# NEW INSIGHTS INTO THE RECOGNITION MECHANISMS OF CHIRAL "BRUSH-TYPE" LIQUID CHROMATOGRAPHIC STATIONARY PHASES

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SUMMARY

It is shown that in some cases the adsorption complex of the enantiomer eluted first is of importance in rationalizing the differences in enantioselectivities between structurally related chiral stationary phases. It is helpful to define the relative capacity factor, $k'^* = k'$(chiral phase)/$k'$(non-chiral phase) and $k'_{A/B} = k'$(chiral phase A)/$k'$(chiral phase B) for each enantiomer of interest.

INTRODUCTION

Chiral stationary phases (CSPs) have proved to be a very useful tool for analytical and preparative separation of enantiomers by liquid chromatography. Chiral recognition mechanisms have been proposed for the various types of CSPs including the "brush-type" phases which consist of silica with covalently bonded chiral functional groups. In these discussions generally a complex between the CSP and the more strongly retained enantiomer is suggested[1–5]. The other enantiomer is assumed to be eluted first because it exhibits a poorer fit with the chiral moiety of the CSP, an idea which is not discussed further. In some cases a rather detailed insight into the CSP–sample complex was obtained which allows assessment of the individual interactions[6–10]. Non-enantioselective interactions with silanol groups can be important[11]. Comparisons with gas chromatographic investigations have been made[12].

We propose to describe the retention of chiral compounds on a "brush-type" CSP, expressed as the capacity factor, $k'$, as proportional to the sum of the non-chiral and chiral interactions (the absolute configuration of the CSP must be known, *e.g.*, R)

$$k'_R = k'_1 \sim \Sigma \text{ interactions with non-chiral sites} + \Sigma \text{ interactions of l complexes}$$
$$k'_S = k'_u \sim \Sigma \text{ interactions with non-chiral sites} + \Sigma \text{ interactions of u complexes}$$

where l and u, meaning like and unlike[13], respectively, describe the relative configuration of the diastereomeric adsorption complex built up from the chiral

moiety of the CSP and the sample. If the CSP and sample both have the $R$ (as above) or $S$ configuration, the complex is called like, if they differ in configuration, the complex is unlike. Enantioselectivity, expressed as the separation factor, $\alpha^*$, then becomes:

$$\alpha^* = \frac{k_l'}{k_u'} = \frac{\Sigma \text{ non-chiral interactions } + \Sigma \text{ l-interactions}}{\Sigma \text{ non-chiral interactions } + \Sigma \text{ u-interactions}}$$

$\alpha^*$ differs from the common definition of the separation factor, $\alpha$, in that it is the ratio of the capacity factors of the $R$ and $S$ enantiomers irrespective of their elution order; therefore, $\alpha^*$ is less than 1 if the $S$ enantiomer is eluted last. For CSPs I, II and IV discussed in this paper, the enantiomer eluted last is always $R$ and therefore $\alpha^* = \alpha$. In the case of CSP III there is an inversion of the elution order, therefore for some enantiomers $\alpha^* = \alpha$, for the others $\alpha^* = 1/\alpha$.

Non-chiral interactions must be taken into consideration when correlating $\alpha$ values with thermodynamic parameters[14]. Retention can be influenced by non-chiral interactions if the loading of the CSP with chiral functional groups is very low. Moreover, if the affinity of the sample to complex with the chiral moiety of the CSP is not so pronounced, $i.e.$, is enhanced only to a low degree relative to the affinity to the non-chiral support, the separation factor, $\alpha$ or $\alpha^*$, can depend on the loading of the stationary phase. This behaviour was observed, $e.g.$, for N-3,5-dinitrobenzoylphenyl-ethylamide on a naphthylethylamide CSP (phase I)[15]. On the contrary, non-chiral interactions can be neglected and the separation factor is independent of the loading of the stationary phase if the sample has an high affinity to complex with the chiral moiety, as has been reported for the same sample on a different CSP[16].

