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Review

Chiral glycosidic surfactants for enantiomeric separation in capillary electrophoresis

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Abstract

Several glycosidic surfactants (GSs) have been shown useful in the separation of enantiomers by capillary electrophoresis. The virtue of GSs is that they can be used as (i) neutral chiral additives in the running electrolyte for the enantioseparation of charged chiral solutes by capillary zone electrophoresis, (ii) as in situ charged micelles for the enantioseparation of neutral and charged chiral solutes by micellar electrokinetic capillary chromatography (MECC), (iii) as anionic chiral surfactants in the MECC mode upon covalently attaching negatively charged groups to their sugar head groups, and (iv) as neutral and anionic chiral surfactants mixed with achiral micelles (e.g., sodium dodecyl sulfate) for MECC of enantiomers. This review article is to provide a comprehensive description of GSs in the chiral separation of various enantiomers over a wide range of operating conditions.

Keywords: Reviews; Enantiomer separation; Capillary electrophoresis; Micellar electrokinetic chromatography; Surfactants; Glycosidic surfactants; Aminonaphthalenedisulfonic acid; Amino acids; Pesticides; Phenoxy acids; Binaphthyldiamine; Binaphthol; Bupivacaine

Contents

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1. Introduction

Capillary electrophoresis (CE) is increasingly employed in the enantiomeric separation of various kinds of racemic mixtures. This is due to the fact that CE is a simple, rapid and practical method yet providing high separation efficiency, and requiring small amounts of samples and reagents.

The importance of chiral separation in CE is manifested by a relatively large number of recent review articles on this topic. Typical recent reviews [1–11] have covered the principles and applications of chiral CE. Thus far, a review article dedicated to the chiral separation in CE with glycosidic surfactants (GSs) is still missing despite their proven potentials in enantioseparations [12–22]. As shown in Fig. 1 chiral GSs are widely differing in their structures, thus providing the ground for the realization of a wide range of enantioselectivity.

The majority of GSs are neutral surfactants that can be charged in situ through borate complexation with their saccharide head groups forming in situ charged micelles. Thus, GSs can be used as neutral chiral selectors for the separation of charged stereoisomers by capillary zone electrophoresis (CZE) [12–14,16–18,22,23] or as in situ charged chiral micelles for the separation of neutral and charged enantiomers by micellar electrokinetic capillary chromatography (MECC) [13,15]. Also, dodecyl-β-D-glucopyranoside monophosphate or monosulfate were synthesized for MECC of enantiomers [19]. Furthermore, other GSs such as digitonin, glycyrrhizic acid and β-escin afforded enantioselectivity in mixed micellar systems [20,21]. Fig. 2 shows the structures of some of the model chiral solutes used in the evaluation of various GSs in enantioseparations by CE.

Our laboratory has conducted several studies concerning not only the potentials of GSs in chiral separations [12–14,16–18,23] but also in the achiral separations of a wide range of solutes [24–31] including natural products, e.g., glucosinolates [32–34].

2. Glycosidic surfactants as neutral chiral selectors – capillary zone electrophoresis mode

2.1. Some basic principles

In the CZE mode, and with only a few exceptions, enantioseparation in the presence of GSs was mainly achieved at surfactant concentration above the critical micellar concentration (CMC) [12–14,17,18,22,23], indicating that the presence of the chiral surfactant in the micellar form is critical for

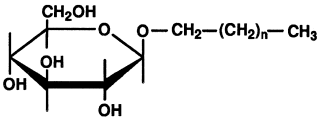
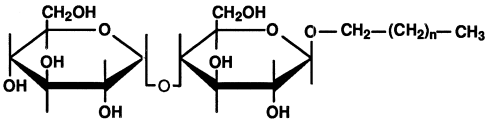
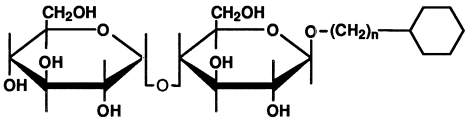
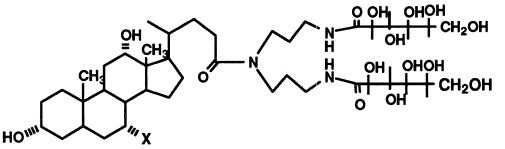
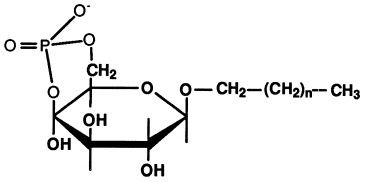
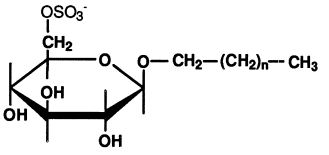
Structure and name of surfactant	Abbreviation	CMC (mM)
		
n = 6: Octyl-β-D-glucopyranoside	OG	25
n = 7: Nonyl-β-D-glucopyranoside	NG	6.5
		
Octyl-β-D-maltopyranoside	OM	23.4
		
n = 1: Cyclohexyl-mehyl-β-D-maltoside	CYMAL-1	340
n =2: Cyclohexyl-ethyl-β-D-maltoside	CYMAL-2	120
n =3: Cyclohexyl-propyl-β-D-maltoside	CYMAL-3	34.5
n =4: Cyclohexyl-butyl-β-D-maltoside	CYMAL-4	7.6
n =5: Cyclohexyl-pentyl-β-D-maltoside	CYMAL-5	2.4
n =6: Cyclohexyl-hexyl-β-D-maltoside	CYMAL-6	0.56
		
X = OH: N,N-Bis-(3-D-glucoamidopropyl)-cholamide	Big CHAP	3.4
X = H: X = OH: N,N-Bis-(3-D-glucoamidopropyl)-deoxycholamide	Deoxy Big CHAP	1.1-1.4
		
n = 10: Dodecyl-β-D-glucopyranoside monophosphate	DGMP	0.5
		
n = 10: Dodecyl-β-D-glucopyranoside monosulfate	DGMS	1.0

Fig. 1. Structures of the various GSs.

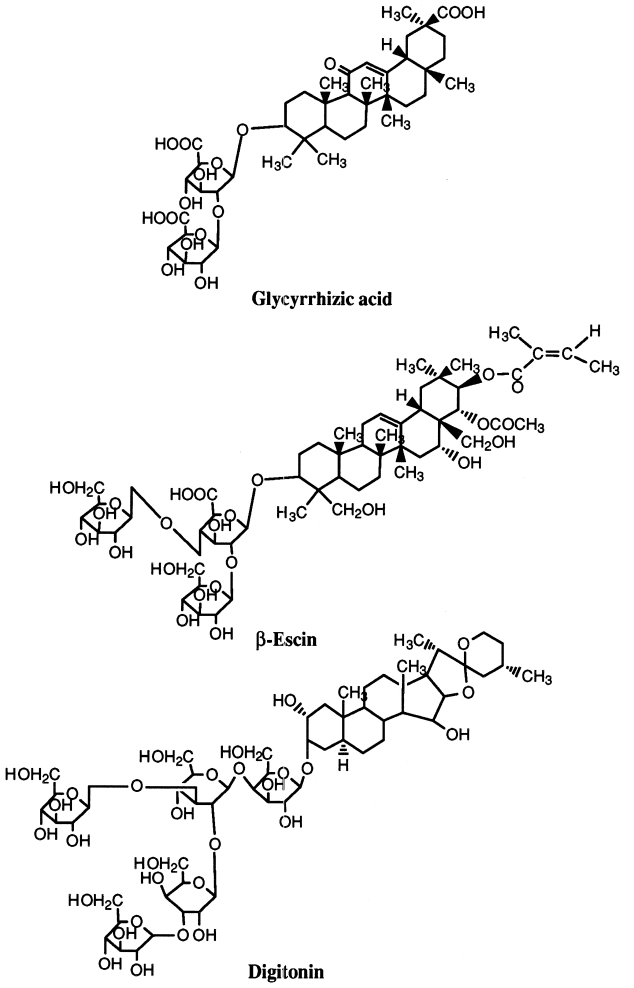


Fig. 1. (*continued*)

chiral recognition. In other words, solute–micelle association via polar and hydrophobic interactions are important components for the enantiomeric resolution.

A schematic of the separation principles of charged solutes in the presence of the GS-based micellar phases using uncoated fused-silica capillaries is depicted in Fig. 3. While the neutral glycosidic micelle migrates at the velocity of the electroosmotic flow (EOF), the electrophoretic mobility of an anionic analyte is opposite in direction to the cathodic EOF. Thus, the effective electrophoretic mobility and in turn the migration time of an anionic solute will decrease as the magnitude of its association with the neutral micelle will increase. In other words, the stronger the interaction between the analyte and the micelle, the higher the apparent mobility of the anionic analyte, and consequently the faster its migration toward the cathode. For a cationic solute, which is migrating in the same direction as the chiral micelle, its association with the micelle will result in decreasing its effective electrophoretic mobility due to a reduction in solute's effective charge density. Consequently, the stronger the interaction between a cationic solute and the neutral GS micelle the slower its migration toward the detection window.

Generally, and with uncharged surfactants, e.g.,

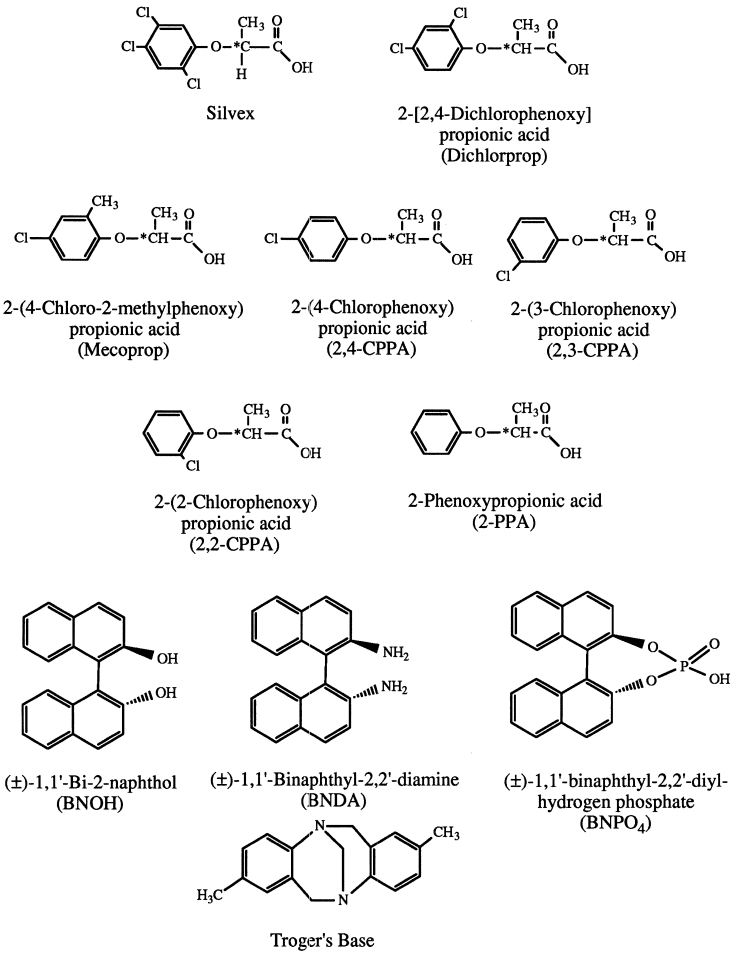


Fig. 2. Structures of some model chiral solutes.

the GSs, the magnitude of solute–micelle association should increase with increasing solute's hydrophobicity. Therefore, and using a positive electric field, the order of elution of cationic solutes should be in the order of increasing the hydrophobicity of the analytes while in the case of anionic solutes the order of elution should follow the order of decreasing hydrophobicity of the analytes.

2.2. Factors affecting enantioseparation with glycosidic surfactants

2.2.1. Surfactant concentration

Two alkylglucosides (OG and NG) [13,22,23], one alkylmaltoside (OM) [12,14] and five cyclohexyl-alkyl-β-D-maltoside (CYMAL) surfactants [16,17] were evaluated in the enantioseparation of various chiral solutes by CZE (see Fig. 1 for structures, CMC and notation of the GSs).

