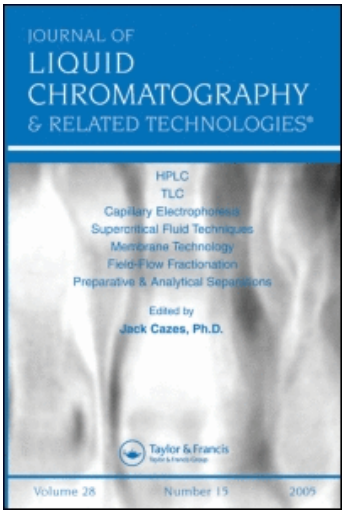

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Enantiomeric Separation of Isochromene Derivatives by Cyclodextrin-Modified Micellar Capillary Electrophoresis

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Abstract: The enantiomeric separations of highly hydrophobic isochromene derivatives were performed and optimized using cyclodextrin-modified micellar capillary electrophoresis. Hydroxypropyl-γ-cyclodextrin proved to be the most effective chiral selector for the enantioseparation of these analytes. The effects of cyclodextrin and sodium dodecyl sulfate concentration and organic modifier were examined in order to optimize the separation conditions. Addition of an organic solvent modifier to the run buffer served to increase the analytes' solubility and enhance the separation efficiency. A highly acidic pH was necessary to effectively suppress the electroosmotic flow when operating in the reverse polarity mode.

Keywords: Capillary electrophoresis, Chiral separation, Cyclodextrin, Isochromene derivatives, Micelle

INTRODUCTION

Isochromene derivatives (Figure 1) exist widely in nature and make up an important class of heterocycles because of their biological

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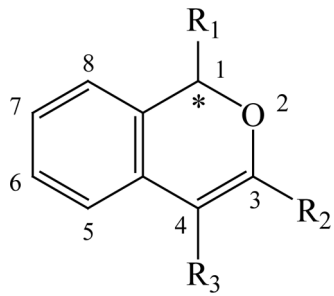


Figure 1. General structures of the chiral isochromene derivatives. $\mathbf{R}_{(1-2)}$ = aromatic or aliphatic group; $\mathbf{R}_3$ = bromine, iodine, sulfur or vinylic group. The carbon marked with an asterisk is the stereogenic center.

activity including antibiotic and anti-tumor properties.[1–6] For example, isochromene carboxamides exhibit excellent activity against the human ovarian cancer cell line SKOV3.[7] Several pyrano-isochromenes display *in vitro* selective cytotoxicity against human lung cancer cell line A549 and liver cancer cell line Bel7402.[8] In addition, isochromene derivatives are versatile intermediates in the synthesis of more complex or important compounds.[9–13]

Recently, Yue, Della Cà, and Larock have synthesized a series of highly hydrophobic chiral isochromenes by electrophilic cyclization of acetylenic aldehydes and ketones with various nucleophiles and electrophiles.[14] In most cases, different enantiomers of a compound have different biological properties. Thus, the enantiomeric separation of these compounds and subsequent evaluation of their properties are necessary.

The highly hydrophobic isochromenes are difficult to separate in the traditional CZE mode due to the aqueous nature of the method. Cyclodextrin-modified micellar capillary electrophoresis (CD-MCE) was, therefore, utilized to separate these neutral and highly hydrophobic compounds.[15,16] First reported in the early 1990s,[17,18] CD-MCE allows the separation of hydrophobic analytes *via* a two-pseudophase system: charged micelles form a pseudophase to enhance the solubility of the neutral hydrophobic analytes, while cyclodextrins form a pseudophase with a different mobility from the micelles to provide enantioselectivity. In order to achieve the separation, the enantiomers must differ in their association with the two pseudophases. To our knowledge, no other CE enantioseparations of isochromene derivatives have yet been reported.

EXPERIMENTAL

Materials

The isochromene derivatives used in this study were synthesized as reported previously, and their structures were confirmed by ^{1}H-NMR and ^{13}C-NMR spectroscopy.[14] Hydroxypropyl-β-cyclodextrin (HP-β-CD) and hydroxypropyl-γ-cyclodextrin (HP-γ-CD) were acquired from Aldrich Chemical Company (Milwaukee, WI, USA), with degrees of substitution of 0.8 and 0.6, respectively. Sodium dodecyl sulfate (SDS), sodium phosphate, sodium hydroxide, 85% phosphoric acid, and acetonitrile were all purchased from Fisher Scientific (St. Louis, MO, USA).

