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Enantiomeric differentiation of a wide range of pharmacologically active substances by cyclodextrin-modified micellar electrokinetic capillary chromatography using a bile salt

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Abstract

This paper shows the versatility of modified charged and non-charged β-cyclodextrins in micellar systems for optically resolving β-agonists, β-antagonists, phenylethylamines stimulants and diclofensine an antidepressant. A total of 22 compounds were optically resolved using hydroxypropyl-β-cyclodextrin with sodium taurodeoxycholate and sodium sulfobutyl ether-β-cyclodextrin with sodium dodecyl sulfate.

1. Introduction

Bile salts are chiral naturally occurring anionic surfactants found in vertebrate species [1]. The primary physiological function of bile salts is to assist in the solubilization of dietary lipids, such as cholesterol, aiding in the excretion of insoluble endogenous lipids [2]. Bile salts have a flat-shaped steroid portion with the hydroxyl groups oriented in the same direction (hydrophilic face) nearly perpendicular to the steroidal frame (hydrophobic face). This induces the formation of small primary micelles [3] possessing a helical structure with the hydrophilic region facing the interior of the micelle [4,5]. Bile salt micelles are more polar, have a lower critical micelle concentration and aggregation number than the commonly used sodium dodecyl sulfate (SDS) [6]. They have been used in micellar electrokinetic capillary chromatography (MECC) for the separation of mainly hydrophobic solutes having rigid planer chemical structures [4,7–21], and optical resolution of racemates [11–17]. The separation principle of MECC using bile salts is based on the large anionically charged micelles electrophoretically migrating towards the anodic end of the capillary; however, since electroosmotic flow (EOF) is in the opposite direction, the net effect is that the micelles are slowly swept towards the detector. The slowly moving micelles create a pseudostationary phase. Analytes in the mobile EOF are separated by differential interactions with the micelles and by

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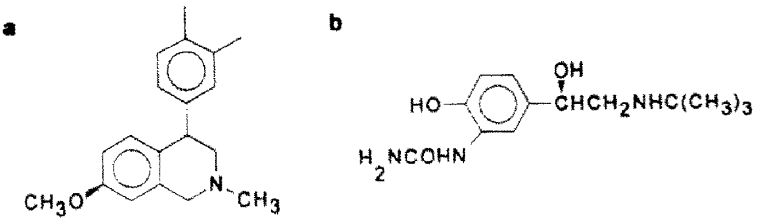

Fig. 1. Chemical structures of (a) diclofensine and (b) carbuterol.

their electrophoretic mobilities. The chiral recognition mechanism for bile micelles in MECC systems appears to be by solute structure compatibility with the helical cavity, enabling a specific interaction with one enantiomer and the micelle polar interior [15,20].

In a previous paper only positively charged drug compounds (β-agonists, β-antagonists, phenylethylamines and alcohols) were optically resolved by cyclodextrin (CD) capillary zone electrophoresis (CZE) [22], although drugs can exist as charged and/or neutral species. Both charged and neutral species can be resolved by incorporating a surfactant into the buffer system [23]. A CD MECC system that optically resolves both phenylethylamines and diclofensine enantiomers has not been reported.

Reported in this paper are CD MECC separation methods using sodium taurodeoxycholate (STDC) and SDS as surfactants and ether derivatives of β-CD to resolve a wide range of pharmacologically active drugs including diclofensine (Fig. 1a) and β-agonists, β-antagonists, phenylethyamines and alcohol stimulants (the chemical structure of carbuterol is illustrated in Fig. 1b, and others have been shown previously [22]). *R,S*-Diclofensine is a tricyclic antidepressant and widely used in the treatment of depression by inhibiting the re-uptake of dopamine, noradrenaline, and serotonin [24].

