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Determination of Stability Constants of the Inclusion Complexes of β-Blockers in Heptakis (2,3-Dimethyl-6-Sulfato)-β-Cyclodextrin

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The β-blockers possess at least one chiral center and the *S*(-)-enantiomer shows higher affinity for binding to the β-adrenergic receptors than antipode. The stability constants of acebutolol, celiprolol, propranolol and terbutaline in the inclusion complexes with single-isomer heptakis (2,3-dimethyl-6-sulfato)-β-cyclodextrin (HDMS-β-CD) were determined by capillary electrophoresis. The approximation and linear double reciprocal methods were adapted with comparable results. Among the β-blockers studied, propranolol had the lowest stability constant but the highest enantioselectivity, indicating that the magnitudes of the stability constants carried little information about enantioseparation. The magnitudes of enantioselectivities between the enantiomer pair were in the order of propranolol > celiprolol > terbutaline > acebutolol.

Key words: Stability constant, β-Blockers, Heptakis (2,3-dimethyl-6-sulfato)-β-cyclodextrin, Capillary electrophoresis

INTRODUCTION

The β-blockers are a family of drugs used to treat cardiovascular disorders such as hypertension, cardiac arrhythmia, or ischemic heart disease, tremors, alcohol withdrawal, glaucoma, and other conditions. They are also used to prevent migraine headaches, stage fright, and second heart attacks (Toda, 2003; Wikstrand *et al.*, 2003; Mehvar *et al.*, 2001; Borchard, 1998). Each of these drugs possesses at least one chiral center, and an inherent high degree of enantioselectivity in binding to the β-adrenergic receptor. For β-blockers with a single chiral center, the *S*-(-)-enantiomer possesses much greater affinity for binding to the β-adrenergic receptors than antipode (Mehvar *et al.*, 2001; Vargas *et al.* 1999; Stoschitzky *et al.*, 1998).

Capillary electrophoresis (CE) has become a very attractive alternative analysis tool for the determination of drug enantiomers. Use of the capillary has numerous advantages, particularly with respect to the detrimental effect of Joule heating. A high voltage is applied with only a low heat generation. Analysis time is short with high efficiency and resolution. In addition, minimum sample volume, on-capillary detection, the potential for quantitative analysis and automation, and high performance provide capillary electrophoresis to be a premier separation technique (Vargas *et al.* 1999).

Chiral analysis by CE usually involves the addition of a chiral selector such as cyclodextrin (CD) to the running buffer. CDs are nonionic cyclic oligosaccharides consisting of six, seven or eight glucose units designated α, β and γ-CD, respectively. CDs have the shape of hollow truncated cone with a cavity diameter determined by the numbers of glucose units. The cavity is relatively hydrophobic while the external surface is hydrophilic. The circumference contains chiral secondary hydroxyl groups. Therefore, the hydrophobic portion of the solute may be included in the cavity while the hydrophilic portion may be interacted with the chiral hydroxyl moieties by a number of mechanisms (e.g., hydrogen bonding, dipole-dipole and/or van der Wals interaction). The resulting complexes may result in a different mobility and, thus, a chiral selectivity (Chankvetadze, 1997).

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drug enantiomers involves the formation of hydrogen bonds between the CDs and molecules. The proximity of the hydrogen bonding moieties can vary markedly for a pair of enantiomers with a concomitant difference in stability. Therefore, stability is a primary property of the complex that is created during the chiral discrimination process. The comparison of differences in the stability constant, which characterize the interaction of host molecules with the analytes, allows a priori selection of the chiral selector. Thus the determination of the stability constants is important in studying chiral separations and in their practical utilization. The stability constants of β-blockers with various chiral selectors were reported (Park *et al.*, 2002, 2003; Kim *et al.*, 1998; Bontchev *et al.*, 1992).

The most commercially available CDs are complicated mixtures of isomers, which differ in their degree of substitution and their substitution patterns. Heptakis (2,3-dimethyl-6-sulfato)-β-cyclodextrin (HDMS-β-CD) is one of a recently developed single-isomer CD family possessing good chiral selectivity and reproducible complexation rate. In this experiment, stability constants of acebutolol, celiprolol, propranolol and terbutaline in the inclusion complexes with HDMS-β-CD have been determined by CE and compared each other.

