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Chiral resolution of functionalized piperidine enantiomers by capillary electrophoresis with native, alkylated and anionic β-cyclodextrins

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Abstract

Anionic sulfobutylether-β-CD (SBE-β-CD) has been found to be more suitable and convenient than native or alkylated β-CDs for the enantiomeric separation of functionalized piperidines. This anionic cyclodextrin derivative facilitates the separation of cationic enantiomers since the mobility of the complexed form is opposed to that of the free cation. The addition of low concentrations of SBE-β-CD (5–20 mg/ml) to the phosphate buffer, pH 2.6, allows a fast and baseline separation of dienomycine C (R_s=1.70) and also of 4-protected 2-alkylated piperidine (R_s=1.26) into their enantiomers. Compared to neutral CDs, this chiral selector offers the benefits of an increased aqueous solubility and a better resolution because this anionic CD acts as a countermigrating complexing agent versus cationic drugs.

Keywords: Enantiomer separation; Chiral selectors; Piperidines; Cyclodextrins

1. Introduction

Molecular recognition is a main concept in the understanding of drug–receptor interactions. In contrast to GC and HPLC techniques, most chiral selectors used in capillary electrophoresis (CE) are added to the carrier electrolyte.

More than two hundred papers have reported the use of cyclodextrins (CDs) as chiral selectors in capillary electrophoresis. Although there are no universal chiral selectors, cyclodextrins are commercially available in numerous types and sizes and can be easily dissolved in the separation buffers. The molecular structure of many pharmaceuticals generally enables cyclodextrins to form inclusion complexes with these substances. Chiral recognition then occurs because of the selective complexation of the enantiomers with these chiral selectors. This complexation process involves the inclusion of the hydrophobic moiety of the solute inside the cyclodextrin cavity; weak enantioseparation will occur if the cyclodextrin cavity is too large and no enantioseparation if the cavity is too small. Many neutral cyclodextrins have already been used as buffer additives for the chiral CE separation of pharmaceuticals [1], such as β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), dimethyl-β-cyclodextrin (DM-β-CD) and more recently, charged cyclodextrins such as anionic carboxymethyl- or carboxyethyl-β-cyclodex-

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trin, sulfobutylether-β-cyclodextrin, and cationic 6A,6D-dimethylamino- or 6A-methylamino-β-cyclodextrin [2–8]. The use of charged CDs involves an additional separation mechanism based on electrostatic interactions which increases the stability of analyte–CD complexes.

A few years ago, Wren et al. [9–11] proposed a simple and convenient model for the separation of pairs of enantiomeric molecules in capillary electrophoresis. The most interesting feature concerned the variation in selectivity as the concentration of chiral selector changed; this theoretical model showed that there is an optimum concentration which depends on the affinity of the enantiomers for the chiral selector. This approach has been supported by a lot of experimental data on the chiral separation of β-blockers [9–11]. In a recent study dealing with the chiral CE separation of benzopyran derivatives [1], the stability constant for the β-CD inclusion complex has been determined for each enantiomer of two 3,4-dihydro-2H-1-benzopyran derivatives; a good agreement has been found between the experimental value of optimum chiral selector concentration (C_{opt}) and the value calculated from the equation $[C]_{opt} = 1/(K_R K_S)^{1/2}$, where K_R and K_S are the experimental inclusion complex constants of the *R*- and *S*-enantiomers. Recently, a more detailed mathematical model from Rawjee [12–14] has considered the complexation process depending on the ionic form and also on the molecular form of the chiral solute.

In this work, the separation of the enantiomers of three functionalized piperidines by capillary electrophoresis has been investigated by using firstly neutral and then anionic charged β-cyclodextrins. For neutral chiral selectors (β-CD, HP-β-CD, DM-β-CD), optimization was by following Rawjee's chiral selectivity model [12–14] and then interpretion by Guttman et al. [15]. Then, the influence of the concentration of the negatively charged SBE-β-CD on the enantioresolution was investigated.