2. Experimental

2.1. Reagents

R,S-Diclofensine hydrochloride was kindly supplied by F. Hoffmann–La Roche, Basel, Switzerland. *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, *R,S*-carbuterol, *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 and *R*-pseudoephedrine were supplied by the Curator of Standards, Australian Government Analytical Laboratories (Pymble, Australia). Hydroxypropyl-β-cyclodextrin [HP-β-CD, average molar substitution (MS) 0.6 and 0.8], dimethyl-β-cyclodextrin, trimethyl-β-cyclodextrin, γ-cyclodextrin, sodium taurodeoxycholate, *R*-propranolol, *S*-propranolol, *R,S*-epinephrine and *S*-epinephrine were supplied by Sigma (St. Louis, MO, USA). *R*-Atenolol, *S*-atenolol, *S*-alprenolol-*S*-tartrate were supplied by Aldrich. Sodium sulfobutyl ether-β-cyclodextrin (SBE-β-CD) was supplied by Isco (Lincoln, NE, USA). SDS was obtained from E. Merck, Kilsyth, Australia. SDS was recrystallised three times from absolute ethanol. Analytical-reagent grade propan-1-ol was redistilled under vacuum from activated charcoal prior to use. All other chemicals and solvents were of analytical-reagent or HPLC grade and were used without further purification.

2.2. Preparation of buffers

A stock buffer solutions of 0.100 *M* sodium

tetraborate adjusted to pH 9.5 with 5.0 *M* sodium hydroxide, 0.100 *M* SDS and 0.100 *M* STDC were used to prepare running buffers. The running buffers were prepared by the appropriate volume of stock buffer and surfactant, propan-1-ol and mass of cyclodextrin. The resulting running buffer was degassed by sonication and filtered through a 0.2-μm 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 at a temperature of 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 electropherogram). 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.

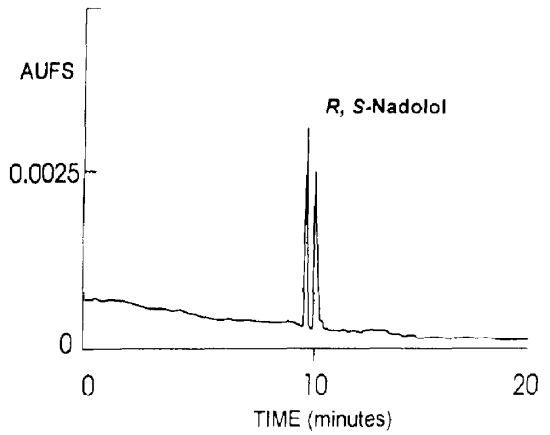

Fig. 2. Electropherogram of a 100 μg/ml *S,R*-nadolol standard. Conditions: 50 kPa/s injection, 30 kV, 220 nm [1.45 m*M* SBE-β-CD, 20 m*M* borate, 20 m*M* SDS, 5% (v/v) propan-1-ol at pH 9.05].

2.4. Procedure for capillary preparation and handling

Prior to extended use, the capillary was filled with 1 *M* sodium hydroxide and allowed to stand for 1 h. This solution was replaced with 0.1 *M* sodium hydroxide, allowed to stand for another hour and washed with deionised water before filling with the running buffer (between sample injections capillary was flushed with 20 μl of running buffer). The capillary was used for a maximum of 40 sample injections before rinsing with 1 *M* sodium hydroxide (200 μl), deionised water (200 μl) and running buffer (200 μl); it was then left filled with running buffer ready for sample injection.

