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Enantioselective reversed-phase and non-aqueous capillary electrochromatography using a teicoplanin chiral stationary phase

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Abstract

Enantiomeric separation of chiral pharmaceuticals is carried out in aqueous and non-aqueous packed capillary electrochromatography (CEC) using a teicoplanin chiral stationary phase (CSP). Capillaries were slurry packed with 5 μm 100-Å porous silica particles modified with teicoplanin and initially evaluated using a non-aqueous polar organic mode system suitability test for the separation of metoprolol enantiomers (R_s=2.3 and 53 000 plates m^{-1}). A number of pharmaceutical drugs were subsequently screened with enantioselectivity obtained for 25 racemic solutes including examples of neutral, acidic and basic molecules such as coumachlor (R_s=3.0 and 86 000 plates m^{-1}) and alprenolol (R_s=3.3 and 135 000 plates m^{-1}) in reversed-phase and polar organic mode, respectively. A statistical experimental design was used to investigate the effects of non-aqueous polar organic mobile phase parameters on the CEC electroosmotic flow, resolution and peak efficiency for two model solutes. Results primarily indicated that higher efficiency and resolution values could be attained at higher methanol contents which is similar to findings obtained on this phase in liquid chromatography.

Keywords: Chiral stationary phases, CEC; Enantiomer separation; Electrochromatography; Experimental design; Teicoplanin

1. Introduction

The majority of chiral selectors previously utilised for chiral chromatographic and electrophoretic separations have now been successfully applied for the separation of enantiomers in either open-tubular, packed or monolithic capillary electrochromatography (CEC) columns [1–4]. The earlier review by Hatajik and Brown [1] has now been updated by others to include the application of additional CSPs and the evaluation of non-aqueous chiral CEC [2–4], but has not included more recent reports [5–10].

Macrocyclic antibiotic CSPs have been applied almost in every technique for the separation of enantiomers since their introduction including LC [11,12], capillary electrophoresis (CE) [13,14], supercritical fluid chromatography [15–17] and CEC [7,18–21]. Their broad enantioselectivity results from their inherent complimentary properties for chiral recognition which primarily include their several stereogenic centers, their numerous functionalities to aid complexation and their overall three-dimensional shape [22]. Although the ansamycin macrocyclic antibiotics, rifamycin B and rifamycin

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SV have been evaluated and shown enantioselectivity [13,23,24], the glycopeptide macrocyclic antibiotics, vancomycin, teicoplanin, ristocetin A and avoparcin have demonstrated broader enantioselectivity and consequently have been applied successfully for a range of compound classes [11,14,22,25–30].

Teicoplanin differs from the other glycopeptide antibiotics in that it contains D-glucosamine and D-mannose carbohydrate groups of which the former is substituted with a long fatty acid chain [28] but shares the aglycone peptide 'basket-like' shape which is considered advantageous for enantioselectivity. It has been applied extensively for chiral discrimination in a number of separation techniques [15,19,25,26,28,31–34] and more recently the enantiorecognition processes have been studied in detail where the direct effects of temperature on enantiomer retention [31] and the precise role of the carbohydrate functionalities have been investigated [35].

Initial studies by Carter-Finch and Smith using a teicoplanin stationary phase in reversed-phase CEC have indicated that at least two racemic molecules, tryptophan and dinitrobenzoyl leucine, could be separated with high resolution [19]. In this study, we investigate this further to extend the knowledge and application of the teicoplanin CSP in CEC. After optimisation and evaluation of reversed-phase mobile phase characteristics, a number of pharmaceutical drugs of interest are screened on this CSP. Additionally, given the multi-modal nature of these antibiotic CSPs, we have investigated the potential of applying the non-aqueous polar organic mode where a statistical approach was adopted to characterise the effects of organic solvent ratio and the influence of acidic and basic additives on the observed electroosmotic flow (EOF), resolution and enantiomeric efficiency. Subsequently, a number of chiral drugs of interest are screened on this CSP in the non-aqueous polar organic mode to determine the broader potential application of this CSP in CEC.

2. Experimental

2.1. Chemicals

The teicoplanin-bonded CSP (5 μm Chirobiotic T™) was a gift from Advanced Separation Technologies (Astec, NJ, USA). Metoprolol, felodipine, alprenolol, thalidomide, bupivacaine and warfarin, were obtained from Medicinal Chemistry, AstraZeneca R&D Mölndal (Mölndal, Sweden). Acetonitrile (MeCN), methanol (MeOH), glacial acetic acid (HOAc) and sodium chloride (NaCl) were purchased from Merck (Darmstadt, Germany). Triethylamine (TEA), pindolol, atenolol, fenoterol, labetalol, sotalol, dopa, 5-(4-methylphenyl)-5-phenylhydantoin, 5,5-phenylhydantoin, 5-(4-hydroxyphenyl)-5-phenylhydantoin, phenylpropanolamine, propranolol, verapamil, ibuprofen, ketoprofen, β-hydroxyphenethylamine, *N*-CBZ-glutamic acid, tryptophan, terbutaline, coumachlor and benzoin were purchased from Sigma–Aldrich Sweden (Stockholm, Sweden). Acetone was purchased from Rathburn Chemicals (Walkerburn, UK). Fused-silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA). Organic solvents were of HPLC grade and deionised water (18.2 MΩ) used throughout the study was taken from a Maxima water purification system (Elga, High Wycombe, UK).

