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Chemically bonded cationic β-cyclodextrin derivatives and their applications in supercritical fluid chromatography

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ABSTRACT

Cationic β-cyclodextrin (CD) perphenylcarbamoylated derivatives were chemically bonded onto vinylized silica using a radical co-polymerization reaction. The derived materials were used as chiral stationary phases (CSP) in supercritical fluid chromatography (SFC). Enantioseparations were successfully demonstrated on 14 racemates encompassing flavanones, thiazides and amino acid derivatives. The electrostatic force between the analytes and the cationic moiety on β-CD derivative was found to be important for retention and enantioseparation of the racemates. Aromatic cationic moiety on β-CD enabled better enantioseparations than aliphatic cationic moiety. It was also found that the presence of acid additives would result in lower retention of the analytes but often assist the chiral resolutions.

1. Introduction

Over the past three decades, drug chirality and its influence *in vivo* have become a well-recognized consideration in clinical pharmacology and chiral drug developments. It is well established that chirality of drugs may influence significantly their pharmacological [1], toxicological [2], pharmacodynamic and pharmacokinetic [3,4] properties. Consequently, obtainment of optically pure enantiomers of the racemic drugs remains an important concern. Meanwhile, driven by the growth of asymmetric organic synthesis leading to chiral drugs, food additives, fragrances, agricultural chemicals and many other important chiral intermediates, the development of chiral selectors has grown rapidly. Many chiral selectors were developed and applied in various chiral resolution technologies. Firstly, Davankov et al. developed metal ion complexes for enantioseparations [5,6]. After that, by linking small chiral molecules onto stationary phase, brush type chiral stationary phases were prepared [3,5]. Most recently, natural chiral macromolecules such as crown ethers [6–8], cyclodextrins (CD) [7,8], celluloses [9,10], macrocyclic glypeptides [11], proteins [12,13] as well as synthetic polymers [14] were modified for the application of enantioselective processes.

The application of charged CD as chiral mobile phase additives in capillary electrophoresis (CE) was first introduced by Terabe [15]. Thereafter, a series of anionic CD derivatives were reported as chiral pseudo-stationary phases useful for the enantioseparation of both neutral and basic enantiomers in CE [16]. Various charged β-CD chiral selectors were then rapidly developed and made commercially available [17–20]. The key advantage of having charged selectors compared with neutral analogues is that they could carry the racemic analytes to provide a higher mobility difference between enantiomers [21]. Tait et al. demonstrated that the use of anionic chiral mobile phase additives would effectively increase the "separation window" as the maximum separation would exist when the analyte and chiral selector migrated in opposite directions [22]. Stalcup et al. developed anionic sulphated β-CD which was applied as chiral mobile phase additive in CE or CZE [23,24]. Meanwhile, the sulphated β-CD derivative was also introduced into CSP and applied in HPLC. The negatively charged β-CD chiral selectors have depicted versatile chiral resolution abilities towards a broad range of racemic analytes. Apparently, both electrostatic and hydrophobic interactions between the chiral selector and the racemic analytes contributed to the final enantioseparation outcome [25]. On the other hand, cationic β-CD CSP is rarely investigated with literature reports only on their application of chiral mobile phase additives in CE and CZE [26,27]. In our previous report [28,29], we prepared coated CSPs based on cationic β-CD derivatives with a 3-alkylimidazolium moiety on the primary rim of β-CD. The remaining hydroxyl groups were fully derivatized into

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phenylcarbamate groups. The cationic β-CD CSPs therein showed excellent chiral selectivities over racemic 2-phenylethanols. However, the coated CSPs have somewhat limited utility as it might be damaged by the applications of higher contents of polar organic modifiers.

Although β-CD derivatives have been universally employed as chiral selectors in HPLC, CE and CEC, relatively little work has been done under SFC conditions [30]. The mobile phase in SFC is mainly CO_2 and it has low viscosity. As a result, the analyte in the mobile phase has higher diffusion coefficient in SFC than when it is in LC mobile phase. Accordingly, higher flow rate of the mobile phase can be applied to shorten the analysis time whilst column pressure is lower [31]. In supporting the development of large scale synthetic manufacture of chiral drugs, it is inevitable to emphasize on the efficiency of developing optimum chiral chromatographic modalities for specific products. Higher flow rate and lower mobile phase viscosity conduce increased peak efficiency and higher resolution in SFC [32]. In the standard gradient elution approach [33], the mobile phase in SFC is normally with only one organic solvent mixed with CO_2. Accordingly, mobile phase optimizations would be much simpler in SFC. In addition, in SFC, the system rapidly attains equilibrium upon changing chromatographic parameters. Consequently, the time needed for condition optimization in SFC would be much shorter than in LC. As the efficiency of finding out optimal separation condition in the analytical grade analysis is highly demanded in the modern pharmaceutical industry, application of SFC instead of LC could effectively shave time off the schedule in a drug development program [34]. Moreover, SFC has higher sensitivity than LC. A comparative investigation between HPLC and SFC has shown that SFC enabled better separation and detection of impurities whereas the peak ascribable to small amount of impurity was invariably obscurred by the major ingredients' peaks in HPLC [35,36]. As a result, SFC is widely employed in the pharmaceutical industry both in high throughput manufacturing and rapid analyses of drugs.

In this report we report on a facile co-polymerization approach for immobilization of 6^A-(3-vinylimidazolium)-6-deoxyperphenylcarbamate-β-cyclodextrin chloride and 6^A-(N,N-allylmethylammonium)-6-deoxyperphenylcarbamate-β-cyclodextrin chloride onto silica. The chemically bonded CSPs were applicable to supercritical fluid chromatographic conditions with high contents of polar modifiers in the mobile phases. Acidic or basic additives are also able to be employed in the mobile phases. Totally 14 pharmaceutical racemates achieved chiral resolutions.

