ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1995) 65–72

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Control of enantiomer migration order in capillary electrophoresis separations using sulfobutyl ether beta-cyclodextrin ☆

L. Liu, M.A. Nussbaum *

Pharmaceutical Sciences Division, Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46285, USA

Received for review 24 April 1995; accepted 6 June 1995

Abstract

The control of enantiomer migration order in capillary electrophoresis (CE) by use of sulfobutyl ether beta-cyclodextrin (SBE-β-CD) was investigated. At high pH, electroosmotic flow (EOF) dominated and the enantiomer most strongly associated with the anionic cyclodextrin was detected last. At low pH (and reversed polarity), EOF was minimal and SBE-β-CD functioned as a carrier. Under such conditions, the enantiomer migration order for a neutral chiral compound was reversed. Factors involved in optimization (cyclodextrin and organic modifier concentration) were studied. The impact of migration order on quantitation of low levels (below 1%) of one enantiomer in the presence of the other was also investigated. The precision of peak area ratios (minor/major enantiomer) was evaluated for samples of both enantiomers run by each method. The migration of minor before major enantiomer yielded better quantitation precision in each case.

Keywords: Capillary electrophoresis; Chiral separation; Cyclodextrins; Enantiomer migration order; Sulfobutyl ether beta-cyclodextrin

1. Introduction

In the pharmaceutical development of chiral compounds, analytical methods for measuring enantiomeric purity are necessary. High performance liquid chromatography (HPLC) is often used to separate and quantitate enantiomeric impurities. However, method development is time-consuming, chiral HPLC columns are often expensive, and resolution is not always adequate. Therefore, a complementary technique such as capillary electrophoresis (CE) is of interest. While not always superior to HPLC, CE has proved to be quite useful for chiral separations [1–4].

Chiral CE typically makes use of one or more of a wide variety of run buffer additives to provide chiral selectivity. By utilizing soluble chiral selectors in the run buffer in combination with simple (and inexpensive) silica capillaries, chiral

☆ Presented at the Sixth International Symposium on Pharmaceutical and Biomedical Analysis, April 1995, St. Louis, MO, USA.

* Corresponding author.

0731-7085/95/$09.50
SSDI 0731-7085(95)01603-1

CE offers a great deal of flexibility in method development. Because of the small volumes involved, only small quantities of a chiral selector are required. In addition, CE provides high efficiencies which can result in adequate resolution even with very low separation factors (α values).

The most common chiral additives in CE are cyclodextrins. Native cyclodextrins are unchanged and therefore inadequate when used alone for separations of neutral compounds. In some cases, native cyclodextrins have been used in combination with micelles (e.g. sodium dodecyl sulfate (SDS)) for such separations [5]. Another approach for separations of neutral enantiomers is to use a derivatized cyclodextrin containing a positively or negatively charged functional group. Depending on the functional group appended, the cyclodextrin may be charged at low pH (quaternary amine) [6], high pH (carboxylic acid) [7,8] or across virtually the entire pH range (sulfated) [9–11].

In chiral HPLC or CE, the elution order of two enantiomers can impact quantitation unless the peaks are widely separated. In HPLC, elution of the minor enantiomer before the major one is preferred. If the minor enantiomer elutes after the major one, then integration is hampered by the tailing from the major peak. However, the order of elution is generally hard to predict or control. It has been suggested that, because of the difference in peak shape between CE and HPLC, quantitation in CE is independent of migration order [12]. However, peak shapes in CE are affected by a variety of factors (e.g. diffusion, adsorption, mobility) such that fronting or tailing can be significant. In cases of severe fronting, elution of the major enantiomer first may be preferable; more commonly, tailing is an issue and migration of the minor before the major enantiomer is likely to improve quantitation, as in HPLC. Control of the enantiomer migration order in CE is, therefore, often desirable.

