# Influence of the degree of substitution of cyclodextrin sulfobutyl ether derivatives on enantioselective separations by electrokinetic chromatography

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## Abstract

Several γ-cyclodextrin sulfobutyl ether (γ-CD-SBE) derivatives, differing in the degree of substitution (DS), were synthesized and characterized by matrix-assisted laser desorption/ionization time-on-flight MS. They were evaluated as chiral selectors for the separation of enantiomers by capillary electrophoresis. The results demonstrate that the number of sulfobutyl groups attached to the cyclodextrin moiety significantly influences the enantioseparation towards the racemic analytes. For the examined racemic solutes, a good resolution was generally obtained when using γ-CD-SBE with an average DS ranging between 5 and 7, except for secobarbital which is only resolved when the average DS is higher than 7. For this compound the migration order of the enantiomers with γ-CD-SBE (DS 7.6) was opposite to that one observed with unmodified γ-cyclodextrin in the presence of sodium dodecyl sulfate micelles. For 1-(9-anthryl)-2,2,2-trifluoroethanol the resolution increases as DS is increasing. These different results clearly demonstrate that it is crucial to use well-characterized CD derivatives. © 1997 Elsevier Science B.V.

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## 1. Introduction

Although most often chiral HPLC is still the method of choice for the analytical separation of enantiomers, capillary electrophoresis (CE) employing chiral additives to the running buffer has gained considerable attention in recent years [1–4]. Major advantages of CE for enantiomer analysis consist in simplicity, high efficiency, low operating costs, and versatility (easy switching from one chiral selector to another). Most of the enantiomeric separations have been accomplished by the use of cyclodextrins and their derviatives. 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 sulfate (SDS).

An alternative approach to the use of neutral cyclodextrins is electrokinetic chromatography (EKC) employing charged cyclodextrins, which have their own electrophoretic mobility [3]. Among them, cyclodextrin sulfobutyl ether (CD-SBE), sulfopropyl ether (β-CD-SPE) and sulfoethyl ether have recently been introduced and proved to be very useful [5–10]. These derivatives, which are negatively charged above pH 2, can be used in lower concentrations and

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permit the separation of the enantiomers of neutral analytes without addition of a micelle agent.

In this paper we describe the preparation, characterization and evaluation of γ-cyclodextrin sulfobutyl ether (γ-CD-SBE) with various degrees of substitution (DS). In a systematic comparison, we have investigated the influence of DS on the chiral separation for a series of racemic analytes.

## 2. Experimental

### 2.1. Chemicals

γ-Cyclodextrin was kindly provided by Wacker (Liestal, Switzerland). 1,4-Butane sultone was purchased from Aldrich (Steinheim, Germany). All racemic samples were commercial products (Sigma or Aldrich). The enantiomers of secobarbital were obtained by semi-preparative chromatographic separation (HPLC) on *meta*-methylbenzoyl-cellulose.

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

γ-CD-SBE was prepared as previously described [11]. As a standard procedure (selector D), the following conditions have been applied. In a 50-ml round-bottom flask equipped 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 matrix-assisted laser desorption/ionization (MALDI) MS (see below), 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 3-fold membrane filtration in a stirred cell (Spec, Basel, Switzerland, exclusion size: $M_r$ 1000; 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 below) and by elemental analysis.

Five other γ-CD-SBE derivatives with different degrees of substitution were prepared by slight modifications of the above procedure. Selector A: only 3 g of butane sultone were added (−30%). Selector B: the reaction temperature was 60°C instead of 47°C. Selector C: the reaction time was 42 h instead of 24 h. Selector E: 6.1 g of butane sultone were added (+30%). Selector F: after 24 h at 47°C, 0.46 g of butane sulfone was added and the reaction mixture was stirred during additional 18 h before work-up.

### 2.3. MALDI-MS

As in our previous work with dextrin sulfopropyl ether [12] and γ-CD-SBE [11], measurements were performed on a linear time-of-flight (TOF) mass spectrometer (prototype of Linear Scientific, Reno, NV, USA) [13]. All spectra represented an accumulation of 30–50 shots. A vacuum in the flight tube of approx. $2\times10^{-6}$ to $7\times10^{-6}$ 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 was prepared as a 3:1 mixture of 2,5-dihydroxybenzoic acid (DHB) and 1-hydroxyisochinolin (HIC) by mixing equal volumes (5 or 10 μl each) of 0.2 $M$ DHB, 0.6 $M$ HIC [both dissolved in water–acetonitrile (50:50, v/v)], 30 m$M$ 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 [14,15].

