HIGH PERFORMANCE MICELLAR LIQUID CHROMATOGRAPHY WITH SILICA-BASED STATIONARY PHASE HAVING SURFACE-BOUND CATIONIC SURFACTANT

BY

YING ZHANG

Bachelor of Science

Peking University

Beijing, China

1986

Submitted to Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment for
the requirements of
the degree of
MASTER OF SCIENCE
July, 1995

HIGH PERFORMANCE MICELLAR LIQUID CHROMATOGRAPHY WITH SILICA-BASED STATIONARY PHASE HAVING SURFACE-BOUND CATIONIC SURFACTANT

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to my major advisor, Dr. Ziad El Rassi for his intelligent supervision, constructive guidance, inspiration and friendship. My sincere appreciation extends to my committee members Dr. Horacio A. Mottola and Dr. Neil Purdie for their support and valuable suggestions.

My sincere gratitude also go to my colleagues and lab-mates, Yehia Mechref, Wassim Nashabeh, Jim Yu, Tim Smith, Ran Pan, Jennifer Postlewait, Kim Wasserkrug and Arron Karcher, for their support and friendship.

I would like to give my special appreciation to my husband, Zhang-Hua, for his precious suggestions to my research, his continuous strong encouragement, love and understanding throughout my study. Thanks also go to my parents, sister and brother for their support and encouragement. I would like to thank my mother-in-law for her unselfish love, understanding and help.

Finally, I would like to thank the Department of Chemistry for support during my study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

α	selectivity
$log\beta$	specific interaction between mobile and stationary phase
v	partial specific volume of the surfactant in the micelle
μ	total concentration of surfactant
φ	the chromatographic phase ratio
Φ_{org}	volume fraction of organic modifier
Brij-35	polyoxyethylene (23) dodecanol
C_{18}	octadecyl silica stationary phase
$C_{18}N^{+}(Me)_2Pr$	the quaternary amine of octadecyl dimethyl propyl bounded C_{18} stationary phase
CMC	critical micellar concentration
CTAB	cetyltrimethylammonium bromide
DoTAB	dodecyltrimethylammonium bromide
Em	Empigen BB (N-dodecyl-N, N-dimethylglycine)
HPLC	high performance liquid chromatography
k'	capacity factor
k_0'	capacity factor of the solute in a purely aqueous or purely micellar mobile phase
k_s'	capacity factor of the free solute
K_{MW}	equilibrium constant for partition of the solute between the mobile phase and micelle

K_{SW}	equilibrium constant for partition of the solute between the mobile phase and stationary phase
LC	liquid chromatography
MLC	micellar liquid chromatography
$[M]$	micellized surfactant concentration
n_c	number of carbons
N	aggregation number
NaTDC	taurodeoxycholate sodium
NE	no elution
P_{MW}	partition coefficient between micelle and water
P_{SM}	partition coefficient between stationary phase and micelle
P_{SW}	partition coefficient between stationary phase and water
RPC	reversed phase chromatography
S	solvent strength parameter
S_{hyb}	solvent strength parameter of the hybrid mobile phase
SDS	sodium dodecyl sulfate
V_e	elution volume of the solute
V_m	volume of the mobile phase
V_s	volume of the stationary phase

CHAPTER I

BACKGROUND AND RATIONALE

Introduction

Micellar liquid chromatography (MLC), as first described by Armstrong et al. [1-4], uses aqueous surfactant solutions at concentrations above the critical micellar concentration (CMC) as the mobile phases. The two main properties of surfactant molecules are adsorption at interfaces and micelle formation above the CMC [5]. For a long time, surfactants have been used in liquid chromatography at concentrations below the CMC in which micelles were not formed (i. e., ion-pair chromatography or soap chromatography). As micelles have been showed to be useful in a wide variety of analytical techniques [6-9], Armstrong and Henry [1] extended the use of micelles into the field of chromatography in 1979 and pioneered the introduction of MLC into high performance liquid chromatography (HPLC) in 1980 [3]. The unique selectivities of such micellar mobile phases was demonstrated by Cline-Love [10], using anionic and cationic micellar mobile phases. Advantages shown by MLC are (i) unique selectivities of the micellar interactions [4, 10, 11], (ii) low cost and non toxicity of surfactants compared to expensive and flammable solvents of chromatographic grade [3, 12], (iii) capability of simultaneous separation of ionic and non-ionic compounds due to some special characteristics of micelles [12], (iv) reproducible and predictable retention behavior and rapid gradient capacity [13], (v) possibility of injecting biological fluids directly into the chromatographic system [14-22], (vi) many solutes show enhanced fluorescences [8, 23-

27], and (vii) in some cases, the possibility of performing room temperature liquid phosphorescence [23, 27, 28] in MLC. One major drawback of MLC is the rather low chromatographic efficiency compared to that obtained in conventional reversed-phase chromatography (RPC) with hydro-organic mobile phase. To overcome this problem, Dorsey et al. [29] as well as other workers [12, 30-34] have introduced ways to improve column efficiency. These involved the addition of a small amount of organic solvent to the micellar system or increasing the working column temperature to improve efficiency. A ternary eluent composed of water-organic solvent-micelles is called a hybrid eluent [35].

The aim of this chapter is to (i) provide an overview of the basic principles of MLC, (ii) discuss the operational parameters that affect retention and selectivity, (iii) describe some of the important features of MLC, (iv) present some selected applications and (v) give the rational behind the present study.

Micelles and Micellar Liquid Chromatography

Surfactants (surface-active agents) are molecules that exhibit both hydrophobic and hydrophilic character [12]. They consist of a long hydrocarbon chain (at least 8 carbon atoms) and a polar head group. Depending on the charge of the head group, surfactants can be classified as non-ionic, anionic, cationic or zwitterionic [36]. Above the CMC value, surfactant molecules dynamically associate to form large aggregates known as micelles. The number of surfactant molecules comprising the micellar entity is called its aggregation number (N). The structure of micelles depends on properties of the solution, such as ionic strength and addition of small amount of an organic solvent. In aqueous media, normal micelles are usually formed whereby the hydrocarbon tails are oriented toward the center of the aggregate and the polar head groups point outward. The aggregation number is typically 30-100 and their size is generally 3-6 nm in diameter for spherical micelles [12, 36]. Further increase in the surfactant concentration can result in the

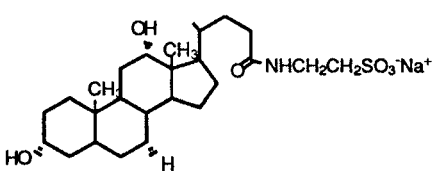
formation of other different types of organized assemblies [37]. Some surfactants can form reversed micelles in nonpolar solvents with the polar head groups oriented toward the interior of the aggregate, and the hydrophobic chains are in contact with the solvent. These reversed micelles are more complex and less studied and understood than normal micelles [38-40]. In addition to these types of micellar-forming surfactants, there is another class of molecules that can associate in water to form micellar aggregates: bile salts. Bile salts are very important biological detergent-like molecules and exhibit a different type of aggregation behavior [37].

From a macroscopic perspective, micellar solutions are homogeneous. They can not be filtered by conventional methods and they do not cause measurable light scattering error in UV-visible absorption spectroscopy [38]. However, from a microscopic perspective, micellar solutions are non-homogeneous in nature and they provide a microenvironment which is distinctly different from the bulk solvent [36]. Micelles are not static, but exist in equilibrium with surfactant monomers above the CMC. Table I lists the surfactants commonly used in MLC with some of their physical properties.

Table I. Surfactants commonly used in MLC and physical parameters[a]

surfactant	CMC (M)[b]	aggregation number[b]	Krafft point (°C)[c]
Anionic			
Sodium dodecyl sulfate (SDS), $CH_3(CH_2)_{11}OSO_3^-Na^+$	0.0081	62	9
Sodium tetradecyl sulfate $CH_3(CH_2)_{13}OSO_3^-Na^+$	0.0021	80	25

Table I Continued:

Cationic			
Cetyltrimethylammonium bromide (CTAB), $CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$	0.0013	78	23
Dodecyltrimethylammonium bromide (DoTAB) $CH_3(CH_2)_{11}N^+(CH_3)_3Br^-$	0.015	50	d
Nonionic			
Polyoxyethylene (23) dodecanol (Brij-35), $CH_3(CH_2)_{11}(OCH_2CH_2)_6OH$	0.0001	40	d
Zwitterionic			
N-Dodecyl-N, N-dimethylammonium-3-propane-1-sulfonate (SB-12), $CH_3(CH_2)_{11}N^+(CH_3)_2(CH_2)_3SO_3^-$	0.003	55	<0
Bile Salt			
Taurodeoxycholate sodium (NaTDC) 	1-4	d	d

a. From references [30, 36, 41].

b. Values for aqueous solution at 25°C.

c. Temperature at which the solubility of an ionic surfactant is equal to the CMC.

d. Not available or not defined.

In MLC, the surfactant monomers adsorb onto the stationary phases in at least two ways [34]: (a) hydrophobic interaction, where the alkyl tail of the surfactant would be

adsorbed on the nonpolar ligand of the stationary phase and the ionic head group would then be in contact with the aqueous mobile phase, giving the stationary phase some ion-exchange capacity with charged solutes (Fig. 1 (a)); (b) silanophilic interaction, where the ionic head group of the surfactant would be adsorbed, and as a result the stationary phase becomes more hydrophobic (Fig. 1 (b)). Also, the surfactant might compete with a solute for adsorption sites on the stationary phase [11]. So, the complexity of MLC is much greater than conventional RPC with hydro-organic solvents, owing to the large number of possible interactions (electrostatic, hydrophobic and steric) with the micellar mobile phase and with the modified stationary phase. In the aqueous mobile phase of RPC, micelles provide both hydrophobic and electrostatic sites for interactions with solutes [36], so that almost any compound can be determined by MLC [34].

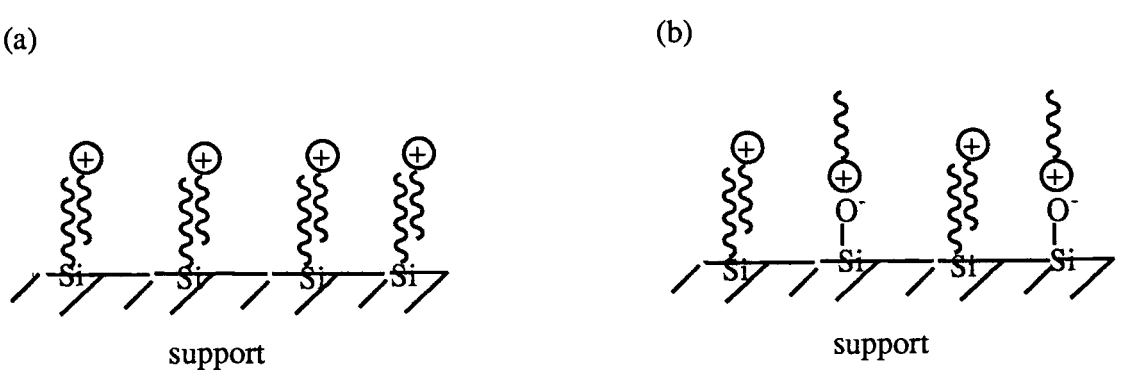


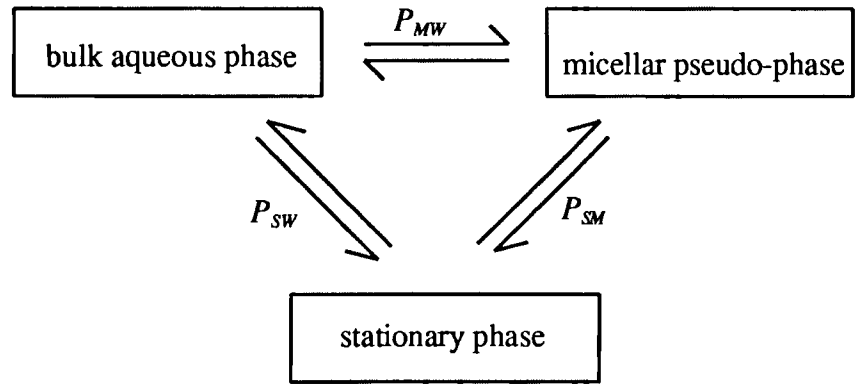
Fig. 1. Adsorption of surfactant monomers onto the stationary phase: (a) hydrophobic interaction; (b) silanophilic interaction.

Modeling of Micellar Liquid Chromatography and Partition Theory

Like any other secondary equilibrium mediated separations, the primary equilibrium is the same as in any RPC separation and is represented by the partition coefficient of the solute between the stationary phase and the bulk mobile phase (P_{SW}). A second equally important solute partition process is the one between the micelle and the bulk mobile phase (P_{MW}). A third partition coefficient of solute is between the stationary phase and the micelle (P_{SM}) [11]. Armstrong and Nome [11] were the first to develop the partition equation using the three-phase model (i. e., stationary phase, bulk aqueous and micellar pseudo-phase as shown in Fig. 2) which accounts for the reversed-phase chromatographic behavior of uncharged solutes. The equation can be written as:

$$\frac{V_S}{V_e - V_m} = \frac{\nu(P_{MW} - 1)}{P_{SW}}[M] + \frac{1}{P_{SW}} \qquad (1)$$

where V_S, V_e, and V_m are the volume of the stationary phase, retention volume of the solute, and the volume of the mobile phase, respectively, ν is the partial specific volume of the surfactant in the micelle; $[M]$ is the micellized surfactant concentration, i.e., concentration of surfactant in micellar form (total surfactant concentration minus the CMC) in moles per liter; and P_{MW} and P_{SW} are the partition coefficients of the solute between the micelle and water and between the stationary phase and water, respectively. By plotting $V_S/(V_e - V_m)$ (the terms of which can be measured) versus $[M]$ (which is known), P_{SW} can be calculated from the intercept and P_{MW} can be calculated from the ratio of the slope over intercept (provided ν is known). The value of P_{MW} obtained in this way is the partition coefficient between the micelle and water per monomer surfactant. To get the true partition coefficient (per micelle), the value of P_{MW} is multiplied by the aggregation number of the micelle. The quantity P_{SM} can be obtained from the ratio of the other two partition coefficients:



P_{MW} = partition coefficient between micelle and water, P_{SW} = partition coefficient between stationary phase and water, P_{SM} = partition coefficient between stationary phase and micelle (ref. [11]).

Fig. 2. Schematic representation of the three-phase model micellar liquid chromatography.

$$P_{SM} = \frac{P_{SW}}{P_{MW}} \qquad (2)$$

The solute-micelle binding constant, the term $v(P_{MW} - 1)$ according to the Berezín treatment [42], can also be determined.

There are some problems with the original derivation; for example, the equation requires a value for the partial specific volume of the surfactant in the micelle, which is often not available, as well as a poor estimate of the stationary phase volume (difference between the empty column volume and the packed column void volume) which affects the calculation of the value of P_{SW}.

Later Arunyanart and Cline-Love [43] proposed an equilibrium model to describe the change in retention of solutes at various micelle concentrations. This model involves two principal equilibrium, one being a reversible equilibrium of solute in the bulk solvent mobile phase (E) with the stationary phase sites (L_S) to form a complex (EL_S) and the second a reversible equilibrium of solute in the bulk solvent mobile phase (E) with the surfactant in the micelle present in the mobile phase (M) to form another complex (EM). A third reversible equilibrium involving the direct transfer of solute in the micelle (EM) to the stationary phase is also possible, but may be neglected. It is assumed that the solute binds independently to the stationary phase and to the micelle in the bulk solvent. The equilibrium expressions are illustrated by the following set of equations in which the concentrations of all species are defined in moles per liter. Of the three equilibria, only two

$$E + L_S \overset{K_{SW}}{\rightleftharpoons} EL_S$$

$$E + M \overset{K_{MW}}{\rightleftharpoons} EM$$

$$EM + L_S \overset{K_{SM}}{\rightleftharpoons} EL_S + M$$

are independent such that K_{SM} can be neglected. Arunyanart and Cline-Love [43] also derived a similar equation that correlates the capacity factor, k', with the micellized surfactant concentration, $[M]$, in the form:

$$\frac{1}{k'} = \frac{K_{MW}}{\varphi[L_S]K_{SW}}[M] + \frac{1}{\varphi[L_S]K_{SW}} \qquad (3)$$

where $[M]$ is the concentration of the surfactant in the micelle (total surfactant concentration minus CMC), φ is the chromatographic phase ratio, i.e., the ratio of the volume of the stationary phase, V_S, to the volume of the mobile phase, V_m, in the column, $[L_S]$ is the concentration of stationary phase "binding sites", K_{SW} and K_{MW} are the equilibrium constants for the partition of the solutes between the mobile and stationary phases and between the mobile phase and micelle, respectively. By plotting $1/k'$ versus $[M]$, the value of K_{MW} can be calculated from the ratio of the slope to the intercept. To obtain the equilibrium constant per micelle, one should multiply the K_{MW} value by the surfactant's aggregation number.

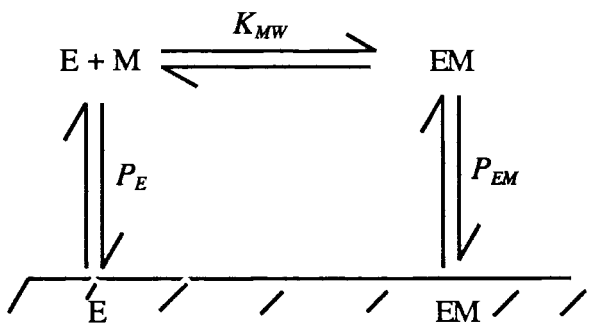
By using the equilibrium model, the volume of the stationary phase and the partial specific volume of the surfactant need not be known in order to calculate equilibrium constants or predict capacity factors. The equation can be used to describe the retention of nonpolar, polar, and even ionic solutes, chromatographed with anionic, cationic and non-ionic surfactants [5, 44]. For high relative molecular mass solutes, intercepts are nearly zero in the $1/k'$ versus $[M]$ plot, and even negative. A zero intercept means that for compounds which have large K_{SW} values, the K_{MW} value may not be obtainable with any accuracy from this type of plot [43]. However, having extremely large K_{SW} is not only physically possible but also consistent with solubility data for compounds that show this behavior (e. g., alkylbenzene homologous compounds beyond butylbenzene are insoluble in water) [45]. For negative intercept, the mechanism that is the direct transfer of these compounds from the micellar pseudo-phase to the surfactant-modified stationary phase, via

reversible adsorption of the solute-occupied micelle onto the "hemimicellar" surfactant-modified stationary phase, has been suggested [46].

Foley [47] developed a retention model for MLC by considering the general model for secondary chemical equilibrium in LC (Fig. 3). Addition of an equilibrant (M, i.e., micelle) to the mobile phase introduces a secondary equilibrium that allows an analyte to exist in two forms, free analyte (E) and the analyte-equilibrant "complex" (EM, i.e., solute-micelle association). The equation derived is:

$$\frac{1}{k'} = \frac{K_{MW}}{k_S'}[M] + \frac{1}{k_S'} \qquad (4)$$

where K_{MW} is the equilibrium binding constant for the formation of the solute-micelle association, k_S' is the capacity factor of the free solute and $[M]$ is the concentration of surfactant in the micelle. The resulting binding constant, K_{MW}, is understood to be per surfactant molecule. By plotting $1/k'$ versus $[M]$, the value of K_{MW} can be calculated from the ratio of the slope to the intercept.



E = solute, M = micelle, EM = solute-micelle complex, K_{MW} = binding constant of the solute-micelle complex, P_E = partition coefficient of solute, P_{EM} = partition coefficient of solute-micelle complex.

Fig. 3. General phenomenological retention model for micellar liquid chromatography. (adapted from ref. [47]).

Chromatographic Characteristics

Retention

Retention of a solute will depend on various types of interactions with the micelle and the surfactant-modified stationary phase. Nonpolar solutes, such as benzene and toluene, should only be affected by hydrophobic interactions (Fig. 4 (a)), but for solutes that are charged, not only hydrophobic interaction but also electrostatic repulsion or attraction can affect the solute retention (Fig. 4 (b) and (c)) [34].

Armstrong and Stine [4] classified solutes into three groups according to their chromatographic properties in MLC: (i) solutes binding to micelles; (ii) non-binding solutes; and (iii) antibinding solutes. Compounds that associate or bind to micelles show decreased retention when the concentration of micelles in the mobile phase is increased. For compounds that do not associate with micelles (non-binding), retention can remain unaltered by the micelle content of the mobile phase or their retention can increase with increasing micelle concentration (anti-binding) [34]. For most cases, solutes can interact with micelles.

According to Armstrong's [11] three-phase model, the retention of a solute in MLC depends on three partition coefficients: P_{SW}, P_{SM}, and P_{MW}, that is how solute molecules interact with the micelles and the stationary phase. So, the retention and separation selectivity can be controlled by several factors [36] such as surfactant type (chain length and head group charge), surfactant concentration, organic co-solvent or other mobile phase additives, temperature, ionic strength, pH (for ionogenic compound), etc.

Effect of micelle concentration on retention. According to equations (1), (3) and (4), the retention of a solute in MLC decreases when micelle concentration in mobile phase increases and the change in retention depends greatly on the nature of the solute. This is in

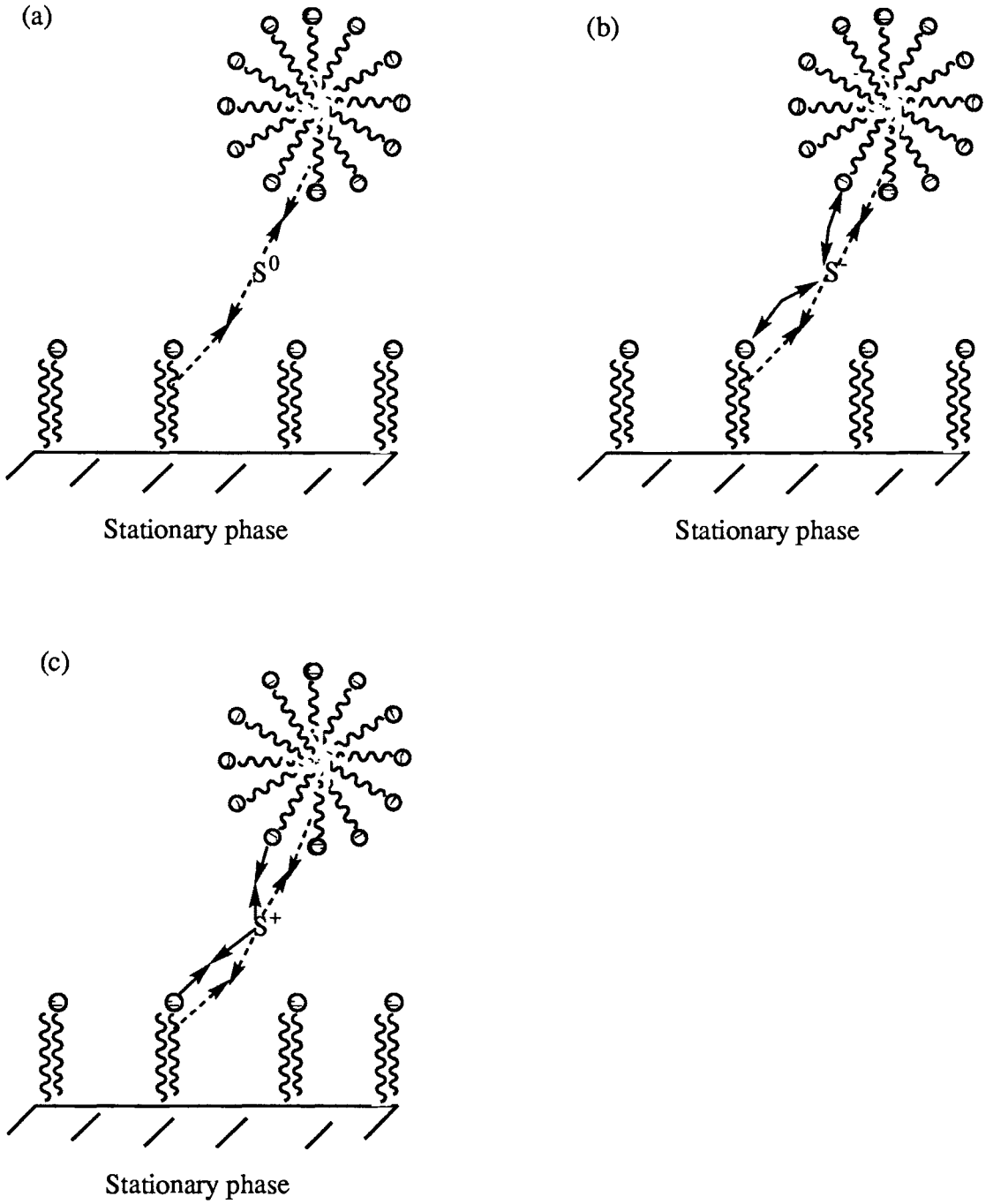


Fig. 4. Solute-micelle and solute-stationary phase hydrophobic (- - ➤) and electrostatic interactions (⟶) with an anionic surfactant: (a) nonpolar solute; (b) anionic solute; and (c) cationic solute (adapted from Ref. [34]).

contrast to reversed-phase ion-pair chromatography where the surfactant concentration is below the CMC (i. e., in the absence of micelles), and the addition of an ionic surfactant will increase retention for compounds which interact electrostatically with it.

