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Di-*n*-amyl L-tartrate–boric acid complex chiral selector *in situ* synthesis and its application in chiral nonaqueous capillary electrophoresis

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ABSTRACT

A chiral selector, di-*n*-amyl L-tartrate–boric acid complex, was *in situ* synthesized by the reaction of di-*n*-amyl L-tartrate with boric acid in a nonaqueous background electrolyte (BGE) using methanol as the medium. And a new method of chiral nonaqueous capillary electrophoresis (NACE) was developed with the complex as the chiral selector. It has been demonstrated that the chiral selector is suitable for the enantioseparation of some β-blockers and β-agonists in NACE. Some chiral analytes that could not be resolved in aqueous microemulsion electrokinetic chromatography (MEEKC) with the same chiral selector obtained baseline resolutions in the NACE system. The enantioseparation mechanism was considered to be ion-pair principle and the nonaqueous system was more favorable for the ion-pair formation which is quite useful for the chiral recognition. The addition of a proper concentration of triethylamine into the BGE to control the apparent pH (pH*) enhanced the enantiomeric discrimination. In order to achieve a good enantioseparation, the effects of di-*n*-amyl L-tartrate and boric acid concentration, triethylamine concentration, applied voltage, as well as capillary length were investigated. Under the optimum conditions, all of the tested chiral analytes including six β-blockers and five β-agonists were baseline resolved.

1. Introduction

In recent years, capillary electrophoresis (CE) has been an important choice among the separation methods in biomedical, environmental, agricultural, and pharmaceutical research. In particular, it has become widely popular for enantioseparations due to its high efficiency and selectivity, simplicity, versatility, and low sample and chiral selector consumption [1–6]. Although the majority of enantioseparations have been carried out in aqueous BGEs, nonaqueous capillary electrophoresis (NACE) has also been proved to be a very powerful tool in enantiomer separations [7–13]. For enantioseparation, NACE has several advantages over traditional aqueous CE [13–17]. First, it may improve the enantioseparation for selectors with a lack of or low enantioselectivity in aqueous BGEs [13,14]. Second, it facilitates the use of chiral selectors with a low solubility in water [15] and enables non or poorly water-soluble substances to be analyzed [16]. Third, the higher volatility of most of the nonaqueous solvents facilitates the hyphenation to mass spectrometry detector [13,17].

Tartrate is a type of widely used chiral selector and many papers were published devoted to their use as chiral selectors [18–24]. Dialkyltartrate has ever been used together with boric acid on enantioselective extraction of some β-blockers [18,25,26] or chiral microemulsion electrokinetic chromatography (MEEKC) of some β-blockers and structurally related compounds [27,28], but there is no published paper reporting the enantioseparation using tartrate chiral selector in NACE up to date.

The aim of this study was to develop a novel NACE method for the enantioseparation of some β-blockers and β-agonists. Di-*n*-amyl L-tartrate–boric acid complex, *in situ* synthesized by the reaction of di-*n*-amyl L-tartrate with boric acid in a nonaqueous BGE using methanol as the medium, was selected as the chiral selector. Some analytes such as sotalol, bisoprolol, atenolol or metoprolol, which could not be enantioseparated with di-*n*-amyl L-tartrate–boric acid complex chiral selector in aqueous MEEKC [27,28], were resolved easily in the new NACE method and the reason was discussed. Like in aqueous media, in addition to the chiral selector, the most significant parameter for modification of the separation selectivity is the composition of the BGE, especially affecting the pH* in NACE [13,29]. Certain concentration of triethylamine was used to control pH* of the BGEs. In order to achieve a good enantioseparation, the effects of di-*n*-amyl L-tartrate and boric acid concentration, triethylamine concentration, applied voltage and capillary length on the enantioseparation were investi-

