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Micellar electrokinetic capillary chromatography of neutral solutes with micelles of adjustable surface charge density

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ABSTRACT

Novel micelles with adjustable surface charge density were introduced for micellar electrokinetic capillary chromatography. These micelles are based on the complexation between octylglucoside surfactant and alkaline borate. The surface charge density of the octylglucoside–borate micelles can be conveniently varied by changing the operating parameters such as borate concentration and/or pH of the running electrolyte. This feature permitted the tuning of the elution range, a parameter that largely influences the peak capacity and resolution in micellar electrokinetic capillary chromatography. Furthermore, with its balanced hydrophile–lipophile character, the octylglucoside–borate micellar system allowed the separation of hydrophobic species including herbicides, *e.g.*, prometon, prometryne, propazine and butachlor, and some polyaromatic hydrocarbons. High separation efficiencies were obtained over a wide range of elution conditions, and consequently the detection limit for the herbicides was in the range of 18–52 fmol using UV detection.

INTRODUCTION

Micellar electrokinetic capillary chromatography (MECC), first reported in 1984 by Terabe *et al.* [1], is increasingly used for the separation of neutral and charged species [1–7]. In MECC, the separation medium consists of an electrolyte containing an ionic surfactant in an amount above its critical micellar concentration. Thus, there are two phases inside the capillary tube, an aqueous mobile phase and a micellar pseudo-stationary phase. Whereas the aqueous mobile phase moves at the velocity of electroosmosis, the micelles migrate much slower due to opposing electrophoretic forces. This creates 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}. Neutral solutes are eluted within the retention window and are separated through their differential distribution between the two phases.

Thus far, most MECC applications have utilized aqueous sodium dodecyl sulphate (SDS) as the micellar phase. Although other surfactants can be used, their potentials have been briefly explored [3,7–10]. This may be due to the fact that these ionic surfactants (*e.g.*, cetyltrimethylammonium bromide, sodium tetradecyl sulphate, etc.) showed little or no improvements over SDS as far as the quality of separation is concerned. Due to the pronounced unbalance in the hydrophile–lipophile character of SDS and similar ionic surfactants, hydrophobic solutes of low water solubility are almost totally incorporated in such micelles and are not separated. Few attempts have been made to alleviate this problem. In one approach, a cyclodextrin (CD)-modified SDS micellar phase was introduced for the separation of hydrophobic compounds [11]. In this system, the water–insoluble compound is partitioned between the CD cavity, which is moving at the velocity of the aqueous phase,

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and the interior of the SDS micelle which is migrating at a slower velocity. Therefore, a more equitable distribution of the solutes can be obtained and consequently improved separation. In another approach, bile salt surfactants in the presence of relatively high methanol content were described for the separation of hydrophobic compounds including polyaromatic hydrocarbons [12].

MECC is characterized by an elution range or retention window that strongly influences peak capacity and resolution. With SDS and other ionic surfactants that have been evaluated thus far [3,7–9], the elution range is rather predetermined and can not be varied systematically. The retention window of MECC can be somewhat elongated by using surfactants having shorter alkyl chains (*e.g.*, sodium decyl sulphate) [13], but its width is still predetermined. Another approach through which the breadth of the retention window can be enlarged has been the surface modification of fused-silica capillaries [13–15]. Under these conditions, both t_{mc} and t_0 increased, which yielded longer analysis time (see Theory section). The only approach through which the retention window can be systematically manipulated seems to be the inclusion of methanol in the micellar system [16,17]. Under these circumstances, however, both t_{mc} and t_0 increased. Such method led to long analysis time, and because methanol induced polydispersity in the micelles, the separation efficiency was significantly reduced.

This paper is concerned with the investigation of the potentials of micelles of adjustable surface charge density. The new micelles are based on the complexation between octylglucoside surfactant and alkaline borate. These micelles provided several advantages over traditionally used surfactants. First, octylglucoside has a relatively short non-polar chain and a large polar head moiety. This balance in the hydrophile–lipophile character of the octylglucoside surfactant is advantageous for the separations of both polar and highly non-polar species. Furthermore, with the octylglucoside–borate micelles, the surface charge density can be varied conveniently by changing the borate concentration and/or the pH of the running electrolyte, and consequently the retention window of the micellar system can be varied systematically over a wider range. These readily tuned features provided a means to manipulate the separation efficiencies, peak capacity and resolution. Other alkylglucoside surfactants are being investigated in our laboratory with a broader range of neutral and charged species. These studies are planned for future papers.

THEORY

Many of the fundamental characteristics of MECC are well understood and have been described by Terabe and co-workers [1,2]. In MECC, retention and resolution are related to the electrokinetic velocities of the aqueous phase (*i.e.*, the electroosmotic velocity) 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} [14,18]:

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

$$= -\frac{\varepsilon E}{\eta}\left[\zeta_c - \frac{2\zeta_{mc}}{3} f(\kappa a)\right] \quad (1)$$

where ζ_c and ζ_{mc} are the ζ potentials of the inner surface of the capillary and of the outer surface of the micelle, respectively, ε and η are the dielectric constant and the viscosity of the electrolyte, respectively, $f(\kappa a)$ depends on the shape of the micelle [18], a is the radius of the micelle, κ is the familiar Debye–Hückel constant and E is the electric field strength. The value of $f(\kappa a)$ varies between 1.0 and 1.50 depending on the dimensions of κa. The negative sign in eqn. 1 is to indicate that when the ζ potential of the capillary is negative the electroosmotic flow is toward the negative electrode [18].

With negatively charged micelles and untreated fused-silica capillaries ζ_{mc} is smaller than ζ_c and both have the same sign [1,2]. Neutral solutes are eluted between t_0 and t_{mc}, which are the retention times of unsolubilized and completely solubilized solute by the micelle, respectively. This is referred to as the elution range or the retention window.

An important variable in MECC is the elution range parameter defined by the ratio [14]:

$$\frac{t_0}{t_{mc}} = \frac{v_{mc}}{v_{eo}} = 1 - \frac{2\zeta_{mc}}{3\zeta_c} f(\kappa a) \quad (2)$$

The ζ potentials can be expressed by the following relationship [19]

$$\zeta = \frac{4\pi\delta\rho}{\varepsilon} \quad (3)$$

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 theory equates δ to $1/\kappa$. Thus, by a rearrangement of eqn. 3

$$\zeta = \frac{4\pi\rho}{\kappa\varepsilon} \propto \frac{1}{\sqrt{I}} \quad (4)$$

It follows then, from eqns. 1 and 4, that the electroosmotic flow of the aqueous phase and the electrophoretic velocity of the micelle will be inversely proportional to the square root of the ionic strength, I.

