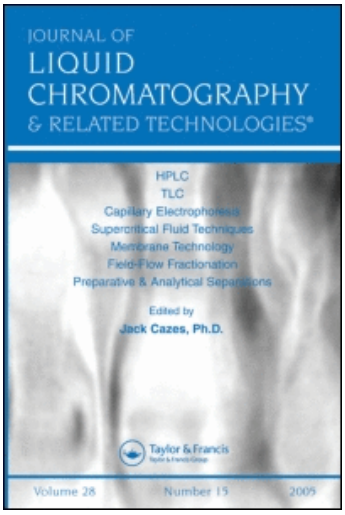

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RESOLUTION OF ENANTIOMERS WITH ACHIRAL PHASE CHROMATOGRAPHY

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ABSTRACT

Resolution of enantiomers is of widely accepted importance. Since there have been sporadic reports on liquid chromatographic resolution of enantiomers using achiral stationary phase in conjunction with a mobile phase having no external chiral compound the present paper focuses on such achiral phase resolutions of enantiomers along with their possible explanations. At the same time it introduces to the present day practice of resolution of enantiomers by liquid chromatography which makes use of a chiral selector either bound to the stationary phase or added to the mobile phase. The mechanisms for chiral phase chromatographic methods are also discussed.

GENERAL CONSIDERATIONS

Most life processes involve chiral molecules and discrimination can be expected to be a common feature of the

interactions. Since Pasteur noted that the (+) asparagine tasted sweet and the other isomer was almost tasteless widely different physiological effects of (+) and (-) forms of various natural and synthetic compounds have been brought to light.

Enantiomers differ not only in the metabolic rates in which they are processed in a biological system but they even be metabolised along different pathways (1), or may bind to different receptor types leading to different biological responses. Enantiomerically pure samples of vast majority of chemical compounds are of value in the fields of organic synthesis, mechanistic studies, catalysis, kinetics, biochemical and geochemical studies. The demand for such compounds is growing in the areas of pharmaceuticals, pesticides, molecular electronics, optical data storage and liquid crystal applications. The enantiomers have very different pharmacodynamic activities therefore attention is required to be paid to the unwanted enantiomer in the drug administered, generally as a racemic mixture, which may even disturb other biological processes and cause catastrophic side effects.

Therefore, the control of resolution procedure of pharmaceuticals and agrochemicals and other compounds exhibiting enantioselectivity in biological action, the control of optical purity of enantiomeric active ingredients, the preparative scale preparation/separation of pure desirable enantiomer, and the determination of the enantiomeric ratio of racemic drugs *etc.* in biological media/environment are the essential features requiring considerable attention. It is a matter of time before law making/enforcing authorities for the pharmaceutical and agrochemical industries that they will insist on detailed information with respect to the presence of adequate drug levels and the relation between drug concentration and therapeutic or toxic effects of individual enantiomers of all new compounds to be marketed.

The importance of optical purity, in the context of a compound's biological activity is gaining increased appreciation and hence efforts are being made to the development of economical methods for the industrial assymetric synthesis of pure enantiomers. In view of the difficulties in the synthesis of enantiomerically pure compounds and the importance of individual enantiomers scientists have been attempting various analytical approaches for the resolution of racemic or enantiomeric mixtures. The introduction of chromatography as a new fascinating tool for separations created the opportunity for efficient enantiomeric separations. Only the liquid chromatography has been reported to be successful in resolving enantiomers even in totally achiral system. The present review, the first of its kind to the best of authors knowledge, deals with chromatographic methods of *chiral resolutions in achiral environment*, along with their possible explanations.

INTRODUCTION TO CHIRAL PHASE RESOLUTIONS

The success of the analytical method used for the resolution of enantiomers, particularly the commercially important ones, should be looked into in terms of costs of substrate and resolving agent, chemical and optical yields, and total number of steps. Since the pioneering discoveries of Louis Pasteur enantioselective crystallization process or enzyme catalysed reactions have been used for the practical resolution of racemic mixtures. In order to achieve a significant separation of enantiomers in these interaction processes with the resolving agents, the discriminating ability of the latter (*i.e.* the degree of enantioselectivity of the process) must be very high; this requirement is met by many crystallization processes and enzymatic reactions.

