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Micellar electrokinetic capillary chromatography with in situ charged micelles
IV. Influence of the nature of the alkylglycoside surfactant

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Abstract

Four different in situ charged micellar phases were evaluated in micellar electrokinetic capillary chromatography (MECC) of neutral and acidic herbicides, and other aromatic compounds. In situ charged micelles refer to dynamically charged entities that are formed via the complexation of borate with surfactants having sugar head groups. These dynamically charged surfactants yield micelles with adjustable surface charge densities which can be conveniently manipulated by changing borate concentration and pH of the running electrolyte. The four surfactants, namely octanoylsucrose (OS), octyl-β-D-glucopyranoside (OG), octyl-β-D-maltopyranoside (OM) and nonanoyl-*N*-methylglucamide (MEGA 9), in the presence of alkaline borate yielded micelles characterized by migration time windows of varying width. The width of the migration time window was largely influenced by the nature of the sugar head group of the polyolic surfactant. The electrochromatographic behavior of OS, OM, OG and MEGA 9 was influenced by both the nature of the sugar head group and the length of the alkyl tail. OS, which differed from the other surfactants by having an alkyl tail with one fewer carbon atom, exhibited the lowest retention. MEGA 9 with its acyclic sugar head group and the presence of a polar amide linkage between the sugar and the alkyl tail showed a medium retentivity towards the various solutes under investigation. OG and OM, which differed from each other by the nature of the sugar head group, exhibited more or less similar retention behavior. Overall, due to differences in their migration time windows and retention behaviors, the four micellar phases afforded different selectivities toward charged and neutral solutes. The separation efficiencies achieved with in situ charged micelles, which exceeded 750 000 plates/m, appear to be superior to those achieved with traditionally used micellar phases.

1. Introduction

Over the last decade, capillary electrophoresis (CE) has developed rapidly and became an important analytical separation technique [1,2] of unsurpassed resolving power. The technique has found applications in the separation of almost all types of compounds. This universal use of CE has been facilitated in part by the fact that a given separation can be performed in several modes thus permitting the achievement of different selectivities. An important development of CE has been the introduction of micellar electrokinetic capillary chromatography (MECC) by

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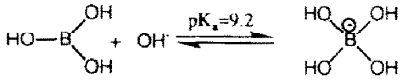
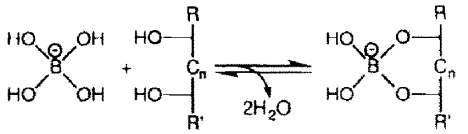
Terabe et al. in 1984 [3], a technique that allows the separation of neutral species under the influence of an electrical field. MECC has shown great promises in the separation of both neutral and charged species [4–8]. The separation is achieved via the differential partitioning of the analytes between an aqueous phase and a charged micellar phase. The basic operational principles and the applications of MECC have recently been reviewed [9,10].

Recently, our laboratory has introduced the concept of in situ charged micelles [11–14] to MECC of neutral and charged species. In situ charged micelles are dynamically charged entities via complexation of a diol group of a polyol surfactant with borate or boronate ion. The generalized reaction scheme of a diol with the borate ion is shown in equilibria 1 and 2 below.

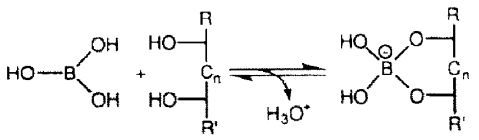


(1)



(2)

Equilibrium 1 represents the ionization of boric acid ($pK_a = 9.2$) to the tetrahydroxy borate ion, i.e., borate. Equilibrium 2 represents complexation between the borate ion and a diol. It is well known that a polyol possessing vicinal diols of the proper geometry can undergo complexation with borate to form an ionized complex upon the loss of two molecules of water [15–17]. In equilibrium 2, n is either 0 or 1, which corresponds to a 1,2- or 1,3-diol, respectively. The 1,2-diol forms a five-membered ring upon complexation and is more stable than the 1,3-diol complex which forms a six-membered ring. Boric acid, in the neutral form, can also complex with diols and is illustrated below in equilibrium 3.



(3)

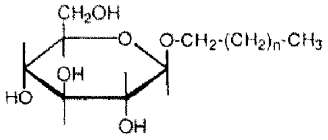
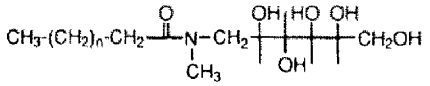
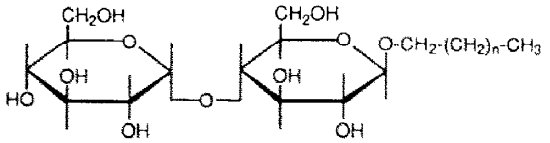
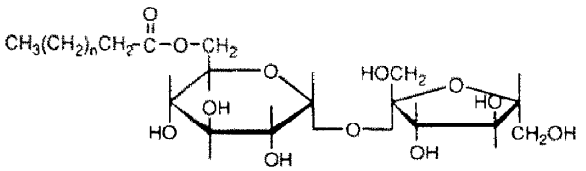
In this reaction, the complex is formed upon the loss of one water molecule and a proton. It is well known that the stability constant of equilibrium 2 is much greater than that of equilibrium 3. The forward rate constant for the complexation (equilibrium 2) is at least 3 orders of magnitude greater than the rate constant for the trigonal boric acid (equilibrium 3) for some polyols [18]. Equilibrium 3, whose rate constants are largely dependent on the geometrical arrangement of diols, is currently being exploited in our laboratory with in situ charged micelles at neutral pH. At alkaline pH, the formation of diol–borate complex is primarily the result of equilibrium 2.

According to the above equilibria, the surface charge density of an alkylglycoside micelle can be easily manipulated by altering the borate concentration, the surfactant concentration, and/or the pH of the running electrolyte. As a result, the migration time window of the in situ charged MECC system can be tailored to suit a given separation problem.