### 2.4. Capillary electrophoresis

All CE experiments were performed on a Beckman P/ACE 5510 series capillary electrophoresis instrument with diode-array UV detection. The depicted chromatograms correspond to the absorption observed at 214 nm with a bandwidth of 4 nm, except for 1-(9-anthryl)-2,2,2-trifluoroethanol which was detected at 254 nm. An untreated 47 cm (40 cm effective length injector to detector)×50 μm I.D. fused-silica capillary was used. The voltage was 20

kV. Injection was achieved by pressure (typically 5 s, 35 mbar) at the anodic end of the capillary and the sample concentration was typically 0.1–0.2 mg/ml in methanol. 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 7, was obtained from the in-house central service department and was diluted to the desired concentration with distilled water. Running buffer was prepared by dissolving the additive in the respective buffer in a 4.5-ml vials or 500-μl minivials, filtering through a 0.45-μm syringe filter (Skan, Basel, Switzerland) and sonicating briefly. The resolution ($R_s$) was calculated applying the usual equation $R_s = (t_2-t_1)/(w_1+w_2)$, where $t_1$ and $t_2$ are the retention times and $w_1$ and $w_2$ are the baseline peak widths of the first and the second peak, respectively.

## 3. Results and discussion

### 3.1. Preparation and characterization of γ-CD-SBE

The described standard procedure is suitable for the easy preparation of γ-CD-SBE in batches of up to ca. 20 g [11]. In order to prepare γ-CD-SBE with different degrees of substitution (DS), reaction temperature, reaction time, and the concentration of butane sultone were varied (see Section 2). As demonstrated previously for dextrin sulfopropyl ether [12] and for γ-CD-SBE [11], MALDI-MS using a mixed DBH/HIC matrix is a powerful tool for the easy and unambiguous characterization of the product. Under the standard conditions (selector D), the average number of SBE groups per molecule is 5.9. From the results listed in Table 1 which are based on

the MALDI determinations of the selectors A–F obtained under various conditions, it can be seen that a lower concentration of butane sultone (A), a higher reaction temperature (B), or a longer reaction time (C), leads to a lower degree of substitution (Table 1) compared to the material obtained under the standard conditions. On the contrary, a higher concentration of butanesultone (E) or a two-step procedure involving a second addition of sultone (10%) and a running time for an additional 18 h (F) leads to an increase of the degree of substitution (Table 1). The average DS values determined by MALDI-MS are in good agreement with those calculated from elemental analysis of sodium. Similar values were calculated from the sulfur content, except for selectors E and F which show a higher average DS. With regard to these two selectors, the average DS calculated from sodium is too low compared to the MALDI results; it can be speculated that they contain some sulfobutyl groups in the protonated form.

### 3.2. Enantiomer separations with γ-CD-SBE

To investigate the influence of the degree of substitution (DS) of γ-CD-SBE on chiral separation, six reference compounds were selected (structures depicted in Fig. 1).

The applied electrophoretic conditions are identical to those used previously [11] and slightly differ for the various racemates (Table 2). For each racemate, these conditions were maintained thorough the whole study with the various γ-CD-SBE derivatives (selectors A–F).

All six γ-CD-SBE derivatives provide a good chiral resolution of the three dansylated amino acids (Fig. 2). However, the resolution reaches a maximum value for a DS 5.5 (selector C) in the case of dansyl-leucine and for a DS 5.9 in the case of

Table 1
Average molecular mass and degree of substitution of γ-CD-SBE selectors A–F

| γ-CD-SBE selector | Average molecular mass | Average DS as given by MALDI | DS calculated from % S | DS calculated from % Na |
|---|---|---|---|---|
| A | 1873 | 3.5 | 4.2 | 3.3 |
| B | 2045 | 4.6 | 5.0 | 4.85 |
| C | 2192 | 5.5 | 5.51 | 5.6 |
| D | 2252 | 5.9 | 5.99 | 5.97 |
| E | 2378 | 6.7 | 7.54 | 6.5 |
| F | 2535 | 7.6 | 8.3 | 6.58 |

D,L Dansyl-leucine

Binaphthol

D,L Dansyl-valine

1-(9-anthryl)-2,2,2-trifluoroethanol

D,L Dansyl-norvaline

Secobarbital

Fig. 1. Structures of the racemic solutes.