Most of the classical methods for separation of enantiomers were characterised by multiple steps and low efficiency; they were time consuming, tedious and required further chemical treatment to reclaim the starting enantiomer. Various modes of chromatography like GC, TLC, and HPLC have been applied to resolution of a variety of compounds. The chromatographic technique can be used for the preparative scale separation of enantiomers and for the qualitative and/or quantitative analysis of the enantiomers in bulk for biological or pharmaceutical preparations.

There have been two basic approaches for the chromatographic resolution of enantiomers: a direct, and an indirect approach; in both the enantiomers separate via their conversion into diastereomers with optically active reagents because the latter have different physico-chemical properties in solution and different adsorption characteristics onto the surface of stationary phase (*e.g.* polar silica gel or hydrophobic reversed phase), and thereby create conditions for resolutions in chromatographic systems.

INDIRECT APPROACH

In the indirect approach diastereomers are formed covalently and prior to chromatography. The enantiomer molecule must possess relatively easily derivatizable functional groups such as carboxyl, amino, alcoholic, carbonyl *etc.*, and the same holds for the chiral derivatizing agent. Following basic considerations are to be taken into account for indirect enantioseparation technique (2) :

(a) The chiral derivatizing agent should be readily available in high and known optical purity and should not racemize during storage.

(b) The chiral derivatizing reagent should react quantitatively or at least the reaction rates with the compounds to be resolved must be kinetically equal; in fact the rates are normally different.
(c) The racemization of chiral centre is negligible under the reaction conditions.
(d) Unequal detector response of the diastereomers must be corrected by standard procedures.

The detection of a diastereomeric pair via the indirect technique is sometimes simpler to perform, particularly in bioanalytical samples where many co-eluting substances might interfere, in comparison to a direct enantiomeric system because the chromatographic conditions are much more easily optimized. The indirect mode of separating enantiomers in GC as well as in HPLC has been used for many years and is still commonly performed for various purposes, and there have appeared several review articles in the area, by Gil-Av and Nurok (3), Lochmüller and Souter (4), Krull (5), Halpern (6), Tamegai *et al.* (7), Davankov (8), Blaschke (9), Lindner (2,10), Pirkle and Finn (11), and Lindner and Pettersson (12).

DIRECT APPROACH

The direct separation approach involves the interaction of enantiomers with a chiral stationary phase or a chiral selector that is either chemically bound to the achiral sorbent or added to the mobile phase, thus no chemical derivatization with a chiral reagent prior to chromatography is required.

A variety of chiral selectors have been immobilised on solid phases to make chiral stationary phases (CSPs). The making of a CSP is generally based on `three point rule´ originally derived by Dalgliesh (13); it suggested a three point interaction (H-

bridge, $\pi-\pi$-interactions, steric repulsion) between the chiral stationary phase and at least one of the enantiomers to distinguish between the two enantiomeric forms. Wainer has proposed a classification scheme for chiral stationary phases based on the type of interactions that are believed to play the main role in the resolution (14). The first successful direct enantiomeric separation using GC was reported by Gil-Av *et al.* (15) in 1966. In 1968 Rogozhin and Davankov (16) showed that ligand exchange chromatography (LEC) can be used for chiral separations by introducing a metal ion, like Cu(II), into a system (amino acid covalently bound to a resin) capable of reversibly coordinating with an assymetric stationary phase and the individual enantiomers, forming complex diastereomeric species dynamically. In this way a whole field of LEC was opened (17,18) which gave chemists further possibilities for enantiomeric separation, particularly when the HPLC was introduced. The principle of LEC was for the first time successfully applied for the TLC resolution of several enantiomeric amino acids and other compounds by Günther, Martens *et al.* (19-23); they developed a new chiral selector (2S, 4R, 2'RS)-N-(2´-hydroxy dodecyl)-4-hydroxy proline and used Cu(II) for complexation. TLC plates using this ligand have been made commercially available by Macherey-Nagel and E.Merck under the trade names of Chiralplate® and Chir® respectively, for enantiomeric resolution via ligand exchange.

Chiral packings have been obtained by the chemical reactions of optically pure compounds such as amines, amino alcohols, acid amides or amino acid with certain supports such as styrene and acryl copolymers and silica gel. A CSP for HPLC was first developed by Pirkle and coworkers (23) consisting of (R)- or (S)-N-(3,5-dinitrobenzoyl)-phenyl glycine attached ionically or covalently to γ-aminopropyl silica gel. Since then, CSPs using a variety of chiral selectors/ supports such as proteins (24,25), cellulose (26,27), synthetic polymers (28) like

d-poly-(triphenyl methyl methacrylate) *etc.* have been developed.

