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Evaluation of the enantioseparation capability of the novel chiral selector clindamycin phosphate towards basic drugs by micellar electrokinetic chromatography

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ABSTRACT

To date, a series of chiral selectors have been utilized successfully in capillary electrophoresis (CE). Among these various chiral selectors, macrocyclic antibiotics have been demonstrated to represent powerful enantioselectivity towards many chiral compounds. Differing from macrocyclic antibiotics, the use of lincosamide antibiotics as chiral selectors has not been reported previously. In our recent work, clindamycin phosphate belonging to the group of lincosamides has been first used as a chiral selector in capillary zone electrophoresis (CZE). In this paper, a micellar electrokinetic chromatography (MEKC) method has been developed for the evaluation of enantioseparation capability of this novel chiral selector towards several racemic basic drugs. As observed during the course of this work, clindamycin phosphate allowed excellent separation of the enantiomers of nefopam, citalopram, tryptophan, chlorphenamine, propranolol and metoprolol, as well as partial enantioresolution of tryptophan methyl ester and cetirizine. In this MEKC chiral separation system, different types of anionic surfactants, organic additives and background electrolytes were tested, and satisfactory enantioseparations of basic drugs above-mentioned were achieved using sodium dodecyl sulfate (SDS) as the surfactant, isopropanol as the organic additive, and phosphate as the background electrolyte. Furthermore, both migration times and enantioseparation of the analytes were influenced by several experimental parameters such as pH of the BGE, clindamycin phosphate and SDS concentrations, phosphate and isopropanol concentrations, and applied voltage. Consequently, the effects of these factors on enantioseparations of the studied basic drugs were systematically investigated in order to evaluate the stereoselectivity of clindamycin phosphate in MEKC.

1. Introduction

Capillary electrophoresis (CE) has been regarded as an attractive separation technique by reason of its high resolution efficiency, short analysis time, and the small amounts of analytes and running solution additives required [1–4]. Over the past decade, CE has also become a powerful technique for the separation of enantiomers [5–10]. Micellar electrokinetic chromatography (MEKC) is an important branch of CE, which has become one of the most popular techniques for the separation of enantiomeric compounds due to its high resolving power and capability of separating both ionic and neutral compounds [11]. Advantages of MEKC include high efficiency, fast analysis, and a powerful flexibility in rapidly tuning or changing the running buffer composition and subsequently the selectivity of the separation [12]. The MEKC technique, first introduced by Terabe et al. [13] for analysis of neutral analytes, uses a surfactant at a concentration above its critical micellar concentration (CMC) in the background electrolyte (BGE). One of the important properties of surfactant is the CMC, defined as a concentration below which the surfactant is in a solution as a monomer and above which practically all additional surfactant forms micelles [14]. The MEKC usually utilizes negatively charged micelles formed from anionic surfactants such as sodium dodecyl sulfate (SDS), which constitutes the pseudostationary phase (PSP). The separation is achieved by differential partitioning of analytes between the PSP and the bulk aqueous phase [15]. Electrostatic, hydrophobic interactions as well as hydrogen bonding with hydrophilic core of the surfactant play important roles in separation of analytes. What's more, chiral separations in MEKC are dependent on the use of different chiral selectors (cyclodextrins and their derivatives, macrocyclic antibiotics, calixarenes, etc. [16–19]) in the BGE in combination with various achiral surfactants. An alternative way

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is to use a chiral surfactant above its CMC in the BGE. The chiral surfactant forms micelles with stereogenic centers at the surface and it acts as the chiral selector. The chiral surfactants used in MEKC include natural chiral surfactants (such as digitonin [20], saponins [21] and bile salts [22]), synthetic chiral surfactants and high-molecular-mass surfactants [23].

Since Armstrong and co-workers [24,25] demonstrated that vancomycin was a useful chiral selector, macrocyclic antibiotics including glycopeptides, ansamycins, aminoglycosides and polypeptides have proved to be a powerful class of chiral selectors for both HPLC and CE. These antibiotics possess several characteristics that allow them to interact with analytes and serve as chiral selectors. They have a number of stereogenic centers and functional groups, allowing them to have multiple interactions with chiral molecules. Electrostatic or charge–charge, dipole–dipole, π–π, hydrophobic interactions, hydrogen bonding and steric repulsion are assumed to be the interactions responsible for chiral recognition [17,18,26]. Additionally, due 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 [26]. Nevertheless, these macrocyclic antibiotics exhibited some drawbacks such as strong absorption in the UV wavelengths, low solubility in the water and adsorption on the capillary wall [27]. On account of this fact, we have been searching for other new types of antibiotics that can be used as chiral selectors for CE. Recently, we have reported the use of a new antibiotic, clindamycin phosphate (CP) belonging to the lincosamides family as a novel chiral selector in capillary zone electrophoresis (CZE) [28]. By comparison to macrocyclic antibiotics, the use of lincosamide antibiotics as chiral selector has not been reported before. CP has a molecular mass of 505, and consists of an amide portion, an amino portion (tertiary amine), a phosphate ester portion and an aglycon portion in each molecule (see Fig. 1). Compared to macrocyclic antibiotics, it possesses not only high solubility but also low viscosity in both water and alcohols. Furthermore, with the lack of aromatic rings in the structure, it exhibits very weak UV absorption.

