Journal of Chromatography, 452(1988) 309–316
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 458

ENANTIOSELECTIVITY OF COMPLEX FORMATION IN LIGAND-EXCHANGE CHROMATOGRAPHIC SYSTEMS WITH CHIRAL STATIONARY AND/OR CHIRAL MOBILE PHASES

V. A. DAVANKOV*, A. A. KURGANOV and T. M. PONOMAREVA
Institute of Organo-Element Compounds, USSR Academy of Sciences, 117813 Moscow (U.S.S.R.)

SUMMARY

Equations are derived describing the retention and separation selectivity of two enantiomeric species in a chromatographic system containing a chiral selector in the stationary and/or in the mobile phase. It is shown that the total column enantioselectivity generally differs from the enantioselectivity of the selector–selectand interaction in solution. In chiral chromatographic systems, there are significant deviations from the principal of reciprocity of mutual chiral selector–selectand recognition.

INTRODUCTION

Impressive achievements of ligand-exchange chromatography (LEC) in separating optical isomers, which have been analysed in two successive reviews[1,2] and the book by Davankov *et al.*[3], have led to the elaboration of three general types of chiral LC systems that employ (i) chiral (bonded) stationary phases (CSPs)[4], (ii) chiral coated stationary phases (CCSPs)[5] and (iii) chiral mobile phases (CMPs)[6,7] respectively. In the last case the chiral selector of the chromatographic system can either largely remain in the mobile phase or partition between the mobile and stationary phases.

The principal interaction mode between two solute enantiomers, A_R and A_S, to be separated and the chiral selector, B, in LEC is the formation of ternary, mixed-ligand complexes with a transition metal cation, M. The two labile ternary complexes formed, A_RMB and A_SMB, are diastereomeric and, therefore, may differ in their thermodynamic stability constants:

$$\beta_{A_RMB} = \frac{[A_RMB]}{[A_R]\,[M]\,[B]} \text{ and } \beta_{A_SMB} = \frac{[A_SMB]}{[A_S]\,[M]\,[B]}$$

The ratio of the two constants, $\alpha^* = \beta_{A_RMB}/\beta_{A_SMB}$, is a convenient quantitative measure for the enantioselectivity of the complexation reaction.

Enantioselectivity of labile complex formation in homogeneous solutions was first demonstrated in nickel(II) bis-histidinato complexes[8] and in copper(II) bis complexes with bidentate α-amino acid ligands[9] and 1,2-diamine-type ligands[10].

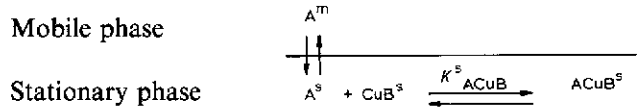
0021-9673/88/$03.50

Several attempts were made to correlate enantioselectivity, $\alpha = k'_{A_R}/k'_{A_S}$, of chiral LEC systems with the enantioselectivity, $\alpha^* = \beta_{A_RMB}/\beta_{A_SMB}$, of complex formation in homogeneous solutions containing the solute enantiomers, A_R and A_S, metal ions, M, and a ligand, B, which would simulate the structure of the chiral selector. Though some chiral polystyrene-type ligand-exchange resins are known for which a qualitative relationship $\alpha \approx \alpha^*$ has been found[1,2,11], such an agreement turned to be an exception, rather than a general rule[12].

It is the purpose of the present paper to analyse theoretically the role of enantioselectivity of ternary complex formation in the discrimination of enantiomers in chiral LEC systems.

CHIRAL STATIONARY PHASES

Independent of whether the chiral selector B is covalently bonded to the sorbent matrix or is permanently adsorbed onto the surface of the packing, the CSP and CCSP systems are very similar in that the chiral selector B and its diastereomeric mixed-ligand complexes with the solute enantiomers A_R and A_S are always located in the stationary phase, whereas the mobile phase only transports the enantiomeric solute species. Supposing A and B are bifunctional amino acids and M is copper(II), a whole series of dissociation and complexation equilibria would be established in the two-phase system. However, the processes that are responsible for the retention and chiral recognition of the solute molecules can be reduced to the following simple scheme



(1)

where the superscripts m and s denote the location of the species in the mobile and stationary phases, respectively.

