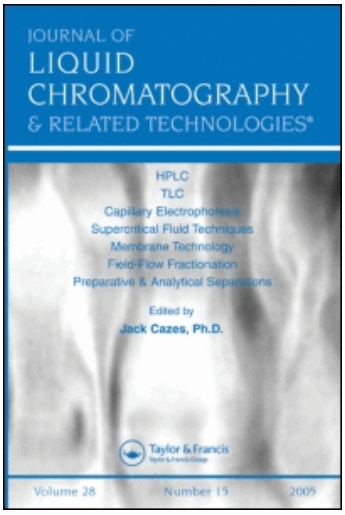

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Micellar Electrokinetic Capillary Chromatography with *In Situ* Charged Micelles. VII. Expanding the Utility of Alkylglycoside-Borate Micelles to Acidic and Neutral pH for Capillary Electrophoresis of Dansyl Amino Acids and Herbicides

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MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY WITH *IN SITU* CHARGED MICELLES. VII. EXPANDING THE UTILITY OF ALKYLGLYCOSIDE-BORATE MICELLES TO ACIDIC AND NEUTRAL pH FOR CAPILLARY ELECTROPHORESIS OF DANSYL AMINO ACIDS AND HERBICIDES[1]

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ABSTRACT

Micellar electrokinetic capillary chromatography (MECC) using micelles based on alkylglycoside-borate complexes, i.e., *in situ* charged micelles, was demonstrated over a wide range of pH including acidic media. The surfactants used in this study were of the alkyl-*N*-methylglucamide (MEGA) types that possess a linear sugar moiety as the polar head group. These surfactants showed the ability to complex with borate even at acidic pH, thus expanding the pH range over which in situ charged micelles can be used in MECC. The electrokinetic behavior of the MEGA-borate micelle at acidic and neutral pH using relatively high borate concentration was different than that observed at alkaline pH using lower borate concentration. These studies which were performed at high borate concentration suggested the formation of polyborates which increased the ionic strength and viscosity of the running electrolyte without further increasing the surface charge density of the micelle. This led to decreasing the electrophoretic mobility of the micelle and the electroosmotic flow with increasing borate concentration. The net result was an increase in the migration time window at increasing borate concentration. Moreover, the magnitude of the migration time window could be adjusted by varying the pH of the running electrolyte over the pH range from 3.5 to 9.0. The MEGA-borate systems proved useful for the separation of phenoxy acid herbicides and their esters, urea herbicides, dansyl amino acids and aromatics.

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INTRODUCTION

Several types of *in situ* charged micelles for use in micellar electrokinetic capillary chromatography (MECC) of neutral and charged species have been recently introduced and characterized by our laboratory [1-7]. These new micellar phases are essentially anionic complexes formed between alkyl- [2-7] or steroidal-glycoside [1] surfactants and borate or boronate anions. *In situ* charged micelles possess many unique and attractive features including: (i) an adjustable surface charge density (ii) a weaker hydrophobic character than the traditional alkyl surfactants, and (iii) chiral selectivity. In addition, and as a result of the variable surface charge density, *in situ* charged micelles allow the manipulation of the migration time window and in turn the optimization of analysis time, resolution and peak capacity *via* changing pH and borate or boronate concentration of the running electrolyte.

Thus far, we have demonstrated the usefulness of *in situ* charged micelles in the alkaline pH range in MECC of chiral and achiral polyaromatics, pesticides, amino acids, barbiturates and medicarpins and precursors [1-7]. In this report, we wish to extend the useful pH range of *in situ* charged micelles to the acidic as well as neutral pH.

In earlier reports on *in situ* charged micelles [2, 3], we have demonstrated by ^{11}B NMR studies that alkylglycoside surfactants with acyclic sugar head groups, such as MEGA surfactants, have 3 to 5 fold higher affinity for borate ions than their counterparts with cyclic sugar head groups (e.g., octylglucoside, octylmaltoside). This observation, which corroborates well previous findings [8] in that linear sugars complex stronger with borate than cyclic ones, prompted us to select the MEGA surfactants for our present studies at lower pH range. The principles of *in situ* charged micelles have been discussed in previous contributions from our laboratory [2, 3, 7], and the complexation of borate with polyols have been briefly described recently by El Rassi [9], El Rassi and Nashabeh [10], and Hoffstetter-Kuhn et al. [11]. For simplicity, the term "borate ions" or "borate" is used in this article to refer to all kinds of borate species. But, whenever a specific borate species is involved the exact term is utilized instead.

EXPERIMENTAL

Instrument

The instrument for capillary electrophoresis was assembled in-house from commercially available components [2, 12]. It was comprised of two high-voltage power supplies of positive and negative polarity, Models MJ30P400 and MJ30N400, respectively, from Glassman High Voltage (Whitehouse Station, NJ, USA) and a UV-Vis variable wavelength detector Model 200, equipped with a cell for on-column detection from Linear Instruments (Reno, NV, USA). The detection wavelength was set at 240 nm for the detection of phenoxy acid herbicides and phenoxy ester herbicides as well as urea herbicides, 254 nm for the detection of alkyl phenyl ketones and dansyl amino acids and 340 nm for the detection of Sudan III. Electropherograms were recorded with a Shimadzu computing integrator Model CR4A (Columbia, MD, USA).

