# Evaluation and Prediction of Stereoisomerizations in Comprehensive Two-Dimensional Chromatography

O. Trapp*

Department of Chemistry, Stanford University, Stanford, California 94305-5080

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Conformational and configurational changes such as isomerizations, epimerizations, diastereomerizations and, enantiomerizations are important for the investigation of a large variety of processes ranging from protein folding to the stereostability of drugs. Under optimized conditions, these processes lead to an elution profile characterized by a plateau formation between the two interconverting species in chromatographic separations in a certain temperature range. By temperature-dependent measurements and subsequent computer simulation of the experimental chromatograms, the forward and backward rate constants $k_1$ and $k_{-1}$, the Gibb's energy $\Delta G^{\ddagger}$, activation enthalpy $\Delta H^{\ddagger}$, and entropy $\Delta S^{\ddagger}$ can be obtained. Due to its high efficiency two-dimensional chromatography is able to resolve the time-dependent distribution of the two species in the second dimension, thereby yielding the precise ratio of stereoisomers. An algorithm for the simulation and evaluation of two-dimensional chromatographic experiments has been developed, based on the theoretical plate model, which allows the determination of rate constants and barriers of isomerization, epimerization, and enantiomerization processes from two-dimensional chromatographic experiments. In the present article a detailed description of the extended theoretical plate model required for the simulation, the methods available, and examples for the evaluation of complex experimental data and the prediction of the separation conditions to observe isomerization, epimerizations, and enantiomerizations in two-dimensional chromatography are given.

## INTRODUCTION

Dynamic chromatography, defined as a method to study reversible conformational and configurational changes proceeding during the time scale of partitioning, is a well-established technique for the investigation of processes such as enantiomerizations, epimerizations, isomerizations, and decompositions. Elution profiles of interconverting species obtained by dynamic chromatography are generally characterized by plateau formation or peak broadening. A prerequisite for the application of this technique is the quantitative on-column separation of the binary mixture into the two stereoisomers in the respective chromatographic setup. One-dimensional dynamic chromatographic experiments have previously been applied for the determination of enantiomerization, epimerization, and isomerization barriers[1] of a wide spectrum of compounds, ranging from spin isomers[2] to drugs,[3] chirotopic nitrogen compounds,[4−9] small peptides,[10] atropisomers,[11,12] and supramolecular structures,[13] only to mention a few. Depending on the physical properties of the analytes different dynamic chromatographic and electrophoretic techniques, i.e., gas chromatography (GC),[14] supercritical fluid chromatography (SFC),[15] high performance liquid chromatography (HPLC),[16−19] capillary electrophoresis (CE),[10] electrokinetic chromatography (EKC),[20] and micellar electrokinetic chromatography (MEKC)[3] were applied.

Recently, two-dimensional GCxGC experiments[21−24] were used to determine the *E*/*Z* isomerization barriers of acetaldoxime and butyraldoxime.[25,26] Depending on the rate constant of the observed intramolecular reaction and the efficiency of the separation method, the peaks of the two interconverting species in the first dimension can be almost baseline separated, showing no noticeable plateau nor exhibit peak coalescence resulting in a broadened single peak. In these cases a two-dimensional experiment reveals the actual distribution of the stereoisomers as fine-structure, provided they can be resolved on the second dimension.

Evaluation of the one-dimensional experimental chromatograms is commonly done by computer simulation and iterative comparison to the experimental data, by fitting the calculated distribution function to the experimental chromatographic profile. By temperature-dependent measurements and computer simulation of the experimental data, the activation enthalpy $\Delta H^{\ddagger}$ and entropy $\Delta S^{\ddagger}$ are obtained from the forward and backward rate constants $k_1$ and $k_{-1}$ by an Eyring plot.

The first simulation program based on the theoretical plate model of chromatography was published in 1984[4] and was later extended to simulations of up to 120000 effective plates running on large computers (SIMUL).[5] The stochastic model, based on the simulation of Gaussian peaks and using a probability distribution, has also been applied for the determination of enantiomerization barriers.[2,16,27−29] Later an approximation function[30,31] for the direct calculation of enantiomerization barriers of racemic mixtures was introduced. Improved algorithms of these methods without restrictions in the plate numbers by using dynamic memory management are implemented in the ChromWin software[32] but can be applied to one-dimensional experiments only.

In the present paper the extended theoretical plate model required for the simulation of two-dimensional experiments

* Corresponding author phone: (650)723-4398; fax: (650)725-0259; e-mail: trapp@stanford.edu.