One method of controlling the enantiomer migration order in CE is by reversing the electroosmotic flow (EOF) via dynamic or permanent coatings on the capillary. Synthetic chiral surfactants may also be used for enantiomer migration reversal by changing the buffer additive from a pure D-surfactant to the L-form [13]. It is also possible in some cases to see a reversal of the migration order when pH and cyclodextrin concentration are changed [14]. Schmitt and Engelhardt [8] used carboxylated cyclodextrins with a coated capillary to reverse the migration order. In that case, the carboxylated cyclodextrin was used in a charged (high pH) or uncharged (low pH) mode in conjunction with cationic analytes. When charged, the cyclodextrin served as a carrier, and the enantiomer more strongly incorporated in the cyclodextrin migrated to the anode first. The capillary coating was necessary to prevent the EOF from overwhelming the cyclodextrin mobility at the high pH. At low pH, the cationic analytes migrated toward the cathode and the enantiomer more strongly incorporated with the uncharged cyclodextrin migrated slowest. The reversal of migration order was not possible with an uncoated capillary or with neutral or anionic analytes.

In this work, anionic sulfobutyl ether beta-cyclodextrin (SBE-β-CD) was used to control the migration order of neutral enantiomers. In silica capillaries at high pH, the migration of all species is dominated by a large EOF and is toward the negative electrode (cathode). Anions (e.g. SBE-β-CD) migrate with a slower net velocity than neutral substances because of their intrinsic mobility in the direction opposite that of the EOF. Thus, if enantiomers are separated using an anionic cyclodextrin at high pH, the enantiomer most strongly associated with the cyclodextrin has the slowest net migration. At low pH, the EOF is very small and the movement of anions is toward the positive electrode (anode) due to their electrophoretic mobility. Therefore, if the same two enantiomers are separated using an anionic cyclodextrin at low pH (using reversed polarity so that the detector is at the anode), the enantiomer most strongly associated with the cyclodextrin now elutes first. A diagram of both modes of separation is shown in Fig. 1. In contrast to carboxylated cyclodextrins, the SBE-β-CD charge and structure remain constant across the pH range. For analytes which also do not change charge with pH, the binding between the cyclodextrin and the analyte remains the same for either order of migration. In such a case, some method parameters (e.g. cyclodextrin or organic

modifier concentration) developed for one order of migration may be appropriate for the opposite order of migration as well.

Finally, while it is reasonable to assume that the migration order can affect quantitation in CE, little data have been provided to support the assumption. To examine the effect in our case, precision data were obtained for the quantitation of less than 1% opposite enantiomer in both the R and S forms of the compound of interest, separated using both orders of migration.

2. Experimental

A P/ACE 5500 capillary electrophoresis instrument (Beckman Instruments, Fullerton, CA, USA) was used for all work. The detector was at the cathode (negative electrode) for work using the pH 8.5 buffer; the polarity was reversed with the pH 2.0 buffer. The capillary was uncoated fused silica (Beckman Instruments), 75 μm × 57 cm (50 cm to detector). The capillary was rinsed with 0.1 N NaOH, water, and run buffer prior to use. The detection wavelength was 215 nm and the capillary temperature was 20 °C. Locally developed software was used for the integration of peaks.

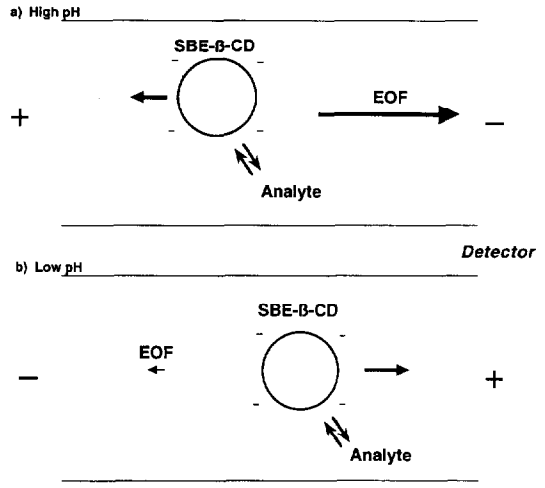

Fig. 1. Reversal of net migration direction and enantiomer migration order using SBE-β-CD at (a) high pH and (b) low pH. At high pH (a), EOF is strong and all substances are carried toward the cathode. The enantiomer spending more time incorporated in SBE-β-CD is detected last. At low pH (b), EOF is minimal and SBE-β-CD migrates toward the anode. The enantiomer spending more time in SBE-β-CD is detected first. Note that the instrument polarity is reversed.

