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Chiral separation of neutral species by capillary electrophoresis Evaluation of a theoretical model

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Abstract

A theoretical model has been developed for the separation of enantiomers of neutral species by employing a combination of charged and neutral cyclodextrins. A neutral compound (LY213829), an ionizable cyclodextrin (sulfobutylether–cyclodextrin), and three neutral cyclodextrins (β-cyclodextrin, trimethyl-β-cyclodextrin, hydroxypropyl-β-cyclodextrin) were chosen to test the model. The model parameters were obtained by performing two specific sets of experiments. Resolution and selectivity can be readily obtained from these model parameters. The validity of the model has been demonstrated by resolving enantiomers of LY213829 and its four isomeric sulfoxide metabolites, and the model was very successful in predicting the migration times and resolution of the LY213829 enantiomers. Baseline separation was achieved for all the analytes.

Keywords: Enantiomer separation; Buffer composition; Cyclodextrins; LY213829

1. Introduction

The importance of stereoselectivity in the biological activity of drugs is well established. It has been shown that chiral drugs display stereoselectivity in both their pharmacokinetic and pharmacodynamic effects [1–4]. Historically, analytical resolution of optical isomers was obtained by high performance liquid chromatography or gas chromatography. Recently, capillary electrophoresis (CE) has gained popularity due to its inherent ability to give higher efficiencies and faster separations. The majority of CE chiral separations reported in the literature employ cyclodextrins (CD) as chiral selectors. Chiral separation in CE is based on differences in mobilities between analyte and analyte–CD complexes and on the equilibrium constants of the analyte–CD complexes, and it is a function of the type and concentration of CD and pH of the background electrolyte (BGE). Though there have been numerous reports on obtaining baseline resolution for a variety of analytes, few reports deal with describing theoretical models for optimization of separation. Wren and Rowe have developed a mathematical model relating mobility differences to the concentrations of CD and organic modifier [5,6]. Penn et al. have extended this treatment to the separation of tioconazole enantiomers [7,8]. These models failed to consider the influence of pH on separation. Rawjee et al. and Biggin et al. have developed a multiple-equilibria based model to account for the effects of pH and CD concentration of buffer for both chiral weak acids and weak bases [9–11].

Chiral separation of neutral species can be accom-

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plished by micellar electrokinetic capillary chromatography (MECC) or by employing charged cyclodextrins. In MECC, chiral resolution is based on differential distribution of the analytes between the micelle and the surrounding aqueous phase and the differential migration of these two phases. There are a number of natural and synthetic chiral surfactants available and these can be used alone or in combination with CD to achieve required separations. Two good reviews of these techniques have been published recently [12,13]. Terabe et al. advocated the use of charged cyclodextrins to separate optical isomers [14]. In this technique, the charged cylodextrin behaves in an analogous manner to the micelles in MECC. However, the separation mechanism is based on inclusion complexation equilibria while the charged cyclodextrin acts like a moving stationary phase. Stella et al. have developed and used an ionizable cyclodextrin, sulfobutylether–cyclodextrin (SBE–CD), to separate the enantiomers of a limited set of cationic drugs [15]. They postulated that the countercurrent mobility exhibited by SBE–CD enhances the separation window and improves the selectivity. Lurie et al. employed a mixture of neutral and ionizable cyclodextrins to enhance the chiral separation of cationic drugs [16]. However, there have been few reports of chiral separation of neutral species employing charged cyclodextrins [17–21]. Smith has used carboxymethylethyl–cyclodextrin to separate neutral positional isomers [17]. Sepaniak et al. have used a mixture of neutral and carboxymethyl–cyclodextrin to separate neutral molecules [18,19]. Recently, Brown et al. have used a mixture of dimethyl–cyclodextrin and SBE–CD to separate 16 polycyclic aromatic hydrocarbons [21]. These studies illustrated the advantage of using a mixture of cyclodextrins to enhance separation. However, the choice and concentration of cyclodextrins is purely empirical and optimization of separation is time consuming. The aim of the present work is to develop a theoretical framework to optimize the chiral separation of neutral species when a combination of neutral and ionizable cyclodextrins are used. The model extends the treatment of simultaneous multiple equilibria developed previously for charged analytes [9–11] to the neutral analytes. This model is based on simultaneous multiple equilibria among neutral analyte, charged CD, and neutral CD. The model presents selectivity and resolution equations and illustrates how to determine these from model parameters by performing a simple set of rapid experiments. The model was then tested experimentally to demonstrate its utility in affording baseline resolution in the shortest possible time.