Non chiral stationary phases can be used for separation of enantiomers when a chiral selector is added to the mobile phase; a stereoselective separation in such a chromatographic system can be due either to one or a combination, of the following mechanisms:

(a) A stereoselective complexation in the mobile phase.
(b) Adsorption of the chiral selector to the solid phase and *in situ* creation of a chiral stationary phase.
(c) Formation of labile diastereomeric complexes with different distribution properties between the mobile and stationary phases.

A two point interaction, such as, an electrostatic attraction and a hydrogen bridge, might be sufficient for resolution as the difference in retention could be due to differential adsorption of the diastereomeric complexes. Chiral selectors used as mobile phase additives include L-amino acids or their simple derivatives, cyclodextrins, crown ethers, albumin, dialkyl-tartaric acid or tartramide, camphor sulphonic acid, quinine, and simple di- or tri-peptides. Formation of inclusion complexes, due to different fittings into the chiral cavity, by crown ethers and cyclodextrins present in the mobile phase has been ascribed to the enantiomeric resolution. Progress in the study of separation of enantiomers of a variety of compounds using liquid chromatography (both HPLC and TLC), with emphasis on direct resolution has been reviewed during the last few years by Feitsma and Drenth (30), Allenmark (31), Bhushan (32), Martens and Bhushan (33-35), Armstrong and Han (36), and Subert *et al.* (37).

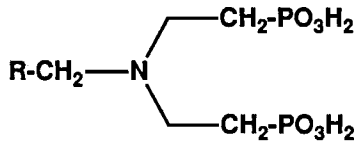

Figure 1. Ion exchanger having methane phosphonic groups.

RESOLUTION WITH BOTH ACHIRAL PHASES

The application of chromatography with achiral adsorbent as well as without a chiral mobile phase additive *i.e.* in a totally achiral system for the purpose of resolution of mixture of enantiomers is not normally brought into consideration, because of the prevalent concept that such an approach does not hold out much promise of success. But, inspite of being contradictory to the generally accepted rules of stereochemistry that no discrimination of optical isomers is possible in an achiral environment, chromatography has been reported to be successful in providing resolution of enantiomeric (or racemic) mixtures of certain compounds.

Szczepaniak and Ciszewska reported, probably for the first time, resolution of DL- amino acid enantiomers in an achiral chromatographic system (38). The separations of DL- tyr, phe, his, cys, try, and met were accomplished using an ion exchanger (**Fig. 1**), packed in simple glass column (of 18 or 36 x 0.7 cm), having iminodi (methane phosphonic) groups in amino copper form.

The ion exchanger with iminodi (methane phosphonic) groups (K-ADMF) was obtained by the exchange of the carboxyl groups of the chelating ion exchanger, Chelex-100 (Bio-Rad Labs), by reacting it with H_3PO_3/PCl_3 ; it was then allowed to sorb ammonium cations from 0.01 M NH_4OH and Cu^{2+} from a 0.01 M $Cu(NO_3)_2$ solution, followed by rinsing with water and

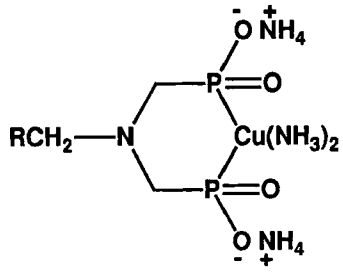

Figure 2 Structure of resin K-ADMF-$Cu(NH_3)_2$.

finally with 0.01 M NH_4OH (39). It was abbreviated as K-ADMF-$Cu(NH_3)_2$ (**Fig.2**) and used for the resolution of DL-amino acids.

Ammonia solutions of DL-amino acids (10^{-3} M) were placed on the top of the column and the column was eluted with *aq* ammonia solutions in the range 0.01 to 0.5 M. The eluates were examined spectrophotometrically at different wavelengths for each amino acid : tyr(225 nm), try(220 nm), his(210 nm), met and cys(205 nm). The resolutions were further confirmed by circular dichroism spectra. It is considered that ligand exchange reaction takes place between the K-ADMF-$Cu(NH_3)_2$ form and the amino acids; the NH_3 groups in the coordination sphere of the Cu atoms are replaced by the amino acid, and the amino acid can be bonded through the amino group or in some cases (his, try) through the aromatic nitrogen (**Fig. 3, 4**).

