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Evaluation of vancomycin-based synergistic system with amino acid ester chiral ionic liquids as additives for enantioseparation of non-steroidal anti-inflamatory drugs by capillary electrophoresis

Jinjing Zhang [a], Yingxiang Du [a,b,c,*], Qi Zhang [a], Yuntao Lei [d]

[a] Department of Analytical Chemistry, China Pharmaceutical University, Nanjing 210009, PR China
[b] Key Laboratory of Drug Quality Control and Pharmacovigilance (Ministry of Education), China Pharmaceutical University, Nanjing 210009, PR China
[c] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, PR China
[d] Department of Physical Chemistry, China Pharmaceutical University, Nanjing 210009, PR China

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ABSTRACT

Recently, chiral ionic liquids (ILs) have drawn increasing attention in chiral separation field; however, few papers reported the application of chiral ILs for chiral separation by capillary electrophoresis (CE), and among the papers, chiral ILs were mainly applied as additives to β-cyclodextrin derivatives systems to establish synergistic systems. The synergistic system based on antibiotics with chiral ILs as additives has never been reported before. In this paper, two chiral ionic liquids (ILs) based on amino acid ester, L-alanine and L-valine *tert* butyl ester bis (trifluoromethane) sulfonamide, were first applied to evaluate the synergistic effect with antibiotic selector for CE chiral separation. The vancomycin-based synergistic system with chiral ILs as additives was successfully established and investigated for the enantioseparation of naproxen, carprofen, ibuprofen, ketoprofen and pranoprofen. Compared to vancomycin-alone cases, significant improvement of separation for all the analytes was observed. Several parameters, such as type and proportion of organic modifier, composition and pH of buffer, concentration of chiral ILs and vancomycin were systematically investigated, and then evaluated by means of Statistical Product and Service Solutions (SPSS) to research the influences on the synergistic effect. Finally, the established method was successfully applied to test the chiral impurity of naproxen sample.

1. Introduction

Chiral separation has always drawn great attention, especially in pharmaceutical field, since it is well known that two enantiomers of a racemic drug usually display different biological activities. Therefore, effective analytical methods for enantiomeric purity are critical for the pharmaceutical industry. Capillary electrophoresis (CE) has proven to be a powerful technique compared to classical chromatographic methods in chiral separation field. Its popularity may be due to its high separation efficiency, short analysis time, low separation cost and small volume requirements for chiral selector. Among different kinds of chiral reagents, native cyclodextrins and their derivatives [1], polysaccharides [2,3], antibiotics [4,5] and surfactants [6,7] have been successfully applied as chiral selector in CE. However, in some cases, it may not provide adequate selectivity and resolution when only one chiral selector is used in separation system. Therefore, combined use of two or more chiral reagents has been widely concerned in recent years.

Vancomycin is one of a family of related macrocyclic glycopeptide antibiotics and has been widely used as chiral selector in CE [8]. However, vancomycin has several limits as a chiral selector for CE chiral separation, such as relatively strong UV absorption below 250 nm (close to the UV absorption of the tested drugs), high solution viscosity at higher concentration and possible adsorption onto the capillary wall. Since the combined use of vancomycin with another chiral reagent may improve the chiral separation and overcome the shortage mentioned above, further research and optimization of the conventional vancomycin separation system is necessary.

Ionic compounds with melting points below 100 °C or more often even lower than room temperature are referred to as ionic liquids (ILs). ILs have unique properties, such as negligible vapor pressure, nonvolatile and nonflammable, miscibility with water and organic solvents, as well as good thermal stability [9,10]. Due

Abbreviations: Chiral ILs, chiral ionic liquids; L-AlaC_4NTf_2, L-alanine *tert* butyl ester bis (trifluoromethane) sulfonamide; L-ValC_4NTf_2, L-valine *tert* butyl ester bis (trifluoromethane) sulfonamide; NSAIDs, non-steroidal anti-inflammatory drugs

* Corresponding author at: Department of Analytical Chemistry, China Pharmaceutical University, No. 24 Tongjiaxiang, Nanjing 210009, Jiangsu, China. Tel./fax: +86 25 83221790.
E-mail address: du_yingxiang@126.com (Y. Du).

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to their unique properties, ILs have been successfully applied in various fields. Concerning analytical applications, ILs have been used as stationary phases in gas chromatography [11,12], mobile phase additives in liquid chromatography [13–15] as well as background electrolyte additives [16–18] or coating reagents of the capillary wall [19] in capillary electrophoresis. In addition, ILs can be modified by introducing one or more chiral centers in cations or anions to form chiral ILs. Chiral ILs not only maintain the same unique properties as ILs, but also have chiral recognition ability, indicating the potential to be used for chiral separation. However, only a few papers have reported the application of chiral ILs for CE enantioseparation [20–24]. Two chiral ILs (ethyl- and phenylcholine of *bis* (trifluoromethylsulfonyl) imide) were evaluated in CE by Francois and coworkers [22], and better resolution results were observed by adding these chiral ILs to classical chiral selectors (di- or trimethyl-β-cyclodextrin) separation system, which suggested synergistic effects. Wang and coworkers [23] enantioseparated five drugs with combined use of trimethyl-β-cyclodextrin and chiral cationic IL (N-undecenoxy-carbonyl-L-leucinol bromide) and evaluated the synergistic effect between chiral IL and chiral selector. Among the mentioned papers, chiral ILs were mainly applied as additives to β-cyclodextrin derivatives systems, while synergistic system based on antibiotics with chiral ILs as additives has never been reported before. Thus their further research for enantioseparation is very valuable and necessary.

Since the structures of amino acids can be easily modified [25], amino acids and their derivatives would be candidates to act as chiral precursors for the synthesis of chiral ILs. Due to their weak UV