2.2. Instrumentation

The pump used for capillary packing was identical to that described earlier [21] and production of retaining frits was carried out using the Advanced Capillary Burner (InnovaTech, Hertfordshire, UK). Electrochromatography experiments were carried out using the Hewlett-Packard 3D-CE system (Hewlett-Packard, Waldbronn, Germany) modified to allow pressure of up to 12 bar to both the inlet and the outlet mobile phase vials. Data were collected and analysed using the HP 3D-CE ChemStation (Rev. A.05.04, Hewlett-Packard).

2.3. Methods

Analyte stock solutions were prepared in MeCN at a concentration of 3.0 mg/ml and stored at 4°C. Samples for reversed-phase and polar organic mode CEC injection were prepared by a 90% dilution of each stock solution with MeCN. Aqueous mobile phases were prepared by combining the desired volume of organic solvent to pH controlled buffer solutions and degassed by sonication or using helium

for at least 10 min. Polar organic mobile phases were prepared by combining the desired ratio of MeOH and MeCN to which trace amounts by volume of HOAc and TEA were added.

2.4. Column preparation

Capillaries (75 μm) were packed with the teicoplanin CSP using a slurry packing technique described previously [21]. Prior to retaining frit production, the capillary was washed with 10 m*M* NaCl for 30 min at 450 bar. The retaining frits were prepared under pressure at 450 bar by threading it through a resistance coil and applying approximately 600°C for 10 s. A salt solution flush (10 m*M* NaCl) as opposed to the traditional water flush prior to frit fabrication was adopted in these studies so that reliable and reproducible frits resulted. This knowledge has been attained from earlier studies with similar materials and described in detail therein [21].

2.5. Column evaluation

Similar to our earlier investigations with vancomycin CSPs, each new column was evaluated using a system suitability test (SST) prior to further exploratory experiments [7,21]. The SST which was carried out in the polar organic mode consisted of (a) the measurement of linear velocities at various voltages for the non-retained marker, acetone and (b) a repeatability study (relative standard deviation (RSD), $n=5$) for the chiral separation of metoprolol. Acetone was chosen as the EOF marker since under identical conditions, it was found to be the least retained molecule when acetone, thiourea and dimethylsulphoxide were simultaneously examined. The conditions employed were: MeOH–MeCN–TEA–HOAc (80:20:0.1:0.1, v/v/v/v); 15 kV; 15°C; electrokinetic injection at 10 kV for 2 s; 200 nm and 10 bar pressurisation over the column.

3. Results and discussion

3.1. Column evaluation

The polar organic mode SST was used to evaluate each new teicoplanin CEC column packed in which the enantiomers of metoprolol were separated ($R_s=$ 2.3) in under 14 min (Fig. 1a) with efficiency values of 53 000 and 37 000 plates m^{-1} for the *S* and *R* enantiomers, respectively. The repeatability of this separation ($n=5$) was found to yield RSD values for retention time (2.8%), resolution (3.3%), area (3.2%) and efficiency (2.0%). A plot of field strength with linear velocity and a van Deemter curve were also constructed for the non-retained marker acetone (Fig. 1b) and results indicate that under these polar organic conditions, thermal and double layer overlap effects can be considered negligible [36], while the van Deemter curve indicates that optimum reduced plate height values may be obtained at relatively low voltages (15 kV). A study of column-to-column repeatability ($n=5$) resulted in RSD values for retention time (8%), resolution (4.5%) and efficiency (8%), respectively, which were considered accept-

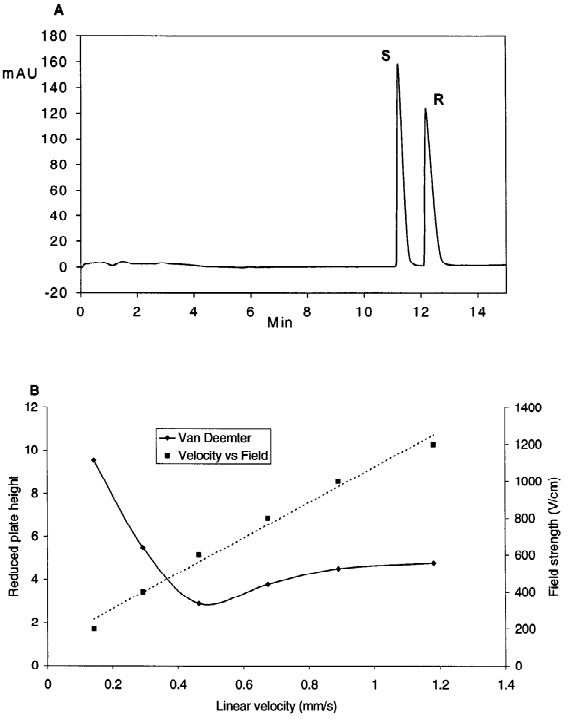


Fig. 1. Polar organic mode SST on the teicoplanin CSP using (a) the chiral CEC separation of metoprolol enantiomers and (b) the measurement of linear velocity with field strength (■) and evaluation of the subsequent van Deemter plot using S enantiomer of metoprolol (♦). Conditions: MeOH–MeCN–TEA–HOAc (80: 20: 0.1: 0.1, v/v/v/v), 335 mm×75 μm I.D. (L_d 250 mm), 15 kV; 15°C; electrokinetic injection at 10 kV for 2 s; 200 nm and 10 bar pressurisation over the column.

able and similar in magnitude to those reported in similar studies [7,19,21].