2. Materials and methods

2.1. Instrumentation

Structures of all compounds were characterized on a Bruker ACF300 FT-NMR spectrometer supplied by Bruker Biospin (Fällanden, Switzerland). Mass spectra of all compounds were obtained using the QSTAR XL LC/MS/MS System, which comprises a high-performance hybrid quadrupole time-of-flight mass spectrometer by Applied Biosystems (Foster City, CA, USA). The loading concentrations of CSPs were determined by TG–DTA supplied by PerkinElmer Thermogravimetric Analyzers Company (USA). Microanalyses of all compounds were performed on Vario EL III universal CHNOS element analyzer supplied by Elementar Analysensysteme (Hanau, Germany). FT-IR results were detected by PerkinElmer FT-IR Spectrometer (Waltham, MA, USA). Melting points were determined on Büchi Melting Point Apparatus B-545 (USA). The SFC setup comprises of a Jasco BP-2080 *plus* automatic back pressure regulator, UV/Vis Detector, column thermostat, rheodyne 6-way valve manual injector 20 μL, HPLC pump, solvent selection unit and a CO_2 delivery pump. Liquid CO_2 is supplied by Singapore Oxygen Air Liquide (SOXAL). In SFC operations, back pressure regulator (BPR) was set beyond 10 MPa, oven temperature 40 °C, total flow rate was set in a range of 1.0–3.0 mL min^{-1} and variable content of 2-propanol or methanol were mixed in mobile phase as organic modifier, UV absorbance was detected at 220 nm wavelength. The samples were prepared at a concentration of 0.1 mg mL^{-1} by dissolving them in pure 2-propanol and the sample injection volume was typically 5 μL.

2.2. Reagents

Phenyl isocyanate and chloroform were obtained from Merck (Schuchardt, Hohenbrunn, Germany). 3-(Methacryloyloxy)-propyltrimethoxysilane, 1-vinylimidazole, N,N-methylallylamine, magnesium sulphate and 2,3-dimethylbutadiene (DMBD) were purchased from Alfa Aesar (Heysham, England). AIBN was supplied by Sinopharm (Shanghai, China). All chemicals were used directly without further purification. Pyridine purchased from Baker Analyzed (Phillipsburg, USA) was distilled with calcium hydride for 15 h before collecting for use. HPLC-grade solvents were purchased from Merck and used directly SFC analysis. Racemic analytes were purchased from Sigma–Aldrich (Switzerland). The Kromasil spherical silica gel was purchased from Eka Chemicals (Bohus, Sweden) with 100 Å pore size, 5 μm particle size and surface area of 319 m^2 g^{-1}.

2.3. Preparation of cationic chiral stationary phase

2.3.1. Synthesis of 6^A-(3-vinylimidazolium)-6-deoxyperphenylcarbamate-β-cyclodextrin chloride (VIMPCCD)

6^A-(3-Vinylimidazolium)-6-deoxyperphenylcarbamate-β-cyclodextrin chloride was synthesized by the method in our former report [28,29]. Yield: 66.16% [4.13 g (1.14 mmol)]. m.p. 197–199 °C. ESI-MS [M^+]: (expected) 3592.16; (found) 3592.07. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) 3.00–6.00 (m, 52H, H-Cyclodextrin, H-Vinyl) 6.00–7.80 (m, 100H, H-Phenyl). Microanalysis for $C_{187}H_{175}ClN_{22}O_{54}$ (expected) C: 61.87%, H: 4.86%, N: 8.49%, (found) C: 60.25%, H: 5.13%, N: 9.11%.

2.3.2. Synthesis of 6^A-(N,N-allylmethylammonium)-6-deoxyperphenylcarbamate-β-cyclodextrin chloride (VAMPCCD)

6^A-(N,N-allylmethylammonium)-6-deoxyperphenylcarbamate-β-cyclodextrin chloride was synthesized by the same method of VIMPCCD. Yield: 89.98% [4.64 g (1.29 mmol)]. m.p. 208–212 °C. ESI-MS [M^+]: (expected) 3569.18; (found) 3569.18. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) 3.00–6.00 (m, 57H, H-Cyclodextrin, H-N-CH_3, and H-N-allyl) 6.00–7.80 (m, 100H, H-Phenyl). Microanalysis for $C_{186}H_{178}ClN_{21}O_{54}$ (expected) C: 61.94%, H: 4.97%, N: 8.15%, (found) C: 61.11%, H: 5.35%, N: 8.92%.

2.3.3. Synthesis of vinylized silica

Synthesis of 3-methacryloyloxypropyltrimethoxysiliane (MPS) functionalized silica gel is described by Chen et al. [37]. The surface coverage of organic functional material on the surface of silica gel was determined by microanalysis: C, 5.81%; H, 1.28%. Accordingly, a surface coverage of MPS on silica gel was calculated as 2.16 μmol m^{-2} based on the carbon content [38,39]. FT-IR (KBr) 2964, 2855 cm^{-1} (C—H) 1705 cm^{-1} (C═O) 1635 cm^{-1} (C═C) 1130 cm^{-1} (C—O and Si—O). The characteristic peaks show the successful bonding of MPS onto silica surface.

