

# Deracemization of a Racemic Compound via Its Conglomerate-forming Salt Using Temperature Cycling

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**Abstract:** The formation of conglomerate salts from chiral molecules, which crystallize as racemic compounds, expands the theoretical application range of Viedma Ripening roughly 10 fold. In the present study, on the use of conglomerate forming salts was studied for temperature cycling, an alternative technique for Viedma ripening. The racemic compound Phenylalanine (Phe) was successfully deracemized via its conglomerate-forming salt with 2,5-xylenesulfonic acid (XSA) by continuous heating-cooling cycles applied to its suspension in glacial acetic acid, coupled with a solution racemization reaction. In addition, the dependence of the deracemization rate on the operational parameters was studied. The results can be used as guidelines for process optimization as well as for the understanding of the mechanism behind temperature cycling. The advantages and disadvantages of temperature cycling and Viedma Ripening, as deracemization methods in an industrial setting are discussed.

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**Key words:** Deracemization, Temperature Cycling, Racemic Compound, Conglomerate-forming  
Salt, Process Optimization

**Abstract:** Salts of chiral molecules, which originally crystallize as racemic compounds, could  
form conglomerates. The utilization of such conglomerate salts, as intermediates for the  
deracemization of corresponding racemic compounds, expands the theoretical application range

of Viedma Ripening by roughly 10 fold. In the present study, the use of temperature cycling on conglomerate forming salts as an alternative technique for Viedma ripening was studied. The racemic compound Phenylalanine (Phe) was successfully deracemized via its conglomerate-forming salt with 2,5-xylenesulfonic acid (XSA) by continuous heating-cooling cycles applied to its suspension in glacial acetic acid, coupled with a solution racemization reaction. In addition, the dependence of the deracemization rate on the operational parameters was studied. Enhanced racemization reaction kinetics, either by a larger amount of free amino acid or a higher concentration of catalyst, was shown to accelerate the deracemization process. It seems to indicate that a concentration difference between the two enantiomers, which could be diminished by a faster racemization rate, behaves as one of the major rate-limiting factors for the deracemization process. A larger mass fraction of solid dissolving and recrystallizing in the heating-cooling cycles, achieved by either a larger temperature swing or a smaller dry mass concentration, also leads to a faster deracemization. A change in cooling rate does not affect the deracemization rate significantly within the range tested, indicating a limited presence of secondary nucleation of the minor enantiomers. The results can be used as a preliminary foundation for process optimization as well as mechanisms investigation. The advantages and disadvantages of temperature cycling and Viedma Ripening, as deracemization methods in an industrial setting are discussed.

## **INTRODUCTION**

Viedma Ripening, the process in which an enantiopure solid phase is obtained from a racemic suspension of conglomerate crystals, under a near-equilibrium condition, by combining

mechanical grinding and solute racemization, has shown to be a reliable method for acquiring the desired enantiomer by means of crystallization, in addition to preferential crystallization.<sup>1-5</sup> Following the success in obtaining enantiopure NaClO<sub>3</sub> crystals from an aqueous solution<sup>6,7</sup> and a conglomerate suspension<sup>8</sup> subjected to a temperature gradient, it was demonstrated that applying a combination of a racemization reaction in solution and heating-cooling cycles rather than grinding of a conglomerate suspension also resulted in full deracemization of the solid phase.<sup>9</sup> Although two major modelling studies have been reported to explain how the deracemization is achieved by the temperature cycles, only a few experimental studies have been conducted to deepen our understandings of the underlying mechanisms.<sup>10, 11</sup>

Though being promising methods for deracemization, Viedma Ripening and the temperature cycling method have the intrinsic drawback of being only applicable to conglomerates, a racemic physical mixture of pure enantiomer crystals.<sup>12</sup> This constraint excludes the utilization of the deracemization methods for roughly 90% of the known chiral molecules since these compounds crystallize as racemic compounds, crystals consisting of both enantiomers.<sup>12</sup> One way around this restriction is by converting a racemic compound into a conglomerate salt: For a series of chiral compounds it has been shown that the probability of forming a conglomerate salt is 2 to 3 times larger than of forming a single component conglomerate.<sup>13</sup> Recently, Spix et al. managed to apply Viedma Ripening to racemic amino acid compounds, e.g. DL-Phenylalanine (Phe), via the formation of its conglomerate salt with 2,5-xylenesulfonic acid (XSA).<sup>14, 15</sup> Their results showed, that using a conglomerate forming salt enables the utilization of Viedma Ripening to racemic compounds. This would expand the application range of Viedma ripening by roughly 10 times, if such conglomerate forming salts exist.

In this study, the model compound system Phe-XSA is used to verify whether deracemization through the temperature cycling method is also successful for a conglomerate salt of an intrinsically racemic compound. In addition, the effects of the operational parameters on the deracemization rate are studied, to achieve more experimental data as a preliminary foundation for process optimization and mechanism investigation. Finally, a qualitative comparison is made between the two deracemization methods.

## EXPERIMENTAL

**Materials.** Solute and catalyst were purchased from Sigma Aldrich (DL-Phenylalanine (DL-Phe, 99%), D-Phenylalanine (D-Phe, 98%) and Salicylaldehyde (SAH, 98%)), Merck (L-Phenylalanine (L-Phe, 99%)) and Alfa Aesar (2,5-Xylenesulfonic acid hydrate (XSA·xH<sub>2</sub>O, 99%)). Glacial acetic acid was purchased from J. T. Baker (99-100%). MilliQ water and Acetonitrile (99%) from Sigma Aldrich were used as effluent for chiral HPLC analysis. Acetone (99%, Sigma Aldrich) was used as washing solvent during sampling.

Phenylalanine can be racemized in glacial acetic acid, catalysed by Salicylaldehyde. This reaction works optimally at a temperature of 100°C but one can still achieve sufficient racemization at lower temperature (e.g. 65°C). It is assumed that Phe was stable through the experiments since decomposition has only been observed in the cases of Tryptophan and Serine.<sup>16</sup> Only free amino acid can be racemized via the formation of a Schiff base.<sup>16</sup> Therefore, less 2,5-Xylenesulfonic acid was added so that the molar ratio between Phe and XSA in the system was between 1.30 and 1.45. The excess amount of Phe exists in the solution phase as free amino acid for the racemization reaction. The Phe:XSA molar ratio in each experiment is shown in Table.1. The molar ratio of Phe over XSA was calculated based on the molar weight of Phe

(165 g/mol) and anhydrous XSA (186 g/mol). From TGA measurement, the purchased XSA·xH<sub>2</sub>O contains approximately 15% wt water. Therefore, the mass of anhydrous XSA was assumed to be 85% of the total XSA·xH<sub>2</sub>O used in preparing the suspensions.