Methods

The CE experiments were performed on a Beckman Coulter P/ACE MDQ with a photodiode array detector (Fullerton, CA, USA). Purchased from Polymicro Technologies (Phoenix, AZ, USA), the bare fused silica capillaries used in these experiments were 40 cm long (30 cm to the detector), with inner diameters of 50 μm and outer diameters of 365 μm. The capillaries were conditioned before their first use by rinsing with 1 M sodium hydroxide for 5 mins, water for 5 mins, sodium hydroxide for 1 min, and finally, water for 1 min. Between each run, 1 min phosphoric acid, sodium hydroxide, water, and run buffer rinses were performed. Buffer solutions of 10 mM sodium phosphate buffer were made from deionized water, and adjusted to pH 2.5 with 85% phosphoric acid, followed by the addition of SDS and the CD. Finally, acetonitrile was added in the buffer solution. This run buffer solution was freshly prepared in order to prevent the slow hydrolysis of SDS. Samples were prepared by dissolving in acetonitrile. All buffer and sample solutions were sonicated for 5 minutes prior to their first run. The capillary was maintained at a temperature of 25°C. All separations were performed in the reverse polarity mode with an applied voltage of −20 kV.

RESULTS AND DISCUSSION

Effect of Different Types of Cyclodextrins

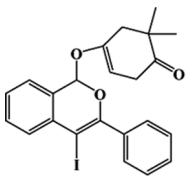
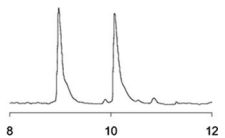
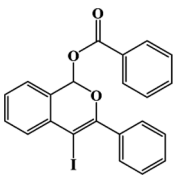
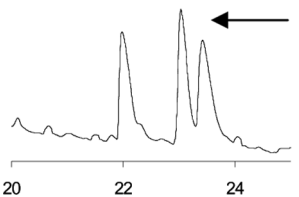
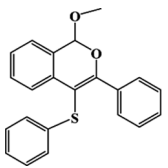
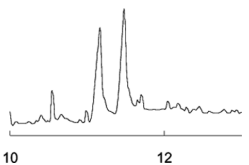
Negatively charged sulfated-β-CD was initially investigated as the chiral selector in CZE. With/without organic modifiers in the run buffer, no enantiomeric separations were observed in either the normal or reverse polarity modes. This result indicated that this CD derivative could

not provide sufficient selectivity to directly separate the enantiomers of isochromene family. Thus, CD-MCE was considered as an alternative approach to separate these highly hydrophobic neutral compounds. HP-β-CD and HP-γ-CD were investigated as potential chiral selectors. These cyclodextrin derivatives are promising because they are neutral and, thus, provide these analytes an enantioselective environment with a very different mobility than the negatively charged SDS micelles. In addition, their water solubility allows them to be used at high concentrations. Nevertheless, the presence of surfactant monomers in the run buffer may also affect the separation. Surfactant monomers exist in dynamic equilibrium between bulk solution and micelles, therefore the hydrophobic tail of free surfactant monomer may compete with the analyte for the CD cavity.[19] The wider γ-CD cavity may allow simultaneous inclusion of the surfactant monomer and the analyte.[20] Separation conditions were optimized by taking into account resolution, efficiency and peak shape, as will be discussed in the following section of this paper. The respective electropherograms using HP-γ-CD and HP-β-CD for the isochromene derivatives are summarized in Tables 1 and 2. Of the ten isochromene derivatives studied, nine compounds were separated with HP-γ-CD and four could be separated with HP-β-CD (Tables 1 and 2). Overall, HP-γ-CD produced better separations presumably due to its having a cavity large enough to allow these 3 or 4-ring compounds to form inclusion complexes.