As the elution range parameter decreases the retention window increases. An elution range parameter of 1 means that the micelle is uncharged and all neutral solutes coelute and migrate at the velocity of the electroosmotic flow. An elution range parameter of zero means an infinite retention window. This corresponds to a situation where the electrophoretic velocity of the micelle is of the same magnitude and opposite in direction to the electroosmotic flow. Since ζ_c and ζ_{mc} are directly proportional to the surface charge density (or the amount of charge per unit surface area, see eqn. 3) of the capillary, ρ_c, and that of the micelle, ρ_{mc}, respectively, the elution range parameter can be varied conveniently by changing the charge density of the micelles and/or that of the capillary inner surface (see eqn. 2). One of the characteristics of the new micellar system under investigation is that while the surface charge density of the capillary can be kept almost constant, the surface charge density of the micelle can be readily adjusted through several operational parameters, see below.

The adjustment of the surface charge density of the surfactant under investigation, and consequently the elution range parameter is based on varying the extent of complexation between the octylglucoside surfactant and borate ions. Fig. 1 is a schematic illustration of the novel MECC system developed and evaluated in this work. It shows the mechanism of retention of neutral solutes and the control of the surface charge density of the micelle through complexation with borate.

It has been known for a long time that polyhydroxy compounds can reversibly form cyclic boronate esters with borate ions in alkaline pH, and the formation of these complexes is dependent on pH, ionic strength, temperature and the nature of the hydroxylated compound [20–22]. Octylglucoside, which is a non-ionic sugar-containing surfactant, can acquire a negative charge upon complexing with borate ions. The following reaction scheme illustrates the complexation of borate across the C-4 and C-6 of the glucose moiety [23] of octylglucoside surfactant:

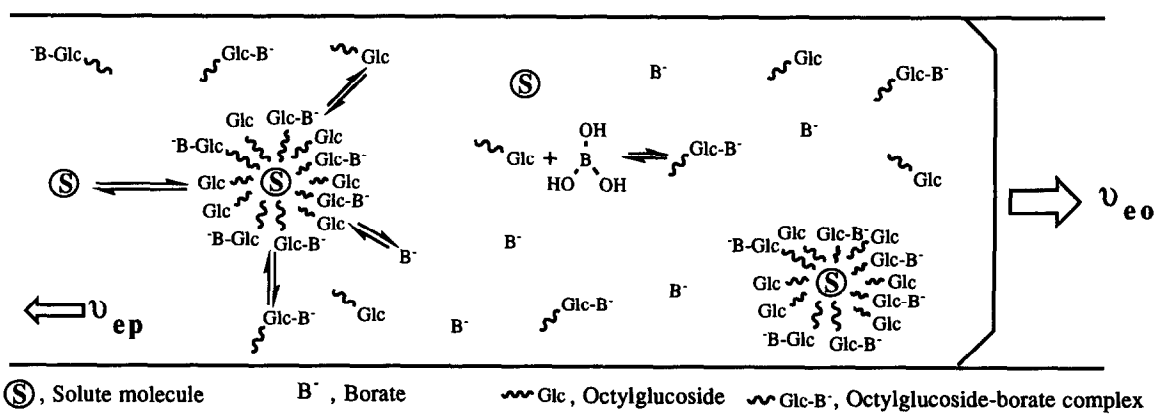
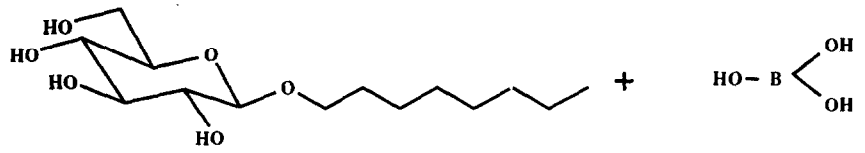
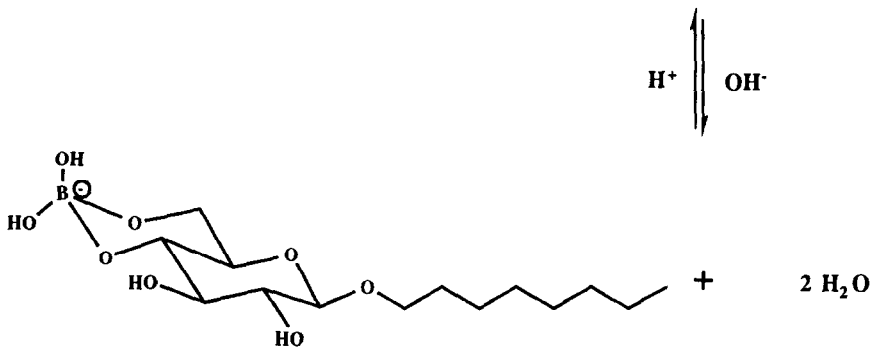


Fig. 1. Schematic illustration of the separation principle in MECC with octylglucoside–borate micellar system.



Octylglucoside



Octylglucoside-borate complex

The alkylglucoside–borate complexation is a reversible reaction, and has an equilibrium constant, K_{eq}, given by

$$K_{eq} = \frac{[\text{OG-Borate}]}{[\text{OG}][\text{Borate}][\text{OH}^-]} \quad (5)$$

where [OG] and [OG-Borate] stand for the total concentrations of the uncomplexed octylglucoside surfactant and octylglucoside–borate surfactant, respectively, and [Borate] and [OH^-] are the borate and hydroxide ions concentrations, respectively. Presumably, it is the negatively charged OG-Borate which migrate in zone electrophoresis. The concentration of OG-Borate in aqueous boric acid is low, and an increase in pH would be expected to raise their concentration and, concomitantly, to result in an increased electrophoretic mobility of the micelle.

As a result of the complexation, the overall surface charge density of the micelle, ρ_{mc}, can be expressed as

$$\rho_{mc} = \frac{[\text{OG-Borate}]}{[\text{OG-Borate}] + [\text{OG}]} \rho_{mc\text{-}c} = \frac{\rho_{mc\text{-}c}}{1 + \frac{[\text{OG}]}{[\text{OG-Borate}]}} \quad (6)$$

where $\rho_{mc\text{-}c}$ is the limiting charge density of the octylglucoside–borate micelle. The higher the charge density the more negative the micelle. There are several operational parameters that can alter ρ_{mc}. These are the concentrations of the surfactant and borate, and pH of the running electrolyte. According to eqn. 5, at constant surfactant concentration, any increase in the borate concentration or pH will result in a decrease in the ratio [OG]/[OG-Borate], and therefore a larger ρ_{mc} (see eqn. 6). At constant pH and borate concentration, an increase in the surfactant concentration will yield an increase in the ratio [OG]/[OG-Borate], and as a result, ρ_{mc} will decrease (see eqns. 5 and 6). According to eqn. 2 these readily tuned features of the micelles would allow the tailoring of the elution range for a given separation problem.