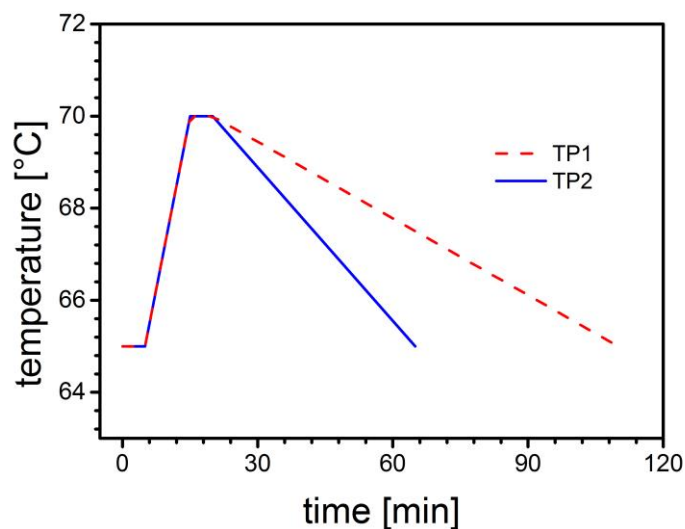
The racemization reagent, SAH, was added in each experiment using a Pasteur pipette. In order to determine the amount of catalyst in the solution phase, 10 separate tests were performed in which the weight of 10 drops of SAH was measured. The average value, 13.2 mg/drop, was used to calculate the concentration of catalyst in each deracemization experiment. The standard deviation for the 10 separate tests was 4%.

**Solubility of DL-Phe-XSA in acetic acid.** The saturation temperature of DL-Phe-XSA was measured in acetic acid at various concentrations by adding a known amount of DL-Phe and XSA·xH<sub>2</sub>O and 1 ml of solvent to a 1.5 ml glass vial. The molar ratio Phe:XSA was always 1.31:1 to mimic the actual suspension composition in the deracemization experiment. The vials were placed in a Crystal16 (Technobis B.V.) and two temperature cycles were applied to all samples. Each cycle involved heating from 0°C to 80°C with a rate of 0.3°C/min, holding at 80°C for 100 min, cooling to 0° with a rate of 0.3°C/min and then holding at 0° for 100 min. The detected clear point was noted as the saturation temperature  $T_s$  of the corresponding concentration. Additional saturation temperatures were measured for which samples contained 13.2 mg/ml and 26.4 mg/ml catalyst, respectively. The concentrations  $c$  (mg/ml) vs. saturation temperature data  $T_s$  were fitted to the empirical function  $c = A \exp(BT_s)$  to interpolate the solubility at different temperatures.

**Verification experiments.** Three experiments were performed to determine whether deracemization can be achieved by using the temperature cycling method. In the standard experiment A1, 580 mg DL-Phe and 589 mg XSA·xH<sub>2</sub>O were mixed with 9 mL glacial acetic

acid in a 25 mL round-bottom flask. In experiments A2 and A3, in addition, respectively 58 mg of D-Phe and 63 mg L-Phe were mixed with DL-Phe into the glacial acetic acid to prepare a scalemic suspension. The suspensions in all experiments were stirred by an oval PTFE-coated magnetic bar (L 20 mm, Ø 10 mm, 300 rpm) at 65°C for 1 hour. Then, two different temperature programs were applied to the suspensions, which are shown in Figure 1. The first temperature profile TP1, which was applied in experiments A1 and A2, consisted of four steps: holding at 65°C for 5 min, heating up to 70°C in 10 min with a linear profile, holding at 70°C for 5 min and finally cooling down to 65°C linearly with a cooling rate of 0.055°C/min. The other temperature profile TP2 was assigned to A3, and its only difference, compared with TP1, was a cooling rate of 0.11°C/min.

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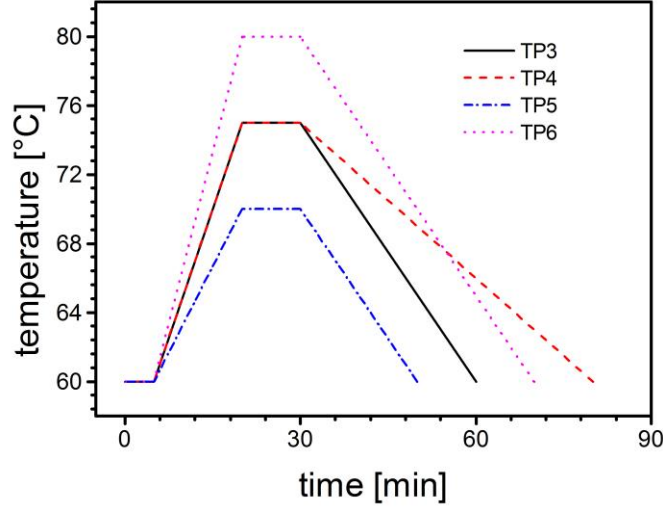
**Figure 1.** Temperature profiles for one temperature cycle applied in the verification experiments A1-A3. A1 and A2 used a cooling rate of 0.055°C/min (TP1), while that of A3 was 0.11°C/min (TP2).

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The initial temperature cycles were applied to the suspension in the absence of the racemization catalyst SAH. The salt precipitation took place rapidly when Phe and XSA were

mixed in the acetic acid, forming large agglomerates. Mixing was therefore not ideal in the initial suspension and a period of dissolution-growth cycles was needed to ensure that the suspension was homogeneous before deracemization experiments were started. After approximately 12 hours of these initial temperature cycles, a sample was taken at the end of the last cycle, at 65°C, the time of which was noted as  $t = 0$ , and then *ca.* 130 mg of SAH was added to the suspensions to start the racemization reaction. After that, samples were taken over time to measure the solid phase enantiomeric excess  $E$  using chiral HPLC. In each case the sample was taken at the end of a cycle.

**Operational Parameter Study.** The dependence of the deracemization rate on five operational parameters was studied: 1) total initial dry mass concentration  $c_t$ , which is the total mass of Phe and XSA·xH<sub>2</sub>O solids before being mixed into the acetic acid per unit of solvent volume; 2) catalyst concentration  $c_{cat}$ ; 3) excess enantiomer concentration  $\Delta c$  which is the additional mass of L- or D-Phe per unit volume of the solvent; 4) the rate of cooling from the highest to the lowest temperature  $R_c$  and 5) the temperature swing  $\Delta T$ , i.e. the difference between the lowest and the highest temperature in a temperature cycle. Four different temperature profiles (TP3-TP6) were used in order to study the effects of temperature swing and cooling rate.



**Figure 2.** Temperature profiles for one temperature cycle applied in the parameter study to investigate the effect of temperature swing and cooling rate.

In this series, experiment B1(see Table 1) was taken as a standard experiment, in which the 9 ml suspension contained 580 mg DL-Phe, 58 mg L-Phe and 589 mg XSA·xH<sub>2</sub>O. The procedure of B1 was the same as that of A1-A3, except that a different temperature profile, TP3, was applied. After B1, experiments B2-B7 were performed, where the value of one of the above-mentioned parameters was changed from that used in B1. Values of these parameters in all deracemization experiments are shown in Table 1, together with the molar weight ratio Phe:XSA, which was used as a measure for the amount of free amino acid in the solution and the mass fraction of solid involved in a cycle  $f_{\Delta T}$ , which was calculated as

$$f_{\Delta T} = \frac{c^*(T_{high}) - c^*(T_{low})}{c_t - c^*(T_{low})} \quad (1)$$

where  $c^*$  is the solubility of DL-Phe-XSA in the presence of a catalyst concentration of 13.2 mg/ml (except for B4, the  $f_{\Delta T}$  of which was calculated based on the Phe-XSA solubility with  $c_{cat}$

of 26.4 mg/ml). The mass fraction  $f_{\Delta T}$  can be controlled in two ways: through the dry mass concentration  $c_t$  and the temperature swing  $\Delta T$ .

