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Diastereomeric Selectivity of Carbon-Coated Zirconia Reversed-Phase Liquid Chromatographic Media

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The determination of enantiomeric excess, that is, the relative amount of any pair of optical antipodes, constitutes a integral part of the work of analytical and synthetic chemists involved in natural products research or pharmaceutical development. Mosher's reagent [α-methoxy-α-(trifluoromethyl)phenylacetyl chloride] has evolved into a major tool for the determination of absolute configuration by NMR. We report here on the separation of diastereomers formed by derivatizing enantiomers with Mosher's reagent. We have shown that reversed-phase liquid−solid adsorption chromatography on carbon surfaces frequently gives considerably superior resolution of diastereomeric pairs than does RPLC on conventional bonded phases. The improved resolution results from the very high sensitivity of solid carbon surfaces to the geometric organization of the solute rather than from differences in column efficiency. We compare the separation of pharmaceutically and biologically important stereoisomeric mixtures, including (±)-warfarin and (±)-amino acid esters, on both conventional bonded phases and carbon surfaces prepared by chemical vapor deposition of organic compounds on porous zirconia microparticles.

Over the past 20 years, increased interest in enantioselective synthesis has led to the demand for accurate, reliable, and convenient methods of measuring enantiomeric purity.[1] Almost simultaneously, regulatory agencies around the world began to impose enantiomeric analysis requirements for chiral drugs on pharmaceutical companies.[2,3] These two actions have generated an enormous interest in analytical methods for the determination of enantiomeric excess.

Chromatographic and nuclear magnetic resonance (NMR) methods are the major techniques used in chiral analyses.[1,4−7] Enantiomers cannot be distinguished in an achiral environment; therefore, a "handed" medium must be used to discriminate between the isomers. Consequently, analysts frequently employ chiral stationary phases in both gas chromatography (GC) and high-performance liquid chromatography (HPLC) for such determinations. Years before chiral auxiliaries were incorporated into polysiloxane films, Gil-Av and co-workers performed the first gas chromatographic chiral separations using an amino acid-modified glass capillary column.[8,9] Since then, chiral separations have developed into both a science and an art. Pirkle et al.,[10−12] Armstrong et al.,[13−15] and Schurig et al.[6,16] made substantial contributions to the current state of chiral separations by introducing grafted chiral donor/acceptor ligands, intrinsically chiral cyclodextrins, macrocyclic antibiotics, and chiral polymeric films to the analytical techniques. Not only can the stationary phase contribute to enantiomeric selectivity, but so can the mobile phase. Chiral additives, both in HPLC and NMR, permit peak resolution and the determination of enantiomeric excess.[7,17−20] However, one does not have to use a chiral environment to achieve an enantiomeric assay.

The derivatization of enantiomers to diastereomers permits the use of achiral media for separations and/or NMR analysis. Chiral derivatizing agents (CDAs) became a mainstay of analytical methodology and CDAs for both NMR and chromatographic analyses evolved over the ensuing decades.[21] *O*-Methylmandelate technology was introduced by Raban and Mislow[22] and later expanded by Parker[23] to include methyl mandelate and *O*-acetylmandelic acid. Camphanic acid and aromatic amines, such as α-phenylethylamine, were used for determinations involving alcohols, carboxylic acids, and carbonyl compounds.[24,25]

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In 1969, Mosher introduced α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (MTPA-Cl). The scientific community rapidly accepted it as a significant advance because there is no hydrogen α to the carbon group; thus, racemization during derivatization is impossible.[26−28] The methoxy(trifluoromethyl)-phenylacetyl (MTPA) derivatives of chiral alcohols or primary amines can be analyzed by either ^{1}H or ^{19}F NMR; the orientation of molecular subunits near the phenyl group of the MTPA moiety results in observable differences in chemical shifts. These differences permit the estimation of enantiomeric excess from integrated peak areas in the NMR spectrum. Furthermore, with a few additional NMR experiments, one can assign the absolute stereochemical configuration to the derivatized enantiomer. Recently, a refined methodology based on the assessment of proton chemical shift differences throughout the molecule demonstrates the advantages of using the ^{1}H NMR chemical shifts rather than the ^{19}F shifts.[29] Commercial availability and high reactivity with primary and secondary amines or alcohols to form the diastereoisomeric amides or esters, coupled with the information content of NMR analysis, give this reagent its popularity.