These equations have been derived and employed with the aim of determining solute-micelle binding constants in purely micellar systems [5, 10, 11, 15, 17, 35, 43, 48-53]. However, most of the reported procedures for the determination of compounds by MLC make use of micellar mobile phases containing an alcohol, and these equations' validity for hybrid eluents have been demonstrated in refs [5, 35, 54, 55]. This has allowed the determination of the solute-micelle binding constants in micellar media modified by alcohols. The addition of an organic modifier to a micellar solution can modify the characteristics of the micellar system (e.g., CMC and aggregation number) and this can lead to a variation of the solute-micelle interactions [56-58] which in turn, can change the chromatographic retention. Solute-micelle interactions generally decrease in media modified by alcohols. In fact, solute-micelle binding constants for a group of benzene and naphthalene derivatives with SDS and CTAB are greater in purely micellar media than in solutions modified by a 5% or 10% n-butanol [54]. This has been attributed to the existence of a competing effect between the solute and the alcohol for interaction with the micelle.

Effect of the percentage of organic modifier on retention. Khaledi et al. [59] proposed the following equation to relate the solute retention (lnk') in MLC and the volume fraction of organic modifier (Φ_{org}):

$$lnk' = -S_{hyb}\Phi_{org} + lnk'_o \qquad (5)$$

where S_{hyb} is the solvent strength parameter and lnk'_o is the retention of the solute in a purely micellar mobile phase. Equation (5) is similar to that used to describe the retention

variation with volume fraction of modifier in RPC where lnk' linearly varies with Φ_{org} over a limited range.

Equation (5) shows how solute retention in MLC decreases when Φ_{org} increases. However, in the same article where equation (5) is proposed, it was observed that the variation of lnk' with Φ_{org} for some amino acids and alkylbenzenes in SDS and CTAB mobile phases is not linear. In another article, a deviation from linearity was also observed as is the case of a group of benzene and naphthalene derivatives in a MLC system with SDS / n-butanol mobile phases [60]. For other groups of solutes, the linear variation of lnk' with Φ_{org} was only found when methanol was used as organic modifier [53, 61].

Recently, Torres-Lapasió et al. [61] have proposed a new model to describe the variation of solute retention in MLC with Φ_{org} where retention can be expressed by the following equation:

$$1/k' = A[M] + B\Phi_{org} + C[M]\Phi_{org} + D \qquad (6)$$

in which $[M]$ and Φ_{org} are the surfactant and alcohol concentrations in mobile phase, respectively. The validity of this model has been shown for several solutes such as catecholamines, amino acids, phenols, and other aromatic compounds with organic modifiers different from methanol [61]. Equation (6) shows that for a constant surfactant concentration in the mobile phase, the term $1/k'$ should linearly vary with Φ_{org}:

$$1/k' = (A[M] + D) + (B + C[M])\Phi_{org} \qquad (7)$$

On the other hand, in a purely micellar mobile phase ($\Phi_{org} = 0$):

$$1/k' = A[M] + D \qquad (8)$$

and an equation similar to equations (1), (3) and (4) is obtained.

More work is required for different solutes, different surfactants, and different organic modifiers to show the validity range of equations (5) and (6).

Effect of pH on retention. The retention of weak organic acids and bases is affected by the pH of the micellar mobile phase. Solute-micelle partition coefficients of the dissociated and undissociated forms are different. Small changes in pH can significantly alter chromatographic retention, particularly when the mobile phase pH is close to the pK_a value of the solute [34]. At different pH, weak acids and bases will yield different retention behavior as the surfactant concentration is varied [50]. For weak acids, such as Bromocresol Green, using a C_{18} column and increasing SDS concentration in the mobile phase, k' values decrease in acidic solution where the neutral form is present, while k' remains constant in more basic solution where the anionic acid form is present, due to electrostatic repulsion by both the negative micelles and the stationary phase [50]. For protonated bases, such as aniline, the positively charged solute will be retained for a longer period of time than the neutral free-base form because of electrostatic attraction from the adsorption of anionic surfactant monomers on the surface of the C_{18} stationary phase. Dependence of k' on pH at a constant value of $[M]$ is sigmoidal if there is no electrostatic repulsion between any of the two acid-base forms and surfactant molecules [50].

Effect of other factors on retention. The charge on the head group of the surfactant also influences the retention of non-ionic compounds, as well as ionogenic solutes, through specific interactions with the functional groups of the solute molecules [36]. Modification of ionic strength can change the solute-micelle interaction behavior, i.e., with increasing ionic strength, most antibinding solutes might transit from antibinding solutes to nonbinding and to binding solutes [62].

Efficiency and Solvent Strength

A problem that must always be addressed when a secondary equilibrium is invoked is the effect of that process on the efficiency of the separation [11]. Although micellar mobile phases offer enhanced selectivity and many other advantages, their chromatographic efficiency is less than traditional hydro-organic mobile phases. The main cause for lower efficiency may include slow mass transfer from the stationary phase, as well as slow exit rates of hydrophobic solutes from micelles [36]. This problem was first addressed by Dorsey et al. [29] who showed that adding small amount of different organic solvents, such as a short chain alcohol, to micellar eluents and operating at higher temperatures, the kinetic problem can be overcome. Other authors suggested working with low flow rates, high temperatures, and low surfactant concentrations in the mobile phase [31]. Surfactant adsorption on the stationary phase may have a great influence on the efficiency [33, 44, 63, 64]. The addition of a short or medium chain alcohol causes surfactant desorption out of the stationary phase [65], reduces the net electrical charge density of the ionic micellar surface and decreases the repulsive barrier [66] to improve efficiency.

Another disadvantage of pure micellar eluents is their weak solvent strength [59]. Micellar eluent strengths depend upon the surfactant type and concentration. Generally, solvent strength increases with an increase in micelle concentration as long as a solute interacts with the micelles. However, an increase in micelle concentration in the mobile phase generally causes a loss of efficiency. At a given concentration, surfactants with longer chain lengths would provide stronger micellar eluents. The surfactant head group can also play a role in contributing to eluent strength depending on the extent of the specific interactions between a solute and the head group [36]. The solvent strength can also be increased by addition of an organic solvent, the effect being larger with more hydrophobic solutes [61].

The addition of organic solvents to micellar mobile phases would cause changes in certain micellar properties, such as the aggregation number and the CMC of the surfactant. However, the observed changes in retention and selectivity in hybrid systems are too large to be explained in terms of changes in micellar properties. The changes might be explained by modification of the micro-environment of the micelles and the stationary phase [34]. Although the exact reason behind the poorer efficiency in MLC and the methods of improving it are still a matter of controversy, the only practical proposals to solve the problem have been to use higher temperature and / or a small percentage of alcohol [36].

Solvent strength (S_{hyb}) in MLC with hybrid eluents has been defined as the slope of the straight line resulting from plots of lnk' vs Φ_{org} [68]. A large value of S_{hyb} indicates that the solvent interact more with micelles and therefore can solvate more effectively and / or can better compete with micelles for solute interactions. The S_{hyb} values obtained by Khaledi et al. [59] for the group compounds studied are smaller than S values in absence of micelles. Therefore, their retentions are less affected by the addition of organic solvents. This is because these compounds strongly interact with micelles and are less accessible to a polar solvent. The S values for a hydro-organic mobile phase change markedly with solute size in a homologous series [68, 69]. Variation in solvent strength with increasing solute size is minimized in the presence of micelles [35]. For a hybrid mobile phase, the solvent strength values are almost constant for a group of homologous compounds. The constancy of solvent strength with the variation in solute size is due to localization of solutes in the micelle environments, which reduces the size factor as far as the solvation of the solute by an alcohol is concerned [34]. In MLC, solutes interact differently with micelles and their own microenvironment in micelle is different, so that the ranking of S_{hyb} for different solutes is different for different organic solvent, such as methanol, propanol and butanol [67]. However, in conventional hydro-organic systems the same ranking of S values can be anticipated for different solutes.

Selectivity

A study of the chromatographic behavior of a homologous series of compounds provides important information that can be used to distinguish retention and selectivity between conventional RPC and MLC [70]. The differences between micellar and organic solvents in acting as the mobile phase modifiers were demonstrated through a comparative study of retention behavior of homologous series and hydrophobic selectivity using micellar, hydro-organic, and hybrid eluents as mobile phases [35]. It was shown that retention of alkylbenzenes and phenyl alkyl ketones in micellar eluents are significantly different from that in hydro-organic solvents. For example, for hydro-organic mobile phases a linear relationship exits between $logk'$ and the number of carbon, n_C, in a homologous series in the following form [71]:

$$log\, k' = n_C \log \alpha_{(CH_2)} + log\beta \qquad (9)$$

where $\alpha_{(CH_2)} = k'_{n+1}/k'_n$ is the hydrophobic or methylene group selectivity, that is, the ratio of the retention factors of two solutes that differ from each other by a methylene group, and $log\beta$ reflects the specific interactions between the functional group of the molecule and the mobile and stationary phases.

For micellar eluents, it is usually the retention factor, k' and not $logk'$, which is linearly dependent on the carbon number [35]:

$$k' = Bn_C + A \qquad (10)$$

where A and B are the intercept and slope, respectively, of the straight line. A plot of $logk'$ versus n_C for these systems has a clear curvature. This is probably due to different solute locations in the micelles for different members of a homologous series, which experience different polarities [46].

Due to the non-homogeneous nature of micelles and their multiple sites of interactions, uncommon separation selectivity such as reversal of elution order with a change in micelle concentration is often observed [4, 10]. The separation selectivity in MLC can be controlled by modifying surfactant nature and concentration [67].

The retention characteristics of the homologous series and methylene group selectivity in hybrid mobile phases are similar to those with the purely aqueous micellar mobile phases. Generally, separation selectivity in MLC is improved in the presence of an organic modifier and increases with the volume fraction of the modifier in the mobile phase [59, 60, 72]. But for some amino acids and peptides, selectivity decreased with the content of isopropanol (2-PrOH) of a SDS micellar mobile phase [72]. The effect of micelle and organic modifier on selectivity could be quite different; therefore, the mutual effects of these two parameters on selectivity require a simultaneous optimization [67].

Gradient Capabilities

One of the advantages of MLC is the uniqueness of performing gradient elution. Gradient elution is a popular HPLC approach to solve general elution problems. Complex mixtures containing compounds with a wide range of retention can be rapidly separated by increasing the eluent strength during the course of the separation. As a result, higher peak capacity, enhanced detection sensitivity, sharper peaks and shorter analysis time can be achieved [73, 74]. However, in most HPLC methods, the composition of the stationary phase is a function of the mobile phase. For repetitive analysis, the stationary phase has to be reequilibrated to the original mobile phase composition, which can greatly increase the total analysis time. In MLC, gradient elution can be performed by increasing the micelle concentration (and/or an organic modifier concentration) during the course of the separation [75].

In micellar solutions, the concentration of free (monomers) surfactants is approximately constant. An increase in total surfactant concentration would simply increase the concentration in the micellar form. For most surfactants and stationary phases, the amount of surfactant adsorbed on the stationary phase remains constant after equilibration once the concentration of surfactant is above the CMC [38, 45] because the alkyl bonded stationary phase is modified only with monomer surfactants, this means a change in the micelle concentration (at least for ionic micelles) in the mobile phase would not affect the composition of the stationary phase. In other words, no column reequilibration step is needed after a micelle concentration gradient elution. This would lead to a great savings in analysis time and solvent cost [36].

Another alternative to perform gradient elution in MLC is to increase the concentration of an organic solvent (e. g., propanol) within a limited range [75]. This has been proved without disturbing the column equilibration with micelle [76]. But in case of adding propanol to the micellar mobile phase, the stationary phase will also be partly modified with the organic modifier as well as with surfactants. Khaledi et al. derived equations for the prediction of gradient retention times in micellar concentration and organic modifier gradient from isocratic data on the basis of the gradient elution theory developed by Snyder [73, 74]. The equations will be useful for efficient development of practical separations by MLC.

Detection Capabilities

Because many common surfactants have saturated hydrocarbon tails and sulfate or quaternary ammonium head groups, they often have no measurable absorbance at common LC detection wavelengths [12] such as 254 nm and 280 nm. In addition, the localization of solutes in micelles on a molecular level would influence their photophysical pathways. This could sometimes leads to improvements in detection capabilities. Typical examples

Table II Continued:

amino acids/peptides	C_{18}-silica	SDS	0.1 M SDS, 15% (v/v) 2-PrOH[a] pH 2.5	[59]
amino acids/peptides	FO[b]	SDS	0.05 M SDS, 14% (v/v) 2-PrOH[a] pH 3.0.	[78]
benzene/naphthalene derivatives	C_{18}-silica	SDS	0.020 M SDS, 5% (v/v) butanol, isocratic	[60]
bumetanide	C_{18}-silica	SDS	0.02 M SDS, 10% (v/v) PrOH, pH 3.5.	[18]
cis/trans Co(III) complexes[c]	methyl or phenyl bonded	CTAB	0.1 M CTAB, isocratic	[79]
dithiocarbamates	C_{18}-silica	CTAB	0.01 M CTAB, 55% (v/v) MeOH, pH 6.8.	[80]
	CN[d]	CTAB	0.0125 M CTAB, 30% (v/v) MeOH, pH 6.8.	[81]
drugs	CN[d]	Brij-35		[22]
drugs(acetaminophen, phenobarbital, chloramphenicol)	C_{18}-silica	SDS	0.02 M SDS, pH 7.0	[21]
hydroxylbenzenes (phenols, quinols, catechols)	C_{18}-silica	SDS	0.09 M SDS	[49]
inorganic anions	C_{18}-silica	CTAC[e]	0.136 M CTAC or 0.01M CTAC 35% (v/v) acetonitrile, pH 6.8.	[52]
nucleosides/bases	PVA[f]	SDS	0.01 M SDS, pH 3.4, isocratic	[15]

Table II Continued:

phenols	C_{18}-silica	CTAB	0.12 M CTAB, 10% (v/v) 2-PrOH[a], pH 7, isocratic.	[59]
	C_{18}-silica	SDS	SDS concentration gradient, pH 2.5.	[82]
phenols/metal ions	C_{18}-silica	SDS	0.1 M SDS, pH 4.05.	[83]
phenols/polynuclear aromatic hydrocarbons	C_{18}-silica	SDS	0.1 or 0.2 M SDS with a flow gradient between 2.0 and 3.0 ml/min.	[3]
proline/hydroxyproline	C_{18}-silica	SDS	aqueous SDS, pH 2.8.	[84]
steroids	C_{18}-silica	SDS	0.1 M SDS, 0.01 M $Tb(NO_3)_3$ 20% (v/v) acetonitrile.	[85]
triglycerides	C_{18}-silica	SDS, CTAB	aqueous SDS or CTAB	[86]

a. Isopropanol.
b. Flurooctyl column.
c. Iminodiacetate.
d. Bondapack CN.
e. Cetyltrimethylammonium chloride.
f. Polyvinyl alcohol column.

Rationale and Objectives of the Study

As can be seen in Table II, the majority of MLC separations have been carried out on traditional reversed phase columns, C_{18}-silica and to a lesser extent CN- or phenyl-silica using sodium dodecyl sulfate (SDS) as the micellar mobile phase. As discussed above, and in all cases the stationary phases, however, become coated with the surfactant (i. e., surfactant-modified stationary phase) during the ensuing chromatographic process. This fact initiated our studies which were aimed at (i) developing a stationary phase that already has a covalently bound surfactant moiety, namely a quaternary amine of octadecyldimethylpropyl function, (ii) characterizing this stationary phase with neutral and ionic solutes under various conditions including surfactant nature and concentration, pH, percentage of organic modifier in the mobile phase, etc. (iii) comparing the selectivity and retention of the new sorbent with the traditionally used C_{18}-silica column and (iv) providing applications of relevance to environmental and biological research.

REFERENCES

[1] D. W. Armstrong, M. McNeely, *Anal. Lett.*, 12 (1979) 1285.

[2] D. W. Armstrong, R. Q. Terrill, *Anal. Chem.*, 51 (1979) 2160.

[3] D. W. Armstrong, S. J. Henry, *J. Liq. Chromatogr.*, 3 (1980) 657.

[4] D. W. Armstrong, G. Y. Stine, *Anal. Chem.*, 55 (1983) 2317.

[5] A. Berthod, I. Girard, C. Gonnet, *Anal. Chem.*, 58 (1986) 1359.

[6] W. L. Hinze, *Colloid and Interface Science*, M. Kerker, Ed., Academic Press, New York (1976), Vol. V, pp. 425-36.

[7] S. Chang, S. Spurlin, W.L. Hinze, *Sci. Ciencia*, 6 (1978) 54.

[8] W. L. Hinze, *Solution Chemistry of surfactants*, K. L. Mittal, Ed., Plenum Press, New York, 1 (1979) 79.

[9] S. Spurlin, W. L. Hinze, D. W. Armsrong, *Anal. Lett.*, 10 (1977) 997.

[10] P. Yarmchuk, R. Weinberger, R. F. Hirsch, L. J. Cline-Love, *Anal. Chem.*, 54 (1982) 2233.

[11] D. W. Armstrong, F. Nome, *Anal. Chem.*, 53 (1981) 1662.

[12] J. G. Dorsey, *Adv. Chromatogr.*, 27 (1987) 167.

[13] J. S. Landy, J. G. Dorsey, *J. Chromatogr. Sci.*, 22 (1984) 68.

[14] M. Arunyanart, L. J. Cline-Love, *J. Chromatogr.*, 342 (1985) 293.

[15] Y. N. Kim, P. R. Brown, *J. Chromatogr.*, 384 (1987) 209.

[16] P. Menéndez Fraga, E. Blanco González, A. Sanz-Medel, *Anal. Chim. Acta*, 212 (1988) 181.

[17] F. Palmisano, A. Guerrieri, P. G. Zambonin, T. R. I. Cataldi, *Anal. Chem.*, 61 (1989) 964.

[18] K. B. Sentell, J. P. Clos, J. G. Dorsey, *BioChromatography*, 4 (1989) 35.

[19] L. J. Cline-Love, J. Fett, *J. Pharm. Biomed. Anal.*, (1991) 323.

[20] I. Carretero, M. Maldonado, J. J. Laserna, E. Bonet, G. Ramis Ramos, *Anal. Chim. Acta*, 259 (1992) 203.

[21] F. J. Deluccia, M. Arunyanart, L. J. Cline-Love, *Anal. Chem.*, 57 (1985) 1564.

[22] L. J. Cline-Love, S. Zibas, J. Noroski, M. Arunynanart, *J. Pharm. Biomed. Anal.*, 3 (1985) 511.

[23] D. W. Armstrong, W. L. Hinze, K. H. Bui, H. N. Singh, *Anal. Lettt.*, 14 (1981) 1659.

[24] F. Fernández-Lucena, M. L. Marina, A. R. Rodríguez, *Vibrational Spectra and Structure,* R. Durig, Ed., Elsevier, Amsterdam, 1991, chap. 3.

[25] H. Singh, W. L. Hinze, *Anal. Lett.*, 15 (1982) 221.

[26] H. Singh, W. L. Hinze, *Analyst* (London), 107 (1982) 1073.

[27] R. Weinberger, P. Yarmchuk, L. J. Cline-Love, *Anal. Chem.*, 54 (1982) 1522.

[28] L. J. Cline-Love, M. Strilee, J. G. Habarta, *Anal. Chem.*, 52 (1980) 754.

[29] J. G. Dorsey, M. T. DeEchegarary, J. S. Landy, *Anal. Chem.*, 55 (1983) 924.

[30] D. W. Armstrong, *Sep. Purif. Methods*, 14 (1985) 213.

[31] P. Yarmchuk, R. Weinberger, R. F. Hirsch, L. J. Cline-Love, *J. Chromatogr.*, 283 (1984) 47.

[32] P. Yarmchuck, R. Weinberger, R. F. Hirsch, L. J. Cline-Love, *Anal. Chem.*, 57 (1985) 1564.

[33] M. F. Borgerding, W. L. Hinze, L. D. Stafford, G. W. Jr. Fulp, W. C. Jr. Hamlin, *Anal. Chem.*, 61 (1989) 1353.

[34] M. J. M. Heránadez, M. C. G. Alvarez-Coque, *Analyst,* 117 (1992) 831.

[35] M. G. Khaledi, E. Penler, J. Ngeh-Ngwainbi, *Anal. Chem.*, 59 (1987) 2738.

[36] M. G. Khaledi, *BioChromatography*, 3 (1988) 20.

[37] W. L. Hinze, D. W. Armstrong, Eds., *Ordered Media in Chemical Separations*, ACS symposium series, Vol. 342, Washington, 1987.

[38] A. Berthod, I. Girard, C. Gonnet, *Anal. Chem.*, 58 (1986) 1356.

[39] H. F. Eicke, *Top. Corr. Chem.*, 87 (1980) 85.

[40] J. H. Fendler, *Membrane Mimetic Chemistry*, Wiley-Interscience, New York, 1982, Chap. 3.

[41] L. J. Cline-Love, J. G. Habarta, J. G. Dorsey, *Anal. Chem.*, 56 (1984) 1132A.

[42] I. V. Berezín, K. Martinek, A. K. Yatsimirskii, *Russ. Chem. Rev.*, Eng. Transl., 42 (1973) 787.

[43] M. Arunyanart, L. J. Cline-Love, *Anal. Chem.*, 56 (1984) 1557.

[44] M. F. Borgerding, W. L. Hinze, *Anal. Chem.*, 57 (1985) 2183.

[45] J. G. Dorsey, M. G. Khaledi, J. S. Landy, J. L. Lin, *J. Chromatogr.*, 316 (1984) 183.

[46] M. F. Borgerding, F. H. Quina, W. L. Hinze, *Anal. Chem.*, 60 (1988) 2520.

[47] J. P. Foley, *Anal. Chim. Acta*, 231 (1990) 237.

[48] M. L. Marina, S. Vera, A. R. Rodríguez, *Chromatographia*, 28 (1989) 379.

[49] E. Pramauro, E. Pelizzetti, *Anal. Chim. Acta*, 154 (1983) 153.

[50] M. Arunyemart, L. J. Cline-Love, *Anal. Chem.*, 57 (1985) 2837.

[51] E. Pramauro, G. Saini, E. Pelizzetti, *Anal. Chim. Acta*, 166 (1984) 233.

[52] F. G. P. Mullins, G. F. Kirkbright, *Analyst* , 109 (1984) 1217.

[53] E. Bonet Domingo, M. J. Medina Hernández, G. Ramis Ramos, M. C. García Alvarez-Coque, *Analyst,* 117 (1992) 843.

[54] M. A. García, S. Vera, M. L. Marina, *Chromatographia*, 32 (1991) 148.

[55] F. P. Tomasella, J. Fett, L. J. Cline-Love, *Anal. Chem.*, 63 (1991) 474.

[56] M. Almgren, S. Swarup, *Surfactants in Solution*, K. L. Mittal, B. Lindman, Eds., Plenum, New York, 1984, pp. 613.

[57] L. G. Zonescu, L. S. Romanesco, F. Nome, *Surfactants in Solution*, K. L. Mittal, B. Lindman, Eds., Plenum, New York, 1984, pp. 789.

[58] A. Berthod, J. Georges, *Nouv. J. Chim.*, 9 (1985) 101.

[59] M. G. Khaledi, J. K. Strasters, A. H Gogers, E. D. Breyer, *Anal. Chem.*, 62 (1990) 130.

[60] M. A. García, S. Vera, M. Bombín, M. L. Marina, *J. Chromatogr.*, 646 (1993) 297.

[61] J. R. Torres-Lapasió, R. M. Villanueva-Camañas, J. M. Sanchís-Mallos, M. J. Medina-Hernández, M. C. García-Alvarez-Coque, *J. Chromatogr.*, 639 (1993) 87.

[62] D. W. Armstrong, G, Y. Stine, *J. Am. Chem. Soc.*, 105 (1983) 6220.

[63] D. W. Armstrong, T. G. Ward, A. Berthod, *Anal. Chem.*, 58 (1986) 579.

[64] R. Bailey, R. M. Cassidy, *Anal. Chem.*, 64 (1992) 2277.

[65] A. Berthod, A. Roussel, *J. Chromatogr.*, 449 (1988) 349.

[66] M. F. Borgerding, R. L. Williams, Jr., W. L. Hinze, F. H. Quina, *J. Liq. Chromatogr.*, 12 (1989) 1367.

[67] M. L. Marina, M. A. García, *J. Liq. Chromatogr.*, 17 (1994) 957.

