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Resolution of non-racemic mixtures in achiral chromatographic systems: a model for the enantioselective effects observed

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ABSTRACT

Resolution of non-racemic samples in achiral chromatographic systems can occur when the solute undergoes self-association, *e.g.*, typically to dimers. In the resulting diastereomeric homo- and hetero-structures, the enantiomers are distributed in a non-symmetric fashion when the sample or sample fraction has an enantiomeric excess (e.e.) larger than 0%. In this paper, a new model for the resolution mechanism is presented. It takes into consideration that all molecules in the sample have capacity factors that are not constant during resolution, but change rapidly with time, owing to the rapid and reversible interconversion of monomers to dimers. Simple equations are given expressing the difference in the capacity factors of the enantiomers in a non-racemic environment on the basis of the time fraction spent by each of the antipodes in the form of the possible molecular species and their respective capacity factors. The process is thus represented in terms of the migration of the enantiomers. This approach leads to a better understanding of the resolution and of certain aspects of the order of elution. Different scenarios are considered with the dimerization taking place either only in the mobile or the solid phase or in both phases. Assuming that association normally also proceeds in the solid phase, both the "dimer distribution" and the "chiral layer" mechanisms can occur simultaneously. Their relative importance will vary with the experimental conditions. Analysis of the equilibrium concentrations showed that resolution can occur only if the dimers differ in stability.

INTRODUCTION

The enantioselective effects observed in non-racemic mixtures using LC with an achiral chromatographic system is of considerable theoretical and practical interest. The first reports on the topic emanated independently and almost simultaneously from three different laboratories [1–3]. The occurrence of this phenomenon has subsequently been confirmed by many more examples and different workers [4–8].

Analogous effects are known to occur in other separation processes, notably in crystallization [9] and, further, in spectroscopy, particularly in NMR [10], and in chemical reactions [11]. However, the above chromatographic results were unexpected [12] and the pertinent observations were made only by chance during the purification of reaction products.

The resolution of optical isomers requires, according to theory [13], a chiral environment. As both the stationary and the mobile phases are achiral, the resolution reported seemed paradoxical at first. In fact, however, the mandatory dissymmetry is provided by the enantiomerically enriched sample itself. It was pointed out [3] that two enantiomers in a non-racemic mixture, when considered with the ensemble of the surrounding

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molecules, are anisometric [14] and, hence, will differ to some extent in their scalar properties and pertinently in their distribution coefficients between two phases. These general symmetry considerations elaborate the theoretical feasibility of the results observed, and are the starting point for the understanding of the phenomenon.

On examining the nature of the racemates resolved thus far in achiral chromatographic systems, it has been found that all are characterized by a potential for self-association, such as hydrogen bonding or dipole–dipole attraction. In a number of instances molecular interactions of this type have, indeed, been confirmed by spectrometric measurements. The various, pertinent resolution mechanisms proposed in the literature [1–4] are based on the diastereomeric nature of the associated structures, derived from the two enantiomers, as typically represented by dimers. These latter can be either homo- (*RR* and *SS*) or hetero- (*RS*) dimers and are in rapid equilibrium with each other and with the "monomers" (Fig. 1).

Some workers [4–6] assume that the excess enantiomer is separated from the racemic residue as a result of the difference in the distribution coefficients of the homo- and heterodimers. Recently, computations were made with a program simulating dynamic processes in chromatography, using sets of assumed values for the equilibrium constants of eqn. 1 and the distribution coefficients of the various molecular species [15]. It was demonstrated that elution curves with good separation of the excess enantiomer from the residual racemate could be obtained.

Other workers [2] have pointed out that, at injection, the adsorbent will be coated with a chiral layer by equilibration with the enantiomerically enriched mixture. Peak separation is pictured to proceed as if the adsorbent were coated with a chiral phase. The resolution coefficient will, however, decrease with the e.e. on the adsorbent surface, and become equal to 1.00 (no enantiomer differentiation) for e.e. = 0%, *i.e.*, under the peak corresponding to the racemate.

The various suggestions as to the possible mechanism of resolution mentioned in the literature [1–6] have been carefully considered and part of them incorporated into the model to be presented in this paper.