Fused-silica capillary columns of 50 μm I.D. and 365 μm O.D. were obtained from Polymicro Technology (Phoenix, AZ, USA). Throughout the present study, the total length of the capillary was 80 cm while the separation length was 50 cm.

Reagents and Materials

Nonanoyl-*N*-methylglucamide (MEGA 9) and decanoyl-*N*-methylglucamide (MEGA 10) were purchased from Calbiochem Corp. (La Jolla, CA, USA). All herbicides were purchased from ChemService (West Chester, PA, USA). Sudan III, which was used as the tracer of the migration time of the micelle, and alkyl phenyl ketones were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Dansyl amino acids were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The chemicals used in the preparation of electrolytes as well as the 0.2 μm Uniprep Syringeless filters used in the filtration of the electrolyte were purchased from Fisher Scientific (Pittsburgh, PA, USA). All solutions were prepared with deionized water.

RESULTS AND DISCUSSION

Dependence of the Magnitude of the Migration Time Window on Borate Concentration

As shown in Fig. 1a, the migration time window of the micellar phase under investigation increased with borate concentration, and to a larger extent at borate concentration higher than 500 mM. This increase is primarily due to the decrease in the electroosmotic flow, EOF, (see Fig. 1b) given the fact that the electrophoretic mobility of the MEGA 9-borate micelle decreased slightly in the range 100 to 600 mM borate or remained almost the same at borate concentrations above 600 mM as shown in Fig. 1b. Since by increasing the borate concentration in the running electrolyte, the values of both the electroosmotic velocity, v_{eo}, and electrophoretic velocity of the micelle, $v_{ep(mc)}$, are decreasing and approaching each other (see Fig. 1b), the difference between the two velocities will decrease with increasing borate concentration. Due to the fact that the electroosmotic velocity and electrophoretic velocity of the micelle are of opposite sign, and according to the following equation, which relates the migration time of the micelle, t_{mc}, to v_{eo} ,$v_{ep(mc)}$ and the separation distance in the capillary, l ,

$$t_{mc} = \frac{l}{v_{eo} + v_{ep(mc)}} \qquad \text{(I)}$$

t_{mc} will increase, and consequently the breadth of the migration time window will enlarge with increasing borate concentration, see Fig. 1a.

Aqueous borate solutions contain tetrahydroxyborate anion, which is formed according to the following equilibrium,

$$B(OH)_3 + OH^- \leftrightarrow B(OH)_4^- \qquad (1)$$

as well as polyborate anions [13-15] such as $B_3O_3(OH)_4^-$, $B_5O_6(OH)_4^-$, $B_3O_3(OH)_5^{2-}$ and $B_4O_5(OH)_4^{2-}$. Singly charged trimer and pentamer borate anions are formed by the following equilibria, respectively [16]:

$$2B(OH)_3 + B(OH)_4^- \leftrightarrow B_3O_3(OH)_4^- + 3H_2O \qquad (2)$$

$$4B(OH)_3 + B(OH)_4^- \leftrightarrow B_5O_6(OH)_4^- + 6H_2O \qquad (3)$$

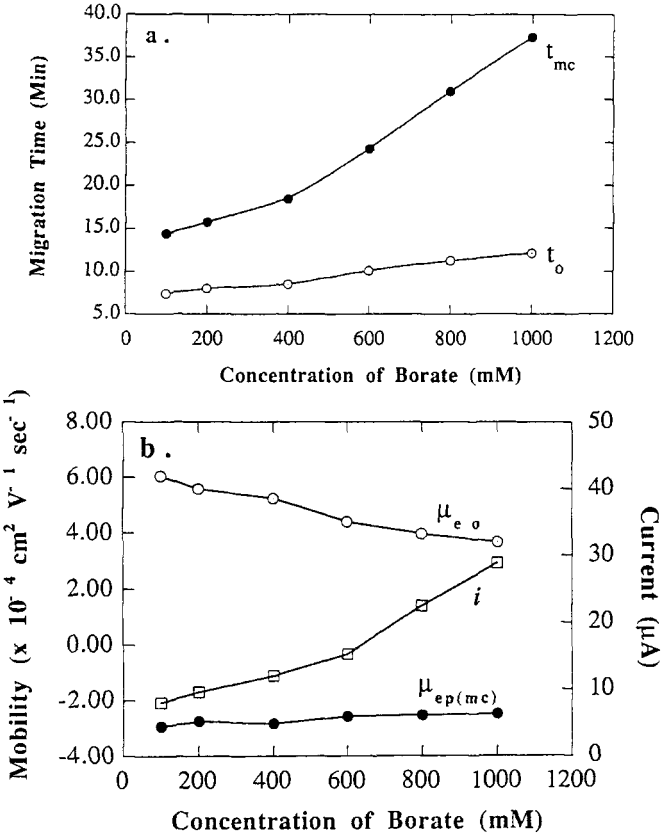


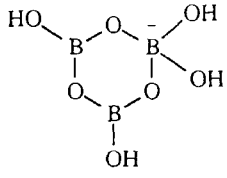
FIGURE 1. Effect of borate concentration on the magnitude of the migration time window in (a) and the electrophoretic mobility of the micelle as well as the electroosmotic mobility and current in (b). Separation capillary, bare fused-silica, 50.0 cm / 80.0 cm x 50 μm i.d.; running electrolyte, 5.0 mM sodium phosphate containing 50.0 mM MEGA 9 and various borate concentration, pH 7.0; running voltage, 15.0 kV; tmc tracer, sudan III; t_0 marker, methanol.

while doubly charged trimer and tetramer anions are produced by the two following equilibria, respectively:

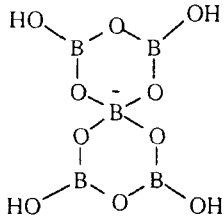
$$3B(OH)_3 + 2(OH)^- \leftrightarrow B_3O_3(OH)_5^{2-} + 3H_2O \quad (4)$$

$$4B(OH)_3 + 2(OH)^- \leftrightarrow B_4O_5(OH)_4^{2-} + 5H_2O \quad (5)$$

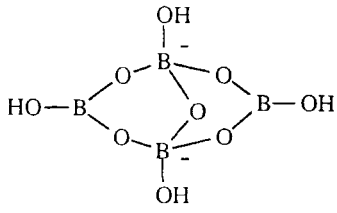
Whereas equilibria 1 and 2 are predominant at low borate concentration (e.g., ≤ 200 mM), the other polyborate anions formed by equilibria 3, 4 and 5 become important species at high borate concentration [15]. For three of these polyborate anions the following structures were suggested [16, 17]:



Cyclic trimer

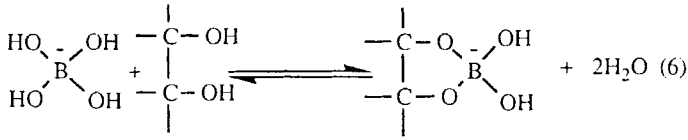


Bicyclic pentamer



Tetramer

The complexation of borate with compounds having vicinal hydroxyl groups, such as the MEGA surfactant used in this study, is thought to occur mainly *via* the reaction of the tetrahydoxyborate ion $B(OH)_4^-$ and the polyolic compound according to the following equilibrium:



(6)

In fact, equilibrium 6 is favored when the O-O distances for the vicinal diol in the polyol compound and for the hydroxyls in the borate ion are on the order of 2.4 Å. This distance is that of the O-O in the tetrahedral boron [18]. On this basis, among polyborate anions, only the cyclic trimer will have the probability to complex with the polyol head group of the MEGA surfactant. However, it can be suggested that due to steric hindrance, the cyclic triborate will complex less than the tetrahydoxyborate anion with a given polyolic compound.

Returning to Fig. 1b, the decrease in the EOF and electrophoretic mobility of the micelle can be explained as follows. At 100 mM borate, pH 7.0, the electrophoretic mobility of the micelle ($\mu_{ep(mc)}$) is quite high which is indicative of the formation of complexing borate ions, e.g., tetrahydoxyborate and cyclic trimer. As the amount of

added boric acid was increased between 200 and 1000 mM, $\mu_{ep(mc)}$ decreased. This may indicate that the concentration of polyborate species such as those shown in equilibria 3, 4 and 5 had increased, a phenomenon that would increase the ionic strength of the running electrolyte without further increasing the surface charge density of the micelle. In fact, and as shown in Fig. 1b, the current i, which is an indirect measure of the magnitude of the ionic strength, has increased with borate concentration over the concentration range studied. As discussed above, the polyborate species such as $B_5O_6(OH)_4^-$, $B_3O_3(OH)_5^{2-}$ and $B_4O_5(OH)_4^{2-}$ are unlikely to complex with vicinal diols. Thus, since increasing the ionic strength of the running electrolyte is accompanied by little or no further increase in the surface charge density of the micelle, the net result is a decrease in the electrophoretic mobility of the MEGA-borate micelle. The decrease in EOF is due to increasing the ionic strength of the running electrolyte. Moreover, the decrease in both parameters (i.e., EOF and electrophoretic mobility of the micelle) may be in part attributed to increasing the viscosity of the running electrolyte at high borate concentration.

Figure 2 displays typical electropherograms of alkyl phenyl ketones obtained at various borate concentrations. As can be seen in this figure, increasing the borate concentration of the running electrolyte have resulted in longer analysis time, i.e., wider migration time window. The rationale of being able to change the migration time window is well illustrated in Fig. 2, whereby the solutes can be completely resolved in less than 15 min using a running electrolyte with borate concentration as low as 100 mM at pH 7.0.