[68] L. R. Snyder, M. A. Quarry, J. L. Glajch, *Chromatographia*, 24 (1987) 33.

[69] P. M. J. Coenegracht, H. J. Metting, A. K. Smilde, P. J. M. Coenegracht-Lamers, *Chromatographia*, 27 (1989) 135.

[70] M. G. Khaledi, *Anal. Chem.*, 60 (1988) 876.

[71] H. Colin, G. Guiochon, Z. Yun, J. C. Diez-Masa, P. Jandera, *J. Chromatogr. Sci.*, 21 (1983) 179.

[72] A. S. Kord, M. G. Khaledi, *Anal. Chem.*, 64 (1992) 1894.

[73] M. A. Quarry, R. L. Grob, L. R. Snyder, *J. Chromatogr.*, 285 (1984) 19.

[74] J. W. Dolan, L. R. Snyder, M. A. Quarry, *Chromatographia*, 24 (1987) 261.

[75] L. S. Madamba-Tan, J. K. Strasters, M. G. Khaledi, *J. Chromatogr. A*, 683 (1994) 321.

[76] L. S. Madamba-Tan, J. K. Strasters, M. G. Khaledi, *J. Chromatogr. A*, 683 (1994) 335.

[77] P.Jandera, *J. Chromatogr.*, 314 (1984) 13.

[78] S. Yang, L. F. R. Kruk, M. G. Khaledi, *J. Chromatogr. A*, 664 (1994) 1.

[79] C. M. Kirkman, C. Zu-Ben, P. C. Uden, W. J. Stratton, *J. Chromatogr.*, 317 (1984) 569.

[80] F. G. P. Mullins, G. F. Kirkbright, *Analyst*, 111(1986) 1273.

[81] G. F. Kirkbright, F. G. P. Mullins, *Analyst*, 109 (1984) 493.

[82] M. G. Khaledi, J. G. Dorsey, *Anal. Chem.*, 57 (1985) 2190.

[83] T. Okada, *Anal.Sci.*, 9 (1993) 59.

[84] S. T. Dydek, J. P. Kehrer, *LC Mag.*, 2 (1984) 536.

[85] M. Amin, K. Harrington, R. von Wandruszka, *Anal. Chem.*, 65 (1993) 2346.

[86] J. A. Singleton, H. E. Pattee, *J. Amer. Oil Chem. Soc.*, 62 (1985) 739.

CHAPTER II

CHROMATOGRAPHIC CHARACTERIZATION OF SILICA-BASED STATIONARY PHASES HAVING SURFACE-BOUND CATIONIC SURFACTANT. COMPARISON WITH OCTADECYLSILICA STATIONARY PHASE

Abstract

The retention behaviors of three different homologous series of *n*-alkylbenzenes, phenyl alkyl ketones and phenylalkyl alcohols were investigated on silica-based stationary phases having surface-bound cationic surfactant ($C_{18}N^+(Me)_2Pr$) via Si-O-Si linkage in micellar liquid chromatography as well as in purely reversed-phase mode. Different selectivities and shorter retention times for the homologous series were observed by using a $C_{18}N^+(Me)_2Pr$-silica column as compared to that of a C_{18}-silica column.

Introduction

Micellar liquid chromatography (MLC) is increasingly used in the simultaneous separation of ionic and non-ionic compounds due to the unique selectivity of micellar interaction [1-4], and to the feasibility of rapid gradient elution schemes [5].

Although recognized for its separation capabilities, MLC is still primarily practiced with octadecyl-silica stationary phases, and no attempts have been made so far to introduce specially designed columns for MLC. In this study, we wish to report on the

characterization of a new stationary phase based on silica with surface-bound cationic surfactant, namely quaternary amine of octadecyldimethylpropyl ligands ($C_{18}N^+(Me)_2Pr$).

The usual practice in characterizing novel stationary phases has been the study of the chromatographic behavior of homologous series because it sheds lights on the mobile and stationary effects on retention and selectivity. In fact, the regular linear increase of retention due to addition of a methylene group in a homologous series is recognized as a measure of hydrophobic interaction in a given RPC system [6]. The existence of a linear relationship between retention factor and a structural parameter (e. g., number of carbons) makes the retention study of homologous series particularly attractive for comparative purposes. Several workers have studied the retention behavior of a variety of homologous series as a function of hydro-organic mobile phase composition, stationary phase nature, and temperature [7-12]. The differences between micellar and organic solvent in acting as the mobile phase modifiers was demonstrated through a comparative study of retention behavior of homologous series and hydrophobic selectivity using micellar, hydro-organic and hybrid eluents as mobile phases [6].

In our study, regarding the chromatographic characterization of the new stationary phase, the retention behaviors of homologous series were investigated with the $C_{18}N^+(Me)_2Pr$ column and compared to conventional C_{18} column. The influence of the stationary phase on retention and selectivity for alkylbenzene, phenyl alkyl ketone and phenylalkyl alcohol homologous series is discussed.

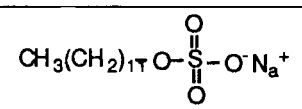
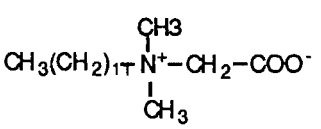
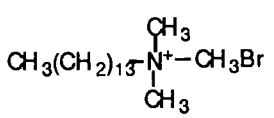
Experimental

Reagents

Zorbax spherical silica was obtained from DuPont (Wilmington, DE). n-Octadecyldimethyl[3-(trimethoxysilyl)propyl]ammonium chloride, n-octadecyldimethyl

chlorosilane were obtained from Hüls America (Bristol, PA). Sodium phosphate monobasic (analytical grade) was from Fisher Scientific (Fair Lawn, NJ). Isopropanol (HPLC grade) used to make the mobile phase solution was from Fisher Scientific or Baxter (McGaw Park, IL). Reagent grade isopropanol used in column packing was from EM Science (Cherry Hill, NJ). The surfactants sodium dodecyl sulfate (SDS), Empigen BB (*N*-dodecyl-*N*,*N*-dimethyl-glycine) (Em) and tetradecyltrimethylammonium bromide (TTAB) were purchased from Calbiochem (LaJolla, CA). The structure, CMC and nature of each surfactant are shown in Table I. Alkylbenzenes, phenyl alkyl ketones, phenylalkyl alcohols, *p*-aminobenzoic acid, pyrogallol, 2-phenylethanol, acetophenone, 2-naphthylamine, 4-cyanophenol and benzoic acid were purchased from Aldrich (Milwaukee, WI).

Table I. Surfactants used in this study.

surfactant	structure	CMC[a] (mM)	nature
SDS		8.2[b]	anionic
Em		1.8[c]	zwittergent,pH>6 cationic, pH<6
TTAB		3.6[b]	cationic

a. The values of CMC were taken from ref. [13]

b. 25 °C.

c. 23 °C.

Apparatus

The chromatograph was assembled from an LDC-Milton Roy (Riviera Beach, FL), Model CM4000 solvent delivery pump with a variable wavelength detector SpectroMonitor 3100, a Rheodyne (Cotati, CA) Model 7125 sampling valve with a 20-μl sample loop, and a C-R5A Chromatopac integrator from Shimadzu (Columbia, MD). The detection wavelength was set at 254 nm for all the solutes. Home-made 100 x 4.6 mm I.D. C_{18} and $C_{18}N^{+}(Me)_2Pr$ columns were used.

Preparation of Stationary Phases

Typically, 5g of Zorbax microspherical silica of 4.6 μm mean particle diameter and 150 Å mean pore diameter were suspended in 50 mL of dry DMF in a round-bottom flask. To this mixture, 7 mL of n-octadecyldimethyl[3-(trimethoxysilyl)propyl]ammonium chloride were added, and the suspension was stirred with a paddle stirrer. The reaction was performed at 120°C for 27 hours. The C_{18}-silica stationary phase was prepared in a similar manner using 2.6 g of dimethyloctadecylchlorosilane, 5g silica and 50 mL toluene. The suspension was heated at 125°C and stirred for 72 hours. In both cases, the stationary phase was washed with acetone and methanol several times and dried at room temperature.

Elemental Analysis and Surface Coverage of Stationary Phase

The percentage of C, H and N for C_{18}-silica and $C_{18}N^{+}(Me)_2Pr$-silica stationary phases were determined by elemental analysis at Galbraith Laboratories, Inc. (Knoxville, TN). The %C and H for C_{18}-silica were 7.28 and 1.38, respectively, while the %C, H and N for $C_{18}N^{+}(Me)_2Pr$-silica were found to be 8.64, 1.71 and 0.53, respectively. These

amounts when converted to surface coverage yielded 2.1 μmoles ligands/m^2 of silica for both silica bonded stationary phases.

Column Packing

The above stationary phases were packed from an isopropanol slurry at 8000 psi using a Shandon column packer (Keystone Scientific, Bellefonte, PA). Isopropanol was used as the solvent for stationary phase suspension and as the packing solvent. All columns were 100 x 4.6 mm I.D., No. 316 stainless steel tubes (Alltech Associates, Deerfield, IL). Column end fittings were also No. 316 stainless steel fitted with 0.5-μm frits and distributor disks from Alltech Associates.

Procedures

Micellar mobile phases were prepared by dissolving the appropriate amounts of surfactants and NaH_2PO_4 in water and then adjusting the pH to the desired value. All mobile phase solutions were filtered through a S/P^{TM} filter paper Grade 360, qualitative from Baxter (McGaw Park, IL). Stock sample solutions were prepared by dissolving pure compounds in 50% (v/v) isopropanol-water solutions. The sample solutions from which μl amounts were injected into the column, were prepared by diluting the stock solutions (in 50% isopropanol) with the mobile phase solutions.

Results and Discussion

Retention and Selectivity of Homologous Series

As stated above, homologous solutes are suitable test compounds for the investigation of retention mechanisms, especially in studying and characterizing new RPC systems [8, 9]. In fact, the linear increase of logarithmic capacity factor (i.e., $\log k'$) due to the addition of a methylene group to homologous series is recognized as a measure of hydrophobic interaction in a given RPC system.

The retention and selectivity of *n*-alkylbenzenes, phenyl alkyl ketones and phenylalkyl alcohols on the $C_{18}N^+(Me)_2Pr$ and C_{18} columns were studied in hybrid and hydro-organic mobile phases. The selectivity factors, α, for the members of the three different homologous series obtained on both C_{18} and $C_{18}N^+(Me)_2Pr$ columns are listed in Tables II and III.

As expected, α decreased with increasing organic content in the mobile phase with both $C_{18}N^+(Me)_2Pr$ and C_{18} columns. In conventional RPC, $\alpha(CH_2)$ is inversely related to solvent strength. This relationship is also observed for $C_{18}N^+(Me)_2Pr$ column in hydro-organic and hybrid mobile phase systems. In general, the $\alpha(CH_2)$ values are smaller on $C_{18}N^+(Me)_2Pr$ column than on the C_{18} column under the same mobile phase conditions (see Tables II and III). This may indicate that the extent of interaction between a $-CH_2$ group and the stationary phase with surface-bound cationic surfactant is less than that with an alkyl bonded stationary phase. Also, some homologous solutes which could not be eluted on C_{18} column were readily eluted on $C_{18}N^+(Me)_2Pr$ column under otherwise the same mobile phase composition.

To further shed light on the chromatographic behavior of the $C_{18}N^+(Me)_2Pr$ stationary phase, the above results were evaluated in terms of the relationship between k'

Table II. Selectivity (α^*) in hydro-organic mobile phase systems. Column, 100 x 4.6 mm; flow rate, 1.0 mL/min; mobile phase, 20 mM sodium phosphate at different concentrations of isopropanol; pH = 3.0.

	C_{18}			$C_{18}N^+(Me)_2Pr$		
2-PrOH% (v/v)	30%	40%	50%	30%	40%	50%
		Alkylbenzenes				
Toluene/benzene	2.10	1.68	1.48	1.79	1.45	1.30
Ethylbenzene/toluene	1.93	1.56	1.37	1.67	1.42	1.23
Propylbenzene/ethylbenzene	NE	1.60	1.41	1.77	1.42	1.27
Butylbenzene/Propylbenzene	NE	1.56	1.39	NE	1.40	1.26
Amylbenzene/Butylbenzene	NE	1.53	1.38	NE	1.39	1.26
		Phenyl alkyl ketones				
Propiophenone/acetophenone	2.05	1.75	1.56	1.75	1.51	1.35
Butyrophenone/propiophenone	1.95	1.63	1.44	1.67	1.42	1.28
Valerophenone/butyrophenone	2.04	1.68	1.47	1.77	1.47	1.30
Hexanophenone/valerophenone	2.04	1.65	1.45	1.79	1.46	1.30
Heptanophenone/hexanophenone	NE	1.60	1.42	1.75	1.43	1.29
		Phenylalkyl alcohols				
2-Phenylethanol/phenylmethanol	2.00	1.57	1.43	1.81		
3-Phenylpropanol/2-phenylethanol	1.87	1.55	1.36	1.66	1.38	1.22
4-Phenylbutanol/3-phenylpropanol	1.81	1.52	1.38	1.61	1.36	1.23
5-Phenylpentanol/4-phenylbutanol	1.88	1.54	1.37	1.65	1.37	1.25
6-Phenylhexanol/5-phenylpentanol	NE	1.54	1.38	NE	1.39	1.25

* The ratio of capacity factors of two compounds differing only in a $-CH_2$ group.
NE, no elution of higher member of the homologous series.

Table III. Selectivity (α) in hybrid eluent systems. Column, 100 x 4.6 mm; flow rate, 1.0 mL/min; mobile phase, 20 mM sodium phosphate and 5 mM Em at different concentration of isopropanol; pH = 3.0.

	C_{18}		$C_{18}N^+(Me)_2Pr$		
2-PrOH% (v/v)	15%	20%	10%	15%	20%
		Phenyl alkyl ketones			
Propiophenone/acetophenone	NE	2.43	2.47	2.40	2.28
Butyrophenone/propiophenone	NE	2.29	2.25	2.21	2.13
		Phenylalkyl alcohols			
2-Phenylethanol/phenylmethanol	2.32	2.38	2.03	2.18	2.25
3-Phenylpropanol/2-phenylethanol	2.25	2.15	2.24	2.12	2.01
4-Phenylbutanol/3-phenylpropanol	2.08	2.02	2.00	1.97	1.89

NE, no elution of higher member of the homologous series.

and the carbon number (n_c) of the solutes for various homologous series. As reported by Colin et al. [7], with hydro-organic mobile phases, $\log k'$ is linearly related to n_c as follows:

$$\log k' = (\log \alpha) n_c + \log \beta \qquad (1)$$

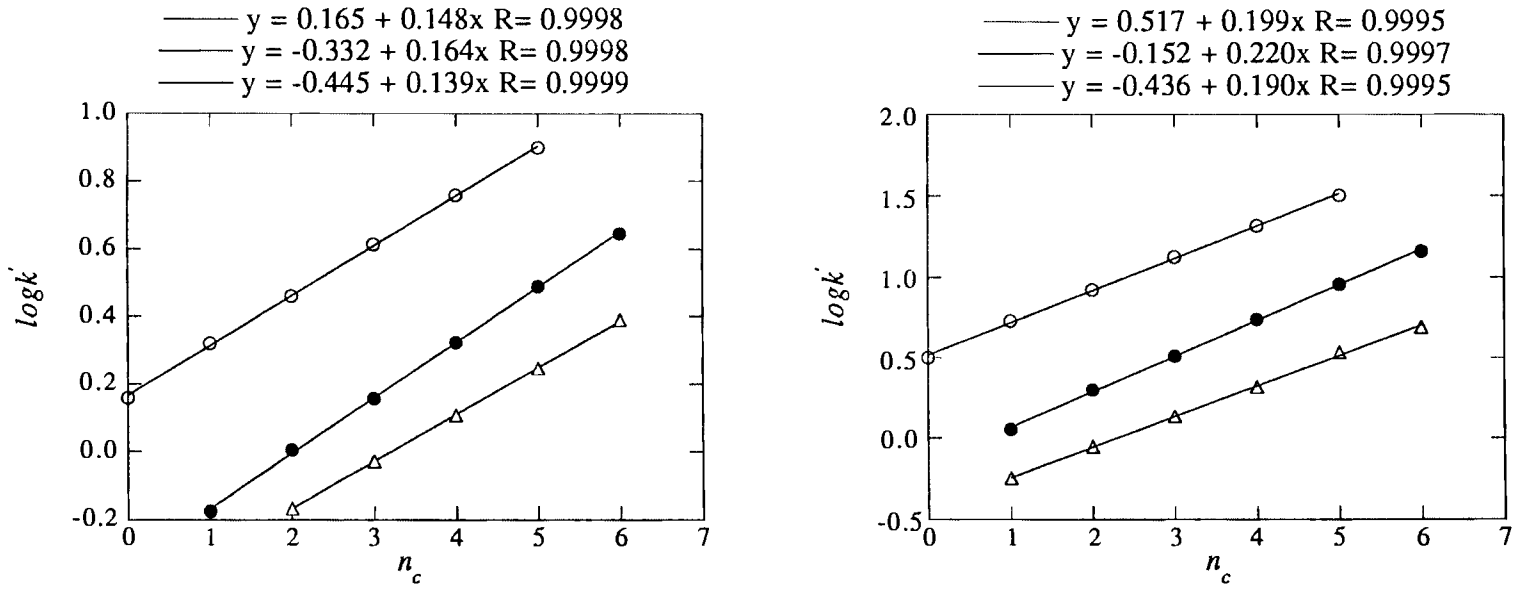
The slope $\log \alpha$ is a measure of methylene or hydrophobic selectivity which characterizes nonspecific interactions. $\log \alpha$ is weakly influenced by the chemical nature of the solute and is usually very close in values for all different homologous series. The intercept $\log \beta$ reflects the specific interactions between the residue of the molecule with the mobile and stationary phase. Khaledi et al. [6] studied the relation between capacity factor and the

carbon number for *n*-alkylbenzenes and phenyl alkyl ketones with SDS, CTAB micellar mobile phases and their hybrid mobile phases. They found that the capacity factor is linearly dependent on the number of carbons instead of $\log k'$ for most of the cases as shown by the following equation:

$$k' = bn_c + a \qquad (2)$$

But for phenyl alkyl ketones with SDS and its hybrid mobile phases, a linear relationship was observed between $\log k'$ and n_c as with pure hydro-organic mobile phases [6].

We studied the retention behaviors of *n*-alkylbenzenes, phenyl alkyl ketones and phenylalkyl alcohols with hydro-organic, SDS and Em hybrid mobile phases on both C_{18} and $C_{18}N^+(Me)_2Pr$ columns. The results are summarized in Tables IV and V. With hydro-organic mobile phases, excellent linearity between $\log k'$ and n_c was observed for all compounds on both columns. Figure 1a and b shows typical plots of $\log k'$ versus n_c in hydro-organic systems with C_{18} and $C_{18}N^+(Me)_2Pr$ columns, respectively. From Table IV, we can see that in a given system (i. e., a given stationary phase and mobile phase system), the nonspecific selectivity term ($\log \alpha$) depends only slightly on the nature of the functional group of the series (i. e., alkylbenzenes, phenyl alkyl ketones and phenylalkyl alcohols). On the other hand, the specific interaction term ($\log \beta$) is very different between different homologous series. Both $\log \alpha$ and $\log \beta$ are smaller on $C_{18}N^+(Me)_2Pr$ column and this means that $C_{18}N^+(Me)_2Pr$ column provides less nonspecific and also specific interaction with solutes than the C_{18} column. This is because the benzene residue of alkylbenzene molecules has a stronger hydrophobic interaction with a nonpolar C_{18} column. In other words, the benzene group has much less hydrophobic interaction with the somewhat polar $C_{18}N^+(Me)_2Pr$ stationary phase than with the C_{18} column. The benzene ketone residue of phenyl alkyl ketone molecules has a less hydrophobic interaction between the benzene ring and the $C_{18}N^+(Me)_2Pr$ stationary phase, but the carbonyl group provides some interaction between the oxygen atom and the $C_{18}N^+(Me)_2Pr$ stationary



o - Alkylbenzenes. ● - Phenyl alkyl ketones. ▲- Phenylalkyl alcohols.

Fig.1. Plots of *logk'* versus n_c using hydro-organic mobile phase. a. $C_{18}N^{+}(Me)_2Pr$ column. b. C_{18} column. Mobile phase, 20 mM sodium phosphate at 40% (v/v) isopropanol; pH 3.0.

Table IV. Correlation between capacity factor and carbon number in hydro-organic mobile phase systems, $\log k' = (\log \alpha) n_c + \log \beta$. Experimental conditions are as in Table II.

	C_{18}			$C_{18}N^+(Me)_2Pr$		
	$\log \alpha$	$\log \beta$	R	$\log \alpha$	$\log \beta$	R
			Alkylbenzenes			
30% 2-PrOH	0.304	0.834	0.9994	0.240	0.412	0.9997
40% 2-PrOH	0.199	0.517	0.9995	0.148	0.165	0.9998
50% 2-PrOH	0.148	0.240	0.9996	0.100	-0.0902	0.9999
			Phenyl alkyl ketones			
15% 2-PrOH	NE			0.339	0.0966	0.9994
30% 2-PrOH	0.305	0.0178	0.9999	0.242	-0.206	0.9998
40% 2-PrOH	0.221	-0.152	0.9997	0.164	-0.332	0.9998
50% 2-PrOH	0.165	-0.308	0.9995	0.114	-0.478	0.9997
			Phenylalkyl alcohols			
15% 2-PrOH	NE			0.299	-0.153	0.9951
30% 2-PrOH	0.274	-0.361	0.9996	0.223	-0.427	0.9991
40% 2-PrOH	0.190	-0.436	0.9995	0.139	-0.445	0.9999
50% 2-PrOH	0.140	-0.555	0.9998	0.093	-0.547	0.9996

NE, no elution of higher member of the homologous series.

Table V. Correlation between capacity factor and carbon number using hybrid mobile phases. SDS hybrid mobile phase, 20 mM sodium phosphate and 80 mM SDS at 10% (v/v) isopropanol; other experimental conditions are the same as Table III.

	C_{18}						$C_{18}N^+(Me)_2Pr$					
	$\log k' = (\log \alpha) n_c + \log \beta$			$k' = bn_c + a$			$\log k' = (\log \alpha) n_c + \log \beta$			$k' = bn_c + a$		
	$\log \alpha$	$\log \beta$	R	b	a	R	$\log \alpha$	$\log \beta$	R	b	a	R
5 mM Em												
Phenyl alkyl ketones												
10% 2-PrOH	NE			NE			0.373	0.503	0.9995	16.9	-11.5	0.9797
15% 2-PrOH	NE			NE			0.363	0.339	0.9996	10.7	-7.01	0.9802
20% 2-PrOH	0.373	0.315	0.9998	11.1	-7.59	0.9776	0.345	0.223	0.9996	7.10	-4.24	0.9820
Phenylalkyl alcohols												
10% 2-PrOH	0.360	0.147	0.9998	6.89	-4.62	0.9712	0.323	0.260	0.9996	10.3	-9.76	0.9594
15% 2-PrOH	0.346	-0.009	0.9995	6.90	-6.95	0.9581	0.321	0.0759	0.9995	6.54	-6.03	0.9637
20% 2-PrOH	0.337	-0.107	0.9988	4.99	-4.83	0.9628	0.310	-0.0280	0.9985	4.57	-3.95	0.9698
80 mM SDS + 10% 2-PrOH												
Phenyl alkyl ketones	0.200	0.598	0.9999	4.78	1.13	0.9899	0.189	0.489	0.9925	3.22	1.41	1.0000
Phenylalkyl alcohols	0.212	0.157	0.9848	2.80	-1.28	0.9337	0.199	0.205	0.9994	1.87	0.530	0.9956

NE, no elution of higher member of the homologous series.

phase, and this compensates somewhat for the reduction of hydrophobic interaction. Therefore, the overall effect makes the $log\beta$ reduced, but not as much as for alkylbenzenes. The phenyl and hydroxyl residues of phenylalkyl alcohols impart similar retention behavior on the $C_{18}N^{+}(Me)_2Pr$ stationary phase than the phenyl alkyl ketones in the sense that the reduction of hydrophobic interactions is compensated by strong interactions with the hydroxyl residue, so that the overall specific interaction will be reduced less. This provides the $C_{18}N^{+}(Me)_2Pr$ column with the capability to separate nonpolar and polar solutes simultaneously in a shorter time.

With hybrid mobile phases (see Table V), plots of $log k'$ vs carbon number showed more linearity than plots of k' vs carbon numbers. This trend is similar to that observed with the hydro-organic mobile phases. The methylene group retention increment (i. e., difference between k' of two compounds differing only in a $-CH_2$ group) is very different between various homologous series in a given system. The nonspecific term $log\alpha$ is always smaller on $C_{18}N^{+}(Me)_2Pr$ column because of its weaker hydrophobicity, but the specific interaction term $log\beta$ depends on the polarity of the solutes, i.e., the solute with polar group (i.e., phenylalkyl alcohols) have larger $log\beta$ on a $C_{18}N^{+}(Me)_2Pr$ column than on a C_{18} column.