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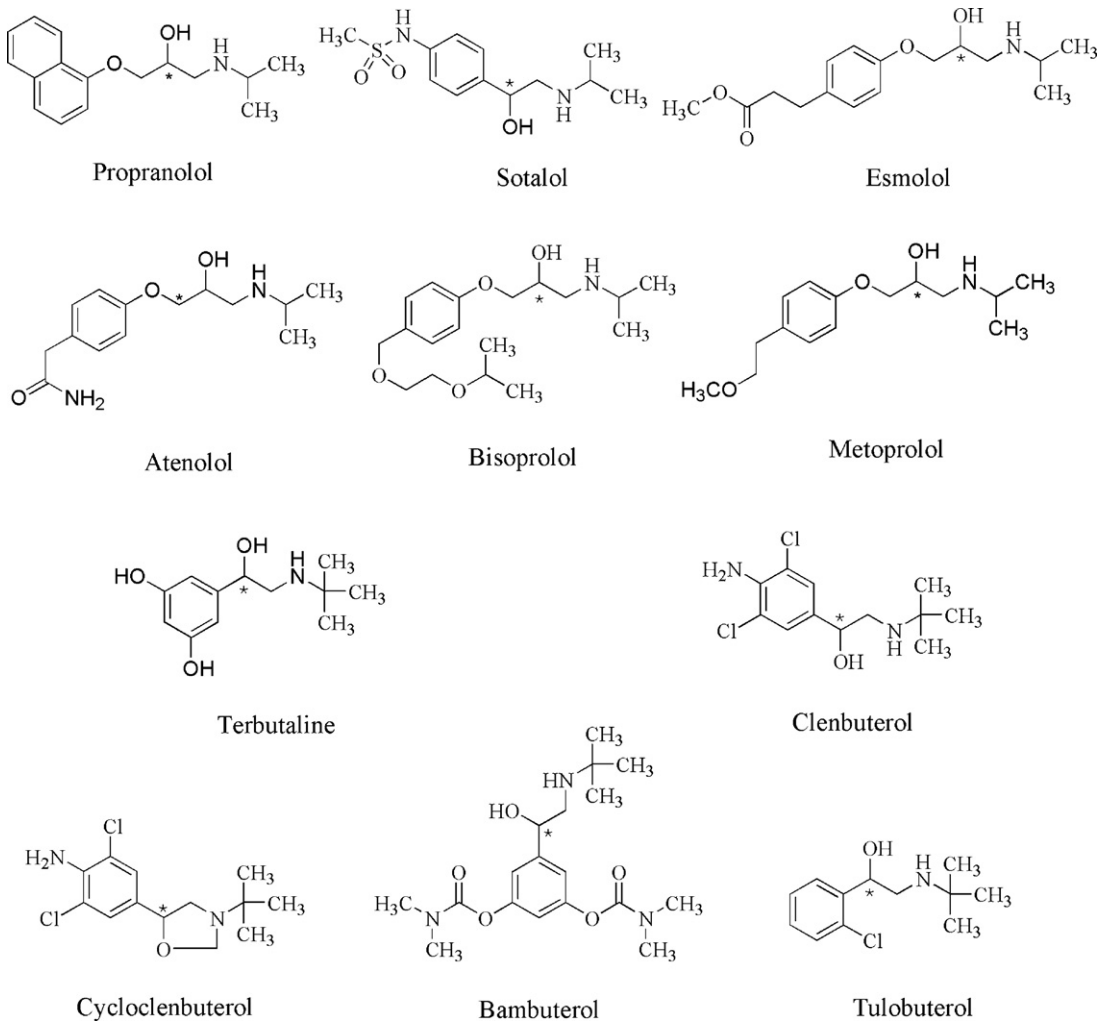

Fig. 1. Structural formulas of chiral analytes.

gated. Baseline separations of six β-blockers including propranolol, sotalol, esmolol, atenolol, bisoprolol and metoprolol, and five β-agonists including terbutaline, clenbuterol, cycloclenbuterol, bambuterol and tulobuterol were achieved under the optimum conditions.

2. Experimental

2.1. Instrumentation

NACE experiments were conducted on a CAILU capillary electrophoresis system (Beijing Cailu Scientific Instrument Co., LTD., Beijing, China), equipped with a UV detector. Data were collected with a QIANPU (HW-2000) chromatography work station. Uncoated fused silica capillaries of 50 μm I.D. (Yongnian Reafine Chromatography Co., LTD., Hebei, China) with a total length (L_{tot}) of 45.0 cm and an effective length (L_{eff}) of 37.0 cm, or L_{tot} 53.0 cm and L_{eff} 45.0 cm were used. All new capillaries were conditioned by flushing with methanol for 10 min, 1.0 M NaOH for 20 min, distilled water for 5 min, 1.0 M hydrochloric acid for 20 min and distilled water for 5 min in sequence. Before each run the capillary was rinsed with running buffer for 3 min. Injections were performed hydrostatically for 5 s at a 10 cm height difference. The experiments were performed at room temperature. The detection wavelength was set at 214 nm.

2.2. Chemicals and reagents

Racemic sotalol hydrochloride, esmolol hydrochloride, clenbuterol hydrochloride, cycloclenbuterol hydrochloride, bambuterol hydrochloride, and tulobuterol hydrochloride were purchased from National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China). The following racemic compounds were extracted by water/methanol (1:1, v/v) from medicine tablets: propranolol hydrochloride (LI®, Tianjin Lisheng Pharmaceuticals Co., Ltd., China), atenolol (YJ®, Beijing Yanjing Pharmaceuticals Co., Ltd., China), bisoprolol fumarate (BOSU®, Wellso Pharmaceuticals Co., Ltd., China), metoprolol tartrate (BETALOC®, AstraZeneca Pharmaceuticals Co., Ltd., China), terbutaline sulphate (BRICANYL®, AstraZeneca Pharmaceuticals Co., Ltd., China).

Di-*n*-amyl L-tartrate (purity: >98%) was synthesized in our laboratory as reported in Ref. [30], and characterized by NMR and IR. 1-Pentanol was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). L-Tartrate was purchased from Tianjin Fuchen Chemical Reagent Factory (Tianjin, China). Boric acid was the product of Baoding Chemical Reagent Factory (Baoding, China). Sodium acetate was the product of Beijing Chemical Reagent Company (Beijing, China). Triethylamine was supplied by Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Methanol, chromatographic reagent grade, was purchased

Table 1
Enantioseparation parameters of analytes under optimum conditions.[a]