3.2. Reversed-phase mode

The effects of reversed-phase organic modifier, buffer concentration, pH and temperature on the EOF, column efficiency and enantioselectivity have been studied earlier for a vancomycin CSP [7]. A number of these parameters have already been investigated on a teicoplanin CSP for their effect on the separation of two racemic compounds by Carter-Finch and Smith [19] which were found to be similar to those observed in the vancomycin study [7]. In this study with the teicoplanin CSP, a broader range of reversed-phase conditions were individually examined in order that a greater number of racemic solutes could be examined effectively. This included mobile phases having different MeCN modifier contents (20–50%) in triethylamine acetate (TEAA) buffers of different concentration (0.5–0.2%) which were each controlled at different pH intervals between 4 and 6. It was found that higher resolution values were obtained at higher buffer concentration and pH but with low modifier content. Similar results were obtained for enantiomeric efficiency with the exception of pH where low values were favoured. Temperature was also evaluated (15–60°C) and results indicated that lower temperatures favoured resolution and efficiency but as expected at the expense of EOF. This was additionally examined and found to be as high as 18.7×10^{-5} cm^2 V^{-1} s^{-1} at pH 6, but could be as low as 5.3×10^{-5} cm^2 V^{-1} s^{-1} at low temperatures (15°C). Twenty-two racemic solutes, including neutral, acidic and basic compounds, were subsequently screened on this CSP in the reversed-phase mode, of which resolution was obtained for 12 (Table 1). Resolution values were found to vary from 3.9 for 5,5-diphenylhydantoin to 0.6 for ibuprofen and bupivacaine, respectively, and shown in Fig. 2 for coumachlor and tryptophan, respectively. In general, column efficiency was excellent, as high as 125 000 plates m^{-1} (tryptophan) with average values almost 60 000 plates m^{-1} when separation was attained.

3.3. Polar organic mode

The polar organic mobile phase which was first introduced for enantioseparations on cyclodextrin CSPs predominantly consisted of the polar solvent MeCN to which trace amounts of MeOH, triethylamine and/or glacial acetic acid additives were added to regulate retention and selectivity [37]. It has been noted in LC studies, however, that when this mode is applied with antibiotic-based CSPs greater performance can be achieved when lower amounts of MeCN are used in the mobile phase, leaving methanol as the predominant solvent with small amounts of acidic and basic additives as normal [38].

The polar organic mode was examined on this teicoplanin CSP and resulted in a cathodic EOF (13.2×10^{-5} cm^2 V^{-1} s^{-1}) which was significantly higher than that obtained with the vancomycin CSP (6.7×10^{-5} cm^2 V^{-1} s^{-1}) under identical SST conditions as outlined above and in Fig. 1 [7]. Given that each CSP is bound to the same support (5 μm, 100 Å silica) it is difficult to understand or attribute these large differences to the selector molecule alone. It is possible that a lower selector coverage was obtained for teicoplanin, due to its size, thus leaving a larger degree of silanol moieties free for EOF generation. The high EOF values obtained with this selector using optimum conditions (16.2×10^{-5} cm^2 V^{-1} s^{-1}) are comparable with those obtained in similar chiral and achiral non-aqueous CEC studies where values of approximately 25, 15.2 and 19×10^{-5} cm^2 V^{-1} s^{-1} were obtained on a weak anion-exchange type (WAX), octadecylated cellulose and an ODS Hypersil stationary phase, respectively, [5,39,40]. These data indicating high electroosmotic mobility in the polar organic mode further outline its potential as an alternative to the traditional aqueous phases applied in chiral or even achiral CEC [5,7,21].

3.4. Evaluation of polar organic mobile phase parameters

Although the effects of polar organic mobile phase factors, MeOH to MeCN ratio and competing base and acid concentrations, have recently been studied in CEC on vancomycin CSPs [7,21] and on a WAX type CSP [5], it can be concluded that relatively little is known since the results have been stationary phase specific. In each study both the modifier ratio and ionic strength were shown to affect the observed EOF, peak efficiency, retention times and resolution

Table 1
Enantioselectivity obtained for pharmaceutical drug enantiomers using the teicoplanin CSP in reversed-phase CEC

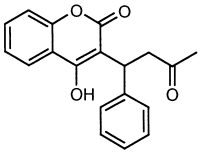
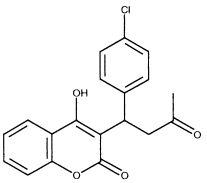
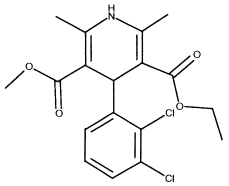
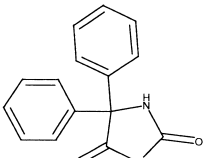
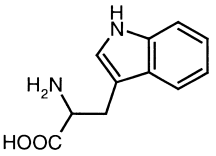
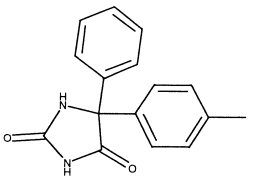
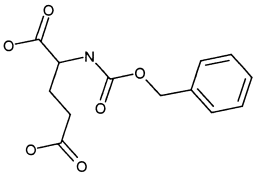
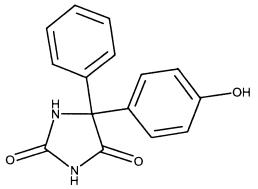
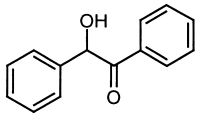
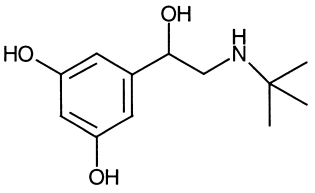
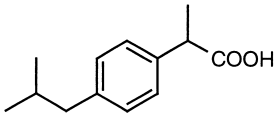
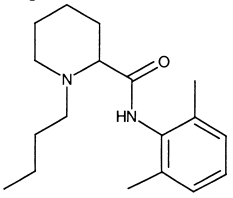
Racemate	Cond.[a]	t_{R1} (min)	N_1 (plates m^{-1})	N_2 (plates m^{-1})	R_s
Warfarin 	a	7.6	89 628	85 752	2.6
Coumachlor 	a	8.1	85 848	81 616	3.0
Felodipine 	b	63.3	57 828	37 524	1.7
5,5-Diphenylhydantoin 	c	10.2	119 336	124 320	3.9
Tryptophan 	c	12.0	125 092	81 680	2.9
5-(4-Methylphenyl)-5-phenylhydantoin 	b	56.7	36 408	28 816	1.1

