ENANTIOMERIC SEPARATIONS IN CHROMATOGRAPHY

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I. INTRODUCTION

The resolution of enantiomers (nonsuperimposable, mirror-image isomers) has traditionally been considered one of the more difficult problems in separation science. Enantiomers have identical physical and chemical properties in an isotropic environment except that they rotate the plane of polarized light in opposite directions. A mixture containing equal amounts of enantiomers is referred to as a racemic mixture. Neither racemic mixtures nor solutions of achiral compounds are able to rotate the plane of polarized light.

In the mid-nineteenth century, the French physicist Biot discovered that certain substances had the unusual property of being able to rotate the plane of polarization of a linearly polarized incident light beam.[1,2] These substances, which were said to be optically active or to possess optical rotatory power, were characterized by a lack of symmetry in their molecular or crystalline structure. In 1848, Pasteur reported the first deliberate separation of enantiomers from a racemic mixture.[3] This separation was possible because saturated, racemic solutions of sodium ammonium tartrate form two types of morphologically distinct crystals, each containing a single enantiomer at temperatures below 27°C. In 1874, Van't Hoff and Le Bel independently deduced that the molecular basis of optical activity lay with the "asymmetric carbon atom".[4,5]

Enantiomeric separations are very important in many fields. Some typical fields include chiral synthesis, mechanistic studies, catalysis, kinetics, geochronology, biology, biochemistry, pharmacology, and medicine. There are many methods for the separation of enantiomers. Several nonchromatographic methods classically have been used to isolate optically pure compounds from racemic mixtures. The most generally useful of these methods involves conversion of the racemic mixture to a pair of diastereomers which have different chemical and physical properties and which may be separated by conventional techniques. Separation of the diastereomers can often be achieved by fractional recrystallization. Enantiomeric excesses have also been obtained via microbiological or enzymatic digestion. In this case, the enzyme must preferentially catalyze the reaction of one enantiomer relative to the other. Crystallizations followed by mechanical separation are useful for the few compounds that segregate into morphologically distinct crystals. Another type of crystallization technique involves seeding a supersaturated racemic solution with a small optically pure crystal. The resulting crystals often contain an enantiomeric excess. While all of these techniques have been utilized successfully, none can be considered generally useful, and all are relatively time consuming and tedious. In addition, these methods often fail to afford total separation of enantiomers. Recently there has been a dramatic increase in the number and type of racemic separations achieved by chromatographic methods. The popularity of the chromat-

ographic approach stems from its relative ease and efficiency. In this review, the enantiomeric separations with gas chromatography (GC), liquid chromatography (LC), and thin layer chromatography (TLC) are discussed. It appears that the most interesting research in this area involves the development of new, highly selective stationary phases. A large number of chiral stationary phases (CSPs) were synthesized, and it was found that minor structural modifications in a stationary phase may have a tremendous influence on an enantiomeric separation.

II. GAS CHROMATOGRAPHY

In GC, the separation of enantiomers is generally achieved in two ways. The first involves the use of CSPs.[6-11] The second involves derivatizing a racemate with a chiral group and making a pair of diastereomers, and then separating those on achiral or chiral stationary phases.[6-11] In 1966, Gil-Av and co-workers[12] first made CSPs for GC and demonstrated their usefulness for separating enantiomers. This approach attracted great attention since it enabled one to precisely measure enantiomeric purities in complex mixtures while utilizing very small samples. The use of chiral derivatization methods has a few limitations: (1) active functional groups for forming diastereomeric derivatives are required, (2) it is not easy to get optically pure chiral reagents, (3) individual enantiomers have different reaction rates, and (4) the diastereomeric mixture must be chemically and stereochemically stable.

A. CSPs

1. Amino Acid Derivatives

Gil-Av and co-workers[12] first introduced an enantioselective GC stationary phase which consisted of *N*-trifluoroacetyl (*N*-TFA) L-isoleucine lauryl ester. This liquid phase was used for the separation of *N*-TFA-α-amino acid esters. It showed excellent resolution for volatile *N*-TFA-α-amino acid esters (e.g., *N*-TFA-valine and leucine esters of 2-butanol), but was not suitable for *N*-TFA-alanine esters, 2-heptyl acetate, and α-acetoxypropionate esters of 2-butanol.

