Complete chiral resolution in a continuous flow crystallizer with recycle stream

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Abstract

Repeated temperature cycling of crystals from a conglomerate forming chiral substance suspended in their saturated solution has shown to be effective in converting a mixture of both enantiomers into an enantiomerically pure state. While by now a large number of different setups has been demonstrated, here we show for the first time how a continuous flow temperature cyclizer with recycle stream is capable of establishing enantiopurity while converting a racemic starting suspension. By capturing the most significant parameters influencing the process kinetics a competitive productivity could be achieved. We show, that fast crystal dissolution at high undersaturations and fast crystal growth at high supersaturations are speeding up the process as long as nucleation can be kept to a minimum or avoided at all. Temperature cycling has shown to result in a shift towards larger sizes for the particle size distribution of the crystals suspended, which is detrimental to the present process governed by size-dependent solubility. By implementing an ultrasound unit recycled material was comminuted, resulting in nearly stable deracemization rates.

Keywords Temperature cycling · Deracemization · Chiral resolution · Tubular crystallizer

Introduction

The driving mechanism for compounds to occur only as a single enantiomeric form was described only a few years ago and is also known as the process of “symmetry breaking” [1]. Prominent examples for single chirality are amino acids and carbohydrates, where one enantiomeric form is preferred in all living organisms. The underlying autocatalytic process starts with crystals from a substance being able to racemize in its dissolved form. The prerequisite of this mechanism is that enantiomers must crystallize as separate crystals, that is, a racemic conglomerate. Ostwald ripening, a process already known for more than 120 years [2] governs the reduction of overall crystal surface, eventually producing one single crystal, which in case of conglomerates, means enantiomeric purity. The rate of this process, though, is inversely proportional to the size of the crystals and therefore slows down extremely

for macroscopic crystals. Repeated or continuous grinding of the crystals has been the first method to greatly accelerate this process [1, 3–5]. Later, cycles of growth and dissolution for crystals suspended in their saturated solution have shown to be equally effective [6]. The preferred route has been to implement cycles between high and low temperatures to induce super- and undersaturation. By starting the process with a scalemic crystal suspension, hence by adding one enantiomer in excess, one can predetermine whether the process evolves towards the (R)- or (S)-form. During crystal growth at low temperatures an existing enantio-imbalance leads to faster re-incorporation of molecules and clusters into the crystals of the “majority” crystalline population. This generates a driving force for molecules of the “minority” population to be converted to the “majority” population via a racemization reaction in solution. “Major” and “minor” in this context refer to the overall surface area present for conglomerates of each handedness. Therefore, particle size distribution plays a major role in this context.

Dissolution at elevated temperatures is generally assumed to be more controlled by thermodynamics, rather than kinetics. Hence, dissolution of crystals depends on their temperature- and size- dependent solubility and therefore proportionally the excess enantiomer dissolves less [6].

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Due to its limitation on conglomerate forming substances symmetry breaking has long been regarded of low interest for pharmaceutical application. In the last years this has changed due to widening its application via transforming non-conglomerate forming substances to conglomerate forming salts [7, 8]. Since then, upscaling of respective processes has been in the center of interest of several publications. Yet, production rates (often disregarding the enantiomeric excess at the start of the experiment) have not exceeded the kg/day scale, being far too low to be applicable for high-demand drugs. By now no continuous process capable of long-term production has been shown to facilitate complete enantiomeric resolution of a racemic mixture of crystals to a selected enantiomeric form. With the present work we show how a simple, fully continuous setup employing a recycle stream is capable of outperforming all other approaches so far by converting all of the counter enantiomer without depending on an enantiomeric excess in the starting suspension.

Materials and methods

Synthesis of NMPA

The compound *N*-(2-methylbenzylidene)-phenylglycine amide (NMPA) has been synthesized according to literature [4] from the following reactants: 2-phenylglycine (95 %, Sigma-Aldrich), *o*-tolualdehyd (96.5 %, Sigma-Aldrich) and thionyl chloride (99 %, Fluka). DBU (1,8-diazabicyclo[5.4.0]undec-7-ene, 98 %, Sigma Aldrich) was used to induce racemization of NMPA in the dissolved state.