2.3.4. Co-polymerization immobilization of VIMPCCD

VIMPCCD was chemically bonded onto vinylized silica gel through co-polymerization in the help of a small molecular monomer DMBD and the initiator AIBN (Fig. 1). The molar ratio for AIBN/VIMPCCD/DMBD was 0.1:1:100. The reaction was conducted

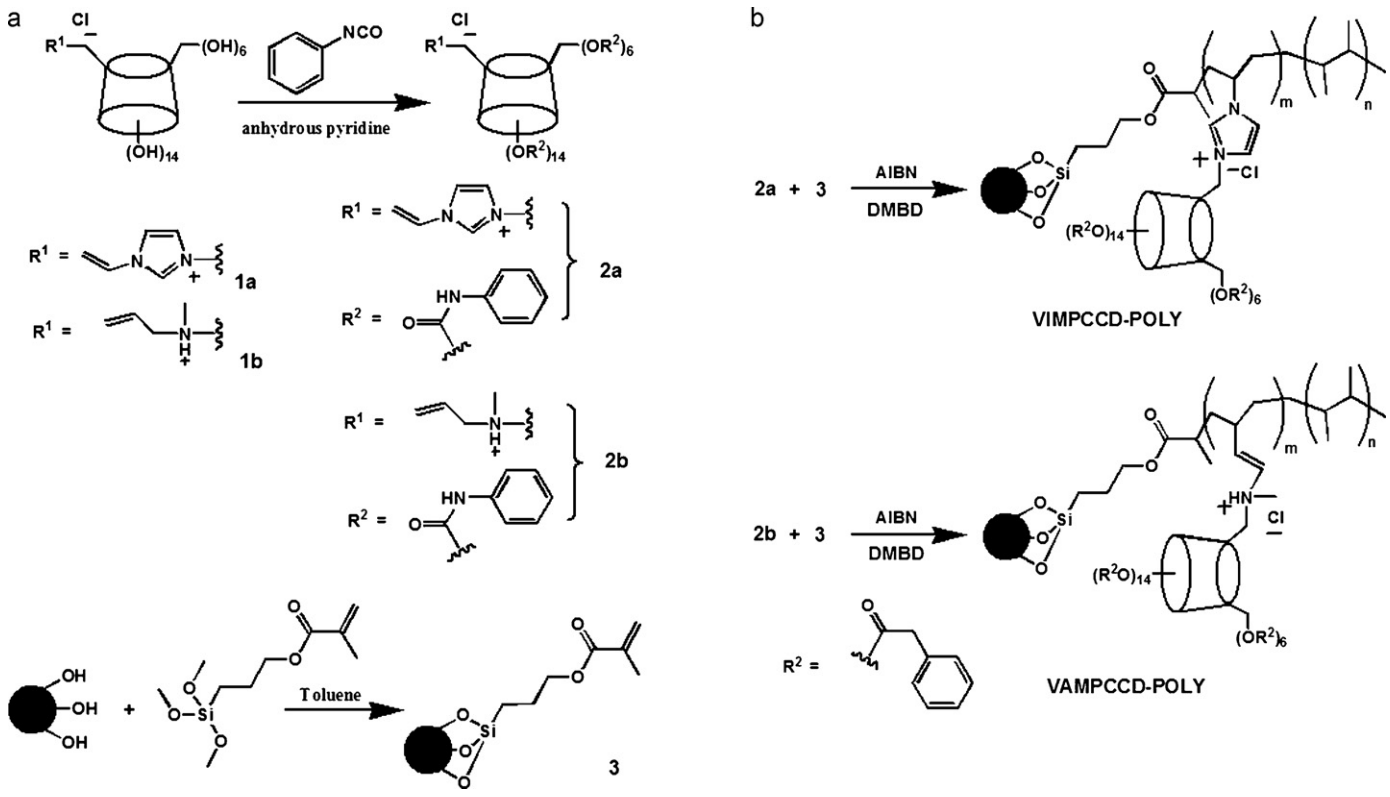


Fig. 1. (a) Synthesis route of cationic β-cyclodextrin derivative and functionalized silica gel. (b) Preparation of chemically bonded cationic β-cyclodextrin on silica surface.

in dried toluene at 80 °C for 18 h. The loading of CD derivative on the surface of silica gel was determined by microanalysis: 12.66, C%; 1.544, H%; 0.785, N%. The cyclodextrin derivatives' grafting coverage was calculated based on the nitrogen content [39], to be 0.088 μmol m^{-2}. FT-IR (KBr) 1720 cm^{-1} (C=O) and 1647, 1558, 1458 cm^{-1} (C=C phenyl group) 1130 cm^{-1} (C—O and Si—O). The characteristic peaks show the CD derivative has been successfully bonded onto silica surface.

2.3.5. Co-polymerization immobilization of VAMPCCD

VAMPCCD were chemically bonded onto stationary phases with the same method. The loading of CD derivative on the surface of silica gel can be determined by microanalysis: 9.432, C%; 1.394, H%; 0.775, N%. The cyclodextrin derivatives' grafting coverage was calculated to be 0.091 μmol m^{-2}. FT-IR (KBr) 2987, 2872 cm^{-1} (C—H), 1708 cm^{-1} (C=O) and 1634, 1541, 1447 cm^{-1} (C=C phenyl group) 1130 cm^{-1} (C—O and Si—O).

2.4. Column packing approach

The cationic chiral stationary phase is slurry packed into stainless steel column (⌀2.1 mm × 150 mm length) with methanol using packing pump from LabAlliance Company (State College, PA, USA). The maximum pressure for packing is 8000 psi (55.16 MPa) and hold for 1 h to ensure the column is packed tightly. The obtained column is applied in SFC directly and conditioned with CO_2 and MeOH (60/40, v/v) at a flow rate of 1.0 mL min^{-1}.