In this paper, we further investigated and evaluated the enantiorecognition capability of this chiral selector towards several basic drugs with anionic surfactant in MEKC. In regard to the use of antibiotics as chiral selectors in MEKC, to date, only the glycopeptides have been used in conjunction with micelles. The composition of the running BGE, containing antibiotic as the chiral selector, can be modified by the addition of micellar phase to the buffer. Let's take glycopeptide antibiotics including vancomycin, teicoplanin and ristocetin A as examples. Rundlett and Armstrong [25] showed that the addition of SDS to the buffer containing vancomycin can be a good approach for improving efficiency, decreasing analysis time and controlling the order of enantiomers elution of chiral anionic

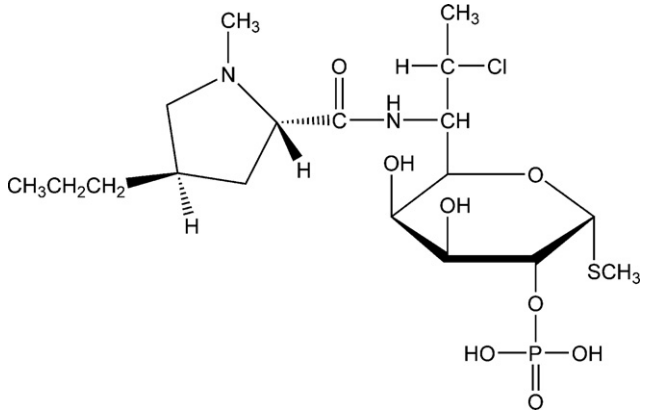

Fig. 1. Structure of clindamycin phosphate (CP) employed in this paper.

analytes. For example, several dansyl-amino acid enantiomers have been separated with 50 mM phosphate buffer (pH 7) containing 2 mM vancomycin and 25 mM SDS; by increasing the SDS concentration up to 50 mM, better resolution was achieved [25]. The addition of SDS to teicoplanin and ristocetin A-based separations has also been investigated. Ristocetin A, like vancomycin, comicellized with SDS to form mixed micelles, with the exception that ristocetin A partitioned to a greater extent than vancomycin to the SDS micelles [29]. In addition, teicoplanin–SDS systems were similar to vancomycin–SDS systems where analysis times decreased, elution orders reversed, and efficiency increased in the micelle-containing teicoplanin systems [29]. On the basis of these valuable results, in this work we presented details of enantioseparations of the studied drugs using CP as a chiral selector with anionic surfactant in MEKC.

2. Experimental

2.1. Chemicals and reagents

CP (purity > 99%, its structure is shown in Fig. 1) was supplied by Jiangsu Institute for Food and Drug Control (Nanjing, China). Propranolol hydrochloride (β-blocker, PRO), tryptophan (a member of α-amino acid, TRY), tryptophan methyl ester (TME), ephedrine hydrochloride (adrenergic agent, EPH), laudanosine (titanic poison, LAU), lomefloxacin hydrochloride (antimicrobial drug, LOM), ketoprofen (analgesic drug, KET), ibuprofen (analgesic drug, IBU), naproxen (analgesic drug, NAP), and pranoprofen (analgesic drug, PRA) were purchased from Sigma (St. Louis, MO, USA). Nefopam hydrochloride (analgesic drug, NEF), citalopram hydrobromide (psychotolytic drug, CIT), chlorphenamine maleate (antihistaminic drug, CHL), metoprolol tartrate (β-blocker, MET), cetirizine hydrochloride (antiallergic agent, CET), oxazepam (psychotolytic drug, OXA), mitiglinide (hypoglycemic agent, MIT), and nateglinide (hypoglycemic agent, NAT) were supplied by Jiangsu Institute for Food and Drug Control. All these drug samples were racemic mixtures.