We have examined several polyol surfactants as possible in situ charged micellar phases including a series of four alkyl-β-D-glucopyranosides [12], a series of three *N*-D-gluco-*N*-methylalkanamides (MEGA) [14], octyl-β-D-maltopyranoside and octanoylsucrose [13]. The structures of these surfactants are listed in Table 1. Fig. 1 illustrates our view of the idealized structure of the alkylglycoside–borate micelle. The micelle consists primarily of the hydrophobic core with hydrophilic sugar residues facing outwards towards the aqueous phase. Borate can complex with the diols of the proper geometry at the surface of the micelle. Since the degree of complexation can be readily controlled, the surface charge density is therefore adjustable.

Due to the nature of the hydrophilic sugar head group, different surfactants have different affinities towards borate. ^{11}B NMR studies [13] have shown that the alkyl-β-D-glucopyranoside surfactants complex through O-4 and O-6 of the glucose residue as suggested by Foster [15]. Similarly, octanoylsucrose and octyl-β-D-maltopyranoside complex with borate primarily via the nonreducing glucose residue and have bind-

Table 1
Structures and CMCs of surfactants used in our studies

Identification	Name	Abbreviation	CMC (m*M*) [a]
Alkylglucosides			
			
$n = 5$	Heptyl-β-D-glucopyranoside	HG	79
$n = 6$	Octyl-β-D-glucopyranoside	OG	25
$n = 7$	Nonyl-β-D-glucopyranoside	NG	6.5
$n = 8$	Decyl-β-D-glucopyranoside	DG	2–3
Alkylglucamides			
			
$n = 5$	Octanoyl-*N*-methylglucamide	MEGA 8	58
$n = 6$	Nonanoyl-*N*-methylglucamide	MEGA 9	19–25
$n = 7$	Decanoyl-*N*-methylglucamide	MEGA 10	6–7
Alkylmaltosides			
			
$n = 6$	Octyl-β-D-maltopyranoside	OM	23.4
Alkanoylsucrose			
			
$n = 5$	*n*-Octanoylsucrose	OS	24.4

[a] Obtained from Ref. [19]

ing affinities very similar to that of the alkyl-β-D-glucopyranosides [14]. The MEGA surfactants possess an acyclic sugar residue which allows the hydroxyl groups to change their conformation freely. This freedom in changing conformation allows the MEGA surfactants to have a much higher affinity towards borate. Previous ^{11}B studies have indicated that the surfactants with acyclic sugar head groups have at least a three-fold higher affinity for borate than surfactants

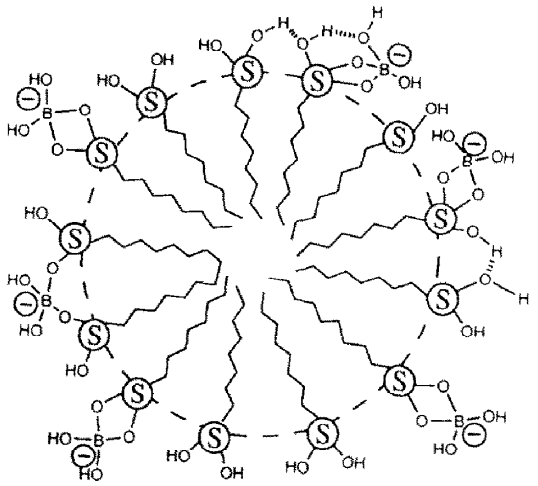


Fig. 1. Idealized structure of alkylglycoside–borate micelle showing borate complexation and hydrogen bonding on the outer surface of the micelle. Circled-S stands for the sugar head group of the surfactant.

possessing a cyclic sugar head group [13]. This feature allows the MEGA micellar phases to form a larger migration time window under similar electrophoretic conditions.

As can be seen in Table 1, the alkylglycoside surfactants under investigation possess a straight chain alkyl tail and a sugar polar head group. We have previously evaluated the influence of the length of the alkyl tail with various types of analytes [12,13], and demonstrated that the differences in hydrophobic character among surfactants with different tails are useful in optimizing selectivity for a given separation.

The aim of this report is to investigate the influence of the nature of the alkylsaccharide surfactant on the electrochromatographic properties of the in situ charged micelles. To achieve this goal, surfactants having different types of sugar head groups and hydrocarbonaceous tails were evaluated, namely, octanoylsucrose (OS), octyl-β-D-maltopyranoside (OM), octyl-β-D-glucopyranoside (OG) and nonanoyl-*N*-methylglucamide (MEGA 9). As can be seen in Table 1, all four of the surfactants have similar CMCs. Three of the surfactants differ in the nature of the sugar head group and have in common an eight-carbon lipophilic tail (i.e., OM, OG and MEGA 9) while the fourth surfactant has a different alkyl tail (a seven-carbon alkyl tail) and sugar head group, i.e., OS. The surfactants were compared in terms of the migration time window, peak capacity, efficiency, retention energetics and selectivity as a function of the nature of the surfactant. The micellar phases were applied to MECC of mixtures of both neutral and acidic herbicides as well as very hydrophobic aromatic compounds.

2. Experimental

2.1. Instrumentation

This work was performed on a capillary electrophoresis system Model HP ^{3D}CE from Hewlett Packard (Waldbronn, Germany) equipped with a real time UV–visible diode array detector (DAD) and accompanying data analysis software. The column temperature was maintained at 30°C. The detection wavelength was set at 240 nm for the detection of herbicides, and at 254 nm for the detection of aromatics and alkyl phenyl ketones. In all the experiments, the electrical field strength was 187.5 V/cm.

Fused-silica capillaries having an inner diameter of 50 μm and an outer diameter of 375 μm were obtained from Polymicro Technology (Phoenix, AZ, USA). The total length of the capillary was 64.0 cm with an effective length of 56.0 cm, i.e., from the injection end to the detection point.

