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Synthesis and application of a novel single-isomer mono-6-deoxy-6-(3R,4R-dihydroxypyrrolidine)-β-cyclodextrin chloride as a chiral selector in capillary electrophoresis

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ABSTRACT

A novel positively charged single-isomer of β-cyclodextrin, mono-6-deoxy-6-(3R,4R-dihydroxypyrrolidine)-β-CD chloride (dhypy-CDCl), was synthesized and employed as a chiral selector for the first time in capillary electrophoresis (CE) for the enantioseparation of anionic and ampholytic acids. The effects of the running buffer pH, chiral selector concentration, analyte structure and organic modifier on the enantioseparation were studied in detail. The chiral selectivity and resolution for most of the studied analytes decreased as the buffer pH increased in the range of 6.0–9.0. Increasing selector concentration led to decreased effective mobility, increased chiral selectivity and resolution for most of the studied analytes. Moreover, the hydroxyl groups located on the dihydroxypyrrolidine substituent of the dhypy-CDCl could have influence on the chiral separation.

1. Introduction

Chiral separation has become an area of considerable interests in modern pharmaceutical industry because different enantiomers may produce critically different pharmacological effects in biological system [1]. In recent years, capillary electrophoresis (CE) has rapidly developed as one of the most attractive and powerful techniques for chiral analysis and separation due to its advantages such as high efficiency, requirement of small amounts of selectors and analytes as well as capability for automation [2–4]. Cyclodextrins (CDs) and their derivatives have been considered as the most widely used chiral selectors in CE because of their ability to form inclusion complexes with a wide range of compounds [5–10]. However, the use of native CDs as chiral selectors in CE is restricted by their limited aqueous solubility and electrically neutral nature [11]. During the last decade, charged CDs have attracted great interests for chiral separation in CE. Compared with native CDs, charged CDs are advantageous not only in high water solubility but also in effective separation of oppositely charged analytes by their strong electrostatic attraction in addition to the effects of inclusion complexation [12]. Furthermore, high enantioselectivity and resolution can be achieved at very low concentration of charged CDs. In the past years, many papers have reported enantioseparation with randomly and selectively substituted charged CDs. However, in the case of randomly substituted charged CDs, the substitution distribution strongly influences the enantioselectivity and efficiency of the chiral separation process. Therefore, single-isomer charged CD is required to offer good reproducibility and resolution for enantioseparations by CE. Numerous works have reported the use of anionic single-isomer CDs, as indicated in several reviews [13–16], while there are fewer reports on the application of cationic single-isomer CDs [17–21].

In view of the increasing interest in cationic CDs, our group introduced a family of single-isomer positively charged CDs, mono-alkylimidazolium-β-cyclodextrin derivatives [22]. These single-isomer charged CDs showed powerful resolution ability to hydroxyl acids, carboxylic acids, and useful to dansyl amino acids [23,24]. However, the UV absorption of the imidazole group could interfere with the detection of analytes. In order to overcome the detection limit and enlarge the scope of single-isomer cationic CDs, our laboratory has systematically developed a family of mono-substituted positively charged CDs through introducing a non-planar pyrrolidine and its derivatives onto the C6 position of the β-CD. Through the introduction of the saturated cyclic amine, the derived CDs have low UV absorption, good water solubility and are expected to exhibit different properties compared to other

