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Research Article

Investigation of enantiomeric separation of basic drugs by capillary electrophoresis using clindamycin phosphate as a novel chiral selector

A wide number of chiral selectors have been employed in CE and among them macrocyclic antibiotics exhibited excellent enantioselective properties toward plenteous racemic drugs. Different from macrocyclic antibiotics, the use of lincomycin antibiotics as chiral selectors has not been reported previously. In this study clindamycin phosphate belonging to the group of lincomycin antibiotics is first used as a novel chiral selector for the enantiomeric separations of several racemic basic drugs, which possess high separability, consisting of nefopam, citalopram, tryptophan, chlorphenamine and propranolol. Other basic drugs giving partial enantioseparation include tryptophan methyl ester, metoprolol and atenolol. Clindamycin phosphate possesses advantages such as high solubility and low viscosity in the water and very weak UV absorption. In the course of this work we observed that both migration time and enantioseparation were influenced by several parameters such as pH of the BGE, clindamycin concentration, capillary temperature, applied voltage and organic modifier. The optimum pH that was in the neutral or weak basic region but varied among drugs, a low capillary temperature and a clindamycin concentration of 60 or 80 mM are recommended as the optimum conditions for chiral separation of these drugs. Moreover, comparison of the influences of the studied parameters was further investigated by means of Statistical Product and Service Solutions in this paper.

Keywords:
Antibiotics / Basic drugs / Capillary electrophoresis / Clindamycin phosphate / Enantiomeric separation
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1 Introduction

Analytical methods used so far for the separation of enantiomeric compounds include GC [1], HPLC [2], TLC [3] and CE [4–9]. HPLC has greatly contributed to this subject and a number of systems have been established for various kinds of chiral drugs. In addition, CE has been demonstrated to be a powerful tool for chiral separations during the past several years, mainly using the direct separation method, where the chiral selector is added to the BGE [10]. Various kinds of chiral selectors have been developed, including cyclodextrins and derivatives, crown ether, antibiotics, proteins, polysaccharides and so on [11–18]. The high resolution (Rs) power and efficiency of CE, combined with the wide number of chiral selectors that can be employed in order to improve the stereoselectivity of the separations, and the low amount of both electrolyte and sample, make this technique complementary or even competitive with HPLC, where expensive chiral columns are requested [19].

Since Armstrong and coworkers [20–22] demonstrated that vancomycin was a useful chiral selector, antibiotics such as rifamycins, streptomycin, ristocetin, teicoplanin, kanamycin and fradiomycin have proved to be a powerful class of chiral selectors for CE. All these antibiotics have many stereogenic centers and functional groups, allowing them to have multiple interactions with chiral molecules. Owing to the presence of ionogenic groups in their structure, they can be either positively or negatively charged as well as uncharged depending on the buffer pH [23]. Two

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Abbreviations: ATE, atenolol; **CP,** clindamycin phosphate; **CHL,** chlorphenamine maleate; **CIT,** citalopram hydrobromide; **IBU,** ibuprofen; **KET,** ketoprofen; **MET,** metoprolol tartrate; **MIT,** mitiglinide; **NAP,** naproxen; **NAT,** nateglinide; **NEP,** nefopam phosphate; **PRA,** pranoprofen; **PRO,** propranolol hydrochloride; **Rs,** resolution; **SPSS,** Statistical Product and Service Solutions; **TME,** tryptophan methyl ester

important interactions are hydrogen bond and ionic interaction [24].