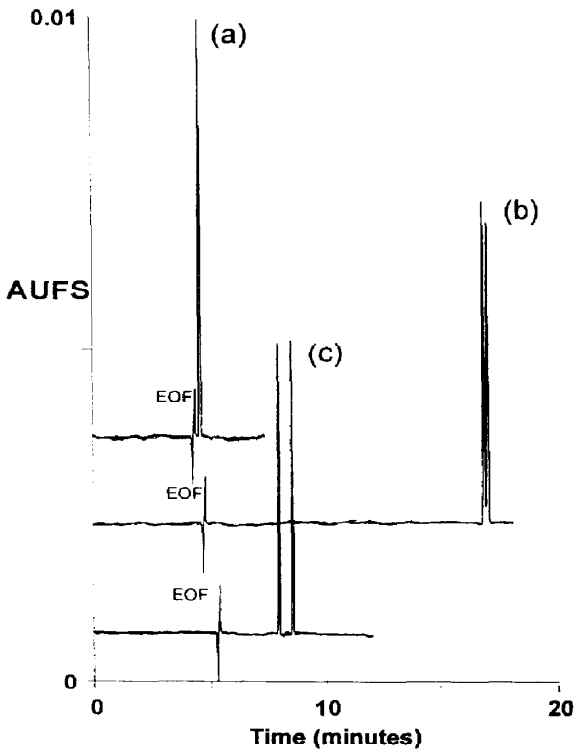

Fig. 3. Electropherogram of a 300 μg/ml *S,R*-diclofensine standard injected into (a) 30 m*M* HP-β-CD (average MS 0.6), 50 m*M* borate buffer at pH 9.5; (b) 2 m*M* HP-β-CD (average MS 0.6), 50 m*M* STDC, 50 m*M* borate buffer at pH 9.5; (c) 30 m*M* HP-β-CD (average MS 0.6), 50 m*M* STDC, 50 m*M* borate buffer at pH 9.5. Other conditions: 2 kPa/s injection, 30 kV, 200 nm.