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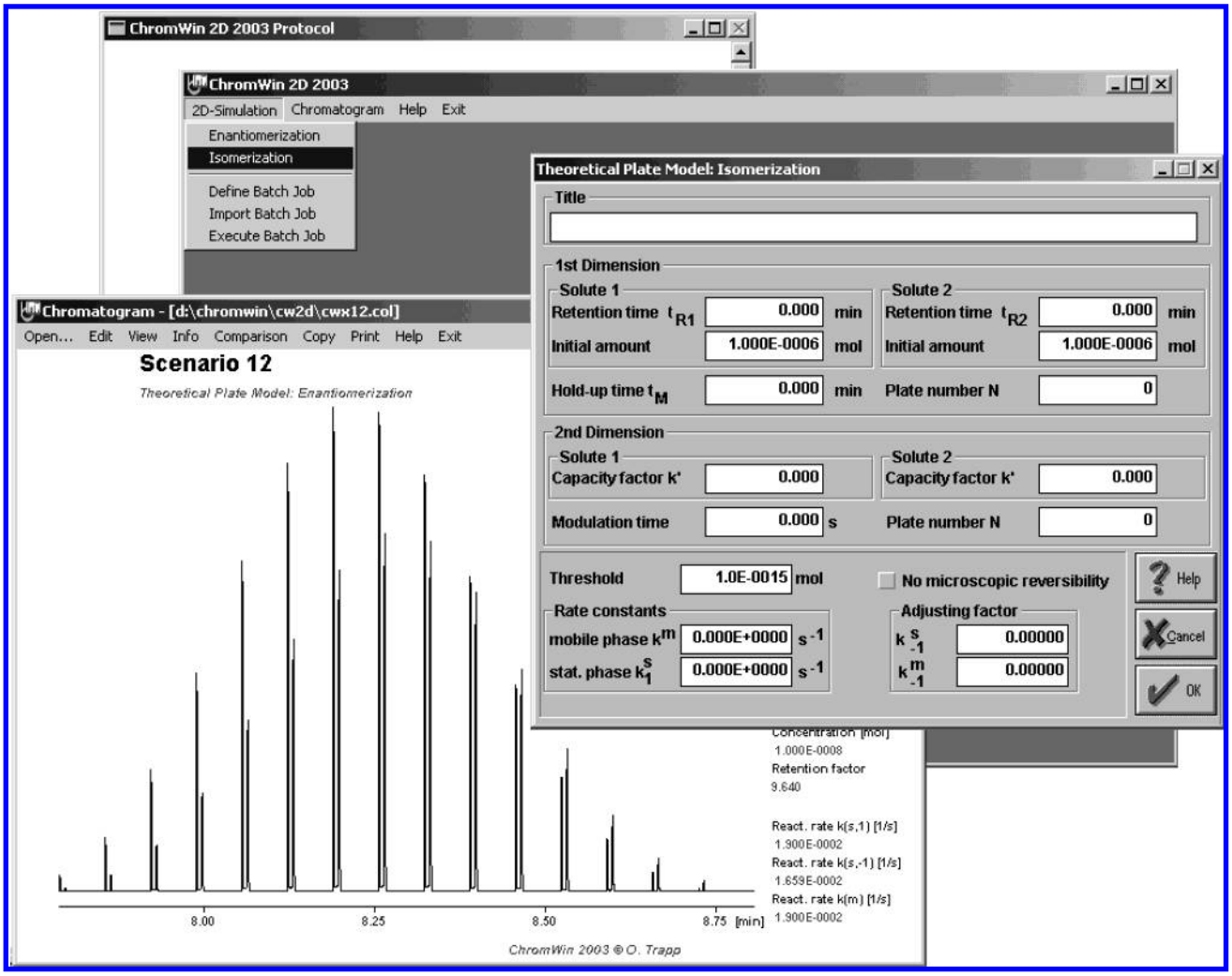


**Figure 1.** User interface of ChromWin 2D. The '2D-Simulation' menu allows one to manually enter data for simulations or to define batch-jobs for automated calculation and efficient use of CPU time. Simulated elution profiles can be visualized with the graphical user interface.

and its implementation in the computer program ChromWin 2D is described. Available methods and examples for the evaluation of experimental data and the prediction of the required separation conditions to visualize epimerizations and enantiomerizations are described.

## MATERIAL AND METHODS

**Structure.** The computer program ChromWin 2D is designed for the fast simulation of dynamic interconversion phenomena in two-dimensional chromatography. It can be used to perform iterative simulations to compare experimental and calculated elution profiles. From this rate constants can be obtained, which allows the determination of activation parameters $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ by temperature dependent measurements. The program has a user-friendly and straightforward graphical interface (cf. Figure 1) and can be run on most personal computer systems and does not effect the computational speed of other programs. The program is completely compatible with the ChromWin simulation software,[6,32] which was designed for the evaluation of one-dimensional elution profiles in chromatography and electrophoresis.

As computer simulation with the theoretical plate model of chromatography (cf. Figure 2) is very powerful this model

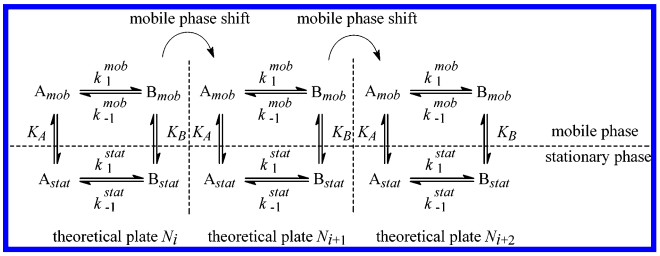


**Figure 2.** Equilibria of interconverting stereoisomers in three consecutive chromatographic theoretical plates. A is the first eluted stereoisomer, B is the second eluted stereoisomer, *k* represents the rate constant, and *K* is the distribution constant of the equilibrium between mobile phase and stationary phase. After distribution of the stereoisomers between the mobile and stationary phase the content of the mobile phase is shifted into the next theoretical plate.

was chosen to perform calculations of dynamic interconversion profiles in two-dimensional chromatography.

To employ the theoretical plate model of chromatography the algorithm had to be extended by the following additional steps: (i) sampling of the analytes after separation on the first dimension during the modulation time $t_{mod}$, (ii) transfer of the analytes into the first theoretical plate of the second dimension, and (iii) separation and interconversion of the analytes on the second dimension. Interconversion of the analytes during the sampling step can be neglected; however,

in case an adsorbent is used to collect the analytes, which are then released at elevated temperature, an appropriate calculation step considering the interconversion in the adsorbent has to be taken into account. The modified theoretical plate model, which is described in detail in the section Theoretical Background (vide infra), can be used for all types of two-dimensional chromatographic experiments where reversible first-order reactions, i.e. enantiomerizations, epimerizations, and isomerizations, occur.

To perform a simulation with ChromWin 2D the following chromatographic parameters of the first as well as the second dimension have to be entered: the hold-up time of the first dimension $t_M$, the retention times $t_R^A$ and $t_R^B$ of the analytes of the first dimension, and the theoretical plate numbers $N^{1st}$ and $N^{2nd}$ of the first and second dimension. The theoretical plate numbers $N$ are obtained from the peak width at half-height $w_h$ and the retention time of the individual peaks as well as the hold-up time and are defined by $N = 5.545 \cdot ((t_R - t_M)/w_h)^2$. In case of an epimerization and isomerization reaction the ratio of the interconverting stereoisomers, which can be obtained by integration of all peaks of the first and second eluted analytes of the second dimension from an experimental chromatogram, is required as input factor $f$, with $f = [A_\infty]/[B_\infty]$, to adjust the reaction rate constant for the backward reaction. It is important to note that the stereoisomers have to be in equilibrium for the determination of $f$. As the precise measurement of the hold-up time of the second dimension is difficult, it is more practical to employ retention factors, which can be correlated to the total retention times of the first dimension if very similar stationary separation phases are used.

$$t_{M,2nd} \approx \frac{k'_{B,1st} t^A_{R,2nd} - k'_{A,1st} t^B_{R,2nd}}{k'_{B,1st} - k'_{A,1st}} \quad (1)$$

In this case the retention factors of the second dimension can be estimated using eq 1, where $k'_{A,1st}$ and $k'_{B,1st}$ are the retention factors of the isomers for the first dimension, $t_{R,2nd}$ are the total retention times of the isomers on the second dimension and $t_{M,2nd}$ is the hold-up time on the second dimension, which can be arbitrarily set to 50 ms as starting point to perform the computer simulations.