Although macrocyclic antibiotics have shown to be an excellent chiral selectors in CE, they exhibited some drawbacks such as strong absorption in the UV wavelengths, low solubility in the water and adsorption on the capillary wall [23]. Owing to this fact, we searched for other new types of antibiotics that can be used as chiral selectors for CE. In this work we find that clindamycin phosphate (CP) belonging to the lincomycin family exhibits good enantioselectivity to some basic drugs for the first time. Different from macrocyclic antibiotics, the use of lincomycin antibiotics as chiral selectors has not been reported previously. CP has a molecular mass of 505 and contains an amide portion, an amino portion and an aglycon portion in each molecules (see Fig. 1). Compared with macrocyclic antibiotics, it possesses not only high solubility but also low viscosity in the water. Moreover, with the lack of aromatic rings in the structure, it exhibits very weak UV absorption. These properties, which have taken researchers a lot of time and effort in improving analytical methods, in this case turn out to be advantages. In this study we investigated the effects of chiral selector concentration, pH of the BGE, running voltage, organic modifier type and concentration as well as capillary temperature on chiral separations of nefopam hydrochloride (analgesic drug, NEF), citalopram hydrobromide (psychotolytic drug, CIT), tryptophan (a member of α-amino acid, TRY), tryptophan methyl ester (TME), metoprolol tartrate (β-blocker, MET), chlorphenamine maleate (antihistaminic drug, CHL), propranolol hydrochloride (β-blocker, PRO) and atenolol (β-blocker, ATE). We also studied the chiral separations of several acidic chiral compounds that were ketoprofen (analgesic drug, KET), ibuprofen (analgesic drug, IBU), naproxen (analgesic drug, NAP), pranoprofen (analgesic drug, PRA), mitiglinide (hypoglycemic agent, MIT) and nateglinide (hypoglycemic agent, NAT). The result suggested that CP exhibited good enantioselectivity to these basic drugs and showed no resolving power to the acidic compounds. Based on this result, we present details of chiral separations of the eight basic enantiomeric pairs with this novel antibiotic selector. Subsequently, comparison of influences of all the parameters on enantioseparation was further investigated using multivariate analysis of variance as a calculation method by means of Statistical Product and Service Solutions (SPSS) in this paper.

2 Materials and methods

2.1 Chemicals and reagents

CP (purity > 99%, Fig. 1) was supplied by Jiangsu Institute For Drug Control (Nanjing, China). TRY, TME, PRO, ATE, KET, IBU, NAP and PRA were purchased from Sigma (St. Louis, MO, USA). NEF, CIT, CHL, MET, MIT and NAT were supplied by Jiangsu Institute For Drug Control. All these drug samples were racemic mixtures. Methanol, ethanol, isopropanol and ACN, all of HPLC grade, were purchased from Jiangsu Hanbon Science and Technologies (Nanjing, China). Sodium hydroxide, hydrochloric acid and sodium tetraborate decahydrate, all of analytical grade, were purchased from Nanjing Chemical Reagent (Nanjing, China). Double distilled water was used throughout all the experiments.

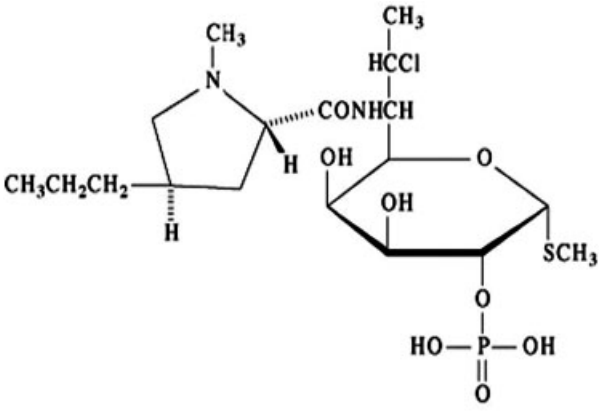

Figure 1. Structure of CP used in this study.

2.2 Apparatus

Electrophoretic experiments were performed with an Agilent 3D CE system (Agilent Technologies, Waldbronn, Germany), which consisted of a sampling device, a power supply, a photodiode array UV detector (wavelength range from 190 to 600 nm) and a data processor. The whole system was driven by Agilent ChemStation software (Revision B.02.01-SR2) for system control, data collection and analysis. It was equipped with a 50 cm (41.5 cm effective length) × 50 μm id uncoated fused-silica capillary (Hebei Yongnian County Reafine Chromatography, Hebei, China). A new capillary was flushed for 10 min with 1 M HCl, 10 min with 1 M NaOH and 10 min with water, respectively. At the end of each day it was flushed successively with 0.1 M NaOH (5 min) and water (5 min). Between consecutive injections the capillary was rinsed with 0.1 M NaOH, water and running buffer for 2 min each. Sample injections were performed by pressure (50 mbar, 4 s). Enantioseparations were performed at a constant voltage in a range of 12–25 kV, and the temperature of capillary was controlled at 14–28°C using an air-cooling system.

2.3 Experimental procedures

Borax buffers were used as the buffers for CE. Buffer solution was a 40 mM borax buffer containing methanol (20% v/v), if not stated otherwise. The running BGE containing CP was freshly prepared by dissolving CP (80 mM or 4 w/v%, if not stated otherwise) in the buffer solution having a specified pH, and then adjusting pH exactly to a desired value by adding 1 M hydrochloric acid or

sodium hydroxide solution. The pH values of these running buffers were adjusted in the range of 6.6–8.0, and then were always checked before and after each run. Each buffer solution was filtered through a 0.45 mm pore membrane filter and degassed by sonication before use.