Feibush[13] reported a highly efficient diamide-liquid stationary phase, *n*-dodecanoyl-L-valine-*tert*-butylamide. This phase was less polar than dipeptide phases but produced relatively high enantioselectivities. Retention times were generally shorter than on the more polar phases. However, this phase was limited to the separation of amino acid and amino alcohol enantiomers. Also, this phase had relatively high column bleeding at the optimum column operating temperature of 130 to 140°C. To reduce the column bleeding, *N*-docosanoyl-L-valine *tert*-butylamide and *N*-lauroyl-L-valine 2-methyl-2-heptadecylamide were used as the active coating.[14] *N*-TFA isopropyl esters of 14 protein amino acids were studied, and 12 of the 14 compounds were resolved on relatively short columns (2 to 4 m). These packed columns could be used at temperatures as high as 190°C (for *N*-docosanoyl-L-valine *tert*-butylamide) and 180°C (for *N*-lauroyl-L-valine 2-methyl 2-hepadecylamide) without losing their efficiency, even after prolonged use. The influence of structural factors on selectivity of CSPs ($R_1CONHCH[CH(CH_3)_2]CONHR_2$) was studied.[15] Hobo and co-workers studied the stereoselectivity of the chiral diamide stationary phase, *N*-lauroyl-L-valine *tert*-butylamide, diluted with diethylene glycol succinate polyester (DEGS) or squalane for the separation of several *N*-trifluoroacetyl isopropyl esters of amino acids.[16] The addition of DEGS greatly reduced the resolution coefficient. It was thought that this resulted from the blocking of the chiral sites of the stationary phase by the polar solvent. When squalane was added, the resolution coefficient either remained the same or increased. Dilution of the chiral liquid stationary phase with achiral additives may lead to the dissolution of the hydrogen-bonded networks of these dipeptide-analog phases. It was concluded that the unblocked monomeric form of the diamide gave higher retention and stereoselectivity.

Table 1
RESOLUTION FACTORS OF *N*-TFA-*O*-ACYL DERIVATIVES OF AMINOALKANOLS

		Acyl group								
		Propionyl			Isobutyryl			Pivaloyl		
Aminoalkanol	Enantiomer	r[a]	$r_{(D:L)}$[b]	T(°C)[c]	r[a]	$r_{(D:L)}$[b]	T(°C)[c]	r[a]	$r_{(D:L)}$[b]	T(°C)[c]
2-Aminopropan-l-ol	L	14.58	1.040	120	9.40	1.051	140	12.00	1.075	140
	D	15.16			9.88			12.90		
2-Aminobutan-l-ol	L	25.00	1.076	120	14.56	1.089	140	15.86	1.112	140
	D	26.90			15.86			17.64		
2-Aminopentan-l-ol	L	42.28	1.089	120	10.94	1.075	160	11.48	1.097	160
	D	46.00			11.76			12.60		
2-Aminohexan-l-ol	L	69.88	1.087	120	16.86	1.071	160	18.14	1.096	160
	D	76.00			18.06			19.88		
2-Aminoheptan-l-ol	L	51.88	1.071	140	19.86	1.062	170	20.88	1.082	170
	D	55.58			21.10			22.60		
2-Aminooctan-l-ol	L	88.80	1.071	140	31.98	1.067	170	33.14	1.083	170
	D	95.10			34.14			35.90		
2-Amino-3-methyl-butan-l-ol	L	28.44	1.094	120	16.90	1.105	140	18.66	1.125	140
	D	31.12			18.68			21.00		
2-Amino-4-methyl-pentan-l-ol	L	24.10	1.057	140	29.84	1.085	140	30.04	1.130	140
	D	25.48			32.38			33.94		

[a] Corrected retention time (minutes).
[b] L/D, resolution factor, i.e., ratio of the corrected retention time of the L over that of the D enantiomer, calculated with r values expressed to the second decimal place.
[c] Temperature at which good peak resolution and relatively short retention were observed.

From Charles, R. and Gil-Av, E., *J. Chromatogr.*, 195, 317, 1980. With permission.

Koenig and co-workers prepared chiral liquid stationary phases by coupling *S*-α-phenylethylamine with *S*-2-hydroxyisopentanoic acid and *S*-2-hydroxyoctanoic acid.[17] The separations of the enantiomers of racemic amines, amino alcohols, and hydroxy acids were achieved using these stationary phases.

