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Carbamate chiral surfactants for capillary electrophoresis

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Abstract

Chiral resolution in capillary electrophoresis (CE) was studied with several novel synthetic surfactants, which are synthesized from an amino acid (L-leucine, L-valine, L-isoleucine, or L-serine) and an alkyl chloroformate with a chain length of C_4 to C_{12}. Several chiral drugs, including atenolol, benzoin, laudanosine, propranolol, ketamine, hydrobenzoin and nefopam were used as test compounds. It was found that resolution can be readily manipulated by varying the chain length, the amino acid and the surfactant concentration. Sulfonated β-cyclodextrin (β-CD) was also studied and compared with these novel surfactants. A different selectivity was found for sulfonated β-CD due to its distinct structure. Neither the new surfactants nor β-CD gave a satisfactory chiral resolution for all the seven drugs. However, a mixture of a surfactant and sulfated β-CD was suitable for chiral resolution of all seven drugs. These mixed reagents were also effective for CE resolution of eight dansyl amino acids.

Keywords: Enantiomer separation; Chiral separation; Buffer compositions; Surfactants

1. Introduction

The importance of enantiomeric differences in biological interactions and reactions has been widely recognized [1]. Analytical techniques for chiral resolution of optically active molecules have undergone extensive development in the past decade, including high-performance liquid chromatography (HPLC), gas chromatography (GC), thin-layer chromatography (TLC), and capillary electrophoresis (CE).

The application of CE for chiral separation has undergone an enormous development over recent years and offers many advantages over conventional analytical methods [2–5], such as fast speed, high efficiency, low cost and small sample volume requirement. Chiral resolution in CE is based on the differential complexation between enantiomers and a chiral selector added to the buffer. The addition of chiral selectors results in the formation of transient non-covalently bound diastereoisomers, which can be easily separated based on their different physical properties.

Cyclodextrins (CDs) including native and derivatized CDs are among the most widely used chiral additives in CE. Nielen [6] studied chiral separations of basic drugs using CDs. Nine out of ten drugs investigated showed resolutions higher than 1.4, which is far more superior than liquid chromatographic methods. He also found that the resolution is strongly dependent on the applied field strength.

Quang and Khaledi [7] improved chiral separation of basic compounds using β-CD and tetraalkylammonium salts. Short-chain tetralkylammonium cations were found more effective in the regard of

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controlling the electroosmotic flow (EOF) and improving resolution of the cationic enantiomers. This is because the short-chain reagents provide better capillary wall coverage, and the short-chain tetraalkylammonium cations are less likely to occupy the hydrophobic cavity of β-CD than the long-chain cationic surfactants, leaving the enantioselective interaction sites more available for the analytes.

Penn et al. [8] utilized a systematic approach to optimize enantiomeric separations using CD selectors. They found that maximum electrophoretic mobility difference between the enantiomers occurs when the concentration of free selector is equal to the reciprocal of the average binding constant.

In the aspect of separation time frame, resolving power and solubilities, derivatized CDs have been demonstrated to be better than the parent CDs. The modifications influence the overall hydrophobic character of the CDs, resulting in changes in the shape and size of their cavities and their hydrogen-bonding ability.

Heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD) is a typical example of an alkylated CD. Yoshinaga and Tanaka [9] applied DM-β-CD in chiral separation of dansyl amino acids. Valko et al. [10] investigated effect of the degree of substitution of (2-hydroxy)-propyl-β-CD on the enantioseparation of organic acids.

Charged CDs, in which hydroxyl groups have been either carboxyalkylated [11], sulfonated [12], sulfoalkylated [13], or aminoalkylated [14], present a distinct advantage over their neutral relatives due to a significant electrophoretic mobility which may be in opposition to EOF. Consequently, a greater separation window may be available, resulting from a better interaction between analytes and CDs.

Mixtures of neutral, DM-β-CD, and anionic CD, sulfobutyl ether β-CD, were used for cationic drugs by Lurie et al. [15]. The resolution and migration speed can be readily adjusted by varying the ratio of the two added CDs provided the anionic CD acted as a countermigrating complexing reagent. Similarly, a dual system of CDs consisting of a cationic mono(6-amino-6-deoxy)-β-CD and a neutral CD (trimethyl-β-CD or dimethyl-β-CD) was studied by Lelievre et al. [16] for separation of arylpropionic acid enantiomers. CD-modified micellar electrokinetic chromatography (CD-MEKC), implementing CDs and a achiral surfactant such as sodium dodecyl sulfate (SDS), were explored by other authors [17,18].

Other chiral selectors such as vancomycin [19], ristocetin A [20], crown ethers [21], bovine serum albumin [22], maltooligosaccharides [23] have been investigated by CE.