Table 1. Continued

Racemate	Cond.[a]	t_{R1} (min)	N_1 (plates m^{-1})	N_2 (plates m^{-1})	R_s
N-CBZ-L-Glutamic acid	b	90.9	30 792	25 900	1.7
					
5-(4-Hydroxyphenyl)-5-phenylhydantoin	b	38.3	51 716	36 360	1.43
					
Benzoin	b	29.5	24 060	23 352	0.5
					
Terbutaline	c	8.5	44 396	17 964	0.5
					
Ibuprofen	b	36.4	61 912	49 944	0.6
					
Bupivacaine	b	23.8	19 072	20 048	0.6
					

[a] Letters in column 2 refer to: (a) MeCN–0.1% TEAA, pH 4 (40:60, v/v); 25 kV; 20°C. (b) MeCN–0.2% TEAA, pH 4 (20:80, v/v); 15 kV; 15°C. (c) MeCN–0.2% TEAA, pH 4 (50:50, v/v); 15 kV; 15°C.

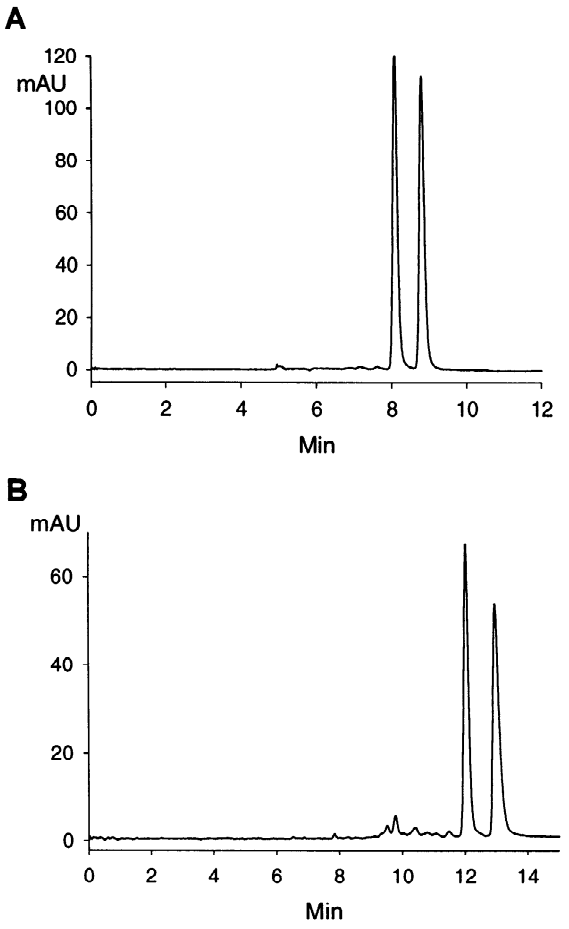


Fig. 2. Reversed-phase chiral CEC separation of (a) coumachlor and (b) tryptophan, on the teicoplanin CSP outlining high efficiency (86 000 and 125 000 plates m^{-1}, respectively, for the first eluting enantiomers) and resolution (3.0 and 2.9, respectively). Conditions: (a) MeCN–0.1% TEAA (pH 4) (40:60, v/v), 25 kV, 20°C and (b) MeCN–0.2% TEAA (pH 4) (50:50, v/v), 15 kV, 15°C; electrokinetic injection at 10 kV for 2 s; 200 nm and 10 bar pressurisation over the column.

but was stronger for the former. Lower MeCN content resulted in higher resolution and lower EOF values on each phase but surprisingly contrasting results were obtained for peak efficiency on the two different CSPs where lower MeCN contents were favoured on the antibiotic CSPs but higher on the WAX CSP. These contrasting results are not altogether surprising since the surface chemistry of each stationary phase examined was different and complex with each containing ionisable functionalities which inevitably contribute to the EOF profile and will thus influence peak efficiency.