MATERIALS AND METHODS

Apparatus and chemicals

CE analysis was performed on a ^{3D}CE (Hewlett Packard, Germany) equipped with a diode array detector. Data were collected and analyzed on a HP Vectra computer equipped with HP Chemstation system. ATI Model 370 (Orion, MA, USA) was used for measuring the pH. HDMS-β-CD sodium salt was purchased from ANTEX® Instruments (TX, USA). Propranolol hydrochloride, (*R*)-, (*S*)- and (*RS*)-propranolol were purchased from Sigma Co. (MI, USA) and other β-blockers such as nadolol, terbutaline, celiprolol, acebutolol, carvedilol, atenolol, metoprolol, arotilolol, bisoprolol were kindly donated from the pharmaceutical companies in Korea. All other reagents and chemicals used were of analytical grade.

Electrophoresis

To separate the enantiomers by CE, a fused silica, uncoated capillary, 50 μm inner diameter, 29.5 cm in length (21 cm effective length) was used with acetate buffer (0.1 M, pH 4) containing 5% isopropyl alcohol as an organic modifier and HDMS-β-CD at various concentration ranging 0-50 mM. The system was programmed to rinse capillary at the beginning and between runs by flush with sodium hydroxide (0.1 M), distilled water and running buffer for 1, 2, and 2 min, respectively. Sample injection was performed by pressure at 50 mbar for 2 s. The electrophoretic procedure was developed at voltage of 10 KV with positive polarity. Detection was performed at the wavelength of 210 nm. Cartridge temperature was set at 20°C.

Calculation of stability constants

The stability constants were calculated as Eq. 1 using the mobility of the enantiomeric analytes and the complexes at a given concentration of chiral selector (Vespalec *et al.*, 2000). This equation allowed the point-by-point calculation of the stability constant.

$$K_{st} = \frac{1}{[C]} \frac{u_D - u_{eff,D}}{u_{eff,D} - u_{DC}} \quad (1)$$

where u_D and u_{DC} are the mobilities of free drug and complexed drug with chiral selector, respectively. $u_{eff,D}$ is the effective mobility of drug. u_{DC} can be determined approximately by experiments or by Lineweaver-Burk reciprocal plot. The Eq. 1 can be transformed into Eq. 2 by double reciprocal method (Rundlett *et al.*, 1996). This equation offers a linear plotting form, where the mobility of drugs-CD complex (u_{DC}) is not required for calculation of stability constants.

$$\frac{1}{u_{eff,D} - u_D} = \frac{1}{(u_{DC} - u_D)K_{st}[C]} + \frac{1}{u_{DC} - u_D} \quad (2)$$

The mobility could be calculated by the Eq. 3, using the migration time of analyte (t) and electroosmosis (t_o) at a given voltage (V), total (L) and effective length (l) of the capillary.

$$u = \left(\frac{1}{t} - \frac{1}{t_o}\right)\frac{Ll}{V} \quad (3)$$

RESULTS

Chiral separation of β-blockers

Enantiomers of ten β-blockers such as nadolol, terbutaline, celiprolol, acebutolol, carvedilol, atenolol, metoprolol, arotilolol, bisoprolol, propranolol were tried to be separated on CE using HDMS-β-CD as chiral selector. Among those, acebutolol, celiprolol, nadolol, propranolol and terbutaline enantiomers could be separated. Nadolol showed four separated peaks on the CE system because of the presence of diastereomers, hence the evaluation of nadolol was not carried out. The resolutions and migration times of the enantiomers were influenced by the concentration of HDMS-β-CD in the electrolyte buffer. In general, the migration was delayed with the CD concentration. To a certain extent, the resolution was increased with the CD concentration, but resolution was decreased at a very high CD concentration. As shown in Fig. 1, the resolution of propranolol enantiomers was increased with the