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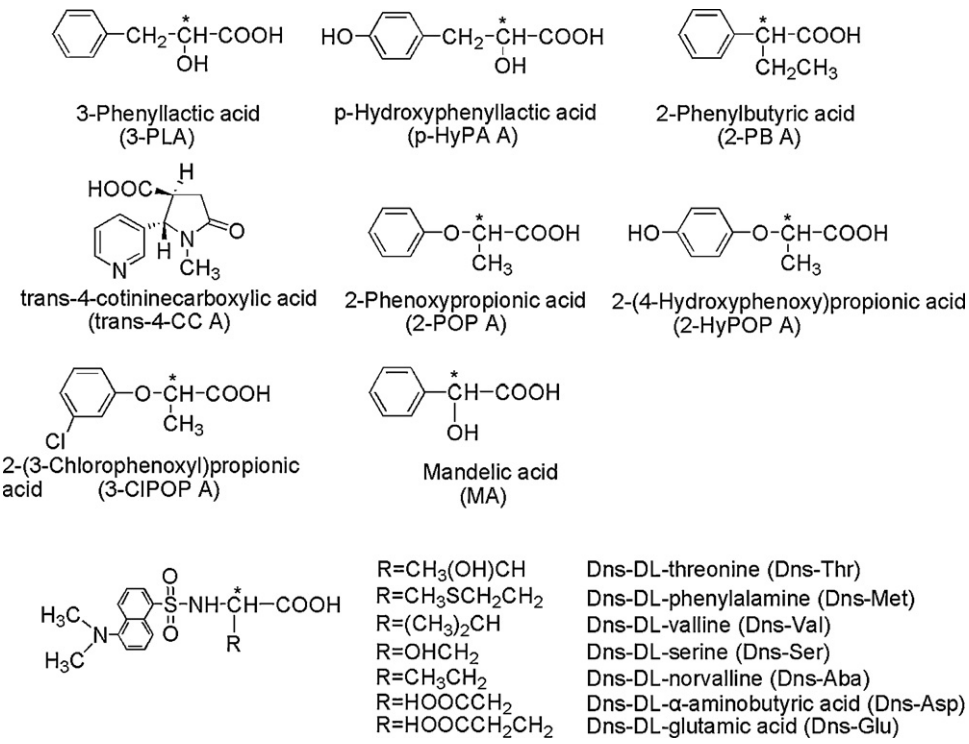

Fig. 1. Structures of anionic and ampholytic acids used in the current work.

cationic CDs. Besides, to the best of the authors' knowledge, there are very limited reports about the saturated heterocyclic group modified single-isomer cationic CDs.

Herein, the synthesis of one member of the newly developed positively charged single-isomer β-CDs, mono-6-deoxy-6-(3R,4R-dihydroxypyrrolidine)-β-CD chloride (dhypy-CDCl), was firstly reported. This novel chiral selector, mono-substituted on the C6 of the CD rim with a non-planar dihydroxypyrrolidine group, was applied for the enantioseparation of anionic and ampholytic acids by capillary electrophoresis. The influences of the buffer pH, chiral selector concentration, analyte structure and organic modifier on the enantioseparation of various acids were studied in detail.

2. Experimental

2.1. Chemicals

All chemicals were of analytical reagent grade unless stated otherwise. Monobasic sodium phosphate (NaH_2PO_4) and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). HPLC-grade methanol (MeOH) and acetonitrile (ACN) were obtained from Fisher (Suwanee, GA, USA). Ultra-pure water was prepared with an Arium 611VF water system supplied by Sartorius Stedim Biotech (Goettingen, Germany). 2-phenoxypropionic acid, 2-phenylbutyric acid, and 2-(3-chlorophenoxyl)propionic acid were purchased from Lancaster Synthesis (Windham, NH, USA). Dansyl amino acids were purchased from Sigma (St. Louis, MO, USA). Other chiral compounds were purchased from Aldrich (Steinheim, Germany). The structures of these racemic compounds are shown in Fig. 1.

2.2. Instrumentation

The NMR spectra were recorded on a Bruker ACF300 (300 MHz) supplied by Bruker Biospin (Fällanden, Switzerland). Mass spectra were obtained on a QSTAR XL LC/MS system purchased from Applied Biosystems (Foster City, CA, USA). All CE separations were performed on a Beckman P/ACE MDQ CE system (Fullerton, CA, USA), equipped with a 50 cm × 50 μm I.D. uncoated fused-silica capillary (effective length 40 cm). The applied voltage is