Sulfobutyl ether beta-cyclodextrin, sodium salt (SBE-β-CD) was provided by CyDex, L.C. (Overland Park, KS) with assistance from the Applied Biosciences Division of Perkin-Elmer Corporation (Foster City, CA). The material used contained an average molecular substitution of four sulfobutyl groups per cyclodextrin molecule.

Borate buffer was prepared by dissolving the required amount of boric acid in water and adjusting the pH to 8.5 with NaOH. A stock solution at 0.2 M was used for subsequent dilution to 40 mM unless otherwise noted. Tris–phosphate buffer was prepared by dissolving sufficient Tris (i.e. tris(hydroxymethyl)aminomethane) for a 0.2 M stock solution in water, and adjusting the pH to 2.0 with phosphoric acid. The stock solution was diluted to 40 mM unless noted otherwise. The concentrations listed for this buffer refer only to Tris; the phosphate concentration was not measured. The SBE-β-CD was either weighed directly into the run buffer at the desired concentration or diluted from a stock solution of 75 mM SBE-β-CD in water. The pH was not remeasured after the dilution of buffer and the addition of SBE-β-CD. All the solutions were filtered through 0.45 μm syringe filters prior to use.

The samples were dissolved in water–methanol (50:50, v/v). All injections were hydrodynamic (1 or 2 s applied pressure). Dimethylformamide (DMF; 1–2% in water) was used as an EOF marker.

3. Results and discussion

Compound I (see Fig. 2) is a chiral compound currently in pharmaceutical development at Lilly Research Laboratories. It is uncharged across the normal pH range and therefore the enantiomers cannot be separated using a neutral cyclodextrin alone. Some work was done using hydroxypropyl-β-cyclodextrin in combination with sodium dodecylsulfate (SDS). Although complexation with the cyclodextrin was observed, no separation of the

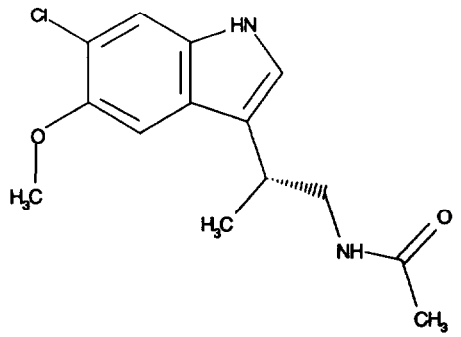

Fig. 2. Structure of compound I (R enantiomer).

enantiomers was obtained. Therefore, SBE-β-CD was investigated.

Initial work was done using 40 mM borate at pH 8.5 containing 10% methanol. A range of SBE-β-CD concentrations was studied, and the resolution of the enantiomers of compound I was found to increase with SBE-β-CD concentration up to about 15 mM. At higher concentrations, migration times increased but the resolution remained almost constant. Therefore, 15 mM SBE-β-CD was used in further work. Fig. 3 shows the effect of SBE-β-CD concentration on migration time and resolution.

The addition of methanol affected the resolution and migration times. Fig. 4 shows the effect of increasing methanol concentration from 0–30% (v/v). The EOF decreased throughout this range (presumably because of increased viscosity), as indicated by the migration times for DMF. Migration times of compound I also increased up to a point. As the methanol concentration reached 25–30%, the migration time of the compound did not increase, despite further decrease in EOF. These results indicate that the compound spent less time in SBE-β-CD as the run buffer became significantly hydrophobic, as would be expected. Resolution between the enantiomers increased with increasing concentrations of methanol, up to 20–25%, then remained fairly constant.

