ELSEVIER

Journal of Chromatography A, 724 (1996) 285–296

JOURNAL OF CHROMATOGRAPHY A

Micellar electrokinetic capillary chromatography with in-situ charged micelles
VI. Evaluation of novel chiral micelles consisting of steroidal–glycoside surfactant–borate complexes[1]

Yehia Mechref, Ziad El Rassi*

Department of Chemistry, Oklahoma State University, Stillwater, OK 74078-3071, USA

Received 30 May 1995; revised 11 August 1995; accepted 18 August 1995

Abstract

Novel in-situ charged micelles having chiral selectivity, namely N,N-bis-(3-D-gluconamidopropyl)-cholamide and -deoxycholamide (denoted by Big CHAP and Deoxy Big CHAP, respectively), were evaluated in micellar electrokinetic capillary chromatography (MECC) of enantiomers. The two neutral steroidal glycoside surfactants (i.e., Big CHAP and Deoxy Big CHAP) could be charged readily via borate complexation, and consequently the surface charge density of their corresponding micelles could be conveniently adjusted by varying the borate concentration and the pH of the running electrolyte. This allowed manipulation of the migration time window of the Big CHAP– and Deoxy Big CHAP–borate micellar systems over a certain range. As a result the enantiomeric resolution of a given racemic mixture could be optimized by varying the pH and borate concentration of the running electrolyte. In addition, the stereoselectivity of the chiral steroidal micelles could be further tuned by changing the concentration and nature of the organic modifier and surfactant as well as by adjusting the temperature of the capillary column. In general, resolution increased with (i) increasing pH and borate concentration of the running electrolyte and (ii) decreasing capillary temperature. On the other hand, there was an optimum organic modifier and surfactant concentration for which maximum resolution was obtained. Big CHAP–borate micelles were less stereoselective than Deoxy Big CHAP–borate micellar systems under otherwise identical conditions. The novel micellar phases were useful for the separation of binaphthyl enantiomers, troger's base, dansyl amino acid enantiomers and silvex herbicide optical isomers.

Keywords: Micellar electrokinetic chromatography; Enantiomer separation; Chiral micelles; Binaphthyl; Silvex; Troger's base; Amino acids

*Corresponding author.

[1]Presented as a part of a lecture at the Seventh International Symposium on High-Performance Capillary Electrophoresis (HPCE '95), Würzburg, Germany, Jan. 29–Feb. 2, 1995, and also as a part of lecture at the 17th International Symposium on Capillary Chromatography and Electrophoresis, Wintergreen, Virginia, May 7–11, 1995.

1. Introduction

Chirality is an important phenomenon in various fields including the pharmaceutical and agrochemical research and industries. Because of the increasing awareness of the different physiological effects of

0021-9673/96/$15.00
SSDI 0021-9673(95)00915-9

the enantiomeric components of racemic mixtures, there is a strong need for various analytical methods for the recognition of molecular chirality and for the separation of enantiomeric compounds. In this regard, various stereoselective separation methods using gas, liquid and supercritical fluid chromatography as well as capillary electrophoresis have been introduced. Very recently, the various aspects of enantiomeric separations by different chromatographic and electrophoretic techniques as well as new developments in this branch of separation sciences have been reviewed and reported in a special issue of the Journal of Chromatography [1].

The potential of capillary electrophoresis in enantiomeric separations was first demonstrated in 1985, i.e. in the early years of CE development [2]. In that first report on CE of enantiomers, several DL-amino acids bearing a dansyl group were resolved by a ligand exchange mechanism and detected by laser-induced fluorescence [2]. This genuine work has paved the way to other important approaches for enantiomeric separations by CE including chiral micelles [3] or chirally derivatized micelles [4], micellar phases with chiral additives [5] or mixed chiral–achiral micelles [6], inclusion complexation (e.g., cyclodextrins and crown ethers) in open tubular format [7,8] or in gel-filled capillaries [9], and affinity interactions involving saccharides [10] or proteins [11,12]. The details of these various approaches have been described in several recent reviews on CE of enantiomers [13–17]. Each of the different methods provided a solution for a limited number of enantiomers, and no single approach or chiral selector exists that can accommodate all enantiomeric separations. This has been the driving force for the search for new chiral selectors [6,17–22] to allow the enantiomeric resolution of a wider range of racemates. Another important aspect of the research on CE of enantiomers has been the optimization of the separation and better understanding of the underlying phenomena [23,24].

The present report describes the potential of novel chiral in-situ charged micelles in micellar electrokinetic capillary chromatography of enantiomers. The novel chiral micellar phases under investigation are essentially based on steroidal glycoside surfactants as borate complexes (see below). The concept of the use of in-situ charged micelles in MECC was

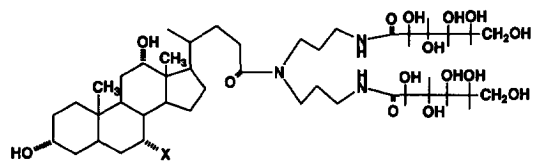

Fig. 1. Structures of Big CHAP (X=OH) and Deoxy Big CHAP (X=H) surfactants.

recently introduced by our laboratory [25–30], and has proved useful in the separations of a wide range of species. However, this recent approach has not been yet exploited in MECC of enantiomers. Thus, the present report is a follow-up on our previous work and is aimed at enlarging the scope of the applications of in-situ charged micelles to include enantiomeric separations.

As shown in Fig. 1, Big CHAP and Deoxy Big CHAP are two nonionic surfactants containing a cholic or deoxycholic steroidal moiety, respectively, and a bisgluconamidopropyl polar group. These chiral, steroidal glycoside surfactants combine both the structural features of bile salts (chiral surfactants) and glycosidic surfactants (in-situ charged, and also chiral surfactants [31]) through the steroidal portion and the polyolic polar groups, respectively. This would make such surfactants very attractive for chiral MECC using the concept of in-situ charged micelles. In analogy with the reported behavior of bile salt surfactants [32], it is believed that (i) the chiral steroidal moieties of Big CHAP and Deoxy Big CHAP give the micelle both hydrophobic and hydrophilic characters and (ii) the steroidal glycoside surfactants would form small micelles, also called primary micelles, via hydrophobic interaction between the non-polar faces of the steroidal monomers [33]. It has been reported that Big CHAP and Deoxy Big CHAP have small aggregation numbers of 10 and 8–16, and CMC values of 3.4 and 1.1–1.4 m*M*, respectively [34]. Furthermore, since both surfactants possess two polar sugar groups, it is believed that their solubilization behavior is different from that of their bile salt counterparts as well as from that of the alkyl type surfactants including alkylglycoside surfactants. The relatively stronger hydrophilic character of Big CHAP and Deoxy Big CHAP should yield an enantioselectivity different from that observed with bile salts and other more hydrophobic chiral

surfactants. Also, because of the presence of two bulky sugar groups, one can envision that these surfactants would be more suitable for the separation of moderately hydrophobic chiral and achiral compounds, which usually associate strongly with alkyl-based micellar phases and do not separate from each other [35]. These issues are under investigation, and will be published in forthcoming articles.