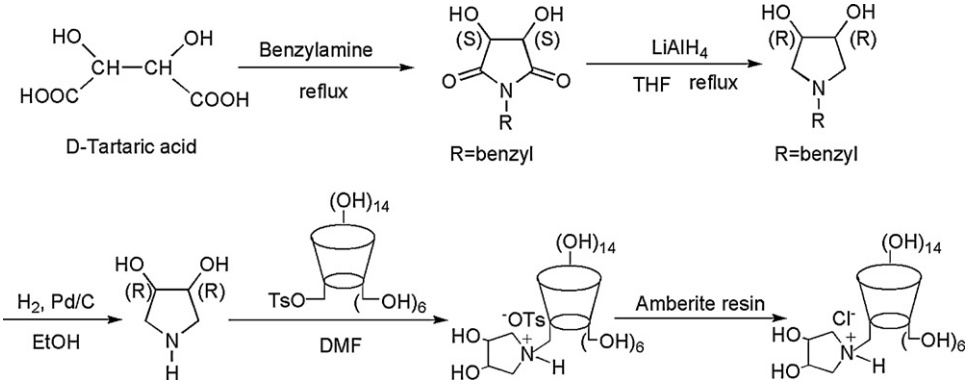

Fig. 2. Schematic synthesis for dhypy-CDCl.

Table 1
Influence of BGEs pH on migration times and effective mobilities (μ_{eff1} ($\times 10^{-5}$) $cm^2\ V^{-1}\ s^{-1}$) of the first enantiomer, selectivities (α) and resolutions (R_s) of analytes using dhypy-CDCl as a chiral selector.

Analytes	pH 6.0				pH 7.0				pH 8.0				pH 9.0			
	t_1	μ_{eff1}	α	R_s	t_1	μ_{eff1}	α	R_s	t_1	μ_{eff1}	α	R_s	t_1	μ_{eff1}	α	R_s
3-PLA	9.28	−12.86	1.079	3.80	8.98	−14.68	1.048	2.63	8.55	−14.84	1.026	1.27	8.48	−15.71	1.020	1.52
p-HyPA A	7.95	−10.51	1.102	3.29	7.63	−12.44	1.061	2.22	7.40	−13.76	1.035	1.72	7.27	−13.80	1.029	1.43
2-PB A	8.78	−11.00	1.051	1.03	8.74	−13.48	1.027	0.77	7.88	−14.10	1.005	<0.5	7.86	−14.96	1.002	<0.5
trans-4-CC A	10.20	−16.77	1.009	<0.5	9.81	−16.97	1.008	<0.5	9.56	−16.42	1.006	<0.5	8.93	−16.43	1.005	<0.5
2-POP A	10.28	−16.82	1.036	2.29	9.87	−17.42	1.028	1.67	9.81	−17.11	1.025	1.63	9.57	−17.47	1.022	1.20
2-HyPOP A	9.32	−13.92	1.051	2.50	8.96	−14.82	1.038	1.93	8.53	−14.72	1.033	1.88	8.24	−15.34	1.028	1.80
3-ClPOP A	8.80	−12.52	1.056	2.56	8.66	−13.63	1.039	1.93	8.19	−13.98	1.030	1.47	7.76	−14.51	1.025	1.39
MA	11.54	−20.30	1.012	1.11	10.32	−20.75	1.008	0.60	10.06	−20.60	1.004	<0.5	9.57	−20.92	1.002	<0.5
Dns-Thr	7.54	−9.98	1.042	1.85	7.40	−10.33	1.039	1.80	7.30	−9.85	1.037	1.73	7.14	−10.29	1.035	1.67
Dns-Met	7.35	−7.49	1.055	1.30	7.16	−8.67	1.038	0.89	6.89	−8.67	1.032	0.79	6.85	−9.04	1.029	0.74
Dns-Ser	7.69	−10.43	1.023	0.88	7.54	−10.68	1.023	0.86	7.35	−10.34	1.021	0.80	7.19	−10.82	1.020	0.77
Dns-Val	7.42	−9.01	1.062	1.97	7.33	−9.60	1.047	1.43	7.07	−9.38	1.044	1.41	6.91	−9.73	1.038	1.37
Dns-Aba	7.32	−7.48	1.156	3.55	7.28	−9.05	1.072	2.30	6.98	−9.22	1.057	1.84	6.90	−9.74	1.037	1.28
Dns-Asp	11.74	−18.73	1.025	2.69	11.05	−19.50	1.028	3.13	10.82	−19.78	1.035	3.75	10.69	−19.94	1.032	3.59
Dns-Glu	11.43	−18.64	1.010	1.00	11.12	−19.83	1.015	1.59	10.86	−20.10	1.021	2.21	10.51	−20.45	1.018	2.13

Conditions: 5 mM CD; 50 mM phosphate buffer; temperature 25 °C; applied voltage 15 kV.