**Sampling.** For sampling, including the initial sample, *ca.* 0.5 mL of the slurry was removed with a Pasteur pipette and vacuum filtered as fast as possible at room temperature on a P4 glass filter ( $\varnothing$  10 mm). The residue was washed with 2 mL of Acetone to remove the adhering racemic solution and dried.

**HPLC.** The enantiomeric excess  $E$  of the sample was measured using chiral HPLC. The analysis of Phe-XSA samples was as follows: 1 mg solid was dissolved in 1 mL Milli Q water; 2.5  $\mu$ L of the solution was injected in the HPLC column Chirobiotic T (250\*4.6 mm ID, 5  $\mu$ m, Astec); An eluent of acetonitrile/water (78/28 v/v) was used with a flow rate of 1 mL/min; The two enantiomers of Phe were detected at retention times of 8.9 min (L) and 9.9 min (D) at a wavelength of 220 nm. The concentrations of L and D enantiomers were determined by integrating the peaks at the two retention times. The enantiomeric excess was calculated as:

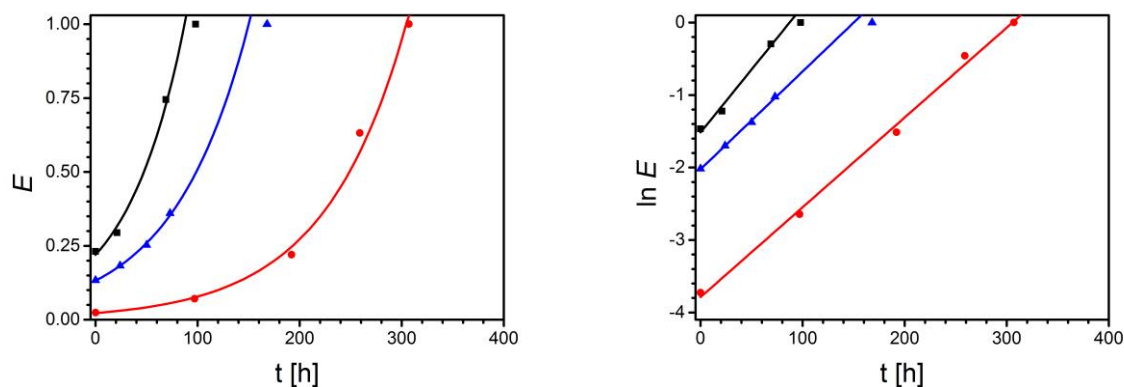
$$E = \frac{|A_L - A_D|}{A_L + A_D} \quad (2)$$

where  $A$  is the area of the peaks of L and D.

**Thermogravimetric Analysis (TGA).** The content of water in XSA was measured with a Perkin Elmer TGA 7 (Perkin Elmer). Samples of *ca.* 32 mg XSA were heated from 15°C – 620°C with a heating rate of 10°C/min, purged by a nitrogen flow of 40 ml/min.

## RESULTS AND DISCUSSION

**Deracemization through Temperature cycling.** Figure 3 depicts the evolution of the solid phase enantiomeric excess  $E$  and its natural logarithm over time in all three verification experiments A1-A3 (for process conditions see Table 1).



**Figure 3.** Left: Enantiomeric excess of the solids  $E$  as a function of time  $t$  in temperature cycling deracemization experiments of Phe-XSA A1 (●), A2 (▲) and A3 (■). The lines are best fits to Equation 3. Right: the natural logarithm of the enantiomeric excess  $\ln E$  as a function of time  $t$ . Solid lines are linear fits of  $\ln(E)$  vs  $t$ . Process conditions for the experiments A1-A3 can be found in Table 1.

The verification experiment A1 started with a racemic suspension. In around 300 hours after adding the catalyst, a complete deracemization towards the L-enantiomer in the solid phase was achieved. The linear correlation between  $\ln E$  and  $t$  in Figure 3 (right) shows that the solid phase enantiomeric excess evolved exponentially during the majority of, if not the whole, deracemization experiment. The two experiments starting with a scalemic mixture, A2 and A3, led to a complete deracemization towards their initial enantiomer in excess, respectively in around 179 hours and 100 hours. These results validate that deracemization of Phe-XSA by programmed heating-cooling cycles can be achieved, as by Viedma ripening. The time needed for deracemization is largest for an initially racemic mixture, while the chiral outcome can be controlled by an initial solid phase enantiomeric excess of the preferred enantiomer.

To quantify the efficiency of the deracemization process Noorduin et al. derived an exponential equation as an approximation for the  $E$  evolution curves of Viedma ripening:

$$E(t) = E(0) \cdot \exp(kt) \quad (3)$$

which describes the enantiomeric excess  $0 \leq E \leq 1$  during Viedma ripening by two parameters:  $E(0)$ , the solid phase enantiomeric excess at  $t=0$  and the deracemization rate constant  $k$ .<sup>17</sup> Since all verification experiments displayed an exponential increase of  $E$  vs  $t$  for the major part of the process,  $k$ , estimated by fitting all experimental points with  $E$  below 1 into equation 3, was used as a measure for the deracemization efficiency. One should note however, that Viedma Ripening takes place at a fixed temperature leading to a consistent rate constant with time, while during temperature cycling deracemization occurs under varying temperatures and herewith the rate constant may also vary.<sup>11</sup> Therefore, the  $k$  -values obtained for temperature cycling deracemization represent an average over the whole experiment.

Moreover, any change to the used temperature cycle (temperature swing or cooling rate) changes the cycle time. When different temperature profiles are employed, a comparison of time-based rate constants  $k$  between experiments is clouded by the different cycle times. Therefore, it is necessary to investigate, in addition to the time-based deracemization rate constant, a cycle-number-based deracemization rate constant. For that equation 3 is modified to describe the evolution of the enantiomeric excess  $E_N$  at the end of each cycle, as a function of the number  $N$  of temperature cycles:

$$E_N(N) = E_N(0) \cdot \exp(k_N N) \quad (4)$$

This enables the study of the deracemization rate as a function of temperature cycle parameters such as temperature swing and cooling rate.

The values for both rate constants are displayed in Table 1 for all experiments. Errors in  $k$  and  $k_N$  values stem from two sources, the standard deviation from fitting experimental data to equations 3 and 4, and the deviation caused by the inaccuracy in catalyst concentration. The sum of these two deviations is shown in Table 1 as an overall error.