3. Results and discussion

3.1. Differences between aromatic and aliphatic cationic substituents on the bonded CSPs in enantioseparations

The bonded CSPs VIMPCCD-POLY and VAMPCCD-POLY have similar structures but different cationic linkers which is an aromatic cationic substituent in VIMPCCD-POLY but an aliphatic cationic substituent in VAMPCCD-POLY. The enantioseparation results of flavanone derivatives and racemic thiazides on VIMPCCD-POLY and VAMPCCD-POLY, using similar conditions, are summarized in Fig. 2. VIMPCCD-POLY depicted higher chiral selectivities for all the racemates tested. It was evident that the cationic CSP with aromatic cationic substituent afforded more favourable enantioseparations than that with aliphatic cationic substituent.

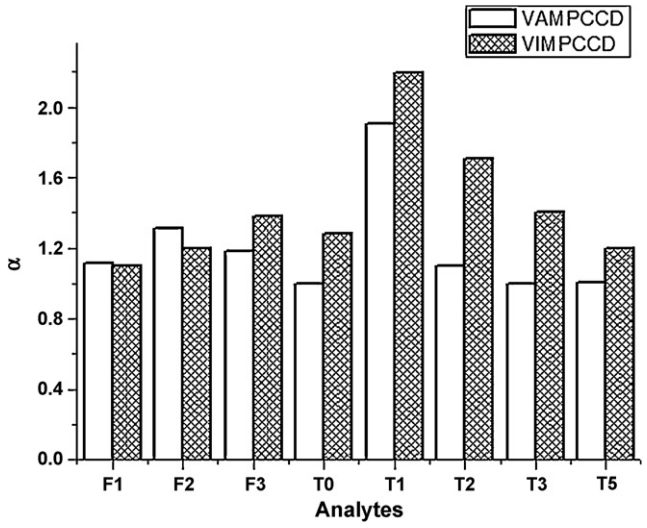


Fig. 2. Comparison between enantioseparations attained on VIMPCCD-POLY and VAMPCCD-POLY (condition: flow rate 1.0 mL min^{-1}, oven temperature 40 °C, BPR 15 MPa; modifier content in CO_2: T1–T3: 30 vol% MeOH; T0: 10 vol% MeOH; T5: 20 vol% MeOH; F1, F2: 1 vol% 2-propanol; F3: 3 vol% 2-propanol. T0: 7-chloro-3-methyl-3,4-dihydro-2H-1,2,4- benzothiadiazine S,S-dioxide (IDRA21), T1: bendroflumethiazide, T2: trichlormethiazide, T3: althiazide, T5: chlorthalidone, F1: 6-methoxyflavanone, F2: 7-methoxyflavanone, F3: 4′-hydroxyflavanone).

In the aromatic cationic substituent, the planarity of the aromatic ring makes for easy interaction with the positive charged substituents and the analytes. In the CSP with aliphatic ammonium linker, however, the substituents on the nitrogen atom are tetrahedron in shape affording less favourable interactions between the analyte and the cationic substituent. Furthermore, the aromatic imidazolium moiety could also interact with analytes through π–π stacking, which is not possible with the aliphatic ammonium group. The aromatic cationic moiety is therefore compatible with multiple interaction types, facilitating various enantioseparation processes. Accordingly, our studies are focused on the performances of CSP VIMPCCD-POLY.

3.2. Evaluation of cationic β-CD bonded CSPs with pharmaceutical drugs

3.2.1. Evaluation of cationic β-CD bonded CSPs with flavanone derivatives

The enantioseparations of 5 flavanone derivatives on VIMPCCD-POLY were studied and the results were summarized in Table 1. The determination of capacity factor and enantioselectivity are the same with our previous paper [28]. As flavanone derivatives with more phenolic groups always depicted higher retention in the experiments, it is suggestive that the attractive interactions between phenolic groups and chiral selectors would account for the retention of the flavanone derivatives. The polar phenolic groups in flavanone derivatives most probably interacted with the substituents on the CD rings, whilst it would be unlikely included into the hydrophobic CD cavity [40]. Although the retention as well as the attractive interaction between phenolic groups and chiral selectors in F3 was not the strongest amongst flavanone derivatives, its chiral selectivity was the best. It is obvious that the stronger interactions between phenolic groups and chiral selectors may not always result in higher stereoselectivity whereas the position of phenolic group on flavanone is important for enantioseparation: cationic CSPs have shown enhanced enantioselectivity if the phenolic group is in 4′-position (F3); their enantioselectivities towards flavanone derivatives with multiple phenolic groups were reduced especially for flavanone derivatives with phenolic groups on both 4′- and 6-positions (F4). The strong influence of 4′-phenolic group on enantioseparations was also mentioned in a comparative study where native β-CD stationary phase (Cyclobond I) was applied for flavanone derivatives' enantioseparations [41].

3.2.2. Evaluation of cationic β-CD bonded CSPs with dansyl-amino acids

SFC enantioseparations of dansyl amino acids were acquired on VIMPCCD-POLY using mobile phase comprising CO_2/2-propanol (60/40, v/v). For dansyl amino acids (Table 1), enantioseparations were obtained using 2-propanol as modifier. However, enantioseparation was not attainable using methanol modifier. As methanol has a higher polarity than 2-propanol, it probably interacted tightly with the substituents on the CD rim, masking the interaction sites on the substituents and thus weakening its enantioseparation ability [31]. On the other hand, comparing the selectivities and resolutions amongst the dansyl amino acids, it was found that when the chain length of the alkyl group ("R") was lengthened from ethyl to *n*-hexyl substituent, CSP VIMPCCD-POLY depicted increased chiral selectivity from 1.09 to 1.18. It can therefore be inferred that hydrophobic inclusion between the CD cavity and the "R" group was involved in the enantioseparation processes for dansyl amino acids. The "R" group with higher hydrophobicity afforded a better interaction with the CD cavity. Moreover, VIMPCCD-POLY afforded dansyl-DL-phenylalanine an enantioselectivity of 1.29. The same CSP depicted a lower chiral selectivity (1.18) towards dansyl-α-aminocaprylic acid which had a *n*-hexyl group. The phenyl group's interaction with CD cavity in VIMPCCD-POLY was apparently more favourable to enantioseparation than an alkyl chain (*n*-hexyl group).