Table 2
Influence of chiral selector concentration on effective mobilities (μ_{eff1} ($\times 10^{-5}$) $cm^2\ V^{-1}\ s^{-1}$) of first enantiomers, selectivities (α) and resolutions (R_s) of analytes using dhypy-CDCl.

Analytes	2.5 mM			5 mM			7.5 mM			10 mM			12.5 mM		
	μ_{eff1}	α	R_s	μ_{eff1}	α	R_s	μ_{eff1}	α	R_s	μ_{eff1}	α	R_s	μ_{eff1}	α	R_s
3-PLA	−16.10	1.048	2.67	−12.86	1.079	3.80	−10.63	1.101	3.95	−9.32	1.113	4.21	−8.27	1.126	4.37
p-HyPA A	−13.56	1.067	2.59	−10.51	1.102	3.29	−8.44	1.128	3.86	−7.33	1.139	3.91	−6.57	1.146	3.97
2-PB A	−14.29	1.033	0.89	−11.00	1.051	1.03	−8.62	1.060	1.15	−7.27	1.065	1.26	−6.24	1.072	1.29
trans-4-CC A	−17.75	1.003	<0.5	−16.77	1.009	<0.5	−16.08	1.012	0.68	−15.54	1.017	1.32	−14.94	1.020	1.58
2-POP A	−18.95	1.021	1.28	−16.82	1.036	2.29	−15.22	1.051	2.30	−13.94	1.060	2.69	−12.95	1.068	3.36
2-HyPOP A	−16.11	1.031	1.80	−13.92	1.051	2.50	−12.11	1.068	3.13	−10.82	1.079	3.16	−9.86	1.089	3.60
3-ClPOP A	−15.44	1.035	1.67	−12.52	1.056	2.56	−10.48	1.071	3.18	−9.31	1.079	3.44	−8.30	1.086	3.44
MA	−21.54	1.006	0.73	−20.30	1.012	1.11	−18.96	1.018	1.65	−17.71	1.023	1.94	−16.33	1.029	2.19
Dns-Thr	−11.77	1.025	1.18	−9.98	1.042	1.85	−8.73	1.044	1.90	−7.89	1.049	1.93	−7.18	1.052	1.97
Dns-Met	−9.81	1.032	0.79	−7.49	1.055	1.30	−5.77	1.073	1.33	−4.80	1.085	1.47	−3.87	1.101	1.52
Dns-Ser	−12.21	1.016	0.58	−10.43	1.023	0.88	−8.95	1.028	1.01	−8.09	1.032	1.09	−7.44	1.034	1.16
Dns-Val	−10.92	1.041	1.44	−9.01	1.062	1.97	−7.60	1.078	2.26	−6.72	1.085	2.37	−5.95	1.094	2.00
Dns-Aba	−10.02	1.100	2.71	−7.48	1.156	3.55	−5.90	1.198	3.81	−4.87	1.218	3.86	−4.08	1.240	4.04
Dns-Asp	−21.37	1.017	2.03	−18.73	1.025	2.69	−16.56	1.028	2.70	−15.21	1.031	2.77	−14.31	1.033	2.67
Dns-Glu	−21.19	1.008	0.75	−18.64	1.010	1.00	−16.71	1.011	1.05	−15.46	1.013	1.09	−14.34	1.015	1.18

Conditions: 50 mM phosphate buffer; pH 6.0, temperature 25 °C; applied voltage 15 kV.

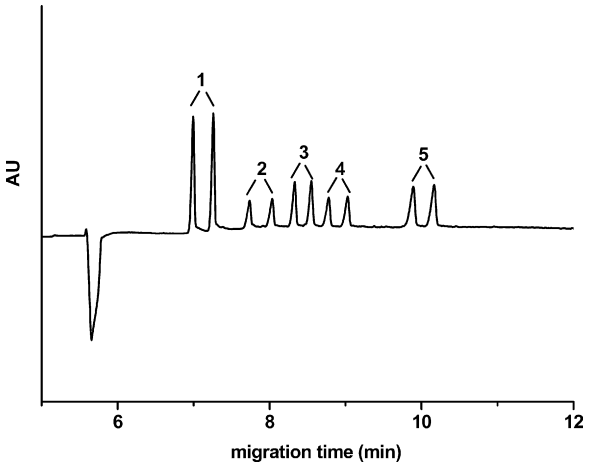

Fig. 3. Separation of a mixture containing five pairs of enantiomers. *Conditions*: 50 mM NaH_2PO_4 buffer; pH 6.0; 5 mM dhypy-CDCl. (1) Dns-Aba, (2) p-HyPA A, (3) 3-ClPOP A, (4) 2-HyPOP A, (5) 2-POP A.