2.2.1.1. Electrophoretic mobility

All GSs exhibited similar behavior concerning the dependence of the average effective electrophoretic mobility of charged enantiomers on the concentration of surfactant [S] in the running electrolyte [12,14,18,22,23]. As a typical example, Fig. 4 shows the variation of the effective electrophoretic mobilities of seven phenoxy acid herbicides (see Fig. 2 for herbicide structures) as a function of OG and NG concentration. As expected, and in all cases, the

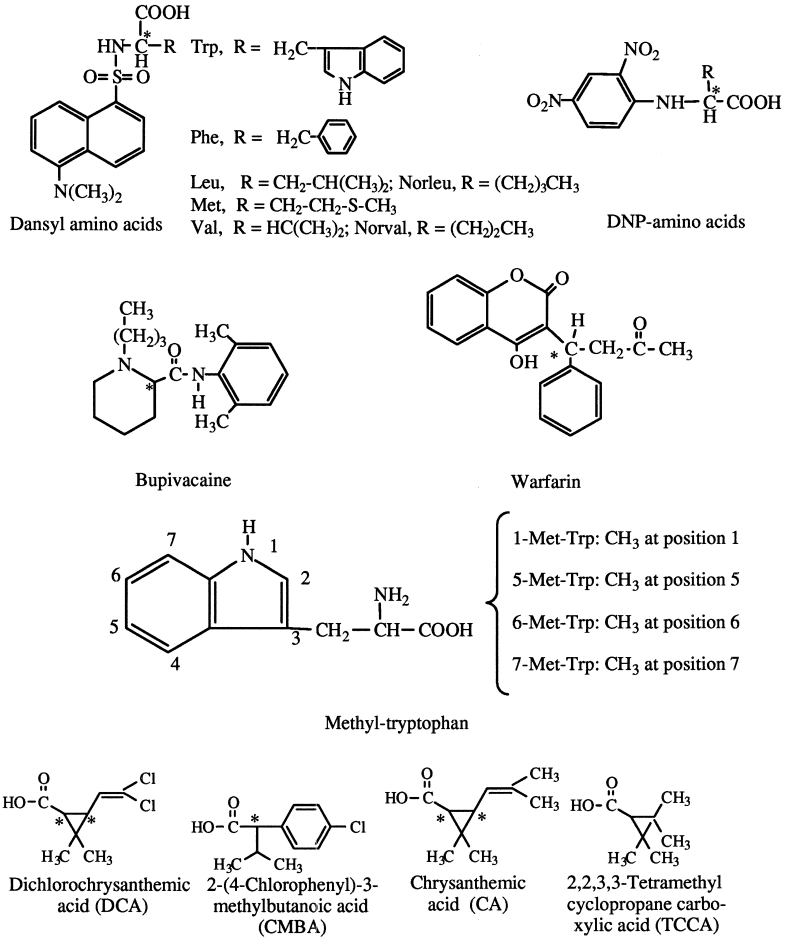


Fig. 2. (*continued*)

effective electrophoretic mobility of the analytes decreased as the concentration of the surfactant increased. This is due to the fact that by increasing [S], the micellized surfactant concentration ([S]−CMC) is increased, and consequently the number of micelles interacting with a given solute at any instant increases. The net result is decreasing the apparent charge-to-mass ratio of the solute. As shown in Fig. 4, silvex, dichlorprop and mecoprop exhibited a sharp decrease in their effective electrophoretic mobilities up to 60 m*M* OG and up to 30 m*M* NG, and this decrease became shallower at higher surfactant concentrations [12]. The effective electrophoretic mobility of the other analytes, 2-(4-chlorophenoxy)propionic acid (2,4-CPPA), 2-(3-chlorophenoxy)propionic acid (2,3-CPPA), and 2-(2chlorophenoxy)propionic acid (2,2-CPPA), decreased almost monotonically with increasing OG and NG concentrations in the concentration range studied. These observations corroborated earlier findings [22] in that the effective electrophoretic mobilities of analyzed molecules in the presence of neutral GSs, e.g., *n*-alkyl-β-D-glucopyranoside (from C_7 to C_{10}), versus the surfactant concentration is an inverse function of the hydrophobicity of the solute.

Assuming a monovalent complex between an enantiomeric solute, E, and a GS micelle, M:

$$M + E \leftrightarrow ME$$

for which the stability constant *K* is given by:

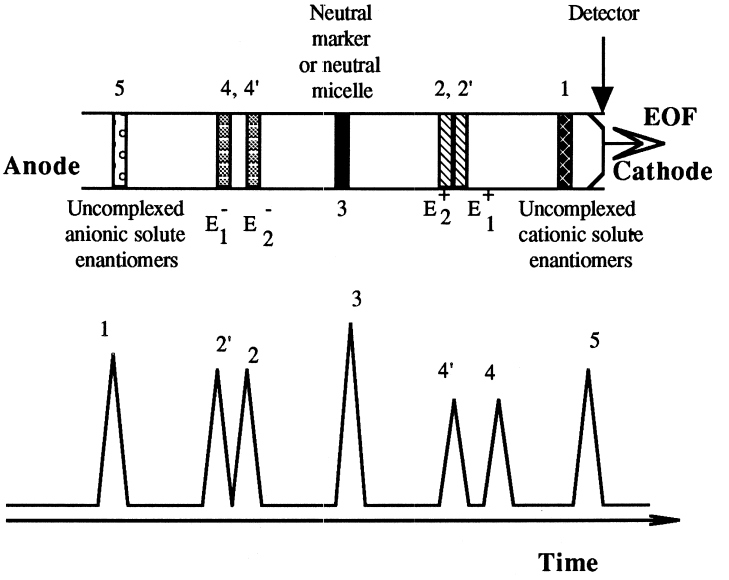


Fig. 3. Principles of separation of charged solutes with neutral GSs.

$$K = \frac{[ME]}{[M][E]} \quad (1)$$

The electrophoretic mobility of the enantiomer, E, in the presence of GS micelle, M, is given by:

$$\mu_{ep} = \mu_{ep}^{0} \cdot \frac{[E]}{[E]+[ME]} + \mu_{eo}^{c} \frac{[ME]}{[E]+[ME]} \quad (2)$$

which can be rearranged to give the following equation:

$$\mu_{ep} = \frac{\mu_{ep}^{0} + K[M]\mu_{eo}^{c}}{1 + K[M]} \quad (3)$$

The concentration of a micelle, [M], is related to the micellized surfactant concentration, [S]−CMC,

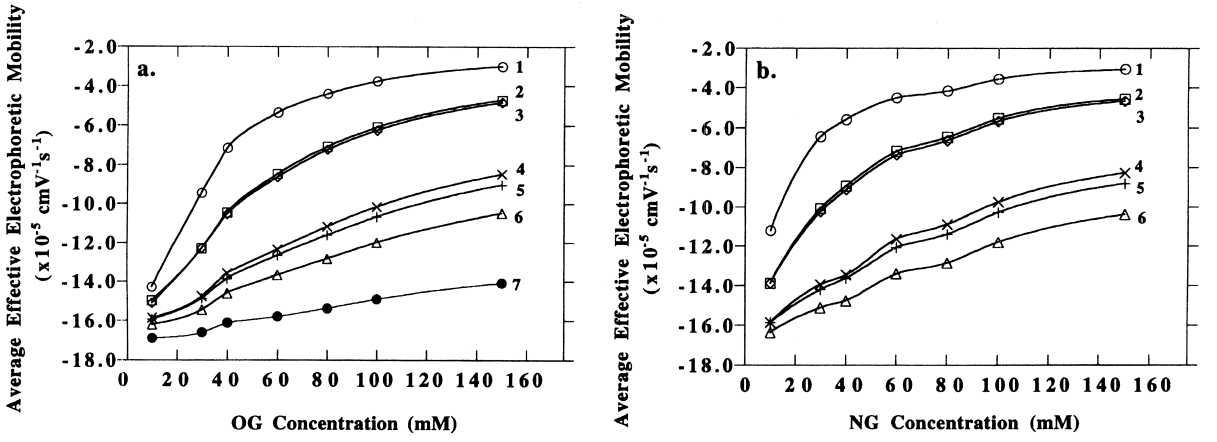


Fig. 4. Plots of the average effective electrophoretic mobility of phenoxy acid herbicides versus the concentration of: (a) OG and (b) NG in the running electrolyte. Conditions: running electrolyte, 200 m*M* sodium phosphate, pH 6.5, containing various concentration of: (a) OG and (b) NG; capillary, 57 cm (50 cm to detection window)×50 μm I.D.; voltage, 20 kV; temperature 15°C. Lines, 1=silvex, 2=dichlorprop, 3=mecoprop, 4=2,4-CPPA, 5=2,3-CPPA, 6=2,2-CPPA, 7=2-PPA. Reproduced with permission from Ref. [12].

through the micelle's aggregation number, *n*, as follows:

$$[M] = \frac{[S] - CMC}{n} \quad (4)$$

Thus, Eq. (3) can be expressed as follows:

$$\mu_{ep} = \frac{\mu_{ep}^{0} + K \cdot \left(\frac{[S] - CMC}{n} \right) \cdot \mu_{eo}^{c}}{1 + K \cdot \left(\frac{[S] - CMC}{n} \right)} \quad (5)$$

Eqs. (3) and (5) are analogous to that describing mobility in the presence of cyclodextrin [35]. μ_{ep}^{0} and μ_{eo}^{c} are the electrophoretic mobilities of an enantiomer at micellized surfactant concentration equal to zero (i.e., [S]=CMC) and infinite (i.e., [S]>>CMC). According to Eqs. (3) and (5), at infinite surfactant concentration, the enantiomer will approach the mobility of the neutral micelle which is that of the EOF. Since the concentration of micelles can be correlated with the concentration of micellized surfactant, see Eq. (4), one can use either Eq. (3) or Eq. (5) in the following discussion.

As stated above, the magnitude of solute association with the GS micelle should increase with increasing solute's hydrophobicity [12]. Therefore, the order of the hydrophobicity of the different phenoxy acid herbicides (for structures, see Fig. 2) seems to decrease in the following order, silvex>> dichlorprop>mecoprop>>2,4-CPPA>2,3-CPPA> 2,2-CPPA>>2-phenoxypropionic acid (2-PPA), which is the reversal of the migration order. This correlates with the number of substituted nonpolar groups (i.e., Cl or CH_3) on the benzene rings. For 2,4-CPPA, 2,3-CPPA and 2,2-CPPA, the position of the chlorine atom seems to influence the net hydrophobicity of the solute. The closer the chloride atom to the oxygen atom the weaker the hydrophobicity of the phenoxy acid herbicides.

Similarly, dansyl amino acids, e.g., dansyl-tryptophan (Dns-Trp), dansyl-phenylalanine (Dns-Phe), dansyl-leucine (Dns-Leu), dansyl-methionine (Dns-Met) and dansyl-valine (Dns-Val), migrated and separated in the order of decreasing hydrophobicity in the presence of OM, OG or NG in the running electrolyte [13]. Also, the same observation was made in the case of CYMAL surfactants [16] with dinitrophenyl (DNP) as well as with Dns-amino acids. With the exception of DNP-lysine (DNP-Lys), the effective electrophoretic mobility of all solutes decreased as the concentration of surfactant increased. The effective electrophoretic mobility of DNP-Lys kept constant in the concentration range studied, indicating that DNP-Lys did not associate with the cyclohexyl-pentyl-β-D-maltoside (CYMAL-5) micelle (i.e., no enantioresolution).

2.2.1.2. Enantioresolution

As stated above, and with only a few exceptions, the presence of the GSs in the micellar form is a prerequisite for chiral recognition. Thus, the enantiomeric separation is achieved when the two enantiomers exhibit different partition coefficients into the chiral micelles. In the presence of an EOF, resolution in CZE is given by [36]:

$$R_s = \frac{\sqrt{N}}{4} \cdot \left(\frac{\Delta \mu_{ep}}{\overline{\mu}_{ep} + \mu_{eo}} \right) \quad (6)$$

where *N* is the number of theoretical plates, $\Delta\mu_{ep}$ is the difference in electrophoretic mobility of two adjacent zones, i.e., two enantiomers, $\overline{\mu}_{ep}$ is the average electrophoretic mobility of two enantiomers and μ_{eo} is the electroosmotic mobility.

