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Stereointegrity of thalidomide: gas-chromatographic determination of the enantiomerization barrier

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Dedicated to Professor Dr Gottfried Blaschke on the occasion of his 65th birthday

Abstract

Enantioselective chromatographic methods, representing the most commonly used techniques for the determination of enantiomeric ratios, can also be used for the evaluation of stereochemical integrity. In the present study, two chromatographic methods, dynamic gas chromatography (DGC) and stopped-flow gas chromatography (SFGC) were used to determine the enantiomerization barrier of thalidomide. In the presence of a chiral stationary phase (CSP), the enantiomers of thalidomide produced characteristic elution profiles exhibiting plateaus and/or peak broadening which were observed between 190 and 220 °C in DGC. To obtain the enantiomerization barrier of thalidomide from experimental data, the fast and efficient simulation program ChromWin was used to simulate the elution profiles and obtain kinetic activation parameters. From temperature-dependent measurements the rate constants k_1 and k_{-1} and the kinetic activation parameters $\Delta G^{\#}$, $\Delta H^{\#}$ and $\Delta S^{\#}$ of the enantiomerization of thalidomide were obtained by DGC. The enantiomerization barrier $\Delta G^{\#}$ was determined to be 154 ± 2 kJ/mol with DGC and 150 ± 3 kJ/mol with the sfGC technique at 200 °C, respectively. The concept of the retention increment R' has been applied to separate the enantiomerization barrier of thalidomide in the dissolved and complexed state of the CSP.

Keywords: Chiral drug; ChromWin; Dynamic gas chromatography; Enantiomerization; Thalidomide; Stop-flow gas chromatography

1. Introduction

Racemic thalidomide was introduced as a sedative and anti-nausea drug in 1957, in a time when toxicity as well as teratogenicity studies were not yet included in the standard protocol of drug safety. The serious consequences of this negligence are known, and this incident had a major impact in the regulation of stereoisomeric drugs as single enantiomers. Moreover the US–American FDA policy for the development of new stereoisomeric drugs requires the submission of unambiguous data concerning the configurational stability of single enantiomers in new drug applications [1]. Therefore, drug manufacturers today

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are bound to assess the 'stereochemical integrity' of enantiomers and to examine the 'potential for interconversion... of the individual isomers'.

After its withdrawal from the market as a sedative, thalidomide has been approved by the FDA in 1998 for the treatment of leprosy. Today thalidomide is still used in many countries as a sedative and in addition as an anti-inflammatory drug and immunosuppressive agent. Since thalidomide is prone to enantiomerization in vitro and in vivo, a racemic switch of thalidomide is clearly not warranted and the drug is administered as a racemate for medical uses [2–7]. Nevertheless, studies concerned with the stereochemical lability of thalidomide and catalytic effects leading to enantiomerization appear as important as they were nearly 40 years ago.

Enantiomerization arises from the interconversion of a stereogenic element in a particular molecule and is defined as a reversible first order reaction. Enantioselective chromatography on chiral stationary phases (CSPs), a technique which is generally used for the determination of enantiomeric ratios (*er*), can also be employed for the evaluation of the stereochemical integrity of chiral compounds. If reversible interconversion occurs during the time scale of enantiomeric separation, characteristic peak profiles are obtained [8]. Depending on the resolution factor and the stereochemical lability of the chiral compound, separated on the CSP, peak distortion (tailing of the first peak and heading of the second peak), plateau formation (between the first and second peak) and finally peak coalescence is observed. Kinetic data of enantiomerization (k, $\Delta G^{\#}(T)$, $\Delta H^{\#}$ and $\Delta S^{\#}$) can be obtained by temperature-dependent studies by this method, referred to as *dynamic chromatography*, via peak-form analysis through the iterative comparison of simulated and experimental chromatograms [9–21]. The first simulation program, was published in 1984 [9] and was based on the theoretical plate model (TPM), later it was extended to simulations of up to 120 000 effective plates (SIMUL) [22,23]. The stochastic model (SM, [24–26]) which treats peaks as a Gaussian curve and utilizes a probability distribution to describe the interconverting species of the enantiomers has also been applied for the determination of enantiomerization barriers. The new program ChromWin [27] allows simulations with the TPM, the SM and the modified stochastic model (SM +). It is running on a personal computer under Windows and permits the simulation of chromatograms with large plate numbers N usually obtained by capillary gas chromatography and capillary electromigration methods.