If non-chiral interactions have been shown experimentally to be negligible, the chiral selectivity, $\alpha^*$, depends only on the l- and u-interactions. Assuming that for each enantiomer only one single complex with the chiral moiety of the CSP is of importance (because other possible complexes are markedly less stable, this assumption is certainly a simplification in many cases), then $\alpha^*$ can be written as follows:

$$\alpha^* = \frac{\text{l-interaction}}{\text{u-interaction}}$$

Within a series of structurally related compounds, $\alpha^*$ is usually not constant. The most simple approach would be a correlation of the capacity factor of the second eluted enantiomer, $k_2'$, with the separation factor. This is generally not the case, see the series of homologues[1,17,18] or of $\pi$-acceptor amides[19,20]. A low separation factor can be due to rather pronounced non-specific complexation of both the $R$ and $S$ enantiomer or due to highly specific like and unlike interactions of the two enantiomers to a similar extent.

For the discrimination of these two cases and a discussion of chiral recognition mechanisms in more detail, we propose to use the relative capacity factor, $k'^*$, which for the two enantiomers is defined as

$$k_u'^* = \frac{k_u'}{k_N'} = \frac{\text{u-interactions}}{\text{non-specific interactions}}$$

$$k_1'^* = \frac{k_1'}{k_N'} = \frac{\text{l-interactions}}{\text{non-specific interactions}}$$

where $k_N'$ is the capactiy factor of the enantiomer on a non-specific, *i.e.*, as low in specificity as possible for the samples investigated, non-chiral reference stationary phase using the same mobile phase, this reflecting mainly the sample polarity.

EXPERIMENTAL

Different non-chiral stationary phases were tested for their suitability as a reference phase: bare silica and silica with chemically bonded nitrile, amine or amide. The silica phase was Spherisorb S5W (Kontron Analytical, Zürich, Switzerland), particle diameter 5 $\mu$m, and the nitrile phase was Nucleosil CN (Macherey-Nagel, Oensingen, Switzerland) with 10-$\mu$m particles; both phases were used as pre-packed columns. The amine and amide phases were laboratory-made from Matrex Silica Si (Grace, Wallisellen, Switzerland). This 5-$\mu$m silica was dried at 100°C and 0.1 mbar for 4 h, then refluxed in a 4% solution of 3-aminopropyltriethoxysilane (Fluka, Buchs, Switzerland) in dry toluene for 16 h; this gave a load of 0.55 mmol/g of aminopropyl groups (calculated with the carbon content obtained by elemental analysis). This amino phase was slurry packed in isopropanol in 25 cm × 4.6 mm I.D. stainless-steel columns. The acetyl amide phase was prepared *in situ* from this amino silica by pumping a solution of 2.5 g of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroxyquinoline (EEDQ, Fluka) and 0.5 g of acetic acid in 50 ml of dichloromethane through an amino column in analogy to a published method[21].

The chromatographic data for the chiral stationary phases used in this study have been published earlier[1,17,18]. The structures of the four different $\pi$-donor amide CSPs, I–IV, are given in Fig. 1. The samples used are an homologous series of
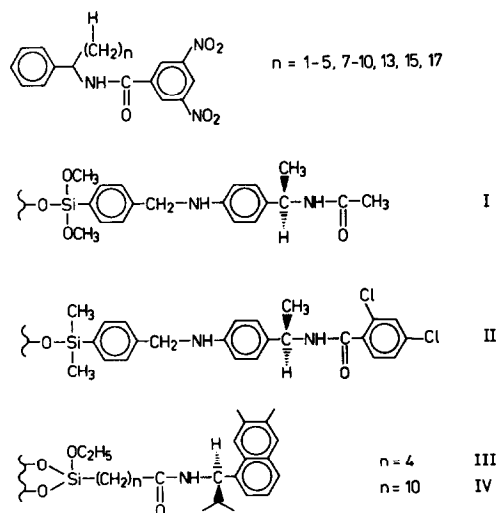


Fig. 1. Structures of the test compounds and of chiral stationary phases I[18], II[18], III[1] and IV[1]. CSP II had been synthesized in the *S* configuration; it is drawn here in the *R* form in accordance with the other phases, and results obtained with it have been adapted.