The ligand exchange reaction has been written as follows:

$$\text{K-ADMF-Cu(NH}_3)_2 + \text{Tyr}^- \rightleftharpoons \text{K-ADMF-Cu(NH}_3\text{Tyr})^- + \text{NH}_4^+$$

Distribution coefficients (K) for D and L enantiomers based on ligand exchange equilibrium were calculated followed by

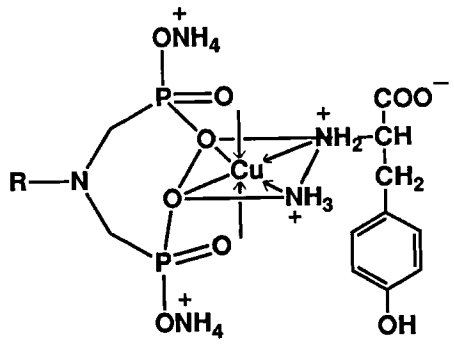

Figure 3. Resin complex with tyrosine (38).

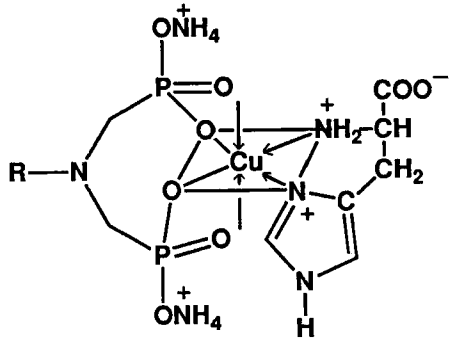

Figure 4. Resin complex with histidine (38).

calculations for enantioselective cofficients $E_{L/D}$ for the L- to -D forms of amino acids, and free energies of formation of the D- and L- complexes with the resin were made. The apparent differences in the distribution coefficients and free energies of formation of the particular enantiomer complex were regarded as the basis for the chromatographic resolution of enantiomeric mixture of amino acids on optically inactive ion exchanger. From the retention volumes of D (V_D) and L (V_L) enantiomers the enantioselectivity coefficients (α), defined as, $\alpha = V_D / V_L$, were obtained, and the apparent differences in the (α) values

Table 1

Values of the Distribution Coefficients (K), Differences Between Free Energies ($\delta\Delta G^0$), and Retention Volumes (V_D, V_L), for Complex Formation for the D and L Amino Acid Enantiomers (38).

Amino acid	H_2O		10^{-2} M NH_4OH		**	
	K	$\delta\Delta G^0_{L/D}$	K	$\delta\Delta G^0_{L/D}$	V_L	V_D
					cm^3	
L-Met	35.2	121	0	–	140	120
D-Met	28.6		0			
L-Tyr	35.3	50	18.6	274	290	275
D-Tyr	32.6		11.6			
L-Phe	43.9	101	36.7	116	270	230
D-Phe	36.7		29.9			
L-His	163.6	91*	43.9	333*	240	290
D-His	192.1		77.8			
L-Try	180.9	116	281.9	208	230	190
D-Try	147.8		196.0			

* $\delta\Delta G^0_{D/L}$

** *Aq* solutions of NH_3 ranging from 0.01M to 0.5M

K values from static experiments, and V_L and V_D values from chromatographic experiments.

further indicated the possibility of a direct resolution of the enantiomers on the achiral resin.

The results of Szczepaniak and Ciszewska (38) are contradictory in terms of the common opinion that *chiral recognition can be effective only by another chiral molecule* (40), and *that with an optically inactive reagent all enantiomers will have the same reaction rate and equilibrium*

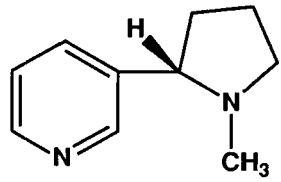

Figure 5. Structure of natural (S)-(-)-Nicotine.