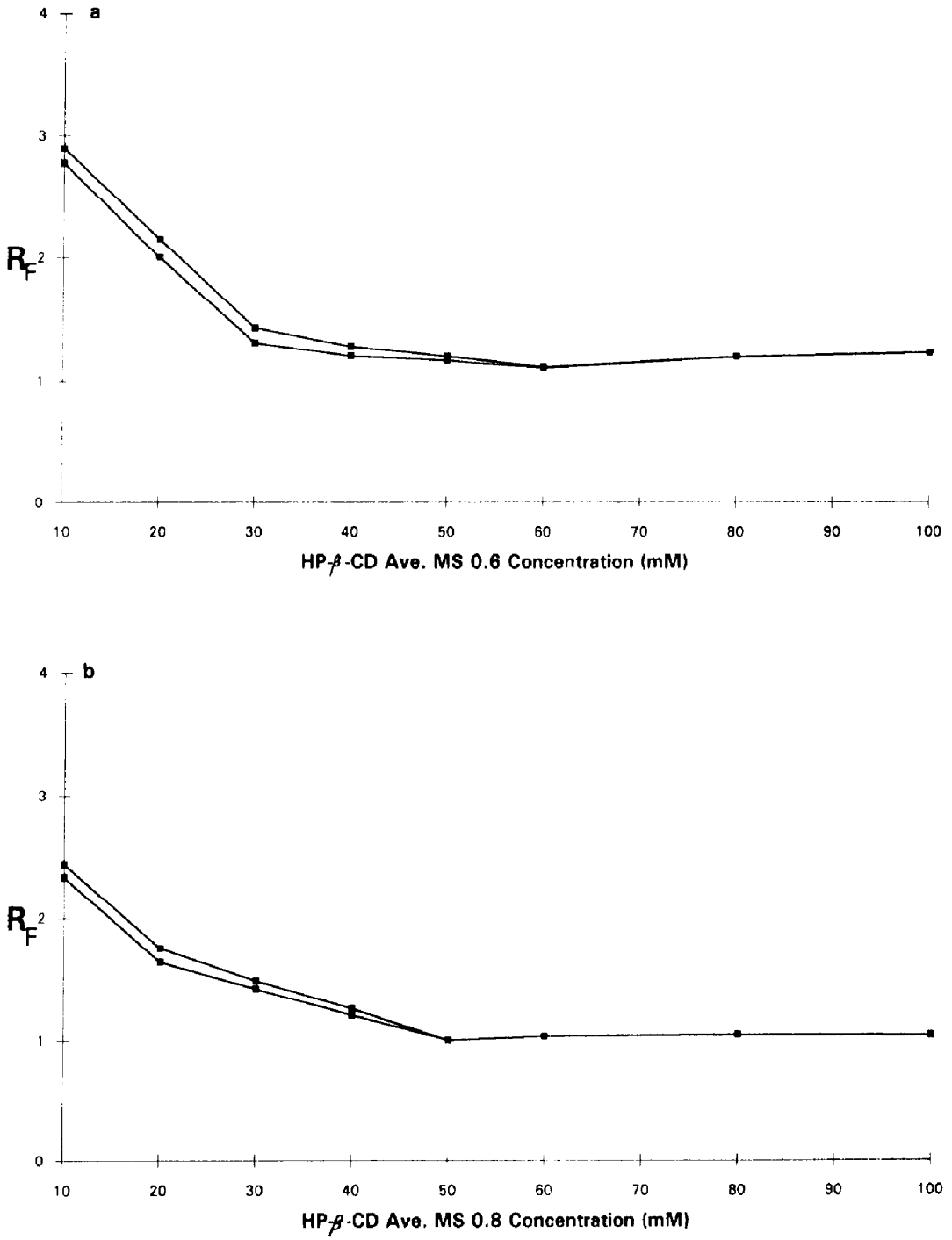

Fig. 4. Plots of *S,R*-diclofensine R_F versus (a) HP-β-CD (average MS 0.6), (b) HP-β-CD (average MS 0.8) and (c) STDC concentration (standard buffer composition 50 m*M* borate at pH 9.5, 50 m*M* STDC and 30 m*M* HP-β-CD).

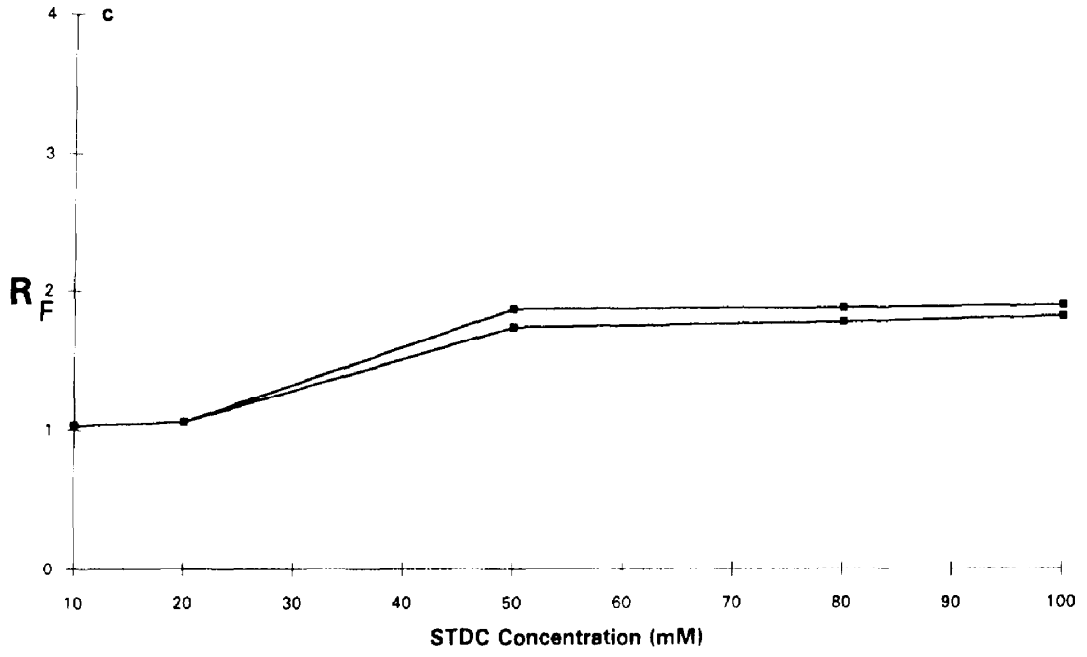

Fig. 4 (*continued*)

Table 1
R_F values of β-agonists and β-antagonists with 60 and 120 m*M* HP-β-CD (average MS 0.6 and 0.8) in 50 m*M* STDC, 5% (v/v) propan-1-ol and 50 m*M* borate buffer at pH 9.5