The capacity factor of the solute amino acid A is related to its adsorption and complexation through

$$k'_A = \varphi \cdot \frac{[A^s] + [ACuB^s]}{[A^m]} \tag{2}$$

where φ is the phase ratio. With the aid of the equilibrium constant, K^s_{ACuB}, of the ternary complex formation reaction 1, eqn. 2 can be written as:

$$k'_A = \varphi \cdot \frac{[A^s] + K^s_{ACuB}[A^s]\,[CuB^s]}{[A^m]}$$

$$= \varphi \cdot \frac{A^s}{A^m} \cdot (1 + K^s_{ACuB}[CuB^s])$$

$$= k''_A(1 + K^s_{ACuB}[CuB^s]) \tag{3}$$

Eqn. 3 is a rather fundamental one. Here, k''_A is the capacity factor of solute A in the absence of complexation reactions ($[CuB^s] = 0$ or $K^s_{ACuB} = 0$); $[CuB^s]$ represents the concentration of chiral sorption sites and K^s_{ACuB} the formation constant of the solute enantiomer–chiral selector adduct in the stationary phase. According to eqn. 3, an increase in both the chiral selector concentration and its complexing ability would result in an enhanced solute retention.

The enantioselectivity of the column is given by

$$\alpha = \frac{k'_{A_R}}{k'_{A_S}} = \frac{k''_{A_R}}{k''_{A_S}} \cdot \frac{1 + K^s_{A_RCuB}[CuB^s]}{1 + K^s_{A_SCuB}[Cub^s]}$$

$$= \frac{1 + K^s_{A_RCuB}[Cub^s]}{1 + K^s_{A_SCuB}[CuB^s]} \tag{4}$$

since the retention of the enantiomers in the absence of complexation reactions with the chiral selector B is identical, *i.e.*, $k''_{A_R} = k''_{A_S}$.

It is obvious that the enantioselectivity of the CSP chromatographic system approaches the value of

$$\alpha \leqslant K^s_{A_RCuB}/K^s_{A_SCuB} \tag{5}$$

if the non-specific adsorption of the solute enantiomers is negligible compared to the complexation reaction with the chiral selector, *i.e.*, $[A^s] \ll [ACuB^s]$ in eqn. 2. Thus, the maximum value of the chiral column enantioselectivity is generally given by the enantioselectivity of the complex formation process in the CSP:

$$\alpha \leqslant \alpha^*, \text{ where } \alpha^* = K_{A_RCuB}/K_{A_SCuB} = \beta_{A_RCuB}/\beta_{A_SCuB}$$

This conclusion is valid for all types of CSPs, not just the ligand-exchange one.

Equations that are similar to 3 and 4 were earlier derived by Feibush *et al.*[13].

CHIRAL MOBILE PHASES

Two cases should be considered here: one with the chiral selector B always remaining in the mobile phase and the other with the selector B partitioning between the two phases.

In the first case, the theoretical treatment very much resembles that of a CSP system:

Mobile phase $A^m + CuB^m \rightleftharpoons ACuB^m$ (K^m_{ACuB})

Stationary phase A^s (6)

$$k'_A = \varphi \cdot \frac{[A^s]}{[A^m] + [ACuB^m]} = \varphi \cdot \frac{[A^s]}{[A^m] + K^m_{ACuB}[A^m][CuB^m]}$$

$$k''_A(1 + K^m_{ACuB}[CuB^m])^{-1} \tag{7}$$

The retention reaction 6 obviously diminishes the solute retention. However, the stronger the complexation, the higher is the enantioselectivity of the CMP system, as follows from eqn. 8:

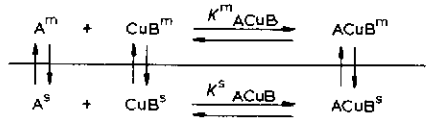
$$\alpha = \frac{k'_{A_R}}{k'_{A_S}} = \frac{k''_{A_R}}{k''_{A_S}} \cdot \frac{1 + K^m_{A_R CuB}[CuB^m]}{1 + K^m_{A_S CuB}[CuB^m]}$$

$$= \frac{1 + K^m_{A_R CuB}[CuB^m]}{1 + K^m_{A_S CuB}[CuB^m]} \tag{8}$$

The limiting enantioselectivity of a CMP is, again, given by the enantioselectivity, α^*, of the complexation reaction 6 with the principal difference from the situation with a CSP column being that the chiral selector B now produces an inversed elution order of the solute enantiomers.

More complex is a CMP chromatographic system where the chiral selector B and its complexes reside both in the mobile phase and stationary phases:

Mobile phase



Stationary phase (9)

$$k'_A = \varphi \cdot \frac{[A^s] + [ACuB^s]}{[A^m] + [ACuB^m]} = \varphi \cdot \frac{[A^s] + K^s_{ACuB}[A^s][CuB^s]}{[A^m] + K^m_{ACuB}[A^m][CuB^m]}$$

$$= k''_A \cdot \frac{1 + K^s_{ACuB}[CuB^s]}{1 + K^m_{ACuB}[CuB^m]} \tag{10}$$

According to eqn. 10, complexation reactions in the stationary phase enhance the solute retention, whereas the complexation in the mobile phase facilitates the elution.

The enantioselectivity of the system is now a complex function of the phase distribution of the chiral selector and the enantioselectivity of the latter in the mobile and stationary phases:

$$\alpha = \frac{k'_{A_R}}{k'_{A_S}} = \frac{k''_{A_R}}{k''_{A_S}} \cdot \frac{(1 + K^s_{A_R CuB}[CuB^s])\,(1 + K^m_{A_S CuB}[CuB^m])}{(1 + K^m_{A_R CuB}[CuB^m])\,(1 + K^s_{A_S CuB}[CuB^s])}$$

$$= \frac{1 + K^s_{A_R CuB}[CuB^s]}{1 + K^s_{A_S CuB}[CuB^s]} \Big/ \frac{1 + K^m_{A_R CuB}[CuB^m]}{1 + K^m_{A_S CuB}[CuB^m]} \tag{11}$$

In the case that the formation constants of ternary complexes are sufficiently high, the enantioselectivity, α, of the chromatographic system is, roughly, given by the ratio of the complexation enantioselectivities in the stationary and mobile phases:

$$\alpha = \frac{K^s_{A_R CuB}}{K^s_{A_S CuB}} \Big/ \frac{K^m_{A_R CuB}}{K^m_{A_S CuB}} = \alpha^*_s/\alpha^*_m$$

ENANTIOSELECTIVITY OF REAL CHROMATOGRAPHIC SYSTEMS

A most important question is whether a rational design of useful chiral chromatographic systems is possible based on experimental data on stereochemical relationships between an appropriate chiral selector and a certain type of racemic compounds which interact in an homogeneous solution; *vice versa*, what kind of information concerning the stereochemistry of a selector–selectand interaction can be inferred from data on occasional successful resolution of a racemic compound in a chiral chromatographic system?