In a complementary technique, referred to as stopped-flow chromatography [28–30], the enantiomers of the racemic mixture are quantitatively separated on a CSP in the first part of the column under conditions of absence of enantiomerization. Afterwards, the flow of the mobile phase is stopped and the temperature is raised until enantiomerization proceeds. Finally, the de novo enantiomeric ratio is determined in the second part of the column after resuming the flow under conditions of absence of enantiomerization. Kinetic data are calculated from the enantiomerization temperature T, time of enantiomerization t and enantiomeric ratio *er*.

In contrast to classical enantiomerization experiments by chiroptical methods (polarimetry), dynamic gas chromatography (DGC) and stopped-flow gas chromatography (sfGC) require only minute amounts of the racemate and allow the measurement of enantiomerization barriers between 80 and 150 $kJ\,mol^{-1}$. If isolated enantiomers are at hand, both methods can also be performed with either one or both of the single enantiomers. An indispensable requirement for the application of DGC and sfGC is the quantitative on-column separation of the enantiomers at elevated temperatures whereby the process of enantiomerization proceeds in the environment of a CSP which may entail catalytic effects on the enantiomerization process in DGC and sfGC. Moreover, the presence of the CSP leads to different forward and backward reaction rates of the interconverting species as the enantiomers possess distinct thermodynamic Gibbs energies ($-\Delta_{B,A}\Delta G$) in the chiral environment as prerequisite for their separation. In sfGC the presence of a CSP can be circumvented by a multidimensional approach [19,31–34] whereby the separation column is separated from the reactor column (stopped-flow multidimensional gas chromatography, sfMDGC).

In the present study, DGC and sfGC were applied for the determination of the enantiomerization barrier of thalidomide (cf. Fig. 1) in the abiotic semipolar liquid environment of a modified cyclodextrin (CSP) dissolved in a dimethyldiphenylpolysiloxane. At first sight and due to its limited volatility thalidomide does not appear to represent a suitable analyte for enantioselective gas chromatographic studies. Also the enantioselectivity of modified cyclodextrins (CDs) toward thalidomide was found to be low. Nevertheless, despite the rather small effect of peak distortions caused by the enantiomerization of thalidomide, the barrier of enantiomerization of thalidomide was successfully determined by DGC using the powerful simulation program ChromWin. The data obtained was validated by the independent method of sfGC.

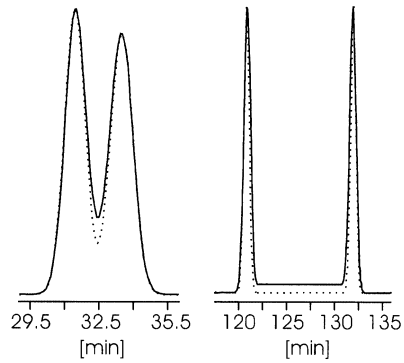


Fig. 2. Comparison of the separation of the enantiomers of thalidomide in presence of interconversion (plain line) and in absence of interconversion (simulated by ChromWin, dotted line) at 200 °C on two columns of different length (Left: 10 m × 0.25 mm i.d. coated with 50% 6-TBDMS-2,3-DM-β-CD dissolved in PS086, 0.25 μm film thickness; Right: 25 m × 0.25 mm i.d. coated with 50% 6-TBDMS-2,3-DM-β-CD dissolved in PS086, 0.25 μm film thickness). Carrier gas: nitrogen.

2. Materials and methods

2.1. Chemicals

The single enantiomers and the racemate of thalidomide (Fig. 1) were obtained from Grünenthal (Aachen, Germany).