From tables IV and V, we can see that the strength of the overall interaction with both columns decreases in the order alkylbenzenes > phenyl alkyl ketones > phenylalkyl alcohols. This means that the $C_{18}N^{+}(Me)_2Pr$ stationary phase is dominated by hydrophobic interaction rather than by polar interactions and that electrostatic (polar) interaction is superposed on the hydrophobic forces.

Evaluation of Retention of Homologous Series on $C_{18}N^{+}(Me)_2Pr$ Column at Various Micelle Concentrations-Comparison with C_{18} Column

As discussed in chapter I, Armstrong and Nome [14] have developed a three-phase model and reported the correlation between retention and micelle concentration by the following equation:

$$\frac{V_s}{V_e - V_m} = \frac{\nu(P_{MW} - 1)}{P_{SW}}[M] + \frac{1}{P_{SW}} \qquad (3)$$

where V_s, V_e, and V_m are the volume of the stationary phase, elution volume of the solute, and the volume of the mobile phase, respectively, ν is the partial specific volume of the surfactant in the micelle, $[M]$ is the micellized surfactant concentration, i.e., concentration of surfactant in micellar form (total surfactant concentration minus the CMC) in moles per liter, and P_{MW} and P_{SW} are the partition coefficients of the solute between the micelle and water and between the stationary phase and water, respectively. By plotting $V_s/(V_e - V_m)$ (the terms of which can be measured) versus $[M]$ (which is known), P_{SW} can be calculated from the intercept and P_{MW} can be calculated from the ratio of the slope over intercept (provided ν is known). The value of P_{MW} obtained in this way is the partition coefficient between the micelle and water per monomer surfactant. To get the true partition coefficient (per micelle), the value of P_{MW} is multiplied by the aggregation number of the micelle. The solute-micelle binding constant, the term $\nu(P_{MW} - 1)$ according to Berezín treatment [15], can also be determined.

The void volume of the system and the time equivalent of the void volume was measured from the injection point to the first deviation from the baseline. The stationary phase volume in equation (3) was estimated by subtracting the void volume from the empty column volume. This poor estimate of the stationary phase volume and the further

requirement for a value for the partial specific volume of the surfactant in the micelle, which is often not available, affect the calculation of the value of P_{SW}.

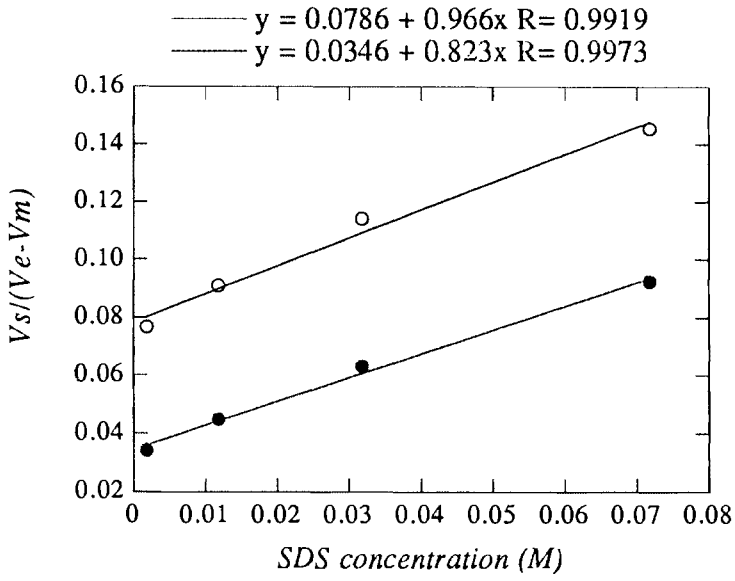
Arunyanart and Cline-Love [16] and later Foley [17] derived a similar equation that related the capacity factor to the concentration of micelle which can be written as:

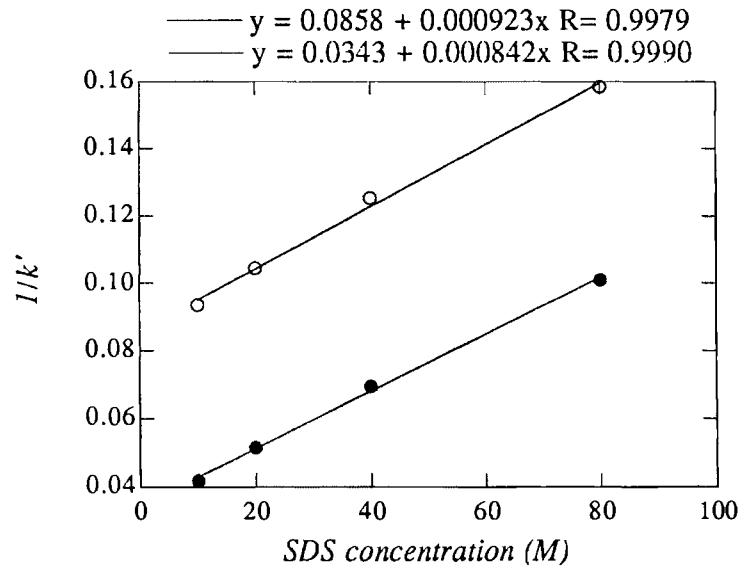
$$\frac{1}{k} = \frac{K_{MW}}{\varphi[L_S]K_{SW}}[M] + \frac{1}{\varphi[L_S]K_{SW}} \qquad (4)$$

where $[M]$ is the concentration of the surfactant in the micelle, i. e., micellized surfactant concentration (total surfactant concentration minus CMC), φ is the phase ratio in the column, i.e., the ratio of the volume of the stationary phase, V_S, to the volume of the mobile phase, V_m, in the column, $[L_S]$ is the concentration of stationary phase "binding sites", K_{SW} and K_{MW} are the equilibrium binding constants for partitioning of the solutes between the mobile and stationary phases and between the mobile phase and micelles, respectively. By plotting $1/k'$ versus $[M]$, the value of K_{MW} can be calculated from the ratio of the slope to the intercept. To obtain the equilibrium constant per micelle, one should multiply the K_{MW} value by the surfactant's aggregation number.

Equations (3) and (4) are derived for purely micellar mobile phases, but they are valid for hybrid eluents [6, 18-20]. The effect of added organic modifier and temperature on the equilibrium constants obtained via equation (3) has been previously examined [6, 21-23]. A change in binding constants can be observed with the addition of organic modifiers.

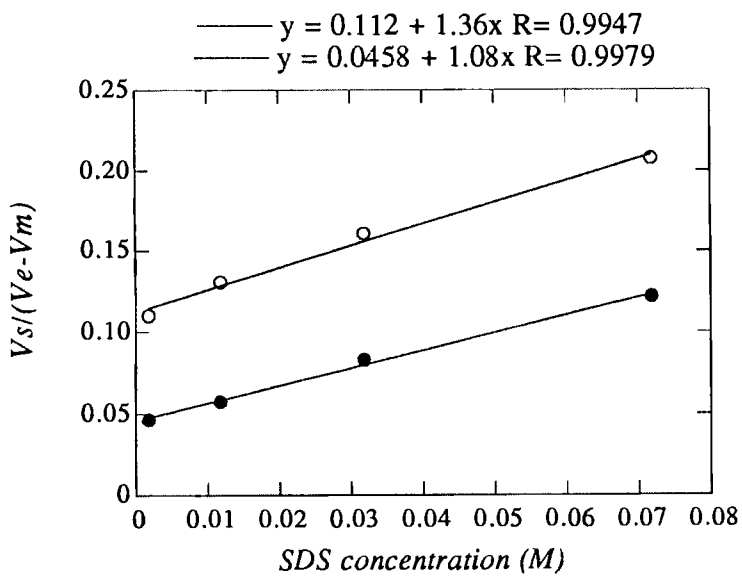
When using both equations, good linearity was observed for all compounds in different hybrid mobile phases as shown by typical examples in Figs 2 and 3. Tables VI-IX list the binding constant values (per monomer surfactant) and partition coefficients between stationary phase and water for some phenyl alkyl ketones and phenylalkyl alcohols in SDS and Em hybrid mobile phase systems obtained with C_{18} and $C_{18}N^{+}(Me)_2Pr$ columns and linear regression coefficients obtained by using both equations (3) and (4).

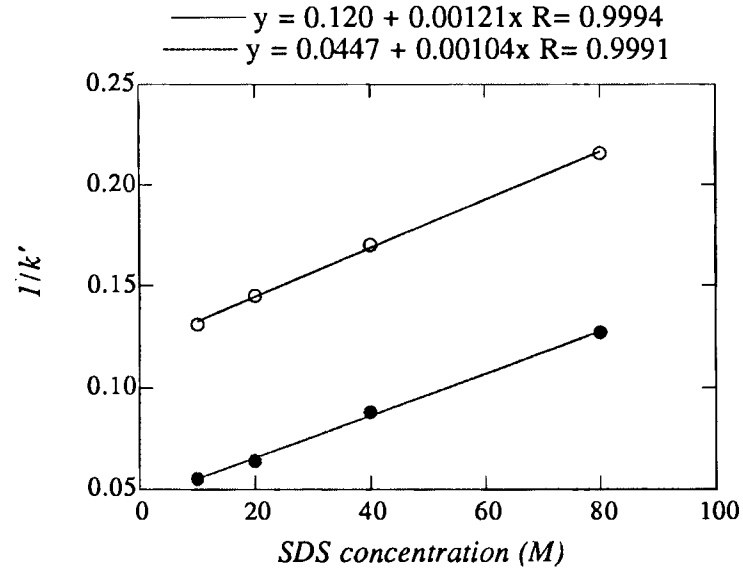




o - Acetophenone. ● - Propiophenone.

Fig. 2. a. Plot of *Vs/(Ve-Vm)* versus *SDS concentration* on the C_{18} column.
b. Plot of $1/k'$ versus *SDS concentration* on the C_{18} column.
Mobile phase, 20 mM sodium phosphate and 10% (v/v) isopropanol at different concentrations of SDS; pH 3.0.





o - Acetophenone. ● - Propiophenone.

Fig. 3. a. Plot of *Vs/(Ve-Vm)* versus *SDS concentration* on the $C_{18}N^{+}(Me)_2Pr$ column.
b. Plot of $1/k'$ versus *SDS concentration* on the $C_{18}N^{+}(Me)_2Pr$ column.
Experimental conditions are the same as Fig. 2.

Table VI. Values of K_{MW} and P_{SW} as measured from equations (3) and (4) using SDS hybrid eluents and C_{18} stationary phase. Column, 100 x 4.6 mm; flow rate, 1.0 mL/min; mobile phase, 20 mM sodium phosphate and 10% (v/v) isopropanol at different concentrations of SDS; pH = 3.0.

	equation (3)			equation (4)	
	K_{MW}	P_{SW}	R	K_{MW}	R
			Phenyl alkyl ketones		
Acetophenone	12.3	12.7	0.9919	9.9	0.9979
Propiophenone	23.8	28.9	0.9973	20.4	0.9991
			Phenylalkyl alcohols		
Phenylmethanol	7.0	4.1	0.9852	4.8	0.9998
2-Phenylethanol	11.3	6.8	0.9966	8.7	0.9987
3-Phenylpropanol	24.0	16.4	0.9984	20.4	0.9992

As can be seen, the values of the binding constant calculated from equations (3) and (4) are not in good agreement for some of the cases. This is probably due to the uncertainties in the measurements of the column void volume and the stationary phase volume. The solute-micelle (i. e., solute-SDS or solute-Em) binding constant values measured on a C_{18} column and a $C_{18}N^{+}(Me)_2Pr$ column are almost the same regardless of the stationary phase, especially for K_{MW} values calculated from equation (4). When compared to phenylalkyl alcohols , phenyl alkyl ketones have more affinity toward both SDS and Em micelles than phenylalkyl alcohols because they are more hydrophobic. The binding constants between solute and micelle and the partitioning coefficient between stationary phase and water increase with the number of carbons in the alkyl chain of the solutes. The solute-SDS binding constant values and the partition coefficients between stationary phase and water

Table VII. Values of K_{MW} and P_{SW} as measured from equations (3) and (4) using Em hybrid eluents and C_{18} stationary phase. Mobile phase, 20 mM sodium phosphate and 10% (v/v) isopropanol at different concentration of Em. Other experimental conditions are the same as Table VI.

	equation (3)			equation (4)	
	K_{MW}	P_{SW}	R	K_{MW}	R
			Phenyl alkyl ketones		
Acetophenone	11.3	11.8	0.9999	9.3	0.9991
Propiophenone	24.2	32.2	0.99997	22.1	0.9996
			Phenylalkyl alcohols		
Phenylmethanol	7.7	5.8	0.9996	5.9	0.9971
2-Phenylethanol	12.3	9.5	0.9996	10.2	0.9970
3-Phenylpropanol	26.6	23.5	1	25.9	0.9971
4-Phenylbutanol	57.0	55.9	0.9991	44.5	0.9991

are much less than those obtained by using pure micellar mobile phase [6] (see Table X) because of the addition of isopropanol to the micellar system. The addition of organic modifier significantly alters the equilibrium of the solute away from the micelle toward the bulk aqueous phase. The bulk aqueous phase is more nonpolar with the addition of an organic modifier. We also found that the K_{MW}/P_{SW} ratio increases in presence of organic modifier. For instance, the K_{MW}/P_{SW} values of acetophenone and propiophenone in a SDS hybrid mobile phase on a C_{18} column which are 0.969 and 0.824, respectively, are much higher than the values obtained in pure SDS micellar mobile phase. Therefore, the elution strength of the mobile phase increases. Similar results were reported by other investigators [20]. From Tables VI to IX, we also can see that the P_{SW} values are different when using

Table VIII. Values of K_{MW} and P_{SW} as measured from equations (3) and (4) using SDS hybrid eluents and $C_{18}N^{+}(Me)_2Pr$ stationary phase. Other experimental conditions are the same as Table VI.

	equation (3)			equation (4)	
	K_{MW}	P_{SW}	R	K_{MW}	R
			Phenyl alkyl ketones		
Acetophenone	12.2	8.9	0.9947	9.3	0.9994
Propiophenone	23.4	21.8	0.9979	19.5	0.9991
			Phenylalkyl alcohols		
Phenylmethanol	7.3	3.9	0.9918	4.7	0.9989
2-Phenylethanol	10.3	7.3	0.9967	7.6	0.9991
3-Phenylpropanol	21.5	16.5	0.9986	17.8	0.9997

different micelles on the same column. This is because the adsorption of surfactant onto the stationary phase makes the stationary phase exhibit some different properties. As can be seen, the P_{SW} values on Em-modified stationary phase are larger than on SDS-modified stationary phase. The adsorption of Em onto stationary phase provide some polar interaction between solutes and stationary phase. When using the same surfactant Em, the P_{SW} values on C_{18} column are larger than $C_{18}N^{+}(Me)_2Pr$ column. This may be due to the higher coating of the C_{18} surface with Em surfactant as compared to $C_{18}N^{+}(Me)_2Pr$ column.

Khaledi et al. [6] derived an equation describing the partition coefficients versus carbon numbers as follows:

$$\log K_{MW}(or P_{SW}) = (slope)n_c + intercept \qquad (5)$$

Table IX. Values of K_{MW} and P_{SW} as measured from equations (3) and (4) using Em hybrid eluents and $C_{18}N^{+}(Me)_2Pr$ stationary phase. Other experimental conditions are the same as Table VII.

	equation (3)			equation (4)	
	K_{MW}	P_{SW}	R	K_{MW}	R
			Phenyl alkyl ketones		
Acetophenone	10.0	9.9	0.9991	10.0	0.9991
Propiophenone	22.9	25.1	0.9998	23.0	0.9998
Butyrophenone	50.6	61.3	0.9993	47.4	0.9995
			Phenylalkyl alcohols		
Phenylmethanol	5.6	5.2	0.9850	5.6	0.9850
2-Phenylethanol	10.5	10.5	0.9967	10.5	0.9967
3-Phenylpropanol	24.4	24.3	0.9993	24.4	0.9993
4-Phenylbutanol	49.3	51.8	0.9999	49.4	0.9999

Table X. Values of K_{MW} and P_{SW} as measured from equations (3) and (4) using SDS pure micellar mobile phases on C_{18} column (50 x 4.6 mm) [6].

	equation (3)			equation (4)
	K_{MW}	P_{SW}	K_{MW}/P_{SW}	K_{MW}
Acetophenone	19.6	52.9	0.371	17.4
Propiophenone	28.3	106	0.267	23.9

where the slope is a measure of the free energy of transfer of a methylene group from the bulk solvent to micelle or from the bulk solvent to the stationary phase, the intercept represents the interaction between the residue of the homologues with the micelle or the stationary phase. Table XI shows the relationship between the K_{MW} (or P_{SW}) and the number of carbons for phenyl alkylalcohols where we can see that the slope of $log\,K_{MW}$ vs n_c are larger for Em micelles than for SDS micelles and the slope of $log\,P_{SW}$ vs n_c are larger for Em-modified stationary phase. These indicate that the methylene group has larger affinity toward the Em micelles and Em-modified stationary phases than SDS-modified stationary phase.

We also tested some other acidic, basic and neutral compounds including *p*-aminobenzoic acid, pyrogallol, 2-phenylethanol, acetophenone, 2-naphthylamine, 4-cyanophenol and benzoic acid with TTAB hybrid mobile phase on both columns. The binding constants and partition coefficients between the stationary phase and water calculated by using equation (3) and equation (4) are listed in Tables XII and XIII. The solute-TTAB binding constant values are almost the same regardless of the stationary phases. The partition coefficients of solute between bulk solvent and stationary phase are less on C_{18} column than on $C_{18}N^{+}(Me)_2Pr$ column for all the compounds except acetophenone. Because TTAB does not adsorb as much as Em onto the stationary phase, the modified $C_{18}N^{+}(Me)_2Pr$ stationary phase is more polar than modified C_{18} stationary phase. All compounds except acetophenone are polar and they should exhibit more affinity to the polar stationary phase. On the C_{18} column, the compounds having -OH group (i. e., p-aminobenzoic acid, pyrogallol, 2-phenylethanol and 4-cyanophenol) are more associated with micelles than the C_{18} stationary phase when compared to the compounds with nonpolar or less polar groups (i. e., acetophenone, 2-naphthylamine and benzoic acid). Among all compounds, *p*-aminobenzoic acid has the least binding constant with TTAB micelles and benzoic acid has the largest binding constant with TTAB micelles. While on the $C_{18}N^{+}(Me)_2Pr$ column, 2-naphthylamine and benzoic acid have more affinity with the

Table XI. Relationship between K_{MW} (or P_{SW}) and number of carbons for phenylalkyl alcohols using both C_{18} and $C_{18}N^{+}(Me)_2Pr$ stationary phases. Experimental conditions are the same as Tables VI and VII.

mobile phase/	$\log K_{MW} = (slope)n_c + intercept$			$\log P_{SW} = (slope)n_c + intercept$		
stationary phase	*slope*	*intercept*	R	*slope*	*intercept*	R
SDS/C_{18}	0.268	0.558	0.9918	0.301	0.285	0.9881
Em/C_{18}	0.294	0.554	0.9943	0.334	0.379	0.9925
SDS/$C_{18}N^{+}(Me)_2Pr$	0.235	0.600	0.9788	0.313	0.264	0.9972
Em/$C_{18}N^{+}(Me)_2Pr$	0.320	0.412	0.9986	0.336	0.369	0.9995

$C_{18}N^+(Me)_2Pr$ stationary phase than TTAB micelles when compared to the other compounds.

For the above compounds, we also determined their capacity factors with pure hydro-organic mobile phase on both columns, see Table XIV. The polar compounds have larger capacity factors on $C_{18}N^+(Me)_2Pr$ column than on C_{18} column and this means the two columns provide different solute-stationary phase interactions, and $C_{18}N^+(Me)_2Pr$ stationary phase is more polar than C_{18} stationary phase.

Table XIV. Capacity factors with pure hydro-organic mobile phase for both C_{18} and $C_{18}N^+(Me)_2Pr$ stationary phases. Mobile phase, 20 mM sodium phosphate and 10% (v/v) isopropanol, pH = 3.0. Other experimental conditions are the same as Table II.

	C_{18} column	$C_{18}N^+(Me)_2Pr$ column
p-Aminobenzoic acid	0.937	9.342
Pyrogallol	0.743	4.296
2-Phenylethanol	6.821	3.832
Acetophenone	12.44	5.421
2-Naphthylamine	3.648	4.404
4-Cyanophenol	5.797	12.34
Benzoic acid	17.18	NE

NE, no elution.

Chromatographic Behavior of Homologous Series at Various Percentage of Organic Modifier

Schoenmakers et al. [24] proposed in conventional RPC the following relationship between capacity factor and volume fraction of organic modifier (Φ_{org}):

$$\log k' = A\Phi_{org}^2 + B\Phi_{org} + C \qquad (6)$$

where A, B and C are constants which depend on the nature of the solute. However, in the usual range $1 < k' < 10$ and a small range of concentrations of modifier, this equation may be approximated to:

$$\log k' = -S\Phi_{org} + \log k_0' \qquad (7)$$

where S is the solvent strength parameter. Khaledi et al. [25] reported that in hybrid MLC, the linear relationship between $\log k'$ and Φ_{org} is conserved. Recently, Torres-Lapasió et al. [26] proposed a model to describe the variation of solute retention in MLC with Φ_{org}:

$$1/k' = A[M] + B\Phi_{org} + C[M]\Phi_{org} + D \qquad (8)$$

where *[M]* and Φ_{org} are surfactant and alcohol concentration, respectively. At constant concentration of surfactant, $1/k'$ is linearly proportional to the concentration of organic modifier:

$$1/k' = A' + B'\Phi_{org} \qquad (9)$$

We studied the dependence of retention behavior of alkylbenzenes, phenyl alkyl ketones and phenylalkyl alcohols on the percentage of organic modifier (Φ_{org}) in hydro-organic and Em hybrid mobile phases. The linear dependence of $\log k'$ vs Φ_{org} was observed in hydro-organic mobile phase system. From Table XV we can see that S values obtained on $C_{18}N^+(Me)_2Pr$ column are smaller than that on a C_{18} column. This means that

Table XV. Relationship between capacity factor and percentage of organic modifier using hydro-organic mobile phases, $\log k' = -S\Phi_{org} + \log k'_0$. Experimental conditions are the same as Table II.

	C_{18}			$C_{18}N^+(Me)_2Pr$		
	S	$\log k'_0$	R	S	$\log k'_0$	R
			Alkylbenzenes			
Benzene	0.028	1.624	0.9982	0.023	1.065	0.9979
Toluene	0.036	2.211	0.9990	0.030	1.551	0.9992
Ethylbenzene	0.045	2.761	0.9964	0.038	2.009	0.9995
Propylbenzene	NE			0.046	2.495	0.9973
			Phenyl alkyl ketones			
Acetophenone	0.030	1.276	0.9916	0.023	0.773	0.9971
Propiophenone	0.036	1.776	0.9938	0.029	1.184	0.9980
Butyrophenone	0.037	2.009	0.9973	0.035	1.597	0.9979
Valerophenone	0.044	2.527	0.9970	0.043	2.076	0.9976
Hexanophenone	0.0512	3.0530	0.9963	0.0457	2.3605	0.9969
Heptanophenone	NE			0.0525	2.7990	0.9959
			Phenylalkyl alcohols			
Phenylmethanol	0.018	0.469	0.9972	0.022	0.453	0.9958
2-Phenylethanol	0.026	1.007	0.9975	0.020	0.628	0.9999
3-Phenylpropanol	0.030	1.381	0.9994	0.028	1.089	0.9993
4-Phenylbutanol	0.036	1.798	0.9967	0.034	1.506	0.9987
5-Phenylpentanol	0.043	2.283	0.9987	0.042	1.982	0.9973

NE, no elution of higher member of the homologous series.

Table XVI. Relationship between capacity factor and percentage of organic modifier using Em hybrid mobile phases. Other experimental conditions are the same as Table III.