The racemic drugs in this study were dissolved in methanol (ATE, KET, IBU, NAP, PRA, MIT and NAT) or water (others). Final sample solutions for injection were all at a concentration of 0.4 mg/ml.

2.4 Calculation of Rs

The Rs of the enantiomer was calculated from $Rs = 2\,(t_2–t_1)/(w_1+w_2)$, where t_1 and t_2 are the migration times of two enantiomers, and w_1 and w_2 are the width of their peaks at the baseline.

3 Results and discussion

3.1 Enantioseparation of basic drugs

As shown in Fig. 2, CP allowed baseline separations of the enantiomers of NEF, CIT, TRY, CHL and PRO as well as partial Rs of TME, MET and ATE under our experimental conditions. Much of the antibiotics' selectivity can be attributed to their ability to form multiple interactions such as hydrogen-bonding, ionic, hydrophobic ones and so on. Two primary interactions are thought to be hydrogen bond

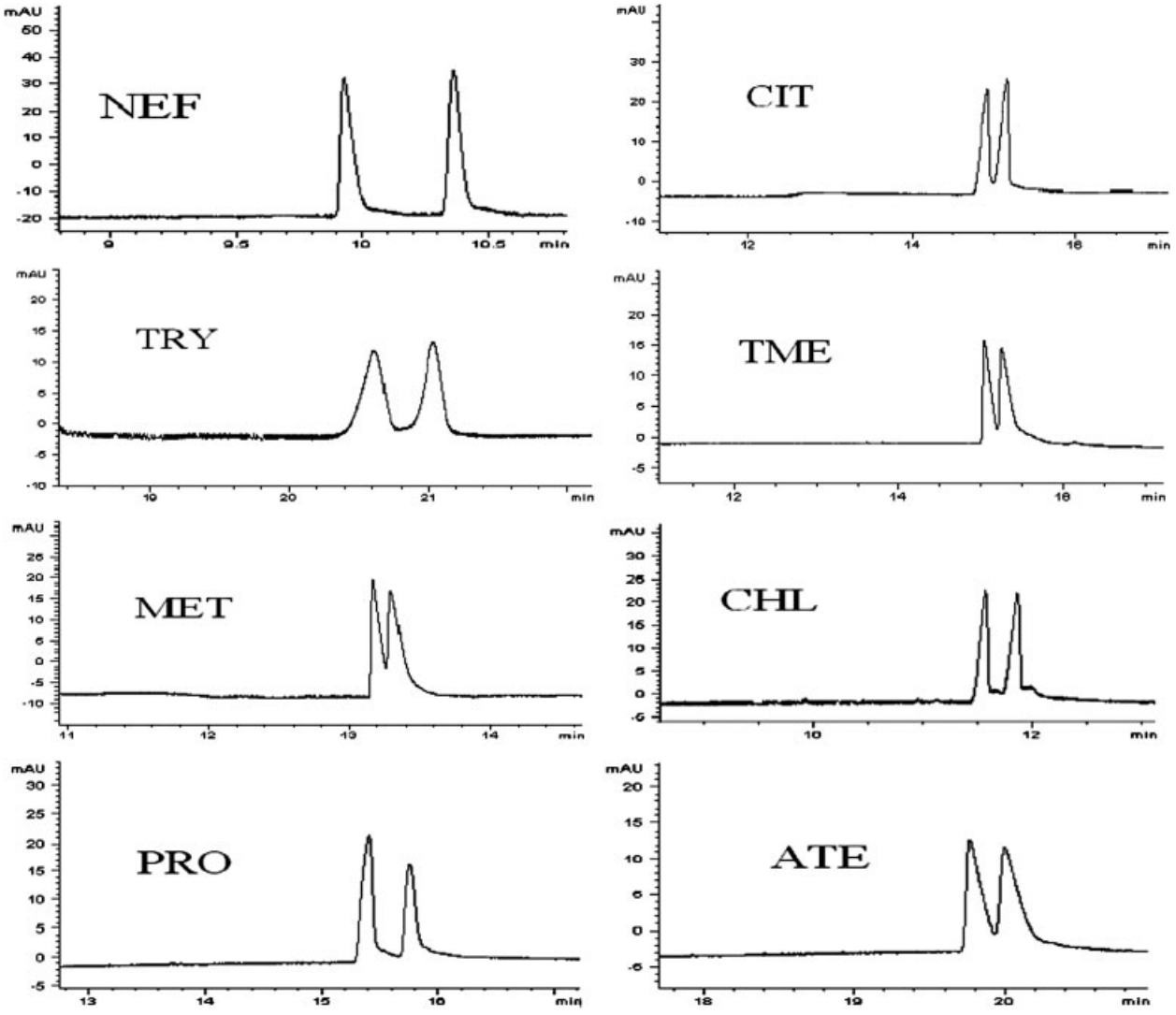

Figure 2. Electropherograms of the chiral separations of NEF, CIT, TRY, TME, MET, CHL, PRO and ATE. Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm id; BGE, 40 mM borax buffer with methanol (20% for NEF, CIT, CHL and MET, 30% for TRY and TME, 50% for ATE, v/v) or ethanol (20% for PRO, v/v) containing CP to a concentration of 60 mM (CHL) or 80 mM (others); buffer pH, 7.0 (CHL and PRO), 7.3 (NEF, CIT and TME) and 7.6 (TRY, MET and ATE); applied voltage, 22 kV (NEF) or 18 kV (others); capillary temperature, 14°C (CIT and MET) or 20°C (others); detection, UV absorption at 215 nm (NEF), 245 nm (CIT), 277 nm (TRY), 277 nm (TME), 221 nm (MET), 265 nm (CHL), 289 nm (PRO) and 225 nm (ATE).

and ionic reaction [25]. In the course of this work, due to the presence of phosphate group (see Fig. 1), which allows CP to be negatively charged at pH 6.6–8.0, CP shows strong ionic interactions with the basic compounds (NEF, CIT, TRY, TME, MET, CHL, PRO and ATE). It is obvious that the hydrogen-bonding exists between the amino and hydroxyl groups in the basic drugs and the amino and hydroxyl groups in CP. As described by Armstrong *et al.*[20–22], antibiotics that were negatively charged exhibited almost no enantioselectivity toward acidic compounds. Based on the structures of KET, IBU, NAP, PRA, HYP and NAT, they all have carboxyl groups. These acidic compounds had no separation compared with the basic drugs investigated in this paper. In this study the chiral separations of basic drugs were achieved depending on several operational parameters. Thus, in order to test this chiral selector for our purposes and to find the optimum experimental conditions we studied the effects of buffer pH, CP concentration, capillary temperature, running voltage and organic modifier.

