Extended Abstract of the Doctoral Thesis: Deracemization via batch temperature cycles - combining racemization and crystallization for chiral resolution

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I. Summary

Enantiomers are chiral molecules that are mirror images of each other and they are characterized by the same physical properties in symmetric conditions, but different in asymmetric environments. Since they can promote different physiological effects, namely therapeutic or toxic, several studies focus on how to isolate the desired enantiomer (eutomer). One of the methods investigated for this purpose is the subject of this thesis: a solid-state deracemization technique defined as *"Deracemization via temperature cycles"*.

Solid-state deracemization techniques enable to increase the maximum theoretical process yield up to 100%, because the racemization reaction promotes the conversion and recovery of the undesired enantiomer (distomer). However, the application of these methods is limited to conglomerate-forming compounds since chiral resolution is fostered by crystal dissolution and growth, which occur thanks to temperature variations.

This study aims at understanding the deracemization via temperature cycle process to set the basis for the implementation of the technology and its variants on a significant scale and commercial level. To do that, we investigated, in experimental and simulation studies, the effect of the many operating conditions on the process performance and we exploited the results to analyze several process variants. The key accomplishments of this doctoral thesis are summarized in the following:

- The operating conditions can be tuned to attain reproducible, robust, and highly productive deracemization processes, characterized by simple operation and reactor configuration, e.g. a thermostated stirred tank reactor.
- The rational and optimal choice of the operating conditions does not require a rigorous mathematical optimization, but it can be based on the parameters that play a major role, i.e. the dissolution factor. Then, the value of the quantities having a minor effect can be adapted depending on the system-specific behavior, revealed by a few screening experiments.
- The deracemization performance is improved when considering a simplified process variant, i.e. crystallization-induced deracemization. However, to attain a high value of final enantiomeric excess the operating conditions must be chosen such that the chiral purity of the initial seeds is preserved.

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As a basis for the understanding of the results of the study, the next section presents the background and the principle of the deracemization via temperature cycle process, while the following ones summarize the work presented in each chapter of the thesis and the corresponding key results. Finally, a closing note highlights the relevance and impact of this doctoral thesis.

II. Background and process principles

Solid-state deracemization methods are crystallization-based technologies where the racemization reaction, that promotes the conversion of one enantiomer into the other, and the crystallization step occur in the same process unit. The process starts with a racemic or enantiomerically enriched suspension that is converted over time to the pure enantiomer. This occurs thanks to dissolution of the crystals of the undesired enantiomer (distomer) and the growth of eutomer crystals, which is fostered by either forming small crystal fragments upon grinding the solid particles, in *attrition-enhanced solid-state deracemization* (or *Viedma ripening*);[1–6] or applying periodical temperature variations between two temperature levels to the entire suspension, in *deracemization via temperature-cycles*.[7–9] This doctoral thesis focuses on the latter method since the process can be carried out in a standard thermostated stirred tank reactor, without the addition of any grinding media or unit. Moreover, the simple operating parameters can be adapted to achieve the desired specifications. Lastly, the potential of this process and its variants as well as a reproducible design

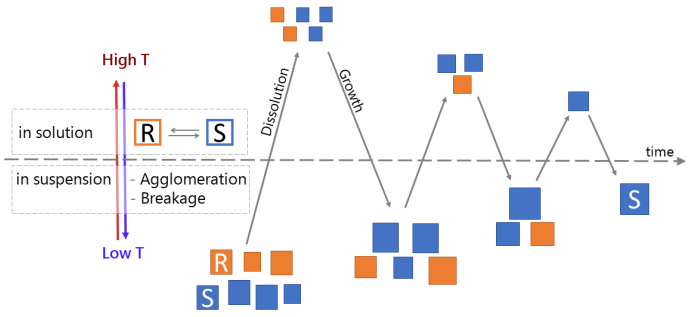


Figure 1: A conceptual representation of deracemization via temperature cycles. (R)- and (S)-enantiomers are depicted in orange and blue, respectively.

approach for a robust and effective deracemization process have not been yet determined.

To perform deracemization via temperature cycles, a thermal profile is defined by combining four steps: an initial heating ramp, isothermal period at a defined high-temperature, $T_{\max}$, a cooling ramp, and a final isothermal period at a given low-temperature, $T_{\min}$. During the process the cycles start periodically one after the other as soon as the last stage of the temperature profile is completed, thus promoting crystals dissolution and growth, while racemization occurs in solution (Fig. 1). At the end of each cycle, the fraction of the desired enantiomer in the solid phase has increased, and a new cycle starts until enantiopurity is attained. During the process, the region in the temperature-concentration plane that the system can explore during every cycle is determined based on the solubility curve and the operating temperature range, which is linked to a maximum concentration change (Fig. 2). Depending on the system's kinetics and the heating and cooling rates selected, the concentration profile will lie closer to or further from the solubility line.

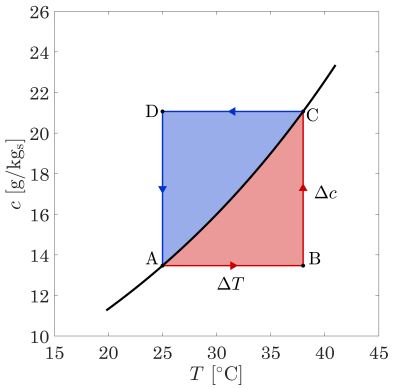


Figure 2: The attainable regions during the heating (red area) and cooling (blue area) phases of the deracemization via temperature cycles with respect to the compound solubility (solid black curve). The red and blue paths represent the changes in temperature during a typical cycle and the corresponding expected change in concentration for heating and cooling periods, respectively.