The effect of surfactant concentration on the enantiomeric resolution of some phenoxy acid herbicides [12] and Dns-amino acid [16] was investigated. As a typical example, see Fig. 5a and b. As can be seen in this figure, and in the presence of a relatively strong EOF, there is an optimum surfactant concentration for maximum enantiomeric resolution. The value of the optimum surfactant concentration seems to decrease as the extent of the analyte solubilization in the OG micelle increases, which in the case of phenoxy acid herbicides and Dns-amino acids seems to correlate with the hydrophobicity of the analyte. Silvex, the most hydrophobic species among the herbicides studied, attained maximum enantiomeric resolution at 40 m*M* OG (Fig. 5a) and 30 m*M* NG (Fig. 5b), while the enantiomeric resolution of the weakly hydrophobic solutes, namely 2,4-CPPA, 2,3-CPPA and 2,2-CPPA, improved as OG concentration increased (see Fig. 5a and b). For the solutes of intermediate hydrophobicity, e.g., mecoprop and dichlorprop, the enantiomeric resolution of these two solutes reached maximum

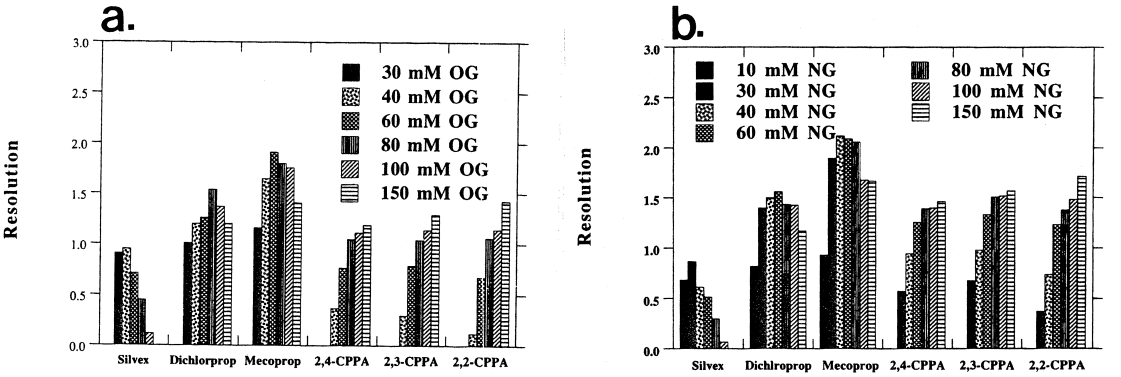


Fig. 5. Bar graphs of the enantiomeric resolution of phenoxy acid herbicides at different concentration of: (a) OG and (b) NG. Experimental conditions: running electrolyte, 200 m*M* sodium phosphate, pH 6.5, containing different concentration of: (a) OG and (b) NG; capillary, 57 cm (50 cm to detection window)×50 μm I.D.; voltage, 20 kV; temperature 15°C. Reproduced with permission from Ref. [12].

values at 60 and 80 m*M* OG (Fig. 5a) and at 40 and 60 m*M* NG (Fig. 5b), respectively [12]. Similarly, the two most hydrophobic Dns-amino acids, namely Dns-Trp and Dns-Phe, reached maximum resolution at 10 to 12 m*M* CYMAL-5 while the enantioresolution of the less hydrophobic Dns-Leu and Dns-Met improved as the CYMAL concentration increased in the concentration range studied [16]. Also, DNP-amino acids, e.g., DNP-Met, DNP-Nle and DNP-Nva, showed a maximum resolution at 10 m*M* CYMAL-5 surfactant [16]. The optimum surfactant concentration for maximum resolution is in the low range for hydrophobic solutes and in the high range for the less hydrophobic ones.

The maximum enantiomeric resolution of 1,1′-binaphthyl-2,2′-diylhydrogen phosphate ($BNPO_4$) was achieved at relatively low surfactant concentration, e.g., 4.0 m*M* CYMAL-5. Because of its relatively high hydrophobicity, the enantioresolution (R_s) of $BNPO_4$ decreased with increasing surfactant concentration from $R_s = 4.0$ at 4 m*M* CYMAL-5 to $R_s = 1.23$ at 18 m*M* CYMAL-5. Baseline separation can be reached even at surfactant concentration lower than the CMC value (e.g., 2 m*M*), but the peaks were broad and asymmetric [16].

The presence of an optimum surfactant concentration for maximum enantioresolution can be attributed primarily to the EOF which moves with it the neutral chiral micellar phase. At relatively low surfactant concentration, increasing the surfactant concentration yields an increase in the micellized surfactant concentration, and in turn an increase in the amount of sites for enantiomeric interaction, thus enhancing enantioresolution via $\Delta\mu_{ep}$ [22] (assuming that N stays the same as the surfactant concentration is changed). Conversely, at elevated surfactant concentration, the solute encounters an increasing number of micelles, and consequently, the effective electrophoretic mobility of the solute approaches that of the EOF, a phenomenon that speeds up the migration of acidic enantiomers and slow down the velocity of positively charged enantiomers. At this point $\Delta\mu_{ep}$ starts decreasing [22] approaching zero at elevated surfactant concentration, and consequently enantiomeric resolution approaches zero too, see Eq. (6). The net result is decreasing enantioresolution at high GS concentration. This is similar to what is usually observed in micellar electrokinetic capillary chromatography (MECC) in the presence of EOF where there is an optimum k' (i.e., surfactant concentration) for maximum resolution of achiral solutes [37,38]. Our results also corroborates the model advanced by Wren and Rowe [39] in that there is an optimum chiral selector concentration for maximum enantiomeric separation in the presence of EOF. However, we strongly believe that the optimum chiral selector concentration for maximum enantioseparation is caused by the presence of EOF as was also demonstrated by Janini et al. [40,41].

In the presence of CYMAL-5, Dns-Trp was enantioseparated only at pH 2.5 [16] (see Fig. 6). As the CYMAL-5 concentration in the running elec-

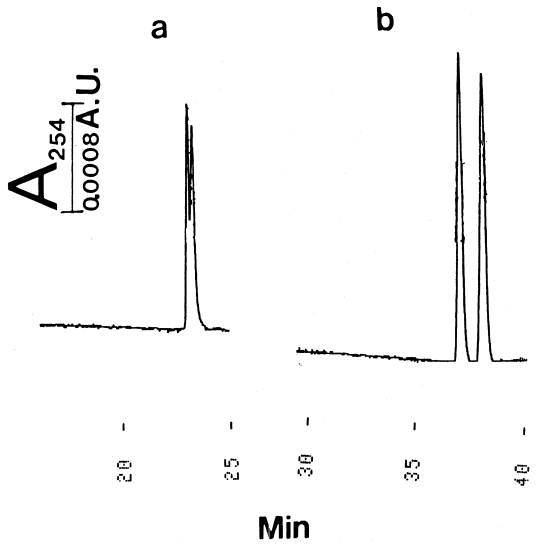


Fig. 6. Electropherograms of Dns-Trp obtained with CYMAL-5. Conditions: running electrolyte, 75 m*M* sodium phosphate, pH 2.5, containing (a) 6 m*M* CYMAL-5, (b) 40 m*M* CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length)× 50 μm I.D. with detection window at 50 cm. Reproduced with permission from Ref. [16].

trolyte was increased, the resolution of Dns-Trp increased from 0.83 to 2.77 when going from 6 to 50 m*M* CYMAL-5. At pH 2.50, the EOF is negligible, and consequently chromatographic conditions prevail in the sense that the neutral CYMAL-5 micelles are virtually not moving. Under these conditions, resolution increases with increasing migration time (i.e., increasing CYMAL-5 concentration) and then level off, as it is usually the case in chromatography [16].

Similarly to Dns-Trp, the methyl-tryptophans (Met-Trps) (see Fig. 2 for structures) were enantioseparated at only low pH, e.g., pH 2.5 [16]. Due to the position of the methyl substituent on the indole ring, 7-Met-Trp, 6-Met-Trp and 5-Met-Trp showed different enantioresolution. 1-Met-Trp can not be resolved enantiomerically indicating that the chiral center of 1-Met-Trp (for structures see Fig. 2) is in a position where the substituted methyl hinders its accessibility. Also, substituting a methyl group at the amine function in 1-Met-Trp suggests that the unsubstituted amino group of the indole ring is essential for achieving chiral recognition. As can be seen in Fig. 7, since the EOF is negligible at pH 2.5, the higher the surfactant concentration the more retarded are the solutes and therefore, resolution keeps increasing with increasing migration time (i.e., chromatographic conditions are prevailing). Fig. 8 illustrates the electropherograms of 6-Met-Trp where it can be seen that at pH 2.5, the enantioresolution increased with a concomitant increase in the migration time from ca. 36 to 43 min for the second enantiomer when going from 60 m*M* to 100 m*M* CYMAL-5.

Other factors were also found to influence the enantiomeric resolution including the nature of the surfactant, the pH and ionic strength of the running electrolyte, the separation temperature, the organic modifier and the modification of the analytes by precolumn derivatization, see below.

2.2.2. Nature of the surfactant

For a given set of enantiomeric solutes, the optimum surfactant concentration depends on the nature of the GSs. For instance, this was illustrated by comparing OG and NG which have the same chiral head group but different alkyl tail. The alkyl chain of NG has one more carbon atom than the alkyl chain of OG, and the CMC value of NG is four-times lower than that of OG (see Fig. 1). As a result, the NG concentration needed to attain enantiomeric resolution is less than that needed for OG (compare Fig. 5a to Fig. 5b) [12]. However, the dependence of enantiomeric resolution on NG concentration followed the same trend as that observed with OG. In other words, optimum enantiomeric resolution was attained for silvex at low NG concentration, for dichlorprop and mecoprop at intermediate NG concentration, and for 2,4-CPPA, 2,3-CPPA, 2,2-CPPA at high NG concentration [12]. Again, no enantiomeric resolution of 2-PPA was observed even at 150 m*M* NG. The electropherograms of the phenoxy acid herbicides obtained at various NG concentrations are illustrated in Fig. 9a, b and c. Note, silvex, dichlorprop and mecoprop were enantiomerically separated using 10 m*M* NG, since the CMC value of NG is 6.5 m*M*. However, at this low surfactant concentration 2,4-CPPA and 2,3-CPPA as well as dichlorprop and mecoprop comigrated. As the concentration of the surfactant increased the migration time of the analytes decreased in a proportion that reflected the hydrophobicity of the analytes. The migration time of

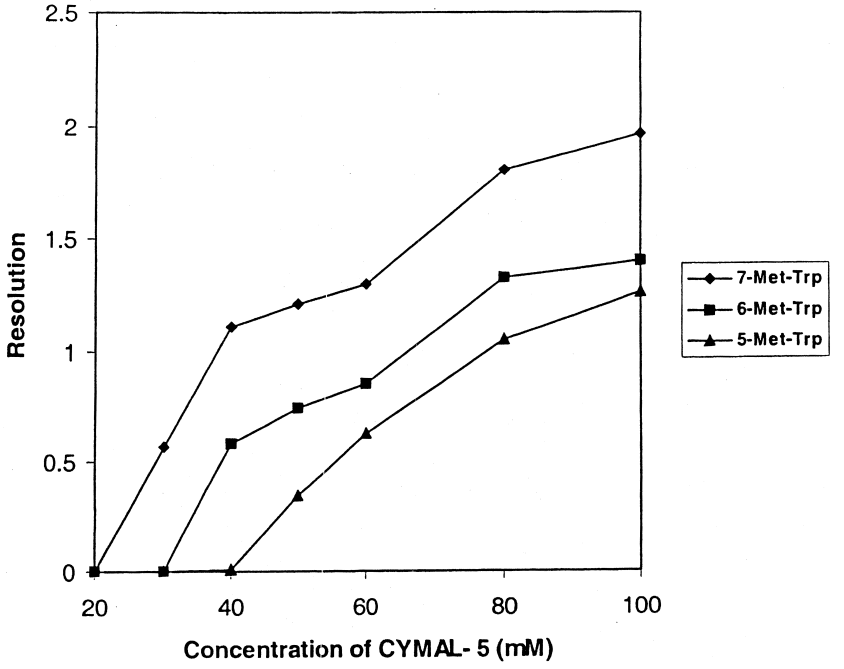


Fig. 7. Effect of CYMAL-5 concentration on the enantiomeric resolution of Met-Trps. Conditions: running electrolyte, 75 m*M* sodium phosphate, pH 2.5, containing various concentrations of CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length)×50 μm I.D. with detection window at 50 cm. Reproduced with permission from Ref. [16].