3.2 Effect of the pH of the BGE

The pH of the BGE is a very important parameter to be studied when chiral CE analysis using antibiotics is carried out. The BGE pH can change the charge of both analytes and chiral selector and thus their electrophoretic mobilities, so that the electrostatic and hydrogen-bonding interactions between CP and analytes are influenced. As a result, we investigated the effect of this parameter on the enantiomeric separation of the studied compounds.

The effects of the pH on Rs and migration times were studied using BGEs at pH 6.6, 7.0, 7.3, 7.6 and 8.0 supplemented with 80 mM (4% w/v) of CP. Table 1 depicts the pH dependence of the separations of all the enantiomers, and the optimum pH values were 7.0 for CHL and PRO; 7.3 for NEF, CIT and TME; 7.6 for TRY, MET and ATE. The variation of the optimum pH for chiral separation among drugs will give important information on the interaction of CP with these drugs. Since the degree of protonation in basic compounds and CP is dependent on pH, the optimum pH will point to the most compatible state of the amino and hydroxyl functions in the drugs for binding or reacting presumably to the amino and hydroxyl groups or the phosphate group in CP. In the present system of the basic drug–CP combination, it is important to know what kinds of bindings exist between this selector and the basic drugs. One of them can be anticipated to be the hydrogen-bonding between the amino and hydroxyl groups in a drug and the amino and hydroxyl groups in CP. Another one is thought to be the ionic interaction between the amino group in a drug and the phosphate group in CP. As a result, over the range of 7.0–7.6 of buffer pH, there was a compatible complexation between the analytes and chiral selector. Whereas, when the pH value was under 7.0, there was an excessive decrease of the negative charge of the phosphate group in CP. On the other hand, when the pH value was over 7.6, there was an excessive decrease of the positive charge of the amino group in a drug. In the two cases as above described, the intensity of the interaction between analytes and chiral selector would become so weak that CP exhibited poor enantioselective properties toward these basic drugs.