TABLE I

CAPACITY FACTORS OF THE SERIES OF HOMOLOGUES ON DIFFERENT NON-CHIRAL STATIONARY PHASES

Mobile phase: $n$-hexane–isopropanol (4:1).

| $n$ | $k'_{Silica}$ | $k'_{Nitrile}$ | $k'_{Amino}$ | $k'_{Amide}$ |
|---|---|---|---|---|
| 1 | 0.41 | 2.41 | 0.57 | 0.90 |
| 2 | 0.32 | 2.40 | 0.48 | 0.87 |
| 3 | 0.24 | 2.19 | 0.41 | 0.74 |
| 4 | 0.20 | 2.03 | 0.38 | 0.70 |
| 5 | 0.17 | 1.88 | 0.35 | 0.65 |
| 7 | 0.13 | 1.63 | 0.31 | 0.57 |
| 8 | 0.12 | 1.52 | 0.29 | 0.54 |
| 9 | 0.11 | 1.44 | 0.28 | 0.51 |
| 10 | 0.10 | 1.36 | 0.26 | 0.48 |
| 13 | 0.08 | 1.17 | 0.23 | 0.40 |
| 15 | 0.07 | 1.08 | 0.21 | 0.37 |
| 17 | 0.06 | 0.99 | 0.19 | 0.34 |

3,5-dinitrobenzoylamides of phenyl-$n$-alkylamines, see Fig. 1. The mobile phase was $n$-hexane–isopropanol (4:1) in all cases.

RESULTS AND DISCUSSION

As seen from Table I, all non-chiral stationary phases tested behave similarly in that the capacity factors of the dinitrobenzoylamides decrease with increasing alkyl chain length. Therefore any of these phases can be used as a reference to calculate $k'^*$; we decided to use the data of the amino phase for reference purposes. The choice is arbitrary and does not influence the quality of the results. A comparison of Tables

TABLE II

CAPACITY FACTORS, SEPARATION FACTORS AND RELATIVE CAPACITY FACTORS, $k'^*$, ON CHIRAL STATIONARY PHASES I AND II

Mobile phase: $n$-hexane–isopropanol (4:1). $k'_u = k'_1$ and $k'_1 = k'_2$ in all cases, therefore $\alpha^* = \alpha$.

