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Journal of Chromatography A, 1050 (2004) 151–157
JOURNAL OF CHROMATOGRAPHY A
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Separation of racemic 2,4-dinitrophenyl amino acids on 9-*O*-(phenyloxycarbonyl)quinine-bonded carbon-clad zirconia in reversed-phase liquid chromatography

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Received 19 April 2004; received in revised form 23 July 2004; accepted 13 August 2004

Abstract

Zirconia is known to be one of the best materials for the chromatographic support due to its excellent chemical, thermal, and mechanical stability. In this work, we report preparation and use of 9-*O*-(phenyloxycarbonyl)quinine-bonded carbon-clad zirconia (QNCZ) as a chiral stationary phase (CSP) for separation of *N*-(2,4-dinitrophenyl) (DNP)-amino acids (AAs) enantiomers in reversed-phase liquid chromatography. Retention and enantioselectivity of the QNCZ CSP were compared with those of quinine 3-triethoxysilylpropylcarbamate-coated zirconia (QNZ) and quinine 3-triethoxysilylpropylcarbamate-bonded silica (QNS). The QNCZ CSP showed in general the better enantioselectivity for most of the amino acids studied.

Keywords: Chiral stationary phases; Enantiomer separation; 9-*O*-(Phenyloxycarbonyl)quinine; Carbon-clad zirconia; DNP-amino acids

1. Introduction

High-performance liquid chromatography (HPLC) separation method based on chiral stationary phases (CSPs) has become one of the most attractive approaches to chiral separations, due to its simplicity for determining enantiomeric purity and easy extension to the semipreparative and preparative scales [1]. One of the major problems in using many CSPs is their narrow range of analyte applicability; they can only discriminate a limited number of specific types of chemical entities, and it is frequently necessary to derivatize the compounds of interest to achieve separation [2]. Quinine has been widely used for enantiomer separation in HPLC as a chiral ion-pairing agent in the mobile phases and as a CSP ligand [3–6]. In recent years, CSPs based on the use of carbamoylated derivatives of quinine and quinidine as selectors were found to be highly stereoselective for the direct resolution of chiral acids using mixtures of aqueous buffers and methanol or acetonitrile as mobile phases [7–18].

Silica is the most popular choice for support of HPLC stationary phase ligands due to the mechanical strength, wide range of particle and pore dimensions, pore structure and well-established silane chemistry. However, silica and bonded phase ligands have stability problems. Silica dissolves in mobile phase buffered at or above pH 8 with loss of bonded phase ligand and column packing [19]. Loss of organosilanes from the silica surface via hydrolysis proceeds rapidly at low pH (<3) and at high temperature (≥40 °C). These deficiencies of the column packing create problems of poor injection reproducibility, poor peak shape, and high backpressure, thus making method development tasks difficult. Siloxane-bonded silica phases with improved hydrolytic stability at extreme pH for use in reversed-phase liquid

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doi:10.1016/j.chroma.2004.08.027

chromatography were introduced [20–23]. However, careful choices of operating conditions such as the use of acetonitrile instead of methanol, the use of boric acid or organic buffers in low concentration and low temperature are still required to achieve acceptable column lifetimes [24–27].

Fast method development, high efficiency, rapid resolution of enantiomers, and robustness are the main criteria for chiral separation methods, especially in the pharmaceutical industry. These priorities require stable CSPs capable of achieving baseline separations in the minimum time, which ultimately means high selectivity and efficiency. Over the last decade, zirconia has received considerable attention as stationary phase support for HPLC [28,29]. Zirconia particles are very robust material; they show no detectable signs of dissolution over the pH range from 1 to 14 and have been used for prolonged periods at temperatures up to 200 °C in chromatographic separations.

We have been working to develop efficient and chemically stable CSPs on zirconia substrate [30–33]. Bare zirconia cannot be covalently modified like silica due to the instability of Zr–C and Zr–O–Si bonds in water [34]. Zirconia-based CSPs reported thus far have been prepared by coating chiral selectors on zirconia surface by utilizing Lewis acid–base chemistry. In this work, we report preliminary results obtained with 9-*O*-(phenyloxycarbonyl)quinine bonded onto 5-μm carbon-clad zirconia particles that combine the excellent enantioselectivity of quinine with the pH and mechanical stability of zirconia. Enantioselectivity of quinine ester-bonded zirconia was compared with that for quinine carbamate-coated zirconia (QNZ) to see if there are any differences in enantioselectivity depending on the type of support material. Hopefully, the quinine ester-bonded zirconia phase could be used as a robust CSP in RPLC separation of variegated enantiomers.