Methanol, ethanol, isopropanol and acetonitrile, all of HPLC grade, were purchased from Jiangsu Hanbon Sci. & Tech. Co., Ltd. (Nanjing, China). SDS, sodium tetradecyl sulfate (STS), sodium octyl sulfate (SOS), sodium hydroxide, hydrochloric acid, phosphoric acid, dipotassium hydrogen phosphate, sodium tetraborate decahydrate, tris(hydroxymethyl)aminomethane (Tris), all of analytical grade, were purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Double distilled water was used throughout all the experiments.

2.2. Apparatus

Electrophoretic experiments were performed with an Agilent 3D capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany), which consisted of a sampling device, a power supply, a photodiode array UV detector (wavelength range from 190 nm to 600 nm) and a data processor. The whole system was driven by Agilent ChemStation software (Revision B.02.01) for system control, data collection, and analysis. It was equipped with a 43.5 cm (35 cm effective length) × 50 μm i.d. uncoated fused-silica capillary (Hebei Yongnian County Reafine Chromatography Ltd., 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 15–30 kV, and the temperature of capillary was controlled at 20 °C using an air-cooling system.

2.3. Procedures

Phosphate buffers were used as the buffers for CE. Buffer solution was a 40 mM phosphate buffer containing isopropanol (25%, v/v). The running background electrolyte containing CP and SDS, was freshly prepared by dissolving CP (60 mM or 3% (w/v), if not stated otherwise) and SDS (40 mM, 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 6.5–8.8, and then were always checked before and after each run. Each buffer solution was filtered through a 0.45 μm pore membrane filter before use.

The racemic drugs in this study were dissolved in methanol (ATE, LAU, OXA, KET, IBU, NAP, PRA, MIT and NAT) or water (NEF, PRO, TRY, CHL, CIT, TME, MET, CET, EPH and LOM). Final sample solutions for injection were all at a concentration of 0.4 mg/mL.

2.4. Calculations

The resolution (Rs) and selectivity factor (α) of the enantiomers were calculated from $Rs = 2(t_2 - t_1)/(w_1 + w_2)$ and $\alpha = t_2/t_1$, where t_1 and t_2 are the migration times of the two enantiomers, and w_1 and w_2 are the widths of their peaks at the baseline.

3. Results and discussion

3.1. Enantioseparation of basic drugs with CP in MEKC

As illustrated in Fig. 2, CP allowed baseline separation of the enantiomers of NEF, PRO, TRY, CHL, CIT and MET, as well as partial resolution of TME and CET under our experimental conditions. The structures of these eight basic drugs are shown in Fig. 3. In addi-