III. Deracemization via temperature cycle of NMPA: operating conditions [10]

The deracemization via temperature cycles showed great potential, but it was still operated in a rather heuristic way since the phenomena on which it relies had not been fully understood, thus there was no rationale for the selection of the operating conditions. Therefore, the experimental analysis presented in this chapter focuses on examining the influence and role of the main process parameters, such as the initial enantiomeric excess, the cooling rate, the temperature range, and the system volume, on the process performance, i.e. reproducibility, purity, and productivity.[10] As model compound, an imine derivative that crystallizes as a conglomerate was selected: N-(2-methylbenzylidene)-phenylglycine amide (NMPA). NMPA racemizes in the presence of the base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in a mixture of isopropanol (95 wt%) and acetonitrile (5 wt%), used as a solvent in all experiments. During the process, the enantiomeric excess in the solid phase was determined by filtrating, washing, and drying samples of the suspension, then analyzed by high-pressure liquid chromatography (HPLC).

Once the system had been characterized, an efficient protocol to perform reproducible experiments at different lab scales was defined and it was found that the operating conditions influence not only the process time but also its reproducibility.[10] Particularly, a high initial enantiomeric excess (ee_0), which consists of the excess amount of the desired enantiomer in the initial enriched mixture, guarantees a high process reproducibility and speeds up the deracemization. Faster cooling rates also lead to shorter process times, with a weak effect on process reproducibility. This result could be a consequence of the relatively high solid density in the suspension used in the experiments, which favors the growth of the crystals already present rather than the formation of new ones by nucleation. Additionally, we observed that both the process time and the number of cycles required to attain deracemization are reduced when the operating temperature is increased, but the amount of crystals dissolved during each cycle is kept constant. Because of the temperature dependence of their kinetics, both growth and racemization likely accelerate at higher temperatures, but further studies, reported in the next chapter, focus on clarifying the interplay among these phenomena. Lastly, the volume of the system has shown a minor effect on the deracemization process, at least for the volume used in these experiments: in the range of 1 to 10 milliliters. Nevertheless, one can expect a significant effect of different mixing conditions on the process at larger scales.

It is worth noting that these trends are valid in the selected operating parameter space, but modeling studies revealed a hidden non-monotonic behavior of the cooling rate effect.[9] Therefore, the appropriate screening of the experimental operating space is suggested for every new compound.

IV. Deracemization via temperature cycle of NMPA: racemization and solvent effects [11]

The racemization reaction is necessary to restore the concentration of the major enantiomer in the solid phase since during the process its crystalline fraction gradually increases, and its concentration in solution would decrease without racemization. If the reaction is too slow, the concentration of the counter enantiomer builds up, thus hindering chiral resolution. This effect was shown for a simple system of atropisomers racemizing spontaneously in solution.[12] For this system, the racemization reaction kinetic varies based on the solvent chosen. By carrying out the deracemization via temperature cycles under conditions where the racemization reaction was the rate-limiting mechanism, the authors showed that the deracemization process was slowed down. They concluded that the racemization reaction controls chiral resolution. However, there might be cases where crystal growth and dissolution are

rate-limiting or the deracemization rate results from a complex interplay among all phenomena.

To analyze these cases, we focused on a more complex system (NMPA) by starting from the results discussed in the previous chapter, which suggests that the effect of the temperature range on the process is related to the temperature dependence of all kinetic phenomena. Therefore, the study aims at clarifying the isolated role of the racemization kinetics by measuring the reaction rates of NMPA at different temperatures and catalyst concentrations in two different solvent systems, namely a mixture of 5 *wt%* acetonitrile in isopropanol and pure acetonitrile.[11] The data was collected by simply measuring the reaction kinetics in solution, then exploited to evaluate the results of the deracemization experiments, based on previous studies demonstrating that the intrinsic racemization rate constant remains unvaried in the presence of the solid phase.[13]

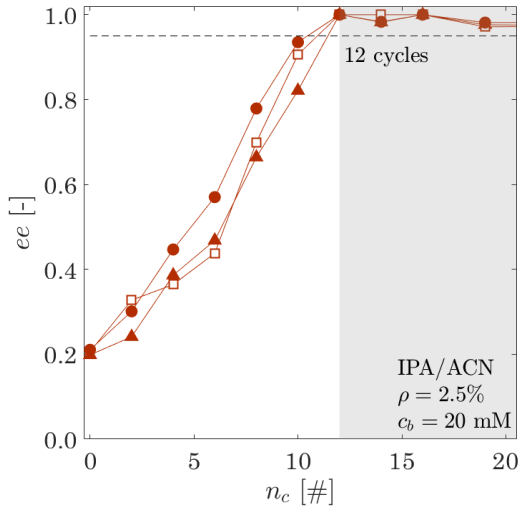
The racemization kinetics measurements were performed by placing a solution of the pure enantiomer and a given amount of catalyst in the desired solvent in the cell of a thermostated polarimeter to monitor the decrease of the enantiomeric excess over time. The data collected at different catalyst concentrations and temperatures were exploited to estimate by linear regression the reaction kinetic constant, that is function of temperature according to the Arrhenius law. Since the racemization reaction is a reversible transformation of one enantiomer into the other, the rate constants are considered the same in both directions.[14,15]

We found that increasing temperature and catalyst concentration promote the reaction, as one can expect. Surprisingly we observed that the reaction kinetic is much faster in pure acetonitrile than in the solvent mixture of isopropanol and acetonitrile. This difference is rationalized in the doctoral thesis based on the solvents' physico-chemical properties, such as polarity, basicity, and viscosity, that affect the reaction mechanism and the stabilization of the transition state.[16,17]