2.2. Reagents

Octyl-β-D-glucopyranoside (OG) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). *n*-Octanoylsucrose (OS), octyl-β-D-maltopyranoside (OM) and nonanoyl-*N*-methylglucamide (MEGA 9) were purchased from Calbiochem (San Diego, CA, U.S.A). For the structures and the critical micelle concentrations (CMCs) of the surfactants, see Table 1. It should be noted that the CMC values given in Table 1 are those obtained in low ionic strength buffer solutions, i.e., 0–0.05 *M* Na^+. All herbicides used in this study were purchased from Chem

Service (West Chester, PA, USA). The series of alkyl phenyl ketones (APK) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Aniline, 2-naphthylamine, naphthalene, biphenyl, 1-chloronaphthalene and anthracene were purchased from Eastman Kodak Co. (Rochester, NY, USA). All chemicals for the preparation of electrolytes were purchased from Fisher Scientific (Pittsburgh, PA, USA). Methanol was purchased from EM Science (Cherry Hill, NJ, USA). All electrolyte solutions were prepared with deionized water and filtered with 0.2 μm Uniprep Syringeless filters from Fisher Scientific to avoid capillary plugging.

2.3. Methods

The running electrolyte was prepared by dissolving proper amounts of boric acid and surfactant in water, and adjusting the pH to the desired value with sodium hydroxide. For all experiments, the running electrolyte was composed of 100 m*M* of the surfactant and 200 m*M* borate at pH 10.0. All sample stock solutions were made by first dissolving pure compounds in methanol. Sample solutions for injection were made by dissolving the proper amount of stock solution in the running electrolyte and adjusting the total volume of methanol in the sample to 20% (v/v). Sample injection was performed by pressurizing the sample reservoir for an appropriate length of time (50–100 mbar · s). Between runs, the capillary was rinsed consecutively with water, 1.0 *M* NaOH, 0.10 *M* NaOH, water and the running electrolyte.

In all calculations involving efficiency, the plate count was estimated from peak standard deviation taken as the half peak width at 0.607 of peak height (i.e., the inflection point) and was reported as the average of at least three runs. Mobilities were determined from average migration times, and again a minimum of three runs were used to calculate the average. The migration time of an unretained species, t_0, was determined by the deflection peak of methanol. The migration time of the micelle, t_{mc}, was determined by the iterative method of a homologous series [8]. Anthracene was used as a marker to visualize t_{mc} but was not used in the calculation of the capacity factor, k'.

3. Results and discussion

3.1. Migration time window

As we have described in previous studies involving in situ charged micellar systems, i.e., alkylglycoside–borate or –boronate micelles [11,13,14], the migration time window can be adjusted over a wide range by adjusting the pH, borate (or boronate) concentration and/or the surfactant concentration. This is because these parameters affect the surface charge density of the micelle.

To provide a meaningful comparison for the results pertaining to the migration time window, the four surfactants, OG, OS, OM and MEGA 9, were evaluated under conditions of constant micellized surfactant concentration. Constant micellized surfactant concentration corresponds to keeping the concentration of surfactant [S] minus the CMC constant, i.e., [S] − CMC = constant. Since the four surfactants all have similar CMC values, all electrolyte solutions used in the present studies contained the same surfactant concentration. All running electrolytes were composed of 100 m*M* surfactant and 200 m*M* borate at pH 10.0. Table 2 lists the migration times for the unretained species, t_0, and those of the micelle, t_{mc}. The elution range parameters (t_0/t_{mc}) were calculated to be 0.65, 0.60, 0.55 and 0.31 for OM, OS, OG and MEGA micellar phases, respectively. The width of migration time window was the smallest for OM–borate micelle (7.70 min) and the highest for MEGA 9 (28.30 min). The value of the width of the migration time window for OS (9.96 min) and OG (10.67 min) was slightly larger than that observed with OM but much smaller than the one exhibited by MEGA 9. These results corroborate well those previously reported with ^{11}B NMR studies [13] in the sense that the MEGA surfactants have two- to three-fold greater affinity for borate than the alkylglucoside surfactants. This translates into a greater electrophoretic

Table 2
Comparison of migration time window, mobility, efficiency and peak capacity for the various micellar phases

Micellar phase	t_o	t_{mc}	$\mu_{ep(mc)}$ ($cm^2V^{-1}s^{-1}$)	N_{av}	n
OS	14.65	24.61	-1.38×10^{-4}	253 960	65
OM	14.27	21.97	-1.22×10^{-4}	337 230	63
OG	12.91	23.58	-1.75×10^{-4}	429 850	99
MEGA 9	12.47	40.77	-2.77×10^{-4}	301 500	163

Conditions: running electrolyte, 200 m*M* borate containing 100 m*M* surfactant, pH 10.0; capillary, untreated fused-silica, 56.0 cm (to detection point), 64.0 cm (total length) × 50 μm I.D.; voltage 15 kV.

mobility, $\mu_{ep(mc)}$, for MEGA–borate micelle, and in turn to a wider migration time window. Also, the small differences in the width of the migration time windows among OS, OM and OG agree with ^{11}B NMR results which revealed that these surfactants have similar affinities for borate [14]. As can be seen in Table 2, the values of t_o were different among the various micellar phases which may due to slight differences in the viscosity of their running electrolyte solutions.

Furthermore, Table 2 lists the $\mu_{ep(mc)}$ for the four surfactant–borate micellar phases under investigation. The $\mu_{ep(mc)}$ is a direct indication of the degree of complexation between borate and the micelle. OS, OM and OG have a somewhat lower $\mu_{ep(mc)}$ as reflected by the migration time window. The larger $\mu_{ep(mc)}$ observed with MEGA 9 is a result of the acyclic nature of the sugar head group of the surfactant. This is because the hydroxyl groups of acyclic sugars, which are not held in a fixed position, are free to move into more favorable binding positions. Due to this conformational freedom found in acyclic sugars, they can possess up to a ten-fold greater affinity for borate than cyclic sugars [20].