For different stationary phases, as they are commonly used in two-dimensional experiments to meet the requirement of orthogonality, the hold-up time of the second dimension should be determined in a separate conventional experiment.

In both cases the total retention times of the analytes of the second dimension can be determined very accurately as the time of each modulation is known.

For a faster calculation the apparent forward rate constants $k_1^{app}$ of a one-dimensional experiment may be used as starting parameter for the 2D calculation. The simulation procedure has to be repeated until the simulated elution profiles coincide with the experimental chromatograms.

The computation time for the simulation of two-dimensional chromatograms is considerably higher as compared to one-dimensional chromatograms, because the calculation of the second dimension has to be performed separately for every single modulation step. Equation 2 gives an estimation for the simulation time increase $t_{sim}$.

$$t_{sim} \approx \frac{120 \cdot (t_R^B - t_R^A)}{t_{\mathrm{mod}}} \cdot \frac{N^{2nd}}{N^{1st}} \quad (2)$$

The simulated comprehensive chromatogram is obtained as output of the calculation. All results (half-width, area, height, rate constants, and resolution) from the simulations are calculated and written in a log-file and can be opened, edited, and printed with the integrated multidocument interface.

ChromWin 2D is available from the author as an executable program upon request.

**Theoretical Background.** The theoretical plate model of chromatography, which was previously applied successfully to the elucidation of enantiomerization, epimerization, and isomerization processes in one-dimensional dynamic chromatography is employed here for the evaluation of two-dimensional dynamic chromatographic experiment. However the one-dimensional model was adapted to simulate isomerization, epimerization, and enantiomerization processes in two-dimensional experiments.

The chromatographic separation is described in the theoretical plate model as a discontinuous process, assuming that all steps proceed repeatedly in separate uniform sections of a multicompartmental column with $N$ theoretical plates each considered as a chemical reactor where partitioning and chemical processes occur (cf. Figure 2).

For the first and second separation dimension with $N^{1st}$ and $N^{2nd}$ theoretical plates, respectively, the following three steps (i, ii, iii) have to be considered in every theoretical plate $N_i$:

(i) Partitioning of the stereoisomers A and B between the mobile phase (mob) and the stationary phase (stat). This is determined according to the eqs 3 and 4 where $A_{mob}$, $B_{mob}$, $A_{stat}$, and $B_{stat}$ are the amounts of the stereoisomers A and B at the equilibrium of partitioning between the mobile and stationary phase, $A^\circ_{mob}$, $B^\circ_{mob}$, $A^\circ_{stat}$, and $B^\circ_{stat}$ are the amounts of A and B before the equilibrium, and $k'_A$ and $k'_B$ are the retention factors of A and B, calculated from the total retention time $t_R$ and the mobile phase hold-up time $t_M$ according to $k' = (t_R\text{-}t_M)/t_M$.

$$A_{mob} = \frac{1}{1 + k'_A}(A^\circ_{mob} + A^\circ_{stat})$$

$$B_{mob} = \frac{1}{1 + k'_B}(B^\circ_{mob} + B^\circ_{stat}) \quad (3)$$

$$A_{stat} = \frac{k'_A}{1 + k'_A}(A^\circ_{mob} + A^\circ_{stat})$$

$$B_{stat} = \frac{k'_B}{1 + k'_B}(B^\circ_{mob} + B^\circ_{stat}) \quad (4)$$

For the second dimension eq 1 is used to correlate the retention factors with the first dimension of the separation and to estimate the hold-up time of the second dimension in case that the stationary phases of both dimensions are very similar. For different stationary phases the hold-up time $t_M$ of the second dimension should be determined in a separate conventional experiment.

(ii) The reversible interconversion process between the stereoisomers during the residence time $\Delta t^{1st} = t_M^{1st}/N^{1st}$ and

$\Delta t^{2nd} = t_M^{2nd}/N^{2nd}$, respectively, in the stationary and in the mobile phase in an individual theoretical plate is determined by the respective rate constants.

For enantiomerization processes the forward and backward rate constants $k_1^{mob}$ and $k_{-1}^{mob}$ in the achiral mobile phase are equal (the equilibrium constant is $K^{mob} = 1$), whereas the equilibrium constant in the stationary phase depends on the two phase distribution constants (partition coefficients) $K_A$ and $K_B$ according to the principle of microscopic reversibility:[4]

$$K^{stat} = \frac{k_1^{stat}}{k_{-1}^{stat}} = \frac{K_B}{K_A} = \frac{k'_B}{k'_A} \tag{5}$$

The application of the principle of microscopic reversibility requires that the rates of interconversion of the corresponding stereoisomers are different in the presence of the chiral stationary phase (CSP). This phenomenon arises from the fact that the stereoisomers are discriminated, and hence separated, due to a different thermodynamic Gibbs energy ($-\Delta_{B,A}\Delta G = RT \ln(K_B/K_A)$) as shown in Figure 2. This implies, that the backward rate constant $k_{-1}^{stat}$ is already determined for given values of $k_1^{stat}$, $k'_A$, and $k'_B$.

The reversible first-order kinetics is described by

$$\frac{dx}{dt} = k_1^{stat}([A_0] - [X]) - k_{-1}^{stat}([B_0] + [X]) \tag{6}$$

where $[X]$ is the change in concentration of A and B. $[A_0]$ and $[B_0]$ are the initial concentrations. Equation 6 is solved by integration, using the initial conditions:

$$[A] = \frac{k_{-1}^{stat}}{k_1^{stat} + k_{-1}^{stat}}([A_0] + [B_0]) + \frac{k_1^{stat}[A_0] - k_{-1}^{stat}[B_0]}{k_1^{stat} + k_{-1}^{stat}} e^{-(k_1^{stat} + k_{-1}^{stat})\Delta t} \tag{7}$$

The amount of $B$ is obtained from the mass balance due to $[A_0] + [B_0] = [A] + [B]$.