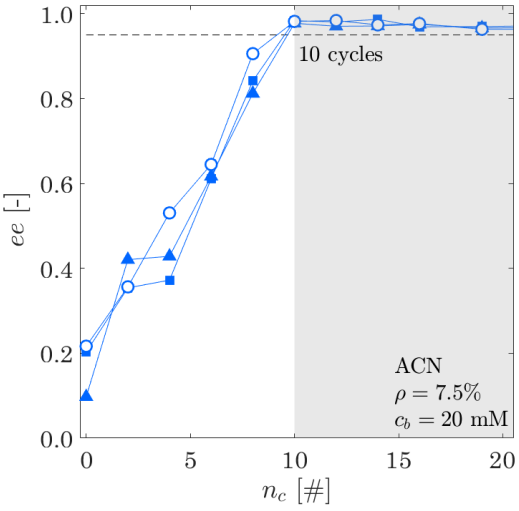
The characterization of the reaction rate as a function of temperature and in different solvent systems enabled us to analyze its effect on the deracemization process. The experiments were carried out on a small lab scale according to the protocol previously developed,[10] such that both the isolated effect of racemization and its interplay with other phenomena could be examined.

The experimental results clarify that the rate of the racemization reaction affects directly the rate of the overall deracemization process via temperature cycles. This can be shown by varying the catalyst concentration only, as we have done in this work in the case of the experiments in the solvent system consisting of a mixture of isopropanol and acetonitrile. Such effect was not obvious in the experiments reported in the previous chapter, because we varied the interval where the temperature cycles were operated, thus affecting the kinetics of all the phenomena involved, i.e. racemization but also growth and dissolution. The fact that all phenomena and mechanisms involved in the deracemization process have an important effect is demonstrated once more in this chapter when deracemization in the solvent mixture is compared to deracemization in pure acetonitrile, where racemization is more than ten times faster. Contrary to the expectation, deracemization is only marginally faster in acetonitrile than in the isopropanol mixture (Fig. 3), most likely because the new solvent affects all the other phenomena involved, and not only the racemization reaction.

Such observation leads to the conclusion that the choice of the solvent system has an important effect on the deracemization process, though in ways that might be difficult to predict a priori, because of the complexity of the deracemization process itself. However, a careful choice based on the thermodynamic (solubility) and kinetic properties (racemization, crystal growth) of the system can promote a successful process design and optimization.



(a)



(b)

Figure 3: Evolution of the enantiomeric excess (ee) of NMPA versus number of cycles (n_c) in (a) solvent mixture of isopropanol and acetonitrile, and in (b) pure acetonitrile. For each set of experimental conditions, three repetitions have been performed (triangles, circles, and boxes).

V. Model-free design of deracemization via temperature cycle of new compounds [18]

The previous work on the deracemization via temperature cycle of a specific compound, namely NMPA, enabled us to improve the understanding of the deracemization process and to clarify the interplay among the phenomena involved. However, this specific example is not sufficient to know if and how the trends identified can be exploited for the design of the deracemization of an additional compound. Therefore, we identified system-specific features by evaluating experimentally the effect of the operating conditions for two new compounds.[18] The parameters investigated are the initial enantiomeric excess, the cooling rate, the temperature range, and the concentration of the racemizing agent. To generalize our conclusion and foster the implementation of the deracemization process, we developed, based on the comparison of old and new results, a model-free strategy for the design of the deracemization process of a new compound.

The investigations were performed with two amino acids imine derivatives, namely 2-(benzylideneamino)-2-(2-chlorophenyl)acetamide (CPG), and 3,3-dimethyl-2-((naphthalen-2-ylmethylene)amino) butanenitrile (tLEU). Both compounds racemize with the same catalyst, DBU, but different solvents were used: a mixture of 5 *wt%* acetonitrile in isopropanol for CPG, while pure methanol was selected for tLEU. The experimental procedure is the same as in the previous works and is extensively described in the corresponding chapter of the doctoral thesis. To achieve comparable conditions for all compounds the process was designed based on their solubility. Particularly, once chosen the minimum temperature level of the cycle, its corresponding maximum was selected as a function of a given variation of solution concentration. This value was computed such that the fraction of solid in suspension dissolved each cycle was constant in all experiments.

When looking at the effect of the initial enantiomeric excess (ee_0), both with CPG and tLEU the deracemization process was accelerated by increasing ee_0, as expected based on previous literature.[4,8,19,20] With large initial enantiomeric excess of the desired enantiomer, the initial asymmetry is more pronounced and the amount of counter enantiomer to convert is smaller, hence, the enantiomeric excess evolves to enantiopurity faster than in the case of smaller values of ee_0. Therefore, the choice of a high value of initial enantiomeric excess is a generally applicable strategy to shorten the deracemization time, select the desired handedness, and improve process reproducibility. This approach is also convenient in terms of productivity, as the reduction of the number of cycles to achieve enantiopurity, i.e. the total process time, compensates for the product (pure enantiomer) invested to generate the desired initial enantiomeric excess, and high productivity is attained.[10,20]

The effect of a second process parameter, namely the cooling rate, can be rationalized by integrating the experimental results presented previously[10] with modeling studies by our group.[9] The former suggests that the number of cycles increases with increasing cooling rates. The latter confirms this experimental trend but also indicates that the process time decreases with fast cooling rates, approaching a minimum, given by the interplay between changes in the number of cycles and in the cycle duration. By further increasing the cooling rate, the process time increases again because of the increasing number of cycles. Depending on the system, the cooling rate value corresponding to this minimum differs, thus the experimental trends depend on the relative position of this point and the cooling rate values tested.

