Comparison of γ-cyclodextrin sulfobutyl ether and unmodified γ-cyclodextrin as chiral selectors in capillary electrophoresis

Martin Jung, Eric Francotte*

Pharmaceutical Research, K-122.P.25, Ciba-Geigy AG, 4002 Basel, Switzerland

Abstract

γ-Cyclodextrin sulfobutyl ether (γ-CD-SBE) was synthesized and evaluated as a chiral selector for capillary electrophoresis. As expected, the new derivative combined the good enantioselectivity of γ-cyclodextrin with the advantages of a negatively charged chiral selector, which have previously been reported also for the β-cyclodextrin analogon. However, it became evident that the attachment of the sulfobutyl ether groups to the periphery of the γ-cyclodextrin torus can markedly alter the enantioselectivity towards some racemic analytes. Thus, there was no general trend towards better separations with the use of γ-CD-SBE rather than unmodified γ-cyclodextrin or vice versa. Some racemates could only be resolved with one or the other system. For two racemates, even the elution order of the enantiomers with γ-CD-SBE was opposite to that one expected from the experiments using unmodified γ-cyclodextrin.

1. Introduction

While in everyday practice chiral HPLC continues to be the number one method for analytical as well as preparative enantiomer separations, capillary electrophoresis (CE) employing cyclodextrins as additives to the running buffer has gained much attention in recent years [1]. Although it appears that in CE chances of success are not as high as in HPLC and problems with peak shape distortions are not uncommon, important advantages of CE for enantiomer analysis consist in simplicity, high efficiency, low operating costs and versatility (easy switching from one chiral selector to another). The majority of enantiomer separations has been accomplished by the use of just a few different cyclodextrins, particularly

β-and γ-cyclodextrin and their hydroxypropyl and 2,6-dimethyl derivatives, which are all readily available [1]. These neutral cyclodextrins are particularly suitable for the enantiomer separation of charged analytes by capillary zone electrophoresis (CZE) and for the chiral resolution of small neutral analytes by micellar electrokinetic chromatography (MEKC), i.e., with addition of a micelle agent such as sodium dodecyl sulphate (SDS).

An alternative approach to the use of neutral cyclodextrins is electrokinetic chromatography (EKC) employing charged cyclodextrins, which have their own electrophoretic mobility [2]. Among them, β-cyclodextrin sulfobutyl ether (β-CD-SBE) and sulfopropyl ether (β-CD-SPE) have recently been introduced and proved to be very useful [3–9]. It was found that these derivatives, which are negatively charged above pH 2, can be used in lower

*Corresponding author.

concentrations (down into the μM range) and yielded better enantiomer resolution for a series of positively charged analytes. In addition, enantiomer separation of neutral analytes became feasible without addition of a micelle agent. Besides β-CD-SBE, linear, negatively charged polysaccharides such as heparin, chondroitin sulphate, dextran sulphate or dextrin sulfopropyl ether have also been suggested as chiral selectors for EKC [10–13].

In this paper we describe the preparation, characterization and evaluation of γ-cyclodextrin sulfobutyl ether (γ-CD-SBE). The question arises whether γ-CD-SBE can generally replace native γ-cyclodextrin in CE. In a systematic comparison, we are trying to evaluate which approach is more useful from a practical point of view and whether the enantioselectivities of the two cyclodextrins follow the same general trend or show distinct differences towards certain analytes.

2. Experimental

2.1. Chemicals

γ-Cyclodextrin was provided by Wacker (Liestal, Switzerland). 1,4-Butane sultone was purchased from Aldrich (Steinheim, Germany). Sodium dodecyl sulphate (SDS) and 2,5-dihydroxybenzoic acid were obtained from Sigma (Buchs, Switzerland), and 3-hydroxyisochinolin was purchased from Fluka (Buchs, Switzerland). Most racemic samples were commercial products (Sigma or Aldrich). Mianserine, butaclamol and the indol derivative were Ciba-Geigy samples. The pure enantiomers of aminoglutethimide had been prepared in-house by preparative resolution of the racemate by HPLC.