Solubility of NMPA in ethanol

The solubility of NMPA in ethanol was determined in the temperature range between 10 and 45 °C by measuring the saturation temperature at various concentrations using a Crystalline® device (Technobis, Netherlands). NMPA and ethanol were filled into vials to get mixtures between 5.5 and 30.0 mg per g ethanol with total volumes of about 5 mL. The suspensions were first cooled to -10 °C and subsequently the samples were heated to up to 45 °C with a rate of 0.5 °C/min. The transmissivity of the samples was detected inline and the clear points obtained correspond to the solubility of the compound. The solution was cooled down again to -10 °C with a cooling rate of 1 °C/min, facilitating the identification of the metastable zone width by detecting the cloud points, hence the onset of nucleation. Two additional temperature cycles proved the reproducibility of results. The mean value of the clear points and cloud points, respectively, from the three temperature cycles are plotted as the resulting solubility curve and metastable zone width in Fig. 1. Higher concentrations of NMPA were tested in a corresponding test

heated up to 70 °C. However, at temperatures > 45 °C a shift of the clear points to lower temperatures occurred during consecutive cycles, as obviously NMPA is unstable in ethanol at higher temperatures.

The metastable range, together with the solubility curve, provided valuable initial indications for the choice of temperatures in the water baths, especially with regard to their temperature difference. Only if a certain critical temperature difference is not exceeded excessively high supersaturations in the cold water bath and hence undesired primary nucleation can be avoided.

Preparation of enantiomerically enriched starting material

In order to obtain enantiomerically enriched NMPA for generating the desired levels of enantio-purity in the starting material of the following continuous experiments, the Crystalline® crystallization system (Technobis) was used. Each vial was loaded with 7 mL saturated ethanolic solution of NMPA, 0.12 g racemic NMPA from synthesis, 0.04 g of enantiomerically enriched (*R*)-NMPA and 30 µL of DBU. The vials were exposed to temperature cycles (based on settings by Breveglieri et al. [9]) while stirring at 500 rpm using a 10 mm stir bar. For each cycle the temperature was increased from 22 to 35 °C (heating rate 1.3 °C/min) followed by a 10 min hold step. Subsequently, the vials were cooled back to 22 °C (1.3 °C/min) followed by another 10 min hold step, giving a total cycle time of 40 min. Up to 320 cycles per vial were performed to obtain sufficient (*R*)-NMPA for preparing scalemic starting suspensions to be used for parameters studies performed in the tubular setup.

Analysis

The key parameter in all the results presented here is the enantiomeric excess *ee* with respect to the *R* enantiomer, which is defined according to Eq. 1.

$$ee = \frac{m_R - m_S}{m_R + m_S} \quad (1)$$

The enantiomeric excess *ee* was measured via HPLC, using a Chiralcel OJ-H column (250 mm x 4.6 mm, pressure = 47 bar, T = 25 °C). Samples of 5.5 mg were dissolved in 8 mL of a mixture of *n*-hexane and isopropanol (1:1 v/v) and volumes of 1 µL were injected. A mixture of 80 % *n*-hexane and 20 % isopropanol (v/v) was used as a mobile phase. Besides spectroscopic analysis of the desired product at 254.4 nm, absorption at 210.4 nm has been measured in order to detect the formation of a potential side product [2-methylphenyl]-5-phenyl-imidazolidin-4-one]. Retention times have been found at 12.0 min for (*S*)-NMPA and at 18.2 min for (*R*)-NMPA.

Fig. 1 Solubility (blue) of NMPA in ethanol (99.8 %) and metastable zone width (orange) for a cooling rate of 1 °C/min. The dotted curves are representations of a logarithmic regression of the data. Solubility curve: $c = 3.795 \cdot e^{0.047 \cdot T}$

Comminution of the starting material

Both the racemic crystals and the enantiomerically enriched crystals were ground using mortar and pestle, followed by optical analysis using light microscopy. The milling was considered sufficient when no crystals larger than 30 µm could be detected. The median particle size for all batches milled and used during cycling experiments was between 10 and 15 µm as confirmed via laser diffraction measurements (HELOS®, Sympatec).