Weinstein and co-workers[18] reported that CSPs containing an amide group and an asymmetric carbon atom, attached to the nitrogen atom [RCONHCH(CH_3)R′], often showed enantiomeric selectivity for amino acids containing *N*-trifluoroacetylamine, *N*-trifluoroacetylamino acid esters, and α-methyl- or α-phenylcarboxylic acid amides. The best efficiency was obtained when R′ was an aromatic group. Particularly good separations were obtained when R′ was an α-naphthyl group, as in *N*-lauroyl-*S*-α-(1-naphthyl)ethylamine. Also, the highest resolution factors were found for aromatic solutes such as *N*-trifluoroacetyl-α-phenylethylamine and α-phenyl butyric acid amides.

Other successful GC separations of enantiomers were reported on modified diamide phases.[19,20] *N*-Docosanoyl-L-valine-2-(2-methyl)-*n*-heptadecyl amide phases[20] were used at column temperatures of up to 200°C. These phases showed excellent stereoselectivity for various compounds (aromatic *N*-TFA amines, *N*-TFA-*O*-acetyl amino alcohol, *N*-TFA-α-methylvaline isopropyl ester, and *N*-TFA-esters of α- and γ-amino acids). Table 1 shows the resolution factors of *N*-TFA-*O*-acetyl derivatives of amino alkanols. Lochmüller and co-workers reported ureide phases (i.e., carbonyl-bis [amino acid esters])[21-24] and examined the separation mechanisms of these phases. The general formula of the ureide phase is shown in Figure 1. Nuclear magnetic resonance (NMR) evidence was interpreted to indicate that only one significant portion of attachment is involved in the formation of diastereoisomeric

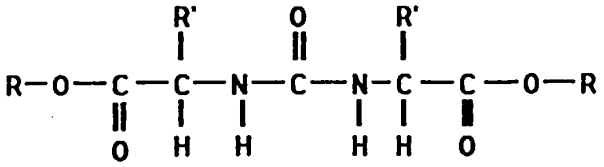


FIGURE 1. General formula of the ureide phase. (From Lochmüller, C. H. and Souter, R. W., *J. Chromatogr.*, 113, 283, 1975. With permission.)

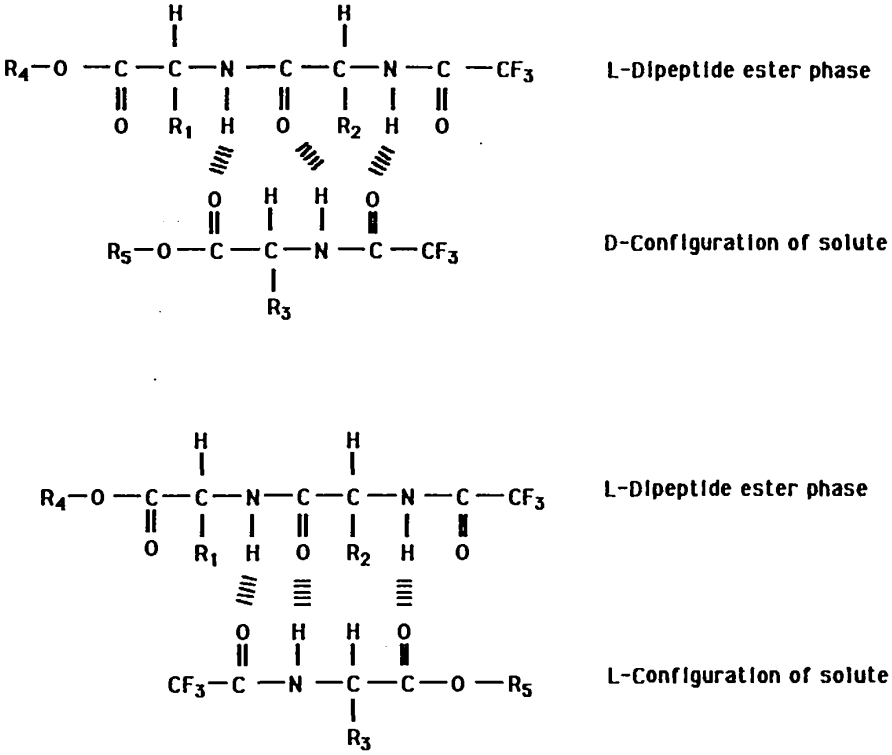


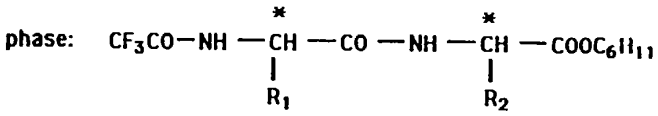
FIGURE 2. Schematic showing the formation of H-bonded diastereomeric association complexes. (From Lochmüller, C. H. and Souter, R. W., *J. Chromatogr.*, 113, 283, 1975. With permission.)