2.2. Preparation of γ-cyclodextrin sulfobutyl ether (γ-CD-SBE)

γ-CD-SBE was prepared in a similar way as previously described for β-CD-SBE [3,4,14–17]. In a 50 ml round-bottom flask equiped with a reflux condenser, 5 g of γ-cyclodextrin were dissolved with vigorous stirring in 10 ml of 25% (w/w) aqueous sodium hydroxide. To the clear, viscous solution, 4.6 g of butane sultone were slowly added through a

dropping funnel, and stirring was continued for 24 h at 47°C. After analyzing a small aliquot of the reaction mixture by MALDI-MS (see Section 2.3), 20 ml of water were added at room temperature, and the solution was neutralized to pH 6 with 4 M hydrochloric acid. Inorganic salt was removed by threefold membrane filtration in a stirred cell (Spec, Basel, Switzerland, exclusion size: 1000 Da; initial volume of the solution, 150 ml; final volume, 20 ml; cell pressure, 4 bar). After concentration in a rotary evaporator, drying overnight at 0.1 Torr/50°C yielded 2.8 g of white powder (1 Torr=133.322 Pa). The product was characterized by MALDI-MS (see Section 2.3).

2.3. MALDI-MS

As in our previous work with dextrin sulfopropyl ether [13], measurements were performed on a linear time-of-flight mass spectrometer (prototype of Linear Scientific, Reno, NV, USA) [18]. All spectra represented an accumulation of 30 to 50 shots. A vacuum in the flight tube of approx. $2 \cdot 10^{-6}$ to $7 \cdot 10^{-6}$ Torr was observed. The length of the tube was 1.7 m. The intensity of the nitrogen laser (337 nm) pulses varied between 3 and 6 μJ. The DHB–HIC matrix [19] was prepared as a 3:1 mixture of 2,5-dihydroxybenzoic acid (DHB) and 1-hydroxyisochinolin (HIC) by mixing equal volumina (5 or 10 μl each) of 0.2 M DHB, 0.6 M HIC [both dissolved in water–acetonitrile 50:50 (v/v)], 30 mM sodium chloride (in water), and aqueous sample solution (approx. 1 mg/ml). For analysis of reaction mixtures, a drop of the solution was desalted prior to preparation of the matrix by placing it for 30 min on the hydrophobic side of a circular cellulose membrane filter (pore size 0.025 μm, Millipore, Volketswil, Switzerland) floating on distilled water in a bowl [20–22].

2.4. Capillary electrophoresis

All CE experiments were performed on a Beckman P/ACE 5000 series capillary electrophoresis instrument with diode-array UV detection. The depicted chromatograms correspond to the absorption observed at 214 nm with a band width of 4 nm. An untreated 47 cm (40 cm injector to detector)×50 μm I.D. fused-silica capillary was used. The voltage was

30 kV. Injection was achieved by pressure (typically 5 s, 35 mbar) and the sample concentration was typically 0.1–0.2 mg/ml. The capillary was rinsed for 1–2 min with buffer before every run and was regenerated for 1–4 min with 0.1 *M* NaOH and for 1–2 min with water after every run. Sodium phosphate buffer pH 6 and 7 were obtained from the in-house central service department and were diluted to the desired concentration with distilled water. The pH 3 buffer was prepared by adjusting the pH 6 buffer with phosphoric acid in the same concentration. Running buffer was prepared by dissolving the additive in the respective buffer in 4.5-ml vials or 500-μl minivials, filtering through a 0.45-μm syringe filter (Skan, Basel, Switzerland) and sonicating briefly.