Chromatographers have long used derivatization as a means to analyze complicated or difficult chemical compounds, especially those involving stereoisomers.[21,30] A racemic mixture or one of unknown optical purity reacts with an enantiomerically pure reagent to yield diastereomeric compounds. The diastereomers possess different physical properties; consequently, they can be separated more easily than the enantiomeric forms.

Only a few reports exist that address the use of MTPA technology in chromatographic analyses. Both gas[31,32] and liquid chromatographic[32−37] techniques have been used for the indirect determination of enantiomers containing hydroxyl or amino functional groups derivatized by MTPA-Cl. Excellent selectivity factors and limits of detection were reported for a range of analytes, including various amphetamines.[32,36] Here we explore a derivatization scheme based on MTPA technology to provide challenging diastereomeric solutes for assessing the stereoselectivity of both carbon and conventional bonded phase RPLC media. The derivatization method makes use of an enantiomerically pure acid chloride capable of reacting with either a primary or a secondary alcohol or amine in the substrate to form an ester or amide linkage. Scheme 1 shows the reactions and conditions used herein to provide the solute set. The chiral amine or alcohol reacts with an excess of MTPA-Cl in the presence of an acylation catalyst to ensure complete derivatization to the amide or ester. Simple extraction of the resulting mixture yields product ready for chromatographic analysis; no further workup is needed.

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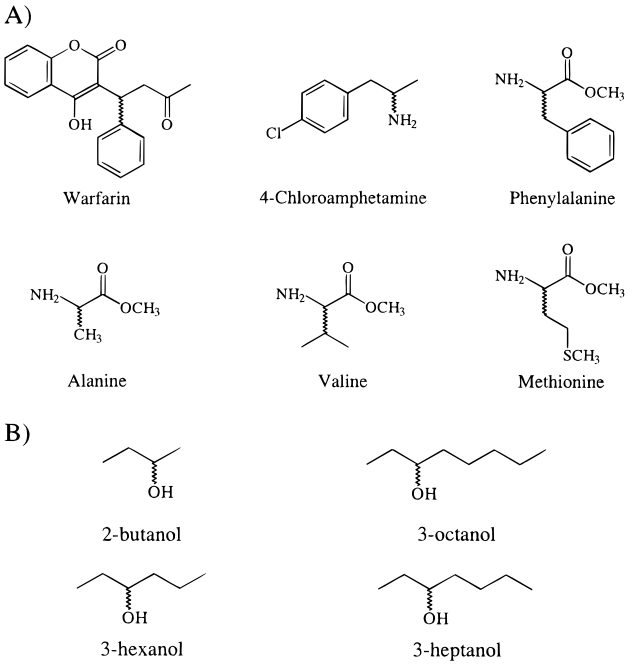
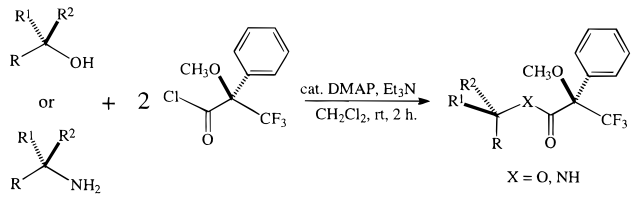


Figure 1. Racemic solutes used for diastereomeric derivatization. (A) Therapeutic agents and amino acid methyl esters; (B) homologue series of racemic aliphatic alcohols.

Scheme 1



We now report the extension of MTPA technology for the indirect determination of enantiomers to liquid−solid chromatography on carbon stationary phases used in reversed-phase liquid chromatography (RPLC). The derivatives of a set of pharmaceutically interesting compounds, consisting of therapeutic agents and amino acid methyl esters, demonstrate the application and use of the method. A second set of aliphatic alcohols probes the practical limits of diastereomeric separations employing different stationary phases. Figure 1 shows the solutes of interest, and the synthetic protocols follow extensive literature precedent.[26−36]

EXPERIMENTAL SECTION

Reagents. The chemicals, reagent grade or better quality, used in this study were obtained from the following suppliers: methylene chloride, acetonitrile (ACN), and tetrahydrofuran (THF) were HPLC grade and obtained from Fisher Scientific (Fairlawn, NJ); 2-propanol and acetone were obtained from Mallinckrodt (Paris, KY); the (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, 3-hexanol, 3-heptanol, 3-octanol, and 4-chloroamphetamine were from ACROS (Fairlawn, NJ); DL-and L-amino acid methyl esters and warfarin were obtained from Sigma Chemical Co. (St. Louis, MO); and all other solutes were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). Unstabilized THF was tested for peroxides before use. Water for the HPLC mobile phase was purified by passing it through a Barnstead/Thermolyne (Dubuque, IA) Nanopure water purification system with an "organic-free" final cartridge followed by a 0.2 μm particle filter.