Although Big CHAP and Deoxy Big CHAP surfactants are neutral, a charge can be introduced into their molecules via complexation of the polyolic polar groups, i.e. the sugar moiety, with borate. This feature confers to the steroidal glycoside micelles an adjustable surface charge density in a way similar to that previously described for in-situ charged micelles [25–30]. This makes the steroidal glycoside surfactants very attractive for difficult separations of e.g. racemates. The possibility of in-situ charging of Big CHAP and Deoxy Big CHAP through borate complexation offers the convenience to adjust the migration time window of the micelles, consequently allowing optimization of the enantiomeric resolutions, analysis time and peak capacity. The migration time window of in-situ charged micelles can be readily adjusted by varying the surface charge density of the micelles through changing the borate concentration and/or the pH of the running electrolyte [26–30].

Thus far, most of the chiral surfactants described for MECC of enantiomers [22,36] do not have a freely adjustable migration time window as the in-situ charged micelles described here; for some of the chiral surfactants a mixed micellar system with SDS was employed either to increase the migration time window [37] or to render the chiral micelles amenable to the separation of neutral enantiomers [6].

2. Experimental

2.1. Instrumentation

The capillary electrophoresis instrument used in this study was a Beckman P/ACE Model 5510 instrument (Beckman Instruments, Fullerton, CA, USA), equipped with a diode-array detector and a data-handling system comprising an IBM personal computer and System Gold software. Detection was performed at 240 nm, and the resulting signal was fed to the computer for storage and real-time display of the electropherogram. Fused-silica capillaries (50 cm to detector, 57 cm total length, I.D. of 50 μm and O.D. of 365 μm) were obtained from Polymicro Technology (Phoenix, AZ, USA). Unless otherwise stated, the temperature of the capillary was maintained at 15°C by the instrument thermostatting system. Samples were injected as methanol–water solutions by applying pressure of 0.034 bar (i.e. 3.5 kPa) for various lengths of time.

2.2. Reagents and materials

The following enantiomers: (*S*)-(−)-1,1′-binaphthyl-2,2′-diamine (BNDA), (*R*)-(+)-1,1′-binaphthyl-2,2′-diamine, (±)-1,1′-bi-2-naphthol (BNOH), (*S*)-(−)-1,1′-binaphthyl-2,2′-diylhydrogen phosphate ($BNPO_4$), (*R*)-(+)-1,1′-binaphthyl-2,2′-diylhydrogen phosphate and troger's base were purchased from Aldrich (Milwaukee, WI, USA). Silvex [2-(2,4,5-trichlorophenoxy)propionic acid] was obtained from Chem Service (West Chester, PA, USA). All dansyl amino acids (Dns-AA), i.e. DL-leucine, DL-methionine, DL-phenylalanine, and DL-tryptophan cyclohexylammonium salts were purchased from Sigma (St. Louis, MO, USA). N,N-Bis-(3-D-gluconamidopropyl)-cholamide (Big CHAP) and N,N-bis-(3-D-gluconamidopropyl)-deoxycholamide (Deoxy Big CHAP) were purchased from Calbiochem (La Jolla, CA, USA). The structures of the model solutes used in this study are given in Fig. 2.

3. Results and discussion

3.1. Variables affecting the chiral recognition and the electrokinetic behavior of the steroidal glycoside–borate micellar phases

The chiral recognition and selectivity of the steroidal glycoside surfactants in chiral MECC were evaluated using three different binaphthylic compounds as model enantiomers and by varying conditions such as the pH of the running electrolyte, borate and surfactant concentration, amount and nature of organic modifier, and capillary temperature.

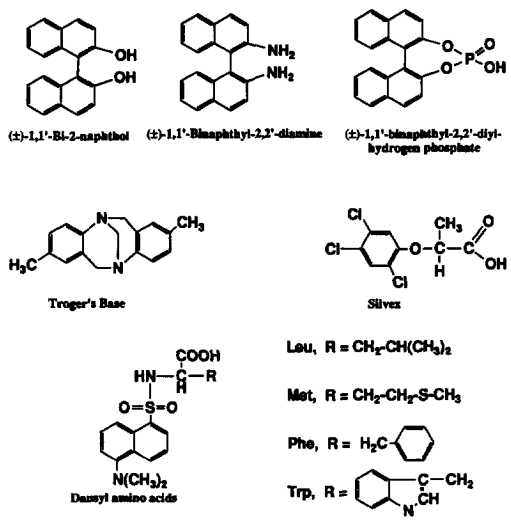

Fig. 2. Structures of the model solutes used in this study.

3.1.1. Electrolyte pH

Fig. 3 illustrates the relationship between the width of the migration time window and the pH of the running electrolyte. As can be seen in Fig. 3, t_{mc} increased sharply with pH due to the increasing surface charge density of the micelle, resulting from the increasing concentration of the Big CHAP–borate complexes at higher pH values. Under this condition, the electrophoretic mobility of the micelle in the direction opposite to that of the electroosmotic flow (EOF) increased, thus yielding an increase in t_{mc}. In addition, the sharp increase in t_{mc} at pH 11.5 might be due in part to ionization of the hydroxyl groups of the sugar moieties of the surfactant. On the other hand, t_0 increased only slightly over the pH range studied due to increase in the ionic strength of the running electrolyte at higher pH. This decreased the zeta potential of the capillary walls causing the EOF to decrease. The silanol groups of the fused-silica surface are fully ionized in the pH range 8–12, and consequently the charge density of the capillary inner surface is virtually constant. Overall, the net result of increasing the pH of the running electrolyte is a significant increase in the migration time window of the in-situ charged chiral micellar phase.

