Journal of Chromatography A, 1224 (2012) 97–103

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

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

Ren-Qi Wang, Teng-Teng Ong, Siu-Choon Ng*

Division of Chemical and Biomolecular Engineering, College of Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459, Singapore

ARTICLE INFO

Article history:
Received 19 October 2011
Received in revised form
13 December 2011
Accepted 15 December 2011
Available online 23 December 2011

Keywords:
β-Cyclodextrin derivatives
Chiral stationary phases
Supercritical fluid chromatography
Enantioseparation
Electrostatic force
Ionic liquid

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

* Corresponding author. Tel.: +65 67904067; fax: +65 67947553.
E-mail address: ngsc@ntu.edu.sg (S.-C. Ng).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.12.053

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