15 kV (normal polarity mode). Detection of analytes was carried out simultaneously at three channels 214, 254 and 280 nm at 25 °C.

2.3. Synthesis and characterization of dhypy-CDCl

The designed synthetic route for the novel positively charged β-CD single-isomer, mono-6-deoxy-6-(3R,4R-dihydroxypyrrolidine)-β-CD chloride is depicted in Fig. 2. Firstly, 3R,4R-dihydroxypyrrolidine was synthesized from D-tartaric acid through three steps according to previous literatures [25,26]. After that, freshly dried 6-monotosyl-β-CD [27,28] and the prepared 3R,4R-dihydroxypyrrolidine in excess amount were dissolved in anhydrous N,N-dimethylformamide (DMF) and the reaction mixture was stirred at 90 °C for 2 days under nitrogen. The resultant solution was cooled to room temperature and slowly added dropwise into analytical-grade acetone with vigorous stirring. After filtration, the collected solid was dissolved in DMF and precipitated from acetone for three times. The obtained solid was thereafter dissolved in water and extracted with ethyl acetate for three times. The aqueous phase was then evaporated under vacuum followed by recrystallization to afford mono-6-deoxy-6-(3R,4R-dihydroxypyrrolidine)-β-CD tosylate (yield, 85.7%). The final pure product mono-6-deoxy-6-(3R,4R-dihydroxypyrrolidine)-β-CD chloride was obtained by exchanging the tosylate anion with chloride using Amberlite resin.

The analytical data for the positively charged β-CD was as follows:

Mono-6-deoxy-6-(3R,4R-dihydroxypyrrolidine)-β-CD: melting point: 247–249 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.61–5.76 (OH-2,3), 4.90 (H-1), 4.84 (H-1), 4.60 (OH-6), 4.51 (OH-6), 3.56–3.78 (H-3,5,6), 3.28–3.34 (H-2,4), 3.14 (H-4′), 2.90 (CH_{py}), 2.83 (H-2′), 2.73 (CH_{2py}), 2.10 (OH_{py}); ^{13}C NMR (75 MHz, DMSO-d_6): 107.9 (C1), 107.2 (C1′), 88.6 (C4′), 86.2 (C4), 73.5 (C2), 72.9 (C3), 72.5 (C5), 60.4 (C6), 50.9 (C6′), 74.5 (CH_{py}), 60.1 (CH_{2py}); elemental analysis: calculated for $C_{46}H_{77}O_{36}NCl{\cdot}6H_2O$ (1363.80) C: 40.51%, H: 6.59%, N: 1.03%, determined C: 40.36%, H: 6.78%, N: 1.02%. ESI MS (m/z): 1220.09 (calcd.) and 1219.56 (found) for [M^+].

The analytical data indicated that the mono-substituted structure was genuinely obtained.

2.4. CE procedure

50 mM NaH_2PO_4 stock solutions were used as the background electrolytes (BGEs). The appropriate amount of the positively charged CD was dissolved into the BGEs and titrated with sodium hydroxide to the required pH value (6.0–9.0). Stocked solutions of 50 μg/mL racemic analytes were prepared with a 50/50 (v/v) methanol/water mixture. All the buffer and sample solutions were filtrated with a 0.45 μm syringe-type Millipore membrane and degassed before use. Samples were introduced into the capillary by a 0.5-psi pressure injection (typically 4 s). The capillary was flushed between injections with 1 M NaOH, water and buffer for 4 min respectively.

The electroosmotic flow (EOF) was measured with methanol as the neutral marker. The separation selectivity (α), was calculated as $\alpha = \mu_2/\mu_1$, where μ_1 and μ_2 are the effective mobilities of two enantiomers. The peak resolution (R_s) was calculated by dividing the migration time difference of the two enantiomers with half of the sum of their peak widths at the baseline.

