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A novel software tool for high throughput measurements of interconversion barriers: DCXplorer☆

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ABSTRACT

The software program DCXplorer is introduced to directly access interconversion rate constants in dynamic chromatography and electrophoresis. The program utilizes the unified equation of chromatography which can evaluate reaction rate constants of all kinds of first order reactions of processes taking place during a separation process. Evaluations with DCXplorer are facilitated by a graphical user interface which allows zooming into the area of interest of an interconversion profile and calculating reaction rate constants without a time consuming simulation process. DCXplorer was applied to determine the enantiomerization barrier of the diuretic drug chlorthalidone by pressure supported dynamic capillary electrokinetic chromatography (DEKC) under acidic conditions at pH 5.00 and pH 3.75. Activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were obtained from temperature dependent measurements between 15.0 and 35.0 °C in 5 K steps at pH 3.75 and between 30.0 and 50.0 °C in 10 K steps at pH 5.00.

1. Introduction

There is a broad interest into the stereodynamics of chiral molecules, in particular of enantiomers, because data about the interconversion kinetics is important in the development of synthetic methods, in catalysis and to ensure that chiral drugs with different effects of the single enantiomers are not prone to racemization [1–3]. Depending on the interconversion barrier several techniques have been developed to study conformational or constitutional changes in chiral molecules. Among the most commonly used techniques are polarimetry, which requires the isolation of mg amounts of pure or at least enantiomerically enriched substance, dynamic NMR, stopped-flow and dynamic chromatographic or electrophoretic techniques.

Very attractive are the dynamic chromatographic and electrophoretic techniques because only minute amounts of the racemic target compound are needed and impurities do not interfere with the determination of exact kinetic data. Furthermore no special analytical equipment is necessary. For the evaluation of elution profiles obtained by dynamic chromatography or electrophoresis sophisticated methods have been developed in the past for the relatively fast and precise determination of reaction rate constants [4–16]. Despite these efforts, there is still a need for improvement in the evaluation process of dynamic elution profiles [17], because using iterative computer simulations, especially for large datasets is computationally expensive and direct calculation with the approximation function [18–20] is only possible for the investigation of enantiomerization processes of racemic mixtures. The recently described unified equation [21–24] allows for the direct calculation of reaction rate constants k_1 and k_{-1} of all types of first order reactions regardless of the initial concentrations of the interconverting analytes A and B and the equilibrium constant $K_{A/B}$. It has been demonstrated that the unified equation is superior in precision compared to iterative simulation procedures, where billions of floating point operations are necessary [21]. Because of the limited precision of these floating point operations some errors are not compensated.

In the present study the software program DCXplorer is introduced, which utilizes the analytical solution of the unified equation. This software was used to evaluate electropherograms of the enantiomerization of the diuretic drug chlorthalidone.

Comparison of the kinetic data obtained from temperature dependent measurements between 15.0 and 40.0 °C at pH 3.75 in

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this study with those at pH 5.0 corroborates the assumption that the enantiomerization is catalyzed under acidic conditions.

2. Materials and methods

2.1. Reagents and materials

Chlorthalidone (2-chloro-5-(1-hydroxy-3-oxoisoindolin-1-yl)-benzenesulfonamide) was a gift from Novartis (Basel, Switzerland). Citric acid, methanol, sodium hydroxide, and sodium monophosphate ($Na_2HPO_4{\cdot}H_2O$) were obtained from Fluka (Deisenhofen, Germany), and were used as is. Carboxymethyl-β-cyclodextrin (Cavasol® W7 CM) and dimethyl-β-cyclodextrin (containing in average 1.8 methyl groups per glucose unit) were a gift from Wacker Chemie (Munich, Germany). Sodium phosphate/citric acid buffer solution was prepared with 18.2 MΩ cm high purity water obtained from a Millipore-Q System (Millipore, Marlborough, Massachusetts, USA). Chlorthalidone was dissolved in methanol (1 mg/ml). Fused-silica capillaries (ID 50 μm, OD 365 μm) were purchased from Microquartz (Munich, Germany).