The differences in borate affinity for the different surfactants offer a variety of migration time windows. It should be emphasized that the $\mu_{ep(mc)}$ for OS, OM and OG can be increased up to $\approx -2.5 \times 10^{-4}$ $cm^2V^{-1}s^{-1}$ by increasing the borate concentration and/or the pH because the surface charge density of the micelle is increased. The MEGA surfactants exhibit a relatively high $\mu_{ep(mc)}$ that can be varied over a wide range with an upper limit approaching that achieved with SDS. A micellar phase consisting of 43 m*M* MEGA 9 and 400 m*M* borate at pH 10.0 produced an $\mu_{ep(mc)}$ of -3.6×10^{-4} $cm^2V^{-1}s^{-1}$ [13] while that of 100 m*M* SDS is reported to be -4.2×10^{-4} $cm^2V^{-1}s^{-1}$ [21]. Many separations do not require a large migration time window in order to separate completely all of the components of a given mixture. If a large retention time window is not required, OS, OM or OG could possibly provide the best results in terms of throughput, but if a complex sample is to be analyzed, a larger migration time window will probably be beneficial and the use of MEGA–borate or –boronate is recommended in this case.

3.2. Efficiency and peak capacity

The separation efficiencies exhibited by the in situ charged micellar phases under investigation are listed in Table 2 in terms of average plate count for the APK homologous series. OG offers the highest separation efficiency with an average plate count of 430 000. The lowest plate count was observed with OS which on the average was 254 000 plates. According to Terabe et al. [22], the dominant intracolumn contributions to band broadening are longitudinal diffusion, sorption–desorption kinetics and electrophoretic homogeneity of the micelles. Since OS–borate micellar phase was the least retentive towards the APKs (see below), the solutes would spend less time associated with the OS micelle than with the other micelles, a condition that would lead to more longitudinal diffusion. This would explain in part the lower separation efficiency obtained

with OS. With the other three alkylglycoside–borate micellar phases under consideration, the contributions to band broadening arising from longitudinal molecular diffusion or electrophoretic homogeneity can be considered to be similar. The observed differences in separation efficiencies among OM, OG and MEGA 9 suggest that the contributions to band spreading arising form sorption–desorption kinetics are different from one micellar phase to another. Davis [23] suggested that nonequilibrium band broadening can be reduced by decreasing the micelles' mobility. In fact, the OM and OG micelles, having the lowest electrophoretic mobilities, yielded higher separation efficiencies than that exhibited by MEGA 9 which is characterized by a higher electrophoretic mobility. Overall, the in situ charged micellar phases produced higher separation efficiencies than those reported with SDS [3,22] or alkyltrimethylammonium halide micelles [24]. The conditions under which these measurements were made produced micelles with mobilities significantly lower than those obtained with SDS and alkyltrimethylammonium halide micelles which, among other things, could explain the higher efficiencies obtained with in situ charged micelles.

As a result of the high plate counts, the number of peaks that can be resolved in a certain time frame is very high as shown in Table 2. The peak capacity, n, is defined as the number of peaks that will fit in a given elution time interval with a resolution of unity. The peak capacity can be calculated experimentally from the values of t_o, t_{mc} and the average plate count [5]. Both OS and OM demonstrated peak capacities greater than 60, but these separations were achieved in a short time frame, i.e., less than 25 min. The peak capacities for OG approached 100 while that of MEGA 9 was in excess of 160. These peak capacities illustrate the tremendous resolving power of MECC in a short time period.

3.3. Retention energetics

To evaluate the influence of the nature of the surfactant on the retention behavior of the various alkylglycoside–borate micellar phases, a series of alkyl phenyl ketone (APK) homologous solutes, ranging from acetophenone to heptanophenone, were electrochromatographed under the above mentioned conditions. In all cases, the six homologous solutes were well resolved, and typical electropherograms obtained with OG- and MEGA 9–borate micellar phases are illustrated in Fig. 2. The last peak is that of anthracene which was used to visualize the migration time window. Table 3 lists the capacity factors, k', of the APK homologous solutes with each micellar phase. As expected, the k' values were the smallest for OS. This surfactant has one fewer carbon atom in its alkyl chain than the other three surfactants, see Table 1. The k' values observed with MEGA 9 were significantly higher than those obtained with OS. OM showed higher retention than any of the other micellar phases for the first three solutes of the homologous series ($n_c = 1$ to 3, where n_c is the number of carbon atoms in the alkyl chain of the solute) while OG was the most retentive for the solutes having $n_c = 4$ to 6.

The retention data of Table 3 were further exploited by plotting logarithmic capacity factor versus n_c for the homologous APKs which yielded a straight line on each micellar phase. This is in agreement with our previous studies [12–14, 24] in which we demonstrated that the relationship between log k' and n_c follows the expression normally found in reversed-phase chromatography:

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

where the slope (log α) is a measure of the methylene group selectivity which characterizes nonspecific hydrophobic interactions, while the intercept (log β) reflects the specific interactions between the residue of the molecule (i.e., benzaldehyde group) and the aqueous and micellar phase. For an in-depth discussion of this data treatment the reader is refereed to previous reports from our laboratory [13,24].

Table 4 lists the results of linear regression for plots of log k' versus n_c for the different micellar phases. The R values obtained with the four plots were either 0.999 or 1.000. The methylene