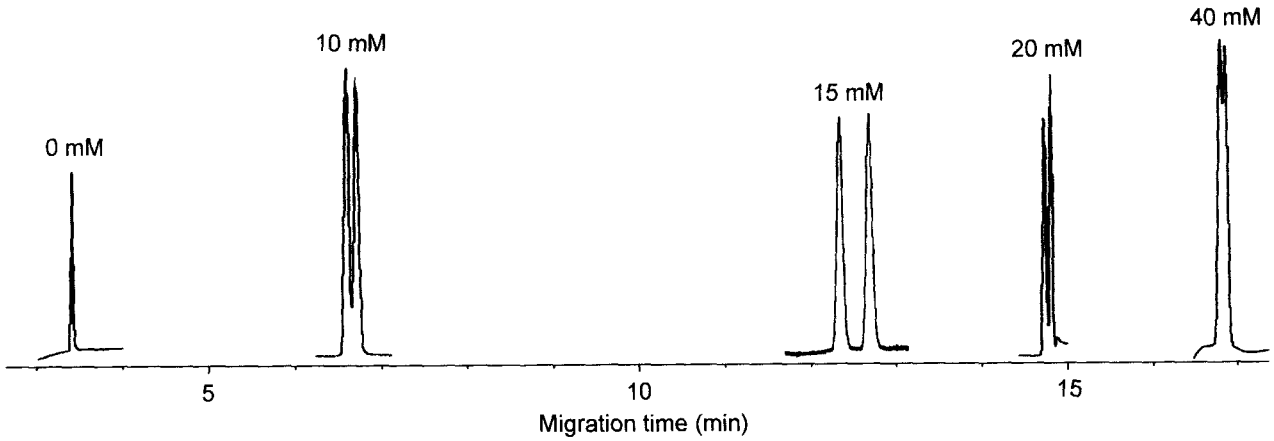

Fig. 1. Effect of HDMS-β-CD concentration on the enantioseparation of propranolol. Fused silica capillary column 29.5 cm, 50 μm I.D.; applied voltage, +10 KV; acetate buffer (0.1 M, pH 4) containing 5% isopropyl alcohol; detection, UV 210 nm; capillary temperature, 20°C.

increased concentration of chiral selector to 15 mM, while the concentration of chiral selector higher than 20 mM resulted in decrease of resolution. In general the migration times of inclusion complexes were shorter than those of electroosmosis up to 50 mM of HDMS-β-CD except for the case of propranolol. Migration time of propranolol-HDMS-β-CD complex was longer than that of electroosmosis when the concentration of HDMS-β-CD was higher than 40 mM.

Stability constants by approximation

The effective mobilities ($u_{eff,D}$) in the electrolyte were calculated using Eq. 3 from the migration time of drugs and electroosmosis in a given concentration of CD. The u_D and u_{DC} are necessary to calculate the stability constants by Eq. 1. While the mobilities of drugs in the absence of the chiral selector in electrolyte, u_D, were readily accessible experimentally, u_{DC} could not be determined directly from experiments, because u_{DC} is the mobility of drug in the electrolyte containing an infinitely high concentration of the chiral selector. Hence u_{DC} was estimated, in this work, by the approximation of the limit approached by effective mobility at increasing CD concentration. Fig. 2 showed the measured and approximated mobilities of β-blockers and their inclusion complexes. The approximation could be extended to infinitely high concentration of HDMS-β-CD, which provided the calculation of u_{DC} value. The optimum concentration of chiral selector (C_{max}), which corresponds to a maximum difference in effective mobilities between two enantiomers, can be obtained either directly from experimental data or from the approximation of the data. However, discrepancies between the measured and approximated optimum concentration were observed (Table I) because the CE experiments were carried out at the discontinuous concentration of HDMS-β-CD.