Dependence of the Magnitude of the Migration Time Window on pH.

The effect of the pH of the running electrolyte on the magnitude of the migration time window is shown in Fig. 3a. Increasing the pH of the running electrolyte between pH 3.5 and 6.5 is accompanied by an increase in EOF and electrophoretic mobility of the micelle (see Fig. 3b). The increase in the EOF with increasing pH is due to the ionization of the silanol groups on the inner capillary walls, while the increase in the electrophoretic mobility of the micelle is indicative of increasing the charge density of the micelle. At 400

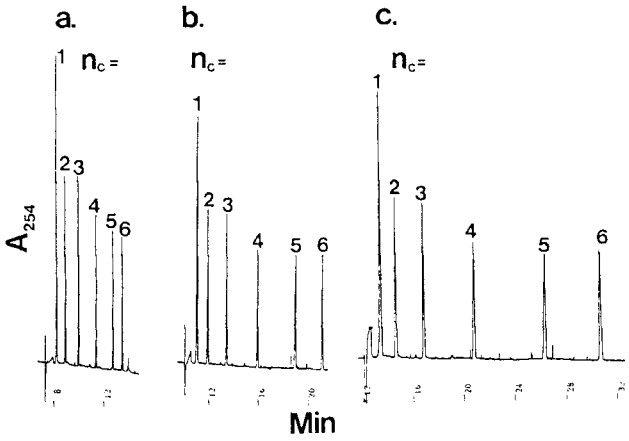


FIGURE 2. Typical electropherograms of alkyl phenyl ketones obtained at different sodium borate concentrations. Running electrolytes, 5.0 mM sodium phosphate containing 50.0 mM MEGA 9, pH 7.0, at various borate concentration: 100 mM (a), 600 mM (b) and 1000 mM (c). Solutes: 1, acetophenone; 2, propiophenone; 3, butyrophenone; 4, valerophenone; 5, hexanophenone; 6, heptanophenone. Other conditions as in Fig. 1.

mM borate in the running electrolyte, increasing the pH between 3.5 and 6.5 may have resulted in yielding higher amounts of complexing borate anions such as tetrahydoxyborate and cyclic trimer anions. This means that equilibria 1 and 2 shifted to the right as the pH was increased (high OH^- concentration).

On the other hand, at pH > 6.5 and using 400 mM boric acid in the running electrolyte, the EOF decreased with increasing pH of the medium. This decrease in the EOF may be attributed to a higher ionic strength at elevated pH as more borate ions are produced. In fact, Fig. 3b shows the sharp increase in the current at pH > 6.5, which is reflective of an increase in the ionic strength of the running electrolyte. Moreover, the electrophoretic mobility of the micelle decreased slightly or stayed the same at pH values greater than 6.5. This may reflect that the magnitude of the increase in the ionic strength is greater than that of the surface charge density of the micelle. It is likely that at 400 mM borate concentration, pH > 6.5, non complexing polyborates start to be produced in a

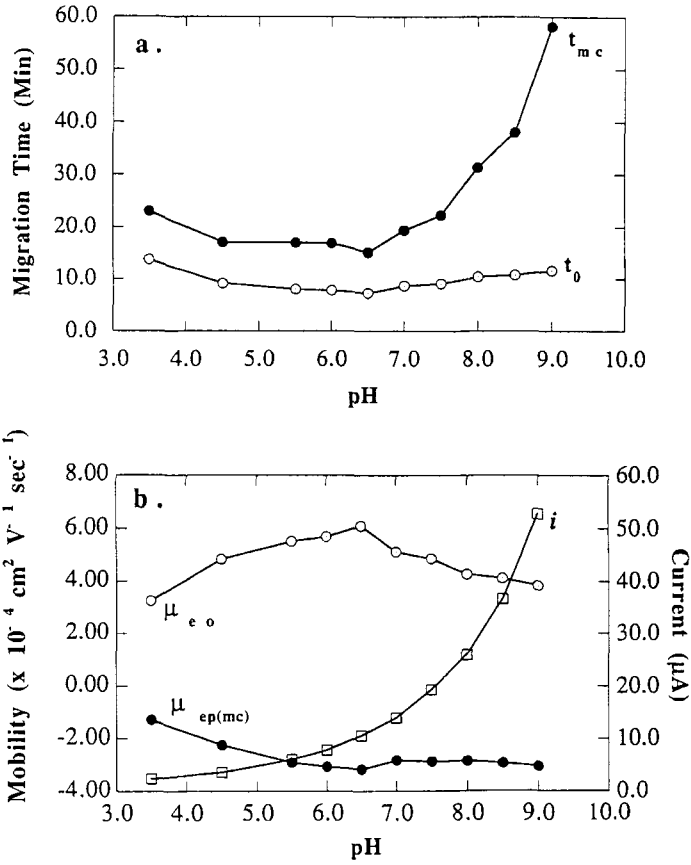


FIGURE 3. Effect of pH on the magnitude of the migration time window in (a) and the electrophoretic mobility of the micelle as well as the electroosmotic mobility and current in (b). Running electrolyte, 5.0 mM sodium phosphate containing 50.0 mM MEGA 9 and 400 mM borate at various pH values. Other conditions as in Fig. 1.

significant amount so that the increase in the ionic strength of the running electrolyte would outweigh the increase in the surface charge density of the micelle.