2. Model

Recently, Rawjee et al. have derived a peak resolution equation to describe chiral resolution as a function of pH of background electrolyte, cyclodextrin concentration, charge of analyte, electroosmotic coefficient, and applied potential [22,23]. In the present investigation, a peak resolution equation is developed to cover the case in which neutral enantiomers (R and S) interact differentially with an ionizable cyclodextrin (ICD) and neutral cyclodextrin (NCD). It is assumed that the electroosmotic flow is negligible at the working pH conditions and the ICD is in its fully charged form. Friedl and Kenndler have derived an equation describing resolution for the general case of multivalent ions and showed that resolution depends on both analyte parameters (mobility and charge number of analyte) and instrumental parameters (temperature and applied voltage) [24]. The resolution equation for enantiomers R and S is:

$$R_{RS} = \frac{\mu_R - \mu_S(z_R z_S)^{1/2}}{\mu_R(z_R)^{1/2} + \mu_S(z_S)^{1/2}} \left(\frac{e_0 U}{8kT}\right)^{1/2} \quad (1)$$

where μ_R is the apparent electrophoretic mobility of the R enantiomer, μ_S is the apparent electrophoretic mobility of the S enantiomer, e_0 is the electric charge, k is the Boltzmann constant, T is the absolute temperature, and z_R and z_S are effective charge number of the R and S enantiomer–ICD complex. Friedl and Kenndler have also defined selectivity as the ratio of the effective mobilities of a pair of analytes [24]. The selectivity equation of R and S enantiomers is:

$$\alpha_{RS} = \frac{\mu_R}{\mu_S} \quad (2)$$

The resolution equation, Eq. (1), can also be expressed as a function of selectivity [24]:

$$R = \frac{\alpha_{RS} - 1}{\frac{1}{z_R^{1/2}} + \frac{\alpha_{RS}}{z_S^{1/2}}} \left(\frac{e_0 U}{8kT}\right)^{1/2} \quad (3)$$

The analytes evaluated herein are neutral species and do not have charge. This requires an additional treatment for the effective charge determination of the analytes. When the analyte forms an inclusion complex with ICD, the analyte–ICD complex assumes the charge of ICD. The charge number of ICD is thus the maximum effective charge number that the analyte–ICD complex can assume if it is incorporated in ICD all the time. Since equilibrium dynamics are involved in the inclusion complexation process, the effective charge number must be corrected for this effect (equilibrium). Thus, the effective charge number Z_R or Z_S, is a function of ionic charge of the ICD and mole fraction of the respective species [23]:

$$z_R = z_{ICD}\varphi_{RICD} \quad (4)$$

$$z_S = z_{ICD}\varphi_{SICD} \quad (5)$$

where z_{ICD} is the charge number of ICD, and φ_{RICD} and φ_{SICD} are the mole fractions of charged R and S enantiomer complexes, respectively. The mole fractions of the charged R and S enantiomers (φ_{RICD} and φ_{SICD}) and the effective mobilities of R and S enantiomers (μ_R and φ_S) can be determined from simultaneous multiple equilibria among neutral analytes, ICD and NCD. Enantiomers can form a complex with both ICD and NCD:

$$R + \text{ICD} \Leftrightarrow R\text{ICD} \quad (6)$$

$$R + \text{NCD} \Leftrightarrow R\text{NCD} \quad (7)$$

The equilibrium constants for Eq. (6) and Eq. (7) are:

$$K_{RICD} = \frac{[R\text{ICD}]}{[R][\text{ICD}]} \quad (8)$$

$$K_{RNCD} = \frac{[R\text{NCD}]}{[R][\text{NCD}]} \quad (9)$$

Since the electroosmotic flow is assumed to be negligible, the neutral analyte is mobile only when it is complexed with the charged cyclodextrin. The mole fraction of the charged analyte complex (φ_{RICD}) is therefore written as:

$$\varphi_{RICD} = \frac{[R\text{ICD}]}{C_R} \quad (10)$$

where $C_R = [R] + [R\text{ICD}] + [R\text{NCD}]$. Substitution of Eq. (8) and Eq. (9) into Eq. (10) and rearrangement yields the following:

$$\varphi_{RICD} = \frac{K_{RICD}[\text{ICD}]}{1 + K_{RICD}[\text{ICD}] + K_{RNCD}[\text{NCD}]} \quad (11)$$

The apparent electrophoretic mobility of the R enantiomer (μ_R) is a function of the mobility of the analyte–ICD complex (μ_{RICD}) and the fraction of the analyte complexed [25]:

$$\mu_R = \mu_{RICD}\varphi_{RICD} \quad (12)$$

Substitution of the terms of Eq. (11) into Eq. (12) gives:

$$\mu_R = \frac{\mu_{RICD}K_{RICD}[\text{ICD}]}{1 + K_{RICD}[\text{ICD}] + K_{RNCD}[\text{NCD}]} \quad (13)$$

By analogous arguments, the mole fraction and apparent electrophoretic mobility expressions for the S enantiomer are:

$$\varphi_{SICD} = \frac{K_{SICD}[\text{ICD}]}{1 + K_{SICD}[\text{ICD}] + K_{SNCD}[\text{NCD}]} \quad (14)$$

$$\mu_S = \frac{\mu_{SICD}K_{SICD}[\text{ICD}]}{1 + K_{SICD}[\text{ICD}] + K_{SNCD}[\text{NCD}]} \quad (15)$$

To test the model experimentally, we have chosen LY213829 (Fig. 1) as a neutral analyte. The prerequisite to the selection of ICD and NCDs is that the analyte must form a complex with the cyclodextrins. An ionizable cyclodextrin, sulfobutylether–cyclodextrin (SBE–CD), and three neutral cyclodextrins, β-cyclodextrin (BCD), trimethyl-β-cyclodextrin (TMCD), and hydroxypropyl-β-cyclodextrin (HPCD) were chosen based on preliminary experimentation.

3. Experimental

3.1. Materials

LY213829 and its sulfoxide metabolites were

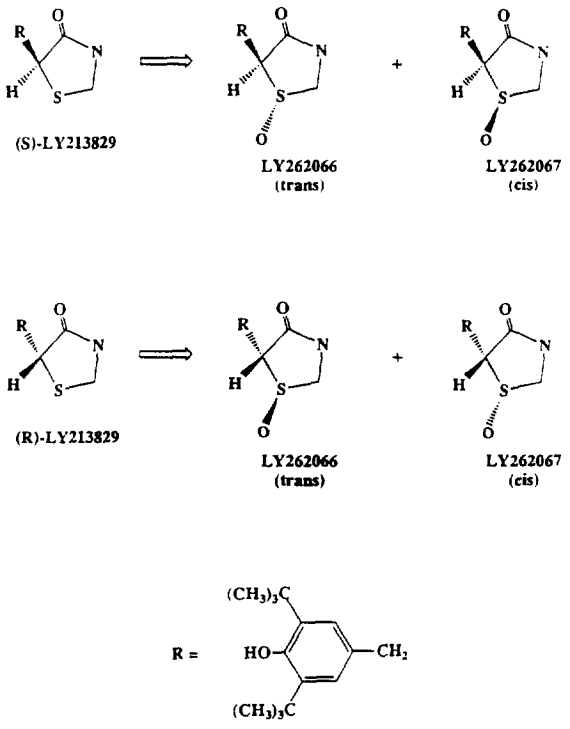

Fig. 1. Structures of the stereoisomers of LY213829 and sulfoxide metabolites.

obtained from Eli Lilly (Indianapolis, IN, USA). HPCD and SBE–CD were gifts from American Maize-Products (Hammond, IN, USA) and CyDex (Overland Park, KS, USA), respectively. BCD was obtained from Aldrich (Milwaukee, WI, USA) and TMCD was purchased from Sigma (St. Louis, MO, USA). All other chemicals used were analytical grade. Buffer solutions were prepared in Milli-Q water (Millipore, Bredford, MA, USA).