2.2. Pressure supported DEKC

Capillary electrophoretic separations were performed on an Agilent ^{3D}CE system (Agilent, Waldbronn, Germany) equipped with a diode array detector (DAD). Fused-silica capillaries with a total length of 80.5 cm and an effective length of 72.0 cm were used. Samples were injected hydrodynamically (50 mbar) at the anodic end of the capillary for 5 s and separated at various temperatures ranging from 15.0 to 40.0 °C using a constant voltage of −25 kV and a pressure of 40 mbar at the cathodic side. The detection wavelength was 214 ± 5 nm. Separations were carried out in a 100 mM sodium phosphate/citric acid buffer solution at pH of 3.75 or 5.00, adjusted with a Schott pH Meter Lab 850 (Schott, Mainz, Germany), containing a mixture of 12.5 mg/ml carboxymethyl-β-cyclodextrin and 12.5 mg/ml dimethyl-β-cyclodextrin as chiral mobile phase additive. New fused-silica capillaries were flushed with 1 M NaOH for 10 min, followed by water and running buffer for 5 min each. Between each run the capillary was rinsed with 1 M NaOH for 1 min, water for 2 min and running buffer for 5 min.

2.3. Determination of rate constants of enantiomerization

Evaluation of the experimental and simulated dynamic interconversion profiles was performed with the recently derived analytical solution of the unified equation of dynamic chromatography [21]. The total retention times of the individual enantiomers t_R, the plateau height h_p, the peak widths at half height w_h, the average number of theoretical plates N, calculated from the retention times and the peak width at half height of the individual peaks were used as data input for the calculation of reaction rate constants. The initial ratio $[A_0/B_0]$ was set to 1, because a racemic mixture of chlorthalidone was used in the experiments. It has to be pointed out that the analytical solution of the unified equation can also be used to perform evaluations of experiments starting with nonracemic mixtures. In cases where the plateau height exceeds 50% the peak width at half height w_h is determined from the peak width of the outer part of the individual peaks.

Eq. (1) is used in case the peak of the first eluted stereoisomer is higher than the peak of the second eluted isomer. Eq. (2) is used in case the second eluted peak is higher than the first eluted peak:

$$k_1^{ue} = -\frac{1}{t_R^A}\left(\ln\left(\frac{100B_0 + A_0(100 - h_p(1+\sqrt{2/\pi N}))}{t_R^B - t_R^A}\right) - \ln\left(B_0\left(\frac{h_p e^{-(t_R^A - t_R^B)^2/2\sigma_B^2} - 100e^{-(t_R^A - t_R^B)^2/8\sigma_B^2}}{\sigma_B\sqrt{2\pi}} + \frac{100}{t_R^B - t_R^A}\right) - A_0\left(\frac{100e^{-(t_R^B - t_R^A)^2/8\sigma_A^2} - h_p}{\sigma_A\sqrt{2\pi}} + \frac{h_p(1+\sqrt{2/\pi N}) - 100}{t_R^B - t_R^A}\right)\right)\right) \quad (1)$$

$$k_1^{ue} = -\frac{1}{t_R^A}\left(\ln\left(\frac{100A_0 + B_0(100 - h_p(1-\sqrt{2/\pi N}))}{t_R^B - t_R^A}\right) - \ln\left(A_0\left(\frac{h_p e^{-(t_R^B - t_R^A)^2/2\sigma_A^2} - 100e^{-(t_R^B - t_R^A)^2/8\sigma_A^2}}{\sigma_A\sqrt{2\pi}} + \frac{100}{t_R^B - t_R^A}\right) - B_0\left(\frac{100e^{-(t_R^A - t_R^B)^2/8\sigma_B^2} - h_p}{\sigma_B\sqrt{2\pi}} + \frac{h_p(1-\sqrt{2/\pi N}) - 100}{t_R^B - t_R^A}\right)\right)\right) \quad (2)$$

with $\sigma_i = w_i/\sqrt{8\ln 2}\quad i = \{A, B\}$.