Evaluation of results

Several groups have studied the behaviour of the process of temperature-cycling-induced deracemization, either experimentally or computationally via the use of population balance equations. While earlier experimental studies relied on simple first-order kinetics expressions to describe the progress and the time-evolution of the enantiomeric excess [8], later more detailed models using population balance equations have been developed [10–13]. In the context of this work, we were looking for a simple way to quantify the efficiency of the deracemization process depending on the process settings of individual trials and relate it to the number of cycles performed. Therefore, we adopt an approach suggested by Li et al. [8] (based on an exponential equation derived by Noorduyn et al. [14]), which assumes that the enantiomeric excess in the product after N temperature cycles ($ee(N)$) is proportional to the enantiomeric excess in the starting suspension $ee(0)$.

$$ee(N) = ee(0) \cdot e^{k_N \cdot N} \quad (2)$$

Instead of using a temperature-based rate constant, here a cycle-number based rate constant (k_N) is used. Thereby we account for constantly changing temperatures and avoid the problem that different flow rates also mean different residence times. In order to quantify the influence of experimental parameters on the deracemization rate we intended to set process

conditions for the temperature cycling unit and to define ee after steady state is reached. Starting from that we discuss discrepancies in light of results from other groups obtained via modelling studies.

Parameter study

Setup and equipment

A setup based on a continuously operated tubular crystallizer [15–17] and similar to our recently described tubular temperature cyclers [18, 19] has been adapted to facilitate continuous deracemization. Polysiloxane tubing ($d_{in} = 2\text{ mm}$, $d_{out} = 4\text{ mm}$) with a total length of 50 m was coiled up into loops of 4 m each and immersed into two water baths to pass these alternately. The resulting 12 cycles with an overall volume of 0.15 L were flanked by 1 m of tube each for inlet and outlet. For each cycle, the tube first passed the warm bath (154 cm), and subsequently the cool water bath (220 cm). $2 \times 13\text{ cm}$ of tubing per loop were used for crossovers from one bath to the other. The unequal partitioning of the tube between the two water baths was used since the dissolution of crystals was expected to be faster than their growth.

NMPA was chosen as a model substance as it has been used in recent publications in the field [9, 20–23]. For each experiment, NMPA was added to ethanol (99.8 %, denat.) in excess to generate a saturated solution. The suspension was stirred at 22 °C for $\geq 2\text{ h}$ and a clear saturated solution was obtained via filtration. This was kept in a 1 L round-bottomed flask starting vessel, agitated using a stir bar (l = 5 cm, 300 rpm). 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was added to catalyse the racemization of NMPA in the dissolved state.

The saturated solution was charged with ground racemic crystals of NMPA, as well as appropriate amounts of enantiomerically enriched (R)-NMPA, resulting in a scalemic mixture of a desired $ee(0)$. The suspension was pumped through the tube by means of a peristaltic pump (Digital

MS-2/6, Ismatec, USA). To achieve controlled particle transport and to minimize backmixing of crystals a segmented flow was established. By connecting two syringe pumps (LA-120, HLL Landgraf, Germany) appropriately, air was continuously supplied to the crystallizer via a T-fitting. Throughout all experiments the ratio of the flow rates of air $\dot{V}_{\text{Air}}$ and suspension $\dot{V}_{\text{Susp}}$ was kept constant at $\dot{V}_{\text{Air}} / \dot{V}_{\text{Susp}} = 0.2$. By keeping one water bath at a temperature below 22 °C and the second one at elevated temperature a sequence of 12 growth- (at $T_1 < RT$) and dissolution stages (at $T_2 > RT$) was generated. In a recent study [20], we reported on a computational approach to model the temperature profile in the tubular crystallizer during heating and cooling. Calculations about the present setup showed, that due to the high heat-transfer rates the respective bath temperatures are reached quickly after entry (see Fig. 2).

A schematic of the setup is shown in Fig. 3. Based on standard settings shown in Table 1, individual parameters were changed to explore their effect on the deracemization rate. During subsequent tests a recycle stream has been added, as discussed in "Implementation of a recycle-stream for complete enantiomeric resolution" section.

