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Computer simulation of three scenarios for the separation of non-racemic mixtures by chromatography on achiral stationary phases

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ABSTRACT

With the aid of a general chromatography simulation program based on the theoretical plate model, three scenarios rationalizing the phenomenon of the separation of non-racemic mixtures into racemate and excess enantiomers by chromatography on achiral stationary phases were simulated. The separation, repeatedly observed in high-performance liquid chromatography and gas chromatography, can be explained by the formation of homo- and heterochiral self-associates (*i.e.* RR, SS and RS). The kinetic calculations were performed using a Runge–Kutta routine. This program offers for the first time a general means of simulating dynamic chromatographic elution profiles involving any type of reaction occurring during chromatographic separation. Thus computer simulation of experimental elution profiles is no longer limited to first-order reactions such as enantiomerizations or isomerizations.

INTRODUCTION

The chromatographic separation of non-racemic mixtures into racemate and excess enantiomers by high-performance liquid chromatography (HPLC) and gas chromatography has been reported by several workers [1–3]. Systematic studies for additional classes of compounds were performed and fractions of high optical purity were isolated [4]. This non-trivial phenomenon, which has gained much interest in the realm of non-linear effects [5] (the EE effect [3]) is caused by diastereomeric interactions between the enantiomers R and S in enriched mixtures, *e.g.* self-association to dimers RR, SS and RS (the latter being identical with SR). Indeed, the chromatographic separation of the racemic mixture from the enantiomer in excess was found to be concentration-dependent and to require stationary or mobile phases, or both, that favour the formation of dimers [4].

Three models have been described as a theoretical basis for the simulation of dynamic chromatographic processes: (i) the discontinuous plate (Craig) model [6–9]; (ii) the stochastic model, assuming a Gauss function for the elution at the end of the column [10,11]; and (iii) the continuous flow model [7,12–14]. There are several reports on the simulation of chromatograms, using models i [15,16], ii [17–19] and iii [12–14]. We have chosen the discontinuous theoretical plate model [15] for the development of an improved, general simulation program* of elution profiles (considering dynamic effects

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* The program (and the variation described here) on the basis of the theoretical plate model, written in FORTRAN 77 and usable on any large computer, is available from the authors on request as a source code and has been accepted by the Quantum Chemistry Program Exchange (QCPE) [24]. It can be adapted to various problems in dynamic chromatography. An unlimited number of solutes may be injected in variable amounts, and a special procedure allows different plate numbers within one chromatographic run.

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during chromatography) because of its mathematical simplicity and the possibility of application to systems with more complicated, higher-order kinetics. The program has been presented and used elsewhere for the determination of rate constants of enantiomerization by computer simulation of experimental elution profiles [16].

Using a variation of this program, three scenarios suitable for the rationalization of the phenomenon of the separation of non-racemic mixtures into racemate and excess enantiomers by chromatography on an achiral stationary phase were simulated. In principle, all combinations of these scenarios are also suitable for the explanation of the phenomenon. It should be noted that these studies are strictly theoretical in character. Rather than simulating experimental chromatograms, the aim of this study was to rationalize non-linear effects between enantiomers in achiral chromatography by a general theoretical approach, often resorting to different or more extreme conditions than those so far observed in practice. For the chromatograms observed experimentally, even different mechanisms might in principle apply [20].

EXPERIMENTAL

Initially it is necessary to assume values for the capacity factors k' of all five species (R, S, RR, SS and RS), the injected amounts of R and S, the theoretical plate number n, the total volumes of mobile and stationary phase, the dead time t_M and the forward and backward rate constants for all equilibria of dimerization occurring. It has to be recalled that a second-order reaction such as this dimerization is concentration-dependent, which means that the injected amounts of R and S and the total volumes of the phases have an influence on the chromatograms, not only their ratios. The capacity factors and rate constants have to be carefully chosen in agreement with the principles described under Theoretical.