The experimental sets presented here enabled us to analyze the two cases. For both CPG and tLEU, the number of cycles to achieve deracemization increases with the cooling rate, similar to what was found with NMPA (Fig. 4 lhs). This trend suggests that with a slow cooling rate, the system stays close to equilibrium, since the supersaturation generated is quickly compensated by crystal growth, and the racemization reaction keeps the solution composition close to racemic. On the contrary, for high cooling rates, the build-up of large supersaturation levels, keeps the system far from equilibrium, thus, promoting not only the growth and the secondary nucleation of the desired enantiomer but also that of the counter enantiomer, hence, leading to more cycles to achieve deracemization.
By looking at the process time, we observed that when the cooling rate increases, the time shortens for CPG, following the trend observed with NMPA, while it becomes longer for tLEU. In the first case, the short absolute deracemization time is determined by the reduction of the cycle time in combination with a similar number of cycles to achieve enantiopurity. In the second case, the cycle time difference between the various experiments is of only 5 or 10 min, due to the temperature range chosen, as a consequence of the steep solubility curve of tLEU. This difference is too small to compensate for the increase in the number of cycles as a function of the cooling rate and to shorten the process time in the same way as in the case of NMPA and CPG. Therefore, the different behavior of the three substances is related to the variation of the cycle duration among experiments, as well as to system-specific kinetics.

The effect of the operating temperature range is system-specific as well, as it was highlighted by the experiments performed with CPG and tLEU. To ensure similar thermodynamic but different kinetic conditions, the temperature range was varied such that the solubility difference between the minimum and the maximum temperature was constant. It follows that with increasing temperature this range becomes smaller, hence the cycle time becomes shorter, due to the non-linearity of the solubility curve. With NMPA we found that increasing temperature levels reduce the number of cycles and the process time, due to the fast racemization, growth, and dissolution kinetics promoted at high temperatures. The same trend is identified for tLEU, while the CPG shows a weak temperature dependence of the kinetic parameters in the range investigated since the number of cycles is similar when the temperature range is varied (Fig. 4 middle). These results suggest that increasing the temperature range can be exploited to attain faster process time for any compound exhibiting a significant temperature dependence on its kinetic parameters. However, the maximum temperature should be carefully selected to

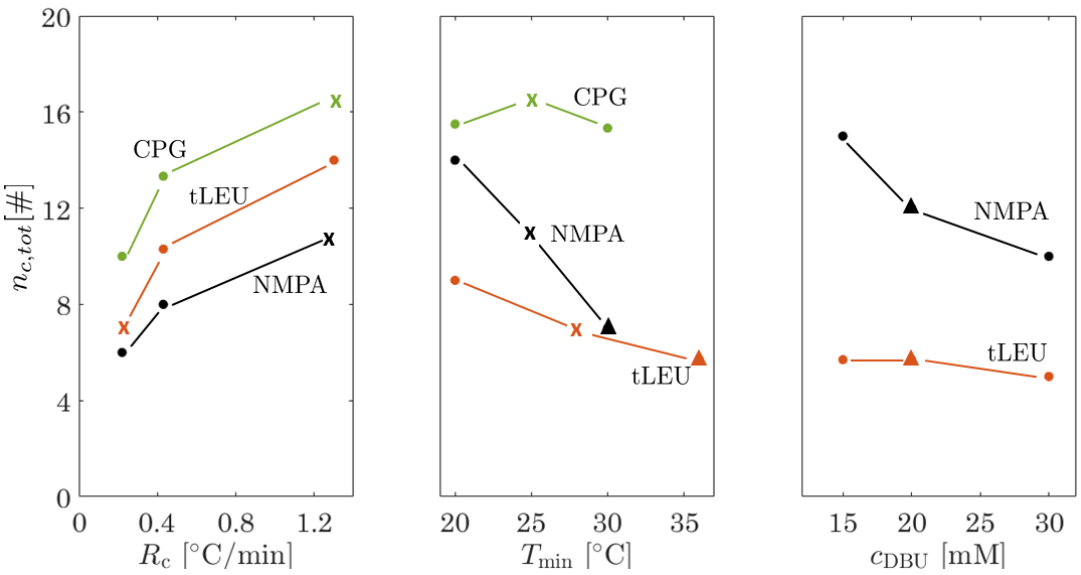


Figure 4: Number of cycles to attain deracemization for the three model compounds NMPA (black symbols), tLEU (orange symbols), CPG (green symbols), as a function of different process parameters: cooling rate (left), temperature range (middle), concentration of the racemization catalyst (right). The reference case is reported with cross symbol (and triangles for the study of the effect of c_b). The lines are a guide to the eye.

ensure the thermal stability of the species involved.

The last parameter investigated was the concentration of catalyst, DBU, that affects the racemization kinetic. Our studies on the deracemization of NMPA indicated that the deracemization rate increases, with an increasing racemization rate, that can be fostered by temperature or catalyst concentration. Now we focus on the effect of the latter and repeat the analysis with tLEU, to verify if the trend is general. Deracemization experiments with increasing concentrations of DBU with this compound resulted in a similar number of cycles, thus suggesting that the racemization reaction is not the rate-limiting phenomenon for tLEU (Fig. 4 rhs). This is expected when looking at the racemization rate constant of tLEU, which is about one order of magnitude larger than that of NMPA.[11,21] It follows that the racemization is fast enough compared to crystal growth and increasing DBU concentration would not be beneficial, but could, on the contrary, lead to product contamination with the catalyst itself or with by-products of possible side reactions. The comparison between NMPA and tLEU showed that the effect of the catalyst concentration depends on the specific system considered, hence the catalyst concentration can be increased in selected cases to accelerate the deracemization process.

