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Summary
The general principles of resolution of enantiomeric species using chiral HPLC systems are briefly summarized, including an extended interpretation of the three-point interaction model and the postulate of reciprocity of chiral recognition. The dependence of chromatographic resolution on the enantiomeric purity of the chiral selector is discussed as well as the manifestation of a possible configurational instability of the chiral selector and solutes. HPLC systems exploiting chiral stationary phases (CSP), chiral coated phases (CCP), and chiral mobile phases (CMP) are compared with respect to their most-appropriate application areas. Selected representative examples of chiral resolutions are given.

Introduction
A remarkably rapid growth of the field of direct chromatographic resolution of enantiomeric species, which probably started about 20 years ago in GC with the introduction of highly efficient capillary columns containing chiral acylamino ester-type stationary phases by Gil-Av et al. [1] and, in LC, with the introduction of highly selective chiral ligand-exchange-type phases by Davankov and Rogozhin [2–3], resulted in a whole series of important theoretical and practical achievements. In addition to Pasteur's classical principles of resolving racemic mixtures into constituent enantiomers, new stereochemical concepts have been formulated and proven experimentally. Some understanding of the mechanisms of intimate intermolecular interactions between solute enantiomers and functional groups of the stationary phase in many chromatographic systems, e.g. ligand-exchanging and charge-transfer-complexing ones, has been gained. Experimental possibilities in biochemical and pharmaceutical research for the determination of the enantiomeric purity of chiral compounds, for the preparation of thousands of new chiral xenobiotics of an unprecedented purity and for the study of metabolic pathways of chiral drugs in living organisms have been dramatically increased. Due to these advances the number of research groups dealing with optically active compounds has multiplied, resulting in a real information explosion at the borders of chromatography with stereochemistry, pharmacology, biochemistry.

Dozens of reviews (e.g. Ref. [4–18]) discuss numerous successful resolutions of enantiomeric compounds in diverse chiral HPLC systems. Therefore, in the present review it is more appropriate to concentrate on the general stereochemical aspects of functioning within chiral HPLC systems.

General Principles of Chiral Chromatographic Separations

1. Thermodynamics of Chiral Resolution
Enantiomeric substances, though consisting of non-superimposable molecular species, display identical* physical and chemical properties both in the gaseous and condensed states as well as in solution. As a basic principle only chiral molecular structures (or chiral irradiation) can distinguish between two enantiomers. Thus, enantiomeric resolutions are only feasible in chromatographic systems that contain an appropriate chiral selector.

* The violation of parity by the weak interactions results [19] in that enantiomeric molecules have inequivalent energies. Thus, L-amino acids appear to be slightly stabilized, by an energy increment of the order of 10^{-14} J/mol, with respect to the D-enantiomers, corresponding to the enantiomeric excess of 10^{-6}% of the L-amino acid in the racemic mixture standing at a thermodynamic equilibrium.
The chiral selector can either be incorporated in the stationary phase — via covalent bonds to the sorbent matrix ("Chiral Stationary Phase", CSP) or permanent adsorption onto the sorbent surface ("Chiral Coated Phase", CCP) — or it can be added to the eluent ("Chiral Mobile Phase", CMP).

The difference in the interaction of the chiral selector with the two enantiomers under resolution is called enantioselectivity [2, 20]. In chromatography we only deal with thermodynamic enantioselectivity effects, i.e. formation of labile diastereomeric adducts AB_R and AB_S of the chiral selector A with the enantiomers B_R and B_S of the solute B, the adducts differing in their stability (in the case of CSP and CCP) and/or in their interphase distribution ratio (in the case of CMP). This difference in stability constants, K_R and K_S, of the two diastereomeric adducts relates to the difference in their free energy, ΔΔG, and the column enantioselectivity, α, through a simple exponential expression

\[ \Delta\Delta G = -RT \ln(K_R/K_S) \approx -RT \ln\alpha \]

A minor thermodynamic enantioselectivity of ΔΔG = 0.024 kJ/mol, corresponding to an α value of 1.01, would already suffice for many enantiomeric pairs to be resolved using modern chromatography techniques. With rising ΔΔG values, the column selectivity rises exponentially. If one could double ΔΔG one would increase α to the square of its initial value.