Table 1. Physical Characteristics of Stationary Phases

phase	d_p[a]	SA[b]	d_{pore}[c]	% C[d]
Hp-C/ZrO_2	2.5	32.5	194	1.7
SB-C18[e]	5	180	80	10

[a] Average particle diameter (μm). [b] Surface area (m^2/g) by BET.[42,43] [c] Average pore diameter (Å) using 4 V/A.[42,43] [d] Weight percent carbon by elemental analysis. [e] Reported by manufacturer.

Carbon Support Preparation. A single lot of porous zirconia particles was used in this work. The particle characteristics are given in Table 1. Chemical vapor deposition (CVD) of heptane created a carbon stationary phase on porous zirconia particles (PICA-7), produced by the polymerization-induced colloid aggregation method.[38] The chemical vapor deposition process utilized a tube furnace in which volatile organic compounds were passed over the porous ZrO_2 particles at an elevated temperature (~700 °C) and at reduced pressure (~5−10 Torr) for approximately 1.5 h.[39−41] The reduced pressure is maintained using a vacuum pump while the carbon source is slowly introduced. After completion of CVD, the carbon-coated particles were rinsed with tetrahydrofuran and Soxhlet-extracted with toluene to remove soluble pyrolysis products. For purposes of comparison, a commercially available conventional octadecyl silica (ODS) chromatographic phase, Zorbax SB-C18 (Rockland Technologies, Newport, DE), was also examined.

Column Packing. Column blanks were Precision Bore 316 stainless steel tubing (Alltech), 5 cm in length having a 0.25 in. o.d., 0.46 cm i.d. Stainless steel (316) column end fittings were used with 2 μm stainless steel frits (Alltech). The columns were packed using an upward stirred slurry technique. For a 5 cm × 0.46 cm column blank, approximately 2 g of particles was slurried in 25 mL of 90:10 hexane/2-propanol, and this mixture was forced into the column using pure 2-propanol at 5500−6000 psi pressure by a Haskel pneumatic pump.

Chromatographic Studies. All studies were conducted on a Hewlett-Packard (Palo Alto, CA) 1090 high-performance liquid chromatograph with a DR5 solvent delivery system and a filter photometric detector. Reported chromatographic parameters of each solute were averages of, at least, triplicate determinations, and detection was at 254 nanometers. Column dead time was measured with acetone. Uracil and sodium nitrate cannot be used as dead volume markers because they either are slightly retained on the carbon support or interact with residual ZrO_2, resulting in broadened peaks. Column efficiencies were calculated from the retention times and the peak height/area ratio reported by the integrator as follows:

$$N = 2\pi\left(\frac{\mathbf{H}t_r}{A}\right)^2 \quad (1)$$

where t_r is the retention time, H is the peak height, and A is the peak area. Resolution factors were calculated according to eq 2,

$$R_s = \frac{\sqrt{N}}{4}\frac{(\alpha - 1)}{\alpha}\frac{k'}{(1 + k')} \quad (2)$$

where R_s is the resolution, N is the number of theoretical plates (efficiency), k' is the retention factor, and α is the selectivity factor.

MTPA-Cl Reaction Conditions. All alcohol- and amine-containing enantiomers were derivatized to their respective diastereomers with (*S*)-MTPA-Cl according to identical procedures. Yields of diastereomeric products were typically 90% or better. The derivatization and characterization of (±)-4-chloroamphetamine is given in detail and is representative of the procedure used. For additional information regarding the synthesis, see ref 26−36.

