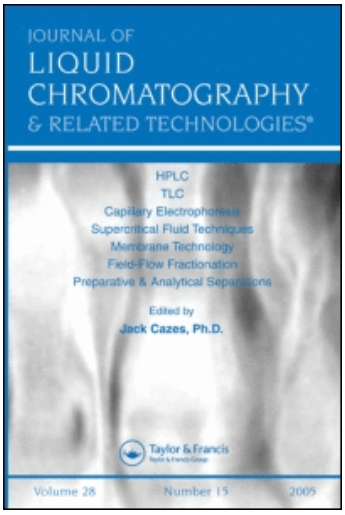

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Optical Resolution of Chlorpheniramine by Cyclodextrin Added Capillary Zone Electrophoresis and Cyclodextrin Modified Micellar Electrokinetic Chromatography

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OPTICAL RESOLUTION OF CHLORPHENIRAMINE BY CYCLODEXTRIN ADDED CAPILLARY ZONE ELECTROPHORESIS AND CYCLODEXTRIN MODIFIED MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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ABSTRACT

Optical resolution of *RS*-chlorpheniramine by cyclodextrin (CD) added capillary zone electrophoresis (CZE) and cyclodextrin modified micellar electrokinetic chromatography (CD-MEKC) was investigated. In CD added CZE, optical resolution was achieved with ß-CD - urea solutions (pH 2.5 - 3.0) with and without methanol. In this system, γ-CD was not effective to achieve enantiomeric separation. In CD-MEKC, similar resolution as in the CD added CZE systems was obtained with sodium dodecyl sulfate (SDS) - ß-CD - urea solutions (pH 3.0).

¶A part of this work was carried out in *Department of Chemistry, Stanford University, Stanford, CA 94305-5080, U.S.A.*

INTRODUCTION

High performance capillary electrophoresis (HPCE) has become a popular separation technique in various analytical fields owing to its high efficiency and developments of fully automated instruments controlled by a microcomputer system which are commercially available. Among some modes of HPCE, capillary zone electrophoresis (CZE) or free solution capillary electrophoresis is the most popular method since it can be easily operated, especially in terms of preparations of capillaries and separation solutions. Micellar electrokinetic chromatography (MEKC) (1), which is a branch of HPCE and uses an ionic micellar solution, has also become a well-known technique to separate small neutral molecules as well as charged solutes.

Optical resolution is one of major objectives in HPCE and some papers on this area have appeared. Zare and co-workers (2,3) first reported chiral separation by CZE using formation of copper (II) complexes. Cyclodextrin (CD) is a popular compound that forms an inclusion complex with various substances, especially with aromatic compounds, and has an ability to achieve chiral recognition. The direct enantiomeric separation by CZE using CD, in which no complexation reagent was used, was first reported by Fanali (4). This CD added CZE system can be applied to optical resolution of charged enantiomers but is hardly effective for neutral enantiomers. Cyclodextrin modified MEKC (CD-MEKC) (5,6), however, can be effective to optical resolution of both neutral and charged enantiomers (7-9).

Chlorpheniramine, the structure is shown in **Figure 1**, is widely used as an antihistaminic component in cold medicines or some other drugs. Here, its *S*-(+) form has such an activity, but *R*-(-) form does not. Then, optical resolution of *RS*-chlorpheniramine might be one of pharmaceutical interests.

In this paper, we briefly describe enantiomeric separation of *RS*-chlorpheniramine by CD added CZE and CD-MEKC using sodium dodecyl sulfate (SDS).

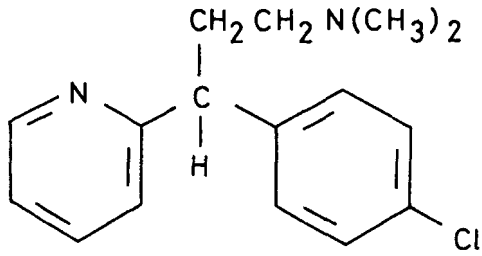

Figure 1. Structure of chlorpheniramine (free).

EXPERIMENTAL

Chlorpheniramines were received as maleates: *RS*– was obtained from Sigma (St. Louis, MO, U.S.A.) and Wako (Osaka, Japan), *R*– and *S*– were from Wako, and injected as methanol–water solutions of *ca.* 0.1 mg mL^{-1}. Sodium dodecyl sulfate (SDS) was received from Sigma and Nacalai Tesque (Kyoto, Japan), γ–cyclodextrin (CD) from Wako, β–CD and methanol from Sigma and Wako. Separation solutions were prepared by dissolving CD – urea or SDS – CD – urea in a 50 mM phosphate buffer adjusted to an appropriate pH. Then, methanol was added to the solution when required. All the chemicals were of analytical reagent grade and used without further purification.