2. Experimental

2.1. Reagents and materials

All reagents used for the preparation of the stationary phase were reagent grade or better. Quinine, 4-nitrophenyl chloroformate, sodium borohydride, sulfur, anhydrous toluene, petroleum ether, *N*,*N*-dimethylformamide, and anhydrous tetrahydrofuran (THF) were obtained from Aldrich (Milwaukee, USA). Methanol was HPLC grade (J.T. Baker, Phillipsburg, USA). *n*-Hexane was purchased from EM Sciences (Gibbstown, USA). D-, L-, and D,L-amino acids (AAs), 2,4-dinitrofluorobenzene were obtained from Sigma (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA). *N*-(2,4-Dinitrophenyl) (DNP) amino acids were obtained according to the procedure described in the literature [35]. HPLC-grade methanol was obtained from J.T. Baker (Phillipsburg, USA). Bare and carbon-clad zirconia, both having spherical shape, particle diameter of 5 μm, pore size of 30 nm, and surface area of 30 m^2/g, were obtained from ZirChrom Separations (Anoka, MN, USA). Nucleosil silica, having spherical shape, particle diameter of 5 μm, pore size of 30 nm, and surface area of 100 m^2/g, was obtained from Macherey-Nagel (Düren, Germany). Water was processed with an Elgastat UHQ water purification system (Bucks, UK). All the chemicals were of the best quality available and used as received without any further purification.

2.2. Preparation of quinine carbamate-coated zirconia

Quinine 3-triethoxysilylpropylcarbamate (QNTEOSPC) was synthesized as previously reported [7]. A 2.0-g amount of quinine (free base) and 1.5 mL of 3-triethoxysilyl isocyanate were refluxed for 4 h in dry toluene using one drop of dibutyl tin dilaurate as catalyst. Evaporation of the solvent and stirring with dry diethyl ether gave QNTEOSPC as white solid in 90% yield. Three hundred micromole amount of QNTEOSPC was dissolved in 4 mL of ethanol and the solution was added slowly to the slurry of 1 g of zirconia in 4 mL of water. The suspension was refluxed for 1 h and the particles were filtered and washed two times with 5 mL of 2/1 (v/v) ethanol/water and once with 3 mL of acetone. The particles were then dried in vacuum at 50 °C. The number of micromoles of quinine carbamate adsorbed on the zirconia surface based on the percent carbon from microanalysis was found to be 2.07 μmol m^{-2}.

2.3. Preparation of quinine carbamate-bonded silica (QNS)

QNTEOSPC was bonded onto silica as previously reported [7]. Chiral selector density based on the percent carbon from microanalysis was found to be 2.00 μmol m^{-2}.

2.4. Preparation of quinine ester-bonded carbon-clad zirconia (QNCZ) (Fig. 1)

2.4.1. 9-O-(4-Nitrophenyloxycarbonyl)quinine hydrochloride

9-*O*-(4-Nitrophenyloxycarbonyl)quinine hydrochloride was prepared according to a procedure in the literature [14].

2.4.2. 9-O-(4-Aminophenyloxycarbonyl)quinine

9-*O*-(4-Aminophenyloxycarbonyl)quinine was prepared by reducing 9-*O*-(4-nitrophenyloxycarbonyl)quinine hydrochloride using sulfurated sodium borohydride ($NaBH_2S_3$) [36] and a modified method of the procedure in the literature [37]. To a suspension of 2.6 g of $NaBH_2S_3$ (19.7 mmol) in 100 mL of tetrahydrofuran being refluxed, 10.0 g of 9-*O*-(4-nitrophenyloxycarbonyl)quinine hydrochloride (19.0 mmol) in 100 mL of THF was added dropwise and allowed to react for 24 h. The reaction mixture was evaporated to dryness and the residual solid was hydrolyzed with 10% hydrochloric acid solution until pH 1 in the presence of 250 mL of diethyl ether. The precipitated sulfur was filtered off and the filtrate was washed repeatedly with diethyl ether. The aqueous fraction was titrated to pH 11 with 20% NaOH solution in the