Two papers [1,4] are of particular relevance to the present discussion. They describe the chromatography of labelled racemic samples mixed with a 5000-fold excess of an unlabelled enantiomer on an achiral column. In the radiochromatogram (Fig. 2, stepped line) the masking effect of the excess cold enantiomer is eliminated (*cf.* the continuous UV absorption line in Fig. 2). Two peaks of roughly equal area are seen to appear corresponding, respectively, to the labelled enantiomer of same configuration as the cold L-additive (first peak) and its labelled antipode (second peak). The elution profile of the

Fig. 1. Equilibria of dimers and monomers. *R* and *S* designate the antipodes and, as suffixes, their respective properties. *RS and SR are identical (*meso*) compounds.

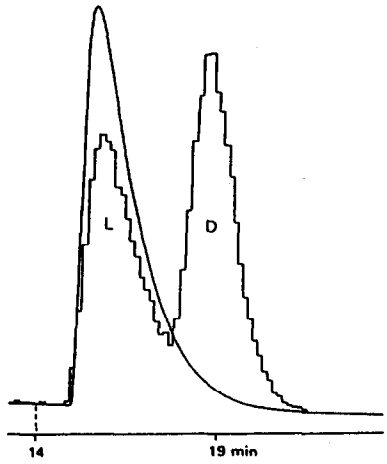

Fig. 2. Separation of racemic [$^{14}COCH_3$]valine *tert.*-butyl ester diluted with the L-enantiomer on a silica gel column. Conditions: mobile phase, 2-propanol–*n*-hexane (7.5:92.5, v/v); column temperature, 9.5°C; flow-rate, 1 ml/min; detection, UV at 230 nm and ^{14}C radioactivity by scintillation counting with a flow cell; mixing ratios of the racemic (2.4 m*M* in $CHCl_3$; 11.7 μCi/ml) and L-enantiomeric solutions (0.095 *M* in $CHCl_3$) by mass, 1:128; injection volume of the mixture, 20 μl; $k'_S = 3.15$, $k'_R = 3.89$; $\alpha = 1.23$. Adapted from Dobashi *et al.* [4].

labelled isomers is thus similar to that of racemates chromatographed on a chiral phase, on which each isomer has a different capacity factor (*i.e.*, migration rate). These results suggest that it should be possible to interpret the resolution of non-racemic samples in an achiral system in terms of the differential migration of the two enantiomers. The equations presented here are an attempt to look at the process from this point of view.

DISCUSSION OF THE MODEL

The resolution model proposed was constructed in analogy with the approach used in NMR for the calculation of chemical shifts in "statistically controlled anisochronism in diastereoisomeric association (SCDA)" [10,11]. These studies include, *inter alia*, determination of the chemical shifts of optical isomers in non-racemic mixtures, particularly relevant to the topic of this paper.

In the compounds considered in the NMR studies, rapid and reversible intermolecular interactions occur just as in the solutes in the chromatographic experiments under discussion. Every isomer finds itself in a rapidly changing molecular environment, as it participates in the dynamic equilibria of Fig. 1, simultaneously also changing its properties (chemical shift in NMR and capacity factor in chromatography).

To define the chromatographic behaviour of the two enantiomers, it is necessary to estimate their averaged capacity factor. Analogously to the NMR precedent [10], this is done by summing the products of the mass fractions (in terms of pure enantiomer in the mobile phase) of each of the molecular species, at equilibrium at a given point x of the column, multiplied by the corresponding capacity factor (see eqn. 1). In the model, it is assumed that the process of distribution between the two phases approaches ideal behaviour, *i.e.*, it is fast and the capacity factor is independent of concentration. It should also be pointed out that the mass fraction at equilibrium of a given species is the same as the time fraction spent by the enantiomer in that form and designated as f^x in writing the following equation for the mobile phase:

$$\Delta_c^x = [k']_S^x - [k']_R^x = k'_S f_S^x + k'_{SS} f_{SS}^x + k'_{SR} f_{SR}^x - k'_R f_R^x - k'_{RR} f_{RR}^x - k'_{RS} f_{RS}^x \quad (1)$$

where the averaged capacity factors are enclosed in square brackets, $[k']$, $\Delta_c = [k']_S - [k']_R$, the capacity factors of the various molecular species are unbracketed, k', the subscripts designate the molecular species and the superscript x is the point of the column considered. It is important to note that the time fractions spent by the *S*- and *R*-isomers as the heterodimer, respectively, f_{SR} and f_{RS}, are *not identical* in non-racemic mixtures (see below; distribution of the enantiomers in a non-racemate).