It was also observed that the increase of the pH of the BGE caused longer migration times (except TRY), because the increase of the pH caused a decrease of the positive charge of analytes. However, taking TRY into consideration, when the pH value was from 6.6 to 7.6, we found its migration time decreased from 18.89 min to 15.99 min on account of the carboxyl group that existed in the molecule as well as the amino group.

3.3 Effect of CP concentration

To obtain the optimum CP concentration for chiral separations of all the analytes, we tested five different concentrations (20, 40, 60, 80 and 100 mM) using optimum

Table 1. Effect of buffer pH on the migration times and resolution (Rs) of basic compounds[a)]

Chiral compounds	pH									
	6.6		7.0		7.3		7.6		8.0	
	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs
NEF	9.87/9.76	0.98	10.75/10.51	2.25	11.80/11.27	5.66	12.77/12.05	5.19	25.17/22.18	3.20
CIT	11.14	<0.5	11.29/11.17	1.16	12.74/12.53	1.63	13.53/13.33	0.89	23.08	<0.5
TRY	18.89	<0.5	17.40/17.26	0.96	16.76/16.50	1.25	16.31/15.99	1.51	34.00/32.25	1.47
TME	9.57/9.48	0.95	11.03/10.88	1.03	12.75/12.60	1.08	13.83/13.68	0.87	21.86	<0.5
MET	9.78	–	10.10	<0.5	10.61/10.53	0.90	11.08/10.97	0.96	15.37/15.17	0.52
CHL	10.33	<0.5	12.21/12.01	1.51	13.46/13.14	1.39	13.67/13.30	1.28	24.87	<0.5
PRO	10.18/10.10	0.58	11.53/11.34	1.66	13.29/12.93	1.52	13.72/13.55	0.87	25.16	<0.5
ATE	9.58	–	9.93	<0.5	10.33/10.27	0.53	10.41/10.34	0.60	13.88	<0.5

a) Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm id; BGE, 40 mM borax buffer with methanol (20% v/v) containing CP to a concentration of 80 mM; buffer pH, as shown in Table 1; applied voltage, 18 kV; capillary temperature, 20°C; detection, as in Fig. 2.

pH values of 7.0, 7.3 and 7.6 (as described in paragraph 3.2). Table 2 shows the effects of CP concentration on the migration times and separations of all enantiomers. It is evident that the Rs of the enantiomers increased in step with CP concentration ascending from 20 mM to 60 mM and 80 mM. In particular, the Rs of almost all the basic compounds studied in this work decreased when CP concentration rose from 80 to 100 mM. The reason may be that at the maximum concentration, Rs decreased presumably due to enhanced peak broadening and saturated complexation between analytes and chiral selector.

In addition, it can be remarked from Table 2 that in parallel with the increase of CP concentration from the minimum (20 mM) to the maximum (100 mM), migration times of all enantiomers increased. This is because complexation between analytes and chiral selector increased, as well as buffer viscosity, as CP concentration rose.

In conclusion, the additive CP concentrations of 60 mM (3% w/v) and 80 mM (4% w/v) were proved to be optimum for the separations of these drug enantiomers.

3.4 Effect of capillary temperature

The effects of capillary temperature on the migration times and separations of enantiomers were investigated over a range of 14–28°C by performing the electrophoretic runs with 80 mM of CP and at each drug's optimum pH. As we observed in Table 3, the increase of capillary temperature brought a general decrease of migration times and Rs. That is because higher temperature caused a decrease of buffer viscosity and thus changes in the mobilities of both analytes and antibiotics.

Table 2. Effect of CP concentration on the migration times and Rs of basic compounds[a)]