2. Experimental

2.1. Apparatus

All open-tube electrokinetic capillary chromatographic separations were performed on a Spectraphoresis 1000 instrument (Spectra-Physics, San Jose, CA, USA) using a silica capillary tube (70 or 44 cm×50 μm I.D.×375 μm O.D.). Data were processed on an IBM PS/2 Model 70 386 computer. Software operating under IBM OS/2 was supplied by Spectra-Physics. The instrument contains a programmable high-speed scanning, multiple-wavelength UV detector. Using the fast scanning mode from 200 to 360 nm, absorption maxima of studied piperidines were determined (226, 259 or 286 nm). Electrophoretic separations were performed at negative or positive voltage. Analytes were injected in the hydrodynamic mode using a 0.75 p.s.i. vacuum for 0.5–3 s (1 p.s.i.=6894.76 Pa).

The capillary was conditioned daily by washing with 0.1 *M* sodium hydroxide solution (5 min) and water (5 min) and then with buffer (5 min).

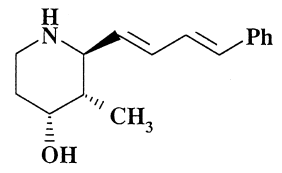
The pK_a values of the analytes were predicted by using Pallas software (CompuDrug Chemistry, Budapest, Hungary). Electrophoretic buffers at fixed pH and ionic strength were predicted and then prepared by using Phoebus software (Centre Analyse, Orleans, France).

2.2. Reagents

All chemicals were of analytical-reagent grade. Water used for dilutions or as buffer preparation was produced by an ELGA apparatus. Borax ($Na_2B_4O_7 \cdot 10\ H_2O$), phosphoric acid and sodium dihydrogenphosphate were obtained from Fluka (Buchs, Switzerland). β-Cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and finally dimethyl-β-cyclodextrin (DM-β-CD) of derivatization degree 1.8 were obtained from Wacker Chemie (Munich, Germany). The anionic charged SBE-β-CD has been synthesized in our laboratory with an average substitution degree of 4.6 (M_r 1678).

Analytical samples of racemic dienomycine C (1), 4-protected 2-alkylated piperidine (2) and 4-hydroxylated 2-alkylated piperidine (3) (Fig. 1) were synthesized by Professor Troin [16]; each enantiomer of these piperidines differs in the configuration of carbon located on position 2.

For free solution capillary electrophoresis under acidic conditions, the buffer composition was 63.5 m*M* H_3PO_4–46.9 m*M* NaOH (pH 2.6); this buffer has an ionic strength of 50 m*M* and a high capacity



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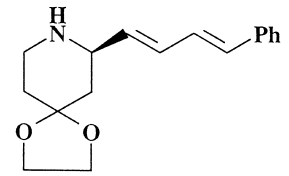

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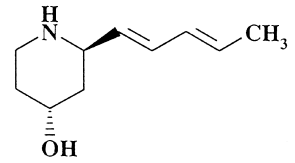

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Fig. 1. Structure of the chiral drugs studied. 1, Dienomycine C; 2, 4-protected 2-alkylated piperidine; 3, 4-hydroxylated 2-alkylated piperidine.

buffer (31.8 m*M*/pH), as indicated by Phoebus software. As chiral agents, native or modified cyclodextrins were dissolved in the buffer at the required final concentration.

3. Results and discussion

Functionalized piperidines are an important class of synthetic intermediates which have been extensively used in the preparation of biologically active compounds [17]. Six membered azaheterocycles represent a common structure in naturally occurring alkaloïds and pharmaceuticals. Racemic dienomycin C (1) and functionalized piperidines (2) and (3) were prepared according to the literature [18]. Dienomycine C has been extracted in enantiomeric form from a *Streptomyces* strain and showed antibiotic properties [19].

3.1. Use of neutral CDs

Most attention has been directed to the use of different neutral CD derivatives to bring about the resolution of these functionalized piperidine enantiomers. Guttmann et al. [15] have recently proposed a step-by-step method development for a cyclodextrin array chiral analysis which reduces the number of runs. We applied this procedure to develop separations of dienomycine C (1) and the functionalized piperidine (2) enantiomers with neutral CDs. These basic solutes (pK_a=9.9) were analysed using the low pH phosphate buffer (pH 2.6) and the cyclodextrin array (3 and 15 m*M* β-CD, 10 and 100 m*M* HP-β-CD, and 10 and 50 m*M* DM-β- CD). The electroosmotic flow was weak ($10 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$) and the electrostatic interactions between positively charged analytes and negative ionized silanol groups were reduced. With neutral CDs, the mobility of the inclusion complex with one cationic solute decreases since the charge/mass ratio is lowered. Under these conditions, the enantiomer forming the most stable complex is the last to elute.

