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Enantiomeric differentiation of a wide range of pharmacologically active substances by capillary electrophoresis using modified β-cyclodextrins

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Abstract

This paper shows the versatility of modified charged and uncharged β-cyclodextrins (CDs) in the direct chiral resolution of β-agonists, β-antagonists, phenylethyamines and alcohol stimulants, and thalidomide and its metabolites. A total of 42 compounds were optically resolved using hydroxypropyl-β-CD and 20 with sodium sulfobutyl ether-β-CD. The degree of enantiomeric separation for most substances is dependent on the modified CD concentration. The separation efficiency reaches a maximum at a particular CD concentration. The separation efficiency reaches a maximum at a particular CD concentration, after which further increases in CD concentration causes a progressive decrease in chiral differentiation. Chiral separation of amphetamine enantiomers indicated that a three-point hydrogen bond interaction between the chiral guest molecule and host CD is not necessary for chiral separation under the conditions used.

1. Introduction

A large number of pharmaceutical drugs contain one or more chiral atoms and can exist in two or more isomeric forms [1]. In most instances, only one of the isomeric forms is highly active therapeutically [1]. The other form can be either much less active, inactive or sometimes even toxic. An example is the drug (*R*,*S*)-thalidomide, which was administered during early pregnancy to alleviate symptoms of morning sickness, with its *R*-enantiomer exhibiting strong activity as a minor tranquillizer, and yet the *S*-enantiomer was found to be toxic [2,3]. More recently, (*R*,*S*)-thalidomide has also been found to suppress the HIV virus, alleviating the symptoms of mass loss, fever and skin lesions associated with HIV [2,3]. The drug regulatory agencies in many countries have expressed an interest in investigations of the stereoisomeric composition of drugs and their associated therapeutic and toxicological consequences. In general, only the highly active isomers are used in the production of drugs [1,4]. Hence there is a real need to develop rapid and sensitive chiral separation methods required in the investigation of drug enantiomer composition.

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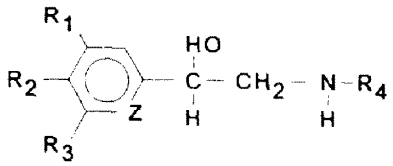
Two common chiral separation techniques used to resolve optically active drugs are gas chromatography (GC) [5] and high-performance liquid chromatography (HPLC) [6]. In the last decade, capillary electrophoresis (CE) has developed into a powerful technique for the separation of small ions, peptides, carbohydrates, oligonucleotides, pharmaceutical drugs and more recently chiral substances [7]. The separation principle of CE is based on the different electrophoretic mobilities of solutes which, in turn, depend on charge densities [8]. Also, cyclodextrin (CD) inclusion complexes can be used for the optical resolution of stereoisomers by CE based on the difference in electrophoretic mobility of the complexes arising from different inclusion formation constants with the analyte [9].

We are interested in the separation and identification of several phenylamine optical isomers of forensic significance, including amphetamines and ephedrines. Amphetamines are illicit drug substances, while ephedrine enantiomers are widely used in a large number of proprietary cough and decongestant remedies. A variety of amphetamine enantiomers possess different pharmacological activities [10]. For example, (*S*)-amphetamine gives greater locomotor stimulation [11] and hyperthermic activity [12] than the *R*-enantiomer [11]. Qualitative analysis of these optical isomers will aid in identifying the synthetic route and may provide additional information of the drug seizer origins as reflected by the impurity profile. We are also interested in the separation and identification of some adrenergic β-receptor agonists (abbreviated as β-agonists here) and β-receptor antagonists (abbreviated as β-antagonists), drugs which are structurally similar to phenylethylamines. In general, β-agonists are used for the relief of reversible bronchospasm in humans with obstructive airway diseases such as asthma, bronchitis and emphysema. On the other hand, β-antagonists are adrenergic β-receptor blocking agents used in the treatment of hypertension and angina. The *S*-enantiomers of many β-antagonist drugs have been demonstrated to be more biologically active than the *R*-enantiomers [13,14]. Pharmacokinetic studies have also shown differences in the adsorption, metabolism and elimination kinetics for the enantiomers of β-antagonists [13,14]. All of these drugs are expressively banned by the

Table 1
Structures of the phenylethyl alcohols studied.



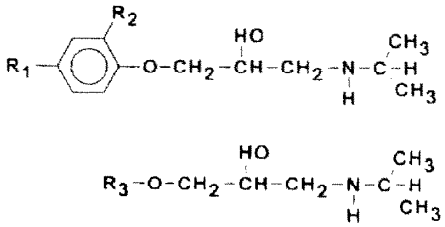
Compound	R_1	R_2	R_3	R_4	Z
Pirbuterol	H	HO	CH_2OH	$C(CH_3)_3$	NH
Salbutamol	H	HO	CH_2OH	$C(CH_3)_3$	C
Cimaterol	H	NH_2	HO	$C(CH_3)_3$	C
Clenbuterol	Cl	NH_2	Cl	$C(CH_3)_3$	C
Terbutaline	HO	H	HO	$C(CH_3)_3$	C
Labetalol	H	HO	H_2NCO	$-CH(CH_3)(CH_2)_2-C_6H_5$	C
Epinephrine	HO	HO	H	CH_3	C

International Olympic Committee [15] because they can be used as doping agents to reduce sympathetic activity in cases when high psychological pressure may impair athletic performance [16].

There have been reports on the use of CDs to separate enantiomers of hydroxyphenylamines [13,14,16–28], β-agonists [27–30] and β-antagonists [20,21,23,29,31–37] by CE. In these chiral CE methods, low-pH buffers with various concentrations of β-CDs (up to 20 m*M*) [7,13,17,18,20,30], and modified β-CDs (up to 40 m*M*) [14,16,19,21–23,26–29,31] were used, and dimethyl-β-CD has been demonstrated to resolve optically the largest number of chiral compounds [14,16,19,26,29,31]. However, to date, no direct chromatographic separation method for the simultaneous separation of phenylethylamines and alcohols enantiomers has been reported.