Based on the above work, 15 mM SBE-β-CD and 20 or 25% methanol were used for further studies. By spiking with the pure R enantiomer, the elution order was found to be R,S using the

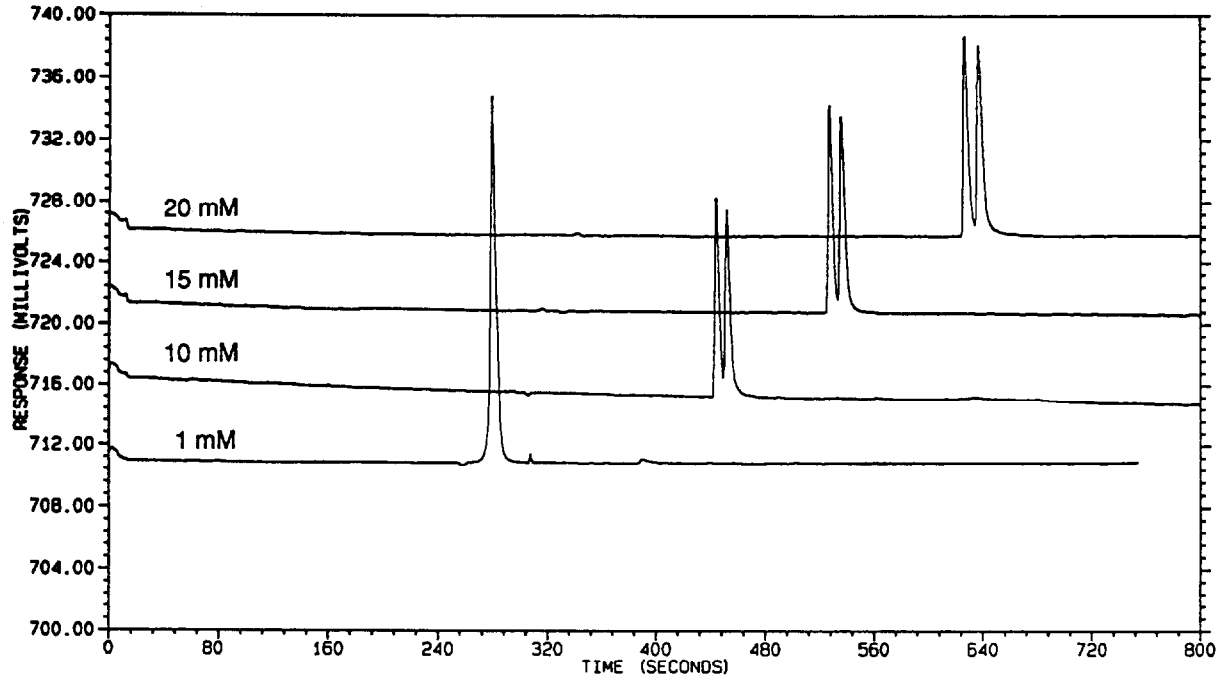

Fig. 3. The effect of SBE-β-CD concentration on the resolution and migration time. Conditions: 40 mM borate (pH 8.5), containing 10% methanol; 25 kV, 2 s injection of racemate (0.4 mg ml^{-1}).