As mentioned above, with alkylglucoside surfactants in alkaline borate, the surface charge density of the micelle can be conveniently manipulated through pH, borate concentration or surfactant concentration without drastically affecting the surface charge density of the fused-silica capillary. In fact, since at alkaline pH (*i.e.*, above pH 8.0) the surface silanols are fully ionized, ρ_c will remain constant. However, increasing the pH or borate

concentration is accompanied by an increase in the ionic strength of the electrolyte, which will decrease the thickness of the electric double layer near the capillary surface, δ_c, and that near the surface of the micelle, δ_{mc}. Furthermore, increasing the surfactant concentration will increase the viscosity of the medium and the amount of surfactant accumulated on the capillary wall. Since ρ_c remains constant, the changes in the ionic strength and viscosity of the medium as well as the variation in the amount of surfactant adsorbed on the capillary wall will be accompanied by some changes in the electroosmotic flow. On the other hand, the magnitude of ρ_{mc} will be largely affected by the operation conditions, which in addition to changes in δ_{mc} and the viscosity of the medium will cause the electrophoretic velocity of the micelle to change considerably. Under these conditions, while the electroosmotic flow velocity will vary over a narrow range, the electrophoretic velocity of the micelle will change over a wider range. According to the following equation (where l is the separation distance):

$$t_{mc} = \frac{l}{v_{eo} + v_{ep}} \tag{7}$$

these processes will lead to large changes in t_{mc}. This is particularly important in manipulating the retention window and the separation behavior of MECC. Stating it differently, in alkaline borate while ζ_c of the capillary will undergo small changes, ζ_{mc} of the micelle will be affected to a much larger extent through borate and surfactant concentrations and the pH of the running electrolyte. According to eqn. 2, this corresponds to tailoring the breadth of the retention window and consequently the magnitude of peak capacity and resolution.

In fact, in MECC both peak capacity, n, and resolution, R_s, are influenced, among other things, by the retention window through the following equations [2]:

$$n = 1 + \frac{\sqrt{N}}{4} \ln \frac{t_{mc}}{t_0} \tag{8}$$

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'_2}{k'_2 + 1} \cdot \frac{1 - \frac{t_0}{t_{mc}}}{1 + \frac{t_0}{t_{mc}} \cdot k'_1} \tag{9}$$

where N is the number of theoretical plates, α is the selectivity factor and k' is the retention factor which is calculated by the following equation [2]:

$$k' = \frac{t_r - t_0}{t_0\left(1 - \frac{t_r}{t_{mc}}\right)} \tag{10}$$

where t_r is the retention time of the solute. For two adjacent peaks, *i.e.*, $k'_1 = k'_2 = k'$, a convenient approximation to eqn. 9 is

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} f(k') \tag{11}$$

where

$$f(k') = \frac{k'}{k' + 1} \cdot \frac{1 - \frac{t_0}{t_{mc}}}{1 + \frac{t_0}{t_{mc}} \cdot k'} \tag{12}$$

For a given surfactant and with neutral solutes, α is virtually independent of k' [6,24,25] (*i.e.*, the surfactant concentration), while N can be approximated as constant since it is slightly dependent on k' in the useful range of surfactant concentration [26–29]. From eqn. 7, it follows that peak capacity, n, is governed by the ratio t_{mc}/t_0, and from eqn. 12, it is clear that resolution is controlled by the retention term, $f(k')$, which encompasses the elution range parameter, t_0/t_{mc}.

A particular feature of the surfactant under consideration is that the elution range can be manipulated through t_{mc} while keeping k' constant. This is readily achieved by varying the pH or the borate concentration at fixed surfactant concentration. In these situations, the peak capacity n, which is another measure of the efficacy of the system [30], can be tailored to accommodate a given separation problem (see later for more details).

It has been shown by Terabe *et al.* [2] that when $f(k')$ is evaluated as a function of k', bell-shapped curves are obtained, each one is valid for a particular value of t_0/t_{mc}. By differentiating eqn. 12 with respect to k' and setting the resulting expression equal to zero, the optimum k' (*i.e.*, optimum surfactant concentration) value for maximum resolution is given by [31,32]

$$k'_{opt} = (t_{mc}/t_0)^{\frac{1}{2}} \tag{13}$$

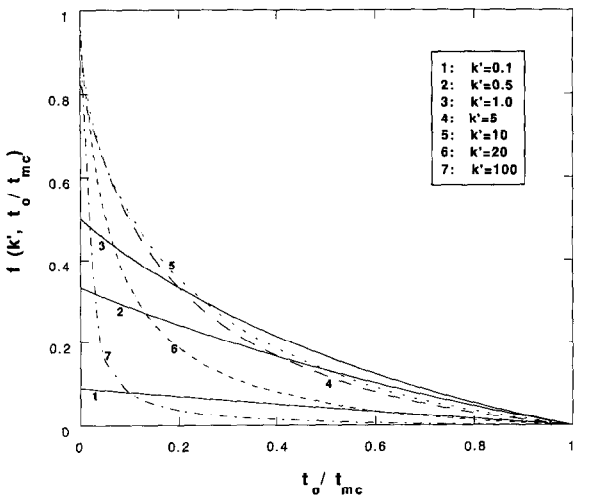


Fig. 2. Plots of $f(k', t_0/t_{mc})$ *versus* t_0/t_{mc} at various k' values.

The retention factor, k', of neutral solutes is determined by the concentration of the surfactant and is independent of the pH. With the traditionally used surfactants t_{mc}/t_0 is largely independent of the pH and the surfactant concentration [2,14]. This means that the retention window is predetermined and cannot be varied systematically. According to eqn. 13, this limits the MECC system to a narrow k' range as far as resolution is concerned.

As mentioned above, the optimum value of $f(k')$ for maximum resolution is influenced by the ratio t_0/t_{mc}. On the other hand, for a given value of k', the higher the retention window (*i.e.*, the smaller the ratio t_0/t_{mc}), the larger the value for the function $f(k')$ will be, and the more satisfactory the resolution. The dependence of $f(k')$ on the ratio t_0/t_{mc} for various values of k' is depicted in Fig. 2. This figure shows that at low values of the retention window (*i.e.*, at high t_0/t_{mc}), a pair of solutes for which k' is in the range 0.5–1.0 has a higher $f(k')$ value (*i.e.*, a better resolution), than those pairs that are 10 times more retained, *e.g.*, $k' = 5.0$–10. Early eluting peaks as well as strongly retained ones are better resolved at relatively high retention window. It takes an infinite retention window (*i.e.*, $t_0/t_{mc} \rightarrow 0$) to affect a good resolution for species that are almost completely dissolved by the micelle.