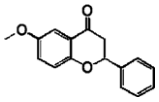
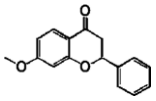
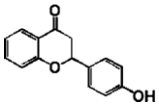
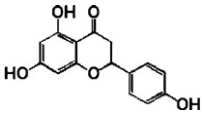
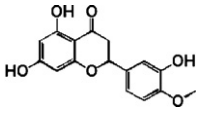
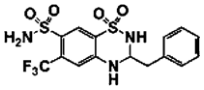
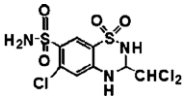
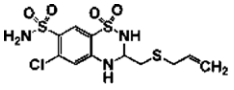
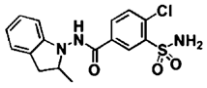
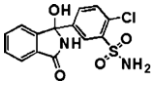
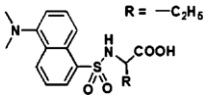
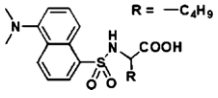
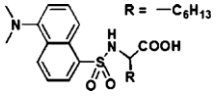
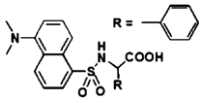
It was envisaged whilst the carboxylic group interacted with the positively charged imidazolium ring tightly, the groups near to the carboxylic group afforded hydrophobic interactions with the other parts of the chiral selector [42]. In this case, the substitute on α position to the carboxylic acid group inserts partially into the cyclodextrin cavity through the smaller cyclodextrin cone nearer to the imidazolium moiety. It is reported that the cyclodextrin cavity is accessible in SFC condition because the non-polar mobile phase CO_2 is small volume molecule [31,43,44]. In the case of dansyl-amino acid, if the substitute on α position to the carboxylic acid group is a short alkyl chain, it forms a relatively loose inclusion complex with the cyclodextrin cavity. Accordingly, the retention and selectivity would be smaller. Conversely, it was found that the longer the alkyl chain, the greater the retention and selectivity. If the substitute is a phenyl group, it forms a tight inclusion complex with the cyclodextrin cavity which explains the highest resolution and capacity factor for dansyl-DL-phenylalanine. The smaller cone of β-cyclodextrin (diameter 6.0–6.5 Å) allows for the inclusion of a benzene ring (dynamic diameter 5.85 Å) [45–47].

3.2.3. Evaluation of cationic β-CD bonded CSPs with diuretic thiazides

Enantioseparations of bendroflumethiazide were previously attainable on bonded native β-CD CSP with a selectivity of 1.11 [48]. It was suggested in these reports that the enantioseparations of the thiazides might require π–π stacking whilst the substituent in their bonded CSP could only form hydrogen bonding with the analytes. The CSPs with O-naphthylethylcarbamoyl substituents on (R)- and (S)-naphthylethylcarbamate-β-CD could provide π–π stacking sites and showed accentuated enantioseparations towards althiazide (α 1.02–1.03), bendroflumethiazide (α 1.10–1.22) and indapamide (α 1.04–1.18) in RPLC and SFC [31,49]. However, our cationic β-CD derivatives contain rather appropriate function groups and accordingly better enantioseparations towards althiazide (α 1.50), bendroflumethiazide (α 1.84) and indapamide (α 1.19) in SFC (Fig. 3).

In previous studies, applications of neutral CD derivatives in CE or RPLC reported weak enantioseparations of diuretic thiazides where the hydrophobic cavity of CD could form only unstable inclusion with the thiazides [50–53]. Comparative studies on neutral CD additives such as native β-CD, (2-hydroxylpropyl)-β-CD and methyl-β-CD had shown that native CD depicted the best enantioseparation towards the thiazide of chlorthalidone, albeit with a modest enantioselectivity of 1.12 [51]. Charged β-CD derivatives were also applied for the enantioseparation of thiazides. A dual additive comprising anionic β-CD (carboxymethyl-β-CD) and neutral β-CD in the mobile phases had shown enantioseparations towards chlorthalidone with an optimized resolution of 1.5. However, the enantioseparation was the combined effect between neutral and charged β-CD additives. It was unclear whether the presence of charged β-CD additive alone would afford better enantioseparation [52]. Cationic β-CD additive, on the other hand, also afforded enantioseparation towards chlorthalidone (α 1.15). Meanwhile, derivatizing β-CD into charged species would also be desirous as it would enhance the solubility of CD additives. With higher concentration of cationic CD derivatives in the mobile phase, the enantioselectivity of chlorthalidone was optimized at 1.19 [53]. However, our bonded cationic β-CD CSP VIMPCCD-POLY, afforded a higher chiral selectivity of 1.29 towards chlorthalidone (T5).

Table 1
Enantioseparations of racemates on VIMPCCD-POLY.