By summarizing these results we concluded that the effect of the initial enantiomeric excess and the cooling rate are generally valid, while that of the temperature range and the catalyst concentration are determined by the kinetics of the different systems (Fig. 4). Based on these outcomes, we suggested a model-free screening strategy for the design of an efficient deracemization via temperature-cycles process. This method indicates how to structure an effective screening experimental campaign by carrying out a few lab-scale experiments around a reference case (Fig. 4 cross and triangle symbols). For an effective selection of the reference settings, the first five operating parameters are chosen once and for all based on experience: a large value of the initial enantiomeric excess to speed up the screening procedure; the suspension density at the minimum temperature for good processability; the fraction of solid dissolved during every cycle to avoid crystals washout at high temperature; the duration of the isothermal steps to let the suspension equilibrate at the new temperature; the heating rate to ensure a constant heating profile for a short cycle time depending on the equipment used. For the complete definition of the benchmark case (cross symbols in Fig. 4), the values of the cooling rate, the minimum temperature, and the catalyst concentration are selected while keeping in mind that they are critical and a few experiments where their values are varied with respect to the reference case will be needed. These reference values are not easy to predict, hence their choice is based on the trends observed in our study. Particularly, the choice of the cooling rate is the outcome of a compromise between the willingness to keep the cycle time short and the need to avoid nucleation. The minimum temperature is selected close to ambient temperature to facilitate the operations and minimize compound degradation. Finally, the catalyst concentration should be preferably low to limit product contamination and costs, but high enough to enable a fast and reproducible process. Once the reference case is defined, the three last parameters can be varied in a reasonable range in a few screening experiments. Their outcome, on the one hand, informs on how effective temperature cycles are in deracemizing the new compound. On the other hand, it suggests how to further improve deracemization performance by varying what and how in the design space of the process, thus serving as a guide for a robust and effective deracemization process.

VI. Periodic non-periodic deracemization via temperature cycle: simplified design [22]

The results presented in the previous chapters set the basis for the design of highly productive operations through the analysis of the effect of the operating parameters on the process performance. Nevertheless, the influence of another parameter, i.e. the fraction of solid amount dissolved per temperature cycle, is only marginally examined in a few works of other research groups.[7,8,23,24] They found that increasing this quantity leads to a faster process because the distomer amount that can convert is larger, but one should consider that also a larger amount of the eutomer can dissolve. To better understand this phenomenon, we elaborated on the amount dissolved only with respect to the undesired enantiomer present, by combining these two pieces of information in a single quantity, i.e. the *dissolution factor* (δ).[22] This parameter quantifies the deracemization capacity of the cycles, and it is defined as follows: $\delta = \rho(1-ee)/2\Delta c_\infty$. The parameters represent (i) the suspension density (ρ, proportional to the total mass), (ii) the enantiomeric excess (ee), that together determine the distomer amount to be converted; and (iii) the amount of solid dissolved every cycle (Δc_∞), that is proportional to the distomer fraction that can be converted each cycle and it is given by the concentration difference between the temperature levels of the cycle. Interestingly, the dissolution factor can be used both as a design parameter (when considering its initial value, indicated by the subscript "0") and as a diagnostic parameter (when considering its cycle-by-cycle definition, indicated by the subscript "n"). The former application is analyzed in the first part of this chapter in both modeling and experimental studies. Based on these results, the latter use is then investigated to rationalize how the deracemization time changes when the period and the amplitude of the temperature cycles are modified over time during the deracemization process, namely when non-periodic temperature cycles are applied.

It is worth noting, that the definition of the dissolution factor derives from a novel re-scaling of the mathematical model previously presented.[9] The new formulation highlights that the process depends on the operating parameters through the dissolution factor, on the temperature-dependent kinetic parameters of racemization and growth, and on the thermodynamic parameters, such as solubility. A detailed analysis of the effect of these factors is reported in the corresponding chapter of the thesis. In this abstract, we focus on describing the key finding of this work, which is the primary role of the dissolution factor, while the system's kinetic and thermodynamic properties play only a secondary role.

The modeling studies were carried out by defining four compounds differing in solubility and growth rate, to investigate if and how the effect of the dissolution factor depends on system-specific properties. The simulation results demonstrated that the dissolution factor affects similarly the deracemization of the four compounds, thus showing a general effect. In all cases, the number of cycles remains constant for a given value of δ_0, when ρ_0 and Δc_∞ vary accordingly. Moreover, the total number of cycles decreases with decreasing δ_0, thus suggesting

