

Amylose Tris(3,5-dimethylphenylcarbamate)-Coated Zirconia as a Chiral Stationary Phase for Micro HPLC

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HPLC separation method based on chiral stationary phases (CSPs) has become one of the most attractive approaches to chiral separations, due to their simplicity for determining optical 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 On the other hand, the polysaccharide derivative-based CSPs developed by Okamoto and co-workers^{3,4,5,6} have proven to be highly versatile and rugged. Okamoto⁷ reported the resolution of 483 racemic mixtures on cellulose and 80% of 483 racemic mixtures were successfully resolved on either the cellulose or amylose tris(3,5-dimethylphenyl carbamate) (ADMPC).

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. Silica is the most popular choice for support for 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.⁸ Loss of organosilanes from the silica surface via hydrolysis proceeds rapidly at low pH (< 3) and at higher 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. Over the last decade, zirconia has received considerable attention as a stationary phase support for HPLC.^{9,10} 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 recently reported preparation of

zirconia based CSPs with cellulose, bovine serum albumin and β -cyclodextrin for use in either normal or reversed-phase LC separation of chiral compounds.^{11,12,13}

In this work we report preliminary results obtained with ADMPC coated on 3- μ m zirconia particles. This CSP is to combine the excellent chiral selectivity of ADMPC with the stability of zirconia on which more efficient, robust, and faster chiral analytical methods can be carried out. We prepared a microbore (1-mm ID) column that leads to many advantages such as low consumption of both mobile and stationary phases *etc.*^{14,15,16} and evaluated chromatographic performance by measuring the chiral discrimination of a set of racemic compounds on the column.

Experimental Section

Reagents. All reagents used for the preparation of the stationary phase were reagent grade or better. Microcrystalline amylose (MW 16,000) was purchased from Nakarai Chemicals (Japan). 3,5-Dimethylphenyl isocyanate, N,N-dimethylacetamide and pyridine were obtained from Aldrich (Milwaukee, USA). Zirconia, having a mean pore size of 30 nm and a mean particle diameter of 3 μ m, was obtained from ZirChrom Separations (Anoka, USA). Acetone, and 2-propanol were HPLC grade (J.T. Baker, Phillipsburg, USA). *n*-Hexane and tetrahydrofuran (THF) were purchased from EM Sciences (Gibbstown, USA). The racemic compounds studied are shown in Figure 1. All are commercially available. Solutions at a concentration of 0.1 mg/mL were prepared by dissolving the compounds in the mobile phase.

Preparation of ADMPC. ADMPC was synthesized as previously reported¹⁷ and was characterized by elemental analysis, IR and NMR spectroscopy. The data indicated that hydroxyl groups of amylose were almost completely converted to the corresponding carbamate groups.

Preparation of ADMPC-coated zirconia. To dehydroxylate zirconia surface, the particles were heated at 750 °C for 5 h and cooled over phosphorus pentoxide before use. Typically, 1.0 g of particles was suspended in 10 mL of THF and sonicated under vacuum for 15 min to eliminate the air from the pores. Polymer loading of 4% by weight was chosen as this loading has been shown to offer excellent

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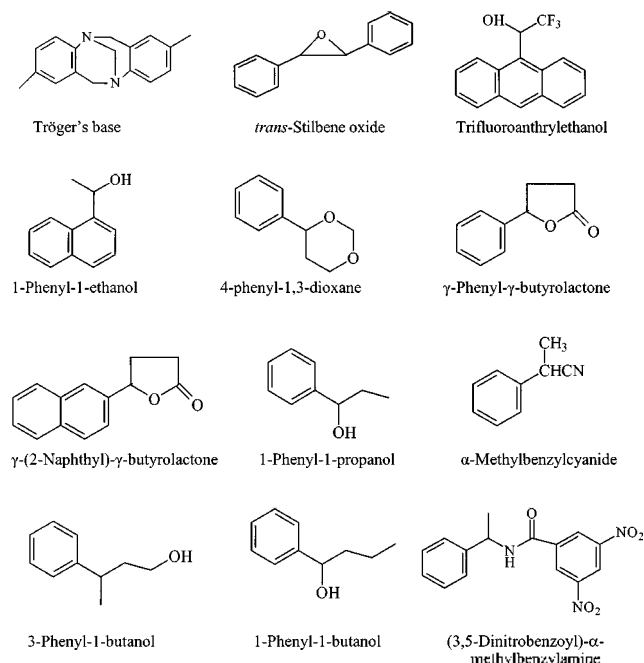


Figure 1. Structures of chiral compounds.

chiral recognition ability and column efficiency.^{11,12} The corresponding amount of ADMPC was dissolved in 10 mL of THF and the solution was added to the slurry of zirconia in THF using a syringe pump at a rate of 0.04 mL/min (~4 h). The suspension was stirred overnight and then the solvent was slowly removed by rotary evaporation at room temperature. Finally, the particles were dried in vacuum at 50 °C.

