EVALUATION OF ALKYLTRIMETHYLAMMONIUM HALIDE SURFACTANTS IN MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1993

OKLAHOMA STATE UNIVERSITY

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ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. Ziad El Rassi for his advise and encouragement throughout my graduate program. I would also like to thank Dr. Richard Bunce and Dr. Horacio Mottola for serving on my graduate committee. Their suggestions were very beneficial throughout the study.

I extend sincere thanks to Amoco Production Company for providing the equipment and supplies necessary for the research. Thanks also to my wife, Chris, for her moral support and typing and proofing skills. I extend a sincere thank you to all of these people.

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LIST OF SYMBOLS AND ABBREVIATIONS

α	selectivity
δ_c	thickness of the diffuse double layer adjacent to the capillary wall
ΔG^0	Gibbs free energy
δ_{mc}	thickness of the diffuse double layer adjacent to the micelle surface
ε	electricial permittivity of the buffer
ζ	zeta potential
ζ_c	zeta potential of the capillary
ζ_{mc}	zeta potential of the micelle
η	viscosity
κ	Debye-Hückel constant
μ_{eo}	electrophoretic mobility
ν_{eo}	electroosmotic velocity
ν_{ep}	electrophoretic velocity of micelle
ν_{mc}	net velocity of the micelle
ρ_c	surface charge density of capillary surface
ρ_{mc}	surface charge density of the micelle
σ^2	peak variances
ϑ	partial specific volume
ϕ	logarithmic phase ratio
φ	phase ratio
φ_B/φ_A	quotient of phase ratios
a	radius of the micelle

CD	cyclodextrins
CD-MECC	CD modified MECC
CE	capillary electrophoresis
CGE	capillary gel electrophoresis
CIF	capillary isoelectric focusing
CITP	capillary isotachophoresis
CMC	critical micellar concentration
CMC_i	critical micellar concentration of the individual surfactant
CMC_{mix}	critical micellar concentration of binary surfactant mixture
CTAB	cetyltrimethylammonium bromide
CTAC	cetyltrimethylammonium chloride
CZE	capillary zone electrophoresis
D_m	longitudinal molecular diffusion
DoTAB	dodecyltrimethylammonium bromide
DoTAC	dodecyltrimethylammonium chloride
DTAB	decyltrimethylammonium bromide
DTAC	decyltrimethylammonium chloride
E	electric field strength
EOF	electroosmotic flow
f_i	activity coefficient
HPCE	high performance capillary electrophoresis
HPLC	high performance liquid chromatography
I	ionic strength
I.D.	inner diameter
K	partition coefficient
k'	capacity factor
kV	kilovolt

L	total length of the capillary
l	effective length of the capillary column (separation length)
log α	hydrophobic selectivity
log β	specific interactions
MECC	micellar electrokinetic chromatrography
MEEKC	microemulsion electrokinetic chromatography
N	separation efficiency (plate number)
n	peak capacity
n_{agg}	aggregation number
N_{av}	average plate number
n_c	number of carbon atoms in an alkyl chain
$n_{c,surf}$	number of carbon atoms in the alkyl chain of the surfactant
O.D.	outer diameter
OTAB	octyltrimethylammonium bromide
OTAC	octyltrimethylammonium chloride
R	gas constant
R_s	resolution
RSD	relative standard deviation
[S]	concentration of the surfactant
SDS	sodium dodecyl sulfate
SDVal	sodium N-dodecanoyl-L-valinate
STS	sodium tetradecyl sulfate
T	absolute temperature
t_0	migration time of an unretained solute
t_{mc}	migration time of micelle
t_r	retention time of solute
TTAB	tetradecyltrimethylammonium bromide

TTAC	tetradecyltrimethylammonium chloride
UV-Vis	ultraviolet and visible
V	potential drop across the capillary
V_m	volume of the mobile phase
V_s	volume of the micellar phase

CHAPTER I

BACKGROUND AND RATIONALE

Introduction and Historical Aspects

High performance capillary electrophoresis (HPCE) is the miniaturized instrumental version of traditional electrophoresis. In fact, electrophoresis is by no means a new concept in separation sciences. It was introduced more than half a century ago by Tiselius (1).

Electrophoresis is one of several separation methods based on rate processes, i.e., separations are attained *via* differences in the kinetic properties of the components in the mixture. Electrically charged species are separated *via* differences in migration velocities through a supporting electrolyte under the influence of a direct current electric field. Thus electrophoresis, like chromatography, is an important member of the class of differential migration methods. The major difference between both methods is that electrophoresis is a single-phase separation process, while chromatography is a two-phase based method. In electrophoresis solutes are separated mainly on the basis of differences in charges and to a lesser extent on the basis of size and shape. In chromatography solutes undergo a series of adsorption-desorption steps while moving down the column and interact with both the mobile and stationary phase *via* a multiplicity of specific and nonspecific interactions.

Electrophoresis in open tubes or gel-filled capillaries using sophisticated instrumentation and advanced detection systems is currently an important microseparation technique featuring high resolution, high speed, automation and a small sample requirement. In fact, HPCE has combined the intrinsic high resolving power of

electrophoresis with the advanced instrumentation and automation of HPLC while developing its own entirely new approaches as an analytical separation method.

Although free solution electrophoresis in open tubular format was first demonstrated by Hjertén (2) in 1967 with 3 mm inner diameter tubes and later by Virtanen (3) and Mikkers *et al.* (4) with narrower 200-500 μm I.D. tubes, the major breakthrough in HPCE in terms of resolution and separation efficiency was first realized by Jorgenson and Lukacs in 1981 (5-8). In this pioneering work, Jorgenson and Lukacs performed the separation of amino acids and peptides in 75 μm capillaries with on-column fluorescence detection, a condition that favored the realization of millions of theoretical plates with minimum detection down to the sub-femtomoles.

Three years later, the applicability of HPCE was extended to the separation of neutral species by Terabe *et al.* (9), who introduced micellar electrokinetic capillary chromatography (MECC). MECC, which is a modification of capillary zone electrophoresis (CZE), allows the separation of neutral species *via* their differential partitioning between a micellar pseudo-stationary phase and an aqueous phase.

With the advent of HPCE, electrophoresis is no longer limited to the separation of biomacromolecules such as proteins and large DNA fragments. HPCE is currently a suitable technique for the separation of small neutral and ionic molecules and small organic and inorganic ions.

The goal of this chapter is to (i) summarize the basic principles of MECC, (ii) provide a description of the operating parameters affecting MECC separation and (iii) give a rationale for the study.

Some Aspects of HPCE Instrumentation

Instrumental Set-up

Figure 1 is a schematic illustration of a home-made HPCE instrument. As shown in Fig. 1, there are six major components: (i) a high voltage power supply capable of delivering 0-30 kV, (ii) two buffer reservoirs, one at each end of the capillary, (iii) a buffer filled fused-silica capillary, typically with an I.D. of 25 to 100 μm, (iv) a plexiglass box with a safety interlock to protect the user from high voltages, (v) an on-column detector, typically a UV-Vis or fluorescence (UV-Vis was used for this study), and (vi) a data collection/processing system. Additional features commonly found in commercial instruments include: automated injectors, capillary rinse and buffer reservoir changers (to enhance reproducibility), fraction collectors, and capillary cooling systems (to reduce band broadening arising from Joule heating).

With the above instrumentation, HPCE may be performed in several different modes to achieve a given separation. The origin of some of these modes of separation is attributed, in part, to the fact that HPCE has developed from a combination of electrophoretic and chromatographic concepts. In addition, HPCE separations are performed in open tubular and gel-filled capillaries. There are at least five distinct HPCE modes: capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromotography (MECC) and its variants, capillary isoelectric focusing (CIF), capillary isotachorphoresis (CITP) and capillary gel electrophoresis (CGE). Whereas CZE, CIF, CITP and CGE have evolved from classical electrophoresis, the development of MECC is closely associated with that of HPCE.

Sample Injections

There are two major approaches for sample introduction, hydrodynamic and electromigration (10). Hydrodynamic injection is the most widely used because it is nondiscriminative, allowing the introduction of all sample components into the separation chamber. There are three different types of hydrodynamic injection: (i) head-space pressurization; (ii) vacuum injection (negative pressure at the opposite end of the capillary); and (iii) gravimetric (siphoning). The latter is used in this study.

With electrokinetic injection the sample is electrophoretically introduced into the capillary. Sample loading is a function of both electroosmotic flow and the migration rate of the solute. Thus, solutes are differently loaded into the capillary because they have different mobilities. This is a problem when trying to inject a low concentration, low mobility solute in conjunction with a high concentration, high mobility solute. Sample introduction by electromigration, is therefore a discriminative approach (11-14). However, electrokinetic injection is useful in concentrating sample solutes (15,16). This pre-concentration step is usually achieved with a sample solution having lower conductivity than the separation buffer. Under these conditions, the electric field in the sample medium is greater than in the capillary and, as a result, the solutes move through the sample buffer until they enter the capillary where they slow down and 'stack' into a narrow zone. Reproducibility is lower than with hydrodynamic injection due to a greater number of variables (i.e., the surface chemistry of the capillary, sample type, sample solution conductivity, etc.).

Principles of Separation in MECC

General Description of MECC

In MECC, the separation medium consists of an electrolyte containing an ionic surfactant above its critical micellar concentration (CMC). Under this condition, the

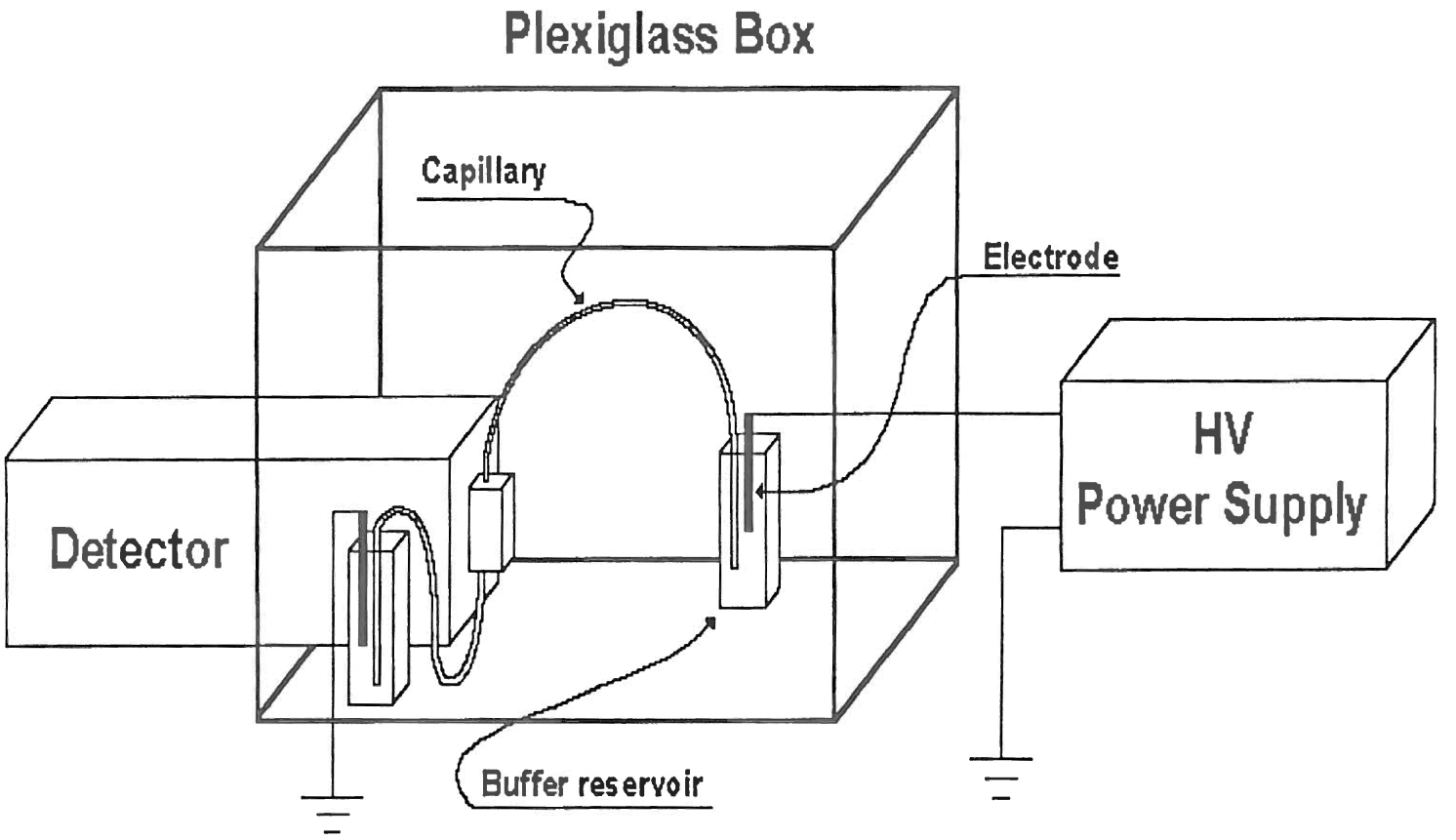


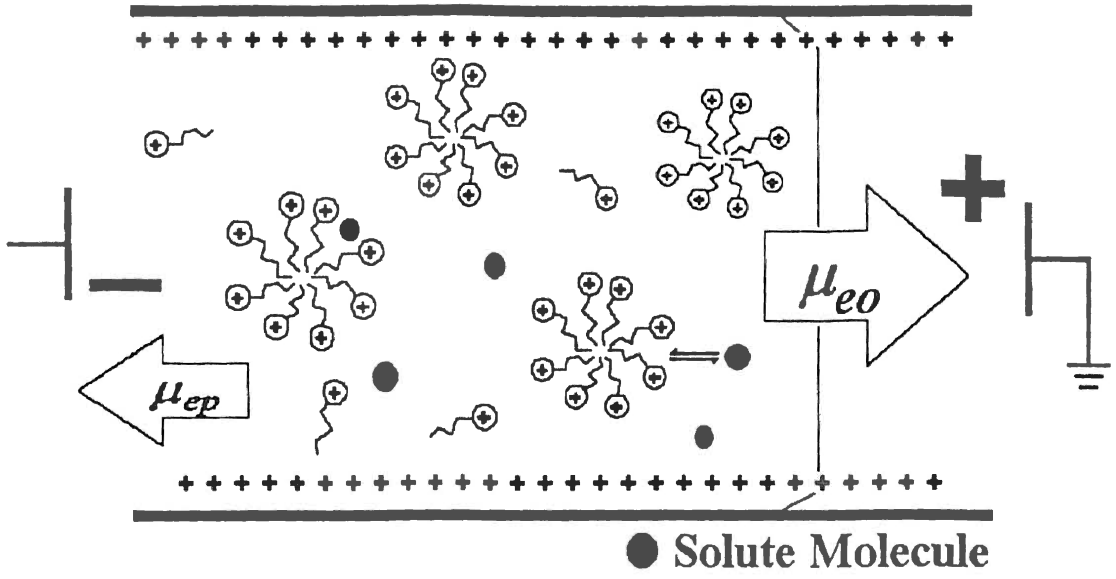
Figure 1. Instrument for capillary electrophoresis

surfactant forms micelles with a hydrophobic interior and highly charged outer surface. Thus, there are two phases inside the capillary tube, an aqueous mobile phase and a micellar pseudo-stationary phase. Upon application of an electric field, the aqueous phase moves at the velocity of the electroosmotic flow (EOF), while the micelles gain a large electrophoretic mobility toward the oppositely charged electrode. The EOF is in the opposite direction and has a greater magnitude than the electrophoretic migration of the micelles. As a result, the micellar pseudo-stationary phase will move in the same direction, but at a slower rate than the aqueous phase, to the electrode with the same charge as the micelle. Solute molecules are then separated *via* their differential partitioning between both phases, and usually elute in the order of increasing hydrophobic character. Polar solutes that do not partition into the micelles are carried by the EOF and elute at time t_0. On the other hand, very hydrophobic solutes that are completely solubilized by the micelle will elute last at time t_{mc} (migration time of the micelle). This creates a retention window that extends from t_0 to t_{mc}. Neutral solutes exhibiting different solubilization with the micelle are eluted and separated within this retention window, see Fig. 2.

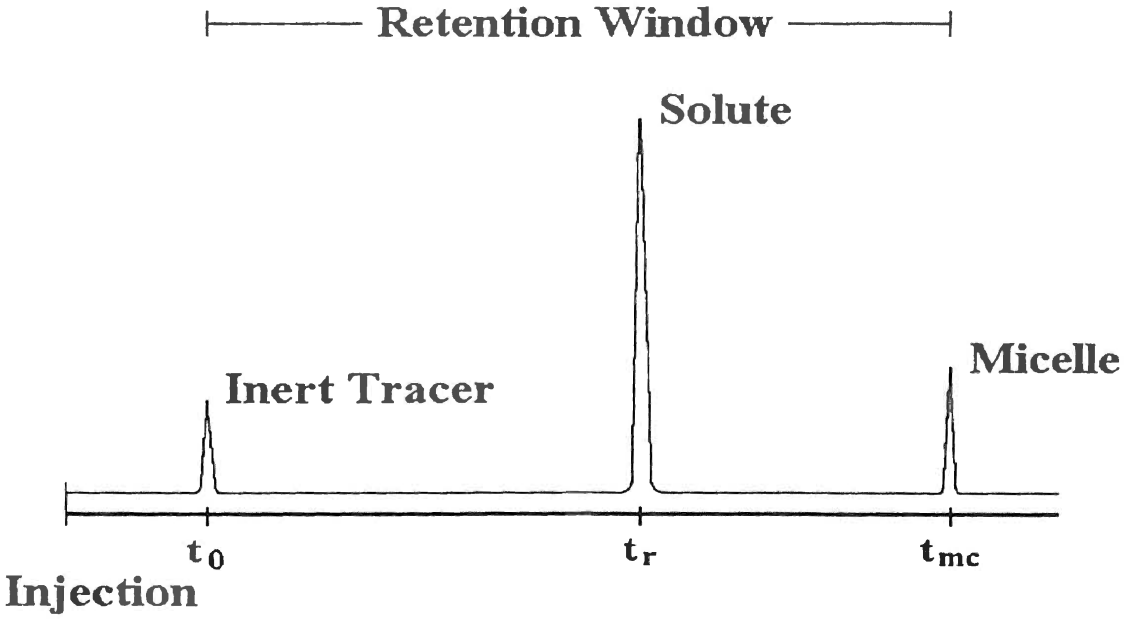
Electroosmosis

As can be seen in Figure 2a, the electroosmotic flow is an important component in MECC. In fact, EOF is the driving force for differential migration and its function draws similarity with the mobile phase in chromatography. The EOF carries the solutes down the capillary tube past the detection point.

In MECC, a fused silica capillary is used as the separation column. Under normal aqueous conditions the capillary wall has an excess of negative charges due to the ionization of the surface silanol groups. Because of this charged surface, electrolyte ions with a similar charge sign (co-ions) are repelled from the surface, while ionic species with



(a)



(b)

Figure 2. Schematic Representation of MECC System (a), and Retention Window in MECC (b)

an opposite sign (counter-ions) are attracted to the capillary wall. This results in the formation of an electric double layer at the silica-solution interface. Some of the counter-ions are tightly bound to the capillary surface by electrostatic interactions and form the compact region of the electric double layer (stagnant layer) region. Other counter-ions (due to thermal motion) reach further into the liquid and make up the diffuse or mobile region of the double layer. Because of this spatial distribution of ions within the electric double layer, an electric potential gradient develops at the solid-liquid interface. The value of this potential at or near the interface between the compact region and the diffuse region of the double layer is termed the zeta potential (ζ) (17).

When an electric field is applied tangentially to the capillary surface, the electrostatic force will cause the hydrated counterions in the diffuse layer to migrate toward the oppositely charged electrode. Because the ions are solvated, they drag solvent with them causing a bulk flow to form, and this is what is termed the electroosmotic flow.

The linear velocity of the EOF, v_{eo}, is given by (18):

$$v_{eo} = \mu_{eo} E = \frac{\varepsilon \zeta}{4\pi\eta} E \tag{1}$$

where μ_{eo} is the electroosmotic mobility, ε is dielectric constant of the medium, η is the viscosity, ζ is the zeta potential across the double layer and E is the electric field strength, which is given by:

$$E = \frac{V}{L} \tag{2}$$

where V is the potential drop across the separation capillary and L is its total length. Two important features of eqn 1 are that the EOF decreases with increasing viscosity of the running buffer and increases with the electric field strength.