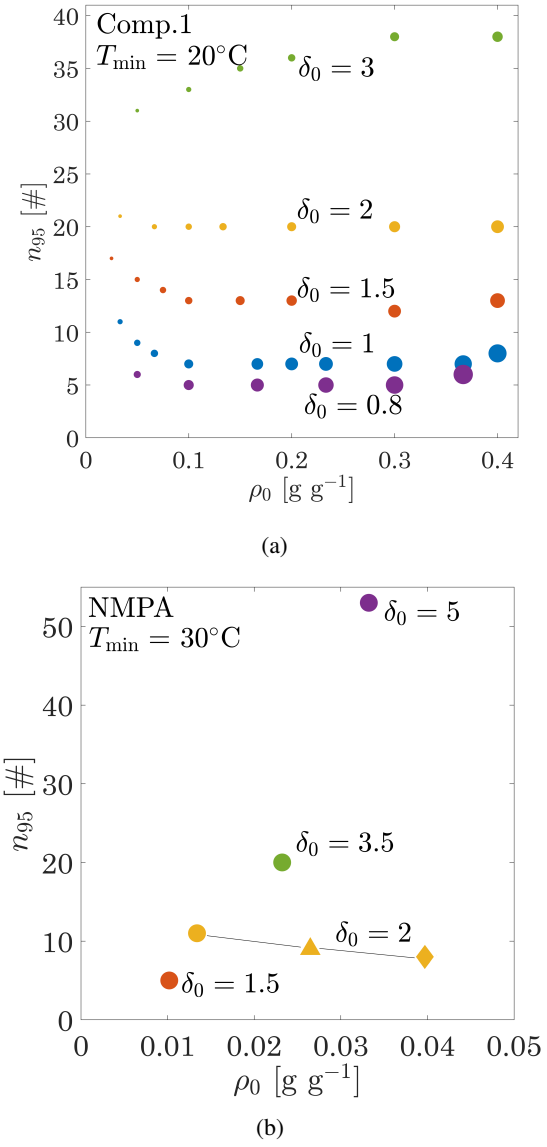
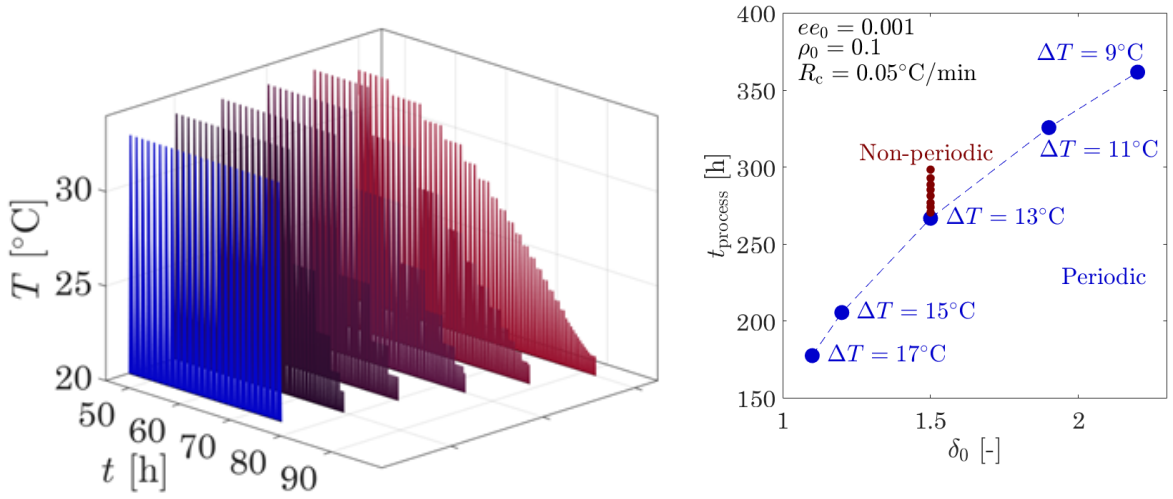


Figure 5: Effect of the initial suspension density, ρ_0, on the total number of cycles, n_{95}, with increasing dissolution factor, δ_0, for (a) the simulated Compound 1, with $ee_0 = 0.4$, and (b) the experimental compound NMPA, with $ee_0 = 0.6$

that the limit case, whereby δ_0=0, may have potential[25] (Fig. 5a). However, from the analysis of the system-specific trends emerged that the growth rate has a stronger influence than solubility, at least when racemization is not the limiting phenomenon.

The experimental runs, performed with the model compounds common throughout the entire doctoral thesis, confirm the general trends identified in the simulation studies (Fig. 5b). First, a similar number of cycles is obtained in experiments where the initial dissolution factor is kept constant for NMPA and tLEU. For CPG a major difference related to the effect of supersaturation is identified, hence analyzed and discussed in detail in a dedicated section of the doctoral thesis. Second, the number of cycles increases with increasing δ_0 for all compounds, thus underlining how general this trend is.



(a) (b)

Figure 6: (a) Example of periodic (blue) and non-periodic (red) temperature profiles. (b) Process time as a function of the dissolution factor (δ_0), that increases with decreasing temperature variation (ΔT, proportional to the amount dissolved) for a given initial enantiomeric excess (ee_0) and suspension density (ρ_0), in the case of periodic (blue) and non-periodic cycles (red).

Based on both simulation and experimental results where a short process time is attained with low δ_0 values, high productivity values are expected under the same conditions. By computing this performance indicator as a function of δ_0, with varying ρ_0 and ee_0 values, we confirm this expectation. Note that the productivity does not increase monotonically with ee_0 and ρ_0, hence the maximum value should be identified to ensure the best process conditions. The results of this analysis, which underlined the key role of the dissolution factor, inspired two more studies with the goal of defining the optimal process. The first consists in operating at the lowest possible value of the dissolution factor, i.e. δ_0=0, when the $ee_0 = 1$ and it is presented in the next chapter. The second, discussed in the following, focuses on the application of non-periodic cycles, for which the minimum temperature is fixed, but the cycle amplitude is reduced during the process such that the dissolution factor is kept to a desired low value for each cycle.