It has to be emphasized that the capacity factors k' of the five species are not directly related to the retention times observed in the chromatogram. They only refer to the phase distribution equilibria, the respective phase distribution coefficients K being given by

$$K = \beta k' \quad (1)$$

where β is the phase ratio. If, in addition, reactions occur between the five species during chromatographic separation, the retention times t_R according to the usual relationship

$$k' = (t_R/t_M) - 1 \quad (2)$$

will not be observed in the resulting chromatogram. For example, if monomer R is injected and dimerization takes place in the stationary phase, but not in the mobile phase, and if the dead time t_M is 1 min and the capacity factor k' for R is 0.001 (implying that R is present only in the mobile phase and not in the stationary phase), then the peak in the chromatogram may be found at a retention time much longer than the expected 1.001 min due to dimerization in the stationary phase.

The chromatographic separation is treated as a discontinuous process by assuming that all processes proceed repeatedly in separate uniform sections of the multicompartmentalized column containing n theoretical plates considered as chemical reactors. The processes taking place in the chromatographic column are separated into three steps: (i) establishment of the distribution (partitioning) equilibrium of all species between the mobile and stationary phases; (ii) reactions between the species during a time period Δt (here the reversible dimerization of the enantiomers R and S in both phases with the respective rate constants); and (iii) transportation (shifting) of the mobile phase to the adjacent section of the column while the stationary phase is retained. The species are initially "injected" into the mobile phase of the first plate (initial amounts in mol), and the content of the mobile phase of the last section (theoretical plate) is recorded in a digital form after every mobile phase shift (in units of mol/min).

In addition to the usual procedure, scenarios 2 and 3 described in the following (involving a permanent interconversion of species with significantly different retentions) were simulated by a slightly modified program. In this instance (to better approach the total equilibrium of all five species in both phases of a theoretical plate) the usual procedure for every plate (step 1, distribution; step 2, association and dissociation, separately for every phase during a time interval Δt; step 3, transportation of the mobile phase) was improved as follows: step 1 and step 2 were performed alternately ten times with only $\Delta t/10$ for step 2, only then does step 3 take place. This procedure is certainly more realistic,

but for the investigated examples it yielded almost identical results.

The kinetic calculations were performed by a fourth-order Runge–Kutta routine [21] with variable step sizes. If too extreme values are chosen for the plate number or the rate constants, the computer may need an excessively long time to perform the calculations. A CONVEX C 220 computer was used. The digitalized chromatograms were transferred to a personal computer for plotting with standard graphics software. The curves shown refer to monomers, *i.e.* a dimer, say RR, that is eluted at the column end is treated like two molecules of R in the detector. Thus the total elution curve is defined as [total] = 2([RS]+[RR]+[SS]) + [R]+[S]. The curve for the racemate is [rac]. (Note that one molecule S together with one molecule R yields two molecules of racemate). The curve of the excess enantiomer is [enant] = [total] − [rac]. For example, let ten molecules RR, five molecules SS, two molecules RS, two molecules R and one molecule S be eluted. This yields a total of $2 \cdot (10 + 5 + 2) + 2 + 1 = 37$ monomer molecules, consisting of 24 molecules R and thirteen molecules S, *i.e.* 26 molecules of racemate and $37 - 26 = 11$ molecules of excess enantiomer R.

THEORETICAL

The simulation is based on the following conditions. All dimerization equilibria are fast and reversible. For the enantiomers R and S and for RR and SS the same rate and equilibrium constants and distribution coefficients are always valid. The kinetics of dimerization (association) is second order and that of dissociation is first order. Thus the following equilibria occur:

mobile phase (m):

$$R_{(m)} + S_{(m)} \underset{k^m_{-1}}{\overset{k^m_1}{\rightleftharpoons}} RS_{(m)}$$

$$2R_{(m)} \underset{k^m_{-2}}{\overset{k^m_2}{\rightleftharpoons}} RR_{(m)}$$

$$2S_{(m)} \underset{k^m_{-2}}{\overset{k^m_2}{\rightleftharpoons}} SS_{(m)}$$

stationary phase (s):

$$R_{(s)} + S_{(s)} \underset{k^s_{-1}}{\overset{k^s_1}{\rightleftharpoons}} RS_{(s)}$$

$$2R_{(s)} \underset{k^s_{-2}}{\overset{k^s_2}{\rightleftharpoons}} RR_{(s)}$$

$$2S_{(s)} \underset{k^s_{-2}}{\overset{k^s_2}{\rightleftharpoons}} SS_{(s)}$$

In addition, the distribution equilibria for all five species occur, so that the system consists of a total of eleven equilibria. The distribution coefficients $K_R = K_S$, $K_{RR} = K_{SS}$ and K_{RS} are determined by the respective capacity factors used as a basis for the simulation (see under Experimental). The rates of dissociation k^m_{-1} and k^m_{-2} are assumed to be equal, as are k^s_{-1} and k^s_{-2}. If RS, RR and SS are assumed to have the same thermodynamic stability in the mobile phase (see under Results and discussion), then it is necessary for statistical reasons[a] that $k^m_1 = 2k^m_2$.