The principle of microscopic reversibility [4,6,25] has been considered in Eqs. (1) and (2) to reflect the reversibility of the degenerated interconversion process in presence of an enantioselective selector. The reaction rate constant k_1^{ue} denotes the conversion of the first eluted enantiomer into the later eluted enantiomer.

All calculations were performed with the software program DCXplorer [26], which utilizes the unified equation to obtain reaction rate constants in dynamic and on-column reaction chromatography. Single elution profiles are directly integrated and evaluated without any iterative step using the unified equation in a graphical interface.

2.4. Calculation of activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$

For the evaluation 9 experiments between 30.0 and 50.0 °C at pH 5.00 and 15 experiments between 15.0 and 35.0 °C at pH 3.75 were considered. At higher temperatures peak coalescence occurred and therefore rate constants could not be determined with sufficient precision. The Gibbs free activation energy $\Delta G^{\neq}(T)$ was calculated according to the Eyring Eq. (3) with k_B as the Boltzmann constant ($k_B = 1.380662 \times 10^{-23}$ J K^{-1}), T as the enantiomerization temperature [K], h as Planck's constant ($h = 6.62617 \times 10^{-34}$ J s), R as the gas constant ($R = 8.31441$ J K^{-1} mol^{-1}). The statistical factor κ was set to

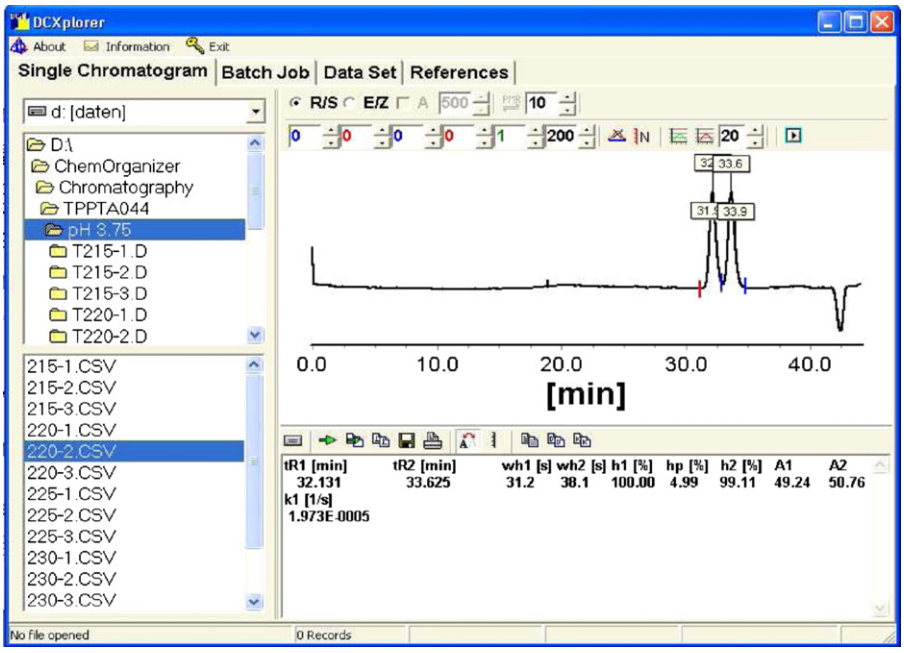

Fig. 1. User interface of the software DCXplorer which utilizes the unified equation of chromatography for the direct evaluation of elution profiles in dynamic chromatography and electrophoresis. All kinds of first order reactions taking place in the time scale of portioning can be analyzed by zooming into the area of interest.

0.5 for a reversible and degenerated interconversion process.

$$\Delta G^{+}(T) = -RT \ln\left(\frac{k_1^{ue} h}{\kappa k_B T}\right) \quad (3)$$

From the temperature dependent studies the activation enthalpy ΔH^{+} for the enantiomerization was obtained via the slope and the activation entropy ΔS^{+} via the intercept of the Eyring plot ($\ln(k_1^{ue}/T$ versus $T^{-1})$).