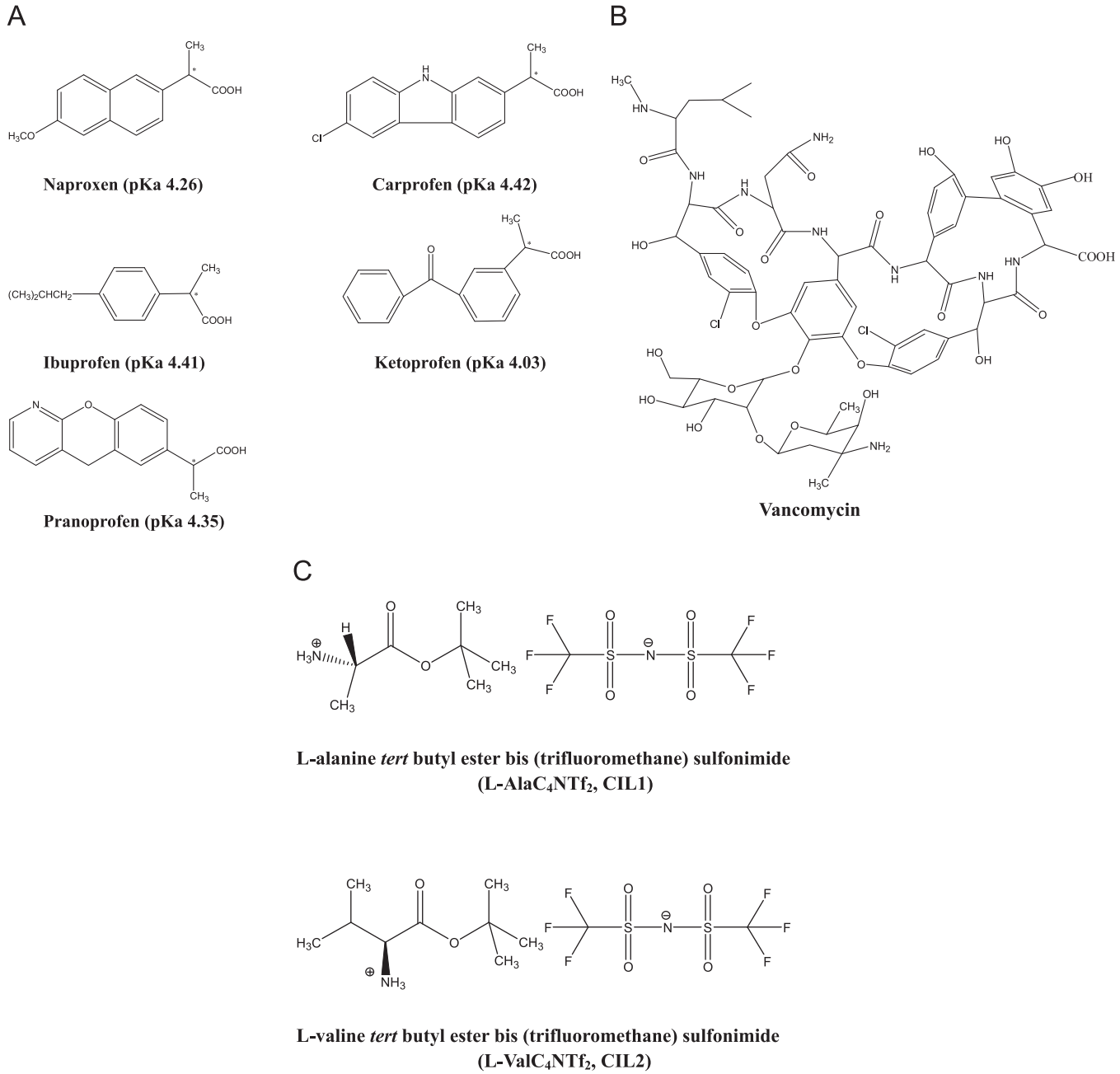

Fig. 1. Structures of (A) five NSAIDs, (B) vancomycin and (C) chiral ionic liquids L-alanine and L-valine *tert* butyl ester bis (trifluoromethane) sulfonimide (L-AlaC$_4$NTf$_2$ (CIL1) and L-ValC$_4$NTf$_2$ (CIL2)).

absorption and low cost, it is meaningful to use chiral ILs based on native amino acid esters as additives in CE separation. In this study, two chiral ILs (L-alanine and L-valine *tert* butyl ester bis (trifluoromethane) sulfonimide, L-AlaC_4NTf_2 and L-ValC_4NTf_2, structures as Fig. 1) were applied to establish the synergistic system with antibiotic selector for chiral separation, which has not been reported so far. With regard to tested racemic drugs, such as naproxen, carprofen, ibuprofen, ketoprofen and pranoprofen, significant improvement of chiral separation was observed in the chiral ILs synergistic system compared to the vancomycin-alone system. Parameters such as type and proportion of organic modifier, composition and pH of buffer, concentration of chiral ILs and vancomycin were systematically investigated, and then evaluated by means of Statistical Product and Service Solutions (SPSS). Finally, the established method was successfully applied to test the chiral impurity of naproxen sample.

2. Experimental

2.1. Chemicals and reagents

Vancomycin hydrochloride was purchased from Changzhou Hangyu Pharmaceutical Technology Co., Ltd. (Jiangsu, China). L-Alanine *tert* butyl ester hydrochloride (L-AlaC_4Cl, $>98.5\%$) and L-valine *tert* butyl ester hydrochloride (L-ValC_4Cl, $>98.5\%$) were purchased from Chengdu Enlai Biological Technology Co., Ltd. (Sichuan, China). Bis (trifluoromethane) sulfonamide lithium salt ($LiNTf_2$) was purchased from Aladdin-reagent Co., Ltd. (Shanghai, China). Naproxen was purchased from xiyashiji.com (Sichuan, China). Carprofen was purchased from Dalian Meilun Biotech Co., Ltd. (Liaoning, China). Ibuprofen and ketoprofen were purchased from Sigma Aldrich (St. Louis, MO, USA). Pranoprofen was purchased from Jiangsu Dr Pharmaceutical Co., Ltd. (Jiangsu, China). Standard R-naproxen was purchased from Sigma Aldrich (St. Louis, MO, USA) and S-naproxen sample (bulk drug) was purchased from Adamas Reagent, Ltd. (Shanghai, China). All these drug samples were racemic mixtures.

Sodium phosphate dibasic ($\geq 99.0\%$) and phosphoric acid were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Jiangsu, China). Methanol, ethanol and acetonitrile, all of HPLC grade, were purchased from Jiangsu Hanbon Sci. & Tech. Co., Ltd. (Jiangsu, China). Double distilled water was used throughout all the experiments.

2.2. Synthesis of chiral ionic liquids

According to the previous literature [26], we synthesized L-alanine *tert* butyl ester bis (trifluoromethane) sulfonamide