The increase in the breadth of the migration time window at pH values of 7.0 and above is the result of the decrease in the difference between the electroosmotic velocity and the electrophoretic velocity of the micelle (see eqn I). As can be seen in Fig. 3a, in the pH range 3.5-6.5 the migration time window exhibits almost constant breadth. This can be

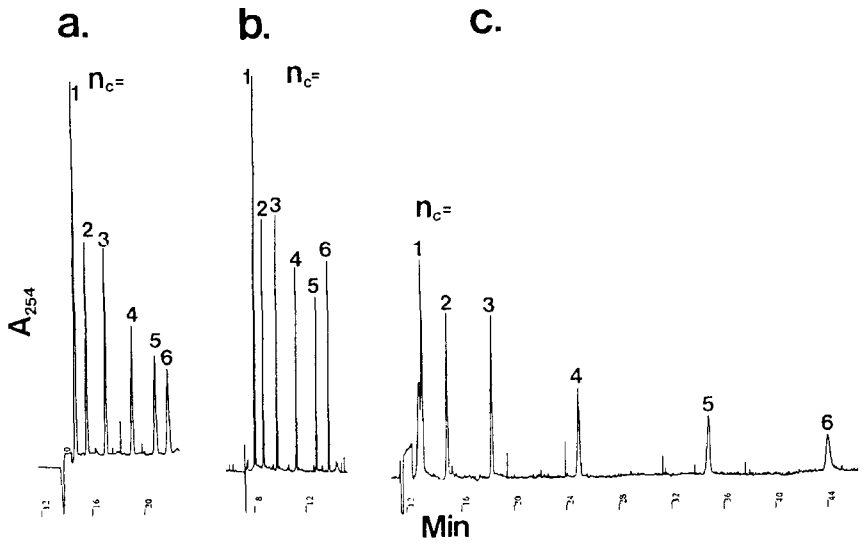


FIGURE 4. Typical electropherograms of alkyl phenyl ketones at various pH. Running electrolyte, 5.0 mM sodium phosphate containing 50.0 mM MEGA 9 and 400 mM borate, at pH 3.5 (a), 6.5 (b) and 9.0 (c). Solutes: 1, acetophenone; 2, propiophenone; 3, butyrophenone; 4, valerophenone; 5, hexanophenone; 6, heptanophenone. Other conditions as in Fig. 1.

attributed to the fact that both the EOF and the electrophoretic mobility of the micelle increased by almost the same factor.

Figure 4 shows representative electropherograms of alkyl phenyl ketones at various pH values. Using 400 mM borate even at a pH as low as 3.5, a negative charge can be introduced into the micelle and the separation of the various solutes can be achieved. As in the case of various borate concentration (see Fig. 2), the results obtained at various pH (see Fig. 4) show the advantage of being able to manipulate the migration time window. Using 400 mM borate at pH 6.5 as the running electrolyte seems to provide the fastest analysis time with excellent resolution among the various homologous solutes.

In summary, using relatively high borate concentration (e.g., 400 mM) in the running electrolyte, the migration time window and the analysis time of the *in situ* charged micellar system can be varied conveniently with changing pH. This represents a definitive

advantage over traditionally used micelles such as sodium dodecyl sulfate (SDS) which are characterized by a fixed surface charge density. In fact, with the MEGA-borate micelles, the EOF remains stronger in magnitude than the electrophoretic mobility of the *in situ* charged micelles, thus allowing the migration of the micelle toward the cathodic end over a wide pH range. This was not the case of the SDS micelle, where it was shown that the direction of the micelle was reversed as the pH was lowered to below pH 5.0 [19]. Under this condition, weakly or non partitioning solutes into the micellar system would be very retarded or would not elute.

Correlation Between Capacity Factor and Carbon Number, n_c, of Homologous Series.

The retention behavior of alkyl phenyl ketones, having alkyl chains with carbon number, n_c, from 1 to 6, was examined using a MEGA 9-borate micellar phase. Plots of the logarithmic capacity factors (log k') of the alkyl phenyl ketone homologous solutes versus n_c of the homologous series at different pH values and borate concentration yielded straight lines, and the linear regression data are listed in Tables 1 and 2. These data are in accordance with those reported previously with MEGA 9-borate micelle phase at pH ≥ 9 [2], and follow the linear expression

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

where the slope $\log\alpha$ is a measure of methylene group (CH_2) 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 homologous series. The data obtained at acidic and neutral pH values were in good agreement with those reported previously at pH ≥ 9 [2] to the extent that the slope and the intercept values are almost identical. This would suggest that the retention behavior of these micellar phases towards uncharged solutes at neutral and acidic pH conditions is similar to that obtained at alkaline conditions.