Analytes		Modifiers	α	Rs
6-Methoxyflavanone (F1)		[a]	1.10	1.22
7-Methoxyflavanone (F2)		[a]	1.20	0.50
4′-Hydroxyflavanone (F3)		[b]	1.38	1.94
Naringenin (F4)		[c]	1.16	1.38
Hesperetin (F5)		[c]	1.22	1.67
Bendroflumethiazide (T1)		[d]	1.84	3.51
Trichlormethiazide (T2)		[d]	1.71	3.94
Althiazide (T3)		[d]	1.50	2.72
Indapamide (T4)		[d]	1.19	1.87
Chlorthalidone (T5)		[d]	1.29	2.44
Dansyl-DL-α-amino-n-butyric acid (D1)		[e]	1.09	0.72
Dansyl-DL-norleucine (D2)		[e]	1.15	1.08
Dansyl-DL-α-aminocaprylic acid (D4)		[e]	1.18	1.29
Dansyl-DL-phenylalanine (D5)		[e]	1.31	1.74

Conditions: flow rate 1.0 mL min^{-1}, oven temperature 40 °C, BPR 15 MPa; modifier content in CO_2.
[a] 1 vol% 2-propanol.
[b] 3 vol% 2-propanol.
[c] 10 vol% 2-propanol.
[d] 10 vol% MeOH.
[e] 40 vol% 2-propanol.

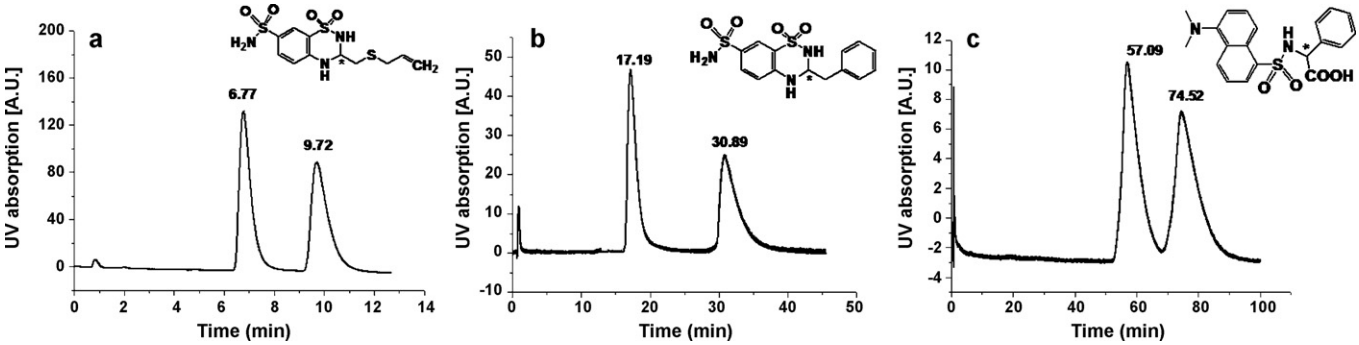


Fig. 3. Representative chromatography achieved on VIMPCCD-POLY (conditions: flow rate 1.0 mL min^{-1}, oven temperature 40 °C, BPR 15 MPa; modifier content in CO_2: (a and b) 30 vol% 2-propanol; (c) 40 vol% 2-propanol).

3.3. Inspection on the influence of acidic additive

In our investigation, acetic acid was added at 1% volume ratio to the organic modifier (2-propanol). The mobile phases with or without added acetic acid (1 vol%) containing the same proportion of organic modifiers were being compared. The presence of acid additive had noticeable impact on the retention and enantioseparation of the racemates in SFC. These differences are illustrated in Fig. 4.

In perusal of the results in Fig. 4, two conclusions can be drawn:

(i) The acid additive shortens strong acidic analytes' retention time significantly but has little or no influence on weakly acidic or neutral analytes.

Thus, in Fig. 4(a), dansyl amino acids exhibited higher retention than weakly acidic (*i.e.* containing phenolic group) and neutral compounds. Acid additive was found to have reduced the retentions of dansyl amino acids significantly whilst the retention times of weakly acidic and neutral compounds were less significantly affected. The acidic groups in the analytes would be attracted electrostatically to the cationic CSP. Acid additive would however compete for the cationic moiety on the chiral selector, thus masking the positive charge on the chiral selectors. Accordingly, the electrostatic forces between analytes and chiral selectors would be weakened, making the influence of acid additive on the retentions of dansyl amino acids clearly evident.

(ii) Acid additive accentuates enantioselectivity of weakly acidic analytes whilst it does not significantly influence the enantioseparations of the strongly acidic analytes and neutral analytes.

In Fig. 4(b), it is noteworthy that the cationic CSP's selectivities towards weakly acidic compounds (F3–F5) were improved by the acid additive, but enantioselectivities of both strongly acidic analytes (D1–D5) and neutral compounds (F1 and F2) were not significantly changed. Acid additive could suppress the dissociation of acidic compounds in mobile phase. The CD cavity may then form stable hydrophobic inclusion with undissociated analytes and acquire higher chiral selectivities. However, neutral compounds (F1, F2 without phenolic groups), which could only interact with CSP through hydrophobic inclusion, attained lower chiral resolutions than weakly acidic compounds (F3–F5 with phenolic groups). It is envisaged that the hydrophobic inclusion alone is not sufficient for enhanced enantioseparations whereas a combination of electrostatic force and hydrophobic inclusion would be more optimal. For strong acids, although the electrostatic force could be weakened by acid additive, it was still excessively strong, thus overwhelming stereoselective interaction which was possibly derived from CD inclusion. Accordingly, the acid additive would only shorten the retention times but did not improved upon the chiral selectivities, and in both cases, the selectivities of strong acids were lower than weak acidic analytes (Fig. 4(b)). In Stalcup's study on anionic β-CD [25], it was also showed that, excessively strong electrostatic force between protonated aminoglutethimide and anionic chiral selector was unfavourable to enantioseparation. On the other hand, weakly electrostatic force could be augmented by the acidic additives to synergize with other interactions better and resulted in a higher enantioselectivity (Fig. 5).