Chiral compounds	CP concentration (mmol/L)									
	20		40		60		80		100	
	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs
NEF	9.18/9.07	1.02	10.39/10.26	2.23	10.87/10.40	4.15	11.80/11.27	5.66	14.13/13.62	2.79
CIT	8.16/8.07	1.01	9.97/9.86	1.39	11.63/11.47	1.46	12.74/12.53	1.63	17.35/17.07	1.03
TRY	8.03	<0.5	10.28/10.09	0.62	14.16/13.95	1.23	16.31/15.99	1.51	28.62/27.87	1.43
TME	10.33	<0.5	11.37/11.25	0.52	12.28/12.04	0.87	12.75/12.60	1.08	16.02/15.76	1.12
MET	7.97	<0.5	9.26/9.20	0.66	10.10/10.01	0.88	11.08/10.97	0.96	15.90	<0.5
CHL	8.25/8.11	0.67	9.94/9.73	1.23	11.86/11.57	2.08	12.21/12.01	1.51	12.53/12.42	0.92
PRO	7.46/7.34	0.73	9.39/9.15	1.33	11.37/11.04	2.11	11.53/11.34	1.66	12.51/12.36	1.20
ATE	7.13	<0.5	8.91/8.88	0.53	10.14/10.06	0.68	10.41/10.34	0.60	14.82	<0.5

a) Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm id; BGE, 40 mM borax buffer with methanol (20% v/v); CP concentration, as shown in Table 2; buffer pH, 7.0 (CHL and PRO), 7.3 (NEF, CIT and TME) and 7.6 (TRY, MET and ATE); applied voltage, 18 kV; capillary temperature, 20°C; detection, as in Fig. 2.

Table 3. Effect of capillary temperature on the migration times and Rs of basic compounds[a)]

Chiral compounds	Capillary temperature (°C)									
	14		17		20		24		28	
	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs
NEF	13.96/13.34	6.23	12.81/12.23	5.99	11.80/11.27	5.66	11.00/10.50	5.40	9.98/9.53	5.01
CIT	15.16/14.91	1.68	14.08/13.84	1.65	12.74/12.53	1.63	11.90/11.73	1.51	10.97/10.83	1.49
TRY	19.86/19.43	1.89	18.29/17.90	1.82	16.31/15.99	1.51	15.01/14.71	1.42	13.69/13.42	1.36
TME	15.09/14.91	1.16	13.96/13.79	1.12	12.75/12.60	1.08	11.88/11.75	1.02	10.97/10.85	0.97
MET	13.29/13.16	1.09	12.19/12.07	1.06	11.08/10.97	0.96	10.33/10.22	0.94	9.35/9.25	0.92
CHL	14.50/14.29	1.57	13.77/13.56	1.54	12.21/12.01	1.51	11.21/11.02	1.50	10.15/9.97	1.48
PRO	14.23/14.01	1.76	13.27/13.05	1.71	11.53/11.34	1.66	10.98/10.77	1.63	10.05/9.86	1.60
ATE	12.42/12.32	0.68	11.42/11.33	0.66	10.41/10.34	0.60	9.52/9.45	0.54	8.68/8.61	0.52

a) Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 ìm id; BGE, 40 mM borax buffer with methanol (20% v/v) containing CP to a concentration of 80 mM; buffer pH, 7.0 (CHL and PRO), 7.3 (NEF, CIT and TME) and 7.6 (TRY, MET and ATE); applied voltage, 18 kV; capillary temperature, as shown in Table 3; detection, as in Fig. 2.

In this system the electrophoretic migration of enantiomers and EOF was commonly toward the cathode, and the electrophoretic migration of chiral selector was toward the anode. Thus, the decrease in migration time with temperature rise demonstrated weakening of the interaction between enantiomers and CP.

3.5 Effect of running voltage

Generally, the effect of applied voltage embodies three aspects described as follows: the efficiency of electrophoresis, Rs and migration time. Higher voltage brings higher CE efficiency, shorter analysis time and more Joule heating (a source of band broadening and migration time reproducibility problems). Thus, as long as no excessive Joule heating is generated during the electrophoresis process, running voltage is supposed to be adopted as highly as possible.

The effects of applied voltage on the migration times and separations of enantiomers were investigated over a range of 12–25 kV. It was observed that the increase in applied voltage brought a general decrease in the migration times of all enantiomers, because high voltage caused high migration velocity of both analytes and EOF. However, the Rs of the enantiomers did not alter obviously, since high voltage caused not only high CE efficiency leading to reducing peak broadening but also short analysis time leading weak complexation between analytes and chiral selector.

3.6 Effect of organic modifier

The use of an organic additive to the BGE can have strong influence on enantiorecognition depending on the antibiotic as well as on analyte type. The organic modifier can influence several parameters such as the viscosity of the BGE, the mobilities of analytes and chiral selector, the inclusion complexation (or competition between solutes and organic modifiers), the solubility of enantiomers and the EOF (variation of the dielectric constant and the zeta potential of the capillary wall) [26]. Furthermore, the presence of the organic modifier seems to enhance the complexation interaction involved in the enantioseparation mechanism when using antibiotics [27].