This paper reports a CE separation method using the ether derivatives of β-CD to resolve a wide range of pharmacologically active drugs including β-agonists, β-antagonists (shown in Tables 1 and 2), phenylethylamines and alcohol stimulants (shown in Table 3) and thalidomide and its metabolites (shown in Fig. 1). The development of such a technique will then per-

Table 2
Structures of the modified phenylethyl alcohols studied



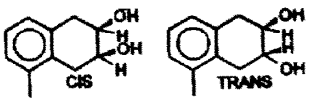
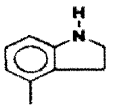
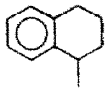
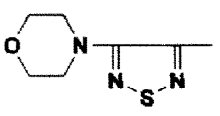
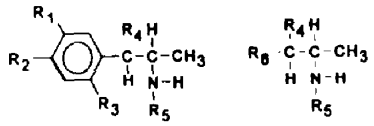
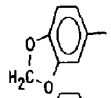
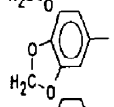
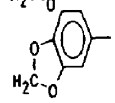
Compound	R_1	R_2	R_3
Nadolol	–	–	
Oxprenolol	H	$OCH_2CH{=}CH_2$	–
Pindolol	–	–	
Atenolol	H_2NCOCH_2	H	–
Alprenolol	H	$CH_2CH{=}CH_2$	–
Propranolol	–	–	
Metoprolol	$CH_3OCH_2CH_2$	H	–
Acebutolol	$CH_3(CH_2)_2CONH$	$COCH_3$	–
Timolol	–	–	

Table 3
Structures of the phenylethylamines studied



Compound	R_1	R_2	R_3	R_4	R_5	R_6
2,5-Dimethoxy-4-methyl amphetamine	CH_3O	CH_3	CH_3O	H	H	–
Ephedrine	H	H	H	HO	CH_3	–
Pseudoephedrine	H	H	H	HO	CH_3	–
Norephedrine	H	H	H	HO	H	–
Amphetamine	H	H	H	H	H	–
Methylamphetamine	H	H	H	H	CH_3	–
4-Bromo-2,5-dimethoxy amphetamine	CH_3O	Br	CH_3O	H	H	–
Methyldimethoxy amphetamine	–	–	–	H	H	
Methyldimethoxymethyl amphetamine	–	–	–	H	CH_3	
Methyldimethoxyethyl amphetamine	–	–	–	H	CH_2CH_3	

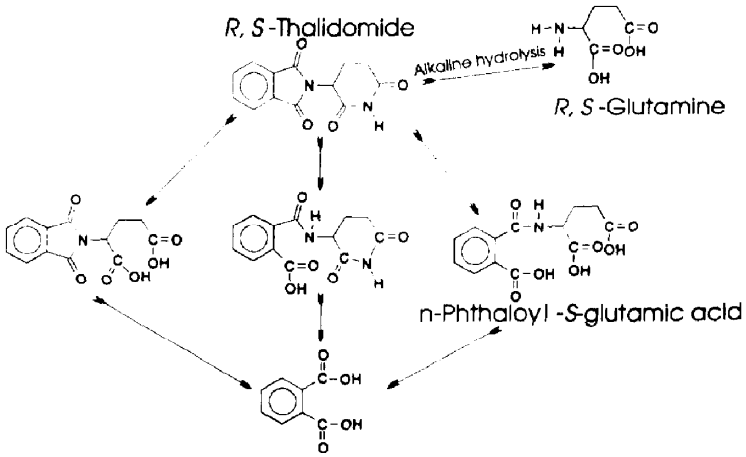


Fig. 1. Major metabolic pathway of thalidomide. Compounds named were resolved in this work.

mit a study of the pharmacokinetic effects of a wide range of optically active drugs directly using a simple chromatographic technique.

2. Experimental

2.1. Reagents

(*R,S*)-Clenbuterol, (*R,S*)-terbutaline, (*R,S*)-cimaterol, (*R,S*)-salbutamol, (*R,S*)-pirbuterol, (*R,S*)-atenolol, (*R,S*)-nadolol, (*R,S*)-propranolol, (*R,S*)-timolol, (*R,S*)-oxprenolol, (*R,S*)-pindolol, (*R,S*)-alprenolol, (*R,S*)-labetalol, (*R,S*)-acebutolol, (*R,S*)-metoprolol, (*R,S*)-amphetamine, (*R*)-amphetamine, (*S*)-amphetamine, (*R*)-methylamphetamine, (*S*)-methylamphetamine, (*R,S*)-methyldimethoxyamphetamine, (*R,S*)-methyldimethoxymethylamphetamine, (*R,S*)-methyldimethoxyethylamphetamine, (*R,S*)-2,5 dimethoxy-4-methylamphetamine, (*R,S*)-4 bromo-2,5-dimethoxyamphetamine, (*R,S*)-norephedrine, (*S*)-ephedrine, (*R*)-ephedrine, (*S*)-pseudoephedrine, (*R*)-pseudoephedrine, (*R,S*)-thalidomide, (*R*)-thalidomide and (*S*)-thalidomide, were supplied by the Curator of Standards, Australian Government Analytical Laboratories (Pymble, NSW, Australia). Hydroxypropyl-β-cyclodextrin (average molar hydroxypropyl group substitution 0.6 and 0.8), (*R*)-propranolol, (*S*)-propranolol, (*R,S*)-epinephrine, (*S*)-epinephrine and (*R,S*)-glutamine were supplied by Sigma (St. Louis, MO, USA). (*R*)-Atenolol, (*S*)-atenolol, (*S*)-alprenolol-(*S*)-tartrate and N-phthaloyl-(*S*)-glutamic acid were supplied by Aldrich. Sodium IV sulfobutyl ether-β-cyclodextrin was supplied by Isco (Lincoln, NE, USA). All other chemicals and solvents were of analytical-reagent or HPLC grade and were used as received.

2.2. Preparation of buffers

A pH 2.5 stock buffer solution of 232 m*M* citric acid–44.7 m*M* disodium hydrogenphosphate was used. The running buffer with 100 m*M* citric acid–19.27 m*M* disodium hydrogenphosphate was prepared using the appropriate volume of stock buffer and mass of modified β-CD. The resulting running buffer was degassed by sonication and filtered through a 0.2-mm PTFE filter (Micro Filtration Systems, Dublin, CA, USA) before use.