From the above considerations, one can conclude that an immediate relationship between the enantioselectivity of a real chromatographic system and that of an homogeneous solution of a model compound only exists in a single case, namely that with the chiral selector residing entirely in the mobile phase. In this case, the column enantioselectivity, α, approaches the value, α^*, for the selector–selectand interaction. The solute enantiomer, which binds most strongly to the chiral selector, is eluted first and, according to eqn. 7, the solute–selector association constant, K^m_{ACuB}, can be easily estimated from the linear relationship between $(k'_A)^{-1}$ and the selector concentration, $[CuB^m]$, in the mobile phase. Unfortunately, the situation when both the chiral selector and its complexes with the solute enantiomers, A_RCuB and A_SCuB, remain entirely in the mobile phase and are not adsorbed onto the column packing is seldom realized. A rare example of this type of chromatographic systems is the chiral resolution of hydrophobic solutes using a reversed-phase (RP) column in combination with a polar eluent that is modified with cyclodextrin[14]. This chiral selector, as well as its inclusion complexes with the solute enantiomers, appear to have but a minimum affinity for the RP packing.

With chiral stationary phases, too, it is sometimes possible to determine the solute–selector association constant and the enantioselectivity of the association. Thus, in the case of ligand-exchange bonded phases, one can vary the copper(II) content from zero to the maximum value of the chiral selector concentration in the stationary phase and then treat the retention parameters according to eqns. 3 and 4. With other types of CSPs, k'_A and K^s_{ACuB} are less readily available, since the sorption site concentration cannot be varied. Another general problem with chiral sorbents is the selection of adequate low-molecular-mass models for the chiral sorption site in the stationary phase, which would simulate in solution all the interactions with the solute enantiomers that are contributing to chiral recognition of the latter in the column. Obviously, this problem does not arise with cyclodextrins and α_1-acid glycoprotein, where the solute molecule appears to be completely enveloped in or, respectively, adsorbed on the rigid surface of the chiral resolving agent. The solute interactions with these chiral selectors are expected to remain unchanged, independent of whether the selector is dissolved or chemically bonded to an insoluble matrix. It is, probably, not difficult to find adequate soluble chiral selector models for Pirkle's type chiral phases, especially when the selector molecule provides all three distinct interaction sites with the solute molecule, which are required for chiral recognition of the latter. The situation becomes much more complicated with the majority of ligand-exchange phases with bonded chiral amino acid type ligands. Here, spacer fragments have to be included in the structure of the selector model, as is the case with N-benzyl-L-proline

taken as a model for the L-proline-incorporating polystyrene type chiral resin[1–3,11]. Finally, only oversimplified models can be designed for coated chiral stationary phases where the solid sorbent surface appears to play a decisive role in the chiral recognition of enantiomers by taking an immediate part in the formation of diastereomeric sorption complexes, as we have shown earlier[5,12].

At least for the time being, the situation with chiral eluent additives distributed between the mobile and stationary phases seems impossible to resolve.. Here, according to eqn. 9, a whole series of labile equilibria exist, each contributing to the overall enantiomeric resolution of solutes on the chromatographic column. Most striking is the conclusion, eqn. 12, that the selector–selectand interaction selectivities in the mobile and stationary phases exert opposite effects on the total resolution. We can arrive at the same conclusion by a purely logical approach: in order to produce maximum chiral discrimination of two enantiomers in a chromatographic column, the chiral selector should bind more strongly and transport more rapidly one enantiomer in the mobile phase, but bind more strongly and retain more strongly the opposite enantiomer when in the stationary phase. This idea has already been exploited by combining a chiral bonded phase with a mobile phase containing the chiral selector of identical chemical structure, but opposite in configuration[15,16].

Be this as it may, a chiral eluent system appears more productive if the solute–selector interaction enantioselectivity changes significantly on transferring the selector–selectand adducts from the solution to the stationary phase. Indeed, the relative stability of the two diastereomeric adducts on the sorbent surface changes dramatically, compared to that in the bulk solution, which makes the CMPs a general powerful approach to chromatographic chiral separations.

However, with a CMP system, it would be extremely difficult to tell which one of the complexing and paritioning equilibria 9 makes the desive contribution to the overall enantioselectivity of the chromatographic column. Moreover, due to the complexity of equilibria 9, CMP systems appear to be much more flexible compared to CSP systems in that changing the chromatographic conditions (pH of the eluent, type and concentration of organic modifiers and inorganic salts) significantly influences the solute retention in both the CMP and CSP systems, but, in the former case, the resolution enantioselectivity would also change dramatically, in addition to the retention parameters.