The effect of pH of the running electrolyte on the resolution of the various chiral analytes was examined using electrolyte systems consisting of 15.0 m*M* Deoxy Big CHAP and 50.0 m*M* sodium borate at different pH. In all cases, the capillary column was thermostatted at 15°C and the applied voltage was 20.0 kV. The enantiomeric resolution of a BNOH racemic mixture increased from R_s=0.90 at pH 8.0 and 9.0 to R_s=1.60 at pH 10.0. $BNPO_4$ enantiomers showed a continuous increase in resolution with increasing pH of the running electrolyte (Fig. 4). R_s increased from 1.95 at pH 8.0 to 2.20 at pH 9.0, then to 2.80 at pH 10.0 and finally to 4.00 at pH 11.0. In addition, the order of migration of the $BNPO_4$

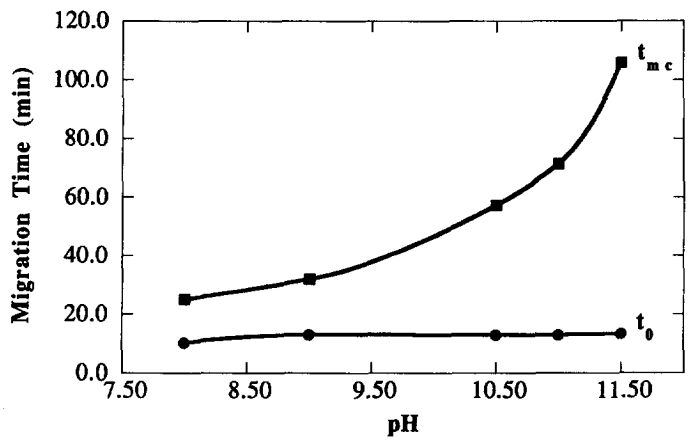

Fig. 3. Effect of pH on the magnitude of the migration time window. Capillary, untreated fused-silica, 50–57 cm×50 μm I.D.; running electrolytes, 46.0 m*M* Big CHAP, 200.0 m*M* borate at various pH; running voltage, 15.0 kV; tracers, Sudan III (for t_{mc}) and methanol (for t_0).

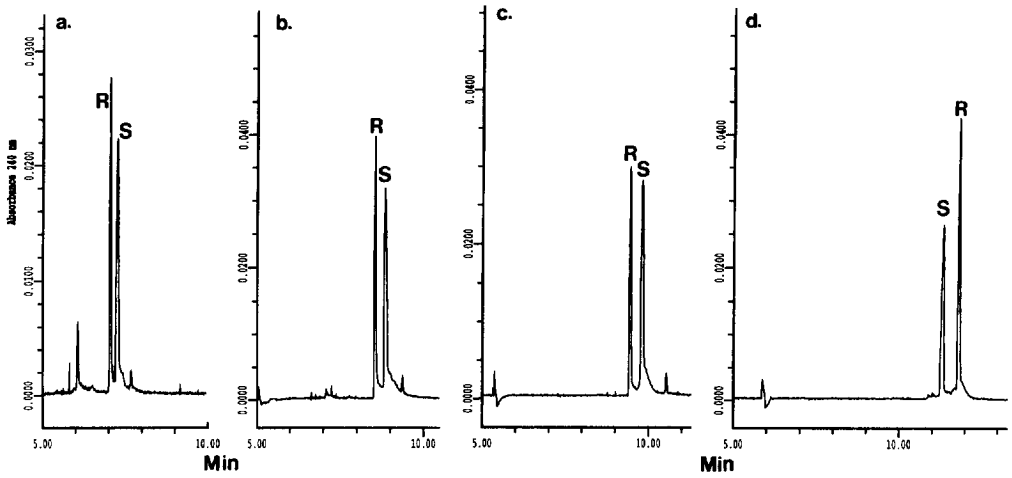

Fig. 4. Electropherograms of standard $BNPO_4$ enantiomers obtained with Deoxy Big CHAP at various pH: 8.0 (a), 9.0 (b), 10.0 (c) and 11.0 (d). Capillary, untreated fused-silica, 50 cm (to detector)–57 cm (total length)×50 μm I.D.; running electrolytes, 15.0 m*M* Deoxy Big CHAP, 50.0 m*M* borate at various pH; capillary temperature, 15.0°C; voltage, 20.0 kV.

enantiomers was inverted at pH 11.0 (see Fig. 4d). In general, the increase in resolution with increasing pH is the result of the increase in migration time window of the micellar system under investigation (see Fig. 2). Since increase of the pH results in increasing electrophoretic velocity of the micelle in the direction opposite to that of the EOF, which in turn leads to increasing t_{mc}, the enantiomer with high affinity to the micelles migrates slower than the one having less affinity to the micelles, and consequently the migration order of the enantiomers is inverted. Note that at pH 11.0, and in the positive polarity mode, i.e. using positive potential at the injection end, the net mobility of the micelles is less than that of $BNPO_4$.

Under the same operating conditions as used in the preceding experiments, Big CHAP showed lower enantiomeric resolution than Deoxy Big CHAP. The enantiomeric resolution of $BNPO_4$ enantiomers increased slightly from R_s=0.5 to R_s=0.6 when going from pH 8.0 to pH 9.0 and then leveled off at a R_s=0.6 at pH 10.0 and 11.0 without inversion of the elution order. Thus, different chiral surfactants would yield different selectivity. Again, in the case of BNOH, the enantiomeric resolution with Big CHAP was much less than that observed with Deoxy Big CHAP. For instance, at pH 10.0 the value of the enantiomeric resolution of BNOH was only 0.8 in the case of Big CHAP as compared to an R_s of 1.6 in the case of Deoxy Big CHAP.