| $n$ | Phase I | | | | | Phase II | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $k'_u$ | $k'_1$ | $\alpha^*$ | $k'_u{}^*$ | $k'_1{}^*$ | $k'_u$ | $k'_1$ | $\alpha^*$ | $k'_u{}^*$ | $k'_1{}^*$ |
| 1 | 6.72 | 8.60 | 1.28 | 11.8 | 15.1 | 13.0 | 16.3 | 1.25 | 22.8 | 28.6 |
| 2 | 6.87 | 9.55 | 1.39 | 14.3 | 19.9 | 13.0 | 16.3 | 1.25 | 27.1 | 34.0 |
| 3 | 6.25 | 8.81 | 1.41 | 15.2 | 21.5 | 13.5 | 16.5 | 1.22 | 32.9 | 40.2 |
| 4 | 6.18 | 8.65 | 1.40 | 16.3 | 22.8 | 12.7 | 15.6 | 1.23 | 33.4 | 41.0 |
| 5 | 5.71 | 8.28 | 1.45 | 16.3 | 23.7 | 12.5 | 15.3 | 1.22 | 35.7 | 43.7 |
| 7 | 4.94 | 7.31 | 1.48 | 15.9 | 23.6 | 10.8 | 13.3 | 1.23 | 34.8 | 42.9 |
| 8 | 4.71 | 7.11 | 1.51 | 16.2 | 24.5 | 10.0 | 12.6 | 1.26 | 34.5 | 43.4 |
| 9 | 4.32 | 6.70 | 1.55 | 15.4 | 23.9 | 9.5 | 12.0 | 1.26 | 34.0 | 42.9 |
| 10 | 4.09 | 6.34 | 1.55 | 15.7 | 24.4 | 8.8 | 11.1 | 1.25 | 34.0 | 42.7 |
| 13 | 3.48 | 5.57 | 1.60 | 15.1 | 24.2 | 7.8 | 9.9 | 1.28 | 33.7 | 43.1 |
| 15 | 3.32 | 5.41 | 1.63 | 15.8 | 25.8 | 7.4 | 9.4 | 1.28 | 35.1 | 45.0 |
| 17 | 3.01 | 4.94 | 1.64 | 15.8 | 26.0 | 6.9 | 9.0 | 1.30 | 36.5 | 47.5 |
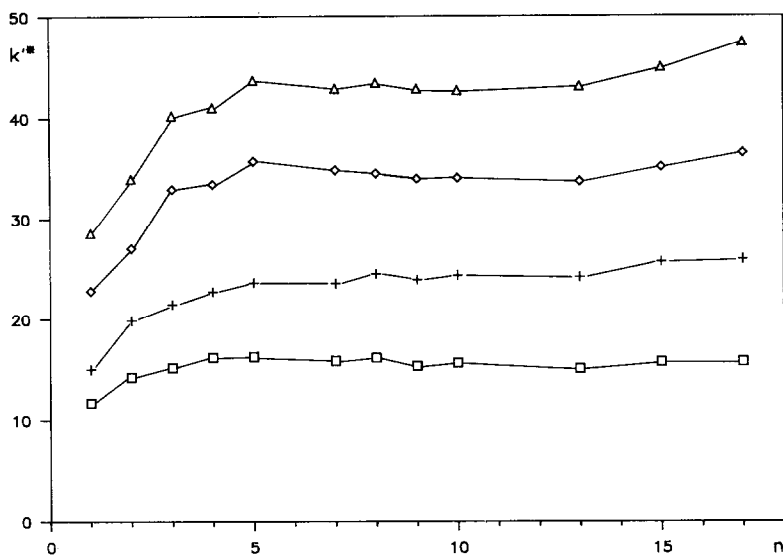
Fig. 2. Relative capacity factors $k'^*$, on chiral stationary phases I and II. $\square = k'_u{}^*$ (phase I); $+ = k'_l{}^*$ (phase I); $\diamond = k'_u{}^*$ (phase II); $\triangle = k'_l{}^*$ (phase II).

I–III shows that the fraction of retention due to the silanol groups or amine groups in the CSPs is negligible.

Table II gives the capacity factors, $k'$, the separation factors, $\alpha^*$, and the relative capacity factors, $k'^*$, for each enantiomer of the samples on CSPs I and II. Relative capacity factors on phase II are almost twice as high as those on phase I. The $k'^*$ values are also presented in Fig. 2. (In Figs. 2–5, points have been connected for clarity, although values between the points have no significance). It is seen that the shapes of the curves are almost the same in all four cases. The decrease of $k'$ with increasing alkyl chain length of the samples is less pronounced on CSPs I and II than on the reference phase. One can speculate that attractive Van der Waals interactions are involved in the retention processes of these samples on phases I and II.

Within a series of compounds, variations of the separation factors are usually rationalized by discussing the complexation of the second enantiomer eluted. This presupposes that the relative capacity factor of the first enantiomer eluted is constant and/or negligible, *i.e.*, that the complexation of this enantiomer does not depend on the chain length (or other parameters) within the series. As is seen from Fig. 2, this is not the case for the homologous series used in this work on CSPs I and II.

Table III and Fig. 3 show the correponding data for CSPs III and IV. The curve for the unlike complexes have similar shapes to those on phases I and II: in contrast, the curves for the like complexes are very different. Beginning at $n = 4$ on phase III or $n = 7$ on phase IV the relative capacity factors decrease drastically instead of reaching a plateau.