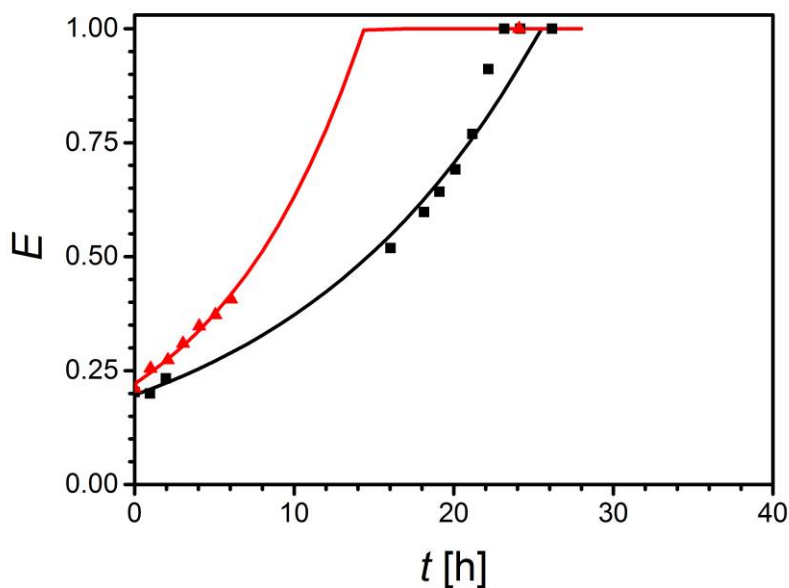
**Table 1.** Deracemization conditions and results: including excess enantiomer concentration  $\Delta c$ , molar ratio Phe: XSA, total dry mass concentration  $c_t$ , catalyst concentration  $c_{cat}$ , temperature swing  $\Delta T$ , cooling rate  $R_c$ , mass fraction of solid involved in a cycle  $f_{\Delta T}$ , temperature profile and the duration of a cycle. The results are represented by the racemization rate constants  $k$  and  $k_N$ .

Experiment	$\Delta c$ (mg/ml)	Molar ratio Phe:XSA <sup>‡</sup>	$c_t$ [mg/ml]	$c_{cat}$ [mg/ml]	$\Delta T$ [°C]	$R_c$ [°C/min]	$f_{\Delta T}^*$	Temperature Profile	Cycle time [min]	$k$ [h <sup>-1</sup> ]	$k_N$ [1/cycle]
A1	0	1.31:1	130	14.7	5	0.055	0.05	TP1	110	0.012 ± 0.001	0.024 ± 0.002
A2	6.4 (D)	1.44:1	135.6	14.7	5	0.055	0.048	TP1	110	0.013 ± 0.001	0.023 ± 0.002
A3	7 (L)	1.45:1	137.8	14.7	5	0.11	0.047	TP2	65	0.017 ± 0.002	0.017 ± 0.002
B1(ref)	6.4 (L)	1.44:1	136.7	14.7	15	0.5	0.14	TP3	60	0.064 ± 0.005	0.064 ± 0.005
B2	6.4 (L)	1.44:1	95.6	14.7	15	0.5	0.222	TP3	60	0.105 ± 0.011	0.105 ± 0.011
B3	6.4 (L)	1.44:1	136.7	7.4	15	0.5	0.14	TP3	60	0.044 ± 0.006	0.044 ± 0.006
B4	6.4 (L)	1.44:1	136.7	29.4	15	0.5	0.14	TP3	60	0.160 ± 0.022	0.160 ± 0.022
B5	0	1.31:1	130	14.7	15	0.5	0.148	TP3	60	0.039 ± 0.003	0.041 ± 0.005
B6	6.4 (L)	1.44:1	136.7	14.7	15	0.3	0.14	TP4	80	0.056 ± 0.005	0.075 ± 0.007
B7	6.4 (L)	1.44:1	136.7	14.7	10	0.5	0.082	TP5	50	0.028 ± 0.004	0.026 ± 0.003
B8	6.4 (L)	1.44:1	136.7	14.7	20	0.5	0.212	TP6	70	0.117 ± 0.015	0.136 ± 0.017

\* $f_{\Delta T}$  of all deracemization experiments were calculated based on the salt solubility in the presence of either a  $c_{cat}$  of 13.2 mg/ml or 26.4 mg/ml (B4). It is assumed that the difference between the  $c_{cat}$  used for  $f_{\Delta T}$  calculation and the actual values used in the deracemization experiments would not lead to a significant deviation in the corresponding  $f_{\Delta T}$ .

**Effects of the Initial Compositions on the deracemization rate constants.** First, the three operation parameters shared by both the temperature cycling method and Viedma Ripening were tested, the dry mass concentration  $c_t$ , the concentration  $c_{cat}$  of catalyst added and the excess enantiomer concentration  $\Delta c$ . Figure 4 shows that in the standard experiment B1 solid phase homochirality was achieved after approximately 23 hours with a deracemization rate constant  $k$  of  $0.066 \text{ h}^{-1}$  and a  $k_N$  value of also 0.066 per cycle because the cycle time was 1 h for that experiment.

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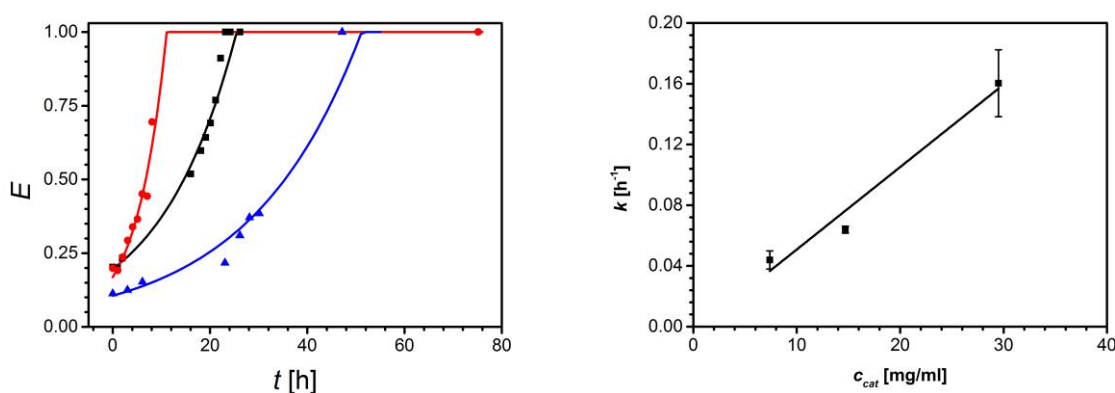
**Figure 4.** Enantiomeric excess  $E$  as a function of time  $t$  with different dry mass concentration:  $c_t = 137.6 \text{ mg/ml}$  (■ standard experiment B1) and  $95.6 \text{ mg/ml}$  (▲ B2). The lines are best fits to equation 3.

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The deracemization experiment B2 differs from B1 by having a roughly 40% lower dry mass concentration  $c_t$  of  $95.6 \text{ mg/ml}$  and is also shown in Figure 4. At a fixed temperature, a lower dry mass concentration  $c_t$  results in a lower suspension density, leading to a faster full deracemization, with a roughly 40% higher  $k$  of  $0.105 \text{ h}^{-1}$ . This faster deracemization rate at a

lower suspension density was also found in both Viedma ripening and the temperature cycling method using other systems.<sup>9, 18</sup>

The next parameter tested was the catalyst concentration  $c_{cat}$  used for the solution phase racemization reaction. Two experiments, B3 and B4, were conducted in which, compared to B1, respectively a higher and a lower  $c_{cat}$  were used. The time development of  $E$  in all three experiments is shown in Figure 5.