Effect of temperature swing

During the first test series, different temperatures of the two water baths were selected and the effect on the deracemization rate was investigated. For each setting a separate experiment was started and samples were taken from the steady state (at least 1.2 residence times after starting the experimental run). Reaching steady-state was verified occasionally by samples taken at later time points during prolonged experiments. This fast establishment of the steady-state is in line with former studies employing segmented flow, owing to significantly reduced axial dispersion [15, 19]. Starting with the standard settings of 17 °C (cold water bath) and 27 °C (warm water bath) (temperature swing $\Delta T = 10$ °C), both larger and smaller temperature differences were tested. In the range between $\Delta T = 2$ °C and $\Delta T = 12$ °C standard deviations from three

experimental runs each were small and a continuous, nearly proportional increase of *ee* with temperature swing was observed. For runs at higher temperature differences ($\Delta T = 14$ –22 °C) the standard deviation of the measured values increased strongly (see Fig. 4a).

Based on the metastable zone width (see Fig. 1), it can be assumed that the stochastic nature of nucleation of the counter-enantiomer is the reason for the wide variation in results at these elevated temperature swings.

During the passage through the cold water bath the supersaturation of the excess enantiomer is preferentially consumed due to its larger available crystal surface area. This favours the nucleation of the counter-enantiomer, drastically decreasing the overall deracemization rate.

Furthermore, the formation of nuclei generates a large surface for the counter enantiomer to grow. If the nuclei formed do not dissolve completely in the warm water bath the *ee* decreases. For even greater temperature swings ($\Delta T = 24$ and 29 °C) our experiments consistently led to *ee*-values decreasing with increasing temperature, which is in line with the increasing amount of racemic crystal mass formed during the cooling stages.

Tubular crystallizers such as the one presented here are characterized by a high surface-to-volume ratio, facilitating fast heat transfer and thus equilibration with the surrounding temperature is achieved after very short times. During the short residence times realized in our setup for each temperature step, no equilibration of the actual concentration with the compound's solubility can occur, as described for similar setups in our earlier publications [18, 19]. Still, the larger the temperature swing is, the more crystal mass is dissolved and regrown per cycle due to accelerated rates of crystal growth and dissolution at higher super- and undersaturations, respectively.

Effect of different pump settings/residence times

In a next step our aim was to characterize the influence of different residence times per cycle on the deracemization rate. We changed the residence time via manipulating the overall

Fig. 2 Modeled temperature profiles of pure ethanol in segmented flow (at total flow rates of 4.8 mL/min (red) and 14.4 mL/min (green), corresponding to the minimum and maximum flow rates used in this study)

during the first six temperature cycles. The blue solid line represents the temperature of the water baths at standard settings

Fig. 3 Schematic representation of the setup (left) and the change of solid mass composition during three temperature cycles (right). While the parameter study was run in a linear setup, a recycle stream was added for

flow rate. As shown in Fig. 4b, the cycle number-based deracemization rate constant decreased only slightly with a higher volume flow, from $k_N = 0.116$ at an overall flow rate of 4.8 mL/min to $k_N = 0.097$ at 14.4 mL/min.

It is assumed, that for a given set of conditions and for low and medium *ee* the same absolute crystal mass is dissolved for each of the two enantiomers. Hence, proportionally the less abundant (counter-)enantiomer dissolved more, implying an increase in the value of *ee* [6, 24]. From this follows, that the longer the residence time is and, therefore the more crystal mass is dissolved, the greater is the increase in *ee*. However, this may trigger an opposite effect on the *ee* in the subsequent cold water bath: The more crystal mass is dissolved, the higher the supersaturation will be, favouring nucleation of the counter-enantiomer, as discussed above.

Our results indicate that the time for the concentration of dissolved NMPA to equilibrate with the temperature is longer than the residence time of the suspension in each water bath for all flow rates tested. The overall flow rate of 4.8 mL/min, leading to the highest k_N , is already quite low and could not be decreased any more to avoid sedimentation.

subsequent experiments. Red and blue color represent high and low temperatures, respectively. Crystals of the two enantiomers are illustrated by silver and gold color cubes

Effect of different solid concentration

Figure 4c shows the dependence of the deracemization constant on the mass of crystals per volume of liquid. A larger solid concentration slows down the process, represented by k_N decreasing from 0.116 to 0.3 % (w/w) to 0.049 at 1 % (w/w). This is consistent with the smaller mass fraction of solid dissolving and re-crystallizing during each temperature cycle. A similar effect was observed before during previous studies [6, 8, 23].

