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Communication

Preparation and chromatographic evaluation of a chiral stationary phase based on carboxymethyl-β-cyclodextrin for high-performance liquid chromatography

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ABSTRACT

A chiral stationary phase (CSP) was prepared by chemically bonding carboxymethyl-β-cyclodextrin (CM-β-CD) onto 3-aminopropyl silica gel through amidation reaction in water solution and was characterized by Fourier transform infrared spectroscopy (FT-IR), element analysis (EA) and thermal gravimetry analysis (TGA). The chromatographic performance was evaluated with 24 racemates under reversed-phase conditions. The effect of salt, organic modifier, mobile phase pH and structures of analytes were discussed. In comparison with native β-CD bonded column, CYCLOBOND I 2000, CM-β-CD CSP exhibited enhanced enantioseparation.

Enantioseparation is important for racemic compounds, especially for many pharmaceuticals, mainly due to their different pharmacological activities and/or toxicities for the two enantiomers of the same compound in biological environment [1]. Various kinds of chromatographic techniques, such as high performance liquid chromatography, gas chromatography, thin layer chromatography, supercritical fluid chromatography, and capillary electrophoresis can be used to detect enantiomers. Among them, HPLC is the most versatile technique for enantiomeric separation. Although numerous chiral selectors have been reported in HPLC, those based on cyclodextrins (CDs) are still attracting research interests. The truncated cone structures of native CDs enable them to form inclusion complexes with a variety of molecules and the natural chirality of CDs is beneficial for chiral separation. Additionally, derivations of hydroxyl groups at C-6 position, C-2 and C-3 positions can remarkably improve enantioselectivity. CDs and their derivatives can be used as chiral additives [2–5] as well as chiral stationary phases (CSPs) [6–8].

Carboxymethyl-β-cyclodextrin (CM-β-CD) as one of the common β-CD derivatives used for chiral mobile phase additive (CMPA) has reached many successful resolutions for chiral drugs [9–13]. However, addition of such chiral selector into mobile phase may suffer from some drawbacks such as the limiting application in normal phase HPLC or reversed phase HPLC with low aqueous composition because of its poor solubility in these mobile phases. In addition, a relatively low chiral recognition is generally found in the CMPA technique unless a large amount of this additive is applied. In order to overcome these problems, CM-β-CD can be bonded onto silica support as a CSP.

Up to now, there are few reports on the application of CM-β-CD CSP. Park and coworkers reported preparation and use of CM-β-CD-coated zirconia as a CSP, where CM-β-CD is just physically coated on zirconia not chemically bonded [14]. Leonelli *et al.* reported to use Orpak CDBS-453 to resolve Wieland-Miescher ketone and derivatives [15], where Orpak CDBS-453 was noted to have silica (5 μm) bonded with CM-β-CD as the packing material. Actually, this column is produced by Shodex, a Japanese chromatographic column manufacturer and the Shodex website (http://shodexhplc.com/product-category/l45/) clearly shows that Orpak CDBS-453 is a *R,S*-hydroxypropyl ether β-cyclodextrin column not a CM-β-CD column. In addition, there is not any report on chemically bonded CM-β-CD CSP.

In the present work, a chiral stationary phase is synthesized by chemically bonding CM-β-CD onto the surface of aminized silica gel through amidation reaction and confirmed by Fourier transform infrared spectroscopy (FT-IR), element analysis (EA) and thermal gravimetry analysis (TGA). Chiral recognition abilities

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are evaluated with some racemic compounds under reversed-phase conditions.