If all the rate constants and thereby all the equilibrium constants for the mobile phase as well as all the distribution coefficients are given, then the equilibrium constants for the stationary phase K^s_1 and K^s_2 can no longer be freely chosen, but are determined by the following expressions according to the principle of microscopic reversibility [15,16]:

$$K^s_1 = K^m_1 \cdot K_{RS}/(K_R K_S) \quad (3)$$

and

$$K^s_2 = K^m_2 \cdot K_{RR}/K^2_R = K^m_2 \cdot K_{SS}/K^2_S \quad (4)$$

This means that the thermodynamic stability of the dimers (K^m_1, K^m_2, K^s_1, K^s_2) is directly connected with the retention behaviour of the species, *i.e.* the distribution coefficients.

RESULTS AND DISCUSSION

If the dimers have significantly longer retention times than the monomers, dimerization takes place mainly in the stationary phase and a peak heading will be observed (see scenario 3 later). In the opposite instance a peak tailing will arise.

In scenario 1 a practically complete dimerization of the enantiomers R and S in both the mobile and stationary phases is assumed. The assumption of significantly different retention times of the heterochiral (RS) and homochiral (RR, SS) species renders a baseline separation (theoretically) feasible if dimer formation and dissociation are sufficiently fast (Fig. 2). Scenario 2 is based on a partial dimerization

[a] This is verified by an experiment: if pairs of two beads are randomly taken out of a container with 100 red and 100 white beads, then on average 50 times the combination red–white and only 25 times each of the combinations white–white and red–red will be obtained.

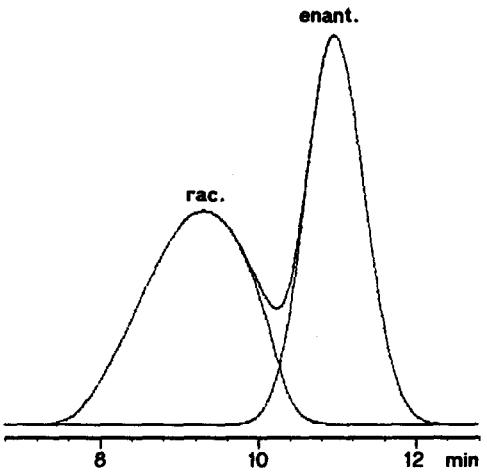

Fig. 1. Simulated chromatogram for scenario 1 (almost complete dimerization in both phases) with higher retention for RR and SS compared with RS. The total elution curve and the curves for the racemate and the excess enantiomer R are shown. The simulation is based on the following data: total volume of the mobile phase, 5 ml; total volume of the stationary phase, 5 ml; injected amounts, $3 \cdot 10^{-9}$ mol R, $1 \cdot 10^{-9}$ mol S, dead time t_M, 1 min; plate number n, 800; capacity factors k' of RS, RR, SS, R and S, 8, 11, 11, 4 and 4; rate constants of dimerization, k_1^m, k_2^m, k_1^s and k_2^s(in 10^{15} l mol^{-1} min^{-1}), 1.0, 2.0, 1.0 and 1.4; rate constants of dissociation of the dimers k_1^m, k_2^m, k_1^s and k_2^s (in min^{-1}), 1, 1, 0.9 and 0.9. At the column end almost exclusively dimers are eluted.