In contrast to enantiomerization processes, where the rate constants of the forward and backward reactions are equal in the achiral mobile phase, step (ii) is more complicated for epimerization and isomerization processes, because of the different ground-state energies of epimers and isomers. This results in different rate constants and different initial amounts for the epimers and isomers in the mobile and stationary phase. This is also valid in case a chiral selector is added to the mobile phase leading to different energies of the diastereomeric complexes due to the thermodynamic enantioselectivity $-\Delta_{B,A}\Delta G = RT \ln(K_B/K_A)$. The ratio of the forward and backward rate constant of the mobile phase depends on the equilibrium of the epimers or isomers A and B according to eq 8.

$$K^{mob} = \frac{k_1^{mob}}{k_{-1}^{mob}} = \frac{[B_\infty]}{[A_\infty]} = f^{-1} \tag{8}$$

The equilibrium constant $K^{stat}$ in the stationary phase depends on the two phase distribution constants (partition coefficients) $K_A$ and $K_B$, according to the principle of microscopic reversibility (cf. Figure 2) and has to be applied for chiral and achiral stationary phases:

$$K^{stat} = \frac{K_B}{K_A} = \frac{k'_B}{k'_A} = \frac{k_1^{stat}}{k_{-1}^{stat}} \frac{k_{-1}^{mob}}{k_1^{mob}} \tag{9}$$

Also in this case the backward reaction rate constant $k_{-1}^{stat}$ is already determined for given values of $k_1^{stat}$, the ratio of $[A]$ to $[B]$, and the retention factors $k'_A$and $k'_B$:

$$k_{-1}^{stat} = \frac{k'_A}{k'_B} k_1^{stat} \frac{[A_\infty]}{[B_\infty]} = \frac{k'_A}{k'_B} k_1^{stat} f \tag{10}$$

However from the computer simulation of the elution profiles of the dynamic chromatographic experiment of enantiomerization, epimerization, and isomerization processes it is a priori not possible to differentiate between the rate constants in the mobile and stationary phase. Only apparent rate constants ($k_1^{app}$ and $k_{-1}^{app}$), which are means of the forward and backward rate constants of the mobile and stationary phase, weighted by the retention factors $k'_A$ and $k'_B$, can be determined. Taking into account that the backward reaction rate can be calculated from the forward reaction rates and the equilibrium constant between A and B, the following equations can be derived for enantiomerization processes (eqs 11) and epimerization or isomerization processes (eqs 12):

$$k_1^{app} = \frac{1}{1 + k'_A} k_1^{mob} + \frac{k'_A}{1 + k'_A} k_1^{stat}$$

$$k_{-1}^{app} = \frac{1}{1 + k'_B} k_{-1}^{mob} + \frac{k'_B}{1 + k'_B} k_{-1}^{stat} \tag{11}$$

$$k_1^{app} = \frac{1}{1 + k'_A} k_1^{mob} + \frac{k'_A}{1 + k'_A} k_1^{stat}$$

$$k_{-1}^{app} = f\left(\frac{1}{1 + k'_B} k_1^{mob} + \frac{k'_A}{1 + k'_B} k_1^{stat}\right) \tag{12}$$

If the rate constants in the gas phase are accessible by an independent method, it is possible to calculate the rate constants in the stationary liquid phase, as previously described.

(iii) After the two steps (i) and (ii) have proceeded, the content of the mobile phase is shifted to the subsequent theoretical plate, whereas the analytes in the stationary phase are retained. While a given amount of the interconverting species A and B is introduced in the first theoretical plate of the first dimension, the content of the mobile phase of the last theoretical plate of the first dimension is sampled during the modulation time period $t_{mod}$. After every modulation period $t_{mod}$ the content of the modulator is shifted to the first theoretical plate of the second dimension, where steps (i), (ii), and (iii) proceed. Finally the mobile phase's content of the last theoretical plate is recorded as a comprehensive chromatogram featuring a time-resolved interconversion profile over the time $t$. The comprehensive chromatogram can be split into discrete sections of the modulation time $t_{mod}$ to represent the elution profile in a 3D-plot.

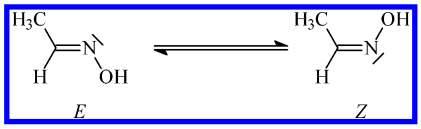


**Figure 3.** *E*/*Z* isomerization of acetaldoxime. Possible interconversion pathways of oximes are inversion at the nitrogen or rotation of the OH group around the C−N bond. The *E*/*Z* isomers of acetaldoxime show pronounced interconversion during gas chromatographic separation (cf. Figure 4a).

**Table 1.** Mean Values of the *E*/*Z*-Isomerization Rate Constants and Barriers of Acetaldoxime in the Presence of Terephthalate Terminated Polyethylene Glycol as Stationary Phase, Determined by Dynamic Gas Chromatography

| T [°C] | $k_{E\to Z}$ [$1 \times 10^{-3}$ s$^{-1}$] | $k_{Z\to E}$ [$1 \times 10^{-3}$ s$^{-1}$] | $\Delta G^{\ddagger}_{E\to Z}$ [kJ·mol$^{-1}$] | $\Delta G^{\ddagger}_{Z\to E}$ [kJ·mol$^{-1}$] |
|---|---|---|---|---|
| 80.0 | 7.91 | 5.83 | 101.2 | 102.1 |
| 90.0 | 13.58 | 9.51 | 102.5 | 103.6 |

It is important for the simulation and comparison of results that the modulation interval, both for the experiment and the simulation, is synchronized with the injection of the analytes on the first dimension.

It also has to be noted that the model described here can only be applied to isothermal, isobaric, and isocratic separation conditions. For processes under nonisothermal, nonisobaric, and nonisocratic separation conditions a step-by-step adaptation of the corresponding chromatographic parameters is necessary, leading to much more complex simulations.

The stationary phases employed in the first and second dimension do not necessarily have to be of the same selectivity toward the analytes. An example for a reversal of selectivity between first and second dimension is given below.