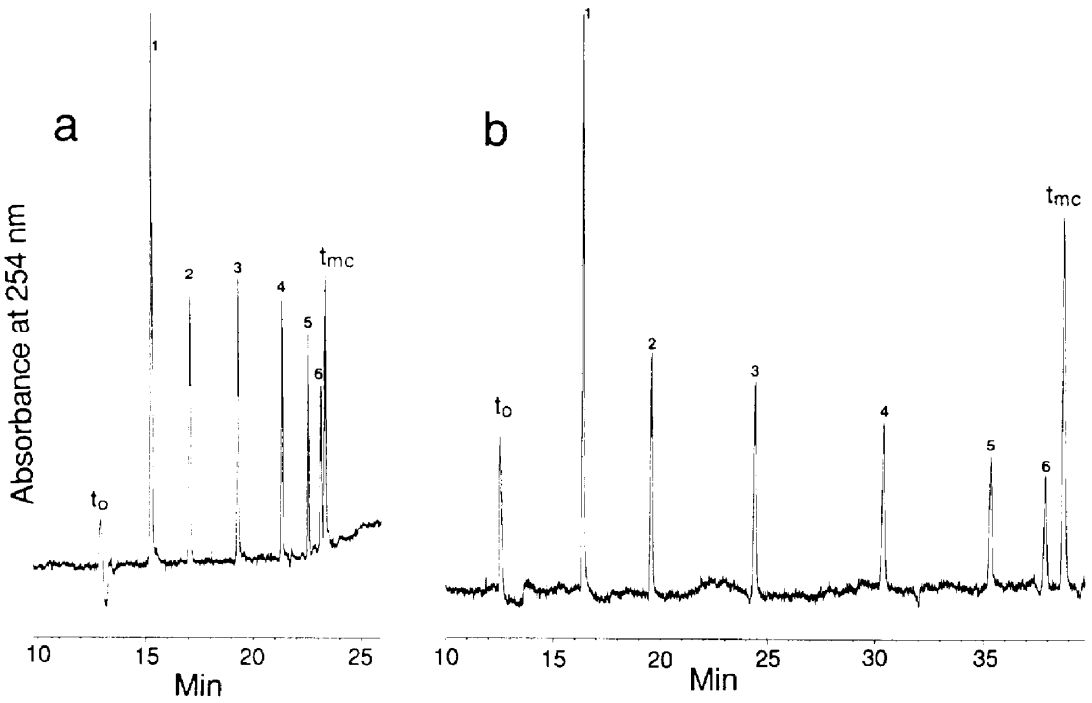


Fig. 2. Electropherograms of APK homologous series. Experimental conditions: running electrolytes, 200 m*M* borate containing 100 m*M* OG in (a) or 100 m*M* MEGA 9 in (b), pH 10.0; capillary, untreated fused silica, 64.0 cm (total length) × 50 μm I.D., 56.0 cm to detection point; voltage, 15 kV. Analytes: 1, acetophenone; 2, propiophenone; 3, butyrophenone; 4, valerophenone; 5, hexanophenone; 6, heptanophenone; t_{mc}, anthracene.

group selectivity for the two surfactants having disaccharide head groups, i.e., OS and OM, are very similar. Both OG and MEGA 9 yielded higher methylene group selectivities, with OG being the highest. On the other hand, there is a significant difference between OS and OM in the magnitude of their specific interactions (i.e., log β values), whereas the specific interactions exhibited by OG and MEGA 9 are similar. OS showed the lowest specific interactions (i.e., the highest negative value for log β) followed by OG and MEGA 9, while OM exhibited the highest specific interactions (i.e., the lowest negative value for log β) with the APK series. These retention behaviors reflect the fact that OM is more retentive towards the smallest

Table 3
Comparison of capacity factors obtained with the various micellar phases

Micellar phase	k'					
	$n_c = 1$	$n_c = 2$	$n_c = 3$	$n_c = 4$	$n_c = 5$	$n_c = 6$
OS	0.37	0.70	1.44	3.17	7.94	15.6
MEGA 9	0.52	1.08	2.37	5.67	14.1	30.4
OM	0.67	1.37	2.90	6.47	14.1	29.7
OG	0.51	1.14	2.69	6.65	16.1	37.2

Conditions as in Table 2. Analytes: $n_c = 1$, acetophenone; $n_c = 2$, propiophenone; $n_c = 3$, butyrophenone; $n_c = 4$, valerophenone; $n_c = 5$, hexanophenone; $n_c = 6$, heptanophenone.

Table 4
Correlation between log k' and n_c of APK homologous series for various micellar phases

Micellar phase	log β	log α	R
OS	−0.805	0.333	0.999
OM	−0.517	0.332	1.000
OG	−0.681	0.375	1.000
MEGA 9	−0.673	0.359	0.999

Conditions as in Table 2.

solutes in the homologous series (i.e., $n_c = 1$ to 3) where the specific interactions are predominant, and OG exhibits the highest retention toward the solutes with $n_c = 4$ to 6 where the effect of the hydrophobic chain of the solute becomes increasingly more significant.

To gain further insight into the retention behavior of the various micellar phases, the retention energetics of these phases were compared by plotting log k' of the APK homologous series obtained with a given micellar phase versus the log k' of the same solutes obtained with a reference micellar phase. Usually, such plots yield straight lines for homologous solutes. If the slope is unity, the differences in Gibbs retention energies of the two micellar phases is zero for all solutes and the retention is termed *homeoenergetic* [25]. In this case, the intercept of the line is equal to the logarithm of the quotient of the phase ratios of the two micellar phases. The quotient of the phase ratios can then be obtained from the antilog of the intercept. If the slope is not unity, then the Gibbs retention energies are proportional by a constant that is equivalent to the slope, and the retention is

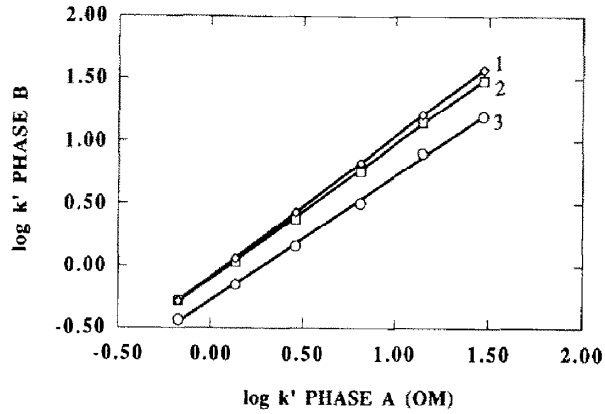


Fig. 3. Plots of log k'–log k' of APK homologous series obtained on one micellar phase versus another reference micellar phase at constant micellized surfactant concentration. Lines: 1, OG micellar phase versus OM micellar phase; 2, MEGA 9 micellar phase versus OM micellar phase; 3, OS micellar phase versus OM micellar phase. Experimental conditions: running electrolytes, 200 m*M* borate containing 100 m*M* surfactant, pH 10.0; other conditions as in Fig. 2.