2.2. Dynamic gas chromatography

DGC of thalidomide was performed on a Carlo-Erba Fractovap 2150 gas chromatograph, equipped with a split injector (350 °C), a flame ionization detector (350 °C) and a Shimadzu C-R 6A integrator, employing a fused silica column (10 m × 0.25 mm i.d.) coated with heptakis-(6-*O*-*tert*-butyldimethylsilyl-2,3-*O*-dimethyl)-β-cyclodextrin (6-TBDMS-2,3-DM-β-CD) [18,19] dissolved in PS086 (85–88%-dimethyl—(12–15%)-diphenylpolysiloxane, Gelest/ABCR Karlsruhe, Germany) (50%, 0.25 μm film thickness). Nitrogen instead of hydrogen was used as the inert carrier gas in order to increase the chromatographic time scale at a given temperature. The measurements displaying interconversion profiles (cf. Figs. 2 and 4) were repeated three times at each temperature.

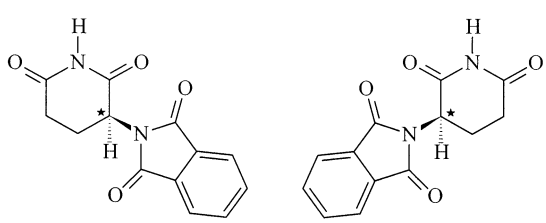


Fig. 1. (*S*)-(−)- and (*R*)-(+)-*N*-(2,6-dioxo-3-piperidyl)phthalimide (thalidomide).

2.3. Computer simulation of interconversion profiles

Computer simulation of the observed interconversion profiles was performed with the SM+ (Find Enantiomerization Barrier II method) of ChromWin [27]. For the calculation of the enantiomerization barrier the plateau height $h_{plateau}$, peak width at half height w_h, the total retention times t_R of the enantiomers and the hold up time t_M (using methane as a hold-up time marker) of the dynamic GC experiment were used as experimental input parameters.

As the enantiomerization process is defined as a reversible first order reaction, a statistical transmission factor κ of 0.5 was used in the Eyring

equation (1) for the calculation of the Gibbs activation energy $\Delta G^{\#}(T)$. Enantiomerization studies were performed at different temperatures and according to the Gibbs–Helmholtz equation the activation enthalpy $\Delta H^{\#}$ was obtained via the slope and the activation entropy $\Delta S^{\#}$ via the intercept of the Eyring plot ($\ln(k_1/T)$ plotted as a function of T^{-1}.

$$\Delta G^{\#}(T) = -RT \ln\left(\frac{k_1 h}{\kappa k_B T}\right) \quad (1)$$

with κ as the transmission factor ($\kappa = 0.5$); k_B as Boltzmann constant ($k_B = 1.380662 \cdot 10^{-23}$ J K^{-1}); T as enantiomerization temperature (in K); h as Planck constant ($h = 6.626176 \cdot 10^{-34}$ J s) and R as gas constant ($R = 8.31441$ J K^{-1} mol^{-1}).

2.4. Stopped-flow gas chromatography

sfGC of thalidomide was performed on a Carlo-Erba VEGA gas chromatograph, equipped with a split injector (350 °C), a flame ionization detector (250 °C) and a Shimadzu C-R 6A integrator, employing a fused silica column (10 m × 0.25 mm i.d.) coated with 6-TBDMS-2,3-DM-β-CD [35,36] dissolved in PS086 (50%, 0.25 μm film thickness). Nitrogen was used as the inert carrier gas. The separation was performed at 170 °C. Five minutes after the injection of one of the enantiomers the gas flow was stopped for a certain reaction time and the column was quickly heated to the reaction temperature. Afterwards, the GC oven was cooled down to the initial separation temperature and the separation of the *de novo* enantiomeric mixture was performed. All measurements were repeated three times at each reaction temperature. The rate constant k of interconversion was calculated from the observed enantiomeric ratio *er* (major to minor peak), the temperature T and the contact time t according to Eq. (2).

$$k = \frac{1}{2t} \ln \frac{er+1}{er-1} \quad (2)$$

The mean values of $\ln(k_1/T)$ were plotted as a function of T^{-1} according to the Eyring equation. From the linear fit, $\Delta H^{\#}$ and $\Delta S^{\#}$ were obtained from the slope and the y-intercept, respectively.