	$\log k' = -S_{hyb}\Phi_{org} + \log k'_0$			$1/k' = A' + B'\Phi_{org}$		
	S_{hyb}	$\log k'_0$	R	B'	A'	R
C_{18}						
			Phenyl alkyl ketones			
Acetophenone	0.031	1.267	0.9919	0.0093	0.0250	0.9995
Propiophenone	0.034	1.721	0.9949	0.0040	0.0051	0.9974
			Phenylalkyl alcohols			
Phenylmethanol	0.036	0.882	0.9898	0.0301	0.0273	0.9994
2-Phenylethanol	0.031	1.180	0.9912	0.0117	0.0292	0.9997
3-Phenylpropanol	0.036	1.598	0.9928	0.0058	0.0048	0.9983
$C_{18}N^+(Me)_2Pr$						
			Phenyl alkyl ketones			
Acetophenone	0.030	1.161	0.9988	0.0119	0.0288	0.9904
Propiophenone	0.033	1.581	0.9991	0.0054	0.0064	0.9868
Butyrophenone	0.036	1.974	0.9994	0.0032	-0.0082	0.9965
			Phenylalkyl alcohols			
Phenylmethanol	0.039	1.001	0.9889	0.0271	0.0013	0.9987
2-Phenylethanol	0.028	1.170	0.9972	0.0102	0.0357	0.9946
3-Phenylpropanol	0.033	1.561	0.9981	0.0055	0.0082	0.9911
4-Phenylbutanol	0.035	1.886	0.9972	0.0035	-0.0072	0.9992

the solvent strength of isopropanol exhibited on a $C_{18}N^{+}(Me)_2Pr$ column is weaker than on a C_{18} column. The retention times of each homologous series are shorter on a $C_{18}N^{+}(Me)_2Pr$ column, especially for nonpolar compounds. Since both columns have about the same surface coverage, the weaker retention on the $C_{18}N^{+}(Me)_2Pr$ column may be attributed to the charged surface of the stationary phase. With Em hybrid mobile phase, the dependence of the capacity factor on the percentage of organic modifier have different relationship on the two columns (Table XVI). For regular C_{18} column, the relationship between $1/k'$ versus Φ_{org} has better linearity, while for the $C_{18}N^{+}(Me)_2Pr$ column, the linearity of the relationship was almost the same whether $\log k'$ vs Φ_{org} or $1/k'$ vs Φ_{org} is considered. Interestingly, the retention times are shorter for phenyl alkyl ketones and are larger for phenylalkyl alcohols on $C_{18}N^{+}(Me)_2Pr$ column than C_{18} column. This may indicate that the $C_{18}N^{+}(Me)_2Pr$ column has a better potential to separate polar and nonpolar solutes simultaneously because it shorten the retention time for nonpolar solutes and increase the retention time for polar solutes. Since the organic modifier does not decrease the equilibrium equally for all solutes tested, the net effect of the modifier is changing the selectivity of the chromatographic system.

Conclusions

The possibility of using $C_{18}N^{+}(Me)_2Pr$-silica based stationary phase in MLC was investigated. This stationary phase can be prepared readily with surface coverage similar to that of traditional C_{18} stationary phase. Different selectivities for phenyl alkyl ketone and phenylalkyl alcohol homologous solutes as well as some acidic and basic compounds were observed. Shorter analysis times for hydrophobic compounds and increased retention for hydrophilic compounds were observed on $C_{18}N^{+}(Me)_2Pr$ column.

REFERENCES

[1] D. W. Armstrong, S. J. Henry, *J. Chromatogr.*, 3 (1980) 657.

[2] P. Yarmchuk, R. Weinberger, R. F. Hirsch, L. J. Cline-Love, *Anal. Chem.*, 54 (1982) 2233.

[3] D. W. Armstrong, R. Q. Terrill, *Anal. Chem.*, 51 (1979) 2160.

[4] J. G. Dorsey, *Adv. Chromatogr.*, 27 (1987) 167.

[5] J. S. Landy, J. G. Dorsey, *J. Chromatogr. Sci.*, 22 (1984) 68.

[6] M. G. Khaledi, E. Peuler, J. Ngeh-Ngwainbi, *Anal. Chem.*, 59 (1987) 2738.

[7] H. Colin, G. Guiochon, *J. Chromatogr. Sci.*, 18 (1980) 54.

[8] H. Colin, G. Guiochon, Z. Yun, J. C. Diez-Masa, P. Jandera, *J. Chromatogr. Sci.*, 21 (1983) 179.

[9] E. Grushka, H. Colin, G. Guiochon, *J. Chromatogr.*, 248 (1982) 325.

[10] P. Jandera, H. Colin, G. Guiochon, *Anal. Chem.*, 54 (1982) 435.

[11] P. Jandera, *J. Chromatogr.*, 314 (1984) 13.

[12] P. Jandera, *Chromatographia*, 19 (1985) 101.

[13] M. J. Rosen, *Surfactants and Interfacial Phenomena,* John Wiley & Sons. New York, 2nd edition., 1988, pp. 109.

[14] D. W. Armstrong, F. Nome, *Anal. Chem.*, 53 (1981) 1662.

[15] I. V. Berezín, K. Martinek, A. K. Yatsimirskii, *Russ. Chem. Rev.,* Eng. Transl., 42 (1973) 787.

[16] M. Arunyanart, L. J. Cline-Love, *Anal. Chem.*, 56 (1984) 1557.

[17] J. P. Foley, *Anal. Chim. Acta*, 231 (1990) 237.

[18] A. Berthod, I. Girard, C. Gonnet, *Anal. Chem.*, 58 (1986) 1356.

[19] M. A. Garca, S. Vera, M. L. Marina, *Chromatographia*, 32 (1991) 148.

[20] F. P. Tomasella, J. Fett, L. J. Cline-Love, *Anal. Chem.*, 63 (1991) 474.

[21] F. P. Tomasella, L. J. Cline-Love, *Anal. Chem.*, 62 (1990) 1315.

[22] P. Yarmchuk, R. Weinberger, R. F. Hirsch, L. J. Cline-Love, *J. Chromatogr.*, 283 (1984) 47.

[23] M. G. Khaledi, *Anal. Chem.*, 60 (1988) 876.

[24] P. J. Schoenmakers, H. A. H. Billiet, L. de Galan, *J. Chromatogr.*, 205 (1981) 13.

[25] M. G. Khaledi, J. K. Strasters, A. H. Godgers, E. D. Breyer, *Anal. Chem.*, 62 (1990) 130.

[26] J. R. Torres-Lapasió, R. M. Villanueva-Camañas, J. M. Sanchis-Mallos, M. J. Medina-Hernández, M. C. García-Alvarez-Coque, *J. Chromatogr.*, 639 (1993) 87.

CHAPTER III

HIGH PERFORMANCE MICELLAR LIQUID CHROMATOGRAPHY WITH SILICA MICROPARTICLES HAVING SURFACE-BOUND CATIONIC SURFACTANT OR OCTADECYL MOIETIES. APPLICATIONS TO THE SEPARATION OF BIOLOGICAL AND ENVIRONMENTAL SPECIES

Abstract

The usefulness of silica microparticles having surface-bound cationic surfactant ligands in MLC is examined and the influence of surfactant type and concentration in the mobile phase on retention and separation selectivity is studied. Different selectivities were observed by using silica microparticles having surface-bound cationic surfactant ligands, such as the quaternary amine of octadecyldimethylpropyl ($C_{18}N^{+}(Me)_2Pr$) when compared to a C_{18}-silica column in the separation of dansyl amino acids (Dns-AA), herbicides, barbiturates and catecholamines by RPC and MLC.

Introduction

Reversed phase chromatography (RPC) using alkyl bonded phases is the most frequently used technique for the separation of non-volatile compounds [1]. As discussed in Chapter I, the use of secondary chemical equilibrium RPC (i.e., micellar liquid

chromatography) as an alternative to conventional RPC, has been proved to be a powerful analytical tool [2-6] since the first report of MLC by Armstrong and Henry [2] in 1980.

In Chapter II, we have characterized chromatographically, over a wide range of conditions, a novel stationary phase which we designed for use in MLC. It is a silica-based stationary phase having surface-bound cationic surfactant. In this Chapter, we wish to report on the utility of this novel stationary phase in the HPLC separation of species of biological and environmental interests, namely dansyl amino acids, some representative barbiturates, catecholamines and some model herbicides. The results are compared to those obtained on the traditional C_{18}-silica stationary phase under otherwise identical conditions.

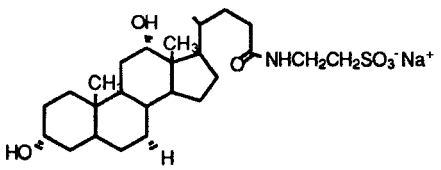
As will be demonstrated in this Chapter, different stationary phases display different retention and selectivity in MLC. This is expected (see Chapter I for more details) since the elution behavior of a solute in MLC is controlled by two competing equilibria [4]: the solute binding to micelles in the mobile phase and its partition onto the stationary phase. In other words, the elution of a solute in MLC depends on three partition coefficients: the partition coefficient between the stationary phase and water (P_{SW}), the partition coefficient between the stationary phase and the micelles (P_{SM}) and the partition coefficient between the micelles and water (P_{MW}). In fact, the monomers of surfactant can adsorb onto the stationary phase through hydrophobic or silanophilic interaction, thus imparting the stationary phase with some ion-exchange capacity or with more hydrophobicity. Since the amount of surfactant molecules (or monomers) adsorbed by the stationary phase is largely influenced by the nature and concentration of the ligand on the surface of the sorbent, P_{SM} and P_{SW} will be affected when varying the nature of the stationary phase ligand. This in turn will affect retention and selectivity in MLC.

To provide a better understanding of the behavior of the novel stationary phase under investigation, the effects of the nature of surfactants and mobile phase composition were also examined.

Experimental

Reagents, stationary phases and column packing

For silica gels used in this study as well as reagents for surface modification and reagents for mobile phase preparation, see Experimental in Chapter II. Taurodeoxycholate sodium (NaTDC) was purchased from Calbiochem (LaJolla, CA). The structure of this anionic surfactant is shown below:



The CMC of NaTDC is reported to be 1-4 mM. The structures, CMCs, and nature of other surfactants used in this work are shown in Table I in Chapter II. Dansyl-L-amino acids (Dns-AA), barbiturates and catecholamines were obtained from Sigma (St. Louis, MO). Herbicides were from Chem Service (West Chester, PA). The preparation of stationary phases and column packings are reported in Chapter II.

Apparatus

The apparatus and columns used in this study are the same as those described in Chapter II. The detection wavelength was set at 230 nm for herbicides, 245 nm for urea herbicides and 254 nm for all the other solutes.

Procedures

Micellar mobile phases were prepared by dissolving the appropriate amount of surfactants and NaH_2PO_4 in water and then adjusting the pH to the desired value. Two mobile phases, A and B, were prepared. They contained the same amount of phosphate buffer and surfactant, but B contained 50% (v/v) isopropanol (2-PrOH). gradient I consisted of 15 min at linearly increasing 2-PrOH concentration from 20% solvent B (i.e., 10% v/v 2-PrOH) to 100% solvent B (i.e., 50% v/v 2-PrOH) while gradient II was performed for 15 min at linearly increasing 2-PrOH concentration from 30% solvent B (i.e., 15% v/v 2-PrOH) to 50% solent B (i.e., 25% v/v 2-PrOH).

Distilled water was used in mobile phase preparations which were filtered through a S/P^{TM} filter paper Grade 360, qualitative from Baxter (McGaw Park, IL). Stock sample solutions were prepared by dissolving pure compounds in 50% (v/v) isopropanol in water. The sample solutions were prepared by diluting the stock solutions with the mobile phase solutions.

Results and Discussion

Since different types of interactions (such as electrostatic and hydrophobic) and competing equilibria are operating in MLC (see Chapter I), it is obvious that the nature and concentration of the surfactant and the type of stationary phase, as well as the concentration and type of organic modifier in the mobile phase have profound effects on retention and selectivity [13, 14].

The retention behavior of dansyl amino acids (Dns-AA), herbicides, catecholamines and barbiturates on $C_{18}N^+(Me)_2Pr$ column were examined under various conditions using hydro-organic eluents with or without micellar phases. In the following sections, the

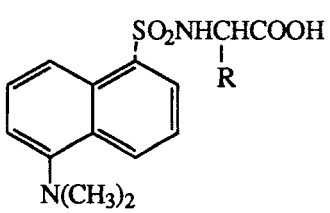
results of these studies are discussed and compared to those obtained on the C_{18} column under the same operating conditions.

Comparison of C_{18} and $C_{18}N^+(Me)_2Pr$ Stationary Phases

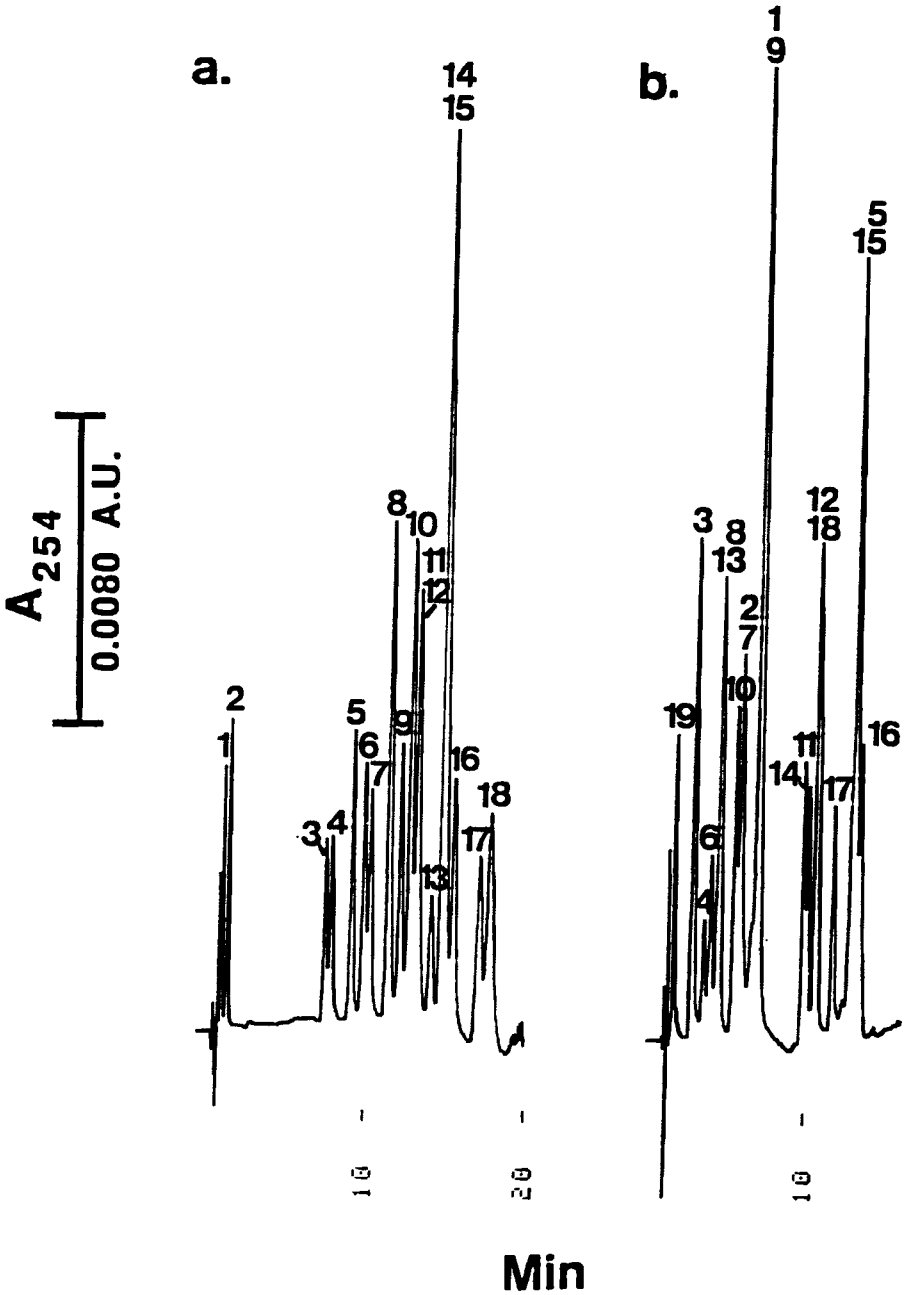
a) Hydro-organic eluents. Dns-AA, and typical herbicides and barbiturates were used as the test solutes to compare the stationary phase with surface-bound cationic surfactant ($C_{18}N^+(Me)_2Pr$) to the traditional C_{18} sorbent in terms of retention, selectivity and the overall elution pattern. As shown in chapter II, both sorbents had the same surface coverage in ligand, i. e., 2.1 μmole ligands/m^2 of silica.

Figure 1a and b shows chromatograms of Dns-AA obtained on $C_{18}N^+(Me)_2Pr$ and C_{18} columns, respectively, by using hydro-organic eluents at pH 2.5. Different elution orders of the solutes, i.e., change in selectivity, can be observed on the $C_{18}N^+(Me)_2Pr$ column when compared to the C_{18} column under otherwise identical elution conditions. In addition, $C_{18}N^+(Me)_2Pr$ column afforded higher peak capacity than traditional C_{18}-silica column, meaning that the number of resolved peaks is higher. This is may be due in part to the fact that with the $C_{18}N^+(Me)_2Pr$ stationary phase, electrostatic interaction are superimposed on hydrophobic interaction.

The general chemical structure of the Dns-AA is



where R is the side chain group. According to studies on the ionization of Dns-AA [15, 16], the pK_a value of the dimethyl amino group of Dns-AA, i.e., for the protonated form $(CH_3)_2N^+HC_{10}H_6SO_2NH$-AA, is between 3.0 and 4.0, and this value is largely



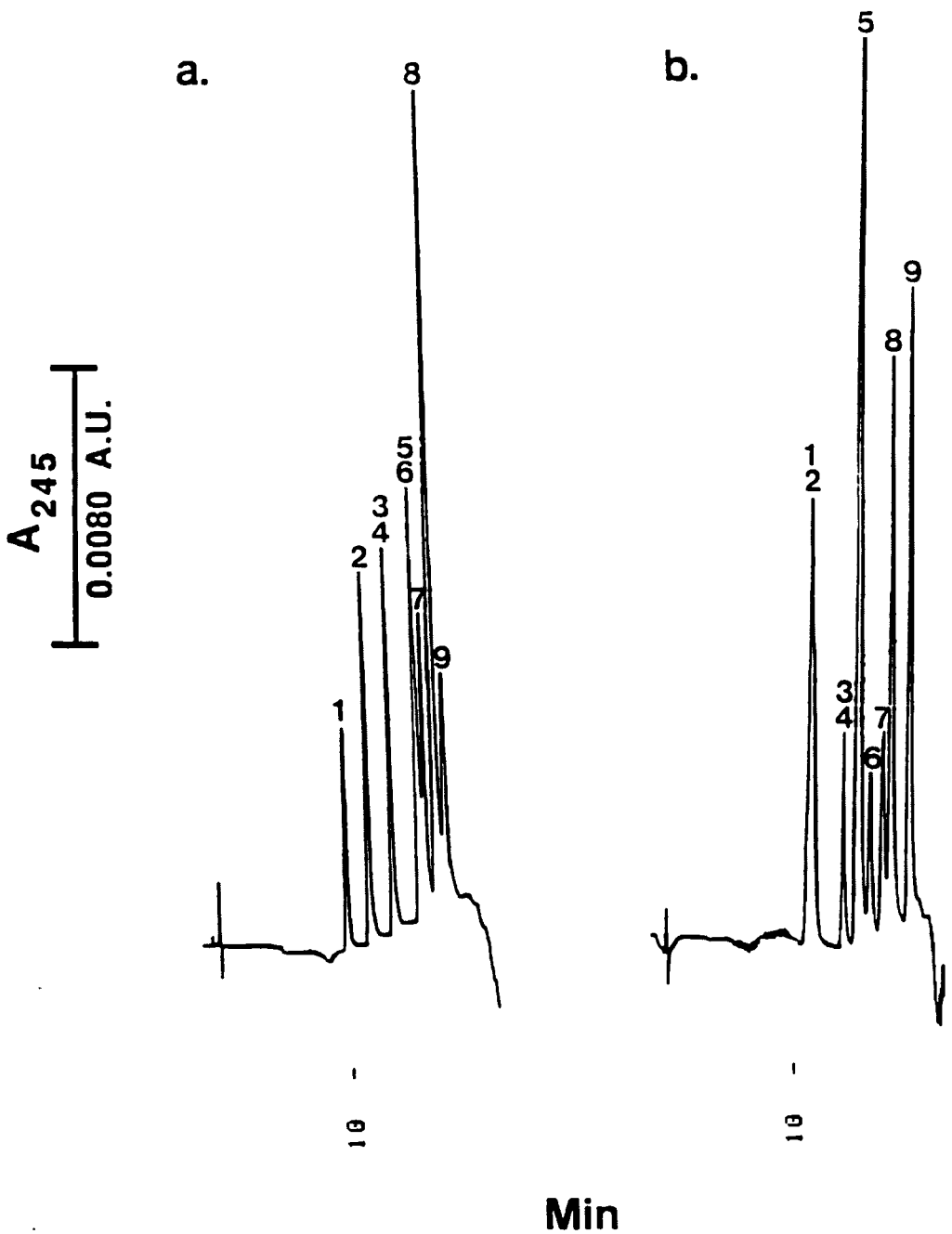
Dns-AA: 1, arginine; 2, lysine; 3, asparagine; 4, glutamine; 5, tyrosine; 6, serine; 7, threonine; 8, glutamic acid; 9, alanine; 10, glycine; 11, proline; 12, valine; 13, aspartic acid; 14, methionine; 15, isoleucine; 16, leucine; 17, phenylalanine; 18, tryptophan; 19, cysteic acid.

Figure 1. Chromatograms of Dns-AA obtained on $C_{18}N^{+}(Me)_2Pr$ column (a) and C_{18} column (b). Columns, 10 x 0.46 cm ID. Linear gradient, 15.0 min from 10 to 50% (v/v), followed by 1.0 min from 50 to 10% and 10 min equilibration time with 10% (v/v) isopropanol in 20.0 mM sodium phosphate, pH 2.5. The mobile phase flow-rate was 1.0 mL/min.

independent of the ionic properties of the side chain of the amino acids. The amino group adjacent to the sulfonyl group of the dansyl moiety has a pK_a value of 11.7, i.e., for the deprotonated form $(CH_3)_2NC_{10}H_6SO_2N^-$-AA, and would dissociate only at extreme alkaline pH. The pK_a value of the α-carboxyl group of each amino acid is around 4.8 [16]. At the pH of the experiment (i. e., pH = 2.5), the dimethyl amino group is positively charged (fully protonated), the amino group adjacent to the sulfonyl group is uncharged and the α-carboxyl group is neutral. As expected, on the C_{18} column (see Fig. 1b), the Dns-AA with nonpolar side chains were eluted after polar amino acids and they emerged out of the column in the order of increasing hydrophobic character of the side chain, i.e., glycine < alanine < valine < leucine. The elution order of the Dns-AA with polar side chain was influenced by the polarity of the solute. Less hydrophobic and charged amino acids, e.g., cysteic acid, eluted first. Asparagine and glutamine each carrying a side chain acetamido group (polar groups) and short alkyl chain eluted thereafter. Although lysine and arginine are doubly positively charged, they were more retarded due to their relatively stronger hydrophobic character.

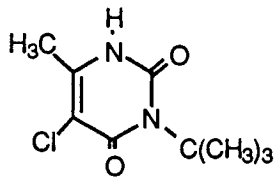
In the case of the $C_{18}N^+(Me)_2Pr$ column (see Fig. 1a), the surface of which is positively charged, cysteic acid whose net charge is zero, but it has a negatively charged group (sulfonic acid group) at the pH of the experiment could not be eluted because of strong electrostatic attraction between positively charged stationary phase and negatively charged sulfonic acid group. The doubly positively charged solutes, i.e., arginine and lysine, eluted first due to their stronger electrostatic repulsion from the positively charged stationary phase. On $C_{18}N^+(Me)_2Pr$ column, most solutes exhibited longer retention time and in turn better separation than on C_{18} column. In addition, the $C_{18}N^+(Me)_2Pr$ column exhibited a unique seletivity toward the Dns-AA.

Figure 2a and b shows the chromatograms of 9 urea herbicides, namely terbacil, monuron, fluometuron, metobromuron, diuron, linuron, chloroxuron and neburon (for structures, see below), obtained on both $C_{18}N^+(Me)_2Pr$ and C_{18} columns at pH 4.0,

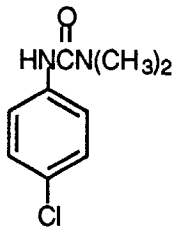


Urea herbicides: 1, terbacil; 2, monuron; 3, fluometuron; 4, metobromuron; 5, diuron; 6, linuron; 7, siduron; 8, chloroxuron; 9, neburon.

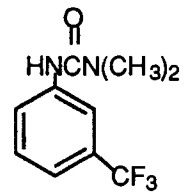
Figure 2. Chromatograms of urea herbicides obtained on $C_{18}N^{+}(Me)_2Pr$ column (a) and C_{18} column (b). Mobile phase solutions pH 4.0. Other conditions are as in Fig. 1.