constants (41), but have sound experimental justification in terms of differences in the distribution coefficients, free energies, and enantioselectivity coefficients for the complexes of the two enantiomers with an achiral resin (**Table-1**). These results have an edge over earlier studies wherein it has been reported on the basis of calorimetric measurements that complexes of certain amino acids (*e.g.* D or L forms of histidine) with metal ions like Zn(II) or Ni(II) of the type M_{DL} were more stable than the M_{DD} or M_{LL} complexes (42). For example,

$$Zn(\text{DL-His}) \quad \Delta H^0_f = \text{-49.2 KJ/mole}$$
$$Zn(\text{LL-His}) \quad \Delta H^0_f = \text{-47.2 KJ/mole}$$

Similarly NMR (43) and potentiometric (44) studies showed that octahedral histidine complexes of Co(II) in the M_{DL} form was more stable than the M_{DD} or M_{LL} forms. But there was no evidence for the difference in the stabilities of the complexes of the type M_{LL} and M_{DD} formed from various metals (Ni II, Cu II, Co II, Zn II) and a wide range of amino acids (45, 46).

In an interesting study of the *in vivo* metabolism of the ^{14}C-labelled nicotine Cundy and Crooks (47) observed resolution of its racemic mixture into enantiomers by HPLC on Partisil SCX cation exchange column and ODS-reversed phase column when co-injected with cold (unlabelled) (–)-nicotine standard (**Fig.5**).

Two different forms of ^{14}C-labelled material were used in the study; [^{14}C-NCH_3]-(±)-nicotine-(+)-bitartrate, and [^{14}C-2′]-(±)-nicotine free base; these racemic radiolabelled compounds exhibited splitting of the radioactivity into two distinct peaks when co-injected with unlabelled (S)-(–)-nicotine or its antipode as standard.

Alone each radiolabelled compound eluted as a single radioactive peak and use of unlabelled racemic nicotine as standard also showed one ^{14}C peak. The resolution behaviour of the above mentioned two forms of (±)-nicotine was examined by variation in: enantiomeric ratio of cold standard; HPLC system *i.e.* a cation exchange column and an octadecylsilane (ODS) reverse phase column; the counter ion of the unlabelled standard; enantiomeric ratio of ^{14}C-labelled nicotine, and by isolation of ^{14}C peaks by preparative HPLC. All these studies (47) confirmed the resolution of enantiomers and also established that diastereomeric salt effect due to optically active tartrate counter ion or the position of ^{14}C label, or the protonation of pyrrolidine nitrogen producing a second chiral centre under the pH used in the study, or some component of the cation exchange column/mobile phase, (0.06M NaOAc-MeOH, or MeOH-H_2O), were not responsible for the enantiomeric resolution.

Charles and Gil-Av (48) reported amplification of optical purity (o.p.) of certain peptides by chromatography on an achiral adsorbent without addition of a chiral reagent; they packed ordinary glass column (80x1.5 cm) with Kieselgur-60 (70-230 mesh, 40g/g sample) deactivated with 6% of water, and the optically active substrate was either a (–)-diamide of varying enantiomeric purity, or (+)- and (–)-N-trifluoroacetyl-valine-valine-cyclohexyl ester in various combinations by mixing (+) and (–) samples, or (–)-N-trifluoroacetyl-alanine-

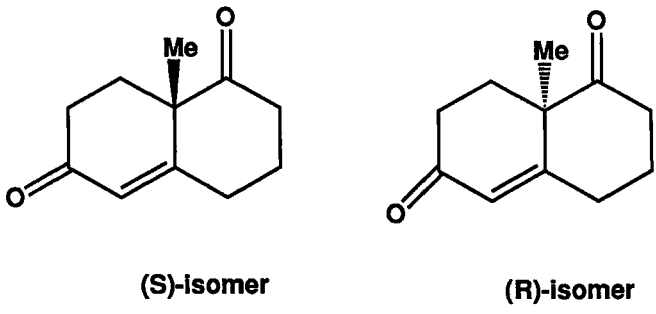

Figure 6. Structures of unsaturated diketone (49).

glycine isopropyl ester and its (±)-sample. The columns were eluted with different compositions of *n*-hexane-ethyl acetate; fractions collected showed maximum enrichment upto 99.8% for a single optical isomer. It was observed that overall depletion of one antipode in part of the eluates was equivalent to its enrichment in the others, as required by the mass balance.

Enantiomeric enrichment, by achiral phase chromatography, of a sample of an unsaturated diketone (**Fig.6**) with a non racemic mixture of its (R) and (S) enantiomers has been reported by Tsai *et al.* (49). The resolution was achieved on a simple silica gel column which was eluted with different compositions of hexane-EtOAc. Samples with varying enantiomeric compositions were analysed and it was found that the enrichment was neither due to racemization nor some decomposition on the chromatography column nor due to undetected impurities in the sample and the column.