Effect of different amounts of catalyst

Previous investigations have shown, that the amount of catalyst present is only critical if the racemization step is rate limiting for at least one part of the multiple-step deracemization process via temperature cycles, and hence is system-specific [21]. In Fig. 4d the dependence of k_N on the catalyst concentration is shown. The process can be accelerated by larger amounts of DBU, as the deracemization constant increases from 0.092 to 20 mM DBU to 0.147 at 44 mM.

One obvious reason for this can be found in the accelerated racemization reaction. This reaction is required to equilibrate

Table 1 Parameters varied during the parameter study

Parameter	Standard setting	Minimum	Maximum
Temperature swing ΔT [°C]	10	2	29
Flow rate (overall) [mL/min]	4.8	4.8	14.4
Solid density [%]	0.3	0.3	1.0
Concentration of catalyst [mM]	30	6.3	44
<i>ee</i> (0) [%]	5	5	20

During investigation of the influence of individual parameters all other parameters were set to the standard settings

Fig. 4 Cycle number-based deracemization rate constants for different settings tested during the parameter study. Influence of **a** the temperature swing, **b** the overall gal-liquid flow rate, **c** the concentration of the

racemization catalyst DBU and **d** the relative crystal mass. Standard deviations are calculated from three experimental runs each

the concentration difference between the two enantiomers in the dissolved state during crystal growth in the cold water bath. Here, an imbalance in their concentrations occurs due to preferential growth of the excess enantiomer. While the racemization rate has shown to be directly proportional to the DBU concentration at constant temperature [22], no proportionality was found in our results. By increasing the DBU concentration by a 7-fold, the overall increase in the overall deracemization rate was only about 1.6-fold. Therefore, we assume that the racemization reaction is rate limiting only for a short time interval of each cycle.

Another reason for the enhanced rate could be that the catalyst has an impact on the solubility of the substance of interest. If the dependence of solubility on temperature is increased, a bigger crystal mass dissolves and re-grows during each cycle, resulting in an effect similar to a larger temperature swing.

To verify this, we measured the solubility of NMPA at different concentrations of DBU. For 10 mg NMPA per g EtOH, no significant influence could be detected in the investigated range between 6 mM and 48 mM DBU.

Effect of initial enantiomeric excess $ee(0)$ and particle size

Existing literature addressing deracemization reveals no general agreement on the impact of various factors governing the shape of ee profiles during temperature cycling. While some

results show an inflection point in the progress of ee with the number of temperature cycles [6, 10, 25, 26], others show an exponential character throughout the evolution of the enantiomeric excess [8, 9, 21].

Further differences in the appearance of the curves were found during studies on the effect of different initial values of the enantiomeric excess ($ee(0)$). Those groups that observed a continuous exponential increase in ee values also show similar dee/dt -values for all values of $ee(0)$ tested. However, those who observed a sigmoidal course and did a variation of $ee(0)$ observed that the gradient does indeed depend on $ee(0)$.

In contrast to Viedma ripening, where particle agglomeration and breakage are essential phenomena for driving the process, for temperature cycling the key factor is size-dependent solubility [6, 10]: Following the Gibbs-Thomson equation the solubility of a certain particle is proportional (but not necessarily equal) to the bulk solubility of the substance (accessible via standard solubility measurements) [27]. The proportionality factor is a function of the particle size (as well as of the interfacial tension and the temperature). For the present purpose this may be written as

$$c^*(L) = c_\infty \cdot \exp\left(\frac{\alpha}{L}\right)$$

where $c^*(L)$ is the solubility of a particle of size L , c_∞ is the bulk solubility and α is the capillary length [10, 28]. Therefore, a critical particle size can be defined. Larger particles will tend to grow at the expense of particles below this

critical value. While being cycled through water baths the temperature of the suspension continuously changes and hence also the critical particle size.

During former studies our group observed the effect of multiple temperature cycles on the particle size distribution (PSD) of crystals and could confirm, that already after a small number of cycles the PSD is shifted to larger values [19]. A corresponding effect was observed during the present study when cycling crystals between a hot and a cold zone. It is likely, that this shift in particle size distributions of the two enantiomeric forms significantly influences the evolution of the enantiomeric excess. The more the PSD is shifted to larger sizes the more the effect of size-dependent solubility fades, since the number of particles falling below the critical size during a heating step continuously decreases. Simultaneously, the specific surface area decreases, leading to lower overall growth and dissolution rates, which results in less crystal mass being dissolved and regrown during each cycle. Therefore, we were interested if the change in the PSD over several cycles leads to a decrease in the deracemization constant if no grinding of the particles is involved. In order to investigate this behaviour in more detail, we carried out tests with different cycle numbers and different $ee(0)$ values. The results are shown in Fig. 5. We observed, that k_N decreases with increasing number of cycles, which correlates with the shift towards larger particles.