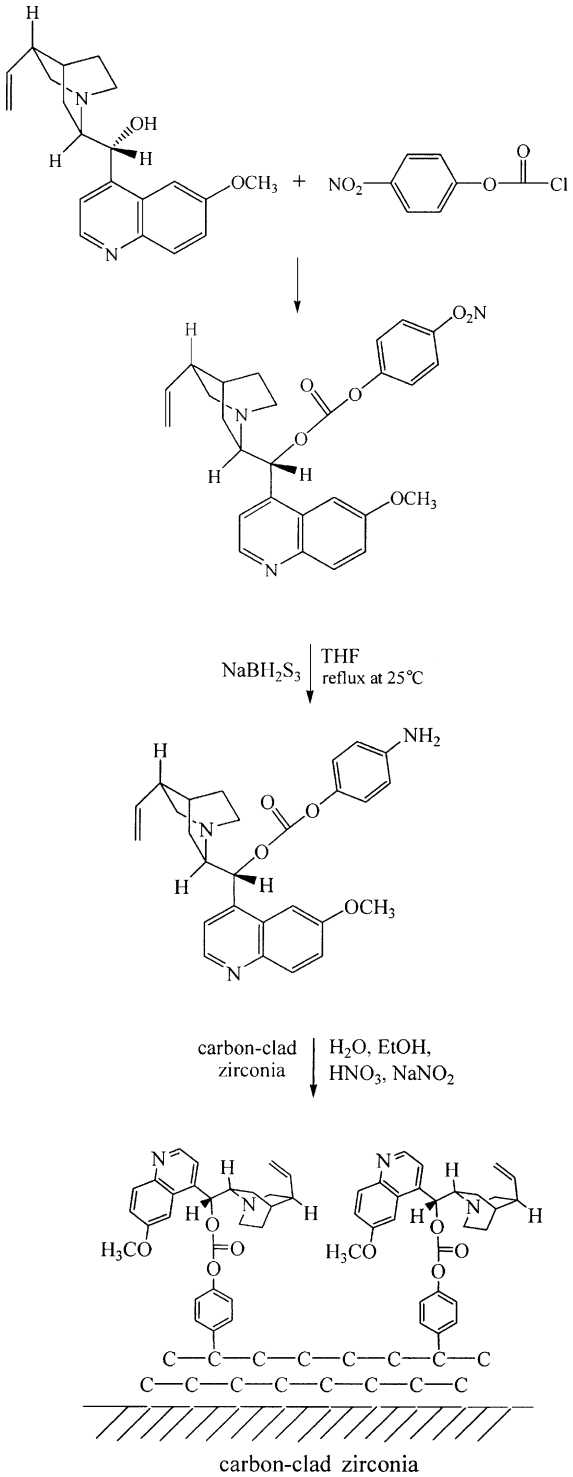

Fig. 1. Reaction scheme for the preparation of 9-*O*-(phenyloxycarbonyl)quinine-bonded carbon-clad zirconia.

presence of 200 mL of diethyl ether. The aqueous layer was extracted again with diethyl ether and the combined ether extracts were washed five times with 100 mL each of 20% NaOH solution. The product in the ether phase was purified by column chromatography on silica gel in 10:1 (v/v) chloroform/methanol (14% yield). IR (cm^{-1}) (KBr): 3384, 2925, 2879, 2854, 1762, 1618, 1591, 1508, 1334, 1263, 1230, 1110, 1031. ^{1}H NMR (ppm) (300 MHz, d_6-DMSO): 8.78 (d, 1H), 7.98 (d, 1H), 7.74 (d, 1H), 7.49 (m, 2H), 7.26 (d, 2H), 6.50 (d, 2H), 5.75 (m, 1H), 5.19 (m, 2H), 3.95 (s, 2H), 3.91 (s, 3H), 3.31 (m, 1H), 2.73 (m, 5H), 1.14 (m, 5H). Anal. Calcd. for $C_{27}H_{29}N_3O_4$: C, 70.57; H, 6.36; N, 9.14. Found: C, 70.71; H, 6.29; N, 8.97.