**Calculation Methods.** ChromWin 2D permits the simulation of the following processes:

*Enantiomerization* simulates two-dimensional elution profile with a given hold-up time of the first dimension $t_M$, total retention times of the analytes A and B on the first dimension $t_R^A$ and $t_R^B$, the initial amounts of the enantiomers A and B, the mean theoretical plate numbers $N^{1st}$ and $N^{2nd}$ of the first and second dimension, the retention factors $k'_A$ and $k'_B$ of the analytes on the second dimension, and the rate constants $k_1^{mob}$ and $k_1^{stat}$. The descriptors A and B of the retention factors refer to the elution order of the first dimension, so that in the case of $k'_A > k'_B$ the elution order and the rate constants $k_1^{mob}$ and $k_1^{stat}$ on the second dimension are reversed.

*Isomerization/Epimerization* simulates reversible first-order reactions with a given hold-up time of the first dimension $t_M$, total retention times of the analytes A and B on the first dimension $t_R^A$ and $t_R^B$, the initial amounts of the stereoisomers A and B, the mean theoretical plate numbers $N^{1st}$ and $N^{2nd}$ of the first and second dimension, the retention factors $k'_A$ and $k'_B$ of the analytes on the second dimension, the rate constants $k_1^{mob}$ and $k_1^{stat}$, and the equilibrium factor $f$. Additionally, the principle of microscopic reversibility can be neglected to study its effect on the elution profile.

The data size of the simulated files is automatically reduced to about 250 KByte per file compared to about 30 KByte for one-dimensional simulations.

For an efficient use of CPU time, ChromWin 2D offers the possibility to define batch jobs. The data can be entered in a spreadsheet, which is provided with the program, saved as an ascii-file, and imported into ChromWin 2D. After the simulation of the entries in a batch job an output file is generated, summarizing all parameters.

## RESULTS AND DISCUSSION

To demonstrate the applicability of the program for the evaluation of isomerization reactions, the *E*/*Z* isomeri-

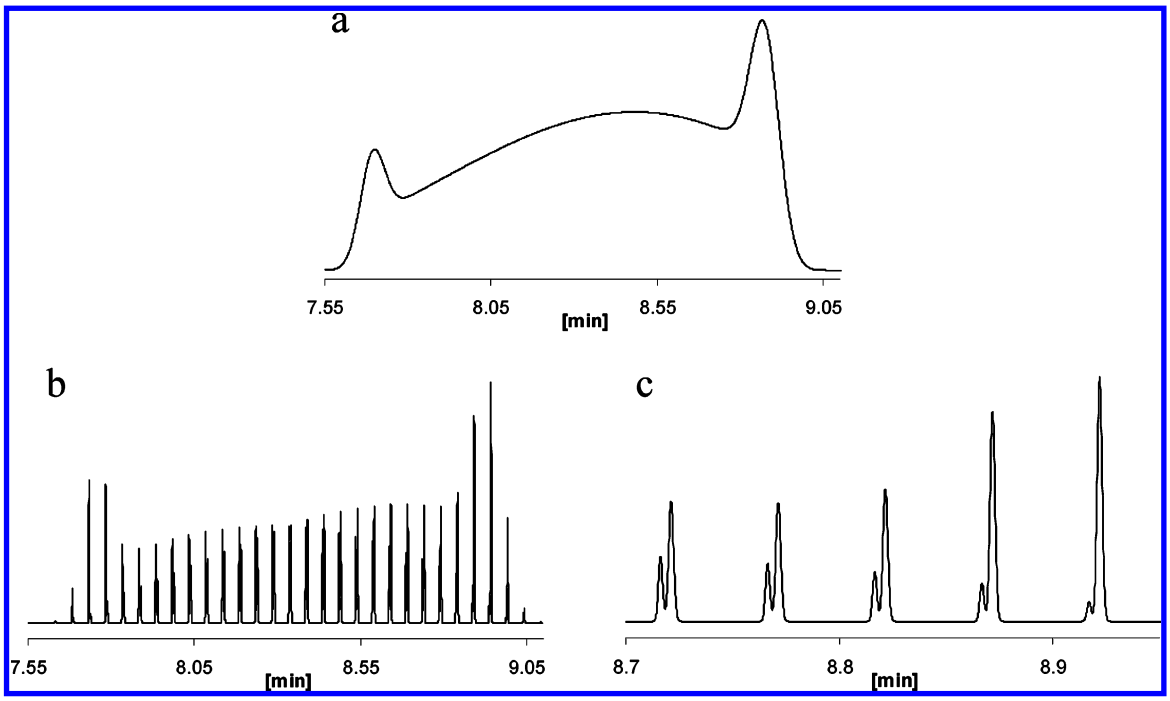


**Figure 4.** Simulated elution profiles of DGC and DGC×DGC experiments of an *E*/*Z* isomerization of acetaldoxime at 80 °C. Simulation parameters: 1st dimension: $t_M = 2.60$ min $t_R^A = 7.69$ min, $t_R^B = 8.88$ min, $N = 30900$, $k_1 = 5.44 \times 10^{-3}$ s$^{-1}$, $f = 0.74$, 2nd dimension: $k'_1 = 12$, $k'_2 = 17$, $N = 1000$, modulation time 3 s. (a) Elution profile, considering simulation parameters of 1st dimension, (b) complete comprehensive two-dimensional elution profile, and (c) zoom in.

