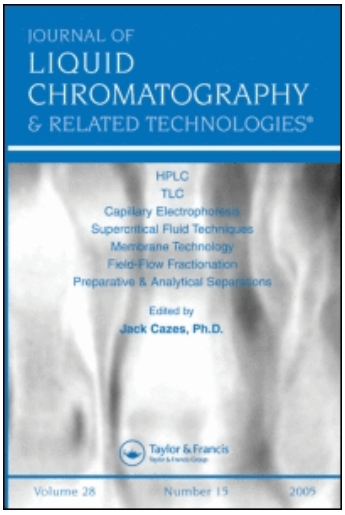

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ENANTIOMER SEPARATION BY ELECTROCHROMATOGRAPHY IN OPEN TUBULAR COLUMNS COATED WITH CHIRASIL-DEX

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ABSTRACT

The possibility of chiral interactions in a 50 μm i.d. open tubular column was created by coating the inner surface with an immobilizable dimethylpolysiloxane containing chemically bonded permethylated β- or γ-cyclodextrin (CHIRASIL-DEX). This chiral stationary phase was employed for the separation of the enantiomers of NSAIDs and small molecules by the principle of electrochromatography under various conditions.

INTRODUCTION

Capillary electrophoresis is a versatile technique for the analysis of charged and uncharged molecules. As compared to pressure driven systems very high efficiencies, resulting from the presence of a nearly plug flow profile in electro-driven systems, are observed. The number of applications, e.g. for biomolecules such as proteins and nucleic acids, but also for small molecules is rapidly in-

creasing. Capillary electrophoresis has recently gained importance in enantiomer analysis of chiral compounds, especially of chiral pharmaceuticals.

The most common method of enantiomer analysis in capillary electrophoresis is based on the use of a chiral complexing agent added to the running buffer system[1]. A pseudostationary phase is thereby created, which moves with the mobile phase and enantioselectivity arises as a consequence of the different stabilities of the diastereomeric complexes formed between the enantiomers and the chiral additive. Thus, Zare et al.[2] achieved the first enantiomer separation in capillary zone electrophoresis by the principle of ligand-exchange using copper(II)-(L)-histidine for the separation of dansylated amino acids.

In micellar electrokinetic chromatography (MEKC) chiral surfactants such as bile salts[3], chiral modified micelles like *N*-dodecanoyl-L-amino acid salts[4] and mixtures of sodium n-dodecylsulfate (SDS) with a nonionic surfactant[5] or cyclodextrins[6] were added to the buffer. Native or derivatized α-, β- or γ-cyclodextrins could also be used as single buffer additives for enantiomer separation in capillary zone electrophoresis[7], capillary isotachophoresis[8] or as charged cyclodextrin derivatives in electrokinetic chromatography.[9] The incorporation of cyclodextrins into gel-filled capillaries led to the separation of dansylated amino acids[10].

The off-column conversion of enantiomers into diastereomers with an optically pure reagent[11], e.g. Marfey's reagent[12], and subsequent separation in achiral capillary electrophoresis is also possible.

In contrast to these methods we explored the direct enantiomer separation on a chiral stationary phase by electrochromatography[13]. The chiral selector, permethylated β- or γ- cyclodextrin, was attached via an octamethylene spacer to a dimethylpolysiloxane (CHIRASIL-DEX, cf. Fig. 1) and coated with various film thicknesses to the inner surface of 50 μm i.d. open tubular columns. Thermal treatment resulted in non-extractable and buffer resistant wall coated open tubular columns (WCOT) containing the chiral stationary phase. Previously,