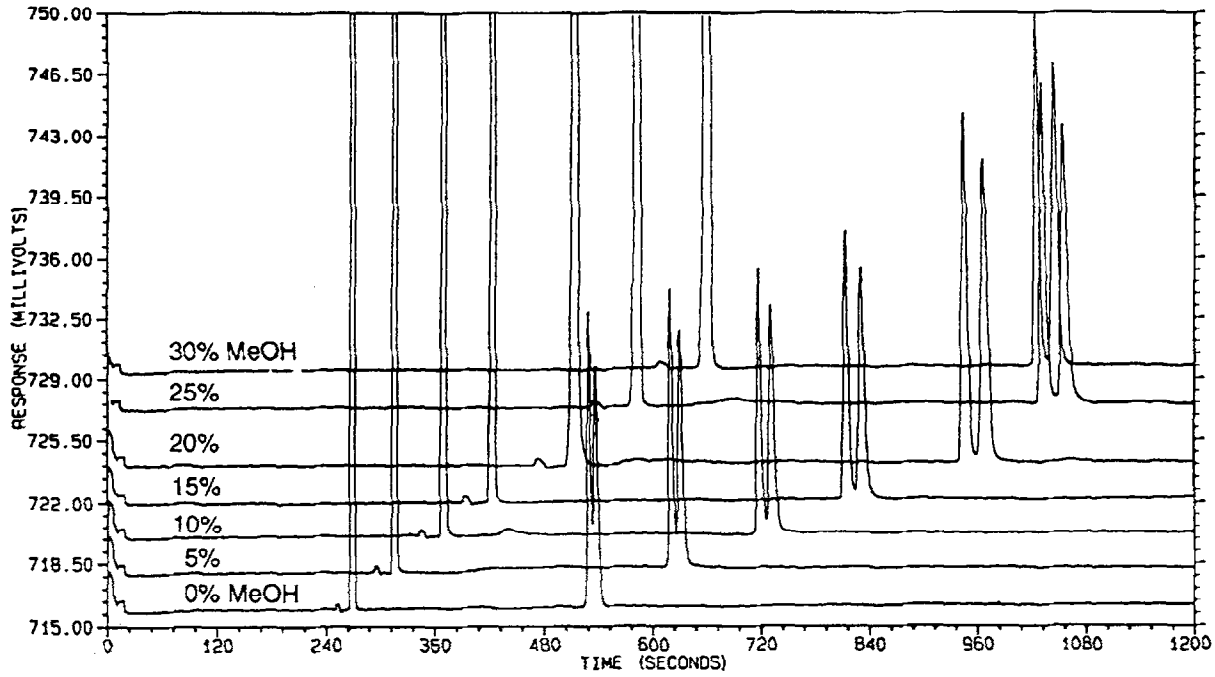

Fig. 4. The effect of methanol concentration (%, v/v) on the resolution and migration time. DMF was included as an EOF marker and is the first major peak in each electropherogram. SBE-β-CD at 15 mM; other conditions as in Fig. 3.

pH 8.5 conditions. The Tris–phosphate buffer (pH 2.0) was then investigated (with reversed electrode polarity). Baseline separation with reversed migration order (i.e. S,R) was obtained. Fig. 5 shows the separation obtained under each set of conditions. The conductivity of the Tris–phosphate buffer was higher than the borate buffer, so the voltage used was typically a few kilovolts less (e.g. 20 kV vs. 25 kV in Fig. 5). The migration time was significantly longer using the pH 2.0 conditions than with the pH 8.5 buffer. No optimization of the buffer concentration was done, but reducing the Tris–phosphate concentration and increasing the voltage would be expected to reduce the migration time. The effect of varying the methanol concentration was briefly investigated using the pH 2.0 buffer and 15 mM SBE-β-CD. Increasing the methanol content from 0 to 25% increased both resolution and migration time, with the resolution at 20 and 25% methanol being similar. No methanol concentrations higher than 25% were tested since migration times were already relatively long and would increase further at higher concentrations.

The effect of enantiomer migration order on quantitation was investigated under both sets of conditions. A sample of the R enantiomer (containing about 0.4% S enantiomer by HPLC) and a sample of S enantiomer (containing about 0.9% R enantiomer by HPLC) were each run by both methods. Peak area ratios (minor/major enantiomer) were measured from six injections in each case and compared. Typical electropherograms are shown in Figs. 6 and 7. Note that peak tailing was seen with both methods. Integration of the minor peak was therefore more difficult when it migrated after the major peak. An impurity peak, in addition to the minor enantiomer, is visible in each electropherogram. Also, some drift in migration times was seen between the two runs shown in Fig. 7.