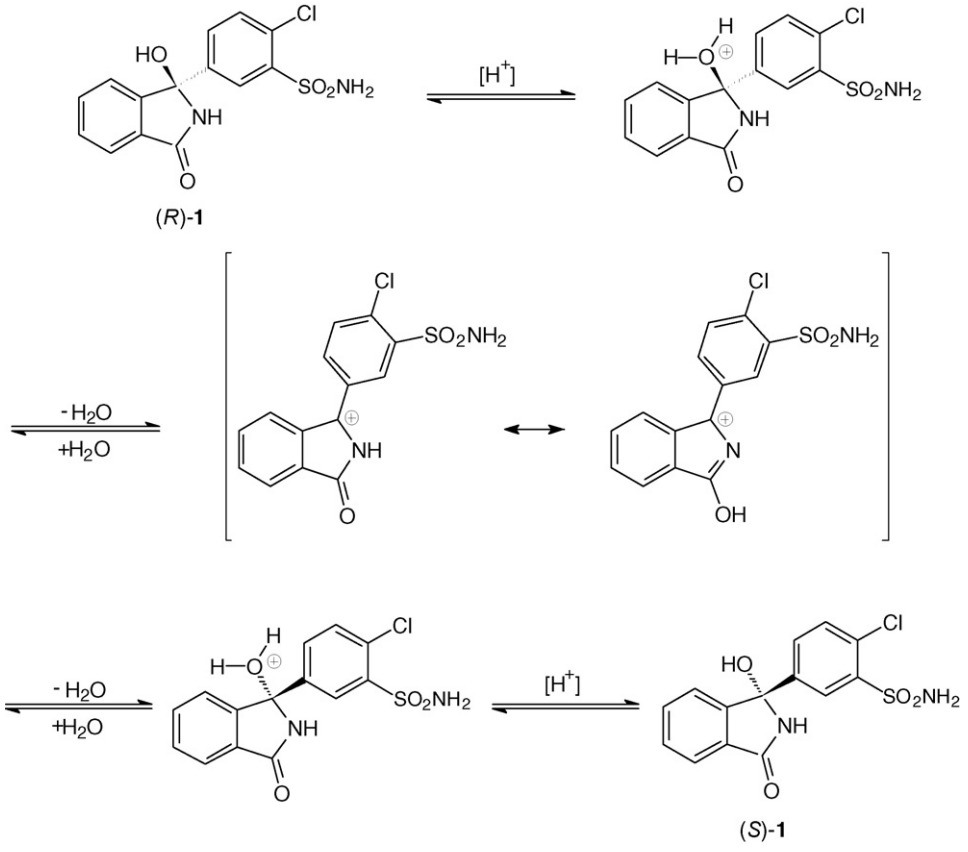

Fig. 2. Proposed acid catalyzed enantiomerization mechanism of chlorthalidone via formation of a benzyl cation as intermediate.