The above discussion underlines the need for surfactants that are less retentive than the existing ones. This article addresses this need by introducing surfactants with balanced hydrophile–lipophile character, the alkylglucoside–borate surfactants. In addition, with the new micellar systems the retention window can be increased by increasing t_{mc} while keeping k' constant. This is readily achieved by increasing the pH or the borate concentration at fixed surfactant concentration. This corresponds to moving up along the bell-shaped curve (*i.e.*, $f(k')$ *vs.* k') and so increasing the contribution of $f(k')$ to resolution. Thus, with the micellar systems under consideration, it is possible to affect simultaneously a double optimization of resolution through k' and t_{mc}. With SDS and other ionic surfactants, window optimization is most often achieved through an increase in both t_0 and t_{mc} by adding an organic solvent to the running electrolyte [17]. But increasing the organic modifier lead to a drastic increase in the analysis time. In addition, SDS micellar system can not tolerate a large amount of organic solvent without disrupting the micelle shape and producing polydispersity that leads to band broadening [28].

EXPERIMENTAL

Instrument and capillaries

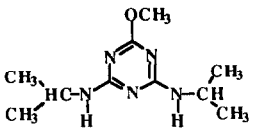
The capillary electrophoresis instrument used in this study is the same as that described previously [33]. It consisted of a 30-kV d.c. power supply Model EH30P03 of positive polarity from Glassman High Voltage (Whitehouse Station, NJ, USA) and a UV–Vis variable-wavelength detector Model 200 from Linear Instrument (Reno, NV, USA) equipped with a cell for on-column detection. The detection wavelength was set at 210 nm. In all the experiments the running voltage was 15 kV. The electropherograms were recorded with a computing integrator Model CR601 from Shimadzu (Columbia MD, USA).

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). In all experiments, the total length of the capillary was 80 cm with 50 cm separation distance, *i.e.*, from the injection end to the detection point.

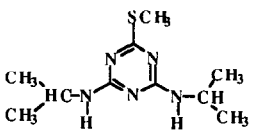
Reagents and materials

Octyl-β-D-glucopyranoside (OG) was obtained from Sigma (St. Louis, MO, USA). Triphenylmeth-

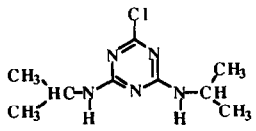
anol, *o*-terphenyl and four herbicides, *i.e.*, prometon, prometryne, butachlor and propazine, were purchased from Chem Service (West Chester, PA, USA). The structures of the four herbicides are shown below:



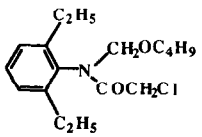
Prometon



Prometryne



Propazine



Butachlor

Sudan III, which was used for the determination of the migration time of the micelles, t_{mc}, was obtained from Aldrich (Milwaukee, WI, USA). All chemicals for the preparation of electrolyte were from Fisher Scientific (Pittsburgh, PA, USA). Methanol was purchased from EM Science (Cherry Hill, NJ, USA). Naphthylamine and naphthalene were from Eastman Kodak (Rochester, NY, USA). All solutions were prepared with deionized water and filtered with 0.2-μm Uniprep Syringeless filters from Fisher Scientific to avoid capillary plugging.

Procedures

The running electrolyte was prepared by dissolving proper amount of boric acid and octylglucoside, and adjusting the pH to the desired value with sodium hydroxide. Sample solutions were made by dissolving pure compounds in the running electrolyte (*i.e.*, micellar solution). Due to the limited solubility of the herbicides in aqueous solvent, the concentrations of the sample solutions were determined form calibration curves that were established with standard solutions prepared by dissolving the pure compounds in water–acetonitrile solvents. The calibration curves were obtained by capillary zone electrophoresis using 10 m*M* phosphate buffer, pH 6.0.

Hydrodynamic sample injection mode, *i.e.*, gravity-driven flow, was used in this study. The sample reservoir was raised to a height of 20 cm above the outlet reservoir for a certain period of time. The following equation was used for the determination of the injected quantities, Q:

$$Q = \frac{\pi r^2 l C t_i}{t_r} \quad (14)$$

where t_i is the injection time, r is the inner radius of the capillary, C is the concentration of the sample, l is the length of the capillary from the injection point to the detection point and t_r is the time it takes for the sample zone to migrate from the injection end to the detection point under the gravity force.

RESULTS AND DISCUSSION

The novel micellar system was characterized with neutral solutes over a wide range of elution conditions including pH of the running electrolyte, borate concentration and surfactant concentration. Various electrokinetic parameters were measured, and the results are discussed in light of the theoretical treatment given above.

Tunable retention window

As demonstrated in the theory section, the reten-

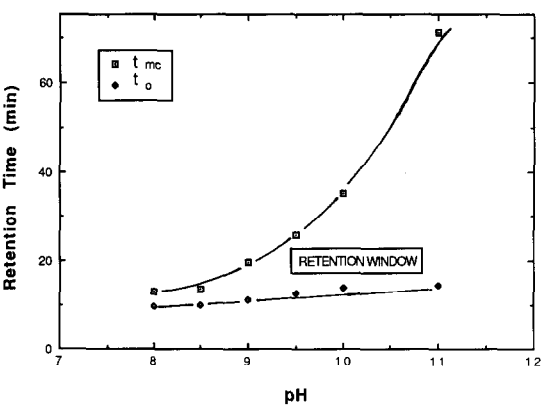


Fig. 3. Effect of pH on the magnitude of retention window. Separation capillary, untreated fused-silica, 50 cm (to the detection point), 80 cm (total length) × 50 μm I.D.; running electrolytes, 50 m*M* octylglucoside, 400 m*M* borate at various pH; sample injection, hydrodynamic, 5 s; running voltage, 15 kV; tracers, Sudan III (for t_{mc}) and methanol (for t_0); detection, 210 nm.

tion window of the micellar system depends on borate concentration, pH of the running electrolyte and the concentration of the surfactant.