The use of chiral surfactants in MEKC is another important separation mode for optically active compounds. The chiral surfactants include naturally occurring compounds, for instance, bile salts [24], and synthetic surfactants derived from simple chiral molecules such as amino acids [25], or glucose [26].

N-Dodecanoyl-L-valinate [25] combined with SDS is one of the earliest example of using synthetic surfactant to resolve enantiomers. Other amino acid ester derivatives, e.g., L-threonine [27] and L-alanine [28] have also been used. Mazzeo et al. [29] introduced (*R*)- and (*S*)-*N*-dodecoxycarbonyl-valines to resolve a range of 12 pharmaceutical drugs including β-blockers, bupivacaine, and homatrophine. Dodecyl-β-D-glucopyranosyl derivatives are another novel class of chiral surfactant introduced by Tickle et al. [26].

One of the advantages of using synthetic surfactants is that the structure can be readily varied. In our study a series of chiral surfactants was prepared from L-amino acids by reaction with alkyl chloroformate ranging from C_4- to C_{12}-. The resulting urethanes were evaluated for the chiral separation of seven model compounds. Satisfactory CE separations were obtained simply by using 25–100 m*M* of the additive in the background electrolyte (BGE). Efficiency of the separations depended strongly on the type of amino acid and on the chain length of the aliphatic chain.

2. Experimental

2.1. Apparatus

CE experiments were performed with a Waters Quanta 4000E CE system (Waters, Milford, MA, USA). The uncoated fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA) were 60 cm (length to detection window from the injector 52.5 cm)×50 μm I.D. Hydrostatic sampling mode was used with a sampling time of 6 s and height of 10

cm. The positive power supply with the detection window on the cathode end was used at 18 kV for each experiment unless indicated. Direct UV detection was employed at either 254 nm or 214 nm. Separations were performed at 25°C. Electropherograms were collected and plotted by the data acquisition system Chromperfect Direct (Justice Innovations, Mountain View, CA, USA).

The capillary was initially rinsed with 0.1 *M* NaOH for 1 h followed by a 1-h rinse with deionized (DI) water, which was subsequently replaced with the running electrolyte. Between each run, the capillary was rinsed with 0.1 *M* NaOH for 2 min, DI water for 3 min, and the electrolyte for 3 min.

2.2. Reagents

2.2.1. Chemicals

All standards and electrolytes were prepared with analytical-reagent grade chemicals and 18 MΩ deionized waters obtained from a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA). The running electrolyte consisted of 0.025 *M* borate and 0.025 *M* phosphate buffer adjusted to pH 8.8 with 0.1 *M* sodium hydroxide and filtered through a 0.45-μm membrane filter (Costar, Cambridge, MA, USA).

Sodium borate, sodium phosphate, sodium hydroxide, calcium chloride, acetonitrile, pyridine, methylene chloride, diethyl ether were obtained from Fisher Scientific (Fairlawn, NJ, USA). Butyl chloroformate, hexyl chloroformate, octyl chloroformate, decyl alcohol, 1-dodecanol, 1-tetradecanol, triphosgene, sulfated β-CD were purchased from Aldrich (Milwaukee, WI, USA). L-Valine, L-leucine, L-isoleucine, L-methionine, L-alanine, L-serine, L-aspartic acid and L-asparagine were obtained from Sigma (St. Louis, MO, USA).

The stock solutions of atenolol, benzoin, laudanosine, propranolol, ketamine, hydrobenzoin, nefopam (Sigma) were prepared at a concentration of 10 000 mg/l (ppm) in methanol, and diluted to 100 ppm in running electrolyte before analysis.

2.2.2. Synthesis of decyl chloroformate [30]

A solution of 3.16 g of decyl alcohol (0.02 mol), 1.98 g of triphosgene (0.0067 mol) and 20 ml methylene chloride was stirred and cooled to 10–15°C. 1.6 g of pyridine (0.02 mol) was added dropwise over a 1-h period. The reaction mixture was stirred for an additional 1 h, then heated in a water bath at 65°C for 15 min until all the methylene chloride evaporated. The residue was washed three times with cold water, and dried over calcium chloride (4–20 mesh), giving 3.66 g (83% yield) of decyl chloroformate [$ClCO_2(CH_2)_9CH_3$]. Other alkyl chloroformates were synthesized in a similar manner.