termed *homeoenergetic* [25]. We have previously reported this approach in the characterization of numerous micellar systems [12–14, 24]. Fig. 3 shows plots of log k' for micellar phase B versus log k' of a reference micellar phase A for the APK homologous series. In these plots, the OM–borate system was chosen as the reference micellar phase, i.e., micellar phase A. The six data points for each line in Fig. 3 are the log k' obtained with the six APK solutes. The slopes, intercepts, and antilog of the intercepts of the log k'–log k' of these plots are listed in Table 5. The R values from the linear regression were all 0.999 or greater. The slope of log k'–log k' plot for OS versus OM was close to unity, thus

Table 5
Slopes, intercepts and antilog of intercepts of log k' – log k' plots for APK homologous series obtained with different micellar phases

Phase A / phase B	Slope	Intercept	R	φ_B/φ_A
OS / OM	1.005	−0.286	0.999	0.52
MEGA 9 / OM	1.084	−0.112	1.000	0.77
OG / OM	1.132	−0.095	1.000	0.80
OM / OM	1.000	0.000	1.000	1.00

Conditions as in Table 2.

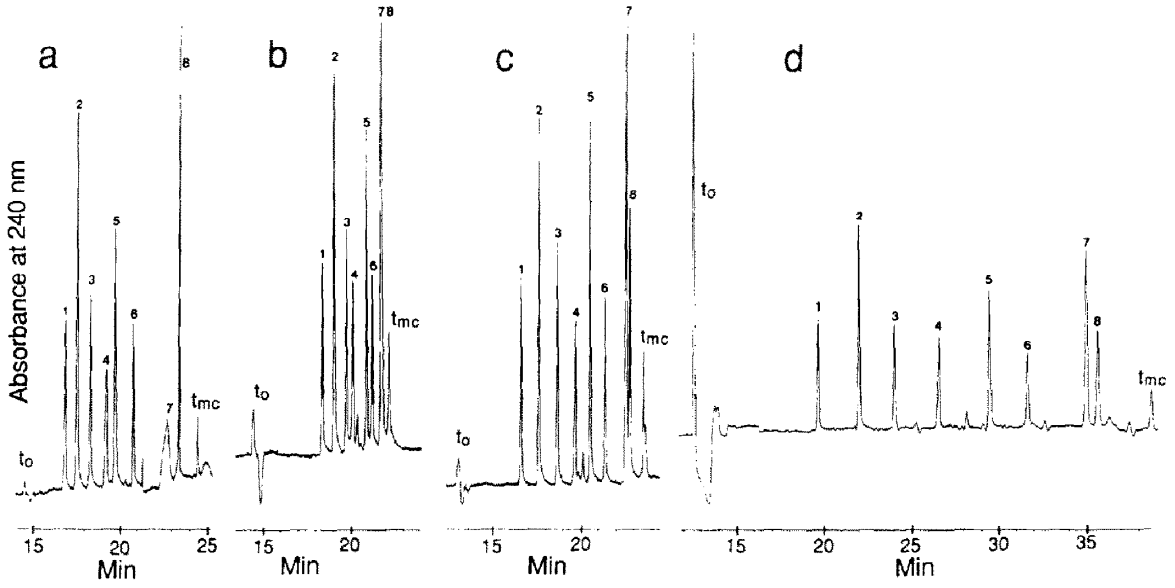


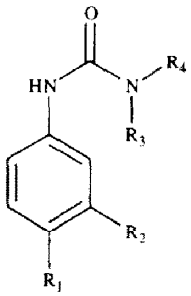
Fig. 4. Electropherograms of urea herbicides obtained with each of the various micellar phases. Experimental conditions: running electrolytes, 200 m*M* borate containing 100 m*M* OS in (a) or 100 m*M* OM (b) or 100 m*M* OG (c) or 100 m*M* MEGA 9 in (d), pH 10.0. Other conditions as in Fig. 2. Solutes: 1, monuron; 2, fluometuron; 3, metobromuron; 4, siduron; 5, diuron; 6, linuron; 7, neburon; 8, chloroxuron; t_{mc}, anthracene.

indicating homoenergetic retention behavior between the two micellar phases, and is consistent with previously reported data [14]. The log k'–log k' plots for MEGA 9/OM and OG/OM have slopes near unity indicating quasi-homoenergetic behaviors between these surfactants. The net influence of the nature of the surfactant (i.e., different hydrophilic sugar head groups and hydrocarbonaceous moieties) is realized by examining the quotient of the phase ratios, φ_B/φ_A. The phase ratio of the OM micellar phase is double that of the OS micellar phase. The phase ratios of MEGA 9 and OG micellar phases are 77% and 80%, respectively, of that obtained on the OM micellar phase.

This method of data treatment has proven to be very useful in the characterization of new micellar phases. The in situ micellar phases we have introduced differ significantly in hydrophobic character. These different characteristics are of great value when selecting a micellar system to perform a given separation. The differences in hydrophobic character allow each micelle to have slightly different selectivities as will be discussed in the following section.

3.4. Selectivity

Fig. 4 illustrates the separation of a mixture of eight closely related urea herbicides obtained on each of the four micellar phases. The structures of the herbicides are listed below.



	R_1	R_2	R_3	R_4
(1) Monuron	Cl	H	CH_3	CH_3
(2) Fluometuron	H	CF_3	CH_3	CH_3
(3) Metobromuron	Br	H	CH_3	OCH_3
(4) Siduron	H	H	H	*
(5) Diuron	Cl	Cl	CH_3	CH_3
(6) Linuron	Cl	Cl	CH_3	OCH_3
(7) Neburon	Cl	Cl	CH_3	C_4H_9
(8) Chloroxuron	**	H	CH_3	CH_3

* 2-methylcyclohexyl

** 4-chlorophenoxy

The average plate counts were 297 360, 329 840, 388 080 and 283 920 for OS, OM, OG and MEGA 9 micellar phases, respectively. The distorted peak shape of neburon obtained with the OS micellar phase (Fig. 4a) may be due to the presence of an impurity.