3.1.2. Borate concentration

The effect of borate concentration on the width of the migration time window is illustrated in Fig. 5. As expected, the migration time window increased as the borate concentration increased. The sharp increase in t_{mc} is due to the increase in the charge density of the micelle as a result of increasing the

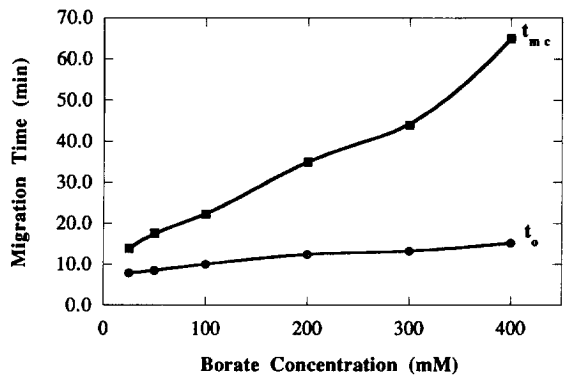

Fig. 5. Effect of borate concentration on the magnitude of the migration time window. Running electrolytes, 9.0 m*M* Big CHAP at various concentrations of borate, pH 10.0. Other conditions as in Fig. 3.

Big CHAP–borate complex concentration caused by the increase in borate concentration. On the other hand, the slight increase in t_0 is due to the higher ionic strength and viscosity of the running electrolyte at high borate concentration.

As expected, the concentration of borate in the running electrolyte largely influenced enantiomeric resolution (see Fig. 6). At 25.0 m*M* borate, the BNDA enantiomers almost coeluted. By doubling the concentration of borate to 50.0 m*M* a significant increase in resolution was realized (R_s=1.26). Enantiomeric resolution kept increasing as the borate concentration increased and finally a resolution of 1.67 was obtained at 150.0 m*M* borate. The same trend was observed for BNOH enantiomers with the difference that even at 25.0 m*M*, R_s approached unity and then kept increasing, reaching a value of almost 2.0 at 150.0 m*M* borate (results not shown). In the case of $BNPO_4$, even at 25.0 m*M* borate the enantiomeric resolution was quite high (R_s=1.86). This is because for a charged solute, such as $BNPO_4$, which is strongly associating with the chiral surfactant, there is virtually no need to charge the micelle to obtain baseline resolution. As can be seen in Fig. 7, charging the micelle through the addition of borate slowed the migration of the enantiomers. The net result is a sharp rise in R_s from 1.86 to 2.80 when going from 25.0 to 50.0 m*M* borate. At a borate concentration higher than 50 m*M* the R_s decreased and then leveled off between 75.0 and 150.0 m*M* at a value of 1.5–1.6. The decrease in R_s at high borate concentration is perhaps due to the slightly broader peaks. Peak broadening here may be caused by a longer residence time in the capillary column, i.e., more longitudinal diffusion.

For the Big CHAP surfactant the effect of borate concentration in the running electrolyte on the enantiomeric resolution of BNOH is illustrated in Fig. 8. At 25.0 m*M* borate no enantiomeric resolution was obtained. By doubling the borate concentration to 50.0 m*M* a resolution of 0.7 is realized. By going to 100 and 150 m*M* borate this resolution stays almost the same. On the other hand, the $BNPO_4$ enantiomeric mixture showed maximum resolution (R_s=0.60) when the 50.0 m*M* borate buffer was used (results not shown). The separation efficiency for this analyte decreased substantially as the concentration of borate was increased to 100 m*M* and above.

3.1.3. Concentration of the surfactant

Since the surfactant is also the chiral selector responsible for the enantiomeric separation, the use

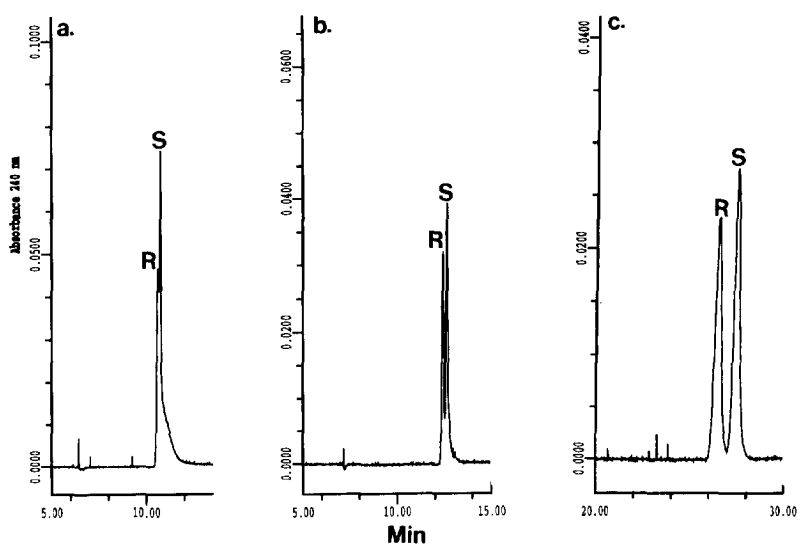

Fig. 6. Electropherograms of BNDA enantiomers obtained with Deoxy Big CHAP at various concentrations of borate in the running electrolytes. Capillary, untreated fused-silica, 50 cm (to detector)–57 cm (total length)×50 μm I.D.; running electrolytes, 25.0 m*M* (a), 50.0 m*M* (b), or 150.0 m*M* (c) sodium borate containing 15.0 m*M* Deoxy Big CHAP (pH 10.0) and 10.0% (v/v) methanol; capillary temperature, 15.0°C; voltage, 20.0 kV.

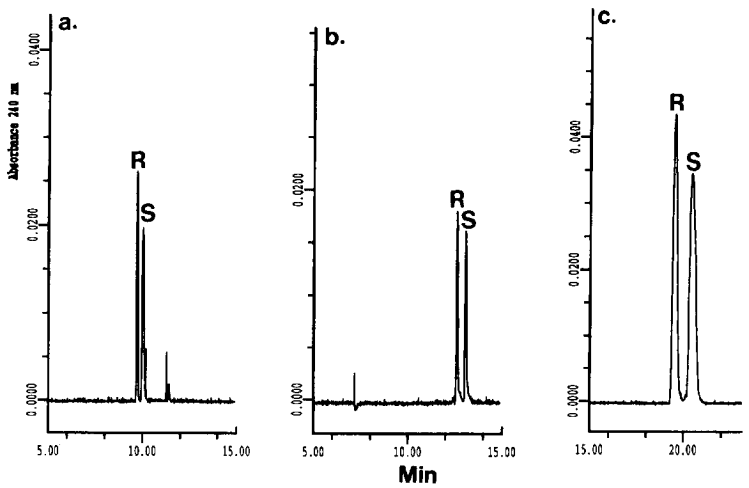

Fig. 7. Electropherograms of $BNPO_4$ enantiomers obtained with Deoxy Big CHAP at various concentrations of borate in the running electrolytes: 25.0 m*M* (a), 50.0 m*M* (b) and 100.0 m*M* (c). Conditions as in Fig. 6.