2.2.3. Synthesis of (S)-(+)-N-decoxycarbonyl-leucine [31]

A solution of 1.31 g (0.01 mol) of L-leucine in 5 ml of 2 *M* sodium hydroxide is placed in a 50-ml three-necked flask fitted with two dropping funnels. The solution is stirred and cooled in an ice bath, and 2.2 g (0.01 mol) of decyl chloroformate and 5 ml of 2 *M* sodium hydroxide are added alternatively to the vigorously stirred solution over 1 h, such that the pH of the mixture is kept at 9–10. The mixture is stirred for an additional 1 h at room temperature. The aqueous solution is acidified with concentrated hydrochloric acid to pH 1–2. Four ml diethyl ether is added to help extract product from the aqueous layer. The upper ether layer is separated, washed with 0.1 *M* hydrochloric acid, and dried overnight, giving 2.4 g (76% yield) of (*S*)-(+)-*N*-decoxycarbonyl-leucine. Other surfactants were synthesized in a similar manner.

3. Results and discussion

The chemical structures of seven test compounds chosen to represent some typical chiral analytes are shown in Fig. 1. The chiral carbon atom is either on a hydrocarbon chain, as in atenolol and propranolol, or within a cyclic structure, as in ketamine and nefopam. The structural variations of the test compounds are likely to result in different stereoselectivities.

(*S*)-*N*-Octoxycarbonyl valine, $(CH_3)_2CHCH(NHCO_2C_8H_{17})CO_2H$ (I), like other chiral surfactants described in this paper, exits as an anionic form in alkaline BGE, and was readily soluble in most basic buffers. Adjusting to acidic condition will make surfactants precipitate thus can be used to

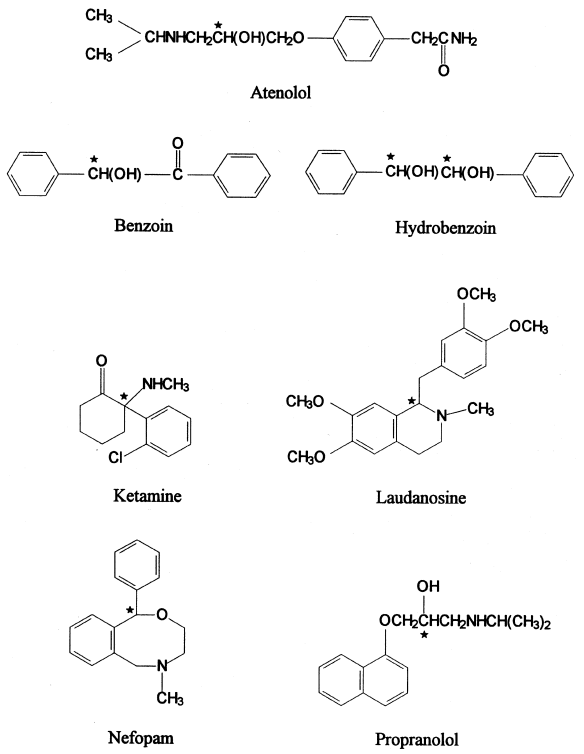

Fig. 1. Structures of seven drugs.

purify these surfactants. In preliminary experiments at pH 8.8 satisfactory separations of several of the chiral test compounds were obtained with this reagent.

3.1. Effect of surfactant concentration

In CD-based chiral separation, the mechanism is quite clear. The chiral nature of the glucose units inside the CD cavity permits the possibility of stereoselective interaction with analytes. Enantiomers which have appropriate structural geometry will fit favorably into CD cavity, resulting in different migration times. Usually, a higher enantiomeric resolution can be obtained by increasing the concentration of CD, due to the formation of stronger inclusion complexes. However, a lower concentration of CD may be needed to obtain a faster and better separation, as Wren [32] indicated that the optimum CD concentration is related to the affinity of the analyte fitting into the CD cavity.

The chiral surfactant (I) has a long hydrocarbon tail, and a chiral functional group which is very close to the charged head. When it forms a micelle, the chiral functional headgroups stick out on the micelle surface, making direct contact with aqueous solution and analytes. The mechanism of chiral discrimination using surfactants is still under investigation. However, it is widely accepted that there may be two steps during the process. First step involves stereoselective interactions between optically active sites on the micelle surface and the chiral groups of analytes. This is followed by inclusion of the preferred enantiomers into the interior hydrophobic core of the micelle. Contrary to CD where chiral interfaces are inside the cavity, chiral surfactants interact with analytes from outside. It is reasonable to assume that some theories which apply well in the CD-case may be inapplicable for chiral surfactants. Nevertheless, these two distinct mechanism may be complementary to each other. For instance, analytes which are too large or too small to fit into the CD cavity may be very well suited for chiral surfactants.