2.4.3. 9-O-(4-Phenyloxycarbonyl)quinine-bonded carbon-clad zirconia

9-*O*-(4-Phenyloxycarbonyl)quinine-bonded carbon-clad zirconia was prepared according to a modified procedure reported in the literature [38]. Briefly, 1 g of carbon-clad zirconia was added to a solution of 4.44 g of 9-*O*-(4-aminophenyloxycarbonyl)quinine dissolved in 83 g of water. The resulting suspension was cooled to 30 °C and 0.70 g of concentrated nitric acid was added. An aqueous solution containing 0.70 g of sodium nitrite was then added gradually with stirring, forming 9-*O*-(phenyloxycarbonyl)quinine diazonium hydroxide inner salt in situ, which reacts with the carbon on zirconia. The resulting product, 9-*O*-(phenyloxycarbonyl)quinine-bonded carbon-clad zirconia, was filtered, washed three times each with water, ethanol, and acetone, and dried in an oven at 125 °C. Chiral selector density based on the percent nitrogen from microanalysis was found to be 1.87 μmol m^{-2}.

2.5. Chromatography

The same packing conditions were employed for packing both zirconia- and silica-based packing materials. Packing materials were suspended in a (1:1) hexane/2-propanol mixture and packed into 20 cm × 1 mm (i.d.) columns using the downward slurry method at ca. 7000 psi. 2-Propanol was employed as the displacing solvent. A chromatographic system consisting of a Model 7520 injector with a 0.5-μL internal loop (Rheodyne, CA, USA), a Model 530 column oven (Alltech, IL, USA) set at 30 °C, and a Linear Model 200 UV/VIS detector (Alltech, IL, USA) with a 0.25-μL flowcell set at 254 nm was used. A Hewlett–Packard (Avondale, CA, USA) Series 3365 integrating recorder was used to record chromatograms. The mobile phases were mixtures (80/20, v/v, %) of methanol and ammonium acetate (0.1 M) or phosphate buffer (0.05 M). They were filtered through a membrane filter of 0.5-μm pore size and degassed prior to use. The flow rate was 80 μL min^{-1}. Deuterated water was used as the dead time marker by noting the baseline disturbance due to the refractive index difference. Peak identification was carried out by injecting solutions of each enantiomer of amino acids.

3. Results and discussion

3.1. Separation on quinine carbamate-coated zirconia

Quinine 3-triethoxysilylpropylcarbamate readily hydrolyzes in aqueous medium to give Lewis basic trisilicate

that strongly adsorbs on to the Lewis acid sites on zirconia surface. This can provide a CSP with immobilized chiral selector moiety. Separation data of 11 DNP-amino acids in 80:20 (v/v, %) methanol/ammonium acetate buffer (pH 4.5, 0.1 M) are listed in Table 1. Most of the amino acids studied were resolved in the eluent except arginine and tyrosine. The performance of columns packed with quinine carbamate-coated zirconia is shown for the resolution of racemic DNP-proline in 80:20 (v/v, %) methanol/ammonium acetate buffer (Fig. 2). Although enantiomers of this amino acid are resolved, the peaks are quite broad and tailed.

Dependence of retention DNP-AAs on the pH of the eluent on the QNZ CSP showed a typical ion exchange behavior (data not shown). Retention decreased upon increasing pH with a slight maximum on the *k*–pH curve at the pH value close to the pK_a of a DNP-AA. Increasing buffer concentration caused a decrease in retention but did not change selectivity values appreciably, as was observed in a previous study on the quinine carbamate-bonded silica CSP [7].

The surface of zirconia, like other metal oxides, is quite complex. The major species have been identified as being Brönsted acid sites, Brönsted base sites, and Lewis acid sites [34]. Although Brönsted base sites may be classified as Lewis base sites, no Brönsted base activity has been observed in liquid solid adsorption sites. The Lewis acid sites are very likely to influence the enantioselective ion-exchange interactions between the quinine carbamate and the analyte by non-selective Lewis acid–base interactions with the isomers of DNP-AAs (see Fig. 3). Strong interactions between Lewis base moiety of DNP-AA and the zirconia surface can disturb chiral-discriminating interactions between DNP-AA and the chiral selector, causing reduced chiral discrimination and increased retention with a tailed peak shape. Undesirable interactions of surface Lewis acid site of zirconia can be reduced if a strong Lewis base such as phosphate ions is present in the eluent to cover the surface Lewis acid sites [34]. Fig. 4 shows a chromatogram for the resolution of DNP-proline in 80:20 (v/v, %) methanol/sodium phosphate buffer (pH 4.5, 0.05 M). Retention of the amino acid is somewhat reduced and peak shape is considerably improved.