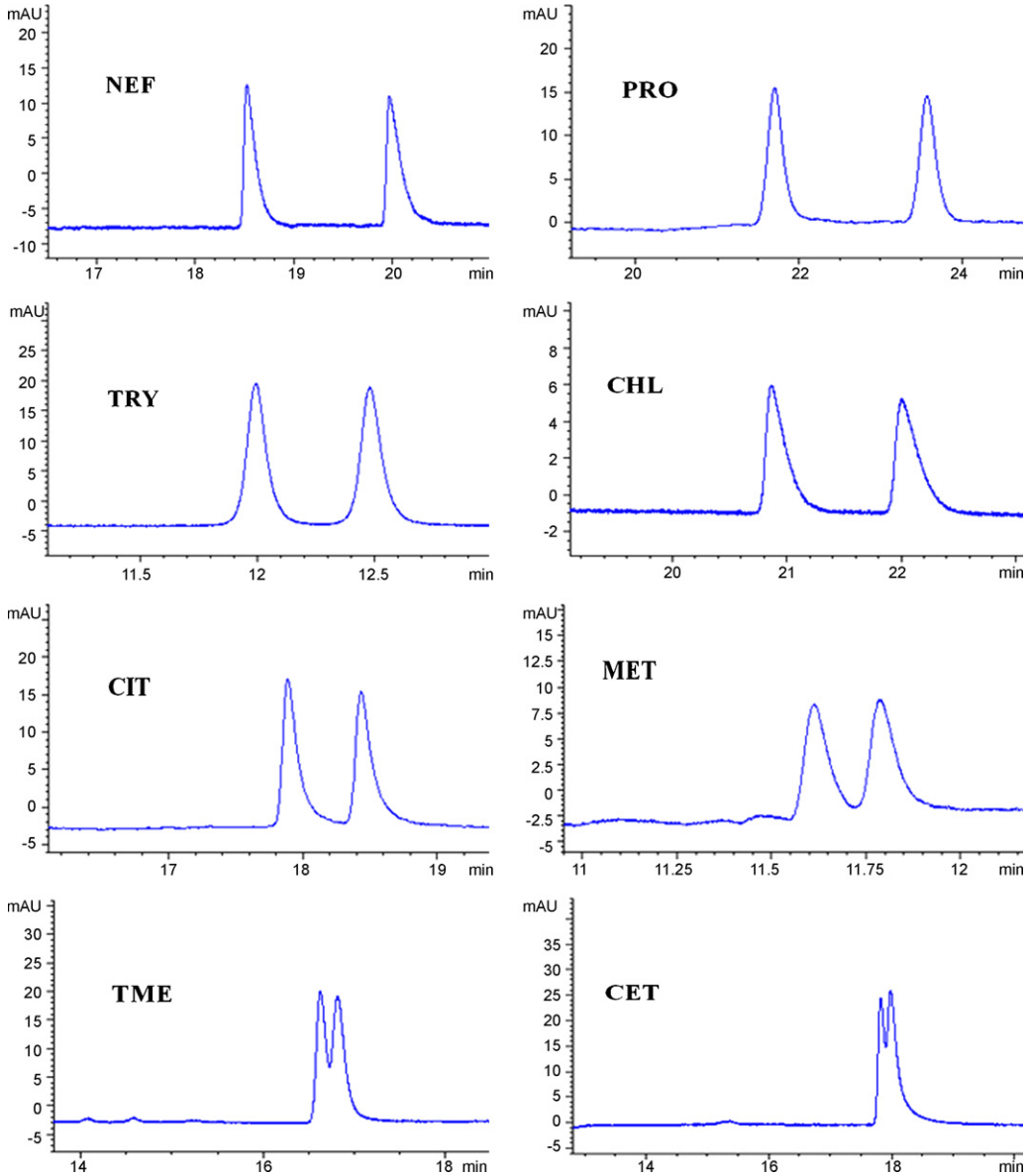

Fig. 2. Electropherograms of the chiral separations of NEF, PRO, TRY, CHL, CIT, MET, TME and CET. *Conditions*: fused-silica capillary, 43.5 cm (35 cm effective length) × 50 μm i.d.; BGE, 40 mM phosphate buffer with isopropanol (25%, v/v) containing CP and SDS; buffer pH, 7.5; CP concentration, 60 mM (NEF, PRO, CHL, TME and CET) and 80 mM (TRY, CIT, MET); SDS concentration, 40 mM; applied voltage, 20 kV (NEF, TRY, MET and TME), 22 kV (CIT), and 24 kV (PRO, CHL and CET); capillary temperature, 20 °C; detection, UV absorption at 220 nm (NEF), 289 nm (PRO), 277 nm (TRY), 265 nm (CHL), 245 nm (CIT), 225 nm (MET), 277 nm (TME), and 237 nm (CET).