The effects of MeOH to MeCN ratio and competing base and acid concentrations were examined on this teicoplanin CSP through the use of a statistical experimental design since in addition to obtaining optimal operating conditions, an estimate of the primary factor effects and potential interactions can also be attained. Factors examined included the ratio of MeOH and MeCN, HOAc and TEA concentrations. During the evaluation, responses used to measure factor effects were observed EOF, peak efficiency and resolution of terbutaline and metoprolol enantiomers. A run sequence generated by the statistical program (Modde version 4.0) was examined experimentally and the results obtained for each response at the factor points are shown for both racemic solutes in Table 2. A summary of fit plot for each response tested indicated that reliable models, calculated using partial least-squares, could be generated for each with average R^2 (coefficient of determination) and Q^2 (cross-validated correlation coefficient) values higher than 0.9 and 0.6, respectively. From these experimental design data it was possible to generate surface plots for each response examined in order to deduce the effect of each factor and to determine if an interaction occurred between them.

The effects of polar organic mobile phase on observed EOF on this teicoplanin CSP are plotted in Fig. 3. It is noted that since each of the three surfaces, indicating low to high volume ratio of HOAc are almost identical and plotted on the same scale, it can be concluded that the effect of HOAc on the EOF is almost negligible. In contrast to this, the effect of both TEA volume ratio and MeOH content on EOF is large where higher values are observed at low MeOH and at low TEA values, respectively. It is not surprising that a higher EOF is observed at low TEA concentrations since the electrophoretic mobility (μ_{eo}) is proportional to the zeta-potential (ξ) but more specifically proportional to the charge density at the plane of shear (σ) [41]. The zeta-potential is also inversely proportional to molar salt concentration (c), according to $\xi \propto -\log c$ [36], but the negligible effect of HOAc, shown in Fig. 3, may indicate that this is less significant for this CSP with polar organic conditions since both TEA and HOAc

Table 2
Statistical design used to evaluate polar organic mobile phase conditions for the separation of enantiomers on the teicoplanin CSP in CEC[a]

MeOH/MeCN/HOAc/TEA	Metoprolol		Terbutaline		μ_{eo}
	R_s	N_1 (plates m^{-1})	R_s	N_1 (plates m^{-1})	($\times 10^{-5}$ cm^2/Vs)
25/75/0.05/0.05	1.6	9564	–[b]	–[b]	16.23
95/5/0.05/0.05	3.2	87 272	6.2	102 160	7.67
50/50/0.2/0.05	1.6	17 420	3.2	15 576	15.31
25/75/0.3/0.05	1.6	11 216	2.4	3592	16.23
95/5/0.3/0.05	2.4	80 196	4.3	137 340	9.21
50/50/0.05/0.2	0.9	9096	3.9	24 708	12.53
95/5/0.2/0.2	2.9	55 316	5.8	62 552	5.06
25/75/0.05/0.3	0.9	13 536	2.3	8 680	15.24
95/5/0.05/0.3	2.1	70 348	5.3	79 160	4.03
25/75/0.3/0.3	1.8	27 076	3.0	7504	11.63
50/50/0.3/0.3	2.2	54 412	3.7	16 928	9.23
95/5/0.3/0.3	2.2	45 136	4.6	44 480	3.79
50/50/0.1/0.1	2.0	26 456	4.1	20 788	13.19
50/50/0.1/0.1	2.1	21 632	4.5	15 016	13.00
50/50/0.1/0.1	2.1	24 252	4.7	12 040	12.22

[a] Experimental factors include: MeOH content in which modifier conditions are calculated as MeOH–MeCN (X:100 − X, v,v) and HOAc–TEA concentrations are also expressed as volume ratios.
[b] No terbutaline peaks observed after 60 min.

will contribute to the overall molar salt concentration term. It is reasonable to assume that the TEA may be acting as a competing base and shielding the silanol groups necessary for EOF generation. In contrast to this, the effect of MeOH content on EOF may be easily interpreted by considering that lower electrophoretic mobilities are obtained with mobile phases having a lower dielectric constant to viscosity ratios (ϵ/η) [42].

Responses surfaces indicating the effects of mobile phase composition on enantiomeric peak efficiency and resolution were also generated for both terbutaline and metoprolol and shown in Fig. 4 for the former since the effects were similar for both solutes. The surfaces for efficiency (Fig. 4a) are plotted for three different MeOH contents as outlined with varying concentrations of acid and base additive. It is clear that higher efficiency values can be attained at higher MeOH contents where approximately 30 000, 5000 and 3000 plates per column are shown for 95, 50 and 25 MeOH volume ratio, respectively. Interestingly, the effects and influence

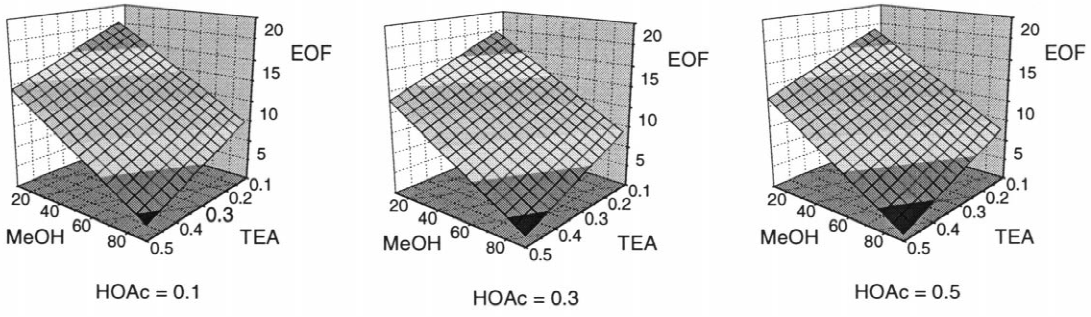


Fig. 3. Response surfaces generated by the Modde 4.0 statistical software from the data in Table 2 to show the effects of organic solvent and TEA–HOAc concentration and volume ratio on the EOF ($\times 10^{-5}$ cm^2 V^{-1} s^{-1}) generated on the teicoplanin CSP in CEC. Conditions are as outlined in Fig. 1 and Table 2.