2.3. Apparatus

Qualitative work was performed with fused-silica capillary tubes (Isco) (100 cm × 50 μm I.D.) with an effective length of 50 cm to the detector window. An Isco Model 3140 electropherograph was used for all analyses. The instrument was operated at 30 kV and thermostated at 23°C with the detector placed on the cathode side. The sample solution was loaded into the capillary under vacuum (vacuum level 4.0 kPa/s for Isco 3140 electropherograph). The compounds were detected at 200 nm and 0.01 AUFS. Electropherograms were recorded and processed with the ICE data management and control software supplied with the Model 3140 electropherograph.

2.4. Procedure for capillary preparation and handling

Prior to extended use, the capillary was filled with 1 *M* sodium hydroxide solution and allowed to stand for 1 h. This solution was replaced with 0.1 *M* sodium hydroxide solution, then the capillary was allowed to stand for a further 1 h and washed with deionized water before filling with the running buffer (between runs the capillary was flushed with 10 μl of running buffer). The capillary was used for a maximum of 40 sample injections before rinsing with 1 *M* sodium hydroxide solution (200 μl), deionized water (200 μl) and running buffer (200 μl); it was then left filled with running buffer ready for sample injection.

The resolution was calculated via the relationship [40]

$$R_s = 2\Delta T/(W_1 + W_2) \quad (1)$$

where ΔT is the difference in migration time between enantiomers and W_1 and W_2 are the widths of the peaks at the baseline.

3. Results and Discussion

3.1. CE separation with hydroxypropyl-β-cyclodextrin (HP-β-CD)

The β-CD water solubility and enantioselectivity can be increased by chemically modifying the CD with alkyl substituents [29,38–42] which increase the flexibility of the CD cavity [39,40]. The orientation of bulky and/or charged substituents on the CD cavity can decrease the enantiosclectivity by causing steric hindrance and/or columbic repulsion, preventing guest–host inclusion complex formation [39,43]. HP-β-CD used in this work is an alkyl-modified β-CD and is generally available in tow grades varying in the molar proportion of hydroxypropyl group substitution on the β-CD (Ave. MS) of 0.6 and 0.8. The physical characteristics of HP-β-CD appear to make it an attractive chiral additive in CE in three respects: (1) it exhibits potentially similar steric restrictions to the CD cavity as alkylated-β-CDs [39,43], resulting in a similar guest–host complex formation; (2) it is highly water soluble [44], in fact well in excess of the parent β-CD; and (3) each grade is a complex cocktail of related substances varying in both the position and the number of hydroxy propyl groups attached [39,43].

HP-β-CD is a complex mixture. where one of these CDs might form guest–host complexes with a certain racemate that approaches the optimum for separation by CE whereas another might be more suitable in the CE separation of another racemate. Further, the high water solubility of the mixed HP-β-CDs enables those CD derivatives which give optimum separation of certain racemates to achieve a sufficiently high concentration in solution to effect that separation. This same argument can be applied to dimethyl-β-CD, explaining the wider range of structural types optically resolved by CE when compared with β-CD. We therefore investigated the use of HP-β-CD Ave. MS 0.6 and 0.8 in the CE separation of a variety of drug racemates.

The drugs studied here are categorized into three main classes (1) β-agonists, amphetamines, ephedrines; (2) β-antagonists; and (3) thalidomide and metabolites. The general structures of classes 1 and 2 are shown in Tables 1–3 and class 3 in Fig. 1. Note that all the drugs studied in this work have a common feature of mono- or bicyclic aromatic rings in their structures.

In order to select the CD and concentration that optically resolve the greatest number of enantiomers, β-agonists, β-antagonists, amphetamines and ephedrines were analysed with various amounts of HP-β-CD Ave. MS 0.6 and 0.8. HP-β-CD Ave. MS 0.6 and 0.8 were added separately to the background electrolyte (100 m*M* citric acid–19.27 m*M* Na_2HPO_4, pH 2.5), by increasing the amount of the chiral additive. The effect of HP-β-CD Ave. MS 0.8 and 0.6 concentration on the migration time of clenbuterol, terbutaline, cimaterol and salbutamol is illustrated in Fig. 2a and b, respectively. An increase in HP-β-CD Ave. MS 0.8 and 0.6 concentration increases not only the buffer viscosity but also the solute migration time. This is more evident when HP-β-CD Ave. MS 0.8 is used as a chiral additive. The optical isomers of clenbuterol and terbutaline co-elute when HP-β-CD Ave. MS 0.6 is used as a chiral additive, but are all well resolved with HP-β-CD Ave. MS 0.8. The distribution and size of the substituent are important to the CD complexation, as the most chemically reactive primary hydroxyls (C_6) are located around the smaller opening of the CD, and the less reactive and optically active secondary hydroxyl (C_2, C_3) are located around the larger opening [43], as illustrated in Fig. 3. Optical selectivity can only be promoted by the juxtaposition with the optically active group or through the stereogenic centre (involving the optically active group indirectly) [45]. The CD complex formed was reported to increase monotonically as a function of the CD concentration [46]. Plots of resolution between each pair of optical isomers (R_s) as a function of CD concentration are shown for a number of β-agonists, β-antagonists, amphetamines and ephedrines in Fig. 4a,b and c. R_s for propranolol, labetalol, clenbuterol, terbutaline, salbutamol, alprenolol, pirbuterol, amphetamine, cimaterol, atenolol, oxprenolol, pindolol, epinephrine and methyl-