Table 1
Separation data for DNP-amino acids on quinine carbamate-coated zirconia (QNZ), quinine carbamate-bonded silica (QNS), and quinine ester-bonded carbon-clad zirconia (QNCZ) columns[a]

DNP-AAs	QNZ		QNS		QNCZ	
	k_1	α	k_1	α	k_1	α
Ileu	7.53	1.41	2.36	1.50	9.17	1.34
Leu	8.10	1.47	1.75	1.57	5.72	1.89
Phe	16.71	1.27	2.94	1.40	7.30	2.71
Arg	10.02	1.00	0.70	1.00	6.87	3.24
His	11.42	1.35	1.97	1.49	6.54	3.42
Met	14.00	1.21	2.62	1.40	5.96	4.66
Gin	6.03	1.69	2.95	1.26	6.12	2.14
Tyr	11.43	1.00	3.98	1.11	6.56	3.63
Val	7.25	1.38	1.83	1.64	5.25	1.51
Pro	6.78	1.61	2.15	2.10	3.90	1.40
Ala	11.63	1.20	2.34	1.44	2.94	1.21

[a] Mobile phase 80/20 methanol/ammonium acetate buffer (pH 4.5, 0.1 M). QNZ: particle size, 5 μm; plate number *N* (unretained marker), 3103; bonding density, 2.07 μmol m^{-2}. QNS: particle size, 5 μm; *N*, 3436; bonding density, 2.00 μmol m^{-2}. QNCZ: particle size, 5 μm; *N*, 2364; bonding density, 1.87 μmol m^{-2}.

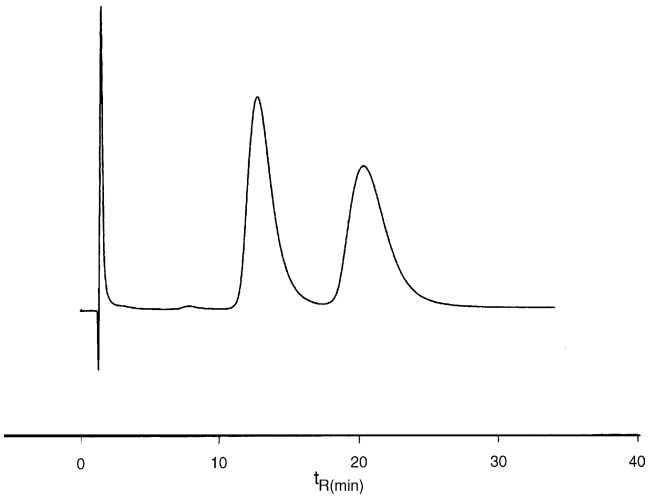

Fig. 2. Chromatogram for the separation of racemic DNP-proline on QNZ in 80:20 (v/v, %) methanol/ammonium acetate buffer (pH 4.5, 0.1 M). Column dimension; 20 cm × 0.1 cm i.d. Flow rate, 80 μL/min. Column temperature, 25 °C.

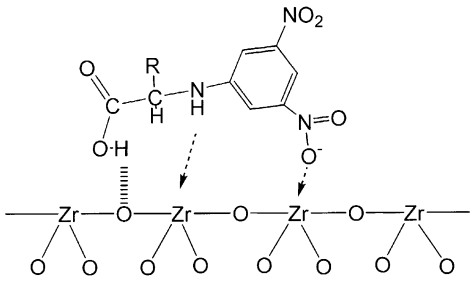

Fig. 3. Possible non-enantioselective interactions between DNP-AA and the zirconia surface.

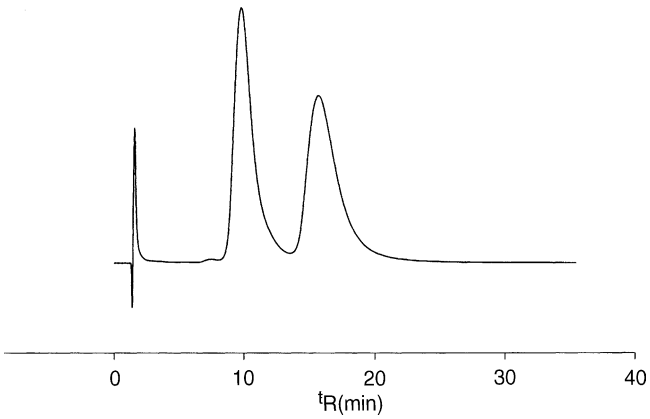

Fig. 4. Chromatogram for the separation of racemic DNP-proline on QNZ in 80:20 (v/v, %) methanol/sodium phosphate buffer (pH 4.5, 0.05 M). Other chromatographic conditions are the same as in Fig. 2.