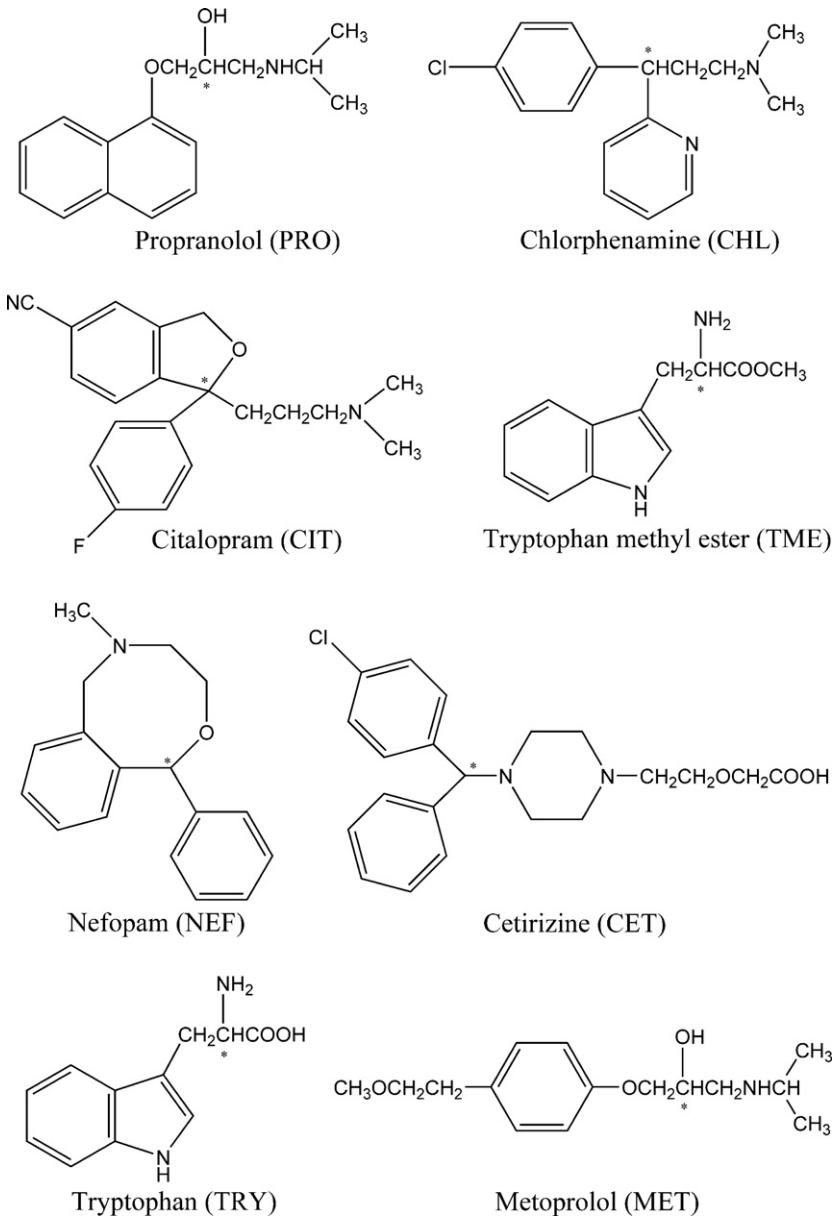

Fig. 3. Structures of the basic drugs studied in this paper.

tion, other 10 drugs consisting of 6 acidic compounds (KET, IBU, NAP, PRA, MIT and NAT) and 4 basic compounds (EPH, LAU, LOM and OXA) have also been tested to demonstrate the enantioseparation capability of CP in MEKC; however, with regard to the 10 compounds, no separation was observed during the process of this work.

In our recent study, we found that CP exhibited enantioselectivity towards some basic compounds in CZE [28]. In comparison with the results obtained by means of CZE, the enantioseparation (e.g. resolution, selectivity factor, and peak shape) of all the studied compounds was almost improved significantly using CP as chiral selector in this MEKC system. Owing to the addition of surfactant to the BGE, the enhancement of enantioseparation was achieved presumably due to differential partitioning of the transient diastereomeric complexes (drug enantiomer-CP complexes) with different stereo-conformation and hydrophobicity between the PSP and the bulk electrolyte phase. In this study the chiral separations of basic drugs were achieved depending on several experimental factors. Thus, in order to evaluate the stereoselectivity of this chiral selector in MEKC, the effects of the composition and concentration of the BGE, surfactant type and concentration, organic modifier type and concentration, buffer pH, selector concentration, and applied voltage on enantioseparations of the eight basic drugs were investigated detailedly, as follows.

3.2. Selections of surfactant type, buffer system and organic modifier

Although cationic surfactants such as cetyltrimethylammonium bromide (CTAB) have been widely used for either slowing down or reversing the electroosmotic flow (EOF) [30,31], the use of cationic surfactants has been avoided because they adsorb on the capillary wall surface by dynamic electrostatic interactions between the negatively charged $Si–O^-$ groups and the positively charged quaternary ammonium ions and thereby affect the enantiomeric separation. Accordingly, three types of anionic surfactants containing STS, SDS and SOS were each added to the BGE above their approximate CMCs (2.4 mM, 8.1 mM and 130 mM, respectively). High electric

current and instable baseline of electropherograms of the tested compounds (NEF, PRO, TRY and CHL) were observed, when SOS was employed. In comparison with STS, better resolutions of the studied four compounds were obtained when SDS was used. Thus, SDS was chosen as the additive surfactant for all the MEKC separations in this study.

The effects of buffer type and concentration on enantioseparation were studied using NEF, PRO, TRY and CHL as tested drugs. Three buffer systems including borax, phosphate and Tris buffers, all at concentration of 20 mM, were tested to find the most applicable one. Compared with borax and Tris, phosphate buffer gave better peak shapes and resolutions of the studied four drugs. Furthermore, different phosphate concentrations (20 mM, 40 mM and 60 mM) were studied to determine an appropriate ionic strength. It was observed that Rs of the tested drug enantiomers increased consistent with phosphate concentration ascending from 20 mM to 40 mM, owing to the decrease of the adsorption of analytes on the inner surface of capillary. However, further increase in phosphate concentration (40–60 mM) caused a slight decrease in Rs, probably because of the effect of Joule heating leading to peak broadening. Hence, 40 mM phosphate buffer was finally selected as the buffer system for all the MEKC separations in this work.