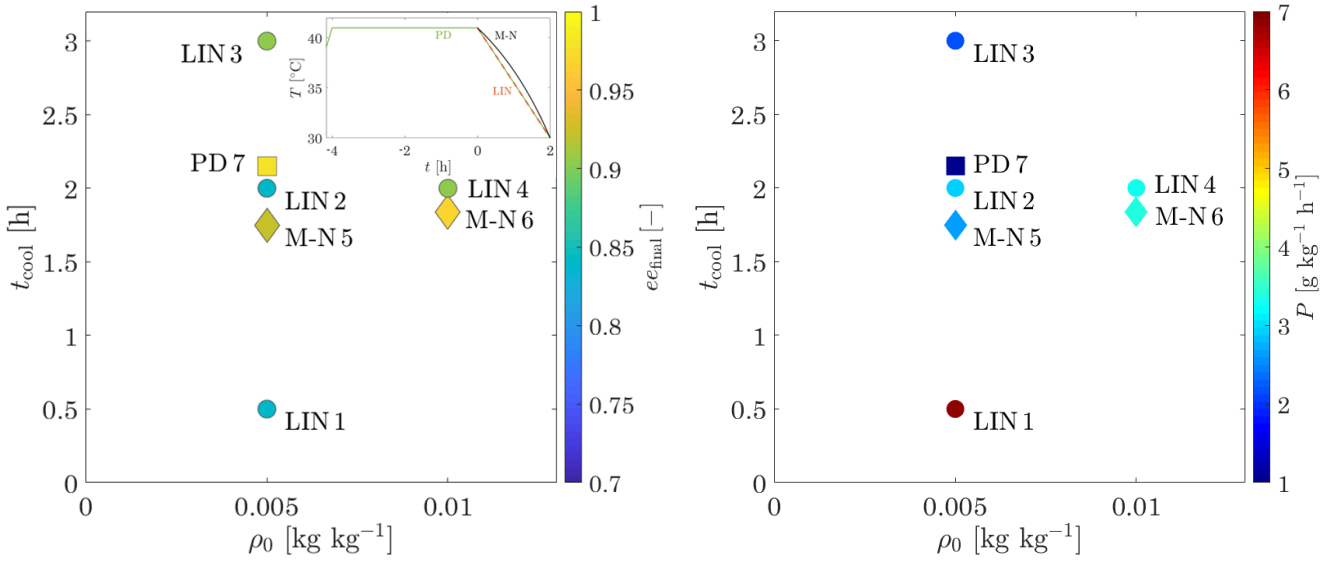
We evaluated the potential of this process variant in modeling and experimental studies, which pointed out once again the leading role of the dissolution factor. We concluded that carrying out non-periodic cycles does not improve the performance of the deracemization via temperature cycles process, contrary to previous studies.[26] To understand and rationalize this apparent controversy, we, first, examined the experiments by Suwannasang et al., thus finding in their design some inconsistencies, of which we were aware in planning our experiments. Then, we ran ad-hoc simulations and compared the results of deracemization with periodic temperature cycles with increasing δ_0 value, to runs where different non-periodic cycles are performed for a fixed value of the dissolution factor (Fig. 6a). They confirm that, even with extremely slow cooling rates (long cycle time), the process takes the shortest time when the δ_0 is the smallest, thus the cycle amplitude (ΔT) is the largest, and that for a given δ_0 the periodic cycles are the most efficient (Fig. 6b).

This outcome clearly suggests that, while reducing the ΔT to values smaller during the process is sub-optimal, periodic temperature cycles perform better than non-periodic ones, as long as the value of δ_0 is appropriately selected. Therefore, we demonstrated that the periodic profiles are preferable because they promote a short deracemization time with a simple model-free process design, based on the information provided by the dissolution factor.

VII. Crystallization-induced deracemization: experiments and modeling[25]

The clarification of the primary role of the dissolution factor motivated the analysis of a simple process variant of deracemization via temperature cycle, which we define as "crystallization-induced deracemization". This is a batch process, where the initial suspension, consisting of pure crystals of the target enantiomer ($ee_0 = 1$) is cooled in a racemic solution to promote crystal growth. The presence during this process of a racemizing agent in solution hinders the nucleation of crystals of the distomer by enabling its conversion into the eutomer. Compared to deracemization via temperature cycles, crystallization-induced deracemization begins at high temperature, the eutomer is crystallized out of the solution by cooling, and no heating steps are performed since distomer crystals are not present when cooling starts. However, they could nucleate upon cooling the suspension, thus decreasing the purity of the final product.

Although "crystallization-induced deracemization" (CID), had already been proposed as a modification of the preferential crystallization process,[27–31] a systematic analysis of the effect of the operating conditions to understand how to tune them targeting high purity and productivity was still missing. Our analysis revealed the relevant trends and showed that the sim-



(a) (b)

Figure 7: Purity (a) and productivity (b) as a function of seed load and cooling time, for the experiments performed with linear (circles), Mullin-Nyvlt based (diamonds), and PD (box symbol) cooling profiles. The latter data points are plotted with slightly shifted coordinates for a better representation.

plicity of this method represents one of the main advantages of such operations, together with the high productivity values attained. However, the trade-off between productivity and purity requires a careful selection of the operating conditions to cope with the presence and the appearance of the distomer, thus obtaining a high-purity product. Specifically, when a simple linear cooling ramp is applied, its rate must be slow enough and the seed load large enough to achieve this goal (Fig. 7 circles). Since long cooling times imply low productivity values, we identified alternative strategies to attain both high productivity and purity.

First, the application of a non-linear cooling profile, defined as "Mullin-Nyvlt-based cooling profile" (Fig. 7a top right corner black line), is proposed because it can be designed as a function of readily available information: the solubility curve and the operating conditions. Even though the Mullin-Nyvlt-based trajectory is an approximation, its simplicity makes this approach very appealing and useful. By comparing experiments and simulations with this and a linear temperature profile with the same seed load and cooling time, we found that the former promotes better product purity (Fig. 7 diamonds vs circles), for similar productivity values. Note that with both temperature profiles, increasing the seed load leads to higher productivity and purity, while increasing the cooling time is only beneficial for product purity.