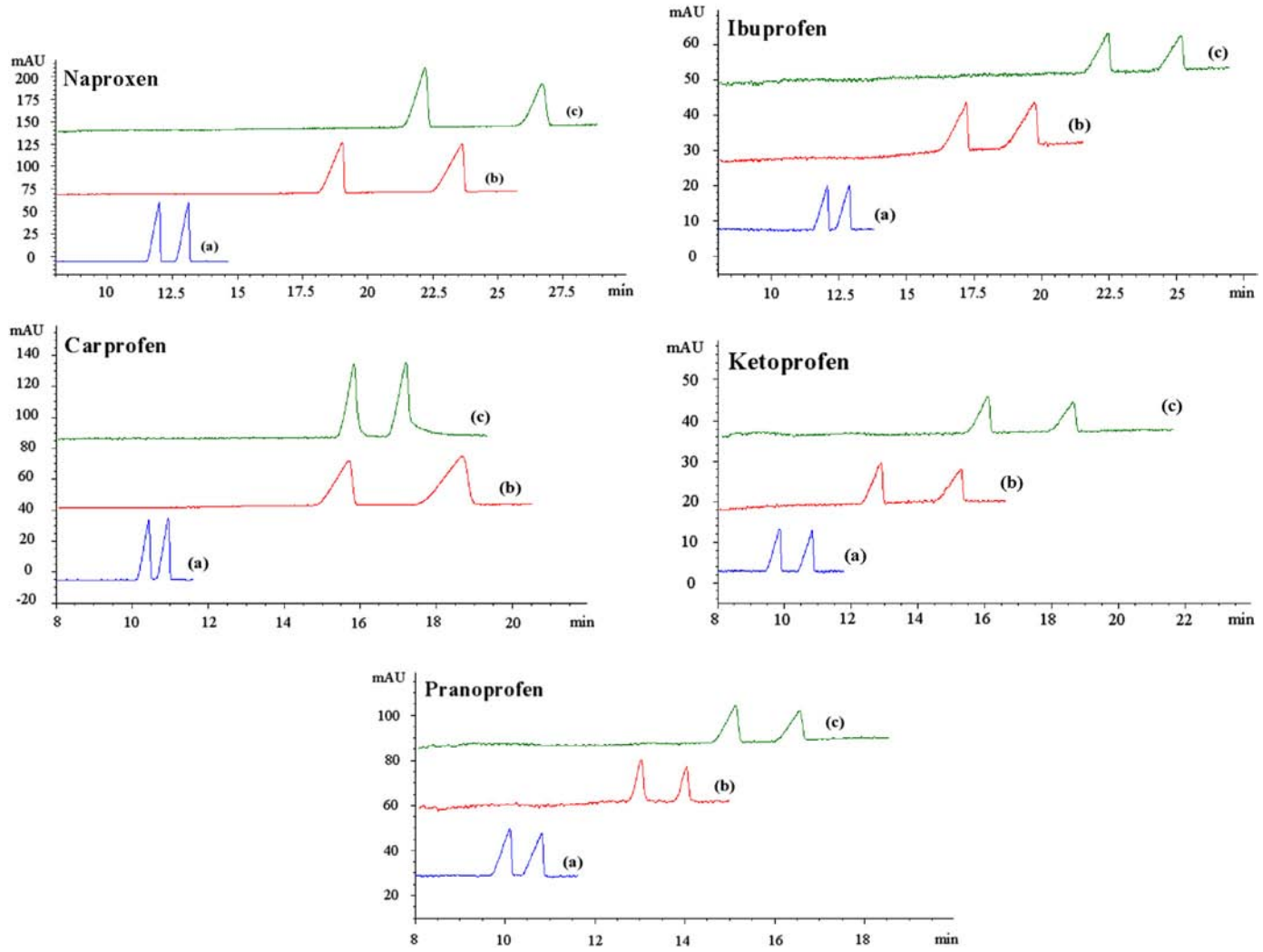

Fig. 2. Typical electropherograms of the chiral separations of five NSAIDs with combined use of vancomycin and CIL1 or CIL2. *Conditions*: fused-silica capillary, 33 cm (24.5 cm effective length) × 50 μm id; 50 mM phosphate buffer (20% (v/v) of methanol included) containing (a) 2 mM vancomycin; (b) 2 mM vancomycin + 15 mM CIL1; (c) 2 mM vancomycin + 15 mM CIL2; pH 7.0; applied voltage, 20 kV; capillary temperature, 25 °C.

(L-AlaC$_4$NTf$_2$) from the responding amino acid ester chloride by one-step anion exchange reaction. In a similar way, L-valine *tert* butyl ester bis (trifluoromethane) sulfonamide (L-ValC$_4$NTf$_2$) was synthesized as the following steps.

0.50 g (2.75 mmol) of L-alanine *tert* butyl ester hydrochloride (L-AlaC$_4$Cl) or 0.58 g (2.75 mmol) of L-valine *tert* butyl ester hydrochloride (L-ValC$_4$Cl) was dissolved in 1.0 ml of distilled water. An equimolar amount (0.79 g, 2.75 mmol) of bis (trifluoromethane) sulfonamide lithium salt (LiNTf$_2$) was dissolved in 1.0 ml of distilled water. Then the two solutions were well mixed in one flask and stirred for 2 h at room temperature. After stirring, the mixed solution was transferred into one separating funnel and stood for about 30 min. The mixture resulted in two layers, of which the lower layer was separated and washed with distilled water for 3–5 times. After dried under vacuum at 70 °C overnight, colorless products were obtained.

2.3. Apparatus

All experiments were performed on an Agilent 3D CE system (Product number G7100A, Agilent Technologies, Waldbronn, Germany) equipped with a sampling device, a power supply, a diode array detector (wavelength range from 190 to 600 nm) for UV detection and a data processor. The system was driven by Agilent Chemstation software for system control and data acquisition. 33 cm Total length (24.5 cm to detection) × 50 μm id. uncoated fused-silica capillary was purchased from Hebei Yongnian County Reafine Chromatography Ltd. (Hebei, China). A new capillary was flushed with 1 M NaOH, 0.1 M NaOH and distilled water for 20 min. Between consecutive injections, the capillary was flushed with 0.1 M NaOH and distilled water for 3 min each and then with running buffer solution till baseline stabilization. All the samples were introduced into the capillary by a 50 mbar pressure for 4 s and were monitored at 235 nm (naproxen), 230 nm (carprofen), 225 nm (ibuprofen and ketoprofen) and 210 nm (pranoprofen), according to cases. The capillary was thermostated at 25 °C, the applied voltage was performed at 20 kV.

2.4. Experimental procedures

The racemic drugs (0.8 mg/ml) in this study were dissolved in a dual solvent mixed with methanol and distilled water (50:50 (v/v)). Thiourea was used as a neutral marker to determine the electroosmotic flow. Buffer solution was 50 mM phosphate buffer containing organic modifier (20% (v/v)). The running buffer was freshly prepared by dissolving vancomycin and chiral ILs in the buffer solution with a specified pH, and then adjusted to a desired pH value by adding a small volume of 5 M sodium hydroxide solution or 10% (v/v) phosphoric acid. All the buffer solutions were filtered through a 0.45 μm pore membrane filter and degassed by sonication before use.

2.5. Calculation for chiral separation

The electroosmotic mobility (μ_{eof}) was calculated by the following Eq. (1):

$$\mu_{eof} = (L \times l)/(V \times t_0) \quad (1)$$

where L, l, V, and t_0 are the total capillary length, the effective capillary length, applied voltage, and the migration time of thiourea (a neutral marker), respectively.

The resolution of the chiral analytes was calculated using Eq. (2):

$$Rs = 2(t_2 - t_1)/(w_1 + w_2) \quad (2)$$

where t_1 and t_2 are the migration times, and w_1 and w_2 are the peak widths at the baseline of each enantiomer.

3. Results and discussion

3.1. Evaluation of the vancomycin-based synergistic system with chiral ILs as additives

It is well documented that vancomycin has chiral separation capability towards these non-steroidal anti-inflammatory drugs (NSAIDs) [7]. Hence, we focused on the establishment of the synergistic system based on vancomycin with chiral ILs as additives. Fig. 2 showed the typical electrophoretograms of chiral separation with the absence and presence of L-AlaC$_4$NTf$_2$ or L-ValC$_4$NTf$_2$ when keeping other conditions unchanged. As observed, combined use of chiral ILs and vancomycin could significantly improve the enantioseparation of five NSAIDs (naproxen, carprofen, ibuprofen, ketoprofen and pranoprofen), suggesting remarkable synergistic effects. It is worth mentioning that no enantioseparation was found for any tested racemic drugs when each chiral ILs was used alone.