Important information on the resolution process can be derived from the value of Δ_c. Separation of the excess isomer from the racemate can obviously occur only if the rates of migration of the enantiomers are different, *i.e.*, $\Delta_c \neq 0$. The magnitude and sign of Δ_c determine the quality of the resolution and the order of peak elution, respectively. In the absence of data on the parameters involved only qualitative conclusions can be drawn from eqn. 1.

The relative amounts of monomers and homo- and heterodimers in a mixture, whether racemic or not, will vary depending on the magnitude of the equilibrium constants of the reactions in Fig. 1. For a racemate, symmetry considerations require that both the capacity factors of the enantiomeric species and the time fractions spent by each enantiomer as a given molecular species be identical, *i.e.*,

$$k'_S = k'_R \qquad f_S^x = f_R^x$$

$$k'_{SS} = k'_{RR} \qquad f_{SS}^x = f_{RR}^x$$

$$k'_{SR} = k'_{RS} \qquad f_{SR}^x = f_{RS}^x$$

(f_{SR}^x and f_{RS}^x are calculated from the mass of $RS/2$).

It follows that all terms corresponding to one isomer in eqn. 2 are cancelled by those of its antipode, so that $\Delta_c = 0$. This conclusion also simply follows from the principle of Curie [13].

When, however, one of the enantiomers is present in excess, assumed here to be the *S*-isomer, such a symmetric distribution of the antipodes is no longer possible. The equilibrium

reached in the racemic mixture will be displaced with the formation of more of the monomer and the homodimer derived from the *S*-isomer, leading to $f^x_S > f^x_R$ and $f^x_{SS} > f^x_{RR}$. As a corollary, the third possible species, the heterodimer, will show the inverse change, $f^x_{RS} > f^x_{SR}$, *i.e.*, the minor isomer (*R*) will spend more time as the *RS* species. For a non-racemic composition Δ_c will therefore be, in general, different from zero, *i.e.*, resolution will occur, except in the special case when the negative and positive terms in eqn. 1 happen to cancel each other.

The corresponding equation is

$$\Delta^x_c = [k']^x_S - [k']^x_R = k'_S(f^x_S - f^x_R) + k'_{SS}(f^x_{SS} - f^x_{RR}) + k'_{SR}(f^x_{SR} - f^x_{RS}) \quad (2)$$

In non-racemic mixtures, the initiating step of resolution is the creation of anisometry, with a consequent statistically controlled dissymmetric distribution of the enantiomers between the different possible molecular species, leading to $\Delta_c \neq 0$. All that is required for this purpose is the excess of one of the enantiomers. It should be that dissymmetry in the distribution of the antipodes will also result when the homo- and the heterodimers have identical stability. However, as recently checked by Dr. M. Jung, (see Note 1), the values of the time fractions (f^x) of the different species are, in this case, such that $\Delta_c = 0$. Thus, a difference in the stability of the dimers is required for resolution to occur.

It should be pointed out that on chiral phases, the difference between the capacity factors of enantiomers is independent of sample composition, strictly speaking on the proviso that the solutes do not self-associate. In contrast, the resolution of non-racemic samples in achiral systems will be dependent on the amount of excess enantiomer present at a certain point of the column. During the separation a gradient of the *S*/*R* ratio forms between the pure enantiomer peak and that corresponding to the racemate. In the corresponding region, the value of Δ^x_c will decrease and tend to zero, as no excess of *S* enantiomer remains at the point *x* considered. In parallel, the difficulty of separating residual excess isomer from the racemate will increase, until further resolution becomes impossible under the column conditions. Hence, baseline separation for non-racemic samples cannot be approached in practice on commonly available columns.