Analytes	Migration time (min)		Effective electrophoretic mobilities ($\times 10^{-5}$ cm^2 V^{-1} s^{-1})		Enantioselectivity (α_{eff})	Resolution (R_s)	Efficiency	
	t_1	t_2	μ_{eff1}	μ_{eff2}			N_1	N_2
Propranolol[b,d]	11.711	12.399	8.869	7.928	1.119	3.00	55,182	36,815
Sotalol[b,d]	11.137	11.628	9.743	8.990	1.084	2.26	58,649	38,826
Esmolol[b,d]	11.192	11.871	9.656	8.641	1.117	2.53	34,911	24,299
Atenolol[b,d]	11.616	12.231	9.007	8.148	1.106	2.36	43,936	29,421
Bisoprolol[b,d]	11.511	12.151	9.164	8.255	1.110	2.30	36,084	26,535
Metoprolol[b,d]	10.632	11.244	10.59	9.574	1.106	2.38	36,963	26,094
Terbutaline[c,e]	10.505	11.461	11.78	10.20	1.155	3.85	43,147	29,044
Clenbuterol[c,e]	10.818	11.992	11.23	9.429	1.191	4.15	36,812	21,550
Cycloclenbuterol[c,e]	10.884	12.061	11.12	9.335	1.191	3.95	32,559	20,427
Bambuterol[c,e]	10.704	11.664	11.42	9.895	1.155	3.15	28,819	19,074
Tulobuterol[c,e]	9.973	11.345	12.78	10.37	1.232	3.63	21,512	9,446

[a] CE conditions: capillary dimensions, L_{tot} 53.0 cm, L_{eff} 45.0 cm, I.D. 50 μm; hydrostatic injection for 5 s at 10 cm height difference; applied voltage, 20 kV; detection wavelength, 214 nm.
[b] $\mu_{EOF} = 8.103 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[c] $\mu_{EOF} = 7.144 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[d] Optimal buffer solution: 100 mM boric acid, 80 mM di-*n*-amyl L-tartrate, 50 mM triethylamine in methanol.
[e] Optimal buffer solution: 120 mM boric acid, 100 mM di-*n*-amyl L-tartrate, 50 mM triethylamine in methanol.

Fig. 2. Electropherograms of analytes under optimum conditions. Experimental conditions are the same as in Table 1.

from Tianjin Concord Technology Co., Ltd. (Tianjin, China). The other reagents and chemicals were all of analytical reagent grade and used as received.

2.3. BGE and sample preparation

CE BGE was prepared by weighing the desired quantities of boric acid, di-*n*-amyl L-tartrate, and dissolving them in methanol to the desired volume in a flask. Then appropriate concentration

Table 2
Effect of boric acid concentration on effective mobility, enantioselectivity and resolution.[a,b]

Analytes	0 mM boric acid				20 mM boric acid				40 mM boric acid				60 mM boric acid			
	μ_{eff1}[c]	μ_{eff2}[c]	α_{eff}	R_s	μ_{eff1}[d]	μ_{eff2}[d]	α_{eff}	R_s	μ_{eff1}[e]	μ_{eff2}[e]	α_{eff}	R_s	μ_{eff1}[f]	μ_{eff2}[f]	α_{eff}	R_s
Propranolol	–	–	–	–	5.587	5.382	1.038	0.74	6.178	5.653	1.093	1.92	7.125	6.428	1.108	2.11
Sotalol	–	–	–	–	5.400	5.314	1.016	0.32	6.673	6.305	1.058	1.29	7.675	7.162	1.072	1.37
Esmolol	–	–	–	–	6.685	6.467	1.034	0.63	7.203	6.661	1.081	1.53	7.929	7.218	1.098	1.64
Atenolol	–	–	–	–	6.434	6.291	1.023	0.45	6.851	6.390	1.072	1.26	7.109	6.491	1.095	1.55
Bisoprolol	–	–	–	–	6.327	6.142	1.030	0.59	6.617	6.135	1.079	1.48	6.943	6.289	1.104	1.72
Metoprolol	–	–	–	–	6.869	6.816	1.008	0.66	7.508	6.960	1.079	1.53	8.062	7.325	1.101	1.74
Terbutaline	–	–	–	–	8.854	8.423	1.051	1.34	9.830	8.890	1.106	2.69	10.22	9.029	1.132	3.16
Clenbuterol	–	–	–	–	8.051	7.562	1.065	1.43	9.228	8.175	1.129	2.68	9.693	8.312	1.166	3.42
Cycloclenbuterol	–	–	–	–	7.878	7.381	1.067	1.56	9.374	8.425	1.113	2.46	9.731	8.359	1.164	3.17
Bambuterol	–	–	–	–	8.113	7.698	1.054	1.17	9.073	8.254	1.099	1.63	9.884	8.727	1.133	2.48
Tulobuterol	–	–	–	–	8.511	7.850	1.084	1.55	10.19	8.932	1.141	2.35	10.94	9.164	1.194	3.11

Analytes	80 mM boric acid				100 mM boric acid				120 mM boric acid			
	μ_{eff1}[g]	μ_{eff2}[g]	α_{eff}	R_s	μ_{eff1}[h]	μ_{eff2}[h]	α_{eff}	R_s	μ_{eff1}[i]	μ_{eff2}[i]	α_{eff}	R_s
Propranolol	7.515	6.700	1.122	2.47	8.156	7.232	1.128	2.95	8.842	7.862	1.125	2.85
Sotalol	8.532	7.946	1.074	1.37	9.011	8.266	1.090	2.02	9.718	8.937	1.087	1.75
Esmolol	8.367	7.706	1.105	1.94	8.682	7.768	1.118	2.55	9.303	8.323	1.118	2.24
Atenolol	7.883	7.185	1.097	1.73	8.106	7.330	1.106	2.16	8.959	8.111	1.105	1.84
Bisoprolol	7.614	6.864	1.109	1.92	8.078	7.256	1.113	2.17	8.780	7.908	1.110	2.16
Metoprolol	8.586	7.752	1.107	1.83	9.317	8.387	1.111	2.15	9.995	8.997	1.111	1.97
Terbutaline	10.95	9.567	1.144	3.15	11.74	1.029	1.141	3.42	11.78	1.020	1.155	3.85
Clenbuterol	10.49	8.940	1.174	3.27	11.39	9.798	1.162	3.83	11.23	9.429	1.191	4.15
Cycloclenbuterol	10.39	8.832	1.176	3.32	10.88	9.313	1.168	3.34	11.12	9.335	1.191	3.95
Bambuterol	10.88	9.610	1.132	2.46	11.48	10.05	1.142	3.05	11.42	9.895	1.155	3.15
Tulobuterol	11.82	9.805	1.206	3.42	12.24	9.962	1.228	3.59	12.78	10.37	1.232	3.63