of the appropriate concentration of the surfactant is crucial for optimum enantiomeric resolution. In fact, there is an optimum chiral selector concentration to achieve maximum enantiomeric resolution [38]. Fig. 9 illustrates the effect of surfactant concentration on the enantiomeric resolution of BNOH. Maximum enantiomeric resolution was achieved when the concentration of Deoxy Big CHAP was 10.0 m*M*. The resolution increased from $R_s = 1.0$ at 5.0 m*M* surfactant to $R_s = 1.7$ at 10.0 m*M* surfactant. Thereafter, R_s decreased to 1.2 at 15 m*M* despite the significant gain in separation efficiency at this surfactant concentration (see Fig. 9c). At 20 m*M* surfactant concentration R_s decreased further to 1.1 and at 25.0

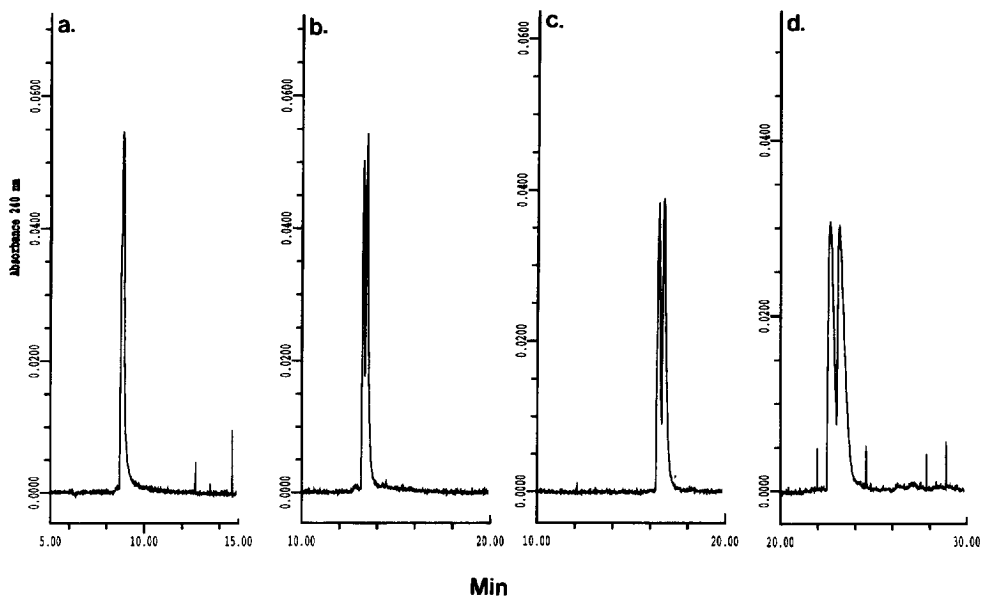

Fig. 8. Electropherograms of BNOH enantiomers obtained with Big CHAP at various concentrations of borate in the running electrolytes: 25.0 m*M* (a), 50.0 m*M* (b), 100.0 m*M* (c) and 150.0 m*M* (d). All electrolytes contained 20 m*M* Big CHAP at pH 10. Conditions as in Fig. 6.

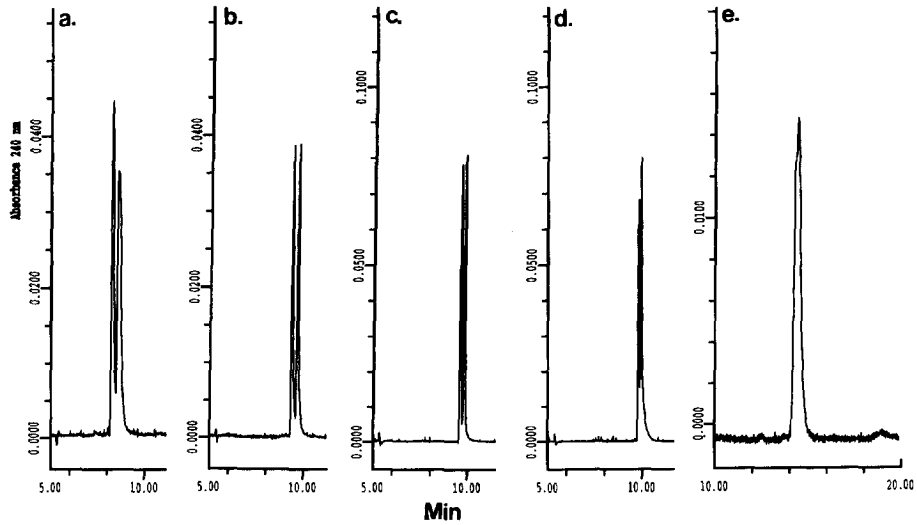

Fig. 9. Electropherograms of BNOH obtained at various concentrations of Deoxy Big CHAP in the running electrolytes. Capillary, untreated fused-silica, 50 cm (to detector)–57 cm (total length)×50 μm I.D.; running electrolytes, 50.0 m*M* sodium borate containing 5.0 m*M* (a), 10.0 m*M* (b), 15.0 m*M* (c), 20.0 m*M* (d) or 25.0 m*M* (e) Deoxy Big CHAP, pH 10.0; capillary temperature, 15.0°C; voltage, 20.0 kV.

m*M* and higher no enantiomeric resolution was observed. $BNPO_4$ showed a maximum enantiomeric resolution ($R_s \approx 2.5$) when 10.0 to 15.0 m*M* Deoxy Big CHAP was used. This is a substantial increase from the value of 1.5 observed at 5.0 m*M* surfactant. The resolution decreased to 1.8 when going to 20 m*M* and reached a value of 1.3 at 30.0 m*M* surfactant. In terms of separation efficiency, the maximum value was obtained in the range 10.0–20.0 m*M* surfactant (N_{av} varied between 100 000 and 140 000 plates/m in this range).

Big CHAP also showed maximum enantiomeric resolution at 20.0 m*M* surfactant in the running electrolyte, but the chiral selectivity of this surfactant was substantially less than that of Deoxy Big CHAP (results not shown).