Compound	R_F [a]	R_F [b]	Compound	R_F [a]	R_F [b]
60 mM HP-β-CD			*120 mM HP-β-CD*		
R,S-Atenolol	0.98	0.98	*R,S*-Timolol	0.95, 1.0	0.98
R,S-Nadolol	0.98	0.98	*R,S*-Atenolol	0.98	0.98
R,S-Oxprenolol	0.98	0.98	*R,S*-Nadolol	0.98	0.96, 0.98
R,S-Cimaterol	0.98	0.98	*R,S*-Oxprenolol	0.98	0.97, 0.98
Methanol	1.0	1.0	*R,S*-Pindolol	0.98	0.98
R,S-Timolol	1.0	0.98, 0.99	*R,S*-Metoprolol	0.98	0.98
R,S-Pindolol	1.0	0.98, 0.99	*R,S*-Terbutaline	0.98	0.98
R,S-Pirbuterol	1.1	1.0	*R,S*-Propranolol	0.98	0.98
R,S-Acebuterol	1.1	1.0	*R,S*-Cimaterol	0.98, 0.99	0.97, 0.98
R,S-Alprenolol	1.1	1.0	Methanol	1.0	1.0
R,S-Clenbuterol	1.05, 1.06	1.0	*R,S*-Clenbuterol	1.01, 1.02	1.02, 1.03
R,S-Metoprolol	1.1	0.97, 0.99	*R,S*-Alprenolol	1.1	1.15
R,S-Terbutaline	1.1	1.1	*R,S*-Pirbuterol	1.1	1.1
R,S-Carbuterol	1.1	1.1	*R,S*-Carbuterol	1.1	1.1
R,S-Salbutamol	1.2	1.2	*R,S*-Diclofensine	1.10, 1.11	1.10, 1.11
R,S-Propranolol	1.2	1.25	*R,S*-Salbutamol	1.2	1.18
R,S-Diclofensine	1.2, 1.3	1.2, 1.3	*R,S*-Labetalol	1.3	1.3
R,S-Labetalol	1.3	1.3			

[a] HP-β-CD: average MS 0.6.
[b] HP-β-CD: average MS 0.8.

3. Results and discussion

Initial attempts to resolve enantiomers of β-agonists, β-antagonists, phenylethylamines and diclofensine were unsuccessful using β-CD, γ-CD, dimethyl- and trimethyl-β-CD, HP-β-CD (average MS 0.6 and 0.8 and SBE-β-CD in association with SDS, propan-1-ol at pH 9.0. Nadolol was only resolved optically using an SDS–propan-1-ol–SBE-β-CD system (Fig. 2).

Propranolol, atenolol, phenylethylamines and alcohols were not optically resolved with CD MECC systems employing SDS or STDC as surfactants at both pH 9 and 11 [25,26]. However, MECC systems employing bile salts and organic modifiers such as alcohols and CDs have optically resolved drugs structurally similar to phenylethylamines and diclofensine [11–17]. Diclofensine has a pK_a value of ca. 7 and is deprotonated at pH 9.5 migrating with the EOF in a CD CZE system (Fig. 3a). Addition of STDC to the CD CZE system optically resolved diclofensine and longer migrations times were observed, shown by Fig. 3a and b. This was attributed to diclofensine partitioning between the slower migrating micellar pseudostationary phase, and resulting in a specific optical interaction between the micelle polar interior and CD buffer phase. Increases in HP-β-CD (average MS 0.6 and 0.8) concentration decreased the relative migration time (R_F = solute migration time/methanol migration time) of diclofensine, and HP-β-CD (average MS 0.6 and 0.8) concentrations greater than 60 and 50 m*M*, respectively did not change diclofensine R_F (Figs. 3b and c and 4a and b). Similar results were observed with other CD MECC systems employing bile surfactants [16,17]. Also, β-agonists and β-antagonists R_F values decreased with increases in HP-β-CD concentration, reflected by $R_F < 1$ for atenolol, nadolol, oxprenolol, cimaterol, timolol and pindolol at 60 m*M* HP-β-CD concentration, and increasing HP-β-CD concentration to 120 m*M* resulted in atenolol, nadolol, oxprenolol, cimaterol, timolol, pindolol, timolol, pindolol, terbutaline and propranolol giving $R_F < 1$ (Table 1). β-Agonists, β-antagonists and phenylethylamines have pK_a values between 9.2 and 11 [25,27] and are protonated at pH 9.5 electromigrating in the same direction as EOF. A number of phenylethylamines, β-agonists and β-antagonists studied gave $R_F < 1$; suggesting weak interactions between the drug and the negatively charged STDC (Tables 1 and 2). Conversely, increases in STDC concentration up to 50 m*M* increased the R_F of diclofensine, and further increases in STDC concentration did not appear to change diclofensine R_F values (Fig. 4c). CD can associate weakly with the hydrophilic face of STDC within the micelle via hydrogen bonding