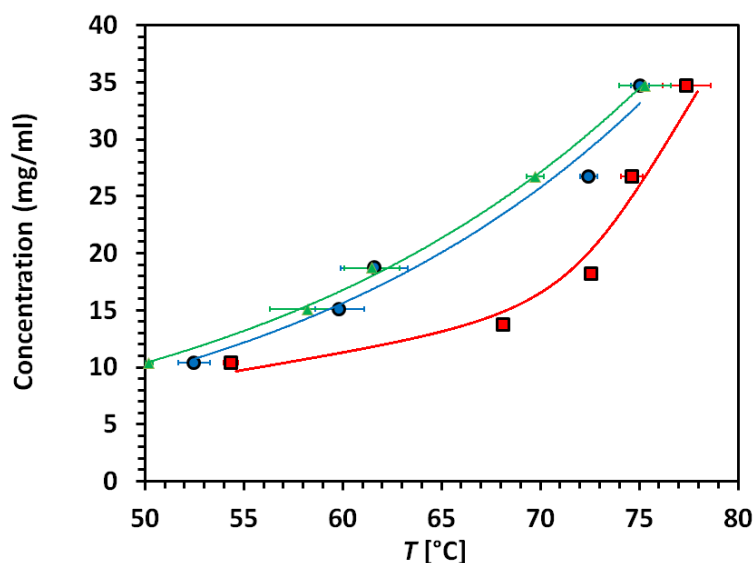
**Figure 5.** Left: Enantiomeric excess  $E$  as a function of time  $t$  with different catalyst concentration:  $c_{cat} = 29.4$  mg/ml (● experiment B4), 14.7 mg/ml (■ standard experiment B1) and 7.4 mg/ml (▲ B3). The lines are best fits to equation 3. Right: Correlation between time-based deracemization rate constant  $k$  and catalyst concentration  $c_{cat}$  from experiment B1, B3 and B4. The solid line is a linear fit of the experimental points (■) and serves as a guide for the eye.

Figure 5 (left) shows that a higher catalyst concentration, such as in B4, increased the deracemization rate significantly. The experiment B3 with a lower catalyst concentration led to a slower deracemization. Figure 5 (right) indicates a roughly linear relationship between the deracemization rate constant  $k$  and the catalyst concentration  $c_{cat}$ , which has also been observed in Viedma Ripening of *N*-(2-methylbenzylidene)-phenylglycine amide by Noorduyn et al.<sup>18</sup> This effect can be attributed to two factors: a faster racemization reaction and a changing solubility.

Yamada et al. tested the effect of catalyst concentration on the racemization kinetics of optical amino acids such as Alanine and Methionine and they found that a higher ratio of catalyst over amino acid led to a faster racemization reaction.<sup>16</sup> The faster racemization reaction in the solution phase might have resulted from the increased concentration of Schiff base formed by the additional catalyst and the amino acid. Spix et al. found that, starting with an enantiopure solution, the racemization reaction of Phe-XSA in acetic acid at 70°C took 1-2 hours to arrive at a racemic solution.<sup>14</sup> This duration is of the same order of magnitude as the cycle time applied in this study. Therefore, when there is an imbalance between the two enantiomers in the solution, it is likely that the relatively slow racemization reaction is not able to eliminate the concentration difference between the two enantiomers in time. A system with a faster racemization reaction would therefore have a smaller, if not negligible, difference in the concentration level of the two enantiomers and is expected to lead to a shorter deracemization time.

Another factor which could influence the deracemization rate constant through  $c_{cat}$  is the solubility change of Phe-XSA salt by the introduction of the catalyst. The presence of a higher concentration of catalyst might lead to an increase in the solubility of Phe-XSA in acetic acid. As has been observed from Figure 4, a higher racemization catalyst concentration facilitates the deracemization process. To verify the solubility dependence of the compound on the catalyst concentration the solubility of Phe-XSA in acetic acid with and without catalyst was measured and is shown in Figure 6. In the presence of a  $c_{cat}$  of 26.4 mg/ml, the saturation temperatures of Phe-XSA in acetic acid were reduced by approximately 2°C compared to those when  $c_{cat}$  was 13.2 mg/ml. This confirms that the solubility of the salt increases due to the presence of the catalyst, which subsequently speeds up the deracemization.

To summarize, when applying a higher  $c_{cat}$ , the combining effect of a faster solution phase racemization and lower solid phase suspension density could lead to a higher deracemization rate constant, as demonstrated in Figure 5. It is worth mentioning that the 4% standard deviation in catalyst amount added by Pasteur Pipette (see the experimental section), according to the correlation shown in Figure 5 (right), could lead to roughly an additional 5% error in  $k$ .

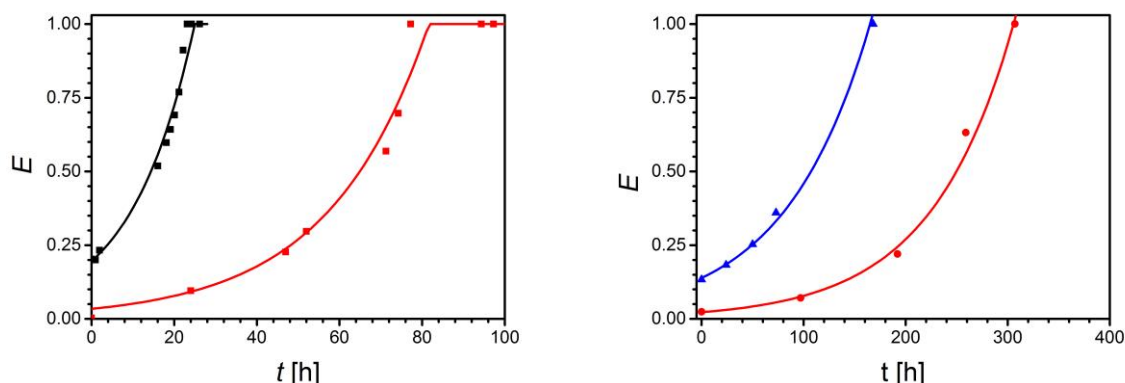


**Figure 6.** Solubility of DL-Phe-XSA salt (1.31:1 molar ratio) in acetic acid with catalyst ( $c_{cat} = 13.2$  mg/ml, ● and 26.4 mg/ml, ▲) and without catalyst (■). The lines are a guide to the eye.

The effect of excess enantiomer concentration  $\Delta c$  was investigated by comparing two pairs of experiments: A1 and A2 as pair one and B1 and B5 as pair two. A1 and B5 have  $\Delta c = 0$  mg/ml while for B1 and A2 additional L-Phe ( $\Delta c = 6.4$  mg/ml) respectively D-Phe ( $\Delta c = 6.4$  mg/ml), was present. Between the two pairs, different temperature profiles were used: TP1, which had a smaller temperature swing of 5°C and a cooling rate of 0.055°C/min, was applied to A1 and A2, while TP3 ( $\Delta T = 15^\circ\text{C}$  and  $R_c = 0.5^\circ\text{C}/\text{min}$ ) was used for B1 and B5.

The results for these two experiment sets are shown in Figure 7. As expected, a longer deracemization time was needed for B5 as compared to B1. On the other hand, in the first

experiment pair, an  $E(0)$  of 0.144 in A2 was obtained while the evolution of  $E$  in A1 starting from 0 shows a similar deracemization rate constant ( $0.0124 \text{ h}^{-1}$  in A1 compared to  $0.0120 \text{ h}^{-1}$  in A2).