To examine the influence of the nature of the

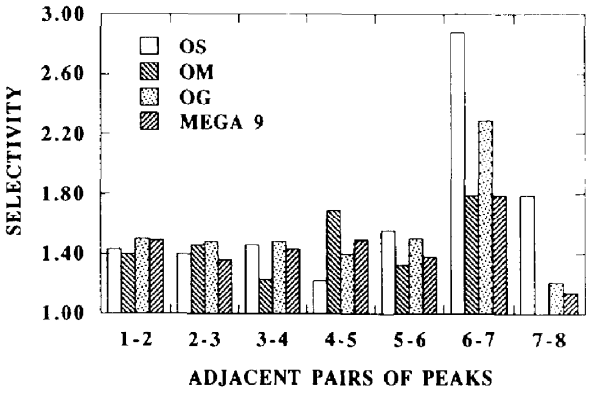


Fig. 5. Bar graphs of the selectivity factor for the urea herbicides with each micellar phase. Experimental conditions as in Fig. 4.

surfactant on the separation of these urea herbicides, the selectivity, $\alpha = k'_2/k'_1$, between adjacent peaks was calculated. Fig. 5 shows bar graphs for the selectivity between each adjacent pair of urea herbicides obtained on each of the four micellar phases. The only case an adjacent pair of peaks is not resolved is with the OM micellar phase where the last two herbicides, neburon and chloroxuron, co-elute. This is attributed to the increased hydrophobic character of OM when compared to the other micellar phases. The selectivities of the first five adjacent pairs are very similar, but with the last two adjacent pairs OS provides notably higher selectivity. This is due to the weaker hydrophobic character of OS when compared to the other three surfactants.

To illustrate further the influence of the nature of the surfactant, a mixture of neutral and acidic herbicides was analyzed. This mixture contained three s-triazine herbicides (i.e., prometon, propazine and prometryne), three chlorinated phenoxy acid herbicides (i.e., silvex, 2,4,5-T and 2,4-D), one organophosphorous pesticide (i.e., parathion) and one sulfur–carbamate herbicide (i.e., aldicarb). The structures of these herbicides are shown below.

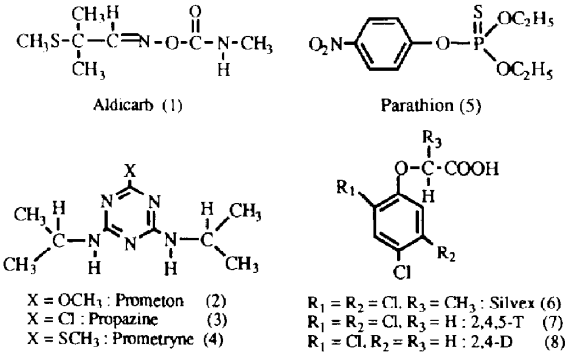


Fig. 6 shows typical electropherograms obtained with 200 mM borate and 100 mM of

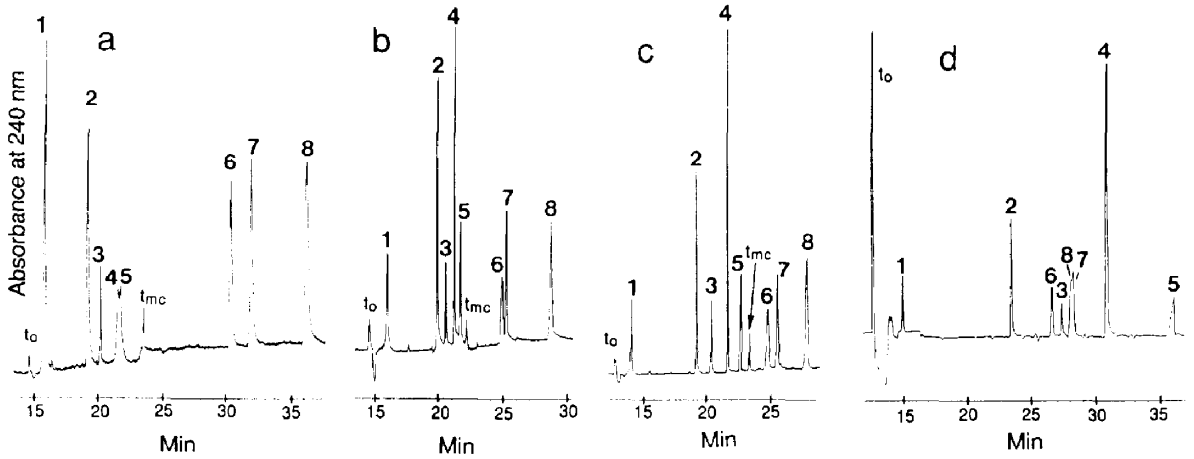


Fig. 6. Electropherograms of neutral and acidic herbicides obtained with each of the various micellar phases. Experimental conditions: running electrolytes, 200 mM borate containing 100 mM OS in (a) or 100 mM OM (b) or 100 mM OG (c) or 100 mM MEGA 9 in (d), pH 10.0. Other condition as in Fig. 2. Solutes: 1, aldicarb; 2, prometon; 3, propazine; 4, prometryne; 5, parathion; 6, silvex; 7, 2,4,5-T; 8, 2,4-D; t_{mc}, anthracene.

surfactant at pH 10.0. At this pH, the acidic herbicides are fully ionized and are migrating primarily by their own electrophoretic mobility. These acidic herbicides elute after t_{mc} with OS, OM and OG micellar phases, but elute within the migration time window with the MEGA 9 micellar phase. In this aspect, the narrower migration time window is advantageous since the neutral components are removed from the acidic components. The average theoretical plates were 176 400 for OS, 257 040 for OM, 283 920 for OG and 222 880 for MEGA 9. The lower average separation efficiencies arise from the much lower plate counts observed with the acidic species. This is because the acidic herbicides are not significantly partitioned in the micelle, a condition that leads to increased longitudinal diffusion.