The results of Tsai *et al.* (49) further confirm the suggestion made by Charles and Gil-Av (48) that simple chromatography may be a useful method for the enrichment of one of the

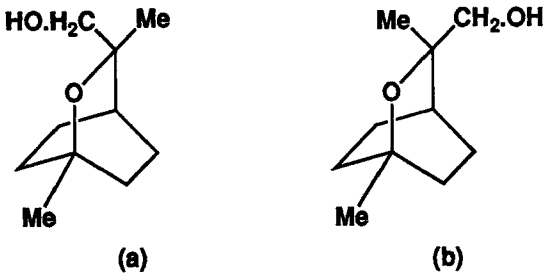

Figure 7. Structures of enantiomeric cineole metabolites.

enantiomers from a mixture in which that enantiomer is in excess, thereby making it possible to resolve racemates.

Recently optical fractionation of a partial racemic mixture of cineole metabolites (**Fig. 7a, 7b**) has been reported by Carman and Klika with achiral phase chromatography (50). For this purpose a preparative HPLC achiral silica column and hexane-ethyl acetate (1:1) were used. These cineole metabolites, though natural products, are also available as optically pure model compounds. Enantiomeric mixtures of varying composition (of 7a and 7b) were examined on a number of columns. The experiments were carried out to show that either spontaneous crystallization leading to inadvertent purification, or some mechanism providing interconversion on acidic silica during chromatographic process, or a column contaminated with a bound chiral compound were not responsible for enantiomeric enrichment.

In an another recent report thin layer chromatography has been claimed to resolve D- and L-lactic acid enantiomers in a totally achiral system (51); glass plates (5x10 cm) coated with silica gel (0.25 mm thick) were from Whatman and were dipped for 10 minutes in 1% *aq* solution of Cu(II) acetate

monohydrate, and then dried. ^{14}C-labelled D- and L-lactic acids were spotted singly and as a mixture, and the chromatogram was developed with dioxane-water (9:1,v/v); the enantiomers resolved nicely with distinct R_f values of 0.92 for D-lactic acid and 0.63 for L-lactic acid. The recovery in terms of the radioactivity as measured by liquid scintillation counter is reported to be 93% for D-, and 95% for L-isomer. The real separation was confirmed by the Boehringer enzymatic test for lactic acid (52) carried out on spots of D-, and L- forms scraped from the TLC plates; the test is based on the oxidation of L/D-lactic acid by NAD^+ to pyruvate and the amount of NADH formed is stoichiometric with the amount of D/L-lactic acid, which is determined spectrophotometrically. The recoveries of D-, and L-lactic acid enantiomers are reported to be 90% and 91% respectively. Thus the TLC procedure of Cecchi and Malaspina (51) can be considered to provide a simple, inexpensive practical method for the direct separation of lactic acid enantiomers as compared to GC and HPLC where GC requires preparation of a derivative *i.e* an ester by means of CH_2N_2 and salt by means of optically active bases, and for HPLC it is necessary to connect a special monitor for radioactivity. Though the complex formation with Cu^{2+} has been held responsible for resolution of enantiomers no efforts to identify or to estimate the difference in stabilities of complexes corresponding to D-, and L-isomers have been made.

EXPLANATIONS FOR RESOLUTIONS IN ACHIRAL SYSTEMS

Though the formation of complexes with D-, and L- isomers having different distribution coefficients and free energies of formation by ion exchange column chromatography (38) has been the reason for resolution of amino acid enantiomers in the achiral system, and the same can be attributed to achiral TLC resolution of DL-lactic acid (51), the real nature for

enantiomeric resolution in achiral systems remain to be established.

Enantiomeric enrichment effects have previously been observed in crystallization (46,53) leading to separation of pure antipodes from initially dissymetric materials (54), sublimation (55), and hydrolysis of peptides (56). The influence of molecular surroundings on enantiomers causing an enantiomeric enrichment effect has been explained by thermodynamically non-ideal behaviour of molecules *i.e* by molecular interactions. In most specific cases these interactions are thought to be associations (57,58).