The macroscopic relative capacity factor, $k'^*$, reflects the microscopic diastereomeric adsorption complex; it takes into account the sample polarity. The similar shapes of the $k'^*$ curves indicate a relationship between the microscopic complexes. From Figs. 2 and 3 it can be concluded that the like and unlike complexes on phases
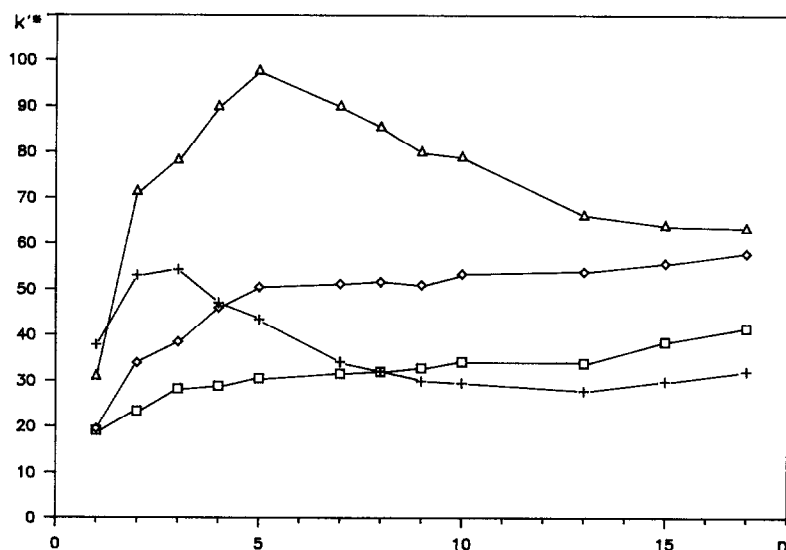
Fig. 3. Relative capacity factors, $k'^*$, on chiral stationary phases III and IV. $\square = k'_u{}^*$ (phase III); $+ = k'_l{}^*$ (phase III); $\diamond = k'_u{}^*$ (phase IV); $\triangle = k'_l{}^*$ (phase IV).

I and II as well as the unlike complexes on phases III and IV behave similarly. Therefore they must be structurally similar. In contrast, the like complexes on phases III and IV are of another kind. It can be concluded that two structurally different retention mechanisms must be taken into consideration when discussing phases III and IV, as has been proposed earlier[1], whereas this is not necessary for CSPs I and II. With a more simplified discussion based only on the size of the groups arranged near the

TABLE III

CAPACITY FACTORS, SEPARATION FACTORS AND RELATIVE CAPACITY FACTORS, $k'^*$, ON CHIRAL STATIONARY PHASES III AND IV

Mobile phase: $n$-hexane–isopropanol (4:1). On phase III there is a reversal of elution order at $\alpha^* = 1.00$ ($n = 8$). For $n = 1$ to 7, $k'_1 = k'_u$ and $k'_2 = k'_l$; for $n = 9$ to 17, $k'_1 = k'_l$ and $k'_2 = k'_u$.