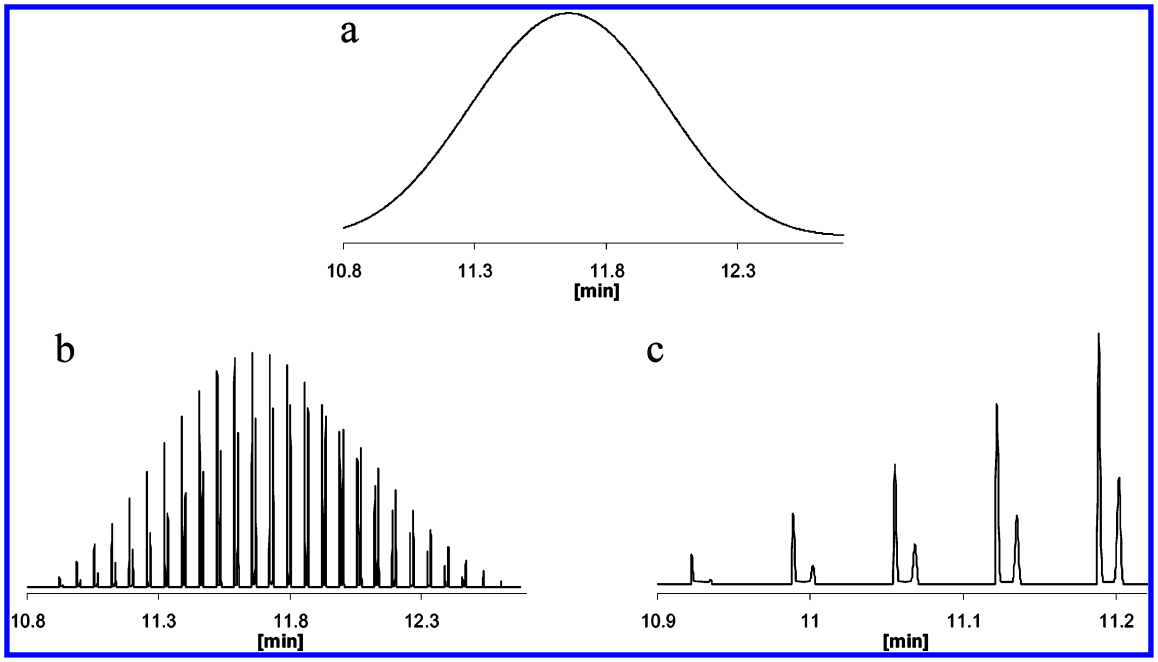


**Figure 5.** Simulated elution profiles of DGC and DGC×DGC experiments of a fast enantiomerization leading to peak coalescence in the first dimension and separation in the second dimension. Simulation parameters: 1st dimension: $t_M = 0.592$ min $t_R^A = 10.69$ min, $t_R^B = 12.62$ min, $N = 30600$, $k_1 = 1.260 \times 10^{-2}$ s$^{-1}$, 2nd dimension: $k'_1 = 17$, $k'_2 = 22$, $N = 3000$, modulation time 4 s. (a) Elution profile, considering simulation parameters of 1st dimension, (b) complete comprehensive two-dimensional elution profile, and (c) zoom in.

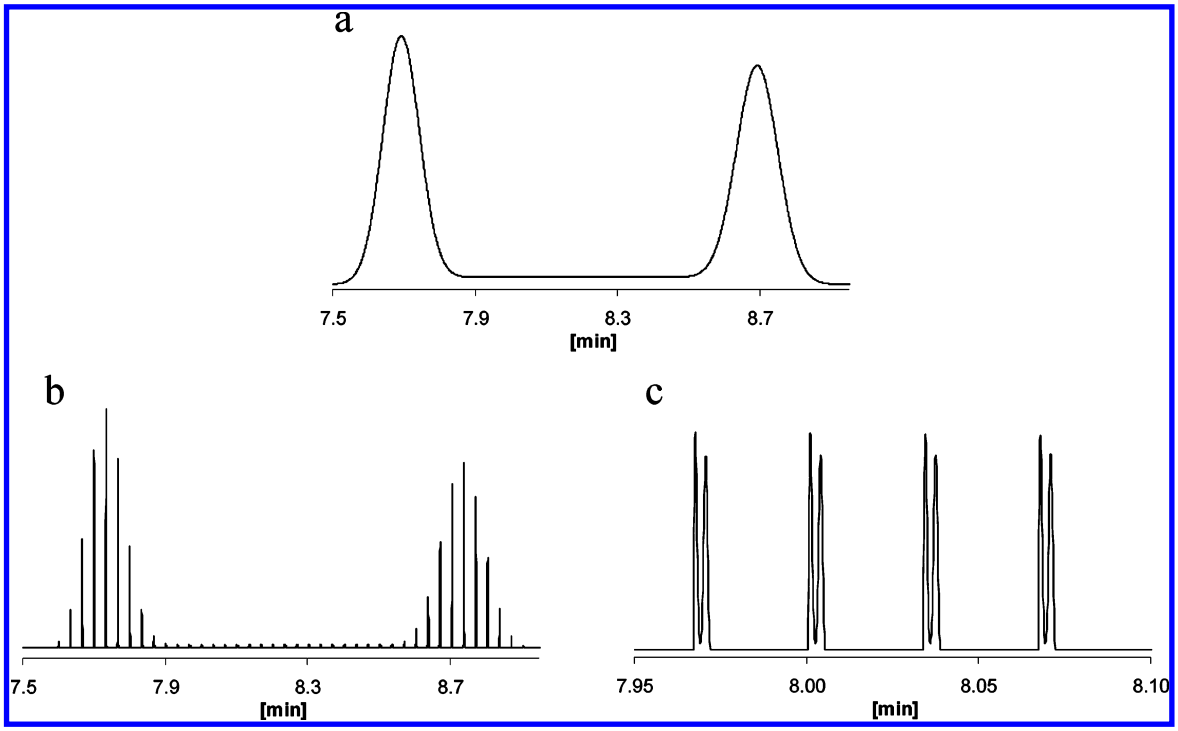


**Figure 6.** Simulated elution profiles of DGC and DGC×DGC experiments of a slow enantiomerization leading to a very low plateau formation in the first dimension. Simulation parameters: 1st dimension: $t_M = 0.817$ min $t_R^A = 7.692$ min, $t_R^B = 8.693$ min, $N = 20200$, $k_1 = 2.270 \times 10^{-4}$ s$^{-1}$, 2nd dimension: $k'_1 = 8.4$, $k'_2 = 9.6$, $N = 2000$, modulation time 2 s. (a) Elution profile, considering simulation parameters of 1st dimension, (b) complete comprehensive two-dimensional elution profile, and (c) zoom in.

zation of acetaldoxime[25,26] (cf. Figure 3) was chosen as an example.