The electric double layer is usually very thin (few hundred nanometers) compared to the inner diameter of the capillary. Therefore, the EOF is considered to originate at the capillary wall, causing the EOF to have a flat or plug profile (see Fig. 2a) as opposed to

the parabolic flow profile observed with pressure driven systems. This flat profile does not cause band broadening, and separation efficiencies in MECC can reach one million theoretical plates.

Fundamental Equations

Many of the fundamental characteristics of MECC are well understood and have been described by Terabe and co-workers (19). In MECC, retention and resolution are related to the electrokinetic velocities of the aqueous phase (i.e., EOF) and the micellar pseudo-stationary phase. The net velocity of the micelle, v_{mc}, is the sum of the electroosmotic velocity of the aqueous phase, v_{eo}, and the electrophoretic velocity of the micelle, v_{ep} (17,20):

$$v_{mc} = v_{eo} + v_{ep} = -\frac{\varepsilon E \zeta_c}{\eta} + \frac{2\varepsilon E \zeta_{mc}}{3\eta} f(\kappa a) = -\frac{\varepsilon E}{\eta}\left(\zeta_c - \frac{2\zeta_{mc}}{3} f(\kappa a)\right) \quad (3)$$

where ζ_{mc} and ζ_c are the zeta potentials of the micelle and the capillary, respectively, $f(\kappa a)$ depends on the shape of the micelle, a is the radius of the micelle, and κ is the familiar Debye-Hückel constant. The value of $f(\kappa a)$ varies between 1.0 and 1.5 depending on the dimensions of κa. The negative sign in eqn 3 indicates that when the zeta potential of the capillary is negative, the EOF is toward the negative electrode.

An important variable in MECC is the elution range parameter defined by the ratio (20):

$$\frac{t_o}{t_{mc}} = \frac{v_{mc}}{v_{eo}} = 1 - \frac{2\zeta_{mc}}{3\zeta_c} f(\kappa a) \quad (4)$$

The zeta potential can be expressed by the relationship (21):

$$\zeta = \frac{4\pi\delta\rho}{\varepsilon} \quad (5)$$

where ρ is the surface charge density of either the capillary surface (ρ_c) or the micelle (ρ_{mc}) and δ is the thickness of the diffuse double layer adjacent to either the capillary wall

(δ_c) or the micelle surface (δ_{mc}). Modern electrolyte theory equates δ to 1/κ. Thus, by a rearrangement of eqn 5:

$$\zeta = \frac{4\pi\rho}{\kappa\varepsilon} \propto \frac{1}{\sqrt{I}} \tag{6}$$

It follows from eqns 3, 5 and 6 that the EOF and electrophoretic velocity of the micelle are inversely proportional to the square root of the ionic strength, I.

As the elution range parameter t_0/t_{mc} decreases, the retention window increases. An elution range parameter of one means that the micelle is uncharged and all solutes coelute and migrate at the velocity of the EOF. A zero elution range parameter means an infinite retention window, a situation where the electrophoretic velocity of the micelle is of the same magnitude and opposite in direction of the EOF. According to eqns 3-6, the retention window is conveniently varied by changing: (i) the charge density of both the capillary and the micelle surfaces, (ii) the viscosity of the medium and (iii) the ionic strength of the running electrolyte.

In MECC, peak capacity, n, and resolution, R_S, are influenced, among other things, by the retention window through the following eqns (10,19):

$$n = 1 + \frac{\sqrt{N}}{4} ln \frac{t_{mc}}{t_0} \tag{7}$$

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2'}{k_2' + 1}\right)\left(\frac{1 - (t_o / t_{mc})}{1 + (t_o / t_{mc})k_2'}\right) \tag{8}$$

where N is the number of theoretical plates, α is the selectivity factor and k' is the capacity (retention) factor. The capacity factor is readily calculated from the electropherogram by

the eqn (19,22,23):

$$k' = \frac{(t_r - t_o)}{t_o\left(1 - \frac{t_r}{t_{mc}}\right)} = K\left(\frac{V_s}{V_m}\right) \tag{9}$$

where t_r is the retention time of the solute, K is the partition coefficient, V_S is the volume of the micellar phase, and V_m is the volume of the aqueous phase. Equation 9 is the conventional chromatographic expression of the capacity factor adjusted to account for movement of the pseudo-stationary phase (the micelle). As t_{mc} approaches infinity, eqn 9 reduces to the conventional form (9):

$$k' = \frac{t_r - t_0}{t_0} \tag{10}$$

whereby the stationary phase is not moving. The selectivity factor α and efficiency N are both readily estimated from the electropherogram by the following equations which are the same as those of chromatography (24):

$$\alpha = \frac{k_2'}{k_1'} = \frac{K_2}{K_1} \tag{11}$$

$$N = 4\left(\frac{t_r}{w_i}\right)^2 = 5.54\left(\frac{t_r}{w_h}\right)^2 = 16\left(\frac{t_r}{w_b}\right)^2 \tag{12}$$

For a Gaussian peak w_i, w_h and w_b are the peak widths at inflection point, half-height and base, respectively.

Some Aspects of Separation Optimization

Referring to eqn 8, resolution is related to three fundamental parameters: efficiency, selectivity and retention. For two adjacent peaks, i.e., $k'_1 = k'_2 = k'$, a convenient

approximation to eqn 8 is:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right) f(k') \tag{13}$$

where (19):

$$f(k') = \left(\frac{k'}{k'+1}\right)\left(\frac{1-\frac{t_0}{t_{mc}}}{1+\left(\frac{t_0}{t_{mc}}\right)k'}\right) \tag{14}$$

Although all three parameters (i.e., efficiency, selectivity and retention) can be manipulated to optimize separation, in MECC as in chromatography, increasing selectivity is the most useful approach. In fact, there is a limit beyond which increasing retention will cause R_S to drop. It was shown by Terabe *et al.* (19) that when *f(k')* is evaluated as a function of k', bell-shaped curves are obtained. Each bell-shaped curve is unique for a particular t_0/t_{mc}. By differentiating eqn 14 with respect to k' and setting the resulting expression to zero, the optimum k' (i.e., optimum surfactant concentration) value for maximum resolution is given by (25,26):

$$k'_{opt} = \left(\frac{t_{mc}}{t_0}\right)^{\frac{1}{2}} \tag{15}$$

In most instances, the retention window is predetermined and cannot be varied systematically. This limits the MECC system to a narrow k' range as far as resolution is concerned. Usually, the capacity factor is changed by adding an organic modifier (27), or by changing the surfactant concentration (19,28). At low surfactant concentrations, the capacity factor is linearly dependent on the amount of surfactant added (19).

Resolution increases in proportion to the square root of the plate number N; see eqn 8. If efficiency is mainly determined by longitudinal molecular diffusion (D_m), the

plate number equation is approximated by (22):

$$N = \frac{t_r^2}{\sigma_t^2} = \frac{L^2}{\sigma_L^2} = \frac{L^2}{2D_m t_r} \tag{16}$$

where σ^2 is the peak variance and L is the total migration distance. According to eqn 16, the higher the applied voltage (i.e., the lower t_r), the higher the plate number, unless conditions are such that the applied voltage generates too much Joule heating. Typically, hydrophobic analytes, which spend most of their migration time in the micelle, yield high theoretical plate numbers because the micelle has a smaller diffusion coefficient. Plate counts on the order of 100,000 to 200,000 are easily generated in MECC, and even one million theoretical plates are often reported (29). But a million theoretical plates is of no value for resolution if the selectivity of the MECC system is not adequate.

Based on the above short discussion, selectivity, α, is the most important and most effective term to maximize resolution. Selectivity is altered by changing the physical properties of the micelle, using aqueous phase modifiers and changing the temperature.

Changing the capillary column temperature will, in principle, produce changes in the distribution coefficients of the solutes. But in capillary electrophoresis Joule heating evolves due to the passage of an electrical current through the medium. This Joule heating must be dissipated to avoid band broadening arising from thermal perturbations of the solute velocity profile. Thus, the capillary column is normally cooled to subambient temperatures. In this temperature range, however, the distribution coefficients of the solutes do not undergo dramatic changes. Therefore, temperature is not used to optimize selectivity. It should be noted that an increase in capillary column temperature is accompanied by a decrease in the viscosity of the buffer, a condition that leads to a decrease in the migration time of the solutes. Thus, to ensure reproducible migration time, it is recommended that the temperature of the capillary be controlled.

The physical properties of the micellar phase are changed by using a different surfactant or by adding a co-surfactant to form a mixed micellar phase. To yield

separation, the surfactant in MECC must be either charged in its own natural environment (i.e., anionic or cationic surfactants) or be converted *in situ* to a charged species such as the nonionic-borate complex surfactants introduced very recently by our laboratory (30).

Surfactants have a hydrophobic tail and hydrophilic head group. When solutes interact with micelles, they may partition (i) in the inner hydrophobic core, (ii) between (sandwiched) the hydrophobic tails of the surfactants, (iii) in the outer hydrophilic head groups, or (iv) adsorb on the micellar surface (31). Since most polar solutes interact with the top two regions of the micelle (i.e., at the surface or with the outer hydrophilic head groups), the hydrophilic head group (or ionic head group) is generally more important in terms of selectivity. This means that changing the nature of the hydrophilic (ionic) head group has a greater impact on MECC selectivity than changing the hydrophobic tail. Thus, exchanging sodium dodecyl sulfate (SDS) with sodium tetradecyl sulfate (STS) will produce very similar selectivity, but going from SDS to sodium *N*-lauroyl-*N*-methyltaurate (LMT) (32) or from SDS to DTAC (33) yields considerably different selectivity.

Some surfactant groups have very specific selectivity. Examples include sodium *N*-dodecanoyl-*L*-valinate (SDVal), bile salts and surfactants with perfluorinated alkyl chains. Perfluorinated surfactants show an enhanced selectivity for fluorinated solutes. Bile salts and SDVal are useful in separating enantiometers (34-37).

Terabe *et al.* (36) demonstrated chiral separations with bile salts. Bile salts are naturally occurring steroidal surfactants that form helical micelles. In his work, Terabe *et al.* (36) demonstrated the chiral separation of pharmaceutical drugs. In addition to chiral separations, Cole *et al.* (38) demonstrated the usefulness of separating very hydrophobic solutes not normally separated by long alkyl chain surfactants.

It should be mentioned that MECC is used to improve the separation of ionic as well as neutral solutes. A solute with a charge opposite in sign to that of the surfactant head group will strongly interact with the micelle *via* electrostatic forces. Thus, hydrophobicity

and charge affect the distribution coefficient of ionic solutes, and consequently anionic and cationic surfactants yield entirely different selectivities.

Besides changing surfactant type, micellar properties are changed by adding a co-surfactant. The addition of a nonionic co-surfactant lowers the surface charge of the micelle, and correspondingly, the electrophoretic mobility of the mixed micelle becomes lower and the retention window is shorter. In addition, the selectivity is significantly affected since the polar head groups of the nonionic and ionic surfactants are different. Rasmussen *et al.* (39) demonstrated changes in selectivity between SDS and SDS/Brig 35 micellar phases.

Modifiers encompass a wide variety of compounds and may affect the micelle or the aqueous phase. Included in the list of modifiers are: (i) cyclodextrins (CD), (ii) ion-pairing agents, (iii) borate, (iv) organic solvents, (v) urea, and (vi) metal salts. One of the more interesting modifiers is the use of an alkaline borate buffer with octylglucoside surfactant introduced by our laboratory (30). The addition of the borate buffer allows the manipulation of the surface charge density of the nonionic micelle and, in turn, the retention window (30).

Organic modifiers such as methanol, acetonitrile and isopropanol change selectivity by shifting the partitioning equilibrium towards the bulk buffer and also alter t_0 and t_{mc} (40-45). There is, however, an upper limit to the amount of added modifier (15%-20%), above which, efficiency decreases dramatically and migration time becomes impractical and the micellar structure itself may break down (22,46).

If heptane is added to micelles, microemulsions form. The core of the micelle contains a heptane droplet that shows a stronger affinity for non-polar solutes than the untreated micelle and is beneficial for the separation of more hydrophobic solutes (47). This form of MECC is known as microemulsion electrokinetic chromatography (MEEKC).

Cyclodextrins have gained interest as chiral separators for racemic and highly hydrophobic solutes (48). This method is known as CD modified MECC (CD-MECC). Cyclodextrins are torus shaped cyclic oligosaccharides with a hydrophilic outer surface and a hydrophobic inner cavity. They are formed with 6 (α-CD), 7 (β-CD) or 8 (γ-CD) glucopyranose units. Since CD's are electrically neutral, they are assumed not to partition in the micelle because of the hydrophilic outer surface. As such, CD's migrate with the electroosmotic flow. The inner cavity of a CD molecule offers a second site for the partitioning of hydrophobic solutes. Complexation with the solute may occur by hydrogen bonding, Van der Waals forces or hydrophobic interactions (10). This shortens the migration time of solutes which formerly would spend most of their time in the micelle. In addtion, the shape of the cavity acts to separate chiral isomers (47,49,50).

Ion-pairing agents such as tetraalkylammonium salts facilitate the separation of ionic solutes (51-53). The alkylammonium salts react with anionic solutes to form ion-pairs. With a SDS micellar phase, an anionic solute-alkylammonium pair exhibits less repulsion with the anionic micellar surface and is more likely to partition in the micelle. Under these conditions, the migration times of anionic solutes increase while that of cationic solutes decrease because the alkylammonium ion competes with the cationic solute for sites on the micellar surface (51).

Metal salts are added to anionic micelles to increase the retention window. Metal ions are attached to the micellar surface *via* electrostatic interactions, thus affording complex formation with solutes. This method has proven useful for the separation of oligonucleotides (54-55).

Like organic modifiers, urea increases the solubility of hydrophobic compounds in the running buffer. Urea breaks hydrogen bonds and deforms the structure of water. This acts to shift the distribution of the analyte towards the aqueous phase and facilitates the separation of highly hydrophobic solutes. In addition, urea slightly reduces t_0 and greatly reduces t_{mc} (56).

Rationale, Significance and Scope of this Study

Micellar electrokinetic capillary chromatography is an important branch of HPCE. The application of micellar electrokinetic chromatography has extended the intrinsic high resolving power of HPCE to the separation of neutral species and chiral racemates which cannot be separated by other conventional modes of capillary electrophoresis. Although significant advances have been made, many aspects of MECC still require further development, and the exploitation of the full potentials of the technique is yet to come.

One of the attractive features of MECC is the ease with which the nature of the micellar pseudo-stationary phase can be altered and/or changed, requiring only that the capillary be rinsed and filled with the new micellar solution. Despite this, most MECC separations have utilized aqueous solutions of sodium dodecyl sulfate (SDS) as the micellar phases and only few other ionic surfactants have been briefly explored.

The broad objective of this research is to further improve the methodology of MECC by (i) evaluating the electrokinetic and chromatographic properties of a series of alkyltrimethylammonium halide surfactants over a wide range of operating conditions and (ii) introducing cationic-cationic mixed micelles. To this end, the present research entails various studies directed toward the following specific aims: (i) to shed light on the energetics of retention of neutral solutes with single and mixed cationic micelles, (ii) to examine the correlation between solute retention and the hydrophobic character of the various alkyltrimethylammonium halide micellar phases, and (iii) to illustrate the dependence of the retention window of MECC on the nature of the micelle. The various micellar phases proved useful for the separation of urea herbicides by MECC.

Overall, this work has contributed to the understanding of the electrokinetic behavior of single and mixed cationic micelles and unveiled MECC capabilities not previously demonstrated.

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CHAPTER II

MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY WITH CATIONIC SURFACTANTS*

Abstract

A series of alkyltrimethylammonium chloride and bromide surfactants were evaluated in micellar electrokinetic capillary chromatography (MECC) of urea herbicides, alkylbenzenes and phenylalkylalcohols. The magnitude of the anodal electroosmotic flow obtained with these cationic micellar phases was largely unaffected by the length of the alkyl chain of the surfactant while the migration time of the micelle increased with decreasing the length of the alkyl tail. The net result was an increase in the retention window as the size of the alkyl tail of the surfactant decreased. The breadth of the retention window stayed almost the same when the micelle counterions were changed from chloride to bromide. At constant micellized surfactant concentration, the capacity factors of neutral solutes increased linearly with increasing alkyl chain length of the surfactant, indicating an increase in the hydrophobic phase ratio of the MECC systems. Under this condition, the value of the methylene group selectivity for the homologous solutes was largely unaffected by the length of the surfactant tail. Also, when the micellized surfactant concentration was held constant, the homologous solutes exhibited quasi-homoenergetic retention on the different cationic micellar phases. In addition, when going from a cationic surfactant to an anionic surfactant while keeping the length of the

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alkyl tail the same, the value of the methylene group selectivity remained unchanged, and the energetics of retention was not affected by the net charge of the micelle. The separation of a mixture of six urea herbicides was best achieved when an MECC system of low hydrophobic phase ratio and wide retention window, such as dodecyl- or decyltrimethylammonium chloride (DoTAC or DTAC), was used as the micellar phase. Tetradecyltrimethylammonium chloride (TTAC) micellar phase having medium hydrophobic character and narrower retention window than DoTAC or DTAC, was slightly less effective in separating the urea herbicide mixture. The overall separation of the urea herbicides could be enhanced by the inclusion of small amounts of octyltrimethylammonium chloride (OTAC) surfactant into the TTAC micellar phase. This is because the addition of OTAC to the TTAC micellar phase decreased the capacity factors and increased the breadth of the retention window.

Introduction

Micellar electrokinetic capillary chromatography (MECC), employing surfactant-rich electrolyte solutions and open tubular fused-silica capillaries, was first introduced in 1984 by Terabe *et al.* (1). MECC, which is a modification of capillary zone electrophoresis (CZE), has extended the utility of CZE to the separation of neutral solutes. Uncharged solutes are separated *via* their differential distribution between a fast moving aqueous phase and a slow moving micellar pseudo-stationary phase, and are eluted within a retention window that extends from the retention time of an unretained solute, t_0, to the retention time of another solute completely solubilized by the micelles, t_{mc}.

Thus far, most MECC separations have utilized aqueous sodium dodecyl sulfate (SDS) as the micellar phase. Other ionic surfactants, such as sodium tetradecyl sulfate (STS), dodecyltrimethylammonium chloride or bromide (DoTAC, DoTAB), cetyltrimethylammonium chloride or bromide (CTAC, CTAB) and bile salts, have been

briefly explored. The types of micellar phases and their applications in MECC have been discussed in recent reviews by Terabe, (2) Sepaniak *et al.* (3) and Janini and Issaq (4).

To add to the armory of useful surfactants in MECC and to further improve the methodology of the technique, our laboratory very recently introduced and evaluated alkylglucoside-borate micelles. The surface charge density of these new micellar phases can be varied conveniently by changing the borate concentration and/or the pH of the running electrolyte (5). As a result, the retention window of the alkylglucoside-borate micellar system can be varied systematically over a wide range. These readily tuned features allowed the manipulation of resolution, separation efficiency and peak capacity. Other micellar phases based on the principle of adjustable surface charge density are being investigated in our laboratory, and the results are planned for future papers.

In this paper, our objectives entail the following: (i) to shed light on the energetics of retention of neutral solutes with various micellar phases, (ii) to examine the correlation between solute retention and the hydrophobic character of the micelles, (iii) to illustrate the dependence of the retention window of MECC on the nature of the surfactant and the composition of the aqueous phase and (iv) to provide selected MECC applications pertaining to species of environmental implications. In this regard, a series of cationic surfactants having alkyl chains of various lengths were evaluated over a wide range of conditions with different neutral homologous series and urea herbicides.