The quantitation precision results are shown in Table 1. Note that quantitation was most precise when the order of migration was minor before

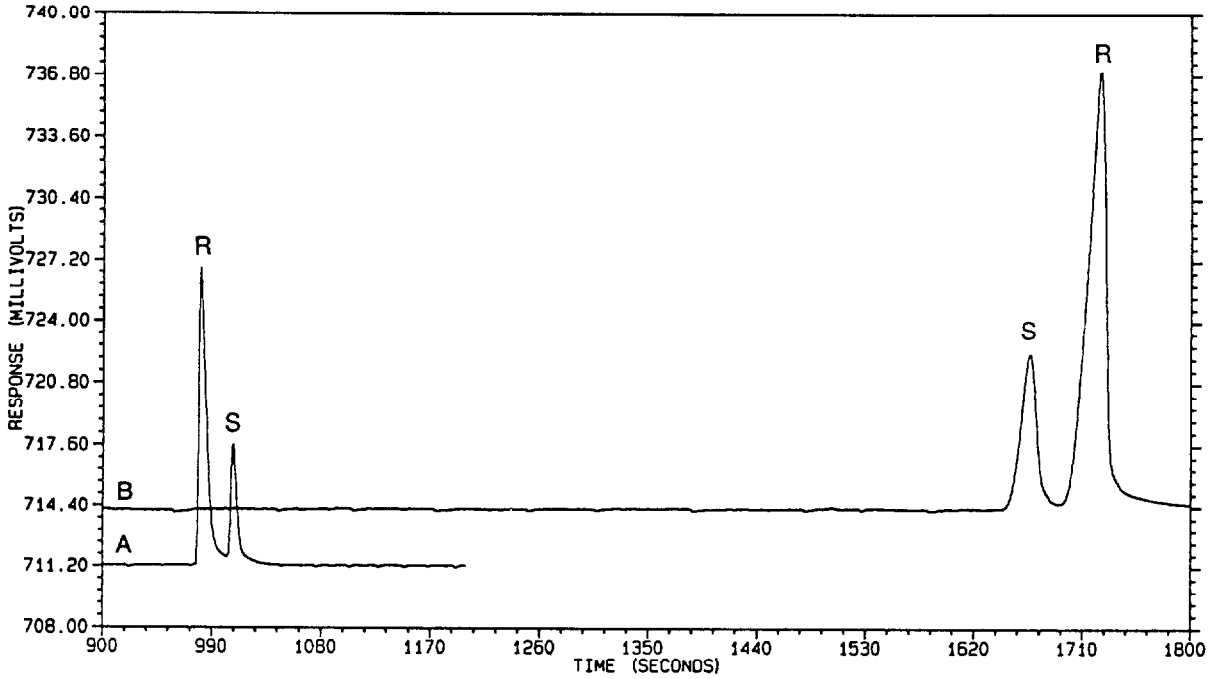

Fig. 5. Reversal of migration order using SBE-β-CD at pH 8.5 and pH 2.0. (a) 40 mM Borate; pH 8.5; 15 mM SBE-β-CD; 20% MeOH; 25 kV. (b) 40 mM Tris; pH 2.0; 15 mM SBE-β-CD; 20% MeOH; 20 kV (reversed polarity). Sample for both (a) and (b): racemate spiked with the R enantiomer. Note that the time axis is from 900 to 1800 s (15–30 min).