(±)-4-Chloroamphetamine-(*R*)-MTPA Amide. In a clean, dry 5 mL pear-shaped flask equipped with a magnetic stirbar and septum were placed (±)-4-chloroamphetamine hydrochloride (20.6 mg, 0.10 mmol), 4-(*N*,*N*-dimethylamino)pyridine (1 mg, 0.008 mmol), triethylamine (200 μL, 2.7 mmol), and 1 mL of dichloromethane. The vessel was completely flushed with nitrogen and capped. An excess of (*S*)-MTPA-Cl (45 μL, 0.24 mmol) was added dropwise via syringe. The stirred mixture was allowed to react for 3 h at room temperature. Within 5 min after the acid chloride addition, the solution changed from clear and colorless to a golden pale yellow, and a precipitate appeared. Upon completion, 20 mL of dichloromethane and 30 mL of 0.10 M phosphate buffer (pH 6.00) were added. The organic layer was extracted with 2 × 30 mL of phosphate buffer, and the aqueous layer was back-extracted with 2 × 30 mL of dichloromethane. Combined organic extracts were dried over sodium sulfate and then filtered through a small bed of zirconia (2 g) to remove free MTPA acid. The filtrate was collected and solvent removed in vacuo to yield the MTPA amide as a yellow oil (51.3 mg, 0.098 mmol, 98%). The residue was then resuspended in either acetonitrile or tetrahydrofuran, depending on the subsequent liquid chromatographic analysis conditions. Table 2 summarizes the GC/MS characterization for the two diastereomers and confirms their identities. The extraction efficiency was not empirically evaluated for all products; however, no residual MTPA products (GC/MS analysis) were found in the aqueous portions of the phenylalanine or the 4-chloroamphetamine extracts. We believe that all MTPA diastereomers were extracted with excellent efficiency.

RESULTS AND DISCUSSION

Stereoisomers and Synthetic Diastereomers. Most of the separations involving carbon-coated zirconia stationary phases have centered around chemically diverse solutes and constitutional isomers.[40,41] We have yet to probe any stereoselectivity of carbon as a stationary phase; therefore, two sets of synthetic products and stilbenes were chromatographed on both carbon and ODS phases. Chart 1 contains representative structures for these solutes, and Table 3 shows the resulting retention, selectivity, and resolution factors.

In the case of the di(phenethyl)amide, a very flexible molecule, both phases show excellent resolution of the two diastereomeric

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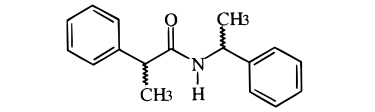
Table 2. GC/MS Data for (±)-4-Chloroamphetamine-(*R*)-MTPA Amide[a]

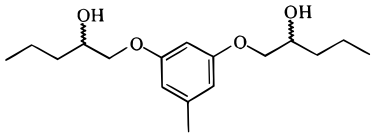
diastereomer 1			diastereomer 2		
time[b]	ion/fragment[c]	abundance[d]	time[b]	ion/fragment[c]	abundance[d]
12.31	385	4	12.47	385	2
	260	31		260	31
	234	10		234	10
	189	100		189	100
	170	8		170	8
	155	9		155	10
	153	28		153	28
	127	18		127	18
	125	50		125	50
	105	14		105	14
	77	8		77	8

[a] Gas chromatographic/mass spectrometric analysis at unit mass resolution. [b] Retention time in minutes; DB-5 column, 6 m × 0.1 mm i.d. × 0.1 μm nominal film thickness. Temperature program 50 °C/2 min/20 °C min^{-1}/250 °C/10 min. [c] Ion or fragment identity (*m*/*z*). [d] Percent of base peak.

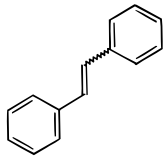
Chart 1



di(phenethyl)amide



resorcinol diether



stilbene

forms. However, the carbon phase shows much greater selectivity for the resorcinol diether compound than ODS, even though the overall resolution is poor. In collaboration with synthetic organic chemists, we discovered that the compound would not separate on normal phase silica media.[44] We speculate that the interaction of the central rigid aromatic resorcinol moiety with the carbon aids in the resolution of the diastereomers. Additionally, the diastereomeric relationship was verified by examination of the UV/visible diode array spectrum of the leading and tailing edges of the peaks as obtained by the HP 1090 LC system. Both showed virtually identical spectra indicative of diastereomeric species. The separation of *cis*- and *trans*-stilbene demonstrates the most profound effect of solute structure and shape on the diastereomeric selectivity exhibited by carbon media. In the cis conformation, the 2-position hydrogen atoms on adjacent phenyl rings sterically interact; that interaction causes one of the aromatic rings to be bent out of the conjugated π system. Thus, each diastereomer has a unique shape and presents a different surface of electron density to the carbon phase. This gives rise to the enormous selectivity factor observed (see Table 3).

(44) Humpal, P., University of Minnesota, personal communication, 1994.