Capillary electrophoresis was performed by a Beckman P/ACE System 2000 (Palo Alto, CA, U.S.A.) with System Gold data processing program and a laboratory built system consisted of a Matsusada HepLL–30P0.08–LS regulated high voltage power supply (Kusatsu, Shiga, Japan), a Shimadzu SPD–6A UV Spectrophotometric Detector (Kyoto, Japan) and a Shimadzu Chromatopac C–R3A data processor. Separation columns were fused silica capillaries purchased from Polymicro Technologies (Phoenix, AZ, U.S.A.) which were of 50 μm i.d. x 260 mm (effective length was 200 mm) for the P/ACE and 50 μm i.d. x 500 mm (effective length 300 mm) for the handmade system.

Sample injection was carried out by the pressurized method in the P/ACE and the manual or hydrodynamic in the handmade system.

Separation was performed under the constant voltage conditions. In the P/ACE temperature was maintained at 25 °C, while ambient in the laboratory built system.

RESULTS AND DISCUSSION

Under separation conditions used chlorpheniramine was positively charged as a maleate was used. Therefore, the electrophoretic migration of chlorpheniramine was toward the negative electrode or cathode, which was the same as the direction of the electroosmotic flow. In those circumstances a reduced electroosmotic flow was required to achieve a long migration time of the solute or good resolution, especially in a CZE system.

In CD added CZE, acidic separation solutions were employed to suppress the electroosmotic flow (10), and detection was carried out at the side of the cathode since the cation was analyzed. Urea was also added to separation solutions to increase the solubility of CD (5) and also to obtain a good peak shape (11,12). With a 100 mM β-CD - 5 M urea solution (pH 3.0) *RS*-chlorpheniramine was successfully resolved as shown in **Figure 2**, which was obtained by using the P/ACE system. In this case, the *R*- form migrated faster than *S*- form. The result indicates that *S*-form was much strongly incorporated into β-CD than *R*-form: When incorporated into CD, the solute migrated with the same velocity as CD or the electroosmosis, while in the aqueous phase it migrated faster than the electroosmotic velocity or the total velocity of the electrophoresis of the solute and electroosmosis.

Similar results were obtained by using the laboratory built instrument, although the capillary length was quite different. As for other lower CD concentrations, *eg.*, 30, 50 and 75 mM β-CD, poorer resolution was attained.

The use of γ-CD was also examined at the same concentrations as the case of β-CD. In this instance, however, no optical resolution was achieved, although Sydor and Mularz reported that γ-CD was also effective (13). Then, we used β-CD only in following experiments.

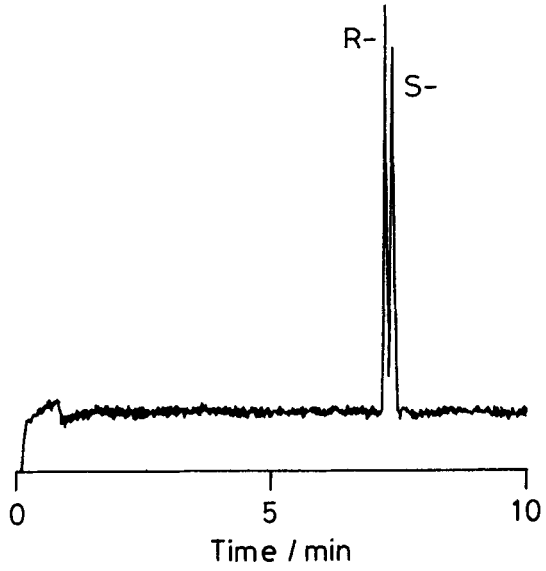

Figure 2. Optical resolution of *RS*-chlorpheniramine by CD added CZE. Separation solution, 100 mM β-CD - 5 M urea (pH 3.0); separation capillary, 50 μm i.d. x 260 mm (effective length, 200 mm); applied voltage, 10 kV; current, 21 μA; detection wavelength, 214 nm, temperature, 25 °C.

To further suppress the electroosmosis and also change the selectivity, methanol was added to CD solutions. The effect of methanol addition on optical resolution of amino acid derivatives in MEKC was discussed in the previous papers (11,12). **Figure 3** shows an example of enantiomeric separation with a 50 mM β-CD - 5 M urea solution (pH 2.5) containing 10% (v/v) methanol obtained by using the laboratory built instrument. In this case, the migration times were *ca.* 16 min, almost the twice of the case in **Figure 2** (*ca.* 7.5 min). The estimated values of the apparent electrophoretic mobilities of both *RS*-pairs in **Figures 2** and **3** are 1.2 x 10^{-4} and 1.6 x 10^{-4} cm^2 V^{-1} s^{-1}, respectively, or the ratio is *ca.* 1 : 1.4. It should be noted that the difference did not directly reflect the effect of methanol addition and/or the change of the CD concentration: The apparent mobility is the sum of the net electrophoretic mobility and