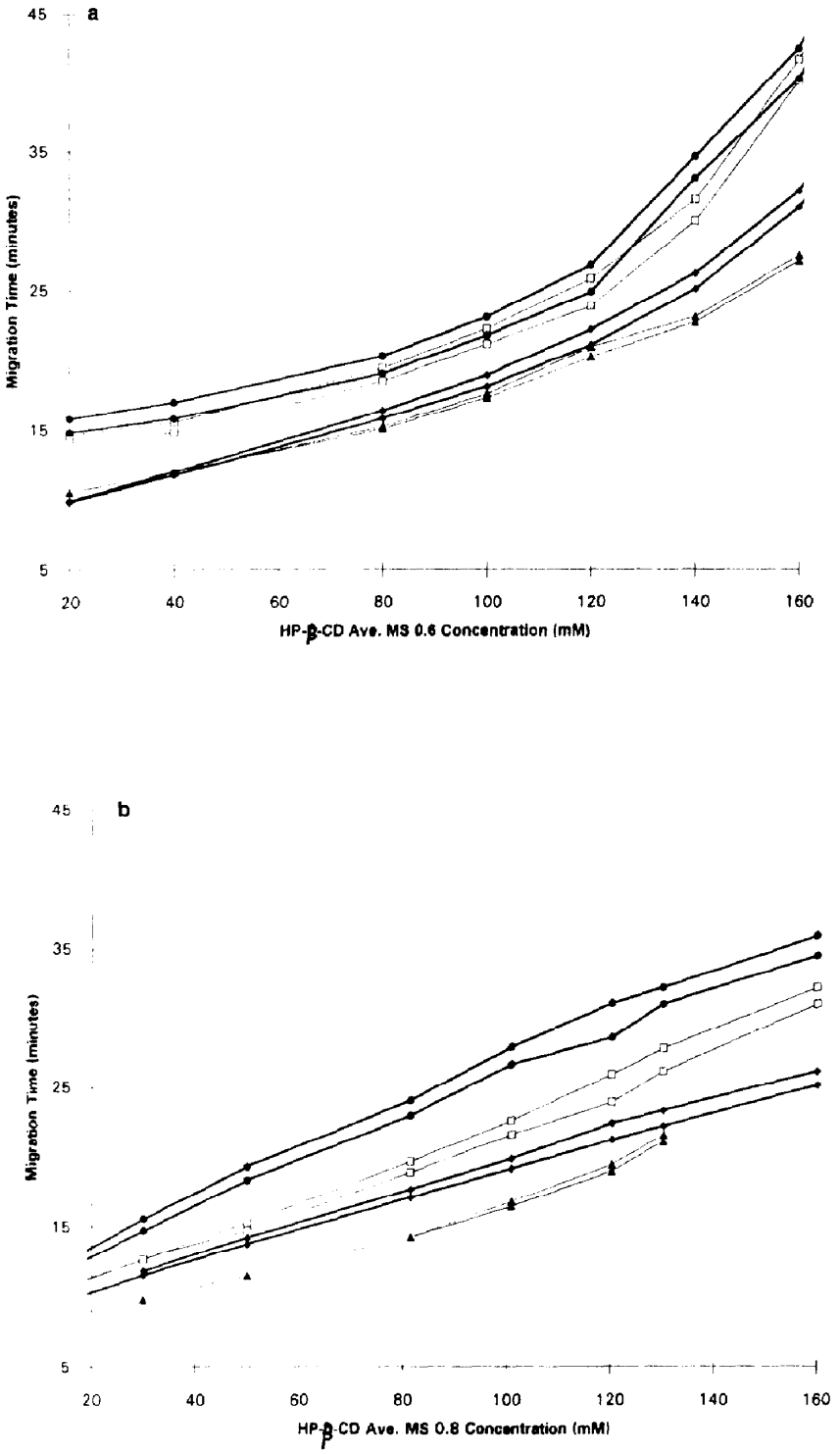


Fig. 2. Plots of β-agonist and β-antagonist migration times versus (a) concentration of HP-β-CD Ave. MS 0.6 and (b) concentration of HP-β-CD Ave. MS 0.8. □ = Clenbuterol; ● = terbutaline; ◆ = cimaterol; ▲ = salbutamol.

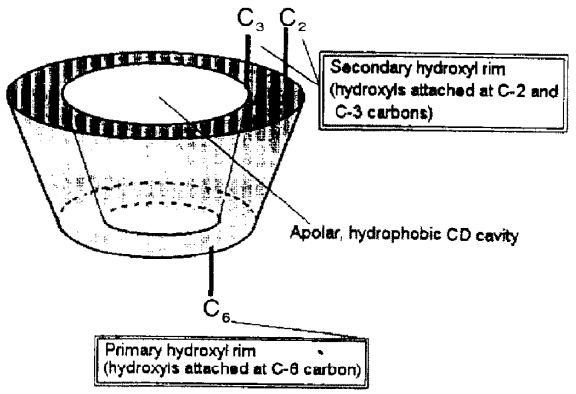


Fig. 3. Representation of "natural" cyclodextrin showing primary and secondary hydroxyl rims forming cyclodextrin complex.

amphetamine increased with HP-β-CD Ave. MS 0.6 and 0.8 concentration, showing an R_s maximum at 120 m*M*, after which R_s decreased with increasing CD concentration (Fig. 4) R_s for methyldioxyamphetamine increased proportionally with HP-β-CD Ave. MS 0.6 concentration, showing no R_s maximum. The HP-β-CD concentration that gave the largest R_s for the β-agonists, β-antagonists, amphetamines and ephedrines studied was 120 m*M* for both HP-β-CD Ave. MS 0.6 and 0.8 (Fig. 4). HP-β-CD Ave. MS 0.8 and 0.6 produced separations with ca. $3 \cdot 10^5$ theoretical plates. Both 120 m*M* HP-β-CD Ave. MS 0.8 and 0.6 resolved equal numbers of compounds, but HP-β-CD Ave. MS 0.8 gave less co-migrations, as shown in Fig. 2a and b.