Table 3. Stereoisomeric Separations on Carbon and ODS[a]

solute	k'_{D1}[b]	k'_{D2}[b]	α[c]	R_s[d]
	ODS			
di(phenethyl)amide[e]	20.92	24.90	1.19	2.07
resorcinol diether[f]	8.35	8.35	1.00	0.00
cis-/*trans*-stilbene[g]	6.37	6.23	1.02	0.15
	C/ZrO_2			
di(phenethyl)amide[h]	5.59	6.72	1.20	1.76
resorcinol diether[i]	10.16	11.57	1.14	0.94
cis-/*trans*-stilbene[j]	0.37	8.37	22.68	6.75

[a] 50 mm × 4.6 mm columns, 1 mL/min, 30 °C, 254 nm detection. [b] Retention factors of diastereomers. [c] Selectivity factor. [d] Resolution factor. [e] 25:75 THF/water, 5 μL injection. [f] 50:50 THF/water, 3 μL injection, 100 mm × 4.6 mm column. [g] 70:30 ACN/water, 3 μL injection, 150 mm × 4.6 mm column. [h] 35:65 THF/water, 3 μL injection. [i] 55:45 THF/water, 3 μL injection. [j] 100% THF, 5 μL injection.

Table 4. Characteristics of Analytical Methods for %ee Determination

	chiral GC	chiral HPLC	diastereomer NMR	diastereomer HPLC
chemical class	volatile	broad	broad	broad
sample prep[a]	+	++	−−	+
sensitivity[b]	0.05	5	≥500	5
% RSD[c]	1	1	2	1
ease	++	+	+	+
time[d]	4 h	4 h	24 h	3 h
overall cost	$	$$	$$$	$

[a] Amount of sample preparation required before enantiomeric assay. ++, little preparation; +, simple derivative with no prior separation; −, simple derivative with some prior separation; −−, complex derivative or extensive separations involved. [b] Micrograms required per sample. [c] Percent relative standard deviation. [d] Includes sample preparation, instrument scheduling, and analysis.

MTPA Diastereomers. Synthetic organic chemists frequently employ derivatization schemes and a variety of analytical methods for the determination of enantiomeric excess and assignment of absolute stereochemical configuration. With the added requirements facing pharmaceutical manufacturers today, the quantitation of stereoisomers and the determination of enantiomeric excess (%ee) increases in importance. Mosher's method of coupling NMR with derivation is the most popular form of stereoisomer analysis for alcohols; however, other analysis methods work well. Some of those methods and their analytical characteristics are shown in Table 4.

Chromatographic analysis has become more popular due to improvements in column lifetimes and method precision. In virtually all the above methods, the analyte must undergo chemical modification prior to analysis, with the possible exception of chiral HPLC. The most desirable method, in terms of sample preparation, total analysis time, sample requirements, and cost, is chiral gas chromatography; however, the analytes must be volatile enough to get into the gas phase without decomposition. This greatly limits the use of GC in pharmaceutical analysis.

Of the remaining broadly applicable methods, derivatization to diastereomers and performing HPLC permits the use of achiral column packings. Thus, cost, method development, and analysis time improve over those of the NMR and chiral HPLC methods. Investment in a new chiral HPLC column is sizable, and there is no general chiral stationary phase that will yield adequate resolution for every analyte. NMR analysis requires the most

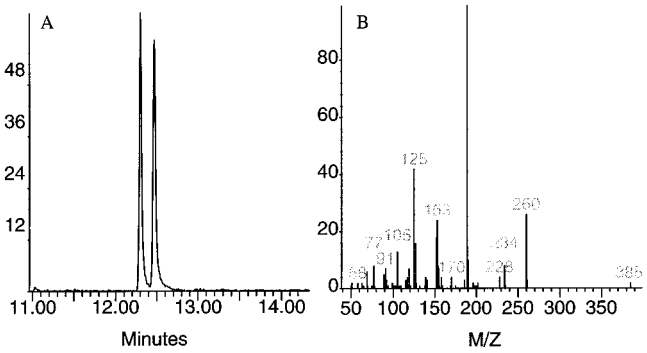


Figure 2. GC/MS analysis of (±)-4-chloroamphetamine-(*R*)-MTPA amide. Conditions: 6 m DB-5 capillary column, HP 5890 GC, HP 7673 autosampler, HP 5970 MSD. Temperature program: hold 50 °C, 2 min; 20 °C/min ramp; hold 250 °C, 10 min. (A) Total ion chromatogram (abundance × 10^{-4}). (B) Mass spectrum normalized to base ion peak.

