Optimizing a Method for Separating Chiral Compounds by Capillary Electrophoresis

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Abstract

This work describes the development of a capillary electrophoresis method for separation of chiral compounds using several β-cyclodextrin derivatives (β-CDs) in the mobile phase. The aims of this work are to demonstrate the effectiveness of β-CDs to separate the chiral compounds and to show how the separations are affected by various buffers and different pH values. The results show that the chiral drugs atropine (alkaloid antidote for cholinesterase-inhibiting compounds), trolox, methylphenyloxazolidinone, ephedrine, and pseudoephedrine can be separated with excellent resolutions by using sulfobutylether β-CDs. Dansyl-DL-amino acids can be separated within 5 min in aqueous solutions. The greatest resolution between L and D amino acids is found at pH 3.0.

Introduction

Capillary electrophoresis (CE) could play a central role in pharmaceutical and biomedical applications today. CE has generated considerable interest in recent years because of its capability of achieving high-speed separation, high efficiency, minimal sample consumption, and simple instrumentation (1,2). CE requires limited sample workup for analysis of drugs in biological matrices and is able to separate analytes at relatively low concentrations. CE has proven to be an excellent method for chiral separations of pharmaceutical compounds because of its wide operational pH range and the capability to separate both ionic and neutral molecules (3–8). The solutes are separated by differences in their net charge, which can be altered by changing the composition or pH of the buffer (1,2). β-Cyclodextrin derivatives (β-CDs) with various functional groups have been reported in previous research in an attempt to study the complexing and catalytic abilities of β-CDs (9,10). Recently, applications including the separation of some chiral resolution of cationic drugs by CE using sulfobutylether β-CDs were reported (11,12).

A different formation constant (K) for the L- and D- forms should result in the resolution of their peaks by the CE method. The formation of inclusion complexes of β-CD and the dansyl-DL-amino acids (Dns-AAs) results from the hydrophobic and hydrophilic interactions between the β-CDs and the guest molecules (13). The apolar Dns-AAs portion of the molecule is bound inside the cavity, and the amino group forms hydrogen bonds with the hydroxyl groups at the edge of the β-CD toroid, resulting in L- or D-AAs having different formation constants due to differences in each enantiomer (10).

It is important to separate the optical isomers of chiral drugs in analytical chemistry, especially in pharmaceutical analysis, because the optical isomers of a given chiral drug often present different pharmacological effects. Because the enantiomers closely resemble each other in their physical and chemical properties, their separation is a challenge for separation scientists.

Herein we report the chiral separation of the chiral drugs atropine, trolox, methyl phenyloxazolidinone, ephedrine, and pseudoephedrine, which can be separated with excellent resolution by using sulfobutylether β-CDs. Also, the results showed that dansyl-DL-amino acids (Dns-AAs) could be separated within 5 min, and as the pH was decreased, the resolution of CE separations of Dns-DL-AAs was enhanced.

Experimental

Apparatus

Chiral drugs were analyzed on an ABI 270A-HT CE system (CT/ABD, Perkin-Elmer, Wilton, CT). Turbochrom 4 software

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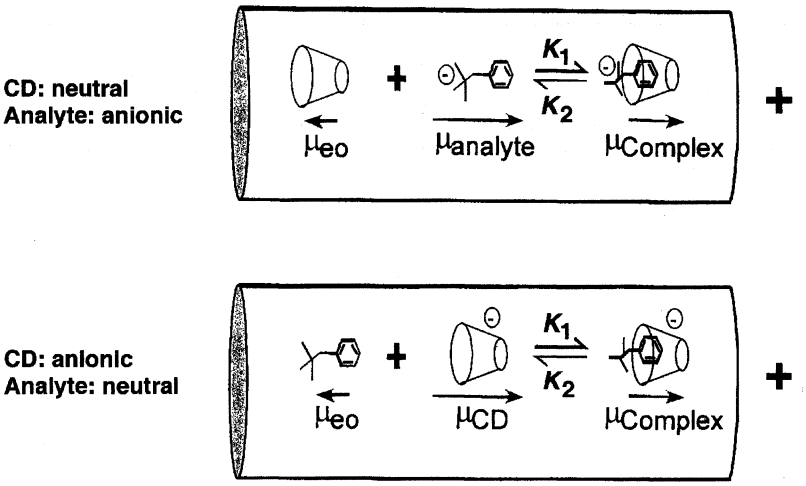

Figure 1. A schematic diagram of chiral separation.