2.5. Determination of the thermodynamic retention increment R'

The thermodynamic retention increment R' separates non-enantioselective contributions to retention arising from the solvent polysiloxane and enantioselective contributions to retention arising from the CD. R' was determined for both enantiomers of thalidomide by using (i) a fused silica capillary coated with neat PS086 (25 m × 0.25 mm i.d., 0.25 μm film thickness) as non-enantioselective (achiral) reference column and (ii) a fused silica capillary coated with 6-TBDMS-2,3-DM-β-CD [35,36] dissolved in PS086 (50%, 25 m × 0.25 mm i.d., 0.25 μm film thickness) as enantioselective (chiral) column. The injected racemate of thalidomide was equally distributed with a Y-glass-connector into the two columns. *n*-Heptadecane and methane were coinjected as a reference standard, and hold-up time marker, respectively, as previously described in detail [37,38,40]. A Carlo Erba Fractovap 2350 gas chromatograph equipped with a liquid injector (350 °C) and two flame-ionization detectors (350 °C) was used. Hydrogen was used as the inert carrier gas.

3. Results and discussion

3.1. Dynamic gas chromatography

Enantiomerization of thalidomide during gas chromatography on the CSP 6-TBDMS-2,3-DM-β-CD at 200 °C is recognized by a slight tailing of the first eluted peak and a slight fronting of the second eluted peak of the resolved enantiomeric pair in Fig. 2(Left) and by plateau formation between the first and second eluted peak of the resolved enantiomeric pair in Fig. 2(Right). The difference of the elution profiles depicted in Fig. 2(Left vs. Right) is due to the different residence time of thalidomide in the columns of varying length (10 m vs. 25 m × 0.25 mm i.d.). For comparison, the dotted lines in Fig. 2 represent chromatograms simulated by ChromWin, which are expected in the absence of interconversion ($k = 0$). Clearly, the interconversion profiles are not very pronounced. But the powerful simulation proce-