3.6.1 The type of the organic modifier

It has been reported that the addition of organic solvents such as methanol (protic) or ACN (aprotic) to the BGE containing antibiotics can play an important role in enantiomer Rs. Four organic modifiers, methanol, ethanol, isopropanol and ACN, were each added to the BGE with the concentration of 20% v/v. Above all, we observed that the separations in the presence of organic modifier were better than the ones in the absence of organic modifier. In particular, we found that isopropanol was the most effective solvent that was used for restraining the EOF. As a result, the peaks of the analytes and solvent (reversal peaks in the chromatograms) were mixed when using isopropanol as the organic additive, and it was difficult to identify the peaks of the enantiomers separately. According to this result, isopropanol was not employed as an organic modifier for the separation of these drug enantiomers in this work.

Figure 3 illustrates the effects of different organic solvent on chiral Rs of the studied basic compounds. Comparing the three organic solvents (methanol, ethanol and ACN), it is obvious that for NEF, CIT, CHL, MET and TME, methanol is the most useful organic modifier, while ethanol is the best choice for the other compounds.

3.6.2 The concentration of the organic modifier

Considering the results obtained, we further investigated the effect of methanol concentration (10–50% v/v) on chiral separation of the studied enantiomers. As can be observed in Table 4, the increase in methanol concentration caused a general increase in the migration times of solutes probably due to the changes of the buffer viscosity, the interactions with the complexing antibiotic and the EOF. Table 4 also shows that a maximum of Rs was recorded at 20% (30%) of methanol for NEF and CHL (CIT and TRY), while the optimum methanol concentration for TME and PRO (MET and ATE) was found at 40% (50%).

3.7 Comparison of influences of all the parameters by means of SPSS

In order to obtain the dominant parameters, we compared the influences of all parameters using SPSS as a calculation tool. An approach performed concretely was entitled multivariate analysis of variance supplied by SPSS. The study was carried out by taking NEF, PRO, TRY and CHL, which possessed higher separability as model drugs. In this method, buffer pH (A), CP concentration (B), capillary temperature (C), applied voltage (D) and methanol

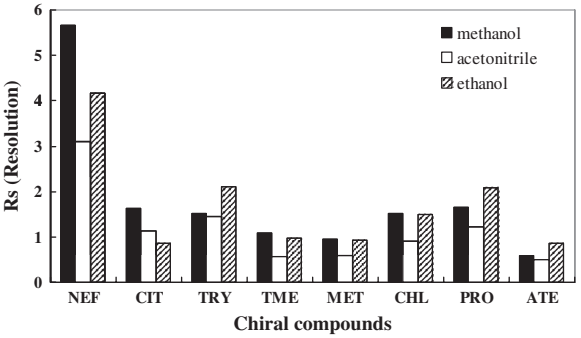

Figure 3. Effect of organic solvent on the Rs of basic compounds. Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm id; BGE, 40 mM borax buffer containing CP to a concentration of 80 mM; organic solvent, as shown in Fig. 3; buffer pH, 7.0 (CHL and PRO), 7.3 (NEF, CIT and TME) and 7.6 (TRY, MET and ATE); applied voltage, 18 kV; capillary temperature, 20°C; detection, as in Fig. 2.

Table 4. Effect of methanol concentration on the migration times and Rs of basic compounds[a)]

Chiral compounds	Methanol concentration (% v/v)									
	10		20		30		40		50	
	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs	t_2/t_1 (min)	Rs
NEF	8.97/8.48	3.68	11.80/11.27	5.66	14.75/14.09	5.13	18.64/17.82	4.85	23.20/22.06	3.48
CIT	11.96/11.71	1.06	12.74/12.53	1.63	15.02/14.81	1.80	18.77/18.50	1.25	21.84/21.57	1.18
TRY	10.50/10.34	1.24	16.31/15.99	1.51	21.03/20.61	1.86	31.91/31.24	1.83	40.12/39.36	1.61
TME	9.41/9.33	0.84	12.75/12.60	1.08	15.25/15.04	1.23	18.49/18.19	1.33	21.92/21.54	1.31
MET	7.99/7.93	0.88	11.08/10.97	0.96	15.05/14.89	1.05	17.37/17.18	1.07	21.30/21.01	1.13
CHL	8.36/8.18	1.18	12.21/12.01	1.51	15.28/15.06	1.43	19.36/18.93	1.38	23.71/23.23	1.24
PRO	8.08/7.93	1.22	11.53/11.34	1.66	15.08/14.79	1.76	18.92/18.32	2.38	20.55/19.91	1.01
ATE	7.21/7.17	0.56	10.41/10.34	0.60	13.14/13.03	0.60	16.58/16.42	1.13	19.99/19.76	1.27