| $n$ | Phase III | | | | | Phase IV | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $k'_1$ | $k'_2$ | $\alpha^*$ | $k'_u{}^*$ | $k'_l{}^*$ | $k'_u$ | $k'_l$ | $\alpha^*$ | $k'_u{}^*$ | $k'_l{}^*$ |
| 1 | 10.9 | 21.6 | 1.98 | 19.1 | 37.9 | 11.1 | 17.8 | 1.60 | 19.5 | 31.2 |
| 2 | 11.2 | 25.5 | 2.28 | 23.3 | 53.1 | 16.3 | 34.4 | 2.11 | 34.0 | 71.7 |
| 3 | 11.6 | 22.3 | 1.92 | 28.3 | 54.4 | 15.8 | 32.2 | 2.04 | 38.5 | 78.5 |
| 4 | 11.0 | 17.9 | 1.63 | 29.0 | 47.1 | 17.5 | 34.3 | 1.96 | 46.0 | 90.3 |
| 5 | 10.7 | 15.2 | 1.42 | 30.6 | 43.4 | 17.7 | 34.3 | 1.94 | 50.6 | 98.0 |
| 7 | 9.8 | 10.6 | 1.08 | 31.6 | 34.2 | 15.9 | 28.0 | 1.76 | 51.3 | 90.3 |
| 8 | 9.3 | 9.3 | 1.00 | 32.1 | 32.1 | 15.0 | 24.9 | 1.66 | 51.7 | 85.9 |
| 9 | 8.4 | 9.2 | 0.91 | 32.9 | 30.0 | 14.3 | 22.5 | 1.57 | 51.0 | 80.4 |
| 10 | 7.7 | 8.9 | 0.87 | 34.2 | 29.6 | 13.9 | 20.6 | 1.48 | 53.5 | 79.2 |
| 13 | 6.4 | 7.8 | 0.82 | 33.9 | 27.8 | 12.4 | 15.3 | 1.23 | 53.9 | 66.5 |
| 15 | 6.3 | 8.1 | 0.78 | 38.6 | 30.0 | 11.7 | 13.5 | 1.15 | 55.7 | 64.3 |
| 17 | 6.1 | 7.9 | 0.77 | 41.6 | 32.1 | 11.0 | 12.1 | 1.10 | 57.9 | 63.7 |
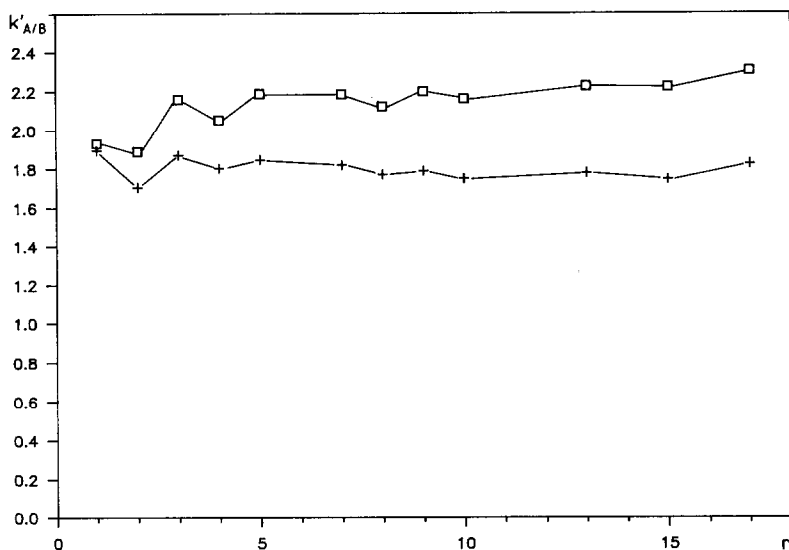
Fig. 4. Relative capacity factors, $k'_{A/B}$ of the unlike (eluted first) and like (eluted last) complexes on chiral stationary phases I and II. $+ = k'_l(II)/k'_l(I)$; $\square = k'_u(II)/k'_u(I)$.

chiral centre or on steric interactions[12], it is more difficult to explain the differences between Figs. 2 and 3.

On phase I, $\alpha^*$ increases with $n$ whereas on phase II $\alpha^*$ remains constant. It is useful to define a relative capacity factor of the second kind, $k'_{A/B}$, which is the ratio of the capacity factors on two different CSPs:

$$k'_{A/B} = \frac{k'(A)}{k'(B)}$$

For our purposes we use:

$$k'_{II/I} = \frac{k'(II)}{k'(I)} \quad \text{and} \quad k'_{IV/III} = \frac{k'(IV)}{k'(III)}$$