2-PPA exceeded 45 min at 10 and 60 m*M* NG and decreased substantially to ca. 27 min at 150 m*M* NG.

The three GSs, e.g., OG, NG and OM (see Fig. 1 for structures) were compared in terms of enantiomeric resolution using various model chiral compounds including Dns-amino acids and $BNPO_4$ [13].

When comparing OG, NG and OM, only NG exhibited some enantiomeric resolution towards some Dns-amino acids at 10 m*M* surfactant concentration [13]. This may be attributed to the fact that NG has a CMC value of 6 m*M* while the CMC values of OM and OG are relatively high reaching 23.4 and 25 m*M*, respectively. While OM allowed the enantiomeric separation of the five tested Dns-amino acids, neither OG nor NG permitted the separation of D- and L-Dns-Trp [13]. This difference in enantioselectivity may be attributed to difference in the extent of solute solubilization in the various GS micelles and/or to the presence of an extra glucose moiety in OM.

In the same report [13], It was noted that the other D,L-Dns-amino acids that were examined including dansyl-aspartate (Dns-Asp), dansyl-glutamate (Dns-Glu), dansyl-serine (Dns-Ser) and dansyl-threonine (Dns-Thr), did not exhibit any enantiomeric resolution over a wide range of surfactant concentration. There seems to be an analyte polarity window for chiral recognition by the various chiral micellar phases. Very polar solutes, which do not solubilize sufficiently in the micelle, are not chirally resolved. Also, strongly nonpolar solutes, which exhibit relatively pronounced solubilization into the inner part of the micelle and in turn less probability to interact with the chiral polar head group of the micelle are not chirally resolved as well.

Using $BNPO_4$ as the chiral model solute, the OG, NG and OM surfactants exhibited optimum enantiomeric resolution at relatively low surfactant concentrations [13]. The relatively strong hydrophobic character of $BNPO_4$ allowed sufficient interactions with the OM monomers thus permitting its enantiomeric resolution even at surfactant concentrations lower than the CMC value of OM. This may be also facilitated by the presence of the maltoside sugar

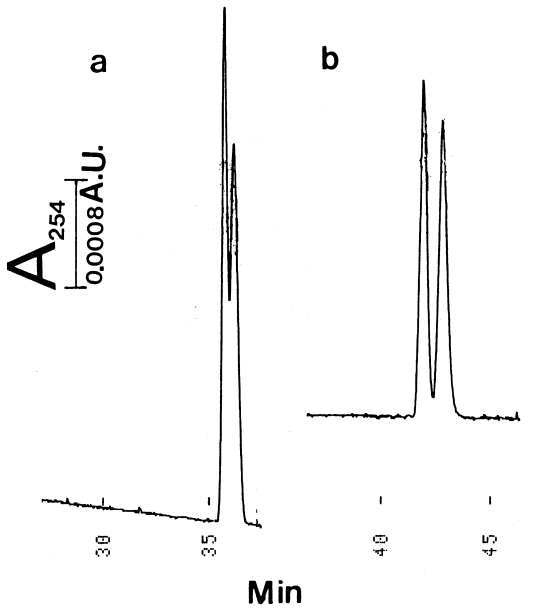


Fig. 8. Electropherograms of 6-Met-Trp obtained with CYMAL-5. Conditions: running electrolyte, 75 m*M* sodium phosphate, pH 2.5, containing (a) 60 m*M* CYMAL-5, (b) 100 m*M* CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length)× 50 μm I.D. with detection window at 50 cm. Reproduced with permission from Ref. [16].

residue in the OM surfactant. However, this was not true for OG and NG where the enantiomeric resolution was only achieved at concentrations above the CMC [13].

2.2.3. pH of the running electrolyte

Since the chiral solutes separated in the presence of the GSs were charged, the pH of the running electrolyte largely influenced enantioresolution. In the case of phenoxy acid herbicides [12], the enantiomeric resolution exhibited by OG and NG decreased as the pH was increased due to the increasing ionization of the phenoxy acid herbicides which is believed to decrease the solute solubilization into the micelle. On the other hand, as the pH increased the EOF increased too, and consequently, the migration time decreased. Thus, pH 6.5 was a compromise in terms of obtaining satisfactory enantiomeric resolution and analysis time [12]. Similar pH effects on the enantiomeric separation of phenoxy acid herbicides were observed with the OM surfactant [23].

A more extensive study on the effect of pH on enantioresolution was performed with CYMAL-5 surfactant using Dns-amino acids, methyl substituted tryptophans (Met-Trps) and $BNPO_4$ as model chiral solutes [16]. Most Dns-amino acids investigated can be separated at pH 6.5. But Dns-Trp can only be resolved at pH 2.5 or 3. Dns-amino acids are ampholytes, and therefore the pH of the running electrolyte will determine their net electric charges. As shown in Fig. 10, at low pH, Dns-amino acids are in the fully protonated form and the predominant ionic species are cations, and as a result, they migrate towards the cathode. At high pH, the Dns-amino acids are in the deprotonated form and the predominant ionic species are anions. Fig. 10 shows the effective electrophoretic mobility at various pH whereby the solute changes from positively to negatively charged passing through neutral as the pH is increased. As can be seen in Fig. 10, the isoelectric points (p*I*) of these solutes seem to be around pH 3.6.

Also, because of the ionic nature of the Met-Trps, the pH had a strong influence on their migration and interaction with the CYMAL-5 micelle. The enantiomeric resolution of Met-Trps is achieved in a narrow pH range, e.g., pH 2.5–3.0. They showed no enantioseparation from pH 6 to 12 over a wide range of CYMAL-5 concentration.

$BNPO_4$ exhibited much higher enantiomeric resolution at low pH (e.g., pH 4.0) than at higher pH (e.g., pH 6.5–8.0). The resolution decreased from 4.6 to 2.5 when going from a pH 4.0 to a pH 6.5–8.0. pH 6.5 seemed to be a good compromise between enantiomeric resolution and analysis time [16].

2.2.4. Ionic strength of the running electrolyte

The effect of the ionic strength of the running electrolyte on the enantiomeric resolution is illustrated in Fig. 11. In all cases, the enantiomeric resolution improved at higher ionic strength [12]. Increasing the ionic strength of the running electrolyte is known to cause a decrease in the CMC of the surfactant and an increase in the aggregation number [42,43]. This will increase the micellized surfactant concentration ([S]−CMC) and in the same time will increase the concentration of chiral centers in the micellar form. In addition, increasing the ionic strength has a salting out effect which

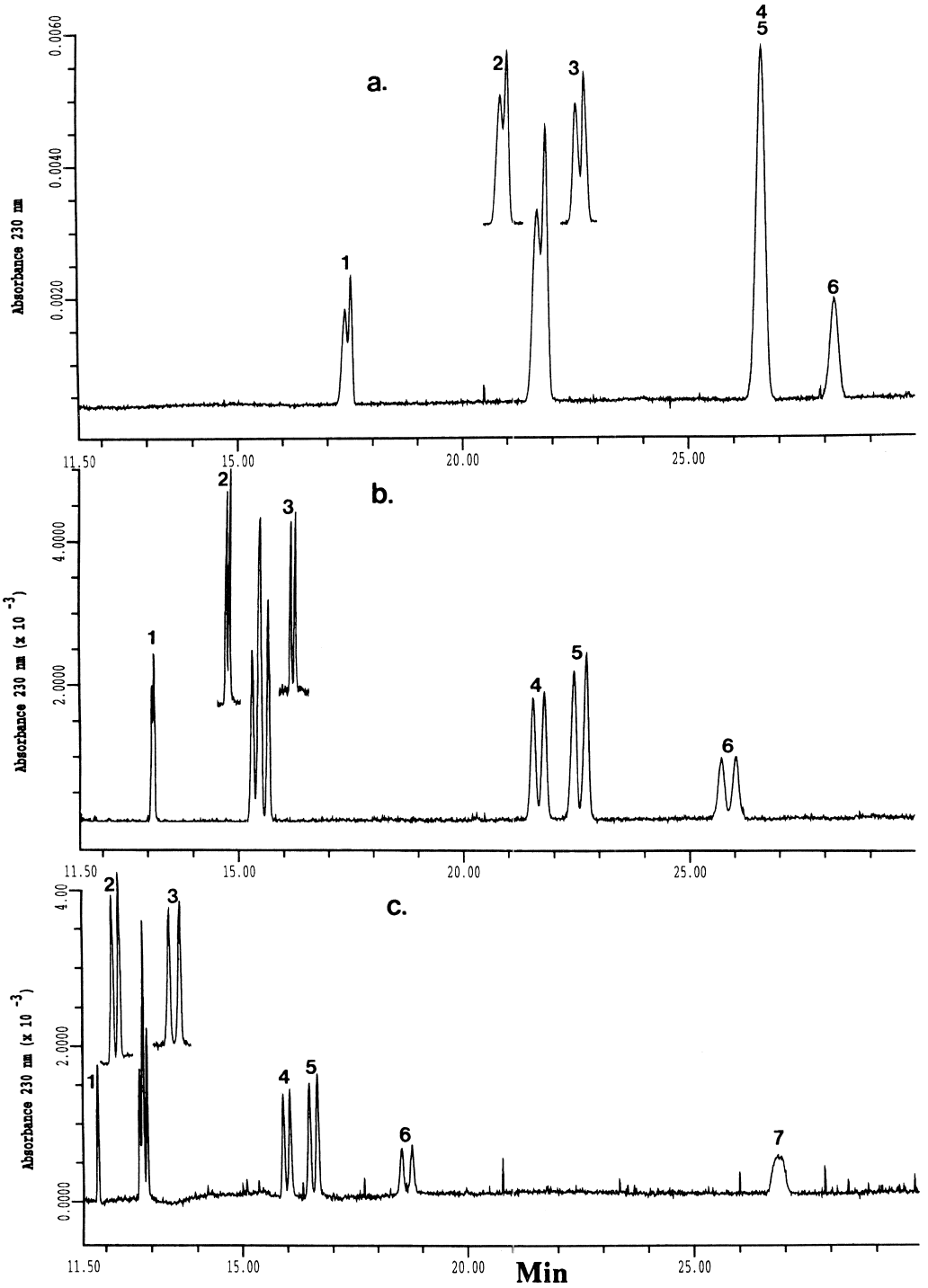


Fig. 9. Electropherograms of phenoxy acid herbicides depicting the effect of NG concentration on enantiomeric resolution. Conditions: running electrolyte, 200 m*M* sodium phosphate, pH 6.5, containing: (a) 10 m*M* NG, (b) 60 m*M* NG and (c) 150 m*M* NG. Other conditions as in Fig. 4. Peaks, 1=silvex, 2=dichlorprop, 3=mecoprop, 4=2,4-CPPA, 5=2,3-CPPA, 6=2,2-CPPA, 7=2-PPA. Reproduced with permission from Ref. [12].