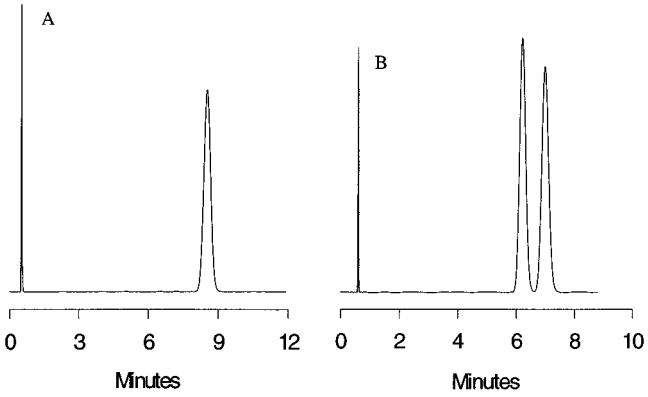


Figure 3. Separation of (±)-warfarin-(*R*)-MTPA ester. Conditions: 50 mm × 4.6 mm, 1 mL/min, 30 °C, 0.1 μL injection, 254 nm detection. (A) Zorbax SB-C18, $d_p = 5$ μm, 40:60 THF/water. (B) Hp-C/ZrO_2, $d_p = 2.5$ μm, 45:55 THF/water.

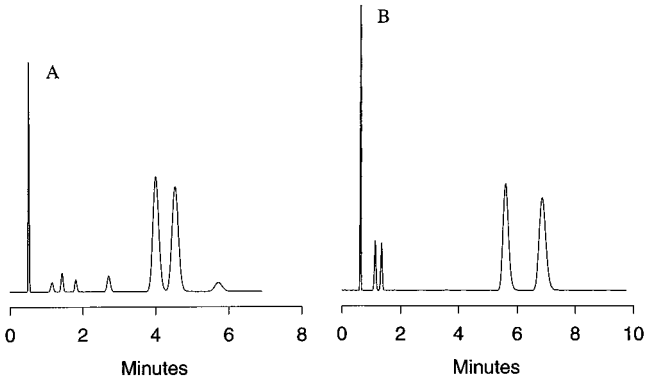


Figure 4. Separation of (±)-4-chloroamphetamine-(*R*)-MTPA amide. Conditions: 50 mm × 4.6 mm, 45:55 THF/water, 1 mL/min, 30 °C, 1.5 μL injection, 254 nm detection. (A) Zorbax SB-C18, $d_p = 5$ μm. (B) Hp-C/ZrO_2, $d_p = 2.5$ μm.

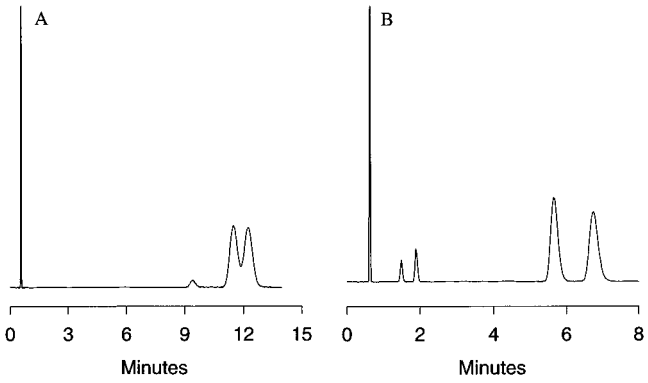


Figure 5. Separation of (*R*)-MTPA amide-(DL)-phenylalanine, methyl ester. Conditions: 50 mm × 4.6 mm, 1 mL/min, 30 °C, 1.5 μL injection, 254 nm detection. (A) Zorbax SB-C18, $d_p = 5$ μm, 35:65 THF/water. (B) Hp-C/ZrO_2, $d_p = 2.5$ μm, 40:60 THF/water. D-Phenylalanine derivative elutes prior to L-phenylalanine derivative.

sample and extensive sample preparation; separation methods are frequently employed before the analytical measurement is taken so as to minimize chemical shift signals arising from impurities. Furthermore, the NMR method necessitates the use of high-field instruments for peak resolution and includes the possibility of multidimensional techniques. The added advantage of NMR comes from the structural information provided and the assignment of absolute stereochemical configuration. However, for the determination of enantiomeric excess, chromatographic methods show good cost-effective behavior and reasonable precision.

Prior to HPLC, for the sake of completeness, all MTPA derivatives used in this work, with the exception of warfarin, were characterized by gas chromatography/mass spectrometry (GC/MS) to confirm their identities. The warfarin MTPA ester did not possess sufficient volatility to pass through the gas chromatograph; thus, gas chromatographic analysis still suffers from volatility problems and limits the applicability of that method. Figure 2 shows a typical total ion chromatogram and mass spectrum obtained for the MTPA derivatives. The signal at *m*/*z* 189 indicates the presence of the MTPA moiety and represents a stable benzyl/oxonium ion. GC/MS not only effectively separates the 4-chloroamphetamine diastereomers but also performed well for all the other derivatives; however, it failed to resolve any of the aliphatic alcohols used in this study. Thus, for volatile, structurally diverse molecules, we suggest using GC/MS as the analytical method; less volatile or thermally labile species require the use of liquid chromatographic analysis.