3. Results and discussion

3.1. Preparation and characterization of γ-CD-SBE

The described procedure is suitable for the easy preparation of γ-CD-SBE in batches of up to ca. 20 g. Amount and concentration of the aqueous sodium hydroxide used as solvent were found to be critical to obtain a homogeneous, but not too viscous solution. Comparison of the proportions of the starting materials and the analysis of the product from the reaction mixture reveiled that a considerable part of the butanesultone did not react, but was lost to hydrolysis. Membrane filtration in a stirred cell proved to be a convenient method for desalting. However, there was a small shift in molecular mass distribution of the product due to a certain loss of molecules with few sulfobutyl ether substituents through the membrane (smallest available exclusion size, M_r 1000). As a consequence, good reproducibility in product composition can only be achieved if reaction temperature (which influences the rate of hydrolysis) and conditions during membrane filtration are carefully controlled. As demonstrated previously for dextrin sulfopropyl ether [13], MALDI-MS using a mixed DBH–HIC matrix is a powerful tool for the easy and unambiguous characterization of the product. Fig. 1 shows the molecular mass distribution of the obtained γ-CD-SBE. The average number
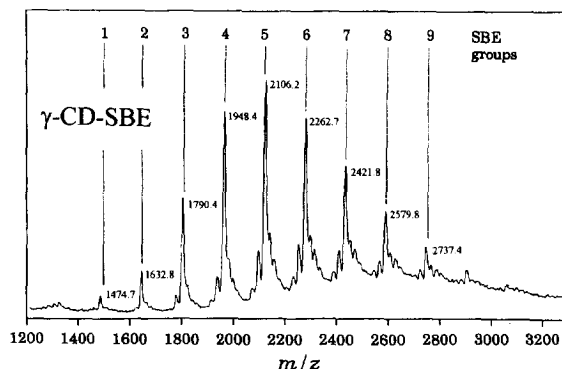

Fig. 1. MALDI-MS spectrum of γ-cyclodextrin sulfobutyl ether (γ-CD-SBE) after preparative desalting in a stirred cell. The spectrum shows the respective [M+Na]$^-$ adduct for every molecular mass. The counterions of the polyanions are also Na$^+$. For further details see Section 2.3.

of SBE groups per molecule indicates that the substituents are not only located on the more reactive primary oxygen atoms in the position-6, but to some extent also in the 2- and 3-positions of the glucose units. The highest peak corresponds to the molecule containing 5 sulfobutyl groups. The β-CD-SBE product used for comparison was prepared according to Refs. [5–8]. Previous characterization of the compounds was performed by CE with indirect UV detection [17], or by electrospray MS [4].

3.2. Enantiomer separations with γ-CD-SBE

As expected from the studies with β-CD-SBE mentioned in the Introduction, the new derivative combined the good enantioselectivity of γ-cyclodextrin with the advantages of a negatively charged chiral selector. Successful enantiomer separations with γ-CD-SBE (structures of the analytes are depicted in Fig. 2) are shown in Figs. 3–10 (the conditions are listed in Table 1). As reported also for β-CD-SBE [8], relatively low cyclodextrin concentrations are often sufficient, particularly for some basic solutes such as mianserine, where a 40 mM concentration of the chiral selector produced a baseline separation. A probable explanation is the large difference in electrophoretic mobilities of the uncomplexed positively charged analyte vs. the complex with the cyclodextrin carrying a multiple negative charge. Unfortunately, this change in elec-
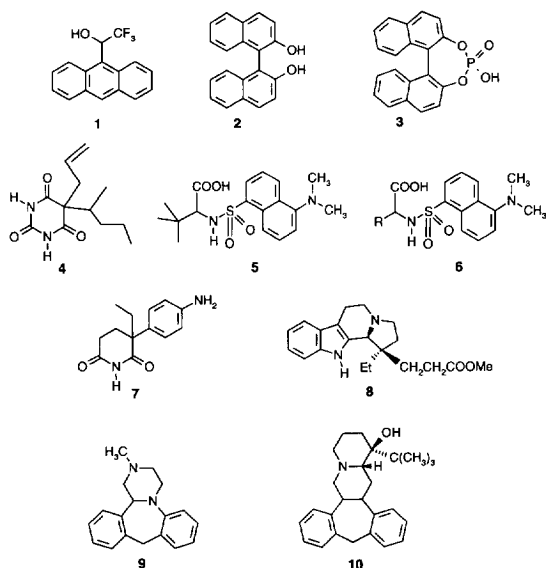
Fig. 2. Structures of the racemic solutes used in this work.