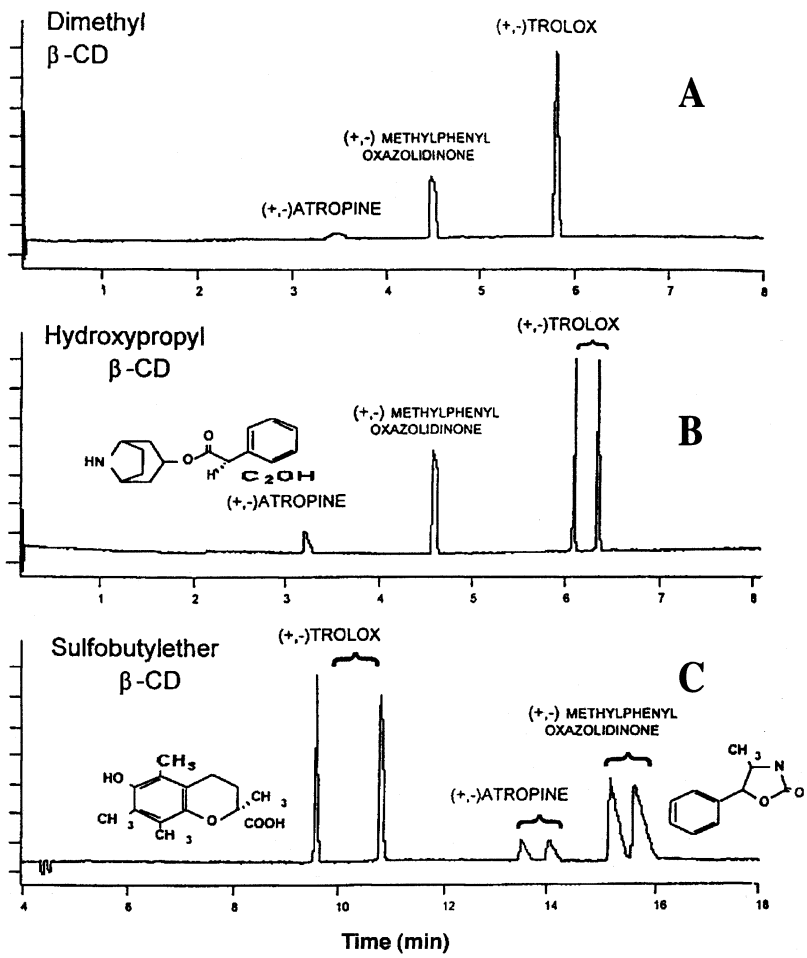

Figure 2. Comparison of chiral drug analyses. Atropine, methylphenyloxazolidinoe, and trolox in 20mM sodium acetate (pH 4.0) at 30 kV and 30°C using (A) dimethyl β-CD, (B) hydroxypropyl β-CD, and (C) SBE.

(PE-Nelson, San Jose, CA) was used for both control of the ABI 270A-HT and processing of the electropherogram data. The capillary was 72 cm × 50-mm i.d.; UV detection was used at 205 nm. For the Dns-DL-AAs analyses, a Waters Quanta 4000 CE system (Milford, MA) with a variable high-voltage power supply (0–30 kV) was used. Electrophoresis was performed in a 60-cm × 75-mm-i.d., fused-silica capillary tube (Waters), and peaks were detected at 214 nm. The temperature of the fused-silica capillary was controlled by a specially designed water-cooling system.

Chemicals and procedures

For chiral drug analysis, a 100.00mM β-sulfobutylether (SBE) stock solution was obtained from Perkin-Elmer/ABD. A 5.00mM SBE solution was prepared by adding 200 mL of 100mM SBE β-CD stock solution and 1.8 mL deionized water to 2 mL of 40mM sodium acetate (Perkin-Elmer/ABD) in a 40-mL glass buffer vial. The chiral drug standard was prepared by adding 500 mL of reagent-grade methanol to the performance evaluation standard (Perkin-Elmer/ABD), which contained 250 mg per compound. Atropine sulfate monohydrate (1,*d*-hyoscyamine), trolox (+,–6 hydroxy-2,5,7,8-tetramethylchroman-2-carboxylicacid), and (+,4S,5R,–,4R,5S)-4-methyl-5-phenyl-2-oxazolidinone were obtained from Perkin-Elmer. The standard stock solution was then diluted 1/10 with deionized water for routine analysis. Dns-DL-AAs were obtained from Sigma (St. Louis, MO). The β-CD derivatives (carboxymethylated β-CD, Na salt) were a gift from Supelco (Bellefonte, PA). Voltage was applied at 30 kV. A volume of 5 nL of each of the AAs was hydrostatically introduced into the capillary for 10 s. The electropherogram signals were recorded using the Waters Maxima software. The β-CD (10mM) was dissolved in three separate buffers: pH 3.0–5.0 (phosphate, 50mM), pH 5.5–6.5 (acetate, 50mM), and pH 7.0–9.0 (tris-HCl, 50mM), each of which contained 5mM NaCl.