However the evaluation of the kinetic parameters from the one-dimensional elution profile is difficult as the precise isomer ratio can only be estimated under these experimental conditions. Due to the high separation efficiency and peak sensitivity of two-dimensional gas chromatography the underlying interconversion profile was time-resolved by cryogenic modulation,[22] allowing the precise determination of the isomeric ratio. The rate constants of the forward and backward reaction were evaluated by iterative comparison between simulated and experimental chromatograms (cf. Figure 4 (parts b and c)) and are summarized in Table 1.

Because of the small temperature range ($\Delta T = 10$ K) the activation parameters could only be estimated: $\Delta H^{\ddagger}_{E\rightarrow Z} = 54.6 \pm 2.0$ kJ·mol$^{-1}$, $\Delta S^{\ddagger}_{E\rightarrow Z} = -132 \pm 12$ J·K$^{-1}$·mol$^{-1}$,

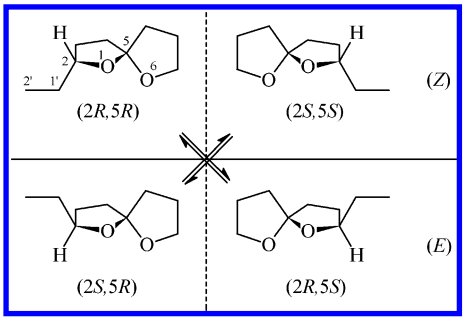


**Figure 7.** Epimeric and enantiomeric pairs of chalcogran. Top left: (*Z*)−(2*R*,5*R*)-2-ethyl-1,6-dioxaspiro[4.4]nonane. Top right: (*Z*)−(2*S*,5*S*)-2-ethyl-1,6-dioxaspiro[4.4]nonane. Bottom left: (*E*)−(2*S*,5*R*)-2-ethyl-1,6-dioxaspiro[4.4]nonane. Bottom right: (*E*)−(2*R*,5*S*)-2-ethyl-1,6-dioxaspiro[4.4]nonane.

$\Delta H^{\ddagger}_{Z\rightarrow E} = 49.2 \pm 2.0$ kJ·mol$^{-1}$, and $\Delta S^{\ddagger}_{Z\rightarrow E} = -150 \pm 12$ J·K$^{-1}$·mol$^{-1}$. These values agree very well with experimental data obtained by independent experiments, i.e. dynamic NMR (DNMR).[33]

In literature experimental examples for enantiomerization processes in two-dimensional chromatography are not yet described. The following two examples predict that two-dimensional dynamic chromatography can enhance the range of determinable rate constants and interconversion barriers. As values for the simulations chromatographic parameters and rate constants similar to those described in the literature[7,8] have been used. It can be expected that the true distribution of enantiomers with low interconversion barriers, normally leading to peak coalescence on the first dimension, can be revealed on the second dimension as the separation process is much faster compared to the interconversion kinetics (cf. Figure 5). A distinct plateau formation is visible for every modulation step on the second dimension.

On the other hand, very slow enantiomerization processes lead to an almost undistinguishable plateau formation in one-dimensional chromatography. As the peak sensitivity is enhanced by the modulation, the interconverted enantiomers can be made visible (cf. Figure 6). Figure 6c also demonstrates that both enantiomers are present in the plateau region which is not expected for a separation of stereostable enantiomers.

The here presented program can also be used to predict complex scenarios such as the epimerization of the four stereoisomers of chalcogran ((2*RS*,5*RS*)-2-ethyl-1,6-dioxaspiro-[4.4]nonane), the principal component of the aggregation pheromone of the bark beetle *pityogenes chalcographus* (cf. Figure 7).[34]

The interconversion takes place at the spiro-center (C5). In diastereo- and enantioselective one-dimensional dynamic gas chromatography (DGC) the epimerization of chalcogran gives rise to two independent interconversion peak profiles, each featuring a plateau between the peaks of the interconverting epimers. Depending on the chiral stationary phase (CSP) the elution order of the stereoisomers leads to a separation or overlapping of the two interconversion peak profiles, respectively. On the CSP nickel(II)-bis[(1*R*)-3-(heptafluorobutanoyl)camphorate], dissolved in OV-101,[35−38] the elution order is (2*S*,5*S*)-chalcogran < (2*R*,5*R*)-chalcogran < (2*R*,5*S*)-chalcogran < (2*S*,5*R*)-chalcogran at 93 °C, leading to a overlapped double plateau formation, where the first and last peak and the second and third peak interconvert, respectively. On Chirasil-$\beta$-Dex (permethylated $\beta$-cyclodextrin linked by a monooctamethylene spacer to poly(dimethylsiloxane))[39,40] the elution order is (2*R*,5*S*)-chalcogran < (2*R*,5*R*)-chalcogran < (2*S*,5*S*)-chalcogran < (2*S*,5*R*)-chalcogran.[41] On the CSP nickel(II)-bis[(1*R*)-3-(heptafluorobu-