Effect of Cyclodextrin and SDS Concentration

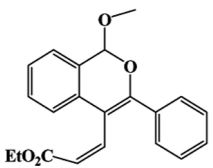
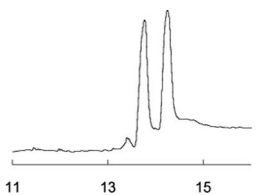
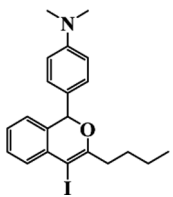
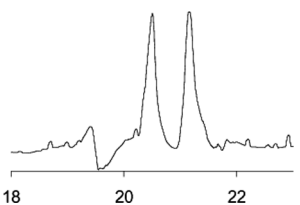
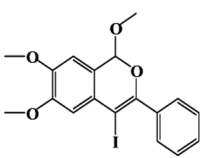
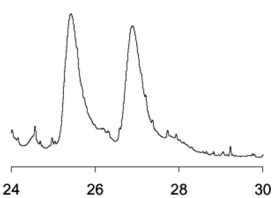
First studied in the early 1990's,[11] CD-MCE allows the separation of neutral hydrophobic analytes via a two-pseudophase system. Anionic micelles provide a negatively charged microenvironment that also serves to increase the solubility of the analytes, and the neutral CDs provide a microenvironment with chiral selectivity. A neutral analyte that partitions between these two environments will have different respective mobilities. The absolute concentrations and the ratio of the cyclodextrin to SDS had a great effect on the enantiomeric separation of all ten compounds studied. In general, at a constant SDS concentration, increased resolution but longer migration times were obtained when the concentration of the hydroxypropyl substituted cyclodextrin was increased. It was also found that an increasing level of CD at a constant SDS concentration eventually resulted in a reversal of the analyte's migration direction in the reverse polarity mode. On the contrary, increasing the SDS concentration at a constant CD concentration decreased the resolution and reduced the migration time. Maintaining the ratio of CD and SDS concentrations constant, and increasing the

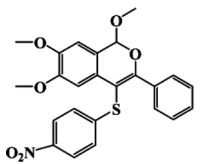
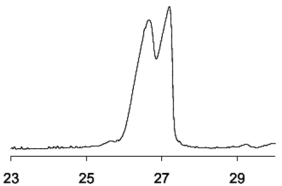
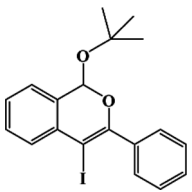
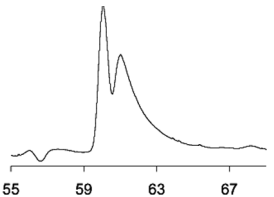
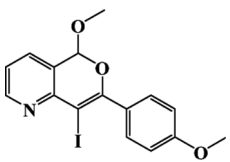
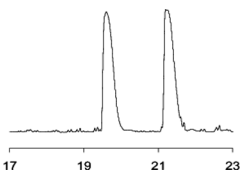
Table 1. Optimized enantioseparations of isochromene derivatives using HP-γ-CD and SDS in 20% ACN run buffer[a]

#	Structure	[SDS] (mM)	[HP-γ-CD] (mM)	[CD]/[SDS]	R_s[b]	t_{m2}/t_{m1}[c]	N[d]	Electropherogram (in mins)
1		120	80	0.67	2.8	1.123	9,000	
2		200	215	1.08	3.5	1.065	49,000	
3		150	150	1.00	1.5	1.028	41,000	

(continued)

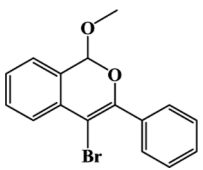
Table 1. Continued

#	Structure	[SDS] (mM)	[HP-γ-CD] (mM)	[CD]/ [SDS]	R_s[b]	t_{m2}/t_{m1}[c]	N[d]	Electropherogram (in mins)
4		150	165	1.10	1.3	1.035	21,000	
5		200	220	1.10	1.5	1.032	44,000	
6		200	220	1.10	1.7	1.058	14,000	

7		200	220	1.10	0.5	1.020	9,000	
8		150	180	1.20	0.5	1.016	47,000	
9		150	120	0.80	3.5	1.084	28,000	