**Figure 7.** Enantiomeric excess  $E$  as a function of time  $t$  for different excess enantiomer concentrations:  $\Delta c = 0 \text{ mg/ml}$  (■ experiment B5, left and ● experiment A1, right),  $6.4 \text{ mg/ml}$  (L) (■ B1, left) and  $6.4 \text{ mg/ml}$  (D) (▲ A2, right). The lines in both figures are best fits to equation 3.

In experiments B1 and A2, the additional L-Phe or D-Phe had three effects on the overall suspension composition, a slightly higher dry mass concentration  $c_t$  which in turn raised the corresponding suspension density, a higher molar ratio Phe:XSA which led to more free amino acid for the solution racemization reaction (see Table 1) and an increased  $E(0)$  (see Figure 7).

Based on the observation from Figure 4, the slightly increased  $c_t$  in B1 and A2 should lead to a slower deracemization, which is opposite to what is observed for the pair of B1 and B5, while the pair of A1 and A2 does not show a change in deracemization rate. From equation 3,  $E(0)$  and  $k$  should be independent from each other, which would exclude an effect from the larger  $E(0)$ . Therefore, the most probable reason for the increased  $k$  in B1 compared to B5 lies in the second effect: an increased free amino acid by the additional L-enantiomer.

Spix et al. have reported that a higher amount of free amino acid in the solution phase increases the racemization reaction kinetics.<sup>14</sup> The racemization reaction was therefore faster in B1 than in B5. As has been discussed before, the solution could end up in more D-enantiomer enriched in B5 compared to B1 and the growth, agglomeration and secondary nucleation of the L-Phe-XSA crystals could be slowed down. As a consequence, the evolution of solid phase *E* was slower when no additional L-Phe was added in B5.

Based on the same reasoning, the racemization reaction was also faster in A2 than in A1. However, in the pair A1 and A2, a smaller  $\Delta T$  and cooling rate was used. The longer cooling period led to a smaller supersaturation of the minor solid phase enantiomer, in this case D-Phe-XSA, compared to the supersaturation of the same enantiomer in B5. Therefore, a shorter, if not negligible, delay in the deracemization process was observed in the pair of A1 and A2.

#### **Effect of the temperature cycling conditions on the deracemization rate constants.**

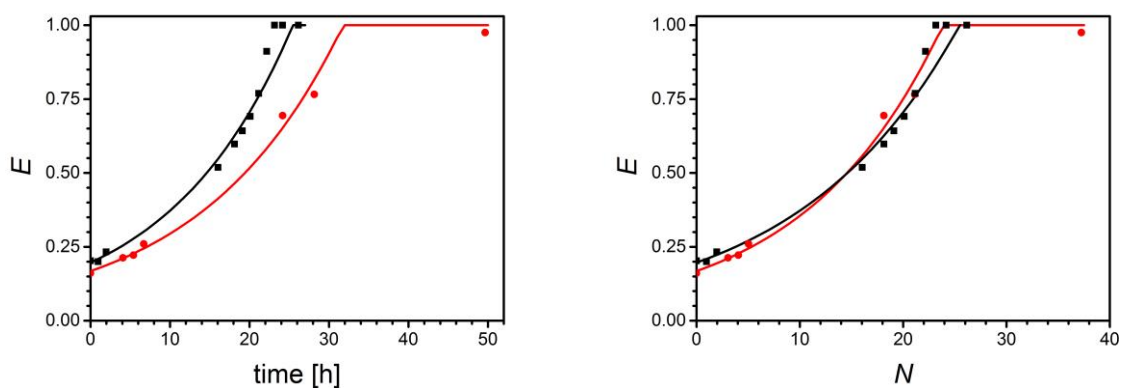
The effects of two parameters related to the temperature profiles were investigated, i.e. the cooling rate  $R_c$  and the temperature swing  $\Delta T$ . Figure 8 shows that experiment B6 with  $R_c = 0.3^\circ\text{C}/\text{min}$  had a slightly smaller deracemization rate constant than the standard experiment B1 for which a higher cooling rate of  $0.5^\circ\text{C}/\text{min}$  was used. Interestingly, the cycle-based deracemization rate constant was slightly larger for B6 than for B1. However, if the errors in  $k$  and  $k_N$  in B1 and B6 are taken into account, the difference in the deracemization rate between these two experiments is negligible (See Table 1.). Therefore, it seems that within the range tested in this study, the cooling rate did not affect the deracemization rate significantly.

Suwannasang et al. found that the cooling rate did not influence the time needed to reach full deracemization, which indicates that in their case the deracemization process with a higher cooling rate needs more cycles to achieve solid phase homochirality. They attributed the less

efficient deracemization per cycle to the increased secondary nucleation rate of the enantiomer of minor solid amount taking place during the cooling part of the temperature cycle. In their study, the lower efficiency in deracemization per cycle was exactly compensated by a shorter cycle time, which led to an unchanging deracemization time with increasing cooling rate.<sup>9</sup>

In the modelling study of Katsuno and Uwaha, a similar reduction in the enantiomeric excess evolution per cycle by faster cooling was obtained while the total time needed for full deracemization was not significantly influenced.<sup>11</sup>

In the present study, the cooling rate showed an impact on neither the time-based nor the cycle-based deracemization rate constants. The results suggest that the secondary nucleation of the minor enantiomer did not delay the deracemization per cycle to the same extent as it was in the literature. Therefore, in future investigations, even higher cooling rate  $R_c$  values can be tested to examine the possibility of speeding up the deracemization process by means of fast cooling.



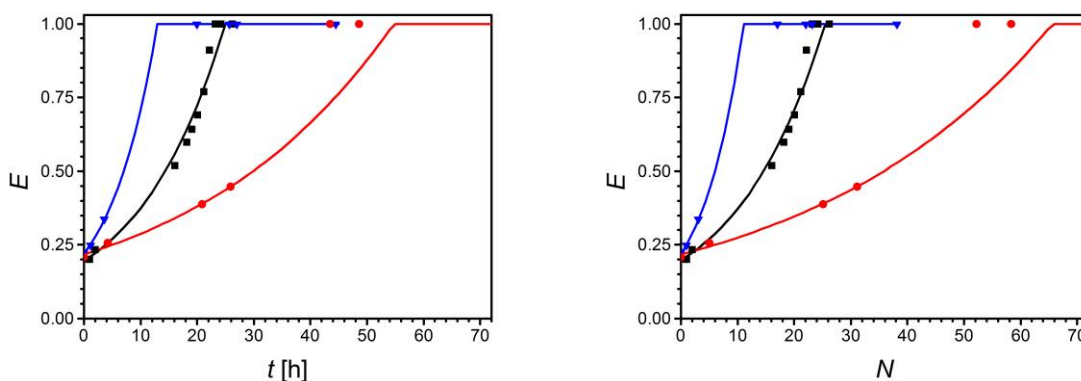
**Figure 8.** Enantiomeric excess  $E$  as a function of time  $t$  (left) and number  $N$  of temperature cycles (right) with different cooling rates:  $R_c = 0.3^\circ\text{C}/\text{min}$  (● experiment B6) and  $0.5^\circ\text{C}/\text{min}$  (■ B1). The lines are best fits to equation 3 (left) and equation 4 (right).