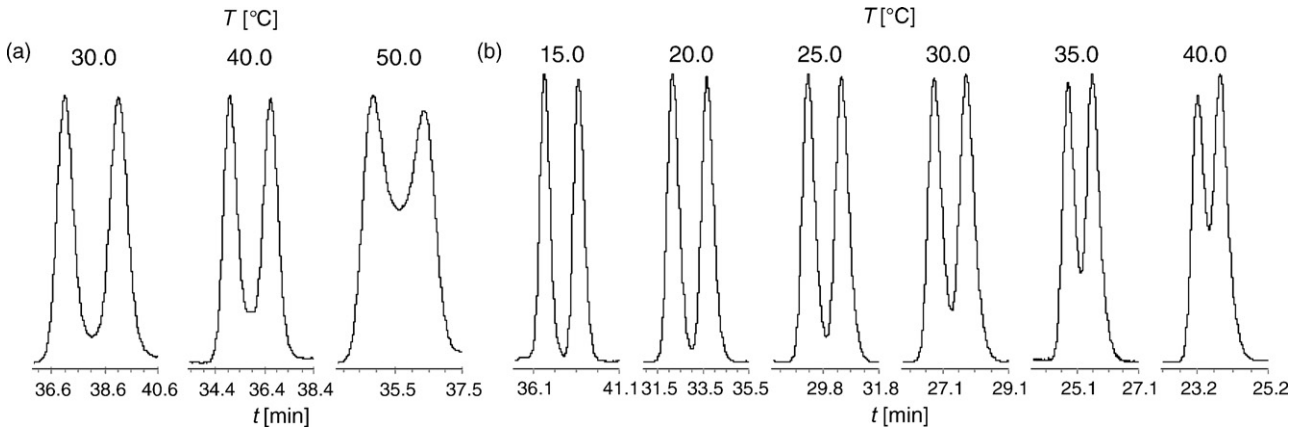

Fig. 3. Selected electropherograms at (a) pH 5.00 and (b) pH 3.75 of the enantiomerization of chlorthalidone at various temperatures. 72 cm (effective length) fused-silica capillary (I.D. 50 μm), 100 mM sodium phosphate/citric acid buffer, 12.5 mg/ml carboxymethyl-β-cyclodextrin, 12.5 mg/ml dimethyl-β-cyclodextrin, $U = -25$ kV, $\lambda = 214 \pm 5$ nm.

3. Results and discussion

Recently, the unified equation of chromatography [21] (Eqs. (1) and (2)) was derived to directly access first order reactions taking place in the time scale of a chromatographic or electrophoretic separation process. These equations are based on the stochastic model reducing an elution profile into Gaussian distribution functions $\Phi(t)$ of the unchanged peaks and a probability density function $\Psi(t)$ to describe the interconverted part in a chromatogram. The unified equation has been completely validated and compared to other evaluation methods based on the theoretical plate model of chromatography and the stochastic model [21,22]. It was found that the unified equation is superior in regard of precision compared to iterative simulation procedures. This can be explained by the fact that for simulation based evaluations billions of iterative calculation steps are necessary which are limited by the precision of the floating point numbers. The unified equation was implemented in the software program DCXplorer [26] to directly evaluate elution profiles in a graphical user interface (cf. Fig. 1). Chromatographic raw data in ASCII can be opened with a file explorer and the elution profiles are evaluated by zooming into the area of the interconverting peaks. All chromatographic parameters are directly determined by an integration method and used to calculate reaction rate constants with the unified equation. For the evaluation process it is important to choose between a degenerated process, where k_1 and k_{-1} are equal, e.g. in enantiomerization processes, or if it is an epimerization or isomerization process where k_1 and k_{-1} are not equal. The kinetic data are apparent rate constants which are weighted means of the individual rate constants in the mobile and (pseudo) stationary phase. However these apparent rate constants from flow dependent experiments or by comparison with stopped-flow experiments can be used to calculate individual rate constants of the mobile and stationary phase [10]. Data can be exported into spreadsheet applications and can be also evaluated in a batch process. Kinetic parameters can be determined in real time also allowing to perform high throughput analysis [27,28] of structurally modified interconverting stereoisomers to get insights into the interconversion mechanism by comparison of activation parameters and structure correlation.

DCXplorer has been used to study the enantiomerization of chlorthalidone **1** (2-chloro-5-(1-hydroxy-3-oxoisoindolin-1-yl)-benzenesulfonamide) [29–32] catalyzed under acidic conditions at pH 5.00 to validate the program with previously reported enantiomerization data [33] and at pH 3.75 to corroborate the