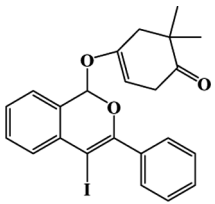
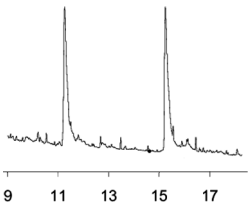
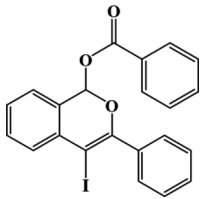
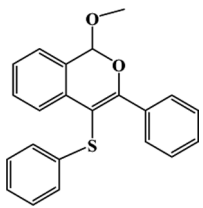
(continued)

Table 1. Continued

#	Structure	[SDS] (mM)	[HP-γ-CD] (mM)	[CD]/[SDS]	R_s[b]	t_{m2}/t_{m1}[c]	N[d]	Electropherogram (in mins)
10		–	–	–	0.0	–	–	No separation

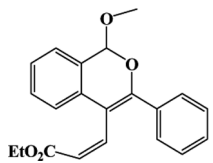
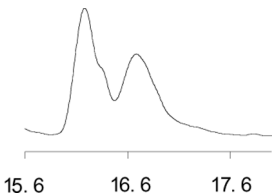
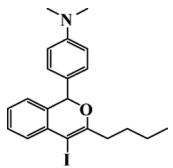
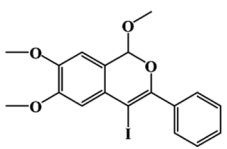
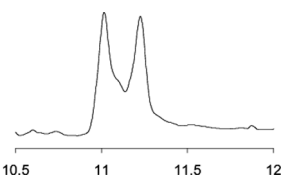
[a]All separations performed with 5 mM phosphate buffer pH 2.5, −20 kV, 37 cm capillary (30 cm to detector) with 50 μm I.D.
[b]R_s: separation resolution.
[c]t_m: migration time. EOF mobility could not be determined due to its suppression at pH 2.5, and therefore α values could not be calculated.
[d]N: the number of theoretical plates obtained for the first detected peak on a 30 cm length (to the detector) capillary.

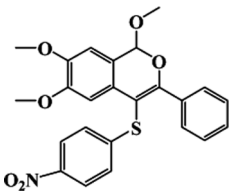
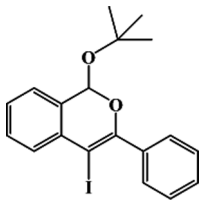
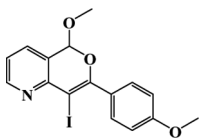
Table 2. Optimized enantioseparations of isochromene derivatives using HP-β-CD and SDS in 20% ACN run buffer[a]

#	Structure	[SDS] (mM)	[HP-β-CD] (mM)	[CD]/[SDS]	R_s[b]	t_{m2}/t_{m1}[c]	N[d]	Electropherogram (in mins)
1		150	100	0.67	12.0	1.355	19,000	
2		–	–	–	0.0	–	–	No separation
3		–	–	–	0.0	–	–	No separation

(continued)

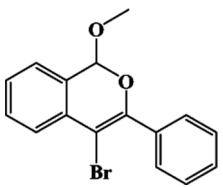
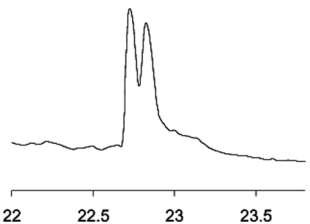
Table 2. Continued

#	Structure	[SDS] (mM)	[HP-β-CD] (mM)	[CD]/[SDS]	R_s[b]	t_{m2}/t_{m1}[c]	N[d]	Electropherogram (in mins)
4		200	180	0.90	1.0	1.031	24,000	
5		–	–	–	0.0	–	–	No separation
6		150	100	0.67	1.2	1.019	82,000	

7		–	–	–	0.0	–	–	No separation
8		–	–	–	0.0	–	–	No separation
9		–	–	–	0.0	–	–	No separation

(continued)

Table 2. Continued

#	Structure	[SDS] (mM)	[HP-β-CD] (mM)	[CD]/ [SDS]	R_s [b]	t_{m2}/t_{m1} [c]	N [d]	Electropherogram (in mins)
10		150	100	0.67	0.7	1.004	614,000	

[a–d]For explanation see Table 1.

concentration of both additives increased the resolution. However, an increase in the migration time was also observed. Figures 2 and 3 give brief illustrations of this point. It is known that chiral selectivity can be manipulated by changing the relative concentration of the chiral selector and surfactant, and thus changing the residence time that an analyte spends with each pseudophase additive.[18] The optimized concentration and ratio of the surfactant and cyclodextrin derivatives are given in Tables 1 and 2.