TABLE 1

Values of Slope, Intercept and R for the Correlation Between Capacity Factor, k', and Carbon Number in the Alkyl Chain of Alkyl Phenyl Ketones [$\log k' = (\log \alpha)\, n_c + \log \beta$] at Various pH Values[a].

pH	$\log \beta$	$\log \alpha$	R
3.5	-1.04	0.360	0.999
4.5	-1.03	0.367	0.999
5.5	-0.97	0.340	0.999
6.5	-0.97	0.330	0.999
7.5	-1.03	0.340	0.999
8.5	-1.09	0.350	0.999

[a][MEGA 9] = 50 mM; [Borate] = 400 mM; [Phosphate] = 5 mM; 15.0 kV.

TABLE 2

Values of Slope, Intercept and R for the Correlation Between Capacity Factor, k', and Carbon Number in the Alkyl Chain of Alkyl Phenyl Ketones [$\log k' = (\log \alpha)\, n_c + \log \beta$] at Various Borate Concentration[a].

[Borate], mM	$\log \beta$	$\log \alpha$	R
100	-0.92	0.34	0.999
200	-1.10	0.37	0.999
400	-1.02	0.33	0.999
600	-1.12	0.34	0.999
800	-1.10	0.34	0.999
1000	-1.15	0.34	0.999

[a] [MEGA 9] = 50 mM; pH 10.0; [Phosphate] = 5 mM; 15.0 kV.

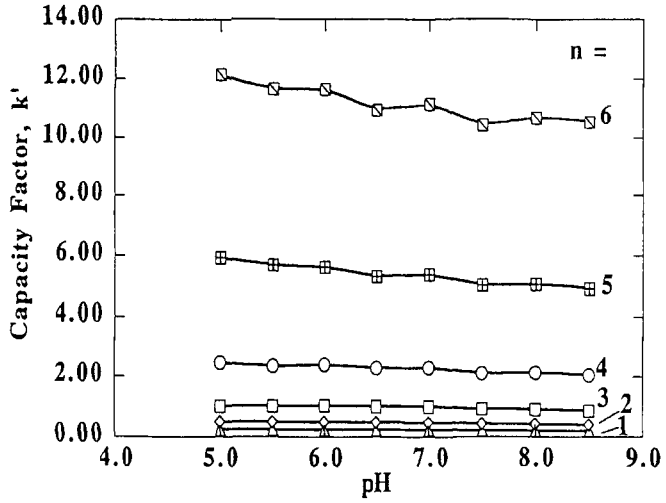


FIGURE 5. Plots of capacity factor k' vs. pH of the running electrolyte. solutes as in Fig. 2. Other conditions as in Fig. 1.

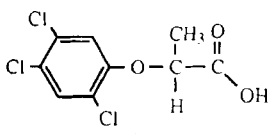
Dependence of Capacity Factor on pH.

Figure 5 shows the dependence of the capacity factor on pH. As expected, the value of the capacity factor remained more or less constant over the pH domain studied. These results agree with those observed at higher pH domain [2]. The capacity factors of the first four solutes of the homologous series were practically unaffected by pH, while the k' of the other two solutes (n_c = 5 and 6) showed slight decrease and some fluctuations as the pH was changed. The slight fluctuations are within the range of experimental errors while the decrease in retention may be attributed to increasing Joule heating as a result of increasing the current with pH.

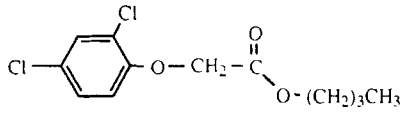
Illustrative Applications

Phenoxy Acid Herbicides and their Esters. An advantage of the MEGA-borate micellar phase at low pH resides in its ability to separate acidic herbicides and their esters. The separation of ester herbicides employing MEGA-borate micellar phases at alkaline pH

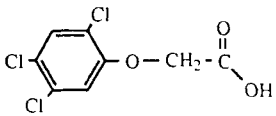
values was not successful due to the fact that esters hydrolyze readily in alkaline media (results not shown). Figure 6 illustrates the separation of a mixture of five herbicides (see below for structures) consisting of two acidic herbicides (silvex and 2,4,5-T) and three ester herbicides namely 2,4-D isopropyl ester, 2,4-D butyl ester and 2,4,5-T isopropyl ester.