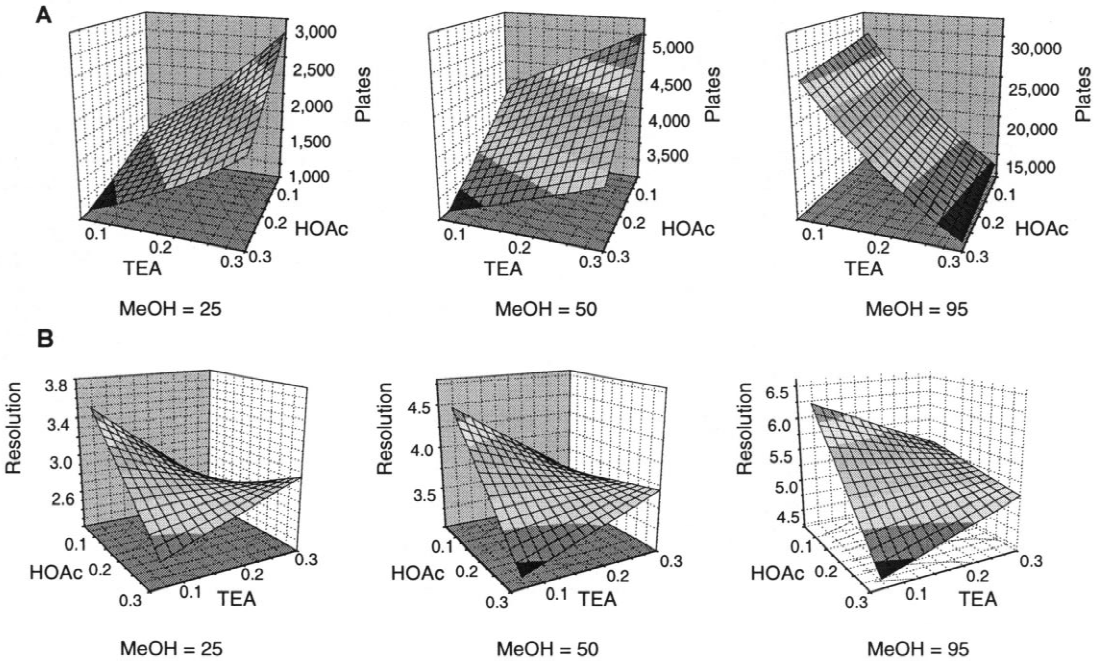


Fig. 4. Response surfaces generated by the Modde 4.0 statistical software from the data in Table 2 to show the effects of organic solvent and TEA–HOAc concentration and volume ratio on (a) the efficiency (plates per column) and (b) the resolution of terbutaline enantiomers on the teicoplanin CSP in CEC. Conditions are as outlined in Fig. 3.

of the TEA–HOAc additives is different at different MeOH contents. At lower contents (25 and 50 volume ratio) both acid and base concentrations play a significant role with higher efficiencies attained at high TEA and low HOAc contents. The opposite is observed, however, at high MeOH content (95 volume ratio) where higher efficiencies are attained at low TEA concentrations while HOAc has little effect. It is difficult to explain this unusual switch in acid–base additive effect on efficiency without equivocation since relatively little is known of the mechanisms by which electrolyte concentration affects column efficiency in CEC. The role of ionic concentration does not directly effect efficiency in CEC but effects the observed EOF as shown above and described by Wan and consequently will effect the resultant flow profile which in turn will strongly effect the peak efficiency [36,43]. The response surfaces for resolution of terbutaline enantiomers is also plotted and shown in Fig. 4b indicating that higher values can be obtained at higher MeOH contents and with low concentrations of TEA and HOAc. These effects on resolution are almost identical to those found on this phase in LC where a starting mobile phase of MeOH with trace amounts of acid and base additives is suggested for optimum results [38].

3.5. Screening of pharmaceutical racemic mixtures

Similar to that described above for reversed-phase operation, a number of pharmaceutical drugs were subsequently examined on this phase in the polar organic mode (Table 3). The majority of these were basic molecules but both neutral and acidic molecules were also included which resulted in high resolution and efficiency values for many analytes. It is interesting to note the difference in enantioselectivity obtained on this teicoplanin phase between reversed-phase (Table 1) and polar organic mode separation (Table 3). The β-blocking drugs could not be separated for example in reversed-phase conditions but easily separated in the polar organic mode and shown in Fig. 5a–c for the *tert*.-butyl analogue

Table 3
Pharmaceutical drug enantiomers examined in the polar organic mode on the teicoplanin CSP in CEC

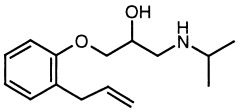
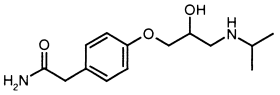
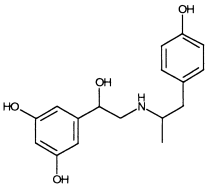
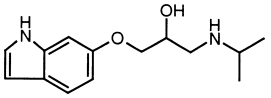
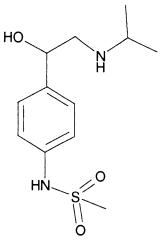
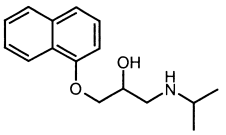
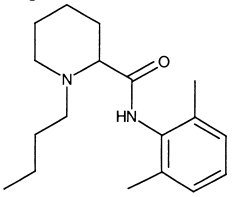
Racemate	Cond.[a]	t_{R1} (min)	N_1 (plates m^{-1})	N_2 (plates m^{-1})	R_s
Alprenolol	a	7.516	135 816	44 824	3.27
					