Fig. 3 shows the electropherograms of β-blockers

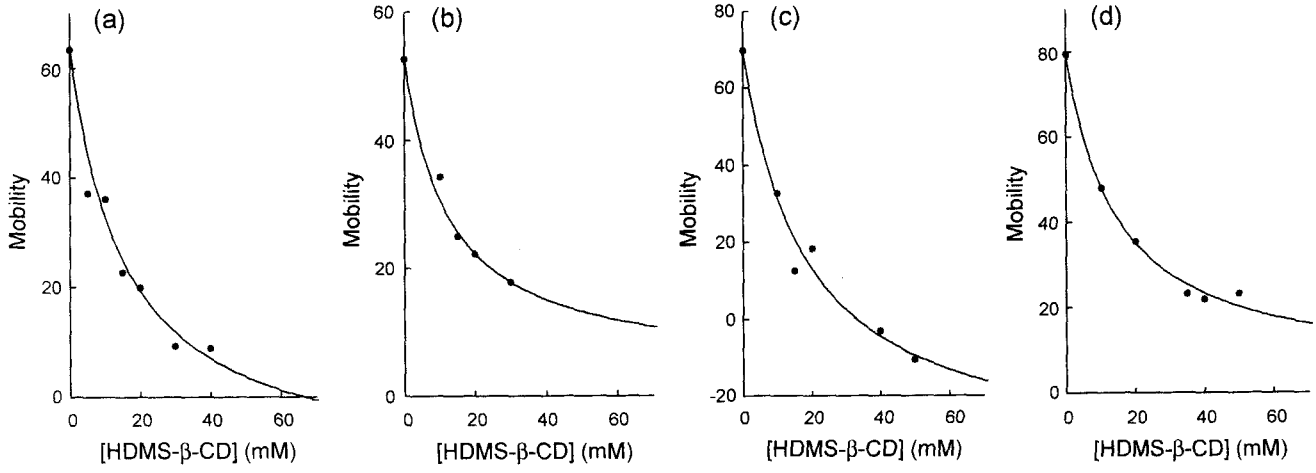

Fig. 2. The measured (closed circle) and approximated (solid line) mobilities of (a) acebutolol, (b) celiprolol, (c) propranolol and (d) terbutaline for the enantiomer having higher stability constant in chiral selector. Mobilities are given in 10^{-4} $cm^2V^{-1}min^{-1}$ unit.

Table I. Optimum concentration of HDMS-β-CD and mobilities[1] of β-blockers

β-Blocker	C_{max}[2]		u_D	u_{DC}
	Observed	Approximated		
Acebutolol	30	–[3]	63.4	-13.8
Celiprolol	20	10	52.6	3.1
Propranolol	15	9	70.0	-42.0
Terbutaline	20	16	79.4	3.5

[1] Mobilities are given in 10^{-4} cm^2 $V^{-1}min^{-1}$ unit. [2] Concentration given in mM. [3] Not available.

separated at observed C_{max} of HDMS-β-CD concentration. Celiprolol and terbutaline showed the imperfect resolution of the enantiomers. The resolutions of the enantiomers of the β-blockers could be increased when the experiment carried out at the approximated C_{max}.

Stability constants by reciprocal plot

Calculation of the stability constant using Eq. 1 was straightforward. This method required u_{DC} value, however the exact determination of this value was not straightforward. The Eq. 2 does not require the u_{DC} value, though more data points for double reciprocal linear plot in $1/(u_{eff,D} - u_D)$ versus 1/[CD] are needed. The stability constant could be calculated from the intercept and slope of the linear plot (K_{st} = intercept/slope). In Fig. 4 the double reciprocal linear plots for β-blockers were presented.

DISCUSSION

The stability constants between β-blocker and HDMS-β-CD and enantioselectivities calculated by two methods were summarized in Table II. Both methods showed the similar results. The weak base enantiomers were pro-