Fig. 4. Examples for enantiomer separations of dansylated amino acids by CE using γ-CD-SBE (upper row) or γ-cyclodextrin (bottom row) as chiral buffer additives in suitable concentrations. For conditions, see Table 1.

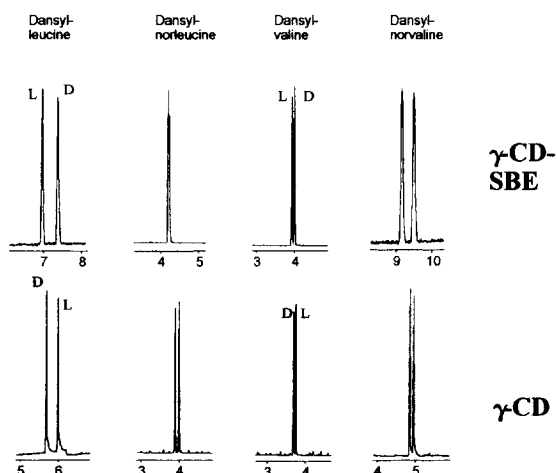trophoretic mobility upon complexation seems to be responsible not only for the observed high enantio-selectivity, but also for the appearance of asymmetric or distorted peaks for a few solutes with higher concentrations of the chiral selector. Using the same background electrolyte and the same experimental conditions, symmetric, sharp peaks are observed for the same analytes when lower concentrations of the

negatively charged cyclodextrin or a native, electrically neutral cyclodextrin are employed. This distortion phenomenon was observed for strongly interacting analytes such as mianserine (200 μM γ-CD-SBE, Fig. 8), butaclamol (100 μM γ-CD-SBE, Fig. 7) and 1,1'-binaphthyl-2,2'-diylhydrogenphosphate (15 mM γ-CD-SBE, Fig. 10). Similar observations have been made with β-CD-SBE [5,8] and with dextrin sulfopropyl ether [13]. Usually the problem could be solved by using the lowest possible concentration of the chiral selector.

An interesting feature is the feasability of enantiomer separations of anionic analytes such as several dansylated amino acids (Figs. 4–6) as well as the
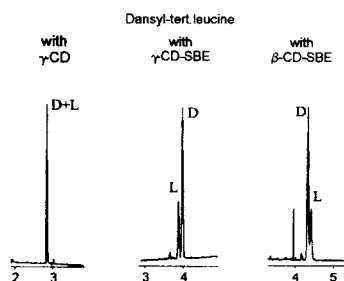

Fig. 3. Examples for enantiomer separations by CE using γ-CD-SBE (upper row) or γ-cyclodextrin (bottom row) as chiral buffer additives in suitable concentrations. For conditions, see Table 1.

Fig. 5. Enantiomer separation of dansyl-tert.-leucine by CE using a 30 mM phosphate buffer (pH 7) containing 20 mM γ-cyclodextrin and 50 mM SDS (left), 10 mM γ-CD-SBE (center) or β-CD-SBE (right) as additives. For other conditions, see Table 1.
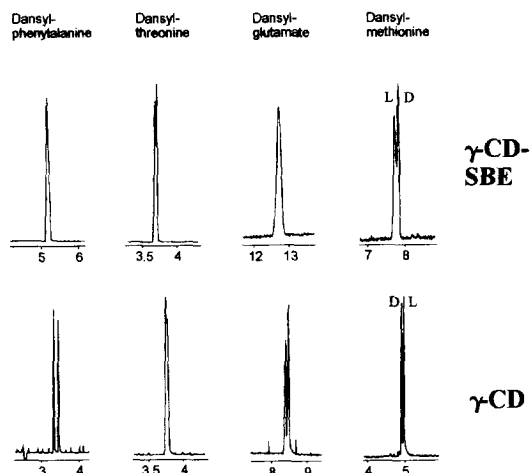
Fig. 6. Examples for enantiomer separations of dansylated amino acids by CE using γ-CD-SBE (upper row) or γ-cyclodextrin (bottom row) as chiral buffer additives in suitable concentrations. For conditions, see Table 1.

