers.[163] They found that the effect of the polymer on chromatographic retention was somewhat similar to that of cyclodextrins. They postulated that the dextran was able to wrap around a variety of organic solutes, providing an environment similar to that of a cyclodextrin.[163] In a somewhat analogous manner, polyethyleneglycol is known to be able to wrap around and complex various metal ions. It is likely, therefore, that they could affect the retention of appropriate

solutes in a manner similar to that of crown ethers or cryptands (vide infra).

Crown ethers and cryptands have been used as mobile phase modifiers to separate solutes containing primary amines and some metal ions.[183-188] Ammonium ion has about the same ionic radius as potassium ion. Both bind strongly to the cavity of 18-crown-6 based crown ethers. Cram and co-workers synthesized a chiral 18-crown-6 analogue which was added to the mobile phase to facilitate the separation of enantiomers of amino acids and amino acid esters.[183] Wiechmann separated groups of biogenic amines using a crown ether containing mobile phase.[184] Nakagawa and co-workers did a considerable amount of work on 18-crown-6 modified mobile phases.[185-188] They derived formulas which accounted for observed pH effects on retention.[185,187] The effectiveness of this technique was demonstrated for β-lactam antibiotics,[186] catecholamines, 187 and alkali metals.[188] Most of these LC separations were done in the reversed phase mode.

Microemulsions can also be used as mobile phases in liquid chromatography.[73] Armstrong and Ward used oil-in-water and water-in-oil microemulsions to separate a series of functionalized aromatic hydrocarbons by LC and TLC. Binding constants or partition coefficients could be calculated for these solutes as well.[73]

VII. CONCLUSIONS

Micelles have been used in a wide variety of separation techniques. In some cases, such as chromatography, a considerable

amount of practical and theoretical work has been done. In others, such as capillary electrokinetic separations, the research is just beginning. The "additional phase" or "pseudophase" concept developed for micellar systems can also be used for a variety of other techniques thereby uniting them through a common theoretical approach. However, deviations from basic theory are known to occur when one or more of the necessary simplifying assumptions are not valid. Theoretical limitations can also result from our incomplete and evolving knowledge of the properties and structure of the micelle. The advantages and disadvantages of micelles in separations are becoming clearer with time. It is doubtful that they will ever replace traditional techniques on a large scale. However, micelles and conceptually related modifiers, will undoubtedly play an important, more specialized role in separations which demand the unique characteristics of these systems. One point that is rarely considered is the impact this work can have outside the field of separations. Scientists interested in colloids, kinetics, catalysis, emulsion polymerization, tertiary oil recovery, enzyme or membrane modeling, and so on, can use many of the aforementioned techniques to study and characterize their systems. Indeed, this may be as important as the more practical aspects of micelles in separations.

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