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The final parameter tested was the temperature swing  $\Delta T$ . In Figure 9, the results of experiment B1 with a temperature swing of  $15^\circ\text{C}$  is compared with those of B7 and B8, in which

a smaller and a larger temperature swing of 10°C and 20°C were employed, respectively. It can be seen clearly that a faster full deracemization was achieved with a larger temperature swing  $\Delta T$  while less cycles were needed. Both the time-based and cycle-based deracemization rate constants increased with increasing temperature swing.

In their temperature cycling deracemizations, Suwannasang et al. observed a significant decrease in the time needed for full deracemization at lower suspension densities and in their following simulation work, in which the authors explored the possibility that the crystal growth kinetics are the cause of the deracemization, the same relation was found.<sup>9, 10</sup> They explained this effect with the mass fraction  $f_{\Delta T}$  of solid involved in the dissolution-growth cycles.

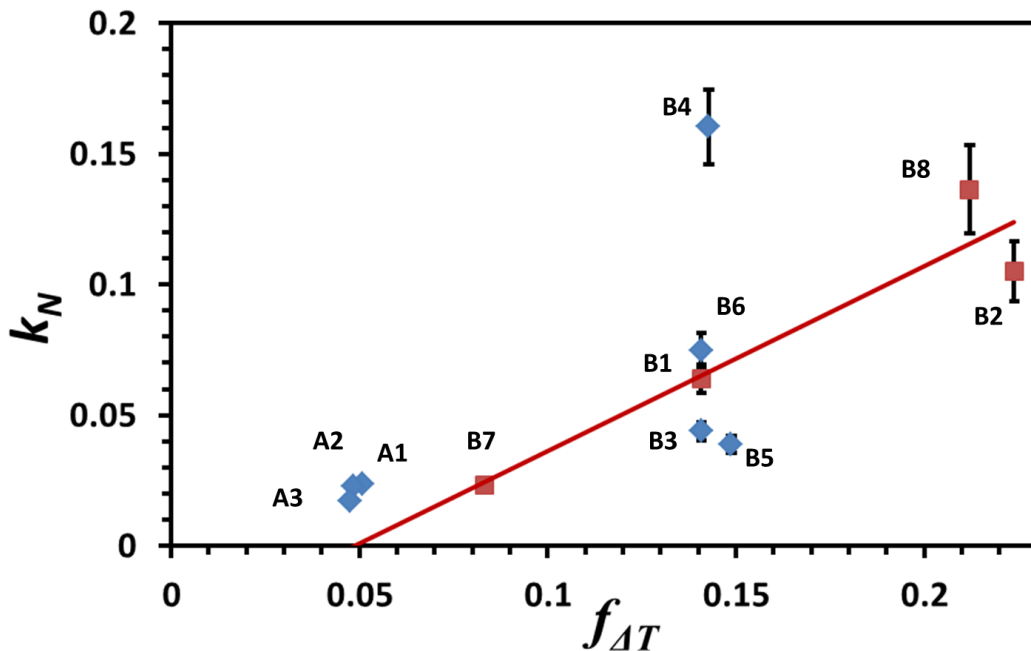


**Figure 9.** Enantiomeric excess  $E$  as a function of time  $t$  (left) and number  $N$  of temperature cycles (right) for different temperature swings:  $\Delta T=10$  (● experiment B7), 15 (■ B1) and 20°C (▼ B8). The lines are best fits to equation 3 (left) and equation 4 (right).

Figure 10 shows the relationship between the temperature cycling-based deracemization rate constant  $k_N$  and the mass fraction  $f_{\Delta T}$  for all the experiments in this study. The cycle-based constant  $k_N$  was used to exclude the influence of the duration of each cycle when different  $\Delta T$  values were used. The four experiments exploiting the adjustment of  $f_{\Delta T}$  by  $\Delta T$  and  $c_i$ , namely

B1, B2, B7 and B8, can be distinguished from the rest of the experiments in Figure 10 by their linear relationship as expressed by the line fitted through the four experimental points of these experiments indicated by the red points.

The other experiments, except B4 with a much higher catalyst concentration of 29.4 mg/ml, also seem to follow the linear trend of  $k_N$  versus  $f_{\Delta T}$ . From the parameter analysis above,  $k_N$  can be influenced by other factors such as  $\Delta c$  as well, however, the trend shown in Figure 10 indicates that among all the factors,  $f_{\Delta T}$  could be one of the most effective.



**Figure 10.** Cycle-number-based deracemization constant  $k_N$  vs mass fraction  $f_{\Delta T}$  of solid involved in the heating-cooling cycles. Experiments exploiting the effect of  $c_i$  and  $\Delta T$  are shown as ■ and other experiments in this study are shown as ♦. The solid line is a linear fit of the experimental points (■) and serves as a guide for the eye.

**Preliminary comparison between Temperature Cycling and Viedma Ripening.** The rate constants  $k$  of all the deracemization experiments performed are compiled in Figure 11. In addition, the rate constant  $k$  in the deracemization of the same salt system using Viedma ripening

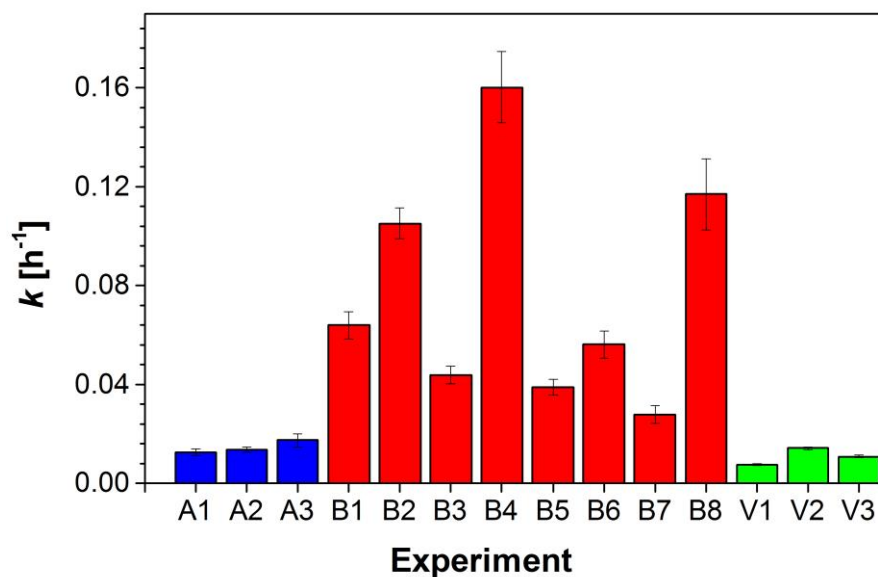
is included.<sup>14</sup> The rate constants in the verification experiments A1 to A3 are in the same range as the Viedma Ripening ones. By modifying some of the operational conditions, especially increasing the temperature swing, the deracemization rate is significantly increased. This comparison suggests that the temperature cycling method is a powerful alternative to Viedma Ripening.