Results and Discussion

Chiral recognition

The chiral separation is dependent on the formation of diastereomers either through covalent or electrostatic interactions. Figure 1 shows the β-CD (SBE) structure, which plays an important role in enhancing resolu-

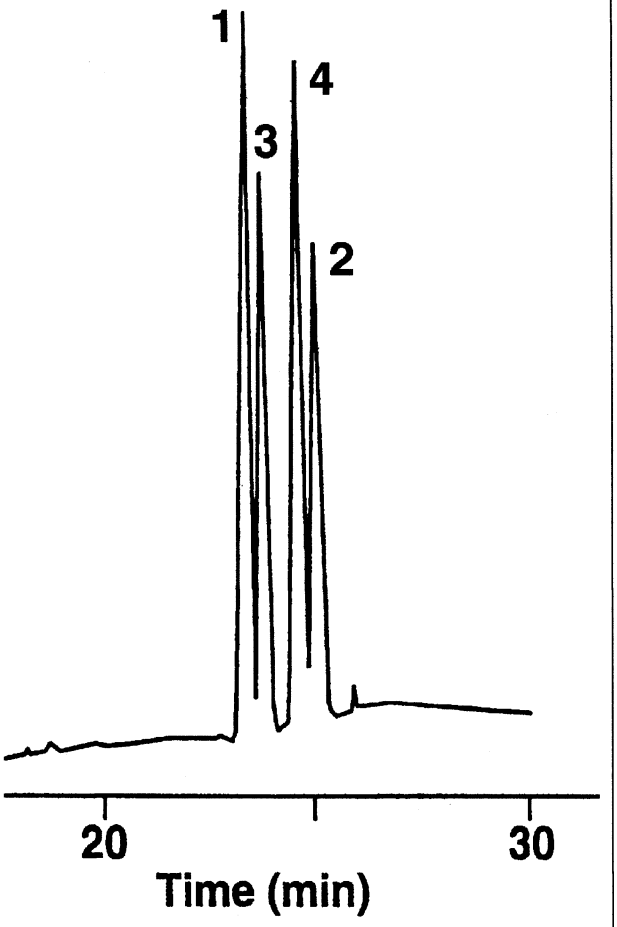

Figure 3. Separation of the stereoisomers of a mixture of pseudoephedrine and ephedrine in 1.5mM sulfobutylether β-CD and 20mM tris-phosphate buffer (pH 2.5) at 30 kV and 30°C. Peaks: 1, (–) pseudoephedrine; 2, (+) ephedrine; 3, (–) ephedrine; 4, (+) pseudoephedrine.

Table I. Chiral Separation of Dansyl-Amino Acids with β-CDs

Dansyl-amino acid	Migration time (min) L-AA	D-AA	pH
Phenylalanine	4.29	4.34	9.0
	4.91	5.05	5.5
	5.33	5.60	3.0
Tryptophan	4.51	4.55	9.0
	5.09	5.22	5.5
	5.42	5.68	3.0
Leucine	4.61	4.62	9.0
	5.23	5.31	5.5
	7.50	7.73	3.0
Methionine	6.06	6.07	9.0
	6.91	7.03	5.5
	7.87	8.04	3.0

tion because the CD derivative stretches the CD cavity, positively affecting the steric interaction between host and guest. The chart shows that the interaction occurs within the molecular cavity by formation of an inclusion complex. Figure 1 shows the attraction of the apolar molecule or segment to the apolar cavity of the β-CD and hydrophilic interaction between the molecule and the β-CD. It is known that the chemical modification of CDs causes changes in the shape and size of their cavities, hydrogen-bonding abilities, and other physical properties.

Figure 2 shows comparisons of the CE analyses of three chiral drugs (atropine, methylphenyloxazolidinoe, and trolox) using dimethyl β-CD (Figure 2A), hydroxypropyl β-CD (Figure 2B), and SBE (Figure 2C). Optimum resolution was achieved with only 5mM SBE in 20mM sodium acetate (pH 4.0) with 30 kV at 30°C. The neutral CDs achieved very little separation of the chiral pairs; only hydroxypropyl β-CD managed to separate trolox. Apparently the relative reverse migration of negatively charged SBE as compared with the migration of the neutral CDs affords more effective host–guest interaction and therefore better separation. Figure 3 illustrates the separation of ephedrine and pseudoephedrine stereoismers at pH 3.0 in 1.5mM sulfodex and 20mM tris-phosphate buffer. It is apparent from Figure 3 that great resolution was obtained for ephedrine and pseudoephedrine stereoisomers when using sulfodex.