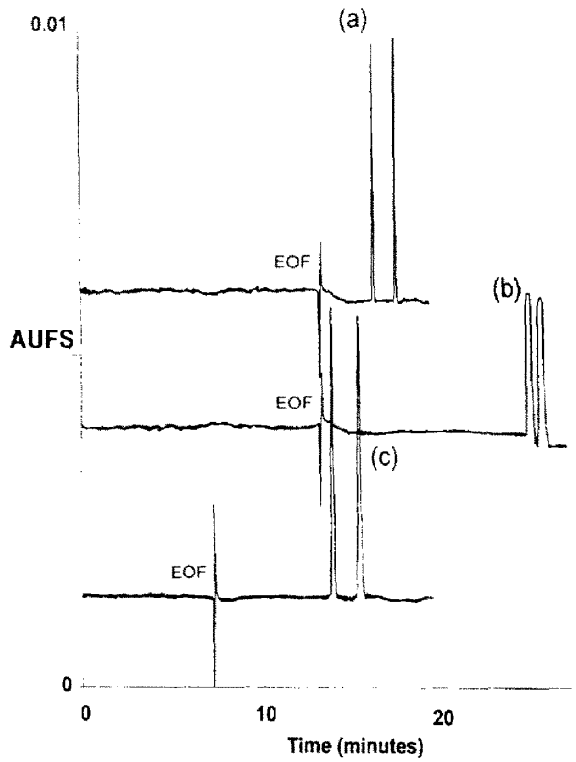

Fig. 5. (a, b) Electropherogram of a 300 μg/ml (a) *S,R*-nadolol and (b) *S,R*-diclofensine standard injected into 20% (v/v) propan-1-ol, 30 m*M* HP-β-CD (average MS 0.6), 50 m*M* STDC, 50 m*M* borate buffer at pH 9.5. (c) Electropherogram of a 300 μg/ml standard *S,R*-diclofensine injected into 5% (v/v) propan-1-ol, 30 m*M* HP-β-CD (average MS 0.6), 50 m*M* STDC, 50 m*M* borate buffer at pH 9.5. Other conditions: 2 kPa/s injection, 30 kV, 200 nm.

Table 2
R_F values of phenylethylamine and alcohols with 60 and 120 m*M* HP-β-CD (average MS 0.6 and 0.8) in 50 m*M* STDC, 5% (v/v) propan-1-ol and 50 m*M* borate buffer at pH 9.5

Compound	R_F [a]	R_F [b]	Compound	R_F [a]	R_F [b]
60 mM HP-β-CD			*120 mM HP-β-CD*		
R,S-Methyldimethoxymethyl-amphetamine	0.92, 0.93	0.96, 0.97	*R,S*-Methylamphetamine	0.92, 0.93	0.90, 0.91
R,S-2,5-Dimethoxy-4-methyl-amphetamine	0.93	0.92	*R,S*-Methyldimethoxymethyl-amphetamine	0.93	0.97, 0.98
R,S-Epinephrine	0.94	0.96	*R,S*-Epinephrine	0.94	0.98
R,S-Methyldimethoxyethyl-amphetamine	0.94, 0.95	0.92, 0.93	*R,S*-4-Bromo-2,5-dimethoxy-amphetamine	0.94	0.95
R,S-Methylamphetamine	0.95, 0.96	0.93, 0.94	*R,S*-2,5-Dimethoxy-4-methyl-amphetamine	0.96	0.97
R,S-Methyldimethoxy-amphetamine	0.95	0.93	*R,S*-Amphetamine	0.98	0.98
R,S-Amphetamine	0.98	0.93	*R,S*-Ephedrine	0.98	0.98
R,S-Ephedrine	0.98	0.98	*R,S*-Pseudoephedrine	0.98	0.98
R,S-Pseudoephedrine	0.98	0.98	*R,S*-Methyldimethoxy-amphetamine	0.98	0.95
R,S-Norephedrine	0.98	0.98	*R,S*-Norephedrine	0.98	0.97
R,S-4-Bromo-2,5-dimethoxy-amphetamine	0.98	0.98	*R,S*-Methyldimethoxyethyl-amphetamine	0.98	0.98

[a] HP-β-CD: average MS 0.6.
[b] HP-β-CD: average MS 0.8.