The suggested six runs using the cyclodextrin array completed with dienomycine C indicate that the resolution of enantiomers seems feasible only with β-CD or HP-β-CD, whereas DM-β-CD fails to resolve the enantiomers of this drug. The second optimization step, as proposed by Guttmann [15], deals with the variation in the concentration of the appropriate cyclodextrins (β-CD and HP-β-CD). The effects of β-CD and HP-β-CD concentrations on the chiral resolution of dienomycine C were studied using the same phosphate buffer (pH 2.6) containing either β-CD at concentrations varying from 1 m*M* up to 9 m*M*, or HP-β-CD in the 5–25 m*M* range. Unfortunately, dienomycin enantiomers were partly resolved at 3 m*M* β-CD (R_s=0.23) and exhibited partial separation (R_s=0.53) at 5 m*M* HP-β-CD (Fig. 2a).

In order to increase the enantioseparation of dienomycine C, methanol was added to the phosphate buffer in the 0–20% (v/v) range; a noticeable

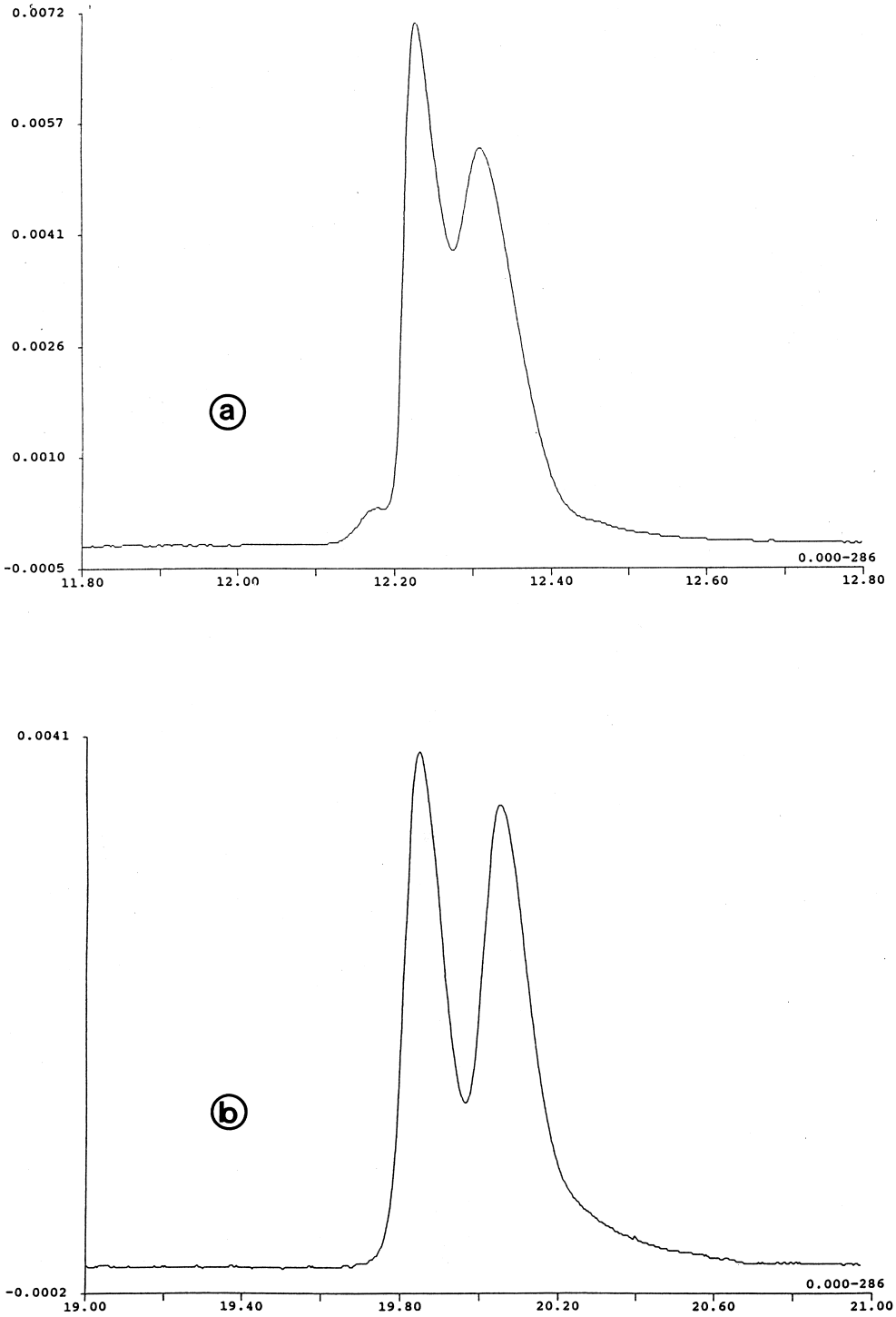

Fig. 2. Influence of the methanol added to HP-β-CD buffer upon the separation of dienomycine C enantiomers. Capillary: 70 cm×50 μm; applied voltage: +30 kV; temperature: 25°C; detection at 286 nm; electrolyte: 63.6 m*M* H_3PO_4–46.9 m*M* NaOH (pH 2.6); hydrodynamic injection time: 1 s; solute concentration: 25 μg/ml. (a) 5 m*M* HP-β-CD, (b) 5 m*M* HP-β-CD, 20% CH_3OH.

improvement in resolution (R_s=0.95) was observed when 20% methanol was added to the electrolyte (Fig. 2b). The addition of methanol to the buffer improves the analyte solubility but should remain limited as it results in a lower selectivity due to a weaker inclusion complexation.