binaphthyl phosphate (Fig. 3) at pH 7 with γ-CD-SBE. The electrostatic repulsion of analyte and chiral selector makes higher selector concentrations necessary, but does not preclude excellent enantioselectivity [9]. The enantiomers of dansyl-leucine can also be separated with reversed elution order in a coated capillary where the electroosmotic flow (EOF) is practically suppressed (see Fig. 9). The insignifi-

Fig. 7. Examples for enantiomer separations of basic solutes by CE using γ-CD-SBE (upper row) or γ-cyclodextrin (bottom row) as chiral buffer additives in suitable concentrations. For conditions, see Table 1.

Fig. 8. Enantiomer separation of mianserine by CE using a 40 mM phosphate buffer (pH 3) containing 100 μM γ-CD-SBE. For other conditions, see Table 1 (cf. Section 3.3).

Fig. 9. Enantiomer separation of dansyl-leucine by CE using a 30 mM phosphate buffer containing 5 mM γ-CD-SBE and 10% (v/v) methanol. Capillary: polyacrylamide coated (Beckman Neutralcap), 37 cm×50 μm I.D., 30 cm injector to detector; voltage, −15 kV (reversed polarity).

cance of EOF was confirmed by measurement using DMF as a marker (UV 220 nm, no SDS added to the buffer). EOF did not yet arrive after 20 min at pH 7.

3.3. Comparison of γ-CD-SBE and native γ-CD

In order to find out whether the novel γ-CD-SBE can possibly replace native γ-CD as a chiral selector in everyday practice, we have directly compared the enantiomer separations that can be achieved for a number of racemates with each system under opti-
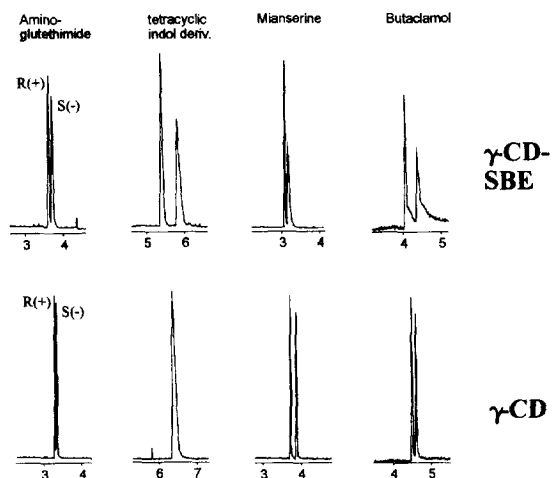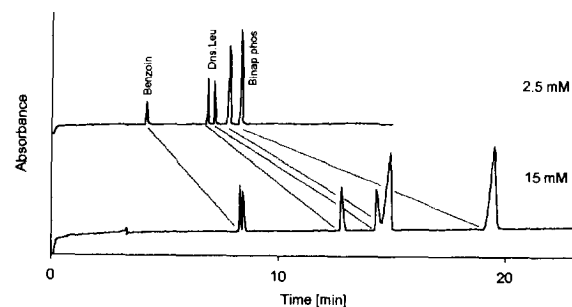

Fig. 10. Enantiomer separation of benzoin, dansyl-leucine and 1,1′-binaphthyl-2,2′-diylhydrogenphosphate by CE using a 30 mM phosphate buffer (pH 7) containing 2.5 mM or 15 mM γ-CD-SBE. For other conditions, see Table 1.