[a] Buffer component in addition to boric acid is 100 mM di-*n*-amyl L-tartrate and 50 mM triethylamine in methanol. Other conditions are the same as in Table 1.
[b] (–)means $t_1 = t_2$, $\mu_{eff1} = \mu_{eff2}$, $\alpha_{eff} = 1$, $R_s = 0$.
[c] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 35.05 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[d] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 11.37 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[e] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 9.395 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[f] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 8.991 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[g] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 8.518 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[h] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 7.573 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[i] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 7.144 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.

of triethylamine was added. The sample solution was prepared by dissolving an appropriate quantity of each racemic sample in methanol/water (1:1, v/v) to a concentration of 0.5 mg/mL. All solutions were filtered through a 0.45 μm syringe type filter prior to use.

2.4. Calculations of performance parameters

The selectivity (α_{eff}) was calculated according to $\alpha_{eff} = \mu_{eff1}/\mu_{eff2}$, where $\mu_{eff} = \mu_{app} - \mu_{EOF} = ((L_{tot}L_{eff})/Vt) - ((L_{tot}L_{eff})/Vt_{EOF})$ (μ_{eff} is the effective mobility, μ_{app} is the apparent mobility, μ_{EOF} is the electroosmotic mobility, V is the applied voltage). Other data including the chiral resolution (R_s) and the theoretical plates number (N) were calculated according to $Rs = (2(t_2 - t_1))/(w_1 + w_2)$ and $N = 5.54(t/w_{1/2})^2$, respectively (t_1 and t_2 are the observed migration times, w_1 and w_2 are the observed peak widths of the enantiomers on the baseline, and $w_{1/2}$ is the peak width at half height), with a QIANPU (HW-2000) chromatography work station.

3. Results and discussion

In this study, six β-blockers and five β-agonists were tested; their molecular structures are listed in Fig. 1. As can be seen in Fig. 2 and Table 1, all the analytes achieved baseline resolutions. Methanol containing di-*n*-amyl L-tartrate, boric acid and triethylamine was used as the BGE.

3.1. Choice of chiral selector and effects of di-n-amyl L-tartrate and boric acid concentrations

Similar to enantioseparation in aqueous CE, the selection of the suitable chiral selectors is very important in NACE enantioseparations. The experimental results showed that the analytes could not be resolved with the BGEs containing only di-*n*-amyl L-tartrate without boric acid. This indicated that boric acid plays an important role for enantioseparation and the real chiral selector is the complex of di-*n*-amyl L-tartrate and boric acid instead of di-*n*-amyl L-tartrate itself.

The effects of the concentration of boric acid and di-*n*-amyl L-tartrate on the chiral separation were investigated in a range of 0–120 mM and 0–100 mM, respectively. Because the reaction of di-*n*-amyl L-tartrate and boric acid is reversible, the increase in the concentration of both of them will promote the production of the chiral selector and thus improve the chiral separation. Of the chromatographic figures of merit, the efficiency was not affected obviously by the concentrations of di-*n*-amyl L-tartrate and boric acid. However, the increase in their concentrations results in the increase in the enantioselectivity (α_{eff}) and resolution (R_s) (Tables 2 and 3). Finally, a boric acid optimal concentration of 100 mM and a di-*n*-amyl L-tartrate optimal concentration of 80 mM were selected for the six β-blockers, and a boric acid optimal concentration of 120 mM and a di-*n*-amyl L-tartrate optimal concentration of 100 mM were selected for the five β-agonists, based on the R_s obtained. Sotalol, bisoprolol, atenolol or metoprolol, which could not be enantioseparated in aqueous MEEKC, were well resolved in this study. The increased discrimination in NACE is assumed to be due to the molecular interaction mech-

Table 3
Effect of di-*n*-amyl L-tartrate concentration on effective mobility, enantioselectivity and resolution.[a,b]

Analytes	0 mM di-*n*-amyl L-tartrate				20 mM di-*n*-amyl L-tartrate				40 mM di-*n*-amyl L-tartrate			
	μ_{eff1}[c]	μ_{eff2}[c]	α_{eff}	R_s	μ_{eff1}[d]	μ_{eff2}[d]	α_{eff}	R_s	μ_{eff1}[e]	μ_{eff2}[e]	α_{eff}	R_s
Propranolol	–	–	–	–	6.706	6.424	1.044	0.99	6.596	6.133	1.075	1.66
Sotalol	–	–	–	–	7.252	7.075	1.025	0.44	6.609	6.306	1.048	0.83
Esmolol	–	–	–	–	7.731	7.412	1.043	0.78	8.108	7.578	1.070	1.40
Atenolol	–	–	–	–	7.538	7.283	1.035	0.61	7.614	7.146	1.066	1.14
Bisoprolol	–	–	–	–	7.490	7.250	1.033	0.63	7.401	6.912	1.071	1.36
Metoprolol	–	–	–	–	8.717	8.426	1.035	0.65	8.339	7.793	1.070	1.43
Terbutaline	–	–	–	–	10.47	10.05	1.042	1.06	10.57	9.787	1.080	1.90
Clenbuterol	–	–	–	–	9.901	9.392	1.054	1.23	10.17	9.232	1.102	2.13
Cycloclenbuterol	–	–	–	–	9.396	8.869	1.059	1.39	9.222	8.289	1.113	2.30
Bambuterol	–	–	–	–	9.516	9.112	1.044	0.86	10.20	9.433	1.081	1.70
Tulobuterol	–	–	–	–	10.18	9.407	1.082	1.42	11.10	9.759	1.137	2.26