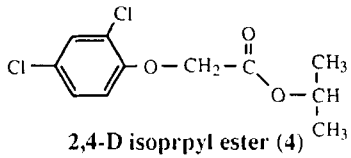
Silvex (1)



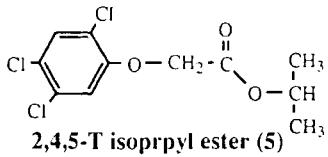
2,4-D butyl ester (3)



2,4,5-T (2)



2,4-D isoprpyl ester (4)



2,4,5-T isoprpyl ester (5)

The separation was carried out using 200 mM sodium borate containing 50 mM MEGA 9 and 5.0 mM sodium phosphate, pH 7.0. Under these separation conditions, silvex and 2,4,5-T are negatively charged and as a result they associate very weakly with the negatively charged MEGA-borate micellar phase, thus migrating mostly by their own electrophoretic mobility faster than the other three analytes. The ester herbicides are neutral and therefore their elution order reflects their relative hydrophobicity. The separation is completed in less than 15 min with an average plate count of 325 000 plates/m.

Urea Herbicides. Fig. 7a and b illustrates the separation of nine urea herbicides (for structures, see below) using MEGA 10-borate micellar phases at pH 5.0 and 7.0, respectively. With the exception of terbacil, the elution order of the components of the standard mixture is the same as that reported in an earlier study [2] using the same micellar phase but at pH 10.0. At pH 5.0 and 7.0, terbacil eluted first while at pH 10.0 it migrated

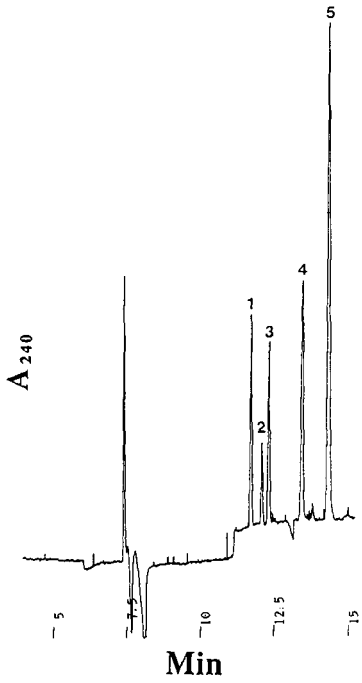
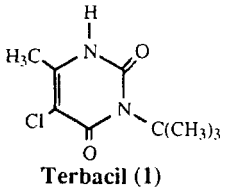
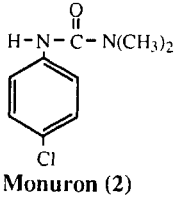


FIGURE 6. Electropherogram of phenoxy acid herbicides and their esters. Running electrolyte, 5.0 mM sodium phosphate containing 50.0 mM MEGA 9 and 400 mM borate, pH 7.0. Solutes: 1, silvex; 2, 2,4,5-T; 3, 2,4-D butyl ester; 4, 2,4-D isopropyl ester; 5, 2,4,5-T isopropyl ester. Other conditions as in Fig. 1.

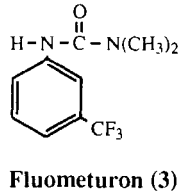
slower than monuron and fluometuron [2]. This illustrates the advantages of being able to utilize a given *in situ* charged micellar phase over a wide range of pH, as far as changing the selectivity of the MECC system is concerned. The average plate counts per meter were 224 000 and 189 000 at pH 5.0 and 6.0, respectively.



Terbacil (1)



Monuron (2)



Fluometuron (3)

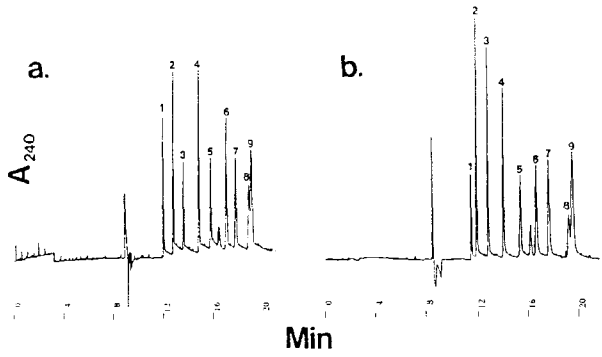
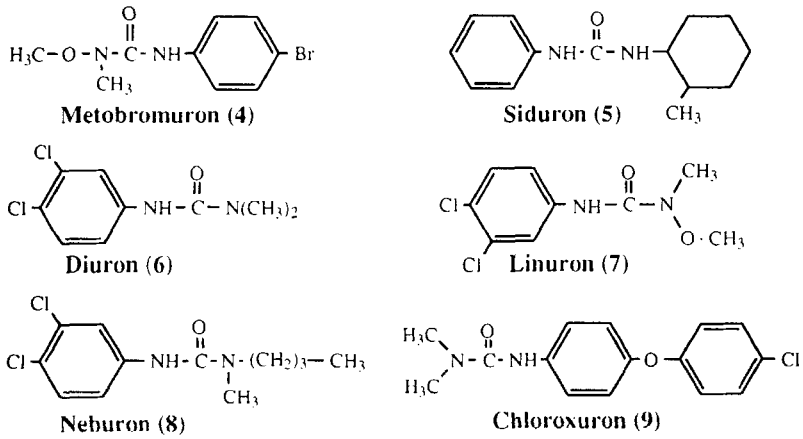


FIGURE 7. Electropherograms of urea herbicides. Running electrolytes, 5.0 mM sodium phosphate containing 50.0 mM MEGA 10 and 400 mM borate, pH 5.0, in (a) or 200 mM borate, pH 7.0 in (b). Solutes: 1, terbacil; 2, monuron; 3, fluometuron; 4, metobromuron; 5, siduron; 6, diuron; 7, linuron; 8, neburon; 9, chloroxuron. Other conditions as in Fig. 1.