3.1.4. Organic modifier

Using Deoxy Big CHAP as the chiral surfactant, the enantiomeric resolution of BNDA increased substantially with increasing % methanol in the running electrolyte. As can be seen in Fig. 10 very little enantiomeric resolution was achieved at 0% and 5.0% methanol in the running electrolyte, and almost baseline resolution is obtained at 10% ($R_s = 1.3$) and 15.0% methanol ($R_s = 1.4$). For $BNPO_4$ and BNOH, which showed relatively high resolution ($R_s = 2.65$ and 1.60, respectively) in the absence of methanol, the addition of methanol up to 10.0% had virtually no effect on resolution (results not shown).

In the case of Big CHAP, the addition of methanol to the running electrolyte increased the resolution of BNOH enantiomers but not that of $BNPO_4$ enantiomers. In both cases, the running electrolyte consisted of 20.0 m*M* Big CHAP and 50.0 m*M* sodium borate, pH 10.0, at various % methanol, and the capillary column was thermostatted at 15°C while the applied voltage was 20 kV. Under these conditions, R_s between BNOH enantiomers more than doubled from 0.30 to 0.70 when going from 0% to 10.0% methanol. On the other hand, the resolution between $BNPO_4$ enantiomers did not change and remained constant at 0.6 when going from 0% to 10.0% methanol. The addition of methanol adjusts the analyte–micelle interaction to give favorable enantiomeric separation conditions.

3.1.5. Capillary temperature

The two glycoside–steroidal surfactants seem to exhibit greater enantioselectivity towards flat and rigid molecules such as the binaphthyls. This observation corroborates earlier findings with chiral bile salt surfactants [14], which have structural features similar to those of Big CHAP and Deoxy

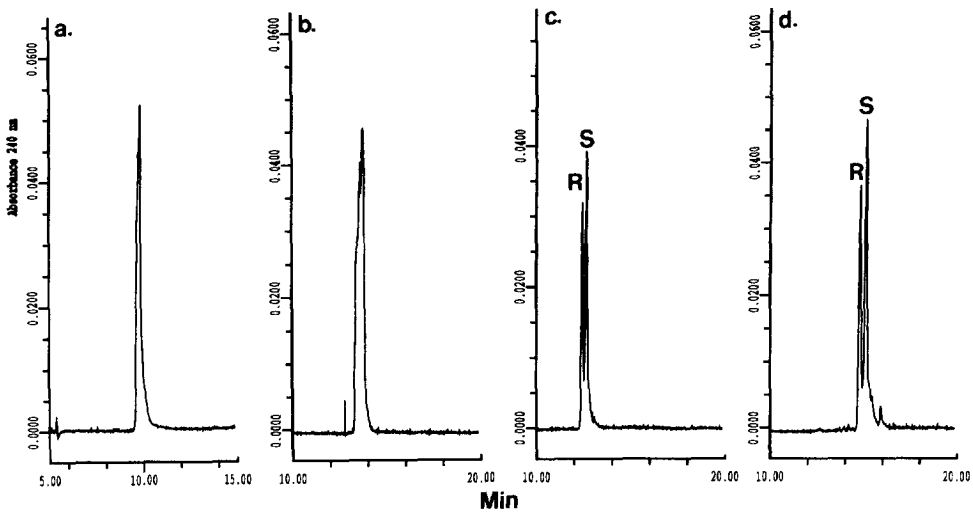

Fig. 10. Electropherograms of BNDA enantiomers obtained with Deoxy Big CHAP at various percentage of methanol in the running electrolytes. Capillary, untreated fused-silica, 50 cm (to detector)–57 cm (total length)×50 μm I.D.; running electrolyte, 50.0 m*M* borate containing 15.0 m*M* Deoxy Big CHAP (pH 10.0) and 0% (a), 5.0% (b), 10.0% (c) or 15.0% (d) methanol; voltage, 20.0 kV.

Big CHAP through the steroidal part of the surfactant molecule. Thus, since temperature affects solute rigidity, temperature should play a major role in the enantiomeric resolution. As shown in Fig. 11, resolution between BNDA enantiomers decreased from 1.67 to 0.89 when going from 15.0°C to 35.0°C passing through $R_s = 1.2$ at 25.0°C. On the other hand, the enantiomeric resolution ($R_s \approx 2.0$) of BNOH was almost unaffected by temperature.

As stated above and under all circumstances, Big CHAP exhibited less enantiomeric resolution than Deoxy Big CHAP for the same analytes. In fact,

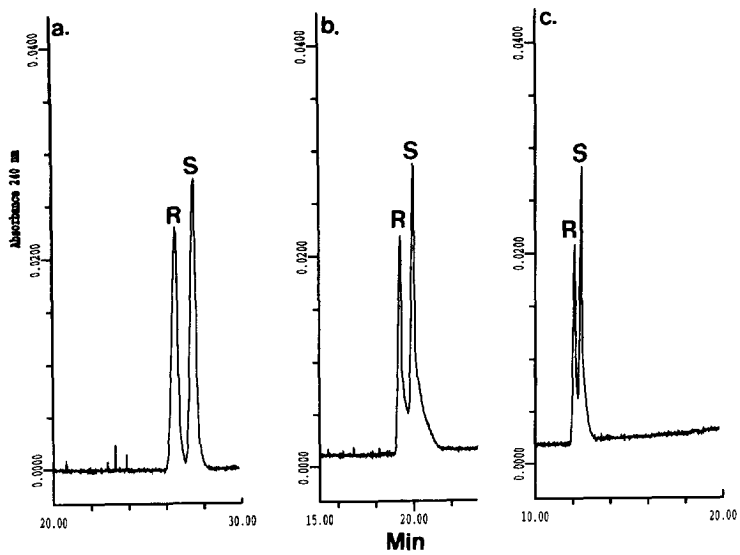

Fig. 11. Electropherograms of BNDA enantiomers obtained with Deoxy Big CHAP at various capillary temperatures. Capillary, untreated fused-silica, 50 cm (to detector)–57 cm (total length)×50 μm I.D.; running electrolytes, 150.0 m*M* sodium borate containing 15.0 m*M* Deoxy Big CHAP, pH 10.0; capillary temperature, 15.0 (a), 25.0 (b) and 35.0°C (c); voltage 20.0 kV.

enantiomeric separation of BNDA was not achieved. In addition, the temperature effect was more pronounced and the resolution of BNOH enantiomers was almost completely lost as the temperature increased from 15.0° to 35°C. In this case, the applied voltage was 20.0 kV and the running electrolyte consisted of 75.0 m*M* sodium borate and 20.0 m*M* Big CHAP pH 10.0, at various % methanol. Using these same operating conditions, $BNPO_4$ showed a substantial decrease in enantiomeric resolution from R_s=0.57 at 15.0°C to R_s=0.34 at 35.0°C. This decrease in resolution might be due to the loss of structural rigidity of the solutes as expected with temperature increase.