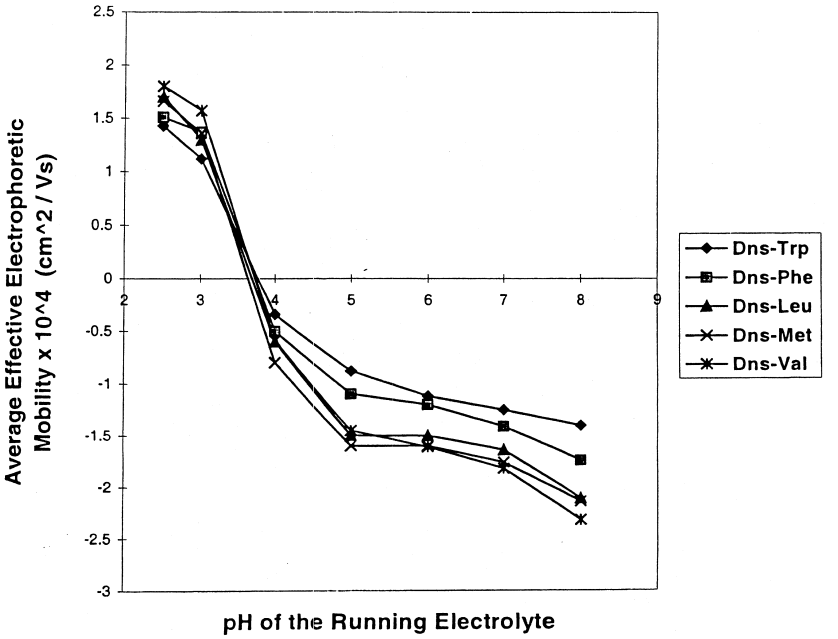


Fig. 10. Effect of pH on the effective electrophoretic mobility of Dns-amino acids. Conditions: running electrolyte, 75 m*M* sodium phosphate, at various pH, containing 6 m*M* CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length)×50 μm I.D. with detection window at 50 cm. Reproduced with permission from Ref. [16].

should afford stronger nonpolar interaction between the solutes and the chiral micelles. The net result is an enhancement in enantioresolution. On the other hand, increasing the ionic strength resulted in longer analysis time [12]. It should be noted that there is a limit for increasing the ionic strength due to the increase in conductivity, and consequently Joule heating at elevated ionic strength. Under the conditions of Fig. 11, 200 m*M* phosphate was the limit to which the ionic strength could be increased without introducing undesirable Joule heating effects.

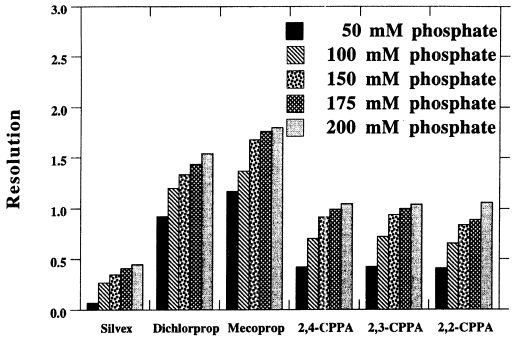


Fig. 11. Bar graphs of the enantiomeric resolution of phenoxy acid herbicides at different ionic strength of the running electrolyte. Conditions: running electrolyte, various sodium phosphate concentrations, pH 6.5, containing 80 m*M* OG. Other experimental conditions as in Fig. 4. Reproduced with permission from Ref. [12].

The same phenomenon (i.e., decrease in CMC, increase in aggregation number and stronger nonpolar interaction) stated above that resulted from increasing the ionic strength yielded different behavior with different solutes and surfactants. In the case of CYMAL surfactants, the ionic strength of the running electrolyte showed an adverse effect on the enantiomeric resolution of Dns-amino acids [16]. That is, increasing the ionic strength of the running electrolyte decreased the enantiomeric resolution of all analytes. With methyl tryptophans, the enantioresolution of 7-Met-Trp, 6-Met-Trp and 5-Met-Trp remained more or less constant at about 1.2, 0.8 and

0.4, respectively, when the ionic strength of the running electrolyte was increased from 75 m*M* to 125 m*M*, and then decreased as the ionic strength continued to increase [16].

2.2.5. Effect of temperature

It has been shown that decreasing the separation temperature increased the enantiomeric resolution of all analytes [12,23]. Temperature has various influences on the electrophoretic system. The effect of temperature on the CMC of surfactants in aqueous medium is complex, the value appearing first to decrease with temperature to some minimum and then to increase with further increase in temperature [44]. The minimum in the CMC–temperature dependence is around 50°C for nonionic surfactants [44] as the GSs. Increasing temperature causes decreased hydration of the hydrophilic group, which favors micellization. This in principle should increase the number of interacting micelles in the temperature range 10 to 30°C and in turn the enantiomeric resolution. However, increasing temperature favors the partitioning of the solute in the aqueous phase, and this may explain the continuous decrease in the enantiomeric resolution of some phenoxy acid herbicides as the temperature was increased from 10 to 30°C. Similar behavior was observed with the OM surfactant toward the phenoxy acid herbicides [23].

2.2.6. Effect of organic modifier

The effect of the percent methanol in the running electrolyte on the enantiomeric resolution of some phenoxy acid herbicides was examined [23]. As expected, enantiomeric resolution was affected differently among the various analytes by the addition of methanol to the separation electrolyte. The effect of methanol correlated satisfactorily with the hydrophobicity of the analytes. For all practical purposes, the CMC and the aggregation number of the micelle can be considered unaffected by the addition of methanol since in the percent range studied (i.e., from 0% to 10%, v/v), there is a slight inhibitory effect of methanol on the micellization process [45]. For silvex, which is the most hydrophobic compound, the enantiomeric resolution improved upon the addition of methanol to the separation electrolyte. This suggests that for this hydrophobic compound, the addition of methanol may have adjusted the degree of solubilization of the solute (probably by a competition effect) into the chiral micelle to favor a better polar interaction with the chiral head group of the surfactant molecule, thus improving the enantioselectivity toward silvex. On the other hand, the least hydrophobic analytes, 2,4-, 2,3- and 2,2-CPPAs, suffered an opposite effect. This would suggest that the addition of methanol has weakened the interaction of the solute with the micelle thus lowering the probability of the solute to encounter the chiral center of the surfactant and associate with. For dichlorprop and mecoprop (solutes of intermediate hydrophobicity), the enantiomeric resolution was slightly affected for dichlorprop and exhibited a maximum for mecoprop. This behavior can be equally explained by the effect of methanol on modulating the degree of solubilization of the solute in the micelle and its subsequent interaction with the chiral polar head group.

At surfactant concentration above the optimal one for maximum enantioresolution, adding a small amount of acetonitrile seems to restore enantioresolution to its maximum probably by a competing effect (see Fig. 12a, b and c) [18]. In other words, the addition of an organic modifier to a surfactant concentration higher than the optimum can have a positive effect on enantioresolution. The electropherogram shown in Fig. 12a contains no organic modifier. Consequently, the CMBA-7-aminonaphthalene-1,3-disulfonic acid (ANDSA) and DCA-ANDSA analytes coeluted (for abbreviation and structures, see Fig. 2). Upon the addition of 10% acetonitrile (Fig. 12c), all three labeled analytes are resolved into their enantiomeric components [18].

2.2.7. Effect of solute tagging with 7-aminonaphthalene-1,3-disulfonic acid on enantiomeric resolution

As mentioned above, it has been observed that there exists a correlation between the extent of solute solubilization into the GS micelle and the enantiomeric resolution [12,23]. In a study involving the behavior of underivatized and ANDSA derivatized phenoxy acid herbicides some significant changes in enantioresolution were observed upon derivatization [14]. The ANDSA derivatized phenoxy acid herbicides acquired a stronger hydrophobic character

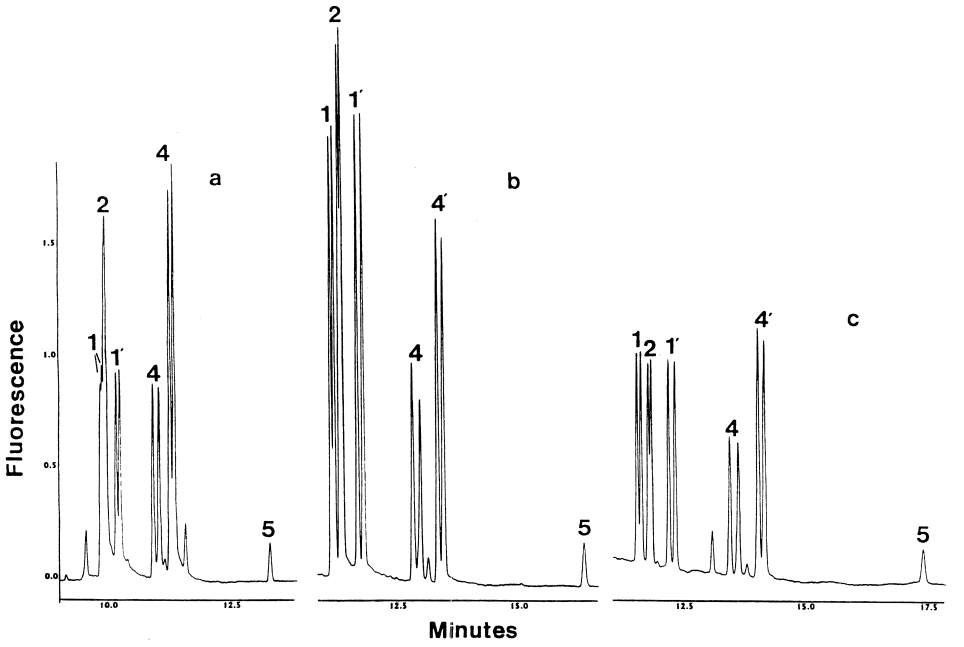


Fig. 12. Electropherograms of the ANDSA derivatives of the hydrolytic products of pyrethroids obtained by laser-induced fluorescence detection. Electrolytes, 100 m*M* sodium phosphate, pH 6.5, containing 70 m*M* OG at 0% in (a), 5% in (b) and 10% (v/v) acetonitrile in (c); 19 kV; 20°C; capillary, fused-silica, 57 cm (50 cm to detection window)×50 μm I.D. Solutes: 1, *cis*-DCA-ANDSA; 1′, *trans*-DCA-ANDSA; 2, CMBA-ANDSA; 4, *cis*-CA-ANDSA; 4′, *trans*-CA-ANDSA; 5, TCCA-ANDSA. Reproduced with permission from Ref. [18].

and two sulfonic acid groups which affected their electrokinetic behavior as well as their chiral recognition by the chiral micelle. One noticeable change is the case of 2-PPA. In fact, underivatized 2-PPA, which is the least hydrophobic phenoxy acid herbicide, was not enantiomerically separated at any OM surfactant concentration while ANDSA-2-PPA resolved enantiomerically very readily. The tagging with ANDSA allowed the molecule to associate with the micelle to a larger extent than in the case of underivatized 2-PPA, a condition that may have favored more encounter among the chiral center of the solute and the chiral head group of the surfactant molecule in the OM micelle, and in turn a better chiral recognition.

As shown in Fig. 13, increasing the hydrophobicity of the analytes through labeling with ANDSA, augmented the analyte–micelle interaction and consequently decreased the migration time as compared to underivatized phenoxy acid herbicides. Although the labeling of the analytes with ANDSA replaced the carboxylic acid group of each phenoxy acid herbicide by two strong sulfonic acid groups thus increasing the negative charges of each analyte from one to two, apparently the increase in hydrophobicity was more pronounced to the extent that the analysis time was almost one half shorter and decreased from ca. 39 min to ca. 22 min, compare Fig. 13a with 13b. As discussed above, another important feature of the derivatization with ANDSA is the fact that 2-PPA which could not be resolved enantiomerically as an underivatized solute at any surfactant concentration is now readily resolved as an ANDSA-2-PPA derivative, see Fig. 13 [14]. Also, 2,4-, 2,3- and 2,2-CPPA which required relatively higher OM concentration to undergo enantiomeric separation [23] are now resolved at lower OM concentration [14]. On the other hand, and as illustrated in Fig. 13, the enantiomeric resolution of dichlorprop and mecoprop decreased as a result of the labeling with ANDSA, and a lower OM concentration is now needed for their enantiomeric resolution. However, unlike underivatized silvex, ANDSA-silvex is not resolved enantiomerically even at very low surfactant concentration [14].