3.2. Influence of the type and proportion of organic modifier

Organic modifier was critical to the chiral separation system because organic additive could improve separation, change the

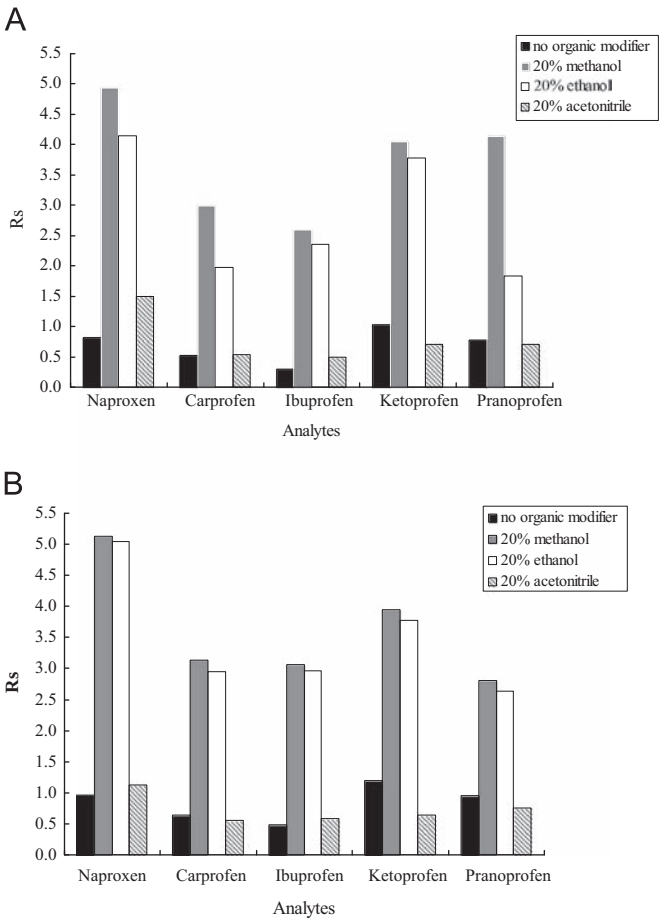

Fig. 3. Effect of organic modifier on the Rs of five NSAIDs: (A) combined use of vancomycin and CIL1, (B) combined use of vancomycin and CIL2. *Conditions*: fused-silica capillary, 33 cm (24.5 cm effective length) × 50 μm id; 50 mM phosphate buffer (20% (v/v) of organic modifier included or no organic modifier) containing 2 mM vancomycin and 15 mM CIL1 or CIL2; pH 7.0; applied voltage, 20 kV; capillary temperature, 25 °C.

viscosity of the running buffer solution and modify the capillary wall [27]. Three types of organic modifier including methanol, ethanol and acetonitrile were studied toward the synergistic system containing chiral ILs and vancomycin. As shown in Fig. 3, the addition of organic modifier obviously increased the separation resolution compared to the systems without organic additive for all the five NSAIDs. In addition, by comparison of three types of organic modifier with the same proportion (20%), methanol was much more suitable to be used as organic additive in consideration of the separation resolution of analytes.

Based on above results, we further investigated the influence of proportion (0–30% (v/v)) of methanol on chiral separation toward tested racemic drugs. As the ratio of methanol increased from 0 to 20%, migration times of analytes prolonged gradually and better separation results (resolution and selectivity factor (α)) were observed (Table 1). It can be noted that when the ratio of methanol reached 30%, no peak was shown in relatively long analysis times (60 min). Furthermore, larger proportion of organic modifier would do harm to the buffer system due to the slight solubility of vancomycin in methanol. So 20% methanol was selected for chiral separation in these synergistic systems.

3.3. Influence of buffer pH

Taking suitable ionic strength and buffer capacity into account, 50 mM phosphate was used as buffer solution. The pH of the buffer solution plays a key role in chiral separation because it may affect the electroosmotic flow, and charge of analytes, vancomycin and chiral ILs in the vancomycin/chiral ILs system. The effect of pH on chiral separation was studied at pH 6.0, 6.5, 7.0, 7.3 and 7.5 with 2 mM vancomycin and 15 mM of each chiral ILs in 50 mM phosphate buffer (20% (v/v) of methanol included). Since the pKa values of these studied NSAIDs are in the range of 4.03–4.42, all the analytes should be in both anionic and undissociated form. As shown in Fig. 4A, when pH increased from 6.0 to 7.5, the electroosmotic mobility (μ_{eof}) went up gradually in vancomycin-alone system and both vancomycin/chiral ILs systems. It is worth noting that the μ_{eof} was lower in vancomycin/chiral ILs system compared to vancomycin-alone system, indicating the adsorption of chiral ILs cation on the capillary wall. In the pH range of 6.0–7.5, separation resolution and α of five NSAIDs reached the maximum values at pH 6.0 in vancomycin-alone system; however, in the cases with L-AlaC_4NTf_2 and L-ValC_4NTf_2, better separation resolution and α were observed when pH was at 6.5 or 7.0 (Table 2).

In general, electrostatic interactions and hydrogen-bonding interactions play a critical part in the chiral recognition. Since pH can affect the dissociation of the carboxyl group in the analytes (naproxen, carprofen, ibuprofen, ketoprofen and pranoprofen), amino and carboxyl groups in vancomycin and amino function in L-AlaC_4NTf_2 and L-ValC_4NTf_2, the electrostatic interactions and hydrogen-bonding interactions among the three entities (analyte, vancomycin and chiral ILs) would be influenced by buffer pH. Resulted from the fact that the optimum buffer pH value in

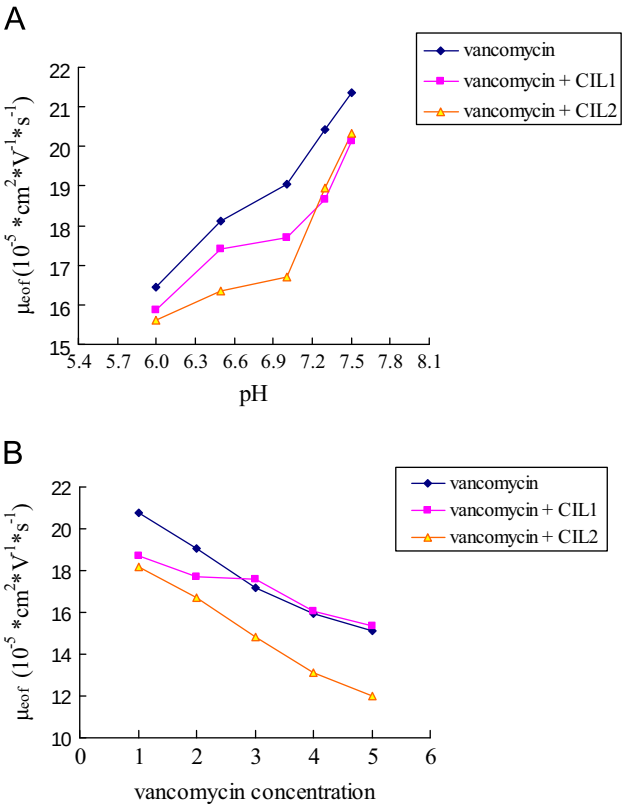

Fig. 4. The variation trend of the electroosmotic mobility as the change of (A) pH and (B) the vancomycin concentration. *Conditions*: fused-silica capillary, 33 cm (24.5 cm effective length) × 50 μm id; 50 mM phosphate buffer (20% (v/v) of methanol included) containing (A) 2 mM vancomycin and 15 mM CIL1 or CIL2, pH 6.0–7.5; (B) 1–4 mM vancomycin and 15 mM CIL1 or CIL2, pH 7.0; applied voltage, 20 kV; capillary temperature, 25 °C.