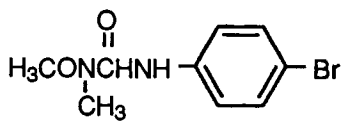
Terbacil



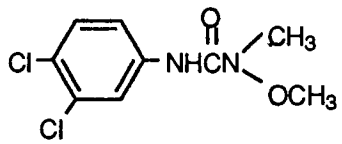
Monuron



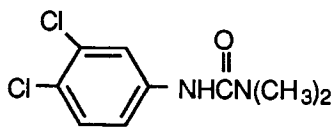
Fluometuron



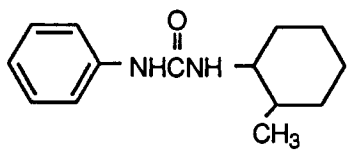
Metobromuron



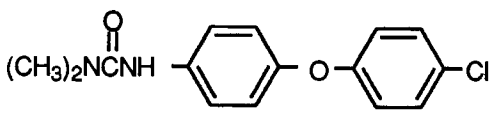
Linuron



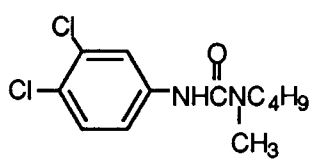
Diuron



Siduron



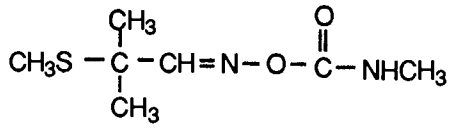
Chloroxuron



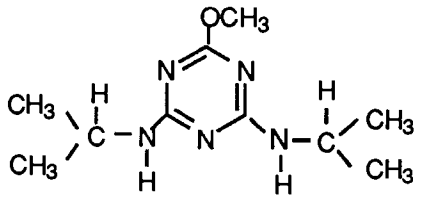
Neburon

respectively. It can be seen that the analysis time is shorter on $C_{18}N^+(Me)_2Pr$ column because of the weaker interaction between these nonionic species and the mildly hydrophobic $C_{18}N^+(Me)_2Pr$ stationary phase. In addition, different selectivities were observed between terbacil and monuron, and between linuron and diuron.

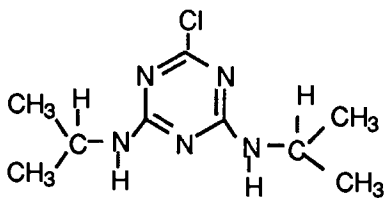
In another set of experiments, seven herbicides including aldicarb, prometon, propazine, prometryne, diazinon, parathion and 2,4-D butyl ester (for structures and pK_a values, see below) can be separated with baseline resolution on $C_{18}N^+(Me)_2Pr$ column (Fig. 3a), while only five solutes can be resolved on C_{18} column with different elution



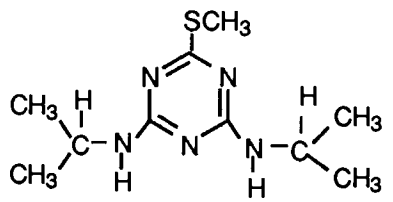
Aldicarb



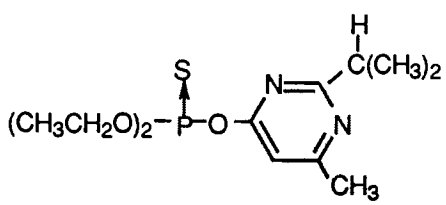
Prometon
$pK_a = 4.2$



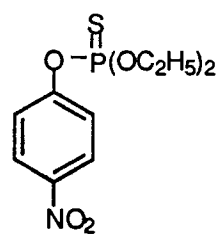
Propazine
$pK_a = 1.85$



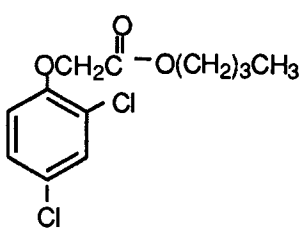
Prometryne
$pK_a = 4.05$



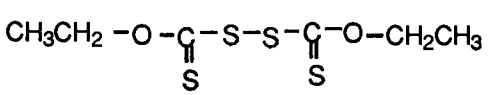
Diazinon



Parathion

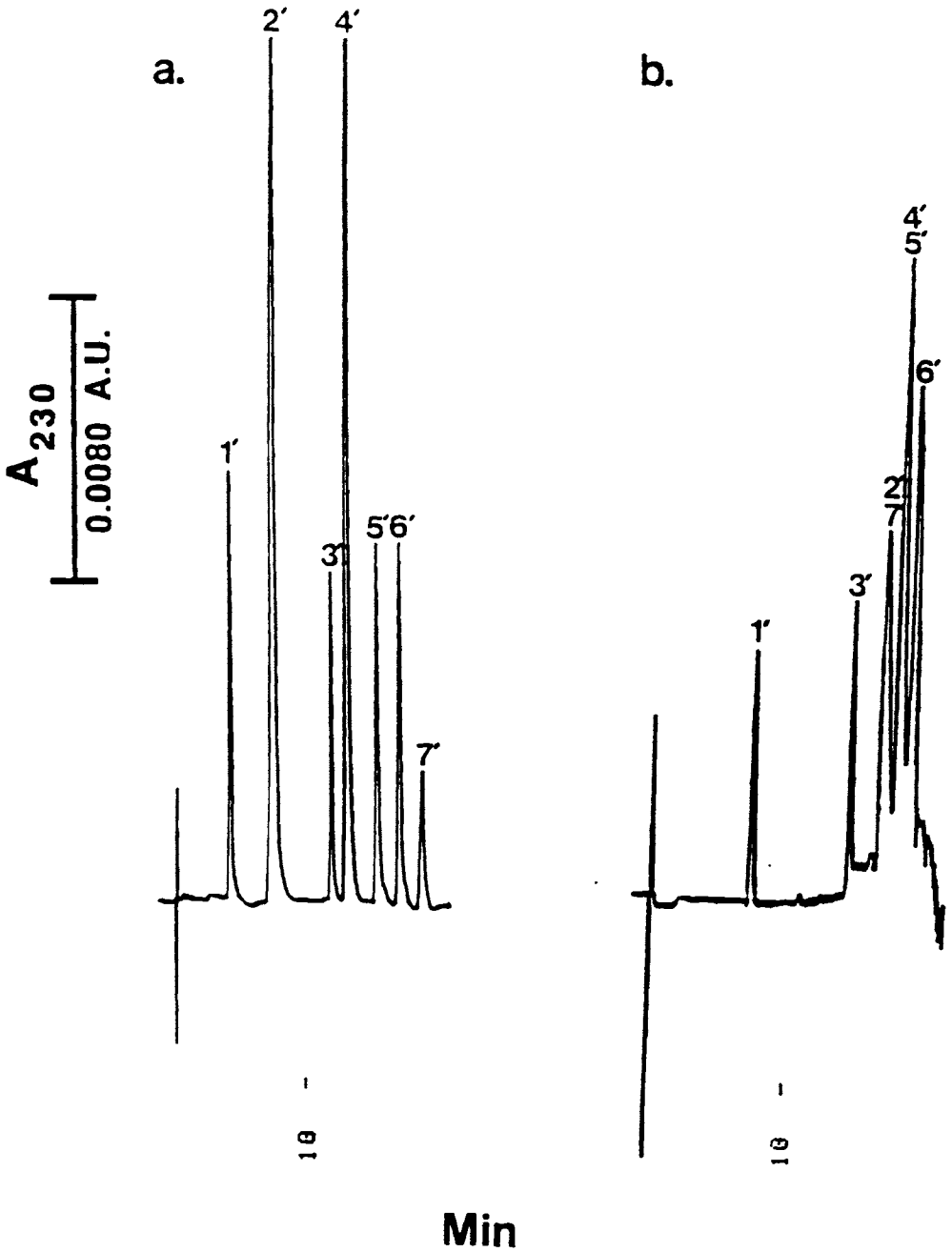


2,4-D butyl ester



Diethyl dithiobis

order and selectivity (Fig. 3b). It should be noted that prometon and 2,4-D butyl ester, prometryne and diazinon coeluted on the C_{18} column. On $C_{18}N^+(Me)_2Pr$ column, with the exception of 2,4-D butyl ester which has larger retention time, all other solutes moved



Herbicides: 1', aldicarb; 2', prometon; 3', propazine; 4', prometryne; 5', diazinon; 6', parathion; 7', 2,4-D butyl ester.

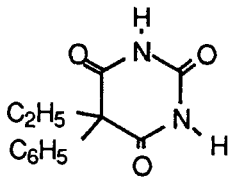
Figure. 3. Chromatograms of herbicides obtained on $C_{18}N^{+}(Me)_2Pr$ column (a) and C_{18} column (b). Experimental conditions are as in Fig. 2.

down the column much faster than on the C_{18} column, especially prometryne and prometon, because these solutes are slightly positively charged at pH 4.0, thus undergoing repulsion from $C_{18}N^+(Me)_2Pr$ stationary phase.

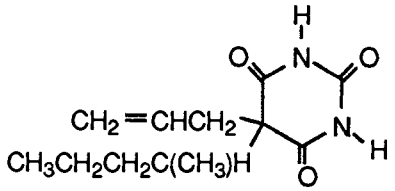
Using a $C_{18}N^+(Me)_2Pr$ with a hydro-organic mobile phase, the separation of barbiturates (for structures and pK_a values, see below), such as barbital sodium, phenobarbital, secobarbital, hexobarbital, butabarbital sodium, amobarbital and pentobarbital sodium can be achieved, see Figure 4. Barbiturates are weak acids having pK_a values higher than 7, so at pH 2.5 they are neutral and expected to be eluted faster than on a C_{18} column.

In summary, a $C_{18}N^+(Me)_2Pr$ stationary phase under investigation has different interactions with solutes than the C_{18} column, thus providing a method to improve separation and selectivity of some ionic and nonionic compounds. The difference in selectivity exhibited by a $C_{18}N^+(Me)_2Pr$ column may be attributed to the superimposition of polar interactions over nonpolar association of the solutes with the positively charged, hydrophobic ligand of the stationary phase.

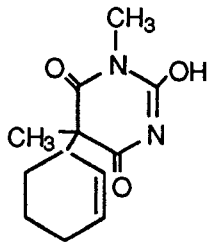
b) Micellar hydro-organic (i. e., hybrid) eluents. When Empigen (Em) was added to the hydro-organic mobile phase at pH 2.5, the retention of various Dns-AA solutes under investigation decreased slightly on the $C_{18}N^+(Me)_2Pr$ column when compared to the retention observed with the hydro-organic mobile phase but without the surfactant (i. e., without Em); compare Fig. 5a to Fig. 1a. This reduction in retention is the result of electrostatic repulsion between equally charged solute and surfactant-modified stationary phase. At pH 2.5, both the surfactant and the Dns-AA are positively charged. The degree of reduction in retention varied among the various solutes and was largely dependent on the hydrophilic-hydrophobic balance of the solute. The retention of Dns-AA solutes of relatively large hydrophobicity such as tryptophan was not as much affected as that of weakly hydrophobic solutes such as serine and asparagine. The difference in the degree of



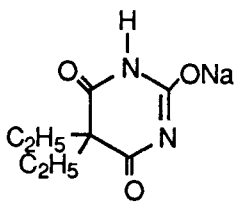
Phenobarbital
$pK_a = 7.4$



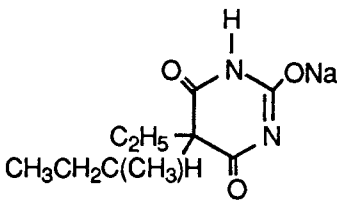
Secobarbital
$pK_a = 8.1$



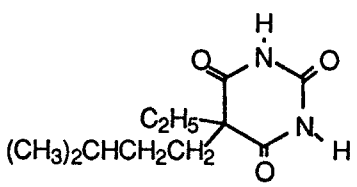
Hexobarbital
$pK_a = 8.4$



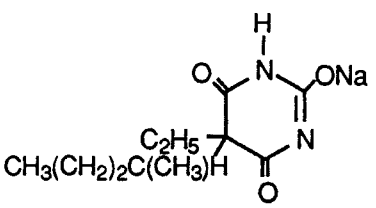
Barbital sodium
$pK_a = 8.2$



Butabarbital sodium



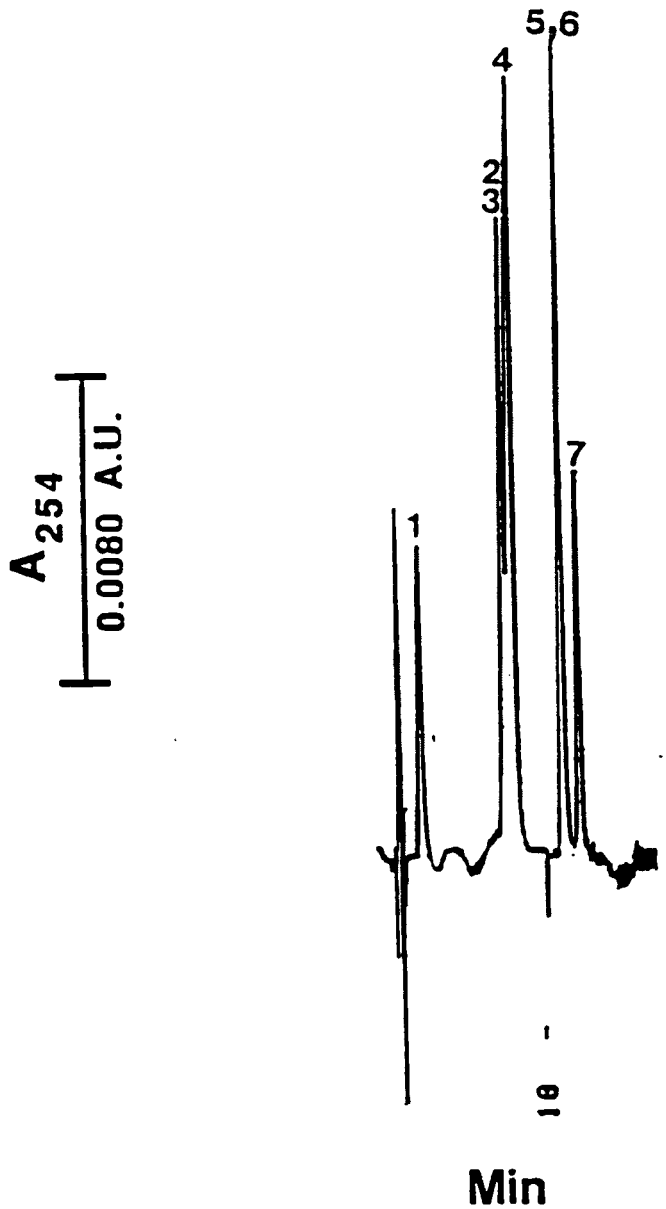
Amobarbital



Pentobarbital sodium
$pK_a = 8.1$

repulsion/hydrophobic interaction may explain why the elution order of valine/aspartic acid and tyrosine/serine were reversed when Em was added to the mobile phase (compare Fig. 5a to Fig. 1a).

Using the same hydro-organic mobile phase containing Em but raising the pH to 6.0, all Dns-AA with nonpolar side chains could not be eluted and only some of the Dns-



Barbiturates: 1, barbital sodium; 2, phenobarbital; 3, butabarbital sodium; 4, hexobarbital; 5, amobarbital; 6, pentobarbital sodium; 7, secobarbital.

Figure 4. Chromatogram of barbiturates obtained on $C_{18}N^{+}(Me)_2Pr$ column. Experimental conditions are as in Fig. 1.

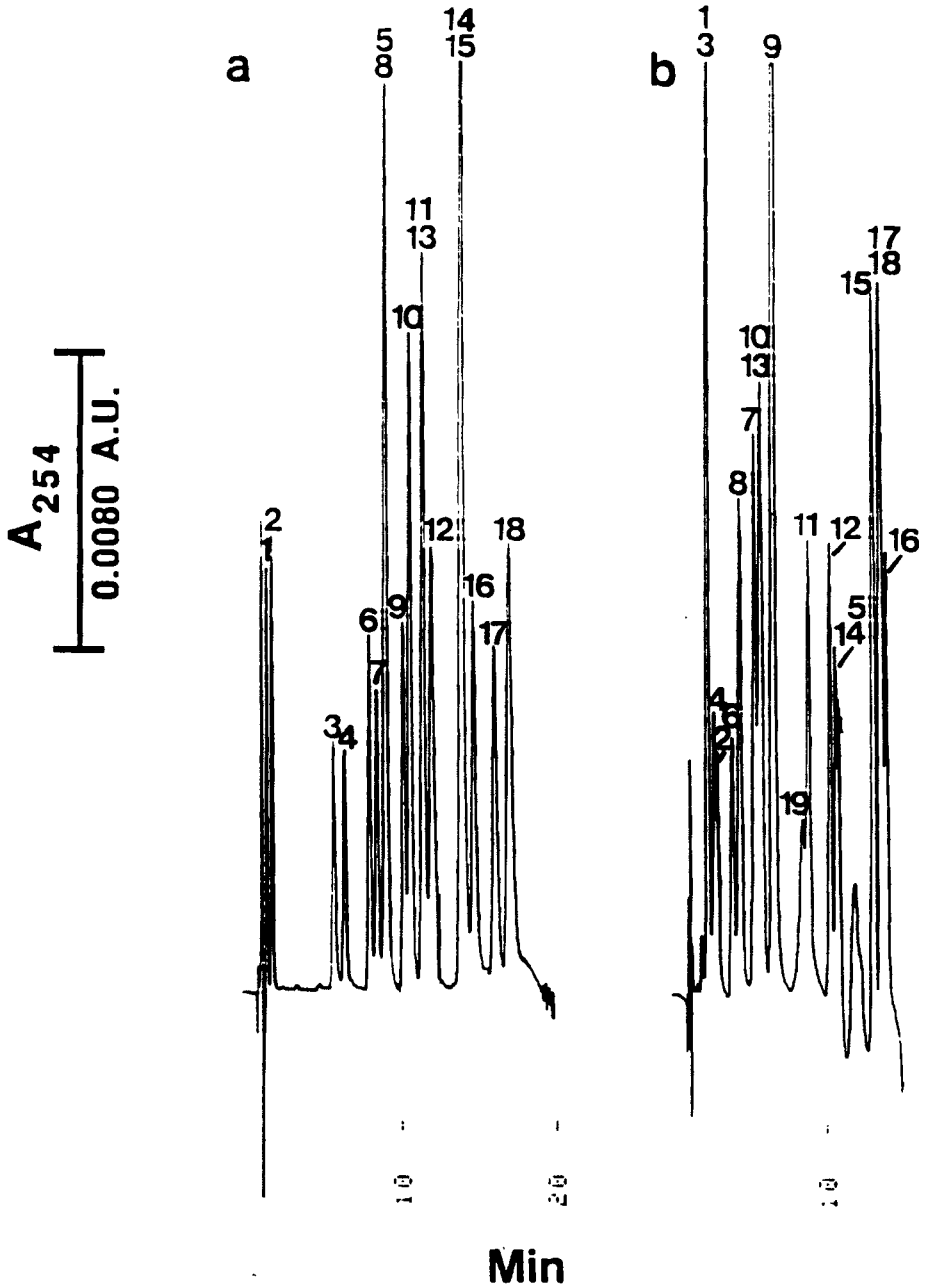


Figure 5. Chromatograms of Dns-AA obtained on $C_{18}N^{+}(Me)_2Pr$ column (a) and C_{18} column (b). Columns, 10 x 0.46 cm ID. Linear gradient, 15.0 min from 10 to 50% (v/v), followed by 1.0 min from 50 to 10% and 10 min equilibration with 10% (v/v) isopropanol in 5.0 mM Em and 20.0 mM sodium phosphate, pH 2.5. The mobile phase flow-rate was 1.0 mL/min. Solutes are as in Fig. 1.

AA with polar side chains, i.e., serine, threonine, asparagine, glutamine, tyrosine, lysine and arginine, were eluted. For the Dns-AA that eluted at pH 6.0, their retention times were longer than at pH 2.5. This indicates that the electrostatic interaction between each dansyl amino acid and stationary phase is stronger at higher pH. At higher pH values, i.e., pH 6.0, the carboxyl group of Dns-AA is negatively charged and has stronger electrostatic attraction with surfactant modified-stationary phase. Under this condition, the hydrophobic Dns-AA with nonpolar side chains underwent both hydrophobic and electrostatic interactions, and as a result they were retarded longer. This dual interaction mechanism provided even stronger interaction when the solute possessed two carboxyl groups (aspartic and glutamic acids) and as a result they could not be eluted. At a lower pH, i.e., pH 2.5, however, where the Dns-AA are positively charged, the electrostaic repulsion from the modified-stationary phase overshadowed the hydrophobic interaction, and were eluted in shorter time.

Figure 5b illustrates the chromatogram of Dns-AA obtained on C_{18} column under the same mobile phase conditions as in Fig. 5a. With C_{18} column, the surfactant (i.e., Em) is adsorbed onto the stationary phase (see Chapter I), thus forming surfactant modified-stationary phase. Under this condition, and comparing to a C_{18} that was used with a hydro-organic mobile phase (Fig. 1b), the doubly positively charged solutes, such as lysine and arginine eluted earlier because of the electrostatic repulsion from the surfactant modified-stationary phase. Also, tyrosine, glutamine and asparagine eluted earlier due to their weak hydrophobicity. On the other hand, Dns-AA with hydrophobic side chains such as phenylalaline and tryptophan were retained more (Fig. 5b). When comparing to a $C_{18}N^{+}(Me)_2Pr$ column that was eluted with an Em hybrid mobile phase (Fig. 5a), most of the Dns-AA yielded reduced retention time on the C_{18} column except tyrosine and lysine. Cysteic acid which can not be eluted on a $C_{18}N^{+}(Me)_2Pr$ column, could be eluted here. As can be seen in Fig. 5, the $C_{18}N^{+}(Me)_2Pr$ column provided a better overall resolution between the various solutes. In fact, 15 Dns-AA were baseline resolved on the

$C_{18}N^+(Me)_2Pr$ column as opposed to only 14 Dns-AA partially resolved on the C_{18} column. This may be attributed to the higher coating of the C_{18}-silica surface with the Em surfactant as compared to the $C_{18}N^+(Me)_2Pr$-silica surface, thus rendering the former column more repulsive than the later. The higher binding of the Em surfactant to the C_{18} surface may be explained by the absence of similarly charged moieties as in the case of the $C_{18}N^+(Me)_2Pr$ column, since both stationary phases (i.e., C_{18} and $C_{18}N^+(Me)_2Pr$) possess the same surface coverage with ligands..

Figure 6a and b displays the chromatograms of herbicides obtained on a $C_{18}N^+(Me)_2Pr$ column using hydro-organic eluents containing Em at pH 4.0. When compared to Fig. 2a, the retention of urea herbicides shown in Fig. 6a was little or not affected since these solutes are nonionic. Also, using the same column and mobile phase, the herbicides shown in Fig. 6b and 3a behaved similarly regardless of the presence or absence of Em in the hydro-organic eluent. At this pH, the weakly basic herbicides (prometon and prometryne) were only slightly ionized and did not undergo extensive electrostatic repulsion with the micelle and/or the surfactant modified-stationary phase. On the C_{18} column and using Em as mobile phase micelles (see Fig. 7), all urea herbicides were less retained when compared to the case of the C_{18} column without the Em surfactant present in the mobile phase. However, the surfactant has no effect on the elution order (compare Fig. 7 to Fig. 2b). When compared to the case of the $C_{18}N^+(Me)_2Pr$ column, the presence of the Em surfactant has different effect on the retention and selectivity of urea herbicides on the C_{18} column (compare Fig. 7 to Fig. 6a).

On the other hand, polar and highly positively charged catecholamines (structures shown below) could not be retained on the $C_{18}N^+(Me)_2Pr$ column.