3.2. Methods

Separations were carried out using a Beckman PACE 5010 capillary electrophoresis system under the following conditions: 57 cm×75 μm I.D. capillary (Beckman, Fullerton, CA, USA) 50 cm to the detector; 15 kV potential; 25°C capillary temperature; and detection at 214 nm. The injection was by pressure mode for 2 s of sample solution containing 0.5 mg/ml LY213829 or sulfoxides in methanol–background electrolyte solution (BGE) (50:50). BGE was prepared by adjusting the pH of a 50 m*M* lithium hydroxide solution to 2.5 with phosphoric acid, and filtering through a 0.2-μm Anotop disposable syringe filter.

3.3. Determination of model parameters

To estimate the model parameters μ_{RICD}, μ_{SICD}, K_{RICD} and K_{SICD}, Eq. (13) and Eq. (15) were modified. Consider a case where the background electrolyte contains only ICD and no NCD. Eq. (13) and Eq. (15) then simplify to the following:

$$\mu_R = \frac{\mu_{RICD} K_{RICD}[\text{ICD}]}{1 + K_{RICD}[\text{ICD}]} \tag{16}$$

$$\mu_S = \frac{\mu_{SICD} K_{SICD}[\text{ICD}]}{1 + K_{SICD}[\text{ICD}]} \tag{17}$$

Reciprocal transformations of Eq. (16) and Eq. (17) give the following equations:

$$\frac{1}{\mu_R} = \frac{1}{\mu_{RICD}} + \frac{1}{\mu_{RICD} K_{RICD}} \frac{1}{[\text{ICD}]} \tag{18}$$

$$\frac{1}{\mu_S} = \frac{1}{\mu_{SICD}} + \frac{1}{\mu_{SICD} K_{SICD}} \frac{1}{[\text{ICD}]} \tag{19}$$

The effective mobilities of the enantiomers (μ_R or μ_S) are obtained by varying the concentration of SBE–CD in the background electrolyte ranging from 1.25 m*M* to 20 m*M*. Then estimates of the model parameters μ_{RICD}, μ_{SICD}, K_{RICD}, and K_{SICD} can be determined by plotting $1/\mu_R$ or $1/\mu_S$ as a function of 1/[ICD]. To determine the model parameters K_{RNCD} and K_{SNCD}, Eq. (13) and Eq. (15) are transformed to the following form:

$$\frac{1}{\mu_R} = \frac{1}{\mu_{RICD}} + \frac{1 + K_{RNCD}[\text{NCD}]}{\mu_{RICD} K_{RICD}} \frac{1}{[\text{ICD}]} \tag{20}$$

$$\frac{1}{\mu_S} = \frac{1}{\mu_{SICD}} + \frac{1 + K_{SNCD}[\text{NCD}]}{\mu_{SICD} K_{SICD}} \frac{1}{[\text{ICD}]} \tag{21}$$

In order to obtain K_{RNCD} and K_{SNCD}, the concentration of SBE–CD was varied from 1.25 m*M* to 20 m*M* while keeping the concentration of NCD constant at 5 m*M*. The reciprocal of the effective mobilities were then plotted against the reciprocal of the SBE–CD concentration. Since the parameters μ_{RICD}, μ_{SICD}, K_{RICD} and K_{SICD} are already known, K_{RNCD} and K_{SNCD} can be determined from the slope

and intercept. The equilibrium constants, K_{RNCD} and K_{SNCD}, for three neutral cyclodextrins BCD, HPCD, and TMCD were determined. These parameters were used in subsequent modeling of resolution and selectivity.