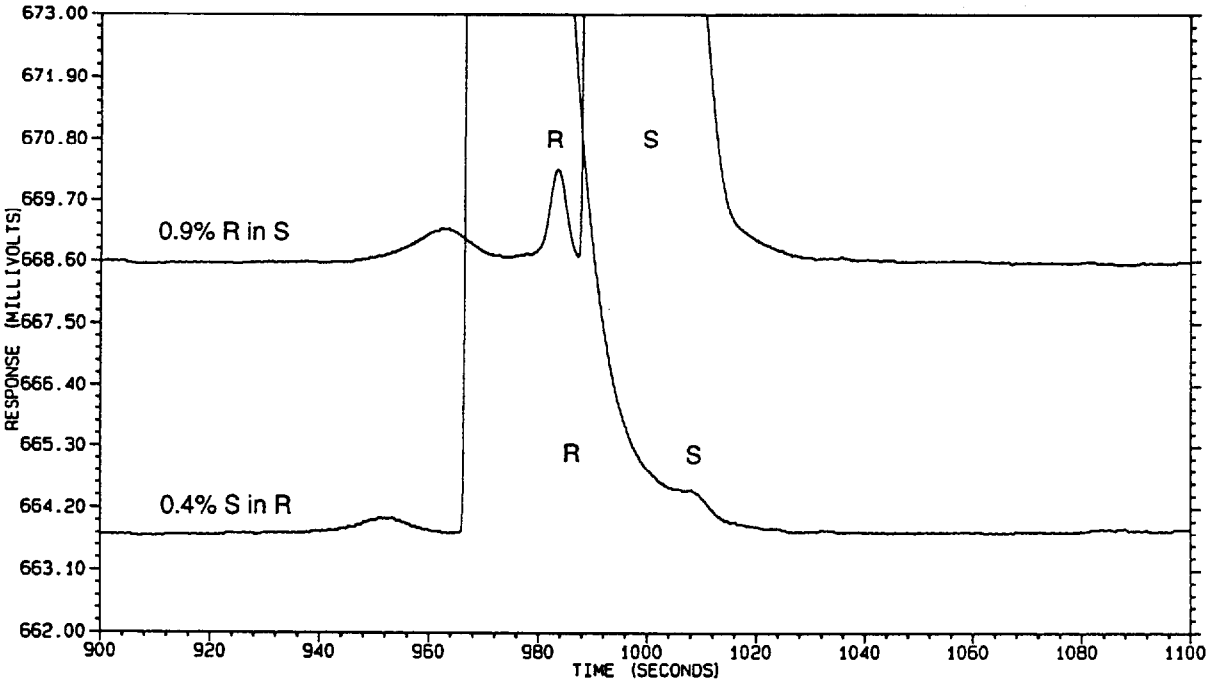

Fig. 6. Electropherograms at pH 8.5 of less than 1% minor enantiomer in the presence of the major enantiomer at 1 mg ml^{-1}. (a) about 0.4% S in R; (b) about 0.9% R in S. Conditions: 40 mM Borate; pH 8.5; 15 mM SBE-β-CD; 25% MeOH; 25 kV; 2 s injections.