With a $C_{18}N^+(Me)_2Pr$ column and adding SDS instead of Em to the mobile phase, the Dns-AA behaved differently (see Fig. 8a). Under this condition, the positively charged quaternary ammonium groups of the stationary phase form ion-pairs with the oppositely charged SDS. In addition, the surfactant is adsorbed onto the stationary phase thus

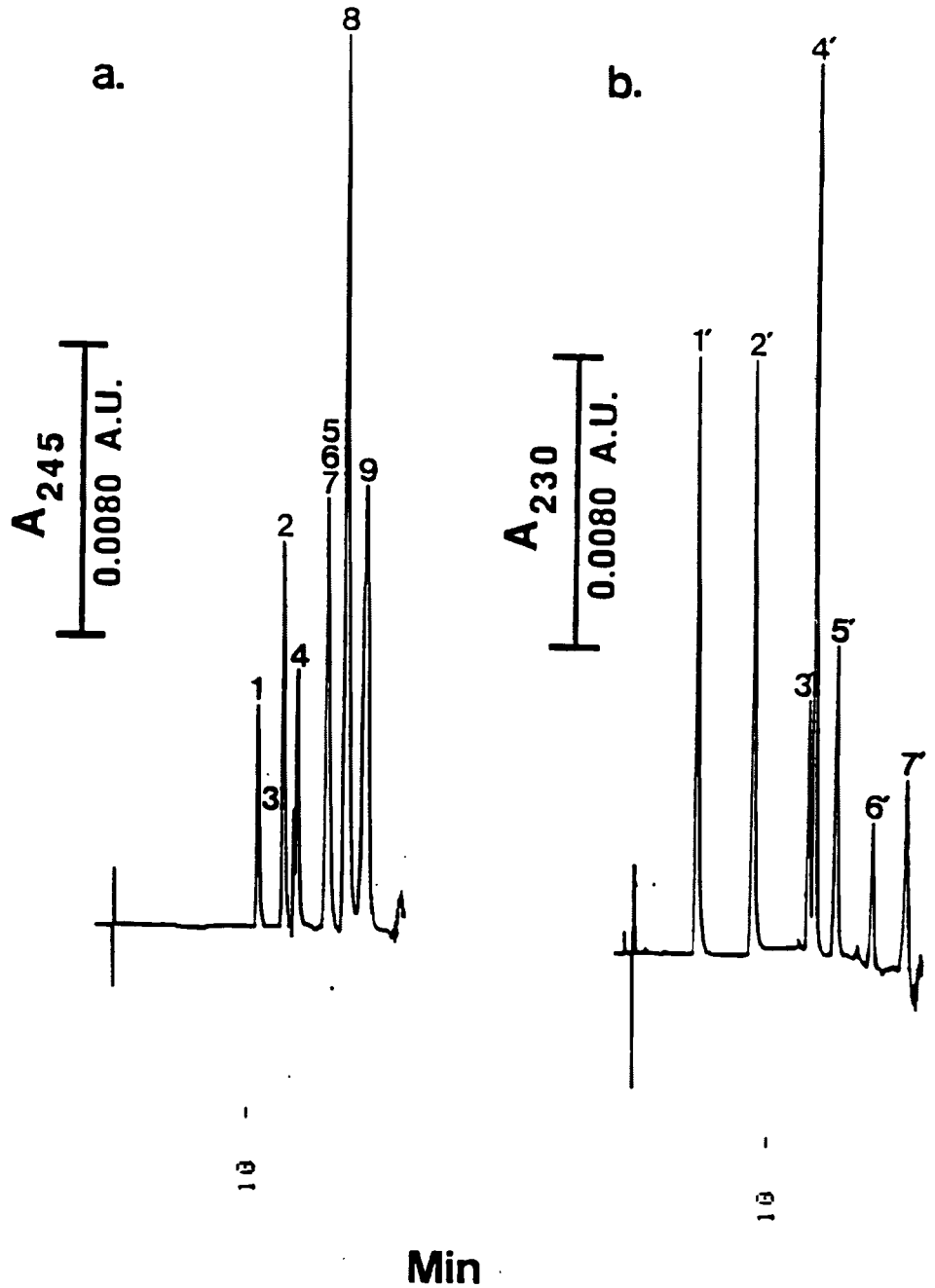


Figure 6. Chromatograms of urea herbicides (a) and other herbicides (b) obtained on $C_{18}N^{+}(Me)_2Pr$ column. Mobile phase solution pH 4.0. Other conditions are as in Fig. 5. Solutes are as in Figs 2 and 3.

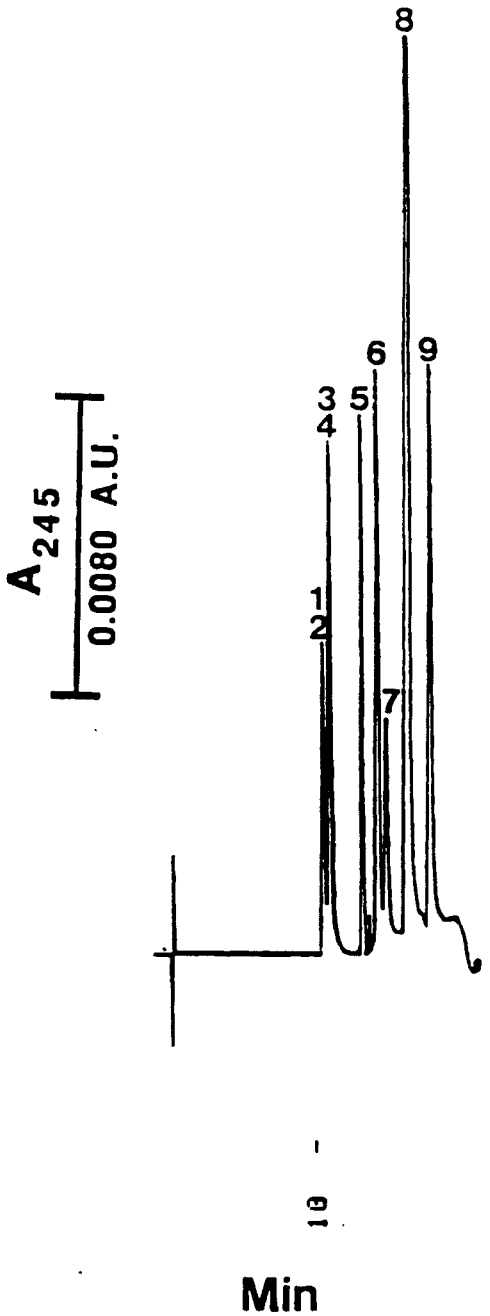
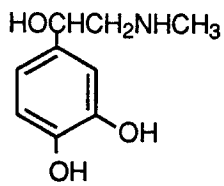
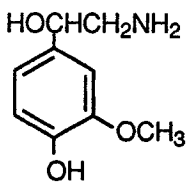


Figure 7. Chromatogram of urea herbicides obtained on C_{18} column. Mobile phase solution pH 4.0. Other conditions are as in Fig. 5 and solutes are as in Fig. 2.

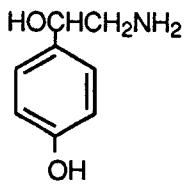


Epinephrine

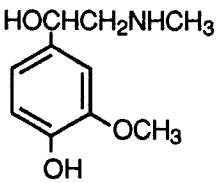
Dopa



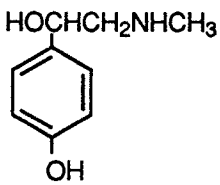
Normetanephrine



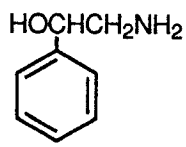
Octopamine



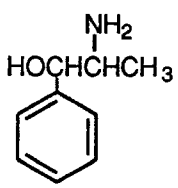
Metanephrine



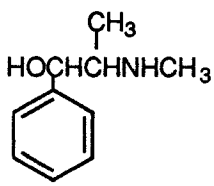
Synephrine



β-hydroxyphenethylamine



Phenylpropanolamine



Ephedrine

yielding an SDS-modified stationary phase. At pH 2.5, Dns-AA with strong side chain acidic groups will repulse from the negatively charged SDS-modified stationary phase, and therefore their retention will be reduced. This is the case of cysteic acid whose strong sulfonic acid group is ionized at any pH. On the other hand, the retention of the doubly positively charged Dns-AA such as lysine and arginine increased and they were retarded much longer in the presence than in the absence of the SDS micellar phase, compare Fig. 8a to Fig. 1a. When comparing with Em, the same reason can explain that most solutes were more retained, such as lysine, arginine, tyrosine and tryptophan while phenylalanine

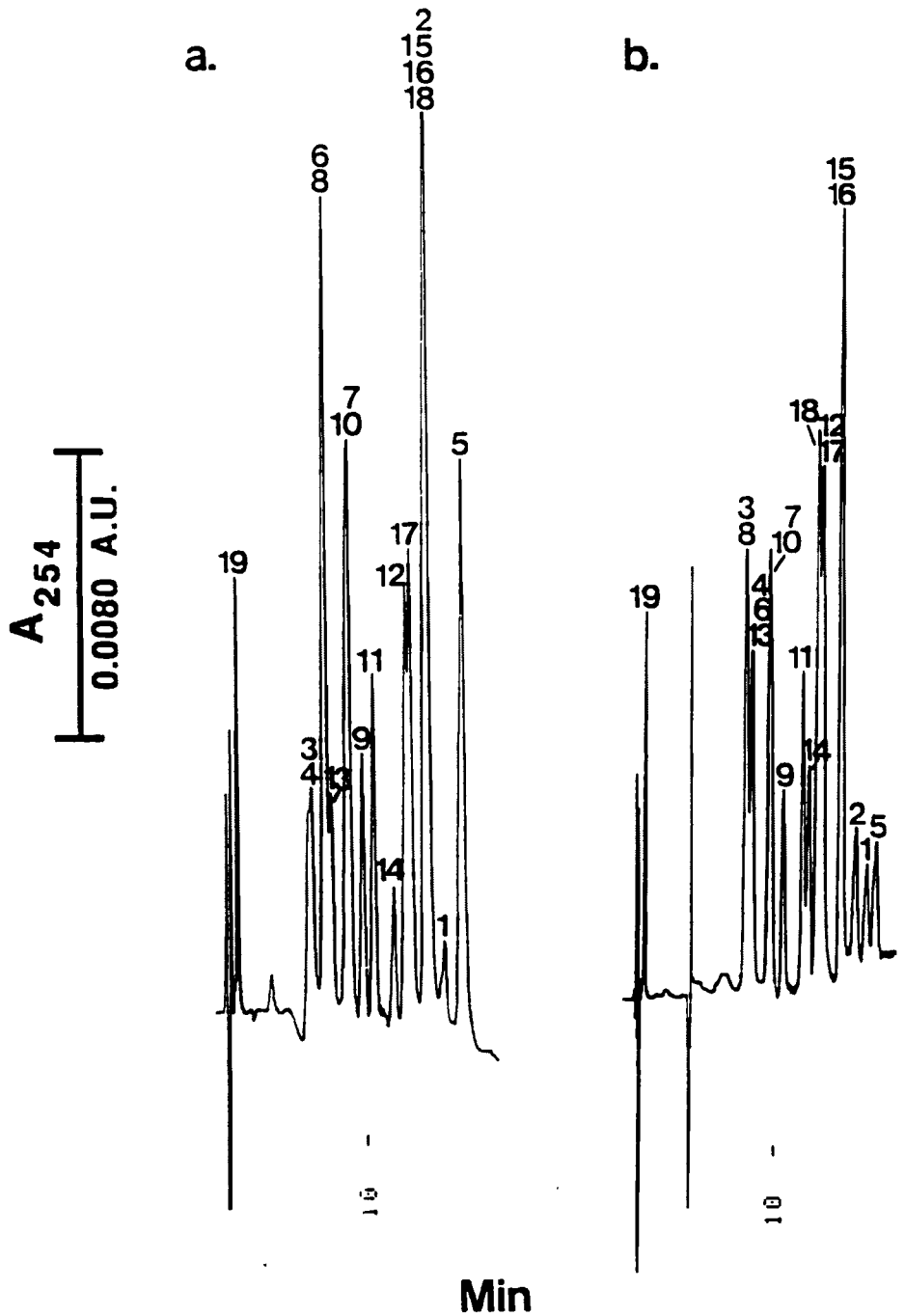


Figure 8. Chromatograms of Dns-AA obtained on $C_{18}N^{+}(Me)_2Pr$ column (a) and C_{18} column (b). Columns, 10 x 0.46 cm ID. Linear gradient, 15.0 min from 10 to 50% (v/v), followed by 1.0 min from 50 to 10% and 10 min equilibration with 10% (v/v) isopropanol in 20.0 mM SDS and 20.0 mM sodium phosphate, pH 2.5. The mobile phase flow-rate was 1.0 mL/min. Solutes are the same as in Fig. 1.

and aspartic acid were slightly less retained. Thus, the chromatography of ionic compounds with anionic micellar eluents can be very different from that with cationic surfactants with the same chain lengths (compare Fig. 8a to Fig. 5a). A similar observation was reported by Yarmchuk and co-workers [17]; they found different selectivities for nitrobenzene, 2-naphthol and toluene, using SDS and dodecyltrimethylammonium bromide (DTAB).

On a C_{18} column, and in the presence of SDS in the hydro-organic eluent, the elution order is almost the same as with a $C_{18}N^+(Me)_2Pr$ column but with different selectivity. An exception is that tryptophan eluted earlier on a C_{18} column (see Fig. 8b)

Using a $C_{18}N^+(Me)_2Pr$ column, urea herbicides terbacil and fluometuron eluted faster while siduron and neburon eluted slower when SDS was present as compared to the absence of SDS, so that fluometuron was separated from metobromuron and elution order of siduron and chloroxuron was reversed (Fig. 9a to Fig. 2b). Prometon and prometryne solutes each carrying a positive charge migrated much slower due to the electrostatic attraction to the surfactant-modified stationary phase and eluted after propazine and diazinon, respectively (Fig. 9b). The overall separation of 16 herbicides is illustrated in Fig. 10 whereby 12 of them were resolved.

On a C_{18} column, nine urea herbicides were completely separated using SDS as the micellar mobile phase (see Fig. 11a). As can be seen not only the retention time of each urea herbicides was reduced, but also monuron and metobromuron were separated from terbacil and fluometuron, respectively, when compared to the case without SDS (Fig. 2b). When comparing to a $C_{18}N^+(Me)_2Pr$ column with an SDS hybrid mobile phase, the retention of diuron and chloroxuron were reduced even more, so that diuron was separated from linuron and chloroxuron was separated from siduron, respectively.

Another set of seven herbicides (namely aldicarb, prometon, propazine, prometryne, diazinon, parathion and 2,4-D butyl ester) was chromatographed on a C_{18} column (Fig. 11b) in the presence of an SDS micellar mobile phase. The weakly ionized

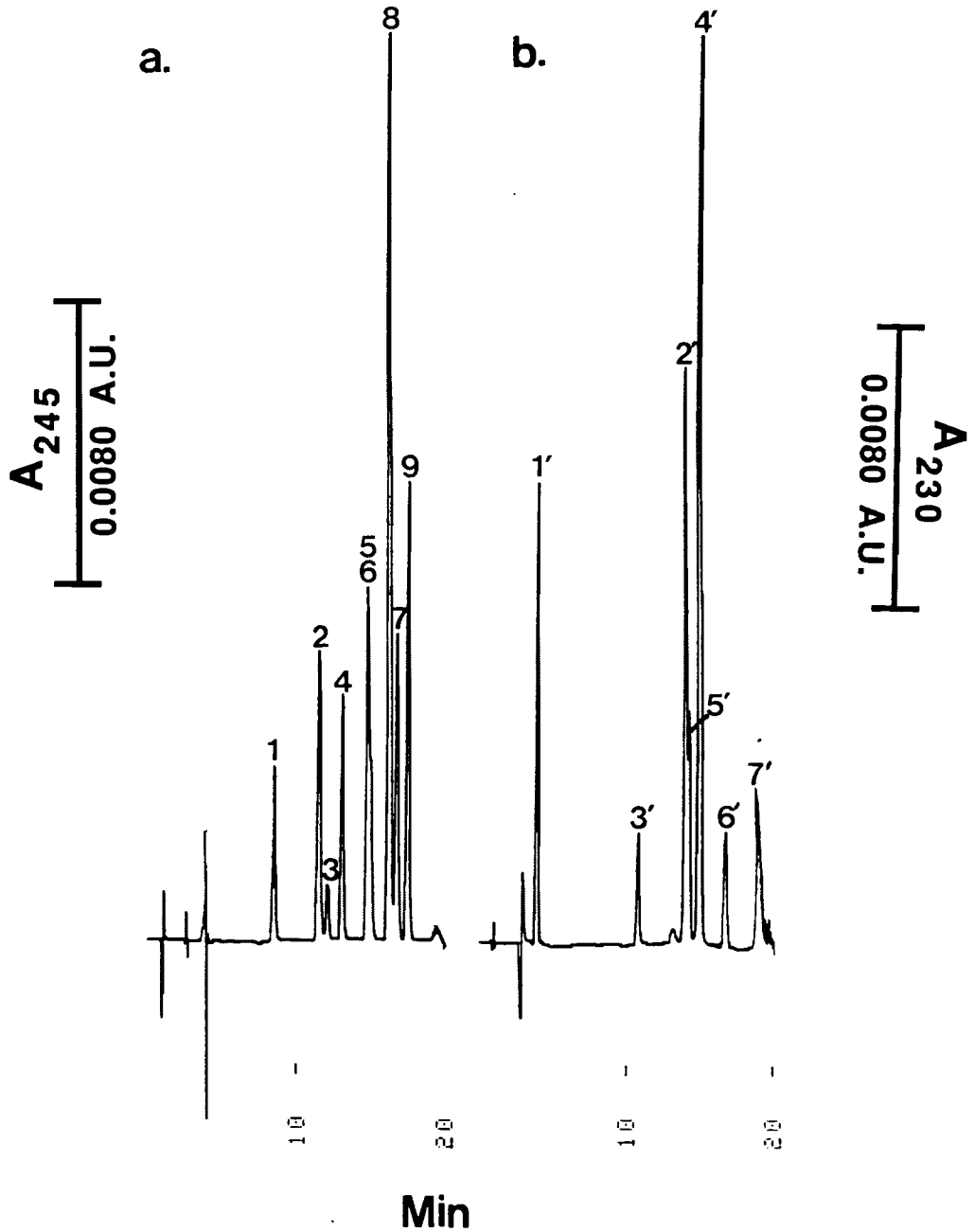


Figure 9. Chromatograms of urea herbicides (a) and other herbicides (b) obtained on $C_{18}N^{+}(Me)_2Pr$ column. Column, 10 x 0 46 cm ID. Linear gradient, 15.0 min from 10 to 50% (v/v), followed by 1.0 min from 50 to 10% and 10 min equilibration with 10% (v/v) isopropanol in 15.0 mM SDS and 20.0 mM sodium phosphate, pH 4.0. The mobile phase flow-rate was 1.0 mL/min. Solutes are as in Figs 2 and 3.

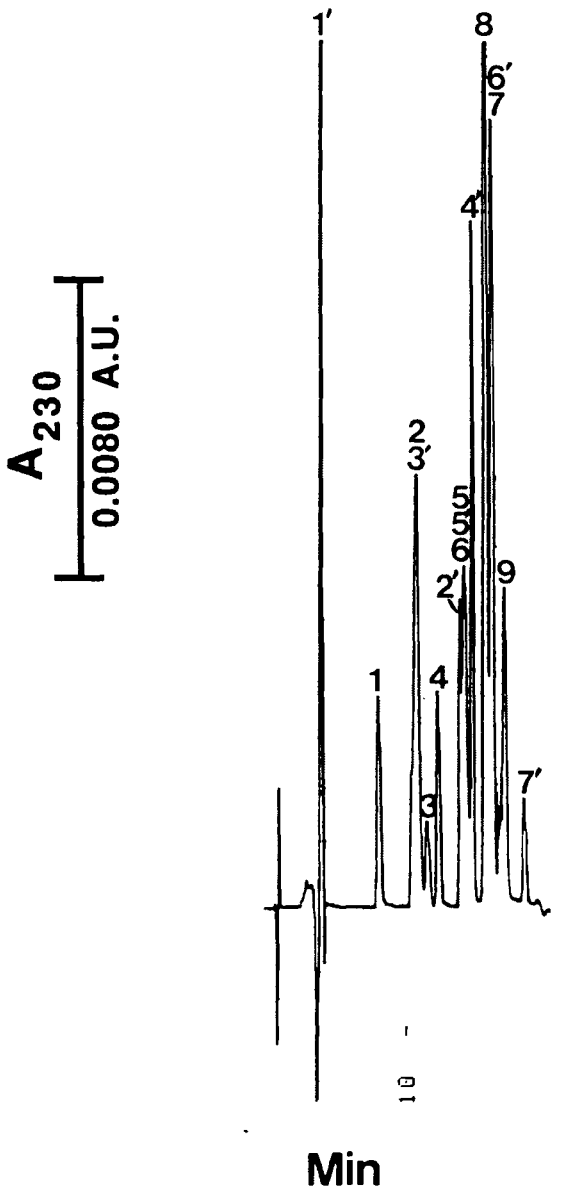


Figure 10. Chromatogram of all herbicides obtained on $C_{18}N^{+}(Me)_2Pr$ column. Experimental conditions and solutes are as in Fig. 9.

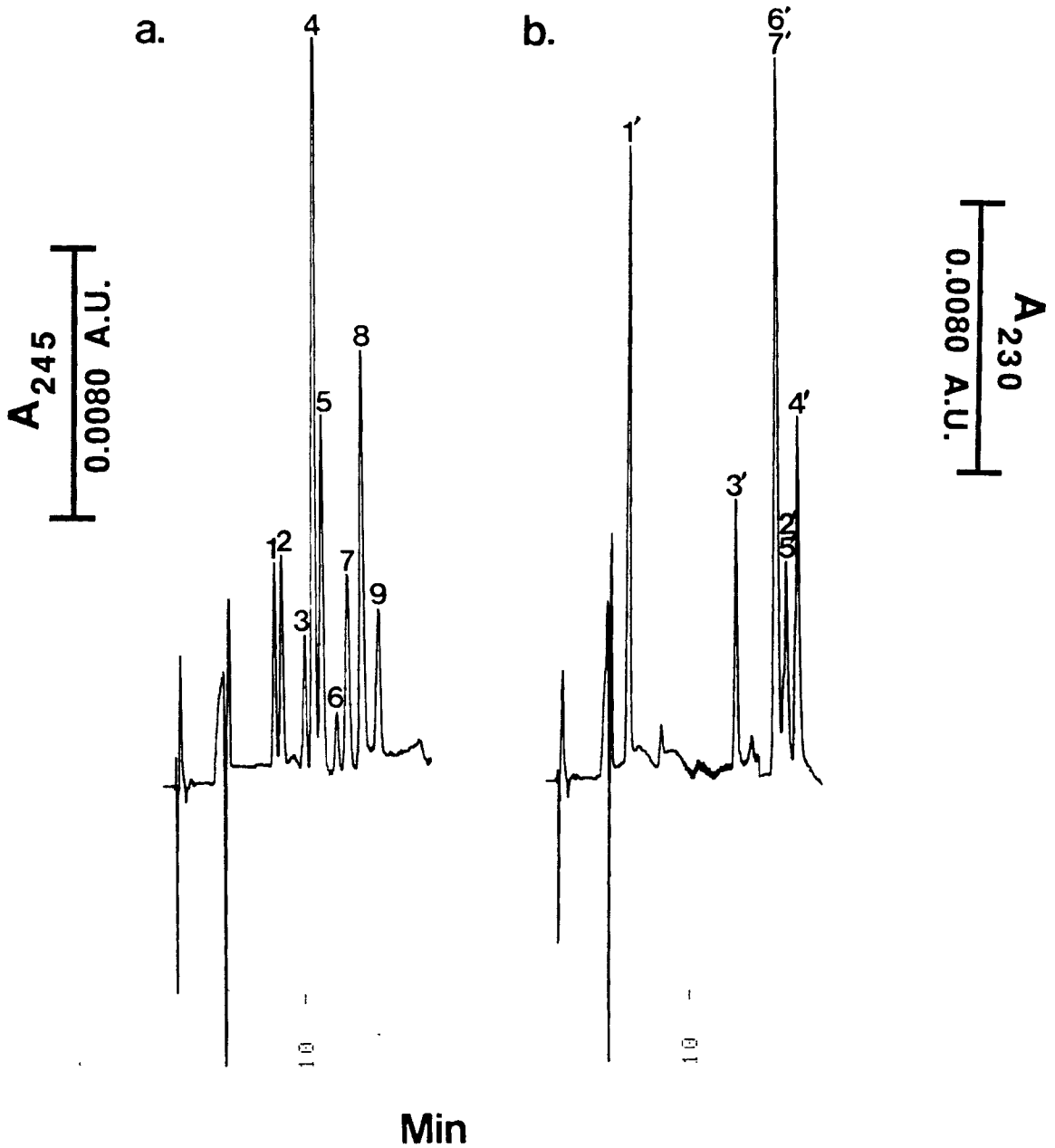


Figure 11. Chromatograms of urea herbicides (a) and other herbicides (b) obtained on C_{18} column. Experimental conditions and solutes are as in Fig. 9.

cationic herbicides prometon and prometryne were retained for longer time because they underwent electrostatic interactions with the oppositely charged surfactant-modified stationary phase. When compared to the result on a $C_{18}N^+(Me)_2Pr$ column (Fig. 9b), the selectivity was completely different. All solutes were more retained except 2,4-D butyl ester, and, in addition, the $C_{18}N^+(Me)_2Pr$ column afforded better overall separation. However, when all the 16 herbicides were chromatographed on a C_{18} column, the overall resolution was better than on a $C_{18}N^+(Me)_2Pr$ column (see Fig. 12) since the C_{18} column seems to better segregate the urea herbicides from the other herbicides, so that less overlapping of peaks occurred.