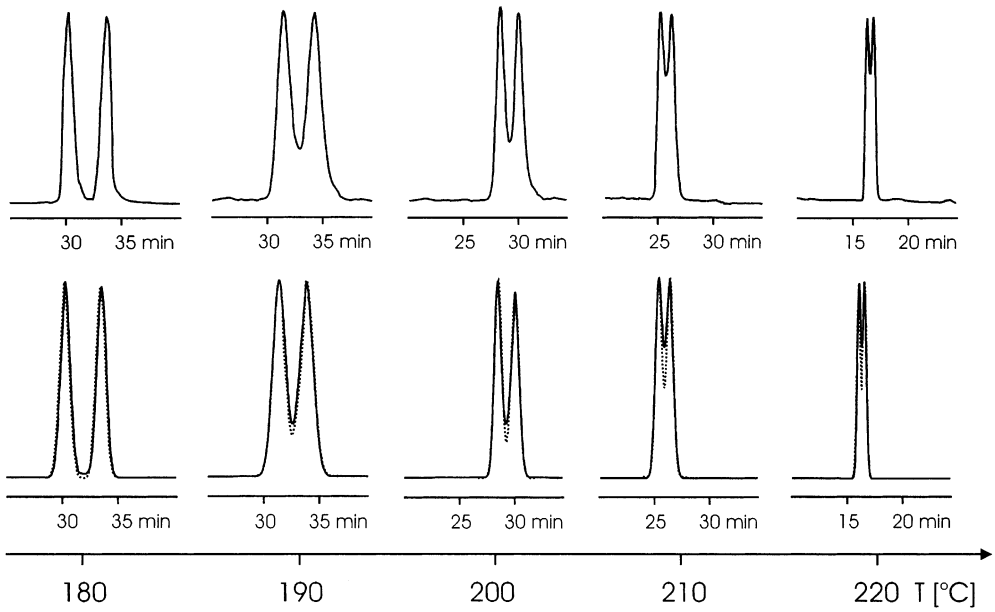


Fig. 3. Experimental (top) and simulated (bottom) chromatograms of the separation of thalidomide at different temperatures by DGC. Fused-silica capillary 10 m × 0.25 mm i.d. coated with 50% 6-TBDMS-2,3-DM-β-CD dissolved in PS086, 0.25 μm film thickness. Carrier gas: nitrogen. The dotted lines represent the separation in absence of interconversion.

dure of ChromWin furnishes reliable rate constants of enantiomerization of thalidomide. The data obtained are equal for both chromatograms within experimental error (Fig. 2, Left: $\Delta G^{\#}(200\ °C) = 154$ kJ mol^{-1} and Fig. 2, Right: $\Delta G^{\#}(200\ °C) = 155$ kJ mol^{-1}).

In order to obtain the activation data $\Delta H^{\#}$ and $\Delta S^{\#}$, temperature-dependent studies were performed. In Fig. 3 experimental and simulated interconversion profiles are depicted. The enantiomerization barrier $\Delta G^{\#}(T)$ was evaluated by iterative comparison of the experimental chromatograms with the simulated chromatograms employing the improved stochastic model (SM +) of ChromWin. The activation parameters of thalidomide were determined from the rate constants according to the Eyring equation. The corresponding diagram is displayed in Fig. 4 (agreement factor: 0.997). The activation parameters were determined to be $\Delta H^{\#} = 42 \pm 2$ kJ mol^{-1} and $\Delta S^{\#} = -246 \pm 16$ J K^{-1} mol^{-1}, respectively. Noteworthy is the highly negative activation entropy $\Delta S^{\#}$ which may be attributed to an enantiomerization mechanism involving charge separation in the transition state of the keto-enol tautomerization.

Enantiomerization of thalidomide was also proven by injection of the neat enantiomers at a column temperature of 220 °C (cf. Fig. 5). At this temperature, the chromatograms show shoulders adjacent to the main peak arising from the de

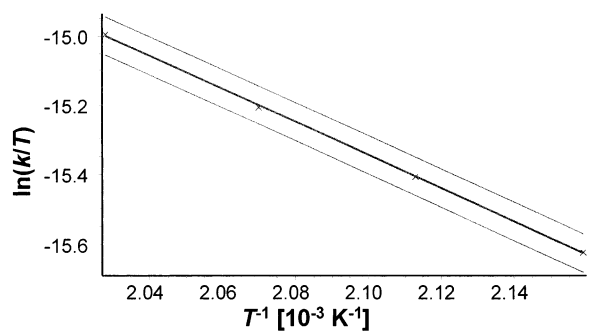


Fig. 4. Eyring plot ($\ln(k_1/T)$ plotted as a function of T^{-1}) of the simulated rate constants k_1 of enantiomerization of thalidomide obtained by DGC. The upper and lower curves represent the error bands of the linear regression with a level of confidence of 95%.

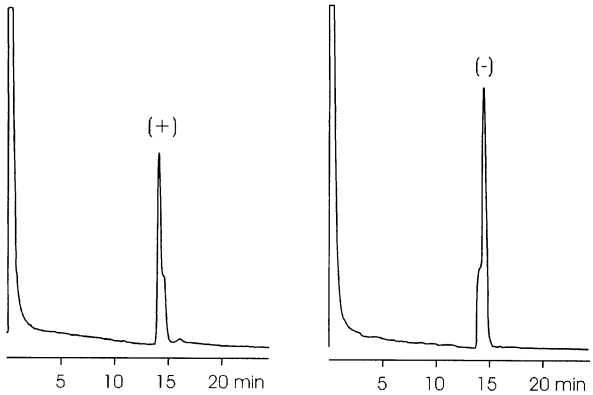


Fig. 5. Dynamic gas chromatographic experiment at 220 °C and 1 bar N_2 with the single enantiomers of thalidomide, displaying shoulders due to enantiomerization during the separation process. (*R*)-(+)-thalidomide is the first eluted enantiomer. Fused-silica capillary 10 m × 0.25 mm i.d. coated with 50% 6-TBDMS-2,3-DM-β-CD dissolved in PS086, 0.25 μm film thickness.