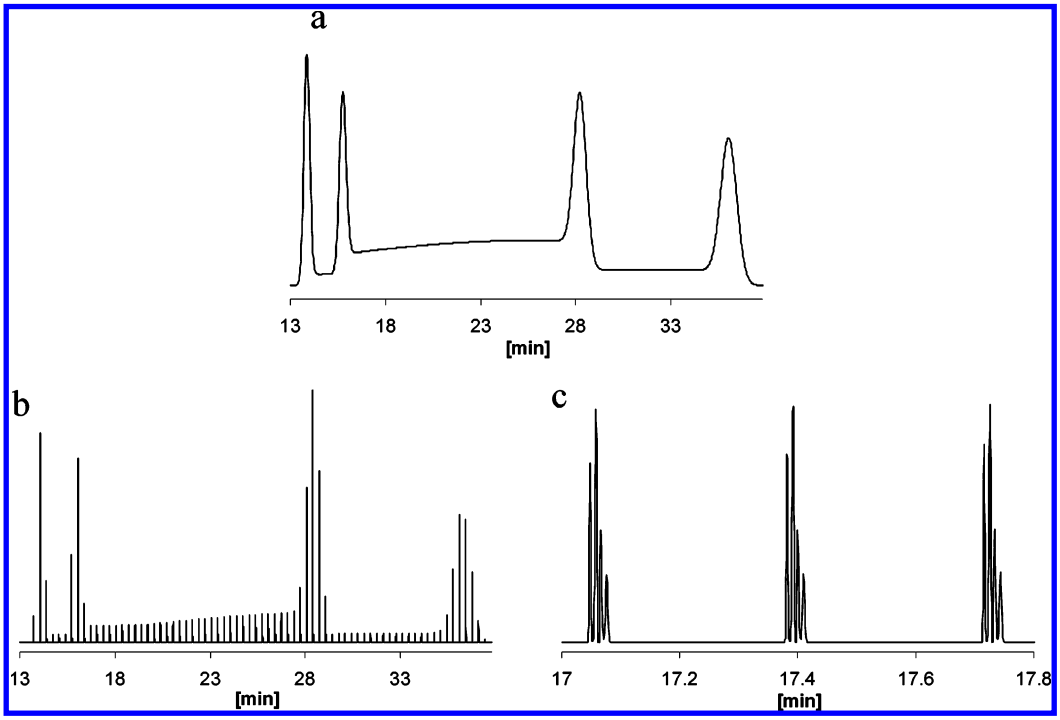


**Figure 8.** Simulated elution profiles of DGC and DGC×DGC experiments of the epimerization of chalcogran at 93 °C. Simulation parameters: 1st dimension: $t_M = 2.02$ min, $N = 7000$, $k_1 = 1.201 \times 10^{-3}$ s$^{-1}$, $f = 0.76$, retention times on the 1st dimension: (2*S*,5*S*)-chalcogran $t_R = 13.87$ min, (2*R*,5*R*)-chalcogran $t_R = 15.76$ min, (2*R*,5*S*)-chalcogran $t_R = 28.24$ min, (2*S*,5*R*)-chalcogran $t_R = 36.06$ min, 2nd dimension: $N = 1500$, modulation time 20 s, retention factors on the 2nd dimension: (2*R*,5*S*)-chalcogran $k' = 17$, (2*R*,5*R*)-chalcogran $k' = 21$, (2*S*,5*S*)-chalcogran $k' = 23$, (2*S*,5*R*)-chalcogran $k' = 27$. (a) Elution profile, considering simulation parameters of 1st dimension, (b) complete comprehensive two-dimensional elution profile, and (c) zoom in.

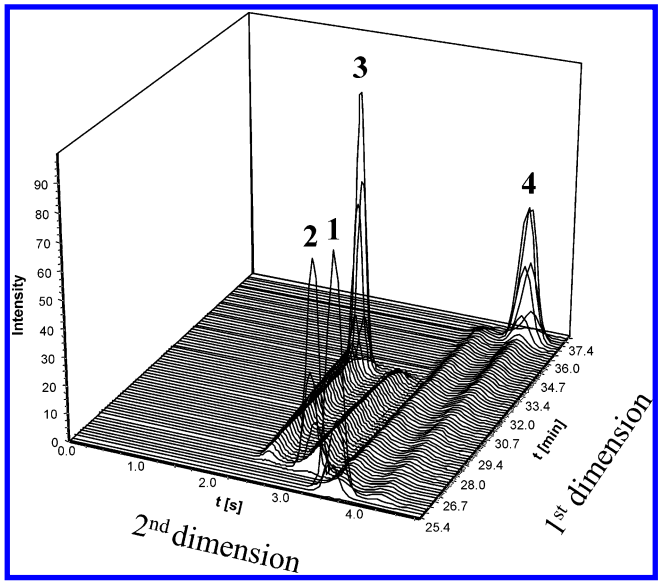


**Figure 9.** 3D-Plot of the simulated elution profiles of a DGC×DGC experiment of the epimerization of chalcogran at 93 °C. The simulation parameters are the same as for Figure 8. 1: (2*S*,5*S*)-chalcogran, 2: (2*R*,5*R*)-chalcogran, 3: (2*R*,5*S*)-chalcogran, 4: (2*S*,5*R*)-chalcogran.

tanoyl)camphorate] a very good separation with a pronounced plateau formation is obtained (cf. Figure 8a). On Chirasil-$\beta$-Dex the single stereoisomers can be separated without overlapping of the epimeric pairs. The rate constants of epimerization in the presence of the chiral stationary phases nickel(II)-bis[(1*R*)-3-(heptafluorobutanoyl)camphorate] and Chirasil-$\beta$-Dex are very similar. Therefore the following scenario in two-dimensional gas chromatography can be envisaged: On the first dimension chalcogran is separated with the CSP nickel(II)-bis[(1*R*)-3-(heptafluorobutanoyl)-camphorate], on the second dimension with Chirasil-$\beta$-Dex, leading to a change in the elution order and complete separation of the single stereoisomers. For the simulation experimentally determined elution and kinetic parameters were employed, the two independent epimerization peak profiles were calculated, and the final elution profile was obtained by superposition of the two epimerization profiles (cf. Figure 8). From Figure 8b,c it can be expected that a complete time-resolved separation of all four stereoisomers can be achieved revealing the amounts of the individual stereoisomers under the interconversion profile. In Figure 9 the elution profile is depicted as a 3D-plot.

## CONCLUSIONS

Simulations based on the extended theoretical plate model are fast and efficient for the evaluation and prediction of elution profiles in two-dimensional dynamic chromatography. It allows for determining and verifying rate constants and activation barriers of interconverting stereoisomers and enhances the potential of dynamic chromatography and electrophoresis as could be shown for the here presented examples. Two-dimensional dynamic chromatographic experiments offer the possibility of visualizing the ratio of the stereoisomers underneath a plateau arising from the interconversion stereoisomeric analytes during the separation process. Two-dimensional dynamic chromatography in combination with computer simulation of elution profiles allows the very precise temperature dependent determination of the ratio of the individual interconverting stereoisomers which is essential to determine kinetic (reaction rate constants $k_1$ and $k_{-1}$, Gibbs activation energy $\Delta G^{\ddagger}$, activation enthalpy $\Delta H^{\ddagger}$, and activation entropy $\Delta S^{\ddagger}$) as well as thermodynamic parameters (isomeric ratio, Gibbs free energy $-\Delta G$, enthalpy $\Delta H$, and entropy $\Delta S$) in a single experimental setup under the same experimental conditions. There is no limit to large plate numbers, which makes the program also suitable for simulations in electromigration systems.

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