Analytes	60 mM di-*n*-amyl L-tartrate				80 mM di-*n*-amyl L-tartrate				100 mM di-*n*-amyl L-tartrate			
	μ_{eff1}[f]	μ_{eff2}[f]	α_{eff}	R_s	μ_{eff1}[g]	μ_{eff2}[g]	α_{eff}	R_s	μ_{eff1}[h]	μ_{eff2}[h]	α_{eff}	R_s
Propranolol	7.277	6.575	1.107	2.56	8.869	7.928	1.119	3.00	8.156	7.232	1.128	2.95
Sotalol	8.162	7.662	1.065	1.30	9.743	8.990	1.084	2.26	9.011	8.266	1.090	2.02
Esmolol	8.065	7.350	1.097	1.90	9.656	8.641	1.117	2.53	8.682	7.768	1.118	2.55
Atenolol	7.458	6.847	1.089	1.56	9.007	8.148	1.106	2.36	8.106	7.330	1.106	2.16
Bisoprolol	7.215	6.582	1.096	1.92	9.164	8.255	1.110	2.30	8.078	7.256	1.113	2.17
Metoprolol	9.049	8.343	1.085	2.05	10.59	9.574	1.106	2.38	9.317	8.387	1.111	2.15
Terbutaline	11.22	10.13	1.108	3.15	13.50	11.95	1.130	3.65	11.74	1.029	1.141	3.42
Clenbuterol	10.85	9.592	1.131	3.38	12.76	10.96	1.164	3.91	11.39	9.798	1.162	3.83
Cycloclenbuterol	10.80	9.533	1.132	3.39	12.66	10.83	1.169	3.83	10.88	9.313	1.168	3.34
Bambuterol	10.95	9.908	1.106	2.78	12.71	11.18	1.137	3.05	11.48	10.05	1.142	3.05
Tulobuterol	12.08	10.34	1.169	3.36	14.07	11.59	1.214	3.60	12.24	9.962	1.228	3.59

[a] Buffer component in addition to di-*n*-amyl L-tartrate is 100 mM boric acid and 50 mM triethylamine in methanol. Other conditions are the same as in Table 1.
[b] (–) means $t_1 = t_2$, $\mu_{eff1} = \mu_{eff2}$, $\alpha_{eff} = 1$, $R_s = 0$.
[c] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 13.23 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[d] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 11.31 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[e] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 9.673 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[f] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 7.547 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[g] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 8.103 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[h] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 7.573 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.

Table 4
Effect of triethylamine concentration on migration time, effective mobility, enantioselectivity and resolution.[a,b]

Analytes	0 mM triethylamine						7.2 mM triethylamine						36 mM triethylamine					
	t_1	t_2	μ_{eff1}[c]	μ_{eff2}[c]	α_{eff}	R_s	t_1	t_2	μ_{eff1}[d]	μ_{eff2}[d]	α_{eff}	R_s	t_1	t_2	μ_{eff1}[e]	μ_{eff2}[e]	α_{eff}	R_s
Propranolol	4.754	4.754	–	–	–	–	5.258	5.494	19.77	18.14	1.089	1.04	9.695	10.252	11.04	9.922	1.112	2.78
Sotalol	3.951	3.951	–	–	–	–	4.943	5.072	22.18	21.16	1.048	0.42	9.067	9.440	12.46	11.59	1.075	1.46
Esmolol	4.455	4.455	–	–	–	–	5.092	5.313	21.00	19.38	1.084	0.68	9.193	9.698	12.15	11.03	1.102	2.11
Atenolol	4.109	4.109	–	–	–	–	5.272	5.475	19.67	18.27	1.077	0.65	9.581	10.062	11.28	10.29	1.096	1.86
Bisoprolol	4.182	4.182	–	–	–	–	5.417	5.624	18.66	17.31	1.078	0.57	9.681	10.203	11.06	10.01	1.105	2.06
Metoprolol	3.475	3.475	–	–	–	–	5.077	5.289	21.12	19.55	1.080	0.59	9.194	9.725	12.15	10.97	1.108	2.18
Terbutaline	4.001	4.001	–	–	–	–	4.923	5.146	22.34	20.59	1.085	0.87	8.554	9.206	13.77	12.12	1.136	3.32
Clenbuterol	3.749	3.749	–	–	–	–	4.862	5.192	22.85	20.25	1.129	1.10	8.749	9.595	13.25	11.25	1.178	3.35
Cycloclenbuterol	3.449	3.449	–	–	–	–	4.780	5.065	23.55	21.21	1.110	1.00	8.593	9.432	13.66	11.61	1.177	3.43
Bambuterol	3.721	3.721	–	–	–	–	5.090	5.410	21.02	18.71	1.123	0.83	8.402	9.085	14.19	12.41	1.143	2.61
Tulobuterol	2.848	2.848	–	–	–	–	4.425	4.786	26.89	23.50	1.144	1.05	8.171	9.198	14.86	12.14	1.224	3.15