Chromatography. Packing materials were suspended in a (1:1) hexane/2-propanol mixture and packed into a 25 cm × 1 mm (ID) column 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- μ L 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- μ L 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 of 2-propanol and hexane (2/98 or 10/90 v/v%). They were filtered through a membrane filter of 0.5- μ m pore size and degassed prior to use. The flow rate was 200 μ L/min. The dead time was estimated by using 1,3,5-tri-*tert*-butylbenzene as unretained compound.¹⁸

Results and Discussion

The performance of a column packed with ADMPC-zirconia is shown for the resolution of Tröger's base in 90:10(v/v) 2-propanol/hexane (Figure 2). Retention factors (*k*) for this analyte under the conditions used are small but its enantiomers are baseline resolved with separation factor of 1.87. Separation data of twelve racemic compounds are listed in Table 1. All the compounds were well resolved

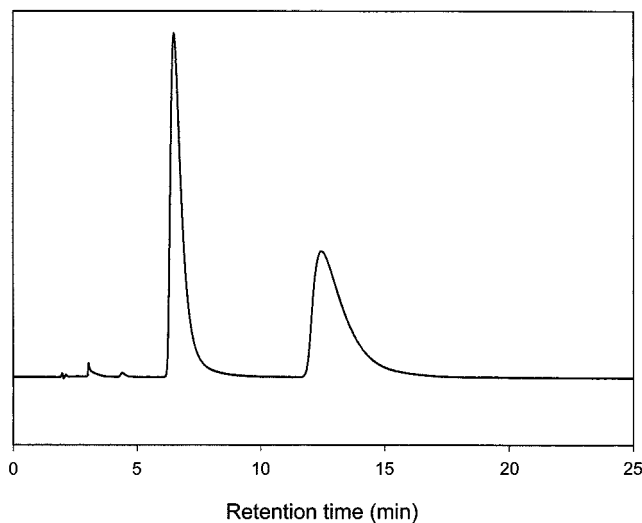


Figure 2. Separation of Tröger's base on ADMPC-zirconia column. Column dimension: 25 × 0.1 cm I.D. Mobile phase: *n*-hexane:2-propanol 90:10 (v/v %). Flow rate: 0.2 mL/min. Column temperature: 30 °C.

except phenyl alcohols, which were only partially separated.

It is interesting to compare the chromatographic performance of ADMPC-coated zirconia with that for an ADMPC-coated silica¹⁷ even though direct comparison of the property of the two materials may not be quite feasible because of the physical differences in the column (column dimension, 1

Table 1. Chromatographic Data on ADMPC-Coated Zirconia in Hexane/2-propanol

Compounds	<i>k</i> ₁ ^a	α ^b	Mobile Phase
Tröger's base	1.82	2.41	98:2
	0.47(0.53)	1.87(1.58)	90:10
<i>trans</i> -Stilbene oxide	0.46	1.34	98:2
	0.25(0.42)	1.20(3.04)	90:10
Trifluoroanthryl ethanol	0.86(1.30)	1.55(1.15)	90:10
α -Methyl-1-naphthalene methanol	8.97	1.08	98:2
4-Phenyl-1,3-dioxane	0.86	1.50	98:2
	0.37	1.35	90:10
γ -Phenyl- γ -butyrolactone	15.06	1.22	98:2
	2.78	1.18	90:10
γ -(2-Naphthyl)- γ -butyrolactone	3.78	1.36	90:10
1-Phenyl-1-propanol	3.20	1.06	98:2
γ -Methylbenzylcyanide	1.68	1.27	98:2
	0.50	1.04	90:10
3-Phenyl-1-butanol	10.02	1.07	98:2
	0.58	1.09	90:10
1-Phenyl-1-butanol	3.17	1.07	98:2
3,5-Dinitrobenzoyl- α -methylbenzylamine	7.82	1.30	90:10

^aRetention factor for the first eluting enantiomer. The values in parentheses are *k* values on an ADMPC-coated silica column (column dimension, 25 cm × 4.6 mm ID; polymer loading, 25% by weight; particle size, 10 μ m; mobile phase, 90:10 hexane-2-propanol).¹⁷

^bSelectivity factor. The values in parentheses are α values on an ADMPC-coated silica column.¹⁷

mm ID vs. 4.6 mm ID; polymer loading, 4% vs. 25% by weight; particle size, 3 μm vs. 10 μm) (see Table 1). Chromatographic data for three compounds, Tröger's base, *trans*-stilbene oxide and trifluoroanthryl ethanol, were available in the literature.¹⁷ Retentive property is represented by retention factor (k), which represents the strength of interaction of the CSP with enantiomers. The k values for the first eluting enantiomers of the three racemic compounds are only slightly smaller on the zirconia column than on the silica column. The resolving power of the CSP can be evaluated from separation factor ($\alpha = k_1/k_2$). The α values of three compounds on the ADMPC-zirconia were compared to those on corresponding silica. Tröger's base and trifluoroanthryl ethanol were better separated on ADMPC-zirconia than ADMPC-silica. In view of the fact that dimension and polymer load were much smaller on zirconia than silica and hence the amount of CSP is smaller in the zirconia column, high enantioselectivity of the zirconia CSP is impressive. Selectivity for *trans*-stilbene oxide on the ADMPC-zirconia is lower than on the ADMPC-silica (a. 1.20 vs. 3.04) but the compound is still well resolved on the zirconia column. The plate number of the ADMPC-zirconia column was found to be 1300 for unretained 1,3,5-tri-*tert*-butylbenzene. Considering the fact that ADMPC-zirconia column was in-house packed and the packing procedure was not at all optimized as the commercial column manufacturer does, it seems that there is a great potential in improving chromatographic performance exhibited by the ADMPC-zirconia column.

The stability of the ADMPC-zirconia column was checked by measuring retention factor of the first eluting enantiomer of Tröger's base after passage of every 500-column volume of the eluent through the column. There was only less than 2% decrease in retention factor of the test solute after 6,000-column volume.

In summary, this study reported the preparation and use of ADMPC-coated zirconia as a stationary phase for chiral HPLC. The ADMPC-coated zirconia column exhibited high enantioselectivity and stability under normal phase conditions. The high enantioselectivity may allow for the use of a

shorter column for reduced analysis time and solvent consumption.

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