The surfactant concentration plays a very important role in chiral discrimination. When the surfactant concentration is below its critical micelle concentration (CMC), there is little or no separation for enantiomers. This may be due to a less interaction between the surfactant monomer and analytes. More detailed study was done using (*S*)-*N*-octoxycarbonylvaline (I) as a chiral selector. Since the hydrocarbon chains in (I) comprises 12 atoms, which is similar to SDS, the CMC for this chiral surfactant is estimated to be close to 10 m*M* provided the CMC for SDS is 8.3 m*M* [33]. CE was done at three different concentrations of this surfactant, e.g., 25, 50, and 100 m*M*, which are well above its CMC. t_0, which is the migration time of the solute that does not interact with the micelle, was found to be 6.17 min using methanol as the EOF marker; t_{mc} was too large to be measured. A similar situation has been described by Ahuja et al. [34] in which a mixed micella was used to achieve an infinite elution range. Therefore, a t_{mc} value of 4 was used to calculate the capacity factors according to Eq. (1):

$$k' = \frac{t_r - t_0}{t_0\left(1 - \frac{t_r}{t_{mc}}\right)} \quad (1)$$

The separation factors α were calculated as the ratios of the k' values of the two enantiomers. Resolution was calculated as Δt divided by the average peak width. The results are summarized in Table 1.

Atenolol, benzoin, laudanosine and propranolol all showed improved chiral resolution when (*S*)-*N*-octoxycarbonylvaline concentration was increased from 25 m*M* to 50 m*M* (see Fig. 2). Atenolol and benzoin displayed an enhancement in resolution by almost 100% and 200%, respectively. Laudanosine increased by only 7%. Increasing the surfactant concentration from 50 m*M* to 100 m*M* did not result in a large improvement in resolution except for atenolol. Laudanosine and propranolol showed decreases in resolution. These two compounds are among the most hydrophobic molecules in this group. A higher surfactant concentration inevitably gives rise to a higher micelle concentration. Highly hydrophobic compounds do not need chiral recognition to participate into the hydrophobic interior of the micelle, resulting in a decrease in chiral resolution.

3.2. Effect of amino acids on the surfactant structure

Several amino acids were reacted with octyl chloroformate to make chiral surfactants, including L-valine, L-leucine, L-isoleucine, L-alanine, L-aspartic acid, L-asparagine, L-methionine and L-serine. Only (*S*)-*N*-octoxycarbonylvaline (I), (*S*)-*N*-octoxycarbonylleucine (II) and (*S*)-*N*-octoxycarbonylisoleucine (III) are readily soluble in aqueous solution. The other products were either difficult to dissolve in water, or produced a bad baseline in CE.

Figs. 3 and 4 show separations of atenolol, benzoin, landanosine and propranolol with 100 m*M* I and II, respectively. Entiomeric resolutions with 50 m*M* I, II and III are shown in Table 2. Even a minor change in surfactant structure brings a definite change in selectivity. For example, resolution for atenolol was 1.77 using 50 m*M* I but 2.17 using 50 m*M* II. There was no chiral discrimination for ketamine and nefopam when either 50 m*M* I or 50 m*M* III was added in the electrolyte, but these two compounds could be enantiomerically separated by 50 m*M* II. The extra methyl group in leucine may change the chiral functional group geometric structure on the micelle surface, resulting in a more appropriate position on which ketamine and nefopam can interact.

Another type of chiral surfactant which has a –NH(CO)R linkage instead of –NH(CO)OR was also synthesized and compared with the carbamate chiral surfactants. In agreement with Mazzeo et al. [29], the latter type was found to be more effective.

3.3. Effect of surfactant chain length

Chain length of the surfactants was varied by reacting L-leucine with alkyl chloroformates ranging from butyl chloroformate to tetradecyl chloroformate. The same concentration (50 m*M*) of surfactants was used, and results are shown in Table 3. Short chain surfactants, e.g., (*S*)-*N*-butoxycarbonylleucine or hexoxycarbonylleucine, are not very effective for enantioseparation. Micelle formation in the electrolyte appears to be critical in chiral recognition. CMC for short chain surfactants is normally in the range of a couple hundred millimolar [35]. Under