Experimental

Instrument and Capillaries

The capillary electrophoresis instrument was assembled in-house from commercially available components. It consisted of a 30-kV dc power supply of dual polarity Model CZE 1000R from Spellman High Voltage Electronics Corp. (Plainview, NY, U.S.A.) and a UV-Vis variable wavelength detector Model 204 from Linear Instrument (Reno, NV,

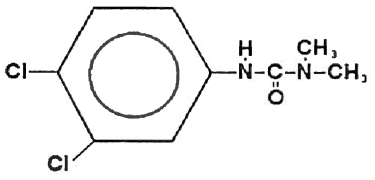
U.S.A.) equipped with a cell for on-column detection. Electropherograms were recorded and processed with Multichrom software (V1.8, VG Data Systems LTD, Cheshire, UK) *via* a VAX 4000-200 minicomputer (DEC, Maynard, MA, U.S.A.).

Fused-silica capillaries with an I.D. of 50 μm and O.D. of 363 μm were purchased from Polymicro Technology (Phoenix, AZ, U.S.A.). The total length of the capillary used in this study was either 55 or 80 cm and the corresponding separation distances were 32.5 or 50 cm.

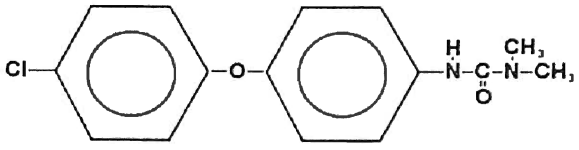
All injections were made by gravity for 10 sec at a differential height of approximately 24 cm between the inlet and the outlet buffer reservoirs. The running voltages were 10-kV for the 55/32.5 cm capillaries and 20-kV for the 80/50 cm capillaries.

Reagents and Materials

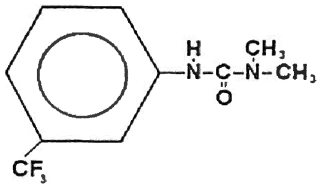
All chemicals used in this study were of analytical grade. Salts used in the preparation of electrolyte solutions were obtained from Fisher Scientific (Pittsburgh, PA, U.S.A) and Mallinchrodt (Saint Louis, MO, U.S.A.). Urea herbicides and ethylbenzene were purchased from Chem Service (West Chester, PA, U.S.A.). The structures of the herbicides are shown below.



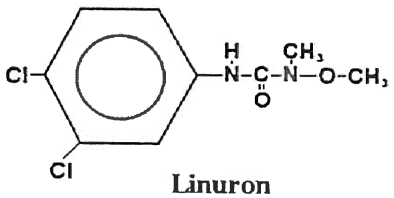
Diuron



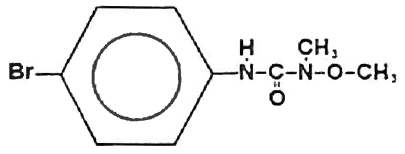
Chloroxuron



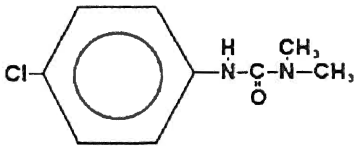
Fluometuron



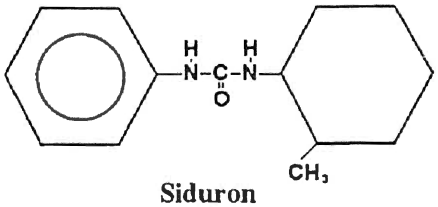
Linuron



Metobromuron



Monuron



Siduron

Table 1 lists all surfactants used in this study with their critical micelle concentration (CMC) and aggregation number (n_{agg}). Octyltrimethylammonium chloride and bromide (OTAC and OTAB, respectively), decyltrimethylammonium chloride and bromide (DTAC and DTAB, respectively), dodecyltrimethylammonium chloride and bromide (DoTAC and DoTAB, respectively), tetradecyltrimethylammonium chloride (TTAC) and 3-phenyl-1-propanol were obtained from TCI America (Portland, OR, U.S.A.). Cetyltrimethylammonium chloride (CTAC) was from Kodak (Rochester, NY, U.S.A.). Tetradecyl- and cetyltrimethylammonium bromide (TTAB and CTAB, respectively) were from Janssen Chimica (Turnhoutseweg, Belgium). n-Propyl- and n-butylbenzene were from Alfa (Danvers, MA, U.S.A.). Methanol (used to measure t_0) and toluene were purchased from J.T. Baker (Phillipsburgh, NJ, U.S.A.). Benzylalcohol and phenethylalcohol were from Schweizerhall (South Plainfield, NJ, U.S.A.). 4-Phenyl-1-butanol, 5-phenyl-1-pentanol, 6-phenyl-1-hexanol and 7-phenyl-1-heptanol were obtained from Lancaster (Windham, NH, U.S.A.). Sudan III (used to measure t_{mc}) was purchased from Aldrich (Milwaukee, WI, U.S.A.). All solutions were filtered with 0.45 μm PTFE Titan syringe filters (SRI, Somerest, NJ, U.S.A.).

TABLE 1

SURFACTANTS USED IN THIS STUDY

Surfactant	Abbreviation	CMC (mM)	n_{agg}
Octyltrimethylammonium chloride	OTAC	--	--
Octyltrimethylammonium bromide	OTAB	140[a]	--
Decyltrimethylammonium chloride	DTAC	61[a]	--
Decyltrimethylammonium bromide	DTAB	68[a]	39[c]
Dodecyltrimethylammonium chloride	DoTAC	20[a]	--
Dodecyltrimethylammonium bromide	DoTAB	16[a]	55[c]
Sodium dodecyl sulfate	SDS	8.2[a]	64[c]
Tetradecyltrimethylammonium chloride	TTAC	45[a]	--
Tetradecyltrimethylammonium bromide	TTAB	3.6[a]	70[c]
Cetyltrimethylammonium chloride	CTAC	1.3[b]	--
Cetyltrimethylammonium bromide	CTAB	0.92[a]	89[c]

[a] 25°C; [b] 30°C; [c] 20°C

The values of CMC and n_{agg} were taken from Ref. 19.

Results and Discussion

Retention Window

Effect of the Length of the Alkyl Tail of the Surfactant. Fig. 1 illustrates the breadth of the retention window obtained with the alkyltrimethylammonium halide surfactants as a function of the number of carbon atoms in the tail of the surfactant molecule. As can be seen in Fig. 1, the width of the retention window increased with decreasing alkyl chain length in the surfactant (6). The migration time of the unretained species t_0 (i.e., the magnitude of the electroosmotic velocity, v_{eo}) was largely unaffected by the length of the alkyl tail. This may indicate that the binding of the various cationic surfactants to the naked wall of the fused-silica capillary occurs to the same extent. In this binding process, it is believed that the surfactant molecules are attracted electrostatically *via* their quaternary ammonium groups to the negatively charged surface silanols, thus forming a primary hydrophobic layer. This tightly bound layer of surfactant molecules 'neutralizes' the negative surface charge, and through its nonpolar chains may undergo hydrophobic interaction with the nonpolar tails of other surfactant molecules, thus leading to the formation of a bilayer (7). In the hydrophobic bilayer, the quaternary ammonium functions of the secondary surfactant layer are oriented toward the aqueous phase. The net charge of the wall becomes positive and, consequently, under the influence of an electric field an anodal electroosmotic flow takes place (i.e., the bulk flow is toward the anode) (7-10). On the other hand, the electrophoretic mobility of the micelle v_{ep}, which is smaller in magnitude than the anodal electroosmotic flow but opposite in direction, increases with decreasing the length of the alkyl tail (i.e., decreasing the size of the micelle). The migration time of the micelle t_{mc} is given by:

$$t_{mc} = l / v_{mc} = l / (v_{eo} + v_{ep}) \qquad (1)$$

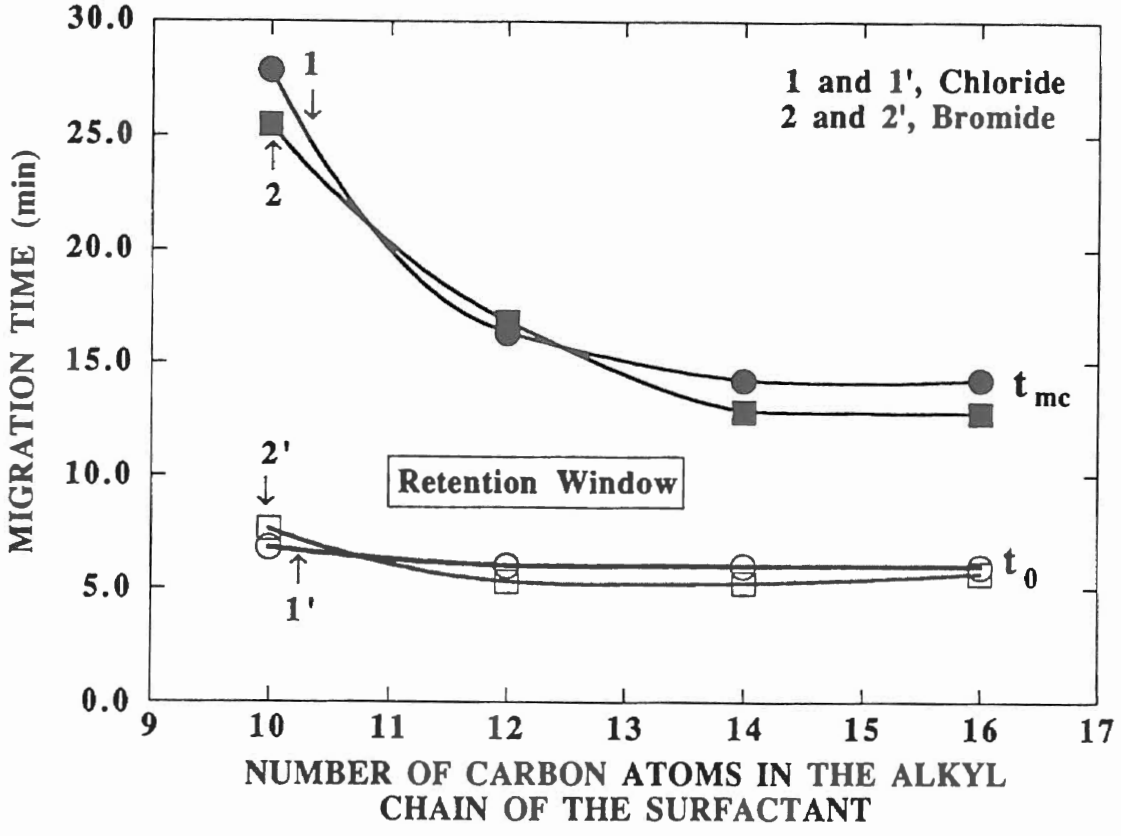


Figure 1. Breadth of the retention window as a function of the alkyl chain length of the surfactant molecule obtained with alkyltrimethylammonium bromide and chloride surfactants at constant micellized surfactant concentration. Capillary, fused-silica, 55 cm total length (32.5 cm separation distance) x 50 μm I.D.; running electrolyte, 50 mM phosphate, pH 7.0. In the case of alkyltrimethylammonium chloride surfactants, the running electrolytes contained 143, 102, 86.5 or 83.3 mM DTAC, DoTAC, TTAC or CTAC, respectively; in the case of alkyltrimethylammonium bromide surfactants, the running electrolytes contained 150, 98, 85.6 or 82.9 mM DTAB, DoTAB, TTAB or CTAB, respectively. Running voltage, 10-kV.

where l is the separation length (i.e., from capillary inlet to detection point) and v_{mc} is the net velocity of the micelle. Since the velocities v_{eo} and v_{ep} are of opposite sign and v_{eo} remains almost unchanged, as v_{ep} increases with decreasing the alkyl chain length of the surfactant, the difference between v_{eo} and v_{ep} decreases and v_{mc} decreases too. Under these conditions, and according to eqn 1, t_{mc} will keep rising, see Fig. 1. The net result is an increase in the retention window as the size of the alkyl tail decreases (6).

As seen in Fig. 1, alkyltrimethylammonium halide surfactants yielded similar retention windows. Thus, by keeping the surfactant tail the same, the micelle counterions can be changed from chloride to bromide without introducing a significant change in the retention window. The CMC of an alkyltrimethylammonium salt has been found to increase slightly when the micelle counterions are changed from BR^- to Cl^-, (11) see Table 1. This is because the polarizability of bromide ions and, consequently, the extent of their binding to the micelle are slightly higher (11). This means that the aggregation number and the size of the micelle remains almost unchanged for the same alkyl tail. This would explain the constancy of the retention window when going from micelles with chloride counterions to micelles with bromide counterions.

pH of the Micellar Phase. To further characterize the cationic micellar phases under investigation, the breadth of the retention window was measured with CTAB surfactant over the pH range 4.5 to 9.0 where the silica surface is negatively charged. Under these conditions, the width of the retention window stayed practically unchanged as the pH of the micellar phase was varied between 4.5 and 9.0. Similar behavior could be predicted for the other cationic micellar phases, since the extent of their binding to the capillary surface was almost the same as observed from the preceding set of experiments. These characteristics of the alkyltrimethylammonium salts may prove useful when such surfactants are applied to the simultaneous separations of neutral and ionizable species. With such mixtures, the migration time of neutral solutes would be unaffected by the pH of the micellar phase, while that of the ionizable species would vary to a large extent, thus

allowing the manipulation of the resolution of the system. This type of behavior can not be attained with a SDS micellar phase. In fact, at pH below 5.0-6.0, the electroosmotic flow decreases while the electrophoretic mobility of SDS micelles, which is in the opposite direction, remains the same (12). Consequently, the net mobility of the SDS micelles is opposite in direction to the electroosmotic flow (12). Thus, only those solutes that partition into the micelles can be eluted and separated.

Effect of Short Tail Surfactant Additives. Although DTAB and DTAC surfactants afforded the widest retention window (see Fig. 1), they must be used at elevated concentration since their CMC is relatively high, see Table 1. These conditions produce relatively high currents and, consequently, would require the use of lower running voltage or longer capillary columns. To provide an adequate retention window without producing excessive currents, an alternative to DTAB or DTAC would be to use cationic surfactants of longer alkyl chains, e.g., TTAC, in the presence of small amounts of short alkyl tail surfactant such as OTAC (i.e., mixed cationic micelles). As can be seen in Fig. 2, the inclusion of small amounts of OTAC surfactant into the TTAC micellar phase at the level of 50-100 mM increased the ratio t_{mc}/t_0 by a factor of 1.4-1.86. In addition, and as will be shown below, the addition of OTAC enhanced the resolution of late eluting peaks. The increase in the breadth of the retention window of the TTAC micellar phase upon adding OTAC surfactant may be explained by the fact that a mixed micelle of smaller size was formed and, consequently, the net anodal migration velocity of the micelle decreased as the amount of added OTAC increased. This is contrary to mixed micelles involving ionic and nonionic surfactants (13), where a lower surface charge and a larger micelle size were obtained, which lowered the electrophoretic mobility of the micelles and caused a narrower retention window (13).

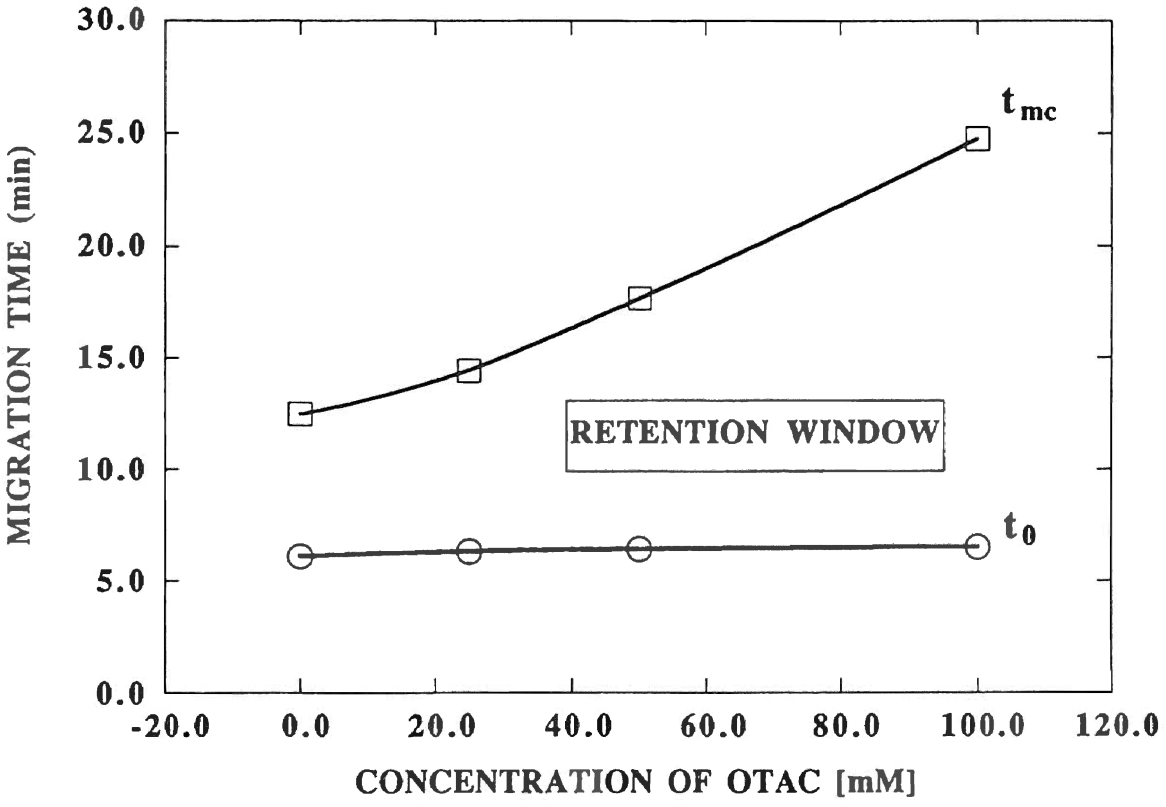


Figure 2. Breadth of the retention window as a function of the amount of OTAC in the TTAC micellar phase. Capillary, fused-silica, 80 cm total length (50 cm separation distance) x 50 μm I.D.; running electrolytes, 50 mM phosphate, pH 7.0, containing 40 mM TTAC and 0, 25, 50 or 100 mM OTAC. Running voltage, 20-kV.

Retention Behavior of Neutral Solutes

Correlation Between Capacity Factor and Carbon Number of a Homologous Series. The retention behavior of two sets of homologous series, namely phenylalkylalcohols and alkylbenzenes, was examined with the various alkyltrimethylammonium bromide and chloride micellar phases. Typical results are depicted in Fig. 3 in terms of plots of logarithmic capacity factors versus the number of carbon atoms, n_C, in the alkyl chains of the homologues. In all cases, the measurements were carried out at constant micellized surfactant concentration, [S] - CMC. As seen in Fig. 3, linear plots were obtained over the range studied. It should be noted that with the exception of DTAC and DTAB, the value of log k' of benzylalcohol solute did not align well with the rest of the homologues. This is why the R values ranged between 0.993-0.996 for surfactants having more than ten carbon atoms in their alkyl chains (see Tables 2 and 3).

Due to the stronger hydrophobic character of the alkylbenzene homologous series, these solutes were resolved only with the decyltrimethylammonium micellar phases (i.e., DTAB and DTAC) for n_C up to 4 under the experimental conditions used in this study. Plots of $log\ k'$ vs. n_C were linear with an R value of 0.999, see Table 4.

From the above results, the relationship between $log\ k'$ and n_C seems to follow the expression normally found in reversed-phase chromatography (14):

$$log\ k' = (log\ \alpha)n_c + log\ \beta \qquad (2)$$

where the slope $log\ \alpha$ is a measure of methylene or hydrophobic selectivity which characterizes nonspecific interactions, while the intercept $log\ \beta$ reflects the specific interactions between the residue of the molecule and the aqueous and micellar phases. This equation implies a constant contribution to the free energy of transfer of the solute between the aqueous phase and the micellar phase with each CH_2 increment in the chain length of the homologue.