3.2. Comparison of quinine carbamate–zirconia with quinine carbamate–silica

The Lewis acid sites found on zirconia surface are not present on silica surface. It would be thus interesting to compare the chiral separation behavior of QNZ with that of quinine carbamate-bonded silica phase. In order for direct comparison to be valid, the chromatographic properties of the two CSPs need to be very similar if they are not exactly the same. Zirconia and silica possess identical particle size and pore diameter. The packing materials were packed into columns of identical dimensions under identical packing conditions. Quinine carbamate density based on the percent carbon from microanalysis was found to be 2.07 and 2.00 μmol m^{-2}, respectively. The plate numbers of zirconia and silica-based materials for unretained marker were also quite similar (3103 and 3436 for QNZ and QNS columns, respectively).

The retention factor k and separation factor α values for the amino acids on the two columns are shown as column graphs in Fig. 5 for comparison. The k values for the first eluted enantiomers of amino acids are much longer while the α values are smaller on the zirconia columns than those on the silica column. It seems that the observed difference in separation behavior may be due to the differences in properties of the two base particles given that zirconia and silica-based CSP columns possess very similar chromatographic properties.

A plausible explanation for longer retention and smaller enantioselectivity for zirconia-based CSP than silica-based CSP is as follows. As described earlier, on zirconia surface exist Brönsted acid sites, Brönsted base sites, and Lewis acid sites, which are not found on silica. The number of surface Lewis acid sites measured by fluoride adsorption was reported to be in the range of 7.9–11.3 μmol m^{-2} in aqueous solutions having pH values between 4.8 and 6.2 [39], which is close to the pH values of the mobile phases used in this work. The Lewis acid sites are not covered completely by quinine moieties like residual silanol groups on silica. These Lewis acid sites are very likely to undergo non-enantioselective Lewis acid–base interactions with each of the enantiomers of analyte to an equal extent. This is analogous to the situation with silica, where surface silanol sites strongly interact with the analyte having Brönsted basic moieties such as proteins, rendering their elution from the silica-based columns very difficult. Gradient elution separation of bovine serum albumin from myoglobin on bare zirconia gave very poor separation. Replicate injections of the protein mixture showed continuous deterioration in column performance due to adsorption of proteins, indicating the strong Lewis acid–base interaction property of zirconia [40]. On CSPs prepared by immobilizing chiral selector ligands with localized chiral centers, enantioselective interactions are much rarer than non-enantioselective interactions [41]. Chiral separations on these CSPs can be achieved only when enantioselective interactions are strong and some strict conditions regarding the relative orientation between the enantioselective group and the analyte molecule, such as the three-point complex interactions, are satisfied. The Lewis acid–base interactions between the zirconia surface and the analyte molecule are very likely to cause geometrical constraints required by chiral recognition not to be satisfied, thus in turn giving diminished chiral selectivity. The quinine carbamate coverage on Lewis acid sites on quinine carbamate-coated zirconia in this work is only 2.07 μmol m^{-2} out of about 10 μmol m^{-2} on zirconia surface [40]. The number of bare Lewis acid sites is much greater than the quinine carbamate-adsorbed sites and contribute quite significantly to the overall retention.