That the total enantioselectivity of CMP systems is not directly related to the enantioselectivity of the selector–selectand interaction in solution can best be demonstrated by the invalidity of the principals of chiral recognition recirocity in these systems.

RECIPROCITY OF CHIRAL RECOGNITION

Chiral recognition is expected to be reciprocal in that if a chiral resolving agent B_R can distinguish between A_R and A_S, then selector A_R may distinguish B_R from B_S.

In LEC of the solute enantiomers A_R and A_S according to the CMP mode, a copper(II) complex of the chiral ligand B_R is added to the eluent. Two diastereomeric ternary complexes, A_RCuB_R and A_SCuB_R, are formed both in the mobile and stationary phases. These complexes can differ in their stabilities and/or phase

TABLE I

ENANTIOSELECTIVITY, $\alpha = k'_R/k'_S$, FOR THE RESOLUTION OF AMINO ACIDS USING A LICHROSORB RP-18 COLUMN AND AN AQUEOUS ACETONITRILE ELUENT MODIFIED WITH 10^{-3} *M* BIS(*S*-AMINO ACIDATO)COPPER(II)COMPLEX

Chiral eluent	*Racemic solute*	k'_R	k'_S	α_1	α_2
S-Pro	Hyp	1.03	2.33	0.44	
S-Hyp	Pro	2.06	3.25		0.63
S-Pro	aHyp	0.80	0.80	1.00	
S-aHyp	Pro	1.37	0.85		1.47
S-Hyp	aHyp	0.82	0.82	1.00	
S-aHyp	Hyp	0.52	0.32		1.63
S-aHyp	BzlaHyp	12.33*	37.89*	0.33	
S-BzlaHyp	aHyp	1.42*	1.17*		1.21
S-BzlPro	Pro	8.38*	2.29*	3.65	
S-Pro	BzlPro	42.50*	27.67*		1.59
S-BzlaHyp	Pro	7.67*	1.75*	4.40	
S-Pro	BzlaHyp	65.16*	19.16*		3.43

* 4% acetonitrile in the eluent.

distributions, which should result in the enantiomers A_R and A_S arriving at the detector cell separately, with an enantioselectivity, α_1, of the chromatographic system.

If now the chiral selector A_R is allowed to play the rôle of the chiral additive to the mobile phase with the aim of resolving racemic solute $B_{R,S}$, then diastereomeric complexes A_RCuB_R and A_RCuB_S should form in the system. Again, these diastereomeric species may behave differently, thus delivering enantiomers B_R and B_S to the detector cell with a total enantioselectivity of α_2.

It is important to note that one of the diastereomeric complexes, namely, A_RCuB_R, appears in both chromatographic systems considered. The two other species, A_SCuB_R and A_RCuB_S, are enantiomeric, *i.e.*, identical in all their properties, including stability and phase distribution. This implies that the two chromatographic systems should produce identical enantioselectivity values, $\alpha_1 = \alpha_2$, exactly as required by the reciprocity rule.

Table I presents enantioselectivity values, $\alpha = k'_R/k'_S$, for chromatographic resolutions of a series of racemic amino acids on a LiChrosorb RP-18, 5-μm colmn using water–acetonitrile eluents containing chiral bis(amino acidato)copper complexes (10^{-3} *M*). Each amino acid appears twice in the table, as a racemic solute to be resolved and as a chiral selector added to the eluent. Accordingly, each pair of amino acids produces two values of the resolution enantioselectivity, α_1 and α_2.