At the opposite, dimethyl-β-CD was found to resolve the enantiomers of functionalized piperidine (2) in less than 13 min. Fig. 3 shows good separation (R_s=1.25) of these enantiomers using the following buffer (63.5 m*M* H_3PO_4–46.9 m*M* NaOH, pH 2.6) to which 20 m*M* dimethyl-β-CD has been added.

Inclusion complexation is probably a stereoselective mechanism and we can assume that the aromatic group bonded to the diene unit of dienomycine C fits the cavity of the CD and that a hydrogen bond is formed between the hydroxyl and nitrogen group located near the chiral carbon on position 2 of dienomycine C.

3.2. Use of anionic sulfobutyl ether-β-CD

Compared to neutral CDs, anionic charged cyclodextrins should facilitate the separation of cationic enantiomers since the mobility of the complexed form is opposed to that of the free cation.

SBE-β-CD is a sulfobutyl ether, sodium salt derivative variably substituted on the 2-, 3- and the 6-position of β-cyclodextrin. This chiral cyclodextrin has been synthesized in our laboratory and used in this study after purification by dialysis to eliminate sodium chloride and some unknown by-products. It was a heterogeneous mixture of different degrees of alkylation with an average value of approximately 4.6 determined by mass spectrometry and capillary electrophoresis. The presence of these sulfonate moieties makes the CD negatively charged over the entire pH range available to CE experiments and more soluble in aqueous buffer than β-CD; conse-