4. Results and discussion

Fig. 1 shows the structure of LY213829 and its sulfoxide metabolites. The model parameters for the *R* and *S* enantiomers of LY213829 were determined according to the procedure in the experimental section and are listed in Table 1. Fig. 2 shows the mobility of the *R* and *S* enantiomers as a function of SBE–CD concentration over the range of 0.5 to 20 m*M*. The dotted and solid curves were predicted from the theoretical equation, and the symbols indicate the experimentally measured values. There was good agreement between the calculated and experimentally determined mobilities. Theoretically predicted (solid line) and experimentally determined (symbols) resolution values are shown in Fig. 3. A resolution of 1.5 is required to obtain baseline separation in chromatography and electrophoresis. It is evident from Fig. 3 that it is difficult to obtain the baseline separation by using SBE–CD as the ICD.

Table 1
Experimentally determined model parameters for LY213829 enantiomer–cyclodextrin complexes

Effective mobility	
$\mu_{RICD}(10^{-4}$ cm^2/V s)	2.32±0.006
$\mu_{SICD}(10^{-4}$ cm^2/V s)	2.31±0.006
Complexation	
SBE–CD	
$K_{RICD}(10^3\ M^{-1})$	6.1832±0.28
$K_{SICD}(10^3\ M^{-1})$	5.7058±0.24
BCD	
$K_{RNCD}(10^3\ M^{-1})$	1.0284±0.016
$K_{SNCD}(10^3\ M^{-1})$	1.1009±0.018
TMCD	
$K_{RNCD}(10^3\ M^{-1})$	0.2446±0.016
$K_{SNCD}(10^3\ M^{-1})$	0.2313±0.014
HPCD	
$K_{RNCD}(10^3\ M^{-1})$	1.9540±0.052
$K_{SNCD}(10^3\ M^{-1})$	1.9127±0.050

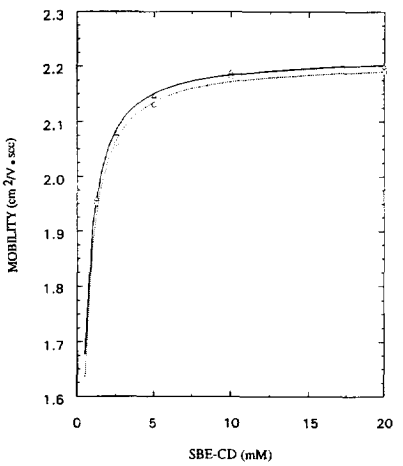

Fig. 2. Mobility of *R* (△) and *S* (○) enantiomers of LY213829 as a function of SBE–CD concentration, solid and dotted lines are mobilities predicted from the model for the *R* and *S* enantiomers, respectively.

Fig. 4 shows the electropherograms of LY213829 at various concentrations of SBE–CD. The experimental evidence is in agreement with the theoretical prediction that resolution is relatively insensitive to SBE–CD concentration. However, the resolution values were approximately 60% of what was predicted (Fig. 3). Similar experimental deviation from theoretical prediction has been observed by others [11,23]. The theoretical calculations are based on the assumption that SBE–CD has a charge of 4. In

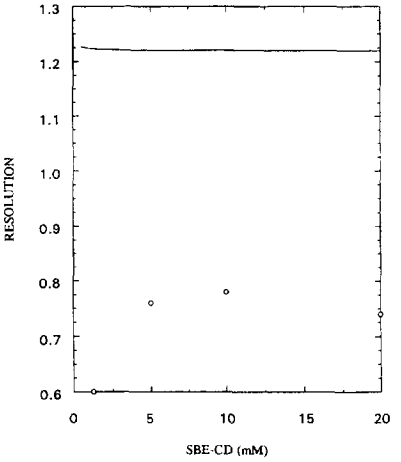

Fig. 3. Theoretical (——) and experimental (○) resolution as a function of SBE–CD concentration.