Table 1
Conditions[a] used for the enantiomer separations shown in [4–7] Fig. 8

Analyte	Background electrolyte	Additives	γ-CD-SBE[b] (mM)	γ-CD+SDS[b] (mM)
1-(9-Anthryl)-2,2,2-trifluroethanol	phosphate pH 7 (30 mM)	urea (2 M)	5	20+50
1,1′-Binaphthyl-2,2′-diol	phosphate pH 7 (30 mM)	methanol (10%, v/v)	2.5	25+40
1.1′-Binaphtyl-2,2′-diylphosphate	phosphate pH 7 (30 mM)		2.5	25+50
Secobarital	phosphate pH 7 (30 mM)	methanol (15%, v/v)	15	35+100
Dansyl-tert.-leucine	phosphate pH 7 (30 mM)		10	20+50
All other dansylated amino acids	phosphate pH 7 (30 mM)	methanol (10%, v/v)	5	20+50
Aminoglutethimide	phosphate pH 3 (40 mM)		1	20+0
Tetracyclic indol deriv.	phosphate pH 3 (40 mM)		1	20+0
Mianserine	phosphate pH 3 (40 mM)		0.04	20+0
Butaclamol	phosphate pH 3 (40 mM)		0.1	20+0
All other basic analytes	phosphate pH 3 (40 mM)		0.1 and 1	20+0

[a] Other conditions: 47 cm×50 μm I.D. untreated fused-silica capillary, 40 cm injector to detector; voltage, 30 kV; detection by UV at 214, 230 or 254 nm.
[b] First buffer: background electrolyte+additive+γ-CD-SBE; second buffer: background electrolyte+additive+γ-CD+SDS.

mized conditions. The successful enantiomer separations are depicted in Figs. 3–7. Since the advantages of γ-CD-SBE seemed to be most pronounced for basic analytes, we focused our study on those solutes. Unfortunately, 16 out of 20 basic analytes could not be resolved by any of the two selectors. Those analytes included a series of drugs such as ephedrin and related compounds, verapamil, propranolol, clenbuterol, labetolol, salbutamol, terbutalin, fenoterol, oxprenolol, sulprimide, formoterol and others. On the other hand, Figs. 3–7 show 9 examples where similar enantiomer separations were achieved with both systems. Obviously, for some racemates the few sulfobutyl ether groups attached to the periphery of the small rim of the cyclodextrin torus seem to have no drastical influence on the inclusion properties of the native cyclodextrin. This assumption has been made for the β-cyclodextrin analogon [4], but has not yet been supported by sufficient experimental data. However, our experiments (Figs. 3–7) clearly demonstrated that the enantioselectivities of γ-cyclodextrin and γ-CD-SBE can also show distinct differences in character. Thus, e.g., among the dansylated amino acids, leucine and valine were well separated with both systems, norvaline was better separated with γ-CD-SBE, norleucine, phenylalanine and glutamate were only separated with γ-cyclodextrin, whereas tert.-leucine could only be separated with γ-CD-SBE. It may be

noted that the enantiomer separation of tert.-leucine with β-CD-SBE was not as good and the elution order of the enantiomers was reversed (Fig. 5). Secobarital was only separated with γ-cyclodextrin, while binaphthol, trifluoroanthrylethanol and aminoglutethimide were similarly well separated with both systems, and only the novel γ-CD-SBE allowed the chiral resolution of the binaphthyl phosphate and the tetracyclic indol derivative (Fig. 8).

The different character of γ-cyclodextrin and γ-CD-SBE with respect to their enantioselectivity led us to two rather surprising examples where even the expected elution order of the enantiomers was not observed. For positively charged analytes, an increasing concentration of either γ-cyclodextrin or γ-CD-SBE has the same effect, leading to longer migration times. Thus, as expected, the same elution order was observed for the enantiomers of aminoglutethimide. The situation is different for neutral or negatively charged analytes that migrate more slowly than the electroosmotic flow. Here, an increasing concentration of the micelle agent SDS leads to longer migration times, and the addition of the neutral γ-cyclodextrin renders the migration times shorter again. If γ-CD-SBE is used, no SDS is required, and an increasing selector concentration results in longer migration times. Consequently, the elution order of the enantiomers is expected to be reversed if the inclusion mechanism of γ-cyclo-