pH of the running electrolyte. To evaluate the relationship between retention window and the pH of the running electrolyte, the electrophoretic experiments were carried out with electrolyte solutions containing 50 m*M* OG and 400 m*M* borate at various pH. Fig. 3 portrays the results in terms of t_0 and t_{mc} *versus* the pH of the running electrolyte. As can be seen in Fig. 3, t_{mc} increased much more than did t_0 over the pH range studied. The larger increase in t_{mc} arises primarily from increasing the extent of complexation of the surfactant with borate at higher pH. Stating it differently, as the pH rises the surface charge density of the micelle increases and consequently the electrophoretic velocity of the micelle in the opposite direction to the electroosmotic flow increases (see Theory section). The slight increase in t_0 or in another word the shallow decrease in the electroosmotic flow is primarily due to increasing the ionic strength with pH, since higher pH values were obtained by adding larger amount of sodium hydroxide to the solution of boric acid. At pH above 8, the charge density of the capillary inner surface is virtually constant since the silanol groups of the siliceous wall are fully ionized in the pH domain investigated. However, as the ionic strength of the running electrolyte increases with increasing pH, the viscosity of the medium would increase and the ζ potential of the capillary wall would decrease. Consequently, the electroosmotic flow is reduced since it is directly proportional to the ζ potential and inversely proportional to viscosity, see eqns. 1, 3 and 4. On the other hand, the decrease in the thickness of the double layer (*i.e.*, the ion atmosphere) surrounding the micelle, δ_{mc}, with increasing pH is outweighed by the larger increase in its surface charge density, ρ_{mc}; reason for which t_{mc} increases. An additional factor that would also contribute to the increase in t_{mc}, is the fact that the electroosmotic flow decreased. According to eqn. 7, the slight decrease in the electroosmotic velocity can result in a dramatic increase in the t_{mc} as the magnitudes of v_{eo} and v_{ep} approach the same value because they are of opposite sign. This phenomenon can explain the continuous and steep rising in t_{mc} at pH values higher than 10. In fact, the electrophoretic mobility of glucose in alkaline borate has been found to level off at pH above 9 in paper-free zone electrophoresis [34], a pH which is above the pK_a value of borate (pK_a = 9.23).

Fig. 4 displays typical electropherograms of three triazine herbicides (*i.e.*, prometon, prometryne and propazine) and butachlor, an acetamide herbicide, obtained at three different pH, *i.e.*, three different retention windows. This figure shows the significance of being able to systematically vary the

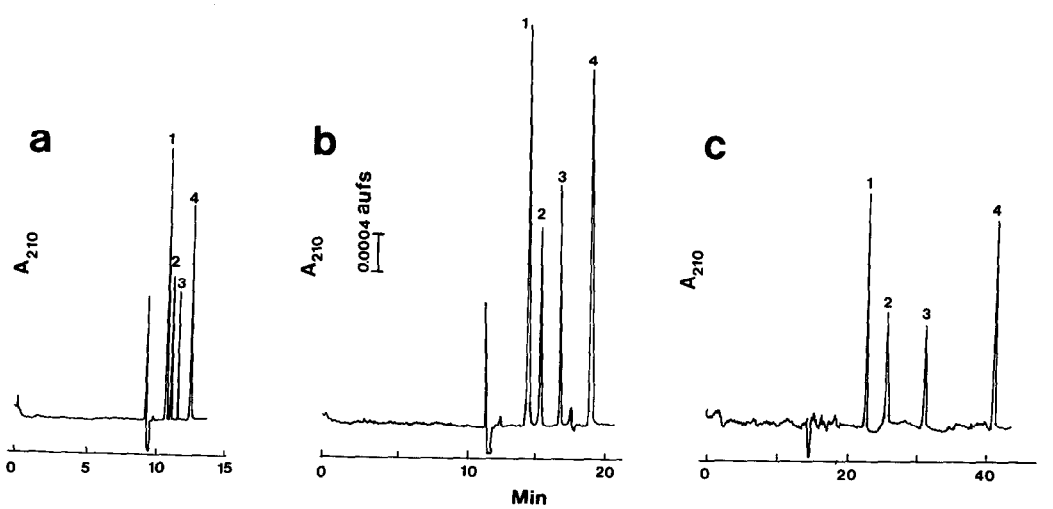


Fig. 4. Typical electropherograms of herbicides at various pH: (a) pH 8; (b) pH 9; (c) pH 10. Electrolytes, 400 m*M* borate containing 50 m*M* OG. Peaks: 1 = prometon; 2 = prometryne; 3 = propazine; 4 = butachlor. Other experimental conditions as in Fig. 3.

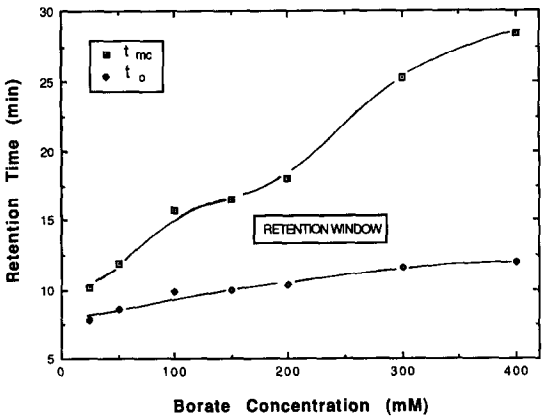


Fig. 5. Effect of borate concentration on the magnitude of the retention window. Running electrolytes, 50 m*M* octylglucoside, pH 10, at various borate concentrations. Other experimental conditions as in Fig. 3.

retention window. In fact, when the separation is satisfactory (Fig. 4a) there is no point to work at high retention window whereby the analysis time is increased. It is therefore important that the retention window stays a freely adjustable parameter. Unlike previously described micellar phases whose retention window is predetermined, octylglucoside–borate micelles offer the advantage of tunable retention window, which can be adjusted to suit a given separation problem, see also below.

Borate concentration. To examine the dependence of retention window on borate concentration, the electrophoretic measurements were performed with running electrolytes of 50 m*M* OG, pH 10, at various borate concentration. Fig. 5 illustrates typical plots of t_0 and t_{mc} *versus* the borate concentration in the running electrolyte. As expected, the retention window increased with borate concentration. The retention time of the micelles, t_{mc}, increased substantially with increasing borate concentration from 25 to 400 m*M* while the retention time of the inert tracer, t_0, increased only slightly in the concentration range studied. The increase in t_{mc} with borate concentration is primarily due to an increase in the charge density of the micelles upon complexation with borate (see eqns. 3, 5 and 6). The slight increase in t_0, which corresponds to a shallow decrease in the electroosmotic flow velocity, may be the result of increasing the ionic strength of the running electrolyte with increasing borate concentration (see eqns. 1, 3 and 4). As discussed in the preceding section, the larger increase in t_{mc} at relatively high borate concentration may be due in part to the slight decrease in v_{eo}.