3.2. Multi-component chiral separation of β-Agonists and β-Antagonists

Fig. 5 shows an electropherogram of twelve racemates of β-agonists and β-antagonists optically resolved in less than 45 min with the order of migration indicated. Note that the optical

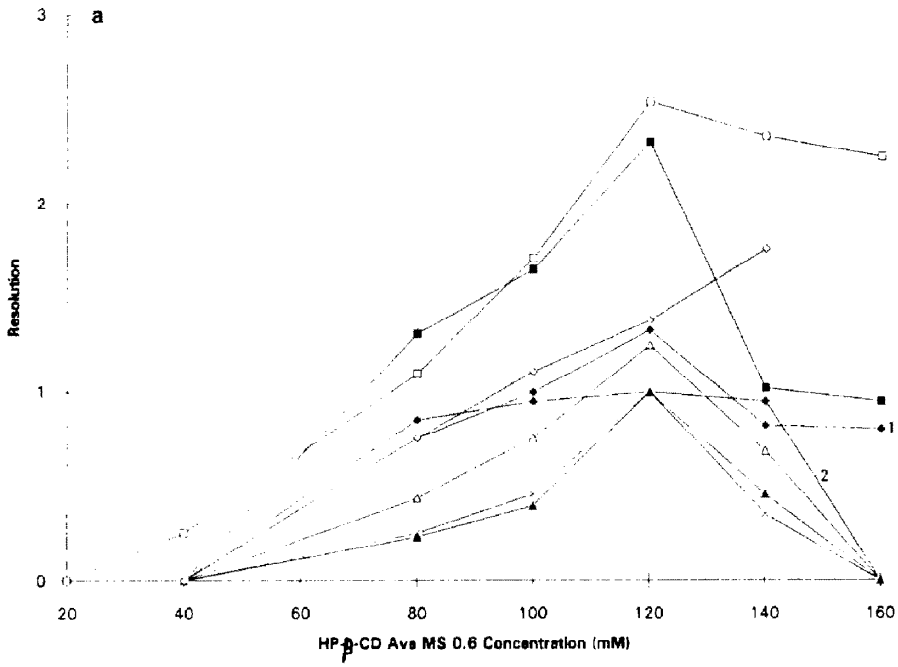
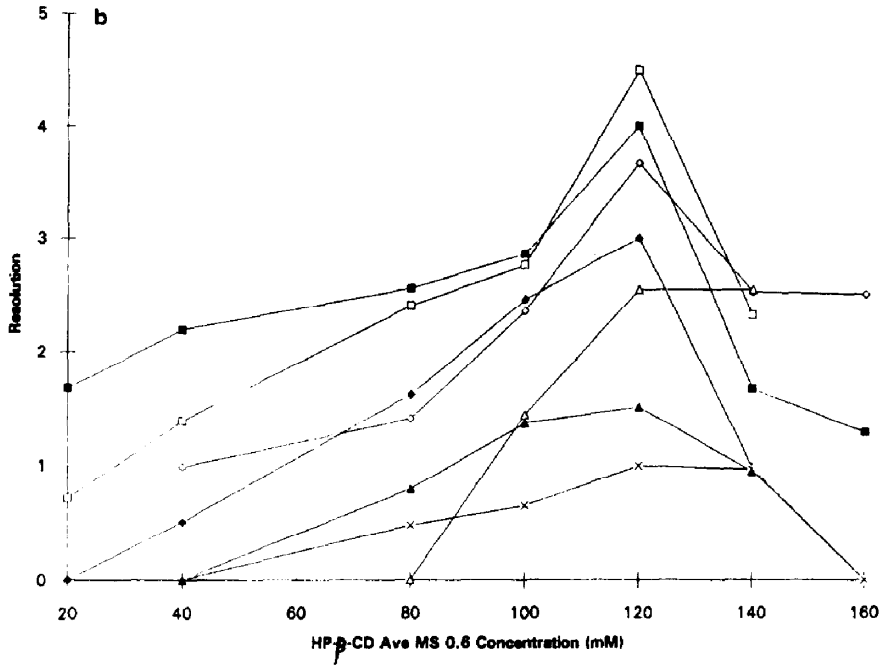


Fig. 4. Resolution of β-agonists, β-antagonists, phenylethylamines and alcohols (ΔT) versus (a) and (b) concentration of HP-β-CD Ave. MS 0.6 and (c) concentration of HP-β-CD Ave. Ms 0.8. (a) △ = Atenolol; ◆ (1) = oxprenolol; ◆ (2) = propranolol; ■ = pindolol; × = alprenolol; ▲ = labetalol; ◇ = methyldimethoxyamphetamine; □ = epinephrine. (b) □ = Clenbuterol; ■ = terbutaline; ◇ = cimaterol; ◆ = salbutamol; × = pirbuterol; △ = amphetamine; ▲ = methylamphetamine; (c) ■ = Clenbuterol; ◇ = terbutaline; □ = cimaterol; △ = salbutamol; ▲ = pirbuterol; ◆ = methylamphetamine; × = amphetamine.



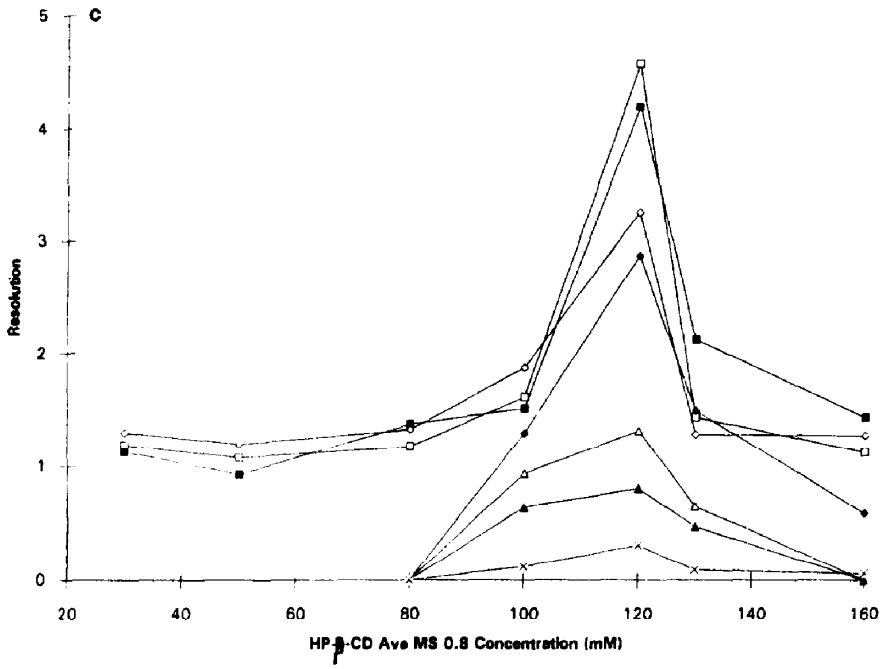


Fig. 4 *(continued)*.