3.2. Illustrative enantiomeric separations

3.2.1. Troger's base

The enantiomeric resolution of troger's base required conditions different from the optimal ones for the separation of the enantiomers of the binaphthyl derivatives. Higher concentrations of surfactant and borate as well as of the organic solvents were needed to induce different enantiomeric associations with the chiral micelle and in turn different enantiomeric mobilities. In addition, troger's base enantiomers were better resolved when acetonitrile was used as the organic modifier instead of methanol (compare Fig. 12b and c).

3.2.2. Dansyl amino acids

Four pairs of DL-dansyl amino acids, i.e. leucine, methionine, phenylalanine and tryptophan, were electrochromatographed with the chiral micelles under investigation. As shown in Fig. 13, Deoxy Big CHAP showed better chiral selectivity than Big CHAP (compare Fig. 13b and c) for all four dansyl amino acid enantiomers. Again, and because of the weaker chiral recognition of the surfactant toward dansyl amino acids, the surfactant concentration needed to achieve the chiral separation was higher than that required for binaphthyl derivatives. As can be seen in Fig. 13, the separation of dansyl amino acid enantiomers could be achieved at pH 10.0. This chiral selectivity is significantly different from that encountered with bile salts, e.g. taurodeoxycholate, which were shown to exhibit chiral recognition toward amino acids only at very acidic pH [3].

As in the case of binaphthyls, here also the concentration of borate was found to be very crucial for the enantiomeric separations of dansyl amino acids. Fig. 13a and b shows the effect of borate

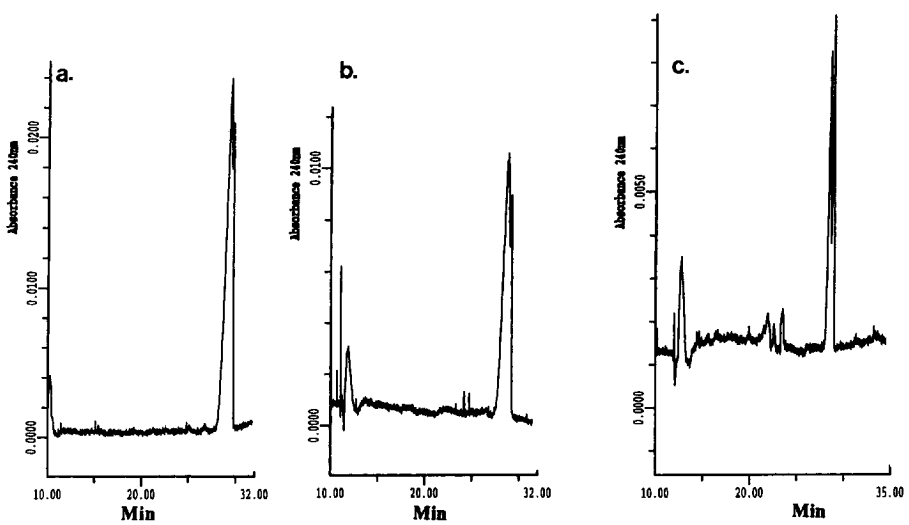

Fig. 12. Electropherograms of troger's base obtained with Deoxy Big CHAP. Capillary, untreated fused-silica, 50 cm (to detector)–57 cm (total length)×50 μm I.D.; running electrolytes, 250.0 m*M* sodium borate containing 30.0 m*M* Deoxy Big CHAP (pH 10.0) and 0% organic solvent (a), 10.0% v/v methanol (b) or 10.0% v/v acetonitrile (c); voltage, 20.0 kV.

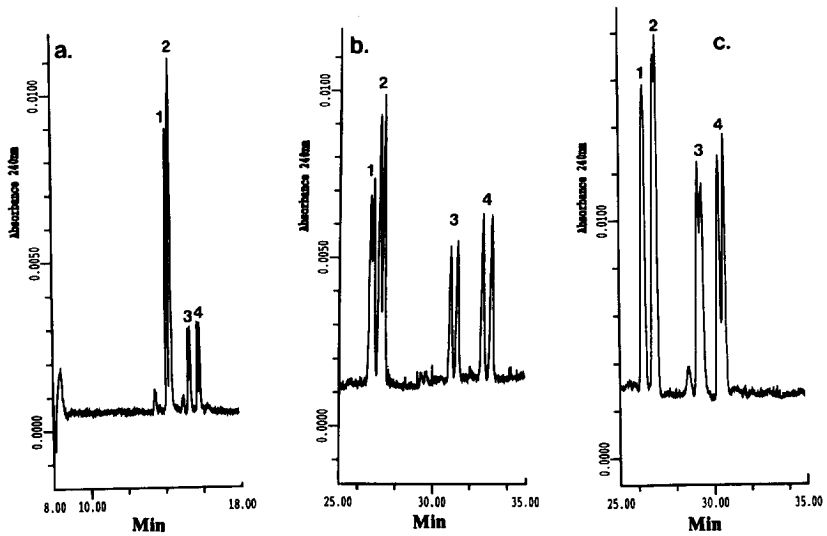

Fig. 13. Electropherograms of dansyl amino acids obtained with Deoxy Big CHAP in (a) and (b) and Big CHAP (c). Capillary, untreated fused-silica, 50 cm (to detector)–57 cm (total length)×50 μm I.D.; running electrolytes, 50.0 m*M* surfactant in 150.0 m*M* sodium borate (a) or in 300.0 *M* sodium borate (b) and (c), pH 10.0; capillary temperature, 15.0°C; voltage, 20.0 kV. Solutes: (1) D- and L-Dns-leucine; (2) D- and L-Dns-methionine; (3) D- and L-Dns-phenylalanine; (4) D- and L-tryptophan.

concentration in the running electrolyte on the enantiomeric separation of dansyl amino acids. The enantiomeric separation increased significantly as the borate concentration was increased from 150.0 m*M* to 300.0 m*M* (compare Fig. 13a and b). Increasing the borate concentration above 300.0 m*M* did not improve the enantiomeric resolution, while the analysis time increased due to the decrease in the EOF and increase in t_{mc}.