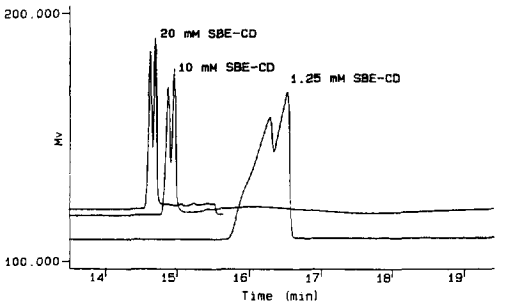

Fig. 4. Electropherograms of LY213829 enantiomers at various concentrations of SBE–CD. For conditions see Section 3.

actuality, this is the average charge with a range from 2 to 10 (certificate of analysis from CyDex). At lower concentrations of SBE–CD, the analyte remains for a longer duration of time in the capillary resulting in greater band broadening. It is evident from Fig. 4 that peaks were broader at lower concentrations than at higher concentrations of SBE–CD under similar experimental conditions.

Complexation constants derived for the neutral cyclodextrins BCD, TMCD and HPCD with LY213829 enantiomers are listed in Table 1. It can be seen that complexation constants vary widely among native and modified cyclodextrins for LY213829. A combination of ionizable and neutral cyclodextrins would result in modification of the mobility of enantiomers due to differences in complexation constants. Using the derived model parameters and Eq. (1), the resolution surfaces have been calculated for SBE–CD in combination with BCD, HPCD, and TMCD. Fig. 5 shows the resolution surface of LY213829 as a function of BCD and SBE–CD concentration. It is apparent from a comparison of Fig. 3 and Fig. 5 that the resolution improved considerably in the presence of BCD; especially at low SBE–CD concentrations. The theoretical model predicts that the resolution increases as concentration of BCD increases while keeping the concentration of SBE–CD low. If the concentration of SBE–CD is allowed to increase while keeping the concentration of BCD constant, the resolution should decrease considerably. The electropherograms of LY213829 at various SBE–CD concentrations and constant BCD concentration (5 m*M*) validate the predicted resolution effect (Fig. 6). The resolution was high at 1.25 m*M* SBE–CD but decreased rapidly as the concentration approached 20 m*M* SBE–CD. This followed the trend predicted by the model. However, the improved resolution at lower concentrations of SBE–CD comes at the cost of longer analysis times and increased band broadening as shown in Fig. 6.

Fig. 7 shows the resolution of LY213829 enantiomers as a function of HPCD and SBE–CD concentration. The resolution surface follows a similar trend to that observed for BCD, though the magnitude of resolution was far less than that which can be achieved with BCD. As the concentration of SBE–CD was increased to 20 m*M*, the resolution was lost. This loss of resolution was also noticed for TMCD. Fig. 8 shows the resolution surface calculated for TMCD and SBE–CD. Again, the resolution surface followed the general trend predicted for BCD and HPCD, though the magnitude of the resolution

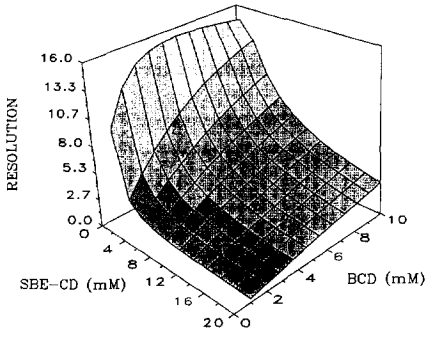

Fig. 5. Resolution surface of LY213829 enantiomers as a function of SBE–CD and HPCD concentrations.

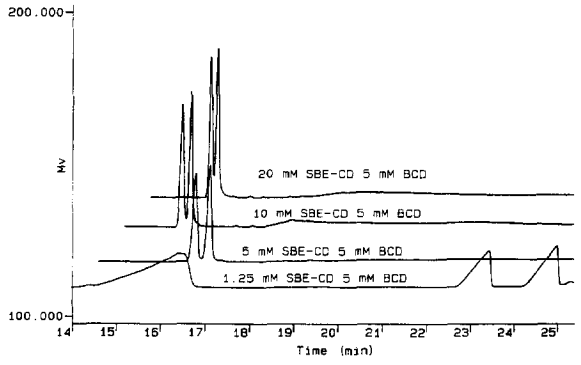

Fig. 6. Electropherograms of LY213829 enantiomers at varying concentrations of SBE–CD and 5 m*M* BCD in BGE. For conditions see Section 3.