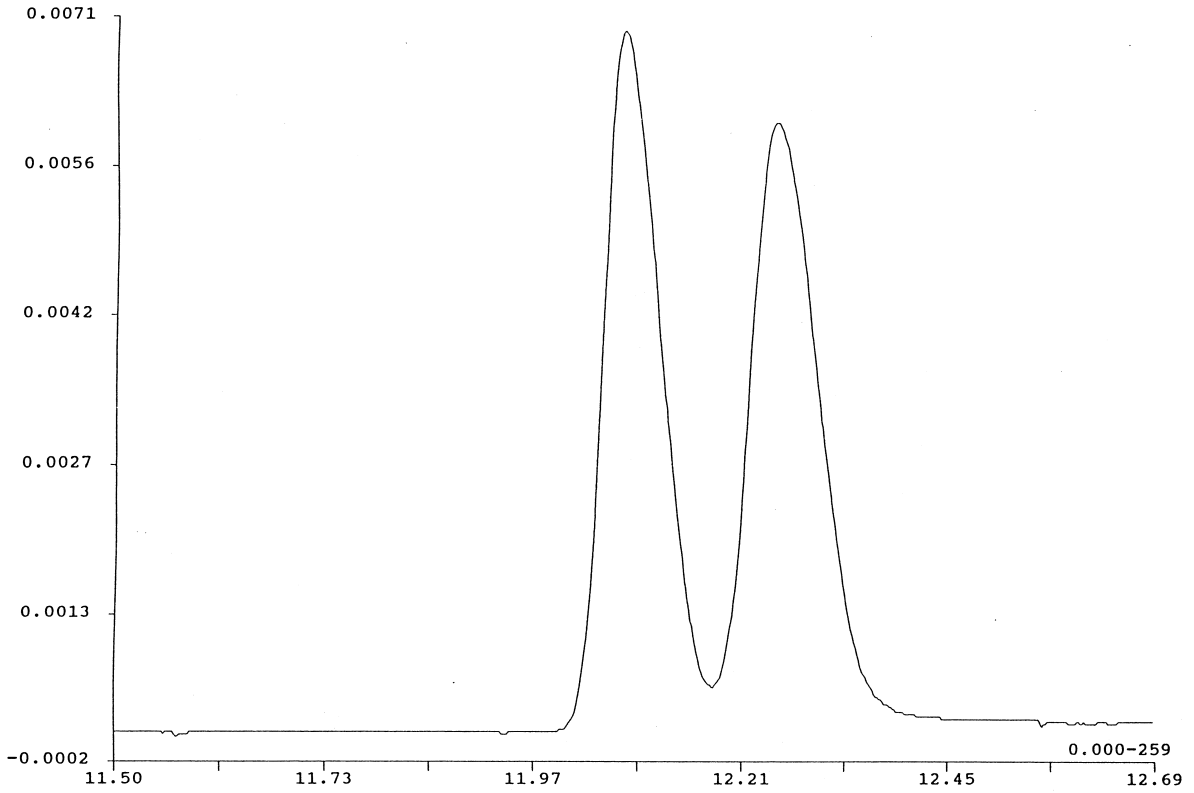

Fig. 3. Separation of the enantiomers of functionalized piperidine (2) by capillary electrophoresis. Capillary: 70 cm×50 μm; applied voltage: +30 kV; temperature: 15°C; detection at 259 nm; electrolyte: 63.6 m*M* H_3PO_4–46.9 m*M* NaOH (pH 2.6), 20 m*M* dimethyl-β-CD (derivatization degree 1.8); hydrodynamic injection time: 0.5 s; solute concentration: 25 μg/ml.