Table 1
Effect of surfactant concentration

Compound	25 m*M* C_8-Valine[a]				50 m*M* C_8-Valine[a]				100 m*M* C_8-Valine[a]			
	t_1	t_2	α	R_s	t_1	t_2	α	R_s	t_1	t_2	α	R_s
Atenolol	5.98	6.02	0.79	0.80	8.55	8.68	1.05	1.77	12.80	13.08	1.04	2.93
Benzoin	8.07	8.10	1.02	0.52	12.77	12.94	1.03	1.65	21.75	22.12	1.02	1.95
Laudanosine	10.35	10.46	1.03	1.06	19.65	19.88	1.02	1.13	32.69	33.02	1.01	1.03
Propranolol	20.72	21.34	1.04	0.97	34.14	34.65	1.02	1.51	51.14	51.71	1.01	1.15
Ketamine	7.88	7.88	1.00	0.00	10.85	10.85	1.00	0.00	13.77	13.77	1.00	0.00
Hydrobenzoin	9.61	9.61	1.00	0.00	12.82	12.82	1.00	0.00	21.99	21.99	1.00	0.00
Nefopam	11.88	11.88	1.00	0.00	24.12	24.12	1.00	0.00	40.59	40.59	1.00	0.00

[a] $t_0 = 6.17$ min, $t_{mc} = 4$.

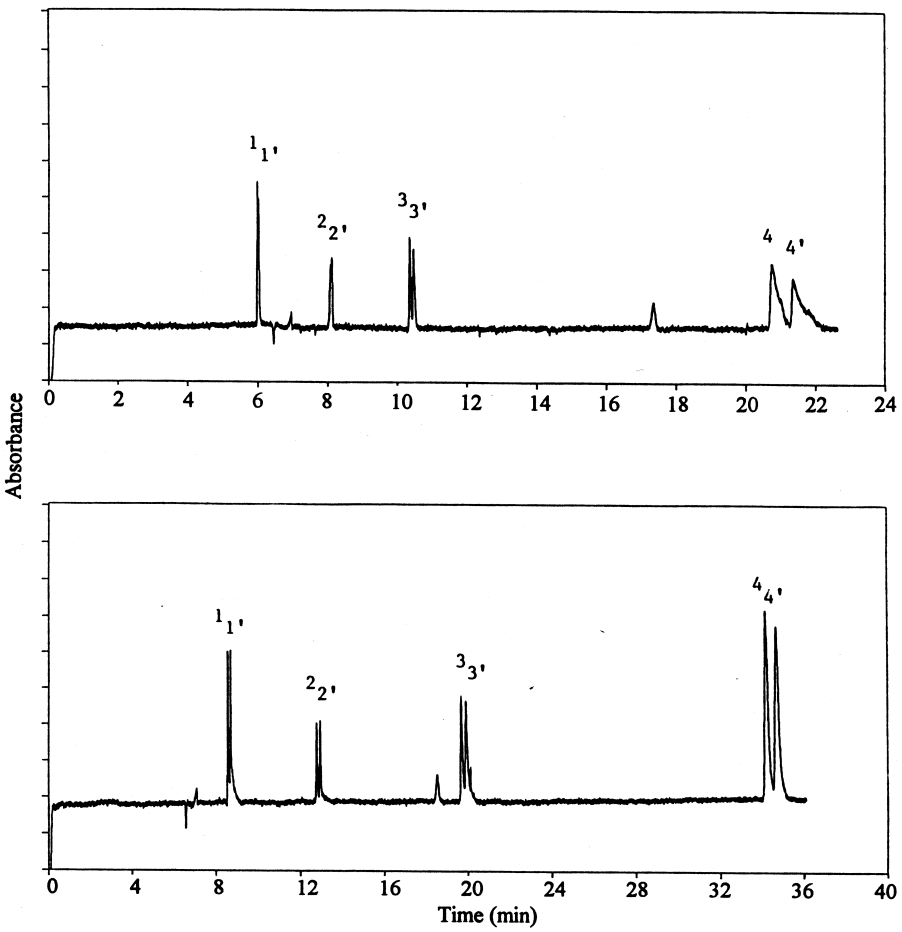

Fig. 2. Enantiomeric separation of four drugs at different surfactant concentration: (A) 25 m*M*; (B) 50 m*M* (*S*)-(+)-*N*-octoxycarbonylvaline. Electrolyte: 25 m*M* borate, 25 m*M* phosphate, pH 8.8; capillary, 60 cm×50 μm I.D.; applied voltage, 18 kV; injection time, 6 s. Peaks: 1=(*S*)-Atenolol; 1′=(*R*)-atenolol; 2=(*S*)-benzoin; 2′=(*R*)-benzoin; 3=(*S*)-laudanosine; 3′=(*R*)-laudanosine; 4=(*S*)-propranolol; 4′=(*R*)-propranolol.

the condition which we investigated (50 m*M*), (*S*)-*N*-butoxycarbonylleucine or hexoxycarbonylleucine existed as monomers. This can explain why there is no chiral resolution in these two surfactants.