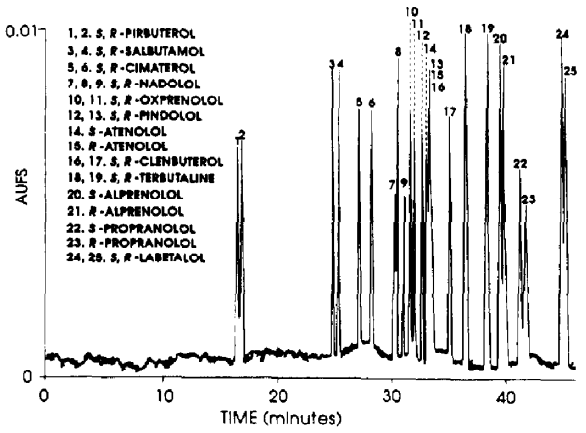


Fig. 5. Electropherogram of phenylamine alcohols optically resolved using 120 m*M* HP β CD Ave. Ms 0.8 (each compound at 600 μg/ml).

migration order is only given for optically pure isomers obtained. (*R*)-Atenolol was observed to co-elute with one of the optical isomers of pindolol and clenbuterol. This is attributed to the large number of structurally related compounds simultaneously resolved into closely migrating bands, depleting the available CD concentration which decreases the resolution [47]. Also, high local solute conductivity can be produced by closely migrating bands causing electrodispersion, which can decrease resolution. This is reflected by larger R_s values for each injected compound compared with a mixture. Peaks for acebutolol and timolol are not shown in Fig. 5 as they were not optically resolved and also metoprolol was only poorly resolved optically. (*R*,*S*)-Nadolol standard appears to contain a mixture of (*R*,*S*)-*cis*- and -*trans*-nadol, as shown in Table 2. With a racemic mixture of (*R*,*S*)-*cis*- and -*trans*-nadolol, an equal concentration of the four isomers exists. A more stable inclusion complex with HP-β-CD will form with the "*cis*" configuration, because of the planar symmetry of the two hydroxy groups in comparison with the "*trans*" conformation [48]. This would be reflected by a large migration time difference between *cis*- and *trans*-nadolol isomers. The existence of three peaks having a peak-height ratio of 1:2:1 shown in Fig. 5 may account for two of the optical isomers being resolved and the other two co-eluting under one peak. Unfortunately, a pure standard of *cis*-nadolol and *trans*-nadolol could not be found.

3.3. Chiral resolution of amphetamines

Fig. 6 shows an electropherogram of eight racemates of phenylethylamines and alcohols optically resolved in less than 45 min with the order of migration indicated. Note that the optical migration order is only given for optically pure isomers obtained. 2,5-Dimethoxy-4-methylamphetamine and 4-bromo-2,5-dimethoxyamphetamine were not optically resolved. 4-Bromo-2,5-dimethoxyamphetamine co-elutes with (*S*)-methylamphetamine and (*R*)-epinephrine with (*S*)-ephedrine (Fig. 6). Peaks for norephedrine are not shown in Fig. 6 as it was not optically resolved.

The optically resolved phenylethylamines migrated in the order of increasing molecular mass within homologous series.

3.4. Chiral resolution limits for HP-β-CD

There are a few compounds that failed to be optically resolved with HP-β-CD, including acebutolol, 2,5-dimethoxy-4-methylamphetamine, 4-bromo-2,5-dimethoxyamphetamine,

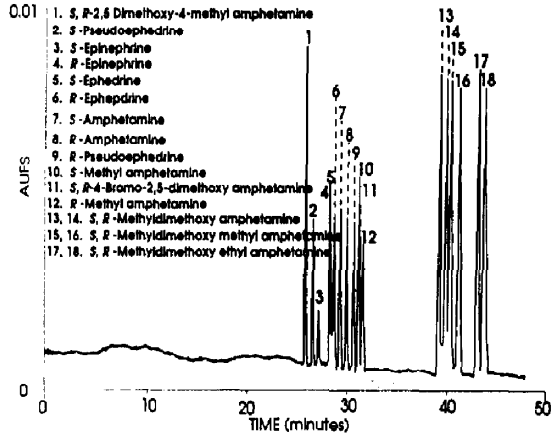


Fig. 6. Electropherogram of phenylamines optically resolved using 120 m*M* HP-β-CD Ave. Ms 0.8 (each compound at 600 μg/ml).

timolol and norephedrine. Chiral resolution for acebutolol and 2,5-dimethoxy-4-methylamphetamine, 4-bromo-2,5-dimethoxyamphetamine appears to be impaired by the protruding aromatic substituent (see Tables 2 and 3). This prevents the aromatic ring from penetrating sufficiently into the CD for hydrogen bonding to occur with the stereogenic centre. Conversely, optical resolution is impaired with timolol by the large distance between the stereogenic centre and hydrogen bonding groups on the CD. A broad peak was obtained with norephedrine. This was attributed to the small difference in hydrogen bond strength between the hydroxyl and primary amine groups in norephedrine and the CD. The difference in hydrogen bond strength between the hydroxyl and primary amine groups in norephedrine and the CD is decreased by substituting a methyl group on the primary amine group, producing a secondary amine (ephedrine). Ephedrine with a hydroxy group and secondary amine group was optically resolved with HP-β-CD, as shown by Fig. 6. Norephedrine is optically resolved with only methyl-substituted β-CD [13,14,16–18, 20,24,26,27]. These results show that the position and type of CD alkyl derivative affect the formation of the guest–host complex.