a) Conditions: fused-silica capillary, 50 cm (41.5 cm effective length) × 50 μm id; BGE, 40 mM borax buffer containing CP to a concentration of 80 mM; Methanol concentration, as shown in Table 4; buffer pH, 7.0 (CHL and PRO), 7.3 (NEF, CIT and TME) and 7.6 (TRY, MET and ATE); applied voltage, 18 kV; capillary temperature, 20°C; detection, as in Fig. 2.

Table 5. Tests of between-subjects effects[a)]

Source	Dependent variable	DF	Mean square	*F*	Sig.
A	Rs (NEF)	4	5.096	3.441	0.129
	Rs (PRO)	4	0.429	5.272	0.068
	Rs (TRY)	4	0.203	4.132	0.099
	Rs (CHL)	4	0.362	7.148	0.002
B	Rs (NEF)	4	5.417	3.657	0.119
	Rs (PRO)	4	0.278	3.415	0.131
	Rs (TRY)	4	0.253	5.157	0.071
	Rs (CHL)	4	0.309	6.258	0.002
C	Rs (NEF)	4	1.197	0.808	0.579
	Rs (PRO)	4	0.004	0.050	0.993
	Rs (TRY)	4	0.105	2.143	0.239
	Rs (CHL)	4	0.001	0.141	0.958
D	Rs (NEF)	4	0.119	0.080	0.984
	Rs (PRO)	4	0.004	0.051	0.993
	Rs (TRY)	4	0.017	0.354	0.831
	Rs (CHL)	4	0.003	0.338	0.841
E	Rs (NEF)	4	0.945	0.638	0.663
	Rs (PRO)	4	0.250	3.080	0.151
	Rs (TRY)	4	0.067	1.356	0.388
	Rs (CHL)	4	0.013	1.423	0.371

a) Calculational method: multivariate analysis of variance supplied by SPSS; A, buffer pH; B, CP concentration; C, capillary temperature; D, applied voltage; E, methanol concentration; DF, degree of freedom; *F*, value of *F*-tests; Sig., the *p*-value of fixed factors.

concentration (E), these five parameters were considered as fixed factors, when the Rs of NEF, PRO, TRY and CHL were regarded as dependent variables. The data operated by SPSS were obtained from the experimental results of the effects of A–E on the chiral separations. A command entitled ''Analyze → General Linear → Multivariate'' was carried out for tests of between-subjects effects. As a result, degree of freedom (DF), Mean Square, *F* (value of *F*-tests) and Sig. (the *P*-value of fixed factors) were presented in Table 5. The meaning of sig. is described as follows: smaller sig. we obtained reflects more remarkable influence of the parameter on the separation.

To sum up, the influence degree of these parameters on the enantiomeric Rs of the model compounds was as follows: B > A > C > E > D for NEF, A > B > E > C = D for PRO, B > A > C > E > D for TRY and A = B > E > D > C for CHL. Based on this result, it was evident that buffer pH and CP concentration were the most significant parameters controlled for the chiral separation.

4 Concluding remarks

We studied the enantioseparation capability of the new chiral selector CP antibiotic toward several basic drugs. It was found that CE could be used for the separations of these drugs and CP showed a strong resolving power to the eight enantiomeric pairs of compounds. It was observed that the enantiomeric separation was strongly influenced by the concentration of the chiral selector and the pH of the BGE. The optimum pH that was in the neutral or weak basic region (7.0–7.6), a low capillary temperature and a CP concentration of 60 or 80 mM with a 40 mM borax buffer were recorded as the optimum conditions. In addition, Rs and migration times of analytes as well as solubility of the chiral selector were also influenced by the organic modifier concentration. And among the different organic solvents used, methanol and ethanol allowed achieving better chiral Rs.

The finding of enantioselectivity by this novel chiral selector will add useful information not only to practical analysis of drug enantiomers but also to studies of chemical and biological functions of antibiotics.

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