CHAPTER II

EXPERIMENTAL

2.1 Chemicals

β-CD and DM-β-CD were purchased from Sigma. The following test analytes in a class of amphetamine drugs were obtained from Aldrich: (±)-amphetamine (AP) sulfate, (±)-nor ephedrine (NE) hydrochloride, *R*- and *S*-methamphetamine (MA) hydrochloride, (1*R*,2*R*)- and (1*S*,2*S*)-pseudoephedrine (PE) hydrochloride and (1*R*,2*S*)- and (1*S*,2*R*)-ephedrine (EP) hydrochloride. Triethanolamine was supplied by Unilab. Phosphoric acid was supplied from Carloerba. Double deionized water was used for preparation of all solutions.

A triethanolammonium-phosphate buffer at pH 3.0 was prepared by titration 100 mM H_3PO_4 with triethanolamine. The appropriate amount of cyclodextrin was added to the triethanolammonium-phosphate buffer to make the BGE. The mixture of AP, MA, PE, EP and NE at 0.15 mM for each isomer was dissolved in double deionized water.

2.2 CE Conditions

All CE separations were performed on a P/ACE system 5010 Beckman CE instrument. An uncoated fused silica capillary used was 57 cm in length (50 cm to detector) × 50 μm I.D., thermostated at 25 °C. Voltage was set at 30 kV and UV detection at 200 nm. A sample solution was injected by 0.5 psi pressure for 2 s. A new capillary was conditioned with 1 M NaOH for 30 min, 0.1 NaOH for 30 min, water for 30 min, 0.1 M H_3PO_4 for 30 min and finally with the BGE for 30 min. Prior to analysis each day, the capillary was rinsed with 0.1

M H_3PO_4 for 15 min and then BGE for 15 min. Between consecutive analyses, the capillary was flushed with 0.1 M H_3PO_4 for 5 min and then BGE for 5 min. All solutions were prepared using water and filtered through 0.45 μm membrane filters prior to analysis. Each experiment was run in duplicate.

2.3 Enantiomeric Separation Using Single Cyclodextrin

Stock solution of 16.0 mM β-CD and 50.0 mM DM-β-CD were separately prepared by weighing an appropriate amount of CD and dissolving in the triethanolammonium-phosphate buffer at pH 3.0. Solutions of β-CD in the concentration range 0 to 16.0 mM were prepared by diluting appropriate amounts of 16.0 mM β-CD with the triethanolammonium phosphate buffer. Solutions of DM-β-CD in the concentration range 0 to 50.0 mM were prepared by diluting appropriate amounts of 50.0 mM DM-β-CD with the triethanolammonium phosphate buffer.

Simultaneous separation of enantiomers were carried out using various concentrations of separately single CD (β-CD or DM-β-CD) and other CE conditions as in Section 2.2. In the case of negligible or no EOF, observed electrophoretic mobility, μ_{obs}, of each enantiomer can be calculated from electropherograms using the equation

$$\mu_{obs} = \frac{lL}{Vt_m} \qquad (2.1)$$

where L and l are the total length of a capillary and the length of a capillary to detector, respectively, V the applied voltage and t_m the migration time of the analyte. Since observed electrophoretic mobility also depends on the viscosity of the BGE containing CD [Penn *et al.* 1993], corrected electrophoretic mobility of analytes, μ, was calculated using the equation

$$\mu = \mu_{obs} \frac{\eta_c}{\eta_o} \qquad (2.2)$$

where η_c/η_o is the relative viscosity of BGE at a given to that at zero CD concentration. Relative viscosity was obtained from Nhujak [2001]

$$\eta_c/\eta_o = 1 + 2.638\times10^{-3}\,[\beta\text{-CD}] + 6.80\times10^{-6}\,[\beta\text{-CD}]^2 \quad (2.3)$$

$$\eta_c/\eta_o = 1 + 3.624\times10^{-3}\,[\text{DM-}\beta\text{-CD}] + 1.41\times10^{-6}\,[\text{DM-}\beta\text{-CD}]^2 \quad (2.4)$$

Equation 1.33 may be presented by the equation

$$\mu = \frac{\mu_o - \mu_\infty}{1 + KC} + \mu_\infty \quad (2.5)$$

The binding constant, K, and μ_∞ were determined by using the CEfit program which is kindly obtained from D.M. Goodall, the University of York, UK. The program is able to fit data points of μ as a function of C [Penn *et al.* 1995, Ruddick 1997], which μ_∞ is assumed to be equal for complexes of CD:enantiomers. Each value of K and μ_∞ was obtained from the mean of μ at two separate runs. Results are shown in Section 3.1.2. The observed and predicted CD concentrations at maximum electrophoretic mobility difference and maximum resolution were compared as shown in Section 3.1.2. Over a wide range of CD concentrations, predicted and observed peak variance, efficiency and resolution were compared as shown in Sections 3.1.3 and 3.1.4.

2.4 Enantiomeric Separation Using Dual Cyclodextrins

Equations and models were proposed for electrophoretic mobility difference of a pair of enantiomers in dual CDs, using data of binding constants and enantioselectivities. Details are discussed in Section 3.2.2. Experimental and predicted values of $\Delta\mu$ for enantiomers in dual CDs were compared as shown in Section 3.2.4. The experiment was carried out using a fixed concentration of one CD type and various concentrations of another CD. Results are shown in Figures 3.12 and 3.13. The observed peak variance and resolution of enantiomers in dual CDs

were compared as shown in Section 3.2.5. It should be noted that the relative vicosity at a given C_β and $C_{D\beta}$ is assumed to be equal to $\eta_\beta/\eta_o \times \eta_{D\beta}/\eta_o$, where C_β and $C_{D\beta}$ are the concentrations of β-CD and DM-β-CD, respectively. η_β/η_o and $\eta_{D\beta}/\eta_o$ are relative viscosities at C_β and $C_{D\beta}$, respectively.