The optimized reversed-phase liquid chromatographic separations for the MTPA derivatives of warfarin, 4-chloroamphetamine, and phenylalanine are shown in Figures 3−5, respectively. In all cases and in accord with prior experience with simple solutes,[45] the conventional bonded phase displayed higher efficiency than the carbon-coated zirconia phase; therefore, any observed resolution differences favoring the carbon media must arise from selectivity and retention (see eq 2). As Table 5 shows, use of the carbon phase results in higher resolution for the first three MTPA derivatives. In each case, the selectivity factor on carbon exceeds that on the ODS; the observed phenomenon must arise from the retention characteristics of carbon and the rigidity of its surface. Prior work with simple solutes in the same mobile phase shows that carbon is frequently superior to ODS in its geometric selectivity.[45,46] However, solute structure still has a significant impact on the selectivity and resolution, as is evident from the last three analytes in Table 5. Both stationary phases yield comparable selectivity and resolution upon optimization. Clearly, the choice of stationary phase for these analyses does not favor one medium over the other. Moreover, the first three analytes all contain an aromatic ring system within their initial structures,

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Table 5. Summary of MTPA Diastereomeric RPLC Analysis[a]

	ODS			carbon		
derivative	α^b	R_s^c	% ee[d]	α^b	R_s^c	% ee[d]
warfarin	1.00	0.00		1.14	1.40	1.3(0.1)
4-chloroamphetamine	1.16	1.48	0.8(0.3)	1.25	2.27	4.5(0.9)
phenylalanine	1.07	0.80	0.5(0.1)	1.22	2.06	0.4(0.4)
valine	1.08	0.89	1.7(1.1)	1.08	0.96	4.1(0.2)
methionine	1.07	0.87		1.07	0.93	7.0(0.5)
alanine	1.12	1.30	1.3(0.2)	1.11	1.30	2.7(0.6)

[a] Optimized separation for all diastereomers. [b] Selectivity factor. [c] Resolution factor. [d] Percent enantiomeric excess with standard deviation in parentheses, calculated as $100 \times ((r-1)/(r+1))$, where r is the ratio of the chromatographic peak areas.

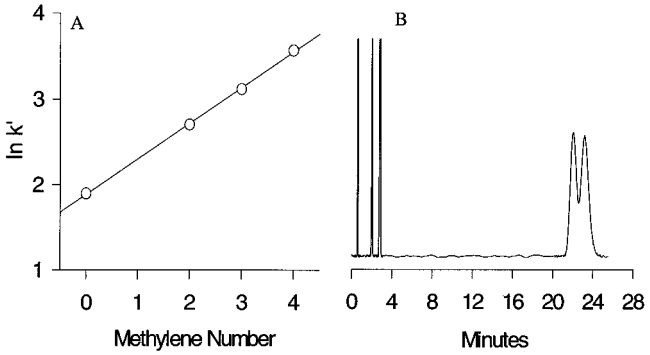

Figure 6. Limits of diastereomeric selectivity on Hp-C/ZrO_2 using MTPA esters of simple alcohol homologues. Conditions: 50 mm × 4.6 mm, 35:65 THF/water, 1 mL/min, 30 °C, 1.5 μL injection, 254 nm detection. (A) All homologues, $\Delta G°(CH_2) = 251$ cal/mol; (B) (±)-3-octanol, MTPA ester, $\Delta\Delta G° = 30$ cal/mol.

and the latter three species do not. This is additional evidence for the participation of aromatic moities in the retention processes on carbon-based stationary phases and, in this case, on the resolution of diastereomers.