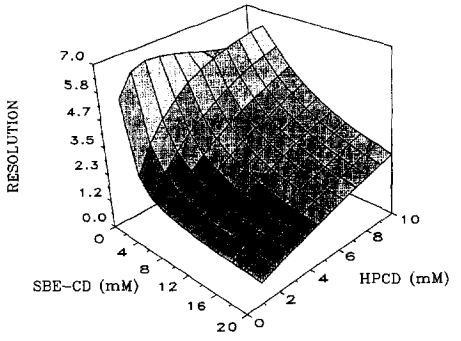

Fig. 7. Resolution surface of LY213829 enantiomers as a function of SBE–CD and HPCD concentrations.

was far inferior to that predicted for either BCD or HPCD. The difference in magnitude of resolution when BCD, HPCD, TMCD were employed in combination with SBE–CD is more appropriately explained by the selectivity Eq. (2). The loss of resolution with high concentrations of SBE–CD can also be explained by the selectivity equation. Substitution of the elements of Eq. (13) and Eq. (15) into Eq. (2) followed by rearrangement of the terms yields the following form:

$$\alpha_{RS} = \frac{\mu_{RICD}}{\mu_{SICD}} \frac{K_{RICD}}{K_{SICD}} \frac{1 + K_{SICD}[\text{ICD}] + K_{SNCD}[\text{NCD}]}{1 + K_{RICD}[\text{ICD}] + K_{RNCD}[\text{NCD}]} \quad (22)$$

The selectivity term (α_{RS}) has three components: the ratio of enantiomer mobilities, the ratio of complexation constants of the enantiomers with the ionizable cyclodextrin, and the ratio of a term involving the concentration of each cyclodextrin

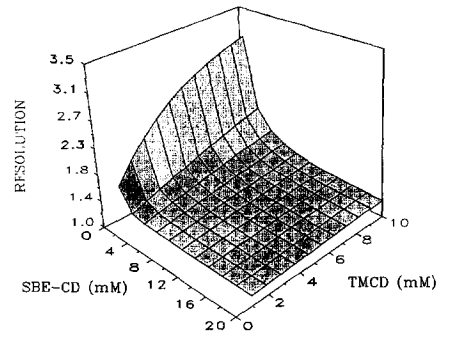

Fig. 8. Resolution surface of LY213829 enantiomers as a function of SBE–CD and TMCD concentrations.

species as well as the complexation constants of each enantiomer with the ionizable and neutral cyclodextrin. The inherent mobilities of the analyte–SBE–CD complex (μ_{RICD} and μ_{SICD}) are equal since the charge to mass ratio is the same for both enantiomers. The experimental data support this conclusion since the μ_{RICD} and μ_{RICD} values in Table 1 are essentially identical. The important aspect of the Eq. (22) is that the *R* enantiomer complexation constant is in the numerator in the second component while it is in the denominator in the third component of the equation. The converse is true in the case of the *S* enantiomer. Therefore, while the value of the second component of Eq. (22) is 1.084 (K_{RICD}/K_{SICD}), the value of the third component is always less than one at all practical concentrations of ICD and NCD (Table 1). Thus, the gain in selectivity resulting from the second component of the Eq. (22) is off set by the third component of the Eq. (22). The selectivity can be optimized when $K_{SNCD} > K_{RNCD}$. It is clear from Table 1 that this requirement is fulfilled when BCD was employed. This explains the maximum selectivity and resolution observed when BCD was employed in combination with SBE–CD. The gain in selectivity and resolution was marginal when HPCD or TMCD is employed in combination with SBE–CD, since the complexation constant $K_{RNCD} > K_{SNCD}$. However, the selectivity dropped sharply as the concentration of SBE–CD was increased. This is due to the differences in magnitude of the complexation constants between SBE–CD and the neutral cyclodextrins. At higher concentrations of SBE–CD, the contribution of neutral cyclodextrin to the third component of Eq. (22) becomes negligible since $K_{SICD}[\text{ICD}]/K_{SNCD}[\text{NCD}] \gg 1$ and $K_{RICD}[\text{ICD}]/K_{RNCD}[\text{NCD}] \gg 1$. Fig. 9 shows an example of the change in selectivity as the concentration of SBE–CD increased at a constant concentration of NCD (5 m*M*). It is clear that maximum selectivity is obtained when BCD is employed as the neutral cyclodextrin with lower concentrations of SBE–CD.