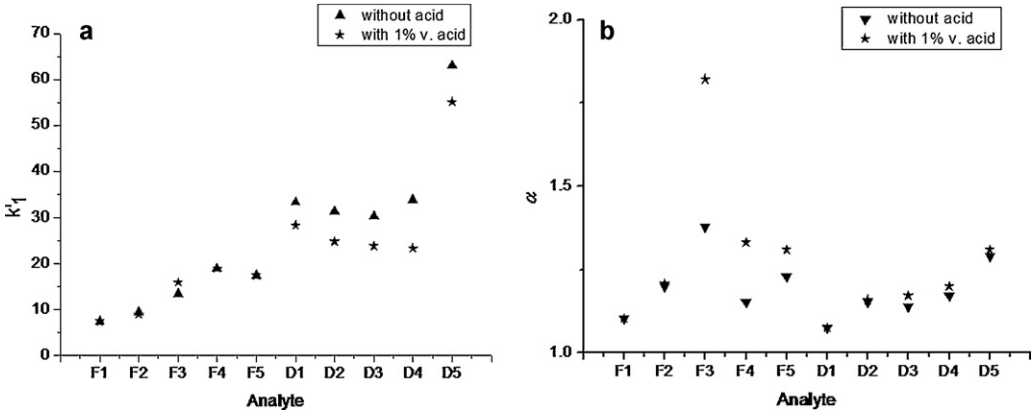


Fig. 4. Change of k'_1 (a) and chiral selectivity (b) of analytes with addition of acetic acids. Conditions: flow rate 1.0 mL min^{-1}, oven temperature 40 °C, BPR 15 MPa; content of 2-propanol in CO_2; F1 and F2: 1 vol%; F3: 3 vol%; F4 and F5: 10 vol%; D1–D5: 40 vol% (F1: 6-methoxyflavanone, F2: 7-methoxyflavanone, F3: 4′-hydroxyflavanone, F4: naringenin, F5: hesperetin; D1: dansyl-DL-α-amino-n-butyric acid, D2: dansyl-DL-norleucine, D3: dansyl-DL-norvaline D4: dansyl-DL-α-aminocaprylic acid, D5: dansyl-DL-phenylalanine).

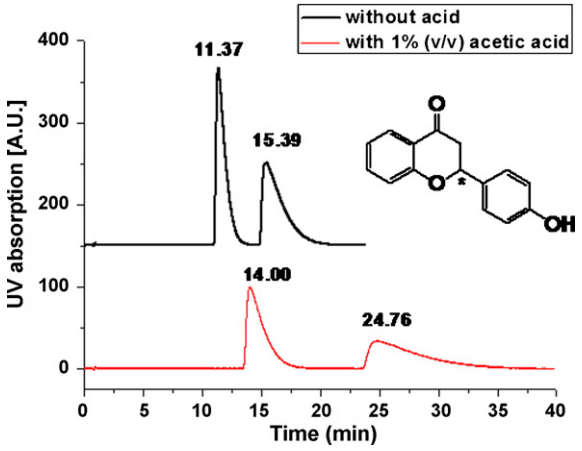


Fig. 5. Chromatography of 4′-hydroxyflavanone (conditions: flow rate 1.0 mL min^{-1}, oven temperature 40 °C, BPR 15 MPa; CO_2: 2-propanol 97:3 (v:v)).

4. Conclusion

The cationic β-cyclodextrin phenylcarbamate derivative has been successfully bonded onto silica gel through facile co-polymerization reaction. The novelty of the cationic β-cyclodextrin derivative lies on the cationic imidazolium or ammonium moiety. Aromatic imidazolium moiety could afford more interaction sites than aliphatic ammonium moiety, thus enabled better enantioseparations. The electrostatic force generated from cationic imidazolium moiety is found important on the retention and chiral separation of the racemates. Enantioseparations of 14 racemates encompassing flavanones, thiazides and amino acid derivatives were affected on the novel chiral stationary phase. The influence of acid additives in SFC was discussed. Herein, competition between the acid additive and analytes weakened the electrostatic force between the analytes and the chiral selector which would decrease retention of the analytes but in several cases enhanced enantioseparation.