3. Results and discussion

3.1. Effect of pH on enantioseparation with dhypy-CDCl

The pH value of BGEs is an important factor for the enantioseparation of charged analytes because it has direct influence on the EOF, the effective charge and mobility of the analytes. Since all the hydroxyl and carboxylic acids have pK_a in the range of 3.0–4.0 [29] and most of the dansyl amino acids have pI below 6.0 [30–32], the effect of BGEs pH on chiral separation was investigated by increasing the pH from 6.0 to 9.0 with 5 mM dhypy-CDCl. In this condition, all the analytes could sufficiently dissociate to ensure the electrostatic interaction between the dhypy-CDCl and the enantiomers. The influence of BGEs pH on enantioseparation is presented in Table 1.

In general, the migration time of the analytes was shortened with the increase of BGEs pH which is mainly due to the enhanced EOF. As a nonselective force, the enhanced EOF could decrease the migration time of the analytes which is beneficial to fast analysis, while some disadvantages like smaller selectivity and resolution could be resulted [33–35]. As shown in Table 1, the separation selectivity (α) and resolution (R_s) for most of the analytes decreased as the BGEs pH changing from 6.0 to 9.0. This observed separation pattern followed the prediction of CHARM model proposed by Vigh and co-workers [36–38]. As for Dns-Asp and Dns-Glu, which bear two carboxylic groups on the side chain, the selectivity and resolution increased towards a local maximum at pH 8.0, then decreased as the BGEs pH was increased further. The increase of selectivity and resolution in pH 6.0–8.0 could be due to the fact that the degree of dissociation of these two analytes is enhanced with the increase of pH which improves the electrostatic interaction with the positively charged CD and results in better resolution [39–41]. The decreased selectivity and resolution at BGEs pH 9.0 could be mainly due to the enhanced EOF. This trend also showed that the enantioselective electrostatic interaction between the analytes and the positively charged CD could contribute to the chiral recognition in addition to the inclusion complexation [17,42–46]. It is noteworthy that the charge density on the current CD may be reduced by the deprotonation of the pyrrolidine nitrogen at BGEs pH 9.0, which could also weaken the enantioselective electrostatic interaction and hence lead to smaller chiral selectivity and resolution. Considering both the separation results and analysis time, we have used BGEs pH 6.0 for the subsequent study.

3.2. Effect of selector concentration on enantioseparation

In order to investigate the influence of chiral selector concentration on the enantioseparation, the racemic acids were separated at pH 6.0 BGEs with varied CD concentration. The effective mobil-