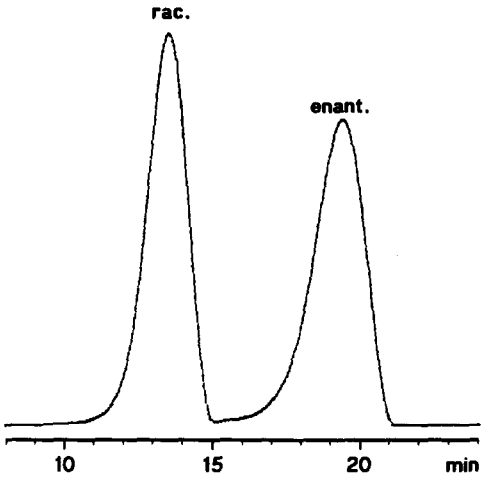

Fig. 2. Simulated chromatogram for scenario 1 (almost complete dimerization in both phases) with higher retention for RR and SS compared with RS. Only the total elution curve is shown. The assumption of significantly different retention times of the heterochiral (RS) and homochiral (RR, SS) species renders a baseline separation (theoretically) feasible if dimer formation and dissociation are sufficiently fast. The simulation is based on the following data: total volume of the mobile phase, 5 ml; total volume of the stationary phase 5 ml; injected amounts, $3 \cdot 10^{-9}$ mol R, $1 \cdot 10^{-9}$ mol S; dead time t_M, 1 min; plate number n, 500; capacity factors k' of RS, RR, SS, R and S, 8, 20, 20, 4 and 4; rate constants of dimerization k_1^m, k_2^m, k_1^s and k_2^s (in 10^{14} l mol^{-1} min^{-1}), 1.0, 2.0, 1.0 and 0.737; rate constants of dissociation of the dimers k_1^m, k_2^m, k_1^s and k_2^s (in min^{-1}), 100, 100, 47.4 and 47.4. At the column end almost exclusively dimers are eluted.

which takes place to a larger extent in the stationary phase than in the mobile phase. In contrast to this, scenario 3 is based on a partial dimerization in the stationry phase only, whereas in the mobile phase practically no dimers are allowed to occur[a].

The simulated separations are shown in Figs. 1–4. The separations are due to the different retention behaviour of the homochiral (RR and SS) and the heterochiral (RS) species. This results in different thermodynamic stabilities within at least one phase; in this treatment, the stationary phase. It is then, however, not necessary that the thermodynamic stabilities of the dimers are also different within the mobile phase.

A scenario 4 with partial dimerization to be assumed only in the mobile phase proved to be not suitable under the condition of equal thermodynamic stabilities of RS, RR and SS in the mobile phase (see earlier), as arithmetic examples of the kinetics of dimerization of a mixture of 75% R and 25% S showed that indeed R mainly forms RR and S mainly forms RS, but the degree of dimerization was always exactly equal for R and S. With this, together with an absence of dimerization in the stationary phase, no separation is feasible. A separation which is based on the different thermodynamic stabilities of RS on the one hand and RR and SS on the other hand in the mobile phase is still expected[a].

[a] In scenario 3 the simulation yields strongly asymmetric elution profiles (peak heading). This can be explained as follows: as the dimer formation (second order) is concentration-dependent, the degree of dimerization is larger in the centre of the elution zone than at the periphery. If the dimers have much longer retention times than the monomers, then the centre of the elution zone, which migrates more slowly, is pushed into the rear periphery, whereas the front periphery drifts apart.

[a] In this instance the dimers have almost no retention, and the separation could only be due to one enantiomer spending more of its time in the dimerized form than the other enantiomer; it thus migrates faster. The racemate is eluted *before* the excess enantiomer if the heterodimers RS are more stable. The separation should be largely independent of the choice of the stationary phase [22].