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References

[1] M.M. Islam, J.G. Mahdi, I.D. Bowen, Drug Saf. 17 (1997) 149.
[2] I. Wainer, Drug Stereochemistry: Analytical Methods and Pharmacology, Marcel Dekker, New York, 1993.
[3] D.E. Drayer, Clin. Pharmacol. Ther. 40 (1986) 125.
[4] K.M. Kamal, M. Gordon, J.R. Maureen, W.H. John, J. Pharm. Sci. 87 (1998) 797.
[5] I.A. Yamskov, B.B. Berezin, V.A. Davankov, Y.A. Zolotarev, I.N. Dostavalov, N.F. Myasoedov, J. Chromatogr. 217 (1981) 539.
[6] S.V. Rogozhin, V.A. Davankov, Usp. Khim. 37 (1968) 1327.
[7] K. Junko, T. Izumi, K. Akari, T. Kiyoshi, Phytochem. Anal. 10 (1999) 175.
[8] H. Nishi, T. Fukuyama, S. Terabe, J. Chromatogr. 553 (1991) 503.
[9] A.F. Fell, T.A. Noctor, J.E. Mama, B.J. Clark, J. Chromatogr. 434 (1988) 377.
[10] Y. Eiji, S. Pennapa, O. Yoshio, Chirality 8 (1996) 446.
[11] D.W. Armstrong, Y. Tang, S. Chen, Y. Zhou, C. Bagwill, J.-R. Chen, Anal. Chem. 66 (2002) 1473.
[12] S. Busch, J.C. Kraak, H. Poppe, J. Chromatogr. 635 (1993) 119.
[13] M. Lienne, M. Caude, R. Rosset, A. Tambut, J. Chromatogr. 472 (1989) 265.
[14] E. Smolková, H. Králová, S. Krýsl, L. Feltl, J. Chromatogr. 241 (1982) 3.
[15] S. Terabe, Trends Anal. Chem. 8 (1989) 129.
[16] Y. Tanaka, M. Yanagawa, S. Terabe, HRC: J. High Resoln. Chromatogr. 19 (1996) 421.
[17] B. Chankvetadze, G. Endresz, G. Blaschke, Chem. Soc. Rev. 25 (1996) 141.
[18] V. Cucinotta, A. Contino, A. Giuffrida, G. Maccarrone, M. Messina, J. Chromatogr. A 1217 (2010) 953.
[19] C. Desiderio, S. Fanali, J. Chromatogr. A 716 (1995) 183.
[20] W. Tang, S.C. Ng, J. Sep. Sci. 31 (2008) 3246.
[21] B. Chankvetadze, Electrophoresis 30 (2009) S211.
[22] R.J. Tait, D.O. Thompson, V.J. Stella, J.F. Stobaugh, Anal. Chem. 66 (1994) 4013.
[23] S.R. Gratz, A.M. Stalcup, Anal. Chem. 70 (1998) 5166.
[24] A.M. Stalcup, K.H. Gahm, Anal. Chem. 68 (1996) 1360.
[25] A.M. Stalcup, K.H. Gahm, Anal. Chem. 68 (1996) 1369.
[26] F. O'Keeffe, S.A. Shamsi, R. Darcy, P. Schwinte, I.M. Warner, Anal. Chem. 69 (1997) 4773.
[27] J.L. Haynes III, S.A. Shamsi, F. O'Keefe, R. Darcey, I.M. Warner, J. Chromatogr. A 803 (1998) 261.
[28] T.T. Ong, R.-Q. Wang, I.W. Muderawan, S.C. Ng, J. Chromatogr. A 1182 (2008) 136.
[29] R.-Q. Wang, T.T. Ong, S.C. Ng, J. Chromatogr. A 1203 (2008) 185.
[30] W. Steuer, M. Schindler, G. Schill, F. Erni, J. Chromatogr. 447 (1988) 287.
[31] K.L. Williams, L.C. Sander, S.A. Wise, J. Chromatogr. A 746 (1996) 91.
[32] L. Svensson, J. Donnecke, K. Karlsson, A. Karlsson, J. Vessman, Chirality 12 (2000) 606.
[33] P. Jandera, J. Chromatogr. A 965 (2002) 239.
[34] T.L. Chester, J.D. Pinkston, Anal. Chem. 74 (2002) 2801.
[35] H. Roy, B. Mirlinda, Z. Jia, M. Bing, F. Kimber, V. Vaso, H. Paul, J.W. Christopher, Chirality 19 (2007) 787.
[36] R.W. Stringham, K.G. Lynam, C.C. Grasso, Anal. Chem. 66 (2002) 1949.
[37] X. Chen, F. Qin, Y. Liu, X. Huang, H. Zou, J. Chromatogr. A 1034 (2004) 109.
[38] K.S. Ahmed, F. Tazerouti, A.Y. Badjah-Hadj-Ahmed, B.Y. Meklati, J. Sep. Sci. 30 (2007) 2025.
[39] B.A. Siles, H. Brian Halsall, J.G. Dorsey, J. Chromatogr. A 704 (1995) 289.
[40] J.A. Yanez, P.K. Andrews, N.M. Davies, J. Chromatogr. B 848 (2007) 159.
[41] M. Krause, R. Galensa, J. Chromatogr. 514 (1990) 147.
[42] S.A.A. Rizvi, S.A. Shamsi, Anal. Chem. 78 (2006) 7061.
[43] K.L. Williams, L.C. Sander, S.A. Wise, Chirality 8 (1996) 325.
[44] P. Macaudiere, M. Caude, R. Rosset, A. Tambute, J. Chromatogr. 405 (1987) 135.
[45] S. Beni, Z. Szakacs, O. Csernak, L. Barcza, B. Noszal, Eur. J. Pharm. Sci. 30 (2007) 167.
[46] C.M. Detz, L.A. Field, U.S. Patent, Chevron Research Company, San Francisco, CA, United States, 1982.
[47] K.A. Connors, Chem. Rev. 97 (1997) 1325.
[48] Y. Zhang, Z. Guo, J. Ye, Q. Xu, X. Liang, A. Lei, J. Chromatogr. A 1191 (2008) 188.
[49] D.W. Armstrong, C.-D. Chang, S. Haing Lee, J. Chromatogr. 539 (1991) 83.
[50] W. Wu, A.M. Stalcup, J. Liq. Chromatogr. 18 (1995) 1289.
[51] H.-H. Rosa, C.-F. Pilar, J. Chromatogr. B 740 (2000) 169.
[52] M. Fillet, L. Fotsing, J. Crommen, J. Chromatogr. A 817 (1998) 113.
[53] C. Roussel, A. Favrou, J. Chromatogr. A 704 (1995) 67.