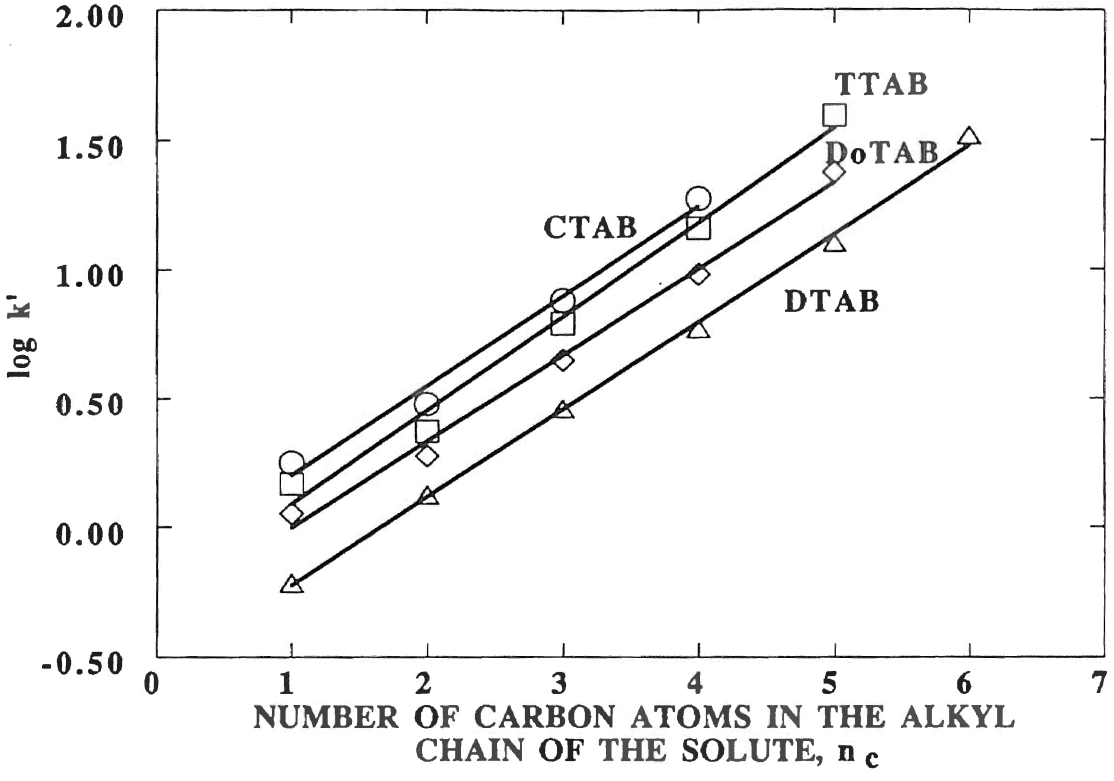


Figure 3. Plots of log k' vs. n_C for a phenylalkylalcohol homologous series obtained with alkyltrimethylammonium chloride surfactants at constant micellized surfactant concentration. Running electrolytes, 50 mM phosphate buffer, pH 7.0, containing 150, 98, 85.6 or 82.9 mM DTAB, DoTAB, TTAB or CTAB, respectively. Other conditions are as in Fig. 1.

TABLE 2

SLOPES, INTERCEPTS AND R VALUES OF PLOTS OF log k' vs. n_c FOR A PHENYLALKYLALCOHOL HOMOLOGOUS SERIES OBTAINED WITH VARIOUS ALKYLTRIMETHYLAMMONIUM CHLORIDE MICELLAR PHASES AS WELL AS WITH SDS

Surfactant	log β	log α	R
DTAC	-0.652	0.318	0.999
DoTAC	-0.313	0.339	0.996
TTAC	-0.162	0.360	0.995
CTAC	-0.159	0.365	0.994
SDS	-0.348	0.346	0.999

Capillary, fused-silica, 55 cm total length (32.5 cm separation distance) x 50 μm I.D.; running electrolyte, 50 mM phosphate, pH 7.0, containing 143, 102, 86.5 or 83.3 mM DTAC, DoTAC, TTAC or CTAC, respectively; running voltage, 10-kV. In the case of SDS surfactant the running electrolyte was 50 mM phosphate buffer, pH 9.2, containing 90.2 mM surfactant.

TABLE 3

SLOPES, INTERCEPTS AND R VALUES OF PLOTS OF log k' vs. n_c FOR A PHENYLALKYLALCOHOL HOMOLOGOUS SERIES OBTAINED WITH THE VARIOUS ALKYLTRIMETHYLAMMONIUM BROMIDE MICELLAR PHASES

Surfactant	log β	log α	R
DTAB	-0.565	0.340	0.999
DoTAB	-0.339	0.336	0.996
TTAB	-0.276	0.365	0.994
CTAB	-0.148	0.348	0.993

Capillary, fused-silica, 55 cm total length (32.5 cm separation distance) x 50 μm I.D.; running electrolyte, 50 mM phosphate, pH 7.0, containing 150, 98, 85.6 or 82.9 mM DTAB, DoTAB, TTAB or CTAB, respectively; running voltage, 10-kV.

TABLE 4

SLOPES, INTERCEPTS AND R VALUES OF PLOTS OF log k' vs. n_c FOR AN ALKYLBENZENE HOMOLOGOUS SERIES OBTAINED WITH DTAB AND DTAC SURFACTANTS

Surfactant	log β	log α	R
DTAC	0.036	0.342	0.999
DTAB	0.217	0.398	0.999

Other experimental conditions are as in Tables 2 and 3.

As seen in Tables 2, 3 and 4, the *log α* values remained almost the same when varying the length of the surfactant tail. The nature of micelle counterion (i.e., chloride or bromide) does not seem to affect the value of the slope. However, as expected, the values of the intercepts increased with the length of the surfactant tail and varied slightly with the nature of the micelle counterions. These findings suggest similar physico-chemical basis for retention on the various cationic micellar phases, and the only difference is the phase ratio.

There are no substantial differences between the log α values obtained with alkylbenzenes and phenylalkylalcohols with the various surfactants. This may suggest that the contribution by a methylene group to the free energy transfer of the solute between the aqueous and micellar phases is largely independent of the rest of the molecule.

When an anionic surfactant such as SDS was used, the log α value was virtually the same as that obtained with the cationic surfactants, see Table 2. This indicates that changing the nature of the ionic head group from cationic to anionic while keeping the size of this group approximately the same, does not change the energetics of retention of neutral, hydrophobic compounds. This may mean that the retention of nonpolar compounds is largely due to their interaction with the hydrophobic core of the various micelles.

Comparison of the Energetics of Retention on the Various Surfactants. For two different MECC systems A and B, the logarithmic capacity factors are written as:

$$\log k'_A = \phi_A - \Delta G^0_A / 2.3RT \tag{3}$$

$$\log k'_B = \phi_B - \Delta G^0_B / 2.3RT \tag{4}$$

where R, T, ϕ and ΔG^0 are the gas constant, absolute temperature, logarithmic phase ratio and Gibbs free energy, respectively. Upon subtraction, eqns 3 and 4 can be rearranged as:

$$\log k'_A = \log k'_B + (\phi_A - \phi_B) + (\Delta G^0_B - \Delta G^0_A) / 2.3RT \tag{5}$$

If the differences in the Gibbs retention energies of the two micellar systems is zero for all solutes, eqn 5 can be simplified to:

$$\log k'_B = \log k'_A - \phi_A + \phi_B \tag{6}$$

A plot of $\log k'_B$ versus $\log k'_A$ should give a straight line with a slope of unity and an intercept equal to the logarithm of the quotient of the phase ratios. In this case, the retention is termed homoenergetic.

On the other hand, if the corresponding Gibbs energies for the two MECC systems are proportional, such as $\Delta G_A^0 = \alpha \Delta G_B^0$, where α is a constant, eqn 6 can be combined with eqns 3 and 4 to yield:

$$log\, k_A^{'} = \alpha\, log\, k_B^{'} + \phi_A - \alpha\phi_B \qquad (7)$$

Equation 7 shows that when the ratio of the Gibbs retention energies in the two micellar phases is constant, linear log k'-log k' plots with slope of α are obtained, and the retention is termed homoenergetic. Equation 6 is a special case of eqn 7 when α is unity.

The above treatment was originally introduced by Horváth *et al.* (15) to evaluate reversed-phase chromatographic retention data obtained on various nonpolar silica-based stationary phases. The same model was also applied by El Rassi and Horváth (16) for the comparison of the reversed-phase chromatographic properties of silica-based stationary phases which were designed for ion-exchange and hydrophobic interaction chromatography of nucleic acids. Very recently, the same treatment allowed the comparison of the energetics of retention on various zirconia-based reversed phase packings introduced by Yu and El Rassi (17). The various surfactants were compared in terms of their energetics of retention using log k'-log k' plots, cf. eqn 6. Typical results are depicted in Fig. 4a and b in terms of plots of $\log k_B^{'}$ versus $\log k_A^{'}$, which show a linear correlation. The values of the slopes and intercepts are summarized in Tables 5 and 6. As seen in Tables 5 and 6, the slopes are close to unity indicating quasi-homoenergetic micellar systems. With the exception of TTAC, the hydrophobic phase ratio decreased monotonically when going from a C_{10} to C_{16} surfactant. The hydrophobic phase ratios of DTAB and DTAC are less than those of CTAB and CTAC by factors of 0.41 and 0.33, respectively, whereas the hydrophobic phase ratios of DoTAB and DoTAC are less than those of CTAB and CTAC by factors of 0.68 and 0.73, respectively. The hydrophobic phase ratio of TTAB is 0.82 that of CTAB. One interesting point is that the hydrophobic

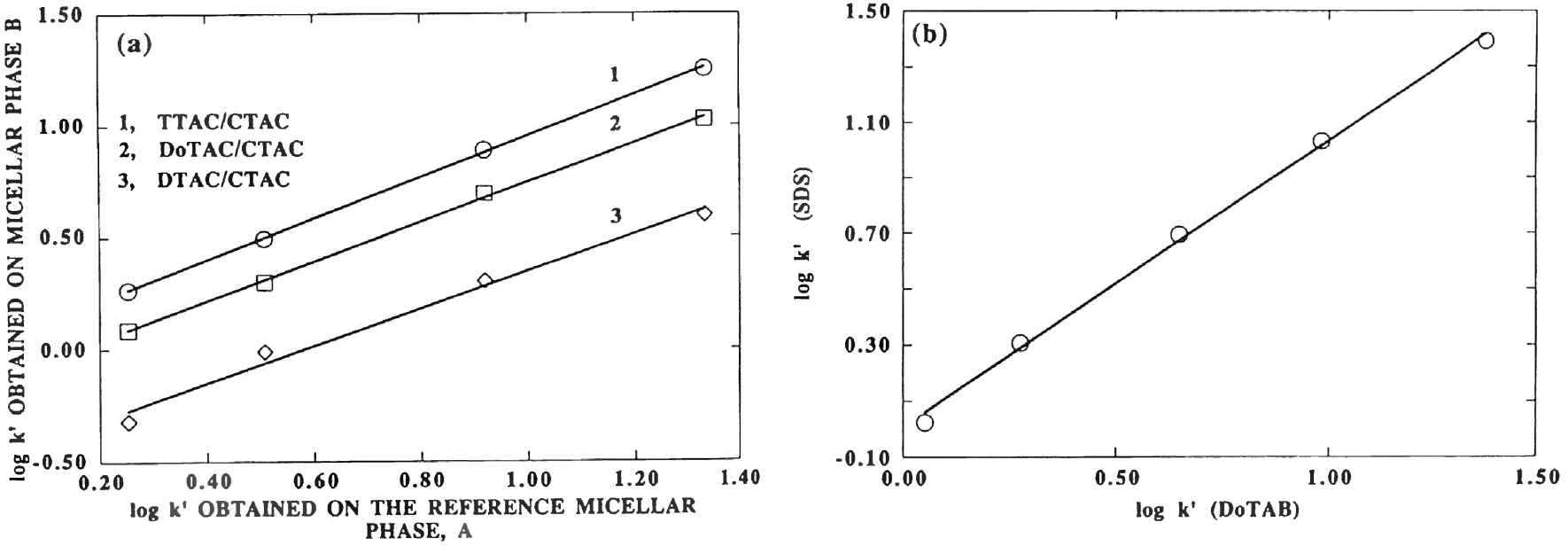


Figure 4. (a) Plots of log k' - log k' of a phenylalkylalcohol homologous series obtained on one micellar phase versus another reference micellar phase at constant micellized surfactant concentration. (b) Plot of log k' - log k' of a phenylalkylalcohol homologous series obtained on SDS versus DoTAB at constant micellized surfactant concentration. Running electrolyte, 50 mM phosphate buffer, pH 7.0, containing 98 mM DoTAC or pH 9.2, containing 90.2 mM SDS. Other conditions are as in Fig. 1.

TABLE 5

SLOPES, INTERCEPTS, R VALUES AND ANTILOG OF INTERCEPTS OF log k' - log k' PLOTS OF A PHENYLALKYLALCOHOL HOMOLOGOUS SERIES OBTAINED ON ALKYLTRIMETHYLAMMONIUM CHLORIDE MICELLAR PHASES

Micellar phase B/ Micellar Phase A	Slope	Intercept	R	φ_B/φ_A
CTAC/CTAC	1.000	0.000	1.000	1.00
TTAC/CTAC	0.920	0.03358	0.9998	1.08
DoTAC/CTAC	0.880	-0.1359	0.9994	0.73
DTAC/CTAC	0.830	-0.4798	0.9933	0.33

All experimental conditions are as in Table 2. The antilog of intercepts is the quotient of phase ratios φ_B/φ_A.

TABLE 6

SLOPES, INTERCEPTS, R VALUES AND ANTILOG OF INTERCEPTS OF log k' - log k' PLOTS OF A PHENYLALKYLALCOHOL HOMOLOGOUS SERIES OBTAINED ON ALKYLTRIMETHYLAMMONIUM BROMIDE AS WELL AS SDS MICELLAR PHASES

Micellar phase B/ Micellar Phase A	Slope	Intercept	R	φ_B/φ_A
CTAB/CTAB	1.000	0.000	1.000	1.00
TTAB/CTAB	0.979	-0.0846	0.9997	0.82
DoTAB/CTAB	0.906	-0.1639	0.9996	0.68
DTAB/CTAB	0.929	-0.3872	0.9913	0.41
SDS/DoTAB	1.027	0.0045	0.9988	1.01

All experimental conditions are as in Table 2. The antilog of intercepts is the quotient of phase ratios φ_B/φ_A.

character of SDS is similar to that of a cationic surfactant of same alkyl tail, e.g., DoTAB, see Table 6 and Fig. 4b.

Correlation Between Capacity Factor and Carbon Number of Surfactant. In MECC, the capacity factor k' is given by (18):

$$k' = \varphi K \approx K\vartheta([S] - CMC) \tag{8}$$

where φ, K, ϑ, $[S]$ and CMC are the phase ratio (i.e., ratio of the volume of the micellar phase to that of the aqueous phase), solute distribution coefficient between micellar and aqueous phases, the partial specific volume of the micelle, the concentration of the surfactant and the critical micellar concentration, respectively. $[S] - CMC$ is the concentration of micellized surfactant.

According to eqn 8, at constant micellized surfactant concentration, the partial specific volume of the micelle ϑ is the parameter that determines the magnitude of the phase ratio φ of the various micellar phases under investigation. In other words, by varying the size of the alkyl tail of the surfactant while keeping $[S] - CMC$ constant, ϑ will vary and the phase ratio φ too. Under these conditions, eqn 8 can be expressed as:

$$k' = \varphi K \approx (Constant) K\vartheta \tag{9}$$

From reported values (19), the aggregation number, n_{agg}, of the surfactants under consideration is a linear function of the number of carbon atoms in the alkyl chain of the surfactant molecule, $n_{c,\ surf}$, see Fig. 5. This quasi-linear relationship is also found with other ionic surfactants having similar alkyl tails such as sodium alkyl sulfates and sulfonates whereby the R values of plots of n_{agg} vs. $n_{c,\ surf}$, were 0.990 and 0.988, respectively (plots not shown). Literature data on micellization (20) of neutral surfactants having aggregation numbers similar to the cationic surfactants under investigation, reveal that there is a linear correlation between the ϑ and n_{agg} of the surfactant, see Fig. 6. It follows then that the partial specific volume of the micelle would also be a linear function of the carbon number of the alkyl tail of the surfactant. Thus,

$$k' = \varphi K \approx (Constant) K\vartheta \propto K.n_{c,surf} \tag{10}$$

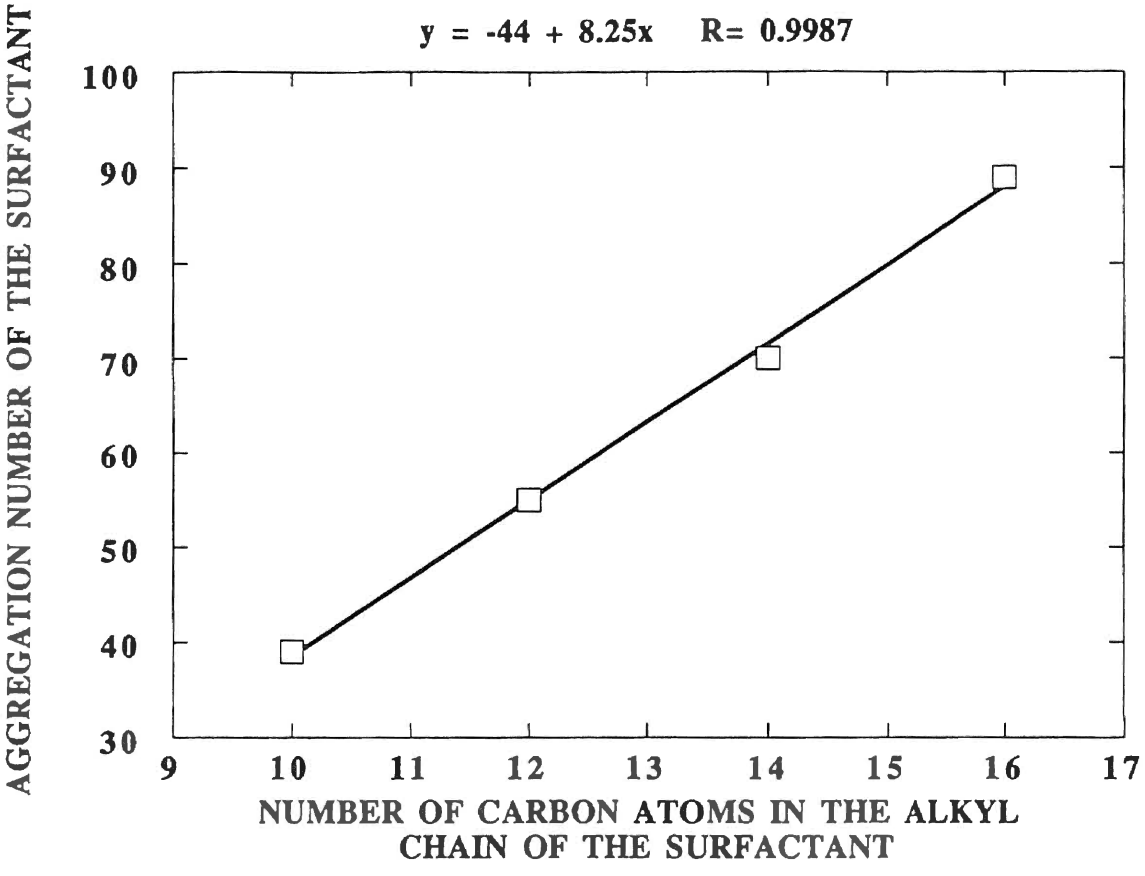


Figure 5. Plot of aggregation number versus the number of carbon atoms in the alkyl chain of the surfactant for alkyltrimethylammonium bromide salts. Data taken from Ref. 16.

According to eqn 10, at constant micellized surfactant concentration, the nature of the surfactant may affect the capacity factor through its effect on either the phase ratio (i.e., ϑ or $n_{c,\ surf}$), the partition coefficient K, or both.

Fig. 7a and b shows plots of capacity factors of phenylalkylalcohol homologues and herbicides versus the number of carbon atoms in the surfactant molecule, respectively. As seen in Fig. 7a and b, the plots are quite linear. This may suggest that the capacity factor depends on the size of the alkyl tail of the surfactant, i.e., $n_{c,surf}$, while the distribution coefficient K remains the same. Stated differently, at constant micellized surfactant concentration, the distribution coefficient of a given solute is largely unaffected by the length of the alkyl tail and the major contributor to retention is the phase ratio (i.e., ϑ or the size of the alkyl tail $n_{c,\ surf}$). These findings corroborate earlier observations by Terabe *et al.* (18) that the distribution coefficients of a series of neutral solutes were found to be almost the same with sodium dodecyl sulfate and sodium tetradecyl sulfate micellar phases.