Table 1
Effect of the proportion of methanol on chiral separation.

	Chiral compounds	Methanol proportion (% v/v)					
		0		10		20	
		t_{R2}/t_{R1} (min)	Rs/α	t_{R2}/t_{R1} (min)	Rs/α	t_{R2}/t_{R1} (min)	Rs/α
Vancomycin + CIL1	Naproxen	4.359/3.877	0.82/1.03	7.020/6.676	1.73/1.05	23.607/19.001	4.93/1.24
	Carprofen	3.428/3.364	0.52/1.02	6.289/6.097	1.18/1.03	18.695/15.713	2.99/1.19
	Ibuprofen	3.703/3.661	< 0.40/1.01	7.153/6.894	0.71/1.04	19.742/17.215	2.59/1.15
	Ketoprofen	3.273/3.015	1.03/1.04	6.300/5.884	2.65/1.07	15.274/12.883	4.05/1.19
	Pranoprofen	3.419/3.251	0.78/1.03	6.415/6.122	1.64/1.05	14.004/12.995	3.51/1.08
Vancomycin + CIL2	Naproxen	3.899/3.786	0.99/1.03	7.266/6.892	1.83/1.05	26.644/22.151	5.13/1.20
	Carprofen	3.624/3.552	0.64/1.02	6.672/6.465	1.37/1.03	17.191/15.829	3.14/1.09
	Ibuprofen	3.915/3.844	0.49/1.02	7.432/7.158	1.23/1.04	25.233/22.521	3.07/1.12
	Ketoprofen	3.322/3.162	1.19/1.05	6.864/6.376	2.14/1.08	18.549/15.992	3.95/1.16
	Pranoprofen	3.621/3.491	0.95/1.04	6.185/5.901	1.53/1.05	16.531/15.107	2.80/1.16

Conditions: fused-silica capillary, 33 cm (24.5 cm effective length) × 50 μm id; 50 mM phosphate buffer (organic modifier included) containing 2 mM vancomycin and 15 mM CIL1 or CIL2; buffer pH, 7.0; applied voltage, 20 kV; capillary temperature, 25 °C.

Table 2
Effect of the pH on migration time and Rs of tested chiral drugs.

Chiral drugs	pH									
	6.0		6.5		7.0		7.3		7.5	
	t_{R2}/t_{R1} (min)	Rs/α	t_{R2}/t_{R1} (min)	Rs/α	t_{R2}/t_{R1} (min)	Rs/α	t_{R2}/t_{R1} (min)	Rs/α	t_{R2}/t_{R1} (min)	Rs/α
Naproxen										
Vancomycin	17.456/15.729	4.34/1.13	16.626/14.66	3.02/1.13	13.097/11.981	2.46/1.09	10.032/9.670	1.85/1.04	9.628/9.214	1.33/1.04
Vancomycin+CIL1	28.330/24.055	4.97/1.22	25.737/22.851	4.89/1.22	23.607/19.001	4.93/1.24	11.003/10.303	2.63/1.07	10.149/9.719	1.91/1.04
Vancomycin+CIL2	37.260/31.774	4.56/1.16	29.719/25.172	4.97/1.18	26.644/22.151	5.13/1.20	12.907/11.917	2.77/1.08	10.534/9.994	1.79/1.05
Carprofen										
Vancomycin	14.166/13.084	2.12/1.08	13.364/12.640	2.00/1.06	10.941/10.427	1.69/1.05	7.808/7.617	1.10/1.03	8.296/8.094	0.95/1.02
Vancomycin+CIL1	26.874/25.261	2.28/1.09	26.413/25.127	2.33/1.09	18.695/15.713	2.99/1.19	9.491/9.138	1.37/1.04	8.708/8.478	0.98/1.03
Vancomycin+CIL2	28.816/27.226	2.62/1.09	27.086/21.166	3.44/1.28	17.191/15.829	3.14/1.09	10.940/10.485	1.81/1.04	8.301/8.088	1.29/1.03
Ibuprofen										
Vancomycin	17.237/15.784	2.06/1.14	14.360/13.509	1.84/1.06	12.863/12.049	1.60/1.07	9.277/8.962	0.91/1.04	8.387/8.085	0.84/1.03
Vancomycin+CIL1	23.348/20.168	2.28/1.14	20.269/18.782	2.48/1.14	19.742/17.215	2.59/1.15	13.233/12.564	1.22/1.05	10.308/9.945	0.75/1.04
Vancomycin+CIL2	32.762/28.816	2.93/1.15	29.317/25.789	3.04/1.12	25.233/22.521	3.07/1.12	12.999/12.267	1.93/1.06	9.044/8.746	1.31/1.03
Ketoprofen										
Vancomycin	16.136/13.754	3.45/1.15	13.170/12.017	3.28/1.10	10.811/9.842	2.45/1.10	8.146/7.755	1.67/1.05	7.501/7.178	1.45/1.04
Vancomycin+CIL1	21.376/18.912	3.71/1.16	18.870/16.146	3.95/1.18	15.274/12.883	4.05/1.19	10.789/9.941	2.74/1.09	8.802/8.372	1.90/1.05
Vancomycin+CIL2	22.197/19.319	3.76/1.16	19.785/17.316	3.97/1.17	18.549/15.992	3.95/1.16	11.267/10.291	2.74/1.09	8.015/7.601	1.71/1.05
Pranoprofen										
Vancomycin	17.014/14.926	2.51/1.10	14.327/13.477	2.44/1.07	10.814/10.103	1.72/1.07	8.293/8.014	1.24/1.03	7.878/7.607	0.95/1.04
Vancomycin+CIL1	21.875/18.254	3.73/1.14	16.784/14.713	3.76/1.15	14.004/12.995	3.51/1.08	9.829/9.273	1.76/1.09	7.931/7.682	1.05/1.04
Vancomycin+CIL2	20.825/18.167	2.67/1.13	18.015/16.769	2.94/1.16	16.531/15.107	2.80/1.16	11.449/10.747	2.06/1.07	8.171/7.859	1.26/1.04

Conditions: fused-silica capillary, 33 cm (24.5 cm effective length) × 50 μm id; 50 mM phosphate buffer (20% methanol included) containing 2 mM vancomycin and 15 mM CIL1 or CIL2;applied voltage, 20 kV; capillary temperature, 25 °C.

vancomycin-alone system and vancomycin/chiral ILs system was different, it can be concluded that chiral ILs take part in chiral recognition process in the vancomycin/chiral ILs system.