FT-IR spectra were performed on a Thermo Fisher Nicolet 6700 FT-IR Spectrometer (Thermo Fisher, U.S.A.). EA was obtained with a vario MICRO cube instrument (Elementar, Germany). High resolution mass spectrometry (HRMS) was carried out on a microTOF-Q II comprising a high-performance hybrid quadrupole time-of-flight mass spectrometer (Bruker, Germany). TGA was recorded on a TG/DSC 2 thermal analyzer (Mettler Toledo, Switzerland). Evaluation of the new column was made on a Shimadzu (Kyoto, Japan) LC-Solution equipped with a LC-20AT binary LC pump, a Shimadzu SPD-20A UV–vis detector and a 7725i manual injector (Rheodyne Inc., U.S.A.) with a 20 μL sample loop. All solvents and reagents used in synthesis and mobile phases were commercially available and directly used without further purification.

The synthetic route is shown in Scheme 1.

17% Chloroacetic acid aqueous solution (9 mL) was added dropwise to a solution of β-CD (5.00 g) in aqueous 20% sodium hydroxide solution (15 mL) on a water bath (60 °C) over 10 min. The resulting mixture was stirred for 4 h. Then hydrochloric acid was added to adjust the pH to 6.0-7.0 while the reaction solution was cooled to room temperature. The obtained product was precipitated by addition of excess MeOH. After filtration, the well white precipitate was washed with 3 × 30 mL aqueous 50% MeOH solution and dried *in vacuo* at 60 °C to afford 3.32 g CM-β-CD. FT-IR (curve B, Fig. S1 in Supporting information) (KBr, cm^{-1}): 3388.9, 2928.6, 1602.4, 1416.8, 1329.9, 1079.9, 1030.5, 579.5; HRMS (Fig. S2 in Supporting information) (*m/z*, negative): 1249.3291, 1271.2849, 1307.3363, 1329.3159, 1365.3399, 1387.3190, 1409.2992 and 1489.2831. The FT-IR spectrum indicates that CM-β-CD exists in the form of sodium CM-β-CD. Two strong peaks at 1602.4 cm^{-1} and 1416.8 cm^{-1} are assigned to the asymmetrical stretching vibration and symmetrical stretching vibration of carboxylic salts, respectively, which are not observed in native β-CD. The HRMS results further corroborate the success of the synthesis procedure. Two peaks at 1249.3291 and 1271.2849 mean that two hydroxyl groups on β-CD reacted with chloroacetic acid; Peaks at 1307.3363, 1329.3159 indicate that three hydroxyl groups on β-CD participated in reaction; Peaks at 1365.3399, 1387.3190, 1409.2992 suggest tetrasubstituted β-CD, and 1489.2831 indicates five. The average substitution degree of CM-β-CD is calculated to be 3 according to the HRMS results.

This new CSP was obtained by chemically bonding CM-β-CD onto 3-aminopropyl silica gel. The CM-β-CD (3.32 g), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (3.51 g), and *N*-hydroxysuccinimide (NHS) (2.03 g) were dissolved in distilled water (70 mL), after which Platisil NH_2 silica gel (3-aminopropyl silica gel) (1.55 g) was added at 0 °C and the reaction was allowed to proceed for 24 h at room temperature, filtrated, washed with water, MeOH, and then dried *in vacuo* at 60 °C for 4 h. CM-β-CD CSP was obtained as white powder (1.94 g, 125%). EA found (%): C 13.02, H 2.68, N 2.93; FT-IR (curve C, Fig. S1 in Supporting information) (KBr, cm^{-1}): 3442.6, 2934.8, 1647.0, 1560.7, 1100.1, 468.5; TGA (curve b, Fig. S3 in Supporting information): Weight loss was 29.9% (w/w). EA shows an increase of carbon content from 7.0% to 13.0%. Surface coverage of CM-β-CD on aminized silica gel was estimated to be about 0.28 μmol/m^2 using the following formula [16]:

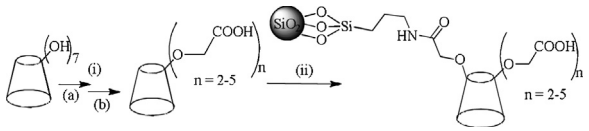
$$\frac{\frac{\%C}{12\,n_c \times 100}}{S\left(1 - \frac{\%C}{12\,n_c \times 100} \times M\right)}$$

where %*C* is the increment of carbon content (6.02), n_c is the number of carbon atoms in the bonded moiety (48), *M* is the molecular weight of the bonded moiety (1309) and *S* is the aminized silica gel special surface (440 m^2/g). The FT-IR data shows a new intense absorption peak at 1647.0 cm^{-1} due to the amide group of the modified silica gel. The loss of weight for CM-β-CD CSP was determined to be 29.9% from 35 °C to 800 °C under air atmosphere at heating rate of 15 °C/min, while the loss of weight for aminized silica gel was only 9.6%, which could also confirm the successful bonding of the CM-β-CD CSP.

The CSP was slurry packed into stainless steel column (150 mm × 4.6 mm i.d.) using a slurry packing method with an Alltech 1666 HPLC Slurry Packer. Carbon tetrachloride/1,4-dioxane = 2/1 (v/v) used as the slurry solvent and MeOH as the packing solvent. Chromatographic performances of this CSP were evaluated with 24 racemic compounds listed in Fig. 1 which were either supplied from pharmaceutical companies or prepared in our laboratory (compounds **6–9**, Supporting information). All the chromatograms were obtained at 1.00 mL/min and 254 nm unless otherwise specified under reversed-phase mode. Mobile phases were filtered with 0.45 μm membranes and degassed with sonication before use. All the working samples were prepared in MeOH at a concentration of about 1.0–2.0 mg/mL, and the injected volume was 3 μL.

In order to investigate the effect of mobile phase (salts, pH and organic modifiers) on chiral separation of CM-β-CD CSP, several compounds were chosen for detailed study. Introduction of salt in the mobile phases could greatly improve separation efficiency and peak shape [17]. The influence of ammonium acetate (NH_4Ac) concentration on the chiral separation was investigated using a neutral (compound **2**), an acidic (compound **7**) and a basic (compound **17**) compound as model analytes (Table 1). As shown in Table 1, in the absence of NH_4Ac in mobile phase, the neutral and acidic compounds could be eluted while the basic compound could not be eluted within 30 min. This is because there is an ion-pairing formation between the protonated basic analyte and the free carboxyl on the CM-β-CD CSP. However, this interaction could not be aroused for the neutral and acidic compounds. It can be noted that the retention factors (*k*) for all the three analytes are reduced with the amount of NH_4Ac in the mobile phase increasing from 0.50% (w/v, 0.0649 mol/L) to 2.00% (w/v, 0.260 mol/L), especially for compound **17**. Meanwhile, the selectivity factors (α) for all of them are kept almost unchanged but the resolutions (*Rs*) decreases slightly. The suitable amount of NH_4Ac is 0.50%.

In addition to NH_4Ac, ammonium chloride (NH_4Cl) and monoammonium phosphate ($NH_4H_2PO_4$) were tested as other two ammonium salt additives, at identical salt concentration (0.0649 mol/L) and MeOH content varied from 50% to 20% (Tables S1–S3 in Supporting information), the representative chromatograms are depicted in Fig. S4 (Supporting information). The results indicate that anion species also affect the retention and the enantioselectivity. $NH_4H_2PO_4$ is found to be more effective in reducing retention factors than NH_4Cl for the three compounds. However, NH_4Ac has different effect in retention for different natural compound. Acidic compound shows the lowest retention in aqueous NH_4Ac while basic compound exhibits the highest retention. It's not surprising because different salt solution has different pH which might vary ionization of solute molecule with acidic or basic functional groups. Among these salt additives,



Scheme 1. Synthesis of CM-β-CD CSP. Reagents and conditions: (i) (a) chloroacetic acid/sodium hydroxide/H_2O/60 °C, (b) hydrochloric acid; (ii) EDCI/NHS/3-aminopropyl silica gel/H_2O.

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