Organic modifier can influence several other parameters such as the viscosity of the BGE, the mobilities of analytes and chiral selector, the solubility of enantiomers, and the EOF. More importantly, the presence of the organic modifier seems to enhance the complexation interaction involved in the enantioseparation mechanism when using antibiotics as chiral selectors [32]. As a result, different types and concentrations of organic additives were investigated for the chiral separation of the tested drugs (NEF, PRO, TRY and CHL). Four organic additives consisting of methanol, ethanol, isopropanol and acetonitrile were each added (20%, v/v) to the BGE to find the most effective one. It could be observed that among the four organic additives, isopropanol was the optimal organic additive for modifying the peak shapes and enantioresolutions of the tested drugs. What's more, an isopropanol concentration range of 0–40% (v/v) was investigated to indicate the effect of organic modifier concentration on separation of drug enantiomers. An increase of isopropanol concentration (0–25%, v/v) brought a general rise in both migration times and Rs of the tested drugs. On the other hand, too long analysis time was unavoidable when higher isopropanol concentration (30–40%, v/v) was employed. Based on the above results, 25% isopropanol was eventually selected as the organic additive for all the MEKC separations in this study.

3.3. *Effect of buffer pH on enantioseparation of basic drugs*

Buffer pH is an important factor in controlling and optimizing the separation. It governs the charge of the chiral selector (ionizable compounds, e.g. antibiotics) and analytes which in turn influences many aspects of the enantioseparation, such as the interactions between the selector and analytes (e.g. electrostatic and hydrogen bonding interactions) as well as the electrophoretic mobilities of EOF, analytes and the selector. Accordingly, we investigated the influence of this factor on the enantiomeric separations of six basic compounds (NEF, PRO, TRY, CHL, CIT and MET), which gave baseline enantioseparations.

As shown in Table 1, the effect of buffer pH on enantioseparation of the studied drugs was investigated over the range of 6.5–8.8 using 40 mM phosphate buffer (containing 25% (v/v) isopropanol) supplemented with 60 mM CP and 40 mM SDS. The six tested pH values were controlled at 6.5, 7.1, 7.5, 7.8, 8.2 and 8.8. It could be observed that as the buffer pH ascended from 6.5 to 7.5 (from 8.2 to 8.8), both α and Rs of all enantiomers tended to increase (to decrease) apparently; consequently, the optimum pH values in terms of Rs were obtained at 7.5 (for NEF, CHL and CIT) and 7.8 (for PRO, TRY and MET). This trend indicated that the pH range of 7.5–8.2 was optimal for the enantioseparation of the studied drugs, and gave important information concerned with the interactions between CP and them. With regard to the structures of these basic drugs and CP (see Figs. 1 and 3), each drug has an amino group and a chiral carbon, while CP has an amino group (tertiary amine), two hydroxyl groups and a phosphate group. Since the degree of both protonation of amino groups (in drugs and CP) and dissociation of phosphate group (in CP) is dependent on pH, the optimum pH range will point to the most compatible state of the amino function in the drugs for binding or reacting presumably to the amino and hydroxyl groups or the phosphate group in CP.

Much of the enantiorecognition capability of antibiotics can be attributed to their ability to form multiple interactions such as electrostatic or charge–charge, hydrogen bonding, dipole–dipole, π–π, hydrophobic interactions, and among them two important ones are probably thought to be electrostatic interaction and hydrogen bonding [17,18,24–26,29]. In the present system of the basic drug–CP combination, it is significant to know what sorts of interactions exist between the basic drugs and this selector. 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. Thus, in the optimum pH range of 7.5–8.2, there was a compatible hydrogen bonding between analytes and chiral selector. Another one is considered to be electrostatic interaction between the amino group in a drug and the phosphate group in CP. When the pH value was under 7.5, there was an excessive decrease of the negative charge of the phosphate group in CP. On the other side, when the pH value was over 8.2, 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 electrostatic interaction between analytes and chiral selector would become somewhat weak, so that CP showed poor enantioselectivity towards these basic drugs.

Table 1
Effect of buffer pH on selectivity factor (α) and resolution (Rs) of basic compounds.

Chiral compounds	Buffer pH											
	6.5		7.1		7.5		7.8		8.2		8.8	
	α	Rs	α	Rs	α	Rs	α	Rs	α	Rs	α	Rs
NEF	1.018	2.13	1.026	2.78	1.086	6.68	1.078	6.61	1.073	6.20	1.037	3.05
PRO	1.017	2.70	1.023	2.99	1.086	5.50	1.088	5.94	1.068	4.03	1.038	2.20
TRY	1.008	0.93	1.010	1.20	1.034	2.81	1.036	3.28	1.039	3.26	1.030	1.59
CHL	1.019	1.27	1.023	1.45	1.055	3.60	1.041	2.81	1.040	2.68	1.022	1.96
CIT	1.006	0.96	1.009	1.15	1.033	2.43	1.028	2.38	1.028	2.27	1.021	1.92
MET	1.000	0.00	1.006	0.51	1.011	1.15	1.012	1.32	1.013	1.29	1.012	0.91

Conditions: fused-silica capillary, 43.5 cm (35 cm effective length) × 50 μm i.d.; BGE, 40 mM phosphate buffer with isopropanol (25%, v/v) containing CP and SDS; buffer pH, as shown in this table; CP concentration, 60 mM; SDS concentration, 40 mM; applied voltage, 20 kV (NEF, TRY and MET), 22 kV (CIT), and 24 kV (PRO and CHL); capillary temperature, 20 °C; detection, as in Fig. 2.