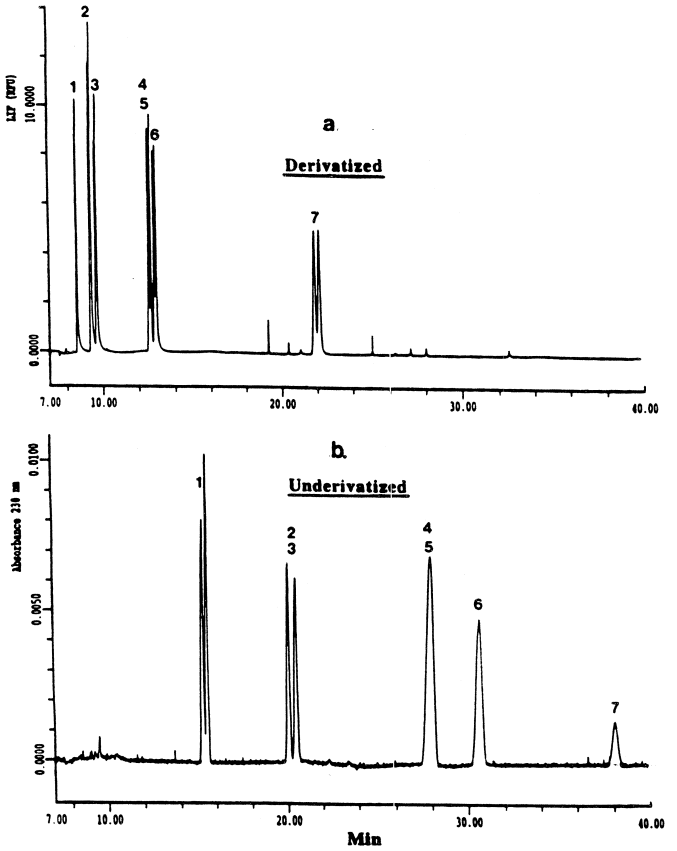
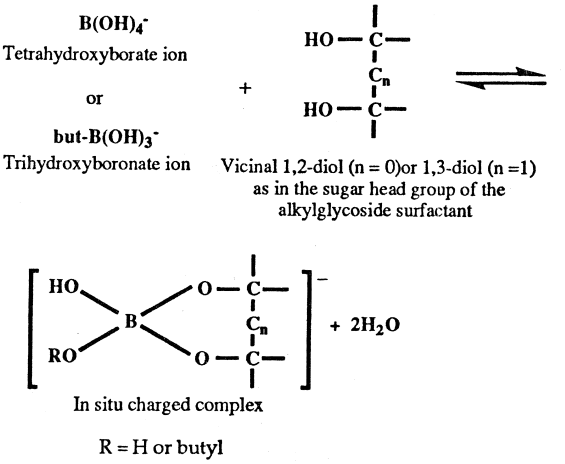


Fig. 13. Electropherograms of derivatized (a) and underivatized (b) phenoxy acid herbicides. Conditions: running electrolyte, 200 m*M* sodium phosphate, pH 6.5, containing 30 m*M* OM; voltage, 25 kV; capillary, bare fused-silica capillary 57 cm (50 cm to detection point)×50 μm I.D.; temperature, 15°C. Peaks in (a), 1=ANDSA-silvex, 2=ANDSA-dichlorprop, 3=ANDSA-mecoprop, 4=ANDSA-2,4-CPPA, 5=ANDSA-2,3-CPPA, 6=ANDSA-2,2-CPPA, 7=ANDSA-2-PPA; peaks in (b), 1=silvex, 2=dichlorprop, 3=mecoprop, 4=2,4-CPPA, 5=2,3-CPPA, 6=2,2-CPPA, 7=2-PPA. Reproduced with permission from Ref. [14].

3. Glycosidic surfactants as in situ charged surfactants – micellar electrokinetic capillary chromatography mode

3.1. Some basic principles

Due to their glycosidic head group, GSs possess the ability to be charged in situ via complexation with borate or boronate (e.g., butylboronic acid, BBA) based electrolyte systems [24–30]. The complex formation between borate/boronate ions and polyhydroxy compounds, e.g., the sugar head group of the GSs, can be represented by the following reaction (I) whose other details were recently summarized in Ref. [46]

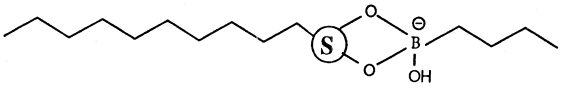


The in situ charging of GSs by borate/boronate complexation allowed the adjustment of the surface charge density of their micelles, and consequently the migration time window of the GS micellar systems. The overall surface charge density of the micelle, ρ_{mc}, can be expressed as [25]

$$\rho_{mc} = \frac{\rho_{mc-c}}{1 + \frac{[GS]}{[GS \cdot B]}} \quad (7)$$

where ρ_{mc-c} is the limiting charge density of the GS–borate (or –boronate) micelle, [GS·B] is the total concentration of the complexed GS, and [GS] is the concentration of uncomplexed surfactant. According to Eq. (7), at constant surfactant concentration, any increase in the borate (or boronate) concentration or pH will result in a decrease in the ratio [GS]/[GS·B], and therefore a larger ρ_{mc}. At constant pH and borate/boronate concentration, an increase in the surfactant concentration will yield an increase in the ratio [GS]/[GS·B] at [B]<[GS], and as a result, ρ_{mc} will decrease, see Eq. (7). On the other hand, at [B]>[GS], an increase in the surfactant concentration will yield an increase in the ratio [GS]/[GS·B], and as a result, ρ_{mc} will increase, see Eq. (7). The effect of surfactant concentration was confirmed by experimental studies using ^{11}B nuclear magnetic resonance (NMR) [27]. These readily tuned features of the micelles allowed the tailoring of the elution range for a given separation problem.

In the case of BBA complexation, both the charge density as well as the hydrophobic character of the micelle can be altered dynamically [28]. Due to the presence of an alkyl tail in BBA, the resulting GS–BBA complex can be viewed as a branched surfactant whose polar head group has dynamically moved to a more central position as follows:



where S represents the sugar polar head group of the surfactant. The CMC of the dynamically branched surfactant–BBA complex will be lower than that of the original surfactant but higher than the CMC of an unbranched surfactant (i.e., all carbon atoms are in one tail) having the same number of carbon atoms [44]. For simplicity, we will use the word borate to designate both borate and boronate ions throughout the article, and when it is necessary, we will make a distinction.

The following summarizes the basic relationships upon which the concept of in situ charged micelles is based. An important variable in MECC is the elution range parameter defined by the ratio [47]:

$$\frac{t_0}{t_{mc}} = \frac{\nu_{mc}}{\nu_{eo}} = 1 - \frac{2\zeta_{mc}}{3\zeta_c} \cdot f(\kappa a) \quad (8)$$

where ν_{mc} is the apparent velocity of the micelle, ν_{eo} is the electroosmotic velocity of the aqueous phase, ζ_c and ζ_{mc} are the zeta potentials of the inner surface of the capillary and of the outer surface of the micelle, respectively, $f(\kappa a)$ depends on the shape of the micelle [48], a is the radius of the micelle and κ is the familiar Debye–Hückel constant. The ζ potentials can be expressed by the following relationship [49]

$$\zeta = \frac{4\pi\delta\rho}{\epsilon} \quad (9)$$

where ρ is the surface charge density of either the capillary surface or the micelle, and δ is the thickness of the diffuse double layer adjacent to either the capillary wall or the micelle surface.

According to Eq. (8), the elution range parameter can be varied conveniently by changing the charge density of the micelle and/or that of the capillary inner surface. One of the characteristics of the GS–borate micellar systems is that the surface charge density of the micelle can be readily adjusted through pH, borate concentration and surfactant concentration [25], see Eq. (7).

With GS–borate complexes, the magnitude of the migration time window can be altered through t_{mc} over a wide range of pH extending from pH 3.5 to pH 11 [24]. Also, the breadth of the migration time window can be varied while keeping the capacity factor, k', of neutral solutes constant. This is readily achieved by varying the pH or the borate concentration at fixed surfactant concentration. Under these conditions, and for a given value of k' (i.e., surfactant concentration), the larger the migration time window (i.e., the smaller the ratio t_0/t_{mc}), the better the resolution and peak capacity.

With in situ charged GSs as with charged surfac-

tants, e.g., sodium dodecyl sulfate (SDS), resolution optimization can be achieved through k' (i.e., surfactant concentration) in accordance with Eq. (10) [50,51]

$$k'_{opt} = \left(\frac{t_{mc}}{t_0}\right)^{1/2} \quad (10)$$

Usually, for a pair of solutes of low retention, the optimum k' for maximum resolution is obtained at relatively high surfactant concentration, whereas the maximum resolution for a pair of solutes of higher retention is at lower surfactant concentration [25]. Thus, the optimization of resolution for various pairs of solutes in a multicomponent mixture cannot be effectively achieved through k', i.e., through surfactant concentration. With the in situ charged GS micellar systems, for each k' value (i.e., surfactant concentration) it is possible to obtain a maximum resolution by adjusting the value of t_{mc} [25] over a wide range via the manipulation of the pH and borate concentration.

Thus far, among the various GSs, only OM and *N*,*N*-bis-(3-D-glucamidopropyl)cholamide (Big CHAP) and *N*,*N*-bis-(3-D-glucamidopropyl)deoxycholamide (Deoxy Big CHAP) have been evaluated in MECC of chiral compounds.

3.2. OM surfactant

The electrokinetic properties of the OM–borate micelle were described in details under various operating conditions including the pH, and borate and surfactant concentration [18,26,29]. The OM–borate micellar system was evaluated in the enantiomeric separation of warfarin and bupivacaine.

Warfarin, an anticoagulant, was enantiomerically resolved using the OM–borate complex surfactant. Warfarin is a weak acid (pK_a of the phenolic group = 5.1 [52]). Since the analysis was performed at pH 6.5, high borate concentration was needed to charge the micelle [24]. Optimum enantiomeric resolution of warfarin was achieved using a buffer consisting of 250 m*M* sodium phosphate and 400 m*M* sodium borate, pH 6.5, containing 60 m*M* OM (Fig. 14).

The enantiomeric resolution of bupivacaine, a

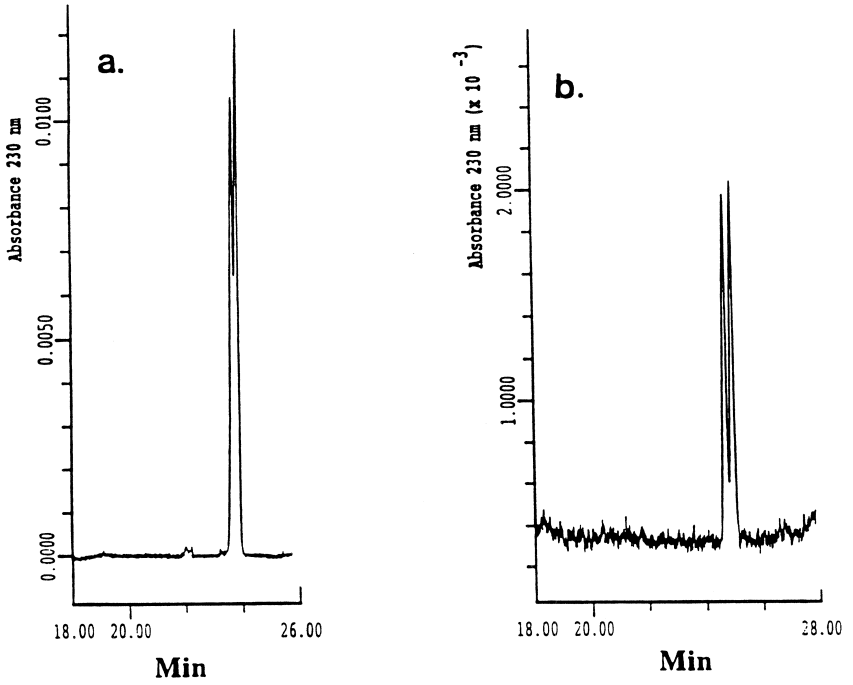


Fig. 14. Electropherograms of warfarin. Conditions: running electrolytes, 400 m*M* sodium borate, pH 6.5, containing 60 m*M* OM and (a) 175 m*M* sodium phosphate or (b) 250 m*M* sodium phosphate; capillary, 57 cm (50 cm to detection window)×50 μm I.D.; temperature, 15°C; voltage, 20 kV. Reproduced with permission from Ref. [13].

local anesthetic solute, was only attainable at a relatively very high OM concentration, i.e., 150 m*M*, and a relatively high ionic strength. As shown in Fig. 15, optimum enantiomeric resolution was attained using a running electrolyte consisting of 200 m*M* sodium phosphate and 400 m*M* sodium borate, pH 6.5, containing 150 m*M* OM. Increasing the ionic strength leads to (i) decreasing the extent of ion-pairing between the positively charged solute and the negatively charged micelle and (ii) to increasing the magnitude of solute nonpolar interaction with the micelle. Also, increasing the ionic strength increases the micellized surfactant concentration. All these effects, arising from the use of a high ionic strength, seem to lead to an adequate chiral interaction between the solute and the OM–borate micelle. Also, increasing the ionic strength slows down the electrophoretic mobility of the bupivacaine solute and the EOF, and as a result the migration time is increased from ca. 7.5 to 11.5 min when going from 50 to 200 m*M* sodium phosphate (Fig. 15).