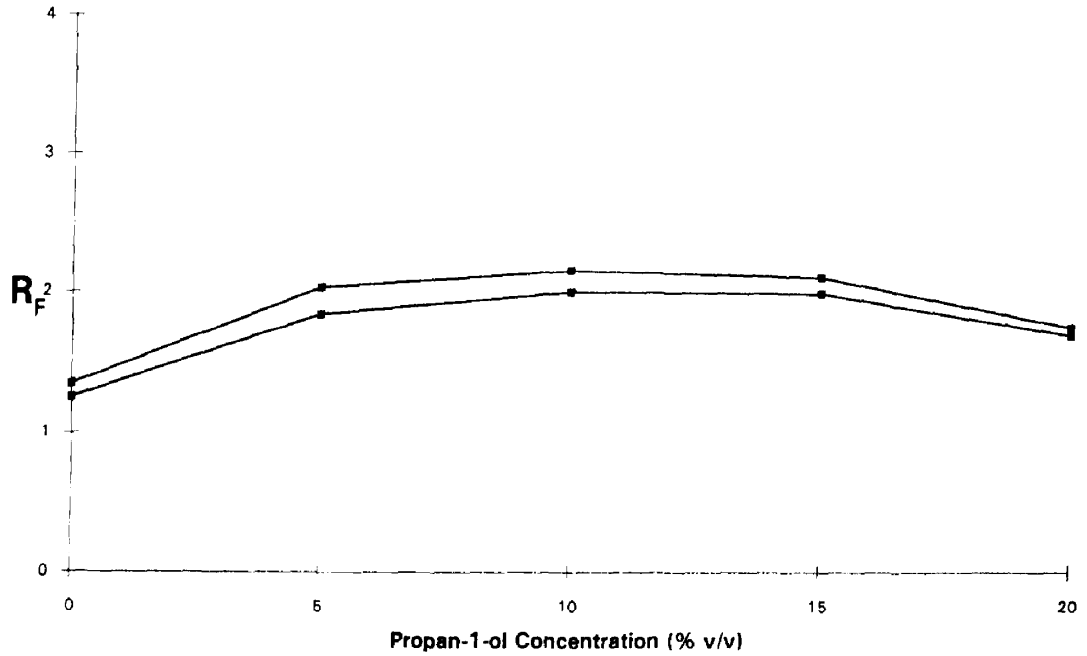

Fig. 6. A plot of *S,R*-diclofensine R_F versus propan-1-ol concentration [50 m*M* STDC, 30 m*M* HP-β-CD (average MS 0.8), 50 m*M* borate buffer at pH 9.5].

[16] decreasing the micelle hydrophobicity, reflected in this work by decreases in R_F values for β-agonists, β-antagonists and diclofensine with increases in CD concentration.

Resolution in MECC can be improved by modifying the buffer with short-chain alcohols, decreasing the EOF and affinity of the hydrophobic solute for the micellar phase [28,29]. The STDC–HP-β-CD system at pH 9.5 studied was modified with propan-1-ol at various concentrations and β-agonists, β-antagonists, phenylethylamines and diclofensine were injected separately to obtain the modifier concentration which optically resolved the greatest number of compounds. The optical resolution of diclofensine was observed to increase with propan-1-ol concentration up to 5%, and further increases in propan-1-ol concentration degraded the chiral resolution (Figs. 3c, 5b and c and 6). Timolol, pindolol, metoprolol, clenbuterol, diclofensine, oxprenolol, cimaterol, methylamphetamine, methyldimethoxymethylamphetamine and methyldimethoxyethylamphetamine were optically resolved with STDC–HP-β-CD modified with 5% (v/v) propan-1-ol (Tables 1 and 2 and Figs. 5c, 7–9. The STDC–HP-β-CD system modified with 20% (v/v) propanol could only resolve optically nadolol and diclofensine (Fig. 5a and b).

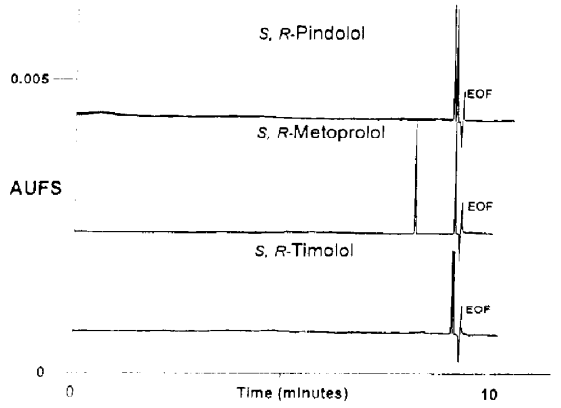

Fig. 8. Electropherogram of a 300 μg/ml *S,R*-pindolol, *S,R*-metoprolol and *S,R*-timolol standard injected into 5% (v/v) propan-1-ol, 60 m*M* HP-β-CD (average MS 0.8), 50 m*M* STDC, 50 m*M* borate buffer at pH 9.5 (2 kPa/s injection, 30 kV, 200 nm).