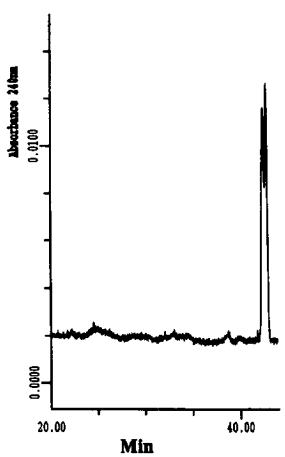

Fig. 14. Electropherogram of a silvex phenoxy acid herbicide. Running electrolyte, 50.0 m*M* Deoxy Big CHAP, 400.0 m*M* borate, pH 10.0. Other conditions as in Fig. 13.

3.2.3. Silvex herbicide

Silvex is a phenoxy acid herbicide with a chiral center. Enantiomeric separation of this analyte was achieved using a high borate concentration in the running electrolyte and a relatively high surfactant concentration. Such chiral separation is illustrated in Fig. 14. Note that the addition of organic modifiers in the case of silvex did not improve the chiral separation, but rather decreased it. This behavior resembles that observed with the dansyl amino acids.

Acknowledgments

This material is based upon work supported by the Cooperative State Research Service, U.S. Department of Agriculture, under Agreement No. 94-37102-0989. ZER acknowledges the partial financial support in form of a travel grant form the Halliburton Fund to travel to HPCE '95 in Würzburg, Germany.

Yehia Mechref is the recipient of the Phillips Petroleum Fellowship in Chemistry.

References

[1] W. Lindner (Editor), Chiral Separations, Fundamental Aspects and Applications, J. Chromatogr. A, 666 (1994).
[2] E. Gassmann, J.E. Kuo and R.N. Zare, Science, 230 (1985) 813.
[3] S. Terabe, M. Shibata and Y. Miyashita, J. Chromatogr., 480 (1989) 403.
[4] A. Dobashi, T. Ono, S. Hara and J. Yamaguchi, J. Chromatogr., 480 (1989) 413.
[5] S. Terabe, Y. Miyashita, Y. Ishihama and O. Shibata, J. Chromatogr., 636 (1993) 47.
[6] K. Otsuka and S. Terabe, J. Chromatogr., 515 (1990) 221.
[7] R. Khun, F. Erni, T. Bereuter and J. Hausler, Anal. Chem., 64 (1992) 2815.
[8] J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, J. Chromatogr., 438 (1988) 211.
[9] A. Guttman, A. Paulus, A.S. Cohen, N. Grinberg and B.L. Karger, J. Chromatogr., 448 (1988) 41.
[10] A. D'Hulst and N. Verbeke, J. Chromatogr., 608 (1992) 275.
[11] S. Busch, J. Kraak and H. Poppe, J. Chromatogr., 635 (1993) 119.
[12] G.E. Barker, P. Russo and A. Hartwick, Anal. Chem., 64 (1992) 3023.
[13] M. Novotny, H. Soini and M. Stefansson, Anal. Chem., 66 (1994) 646.
[14] S. Terabe, K. Otsuka and H. Nishi, J. Chromatogr. A, 666 (1994) 295.
[15] S. Terabe, N. Chen and K. Otsuka, Adv. Electrophoresis, 7 (1994) 87.
[16] J.W. Ward, Anal. Chem., 66 (1994) 633.
[17] S. Fanali, M. Cristalli, R. Vespalec and P. Bocek, Adv. Electrophoresis, 7 (1994) 1.
[18] A.M. Stalcup and N.M. Agyel, Anal. Chem., 66 (1994) 3054.
[19] H. Soini, M. Stefansson, M.-L. Riekkola and M.V. Novotny, Anal. Chem., 66 (1994) 3477.
[20] J. Wang and I.M. Warner, Anal. Chem., 66 (1994) 3773.
[21] R.J. Talt, D.O. Thompson, V.J. Stella and J.F. Stobaugh, Anal. Chem., 66 (1994) 4013.
[22] K. Otsuka, K. Karuhaka, M. Higashimori and S. Terabe, J. Chromatogr. A, 680 (1994) 317.
[23] S.G. Penn, E.T. Bergström, D.M. Goodall and J.S. Loran, Anal. Chem., 66 (1994) 2866.
[24] Y.Y. Rawjee, R.L. Williams and G. Vigh, Anal. Chem., 66 (1994) 3777.
[25] J.T. Smith and Z. El Rassi, J. Capillary Electrophoresis, 1 (1994) 136.
[26] J.T. Smith and Z. El Rassi, J. Chromatogr. A., 685 (1994) 131.
[27] J.T. Smith and Z. El Rassi, Electrophoresis, 15 (1994) 1248.
[28] J.T. Smith, W. Nashabeh and Z. El Rassi, Anal. Chem., 66 (1994) 1119.
[29] J.T. Smith and Z. El Rassi, J. Microsep. Sep., 6 (1994) 127.
[30] J. Cai and Z. El Rassi, J. Chromatogr., 608 (1992) 31.
[31] Y. Mechref and Z. El Rassi, in preparation.
[32] L. Fisher and D. Oakenfall, Aust. J. Chem., 32 (1979) 31.
[33] D. Attwood and A.T. Florence, Surfactant Systems, Their Chemistry Pharmacy and Biology, Chapman and Hall, London, 1983.
[34] L.M. Hjelmeland, W.A. Klee and J. Osborne, Anal. Biochem., 130 (1983) 485.
[35] S. Terabe, K. Otsuka and T. Ando, Anal. Chem., 57 (1985) 834.
[36] J.R. Mazzeo, E.R. Grover, M.E. Swartz and J.S. Peterson, J. Chromatogr. A, 680 (1994) 125.
[37] K. Otsuka, J. Kawahara, K. Tatekawa and S. Terabe, J. Chromatogr., 559 (1991) 209.
[38] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 603 (1992) 235.