Compared to Viedma Ripening, the industrial application of the temperature cycling method is likely to be more convenient: 1) It is easier to scale-up than Viedma Ripening. In order to achieve sufficient grinding, the total volume of glass beads and the slurry will require a significantly larger crystallizer, which will increase the capital investment. Though recent studies have proved that glass beads can be replaced by ultrasound,<sup>19, 20</sup> novel industrial crystallizers need to be developed to host the ultrasound generator. The crystallizers should be able to cope with technical issues such as the nonhomogeneity of the acoustic field in the suspension.<sup>21</sup> 2) The solid phase from a Viedma ripening process still needs additional steps to separate the crystals from the grinding beads, which might lead to product loss.

In any case, both the Viedma ripening and the temperature cycling method need to be further optimized in order to facilitate the deracemization efficiency. In the case of Viedma ripening, parameters such as the suspension density, the catalyst concentration and the intensity of grinding have been found influential to the deracemization efficiency.<sup>18</sup> While the temperature cycling method can be potentially tuned by these parameters, except the grinding intensity, it can also be further optimized by applying a larger temperature swing, which is proved in this study to be a major contributor for speeding up the deracemization process. Moreover, a change in  $\Delta T$  goes without penalty on the yield as is the case when adjusting the suspension composition. A larger temperature swing could even be counterbalanced by a faster cooling rate resulting in the

same cycle time, provided that the cooling rate  $R_c$  is still small enough to avoid significant secondary nucleation of the enantiomer present in minority.

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**Figure 11.** Time-based deracemization rate constant  $k$  of the temperature cycling method (A1-B8) and the grinding method (V1-V3).<sup>14</sup> Two different  $\Delta c$  were used in Viedma Ripening, 0 mg/ml (V1) and 6.4 mg/ml (L) (V2 and V3).

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## CONCLUSION

The successful deracemization of the racemic compound Phenylalanine by the temperature cycling method, via its conglomerate-forming salt, shows that the theoretical application range of this technique can be expanded likewise as was shown earlier for Viedma ripening. Moreover, the studied effects of operational parameters give insight in how to optimize the deracemization process. For the Phe-XSA system under investigation, the deracemization rate is mainly influenced by the kinetics of the solution racemization and the mass fraction  $f_{\Delta T}$  of solid involved in the temperature cycles. This information will benefit the design and optimization of an

industrial application. A comparison of the deracemization rate using Viedma Ripening and the temperature cycling method indicates that the latter one is a powerful alternative tool for deracemization with additional parameters, such as the temperature swing  $\Delta T$ , to control and optimize the process.

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### **Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

### **Notes**

The authors declare no competing financial interest.

## **ACKNOWLEDGEMENT**

JtH thanks the EPSRC Centre for Innovative Manufacturing in Continuous Manufacturing and Crystallisation (<http://www.cmac.ac.uk>) for supporting this work (EPSRC funding under grant reference: EP/I033459/1).

This work is part of the CW Programme Grant on “Chiral purification of racemic compounds: a grinding approach” and was made possible by financial support from the Netherlands Organization for Scientific Research (Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO)).

The authors thank the help of Ben Norder from TU Delft for the TGA measurement of water content in XSA. The authors also thank the help of Erik de Ronde from Radboud University for the chiral HPLC analysis of enantiomeric excess.

The author(s) would like to acknowledge networking support by the COST Action CM1402 Crystallize.

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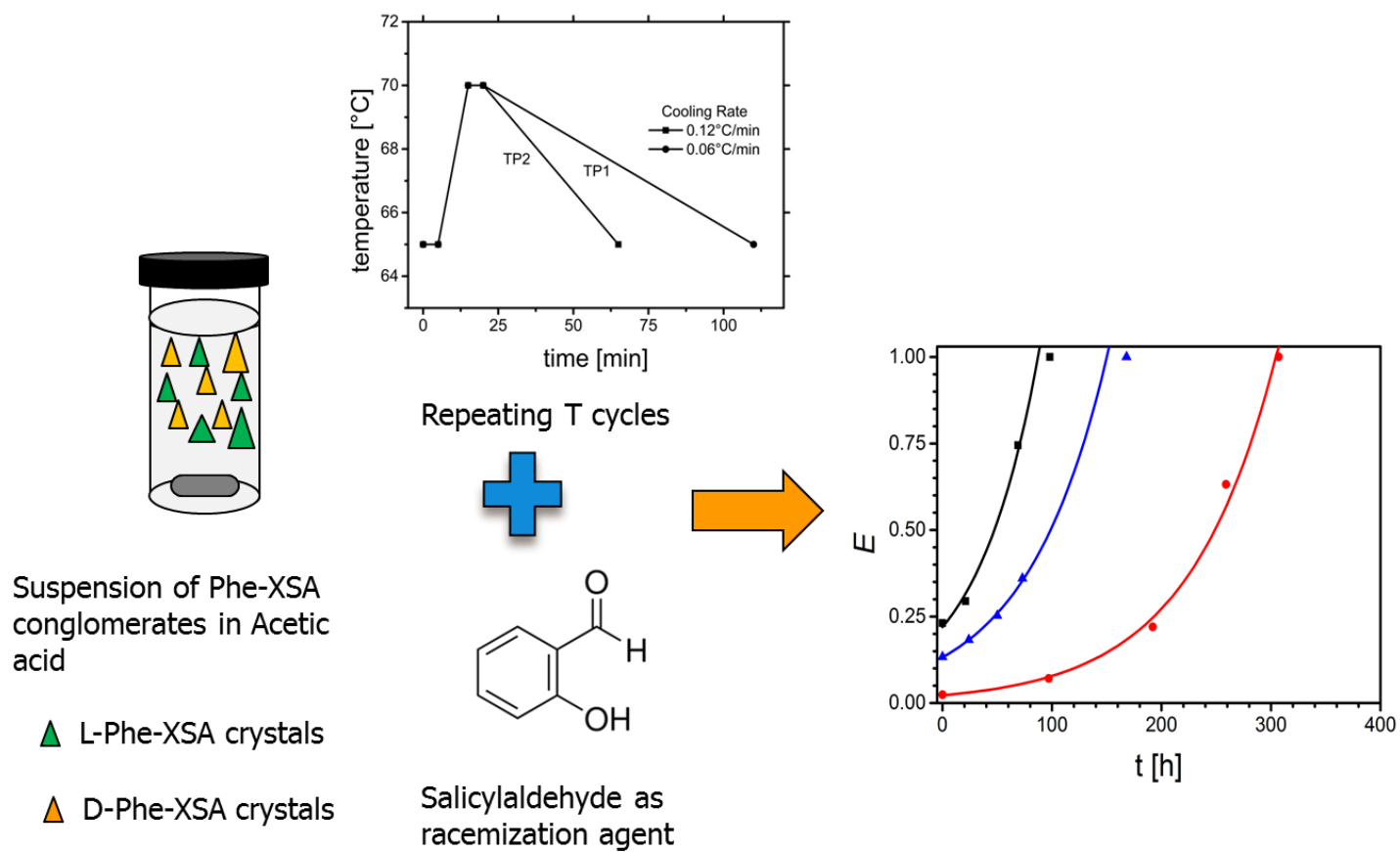
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## Table of Contents



## **Synopsis**

The present paper describes the deracemization of a racemic compound via its conglomerate-forming salt by the temperature cycling method. Additional parameter test identifies the key factors of the operation condition on the deracemization rate. Moreover, advantages and disadvantages of the temperature cycling method and Viedma Ripening are discussed.