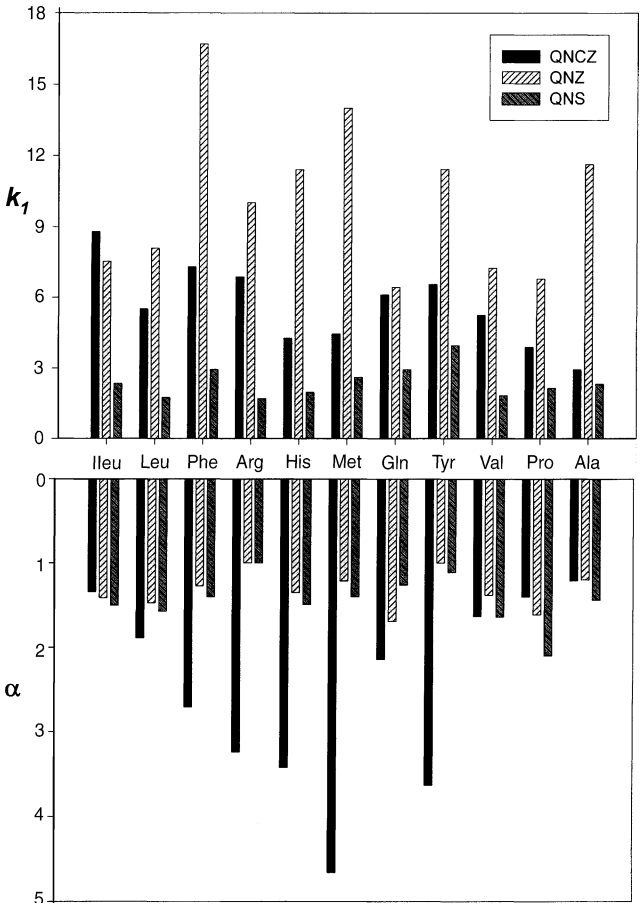

Fig. 5. Comparison of retention and chiral selectivity of quinine ester-bonded carbon-clad zirconia (QNCZ), quinine carbamate-coated zirconia (QNZ), and quinine carbamate-bonded silica (QNS). Mobile phase, 80/20 methanol/ammonium acetate buffer (pH 4.5, 0.1 M). Solid bar, QNCZ; light gray bar, QNZ; dark gray bar, QNS.

In order for zirconia to be useful base material for CSP, it seems necessary to increase surface density of the chiral selector while decreasing undesirable non-enantioselective interactions of surface Lewis acid sites on zirconia. Bonding chiral selector moieties onto carbon-clad zirconia may be a solution to alleviate this problem.

3.3. Separation on quinine ester-bonded carbon-clad zirconia

Carbon-clad zirconia is made by passing organic vapors over very hot porous zirconia [42]. Carbon-clad zirconia particles show similar mechanical, thermal, and chemical stability to bare zirconia particles but no appreciable Lewis acidity. They neither exhibit peak tailing for amines nor do they adsorb phosphates or carboxylates [43].

There are several possible intermolecular site reactions of the diazonium salt of 9-*O*-(4-aminophenyloxycarbonyl)quinine that may reduce the bonding yield to the carbon layer on the zirconia or cause changes in the structure of the selector molecule. Reaction of the diazonium salt of 9-*O*-(4-aminophenyloxycarbonyl)quinine with the amino group of 9-*O*-(4-aminophenyloxycarbonyl)quinine is possible but this will only reduce the yield of bonding to the carbon layer of the zirconia. Aryl dimerization between the diazonium ions of 9-*O*-(4-aminophenyloxycarbonyl)quinine is possible but this will also reduce the yield of bonding to the carbon layer of the zirconia. The Meerwein acylation by the diazonium salt of 9-*O*-(4-aminophenyloxycarbonyl)quinine of the vinyl group of the quinuclidine ring is possible but in the absence of a proper catalyst the yield of this reaction would be very low [44]. Cleavage of the vinyl group of the quinuclidine ring by the diazonium salt of 9-*O*-(4-aminophenyloxycarbonyl)quinine is possible but the reaction yield is appreciable only with those having the structure of PhCH=CHR [45]. The latter two reactions, if occurring to an appreciable extent, could lead to some modification of the vinyl groups of the chiral selector molecule but it is not likely that this will result in poorly defined stationary phase materials.

9-*O*-(4-Phenyloxycarbonyl)quinine-bonded carbon-clad zirconia was packed into a column of the identical dimension under the identical packing conditions to the QNZ column. Quinine ester density based on the percent nitrogen from microanalysis was found to be 1.87 μmol m^{-2}. The plate number of the QNCZ column for the unretained marker was 2364. Fig. 6 shows a chromatogram for the resolution of DNP-proline in 80:20 (v/v, %) methanol/ammonium acetate buffer (0.1 M, pH 4.5) on a column (1 mm i.d. $\times$ 20 cm length) packed with 9-*O*-(4-phenyloxycarbonyl)quinine-bonded carbon-clad zirconia. Retention of the AA was somewhat shortened compared to that on QNZ, which is indicative of a decrease in (or lack of) undesirable Lewis acid–base interactions between DNP-AA and the zirconia surface. The peak shape for DNP-proline does not look much better than that on QNZ. This is probably due to the fact that the QNCZ column was not as well packed as the QNZ column in view of the fact that the plate numbers for QNCZ and QNZ columns are 2364 and 3103, respectively.