3.5. Solute cyclodextrin interactions

The optical isomers of β-agonists, β-antagonists, ephedrines and amphetamines resolved with HP-β-CD were observed to elute with the *S*-form first, followed by the *R*-form, suggesting a common mechanism operative with the sixteen optically pure isomers studied. In order to obtain a direct separation with CD, it is necessary for three different interactions to take place between the enantiomer and the CD, as proposed in the three-point interaction model of Dalgliesh [48]. The three points of contact proposed as being necessary for enantioselectivity to occur for β-agonists, β-antagonists and hydroxyphenylamines are illustrated in Fig. 7A. The aromatic portion (hydrophobic end) of the molecule is likely to fit into the CD cavity (Fig. 7a, point 1) [45]. The molecule requires a hydrogen bonding site near the chiral centre for hydrogen bonding

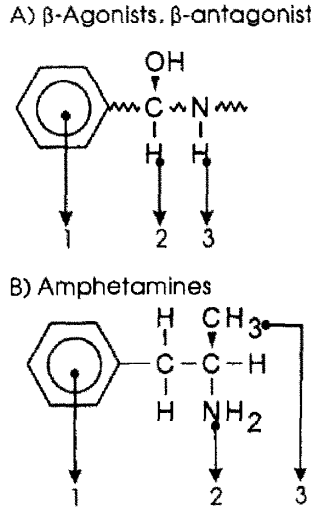


Fig. 7. The three points of CD interaction with (A) β-agonists, β-antagonist and ephedrines, and (B) amphetamines.

with the CD, and the chiral centre is close enough to the CD activity for such bonding to be possible. The three points of contact necessary for enantioselectivity to occur with amphetamines is illustrated in Fig. 7B. The aromatic portion (hydrophobic end) of the molecule is likely to fit into the CD cavity (Fig. 7B, point 1). CD hydrogen bonding occurs with the amphetamine primary amine group near the chiral centre. The optically active methyl substituent in amphetamine is the third point of contact with HP-β-CD. Optical differentiation of (*R*)- and (*S*)-amphetamines is made possible by steric hindrance between the hydroxypropyl groups in HP-β-CD and the optically active methyl substituent in amphetamine.

3.6. Sodium sulfobutyl ether-β-CD Ave. MS 0.4 (SBE-β-CD)

We postulated that increasing the alkyl substituent from hydroxypropyl to hydroxybutyl on the CD would further increase the flexibility and hydrophobicity of the CD cavity. Also, migration of a charged CD in the opposite direction to the analyte would increase the partition per unit volume between the molecule and charged CD, resulting in smaller amounts of CD being re-

quired to achieve a separation. This in part was obtained using SBE-β-CD as a chiral additive. The versatility of such a system was shown with SBE-β-CD optically resolving β-agonist, β-antagonist and amphetamines using a citrate buffer at pH 2.5. In an attempt to study the range of compounds that could be resolved with SBE-β-CD, thalidomide and some metabolites were employed in the experiment.

Fig. 8 shows an electropherogram of six racemates of β-agonists, β-antagonist and thalidomide optically resolved in less than 25 min with 2 m*M* SBE-β-CD, with retention times and R_s values given in Table 4. Note that the optical migration order is only given for optically pure isomers obtained. Increasing the SBE-β-CD concentration from 2 to 4.6 m*M* causes the racemates to elute within 15 min in the order of N-phthaloyl-(*S*)-glutamic acid, terbutaline, clenbuterol, (*R*)-thalidomide, (*R*,*S*)-glutamine and (*S*)-thalidomide, as shown in Figs. 9 and 10, with the corresponding retention times and R_s values given in Table 4. Increasing the SBEβ-CD concentration in the CE running buffer would have increased the ionic strength and conductivity of the medium, hence reducing the migration time of the solutes. This is most pronounced with thalidomide, where the migration time is halved and R_s decreased with increases in SBE-β-CD concentration from 2.0 to 4.6 m*M*, as shown in Table 4.

In order to improve the chiral separation of

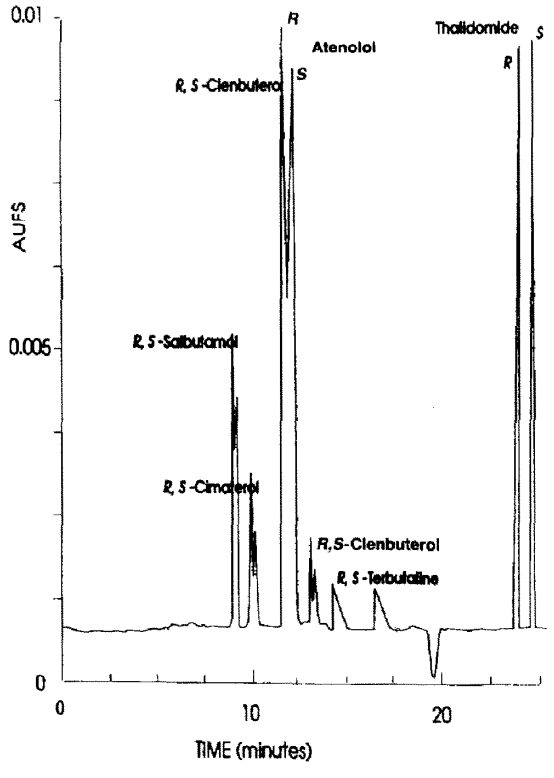


Fig. 8. Electropherogram of (*R*,*S*)-salbutamol, (*R*,*S*)-cimaterol, (*R*,*S*)-clenbuterol, (*R*,*S*)-atenolol, (*R*,*S*)-terbutaline and (*R*,*S*)-thalidomide optically resolved. Conditions: 2 m*M* SBE-β-CD, 20 m*M* citric acid–phosphate buffer (pH 2.5), anode at injection side, 20 kV, 220 nm, 4 kPa injection of 300 μg/ml solution.