The optical isomers of β-agonists, β-antagonists, ephedrines and amphetamines resolved at pH 2.5 with HP-β-CD migrated with the "*S*" form first, followed by the "*R*" [22]. In this work methylamphetamine migrated with the "*S*" form

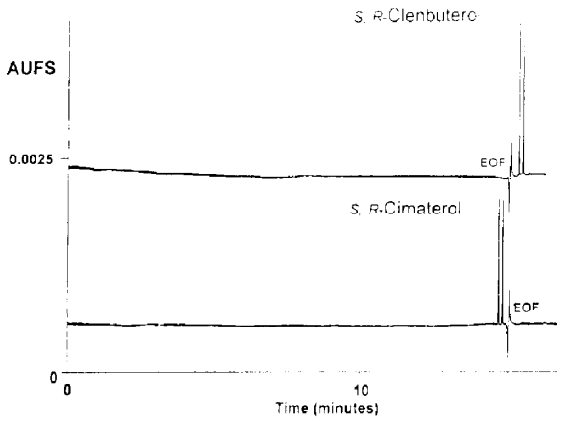

Fig. 7. Electropherogram of a 300 μg/ml *S,R*-clenbuterol and *S,R*-cimaterol standard injected into 5% (v/v) propan-1-ol, 30 m*M* HP-β-CD (average MS 0.8), 120 m*M* STDC, 50 m*M* borate buffer at pH 9.5 (2 kPa/s injection, 30 kV, 200 nm).

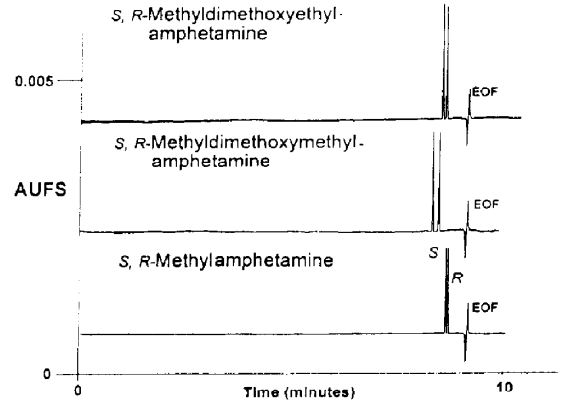

Fig. 9. Electropherogram of a 300 μg/ml *S,R*-methyldimethoxyethylamphetamine, *S,R*-methyldimethoxymethylamphetamine and *S,R*-methylamphetamine standard injected into 5% (v/v) propan-1-ol, 60 m*M* HP-β-CD (average MS 0.8), 50 m*M* STDC, 50 m*M* borate buffer at pH 9.5 (2 kPa/s injection, 30 kV, 200 nm).

first, followed by the "*R*" (Fig. 9), suggesting a common mechanism to the HP-β-CD CZE system [22]. Chiral resolution for metoprolol at pH 2.5 with HP-β-CD appeared to be impaired by the protruding aromatic substituent preventing the aromatic ring from sufficient penetration into CD for hydrogen bonding to occur with the stereogenic centre [22]. Conversely, optical resolution appeared impaired with timolol by the large distance between the stereogenic centre and hydrogen bonding groups on the CD [22]. In this work, the combination of HP-β-CD with STDC appeared to produce a synergetic effect optically resolving metoprolol and timolol. Similar effects have appeared using dansylamino acid derivatives with STDC–CD systems [17,18].

4. Conclusions

Mixtures of modified charged and non-charged β-CDs in association with SDS or STDC at pH 9.0 and 9.5 have shown to be useful in optically resolving a wide variety of pharmacologically active drugs by CD MECC. These systems are further being evaluated for resolving a number of other drugs.

Acknowledgement

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