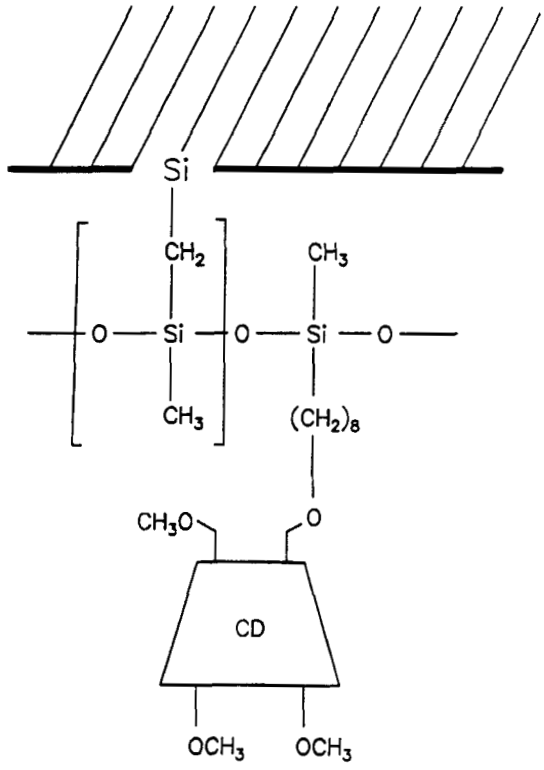

FIGURE 1. Structure of CHIRASIL-DEX (CD: either β- or γ-cyclodextrin).

CHIRASIL-DEX has found numerous applications in gas chromatography[14] and supercritical fluid chromatography[15].

The aim of the present work was to combine the widely applicable chromatographic separation of enantiomers on a chiral stationary phase with the high efficiency of capillary electrophoresis. While the analyte is carried along the column through an electric field by a buffer as mobile phase, the observed separation of the enantiomers is accomplished by diastereomeric interactions in the wall region.

Electrochromatography applied for WCOT columns has already been described for *achiral* coatings. Tsuda et al.[16] used an octadecylsilane capillary column of 30 μm i.d. for the separation of some aromatic compounds under reversed phase conditions (acetonitrile-water). Bruin et al.[17] compared electrically and pressure driven liquid chromatography in regard to theoretical plate heights for columns with different inner diameters. Polycyclic aromatics were separated with a 10 μm i.d. octadecylsilane capillary using a buffer-methanol mixture. Capillaries coated with methyl- and cyanopropylsiloxane were employed for the separation of anions having similar electrophoretic mobilities with the aid of a ion-pairing agent[18].

EXPERIMENTAL

Instrumentation

Electrophoretic experiments were performed with a "Kapillar- Elektrophorese-System 100" (Grom, Herrenberg, Germany) equipped with an on-column UV-detector. The electropherograms were recorded with a Shimadzu C-R3A Chromatopac integrator (Bischoff, Leonberg, Germany).

Materials

Fused silica capillaries of 50 μm i.d. were obtained from Chrompack (Middelburg, The Netherlands). All described columns had an effective length (L_{eff}) of 80 cm and a total length of 97 cm. The optical window, located at a distance of 80 cm from the injector was prepared by burning off a section of about 3 mm of the polyimide outer coating. The synthesis of CHIRASIL-DEX, the coating of the capillaries and the immobilization procedure is described elsewhere[19]. The film thickness was determined by GC measurements.

The coated columns were conditioned with buffer for half a day. All measurements were carried out with a 20 mM borate-phosphate buffer, which was degassed and filtered through a 0.45 μm pore size filter (Machery-Nagel, Düren, Germany). The CHIRASIL-DEX coated capillaries were rinsed between each chromatographic run with water (HPLC-grade, Merck, Darmstadt, Germany) followed by the operating buffer. All analytes were injected as solutions in methanol (about 0.01mg/ml). The samples were obtained from Aldrich, Steinheim, Germany (ibuprofen, 1,1'-binaphthyl-2,2'-diylhydrogenphosphate), Fluka, Buchs, Switzerland (1-phenylethanol), Prof. Dr. G. Blaschke, Münster, Germany (flurbiprofen) and Prof. Dr. W. H. Pirkle, Urbana, USA (cicloprofen, etodolac). All solutes were injected by the hydrostatic method (5 s) and detected at 220 nm.