Analytes	50 mM triethylamine						72 mM triethylamine					
	t_1	t_2	μ_{eff1}[f]	μ_{eff2}[e]	α_{eff}	R_s	t_1	t_2	μ_{eff1}[g]	μ_{eff2}[g]	α_{eff}	R_s
Propranolol	11.711	12.399	8.869	7.928	1.119	3.00	13.324	13.916	7.495	6.860	1.092	2.25
Sotalol	11.137	11.628	9.743	8.990	1.084	2.26	13.002	13.409	7.864	7.400	1.063	1.67
Esmolol	11.192	11.871	9.656	8.641	1.117	2.49	13.006	13.600	7.859	7.192	1.093	2.09
Atenolol	11.616	12.231	9.007	8.148	1.106	2.36	13.552	14.111	7.244	6.663	1.087	1.86
Bisoprolol	11.511	12.151	9.164	8.255	1.110	2.30	13.806	14.416	6.974	6.365	1.096	2.07
Metoprolol	10.632	11.244	10.59	9.574	1.106	2.38	12.878	13.497	8.012	7.304	1.097	2.12
Terbutaline	9.201	9.912	13.50	11.95	1.130	3.65	11.156	11.901	1.039	9.278	1.120	3.24
Clenbuterol	9.527	10.427	12.76	10.96	1.164	3.91	11.594	12.516	9.721	8.458	1.149	3.13
Cycloclenbuterol	9.571	10.497	12.66	10.83	1.169	3.83	11.580	12.510	9.741	8.466	1.151	3.17
Bambuterol	9.551	10.308	12.71	11.18	1.137	3.05	11.449	12.209	9.937	8.857	1.122	2.82
Tulobuterol	8.966	10.094	14.07	11.59	1.214	3.60	11.152	12.308	10.40	8.726	1.192	3.56

[a] Buffer component in addition to triethylamine is 80 mM di-*n*-amyl L-tartrate and 100 mM boric sodium in methanol. Other conditions are the same as in Table 1.
[b] (–) means $\alpha_{eff} = 1$, $\mu_{eff1} = \mu_{eff2}$, $R_s = 0$.
[c] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 23.20 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[d] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 18.03 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[e] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 9.466 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[f] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 8.103 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
[g] $\times 10^{-5}$ cm^2 V^{-1} s^{-1}, $\mu_{EOF} = 7.422 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.

anisms that might be very different in aqueous and nonaqueous BGEs [31].

The present enantioseparations are assumed to be based on a reversible formation of diastereomeric ion-pairs between the negatively charged chiral counter-ion di-*n*-amyl L-tartrate–boric acid and positively charged enantiomeric aminoalcohols. The electrophoretic mobilities of free enantiomeric aminoalcohols are equal whereas the uncharged diastereomeric ion-pairs have no electrophoretic mobility. Thus, the enantiomeric mobility difference ($\Delta\mu$) or the enantioselectivity (α_{eff}) is based on the differences in the equilibrium constants for ion-pair formation, the mobility of the free forms of the analyte and the concentration of the chiral selector [14]. Since the lower dielectric constants in organic solvents promote the ion-pair formation, the improvement of chiral recognition performance in nonaqueous BGEs than aqueous ones can be elucidated [13].

3.2. Effect of the pH* of the BGE

The utilized chiral selector is di-*n*-amyl L-tartrate–boric acid complex which is an acidic protolyte, and the proper pH* is important for it to become negatively charged through deprotonation. In this paper, triethylamine used to control the pH* of the BGE was added along with the chiral selector. In most cases, the charge of protolytes is controlled using a suitable buffer. However, when using ion-pairing chiral selectors, ions from the buffer might form competing ion-pairs with the selector and/or