3.4. Influence of concentration of chiral ILs

With a fixed concentration of vancomycin (2 mM), a range of concentrations (from 0 to 25 mM) of L-AlaC$_4$NTf$_2$ and L-ValC$_4$NTf$_2$ was investigated to research on the role chiral ILs played in the synergistic system. As observed in Fig. 5, when the concentration of both chiral ILs increased from 0 to 15 mM, resolution (Rs) of all the five analytes were in an escalating trend, indicating the increased synergistic effect in the vancomycin/chiral ILs system. However, when the concentration further ascended, separation resolution went down due to the gradually saturated complexation. Finally, 15 mM of each chiral ILs was regarded as the optimal concentration of chiral ILs in the synergistic system.

3.5. Influence of the concentration of vancomycin

With an optimal concentration of each chiral ILs, we further investigated the effect of concentration of vancomycin on the chiral separation of five analytes (naproxen, carprofen, ibuprofen, ketoprofen and pranoprofen). A range of concentrations (from 1 mM to 5 mM) of vancomycin was studied. As the concentration of vancomycin increased from 1 mM to 5 mM, the electroosmotic mobility (μ_{eof}) gradually went down (Fig. 4B) in both of the vancomycin-alone system and vancomycin/chiral ILs system, indicating the adsorption of vancomycin on the capillary wall. Improvement of resolution and α of all five drugs were observed with the increasing concentration (1–4 mM) of vancomycin (Table 3) in all above systems, resulting from the increased complexation between chiral selector and enantiomers. When the concentration of vancomycin went up to 5 mM, no peak was found in long analysis times (more than 100 min). Unstable baselines caused by strong UV absorption of vancomycin tended to gradually severe when more than 3 mM of vancomycin was added in the running buffer. Taking resolution, small consumption of chiral selector and short analysis times for further consideration, lower concentration of vancomycin (2–3 mM) was more suitable for the synergistic system.

Compared to vancomycin-alone system, satisfied separation could be achieved for all the analytes with relatively low concentration of vancomycin in vancomycin/chiral ILs system. It is obvious that the establishment of synergistic systems could overcome several disadvantages of vancomycin, such as strong UV absorption, high solution viscosity and adsorption onto the capillary wall at higher concentrations.

3.6. Evaluation of the influences of the parameters on the synergistic system by SPSS

With a goal to study the effects of each parameter on the dual separation system, Statistical Product and Service Solutions (SPSS) was used as a calculation tool. The vancomycin/L-ValC$_4$NTf$_2$ synergistic system was taken as the model system, and five analytes were regarded as model drugs. In this method, methanol proportion ($X1$), the buffer pH ($X2$), the absence and presence of L-ValC$_4$NTf$_2$ ($X3$) and vancomycin concentration ($X4$) were considered as independent variables, while the separation resolution (Rs) of five analytes was regarded as dependent variable. The approach entitled multiple linear regression was performed by SPSS and the data operated by SPSS was obtained from the experimental results of the influences of these four parameters on enantioseparation by CE. A command entitled "Analyze→Regression→Linear" was clicked and then each Rs of five model drugs was selected as "Dependent" and $X1$, $X2$, $X3$, $X4$ were chosen as "Independent(s)". As a result, R (multiple correlation coefficient), standardized regression coefficient and sig. (the p-value of independent variables) were shown in Table 4.

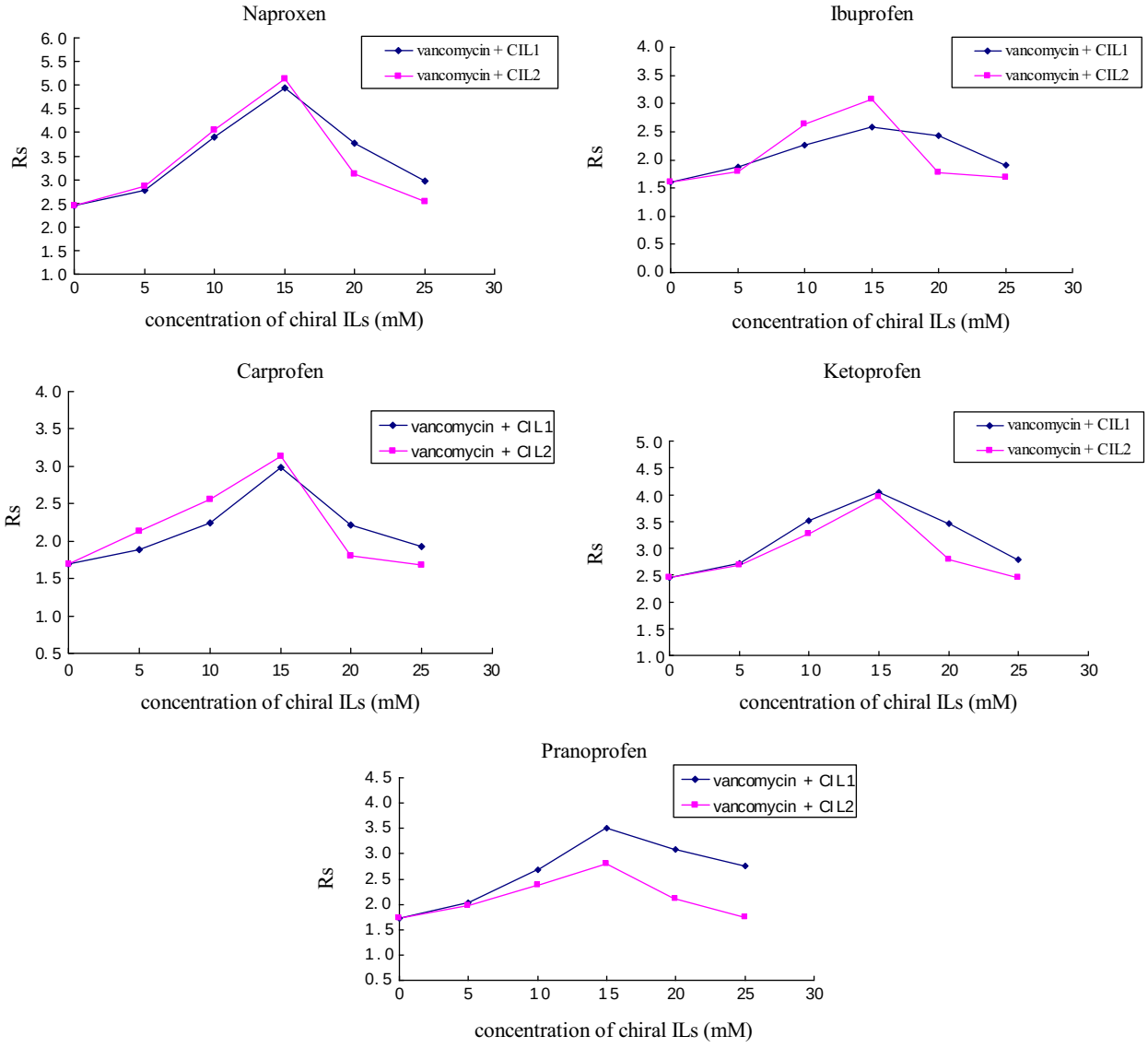

Fig. 5. Effect of the concentration of chiral ILs on resolution (Rs) of five NSAIDs. *Conditions*: fused-silica capillary, 33 cm (24.5 cm effective length) × 50 μm id; 50 mM phosphate buffer (20% (v/v) of methanol included) containing 2 mM vancomycin and 0–25 mM CIL1 or CIL2; pH 7.0; applied voltage, 20 kV; capillary temperature, 25 °C.