RESULTS AND DISCUSSION

The enantiomer separation of the underivatized non-steroidal antiinflammatory drugs (NSAIDs) ibuprofen, cicloprofen, flurbiprofen and etodolac is shown in Fig. 2-5. These important pharmaceutical compounds are derived from free arylpropionic acids and exhibit antiinflammatory and analgesic effects depending on the dosage. Etodolac, having a slightly different chemical structure, could not be separated on the CHIRASIL-DEX derived from permethylated β-cyclodextrin. However, baseline resolution was achieved by employing a new immobilized CHIRASIL-DEX derived from permethylated γ-cyclodextrin, which was prepared in an similar manner[14]. As can be seen from Figure 5 the peak width of the second eluted enantiomer is increased more than expected. This is probably due to either slower kinetics of the formation of the diastereomeric complex or a different separation mechanism for each of the enantiomers.

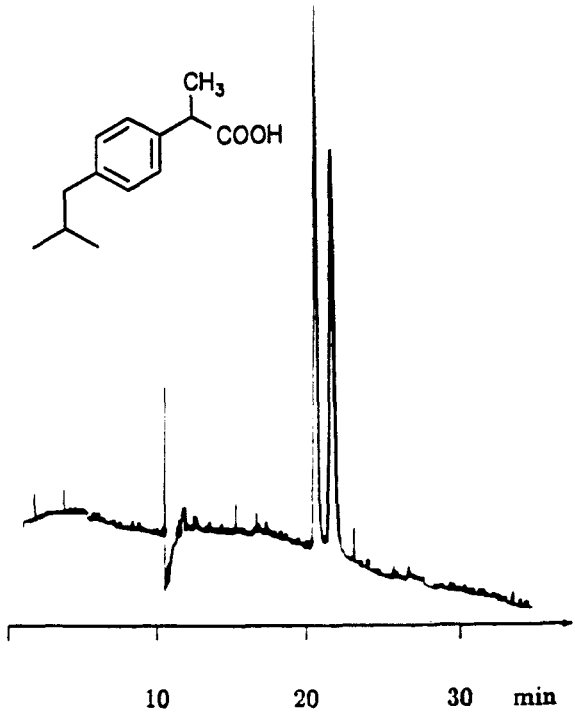

FIGURE 2. Enantiomer separation of racemic ibuprofen; fused silica capillary coated with CHIRASIL-DEX based on permethylated β-cyclodextrin (d_f = 0.4 μm); pH 7.0; applied voltage: 30 kV.

The negative slope of the baseline in the chromatograms of flurbiprofen and cicloprofen (Fig. 3 and 4) may result from an incomplete equilibration since the coated capillary was conditioned only for a short period of time.

In order to get a more precise understanding of the separation process and to find out optimum working conditions, the influence of the most important variables such as the applied voltage, buffer pH and film thickness on electroosmotic flow u_{eo}, separation factor α for the enantiomers, their capacity factors k' and the efficiency of the system has been examined. The enantiomer

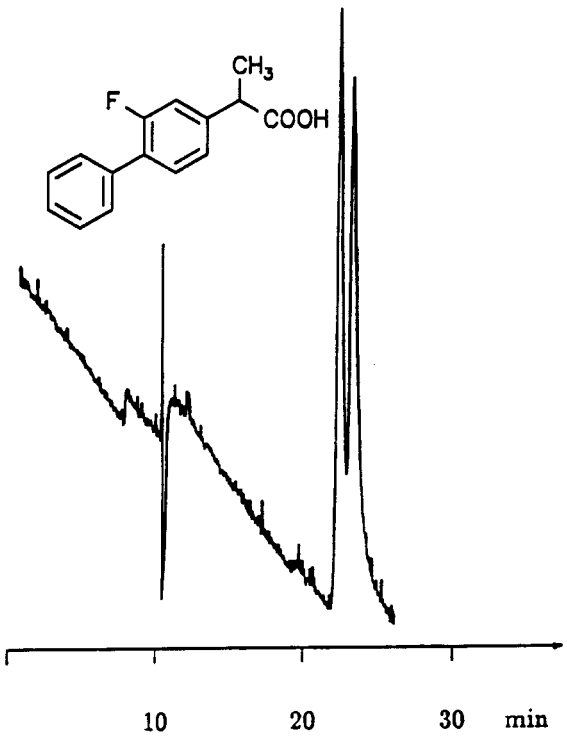

FIGURE 3. Enantiomer separation of racemic flurbiprofen (Conditions see Fig. 2).