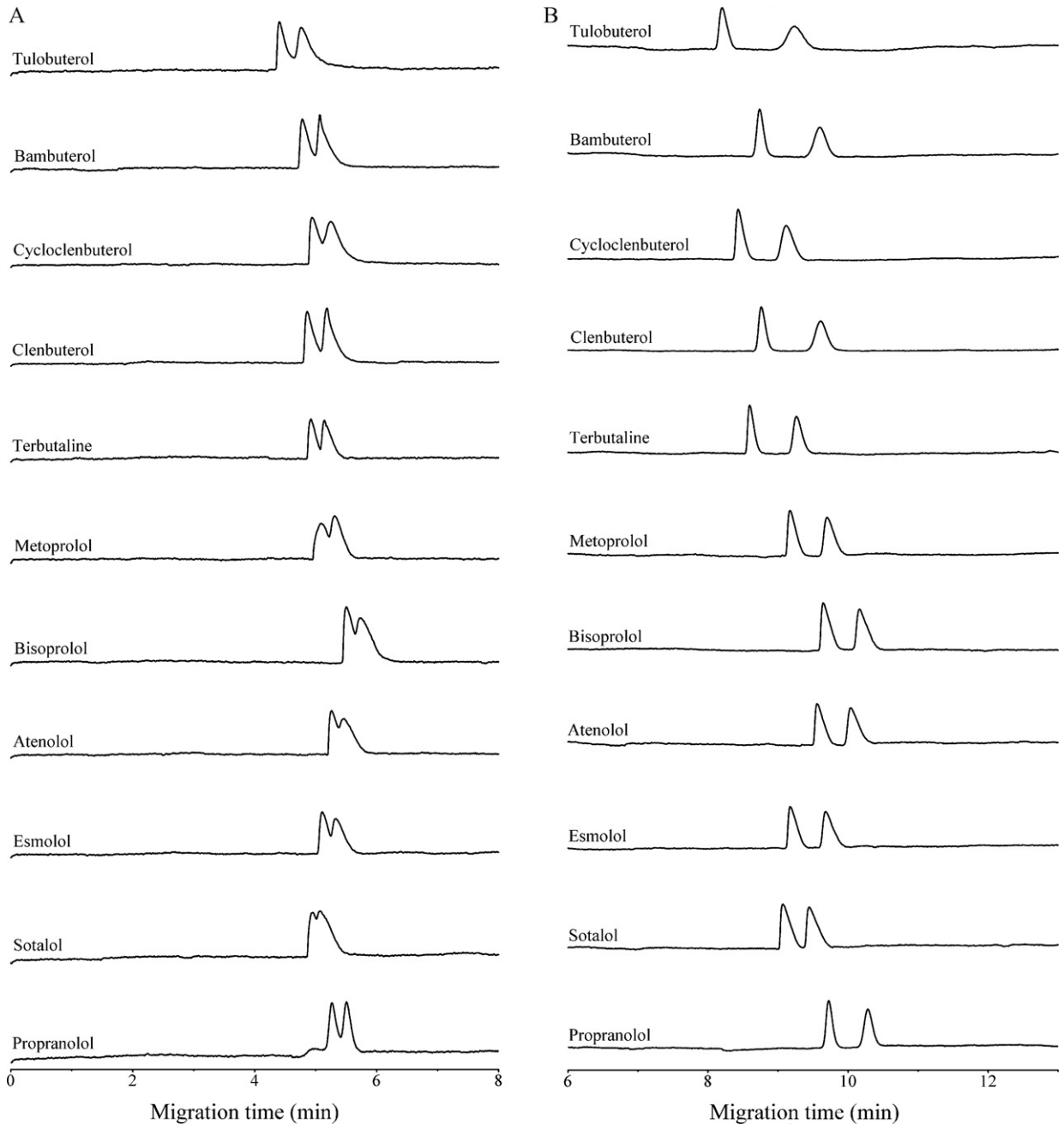

Fig. 3. Effect of triethylamine concentration on NACE chiral separation. BGE composition: 80 mM di-*n*-amyl L-tartrate, 100 mM boric acid and 7.2 mM triethylamine (A), 36 mM triethylamine (B), 72 mM triethylamine (C) in methanol. Other conditions are the same as in Table 1.