(*S*)-*N*-Tetradecoxycarbonylleucine has low a solubility in water, so no data for it are shown in Table 3. There is a decrease in resolution from (*S*)-*N*-decoxycarbonylleucine to dodecoxycarbonylleucine. The hydrophobic interaction between micelle and analyte becomes stronger in longer chain surfactants, and it competes with chiral interaction between analytes and chiral functional headgroups, resulting in a decrease in chiral recognition.

3.4. A mixed system containing sulfated β-CD and chiral surfactant

Sulfated β-CD was first used by Stalcup and Gahm [12] to separate enantiomers at acidic conditions in CE. We used this chiral selector under basic conditions and compared the results with those obtained from using 100 m*M* (*S*)-*N*-octoxycarbonylleucine (see Table 4). Separations were faster with sulfated β-CD and the resolution of benzoin and hydrobenzoin was much better than with C_8-leucine. Apparently the β-CD cavity is well suited for the benzene rings in these compounds. The other test

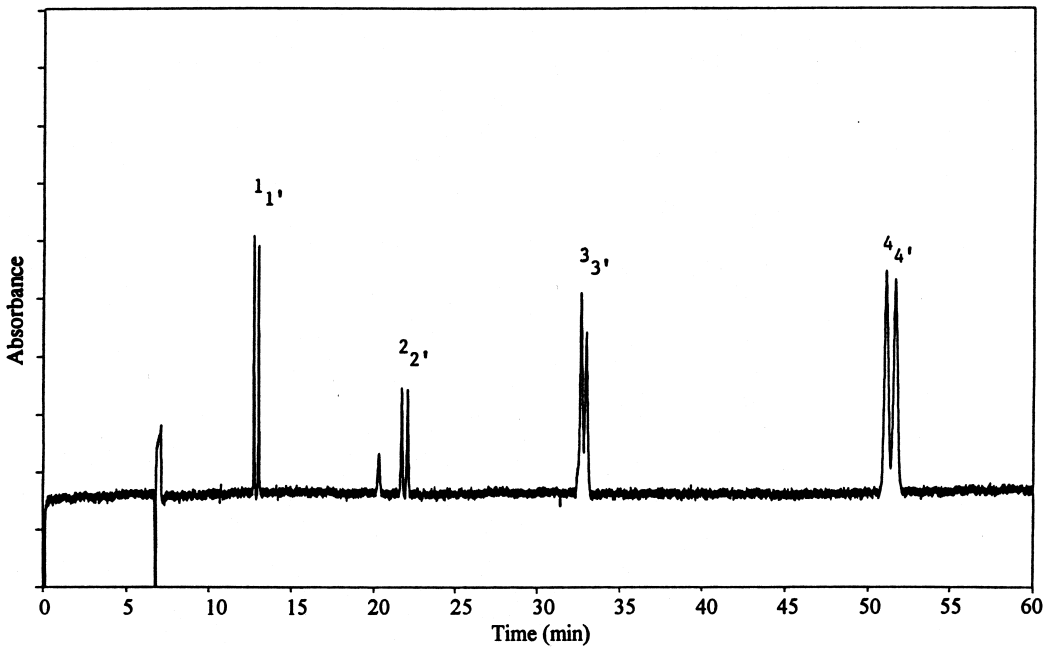

Fig. 3. Enantiomeric separation of drugs using 100 m*M* (*S*)-(+)-*N*-octoxycarbonylvaline. Conditions and peak identities: see Fig. 2.