Separation data of 11 DNP-AAs in 80:20 (v/v, %) methanol/acetate buffer (pH 4.5, 0.1 M) on QNCZ are listed in Table 1. For most of DNP-AAs studied retentions are shorter but enantioselectivity factors are greater on QNCZ than on QNZ. Retention factors for seven AAs on QNCZ are still longer than those on QNS but their enantioselectivity factors are greater on QNCZ than on QNS (Fig. 5). For the remaining four AAs (Ileu, Val, Pro, and Ala) selectivity was lower on QNCZ than on QNS. We do not have a clear explanation for why QNCZ CSP shows better enantioselectivity for some DNP-AAs while showing inferior selectivity for the other AAs at present. One plausible explanation for the different selectivity of the QNS, QNZ, and QNCZ CSP is as follows: on QNZ and QNS quinine carbamate is immobilized while on QNCZ quinine ester is immobilized. The carbamate group on QNS (and QNZ) can be available as both hydrogen-bond donor and acceptor, and also undergoes dipole–dipole interaction. The ester group (phenyloxycarbonyl) on QNCZ can act as a hydrogen-bond acceptor but not a hydrogen-bond donor. The lack of hydrogen-bond donor ability and presence of an extra phenyl group on QNCZ may significantly influence the chiral recognition processes occurring, exhibiting different selectivity behavior for the QNCZ phase than the QNS phase. In addition, the ability for dipole–dipole interaction for the phenyloxycarbonyl moiety will be also different from that for the carbamate group. This seems in accordance with the fact that enantioselectivity on the *O*-underivatized quinine CSP is mainly dependent on the hydrogen bonding and dipolar interaction ability of the eluent [46]. On the whole, it can be said that QNCZ gives better chiral separation for DNP-AAs than QNZ and QNS.

The stability of the QNCZ, QNZ, and QNS columns was checked by measuring retention factor of the first eluted enantiomer of DNP-proline after passage of every 500 column volume of the eluent through the column. After 6000 column volumes only less than 2% decrease in retention factor of the test solute was observed on the QNCZ and QNS columns but on the QNZ column about 3% decrease in retention factor of the test solute was observed.

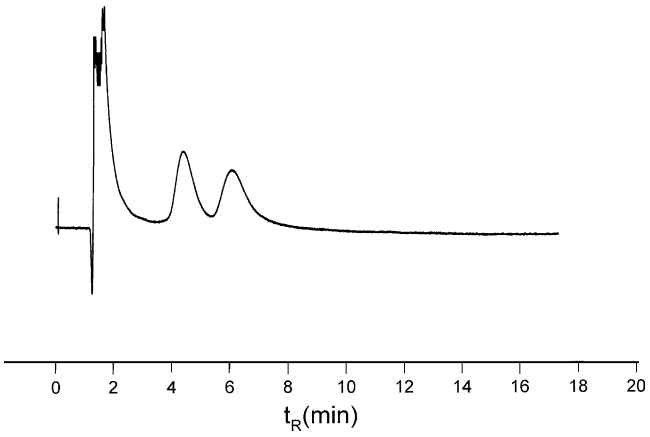

Fig. 6. Chromatogram for the separation of racemic DNP-proline on QNCZ in 80:20 (v/v, %) methanol/ammonium acetate buffer (pH 4.5, 0.1 M). Other chromatographic conditions are the same as in Fig. 2.

4. Conclusions

Quinine ester-bonded carbon-clad zirconia exhibited quite good enantioselectivity for the amino acids studied and its stability was satisfactory under RPLC conditions. The QNCZ CSP, on which strong Lewis acid sites are roofed with carbon layer, showed shorter retention but much improved chiral selectivity than the QNZ CSP. Retention is somewhat longer but enantioselectivity is better on QNCZ than QNS for most of DNP-AAs investigated. Considering the fact that the QNCZ column was in-house packed and the packing procedure was not at all as optimized as the commercial column manufacturer does, it seems that there is a great potential in improving chromatographic performance exhibited by the zirconia column. However, it should be noted that although highly stable under extreme pH conditions, zirconia surfaces lack straightforward ligand immobilization chemistries being in efficiency comparable to that established for silica. At present, zirconia materials may not compete with silica materials until suitably mild and generally applicable ligand grafting methodologies have been advanced.

Acknowledgment

This work was supported by a grant (R-01-2002-000-00150-0) from the Korea Science & Engineering Foundation.

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