3.3. Steroidal-glycosidic surfactants

Like all other GSs, Big CHAP and Deoxy Big CHAP surfactants can be charged in situ via the complexation of their polyolic polar head groups with borate. This have rendered the steroidal glycoside surfactants very attractive for difficult separations such as racemates [15].

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

The chiral recognition and selectivity of the steroidal GSs in chiral MECC were evaluated using three different binaphthylic compounds as the model enantiomers under various conditions including the

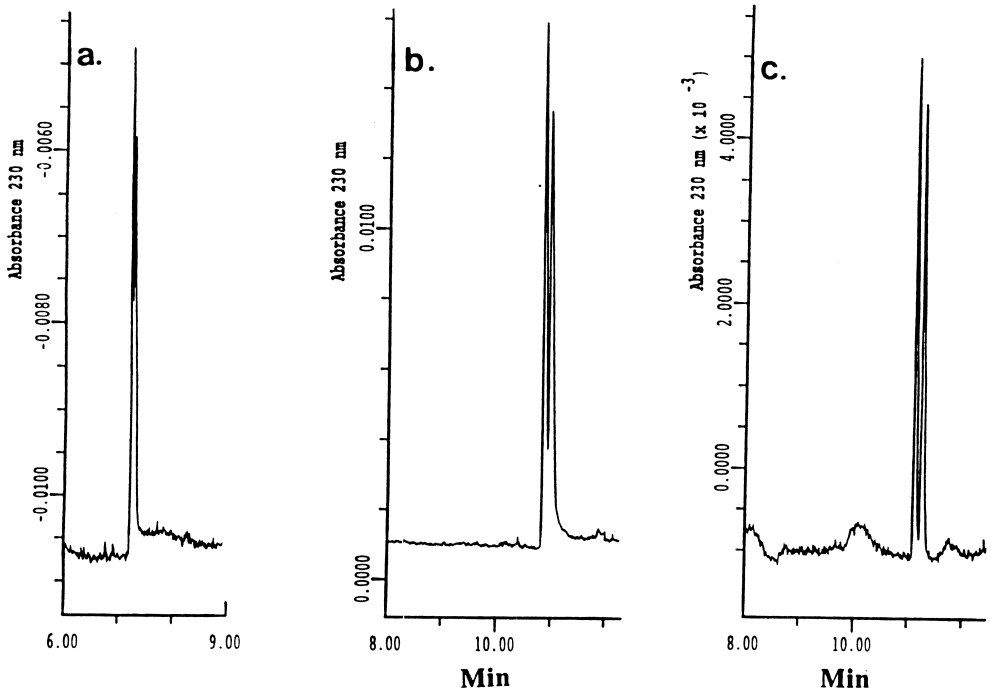


Fig. 15. Electropherograms of bupivacaine. Conditions, running electrolytes, 400 m*M* borate, pH 6.5, containing 150 m*M* OM and (a) 50 m*M* sodium phosphate, (b) 150 m*M* sodium phosphate or (c) 200 m*M* sodium phosphate; voltage, 20 kV. Other experimental conditions as in Fig. 14. Reproduced with permission from Ref. [13].

pH of the running electrolyte, borate and surfactant concentration, amount and nature of organic modifier and capillary temperature [15].

3.3.1.1. Electrolyte pH

Fig. 16 illustrates the relationship between the breadth of the migration time window and the pH of the running electrolyte. t_{mc} increased sharply with pH due to increasing the surface charge density of the micelle, which resulted from increasing the concentration of the Big CHAP–borate complexes at higher pH values, see reaction scheme I. On the other hand, t_0 increased only slightly over the pH range studied (pH 8.0 to 11.5) due to the increase in the ionic strength of the running electrolyte at higher pH. 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 [24–29].

The effect of pH of the running electrolyte on the enantiomeric resolution of BNOH using the Deoxy Big CHAP surfactant was examined [15]. The enantiomeric resolution of BNOH racemic mixture increased from an $R_s = 0.90$ 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 as the pH of the running electrolyte increased (Fig. 17). R_s increased from 1.95 at pH 8.0 to 2.20 at pH 9, 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 $BNPO_4$ enantiomers was inverted at pH 11.0 (see Fig. 17). In general, the increase in resolution as the pH is increased is the result of increasing the magnitude of the migration time window of the micellar system. Since increasing the pH results in increasing the electrophoretic velocity of the micelle in the opposite direction to the EOF, which in turn leads to increasing t_{mc}, the enantiomer which associates more with the micelle migrates slower than the one showing less affinity to the micelle, and consequently the migration order of the enantiomers is inverted.

Under the same operating conditions as in the preceding experiments, Big CHAP showed lower enantiomeric resolution than Deoxy Big CHAP [15].

3.3.1.2. Borate concentration

The effect of borate concentration on the breadth of the migration time window is illustrated in Fig. 18. The sharp increase in t_{mc} is due to the increase in the charge density of the micelle as a result of increasing the Big CHAP–borate complex concen-

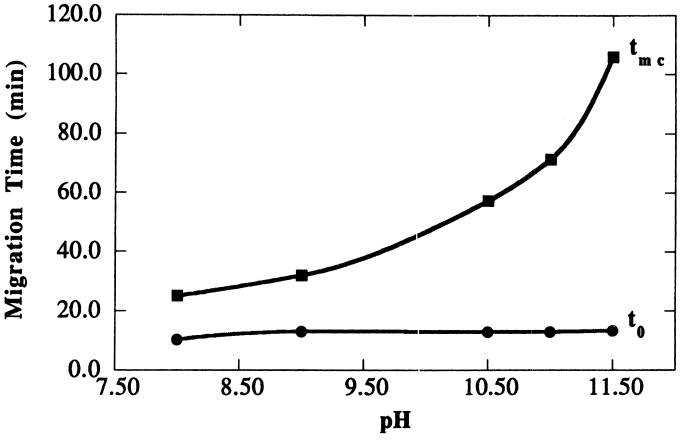


Fig. 16. Effect of pH on the magnitude of the migration time window. Capillary, untreated fused-silica, 57 cm (50 cm to detection window)×50 μm I.D.; running electrolytes, 46 m*M* Big CHAP, 200 m*M* borate at various pH; running voltage, 15.0 kV; tracers, Sudan III (for t_{mc}) and methanol (for t_0). Reproduced with permission from Ref. [15].

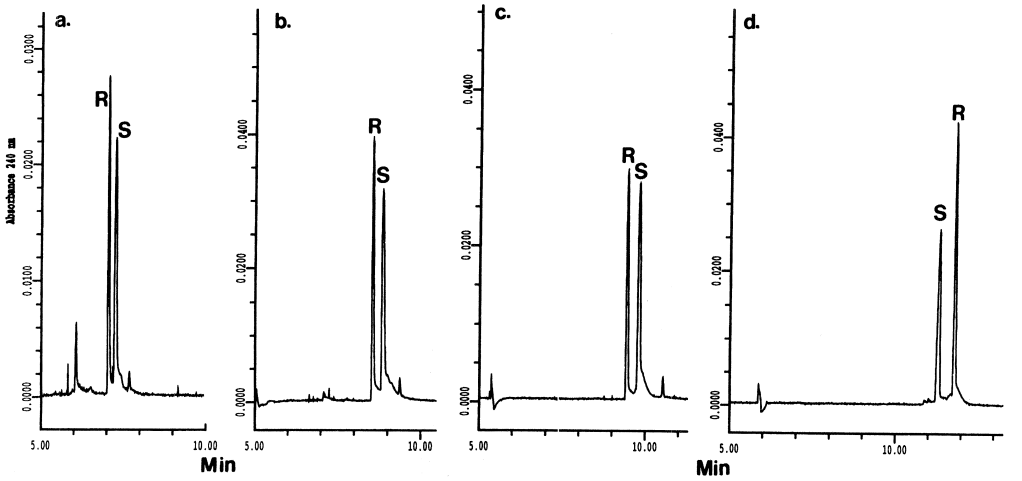


Fig. 17. Electropherograms of standard $BNPO_4$ enantiomers obtained with Deoxy Big CHAP at various pH: 8.0 (a), 9.0 (b), 10.0 (c) and 11.0 (d). Capillary, untreated fused-silica, 57 cm (50 cm to detection window)×50 μm I.D.; running electrolytes, 15 m*M* Deoxy Big CHAP, 50 m*M* borate at pH 8.0 (a), 9.0 (b) and 10.0 (c); capillary temperature, 15°C; voltage, 20 kV. Reproduced with permission from Ref. [15].

tration caused by the increase in borate concentration, see reaction scheme I. 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 [15].

As shown in Fig. 19, the concentration of borate in the running electrolyte largely influenced enantiomeric resolution. At 25 m*M* borate, 1,1′-binaphthyl-2,2′-diamine (BNDA) enantiomers almost coeluted. By doubling the concentration of borate to 50 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 m*M* borate. The same trend was observed for BNOH enantiomers with the difference that even at 25 m*M*, R_s was nearing unity and then kept rising and reached a value of almost 2.0 at 150 m*M* borate. In the case of $BNPO_4$, even at 25 m*M* borate the enantiomeric resolution was quite high (R_s = 1.86). This is because for a charged solute, such as $BNPO_4$, that is strongly associating with the chiral surfactant, there is virtually no need to charge the micelle to obtain baseline resolution.

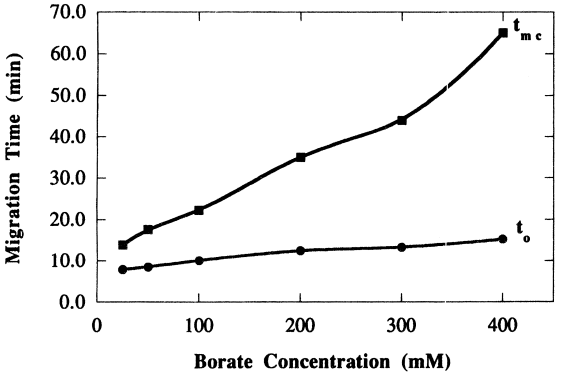


Fig. 18. Effect of borate concentration on the magnitude of the migration time window. Running electrolytes, 9 m*M* Big CHAP at various concentrations of borate, pH 10. Other conditions as in Fig. 16. Reproduced with permission from Ref. [15].

Again, Big CHAP surfactant afforded lower enantioselectivity than Deoxy Big CHAP at various borate concentration under otherwise the same running conditions [15].