novo formed enantiomers, whereas at 170 °C, the shoulders are absent.

3.2. Stopped-flow gas chromatography

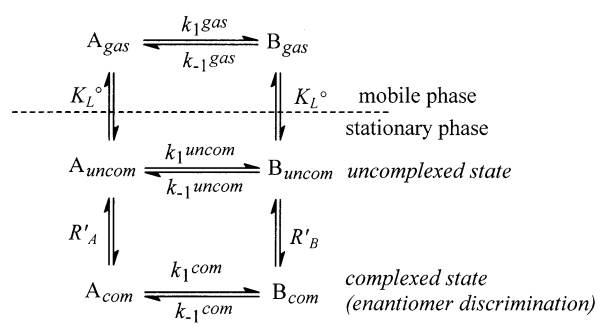
To corroborate the enantiomerization barrier obtained by DGC additional experiments were performed by sfGC. Since single enantiomers of thalidomide were at hand, the measurements were not performed with the racemic mixture. Thus, either of the enantiomerically pure stereoisomers of thalidomide was injected into the first part of the separation column containing the CSP 6-TB-DMS-2,3-DM-β-CD at 170 °C. After ≈5 min, the gas flow was stopped for a given reaction time *t* and the column was quickly heated to the respective enantiomerization temperature *T* (200, 210 and 220 °C) after ≈30 min. Subsequently, the column was cooled down to the initial separation temperature and the separation of the de novo enantiomeric mixture was performed in the second part of the column to yield the enantiomeric ratio *er*. The results (cf. Table 1) obtained by these experiments are in a reasonably good agreement with the dynamic gas chromatographic experiment. No side products or degradation of thalidomide were observed.

Table 1
Comparison of the enantiomerization barriers of thalidomide obtained by dynamic chromatography and computer simulation with ChromWin and by sfGC

T (°C)	$\Delta G^{\#}$ (DGC) (kJ mol^{-1})	$\Delta G^{\#}$ (sfGC) (kJ mol^{-1})
200	154 ± 2	150 ± 3
210	157 ± 2	154 ± 2
220	159 ± 2	155 ± 3

3.3. Determination of retention increment *R′*

Enantiomerization occurring in the liquid stationary phase should preferentially be separated into two contributions [19,23]. A non-enantioselective contribution to retention of thalidomide arises from the achiral matrix dimethyldiphenylpolysiloxane (uncomplexed state), whereas an enantioselective contribution to retention of thalidomide is due to the presence of the CD (complexed state). Moreover, the rate constants in the achiral solvent poylsiloxane k^{uncom} will be equal for both enantiomers and, in principle, be different from the rate constants k^{com} (k_1 for the first eluted enantiomer A and k_{-1} for the second eluted enantiomer B) (cf. Scheme 1). In order to separate the contribution of the uncomplexed vs. complexed state to determine the thermodynamic enantioselectivity and the kinetic enantiomerization rate constant in the liquid stationary phase (6-TBDMS-2,3-DM-β-CD in PS086), the concept



Scheme 1. Equilibria in a theoretical plate involving nonenantioselective and enantioselective contributions to retention in the separation of enantiomers A and B in the liquid stationary phase (K_L° denotes the distribution equilibrium between pure A_{gas} and A_{uncom} (and B, respectively)).