Atenolol	a	17.729	27 861	9,124	1.87
					
Fenoterol	b	10.322	48 784	54 028	2.56
					
Pindolol	c	10.868	76 936	54 028	2.43
					
Sotalol	b	11 789	43 308	38 340	2.13
					
Propranolol	b	9.450	59 604	59 908	2.40
					
Bupivacaine	b	9.774	42 520	62 392	0.63
					

Table 3. Continued

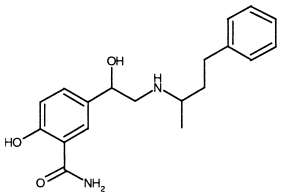
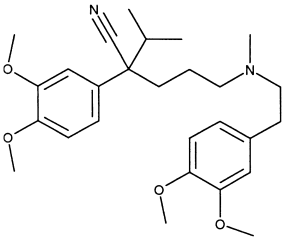
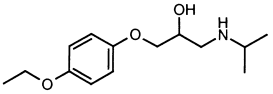
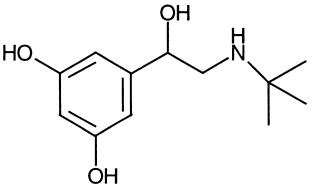
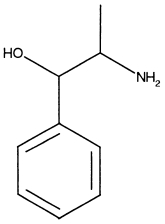
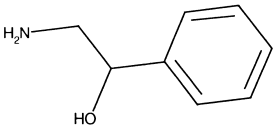
Racemate	Cond.[a]	t_{R1} (min)	N_1 (plates m^{-1})	N_2 (plates m^{-1})	R_s
Labetalol	b	15 804	129 584	34 768	4.89, 0.53
					
Verapamil	b	15.707	27 000	10 080	1.15
					
Metoprolol	d	8.749	77 100	52 024	3.17
					
Terbutaline	e	9.185	137 340	58 564	4.3
					
Phenylpropanolamine	a	8.256	71 332	17 688	0.45, 9.76
					
β-Hydroxy-phenethylamine	a	9.593	21 248	10 840	0.51
					

Table 3. Continued

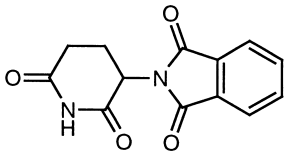
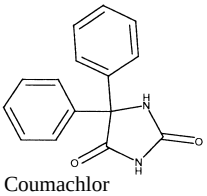
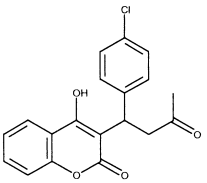
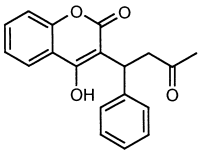
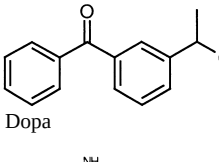
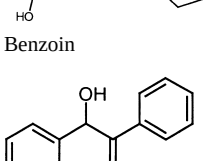
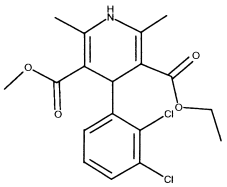
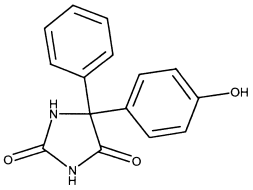
Racemate	Cond.[a]	t_{R1} (min)	N_1 (plates m^{-1})	N_2 (plates m^{-1})	R_s
Thalidomide 	a	7.115	81 484	–	–
5,5-Diphenyl hydantoin 	a	7.328	125 624	–	–
Coumachlor 	a	11.748	39 008	–	–
Warfarin 	a	9.428	67 280	–	–
Ketoprofen 	a	9.330	56 700	–	–
Dopa 	a	6.704	23 072	–	–
Benzoin	a	6.480	100 952	–	–

Table 3. Continued

Racemate	Cond.[a]	t_{R1} (min)	N_1 (plates m^{-1})	N_2 (plates m^{-1})	R_s
Felodipine	a	6.248	229 932	–	–
					
5-(4-Hydroxyphenyl)-5-phenylhydantoin	a	6.983	22 324	–	–
					

[a] Letters in column 2 refer to: (a) MeOH–MeCN–TEA–HOAc (80:20:0.1:0.1, v/v/v/v); 20 kV; 15°C; (b) MeOH–MeCN–TEA–HOAc (95:5:0.05:0.3, v/v/v/v); 15 kV; 15°C. (c) MeOH–MeCN–TEA–HOAc (95:5:0.3:0.3, v/v/v/v); 25 kV; 20°C. (d) MeOH–MeCN–TEA–HOAc (80:20:0.1:0.1, v/v/v/v); 25 kV; 15°C. (e) MeOH–MeCN–TEA–HOAc (95:5:0.05:0.3, v/v/v/v); 25 kV; 15°C.