Contrary to the requirements of the above reciprocity rule, the two corresponding enantioselectivity values never coincide. Most striking is the situation with the pair *allo*-hydroxyproline–N-benzyl-*allo*-hydroxyproline. If Cu(*S*-aHyp)$_2$ is present in the eluent as the chiral selector, then the ternary complex Cu(*S*-aHyp)

(*S*-BzlaHyp) is the most strongly retained diastereomeric species, so that the elution sequence of BzlaHyp enantiomers is *S* after *R* with $\alpha_1 = 0.33$. If now Cu(*S*-BzlaHyp)$_2$ is added to the eluent, the elution order of the enantiomers of aHyp is observed to be *S* ahead of *R*, $\alpha_2 = 1.21$, which implies that the complex Cu(*S*-aHyp) (*S*-BzlaHyp) is less strongly retained than its diastereomer. This situation can be understood only with the assumption[17] that, after conditioning the column with the corresponding chiral eluents, the hydrophobic complex Cu(*S*-BzlaHyp)$_2$ covers the RP packing material much more densly than is the case with the hydrophilic complex Cu(*S*-aHyp)$_2$. Therefore, the phase partitioning conditions for the two diastereomeric ternary complexes formed differ drastically in the two chromatographic systems concerned, producing opposite signs of the total enantioselectivity effects, *i.e.*, inversed elution order of the amino acid enantiomers.

CONCLUSION

Enantioselectivity of chiral chromatographic systems appears to be a complex function of the enantioselectivity effects of the selector–selectand adduct formation in both the mobile and stationary phases, as well as of the phase distribution of these adducts, unless the chiral selector resides entirely in one of these phases. The microenvironment of the diastereomeric adducts in the stationary phase can influence significantly the association enantioselectivity. For these reasons, the reciprocity relationships for mutual chiral selector–selectand recognition, which are known to be valid for their association in solutions as well as for diastereomeric salt crystallization, do not necessarily hold for chiral chromatographic systems.

REFERENCES

1 V. A. Davankov, *Adv. Chromatogr. (N.Y.)*, 18 (1980) 139.
2 V. A. Davankov, A. A. Kurganov and A. S. Bochkov, *Adv. Chromatogr. (N.Y.)*, 22 (1983) 71.
3 V. A. Davankov, J. D. Navratil and H. F. Walton, *Ligand Exchange Chromatography*, CRC Press, Boca Raton, FL, 1988.
4 S. V. Rogozhin and V. A. Davankov, *Dokl. Akad. Nauk SSSR*, 192 (1970) 1288.
5 V. A. Davankov, A. S. Bochkov, A. A. Kurganov, P. Roumeliotis and K. K. Unger, *Chromatographia*, 13 (1980) 677.
6 P. E. Hare and E. Gil-Av, *Science (Washington, D.C.)*, 204 (1979) 1226.
7 J. N. LePage, W. Lidner, G. Davies, D. E. Seitz and B. L. Karger, *Anal. Chem.*, 51 (1979) 433.
8 C. C. McDonald and W. D. Phillips, *J. Am. Chem. Soc.*, 85 (1963) 3736.
9 V. A. Davankov, S. V. Rogozhin and A. A. Kurganov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1971) 204.
10 A. A. Kurganov, T. M. Ponomareva and V. A. Davankov, *Inorg. Chim. Acta*, 86 (1984) 145.
11 V. A. Davankov and P. R. Mitchell, *J. Chem. Soc. Dalton Trans.*, (1972) 1012.
12 V. A. Davankov and A. A. Kurganov, *Chromatographia*,17 (1983) 686.
13 B. Feibush, M. J. Cohen and B. L. Karger, *J. Chromatogr.*, 282 (1983) 3.
14 J. Debowski, D. Sybilska and J. Jurczak, *Chromatographia*, 16 (1982) 198.
15 M. Fujita, Y. Yoshikawa and H. Yamatera, *Chem. Lett.*, 11 (1975) 473.
16 C. Pettersson and C. Gioeli, *J. Chromatogr.*, 435 (1988) 225.
17 A. A. Kurganov, T. M. Ponomareva and V. A. Davankov, *6th Int. Symp. Capillary Chromatography, Riva del Garda, 1985*, p. 871.