3.3.1.3. Surfactant concentration

Fig. 20 illustrates the effect of the surfactant concentration on the enantiomeric resolution of BNOH. Maximum enantiomeric resolution was achieved when the concentration of Deoxy Big CHAP was 10 m*M*. It increased from an R_s = 1.0 at 5 m*M* surfactant to an R_s = 1.7 at 10 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. 20c. At 20 m*M* R_s

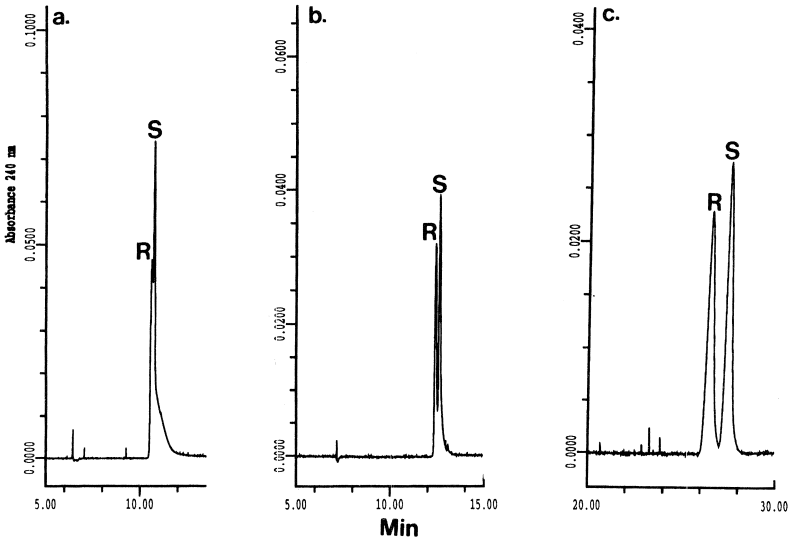


Fig. 19. Electropherograms of BNDA enantiomers obtained with Deoxy Big CHAP at various concentrations of borate in the running electrolytes. Capillary, untreated fused-silica, 57 cm (50 cm to detection window)×50 μm I.D.; running electrolytes, 25 m*M* (a), 50 m*M* (b), or 150 m*M* (c) sodium borate containing 15 m*M* Deoxy Big CHAP (pH 10.0) and 10% (v/v) methanol; capillary temperature, 15°C; voltage, 20 kV. Reproduced with permission from Ref. [15].

decreased further to 1.1 and at 25 m*M* and above no enantiomeric resolution was observed. $BNPO_4$ showed a maximum enantiomeric resolution ($R_s \approx$ 2.5) when 10 to 15 m*M* Deoxy Big CHAP was used. This is a substantial increase from a value of 1.5 that was observed at 5 m*M* surfactant. The resolution

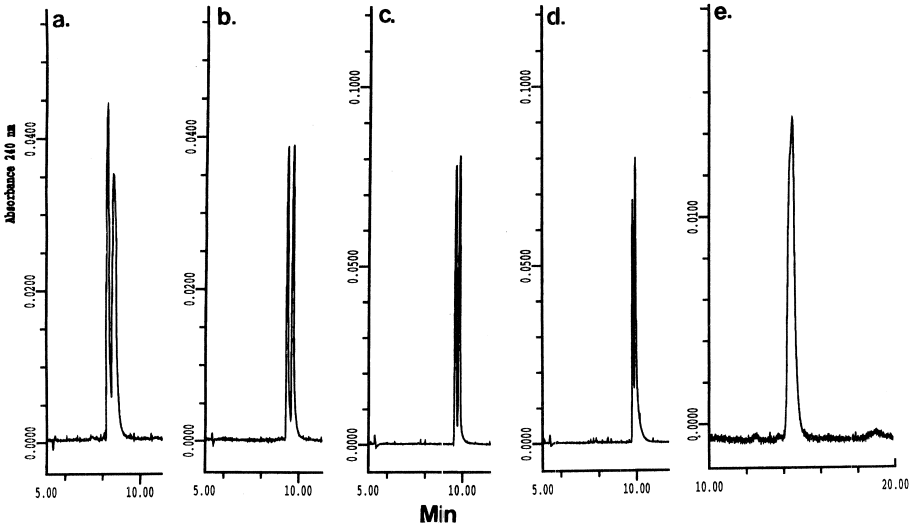


Fig. 20. Electropherograms of BNOH obtained at various concentrations of Deoxy Big CHAP in the running electrolytes. Capillary, untreated fused-silica, 57 cm (50 cm to detector)×50 μm I.D.; running electrolytes, 50.0 m*M* sodium borate containing 5.0 m*M* (a), 10.0 m*M* (b), 15.0 m*M* (c), 20.0 m*M* (d) or 25.0 m*M* (e) Deoxy Big CHAP, pH 10.0; capillary temperature, 15.0°C; voltage, 20.0 kV. Reproduced with permission from Ref. [15].

decreased to 1.8 when going to 20 m*M* and reached a value of 1.3 at 30 m*M* surfactant [15].

Big CHAP also showed maximum enantiomeric resolution at 20 m*M* surfactant in the running electrolyte, but the chiral selectivity of this surfactant was substantially less than that of the Deoxy Big CHAP [15].

3.3.1.4. Organic modifier

Using Deoxy Big CHAP as the chiral surfactant, the enantiomeric resolution of BNDA increased substantially with increasing the percentage of methanol in the running electrolyte. Very little enantiomeric resolution was achieved at 0% and 5% methanol in the running electrolyte, and an almost baseline resolution was obtained at 10% ($R_s = 1.3$) and 15% 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 MeOH for up to 10% had virtually no effect on resolution [15].

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 [15].

3.3.1.5. Capillary temperature

The two steroidal GSs seem to exhibit greater enantioselectivity towards flat and rigid molecules such as the binaphthyls. This observation corroborates earlier findings with chiral bile salt surfactants [54], which have structural features similar to Big CHAP and Deoxy Big CHAP through the steroidal part of the surfactant molecule. Thus, temperature should play a major role in the enantiomeric resolution since temperature affects solute rigidity. In fact, the resolution between BNDA enantiomers decreased form 1.67 to 0.89 when going from 15°C to 35°C passing through $R_s = 1.2$ at 25°C. On the other hand, the enantiomeric resolution ($R_s \approx 2.0$) of BNOH was almost unaffected by the temperature [15].

3.3.2. Illustrative enantiomeric separations

3.3.2.1. Troger's base

The enantiomeric resolution of Troger's base required different conditions than those optimal for the separations of the enantiomers of the binaphthyl derivatives described above. Higher surfactant and borate concentrations as well as organic solvents were needed to induce different enantiomeric association 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 as compared to methanol [15].

3.3.2.2. Dansyl amino acids

Four pairs of DL-Dns-amino acids, namely leucine, methionine, phenylalanine and tryptophan, were enantioseparated with Deoxy Big CHAP [15]. The separation of Dns-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 at very acidic pH only [53].

3.3.2.3. Silvex herbicide

Enantiomeric separation of silvex was achieved using high borate concentration in the running electrolyte and relatively high surfactant concentration. 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 Dns-amino acids [15].

4. Modified glycosidic surfactants with monophosphate or monosulfate groups – micellar electrokinetic capillary chromatography mode

Tickle et al. [19] synthesized dodecyl-β-D-glucopyranoside monophosphate (DGMP) and monosulfate (DGMS) anionic surfactants for use in MECC for the separation of enantiomers (for structures, see Fig. 1a). DGMP and DGMS were found to have a relatively low CMC of 0.5 and 1.0 m*M*, respectively. The anionic character of these two surfactants is imparted by the replacement of the hydroxy group at C6 in the glucose ring with either a sulfate or a phosphate group. Linking the phosphate group to C4 of the glucose residue in DGMP resulted in a more rigid bicyclic structure and a dialkyl phosphate with

a low pK_a value in the range 1 to 2 which is comparable with that of the sulfate group in DGMS [19].

The potentials of DGMP and DGMS were evaluated with various enantiomeric solutes varying in structural complexity, hydrophobicity and ionic character including some Dns-amino acids, $BNPO_4$, BNOH, Troger's base, ephedrine, metoprolol, hexobarbital, phenobarbital and fenoldopam. In all cases, DGMP exhibited a greater potential as a chiral selector than DGMS. However, the two chiral surfactants were complimentary to each other in the sense that enantiomers that were not resolved with DGMP were successfully separated with DGMS [19].

5. Other glycosidic surfactants used in mixed micellar systems – micellar electrokinetic capillary chromatography mode

Digitonin [21], and saponins such as glycyrrhizic acid and β-escin [20] have found limited use in MECC of enantiomers. These three surfactants have a steroidal hydrophobic core structure bonded to carbohydrates (for structures, see Fig. 1b). Digitonin is a neutral surfactant over a wide range of pH, and consequently it is practically insoluble in water. However, digitonin can be solubilized in an SDS micellar phase. The resulting mixed micelle has been used at pH 3.0 for the enantiomeric separations of D- and L-phenylhydantoin amino acids (D,L-PTH-amino acids) [21]. On the other hand, glycyrrhizic acid and β-escin are anionic surfactants. They have been used in mixed micelle systems containing OG and SDS in phosphate–borate buffers [20] in the separation of some Dns-amino acids and PTH-amino acids. The major disadvantage of such mixed micellar systems is their tendency to form gels.

6. Conclusions

This review article summarized the progress made so far in the area of chiral separation by CE in the presence of chiral GSs. Clearly, the GSs have offered a wide range of enantioselectivity due to the diversity of their structures. Also, the GSs have the virtue to be used in the CZE and the MECC modes over a wide range of operating conditions, thus facilitating the separations of various types of enantiomers. This represents an advance in the area of chiral CE by enlarging its scope of applications.

7. Abbreviations

ANDSA	7-Aminonaphthalene-1,3-disulfonic acid
Asp	Aspartate
BBA	Butylboronic acid
Big CHAP	*N*,*N*-Bis-(3-D-glucoamidopropyl)-cholamide
BNDA	1,1′-Binaphthyl-2,2′-diamine
BNOH	1,1′-Bi-2-naphthol
$BNPO_4$	1,1′-Binaphthyl-2,2′-diylhydrogen phosphate
CE	Capillary electrophoresis
CMBA	2-(4-Chlorophenyl)-3-methylbutanoic acid
CMC	Critical micellar concentration
2,2-CPPA	2-(2-Chlorophenoxy)propionic acid
2,3-CPPA	2-(3-Chlorophenoxy)propionic acid
2,4-CPPA	2-(4-Chlorophenoxy)propionic acid
CYMAL	Cycloalkyl-β-D-maltoside
CZE	Capillary zone electrophoresis
Deoxy Big CHAP	*N*,*N*-Bis-(3-D-glucoamidopropyl)-deoxycholamide
DCA	Dichlorochrysanthemic acid
DGMP	Dodecyl-β-D-glucopyranoside monophosphate
DGMS	Dodecyl-β-D-glucopyranoside monosulfate
Dns-amino acids	Dansyl (=5-dimethylamino-naphthalene-1-sulfonyl) amino acids
DNP-amino acids	Dinitrophenyl amino acids
E	Enantiomer
EOF	Electroosmotic flow
Glu	Glutamate
GSs	Glycosidic surfactant(s)
GS·B	Glycosidic surfactant–borate complex
Leu	Leucine

Lys	Lysine
[M]	Micelle concentration
MECC	Micellar electrokinetic capillary chromatography
Met	Methionine
Met-Trps	Methyl tryptophans
MeOH	Methanol
NG	Nonyl-β-D-glucopyranoside
Nle	Norleucine
Nva	Norvaline
OG	Octyl-β-D-glucopyranoside
OM	Octyl-β-D-maltopyranoside
2-PPA	2-Phenoxypropionic acid
PTH-amino acids	Phenylhydantoin amino acids
R_s	Resolution
[S]	Surfactant concentration
SDS	Sodium dodecyl sulfate
Ser	Serine
[S]−CMC	Micellized surfactant concentration
Thr	Threonine
Trp	Tryptophan
Val	Valine

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