separation of racemic 1,1'-binaphthyl-2,2'-diylhydrogenphosphate at different applied voltages was investigated. The separation factor α and the capacity factors k' remain nearly constant ($\alpha = 1.020 \pm 0.002$, $k'_2 = 0.74 \pm 0.02$) by raising the applied voltage from 10 kV to 30 kV, whereas the total retention time decreases [13]. The theoretical plate heights calculated according to

$$H = L_{eff} / N$$

and

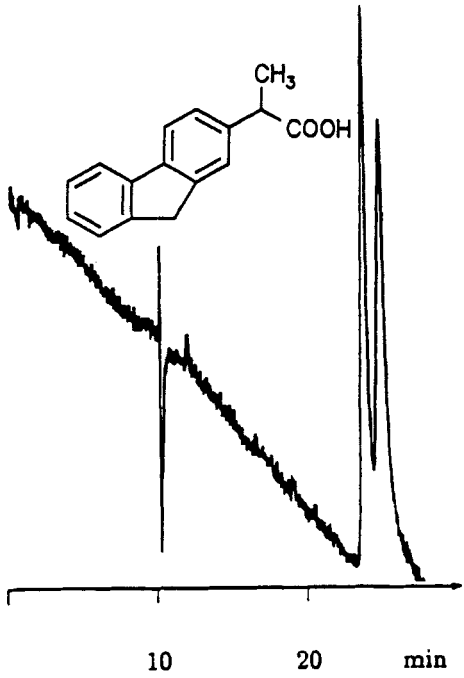

FIGURE 4. Enantiomer separation of racemic cicloprofen (Conditions see Fig. 2).

$$N = 5.54 \cdot (t_R (2) / b_{0.5} (2))^2$$

where t_R (2) is the overall retention time and $b_{0.5}$ the peak width at half height of the second eluted enantiomer, versus electroosmotic flow are shown in Figure 6. The minimum observed plate height for 1,1'-binaphthyl-2,2'-diylhydrogenphosphate was 3.9 μm (30 kV) corresponding to 250.000 theoretical plates per meter, which amounts to about 70 % of the value found for this compound on an uncoated capillary under the same conditions. The loss of efficiency for the coated capillary is probably due not only to the presence of the diastereomeric interaction of the analyte with the cyclodextrin selector,

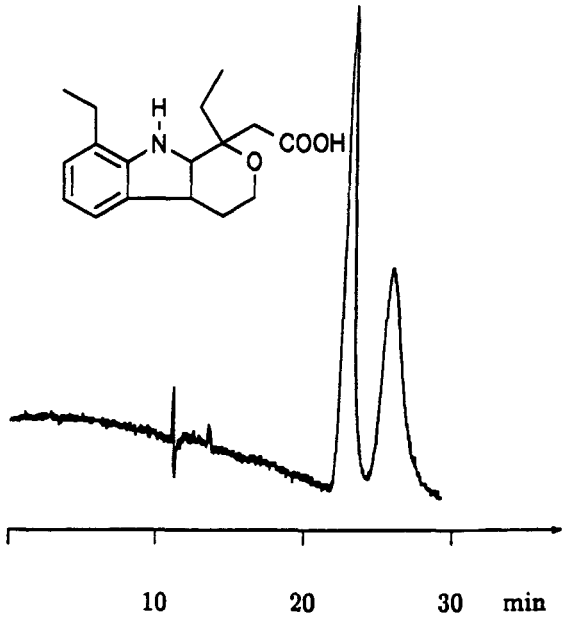

FIGURE 5. Enantiomer separation of racemic etodolac; fused silica capillary coated with CHIRASIL-DEX based on permethylated γ-cyclodextrin (d_f = 0.4 μm); pH 7.0; applied voltage: 30 kV.

but also to an increased C-term, caused by a larger resistance to mass tansfer in the mobile phase[20].