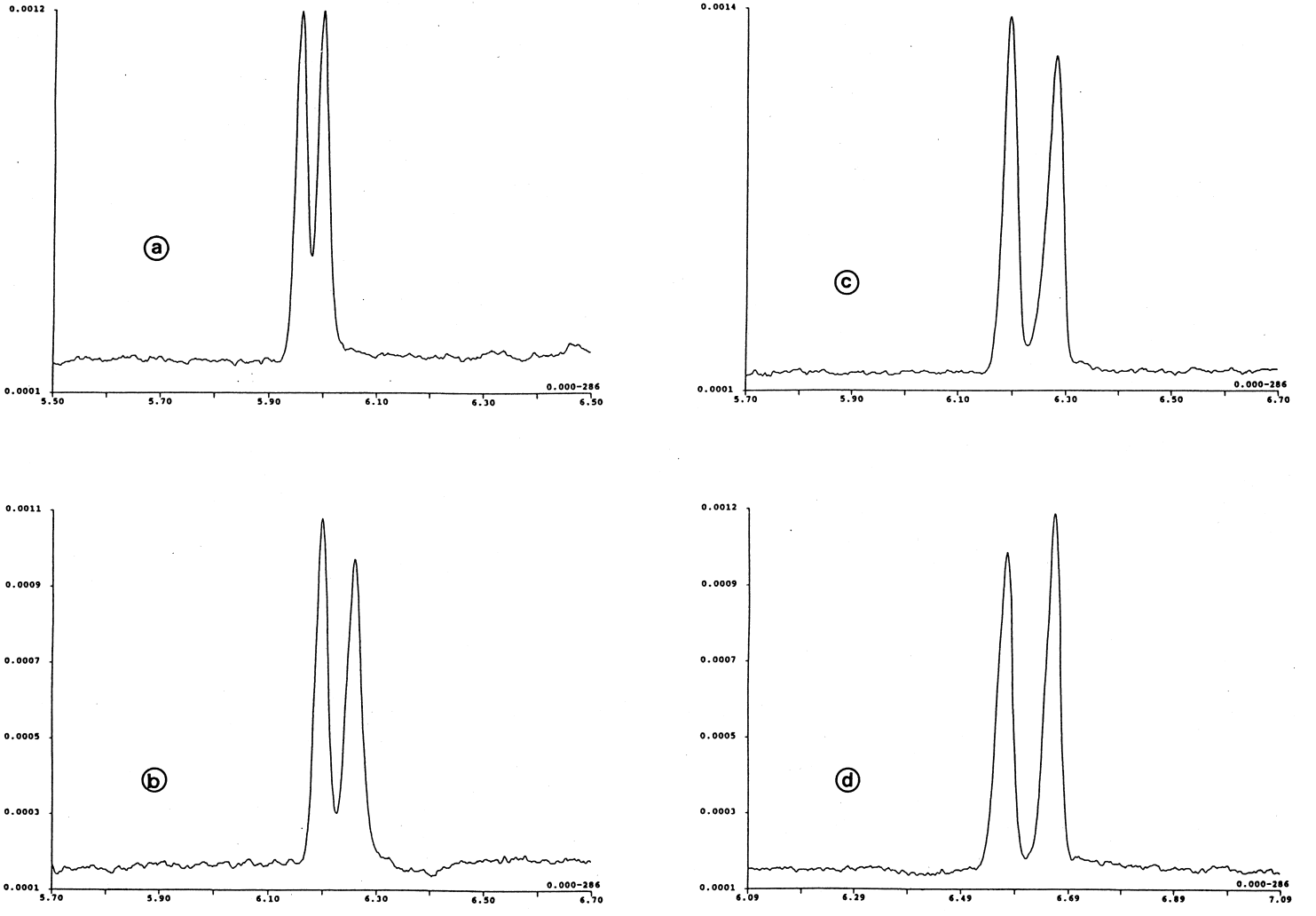

Fig. 4. Electropherograms of dienomycin with increasing concentrations of sulfobutyl ether β-CD. Capillary: 44 cm×50 μm; applied voltage: −15 kV; temperature: 25°C; detection at 286 nm; electrolyte: 63.6 m*M* H_3PO_4–46.9 m*M* NaOH (pH 2.6), hydrodynamic injection time: 1 s; solute concentration: 25 μg/ml. SBE-β-CD concentration (mg/ml): (a) 20; (b) 10; (c) 7; (d) 5.

Table 1
Influence of SBE-β-CD concentration during the separation of dienomycin C enantiomers

	SBE-β-CD concentration (mg/ml)				
	20	10	7	5	3
Resolution	0.93	1.05	1.51	1.70	1.93

Experimental conditions as in Fig. 4.

quently, the negatively charged CD migrates towards the anode whether it is free or complexed.

Fig. 4 illustrates the effect of the concentration of SBE-β-CD, added to the background electrolyte, on the separation of dienomycin C enantiomers; Table 1 gives the resolution when the separations were run at pH 2.6 and the electrolyte contained increasing concentrations of SBE-β-CD. Enantiomers of dienomycin C are perfectly resolved (R_s=1.70, 360 000 theoretical plates) in 7 min by adding 5 mg/ml of SBE-β-CD in the phosphate buffer pH 2.6 (Fig. 5). Moreover, a smaller cyclodextrin concentration induced increased peak tailing probably due to electrodispersion [7]; indeed, the asymmetric factor decreases from 1.0 at 5 mg/ml down to 0.26 at 3 mg/ml.