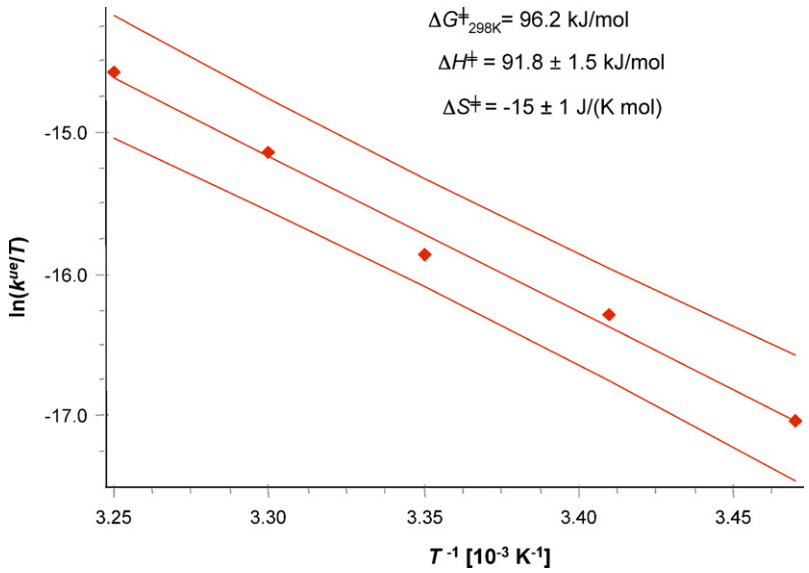

Fig. 4. Eyring plot for the determination of the activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of the enantiomerization of chlorthalidone from the DEKC experiment at pH 3.75. The upper and lower curves represent the error bands of the linear regression with a level of confidence of 95%. For the linear regression 15 data points were considered.

Table 1
Selected experimental data (100 mM sodium phosphate/citric acid buffer, 12.5 mg/ml carboxymethyl-β-cyclodextrin and 12.5 mg/ml dimethyl-β-cyclodextrin, $U = -25$ kV, $p = 40$ mbar) of chlorthalidone at pH 5.00 and pH 3.75

pH	T (°C)	t_R^A (min)	t_R^B (min)	N	h_A (%)	h_p (%)	h_B (%)	k_1^{ue} (10^{-5} s^{-1})
5.00	30.0	37.3	39.3	18300	100.0	6.2	99.2	3.27
5.00	40.0	35.0	36.6	17800	100.0	18.2	98.1	9.77
5.00	50.0	34.2	35.7	13700	100.0	51.8	95.9	24.8
3.75	15.0	36.7	38.7	20000	100.0	2.1	98.4	1.15
3.75	20.0	32.1	33.6	21200	100.0	4.3	99.2	2.30
3.75	25.0	29.3	30.5	22100	100.0	8.8	99.4	3.85
3.75	30.0	26.8	27.8	24300	99.3	18.5	100.0	6.70
3.75	35.0	24.8	25.7	25400	97.6	30.7	100.0	14.5

Rate constants were obtained by direct calculation with the analytical solution of the unified equation of dynamic chromatography using the software DCXplorer.

proposed enantiomerization mechanism involving acid catalysis (cf. Fig. 2). The enantiomer separation of chlorthalidone **1** was achieved by pressure supported EKC (−25 kV and 40 mbar pressure applied at the cathodic electrode) using 100 mM sodium phosphate/citric acid buffer and 12.5 mg/ml carboxymethyl-β-cyclodextrin and 12.5 mg/ml dimethyl-β-cyclodextrin [34–36] as chiral mobile phase additives (CMPA) at a pH value of 5.00 or 3.75, respectively.

Pronounced peak broadening between the separated peaks of the enantiomers was observed at pH 3.75 by varying the separation temperature between 15.0 and 40.0 °C in 5 K steps (cf. Fig. 3b). However, only a slight plateau formation between the separated peaks could be observed. Peak broadening towards the individual peaks (peak width at the inner side of the peaks is larger than at the outer part of the peaks) at a good peak resolution indicates an interconversion process without the occurrence of a pronounced plateau formation. At higher temperature peak coalescence occurred. The dynamic capillary electrokinetic chromatography (DEKC) experiments were repeated at least three times at exactly the same experimental conditions.