Effect of Organic Modifier and Run Buffer pH

A pH of 2.5 was chosen to suppress the electroosmotic flow (EOF) so that the EOF was nearly zero. This allowed the use of a high

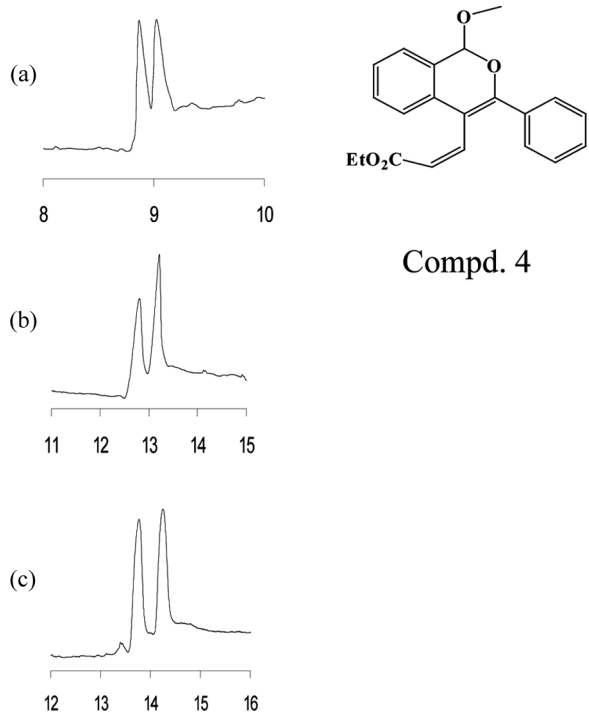


Figure 2. Comparison of the enantiomeric separations of compound 4 (Table 1) with constant 150 mM SDS concentration and different selector concentrations in the run buffer. a) 120 mM HP-γ-CD; b) 150 mM HP-γ-CD; c) 165 mM HP-γ-CD.

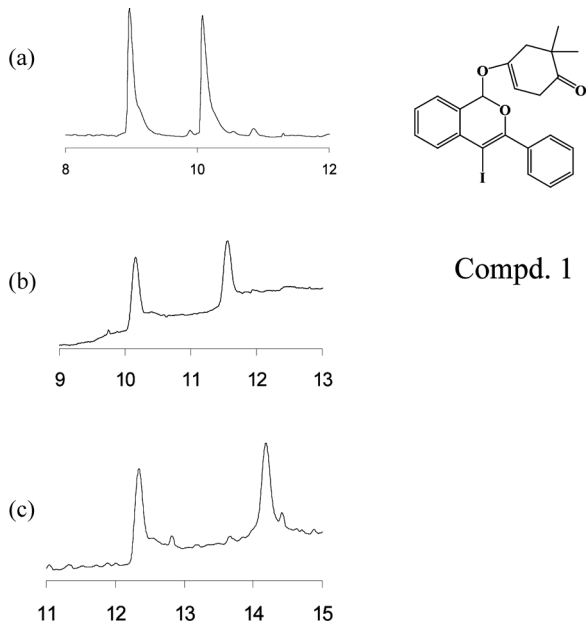


Figure 3. Comparison of the enantiomeric separations of compound 1 (Table 1) at a constant ratio of [SDS]/[HP-γ-CD] = 1.5, but at different absolute concentrations in the run buffer. a) 120 mM SDS, 80 mM HP-γ-CD; b) 150 mM SDS, 100 mM HP-γ-CD; c) 180 mM SDS, 120 mM HP-γ-CD.