Effect of pH on separations

pH plays an important role in the CE separation of the Dns-DL-AAs. The change in pH can affect solute charge and change electroosmotic flow (EOF), thus influencing resolution. At low pH, the EOF is slower than at high pH because at low pH, the negatively charged wall can cause adsorption of cationic solutes through coulombic interactions. The latter effect (double-layer) has been established theoretically (14,15) and experimentally (16) from electrochemistry and colloid and surface chemistry. Over the pH range of 3.0–9.0, strong influences on the migration time of Dns-DL-AAs were observed. Table I shows that lower pH levels produced the greatest resolution between L and D for the four amino acids. At low pH, the EOF was smaller than at higher pH, which provided analytes with more time for interaction with the CD cavity; the migration time was longer, but the resolution was improved. It is important to observe that in acidic solution, the amino group of the Dns AAs is protonated and forms a strong hydrogen bond with β-CD, producing greater resolution. The opposite is also true; at high pH, the amino group was not protonated, and thus the hydrogen bond was apparently weaker, which then reduced the resolution.

Effect of applied voltage on separations

It is well-known that the rate of voltage application across the capillary significantly affects the resolution. An increase in voltage will increase the temperature, which will decrease the viscosity of the eluent in the capillary and thus cause mobilities to increase and migration times to decrease. Figure 4 shows that the greatest resolution was found at 30 kV (the apparatus's maximum value).

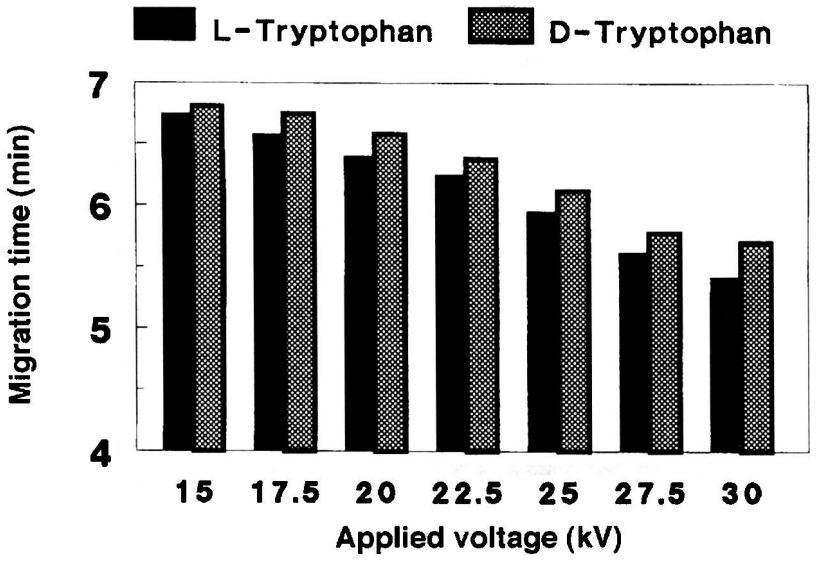

Figure 4. Influence of voltage on peak resolution of Dns-DL-tryptophan. The mobile phase contained 100mM β-CD, 5.0mM NaCl, and 10mM buffer (pH 3.0). The column was a 60-cm × 75-mm-i.d. fused-silica capillary.

Conclusion

The results show that chiral drugs (atropine, trolox, and methylphenyloxazolidinone) could be separated with the greatest resolution by using SBE. This was accomplished by virtue of SBE's negative charge slowing its migration, therefore affording longer, more effective interaction with the chiral analytes. This method may have wide applications in the pharmacological evaluation of enantiomeric drugs. The results also show that separations were achieved with Dns-DL-AAs in less than 5 min and were strongly dependent on pH. The optimization of pH was extremely essential to achieve the ionic states for both the analyte and CDs, which lead to the greatest resolution. CE was found to be very sensitive and selective in separating Dns-DL-AAs with β-CDs. CE can provide several advantages over other analytical techniques for enantiomeric separation (e.g., high resolution and shorter analysis times). β-CD derivatives play an important role in enhancing chiral resolution, either by sterically modifying the CD cavity or by employing negatively charged CDs that effectively enhance the interaction potential between the analyte and the CD.

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