dextrin and γ-CD-SBE are similar. This behaviour was observed for dansyl-leucine and dansyl-valine (Fig. 4). For binaphthol and trifluoroanthrylethanol, however, the elution order remained the same, which was rather unexpected (Fig. 3). This means that for these two analytes, the (−)-enantiomers complex more strongly to γ-cyclodextrin than the (+)-enantiomers, whereas the (+)-enantiomers complex more strongly to γ-CD-SBE than the (−)-enantiomers, pointing to a modification of the mechanism of complexation.

3.4. Effect of the concentration of γ-CD-SBE

As it can be seen from Fig. 11 and Fig. 12, the selector concentration drastically influences migration times and enantiomer resolution and has to be optimized for every class of analytes. Whereas a 40 μM concentration is useful for mianserine, 2.5 mM are recommended for dansyl-leucine and the binaphthyl phosphate, and 15 mM are necessary to achieve a chiral resolution of benzoin. In order to

Fig. 12. Migration times (top) or enantiomer resolution R_s (bottom) for mianserine vs. concentration of γ-CD-SBE in the running buffer, i.e., 40 mM phosphate buffer (pH 3). EOF approx. 12 min.
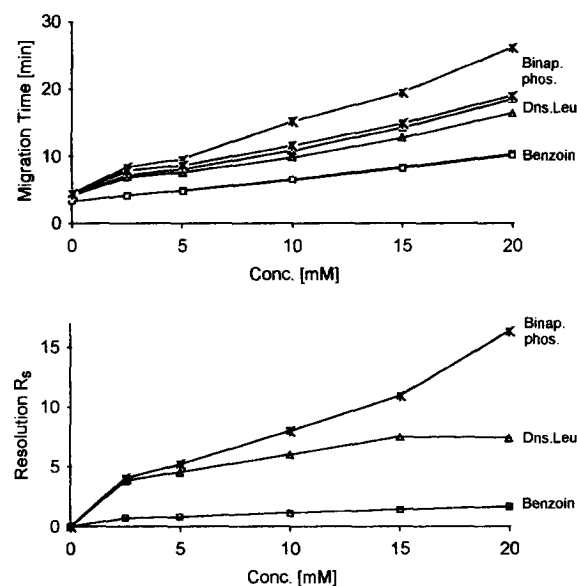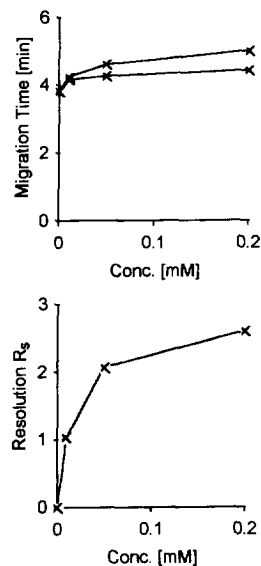
Fig. 11. Migration times (top) or enantiomer resolution R_s (bottom) for benzoin, dansyl-leucine and 1,1'-binaphthyl-2,2'-diylhydrogenphosphate vs. concentration of γ-CD-SBE in the running buffer, i.e., 30 mM phosphate buffer (pH 7). EOF approx. 3 min.

obtain a baseline separation within the shortest possible analysis time while avoiding peak shape distortions, the lowest possible selector concentration should be used. It is interesting to note, however, that the very high enantiomer resolution of the binaphthyl phosphate still keeps on increasing beyond a concentration of 15 mM γ-CD-SBE (see Fig. 11). Joule heating resulting from an excessively high current limits the use of γ-CD-SBE to maximum concentrations of ca. 15–20 mM, depending on the capillary dimensions, as illustrated in Fig. 13.