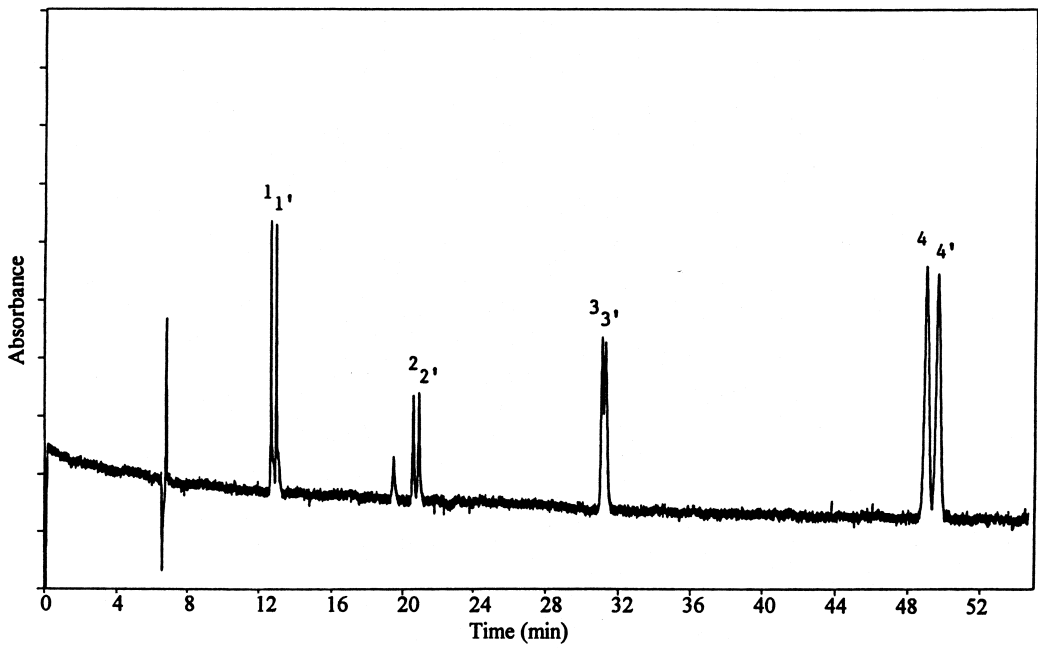

Fig. 4. Enantiomeric separation of drugs using 100 m*M* (*S*)-(+)-*N*-octoxycarbonylleucine. Conditions and peak identities: see Fig. 2.

Table 2
Effect of amino acid structure

Compound	50 m*M* C_8-Valine				50 m*M* C_8-Leucine				50 m*M* C_8-Isoleucine			
	t_1	t_2	α	R_s	t_1	t_2	α	R_s	t_1	t_2	α	R_s
Atenolol	8.55	8.68	1.05	1.77	8.94	9.10	1.06	2.17	14.94	15.20	1.03	1.93
Benzoin	12.77	12.94	1.03	1.65	12.79	12.95	1.02	1.56	20.54	20.81	1.02	1.59
Laudanosine	19.65	19.88	1.02	1.13	19.60	19.84	1.01	0.85	32.65	33.33	1.03	1.79
Propranolol	34.14	34.65	1.02	1.51	32.16	32.64	1.02	1.55	50.39	50.85	1.01	0.95
Ketamine	10.85	10.85	1.00	0.00	11.69	12.20	1.09	0.71	22.96	22.96	1.00	0.00
Hydrobenzoin	12.82	12.82	1.00	0.00	10.48	10.48	1.00	0.00	17.50	17.50	1.00	0.00
Nefopam	24.12	24.12	1.00	0.00	23.02	23.20	1.01	0.79	43.60	43.60	1.00	0.00

Table 3
Effect of surfactant chain length on chiral resolution

Compound	Resolution (R_s)			
	50 m*M* C_6-Leucine	50 m*M* C_8-Leucine	50 m*M* C_{10}-Leucine	50 m*M* C_{12}-Leucine
Atenolol	0.00	2.17	2.36	1.80
Benzoin	0.00	1.56	1.75	1.04
Laudanosine	0.00	0.85	0.59	0.00
Propranolol	0.00	1.55	0.39	0.00
Ketamine	0.00	0.71	1.33	0.92
Hydrobenzoin	0.00	0.00	0.86	1.00
Nefopam	0.00	0.79	0.29	0.10

compounds showed better resolution with the C_8-leucine additive.

It is still difficult to predict which chiral compound can be separated by which chiral selector. We therefore tried a mixture of sulfated β-CD and (*S*)-*N*-octoxycarbonylleucine (II) which have different structures and may have different separation mechanisms. The results are shown in Table 4. Because of the high current encountered, lower concentrations of sulfated β-CD and (*S*)-*N*-octoxycarbonylleucine were utilized. However, it is enough to illustrate the idea. Chiral resolutions are greatly improved by using this mixture of selectors, and most of them are even better than just a simple adding-effect. The enhancement in chiral resolution and enantioselectivity can be explained by the prolongation of the analyte interaction time with anionic CD and chiral micelles. Enantioselectivity is boosted by multiple stereoselective interactions with both CDs and chiral micellar surface.

This concept was demonstrated further by another application. An attempted separation of 12 DL-dansyl amino acids in alkaline solution with 10 m*M* sulfated β-CE gave almost no resolution. An electropherog-

Table 4
Comparison single surfactant with mixed surfactants

Compound	Resolution (R_s)		
	100 m*M* C_8-Leucine	25 m*M* β-CD Sulfate	10 m*M* β-CD Sulfate–50 m*M* C_8-leucine
Atenolol	2.93	0.92	2.34
Benzoin	1.75	5.21	2.61
Laudanosine	0.59	0.45	0.53
Propranolol	1.35	1.31	3.05
Ketamine	0.43	0.00	0.81
Hydrobenzoin	0.91	2.75	2.05
Nefopam	0.58	N/A	8.03