association complexes. This, of course, is highly unlikely given the well-known geometrical requirements (i.e., three-point attachment) for chiral recognition. Also studied were the effects of stationary phase structure on selectivity.[23]

2. Peptide Phases

Gil-Av and co-workers also developed optically active stationary phases composed of *N*-TFA-α-amino acids (e.g., *N*-L-valyl-L-valine isopropylester, *N*-TFA-L-valyl-L-valine cyclohexyl ester,[25] *N*-acetyl-L-valyl-L-valine isopropyl ester,[26] and *N*-TFA-(L-valyl)$_2$-L-valine isopropyl ester[26]). However, these phases had low efficiencies and long analysis times because of limited thermal stability. For example, a *N*-TFA-L-valyl-L-valine cyclohexyl ester stationary phase was used at 110°C. Above 110°C, the column began to bleed, and at temperatures below 110°C, the column efficiency was very low and the peaks were broad.

In peptide phases, the resolution of enantiomers is thought to be through the formation of hydrogen-bonded diastereomeric association complexes between the enantiomeric compounds and asymmetric chiral phases. Figure 2 shows this interaction on a dipeptide ester



	R_1, R_2	abbreviation
N-TFA-L-alanyl-L-alanine cyclohexyl ester	$-CH_3$	ala-ala
N-TFA-L-α-amino-n-butyryl-L-α-amino-n-butyric acid cyclohexyl ester	$-CH_2-CH_3$	aba-aba
N-TFA-L-norvalyl-L-norvaline cyclohexyl ester	$-CH_2-CH_2-CH_3$	nval-nval
N-TFA-L-norleucyl-L-norleucine cyclohexyl ester	$-CH_2-CH_2-CH_2-CH_3$	nleu-nleu

FIGURE 3. Homologous dipeptide stationary phases. (From Parr, W. and Howard P. Y., *Anal. Chem.*, 45, 711, 1973. With permission.)

phase.[24] Parr and co-workers synthesized many dipeptide ester phases.[27-31] They also studied the structural effects on selected dipeptides as stationary phases for the enantiomeric separation of amino acids.[32] Four optically-active dipeptide cyclohexyl esters (*N*-TFA-L-alanyl-L-alanine cyclohexyl ester, *N*-TFA-L-α-amino-*n*-butyryl-L-α-amino-*n*-butyric acid cyclohexyl ester, *N*-TFA-L-norvalyl-L-norvaline cyclohexyl ester, and *N*-TFA-L-norleucyl-L-norleucine cyclohexyl ester) were used (see Figure 3). Separation factors and thermodynamic properties of liquid stationary phase-solute interactions were studied. Table 2 shows relative retention times and separation factors for *N*-TFA-D,L-amino acid isopropyl esters. An increase in the size of the alkyl substituent on the asymmetric centers of the dipeptide solvent produced a greater liquid stationary phase-solute interaction. When the analogous modification was made with the side chain on the α carbon, the interaction decreased.

In order to obtain a dipeptide ester phase of greater thermal stability and higher molecular weight, aromatic amino acids such as phenylalanine were employed (i.e., *N*-TFA-L-phenylalanyl-L-leucine cyclohexyl ester,[29,31,33,34] *N*-TFA-L-phenylalanyl-L-phenylalanine cyclohexyl ester,[35,36] and *N*-TFA-L-phenyl-L-aspartic acid bis [cyclohexyl] ester[35-37]). These phases were able to be used at higher temperatures (130 to 165°C) than the original dipeptide phases and therefore were more effective for separating less volatile enantiomeric amino acid derivatives.

Koenig and co-workers used glass capillaries because they found partial decomposition of some compounds during chromatography with steel capillaries. They reported that steel capillaries promoted the decomposition of solutes (cysteine, serine, and threonine derivatives) to a greater extent than glass capillaries did. The glass capillary columns also possessed higher efficiencies compared to analogous stainless steel capillaries. Some of the stationary phases synthesized and used with glass capillaries include *N*-TFA-L-phenylalanyl-L-phenylalanine cyclohexyl ester, *N*-TFA-L-phenylalanyl-L-leucine cyclohexyl ester, and *N*-TFA-L-phenylalanyl-L-aspartic acid bis (cyclohexyl)ester.[35] Figure 4 shows the separation of racemic TFA-amino acid isopropyl esters on a glass capillary coated with *N*-TFA-L-valine-L-valine cyclohexyl ester.

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