The second approach presented consists in getting rid of the distomer impurities by dissolving part of the seed crystals, thus increasing the ee_0 and preventing the growth of distomer crystals. This is achieved by increasing the temperature of the suspension by a few degrees and starting the linear cooling ramp once the solution is re-equilibrated (Fig. 7a top right corner green line). By running experiments with this temperature profile at different catalyst concentrations, we observed a peculiar behavior: the ee increases upon heating when the catalyst concentration is low, while the ee decreases when it is high. Through simulation studies, we proved that a fast racemization reaction converts the eutomer into the distomer, as soon as the former dissolves, thus hindering the dissolution of the latter and leading to the decrease of the ee. On the other hand, slow reaction rates promote high eutomer concentration in solution, hence hampering its dissolution. However, if racemization is too slow during the cooling step, the distomer nucleates and the product purity decreases. Based on these experimental and simulation studies, we showed that by carrying out the dissolution step at a low catalyst concentration, which is increased before cooling starts, the enantiopurity of the seed mixture increases, due to slow racemization, and it is preserved during the cooling ramp, thanks to fast racemization (Fig. 7a box symbol). It is worth noting that the long equilibration time needed after heating (4 hours) leads to low productivity values (Fig. 7b box symbol), but this holding time can be reduced depending on the dissolution kinetics of the compound, thus providing more competitive productivity values.

When focusing on the productivity values we observed that they vary from about 1 g kg^{-1}h^{-1} when partial seeds dissolution is performed, up to 3.4 g kg^{-1}h^{-1} by operating at conditions that enable to collect a highly enantiopure product, i.e. by implementing the Mullin-Nyvlt-based temperature profile with larger seed load. Even higher values of productivity can be attained at the expense of the final ee when fast cooling is implemented. By comparing these values with those obtained with deracemization via temperature cycles, achieving a productivity value of 2 g kg^{-1}h^{-1} under optimal conditions, we concluded that crystallization-induce deracemization is an attractive and competitive method, since it is simple to implement and to scale-up because of its analogy with cooling crystallization processes (e.g. preferen-

tial crystallization). Moreover, the desired product purity can be attained by implementing the strategies presented in this work. However, deracemization via temperature cycles should be preferred in the case nucleation of the counter enantiomer cannot be avoided, but also when the process yield is limited by the compound properties, such as the solubility or the thermal stability.

VIII. Relevance and Impact of the Thesis

The extensive study on the deracemization via temperature cycles process presented in this doctoral thesis exploits reliable experimental and modeling[20,25] frameworks to demonstrate the reproducibility and robustness of the deracemization of multiple compounds,[10,18] as well as to rationalize the interplay among the different phenomena during the process. The thorough understanding of the technology gained within this work enabled us to master the process design for a new compound without the need for a rigorous mathematical optimization, that would require tedious measurements of the system's kinetic properties. Additionally, the analysis of several process variants shed light on their advantages and disadvantages, thus underlining which techniques to consider because they can foster process performance. These findings demonstrated that deracemization via temperature cycles is a promising novel method to attain enantiomer purification. By doing this, the studies provide a solid ground for the application of the deracemization via temperature cycle process and its variants on an industrial scale. Nevertheless, this work clarified aspects related only to the batch processes, but there is great scope for improvement of both batch and continuous deracemization processes.[32–34] Such processes differ in operation modes and temperature profiles applied, as indicated in the table below.

Mode / Temp.	Batch process	Continuous process
Temperature Cycles	Deracemization via temperature cycles	Periodic forcing via temperature cycles
Cooling	Crystallization-induced deracemization	MSMPR-type deracemization

In all cases, racemization keeps the solution composition close to racemic, and one can foster the conversion of the distomer into the eutomer by performing periodic temperature variations in batch (deracemization via temperature cycles) or continuous mode (periodic forcing via temperature cycles). Alternatively, one can promote the growth of pure particles of the target enantiomer in a simple cooling crystallization process such as crystallization-induced deracemization (batch) and mixed-suspension-mixed-product-removal-type deracemization (MSMPR-type, continuous).

Although the focus of this thesis is on batch operations, it encouraged the modeling and experimental investigation of continuous process alternatives in collaboration with other doctoral students in Prof. Mazzotti's research group. Part of this work consists of a detailed model-based analysis of these techniques and culminates with the development of a simulation tool for the fair comparison of the four technologies.[34,35] Moreover, experimental studies on continuous processes have started in a follow-up work to complete the picture. In the frame of continuous operations, it is worth mentioning a side project that aimed at expanding the range of opportunities for chiral purification by looking at chromatographic methods combined with a racemization reaction.[36,37] The experimental and modeling analyses were carried out with NMPA as a model compound to provide the basis of a fair and consistent comparative assessment, which is very challenging due to the fundamental differences among the crystallization- and chromatography-based technologies. Nevertheless, a detailed investigation of the role of the racemization reaction when combined with a chromatographic process and the implications of slow racemization kinetics was carried out with the support of a younger student.

Summarizing, the work presented in this thesis examines extensively the batch deracemization methods in experimental analysis supported by model-based rationalizations. Moreover, the advanced understanding of batch operations motivated the investigation of the corresponding continuous variants. Although for the industrial application of batch and continuous deracemization, more studies on process scale-up, as well as monitoring methods, are necessary, the achievements reported in this thesis led to significant progress in process understanding and optimization, thus paving the way for enhanced and facilitated development of both batch and continuous deracemization processes.

Notes

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