Both on the basis of symmetry consideration as well as on the basis of a free energy argument it is evident that the molecular surroundings of a (+)-isomer in solution is different from the molecular surrounding of that (+)-isomer in a (±)-mixture (59); the implication of this difference in chiral surroundings can influence reaction rates and products ratios. For example, when the reaction between (+) and (+) takes place in *optically pure* (+), some interactions of the reacting molecules during the entire sequence are with other (+)-isomers (*homo*-interaction), if on the other hand the reaction between (+) and (+) takes place in a medium containing (–) as well (*i.e.* the enantiomeric mixture) then some of the interactions of the reacting molecules will be with (–) isomers (*cross*-interaction) and some with (+) isomers, as depicted below:

(+) + (+) or (–) + (–) = (+) (+) or (–) (–) ≡ *homo*-interaction
(+) + (–) or (–) + (+) = (+) (–) or (–) (+) ≡ *cross*-interaction

The thermodynamics of these interactions may or may not be the same but the proportion of *homo*-, or *cross*-interacted

reaction products (say dimers) would certainly be under statistical control.

In the achiral phase chromatographic resolutions, the differences in rates of migration *i.e.* partition coefficients, reported for the enantiomers, have been attributed to self association of the solutes as noted above. It is assumed (48) that self association occurs only in the adsorbed layer on the silica gel whereas in the eluent it is negligible due to H-bridging of the solute with its polar component, and *e.g.*, if L-isomer is in excess in the sample chromatographed formation of L,L-hydrogen bonded dimer is preferred over that of L,D-diastereomeric associates leading to the adsorbed layer enriched in the L-isomer; this means that the partition coefficient of the L-isomer between the stationary and the mobile phase will be larger than that of the D-isomer, and hence the first fractions will be depleted in the L-isomer. Correspondingly, if under the same set of assumptions the L,D-associate is formed preferentially, the relative rates of migration of the enantiomers will be reversed. Tsai *et al.* (49), also concluded that associations between molecules of the diketone (**Fig.6**) occur in relatively dense solutions and therefore enantiomeric enrichment effect become noticeable only at higher concentrations which cannot be excluded at the surface of the adsorbent. This view has further been supported by the studies made by Carman and Klika (50) who observed that the chromatography of more dilute fractions of the partial racemic mixtures of the cineole metabolites (**Fig. 7a, 7b**) afforded a less effective enantiomeric separation.

The resolution of ^{14}C-labelled nicotine enantiomer has also been considered on similar dimeric association that an association between two like enantiomers (say, ++) produces a dimer with slightly different properties than that one consisting of unlike (say, +–) enantiomers (47). Thus, with an excess of (–)-

nicotine both isomers of the radiolabelled material would be predominantly associated with the cold material and the activity due to radiolabelled (+)-nicotine would entirely be associated in *cross*-dimers, whereas that due to radiolabelled (–)-nicotine would entirely be in *homo*-dimers. These two dimers produce sufficiently unequal and different affinities for the achiral column/mobile phase and the two radiolabelled enantiomers get separated, by HPLC in this case. The converse applies when (+)-nicotine is the cold standard *i.e.* activity due to radiolabelled (+)-nicotine would predominantly be in *homo*-dimers, and that due to (–)-nicotine in *cross* -dimers. The behaviour of radiolabelled (±)-nicotine when injected alone or with cold racemic standard suggests that *homo*-association is preferred to *cross* -association.

The intermolecular H-bridging may indeed be a factor for most of the enantiomeric enrichment effects because it can cause molecular associations already in relatively dilute solutions, other associations such as due to van der Waals forces cannot be ruled out (49).

Since no evidence to confirm such dimeric associations have been provided these explanations of the phenomenon remain purely speculative. Nevertheless, amplification of optical activity of initially enriched samples involving an achiral adsorbent is not excluded theoretically and its feasibility is confirmed by such reports. Amplification of the optical activity of non-racemic mixtures in an achiral system further gets support from the stereoselective adsorption of racemic amino acids from their *aq* solutions on clay (60), though certain assumptions have to be made to explain the formation of chiral sites on clay.