Fig. 7 shows plots of the selectivities between each adjacent pair of peaks for neutral herbicides (i.e., aldicarb, prometon, propazine, prometryne and parathion) for the various micellar phases. The selectivity for pairs 2–3 and 3–4 are very similar for all the micellar phases. The selectivity of the first adjacent pair, 1–2, is the highest with OM and OG micellar phases, whereas the selectivity for the last adjacent pair, 3–4, is greatest with OG and MEGA 9 micellar phases. For this mixture of herbicides, the OG micellar phase yielded the best separation in terms of resolution and separation efficiency.

As a final illustration of the different selectivities obtained with the different micellar phases, a mixture of hydrophobic aromatic compounds containing one, two or three fused rings was electrochromatographed, see Fig. 8. The OS micellar phase provides better separation than OM or OG micellar phases, but still the most hydrophobic species are only partially resolved. The wider migration time window of the MEGA 9 micellar phase allowed the best separation of the aromatic species, but at the cost of longer analysis time. The average plate counts were 233 520, 248 080, 287 280 and 246 400 for OS, OM, OG, and MEGA 9 micellar phases, respectively.

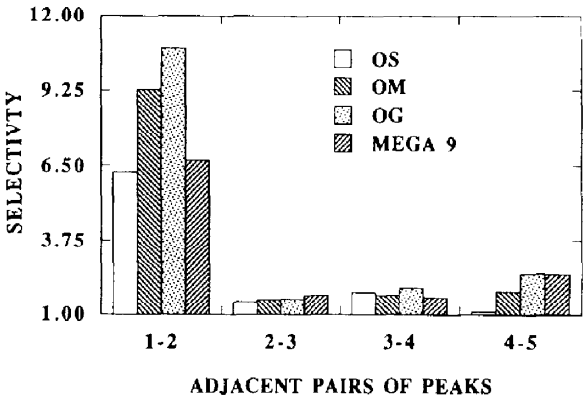


Fig. 7. Bar graphs of the selectivity factor for neutral herbicides with each micellar phase. Experimental conditions as in Fig. 6.

4. Conclusions

In situ charged micellar phases have proven very effective in the separation of both neutral and acidic species. These micellar phases offer an adjustable migration time window that is largely influenced by the nature of the hydrophilic sugar head group of the individual surfactants. The acyclic sugar head group present in the MEGA 9 micellar phase provides the widest migration time window due its increased affinity to borate. Retention energetic studies show that OM micellar phase possesses the greatest hydrophobic character followed by OG and MEGA 9 micellar phases. Having one fewer carbon atom in its alkyl tail and possessing a disaccharide head group, the OS micellar phase displayed the least hydrophobic character making it most suitable for MECC of very hydrophobic species. In all cases, separation efficiencies ranged from 170 000 to 400 000 theoretical plates.

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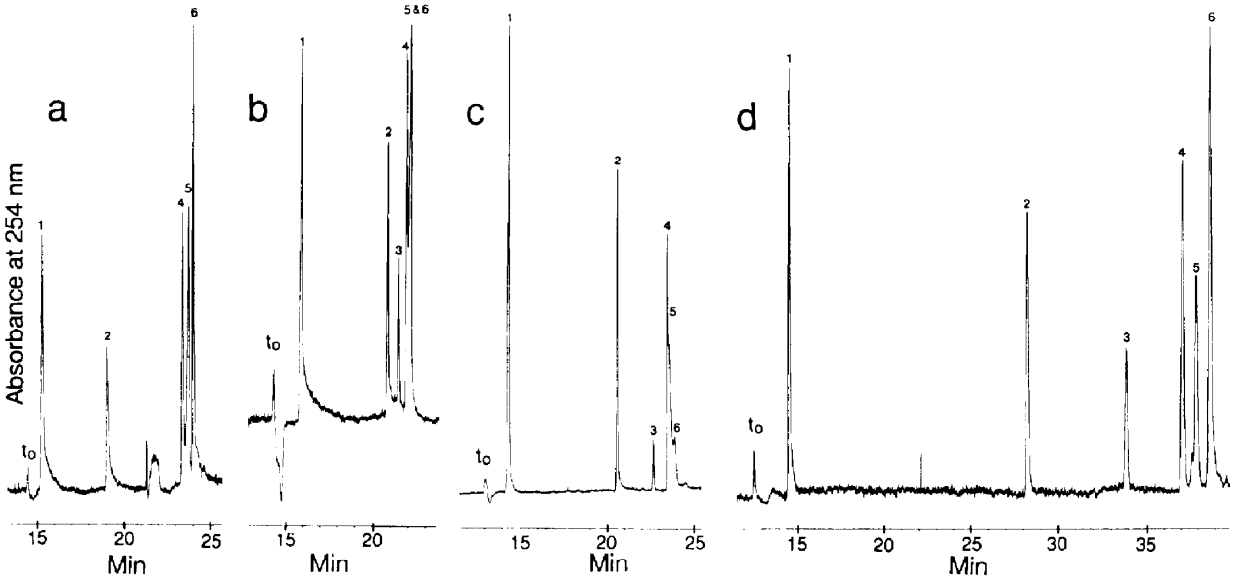

Fig. 8. Electropherograms of aromatic species obtained with each of the various micellar phases. Experimental conditions: running electrolytes, 200 m*M* borate containing 100 m*M* OS in (a) or 100 m*M* OM (b) or 100 m*M* OG (c) or 100 m*M* MEGA 9 in (d), pH 10.0. Other condition as in Fig. 2. Solutes: 1, aniline; 2, 1-naphthylamine; 3, naphthalene; 4, biphenyl; 5, 1-chloronaphthalene; 6, anthracene.

capillary electrophoresis instrument from Hewlett Packard is greatly appreciated.

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