In another instance where the C_{18} column seems to afford superior separation is with polar catecholamines (see Fig. 13). This reflects the larger hydrophobicity of the C_{18} column. In fact, in the presence of SDS as micellar mobile phase, catecholamines including epinephrine, dopa, normetanephrine, octopamine, metanephrine, synephrine, β-hydroxyphenethylamine, phenylpropanolamine and ephedrine, can be retained and separated using gradient elution II (Fig. 13b). The elution order depends on the hydrophobic character of the solutes. The solutes with two polar hydroxyl groups on their benzene ring, i.e., epinephrine and dopamine, eluted first. The solutes without hydroxyl group on their benzene rings, i.e., phenylpropanolamine and ephedrine, eluted last. Catecholamines which could not be retained on a $C_{18}N^+(Me)_2Pr$ column in the absence of SDS in the mobile phase, they were retained on this column in the presence of SDS as the micellar mobile phase (see Fig. 13a). However, since the $C_{18}N^+(Me)_2Pr$ column is less hydrophobic than the C_{18} column, the overall resolution among the catecholamine solutes was less satisfactory on the former column than on the latter.

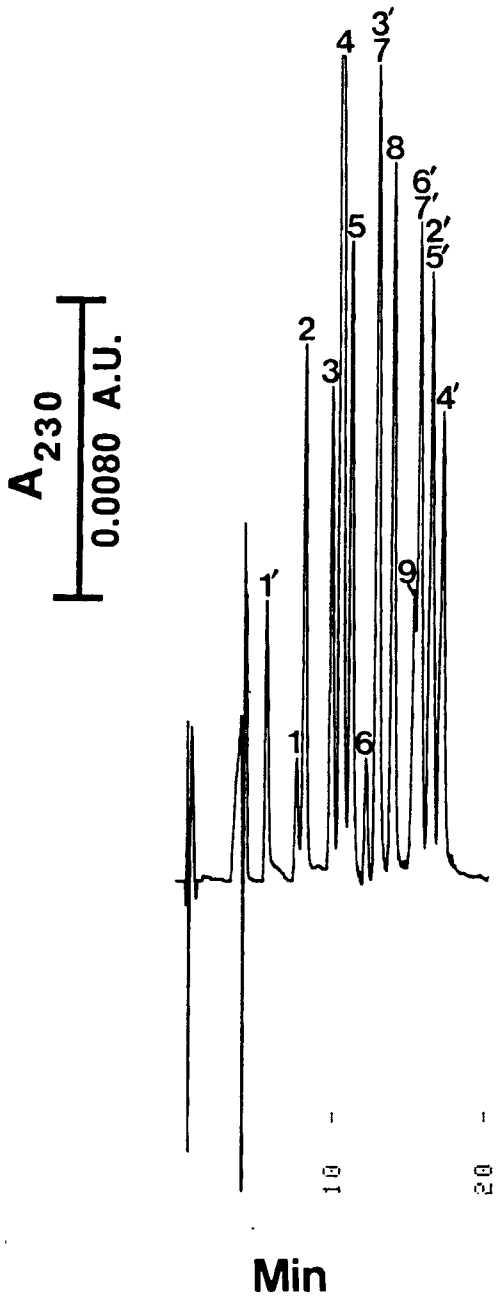
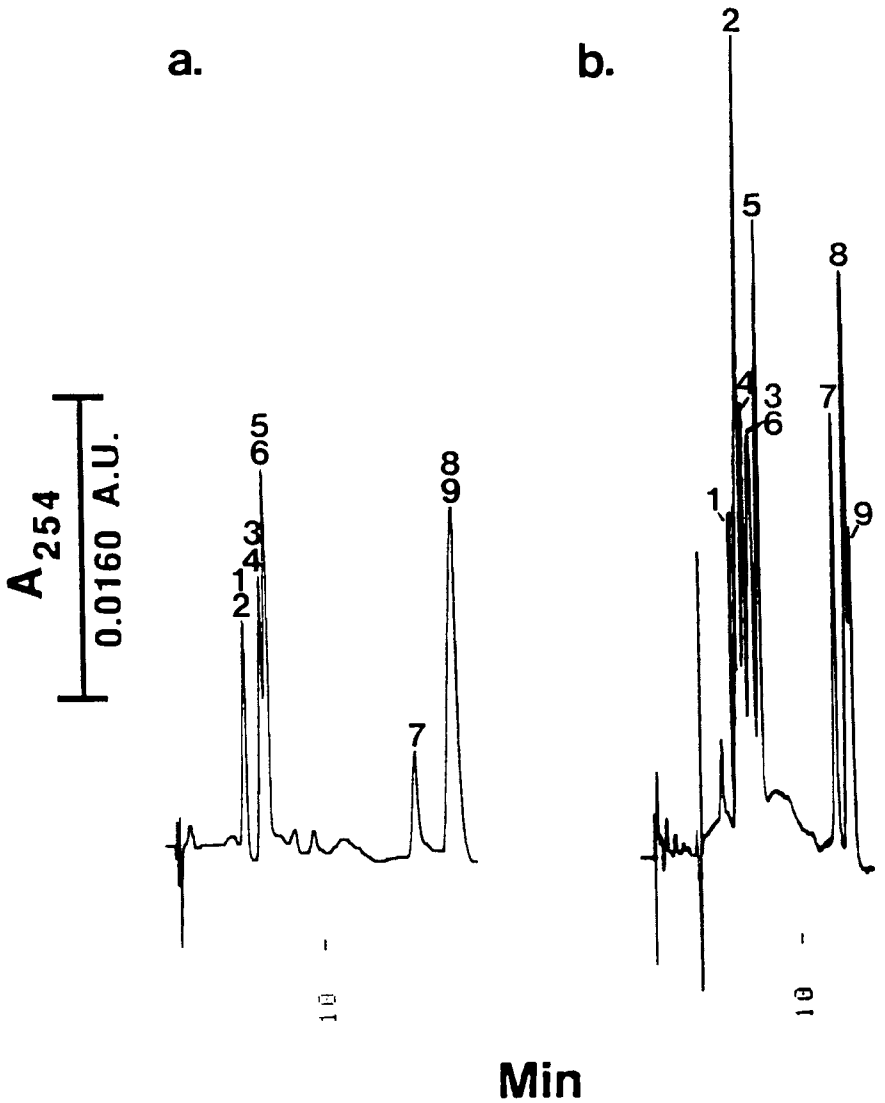


Figure 12. Chromatogram of all herbicides obtained on C_{18} column. Experimental conditions and solutes are as in Fig. 9.



Catecholamines: 1, dopa; 2, epinephrine; 3, normetanephrine; 4, octopamine; 5, metanephrine; 6, synephrine; 7, β-hydroxyphenethylamine; 8, phenylpropanolamine; 9, ephedrine.

Figure 13. Chromatogram of catecholamines obtained on $C_{18}N^{+}(Me)_2Pr$ column (a) and C_{18} column (b). Columns, 10 x 0.46 cm ID. Linear gradient, 15.0 min from 15 to 25% (v/v), followed by 1.0 min from 25 to 15% and equilibration with 10 min 15% (v/v) isopropanol in 20.0 mM SDS and 20.0 mM sodium phosphate, pH 2.5. The mobile phase flow-rate was 1.0 mL/min.

Effects of Micelle Concentration

Due to the non-homogeneous nature of micelles and their multiple sites of interactions, different separation selectivity, such as reversal of elution order with a change in micelle concentration is often observed [17, 18]. Retention of non-ionic compounds decreases with increase in micelle concentration [17], and under certain conditions retention of ionogenic compounds can increase with micelle concentration due to electrostatic repulsion in the mobile phase which forces the solutes into the stationary phase [18].

Figure 14 shows the dependence of retention and selectivity of Dns-AA on Em concentration using a $C_{18}N^{+}(Me)_2Pr$ column. It is obvious from Fig. 14a and b that increasing the Em concentration from 3 mM to 5 mM (by a factor of less than 2.0), an improvement in the overall separation is obtained. A further increase in Em concentration (see Fig. 14c and d) resulted in a decrease in retention of all solutes and, in turn, less resolution. In summary, 5 mM of Em seems to be an optimum concentration.

Mixed Micellar Hydro-Organic Eluents

The third surfactant used in this study is one of the bile salts, taurodeoxycholate sodium (NaTDC), which has helical structure above CMC. In many instances, the use of a single surfactant in the micellar mobile phase may not yield the desired resolution and selectivity. Under this circumstance, a mixed micellar phase may be the option. We used mixed micellar solutions as mobile phases, such as NaTDC mixed with Em and NaTDC mixed with SDS. When using mixed NaTDC and SDS, the elution order of Dns-AA are not changed when compared with SDS, but the total analysis time is shortened from 16.4 min to 14.6 min (Fig. 15). The analysis time of catecholamines is also reduced in the presence of NaTDC (Fig. 16) because the solutes can associate more easily with NaTDC than with SDS. When using NaTDC mixed with Em, not only total analysis time of Dns-AA was shortened from 17.2 min to 11.2 min when compared with Em alone, but also the

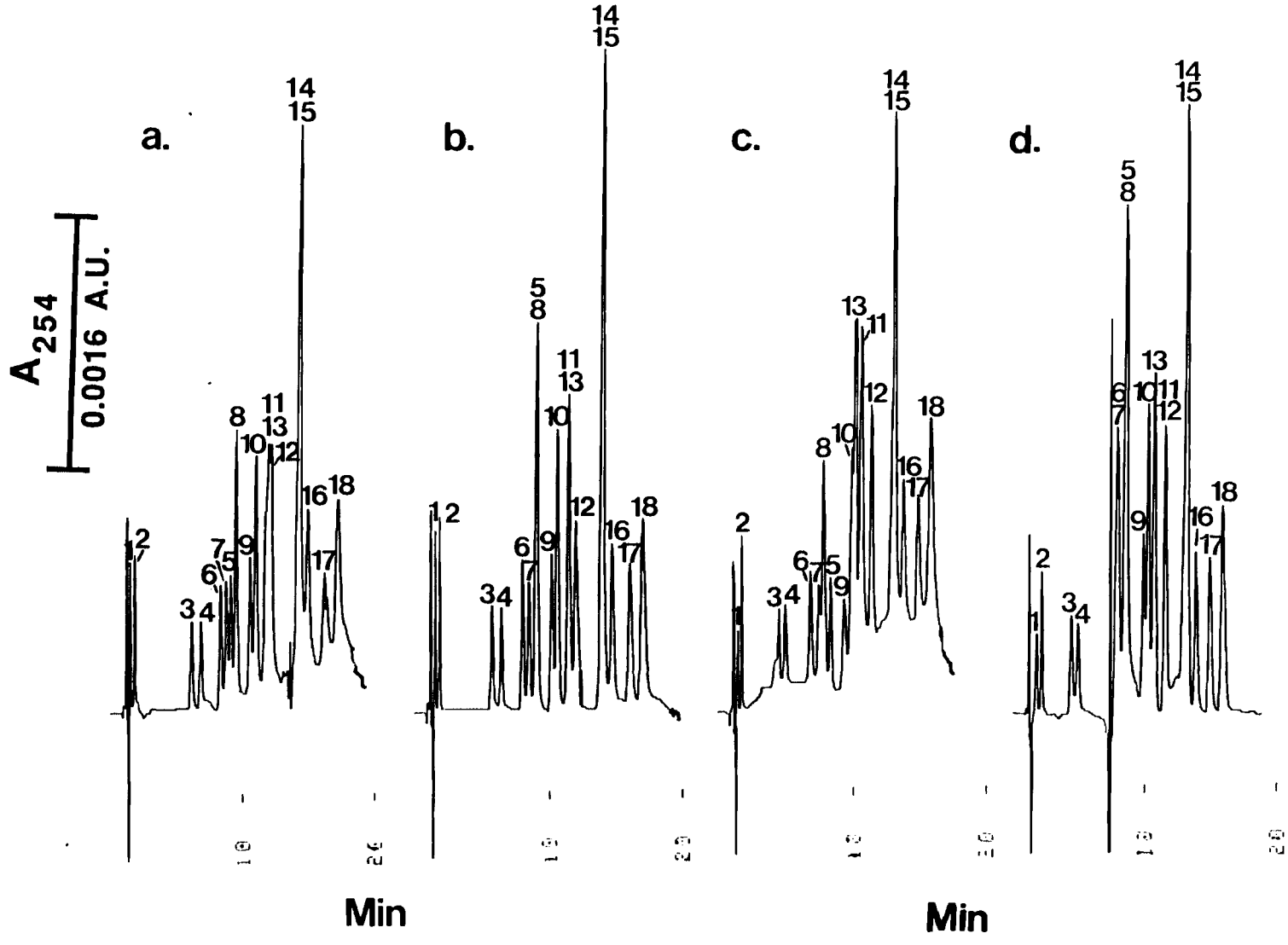


Figure 14. Chromatograms of Dns-AA obtained on $C_{18}N^{+}(Me)_2Pr$ column using Em hybrid mobile phases.
a. Em = 3 mM. b. Em = 5 mM. c. Em = 10 mM. d. Em = 20 mM. Other conditions and solutes are as in Fig. 5.

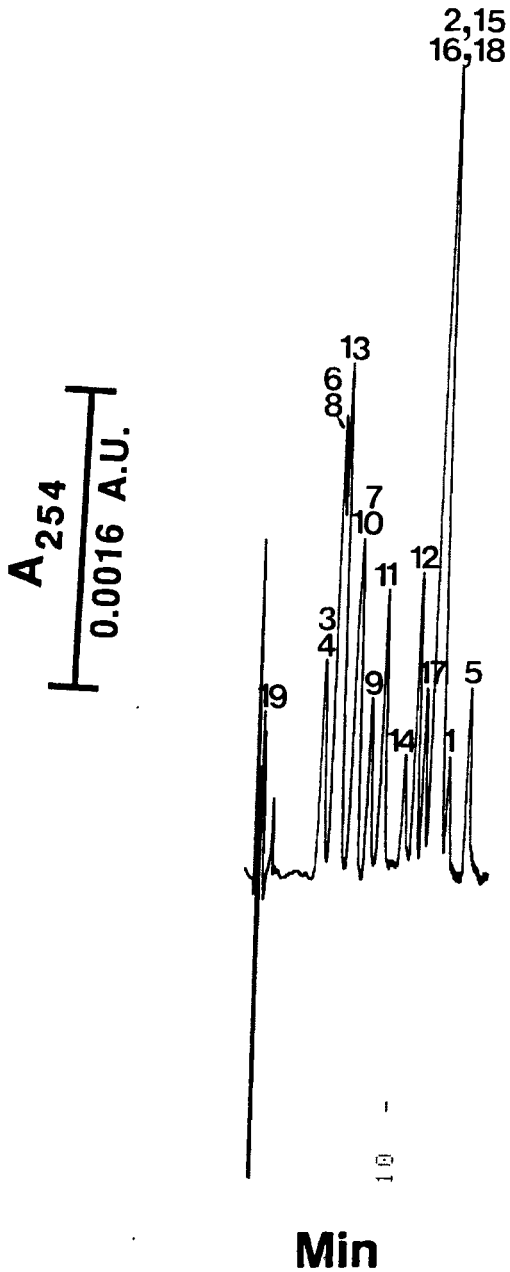


Figure 15. Chromatogram of Dns-AA obtained on $C_{18}N^{+}(Me)_2Pr$ column. Column, 10 x 0.46 cm ID. Linear gradient, 15.0 min from 10 to 50% (v/v), followed by 1.0 min from 50 to 10% and 10 min equilibration with 10% (v/v) isopropanol in 20.0 mM SDS, 25 mM NaTDC and 20.0 mM sodium phosphate, pH 2.5. The mobile phase flow-rate was 1.0 mL/min. Solutes are as in Fig. 1.

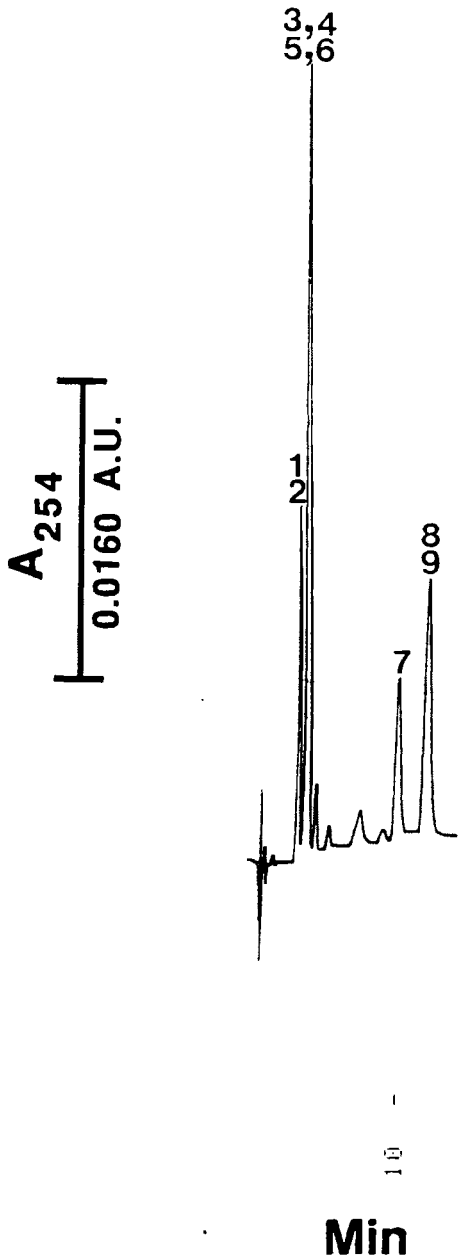


Figure 16. Chromatogram of catecholamines obtained on $C_{18}N^{+}(Me)_2Pr$ column. Experimental conditions are as in Fig. 15 and solutes are as in Fig. 13.

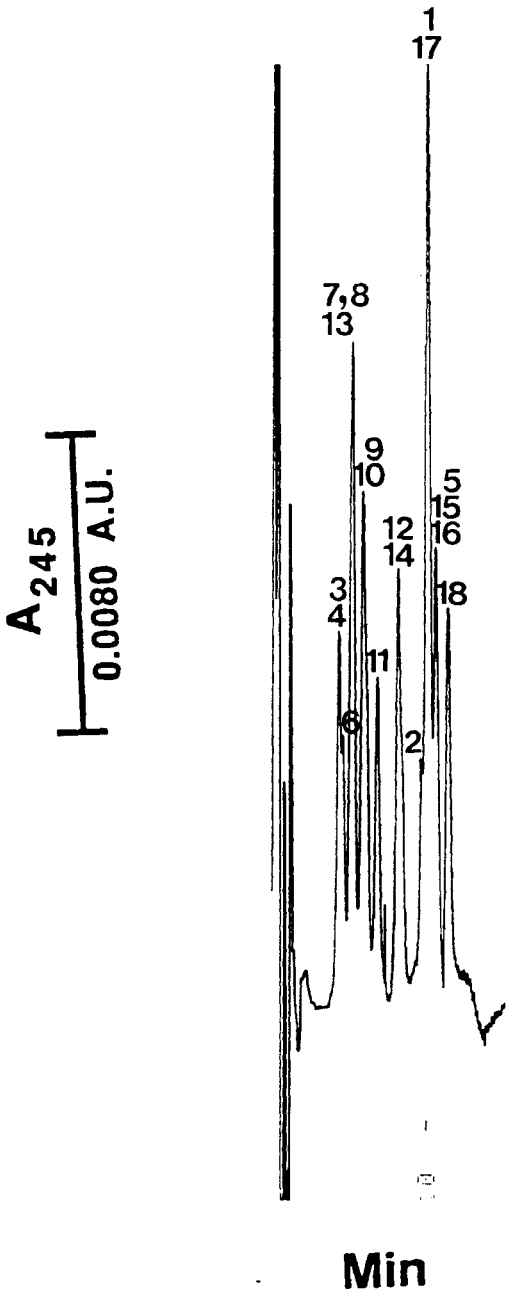


Figure 17. Chromatogram of Dns-AA obtained on $C_{18}N^{+}(Me)_2Pr$ column. Column, 10 x 0.46 cm ID. Linear gradient, 15.0 min from 10 to 50% (v/v), followed by 1.0 min from 50 to 10% and 10 min equilibration with 10% (v/v) isopropanol in 5 mM Em, 25 mM NaTDC and 20.0 mM sodium phosphate, pH 2.5. The mobile phase flow-rate was 1.0 mL/min. Solutes are as in Fig. 1.

elution order changed. Most solutes are less retained except arginine, lysine and tyrosine, which were more retained because of the adsorption of NaTDC making the electrostatic interaction between stationary phase and cationic solutes weaker, and the hydrophobic interaction stronger. The same reasoning can explain why phenylalanine eluted earlier than isoleucine, leucine and tyrosine (Fig. 17). Therefore, the combination of surfactants can improve solvent strength, but alter the selectivity.

In this study, up to 50% of 2-PrOH has been used during gradient. It is well established that the addition of alcohols to micellar mobile phases would cause changes in certain micellar properties, such as the aggregation number and the CMC of the surfactant. However, the observed changes in retention and selectivity in hybrid system are difficult to be explained in terms of changes by micellar properties, especially for this work.

Conclusions

The possibility of using silica microparticles having surface bound cationic surfactant ligands has been examined. Better separation can be obtained on the $C_{18}N^{+}(Me)_2Pr$ column for Dns-AA and herbicides. The presence of micelles in the mobile phase has a great influence on chromatographic selectivity depending on the nature of the micelle. For the Dns-AA, the Em suractant seems to give a better separation selectivity than the SDS surfactant on the $C_{18}N^{+}(Me)_2Pr$ column. While for urea herbicides, it seems that SDS yielded a better separation than Em on the C_{18} column. In addition, The combination of micelles can provide different selectivity and improve solvent strength.

REFERENCES

[1] N. Tanaka, H. Goodel and B. L. Karger, *J.* Chromatogr., 158 (1987) 233.

[2] D. W. Armstrong and S. J. Henry, *J. Liq. Chromatogr.*, 3 (1980) 657.

[3] M. G. Khaledi, *Trends Anal. Chem.*, 7 (1988) 293.

[4] D. W. Armstrong and F. Nome, *Anal. Chem.*, 53 (1981) 1662.

[5] D. W. Armstrong, *Sep. Purif. Methods,* 14 (1985) 213.

[6] J. G. Dorsey, *Adv. Chromatogr.*, 27 (1987) 167.

[7] J. G. Dorsey, M. T. DeEchegaray and J. S. Landy, *Anal. Chem.*, 55 (1983) 924.

[8] P. Yarmchuck, R. Weinberger, R. F. Hirsch and L. G. Cline Love, *Anal. Chem.*, 57 (1985) 1564.

[9] M. F. Borgerding, R. L. Williams, W. L. Jr. Hinze and F. H. Quina, *J. Liq. Chromatogr.*, 12 (1989) 1367.

[10] P. Yarmchuk, R. Weinberger, R. F. Hirsch and L. J. Cline Love, *J. Chromatogr.*, 283 (1984) 47.

[11] M. F. Borgerding, W. L. Hinze, L. D. Stafford, G. W. Jr. Fulp and W. C. Jr. Hamlin, *Anal. Chem.*, 61 (1989) 1353.

[12] M. J. M. Heránadez and M. C. G. Alvarez-Coque, *Analyst*, 117 (1992) 831.

[13] M. G. Khaledi, J. K. Strasters, A. H. Rodgers and E. D. Breyer, *Anal. Chem.*, 62 (1990) 130.

[14] M. G. Khaledi, *Anal. Chem.*, 60 (1988) 876.

[15] I. M. Klotz, H. A. Fiess, *Biochim. Biophys. Acta*, 38 (1960) 57.

[16] G. B. Gavioli, G. Grandi, L. Menabue, G. C. Pellacani, M. Sola, *J. Chem. Soc. Dalton Trans.*, (1985) 2363.

[17] P. Yarmchuk, R. Weinberger, R. F. Hirsch and L. J. Cline Love, *Anal. Chem.*, 54 (1982) 2233.

[18] D. W. Armstrong and G. Y. Stine, *Anal. Chem.*, 55 (1983) 2317.

VITA

Ying Zhang

Candidate for the Degree of

Master of Science

Thesis: HIGH PERFORMANCE MICELLAR LIQUID CHROMATOGRAPHY WITH SILICA-BASED STATIONARY PHASE HAVING SURFACE-BOUND CATIONIC SURFACTANT

Major Field: Chemistry

Biographical:

Personal Data: Born in Hebei, China, August 26, 1963.

Education: Received Bachelor of Science Degree in Chemistry from Peking University, P. R. China, in July, 1986; received Master of Science Degree in Environmental Chemistry from Peking University, P. R. China, in July, 1989; completed requirements for the Master of Science degree at Oklahoma State University in July, 1995.

Professional Experience: July 1986 to July 1989, graduate research assistant, Center of Environmental Sciences, Peking University. July 1989 to November 1992, assistant engineer, China National Environmental Monitoring Center. August 1992 to present, graduate research and teaching assistant, Department of Chemistry, Oklahoma State University.

Honors: Johnston Fellowship, Summer 1993 and Summer 1994.