Surfactant concentration. The effect of alkylglucoside concentration on the electrokinetic behavior of the MECC system was investigated with electrolytes containing 200 or 400 m*M* borate, pH 10 at various concentration of OG. Fig. 6a and b shows the dependence of t_{mc} and t_0 on surfactant concentration. In general, the retention window decreased slightly with increasing surfactant concentration. At constant pH and borate concentration, increasing surfactant concentration will increase the ratio [OG]/[OG-Borate], which then lead to a monotonic decrease in the surface charge density of the micelle

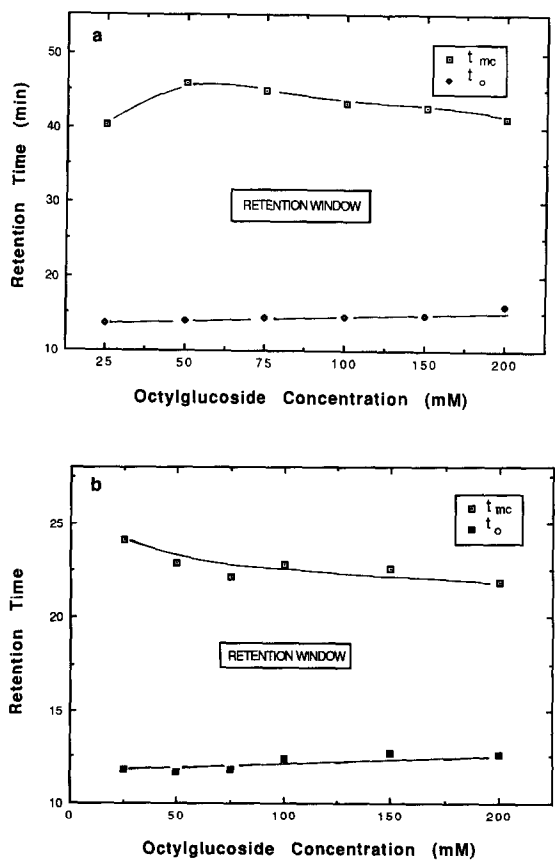


Fig. 6. Effect of octylglucoside concentration on the magnitude of retention window. Running electrolytes, 400 m*M* borate in (a), 200 m*M* in (b), pH 10 at various concentration of OG. Other experimental conditions as in Fig. 3.

TABLE I

COMPARISON OF THE RETENTION FACTORS, k', OF NITROBENZENE AND HERBICIDES OBTAINED WITH SDS AND OG–BORATE MICELLAR PHASES

Conditions: 25 m*M* SDS in 25 m*M* phosphate, pH 9.0; 50 m*M* OG in 400 m*M* borate, pH 9.0; running voltage, 15 kV.

Analyte	k'	
	SDS	OG
Nitrobenzene	0.53	0.88
Prometon	6.49	1.38
Prometryne	7.45	2.73
Propazine	14.79	12.73
Butachlor	192.30	59.00

TABLE II

CMC VALUES OF OCTYLGLUCOSIDE

Conditions as in Fig. 6.

Surfactant	CMC values [m*M*]		
	Pure water	200 m*M* borate, pH 10	400 m*M* borate, pH 10
Octylglucoside	25[a]	23	20

[a] From ref. 38.

and consequently in t_{mc} (see eqns. 5 and 6). The electroosmotic velocity decreased slightly, which was probably due to the increase in the viscosity of the running electrolyte as a result of high concentration of the surfactant, and perhaps to the adsorption of the surfactant to the capillary walls. This shallow decrease in v_{eo} (*i.e.*, increase in t_0) was may be responsible for the slight decrease in t_{mc} despite the fact that the surface charge density of the micelle decreased.

The above studies show that the retention window of the micellar system can be readily tuned by borate concentration and pH of the running electrolyte, and to a lesser extent, by varying the concentration of the surfactant.

Retention factor

To further characterize the new micellar system under investigation, the retention factors, k', of neutral model solutes were measured using eqn. 10 under various elution conditions with OG–borate micelles. Also, the OG–borate micellar phase was compared to SDS.

Comparison with SDS. Table I presents the k' values of five model solutes obtained with SDS and OG–borate micelles. Due to the more balanced hydrophile–lipophile character of OG–borate micelles, the k' values obtained with the alkylglucoside micellar phase were lower than those obtained with SDS. As expected, nitrobenzene, which is a relatively more polar species than the other model solutes, exhibited higher partitioning in the OG–borate micelles, whereas butachlor the more hydrophobic solute in the test mixture was almost completely solubilized by SDS. These results demonstrate that OG–borate micellar system allows more equitable distribution of polar and non-polar solutes between the micelles and the aqueous phase containing the monomers.

To further illustrate the utility of OG–borate micelles, some polyaromatic hydrocarbons were analyzed with the new micellar phase as illustrated in Fig. 7. The OG–borate micellar system permitted

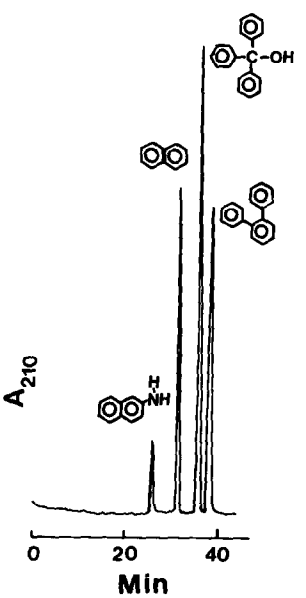


Fig. 7. Typical electropherogram of polyaromatic hydrocarbons. Electrolyte, 200 m*M* borate, containing 50 m*M* OG, pH 10. Samples from left to right: 2-naphthylamine, naphthalene, triphenylmethanol and *o*-terphenyl. Other experimental conditions as in Fig. 3.

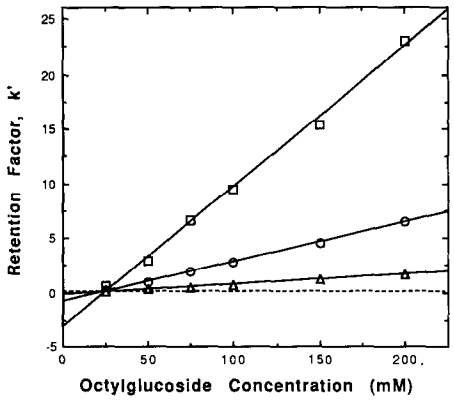


Fig. 8. Retention factor, k', *versus* octylglucoside concentration in the running electrolyte, 200 mM borate, pH 10, at various OG concentrations. Other experimental conditions as in Fig. 3. □ = Prometryne; ○ = prometon; △ = nitrobenzene.

the baseline resolution of these water-insoluble compounds without the addition of organic solvent to the running electrolyte.

Concentration of surfactants. Fig. 8 portrays typical plots of the retention factor, k', *versus* octylglucoside concentration in the running electrolyte. The results were obtained with electrolytes containing 200 mM borate, pH 10, at various OG concentrations. As expected, the retention factors of the model solutes increased linearly with the surfactant concentration in the range studied. Increasing the octylglucoside concentration in the running electrolyte corresponds to increasing the phase ratio ϕ, *i.e.*, ratio of the volume of the micellar pseudo-stationary phase to that of the aqueous phase. According to the following equation the retention factor is a linear function of the surfactant concentration [2]:

$$k' = \phi K \approx Kv([S] - CMC) \quad (15)$$

where K is the distribution coefficient of solute between micellar and aqueous phases, v is partial specific volume of the micelle, [S] is the concentration of the surfactant and CMC is the critical micellar concentration.