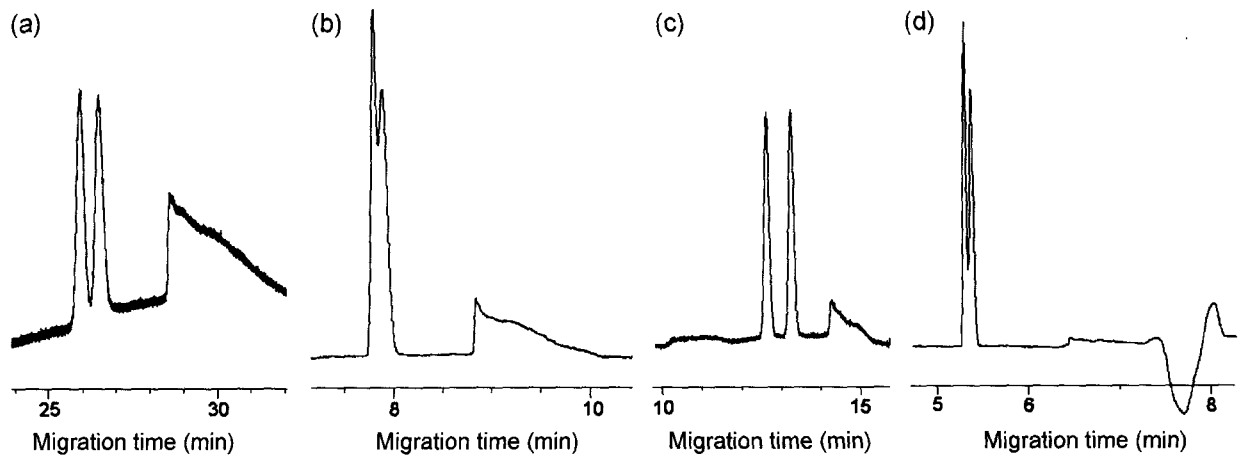

Fig. 3. Electropherograms of β-blockers separated at a HDMS-β-CD concentration of maximum mobility difference between the enantiomers for (a) acebutolol, (b) celiprolol, (c) Propranolol and (d) terbutaline. Fused silica capillary column, 29.5 cm×50 μm I.D.; applied voltage, 10 KV; acetate buffer (0.1 M, pH 4) containing 5% isopropyl alcohol and 30 mM (a), 20 mM (b, d) or 15 mM (c) of HDMS-β-CD; detection, UV 210 nm; capillary temperature, 20°C.

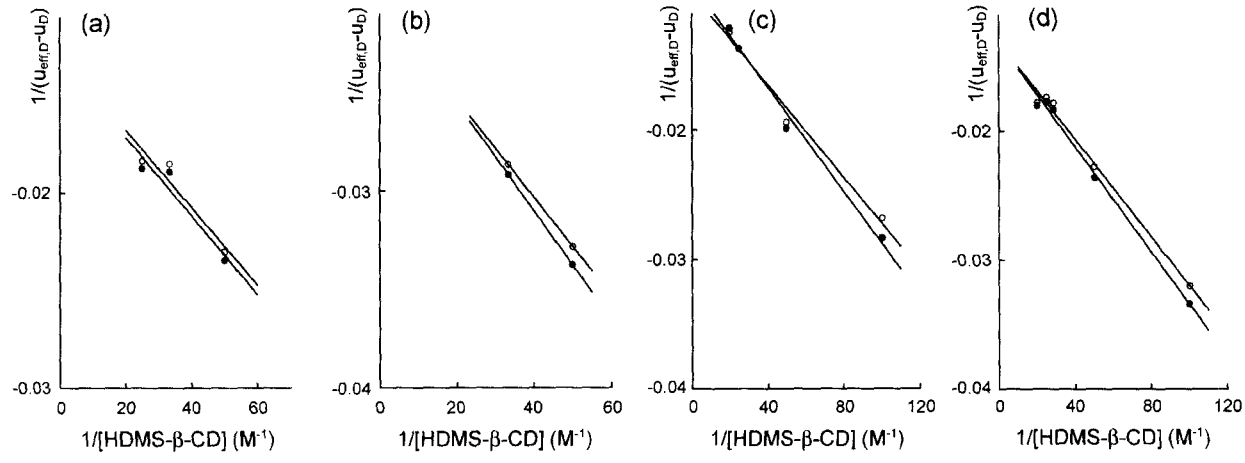

Fig. 4. Linear double reciprocal plots for (a) acebutolol, (b) celiprolol, (c) propranolol and (d) terbutaline for the first (closed) and last (open) eluted enantiomers. Mobilities are given in 10^{-4} cm^2 $V^{-1}min^{-1}$ unit.

Table II. Stability constants[1] of β-blockers and between HDMS-β-CD complexes determined by capillary electrophoresis

β-Blocker	Approximation of u_{DC}			Double reciprocal plot		
	K_2	K_1	$\alpha^{2)}$	K_2	K_1	α
Acebutolol	74.3	73.0	1.02	65.0	64.7	1.00
Celiprolol	77.5	71.8	1.08	81.8	72.9	1.12
Propranolol	56.1	46.0	1.22	52.6	43.4	1.21
Terbutaline	70.5	65.2	1.07	70.0	65.3	1.07