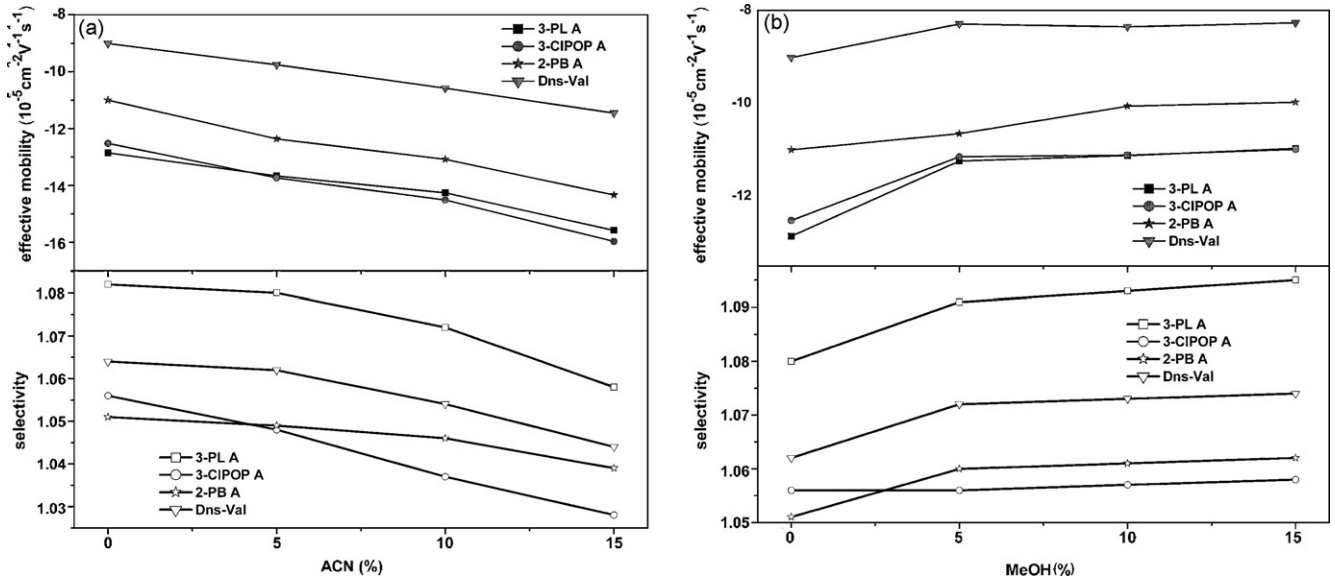

Fig. 4. Effective mobility (top panel) and separation selectivity (bottom panel) for the model analytes in pH 6.0 BGEs with 5 mM dhypy-CDCl. (a) ACN as the organic modifier; (b) MeOH as the organic modifier.

ities of the first enantiomers, the separation selectivities (α) and resolutions (R_s) are summarized in Table 2.

As shown in Table 2, the increase in CD concentration led to smaller effective mobility for all the analytes. This is the consequence of the increased BGEs viscosity, ionic strength and degree of complexation as the CD concentration increased [47]. In general, the effective mobilities of hydroxyl and carboxylic acids were higher than those of most dansyl amino acids except Dns-Asp and Dns-Glu. However, these data does not reflect the true binding degree between the analytes and selector. This is because the effective mobilities are strongly influenced by the ionic strength and viscosity of BGEs so that the measured effective mobilities only permit qualitative comparison of the migration behavior of the analytes [48,49].

The chiral selectivities (α) of all the racemic acids increased with increasing CD concentration ranging from 2.5 to 12.5 mM, which is in agreement with the prediction of the CHARM model [36–38,40]. In addition, the positively charged dhypy-CDCl is expected to undergo adsorption onto the negatively charged inner wall of the uncoated capillary. Therefore, as the concentration of positively charged CD increased, the migration time was extended due to the reduced cationic EOF, which could also do favor to chiral selectivity [16,50,51]. The effects of CD concentration on the chiral resolution (R_s) showed a similar trend as that on selectivity (α). However, the peak resolutions depend not only on the separation selectivity but also very strongly on the operation parameters (effective portion of the applied potential, dimensionless electroosmotical flow) and peak shapes [47,52].

Compared with its analog mono-6-deoxy-6-pyrrolidine-β-CD chloride (pyCDCl) [53], the current selector dhypy-CDCl presented larger anionic effective mobility, relatively lower selectivity and resolution. This result suggested the participation of the two hydroxyl groups located on the dihydroxypyrrolidine substituent in the chiral recognition process. These two hydroxyl groups might bring about steric hindrance to weaken the degree of complexation and result in a decreased binding constant hence more anionic effective mobility, less selectivity and resolution. In addition, these hydroxyl groups endowed chirality to the C3 and C4 of the pyrrolidine ring, which might provided another chiral recognition site in addition to the cavity of β-CD in the dhypy-CDCl. This chiral recognition centre might also play a role in the enantioseparation, which is under further investigation.

The chiral recognition ability of dhypy-CDCl was further examined with a mixture containing five pairs of racemic acids (Dns-Aba, p-HyPA A, 3-ClPOP A, 2-HyPOP A, 2-POP A). All the enantiomers in the mixture can be base-line separated within 12 min with 5 mM dhypy-CDCl at BGEs pH 6.0 (Fig. 3). The migration order of the model acids in the mixture was verified by injecting each enantiomers individually.