The influence of the CHIRASIL-DEX coating on the capillary wall was investigated by comparing the electroosmotic flow (measured for methanol) between a coated column and an uncoated and untreated fused silica capillary of the same batch (both 50 μm i.d. and L_{eff} = 0.80 m) at neutral pH (Fig. 7). For both columns a linear relationship between applied voltage and electroosmotic flow was observed. For the CHIRASIL-DEX coated capillary the electroosmotic flow is likely reduced as a consequence of the effective shielding of the silanol groups on the surface of the untreated fused silica capillary after the immobi-

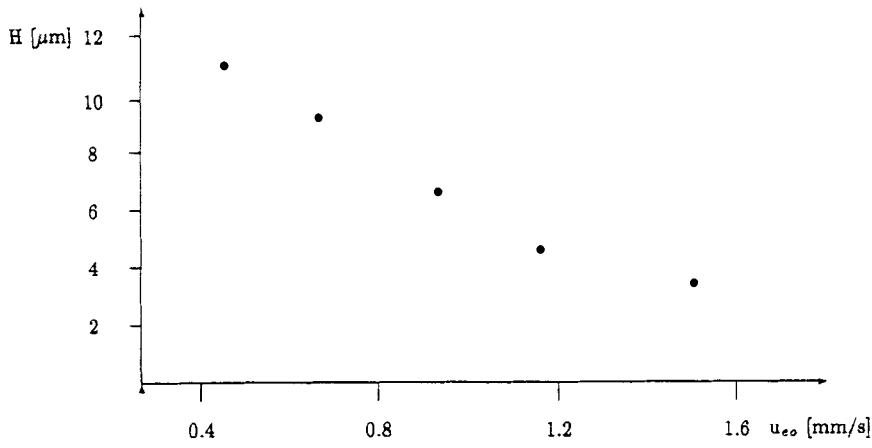

FIGURE 6. Theoretical plate heights H versus electroosmotic flow u_{eo} for the second eluted enantiomer of 1,1'-binaphthyl-2,2'-diylhydrogenphosphate; fused silica capillary coated with CHIRASIL-DEX based on permethylated β-cyclodextrin ($d_f = 0.2$ μm); pH 7.0.

lization of the polysiloxane backbone. Thus the retention times on the coated column are higher as compared to the uncoated column[13].

The effect of pH changes at constant buffer concentration on the electroosmotic flow, the retention time and the separation factor α was investigated for 1-phenylethanol (cf. Fig. 8). The separation factor remains nearly constant over the range of pH 5 - 9 ($\alpha = 1.17 \pm 0.02$). The electroosmotic flow and thereby the retention times of the enantiomers are affected such, that an increase in pH leads to a higher electroosmotic flow, e.g. from pH 7 to pH 9 the electroosmotic flow is changed from 1.60 to 1.74 mm $\cdot$ s^{-1} (Fig. 9). This observation may be rationalized by a partial and reversible ionization of the polysiloxane backbone. The stability of CHIRASIL-DEX coated columns towards the pH of the buffer system was tested by treating them with borate-phosphate buffer