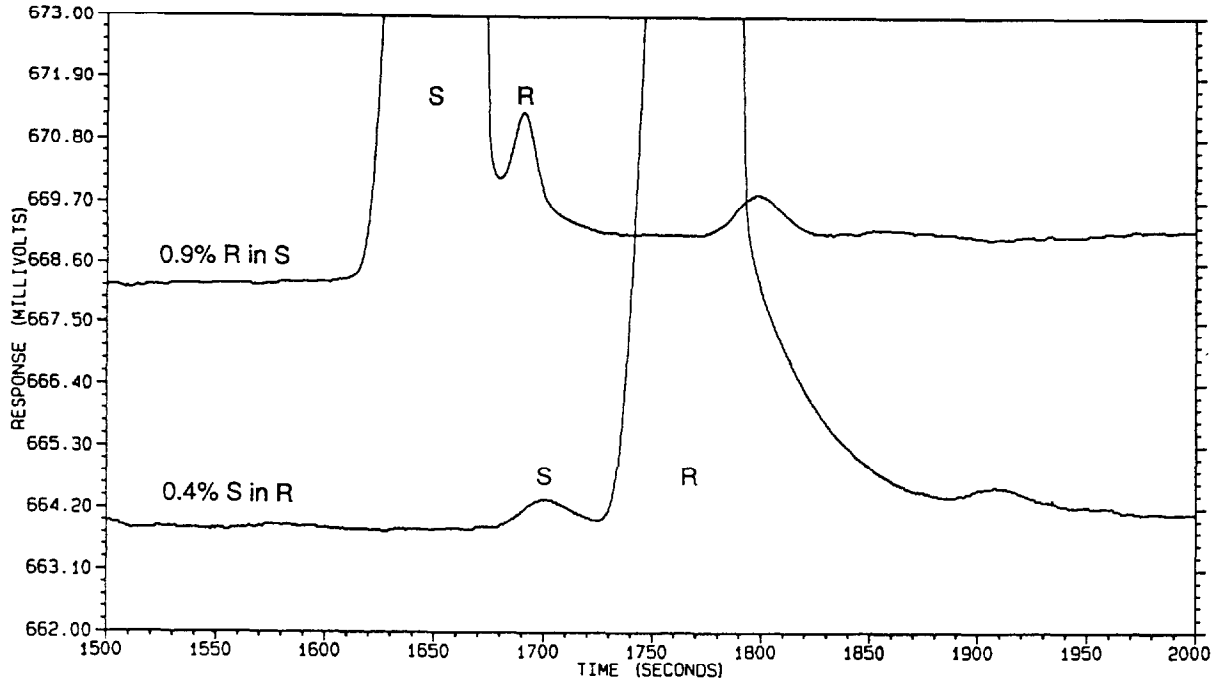

Fig. 7. Electropherograms at pH 2.0 of less than 1% minor enantiomer in the presence of major enantiomer at 1 mg/ml^{-1}: (a) about 0.4% S in R; (b) about 0.9% R in S. Conditions: 40 mM Tris; pH 2.0; 15 mM SBE-β-CD; 25% MeOH; 23 kV; 2 s injections.

Table 1
Precision (% RSD) of peak area ratios (minor/major enantiomer)

Sample	RSD[a] (%)	
	pH 8.5 (order: R,S)	pH 2.0 (order: S,R)
Approx. 0.9% R in S	5.4	15.0
Approx. 0.4% S in R	23.8	3.8

[a] $n = 6$.

major enantiomer in each case. That is, quantitation of low levels of R in S was most precise at pH 8.5, while quantitation of S in R was most precise at pH 2.0. These results clearly indicate that it was the migration order, not other difference between the methods, that had the most significant impact on the precision of quantitation of the minor enantiomer.

4. Conclusions

The use of SBE-β-CD at either high pH or low pH (with reversed polarity) has been shown to be an effective means of controlling enantiomer migration order for a neutral chiral compound. Furthermore, the utility of such migration order control in the quantitation of low levels of one enantiomer in the presence of the other has been demonstrated. In the cases observed here, where peak tailing was present, migration of minor before major enantiomer resulted in improved integration precision. Therefore, when low limits of quantitation are required, control of enantiomer migration order by this or other means should be considered.

Acknowledgments

We gratefully acknowledge the generosity and assistance of the Higuchi Biosciences Center

(Lawrence, KS), Diane Thompson of CyDex, L.C., and Steve Moring of Perkin-Elmer (Applied Biosystems Div.) in providing samples of sulfobutyl ether beta-cyclodextrin.

References

[1] T.J. Ward, Anal. Chem., 66 (1994) 632A–640A.
[2] M. Novotny, H. Soini and M. Stefansson, Anal. Chem., 66 (1994) 646A–655A.
[3] R. Vespalec and P. Bocek, Electrophoresis, 15 (1994) 755–762.
[4] R. Kuhn and S. Hoffstetter-Kuhn, Chromatographia, 34 (1992) 505–512.
[5] S. Terabe, Y. Miyashita, Y. Ishihama and O. Shibata, J. Chromatogr., 636 (1993) 47–55.
[6] A. Nardi, A. Eliseev, P. Bocek and S. Fanali, J. Chromatogr., 638 (1993) 247–253.
[7] N.W. Smith, J. Chromatogr. A, 652 (1993) 259–262.
[8] T. Schmitt and H. Engelhardt, Chromatographia, 37 (1993) 475–481.
[9] C. Dette, S. Ebel and S. Terabe, Electrophoresis, 15 (1994) 799–803.
[10] R.J. Tait, D.O. Thompson, V.J. Stella and J.F. Stobaugh, Anal. Chem., 66 (1994) 4013–4018.
[11] B. Chankvetadze, G. Endresz and G. Blaschke, Electrophoresis, 15 (1994) 804–807.
[12] A. D'Hulst and N. Verbeke, Electrophoresis, 15 (1994) 854–863.
[13] J.R. Mazzeo, E.R. Grover, M.E. Swartz and J.S. Peterson, J. Chromatogr. A., 680 (1994) 125–135.
[14] Y.Y. Rawjee and G. Vigh, Anal. Chem., 66 (1994) 619–627.