Effect of Short Tail Surfactant Additive. Besides affecting the retention window (see Fig. 2), a short alkyl chain surfactant, such as OTAC, produced a monotonic decrease in the retention of neutral species when added in small amounts to the TTAC micellar phase as illustrated in Fig. 8a and b. This may be attributed to the formation of a mixed micelle of smaller size than that of the TTAC micelle. The correlation between log k' and the concentration of OTAC additive is quasi-linear and yields parallel lines for the homologues and the herbicides. This may mean that the addition of OTAC to the TTAC micellar phase does not produce any significant change in selectivity. Instead, the addition of OTAC resulted in enhancing the resolution of late eluting peaks (i.e., very hydrophobic solutes) by decreasing the capacity factors and enlarging the retention window of the MECC system, see below.

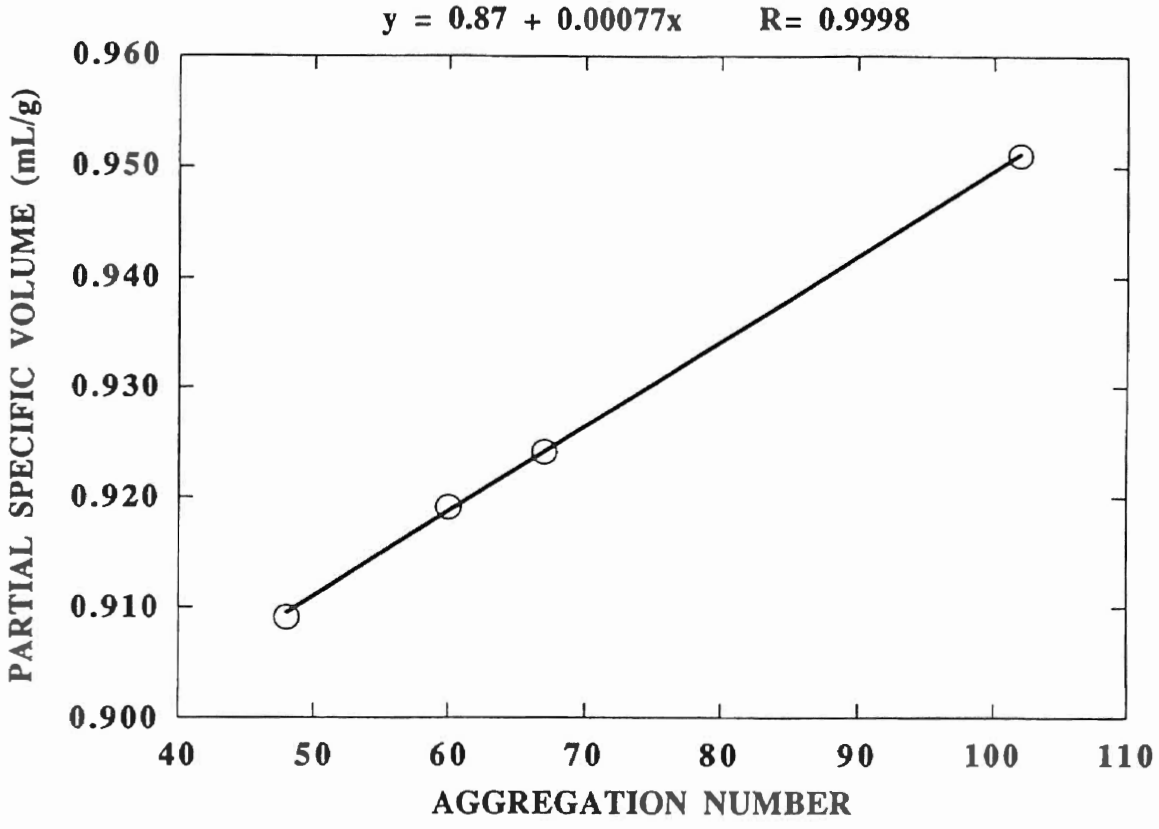


Figure 6. Plot of partial specific volume versus the aggregation number of methoxypolyoxyethylene decanoate and dodecanoate surfactants. Data taken from Ref. 17.

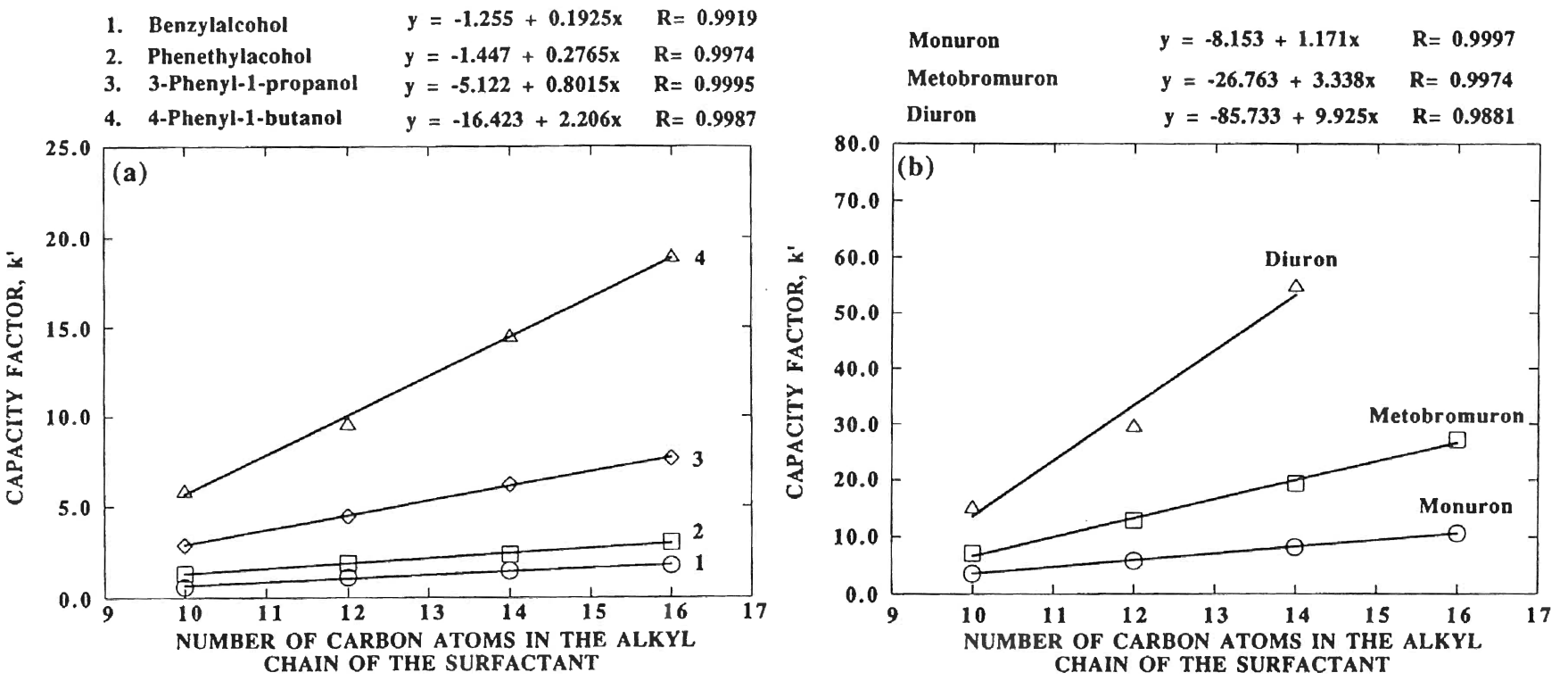


Figure 7. Plots of capacity factors of phenylalkylalcohols in (a) and urea herbicides in (b) versus the number of carbon atoms in the alkyl chain of the alkyltrimethylammonium bromide surfactants at constant micellized surfactant concentration. Conditions are as in Fig. 1.

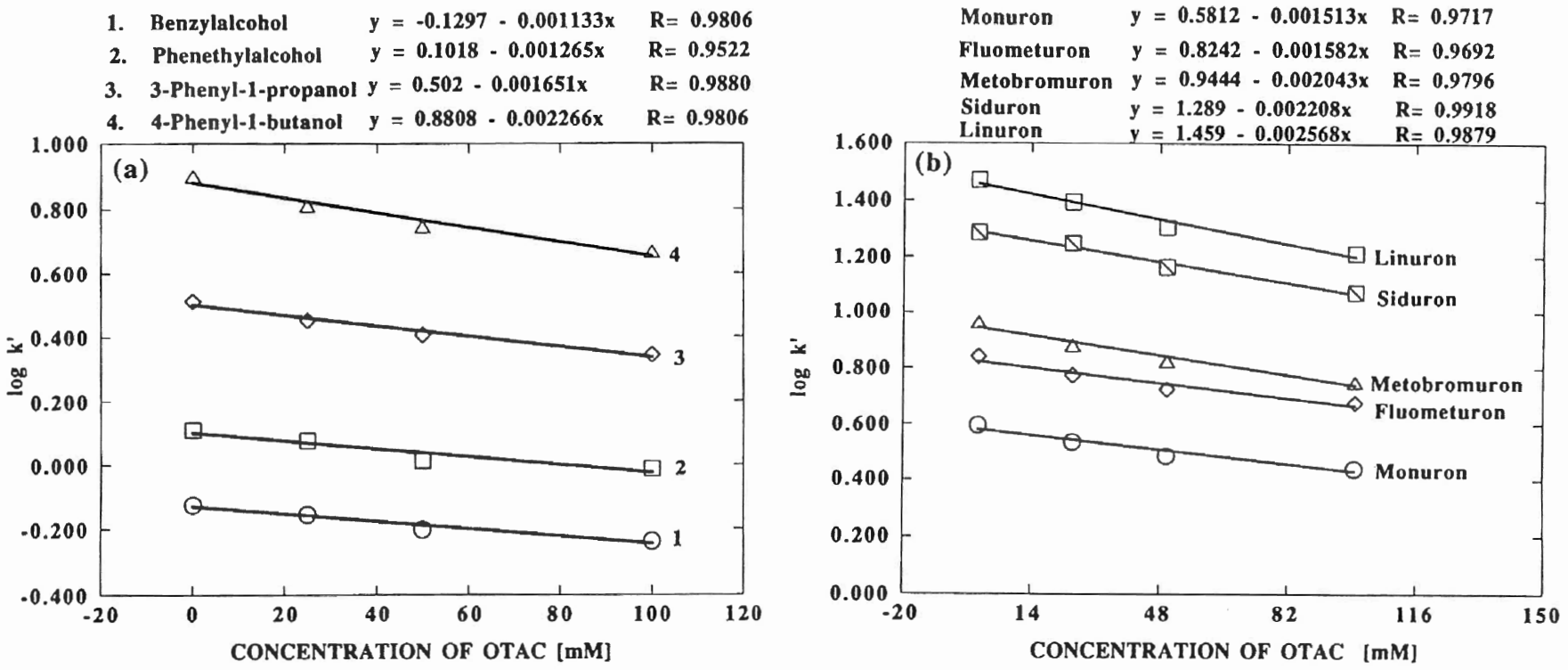


Figure 8. Plots of log k' of a phenylalkylalcohol homologous series in (a) and urea herbicides in (b) versus the concentration of OTAC in TTAC micellar phase. Conditions are as in Fig. 2.

Selected Applications

To illustrate the potentials of the various alkyltrimethylammonium halide surfactants in MECC of neutral species of environmental significance, we have selected a series of six urea herbicides, namely monuron, fluometuron, metobromuron, siduron, linuron and chloroxuron, the structures of which are given in the Experimental.

As seen in Fig. 9a, the CTAC surfactant at a concentration of 31.2 mM (i.e., 24 times the CMC) in the running electrolyte allowed the separation of only five herbicides with an average plate count of 120,000 plates/m. At this surfactant concentration, linuron almost co-eluted with chloroxuron. At CTAC concentration below 31.2 mM, the overall separation did not improve and broad peaks were observed. This may be attributed to the fact that the packing density of the capillary column with micelles decreased at lower amount of surfactant in the running electrolyte. This would lead to longer intermicellar distances which would give rise to slower mass transfer in the aqueous phase and concomitantly lower separation efficiencies (21).

The TTAC surfactant at a concentration of 27 mM (i.e., 6 times the CMC) in the running electrolyte proved to be useful for the separation of the herbicide mixture, see Fig. 9b. Chloroxuron was slightly resolved from the Sudan III (i.e., the migration time of the micelle), and the separation efficiency was 63% of that obtained with CTAC (N_{av} = 75,600 plates/m). Increasing the concentration of TTAC in the running electrolyte to 40 mM increased the separation efficiency by a factor of 2.65 (N_{av} = 201,000 plates/m), see Fig. 10a. This may be due to shortening the intermicellar distances and, consequently, to faster mass transfer in the aqueous phase. However, at this surfactant concentration (i.e., 40 mM) chloroxuron co-eluted with Sudan III. To provide an adequate retention window, OTAC surfactant was added to the 40 mM TTAC micellar phase at concentrations of 25, 50 or 100 mM. As can be seen in Fig. 10b and c, the addition of 25 or 50 mM OTAC enlarged the retention window and, consequently, the resolution

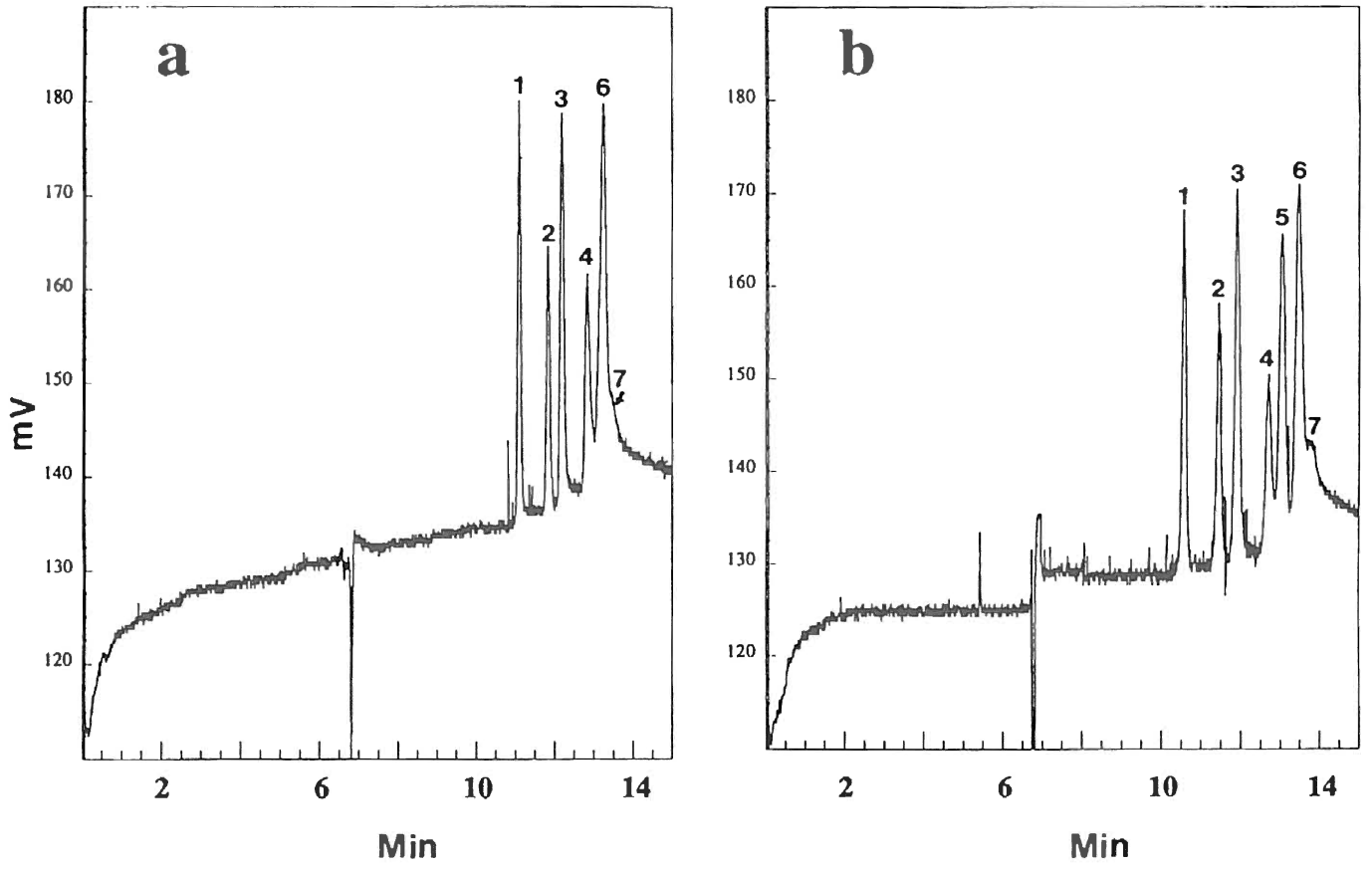


Figure 9. Electropherograms of urea herbicides obtained with CTAC in (a) and TTAC in (b). Capillary, fused-silica, 80 cm total length (50 cm separation distance) x 50 μm I.D.; running electrolyte, 50 mM phosphate, pH 7.0, containing 31.2 mM CTAC or 27 mM TTAC; running voltage, 20-kV; current, ca. 27 μA. Solutes: 1, monuron; 2, fluometuron; 3, metobromuron; 4, siduron; 5, linuron; 6, chloroxuron; 7, Sudan III.

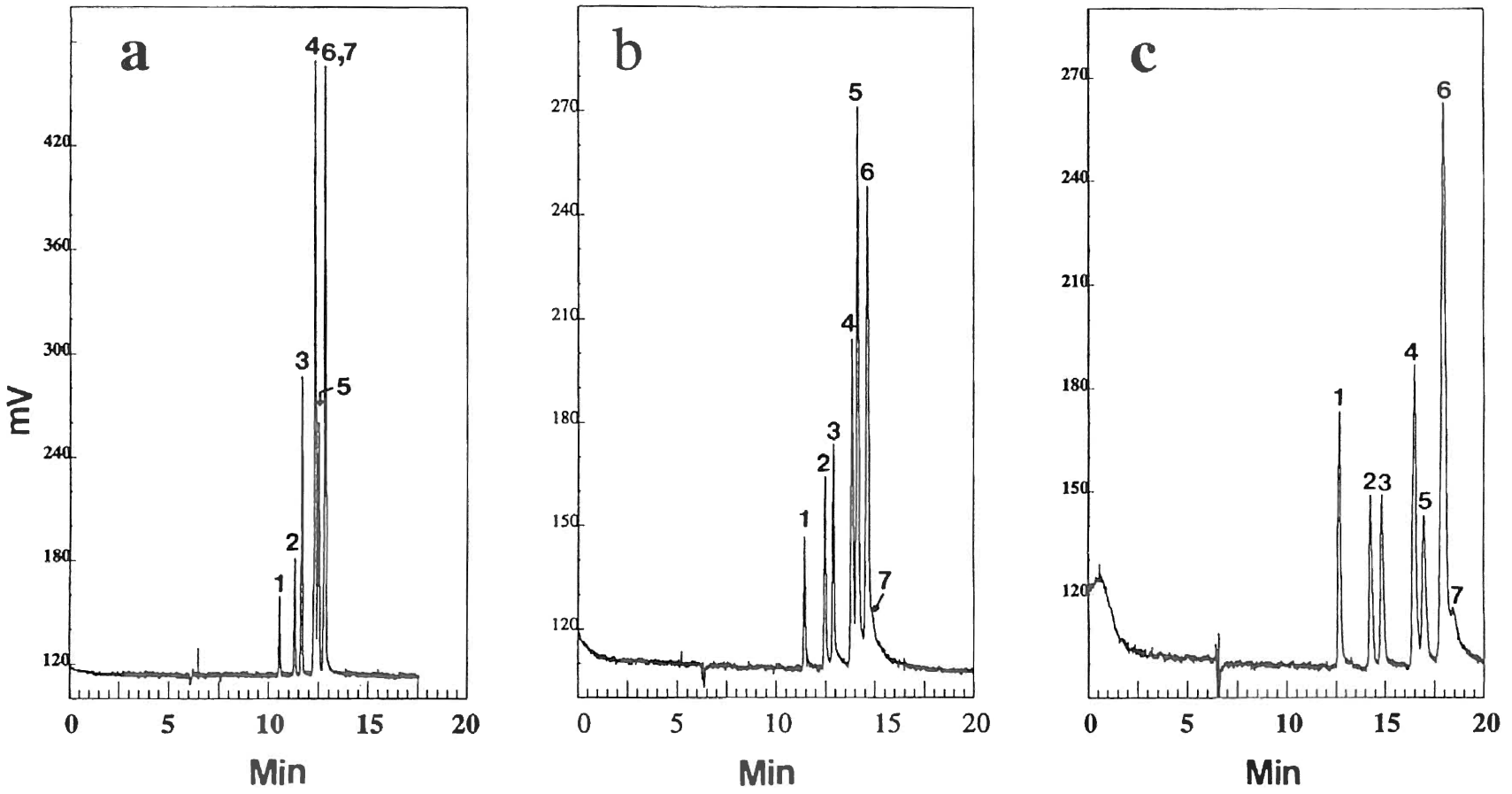


Figure 10. Electropherograms of urea herbicides. Running electrolytes, 50 mM phosphate, pH 7.0, containing 40 mM TTAC and 0 or 25 or 50 mM OTAC in (a), (b) and (c), respectively. Currents, 29, μA in (a), 44, μA in (b) and 60 μA in (c). Solutes and other conditions are as in Fig. 9.

between chloroxuron and Sudan III increased, but at the expense of decreasing the overall separation efficiency by factors of 0.83 (N_{av} = 167,000 plates/m) and 2.48 (N_{av} = 84, 200 plates/m), respectively. By adding 100 mM OTAC to the TTAC micellar phase (results not shown), the chloroxuron peak was completely resolved from the Sudan III peak, but the separation efficiency decreased even further to 47,160 plates/m (i.e., by a factor of 4.26). From these results, the addition of OTAC to the TTAC micellar phase in the concentration range of 25-50 mM seems to provide an adequate retention window (also resolution) with sufficient plate count.