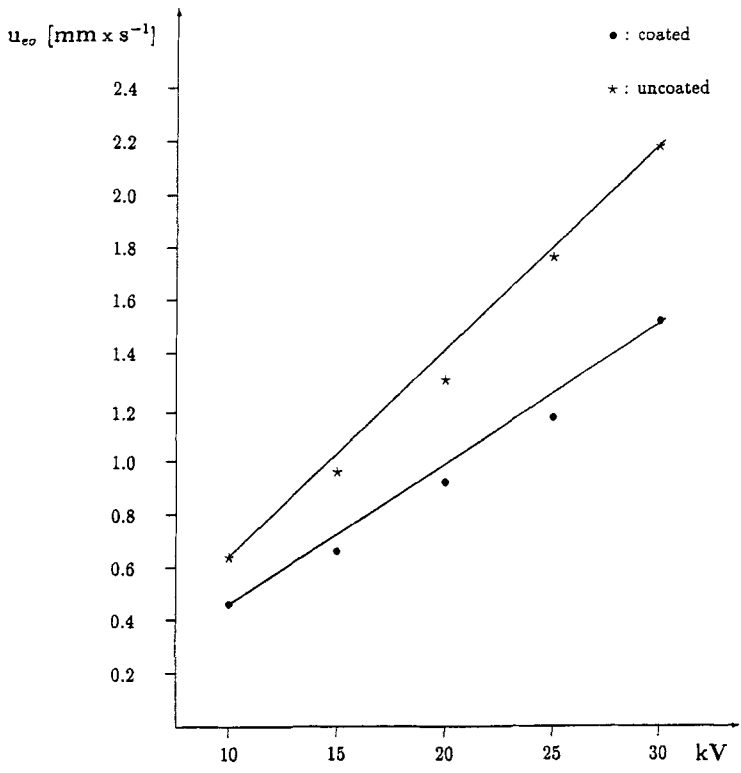

FIGURE 7. Electroosmotic flow u_{eo} measured for methanol versus applied voltage; fused silica capillary uncoated or coated with CHIRASIL-DEX based on permethylated β-cyclodextrin ($d_f = 0.2$ μm); pH 7.0.

at pH 5, 6, 8 and 9 for about two days each. No loss in efficiency or change in retention times were observed for 1-phenylethanol. Subsequent GC measurements of the capacity factors and efficiencies for n-alkanes (n-dodecane and n-tridecane) and 1-phenylethanol showed no reduction of capacity factors but efficiency was reduced to 20% for the n-alkanes, when compared to values obtained by GC before using the capillary in electrochromatography. The

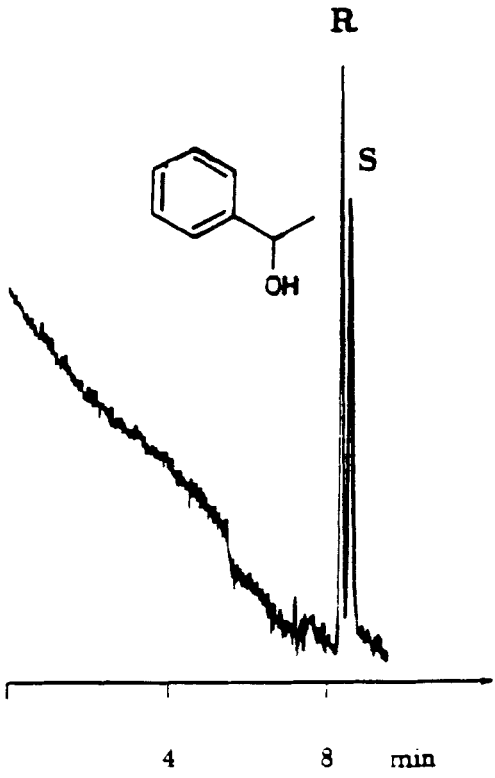

FIGURE 8. Enantiomer separation of racemic 1-phenylethanol; fused silica capillary coated with CHIRASIL-DEX based on permethylated β-cyclodextrin ($d_f = 0.2\ \mu m$); pH 7.0, applied voltage: 30 kV.

enantioselectivity towards 1-phenylethanol remained constant, wheras resolution R_s was reduced significantly (from 1.4 to 0.45). These effects indicate changes only in the chemical structure of the polysiloxane backbone, since the amount of the cyclodextrin selector was not reduced. Under neutral buffer conditions the coating proved stable for at least five months in electrochromatography.