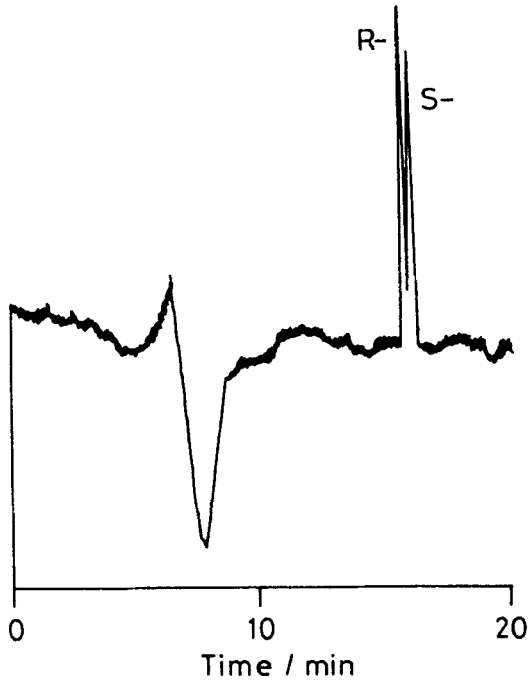

Figure 3. Enantiomeric resolution of *RS*-chlorpheniramine by CD added CZE. Separation solution, 50 mM β-CD - 5 M urea (pH 2.5) containing 10% (v/v) methanol; separation capillary, 50 μm i.d. x 500 mm (effective length, 300 mm); applied voltage, 10 kV; current, 7 μA; detection wavelength, 220 nm; temperature, ambient.

the electroosmotic mobility. Both mobilities could be affected not only by the addition and/or the concentration change but also by the difference of the other experimental conditions such as the capillary properties, current, pH and temperature.

In CD-MEKC, on the other hand, acidic SDS - β-CD solutions containing urea were employed. Effects of urea addition in MEKC have been discussed previously (11,12,14). When an acidic solution is used, the electroosmotic flow, which is toward the cathode, is much smaller than the electrophoresis of the SDS micelle, which is toward the positive electrode or anode, and hence, the SDS micelle migrates toward the

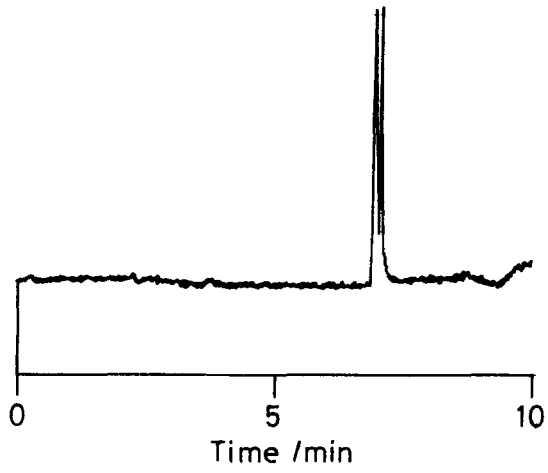

Figure 4. Optical resolution of *RS*-chlorpheniramine by CD-MEKC. Separation solution, 50 mM SDS - 100 mM β-CD - 5 M urea (pH 3.0); current, 38 μA. Other conditions as in **Figure 2.**

anode (10). A positively charged solute, however, is forced to the cathode when it exists in the aqueous phase and also is incorporated into CD: CD has no charge and migrates with the same velocity as the electroosmosis although the electroosmotic flow is quite small. The solute is only forced to the anode when it is in the micelle. The migration direction of the solute is dependent on the degree of the solubilization by the micelle (10). Under the experimental condition, chlorpheniramine migrated toward the cathode, which was the same as in CD added CZE systems mentioned above.

By using a 50 mM SDS - 100 mM β-CD - 5 M urea solution (pH 3.0), *RS*-chlorpheniramine was optically resolved as shown in **Figure 4**, which was obtained by the P/ACE system. The result was almost similar to that obtained in CD added CZE system (**Figure 2**). In this case, the migration order was not assigned but expected to be the same as in CD added CZE.

It seems that CD-MEKC is not necessary as long as the purpose of enantiomeric resolution of *RS*-chlorpheniramine. But it should be

noted that in CD–MEKC system both the CD and micellar concentrations can be used to maintain the migration time or capacity factor (5,6). This implies that an easier manipulation of the separation in CD–MEKC is possible than in CD added CZE in which only the CD concentration can be altered.

In conclusion, CD added CZE and CD–MEKC provided almost the same results on optical resolution of *RS*–chlorpheniramine and both systems were effective to that separation. Further investigations on enantiomeric resolution of some drug components by both systems and also by MEKC with chiral surfactants or additives are being preceded.

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