The low pH running electrolyte used minimizes electrosmotic flow so the separation mechanism was based upon the competing electrophoretic mobilities of the free cationic analyte and the anionic complex. Dienomycin C migrates towards the anode (detection side) in the presence of SBE-β-CD which is consistent with very strong drug–CD interactions. Positively-charged drugs are more strongly bound to SBE-β-CD compared to neutral cylcodextrins due to the positive influence of its charge on the complexation process. Besides, hydrophobicity, ion-pair formation, and molecular size are all potential contributing factors. The effects of countermigration

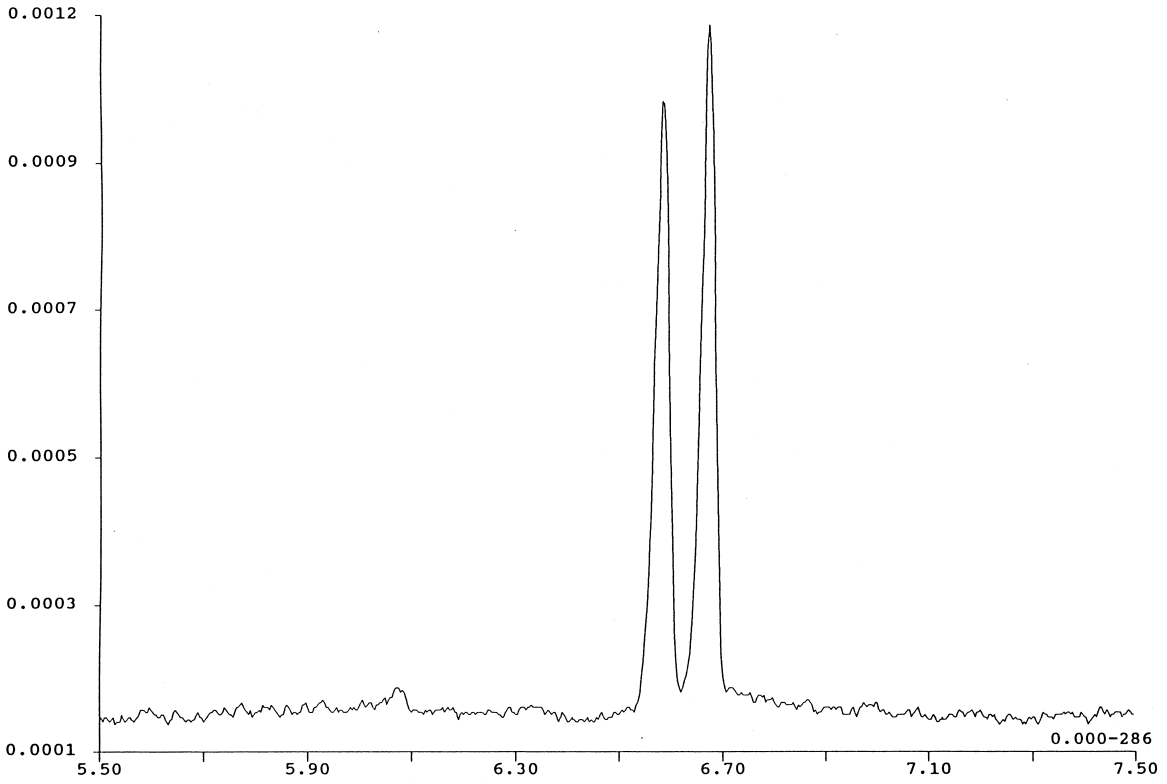

Fig. 5. Optimized separation of dienomycine C enantiomers by capillary electrophoresis by using anionic sulfobutyl-ether-β-CD as chiral selector. Capillary: 44 cm×50 μm; applied voltage: −15 kV; temperature: 25°C; detection at 286 nm; electrolyte: 63.6 m*M* H_3PO_4–46.9 m*M* NaOH (pH 2.6), 5 mg/ml SBE-β-CD; hydrodynamic injection time: 1 s; solute concentration: 25 μg/ml.

were pronounced with favorable peak shapes and efficiencies; no band broadening appears which would be caused by the presence of multiple species in the SBE-β-CD sample.

At last, the separation of the racemic functionalized piperidine (3) into its enantiomers has been optimized at pH 2.5 (Fig. 6); a good enantiomeric resolution (R_s=1.26) was also obtained by adding 25 mg/ml of SBE-β-CD to the phosphate buffer (pH 2.6, 50 m*M* ionic strength).

In conclusion, the use of anionic SBE-β-CD was found to be more suitable and convenient for the enantiomeric separation of functionalized piperidines rather than native or alkylated β-CDs. This anionic cyclodextrin derivative facilitates the separation of cationic enantiomers since the mobility of the complexed form is opposed to the free cation. Compared to neutral CDs, it offers the benefits of an increased aqueous solubility and a better resolution because this anionic CD acts as a countermigrating complexing agent against cationic drugs.