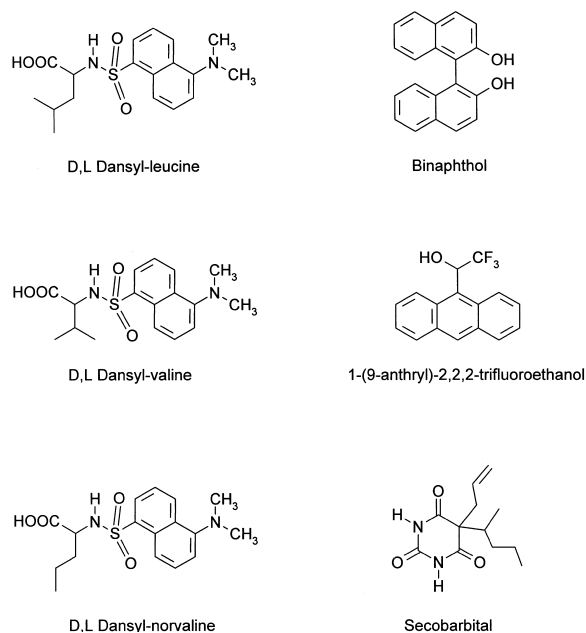Fig. 2. Influence of DS of γ-CD-SBE on resolution ($R_s$) of the enantiomers of the dansylated amino acids. For conditions, see Table 2.

dansyl-valine and dansyl-norvaline. These results show that the resolution is strongly influenced by the DS of the cyclodextrin. An optimum value exists for each amino acid derivative, but a DS lower than 4.5 or higher than 7 seems to be detrimental to the chiral recognition. The electropherogram obtained for the three amino acid derivatives with the γ-CD-SBE/DS=5.5 is shown in Fig. 3. With all γ-CD-SBE and for the three dansylated amino acids, the L enantiomer elutes first.

For binaphthol, a similar behavior was observed. The resolution increases from 1.93 for DS 3.5 and reaches a maximum of 2.53 for DS 5.9 (Figs. 4 and 5). A higher DS induces a decrease of resolution. The electropherograms obtained with the γ-CD-SBE/DS=5.9 and 6.7 are shown in Fig. 5. With all γ-CD-SBE, the S enantiomer elutes first.
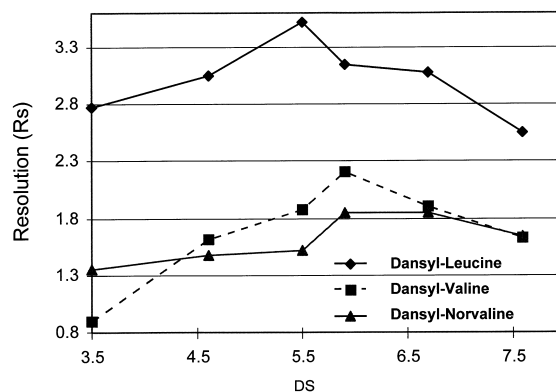
For the chiral solvating agent 1-(9-anthryl)-2,2,2-trifluoroethanol, no separation was observed with the γ-CD-SBE having an average DS of 3.5, but from a DS value of 4.6 up to 6.7 the resolution continuously improves as DS increases (Fig. 4). However, with γ-CD-SBE/DS=6.7 the retention time becomes very high ($t_2$=45.9 min, Fig. 5), and with γ-CD-SBE/DS=7.6 the substance does not elute further under the applied conditions. With all γ-CD-SBE, the R enantiomer of 1-(9-anthryl)-2,2,2-trifluoroethanol elutes first (Fig. 5). The strong increase of the migration time of the enantiomers of this neutral analyte with increasing DS indicates that the electrophoretic mobility of the chiral selector is considerably affected by the degree of substitution. In this particular case the reduced mobility of the chiral selector has a positive effect on the separation, but as shown by the results obtained with the previous examples, this is not a general rule, and it seems to be difficult to predict whether this change of mobility of the chiral selector will have a positive or an adverse effect on the separation. These five examples show that no general rule can be applied in predict-