3.3. *Effect of analyte structure on the enantioseparation*

It is generally assumed that the enantioseparation occurs mainly via host–guest inclusion complexation with the CDs. Therefore, the molecular structure of the analytes plays an important role in the chiral recognition process. As shown in Table 2, most of the analytes could be resolved effectively with the current cationic CD. This could be explained by the mechanism of the chiral recognition. The common feature in the structure of all the analytes was the presence of aromatic ring and carboxylic group attached to the chiral carbon. In the chiral recognition process, these analytes could be expected to interact with the hydrophobic cavity of the dhypy-CDCl through inclusion of the aromatic ring. In addition, the carboxylic acids attached to the chiral centre could undergo enantioselective electrostatic attraction with the positively charged dihydroxypyrrolidine group on the CD rim of dhypy-CDCl at the pH condition in this work. A combination of the inclusion complexation and enantioselective electrostatic interaction could be responsible for the effective enantioseparation in the current study. Besides, the hydroxyl groups on the CD rims could also supply hydrogen bonding effects with the carboxylic group.

The high resolution for the analytes with a hydroxyl group as the substituent of the chiral carbon (3-PL A, p-HyPA A) suggests a positive contribution of this hydroxyl group to the chiral recognition, probably through the formation of hydrogen bonds with the secondary hydroxyl groups on the CD rim [14,54,55]. The analytes with ether linkage (2-POP A, 2-HyPOP A and 3-Cl POP A) also presented high resolution, which could be ascribed to the fact that the oxygen could afford proton acceptor for the formation of hydrogen bonds with the CD selector [24]. The relatively low resolution for MA, whose chiral carbon linked to the aromatic ring directly, could be attributed to less flexibility of the MA due to the short alkyl chain [54]. For the dansyl amino acids, the complexation between the dansyl group and CD cavity could be highly favorable [56], while the

resolution decreased as the length of alkyl chain of the chiral carbon is increased (Dns-Aba vs. Dns-Val, Dns-Asp vs. Dns-Glu), suggesting a steric hindrance due to the bulky substituents [50,51]. It is rather surprising that the Dns-Thr presented better resolution than its analog Dns-Ser which bears a shorter alkyl chain. This result was also observed in the study using alkylimidazolium-β-cyclodextrin derivatives as chiral selectors [57].

3.4. Effect of organic modifier on the enantioseparation

In the enantioseparation with CDs by CE, the addition of organic modifier in BGEs could affect the strength of the inclusion complexation, the EOF, and the viscosity as well as the conductivity of the BGEs [55,58,59]. In this study, the effects of two typical organic modifiers, ACN and MeOH, were investigated in a range of 0–15% (v/v) at 5 mM dhypy-CDCl BGEs (pH 6.0) with 3-PL A, 3-ClPOP A, 2-PB A and Dns-Val as model analytes (Fig. 4).

As shown in Fig. 4(a), the effective mobilities of the analytes increased as the ACN content increased from 0% to 15% (v/v). ACN is known to compete strongly with the hydrophobic inclusion complexation [60]. The binding constant of the complexation equilibrium decreased and the interaction of the analytes with the CD was less favored which led to increased anionic effective mobility [61]. This is further supported by the reduced selectivity and resolution with the increased ACN content. As for MeOH, a general slight decrease in effective mobility of all the model analytes was observed upon the addition of MeOH into the BGEs (Fig. 4(b)). Although the addition of MeOH could generally reduce the affinity between analytes and the CD hydrophobic cavity [59,62], it seems that the increased viscosity of BGEs is the predominant effect in the current study. A general improvement of the chiral selectivity and resolution might be attributed to the reduced EOF and extended analysis time.

4. Conclusion

A novel single-isomer cationic chiral selector mono-6-deoxy-6-(3R,4R-dihydroxy pyrrolidine)-β-CD chloride was synthesized and applied as a chiral selector for enantioseparation of anionic and ampholytic acids by CE. The selectivity and resolution declined as the BGEs pH changing from 6.0 to 9.0 for most of the analytes except Dns-Asp and Dns-Glu which have two carboxylic groups. Increase of selector concentration led to decreased effective mobility, incremental chiral selectivity and resolution for most of the analytes. The enantioseparation was improved with the increase of methanol content in BGEs while weakened as ACN was added. The two hydroxyl groups located on the pyrrolidine substituent of the dhypy-CDCl might also influence the chiral recognition process. In conclusion, dhypy-CDCl displayed fast and effective enantioseparation towards the selected analytes, which could favorably broaden the scope of CE enantioseparation with single-isomer cationic CDs as selectors.

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