CD concentration without reversing the migration direction of the analyte in the reverse polarity mode. In this operation mode, without CDs the highly hydrophobic analytes tend to reside in micelles and rapidly comigrate towards the anode where the detector is located. The addition of neutral CDs forms a pseudophase moving at the same speed and direction as the EOF, towards the cathode. With increasing concentration of CD in the buffer, the analytes will eventually favor the CD pseudophase, which increases the time it takes them to reach the detector. Hence, a very slow EOF is preferred to ensure the detection of analytes at high CD concentrations. The data in Tables 1 and 2 indicate that a relatively high CD concentration was necessary for most enantioseparations.

Although micellar capillary electrophoresis is particularly useful for highly hydrophobic analytes, it has been observed that systems utilizing pseudophases sometimes have a lower efficiency than traditional capillary zone electrophoresis. This effect may be due to both a thermal effect[21] and the slow mass transfer of the analytes between the micelle

and the analyte-cyclodextrin complex.[22] Asymmetrical peaks, which were observed in the experiment reported in Table 1, are often seen for solutes that are poorly soluble or insoluble in the bulk solution. Hence, the solutes can only reside in the micelle and/or cyclodextrins, and the bulk solution inhibits or forms a barrier to the transfer of solutes between the two pseudophases. In our study, the addition of ACN in the run buffer generally produced increased separation efficiencies and reduced migration times. This may be due to the combined effects of ACN competing with the analyte for the CD cavity and increasing the analyte solubility in the bulk solvent, thus enhancing the mass transfer. However, it was also observed that a slightly higher CD concentration was needed to retain the same resolution when the run buffer contained ACN. Figure 4 gives one example of how ACN affected the efficiency and migration time in the separation of isochromene enantiomers (compound 9 in Table 1).

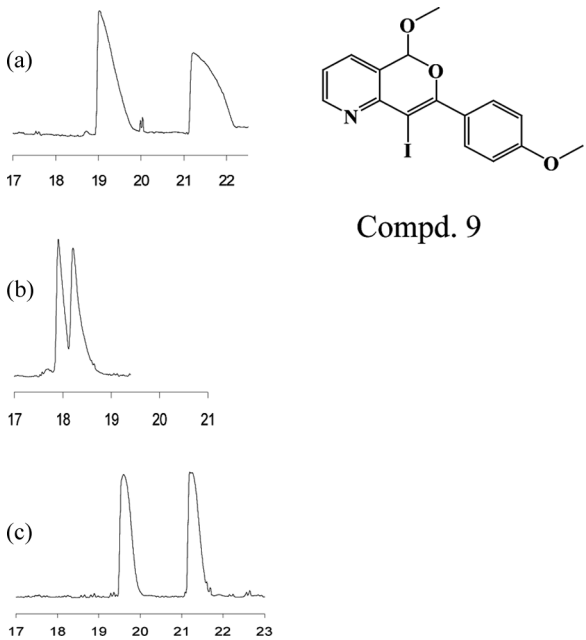


Figure 4. Comparison of the enantioseparations of compound 9 (Table 1) with/without ACN in the run buffer. a) 150 mM SDS, 115 mM HP-γ-CD; b) 150 mM SDS, 115 mM HP-γ-CD with 20% ACN; c) 150 mM SDS, 120 mM HP-γ-CD with 20% ACN in the run buffer.

CONCLUSIONS

Ten chiral isochromene derivatives were separated using either HP-γ-CD or HP-β-CD in conjunction with SDS micelles. In general, HP-γ-CD separated more of these relatively large hydrophobic molecules presumably due to its larger cavity. An organic modifier played an important role in enhancing the separation by improving the separation efficiency. Without an organic modifier, very slow mass transfer and precipitation of these highly hydrophobic analytes may occur. The addition of acetonitrile to the run buffer also increased the solubility of all analytes and, therefore, improved the UV detectability as well. Higher efficiencies were usually achieved with lower concentrations of SDS and CDs. However, optimized enantiomeric separations sometimes required higher concentrations to be used.

ABBREVIATIONS

MCE, micellar capillary electrophoresis; CD-MCE, cyclodextrin-modified micellar capillary electrophoresis; CD, cyclodextrin; HP-β-CD, hydroxypropyl-β-cyclodextrin; HP-γ-CD, hydroxypropyl-γ-cyclodextrin; SDS, sodium dodecyl sulfate; EOF, electroosmotic flow; ACN, acetonitrile

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