Some support to the phenomenon of chiral recognition based on dimeric associations can be taken into consideration

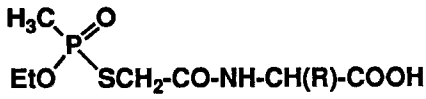

Figure 8. P-ethoxy-P-methyl phosphinothioylthioglycolloylvaline, (R= *i*- C3H7).

from the calorimetric studies of the interaction among enantiomeric α-amino acids. Recent reports (61, 62) on such studies showed that values for enthalpic interaction coefficients for *homo*-, and *hetero*-, (h_{DD} or h_{LL}, and h_{DL} or h_{LD}) interactions were different and varied with alkyl chain length. For leucine the value of enthalpic coefficient for *hetero*-interaction (h_{DL}) was smaller than the value for *homo*-interaction (h_{LL}). Such a difference in the enthalpic coefficients clearly indicates that chiral recognition occurs.

Differences between the NMR spectra of racemates and pure enantiomers (57,63-64) of certain optically active compounds, *e.g.* dihydroquinone(64), have been reported; whenever there is an excess of one enantiomer in a sample the NMR spectrum splits with a relative intensity corresponding to the proportion of that enantiomer in the mixture, and as the sample approaches racemic composition the signals of each enantiomer become equal in intensity and superimpose. The NMR spectrum of RR and SS enantiomers of P-ethoxy-P-methyl phosphinothioyl-thioglycolloylvaline (**Fig.8**) in chloroform without any chiral shift reagent provided interesting results (65) and supporting explanations to the assumption of the molecular association.

The NMR of individual enantiomers showed a narrow singlet signal and that of a racemic mixture showed a single singlet at a position different by 0.35 ppm, while the nmr spectrum with

the concentration ratio of (RR) : (SS) = 7:3 showed two signals whose integral intensities were in the ratio of 7:3; an identical spectrum was observed with concentration ratio of 3:7. It was considered that every molecule in association complex is surrounded solely by identical molecules and rapid exchange takes place between the complexes so narrow singlet signals are observed in solutions of individual enantiomers; in a solution of racemic mixture the molecules of each enantiomer are surrounded both by identical and antipodal molecules with rapid exchange resulting into molecular associations providing *homo*-interactions or *cross*-interactions at each enantiomer- this changes the resultant local field at the indicator nucleus of the enantiomer molecule and hence a chemical shift of the nmr signal, but because the situations for the two enantiomers have a mirror image relation the chemical shifts of their signals are identical and they merge into a single signal. When the concentration of antipodes in the solution are unequal they exhibit different extents of *homo*-, or *cross*- interactions; if *homo*-association predomonates at one antipode, *cross*-association predominates at the other, and the situations are not now those of mirror images resulting into a doublet in the spectrum with intensity ratio equal to the concentration of ratio of antipodes.

Thus the appearence of an elementary diastereomeric doublet in the spectra of solutions of unequal concentrations of antipodes is due to non-equivalence of the stereochemical environment of antipodal molecules under conditions of statistical control with rapid exchange between association complexes. This non-equivalence is caused by the unequal concentration of antipodes. It depends also on energy parameters of association but may arise even in the absence of energy differences for identical and diastereomeric contacts.

CONCLUSION

Chromatography, specially the liquid chromatography, has provided successful methods for direct resolution of a variety of enantiomers by using chiral stationary or mobile phase. At the same time chromatography has been successful in resolving enantiomers with both stationary and mobile phases being achiral. Such achiral phase resolutions could potentially be developed into sensitive methods for the detection of small quantities of (+) or (-) enantiomer in biological fluids, resolution of a number of racemic radiolabelled compounds, and without the involvement of an extra compound *i.e.* a chiral selector. The direct liquid chromatographic enantiomeric resolution methods have relieved of derivatization problems and the enantiomeric resolutions by achiral phase chromatography would relieve from the limitations posed by the choice and availability of chiral selector for a particular resolution problem.

In view of the observations made by Carman and Klika (50) it looks appropriate to take into consideration the problems of optical non-purity, and of enantiomeric enrichment, during purification of natural products and the analysis of products of assymetric syntheses. The experimental data pertaining to the resolution of enantiomers from non-racemic mixtures can be considered as affirmative in view of the theoretical justification and evidence provided by the NMR spectroscopy while the results for resolution of racemic mixtures in achiral chromatographic systems do not go along these justifications. One possibility for the resolution of enantiomers from *these racemic mixtures* could be that these samples were non-racemic and were inadvertently considered to be racemic. Different kinds of explanations follow a significant experimental observation! Therefore extensive studies to obtain stronger evidences to explain the chiral resolutions in achiral systems are required.

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