Dansyl Amino Acids. Figure 8 illustrates the electropherogram of 15 dansyl amino acids using MEGA 10-borate micellar phase at pH 7.0. As can be seen in this figure, the system provided a baseline separation of 13 dansyl amino acids. The order of migration of the different analytes was the same as that observed in an earlier study using the same

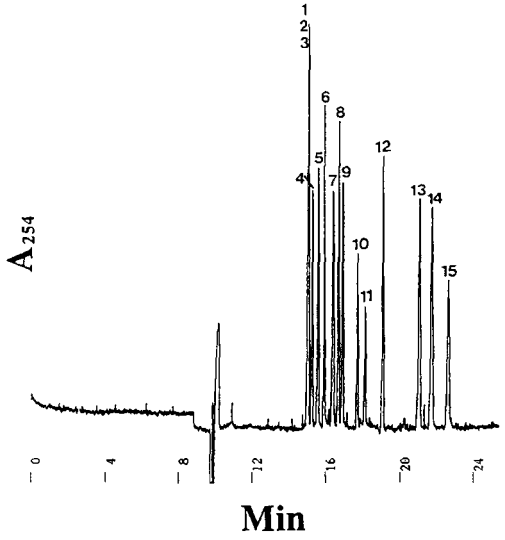


FIGURE 8. Electropherogram of dansyl amino acids. Running electrolyte, 400 mM borate containing 100 mM MEGA 10, pH 7.0. Solutes: 1, glutamine; 2, asparagine; 3, threonine; 4, serine; 5, valine; 6, methionine; 7, glycine; 8, isoleucine; 9, leucine; 10, arginine; 11, phenylalanine; 12, tryptophan; 13, glutamic acid; 14, aspartic acid; 15, cysteic acid. Other conditions as in Fig. 1.

micellar phase but at pH 10.0 [2]. Although, dansylated glutamine, asparagine and threonine co-migrated at pH 7.0, the resolution among the other thirteen dansyl amino acids is much better than that obtained at pH 10.0 [2]. The difference between the two separations may be attributed to differences in the degree of ionization of the solutes at pH 7.0 and 10.0. The separation was achieved in less than 24 minutes with an average plate count of 332 000 plates/meter.

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REFERENCES

[1] Y. Mechref and Z. El Rassi, *J. Chromatogr. A*, in press (1995)

[2] J. T. Smith, W. Nashabeh and Z. El Rassi, *Anal. Chem.*, 66: 1119 (1994)

[3] J. T. Smith and Z. El Rassi, *J. Microsep. Sep.*, 6: 127 (1994)

[4] J. T. Smith and Z. El Rassi, *Electrophoresis*, 15: 1248 (1994)

[5] J. T. Smith and Z. El Rassi, *J. Chromatogr. A*, 685: 131 (1994)

[6] J. T. Smith and Z. El Rassi, *J. Cap. Elec.*, 1: 136 (1994)

[7] J. Cai and Z. El Rassi, *J. Chromatogr.*, 608: 31 (1992)

[8] H. B. Davis and C. J. B. Mott, *J. Chem. Soc. Faraday I*, 76: 1991 (1980)

[9] Z. El Rassi, *Adv.. Chromatogr.*, 34: 177 (1994)

[10] Z. El Rassi and W. Nashabeh, in Z. El Rassi (Z. El Rassis), *Carbohydrate Analysis: High Performance Liquid Chromatography and Capillary Electrophoresis*, Elsevier, Amsterdam, 1995, 267.

[11] S. Hoffstetter-Kuh, A. Paulus, E. Gassmann and H. M. Widmer, *Anal. Chem.*, 63: 1541 (1991)

[12] Y. Mechref, G. K. Ostrander and Z. El Rassi, *J. Chromatogr. A*, 695: 83 (1995)

[13] N. Ingri, *Acta Chem. Scand.*, 16: 439 (1962)

[14] N. Ingri, *Acta Chem. Scand.*, 17: 581 (1963)

[15] J. L. Anderson, E. M. Eyring and M. P. Whittaker, *J. Phys. Chem.*, 68: 1128 (1964)

[16] R. K. Momii and N. H. Nachtrieb, *Inorg. Chem.*, 6: 1189 (1967)

[17] N. Ingri, *Sv. Kem. Tidskr.*, 75: 199 (1963)

[18] C. C. Christ, J. R. Clark and H. T. Evans, *Acta Cryst.*, 11: 761 (1958)

[19] K. Otsuka and S. Terabe, *J. Microcol. Sep.*, 1: 150(1989)

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