4. Conclusion

The novel γ-CD-SBE is a powerful chiral selector for CE. It can be used in lower concentrations than native γ-cyclodextrin, particularly for cationic analytes. However, it cannot generally replace γ-cyclodextrin in practice since the enantioselectivities of the two selectors are sometimes distinctly different. For most neutral or anionic analytes, the switching between γ-CD and γ-CD-SBE can be employed to inverse the elution order.

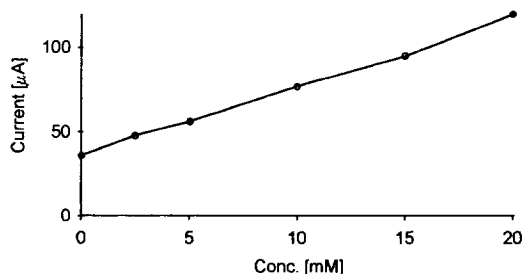Fig. 13. Current vs. concentration of γ-CD-SBE in the running buffer, i.e., 30 mM phosphate buffer (pH 7). Capillary: 47 cm×50 μm I.D.; voltage, 30 kV.

References

[1] H. Nishi and S. Terabe, J. Chromatogr. A, 694 (1994) 245–276, and references cited therein.

[2] S. Terabe, Trends Anal. Chem., 8 (1989) 129–134.

[3] S. Mayer, Ph.D. Thesis, University of Tübingen, 1993.

[4] S. Mayer, M. Schleimer and V. Schurig, J. Microcol. Sep., 6 (1994) 43–48.

[5] R.J. Tait, D.O. Thompson, V.J. Stella and J.F. Stobaugh, Anal. Chem., 66 (1994) 4013–4018.

[6] I.S. Lurie, R.F.X. Klein, T.A. Del Cason, M.J. LeBelle, R. Brenneisen and R.E. Weinberger, Anal. Chem., 66 (1994) 4019–4026.

[7] C. Dette, S. Ebel and S. Terabe, Electrophoresis, 15 (1994) 799–803.

[8] B. Chankvetadze, G. Endresz and G. Blaschke, Electrophoresis, 15 (1994) 804–807.

[9] B. Chankvetadze, G. Endresz and G. Blaschke, J. Chromatogr. A, 704 (1995) 234–237.

[10] A.M. Stalcup and N.M. Agyei, Anal. Chem., 66 (1994) 3054–3059.

[11] H. Nishi, K. Nakamura, H. Nakai, T. Sato and S. Terabe, Electrophoresis, 15 (1994) 1335–1340.

[12] H. Nishi, K. Nakamura, H. Nakai and T. Sato, Anal. Chem., 67 (1995) 2334–2341.

[13] M. Jung, K. O. Börnsen and E. Francotte, Electrophoresis, 17 (1996) 130–136.

[14] S.M. Parmerter, E.E. Allen and G.A. Hull, US Pat., No. 3 426 011 (1967); Chem. Abstr., 71 (1969) 13 331.

[15] J.N.J.J. Lammers, J.L. Koole and J. Hurkmans, Stärke (Starch), 23 (1971) 167–171.

[16] R.A. Rajewski and V.J. Stella, US Pat. No. 5 134 127 (1992); Chem. Abstr., 115 (1993) 189787u.

[17] R.J. Tait, D.J. Skanchy, D.P. Thompson, N.C. Chetwyn, D.A. Dunshee, R.A. Rajewski, V.J. Stella and J.F. Stobaugh, J. Pharm. Biomed. Anal., 10 (1992) 615–622.

[18] K.O. Börnsen, M. Schär and E. Gassmann, Biol. Mass Spectrom., 20 (1991) 471–478.

[19] M.D. Mohr, K.O. Börnsen and H.M. Widmer, Rapid Commun. Mass Spectrom., 9 (1995) 809–814.

[20] R. Marusyk and A. Sergeant, Anal. Biochem., 105 (1980) 403–404.

[21] H. Görisch, Anal. Biochem., 173 (1988) 393–398.

[22] K.O. Börnsen, M.D. Mohr and H.M. Widmer, Rapid Commun. Mass Spectrom., 9 (1995) 1031–1034.