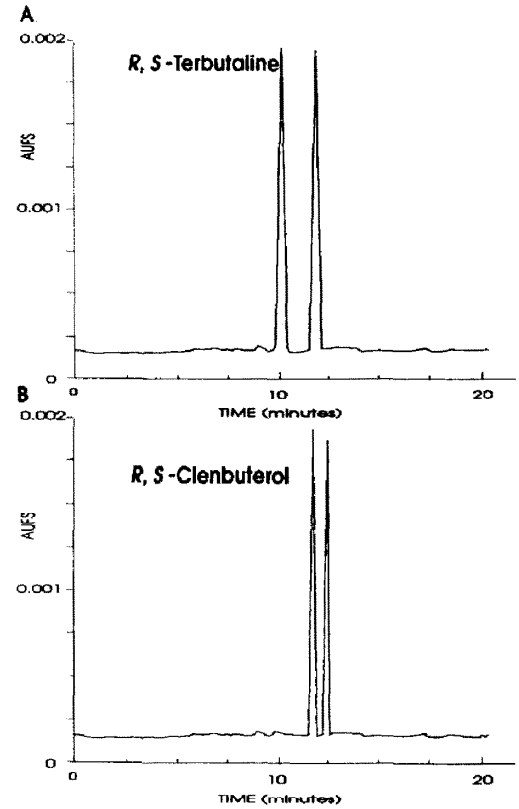


Fig. 9. Electrophereograms of (A) (*R*,*S*)-terbutaline and (B) (*R*,*S*)-clenbuterol optically resolved. Conditions: 4.6 m*M* SBE-β-CD, 20 m*M* citric acid–phosphate buffer (pH 2.5), cathode at injection side, 20 kV, 220 nm, 4 kPa injection of 300 μg/ml solution.

Table 4
Compounds optically resolved with various SBE-β-CD concentrations in 20 m*M* citric acid–phosphate buffer (pH 2.5) using 1-propanol as organic modifier

SBE-β-CD concentration (mM)	Compound optically resolved	Retention time (min)		Resolution
2.0	(*R,S*)-Salbutamol	8.81	8.93	1.00
2.0	(*R,S*)-Cimaterol	10.04	10.22	0.75
2.0	(*R,S*)-Atenolol	13.03	13.25	0.67
2.0	(*R,S*)-Clenbuterol	14.53	14.88	1.31
2.0	(*R,S*)-Terbutaline	14.68	16.88	2.80
2.0	(*R,S*)-Thaldiomide	23.55	24.87	2.25
4.6	N-Phthaloyl-(*S*)-glutamic acid		9.89	
4.6	(*R,S*)-Terbutaline	10.31	12.09	2.88
4.6	(*R,S*)-Methylamphetamine	12.21	13.01	1.78
4.6	(*R,S*)-Clenbuterol	12.23	12.57	1.67
4.6	(*R,S*)-Amphetamine	13.98	15.13	1.33
4.6	(*R,S*)-Glutamine		14.57	
4.6	(*R,S*)-Thalidomide	14.14	14.69	2.00
4.6	(*R,S*)-Atenolol	15.77	16.44	3.13
Addition of 5% (V/V) 1-propanol				
2.0	(*R,S*)-Clenbuterol	12.33	12.44	0.40
2.0	(*R,S*)-Methyldimethoxyamphetamine	14.37	14.71	0.71

the compounds studied at 2.0 m*M* SBE-β-CD, an organic modifier was used to modify the host–guest complex. Based on previous work [38], the buffer system was modified with 5% (v/v) of 1-propanol. Compounds which normally resolved without organic modifier failed to do so; only methyldioxyamphetamine was resolved and clenbuterol poorly, as shown in Table 4.

At pH 2.5, SBE-β-CD can optically resolve only positively charged compounds. This is probably because they migrate under the influence of an applied voltage. However, under these conditions, carboxylic acids are protonated and hence do not migrate electrophoretically.

4. Conclusions

The highly water-soluble HP-β-CD was used at elevated concentrations enabling those CD derivatives to give the optimum separation of certain racemates to achieve a high enough concentration in solution to effect that separation. This provided the means to resolve optically many β-agonists, β-antagonists, amphetamines and ephedrines with a single buffer system. The native CD is made more size dependent by introducing a bulky hydroxypropyl substituent. Steric factors are also relevant for chiral recognition; at least one polar group with the proper hydrogen bonding ability must be in proximity to the stereogenic centre, such as hydroxyl groups and/or secondary amines. This work has shown the versatility of CE in the simultaneous multi-component chiral separation of illicit and pharmaceutical drug substances for direct pharmacokinetic studies of a wide range of optically active drugs.

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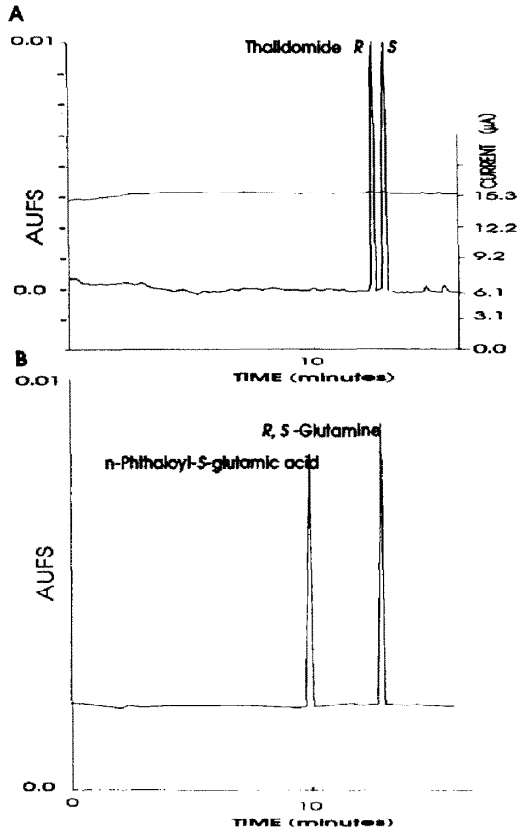

Fig. 10. Electropherograms of (A) (*R*)- and (*S*)-thalidomide and (B) (*R*,*S*)-glutamine and phthaloyl-(*S*)-glutamic acid. Conditions: 4.6 m*M* SBE-β-CD, 20 m*M* citric acid–phosphate buffer (pH 2.5), 20 kV, anode at injection side, 220 nm, 4 kPa injection of 300 μg/ml solution.

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