The octylglucoside–borate surfactant can be considered as an anionic surfactant. This surfactant will associate at monomer concentration different than the uncomplexed octylglucoside, and the value of its CMC should be different. The CMC values of the alkylglycoside–borate surfactant were determined from the MECC measurements at two borate concentration using the linear plots of k' *versus* the surfactant concentration, *i.e.*, eqn. 15. The results are summarized in Table II. The CMC of OG–borate surfactant decreased by a factor of *ca.* 0.9 and 0.8 at 200 and 400 mM borate, respectively, with respect to the CMC of OG in pure water. Due to electrostatic repulsion between their charged polar head groups, anionic surfactants are characterized by a higher CMC than neutral surfactants having the same length of the alkyl tail [35]. But in the case of OG–borate surfactant, at relatively high ionic strength, the concentration of counter ions becomes

TABLE III

LIMITS OF DETECTION

Electrolyte, 40 mM OG in 200 mM borate, pH 10. Other conditions as in Fig. 3. The injected quantities were determined by eqn. 14.

Sample solute	Limit of detection		
	Concentration (μM)	Injected quantity	
		(pg)	(fmol)
Prometon	4.4	6.0	26.5
Prometryne	8.3	12	49.9
Propazine	3.0	4.2	18.0
Butachlor	8.7	16	52.3

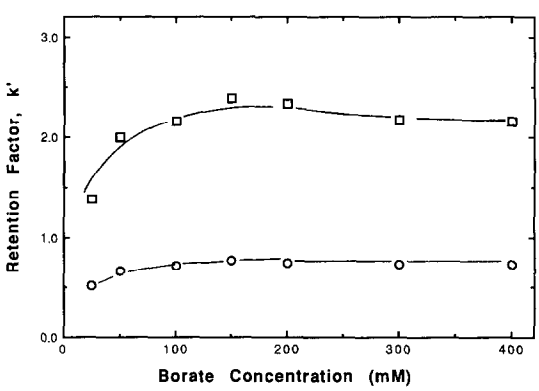


Fig. 9. Retention factor, k', *versus* borate concentration in the running electrolyte. Electrolytes, 50 mM OG, at various borate concentration, pH 9.0. Samples, prometon (○) and prometryne (□). Other experimental conditions as in Fig. 3.

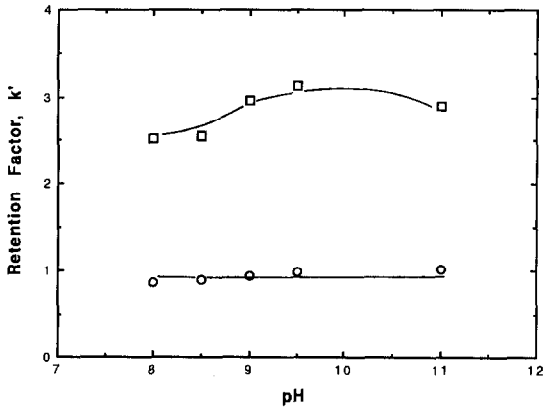


Fig. 10. Retention factor, k', *versus* pH of the running electrolyte. Electrolytes, 200 mM borate containing 50 mM OG, at various pH. Samples, prometon (○) and promctryne (□). Other experimental conditions as in Fig. 3.

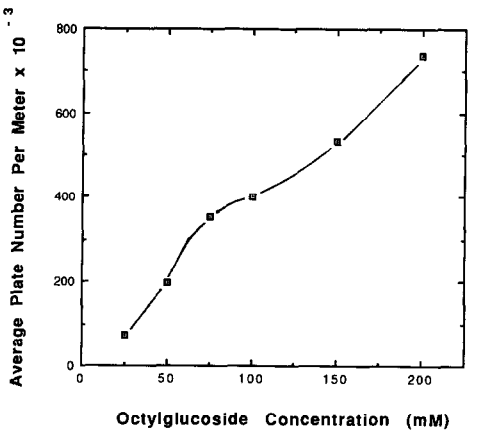


Fig. 11. Average plate number per meter *versus* octylglucoside concentration in the running electrolyte. Electrolytes, 200 mM borate, pH 10, at various OG concentrations. The average plate number was measured from the peaks of nitrobenzene, prometon and prometryne. Other experimental conditions as in Fig. 3.

high, thus reducing the electrostatic repulsion between the charged head groups (*i.e.*, borate-sugar moieties) and consequently allowing the association of the OG–borate surfactant at lower monomer concentration. This corroborate earlier findings with ionic surfactants [36].

Borate concentration. Fig. 9 shows the change in k' values of the neutral model solutes with borate concentration at constant pH and OG concentration. Although, the surfactant concentration was kept the same and the solute are neutral at the pH of the experiments (pK_a values of prometon, prometryne and propazine are 4.20, 4.05 and 1.85, respectively [37]), the k' values first increased at low borate concentration (*ca.* 25–100 mM) and then leveled off at high borate concentration. This may be explained by a salting-out effect in the sense that increasing the ionic strength of the running electrolyte would increase the extent of solubilization of neutral solute in the inner core of the micelle [35]. The effect of increasing the ionic strength with increasing borate concentration is to decrease the repulsion between the similarly charged ionic head groups of the OG–borate surfactant, thereby decreasing the CMC and increasing the aggregation number and volume of the micelles. The increase in the aggregation number of the micelles presumably results in an increase in the solubilization of neutral solutes in the inner core of the micelle [35]. On the other hand, at relatively high borate concentration, the micelle configuration would stabilize and the k' value too.

pH of the running electrolyte. Fig. 10 shows the dependence of the retention factor on pH. As expected, since the borate and surfactant concentrations were kept constant, the k' values of prometon and prometryne did not change in the pH domain studied. The slight fluctuations in the k' values of prometryne are within the range of experimental errors.

Efficiency and peak capacity

Fig. 11 presents typical data on separation efficiencies in terms of average plate number per meter *versus* the surfactant concentration. As can be seen in Fig. 11, N increased sharply with increasing OG concentration in the running electrolyte. In MECC, the micelles are so small that the mass transfer resistance in the pseudo-stationary phase is insignificant [26,27]. In the absence of excessive joule heating longitudinal molecular diffusion is the ultimate limitation [26,27]. Under these conditions, increasing the surfactant concentration would lead to more densely packed capillary with micelles so that the intermicellar diffusion distances become shorter which would give rise to faster mass transfer in the mobile phase and concomitantly higher separation efficiencies [26]. In most cases high separation efficiencies were obtained with the new

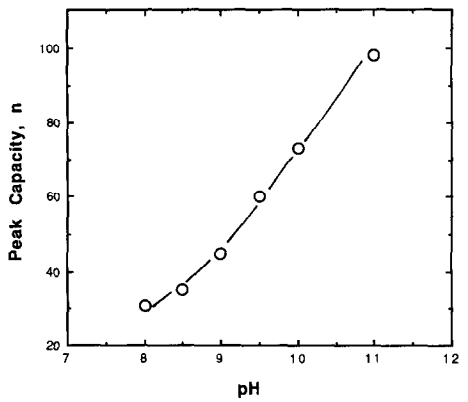


Fig. 12. Peak capacity, *n*, *versus* the pH of the running electrolyte. Electrolytes, 400 m*M* borate containing 50 m*M* OG, at various pH. Other experimental conditions as in Fig. 3.

micellar system, and the average theoretical plate number per meter was normally above 150 000 plates. As can be seen in Fig. 11, as high as 700 000 theoretical plates per meter was achieved.