The theoretical model predicted, and the experimental evidence demonstrated that high resolution of LY213829 enantiomers is obtained by employing a combination of BCD and SBE–CD. To test the theoretical model, a BGE system consisting of 10 m*M* SBE–CD and 7 m*M* BCD was chosen. Though high resolution can be obtained at lower SBE–CD

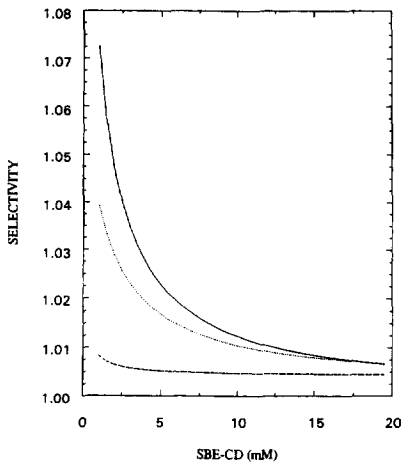

Fig. 9. Change in selectivity as a function of SBE–CD concentration at 5 m*M* neutral cyclodextrin; BCD (solid line), HPCD (dotted line), and TMCD (dashed line).

and higher BCD concentrations, it is not a favorable choice owing to increased analysis time and peak broadening. Primary considerations for the selection of background electrolyte are: (a) baseline resolution of enantiomers, (b) short analysis time, and (c) reproducible migration times. Fig. 10 shows the electropherograms obtained under these experimental conditions. Baseline separation of LY213829 enantiomers was achieved under the conditions predicted by the model parameters. The migration times predicted from the mobility equation (indicated by arrows in electropherogram Fig. 10A are in close

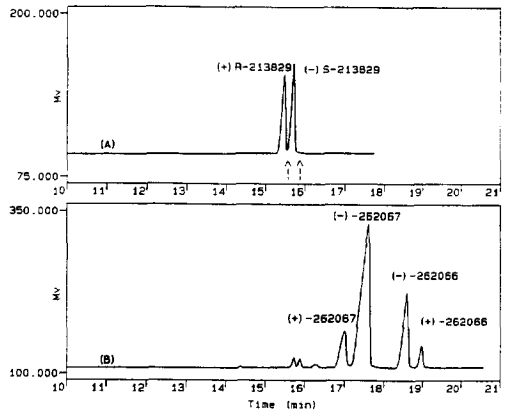

Fig. 10. Electropherograms of LY213829 (A) and sulfoxide metabolites (B) in BGE containing 10 m*M* SBE–CD and 7 m*M* BCD. For conditions see Section 3.

agreement with the experimentally obtained migration times. It can also be seen from Fig. 10B that the sulfoxide metabolites were resolved under the same experimental conditions. The theoretical model was successful in predicting the resolution as well as migration times of LY213829 enantiomers.

5. Conclusions

A theoretical model has been developed for separation of enantiomers of neutral species by employing a combination of charged and neutral cyclodextrins. Resolution and selectivity can be calculated based on model parameters. The validity of the model has been demonstrated by resolving the stereoisomers of LY213829 and its sulfoxide metabolites with a BGE containing 10 m*M* SBE–CD and 7 m*M* BCD. It has been demonstrated that the model is very useful in predicting the migration times and resolution of test substances, and baseline separation was achieved for all species. Further investigations will be conducted to test the usefulness of other charged cyclodextrins in resolving neutral species and the effect of charge on resolution.

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