Table 2
Experimental retention increment $R' = (r/r_0) - 1$ of the enantiomers of thalidomide on 6-TBDMS-2,3-DM-β-CD dissolved in PS086 (reference standard *n*-heptadecane; $r_A = t'_{R,A}/t'_{R,standard}$ and $r_B = t'_{R,B}/t'_{R,standard}$ are the relative retention of the thalidomide enantiomers on 6-TBDMS-2,3-DM-β-CD dissolved in PS086, $r_0 = t'_{R,A/B}/t'_{R,standard}$ relative retention of thalidomide on PS086)

T (°C)	r_0	r_A	r_B	R'_A	R'_B	$-\Delta_{B,A}\Delta G$ (kJ mol^{-1})
179.8	1.62	4.61	5.08	1.85	2.14	−0.55
189.0	1.55	4.07	4.44	1.63	1.88	−0.53
197.0	1.51	3.78	4.08	1.51	1.71	−0.48
203.0	1.51	3.51	3.76	1.33	1.49	−0.46
210.0	1.49	3.41	3.61	1.29	1.43	−0.40

of the retention increment R' [37–40] has been applied [19,23].

The retention increment R' (i.e. $R' = (k'/k'^{achiral}) - 1$), is easily accessible experimentally and is obtained by relating the retention factors of the enantiomers k'_A and k'_B on a separation column containing the CD selector in the polysiloxane solvent with the retention factors $k'^{achiral}{}_A = k'^{achiral}{}_B$ observed on an achiral reference column containing only the polysiloxane solvent devoid of the CD selector. The measurement of the retention increment necessitates the coinjection of an essentially inert reference standard not complexing with the CD selector. A retention increment R' always arises from selective complexation of a selectand and a selector. The retention increment R' is directly related to the thermodynamic complexation constant, i.e. $R'_A = K_{CD,A}m_{CD}$ (m_{CD} refers to the molality of the CD selector in the polysiloxane) and thus represents the fraction of the uncomplexed versus complexed enantiomer A, $1/(R'_A + 1)$ and $R'_A/(R'_A + 1)$, in the stationary phase [19,23]. The same expression refers to enantiomer B. Thus, for competing stereoisomers, the ratio of the retention increments are directly related to the thermodynamic enantioselectivity $-\Delta_{B,A}\Delta G_{CD} = RT \ln(K_{CD,B}/K_{CD,A}) = RT \ln(R'_B/R'_A)$).

In temperature-dependent studies, $-\Delta_{B,A}\Delta H_{CD}$ and $\Delta_{B,A}\Delta S_{CD}$ can be obtained from the Gibbs–Helmholtz equation [38–40]. In Table 2, data for the retention increment R' for the thalidomide enantiomers A and B are recorded. From the linear fit of the van't Hoff plot of $\ln(R'_B/R'_A)$ as a function of T^{-1} (cf. Fig. 6; agreement factor 0.9733) $-\Delta_{B,A}\Delta H_{CD}$ and $\Delta_{B,A}\Delta S_{CD}$ were calculated from the slope and the *y*-intercept, respectively. $-\Delta_{B,A}\Delta H_{CiD}$ was found to be 2.8 ± 0.1 kJ mol^{-1} and $\Delta_{B,A}\Delta S_{CD} = -5.0 \pm 0.2$ J (K mol)$^{-1}$. At the isoenantioselective temperature $T_{iso} = 560$ K ($T_{iso} = \Delta_{B,A}\Delta H_{CD}/\Delta_{B,A}\Delta S_{CD}$) peak coalescence [41] would be expected (enantiomer separation of thalidomide is not possible at this temperature).