Pirkle and Pochapsky [21] demonstrated generation of extreme selectivity in chiral recognition on the basis of the above considerations. On a chiral stationary phase of the following structure

\[
\begin{align*}
C_{10}H_7-NH \text{--COO}(CH_2)_{11}-Si & \text{--CH}_3 \\
& (O_2N)_{2}C_6H_3CO-NH \text{--CO-NH-(CH}_2)_6CH_3
\end{align*}
\]

the authors observed resolution of N-(3,5-dinitrobenzoyl)-leucine n-hexylamide

\[
\begin{align*}
& H \text{--CH}_2CH(CH_3)_2 \text{--CH}_2CH(CH_3)_2 \\
& (O_2N)_{2}C_6H_3CO-NH \text{--CO-NH-(CH}_2)_6CH_3
\end{align*}
\]

with a selectivity value of α = 10.5 (in 30% 2-propanol in hexane). This selectivity corresponded to stronger binding of the S-solute by a value of ΔΔG = 5.93 kJ/mol. When a bis-solute was prepared that contained two asymmetric centers

\[
\begin{align*}
& H \text{--CH}_2CH(CH_3)_2 \text{--CH}_2CH(CH_3)_2 \text{--CH}_2CH(CH_3)_2 \text{--CH}_2CH(CH_3)_2 \\
& (O_2N)_{2}C_6H_3CO-NH \text{--CO-NH-(CH}_2)_6NH-OC \text{--CO}-C_6H_3(NO_2)_2
\end{align*}
\]

situated at a distance that would allow simultaneous interaction of the bis-solute with two chiral sorption sites of the bonded phase, the value of ΔΔG was doubled. Indeed, selectivity of the column with respect to the SS- and RR-enantiomers of the bis-solute was observed to rise to the square of the initial value and reach the record value of α = 121.

As a thermodynamic quantity, the stability constant, K, of each of the two diastereomeric sorption complexes should be temperature dependent: -RT ln K = ΔG = ΔH - TΔS. This leads [22, 23] to a linear dependence of the enantioselectivity ΔΔG and lnα on the inverse of temperature 1/T, as shown in Fig. 1. It is important to note, that the resolution selectivity may both fall or rise with rising temperature, depending on the position of the characteristic temperature, T_inv, at which the stability constants of the two diastereomeric sorption complexes appear equal and, consequently, the resolution vanishes. On passing this temperature the sign of the enantioselectivity effect should reverse [23]. Such an inversion of elution order of two enantiomers has been observed in GC experiments by Watanabe et al. [24] and more recently by Koppenhoefer et al. [25] and Schurig et al. [26, 27]. (The latter authors discuss the competition of two complexation mechanisms as an alternative to the above thermodynamic explanation of the enantioselectivity inversion). In liquid chromatography, reversal of the elution order of 2,4,2',4'-tetrahydroxy-6,6'-dimethyl-biphenyl enantiomers from a starch column on raising the temperature from 20 to 59°C was reported [28].

Usually, the temperature range accessible in LC is too small to allow the enantioselectivity inversion temperature to be located experimentally. As a rule, the selectivity falls with increasing temperature, which is especially remarkable in the case of the crown ether-incorporating CSP "Crownpack CR". There is also one example described, namely, the resolution of racemic N-benzylproline on the Cu(II) form of a L-proline-containing polystyrene-type ligand exchanger, where higher column temperatures resulted in increased α-values [29]. It was shown [30] that the enantioselectivity of bis-(N-benzyl prolinato)copper formation is governed by the entropic contribution abnormally dominating over the enthalpic one.

2. Enantiomeric Purity of the Chiral Selector

In all the classical Pasteur procedures for resolving racemates an insufficient enantiomeric purity of the chiral selector necessarily led to an equivalent loss in the enantio-