The results were explained as follows: R elucidates the fitting degree of the linear regression relationship between dependent variable and all the four independent variables. The value of R is between 0 and 1, and the closer to 1, the better linear regression can be found. As shown in Table 4, the R values of all the five model drugs are higher than 0.950, indicating the success establishment of the linear regression models. As for p-value, it is statistically significant when $p < 0.05$. From Table 4, all the p-values of all the independent variables in five models are much smaller than 0.05, which indicates that all the four factors have statistical significance in this linear regression model. It is worth mentioning that addition of chiral IL ($X3$) had a significant impact on chiral separation in the established separation system. The standardized regression coefficient provides a useful way to see the impact of changing each independent variable on the regression model. By comparison of the absolute value of standardized regression coefficient of each independent variable ($X1$, $X2$, $X3$, $X4$), we can figure out which parameter(s) is (are) more important for the synergistic system. The influence order was arranged as the results: $X4 > X1 > X2 > X3$ for naproxen, $X4 > X1 > X3 > X2$ for carprofen and ibuprofen, $X4 > X2 > X1 > X3$ for ketoprofen and pranoprofen. It can be noted that the vancomycin concentration played a key role in the separation system; however, chiral ILs is also an important parameter which can not be ignored.

3.7. Method application

Based on the above studies, we validated the method with respect to the LOD and LOQ of (R)-naproxen, linearity range of (R)-naproxen, stability of standard (R)-naproxen solution and (S)-naproxen sample solution and precision of (R)-naproxen optical impurity determination in (S)-naproxen sample, for testing the chiral purity. The electropherogram of chiral separation of 800 μg/mL (RS)-naproxen solution (400 μg/mL of each enantiomer) has been shown in Fig. 2. The peak identification was achieved by adding extra (R)-naproxen in the racemic naproxen. The first eluting peak was identified as the (R)-naproxen (impurity) and the second eluting peak was the (S)-naproxen. LODs and LOQs of (R)-naproxen were determined based on signal to noise ratio, 3:1 and 10:1, respectively. For vancomycin/CIL1 system, the LOD and LOQ for (R)-naproxen were 9.0 μg/mL and 30 μg/mL, corresponding to 0.18% and 0.60% impurity in (S)-naproxen sample (5.0 mg/ml in methanol/water solution, 50:50 v/

Table 3
Effect of the vancomycin concentration on migration time and Rs of tested chiral drugs.

Chiral drugs	Vancomycin concentration (mM)							
	1		2		3		4	
	t_{R2}/t_{R1} (min)	Rs/α	t_{R2}/t_{R1} (min)	Rs/α	t_{R2}/t_{R1} (min)	Rs/α	t_{R2}/t_{R1} (min)	Rs/α
Naproxen								
Vancomycin	9.154/8.812	1.47/1.04	13.097/11.981	2.46/1.09	22.263/18.692	4.74/1.19	28.043/22.850	6.42/1.23
Vancomycin+CIL1	10.872/10.145	1.72/1.05	23.607/19.001	4.93/1.24	24.145/19.641	7.37/1.16	66.903/32.911	8.86/2.03
Vancomycin+CIL2	12.220/11.568	1.80/1.06	26.644/22.151	5.13/1.20	27.810/23.125	6.19/1.22	48.360/29.256	8.17/1.65
Carprofen								
Vancomycin	7.318/7.712	0.91/1.02	10.941/10.427	1.69/1.05	15.399/14.206	2.88/1.08	24.449/20.345	5.32/1.20
Vancomycin+CIL1	9.721/9.418	1.17/1.03	18.695/15.713	2.99/1.19	19.750/18.194	3.31/1.19	21.219/18.481	5.46/1.21
Vancomycin +CIL2	10.311/10.001	1.21/1.03	17.191/15.829	3.14/1.09	21.638/19.064	3.69/1.14	26.549/21.774	5.52/1.22
Ibuprofen								
Vancomycin	7.736/7.549	0.96/1.02	12.863/12.049	1.60/1.07	20.671/18.411	3.06/1.12	25.221/21.295	4.14/1.18
Vancomycin+CIL1	10.734/10.217	1.24/1.03	19.742/17.215	2.59/1.15	22.021/19.102	3.25/1.16	23.409/19.921	5.04/1.18
Vancomycin +CIL2	11.995/11.498	1.30/1.04	25.233/22.521	3.07/1.12	28.833/26.063	3.38/1.13	30.989/28.451	4.88/1.14
Ketoprofen								
Vancomycin	6.943/6.671	1.22/1.04	10.811/9.842	2.45/1.10	15.794/13.675	3.29/1.15	16.834/14.112	5.17/1.19
Vancomycin+CIL1	9.986/9.315	1.78/1.06	15.274/12.883	4.05/1.19	18.145/15.813	4.24/1.18	22.486/19.002	5.70/1.23
Vancomycin +CIL2	10.712/10.044	1.62/1.07	18.549/15.992	3.95/1.16	21.727/19.308	4.37/1.17	25.734/23.318	5.26/1.18
Pranoprofen								
Vancomycin	7.020/6.809	0.74/1.03	10.814/10.103	1.72/1.07	15.335/13.917	2.62/1.10	16.543/14.516	4.00/1.14
Vancomycin+CIL1	9.712/9.178	1.37/1.05	14.004/12.995	3.51/1.07	15.832/14.271	3.89/1.12	18.511/16.963	5.20/1.15
Vancomycin+CIL2	10.986/10.487	1.26/1.05	16.531/15.107	2.80/1.16	17.784/16.286	3.40/1.16	20.680/18.845	4.40/1.17

Conditions: fused-silica capillary, 33 cm (24.5 cm effective length) × 50 μm id; 50 mM phosphate buffer (20% methanol included) containing 1–4 mM vancomycin with or without 15 mM CIL1 or CIL2; pH 7.0; applied voltage, 20 kV; capillary temperature, 25 °C.

Table 4
Tests of effects of each independent variable.