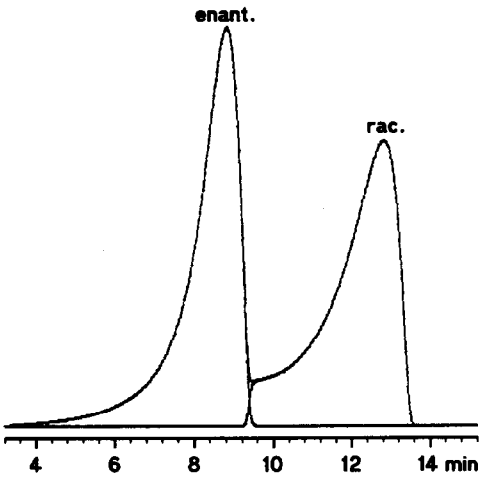

Fig. 3. Simulated chromatogram for scenario 2 (partial dimerization in both phases) with higher retention for RS compared with RR and SS. The total elution curve and the curves for the racemate and the excess enantiomer R are shown. The simulation is based on the following data: total volume of the mobile phase, 5 ml; total volume of the stationary phase, 5 ml; injected amounts, $3 \cdot 10^{-9}$ mol R, $1 \cdot 10^{-9}$ mol S; dead time t_M, 1 min; plate number n, 300; capacity factors k' of RS, SS, R and S, 20, 10, 10, 3 and 3; rate constants of dimerization k_1^m, k_2^m, k_1^s and k_2^s (in 10^{13} l mol^{-1} min^{-1}), 1, 2, 10 and 42.2; rate constants of dissociation of the dimers k_1^m, k_2^m, k_1^s and k_2^s (in min^{-1}), 400, 400, 1778 and 1778. A total of 33% of the monomers R and S injected are eluted in the form of monomers at the column end, the rest in the form of dimers.

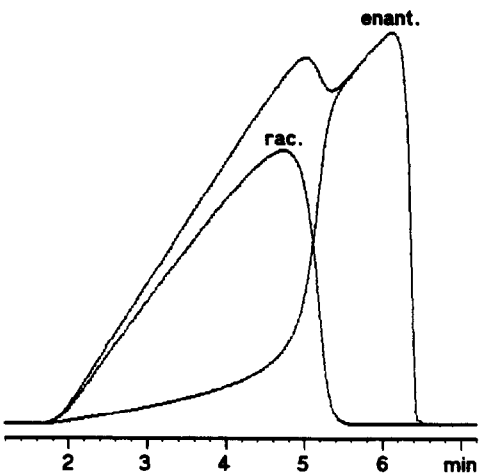

Fig. 4. Simulated chromatogram for scenario 3 (partial dimerization in the stationary phase, negligible dimerization in the mobile phase) with higher retention for RR and SS compared with RS. The total elution curve and the curves for the racemate and the excess enantiomer R are shown. The simulation is based on the following data: total volume of the mobile phase, 5 ml; total volume of the stationary phase, 5 ml; injected amounts, $3 \cdot 10^{-9}$ mol R, $1 \cdot 10^{-9}$ mol S; dead time t_M, 1 min; plate number n, 200; capacity factors k' of RS, RR, SS, R and S, 2 000 000, 4 000 000, 4 000 000, 2 and 2; rate constants of dimerization k_1^m, k_2^m, k_1^s and k_2^s (in l^{-1} min^{-1}), 10^4, $2 \cdot 1^{\cdot 4}$, 10^{12}, 10^{12}; rate constants of dissociation of the dimers k_1^m, k_2^m, k_1^s and k_2^s (in min^{-1}); 10 000, 10 000, 25 000 and 25 000. At the column end almost exclusively monomers are eluted. As in this instance no dimers are supposed to exist within the mobile phase, the phase distribution equilibria of the dimer species must be in favour of the stationary phase, *i.e.* the capacity factors of the dimers must be extremely high.

In the simulation of the scenarios 1–3 the elution order, determining whether the racemate or the excess pure enantiomer is eluted first, can freely be chosen in accordance with the retention behaviour of RS, RR and SS. In practice, however, the pure enantiomer is almost exclusively found to elute first [4]. In our theoretical treatment this reflects the fact that the heterochiral dimers RS are more stable than the homochiral dimers RR and SS within the stationary phase. This conclusion is reminescent to the conditions observed in the crystallization of racemic mixtures, where the formation of racemates is favoured whereas that of conglomerates is less often observed [23].

Unfortunately, with so many unknown constants needed for the simulation, the procedure is in this instance not suitable for the simulation of the experimental elution curves or even the determination of kinetic constants (in analogy to the study of enantiomerization during chromatographic separation [16]). However, the program would of course be capable of a correct simulation of the experimental curves if all the constants were known.

CONCLUSIONS

This program successfully simulates elution profiles featuring the separation of non-racemic mixtures into racemate and excess enantiomers by chromatography on achiral stationary phases. The program allows the possibility of studying not only simple interconversions such as enantiomerizations or isomerizations, but also more complicated kinetics by computer simulation of chromatographic elution profiles caused by dynamic reaction chromatography. The program is suitable not only for dimerizations, but can easily be adapted to any kind of chemical transformation.

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