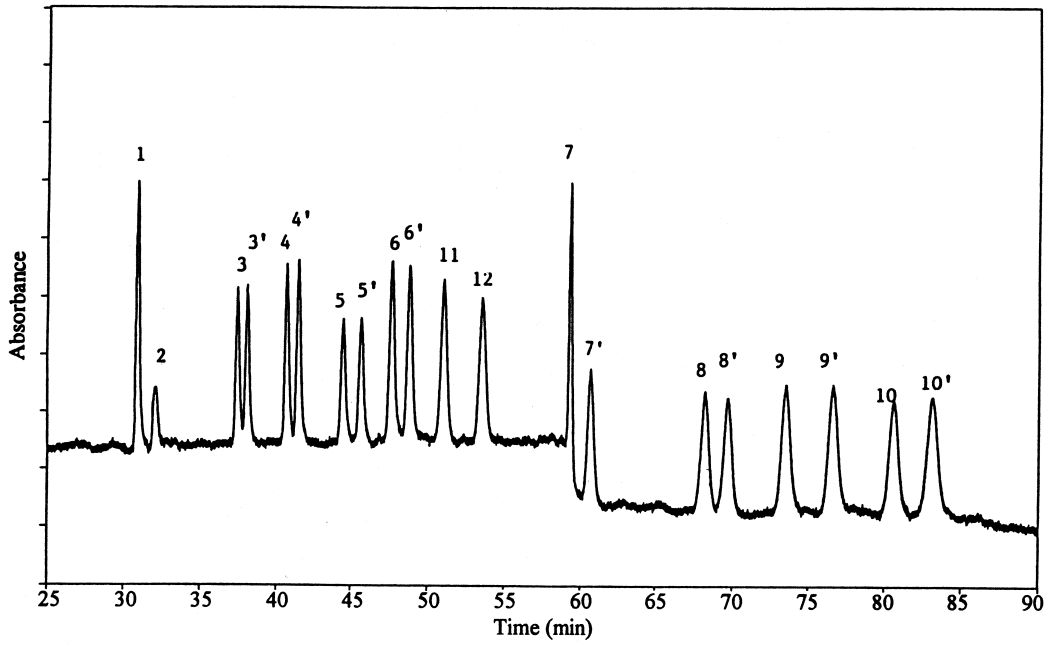

Fig. 5. Electropherogram of dansyl amino acids using 100 m*M* (*S*)-(+)-*N*-octoxycarbonylleucine and 10 m*M* β-CD sulfate. Conditions: as in Fig. 2 except applied voltage is 12 kV. Peaks: 1=Threonine; 2=serine; 3=(*S*)-α-amino-*n*-butyric acid; 3′=(*R*)-α-amino-*n*-butyric acid; 4=(*S*)-valine; 4′=(*R*)-valine; 5=(*S*)-methionine; 5′=(*R*)-methionine; 6=(*S*)-norvaline; 6′=(*R*)-norvaline; 7=(*S*)-leucine; 7′=(*R*)-leucine; 8=(*S*)-tryptophan; 8′=(*R*)-tryptophan, 9=(*S*)-phenylalanine; 9′=(*R*)-phenylalanine; 10=(*S*)-norleucine; 10′=(*R*)-norleucine; 11=glutamic acid; 12=aspartic acid.

ram with 50 m*M* (*S*)-(+)-*N*-octoxycarbonylleucine gave peaks for the individual amino acids but almost no resolution of the optical isomers. Partial resolution of optical isomers was obtained with 10 m*M* sulfated β-CD plus 50 m*M* (*S*)-(+)-*N*-octoxycarbonylleucine in 19.5 min while baseline resolution of most of the isomers was obtained with 10 m*M* β-CD and 100 m*M* of the C_8-leucine additive (see Fig. 5).

4. Conclusions

The selectors probably form a micelle when added to the BGE in CE with chiral recognition depending on interaction of the analytes with the chiral part of the selector which is near the outside of the micelle. Varying the type of amino acid, the chain length of the R group and the concentration of the reagent are effective ways to manipulate the chiral selectivity. Even a small change in the amino acid, as in valine and leucine, can have a major effect on chiral selectivity. A chain length of C_8 or C_{10} was found to be the most effective.

Sulfated β-CD was compared to our synthetic chiral additives, and it showed different selectivities resulting form its unique chiral recognition mechanism. A combination of these two different additives was applied to enantiomeric compounds, including the seven test drugs and dansyl amino acids. Better results were obtained using this duo-chiral system than either one alone, and chiral resolution in most cases was largely enhanced. This new system provides a fast and effective way for chiral method development and preliminary studies.

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