Table 2
Conditions used for the enantiomer separations

| Analyte | Background electrolyte | Additive | γ-CD-SBE concentration (m$M$) |
|---|---|---|---|
| 1-(9-Anthryl)-2,2,2-trifluoroethanol | Phosphate, pH 7(20 m$M$) | Urea (2 $M$) | 15 |
| Binaphthol | Phosphate, pH 7 (20 m$M$) | Methanol (10%, v/v) | 2.5 |
| Dansylated amino acids | Phosphate, pH 7 (20 m$M$) | Methanol (10%, v/v) | 2.5 |
| Secobarbital | Phosphate, pH 7 (20 m$M$) | Methanol (15%, v/v) | 15 |

**D,L Dansyl-leucine**

8.96 (L)

9.37 (D)

8.6    9.0    9.4    9.8 min

**D,L Dansyl-valine**

8.81 (L)

9.01 (D)

8.5    9.0    9.5 min

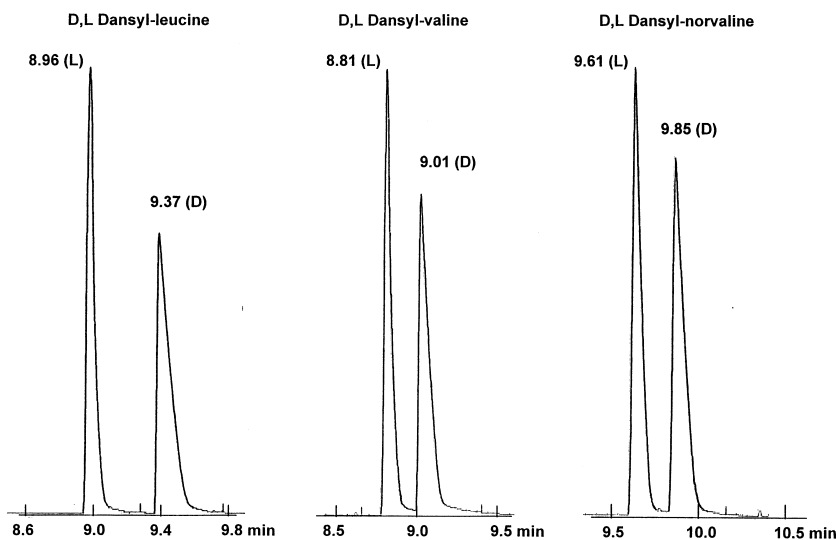**D,L Dansyl-norvaline**

9.61 (L)

9.85 (D)

9.5    10.0    10.5 min

Fig. 3. Enantiomeric separation of the dansylated amino acids by CE using γ-CD-SBE (DS=5.9) as chiral buffer additives. For conditions, see Table 2.

ing the optimal DS for a particular compound. Similar results were found for other chiral analytes using carboxymethyl-β-cyclodextrin with various degrees of substitution as chiral selectors [16].

For secobarbital, we have shown in a previous work that the enantiomers could be well separated with unmodified γ-cyclodextrin in the presence of SDS micelles, whereas no separation could be achieved with a γ-CD-SBE having an average DS of 5.9 [11]. This was confirmed in this study with the
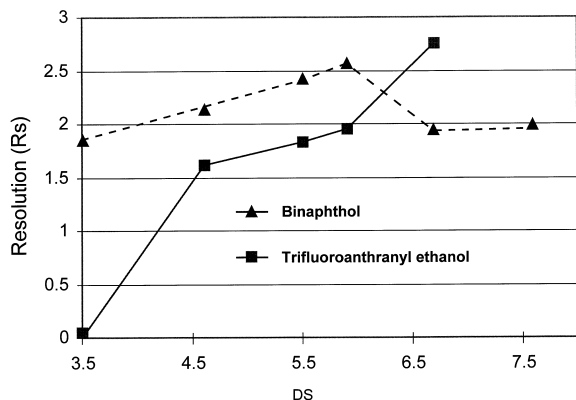


Fig. 4. Influence of DS of γ-CD-SBE on Resolution ($R_s$) of the enantiomers of binaphthol and of 1-(9-anthryl)-2,2,2-trifluoro-ethanol. For conditions, see Table 2.