As shown above (cf. Fig. 1 and Table 5), the DoTAC micellar phase exhibited a wider retention window and a lesser hydrophobic phase ratio than CTAC or TTAC. These two features of the DoTAC micellar phase yielded a better overall separation of the urea herbicide mixture than CTAC or TTAC, see Fig. 11a. The concentration of DoTAC surfactant in the running electrolyte was 80 mM (i.e., 4 times the CMC), and the average plate count was approximately 164,000 plates/m. All peaks were well resolved and the most hydrophobic herbicide (i.e., chloroxuron) was quite separated from Sudan III.

Fig. 11b displays the separation of the same herbicide mixture with DTAC micellar phase. As seen in Fig. 11b, the DTAC surfactant at a concentration of 170 mM (i.e., ca. 2.8 times the CMC) in the running electrolyte yielded an overall separation that was the best among the various alkyltrimethylammonium halide micellar phases. This is not surprising since DTAC afforded the widest retention window and the lowest hydrophobic phase ratio (see Fig. 1 and Table 5). The average plate count was approximately 115,000 plates/m.

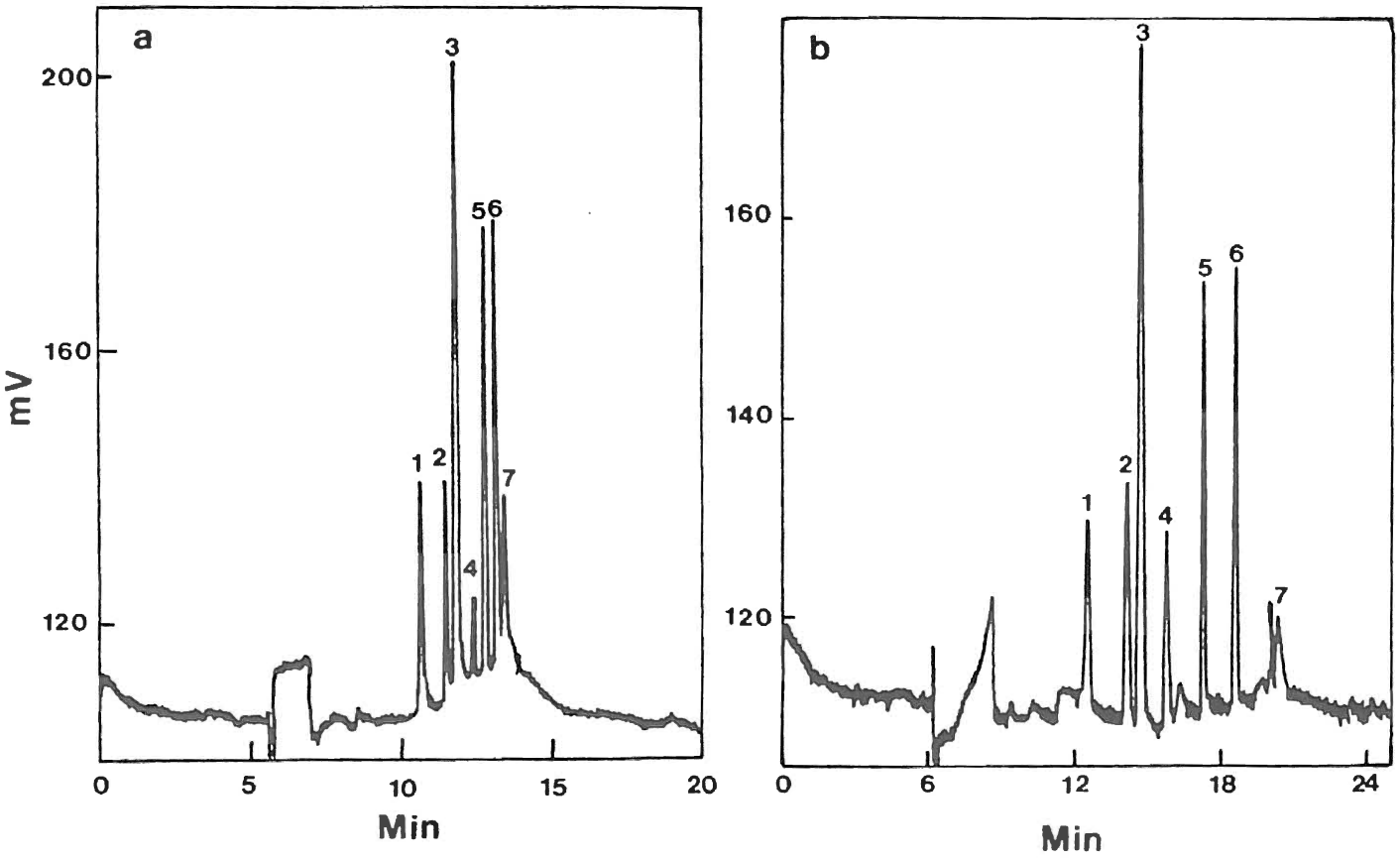


Figure 11. Electropherograms of urea herbicides obtained with DoTAC in (a) and DTAC in (b). Running electrolyte, 30 mM phosphate, pH 7.0, containing 80 mM DoTAC or 170 mM DTAC. Running voltages, 20-kV in (a) and 19-kV in (b); current, ca. 38 μA in (a) and 95 μA in (b). Solutes and other conditions are as in Fig. 9.

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CHAPTER III

MIXED CATIONIC-CATIONIC MICELLAR PHASES

Introduction

In MECC, several experimental conditions can be controlled to manipulate retention and selectivity. The distribution coefficient and, in turn, retention and selectivity can be altered through at lease five different operating conditions: (i) capillary temperature, (ii) nature of the surfactant, (iii) composition of the micelle, (iv) choice of the aqueous phase and (v) aqueous phase additives. As discussed in Chapter I, temperature is not used to optimize selectivity, but should be controlled during runs to allow reproducible separations. For neutral solutes, the pH and the ionic strength of the electrolyte (aqueous phase) have little or no effect on solute partitioning into the micelles. Thus, manipulating retention and selectivity by the choice of the aqueous phase (i.e., pH and ionic strength) is only meaningful for ionizable solutes whose interaction with the micelle is largely dependent on the pH and the ionic strength of the medium. This is because polar, ionic solutes undergo association mostly with the ionic polar head of the surfactant. Of course, the partitioning of all types of solutes (neutral or charged) can be influenced by additives such as organic modifiers. However, the presence of organic modifiers has two adverse effects. The addition of organic modifiers (acetonitrile or methanol) is accompanied by a decrease in separation efficiencies due to the partial breakdown of the micelle and by an increase in separation time due to a decrease in the electroosmotic flow (1).

The nature of the surfactant has an influence on MECC selectivity. Changing the length of the alkyl tail while keeping the polar head group the same will obviously change

retention but not selectivity. Chapter II provides the first systematic study in this direction. To change selectivity through the nature of the surfactant, a surfactant with a different polar head must be selected. For instance, going from a MECC system consisting of SDS micelles to another MECC system made up of bile salts is shown to produce significant changes in selectivities under otherwise identical conditions (2). The modification of the micelle by adding a second surfactant to form a mixed micelle is utilized to manipulate retention in MECC. Thusfar, only a few attempts involving ionic-nonionic mixed micelles have been reported (3). Since a mixed micelle of ionic and nonionic surfactant has a lower surface charge and a larger size, its electrophoretic mobility is lower than a single ionic micelle, and the net result is a narrower retention window. In this chapter, our aim is to provide a systematic study involving cationic-cationic mixed micelles and to evalute the electrokinetic behavior of such micelles as well as the retention behavior of homologous solutes and neutral urea herbicides.

Experimental

Instrumentation and Capillaries

The capillary electrophoresis instrument was assembled in-house from commercially available components. It consisted of a 30-kV dc dual polarity power supply, Model CZE 1000R, from Spellman High Voltage Electronics Corp. (Plainview, NY, U.S.A.) and a Model 204 UV-Vis variable wavelength detector with an on-column capillary detection cell from Linear Instrument (Reno, NV, U.S.A.). Electropherograms were recorded and processed with Multichrom software (V1.8, VG Data Systems LTD, Cheshire, UK) *via* a VAX 4000-200 minicomputer (DEC, Maynard, MA, U.S.A.).

Fused-silica capillaries (50 μm I.D., 363 μm O.D.) were purchased from Polymicro Technology (Phoenix, AZ, U.S.A.). The total length of the capillary used in this study

was 80 cm and the separation distance was 50 cm. All capillaries were first treated with 1.0 M NaOH and then 3.0 M HNO_3 and finally conditioned for 3 hours at 20-kV with the running electrolyte before use. Capillaries were washed with methanol and the running electrolyte between injections.

All injections were made hydrodynamically (i.e., by gravity) for 3 seconds at a differential height of approximately 22 cm between the inlet and the outlet buffer reservoirs. The running voltage was 20-kV and detection wavelength was 210 nm. In cases where Sudan III produced a split peak, the retention time of the taller peak was used as the t_{mc}.

Reagents and Materials

Analytical grade reagents were used throughout this study. Salts used to prepare the electrolyte solutions were obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.) and Mallinchrodt (St. Louis, MO, U.S.A.). The urea herbicides were purchased from Chem. Service (West Chester, PA, U.S.A.), and their structures are diagrammed in the previous chapter. Decyltrimethylammonium chloride (DTAC), dodecyltrimethylammonium chloride (DoTAC), tetradecyltrimethylammonium chloride (TTAC) and 3-phenyl-1-propanol were obtained from TCI America (Portland, OR, U.S.A.). Cetyltrimethylammonium chloride (CTAC) was obtained from Kodak (Rochester, NY, U.S.A.). HPLC grade methanol, used to measure t_0, was purchased from J.T. Baker (Phillipsburgh, NJ, U.S.A.). Benzylalcohol and phenethylalcohol were obtained from Schweizerhall (South Plainfield, NJ, U.S.A.). 4-Phenyl-1-butanol and 5-phenyl-1-pentanol were obtained from Lancaster (Windham, NH, U.S.A.). Nitromethane, nitroethane, nitropropane, nitrobutane, nitropentane, nitrohexane and Sudan III were obtained from Aldrich (Milwaukee, WI, U.S.A.). All solutions were filtered with 0.45 μm PTFE Titan syringe filters (SRI, Somerest, NJ, U.S.A.).

Results and Discussion

Some General Aspects of Mixed Micelles

Binary cationic surfactant mixtures at concentrations above their CMC's were used to adjust the capacity factors of the solutes under investigation. It is well established, from studies in free solutions (4,5), that the addition of a co-surfactant changes the physical properties of the micelle (e.g., CMC, aggregation number, partial specific volume, etc.), a condition that should lead to changes in the partitioning of the solutes to the micelle, thus allowing the adjustment of their retention.

The critical micellar concentration of binary surfactant mixtures (CMC_{mix}) may be less than (synergism), an intermediate of, or greater than (negative synergism) the CMC of the two components (4,5). For cationic-cationic mixed micellar systems, the CMC_{mix} is usually an intermediate value between the two individual surfactants. However, the value of CMC_{mix} is disproportionally influenced by the lower CMC component, i.e., by the surfactant with the longer hydrophobic tail. For example, a 1:1 molar ratio of DoTAC, with a CMC of 20.3, and TTAC, with a CMC of 4.5, has a mixed CMC of 7.3 mM (4).

Mixed micelle formation in aqueous solutions arise from two types of interactions (4,6-9). The first type is the hydrophobic effect, which is the free energy driving force for the formation of aggregates in solution. Since the hydrophobic effect is not specific to head groups, it favors the formation of randomly mixed surfactant molecules in the same way as it does in pure surfactant systems. Therefore, the hydrophobic effect can be viewed as the force responsible for 'ideal' mixtures of surfactants in the aggregate.

The second driving force for mixed aggregates involves interactions between unlike head groups of different surfactant molecules in the mixed aggregate (or micelle) itself. Since this type of interaction occurs within the micelle, it can be viewed as an excess free energy of mixing which measures 'nonideal' mixing in the micelle. There is ample evidence (4) that the primary cause of nonideal mixing behavior is the electrostatic interaction

between head groups of the surfactant molecules in the mixed micelle. Mixed cationic-anionic micelles are termed 'nonideal' because of electrostatic attraction between dissimilar charges, whereas mixed micelles formed from surfactants of like charges behave ideally. For mixed ionic-nonionic surfactant systems, charge separation resulting from the interaction between ionic and nonionic surfactant molecules as well as the relative head group sizes are the major factors in determining the strength of interactions (9).

TABLE 1

CALCULATED CMC's AND MICELLIZED SURFACTANT CONCENTRATIONS FOR VARIOUS ALKYLTRIMETHYLAMMONIUM CHLORIDE MIXTURES

Surfactant Mix	Set #	CMC (mM)	[S]-CMC (mM)
122 mM DTAC		61	61
112 mM DTAC/10 mM DoTAC	Set 1	52	70
82 mM DTAC/40 mM DoTAC		36	86
62 mM DTAC/60 mM DoTAC		30	92
60 mM DoTAC		20	40
55 mM DoTAC/5 mM TTAC	Set 2	16	44
50 mM DoTAC/10 mM TTAC		13	47
45 mM DoTAC/15 mM TTAC		11	49
60 mM DoTAC		20	40
59 mM DoTAC/1 mM CTAC	Set 3	16	44
55 mM DoTAC/5 mM CTAC		9	51
20 mM DoTAC/40 mM CTAC		2	58

The micellar systems evaluated in this study are cationic-cationic mixed micelles, and therefore ideal mixing behavior may prevail. The generalized formula for calculating the CMC of mixed surfactant micelles is (4):

$$\frac{1}{CMC_{mix}} = \sum_{i=1}^{n} \frac{\alpha}{f_i CMC_i} \qquad (1)$$

where α is the mole fraction of an individual surfactant, CMC_i is the critical micellar

concentration of the individual surfactant and f_i is the activity coefficient. For ideal systems, such as cationic-cationic mixtures, the activity coefficient is unity (5). This reduces eqn 1 for binary mixtures to:

$$CMC_{mix} = \frac{CMC_1 * CMC_2}{CMC_1(1-\alpha) + CMC_2\alpha} \quad (2)$$

The calculated CMC_{mix} and the mixed micellized surfactant concentrations for the mixed micelles used in this study are listed in Table 1. In this Table, the different mixed micelles are grouped into 'sets' each of which corresponds to mixtures of two given surfactants at various molar ratios.

Electrokinetic Behavior of the Mixed Micellar Systems

Figure 1 illustrates the electrokinetic behavior observed with the various mixed micelles consisting of binary mixtures of alkyltrimethylammonium chloride surfactants. The results are plotted in terms of the migration time of an unretained solute (t_0, methanol) and that of a solute fully retained in the micelle (t_{mc}, Sudan III) versus the molar ratio of the surfactants. The breadth of the retention window decreases with increasing concentration of the longer alkyl chain co-surfactant. The migration time of methanol, the unretained species, and therefore the magnitude of the electroosmotic velocity (v_{eo}) was relatively unaffected by the molar ratio of the mixed surfactants, indicating that the adsorbed surfactant layer from the various cationic surfactant mixtures imparts to the capillary wall similar characteristic charges (i.e., similar zeta potential). In this adsorption process, cationic surfactant molecules are attracted electrostatically *via* their quaternary ammonium groups to the negatively charged silanols on the surface of the capillary.

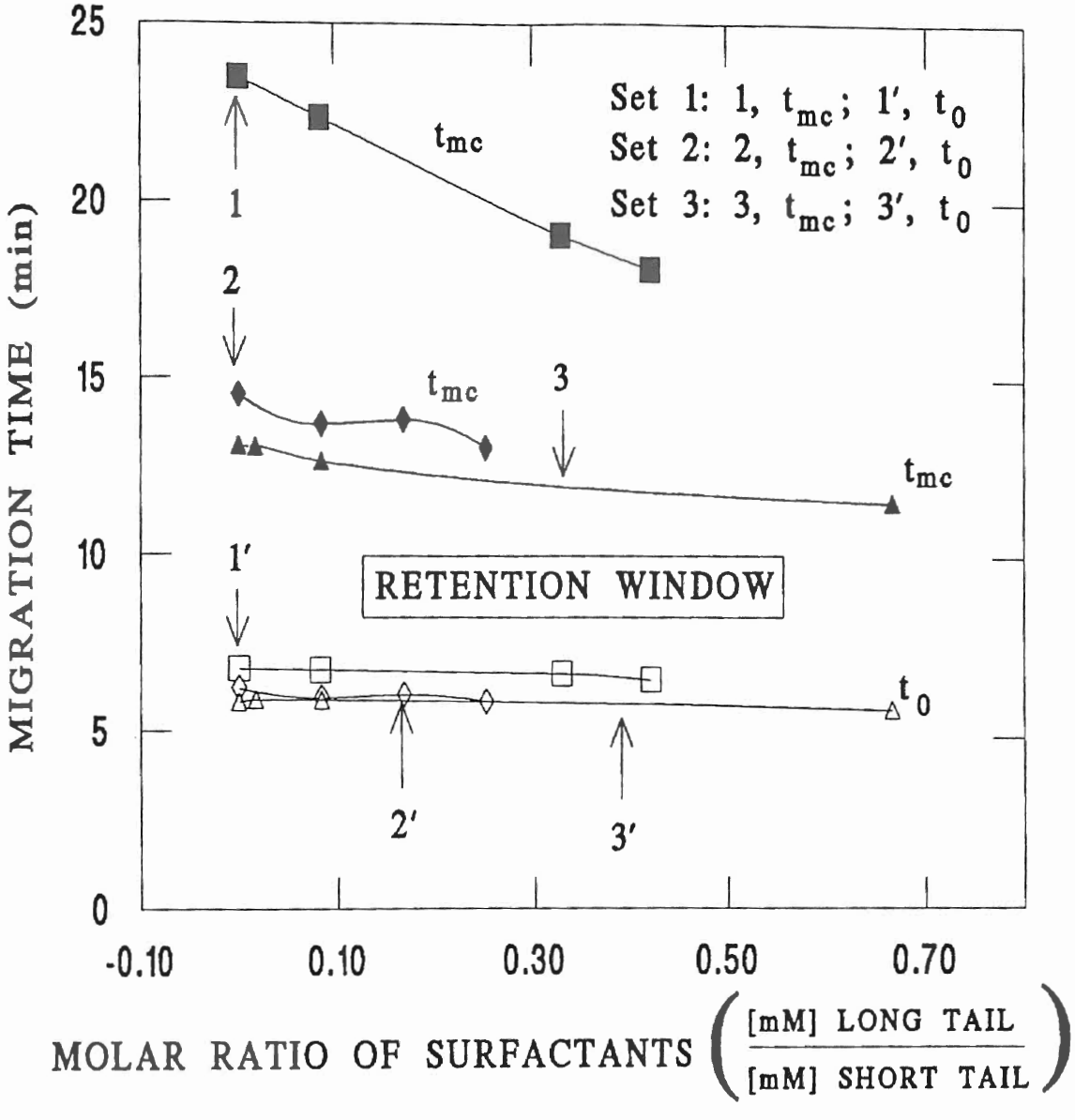

Figure 1. Breadth of the retention window as a function of the molar ratio of the co-surfactants obtained with various mixed alkyltrimethylammonium chloride micelles. Capillary, fused-silica, 80 cm total length (50 cm separation distance) x 50 μm I.D.; running electrolyte, 25 mM phosphate, pH 7.0. Running voltage, 20-kV.