While these thermodynamic data of enantioselectivity of the CD selector toward the thalidomide enantiomers are of interest in its own right, the concept of the retention increment R' can also be used to separate the rate constants of the interconverting enantiomers of thalidomide in the liquid stationary phase between the polysiloxane k^{uncom} (uncomplexed state) and the CD selector k^{com} (complexed state, cf. Scheme 1). It is important to note that $k_1{}^{com}$ is rendered different from $k_{-1}{}^{com}$ in the presence of a CSP [23]. The following equations have been applied [23]:

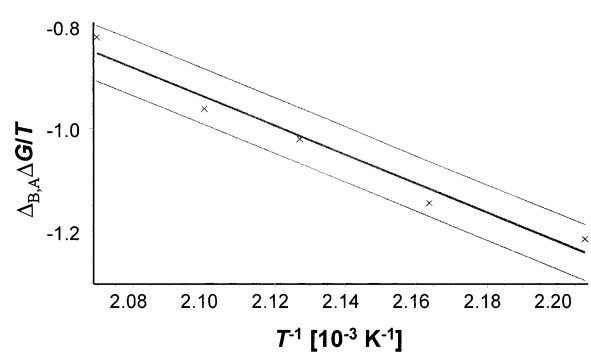


Fig. 6. Van't Hoff plot of the thermodynamic enantioselectivity $\Delta_{B,A}\Delta G_{CD}/T$ as a function of $1/T$. The upper and lower curves represent the error bands of the linear regression with a level of confidence of 95%.

$$k_1^{\mathrm{liq}} = \frac{1}{1+R'_A}k^{\mathrm{uncom}} + \frac{R'_A}{1+R'_A}k_1^{\mathrm{com}}$$

$$k_{-1}^{\mathrm{liq}} = \frac{1}{1+R'_B}k^{\mathrm{uncom}} + \frac{R'_B}{1+R'_B}k_{-1}^{\mathrm{com}} \quad (3)$$

Because of the very high retention factors (50–250) observed, implying only a very brief residence of the enantiomers in the gas phase, only the enantiomerization process taking place in the stationary liquid phase is considered.

Since the difference of the reaction rate of thalidomide in the complexed and uncomplexed state are a priori unknown, two borderline cases can be considered. (i) The enantiomerization process takes place only in the complexed state ($k_1^{\mathrm{uncom}} = 0$) and (ii) the enantiomerization process takes place only in the uncomplexed state ($k_1^{\mathrm{com}} = 0$). For (i), the calculated rate constant at 220 °C is $k_1^{\mathrm{com}} = 1.510^{-4}$ s^{-1} ($\Delta G^{\#} = 156$ kJ mol^{-1}), and for (ii), $k_1^{\mathrm{uncom}} = 1.4{\cdot}10^{-4}$ s^{-1} ($\Delta G^{\#} = 156$ kJ mol^{-1}), respectively, applying the rate constant from the DGC experiment at 220 °C ($k_1^{\mathrm{liq}} = 7{,}4{\cdot}10^{-5}$ s^{-1}; $\Delta G^{\#} = 159$ kJ mol^{-1}).

4. Conclusion

Thalidomide represents a classical example for the importance of investigations of the stereolability of chiral drugs. Although gas chromatography is clearly not the method of choice for the separation of the enantiomers of thalidomide, DGC and sfGC were successfully employed for the determination of kinetic activation parameters of enantiomerization in an abiotic, aprotic and pH-neutral semipolar environment. Compared to measurements of the enantiomerization barrier of thalidomide in liquid systems (aqueous buffer, pH 8), where barriers of 102 ± 1 kJ mol^{-1} were found [21], a rather high barrier of 159 ± 2 kJ mol^{-1} was determined at 220 °C by DGC. The barrier of the enantiomerization was accessible with ChromWin despite the absence of appreciable plateau formation. Yet the observed peak-tailing, peak-heading and peak overlapping could be differentiated mathematically. Applying the concept of the retention increment, boundary values of the enantiomerization barriers in the complexed state and the uncomplexed state have been estimated to be about 156 kJ mol^{-1} at 220 °C. By comparison of the activation parameters obtained by DGC with those of the sfGC experiment the accuracy of the simulation program and the reliability of both methods could be validated.

The still elusive data of enantiomerization of thalidomide in the pure inert gas phase may in principle be accessible by sfMDGC. Preliminary experiments were, however, unsuccessful in our hands due to the low volatility of thalidomide.

Acknowledgements

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