of metoprolol, alprenolol and pindolol where resolution values of 2.7, 2.5 and 2.0 with efficiency values of 57 000, 96 000 and 58 000 plates m^{-1} for the first eluted enantiomer are demonstrated, respectively. Interestingly, this was also found to be the situation in our earlier studies with the vancomycin CSP [7]. In addition to the differences observed for the β-blocking drugs in aqueous and non-aqueous modes, there were also a number of drugs that could not be separated in the polar organic mode (Table 3) that were easily separated in the reversed-phase mode (Table 1). Warfarin and coumachlor for example were separated with high resolution and efficiency values (2.6 and 3.0 and 89 000 and 86 000 plates m^{-1}, respectively) in reversed-phase but not at all in the polar organic mode despite having longer

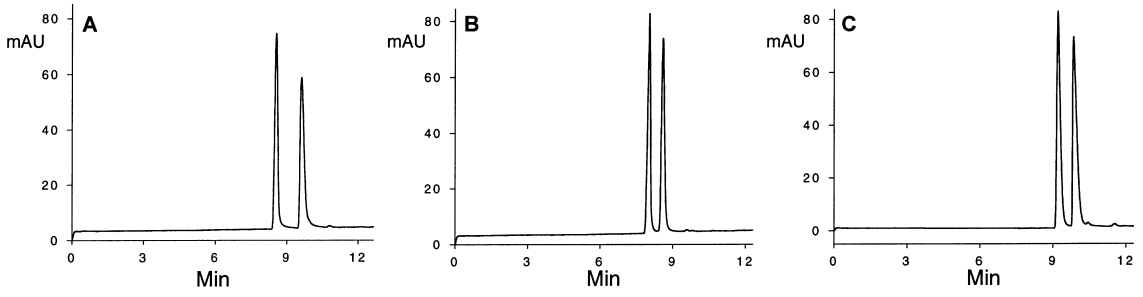


Fig. 5. Polar organic mode chiral CEC separation of (a) the *tert*.-butyl analogue of metoprolol (b) alprenolol and (c) pindolol where resolution values of 2.7, 2.5 and 2.0 with efficiency values of 57 000, 96 000 and 58 000 plates m^{-1} for the first eluting enantiomer were obtained. Conditions: MeOH–MeCN–TEA–HOAc, (a) (95:5:0.05:0.3, v/v/v/v), 335 mm×75 μm I.D. (L_d=250 mm), 15 kV; 15°C; electrokinetic injection at 10 kV for 2 s; 200 nm and 10 bar pressurisation over the column.

retention times. These results indicating the complementary multi-modal nature of these antibiotic chiral selectors are in agreement with earlier LC data [26] but further indicate their usefulness as chiral or achiral chromatographic supports for separating a range of compound classes in drug discovery processes.

3.6. Influence of temperature

Since temperature has been shown to have a significant effect on both achiral CEC parameters and enantioselectivity with this phase in LC, it was considered important to examine its influence for this CSP when operated in the polar organic mode. The EOF was found to increase by almost 100% from 5.7 to 10.3×10^{-5} cm^2 V^{-1} s^{-1} with an increase in column cassette temperature from 15 to 60°C (Fig. 6). This trend has been previously observed in chiral CEC studies but not to the extent described above [5,7,10,19]. In addition to these changes, the resolution of terbutaline was found to decrease as expected from corresponding LC data. Surprisingly, the efficiency of terbutaline enantiomers was not significantly affected by temperature and difficult to interpret since we and others have recently demonstrated higher enantiomeric efficiency at higher temperatures in chiral CEC [5,7].

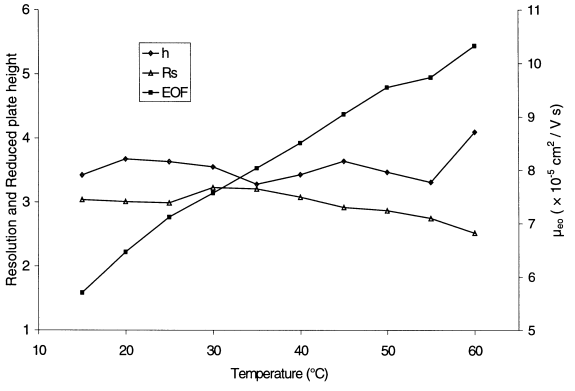


Fig. 6. Effect of column temperature on the observed EOF, efficiency and resolution of terbutaline enantiomers on the teicoplanin CSP in polar organic mode CEC. Conditions: MeOH–MeCN–TEA–HOAc, (a) (95:5:0.05:0.3, v/v/v/v), 335 mm×75 μm I.D. (L_d 250 mm), 25 kV; electrokinetic injection at 10 kV for 2 s; 200 nm and 10 bar pressurisation over the column.

4. Conclusions

The application of a teicoplanin CSP for high resolution and efficiency chiral separations in CEC is demonstrated for a number of pharmaceutical drugs of interest in either reversed-phase or the non-aqueous polar organic mode. Enantioselectivity was obtained for 25 racemic solutes including examples of neutral, acidic and basic molecules. The effects of the non-aqueous polar organic conditions on the observed EOF, resolution and efficiency indicated that MeOH content of the mobile phase was the predominant factor for obtaining high values. The effects of temperature were additionally found, as expected, to influence these factors and optimum results were obtained at lower temperature values. Overall this paper indicates that a teicoplanin CSP can be effectively applied in CEC offering complementary selectivity for a number of racemic drugs in reversed-phase and polar organic conditions and it is hoped that further work will extend its application to more traditional normal-phase conditions using this electrophoretic technique.

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