Three different models have been proposed for the adsorption of ionic surfactants on polar adsorbents (10-12). One model suggests the formation of a surfactant bilayer called an admicelle (10,12). The admicelle consists of two tightly-packed parallel surfactant layers with the polar heads facing out and the hydrophobic tails intertwined. The admicelle produces a hydrophilic surface. In the second model, a surfactant monolayer called a hemimicelle (10,12) is proposed. The hemimicelle is a tightly-packed surfactant layer with the hydrophobic tails sticking out into the liquid interface and the ionic heads attached to the polar adsorbent, i.e., one half of the admicelle. The hemimicelle produces a hydrophobic surface. The third model assumes the formation of surface micelles (10,11). Surface micelles are preceded by the formation of a loosely-packed monolayer of surfactants. Surfactants in this monolayer serve as anchors to which other surfactants attach themselves to form structures similar to free-floating micelles.

The appropriate adsorption model is actively debated in the literature. According to Cases and Villeras (10), the adsorption of surfactants starts below the CMC with a loosely-packed monolayer. This loosely-packed monolayer may evolve into a hemimicelle as the surfactant concentration increases. An admicelle forms at the saturation concentration of the surfactant solution if the adsorbate-adsorbent system is below the Krafft point (i.e., the temperature were the solubility of the surfactant equals the CMC), which means in the absence of micelles. If the adsorbate-adsorbent system is above the Krafft point, surface micelles are obtained. For MECC, surfactant concentrations are well above the CMC and the temperature is above the Krafft point. These conditions preclude the formation of a hemimicellar capillary wall. In fact, if this phenomenon prevails, each silanol group would undergo ion-pairing with a surfactant molecule, and consequently a zero flow would be obtained. This is not the case, and all mixed micelles yielded anodal electroosmotic flow. Thus, the capillary surface can be pictured as an admicelle and/or a surface micelle. In both models, the charge on the capillary wall changes from negative to positive and for this reason an anodal electroosmotic flow is established (13-16).

It was observed in Chapter II that the retention window decreases with increasing length of the surfactant alkyl tail for unisurfactant micelles. This trend is also seen for mixed micelles, see Fig. 1. The migration time of the micelle (t_{mc}) is given by:

$$t_{mc} = l / v_{mc} = l / (v_{eo} + v_{ep}) \quad (3)$$

where l is the separation length of the capillary, v_{ep} is the electrophoretic mobility of the micelle and v_{mc} is the net velocity of the micelle. For cationic MECC, the velocity v_{eo} is relatively constant and in the opposite direction of v_{ep}. The velocity v_{ep} decreases with increasing concentration of the longer chain co-surfactant due to increasing size of the mixed micelle. Thus, the difference of v_{eo} and v_{ep} (i.e., v_{mc}) will increase. Under these conditions, the t_{mc} will decrease, and the net result is a decrease in the retention window as the concentration of the longer chain co-surfactant increases.

Retention Behavior of Neutral Solutes

Correlation Between Capacity Factor and the Number of Methylene Groups in a Homologous Series. The retention behavior of the two homologous series, phenylalkylalcohols and nitroalkanes, was examined with various mixed cationic micellar phases (see Table 1) in terms of plots of log k' against the number of carbon atoms in the homologues. In all cases, log k' was a linear function of n_c and followed the relationship usually found in reversed phase chromatography (17), see Chapter II. Typical plots are depicted in Figure 2a and b. The slope ($log\ \alpha$), intercept ($log\ \beta$) and correlation coefficient (R) are listed in Tables 2 and 3. A linear least squares regression was used to fit the retention data. Benzylalcohol and, to a lesser extent, nitromethane did not fit the linear relationship between $log\ k'$ and n_c. This type of non-linearity is also observed in liquid chromatography (17) for homologous species below a 'critical carbon number' and is attributed to functional group(s) in the homologous series. Below the critical carbon

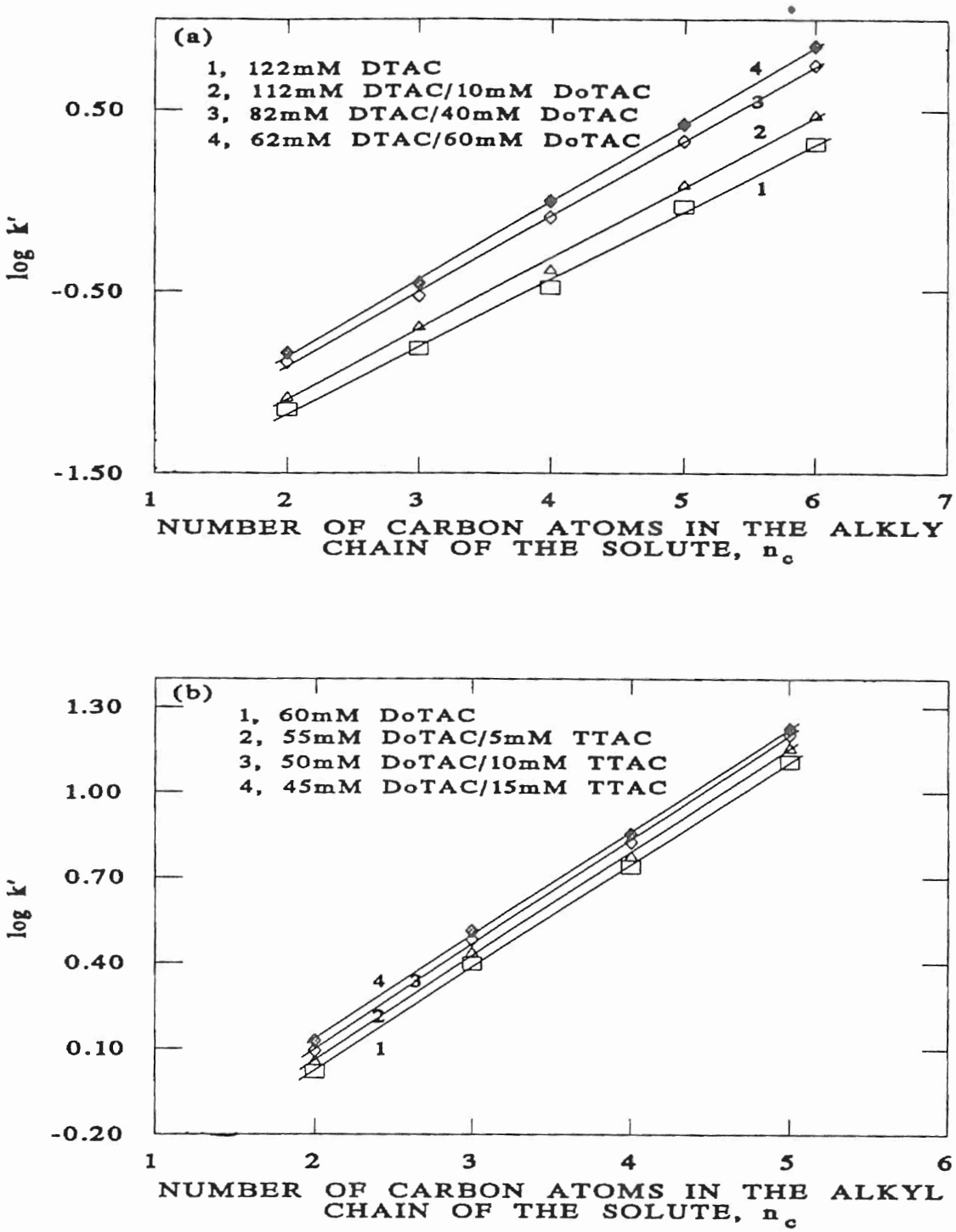

Figure 2. Plots of *log k' vs.* n_C for a nitroalkane homologous series in (a) and for a phenylalkylalcohol homologous series in (b) obtained with Set 1 and Set 2 binary alkyltrimethylammonium chloride surfactants, respectively. Running conditions are the same as in Fig. 1.

TABLE 2

SLOPES, INTERCEPTS AND R VALUES FOR *log k' vs.* n_C PLOTS OF A NITROALKANE HOMOLOGOUS SERIES USING VARIOUS ALKYLTRIMETHYLAMMONIUM CHLORIDE MIXED MICELLAR PHASES

Surfactant Mix	Set #		log β	log α	R
122 mM DTAC			-1.93	0.37	0.9992
112 mM DTAC/10 mM DoTAC	Set 1		-1.87	0.39	0.9991
82 mM DTAC/40 mM DoTAC			-1.71	0.41	0.9996
62 mM DTAC/60 mM DoTAC			-1.67	0.42	0.9995
		Mean	-1.79	0.40	
		%RSD	6.05	4.24	
60 mM DoTAC			-1.95	0.43	0.9999
55 mM DoTAC/5 mM TTAC	Set 2		-1.91	0.44	1.0000
50 mM DoTAC/10 mM TTAC			-1.69	0.40	0.9979
45 mM DoTAC/15 mM TTAC			-1.78	0.42	0.9989
		Mean	-1.83	0.42	
		%RSD	5.65	3.42	
60 mM DoTAC			-1.89	0.41	0.9991
59 mM DoTAC/1 mM CTAC	Set 3		-1.86	0.42	0.9989
55 mM DoTAC/5 mM CTAC			-1.92	0.44	0.9993
20 mM DoTAC/40 mM CTAC			-1.71	0.45	0.9986
		Mean	-1.85	0.43	
		%RSD	4.31	3.46	

Capillary, fused-silica, 80 cm total length (50 cm separation distance) x 50 μm I.D.; running electrolyte, 25 mM phosphate, pH 7.0; running voltage, 20-kV.

TABLE 3

SLOPES, INTERCEPTS AND VALUES R FOR *log k' vs.* n_C PLOTS OF A PHENYLALKYLALCOHOL HOMOLOGOUS SERIES USING VARIOUS ALKYLTRIMETHYLAMMONIUM CHLORIDE MIXED MICELLAR PHASES

Surfactant Mix	Set #		log β	log α	R
122 mM DTAC			-0.99	0.32	0.9965
112 mM DTAC/10 mM DoTAC	Set 1		-0.79	0.33	0.9993
82 mM DTAC/40 mM DoTAC			-0.58	0.34	0.9997
62 mM DTAC/60 mM DoTAC			-0.51	0.35	0.9997
		Mean	-0.72	0.34	
		%RSD	26.24	3.93	
60 mM DoTAC			-0.70	0.36	0.9998
55 mM DoTAC/5 mM TTAC	Set 2		-0.67	0.36	0.9997
50 mM DoTAC/10 mM TTAC			-0.64	0.37	0.9998
45 mM DoTAC/15 mM TTAC			-0.59	0.36	0.9997
		Mean	-0.65	0.36	
		%RSD	5.99	0.74	
60 mM DoTAC			-0.71	0.36	0.9998
59 mM DoTAC/1 mM CTAC	Set 3		-0.70	0.36	0.9998
55 mM DoTAC/5 mM CTAC			-0.67	0.37	0.9999
20 mM DoTAC/40 mM CTAC			-0.60	0.40	0.9976
		Mean	-0.67	0.37	
		%RSD	6.42	4.56	

Capillary, fused-silica, 80 cm total length (50 cm separation distance) x 50 μm I.D.; running electrolyte, 25 mM phosphate, pH 7.0; running voltage, 20-kV.

number the influence of a methylene group is overshadowed by the influence of an adjacent functional group. In this study, the functional groups are OH and NO_2. Accordingly, k' values of benzylalcohol and nitromethane were not used in computing the regression data listed in Tables 2 and 3.

Three relationships need to be emphasized when reviewing the *log* α results: (i) agreement between two homologous series for a given mixed micelle, (ii) agreement between the same homologous series within a set of mixed micelles and (iii) agreement between the same homologous series for different sets of mixed micelles. The agreement of *log* α between the nitroalkanes and phenylalkylalcohols for a given mixed micelle is good (15% RSD or better). The agreement of *log* α between the nitroalkanes or the phenylalkylalcohols within a set of mixed micelles is excellent (5% RSD or less). The agreement of *log* α for the two homologous series among the various sets of mixed micelles is excellent (4% RSD or less). This means that *log* α for mixed cationic MECC is primarily a solvophobic effect. Thus, the free energy transfer of the solute between the aqueous and micellar phases is dependent on the methylene group and independent of the alkyltrimethylammonium chloride co-surfactant. The change in the capacity factor for a solute with different alkyltrimethylammonium chloride co-surfactants is thus due to a change in the phase ratio of the various mixed micelles.

The value *log* β is characteristic of the functional group(s) of the homologous series. Thus, the intercept for the two homologous series is expected to be different. Of interest is the good agreement between the intercepts within a set of mixed micelles (7% RSD or better except for the phenylalkylalcohols of Set 1) and the good agreement between different sets of mixed micelles (6% RSD or less). This implies that, like nonspecific interactions, the specific interactions are largely independent of the alkyltrimethylammonium chloride co-surfactant.

Comparison of the Energetics of Retention with the Various Binary Mixed Micelles. In the previous chapter, a model introduced by Horvath *et al.* (18) was used to examine

the energetics of solute retention with different surfactant micelles. Here Horvath's model is used to compare the different binary mixed micelles. Typical *log k'-log k'* plots are depicted in Fig. 3a and b. The results of a linear least squares regression of all the retention data obtained with the various micellar mixtures are summarized in Tables 4 and 5. The slopes of all the straight lines are close to unity, indicating quasi-homoenergetic systems. As expected, the addition of the longer chain co-surfactant resulted in a monotonic increase of the quotients of hydrophobic phase ratio, φ_B/φ_A, which is the antilog of the intercept of the *log k'-log k'* plots.

Effect of Long Tail Surfactant Additive. The effect of the long tail co-surfactant on the retention of neutral solutes is illustrated in Figure 4a to c. The results of a linear least squares curve fit are listed in Tables 6 to 8. The long tail co-surfactants produce monotonic increases in the retention of neutral species. The correlation between *log k'* and the concentration of the longer alkyl chain co-surfactant is quasi-linear and yields parallel lines for all of the test solutes. Nitromethane and phenylmethanol are not considered part of this data set for the same reasons stated earlier in this chapter. This mirrors the results of the previous chapter where OTAC, a shorter alkyl chain co-surfactant, was added to TTAC. For the TTAC/OTAC micellar phase, plots of *log k'* versus the concentration of the octyltrimethylammonium chloride (OTAC) co-surfactant produced a quasi-linear decrease in the retention of neutral solutes. Here the correlation between *log k'* and concentration of co-surfactant is enlarged upon by (i) increasing the number of different cationic-cationic mixed micelles, (ii) changing the length of the alkyl chain of the two mixed surfactants, (iii) using different molar ratios and (iv) including different solutes. The results, therefore, reinforce the idea that binary surfactant micelles do not significantly change the selectivity, compared to a single micelle; instead, they cause changes in the breadth of the retention window as well as in retention by altering the solute capacity factor.

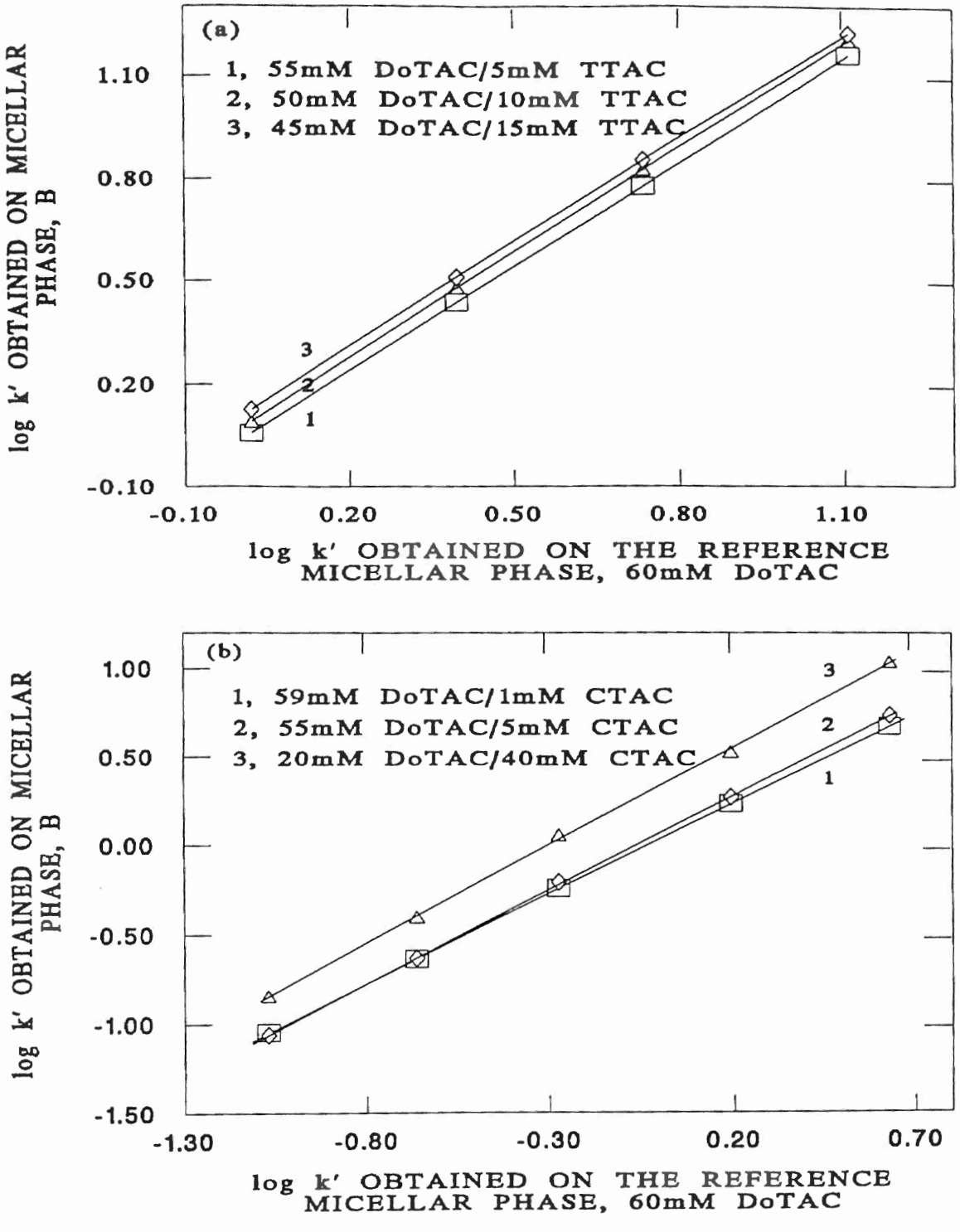

Figure 3. Plots of *log k' - log k'* for a phenylalkylalcohol homologous series in (a) and for a nitroalkane homologous series in (b) obtained on a unimicellar phase *versus* the binary micellar phases of Set 2 and Set 3, respectively. Other conditions are the same as in Fig. 1.