In summary, both hydrogen bonding and electrostatic interaction have contributed to the complexation between drug enantiomers and the selector, which resulted in forming the transient diastereomeric complexes (drug enantiomer–CP complexes) exhibiting different stereo-conformation and hydrophobicity. In this MEKC separation system here, aqueous micelles provided a transient hydrophobic environment distinct from bulk water, in which hydrophobic interaction between the transient diastereomeric complexes and the micellar phase took effect. This hydrophobic interaction could be expected to improve the enantioseparations of the studied basic drugs.

3.4. Effect of CP concentration on enantioseparation of basic drugs

The concentration of chiral selector is an easily adjusted factor that has a profound impact on the enantioseparation of analyte. Generally, Rs of the enantiomers tends to increase in conjunction with the rise of chiral selector concentration for such combination as basic drug–CP. The reason for this behavior might be expected that as the selector concentration is increased, the molar fraction of drug-selector adduct increases leading to the rise of Rs.

In order to acquire the optimum CP concentration for the chiral separations of the studied drugs (NEF, PRO, TRY, CHL, CIT and MET), we tested five different values (20 mM, 40 mM, 60 mM, 80 mM and 100 mM). Fig. 4 depicts the effect of CP concentration on the Rs of these drugs with 40 mM phosphate buffer (pH 7.5) containing 25% (v/v) isopropanol and 40 mM SDS. It is evident that Rs of the drug enantiomers increased apparently in step with CP concentration ascending from 20 mM to 60 mM (for PRO and CHL) and to 80 mM (NEF, TRY, CIT and MET), and subsequently tended to decrease slightly at higher CP concentration presumably due to enhanced peak broadening and saturated complexation between analytes and chiral selector.

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

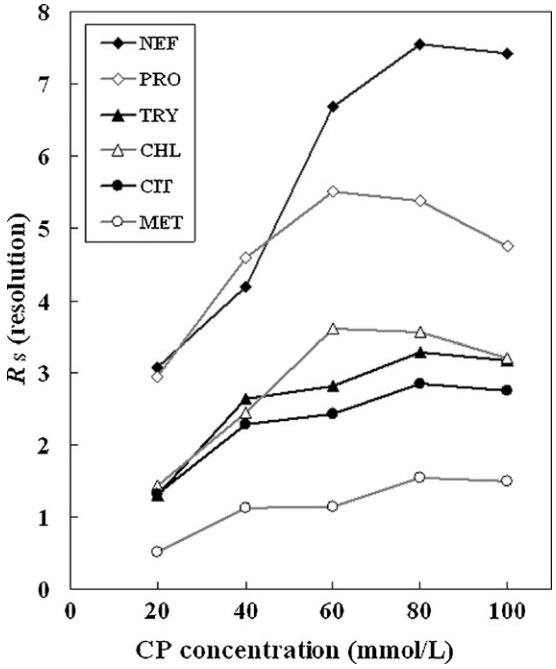

Fig. 4. Effect of CP concentration on the resolution (Rs) of basic compounds. *Conditions*: fused-silica capillary, 43.5 cm (35 cm effective length) × 50 μm i.d.; BGE, 40 mM phosphate buffer with isopropanol (25%, v/v) containing CP and SDS; buffer pH, 7.5; CP concentration, 20–100 mM; SDS concentration, 40 mM; applied voltage, 20 kV (NEF, TRY and MET), 22 kV (CIT), and 24 kV (PRO and CHL); capillary temperature, 20 °C; detection, as in Fig. 2.

3.5. Effect of SDS concentration on enantioseparation of basic drugs

The complexation between drug enantiomers and the selector formed the transient diastereomeric complexes (selector-selectand complexes) exhibiting different stereo-conformation and hydrophobicity. In consideration of the addition of SDS micelles to the BGE, differential partitioning of the diastereomer complexes between the bulk aqueous phase and the SDS micellar phase, resulted in the improvement of enantiorecognition for the studied basic drugs (NEF, PRO, TRY, CHL, CIT and MET). Accordingly, different SDS concentrations (20 mM, 40 mM and 60 mM) were investigated for the chiral separation of these drugs in MEKC system.