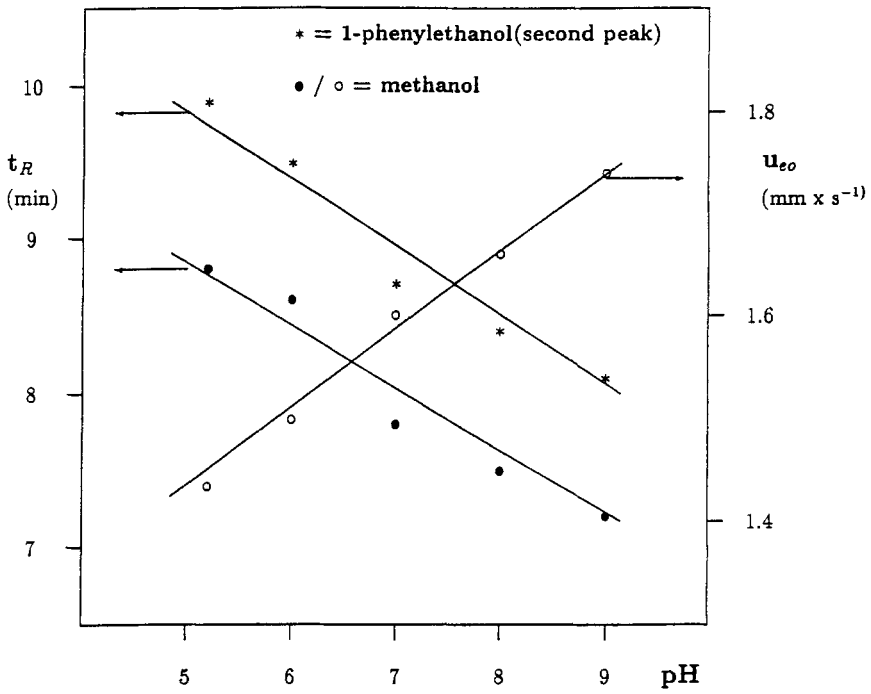

FIGURE 9. Electroosmotic flow u_{eo} measured for methanol and retention times of methanol and 1-phenylethanol versus pH of buffer; fused silica capillary coated with CHIRASIL-DEX based on permethylated β-cyclodextrin (d_f = 0.2 μm); applied voltage 30 kV.

TABLE I

Analyte	d_f (μm)	k'$_2$	α	N
1-phenylethanol	0.2	0.21	1.17	30500
	0.4	0.32	1.19	24016
	0.8	1.10	1.12	9507
flurbiprofen	0.2	1.03	1.04	31162
	0.4	1.36	1.05	12492
cicloprofen	0.2	1.14	1.06	19828
	0.4	1.43	1.08	9939

For evaluation of the influence of film thickness on retention time, enantioselectivity and efficiency, three columns with different film thicknesses of the immobilized CHIRASIL-DEX stationary phase were compared (Table I), demonstrating a dramatic loss of efficiency as film thickness is increased. This observation is explained by the chromatographic partitioning process superimposed to electroosmosis whereby the entire film is accessible for the solutes which then suffer from the low speed of mass transfer in the stationary phase, well known for polymeric coatings[21].

CONCLUSION

We have shown that a chiral surface in capillary electrophoresis is capable to induce chiral recognition towards racemic solutes. The parameters affecting enantiomer separation by electrochromatography have been evaluated. Further studies will be concerned with the use of film thicknesses below 0.2 μm and the variation of the permethylated β-cyclodextrin to α- and γ-cyclodextrin in order to optimize efficiency and enantioselectivity of the system.

ACKNOWLEDGEMENTS

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REFERENCES

(1) Snopek, J., Jelinek, I., Smolkova-Keulemansova, E., Micellar, inclusion and metal-complex enantioselective pseudophases in high-performance electromigration methods, J. Chromatogr., 452, 571, 1988.

(2) a) Gassmann, E., Kuo, J., Zare, R. J., Electrokinetic separation of chiral compounds, Science, 230, 813, 1985; b) Gozel, P., Gassmann, E., Michelson, H., Zare, R. J., Electrokinetic resolution of amino acid enantiomers with copper(II)-aspartame support electrolyte, Anal. Chem. 59, 44, 1987.

(3) Nishi, H., Fukuyama, T., Matsuko, M., Terabe, S., Chiral separation of diltiazam, trimetoquinol and related compounds by micellar electrokinetic chromatography with bile salts, J. Chromatogr., 515, 233, 1990.

(4) a) Dobashi, A., Ono, T., Hara, S., Yamaguchi, J., Optical resolution of enantiomers with chiral mixed micelles by electrokinetic chromatography, Anal. Chem., 61, 1984, 1989; b) Dobashi, A., Ono, T., Hara, S., Yamaguchi, J., Enantioselective hydrophobic entaglement of enantiomeric solutes with chiral functionalized micelles by electrokinetic chromatography, J. Chromatogr., 480, 413, 1989.

(5) Otsuka, K., Terabe, S., Enantiomeric resolution by micellar electrokinetic chromatography with chiral surfactants, J. Chromatogr., 515, 221, 1990.