[1] Stability constant were given in M^{-1} unit. [2] a = K_2 / K_1, for K_1 and K_2 first and last eluting enantiomers, respectively

tonated while HDMS-β-CD deprotonated at the pH of the used electrolyte. The deprotonated HDMS-β-CD moved opposite direction of electroosmosis, hence, an enantiomer with higher stability eluted later than that with lower stability. Among the β-blockers, propranolol showed the lowest stability constant but the highest enantioselectivity, indicating that the magnitudes of the stability constants contained little information about enantioseparation. The magnitudes of enantioselectivities between the enantiomer pair were in the order of propranolol > celiprolol > terbutaline > acebutolol. On the other hand the resolution was in the order of propranolol > acebutolol > terbutaline > celiprolol. The discrepancy between the stereoselectivities, calculated from stability constant and obtained by CE experiments in acebutolol and terbutaline, might be caused by the similar magnitudes of the stability constants of both enantiomers. In principle, the stability constant of the enantiomer eluted first should be lower than that of the one eluted last. The stability constant of propranolol-HDMS-β-CD complex was lower than that of propranolol-carboxymethyl-β-CD complex (Park *et al.*, 2003). However, the stereoselectivities of propranolol enantiomers for both chiral selector were comparable each other. The observation suggests that the efficiency of HDMS-β-CD is comparable to carboxymethyl-β-CD as chiral selector for the enantioseparation of propranolol.

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REFERENCES

Bontchev, P. R., Kadum, H., Evtimova, B., Nachev, C., Ivanov, D., Zhecheva, E., and Mehandjiev, E., Copper(II) complexes of (1-[isopropylamine]-3-[1-naphthyloxy]-2-propanol) (propranolol). *J. Inorg. Biochem.*, 48, 153-161 (1992).

Borchard, U., Pharmacological prosperities of β-adrenoceptor blocking drugs. *J. Clin. Bas. Cardiol.*, 1, 5-9 (1988).

Chankvetadze, B., Capillary electrophoresis in chiral analysis. John Wiley & Son, pp. 145-146 (1997).

Kim, K. H. and Park, Y. H., Enantioselective inclusion between terbutaline enantiomers and hydroxypropyl-β-cyclodextrin. *Int. J. Pharm.*, 175, 247-253 (1998).

Mehvar R. and Brocks, D. R., Stereospecific pharmacokinetics and pharmacodynamics of β-adrenergic blockers in humans. *J. Pharm. Pharmaceut. Sci.*, 4, 185-200 (2001).

Park, K. L., Kim, K. H., Jung, S. H., Lim, H. M., Hong, C. H., and Kang, J. S., Enantioselective stabilization of inclusion complexes of metoprolol in carboxymethylated β-cyclodextrin. *J. Pharm. Biomed. Anal.*, 27, 569-576 (2002).

Park, K. R., Lim, H. M., Phuong, N. T., Kim, K. H., and Kang, J. S., Determination of stability constants for β-blocker and carboxymethyl-β-cyclodextrin complexes by capillary electrophoresis. *Yakhak Hoeji*, 47, 200-205 (2003).

Rundlett, K. L. and Arms, D. W., Examination of the origin, variation, and proper use of expression for the estimation of association constants by capillary electrophoresis. *J. Chromatogr. A*, 721, 173-186 (1996).

Stoschizly, K., Zernig, G., and Lindner, W., Racemic β-blockers-fixed combinations of different drugs. *J. Clin. Bas. Cardiol.*, 1, 14-18 (1998).

Toda, N., Vasodilating β-adrenoceptor blockers as cardiovascular therapeutics. *Pharm. Therapeu.*, 100, 215-234 (2003).

Vargas, M. G., Heyden, Y. V., Maftouh, M., and Massart, D. L., Rapid development of the enantiomeric separation of β-blockers by capillary electrophoresis using an experimental design approach. *J. Chromatogr. A*, 855, 681-693 (1999).

Vespalec, R. and Bocek, P., Calculation of stability constants for the chiral selector-enantiomer interaction from electrophoretic mobilities. *J. Chromatogr. A*, 875, 431-445 (2000).

Wikstrand, J., Berglund, G., Hedblad, B., and Hulthe, J., Antiatherosclerotic effects of β-blockers. *Am. J. Cardiol.*, 12, 25-29 (2003).

Williams, B. A. and Vigh, G., Dry look at the CHARM (charge resolving agent migration) model of enantiomer separations by capillary electrophoresis. *J. Chromatogr. A*, 777, 295-309 (1997).