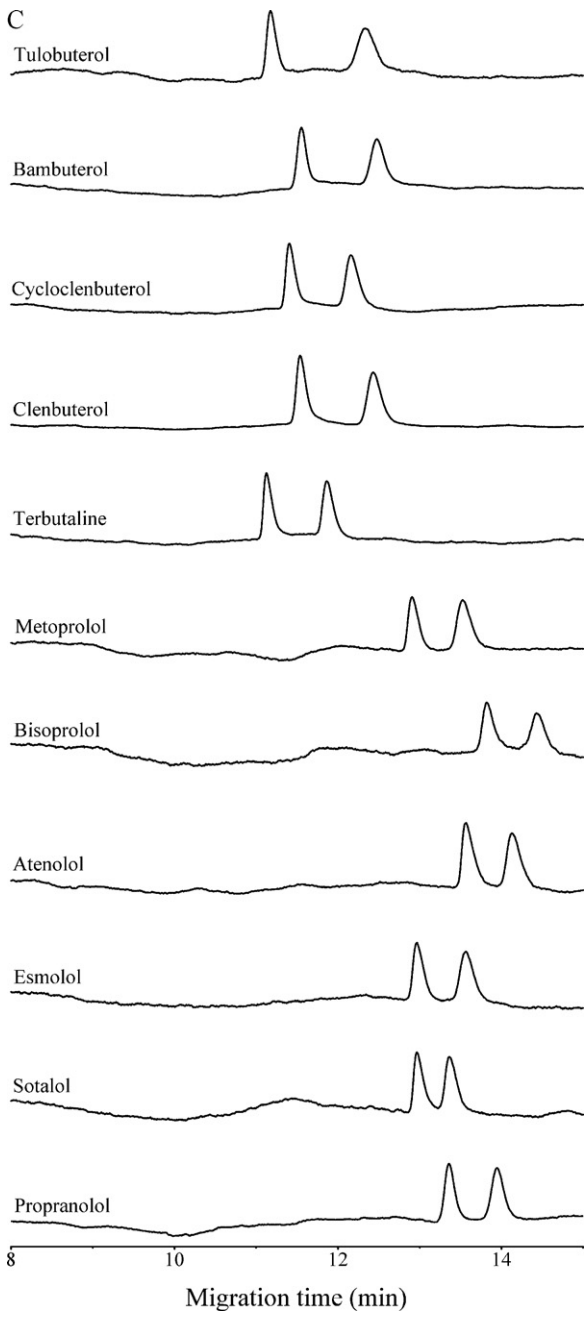

Fig. 3. *Continued.*

the analyte [32–34]. Therefore, only triethylamine was added to the BGE to deprotonate the chiral counter-ion in the investigations reported here. The effect of triethylamine concentration on enantioseparation was investigated from 0 to 72 mM. It was found that the BGE containing chiral selector without triethylamine gave short migration times, but no enantioselectivity. The migration times, enantioselectivities as well as resolutions increased with the concentration of triethylamine from 0 to 50 mM. When triethylamine concentration increased from 50 mM to 72 mM, the migration times increased, but enantioselectivities and resolutions decreased. As shown in Fig. 3(A), when 7.2 mM triethylamine was added to the BGE, the analytes could not be enantioseparated very well. In Fig. 3(B), when 36 mM triethylamine was added, all the chiral analytes could be baseline separated with a little tailing. In Fig. 2, 50 mM triethylamine was used, and good resolutions were obtained with more symmetrical peaks. In Fig. 3(C), when 72 mM triethylamine was added, all the analytes could be baseline separated and more symmetrical peaks were obtained, but the migration times were prolonged and the enantioselectivities and resolutions decreased.

With the increase of triethylamine concentration from 0 to 50 mM, the pH* of the BGE increases, promoting the production of the chiral counter-ion, and thus facilitates the ion-pair formation for chiral separation. The increase of triethylamine concentration also decreases μ_{EOF}. This may be advantageous because a decrease of μ_{EOF} should increase the difference of μ_{eff} of the enantiomers [35]. However, when triethylamine concentration increases to 72 mM, the high pH* decreases the degree of the ionization of ana-

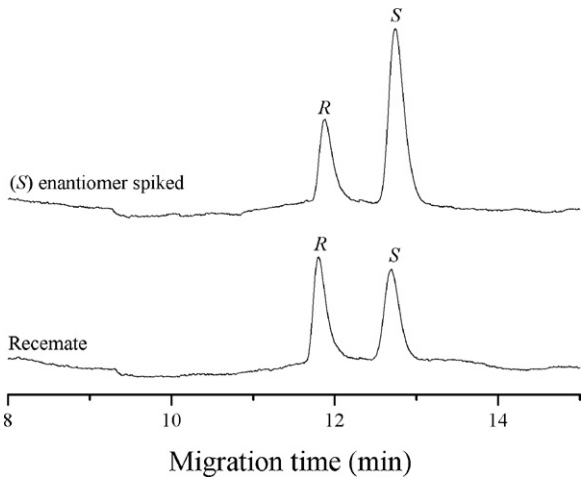

Fig. 4. Migration orders of propranolol enantiomers. BGE composition: 80 mM di-*n*-amyl L-tartrate, 100 mM boric sodium and 50 mM triethylamine in methanol. Other conditions are the same as in Table 1.

lytes and weakens their interactions with negatively charged chiral selector, so that it is unfavorable for chiral separation. Another possibility is that when the migration time was relatively long caused by the much lower μ_{EOF} and μ_{eff} with a high triethylamine concentration, diffusion effect became an important factor affecting the enantioseparation.

In this study, it was found that the enantioselectivity was the major factor affecting the resolution as the concentration of triethylamine changed. As shown in Table 4, the enantioselectivities and resolutions change in the same trend for most analytes, i.e., they increase to a maximum value, then decrease with the increase of triethylamine concentration. Since most analytes obtained a relatively better resolution at 50 mM (Table 4 and Fig. 2), it was selected as the optimal concentration and the effects of other experiment conditions were investigated at this concentration.

3.3. Effects of applied voltage and capillary length

In capillary electrophoresis, the applied voltage has a large effect on resolution and efficiency. In our experiments the effect of applied voltage on the migration time, enantioselectivity, and resolution was investigated at 15, 20, 25, and 30 kV. All of the analytes could be baseline separated at 15, 20, 25, and 30 kV, and 20 kV was selected in all of the experiments as a compromise between the analysis time and the baseline appearance.

Capillary length is also a very important parameter in this study and two capillary lengths were investigated. Under the current conditions, when 45 cm effective length capillary was used, all of the eleven analytes were well resolved, but only two analytes could be baseline separated using 37 cm effective length capillary.

3.4. Migration orders of two enantiomers

The identity of the peaks of propranolol enantiomers is assigned by spiking a single pure (*S*)-enantiomer into the solution of its racemate. The (*S*)-enantiomer of propranolol migrates later as shown in Fig. 4, indicating that it interacts more strongly with the chiral selector than the (*R*)-enantiomer. Owing to the lack of optical pure standard materials, the migration orders of other enantiomers have not been determined.

4. Conclusions

This paper reported a novel method for the chiral separation of some β-blockers and β-agonists in NACE using di-*n*-amyl L-tartrate–boric acid complex as the chiral selector. The chiral selector, having a better chiral recognition capability for the studied analytes in NACE, was *in situ* synthesized by the reaction of di-*n*-amyl L-tartrate with boric acid in a nonaqueous BGE using methanol as the medium. Some chiral analytes that could not be resolved in aqueous MEEKC with the same chiral selector could be separated with a baseline resolution in NACE. The enantioseparation mechanism was considered to be ion-pair principle and the nonaqueous system was more favorable for the ion-pair formation which is quite useful for the chiral recognition. The addition of a proper concentration of triethylamine into the BGE to control the pH* enhanced the enantiomeric discrimination. This work is a significant advance in the application of tartrate chiral selector in NACE, and the applicability of the established method for other kinds of chiral drugs and the chiral recognition mechanism will be further studied.

Conflict of interest

The authors declared no conflict of interest.

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