(6) Nishi, H., Fukuyama, T., Terabe, S., Chiral separation by cyclodextrin-modified micellar electrokinetic chromatography, J. Chromatogr., 553, 505, 1991.

(7) Fanali, S., Flieger, M., Steinerova, N., Nardi, A., Use of cyclodextrins for the enantioselective separation of ergot alkaloids by capillary zone electrophoresis, Electrophoresis, 13, 39, 1992.

(8) a) Snopek, J., Jelinek, I., Smolkova-Keulemansova, E., Use of cyclodextrins in isotachoporesis. IV. The influence of cyclodextrins on the chiral resolution of ephedrin alkaloid enantiomers, J. Chromatogr., 438, 211, 1988; b) Snopek, J., Jelinek, I., Smolkova-Keulemansova, E., Use of cyclodextrins in isotachophoresis. VIII. Two-dimensional chiral separation in isotachophoresis, J. Chromatogr., 472, 308, 1989.

(9) Terabe, S., Electrokinetic chromatography: An interface between electrophoresis and chromatography, Trends Anal. Chem., 8, 129, 1989.

(10) Guttman, A., Paulus, A., Cohen, A., Grinberg, N., Karger, B., Use of complexing agents for selective separation in high performance capillary

electrophoresis. Chiral resolution via cyclodextrin incorporated within polyacrylamide gel columns, J. Chromatogr., 448, 41, 1988.

(11) Nishi, H., Fukuyama, T., Matsuo, M., Resolution of optical isomers of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC)-derivatized DL-amino acids by micellar electrokinetic chromatography, J. Microcol. Sep., 2, 234, 1990.

(12) Tran, A. D., Blanc, T., Leopold, E., Free solution capillary electrophoresis and micellar electrokinetic resolution of amino acids enantiomers with L- and D- Marfey's reagent, J. Chromatogr., 516, 241, 1990.

(13) a) Mayer, S., Schurig, V., Enantiomer separation by electrochromatography on capillaries coated with CHIRASIL-DEX, J. High Resolut. Chromatogr., 15, 129, 1992; b) Schurig, V., Mayer, S., Proceedings of the 14th Int. Symp. on Capillary Chromatography, P. Sandra, M. L. Lee, eds., Baltimore, Maryland, USA, 1992, p. 587.

(14) Schurig, V., Schmalzing, D., Mühleck, U., Jung, M., Schleimer, M., Mussche, P., Duvekot, C., Buyten, J., Gas chromatographic enantiomer separation on polysiloxane-anchored permethyl-β-cyclodextrin (CHIRASIL-DEX), J. High Resolut. Chromatogr., 13, 713, 1990.

(15) Schmalzing, D., Nicholson, G., Jung, M., Schurig, V., Enantiomer separation by capillary SFC and GC on immobilized permethylated β-cyclodextrin, J. Microcol. Sep., 4, 23, 1992.

(16) Tsuda, T., Nomura, K., Nakagawa, G., Open-tubular microcapillary liquid chromatography with electroosmosis flow using a UV-detector, J. Chromatogr., 248, 241, 1982.

(17) Bruin, G., Tock, P., Kraak, J., Poppe, H., Electrically driven open-tubular liquid chromatography, J. Chromatogr., 517, 557, 1990.

(18) Pfeffer, W., Yeung, E., Electroosmotically driven electrochromatography of anions having similar electrophoretic mobilities by ion pairing, Anal. Chem., 62, 2178, 1990.

(19) Schurig, V., Juvancz, Z., Nicholson, G., Schmalzing, D., Separation of enantiomers on immobilized polysiloxane-anchored permethylated β-cyclodextrin (CHIRASIL-DEX) by supercritical fluid chromatography, J. High Resolut. Chromatogr., 14, 58, 1991.

(20) Martin, M., Guiochon, G., Axial diffusion in open-tubular capillary liquid chromatography with electroosmotic flow, Anal. Chem., 56, 614, 1984.

(21) Engelhardt, H., Löw, H., Eberhardt, W., Mauß, M., Polymer encapsulated stationary phases: Advantages, properties and selectivities, Chromatographia, 27, 535, 1989.