γ-CD-SBE having an average DS lower than 7, but using the γ-CD-SBE selector F (DS=7.6), a partial separation was obtained (Fig. 6). As expected, injection of the single enantiomers under the same conditions confirmed the inversion of elution order of the enantiomers of secobarbital with unmodified γ-cyclodextrin and with γ-CD-SBE/DS=7.6. A loss of chiral recognition was also observed by using an equimolar mixture (15 m$M$) of unmodified γ-cyclo-dextrin and of γ-CD-SBE (DS=7.6), resulting from the antagonist effects of both types of selectors which exhibit opposite migration order in the normal polarity mode. In the reversed polarity mode, it has been demonstrated that such a combination of chiral selectors may considerably enhance the enantiosepa-ration of racemic compounds [17,18].

## 4. Conclusion

γ-CD-SBE is a powerful chiral selector for CE. However, the degree of substitution (DS) signifi-cantly influences the resolution and the optimal value varies for each analyte, even among a series of structurally related ones such as dansylated amino acids, for example. A DS of ca. 5–6 seems to be a good overall compromise. Anyway, the strong in-
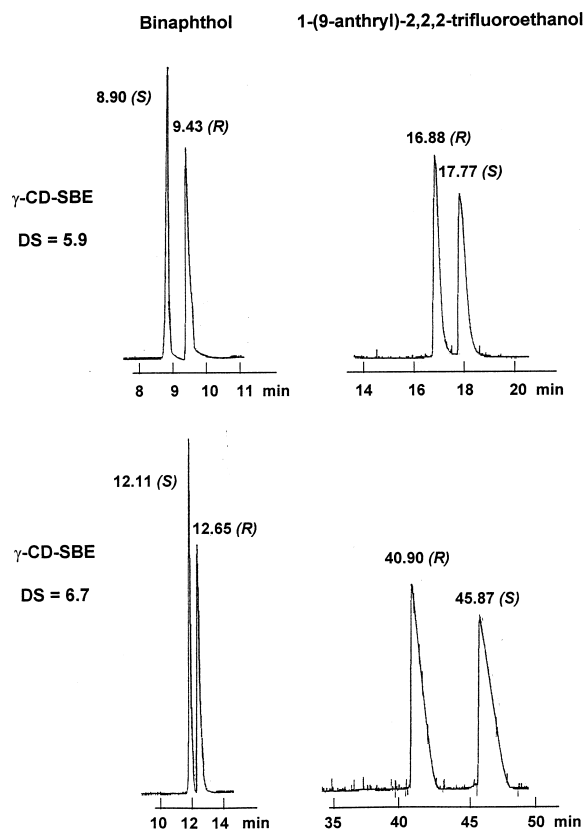
fluence of DS on chiral separation points to the importance of the full characterization of the used chiral selector.



Fig. 5. Enantiomeric separation of racemic binaphthol and racemic 1-(9-anthryl)-2,2,2-trifluoroethanol by CE using γ-CD-SBE (DS= 5.9 and 6.7) as chiral buffer additives. For conditions, see Table 2.

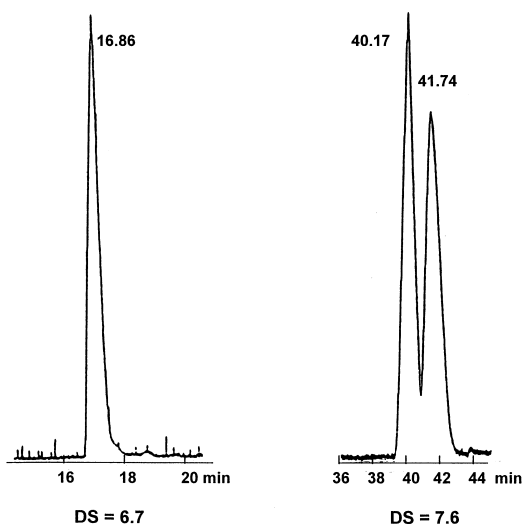

Fig. 6. Enantiomeric separation of racemic secobarbital by CE using γ-CD-SBE having an average DS=6.7 and 7.6 as chiral buffer additives. For conditions, see Table 2.

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