At constant surfactant concentration, the separation efficiency was practically independent of borate concentration and pH in the range studied. On the other hand, peak capacity, which is another measure of the efficacy of the system, almost always increased with borate concentration and pH of the running electrolyte. Typical results are shown in Fig. 12, whereby peak capacity increased from 30 to 100 when pH changed from 8 to 11. This is because of the large increase in the retention window.

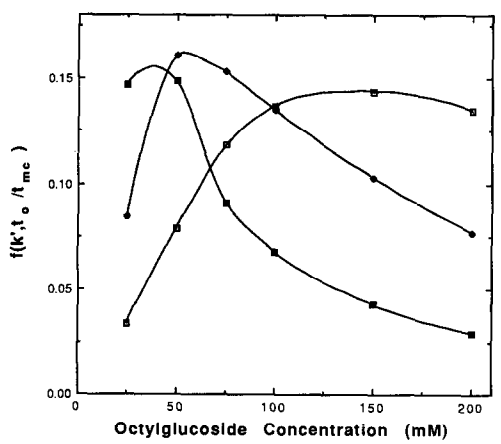


Fig. 13. Dependence of $f(k', t_0/t_{mc})$ *versus* octylglucoside concentration in the running electrolyte. Electrolytes, 200 m*M* borate containing various OG concentration, pH 10. Samples, nitrobenzene (□), prometon (◆) and prometryne (■). Other experimental conditions as in Fig. 3.

Resolution

As in chromatography, the resolution in MECC is a function of retention [*i.e.*, $f(k')$], selectivity and separation efficiencies (see eqn. 11). With OG borate surfactant, the selectivity for the neutral solutes under investigation did not vary to any significant extent by changing the surfactant concentration, borate concentration or pH of the running electrolyte. Although, separation efficiencies can be increased with increasing OG concentration (see above), which in turn would increase resolution, there is a limit beyond which increasing the amount of surfactant would cause the contribution of $f(k')$ for resolution to decline. Fig. 13 displays the dependence of $f(k')$ on surfactant concentration under conditions of relatively constant retention window, *i.e.*, constant t_0/t_{mc}. The optimum surfactant concentrations at which maximum $f(k')$ values are obtained decreased with increasing k'. For a pair of solutes having an average retention as high as that of prometryne, the optimum surfactant concentration is low, whereas, for a pair of solutes whose average k' is similar to that of nitrobenzene, the optimum surfactant concentration corresponding to the maximum $f(k')$ is located at higher values. This means that for a multicomponent mixture, the optimization of resolution for the various pairs of solutes cannot be effectively achieved through k', *i.e.*, surfactant concentration. High surfactant concentrations are unfavorable for good resolution, because $f(k')$ will drop considerably.

However, one of the unique characteristics of the OG–borate micelles is that the retention window can be adjusted over a certain range to any desired level without affecting k', by keeping the surfactant concentration constant while varying borate concentration or pH of the running electrolyte. Under these conditions, the contribution of $f(k')$ to resolution can be increased through increasing the retention window. To illustrate the dependence of $f(k')$ on the elution range parameter at constant k', $f(k')$ of a pair of solute having similar retention behavior to the model solute prometon was plotted against the ratio t_0/t_{mc}, for several different studies of borate

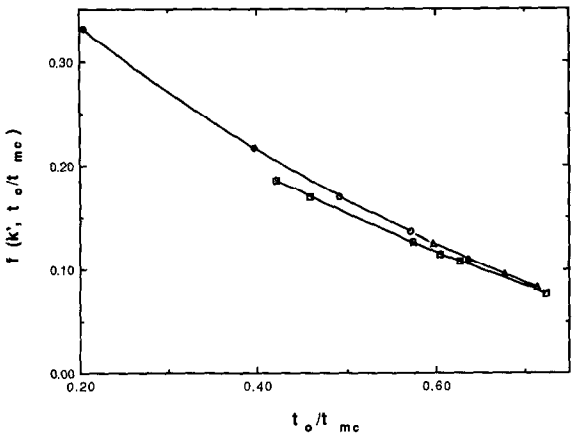


Fig. 14. Dependence of $f(k',t_0/t_{mc})$ *versus* the elution range parameter, t_0/t_{mc}. Solute, prometon. Values were obtained at different borate concentration and pH but at constant OG concentration (50 m*M*). □ = Various borate concentrations, pH 10; △ = various borate concentrations, pH 9.0; ○ = 400 m*M* borate at various pH.

concentration and pH of the running electrolyte, see Fig. 14. In all cases, average k' values were used for the calculation of $f(k')$ over the range in which the retention factors of the solutes were almost constant regardless of the change in borate concentration or pH. As the retention window increased, *i.e.*, t_0/t_{mc} decreased, the $f(k')$ contribution to resolution increased (see Fig. 14). Thus, with OG–borate micelles a suitable compromise between analysis time and resolution can be readily reached by first selecting a moderate surfactant concentration and subsequently either increase or decrease t_{mc} by varying the pH or the borate concentration of the running electrolyte while k' remains practically unchanged.

Limit of detection

The limits of detection of herbicides obtained in this study are listed in Table III. The data were determined by injecting several dilutions of a relatively concentrated standard mixture using an electrolyte of 200 m*M* borate containing 40 m*M* OG, pH 10. The concentration limits correspond to a signal-to-noise ratio of 3. The detection limits show that as low as few micromolar in terms of concentration or a few picograms in terms of absolute mass of solute injected can be determined. The amounts injected were calculated using eqn. 14.

CONCLUSIONS

MECC with octylglucoside–borate micellar phases has shown promise for the determination of neutral organics at low level. The new MECC method with micelles of adjustable surface charge density offered tunable retention window by simply altering some of the operational parameters, and thus allowed the manipulation of peak capacity and resolution of the system. Very high theoretical plate numbers were obtained, and consequently the detection limits were quite promising with a UV detector. In addition, due to the balanced hydrophile–lipophile character of the surfactant, the OG–borate micellar phase exhibited decreased retention toward hydrophobic solute and promoted more equitable distribution of relatively polar compounds, *e.g.*, nitrobenzene.

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