Reacting (*S*)-MTPA-Cl with only the L-amino acid methyl ester and performing the same chromatographic assays as reported above allows us to examine the elution order of the amino acid-based diastereomers. We expect to see some variability in the order of elution since the stationary phases are so structurally diverse. Surprisingly, both the ODS and carbon phases yield the same elution sequence for these solutes. Since only four compounds contributed to this part of the study, we cannot state with any certainty that the elution of MTPA diastereomers on carbon and ODS will always be identical; however, previous work indicates that the selection of an appropriate derivatizing agent can control the elution pattern.[21]

Table 5 also gives the results of the enantiomeric excess assays. The estimate of enantiomeric excess is based on the peak areas of the diastereomers eluting from the column. No additional signal processing or peak deconvolution methods were employed; therefore, we expect significant errors in the %ee reported for unresolved diastereomers. Furthermore, the small degree of peak tailing on the carbon phase complicates peak integration.[47−50]

Since the suppliers report all the starting compounds as racemates, we anticipate small values for the calculated enantiomeric excesses. The values reported in Table 5 fall within our expectations. However, the calculated %ee for 4-chloroamphetamine does not agree between analyses on the two liquid chromatographic phases, even though both separations yield near-baseline resolution. Subsequent GC/MS work with the same sample shows a %ee of 3.5; thus, the results of the enantiomeric assay on carbon were confirmed by an independent method. Furthermore, this suggests that we can use diastereomeric derivatization schemes coupled with a rigid, selective carbon stationary phase to analyze for enantiomeric excess and perform separations on structurally similar analytes.

The reproducibility of the separations across multiple C/ZrO_2 columns cannot be addressed in this study due to the limited amount of chromatographic packing material available. However, previous data suggest that the separations developed on the C/ZrO_2 phase should be transferable and reproducible across multiple columns.[39−41,45]

Homologues of MTPA Esters. To extend and further test the selectivity of carbon HPLC media, we analyzed a homologous series of alcohol MTPA esters. Three substitutents on the carbon-based stereocenter were fixed (see Figure 2B): a hydrogen, a MTPA-derivatized hydroxyl, and an ethyl group. The fourth substituent consists of an alkyl chain whose length was systematically varied. These diastereomers were not separable on the ODS phase in either THF/water or ACN/water mobile phases. Additionally, no resolution was observed for these same diastereomers on the carbon phase, with the exception of the longest alkyl chain MTPA ester. The carbon phase partially resolved the (±)-3-octanol, MTPA ester.

The separation of the (±)-3-octanol, MTPA ester defines the limits of diastereomeric selectivity for the carbon system; we have discovered the conditions under which differentiation between alkyl groups defining a stereocenter becomes possible. From the chromatogram and homologue plot, shown in Figure 6, the free energies of methylene transfer and diastereomeric differentiation were calculated. The slope of the homologue plot yields the free energy of transfer for a methylene group from the mobile phase to the stationary phase of 251 cal/mol. Since the difference between alkyl segments in the molecule is three methylene units, the $\Delta\Delta G°$ for the alkyl segment is 753 cal/mol. Furthermore, the $\Delta\Delta G°$ based on the selectivity factor for the octanol diastereomers at 30 °C is 30 cal/mol. The fraction of free energy required for diastereomeric differentiation relative to the transfer energy of the alkyl subunits is the ratio of the later two numbers; evidently only 4% of the differential alkyl free energy of transfer provides the separation. This reflects the difficulty in stereoisomer separations. In particular, very small relative differences in energy exist between chemically similar molecules, and those features lead to extremely difficult separations. Furthermore, these types of separations require stationary phase media that are particularly sensitive to the geometric orientation of the solutes. With recent reports on new chiral anisotropic reagents, similar to MTPA but possessing larger aromatic moieties,[51−53] one may be able to extend the stereoselectivity of carbon stationary phases. A

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naphthyl or anthryl ring will strongly interact with the carbonaceous surface and potentially lead to greater selectivities than those exhibited by the MTPA derivatives examined here. Unfortunately, these reagents have yet to be made commercially available, but the study of such derivatives for rapid chromatographic analysis may prove fruitful.

CONCLUSIONS

For the analysis of stereoisomers, and in particular diastereomers, carbon-coated zirconia HPLC media can provide excellent resolving power for a wide range of analytes. Since the conventional bonded phase possesses better efficiency, carbon's ability to resolve closely related chemical species arises from its retention and selectivity. Consequently, the rigid, polarizable carbon surface is sensitive to the geometric orientation of analytes and leads to resolution. This sensitivity extends the capabilities of RPLC to provide separations at small relative energy differences.

Chromatographic methods cannot compete with the ability of NMR analysis in the assignment of absolute stereochemical configuration but do satisfy the need for rapid determination of enantiomeric excess, either with chiral stationary phases or, in this case, derivatization to diastereomers. Volatility and selectivity remain major concerns with gas chromatographic analyses, and the cost of chiral stationary phases in gas or liquid chromatography can be substantial. Therefore, we recommend carbon stationary phases as a first choice for the cost-effective separation of chemically similar analytes, especially diastereomers.

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