Dependent variable	*R*	Standardized regression coefficient				Sig.			
Rs		***X*1**	***X*2**	***X*3**	***X*4**	***X*1**	***X*2**	***X*3**	***X*4**
Rs (Naproxen)	0.958	0.365	−0.356	0.292	0.784	0.001	0.001	0.004	< 0.001
Rs (Carprofen)	0.950	0.320	−0.242	0.251	0.838	0.004	0.015	0.016	< 0.001
Rs (Ibuprofen)	0.960	0.400	−0.299	0.327	0.784	< 0.001	0.002	0.001	< 0.001
Rs (Ketoprofen)	0.961	0.348	−0.426	0.262	0.768	0.001	< 0.001	0.006	< 0.001
Rs (Pranoprofen)	0.970	0.315	−0.378	0.270	0.815	0.001	< 0.001	0.002	< 0.001

Calculation method: multiple linear regression supplied by SPSS; *X*1 (scale), methanol proportion (0%, 10%, 20%); *X*2 (scale), buffer pH (6.0, 6.5, 7.0, 7.3, 7.5); *X*3 (ordinal), addition of CIL2 (0—without L-ValC$_4$NTf$_2$, 1—with L-ValC$_4$NTf$_2$ in an optimal concentration); *X*4 (scale), vancomycin concentration (1 mM, 2 mM, 3 mM, 4 mM). *R*, multiple correlation coefficient; Sig., the *p*-value of independent variables.

v). For vancomycin/CIL2 system, the LOD and LOQ for (R)-naproxen were 4.5 μg/mL and 15 μg/mL, corresponding to 0.09% and 0.30% impurity in (S)-naproxen sample (5.0 mg/ml in methanol/water solution, 50:50 v/v). The linearity of the (R)-naproxen was evaluated at optimal conditions with two vancomycin/chiral ILs systems. Linear regression analysis ($Y=aX+b$) was performed over the range of 30–400 μg/mL R-naproxen solution (methanol/water, 50:50 v/v) for vancomycin/CIL1 system and 15–400 μg/mL R-naproxen solution (methanol/water, 50:50 v/v) for vancomycin/CIL2 system. The following equation and correlation coefficient (r^2) were obtained: $Y=1.1111X+0.91$ and 0.9968 for vancomycin/CIL1 system, $Y=1.1568X+1.7445$ and 0.9961 for vancomycin/CIL2 system (X represents the concentration of (R)-naproxen, μg/mL; Y represents peak area of (R)-naproxen). The stability of standard (R)-naproxen solution (400 μg/mL in methanol/water solution, 50:50 v/v) and (S)-naproxen sample (real commercial bulk drug) solution (5.0 mg/ml in methanol/water solution, 50:50 v/v) were also evaluated by six measurements at 0 h, 2 h, 4 h, 8 h, 12 h and 24 h. For both vancomycin/CILs systems, the RSD of peak area of (R)-naproxen in standard solution were less than 2.9%, and the RSD of peak area of (R)-naproxen and (S)-naproxen in sample solution were less than 2.7% and 3.6%, respectively. Then, the established method was used to determine the (R)-naproxen impurity in 5.0 mg/ml (S)-naproxen sample solution by area normalization method, while the precision of optical impurity determination was also evaluated by six replicate testing during a single day and by duplicating the experiments on six successive days. The average of impurity content of (S)-naproxen sample was 0.9867% for vancomycin/CIL1 system and 0.9927% for vancomycin/CIL2 system. The RSD of intraday of impurity content were 3.1% for vancomycin/CIL1 system and 3.7% for vancomycin/CIL2 system, and the RSD of interday were 3.8% for vancomycin/CIL1 system and 3.6% for vancomycin/CIL2 system. The typical electrophoretograms of optical purity test with both vancomycin/CILs systems were seen in Fig. 6.

4. Concluding remarks

This paper demonstrated that two chiral ILs based on amino acid ester, L-AlaC$_4$NTf$_2$ and L-ValC$_4$NTf$_2$, can be successfully applied as additives for chiral separation in CE. The vancomycin-based synergistic system with chiral ILs as additives were first established and systematically investigated for chiral separation by CE. Significant

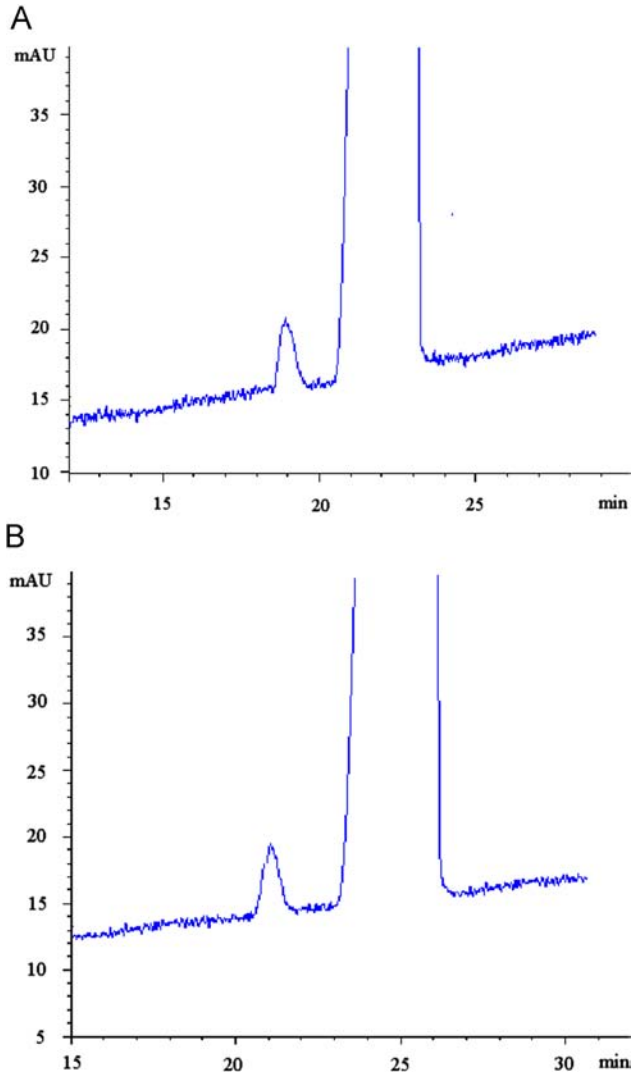

Fig. 6. Typical electrophoretograms of optical purity test of (S)-naproxen sample with (A) combined use of vancomycin and CIL1, (B) combined use of vancomycin and CIL2. *Conditions*: fused-silica capillary, 33 cm (24.5 cm effective length) × 50 μm id; 50 mM phosphate buffer (20% (v/v) of methanol included) containing 2 mM vancomycin and 15 mM CIL1 or CIL2; pH 7.0; applied voltage, 20 kV; capillary temperature, 25 °C.

improvements of chiral separation for analytes were observed after adding chiral ILs compared to vancomycin-alone cases with other conditions unchanged. In addition, the establishment of synergistic system could also overcome several disadvantages of vancomycin selector in CE chiral separation.

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