TABLE 4

SLOPES, INTERCEPTS, R VALUES AND THE ANTILOG OF INTERCEPTS FOR *log k'* - *log k'* PLOTS FOR A NITROALKANE HOMOLOGOUS SERIES OBTAINED ON MIXED ALKYLTRIMETHYLAMMONIUM CHLORIDE MICELLES

(Micellar Phase B)/Micellar Phase A	Set #	Slope	Intercept	R	φ_B/φ_A
(112mM DTAC/10mM DoTAC)/122mM DTAC	Set 1	1.039	0.137	0.9997	1.37
(82mM DTAC/40mM DoTAC)/122mM DTAC		1.087	0.391	0.9991	2.46
(62mM DTAC/60mM DoTAC)/122mM DTAC		1.115	0.486	0.9987	3.06
(55mM DoTAC/5mM TTAC)/60mM DoTAC	Set 2	1.006	0.059	0.9999	1.15
(50mM DoTAC/10mM TTAC)/60mM DoTAC		0.921	0.110	0.9977	1.27
(45mM DoTAC/15mM TTAC)/60mM DoTAC		0.975	0.124	0.9987	1.33
(59mM DoTAC/1mM CTAC)/60mM DoTAC	Set 3	1.007	0.036	1.0000	1.09
(55mM DoTAC/5mM CTAC)/60mM DoTAC		1.056	0.071	1.0000	1.18
(20mM DoTAC/40mM CTAC)/60mM DoTAC		1.087	0.336	0.9997	2.17

The antilog of intercepts is the quotient of phase ratios φ_B/φ_A. See Table 2 for experimental conditions.

TABLE 5

SLOPES, INTERCEPTS, R VALUES AND THE ANTILOG OF INTERCEPTS FOR *log k'* - *log k'* PLOTS FOR A PHENYLALKYLALCOHOL HOMOLOGOUS SERIES OBTAINED ON MIXED ALKYLTRIMETHYLAMMONIUM CHLORIDE MICELLES

(Micellar Phase B)/Micellar Phase A	Set #	Slope	Intercept	R	φ_B/φ_A
(112mM DTAC/10mM DoTAC)/122mM DTAC	Set 1	1.053	0.194	0.9980	1.56
(82mM DTAC/40mM DoTAC)/122mM DTAC		1.069	0.462	0.9980	2.90
(62mM DTAC/60mM DoTAC)/122mM DTAC		1.098	0.581	0.9982	3.81
(55mM DoTAC/5mM TTAC)/60mM DoTAC	Set 2	1.000	0.045	0.9999	1.11
(50mM DoTAC/10mM TTAC)/60mM DoTAC		1.012	0.080	0.9999	1.20
(45mM DoTAC/15mM TTAC)/60mM DoTAC		0.999	0.116	0.9999	1.31
(59mM DoTAC/1mM CTAC)/60mM DoTAC	Set 3	1.000	0.035	0.9998	1.04
(55mM DoTAC/5mM CTAC)/60mM DoTAC		1.035	0.083	0.9998	1.21
(20mM DoTAC/40mM CTAC)/60mM DoTAC		1.077	0.321	0.9987	2.09

The antilog of intercepts is the quotient of phase ratios φ_B/φ_A. See Table 2 for experimental conditions.

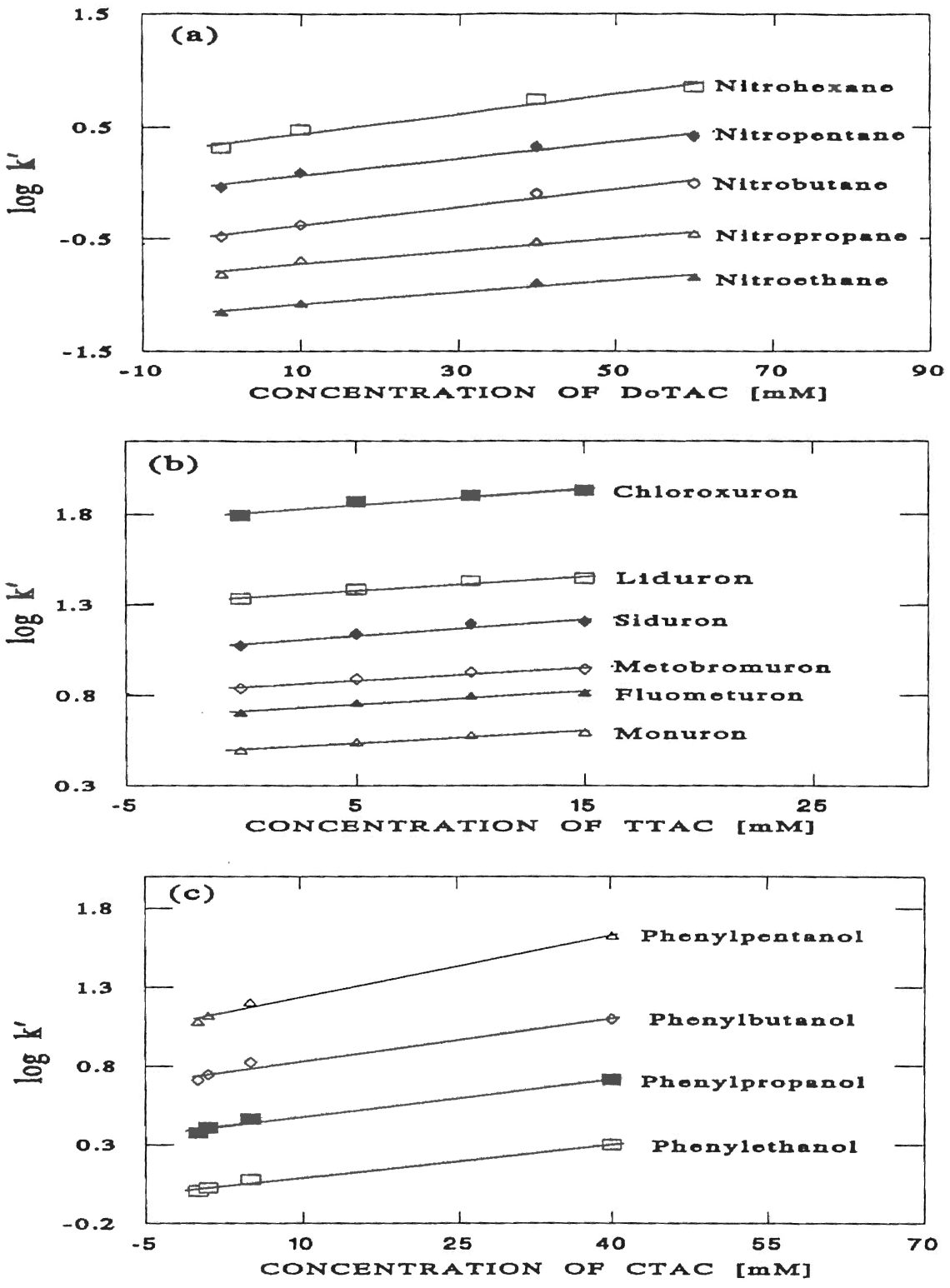

Figure 4. Plots of *log k'* for a nitroalkane homologous series in (a), of urea herbicides in (b) and for a phenylalkylalcohol homologous series in (c) versus the concentration of the long tail surfactant of Set 1, Set 2 and Set 3, respectively. Conditions are as in Fig. 1.

TABLE 6

SLOPES, INTERCEPTS AND R VALUES OF PLOTS *log k' vs. mM of Co-Surfactant* FOR A NITROALKANE HOMOLOGOUS SERIES USING VARIOUS ALKYLTRIMETHYLAMMONIUM CHLORIDE MIXED MICELLAR PHASES

Solute	Set #	Intercept	Slope	R
Nitroethane	Set 1	-1.134	0.0053	0.9857
Nitropropane		-0.780	0.0058	0.9791
Nitrobutane		-0.462	0.0081	0.9900
Nitropentane		-0.006	0.0076	0.9884
Nitrohexane		0.353	0.0089	0.9853
Nitroethane	Set 2	-1.087	0.0108	0.8672
Nitropropane		-0.632	0.0061	0.9484
Nitrobutane		-0.217	0.0088	0.9734
Nitropentane		0.226	0.0080	0.9828
Nitrohexane		0.665	0.0087	0.9859
Nitroethane	Set 3	-1.070	0.0056	0.9905
Nitropropane		-0.656	0.0064	0.9966
Nitrobutane		-0.257	0.0079	0.9967
Nitropentane		0.216	0.0078	0.9939
Nitrohexane		0.660	0.0095	0.9916

Capillary, fused-silica, 80 cm total length (50 cm separation distance) x 50 μm I.D.; running electrolyte, 25 mM phosphate, pH 7.0; running voltage, 20-kV.

TABLE 7

SLOPES, INTERCEPTS AND R VALUES OF PLOTS *log k' vs. mM of Co-Surfactant* FOR A PHENYLALKYLALCOHOL HOMOLOGOUS SERIES USING VARIOUS ALKYLTRIMETHYLAMMONIUM CHLORIDE MIXED MICELLAR PHASES

Solute	Set #	Intercept	Slope	R
Phenylethanol	Set 1	-0.306	0.0091	0.9697
Phenylpropanol		0.077	0.0086	0.9824
Phenybutanol		0.354	0.0096	0.9798
Phenylpentanol		0.670	0.0107	0.9820
Phenylethanol	Set 2	0.021	0.0071	0.9999
Phenylpropanol		0.398	0.0078	0.9980
Phenybutanol		0.739	0.0080	0.9950
Phenylpentanol		1.116	0.0078	0.9828
Phenylethanol	Set 3	0.021	0.0070	0.9930
Phenylpropanol		0.398	0.0080	0.9918
Phenybutanol		0.740	0.0091	0.9875
Phenylpentanol		1.108	0.0131	0.9953

Capillary, fused-silica, 80 cm total length (50 cm separation distance) x 50 μm I.D.; running electrolyte, 25 mM phosphate, pH 7.0; running voltage, 20-kV.

TABLE 8

SLOPES, INTERCEPTS AND R VALUES OF PLOTS *log k' vs. mM of Co-Surfactant* FOR HERBICIDE UREAS USING VARIOUS ALKYLTRIMETHYLAMMONIUM CHLORIDE MIXED MICELLAR PHASES

Solute	Set #	Intercept	Slope	R
Monuron		0.111	0.0097	0.9781
Fluometuron	Set 1	0.296	0.0103	0.9875
Metobromuron		0.382	0.0107	0.9902
Siduron		0.515	0.0125	0.9888
Linuron		0.754	0.0127	0.9888
Chloroxuron		1.097	0.0139	0.9856
Monuron		0.501	0.0069	0.9802
Fluometuron	Set 2	0.714	0.0074	0.9730
Metobromuron		0.846	0.0071	0.9764
Siduron		1.084	0.0091	0.9668
Linuron		1.339	0.0077	0.9751
Chloroxuron		1.806	0.0089	0.9655
Monuron		0.498	0.0088	0.9848
Fluometuron	Set 3	0.716	0.0098	0.9825
Metobromuron		0.852	0.0104	0.9870

Capillary, fused-silica, 80 cm total length (50 cm separation distance) x 50 μm I.D.; running electrolyte, 25 mM phosphate, pH 7.0; running voltage, 20-kV.

Effect of Added Co-surfactant on Efficiency

Figure 5 illustrates plots of the average theoretical plate number of the test solutes against the molar ratio of mixed surfactants. With the exception of Set 3, increasing co-surfactant concentration showed a modest increase in plate number. In all cases, however, small amounts (<20%) of co-surfactant have little effect on efficiency. In principle, increasing the size of the micelle increases the resonance time of the solute in the micellar phase; a condition that favors less longitudinal molecular diffusion, since the micelle has a smaller diffusion coefficient. The utility of mixed micelles in MECC of neutral solutes is shown in Fig. 6a to d for the separation of six urea herbicides. As seen in Figure 6c, a mixed surfactant of 82mM DTAC/40mM DoTAC provides the best compromise in terms of separation efficiency and breadth of the retention window. All peaks are still baseline resolved, and chloroxuron, the hydrophobic solute, elutes ahead of the migration time of the micelle, see Fig. 6c.

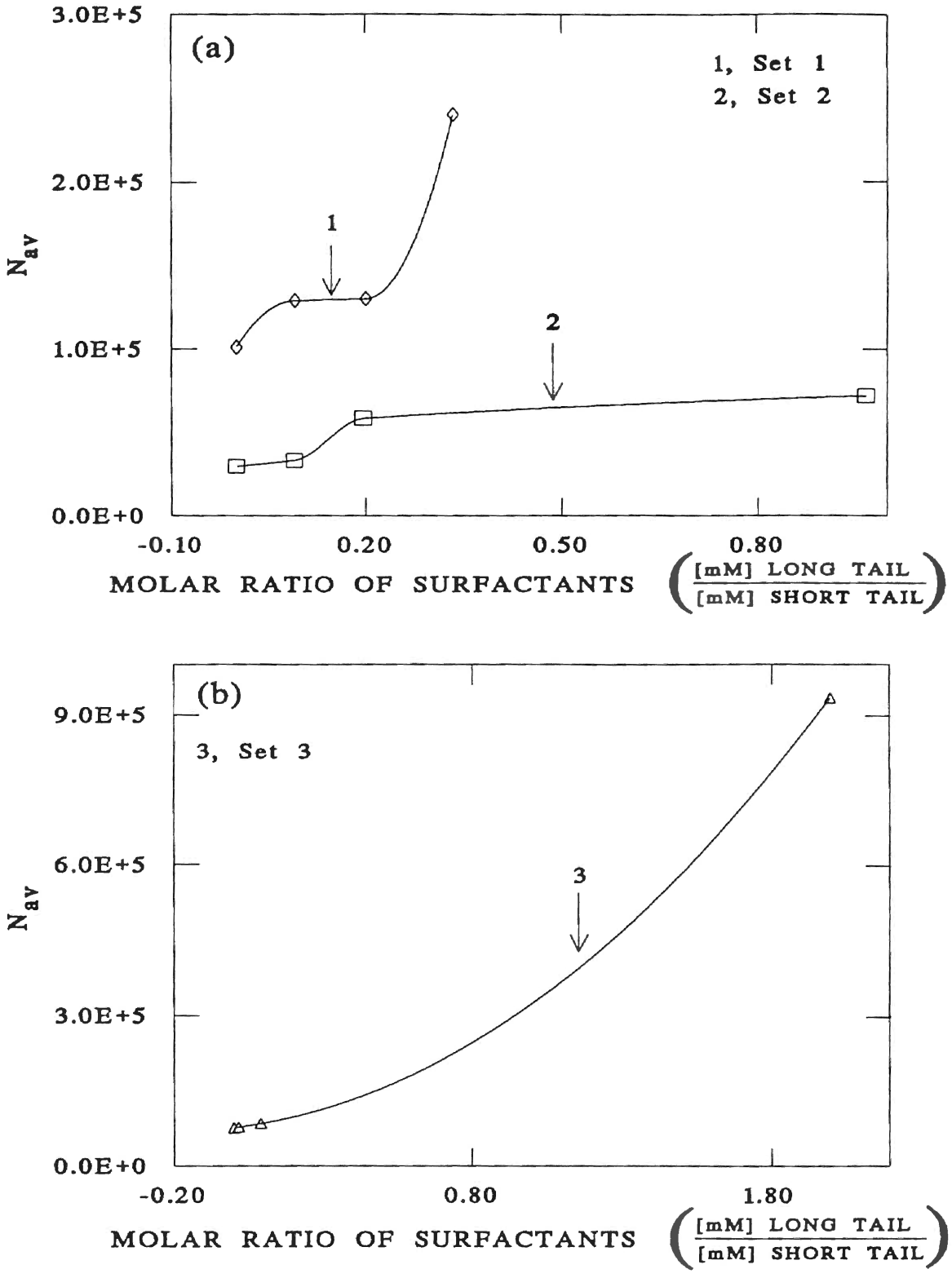

Figure 5. Plot of N_{av} of urea herbicides as a function of the molar ratio of the surfactants obtained with various mixed alkyltrimethylammonium chloride micelles. Other conditions are the same as in Fig. 1.

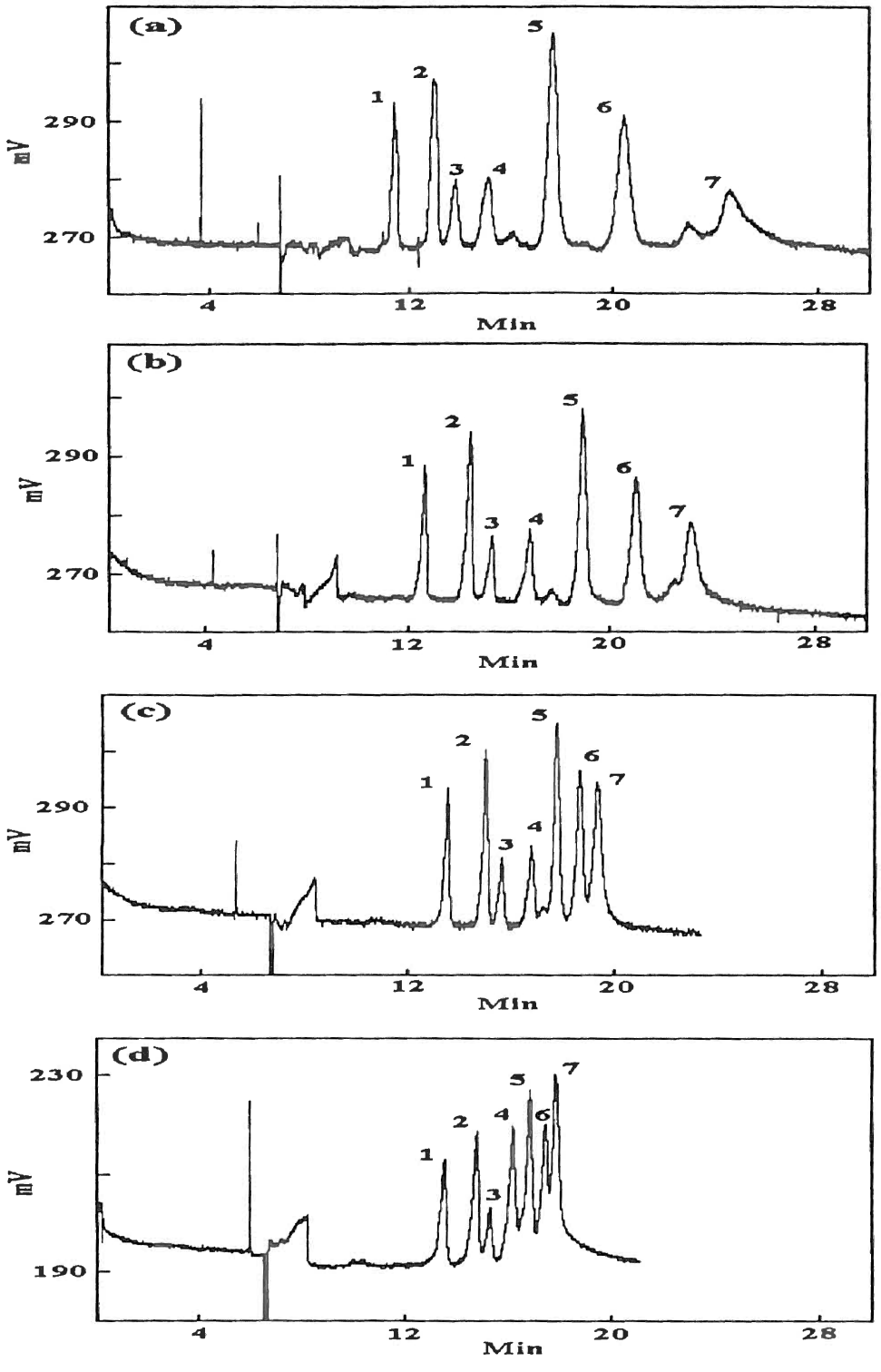

Figure 6. Electropherograms of urea herbicides obtained with (a) 122mM DTAC, (b) 112mM DTAC/10mM DoTAC, (c) 82mM DTAC/40mM DoTAC, and (d) 62mM DTAC/60mM DoTAC. Conditions are as in Fig. 1. Solutes: 1, monuron; 2, fluometuron; 3, metobromuron; 4, siduron; 5, linuron; 6, chloroxuron; 7, Sudan III.

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VITA 2

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