With the help of this model, certain additional aspects of the resolution process can be clarified, as illustrated by the following examples. In a first scenario (I), the dimers are assumed to be present only in the mobile phase and not on the adsorbent, *e.g.*, because of instantaneous dissociation in contact with the stationary phase and the formation of, *e.g.*, a monomer–adsorbent associate. In this case, $k'_{SS} = k'_{RS} = 0$, *i.e.*, the dimers both travel at the speed of the mobile phase and do not directly affect the difference in the rate of migration of the enantiomers, which is $[k']^x_S - [k']^x_R = k'(f^x_S - f^x_R)$. As association is assumed not to occur on the adsorbent, the capacity factors of the enantiomers will not be affected by stereoselective interactions and will be identical, *i.e.*, $k'_S = k'_R$. The self-association in the mobile phase will, however, bring about resolution by changing the relative time fractions spent as the monomer by each antipode if the dimers are of unequal stability. As, at equilibrium, the minor enantiomer spends less time as the monomer than *S* (see above), and as $k'_S = k'_R$, it follows that $k'_R f^x_R$ will be smaller than $k'_S f^x_S$. Accordingly, the *R*-isomer will have a smaller capacity factor and, therefore, migrate more rapidly, meaning that the racemate, which contains the minor enantiomer, should be eluted with the head fraction. This scenario is, admittedly not very realistic, but it exemplifies one of the main features of the model, namely how anisometry, in combination with dimerization, brings about changes in the capacity factors of enantiomers.

In a second scenario (II), dimerization is considered to take place only on the adsorbent and not in the mobile phase. This situation can arise when the self-association of the solute is reduced to negligible proportions, *e.g.*, by strong association with a polar solvent. In this case, f^x_{SS}, f^x_{RR} and f^x_{SR}, f^x_{RSS} will be zero in the mobile phase, *i.e.*, the movement of the enantiomers through the column will not proceed at any time as dimers, but only as monomers, so that $f^x_S = f^x_R = 1.0$ in the mobile phase. Under these con-

ditions, resolution is possible only if the capacity factors of the enantiomers differ. In this scenario, self-association with consequent enantioselective interactions can occur on the stationary phase provided that at the point of the column considered there is an excess of one enantiomer, so that there is dissymmetry in both phases, and that, further, the stabilities of the hetero- and homodimers are unequal. In fact, scenario II resembles the chromatography on chiral phases, where the capacity factors of the enantiomers are different owing to the stereoselective interactions with the selector. Scenario II is, indeed, the "adsorbed chiral layer" model proposed [2] to explain the phenomenon discussed. The difference between the migration rate of the enantiomers will depend on the relative stability of the homo- and heterodimers. It should be pointed out, however, that the initiation of the resolution process is due to the creation of an anisometric medium by the excess enantiomer, leading to dissymmetry in the system.

The extreme assumptions made in scenarios I and II are hardly ever approached in practice and it is more realistic to assume (III) that self-association occurs in both phases. In this scenario, all the equilibria, described in Fig. 3, have to be taken into account. Fig. 3 shows that dimers in the stationary phase can be formed by two thermodynamically equivalent pathways.

In route A (A') the dimers are formed in the mobile phase and, subsequently, partition between the phases. In terms of the model presented here, the mechanism of recognition of the enantiomers resembles, in principle, that proposed for scenario I, and is related to the "dimer distribution" mechanism mentioned in the literature [5].

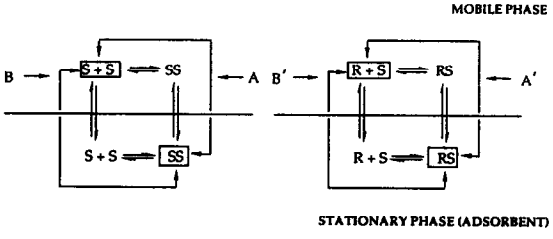

Fig. 3. Equilibria between monomers and dimers in a two-phase system. *RR* is not shown, as it is assumed that only a negligible amount of homodimer forms from the minor dimer.

In route B (B'), on the other hand, the monomers first distribute themselves between the two phases, and then dimerize on the adsorbent. Recognition of enantiomers proceeds, in this case, as in the "chiral layer" model [2], discussed in scenario II.

In principle, the process could evolve along both pathways simultaneously; however, depending on the experimental conditions, such as sample size, one route could predominate over the other.