To determine the enantiomerization rate constants and to obtain the activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ from the temperature dependent measurements of chlorthalidone **1** the computer program DCXplorer was employed. Selected experimental data at pH 5.00 and pH 3.75 and rate constants obtained from the unified equation are summarized in Table 1.

The activation enthalpies $\Delta H^{\neq}$ for the enantiomerization of chlorthalidone **1** were obtained via the slope and the activation entropies $\Delta S^{\neq}$ via the intercept of the Eyring plots (T^{-1} versus $\ln(k_1^{ue}/T)$) (cf. Fig. 4 for pH 3.75).

An error band analysis of the linear regression with a level of confidence of 95% (pH 3.75: agreement factor $r = 0.9959$, residual SD $s_y = 0.0871$; pH 5.00: agreement factor $r = 0.9956$, residual SD $s_y = 0.0684$) of the activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ has been performed to determine deviations. The repeatability of the method is satisfactory (2.93 σ (SD) with a mean error of $\Delta G^{\neq}$ (20 °C) of 0.22 kJ mol^{-1} at pH 3.75).

Activation parameters obtained from the experiments at pH 5.00 ($\Delta G^{\neq}_{298\,K} = 97.6$ kJ mol^{-1}; $\Delta H^{\neq} = 70.2 \pm 1.2$ kJ mol^{-1}; $\Delta S^{\neq} = -92 \pm 6$ J K^{-1} mol^{-1}) agree very well with previously reported activation parameters determined under similar separation conditions by DEKC at pH 5.0 ($\Delta G^{\neq}_{298\,K} = 99.3$ kJ/mol; $\Delta H^{\neq} = 69.2 \pm 0.2$ kJ/mol; $\Delta S^{\neq} = -101 \pm 6$ J K^{-1} mol^{-1}) [33]. These previously reported data were evaluated by computer simulation [37] and validate the here determined activation parameters. It has to be pointed out that evaluation procedures based on iterative comparison of experimental elution profiles and simulated profiles are limited in precision due to termination criteria and precision in calculation.

The results of the activation parameter obtained at a pH of 3.75 ($\Delta G^{\neq}_{298\,K} = 96.2$ kJ mol^{-1}; $\Delta H^{\neq} = 91.8 \pm 1.5$ kJ mol^{-1}; $\Delta S^{\neq} = -15 \pm 1$ J K^{-1} mol^{-1}) show that the barrier has been lowered by $\Delta\Delta G^{\neq} \sim 1.5$ kJ mol^{-1} which can be attributed to acid catalysis at a lower pH value. However there is a strong change in the activation enthalpy $\Delta H^{\neq}$ and activation entropy $\Delta S^{\neq}$. It seems that at a lower pH value the transition state can be reached more easily, which leads to an increasing activation entropy $\Delta S^{\neq}$. This data and the fact that enantiomerization is facilitated at lower pH value leading to higher enantiomerization rate constants at lower temperatures (cf. Table 1) corroborates the proposed acid catalyzed enantiomerization mechanism.

4. Conclusions

The here presented software DCXplorer is a versatile tool to quickly evaluate elution profiles of interconverting stereoisomers employing the unified equation of chromatography. It is no longer necessary to separately determine chromatographic data which is used in a simulation process, but this data can now be directly accessed and evaluated within the user interface by visually zooming into the area of the interconverting species. This software is not limited to interconversion processes, but can be applied to all kinds of first order reactions as demonstrated also for a pseudo-first order reaction (acid catalysis) of the enantiomerization of chlorthalidone in DEKC, which helps to understand interconversion mechanisms.