The effects of SDS concentration (20 mM, 40 mM and 60 mM) on the migration times, α and Rs of the studied drugs were investigated with 40 mM phosphate buffer (pH 7.5) containing 25% (v/v) isopropanol and 60 mM CP. As seen in Table 2, α and Rs of all enantiomers increased in parallel with the rise of SDS concentration (20–40 mM), and then reached the maximum values at 40 mM SDS concentration. Further increase of SDS concentration (40–60 mM) caused the decrease in both α and Rs. This is probably due to the competition of SDS micellar phase with CP for binding or reacting to the analytes at high SDS concentration. In addition, a slight decrease of the separation efficiency resulting from the Joule heating effect should be also responsible for the decrease of α and Rs, when high SDS concentration caused more Joule heating. It was also observed the migration times of the studied drugs increased apparently in company with the rise of SDS concentration (20–40 mM),

Table 2
Effect of SDS concentration on the migration times, selectivity factor (α) and resolution (Rs) of basic compounds.

Chiral compounds	SDS concentration (mmol/L)								
	20			40			60		
	t_2/t_1 (min)	α	Rs	t_2/t_1(min)	α	Rs	t_2/t_1 (min)	α	Rs
NEF	15.52/14.42	1.076	6.32	21.16/19.49	1.086	6.68	21.77/20.30	1.072	5.38
PRO	15.72/14.68	1.071	5.41	23.57/21.70	1.086	5.50	23.46/21.88	1.072	4.74
TRY	12.69/12.30	1.032	2.56	13.90/13.44	1.034	2.81	13.76/13.31	1.034	2.79
CHL	15.36/14.69	1.046	2.89	22.00/20.86	1.055	3.60	22.23/21.16	1.051	3.48
CIT	15.74/15.31	1.028	2.35	23.12/22.39	1.033	2.43	25.32/24.62	1.028	2.15
MET	11.25/11.15	1.009	0.98	13.09/12.95	1.011	1.15	12.82/12.67	1.012	1.01

Conditions: fused-silica capillary, 43.5 cm (35 cm effective length) × 50 μm i.d.; BGE, 40 mM phosphate buffer with isopropanol (25%, v/v) containing CP and SDS; buffer pH, 7.5; CP concentration, 60 mM; SDS concentration, as shown in this table; applied voltage, 20 kV (NEF, TRY and MET), 22 kV (CIT), and 24 kV (PRO and CHL); capillary temperature, 20 °C; detection, as in Fig. 2.

and subsequently tended to increase (for NEF, PRO, CHL and CIT) or decrease (for TRY and MET) slightly with the further increase of SDS concentration (40–60 mM).

Therefore, the additive SDS concentration of 40 mM was demonstrated to be optimal for the chiral separations of these drugs.

3.6. Effect of applied voltage on enantioseparation of basic drugs

The effects of applied voltage on the migration time and enantioseparation of the studied drugs (NEF, PRO, TRY, CHL, CIT, MET, TEM and CET) were investigated over a range of 15–30 kV. It was observed that an increase of applied voltage brought a general decrease of the migration times of all the enantiomers, naturally because high voltage caused high migration velocity of both analytes and EOF. However, Rs of the enantiomers did not vary obviously, since higher voltage probably caused not only higher seperation efficiency but also shorter reaction time and more Joule heating. Taking account of satisfactory enantioseparation and short analysis time simultaneously, applied voltages of 20 kV (NEF, TRY, MET and TEM), 22 kV (CIT), and 24 kV (PRO, CHL and CET) were determined to be appropriate for the separations of the eight basic compounds.

4. Conclusions

In this paper, an effective MEKC method has been developed for the evaluation of enantioseparation capability of the novel chiral selector clindamycin phosphate towards several racemic basic drugs. Among these basic drugs, CP brought baseline separation of nefopam, citalopram, tryptophan, chlorphenamine, propranolol and metoprolol, as well as partial resolution of tryptophan methyl ester and cetirizine under the experimental conditions. Furthermore, some factors including the composition and concentration of the BGE, surfactant type and concentration, organic modifier type and concentration, buffer pH, selector concentration and applied voltage, have been observed to influence the separation of these drugs. In the course of this work, satisfactory enantioseparations of the studied drugs were achieved at the buffer pH range of 7.2–8.2 using 40 mM phosphate buffer with the additions of 25% (v/v) isopropanol, 60 mM or 80 mM CP, and 40 mM SDS. Our study focusing on the exploration of new antibiotics used as chiral selectors will enhance the scope of enantioseparation of drug enantiomers and will ensure the continued growth of chiral analysis using CE as the separation technique.

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