To discuss the possible order of elution in scenario III, let us scrutinize separately pathways A (A') and B (B'). Eqn. 1 can be applied to A (A') and as has been pointed out above, $f^x_{RS} > f^x_{SR}$, if the *S*-isomer is in excess. As the term in eqn. 1 due to the heterodimer, $k'_{SR}(f^x_{SR} - f^x_{RS})$, will be negative, it will tend to decrease Δ_c. The extent of its effect on Δ_c will depend on the absolute magnitude of the term. If, moreover, the heterodimer is more stable than the homocompound, f^x_{RS} will tend to have a maximum value, which is 1.0. Under these conditions Δ_c could become negative, meaning a higher averaged capacity factor for *R*, and later elution of the minor isomer (as *RS*). Hence the relative stability of the dimers is seen to effect on the order of elution, also in the case of A (A').

In pathway B (B'), which corresponds to the "chiral layer" mechanism, a higher stability of the heterodimer will lead to a stronger interaction, *i.e.*, retardation, of the *R*-isomer by the excess of *S*-isomer in the stationary phase; this clearly determines the later elution of the minor enantiomer as the second (racemate) peak. Pathway B (B'), to the extent that it participates in scenario III, will strengthen the effect of the higher stability of the heterodimer as attributed to A (A').

As scenario III is a realistic one, the above considerations have particular importance. It should be emphasized, however, that, in the absence of actual data on the various parameters involved in eqns. 1 and 2, it is not possible to predict with certainty the elution order. Even the

possibility that the values of the different terms of the equation could compensate each other and lead to $\Delta_c = 0$ has to be taken into account. Empirically, it has been found that in most reported results published thus far, the minor isomer elutes last. However, in two studies [2,8] the reverse order was observed. This should suffice to establish that elution order may change from case to case (see Note 2).

CONCLUSIONS

A new approach to the interpretation of the resolution of non-racemic mixtures on achiral columns is proposed. The model developed is based on the idea that in the rapid and reversible association of monomers to dimers each molecule cannot have a constant capacity factor, but will change its partitioning behaviour rapidly as a function of time. Eqns. 1 and 2 have been written to represent the difference between the resulting averaged capacity factors of the enantiomers (Δ_c). It is believed that this approach is useful for a better understanding of the resolution of non-racemic samples on achiral columns.

It has further been attempted to demonstrate that certain predictions can be made as to the elution order by the use of the above equations. Only further study of the phenomenon, preferentially on labelled racemates and including the determination of the relevant constants involved in eqns. 1 and 2, could show to what extent the model proposed fits experiment.

It should also be established whether eqn. 1, which varies with x, can be used to calculate elution curves starting with simulated values for the parameters involved.

NOTES ADDED IN PROOF

Note 1

Dr. M. Jung, at the University of Tübingen, has calculated the distribution of the various molecular species in self-associating non-racemic mixtures of enantiomers, for the case of equal stability of the dimers. He dealt with five cases, in which the stability constants of dimerization varied over 13 orders of magnitude and the S/R ratio varied from 3 to 100. Designating the concentration of the species by R, S, RR, SS and RS and the total amount of the enantiomers in the system by tR and tS, he found that throughout $R/tR = S/tS$ and $(RR/tR - SS/tS) = (SR/2tS - RS/2tR)$. Introducing these relationships into eqn. 2 and remembering that $k'_{SS} = k'_{RS}$ for coupled equilibria in the two-phase chromatographic system and equal stability of the homo- and hetero-dimers, it follows that $\Delta_c = 0$, *i.e.* no resolution can occur.

The authors are indebted to Dr. M. Jung for his invaluable contribution.

Note 2

P. Diter, S. Taudien, O. Samuel and H.B. Kagan (*J. Org. Chem.*, 1994, in press) have found that *R*-ferrocenyl-phenyl-sulphoxide of e.e. = 65% gives, when using flush chromatography on silica gel, with EtOAc–cyclohexane as eluent, a first fraction enriched with the minor *S* enantiomer. This finding confirms our conclusion that the elution order in the resolution of non-racemic mixtures is not a fixed one, and that it has not to be taken for granted that the minor enantiomer always has to elute last.

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