The corresponding data are given in Table IV and the plots are in Figs. 4 and 5. Note that for CSPs I and II, $k'_{A/B}$ of the like complexes (the enantiomers eluted last) in Fig. 4 is nearly constant whereas the unlike complexes (the enantiomes eluted first) show a slight increase. Any rationalization for the different selectivities of phases I and II for this homologous series therefore needs to be based on the retention of the enantiomer eluted first with the unlike complex. With CSPs III and IV (Fig. 5) the situation is different in that the like complexes show a markedly stronger dependence on $n$ than the unlike ones. Therefore the like complexes must be discussed to explain the different selectivities of phases III and IV.
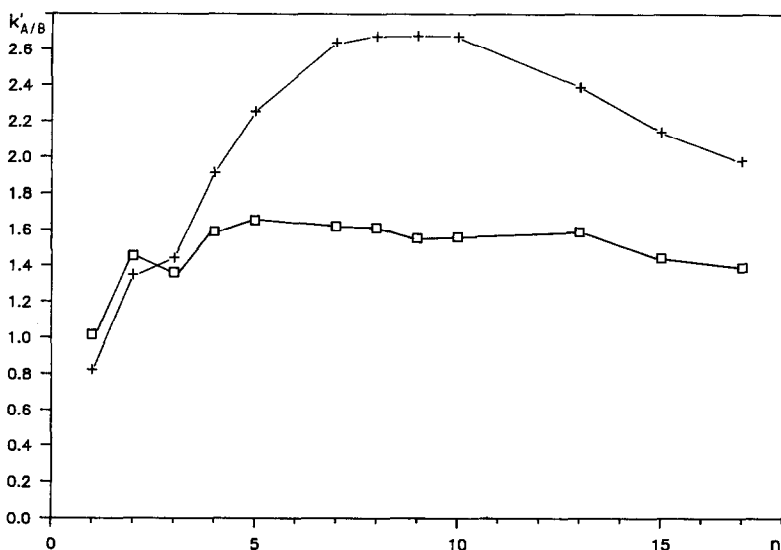
Fig. 5. Relative capacity factors, $k'_{A/B}$, of the unlike and like complexes on chiral stationary phases III and IV.
$+ = k'_1(IV)/k'_1(III); \square = k'_u(IV)/k'_u(III)$.

CONCLUSIONS

The here defined relative capacity factors, $k'^*$, which is the ratio of capacity factors on a chiral and on a non-chiral stationary phase for the same enantiomer, or $k'_{A/B}$, which is the ratio of capacity factors on different CSPs, allow a more detailed discussion of chiral recognition mechanisms than is possible by merely using the separation factor, $\alpha$. It may be necessary to consider the complexation of the enantiomer eluted first to explain the differences in enantioselectivity of various CSPs towards a series of samples of related structure; this is the case for CSPs I and II used in

TABLE IV

RELATIVE CAPACITY FACTORS, $k'_{A/B}$

| $n$ | $k'_u(II)/k'_u(I)$ unlike | $k'_1(II)/k'_1(I)$ like | $k'_u(IV)/k'_u(III)$ unlike | $k'_1(IV)/k'_1(III)$ like |
|---|---|---|---|---|
| 1 | 1.93 | 1.90 | 1.02 | 0.82 |
| 2 | 1.89 | 1.71 | 1.46 | 1.35 |
| 3 | 2.16 | 1.87 | 1.36 | 1.44 |
| 4 | 2.06 | 1.80 | 1.59 | 1.92 |
| 5 | 2.19 | 1.85 | 1.65 | 2.26 |
| 7 | 2.19 | 1.82 | 1.62 | 2.64 |
| 8 | 2.12 | 1.77 | 1.61 | 2.68 |
| 9 | 2.20 | 1.79 | 1.55 | 2.68 |
| 10 | 2.16 | 1.75 | 1.56 | 2.68 |
| 13 | 2.23 | 1.78 | 1.59 | 2.39 |
| 15 | 2.22 | 1.75 | 1.44 | 2.14 |
| 17 | 2.31 | 1.83 | 1.39 | 1.98 |

this study. This means that the discussion of the retention mechanism of the enantiomer eluted second alone does not necessarily include the relevant part of a separation.

Possible structures of a complex of the enantiomer eluted first are not given in this paper. It was the aim to propose the concept of relative capacity factors which can help to validate any proposed complexes. These complexes can be found experimentally or theoretically by chromatographic investigations, spectroscopic data[22] or computer methods[3,8,9]. Any structures found by these methods must not be in contradiction with the concept proposed by this paper.

ACKNOWLEDGEMENT

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