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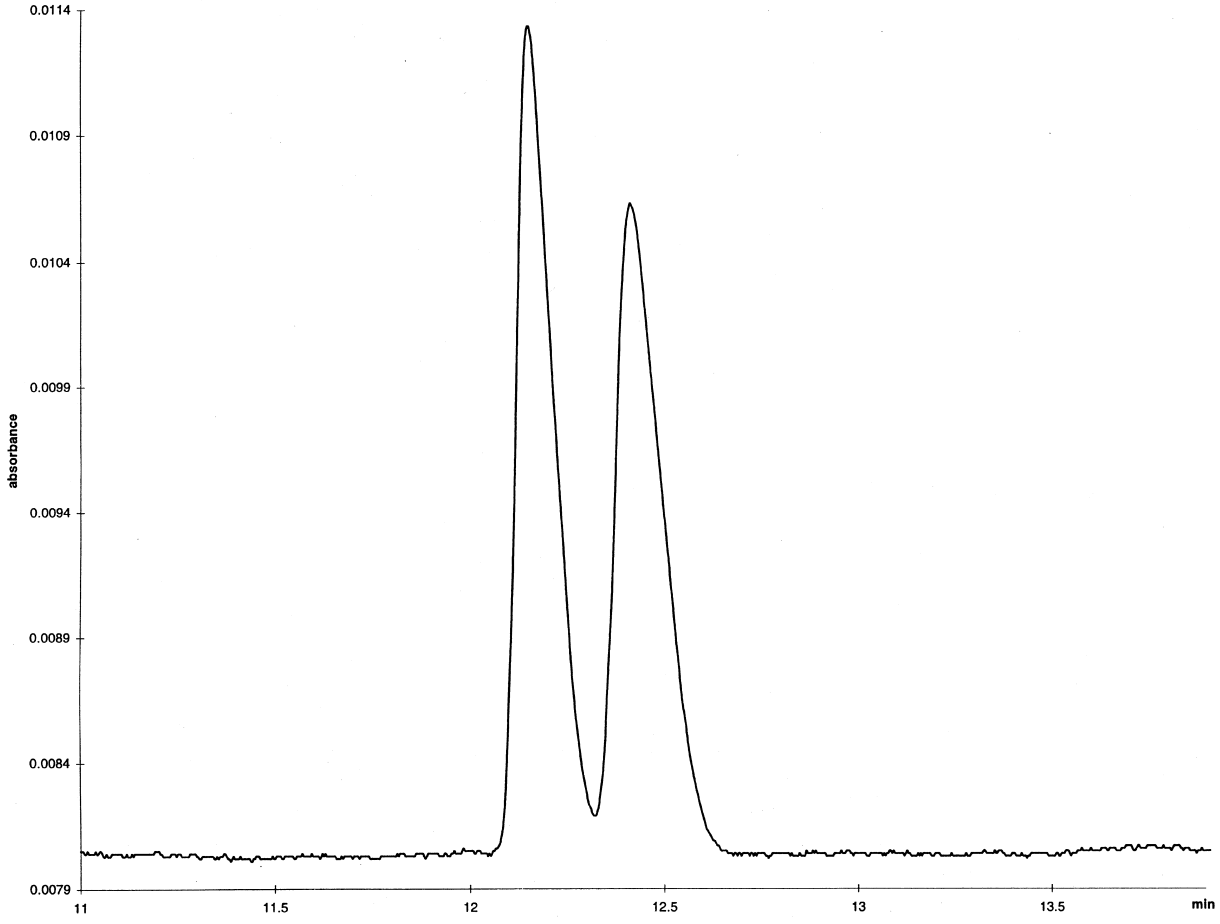

Fig. 6. Separation of the enantiomers of functionalized piperidine (3) by capillary electrophoresis using anionic sulfobutyl ether β-CD as chiral selector. Capillary: 44 cm×50 μm; applied voltage: −15 kV; temperature: 25°C; detection at 226 nm; electrolyte: 63.6 m*M* H_3PO_4–46.9 m*M* NaOH (pH 2.6), 25 mg/ml SBE-β-CD; hydrodynamic injection time: 3 s; solute concentration: 25 μg/ml.

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