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References

[1] M. Reist, B. Testa, P.A. Carrupt, Enantiomer 2 (1997) 147.
[2] M. Reist, P.-A. Carrupt, E. Francotte, B. Testa, Chem. Res. Toxicol. 11 (1998) 1521.
[3] N.H.H. Heegaard, T.J.D. Jorgensen, L. Cheng, C. Schou, M.H. Nissen, O. Trapp, Anal. Chem. 78 (2006) 3667.
[4] W. Bürkle, H. Karfunkel, V. Schurig, J. Chromatogr. 288 (1984) 1.
[5] J. Veciana, M.I. Crespo, Angew. Chem. 103 (1991) 85; J. Veciana, M.I. Crespo, Angew. Chem. Int. Ed. 30 (1991) 74.
[6] M. Jung, V. Schurig, J. Am. Chem. Soc. 114 (1992) 529.
[7] J. Oxelbark, S. Allenmark, J. Org. Chem. 64 (1999) 1483.
[8] G. Weseloh, C. Wolf, W.A. König, Angew. Chem. 107 (1995) 1771; G. Weseloh, C. Wolf, W.A. König, Angew. Chem. Int. Ed. 34 (1995) 1635.
[9] D.H. Hochmuth, W.A. König, Liebigs Ann. (1996) 947.
[10] P. Dubsky, E. Tesarová, B. Gas, Electrophoresis 25 (2004) 733.
[11] O. Trapp, G. Schoetz, V. Schurig, Chirality 13 (2001) 403.
[12] G. Cannazza, D. Braghiroli, A. Tait, M. Baraldi, C. Parenti, W. Lindner, Chirality 13 (2001) 94.
[13] J. Krupcik, P. Oswald, P. Majek, P. Sandra, D.W. Armstrong, J. Chromatogr. A 1000 (2003) 779.
[14] C.I.D. Newman, V.L. McGuffin, Electrophoresis 26 (2005) 537.

[15] C. Wolf, Chem. Soc. Rev. 34 (2005) 595.
[16] I. D'Acquarica, F. Gasparrini, M. Pierini, C. Villani, G. Zappia, J. Sep. Sci. 29 (2006) 1508.
[17] O. Trapp, Electrophoresis 26 (2005) 487.
[18] O. Trapp, V. Schurig, J. Chromatogr. A 911 (2001) 167.
[19] O. Trapp, V. Schurig, Chirality 14 (2002) 465.
[20] A.M. Skelley, R.A. Mathies, J. Chromatogr. A 1132 (2006) 304.
[21] O. Trapp, Anal. Chem. 78 (2006) 189.
[22] O. Trapp, Chirality 18 (2006) 489.
[23] O. Trapp, Electrophoresis 27 (2006) 534.
[24] O. Trapp, Electrophoresis 28 (2007) 691.
[25] O. Trapp, V. Schurig, J. Am. Chem. Soc. 122 (2000) 1424.
[26] O. Trapp, DCXplorer is available from the author as executable programs, running under Microsoft Windows 2000, XP and Vista.
[27] O. Trapp, S.K. Weber, S. Bauch, W. Hofstadt, Angew. Chem. 119 (2007) 7447; O. Trapp, S.K. Weber, S. Bauch, W. Hofstadt, Angew. Chem. Int. Ed. 46 (2007) 7307.
[28] O. Trapp, J. Chromatogr. A 1184 (2008) 160.
[29] G. Blaschke, H. Markgraf, Chem. Ber. 113 (1980) 2031.
[30] G. Severin, Chirality 4 (1992) 222.
[31] K. Cabrera, M. Jung, M. Fluck, V. Schurig, J. Chromatogr. A 731 (1996) 315.
[32] H. Jakubetz, M. Juza, V. Schurig, Electrophoresis 18 (1997) 897.
[33] G. Schoetz, O. Trapp, V. Schurig, J. Cap. Elec. 6 (1999) 169.
[34] M. Fillet, L. Fotsing, J. Crommen, J. Chromatogr. A 817 (1998) 113.
[35] J. Lötter, H.M. Krieg, K. Keizer, J.C. Breytenbach, Drug Dev. Ind. Pharm. 25 (1999) 879.
[36] R. Herraez-Hernandez, P. Campins-Falco, J. Chromatogr. B 740 (2000) 169.
[37] O. Trapp, V. Schurig, Comput. Chem. 25 (2001) 187.