We suspect that this effect is mainly responsible for the sigmoidal shape of the ee profiles from other studies, where an exponential increase of ee was found initially, which slows down considerably after several temperature cycles.

Implementation of a recycle-stream for complete enantiomeric resolution

For ordinary n th-order reactions ($n > 0$) the PFR reactor is generally more efficient as it operates at highest reactant concentrations. A different setup is usually chosen for autocatalytic reactions, since in this case faster reaction rates are found at high product concentrations. Therefore, continuous stirred tank reactors that provide complete backmixing are expected to perform better when operated at high conversion. Limitations usually only arise from depletion of the reactant(s) [29]. Consequently, linearly operated PFRs are generally not well suited for autocatalytic reactions, that are approximately independent of the reactant concentration. For two reasons, the tubular crystallizer presented here is nevertheless superior to other methods found in the literature so far: On the one hand, productivity is increased by extremely fast temperature changes which keep the growth and dissolution rates to a maximum and can hardly be realized in tank reactors. On the other hand, it facilitates the straightforward implementation of a recycle stream, allowing operation at high turnover by careful selection of the recycle ratio. A prerequisite for

efficient operation is having information about the deracemization rate for the selected process parameters.

Experimental setup

In order to realize a fully continuous setup with recycle stream a second peristaltic pump was installed to transport fractions of the product stream from the exit of the temperature cycling unit back to the entrance. To enable efficient degassing of the product stream a stirred product collecting vessel with level drain was implemented. From this, the recycle stream was withdrawn. To minimize effects of backmixing the size of the collecting vessel was small (5 mL) and the average residence time was 25 s, which is less than half of the residence time in a single temperature cycle (52 s). In order to obtain further information on the influence of particle size on the deracemization rate, an ultrasonic probe (Sonoplus mini20, Bandelin, Germany, max. 20 W_{eff}) was installed in the collecting vessel, enabling crystal breakage and hence shifting the product PSD to smaller sizes before recycling.

The settings of experiments involving a recycle stream were based on the standard settings described above, but with an overall flow rate of 14.4 mL/min. The reason for choosing these parameters was the excellent long-term stability obtained with these settings on the one hand and the highest production rate achieved for the 12-cycles-setup within all parameters tested in this study. For each experiment a starting pulse was required to ensure evolution towards the same enantiomer for each liquid segment moving through the tube and establish reproducibility. This was realized by starting the recycle experiments using a scalemic feed suspension. Here, the enantiomeric excess of the R enantiomer was chosen to be $ee(F)_0 = 0.1$. As soon as the temperature cycling unit had reached the steady-state (determined by HPLC measurements as described above) the scalemic starting suspension was replaced by a racemic one, the recycle pump was turned on and set to the desired pump rate. The pump rate of the racemic starting suspension was set to result in the same overall flow rate after the mixer as before. Tube lengths for transporting the recycle stream have been realized as short as possible and with a slight slope downhill to avoid sedimentation of particles at low recycle ratios and hence low flow rates.

During experiments, different recycle ratios were tested to identify the optimal recycle ratio, identified as offering both enantiopurity and highest productivity. Samples were drawn from the collecting vessel immediately after switching to the racemic starting suspension (t_0) and from this time point after every 10.4 min, corresponding to the average time for a single run from the mixer through the temperature cycling unit to the collecting vessel and back to the mixer ($t_{residence \text{ per run}}$).

In order to evaluate experimental results, we also aimed for a mathematical solution by setting up mass balances for a temperature cycling unit flanked by an upstream mixer and a downstream splitter (see Fig. 6). Just like in the experimental

Fig. 5 Values of k_N determined for different numbers of temperature cycles and for three different $ee(0)$ values of the starting suspension ($ee(0)$). Other settings correspond to the standard settings (see Table 1)

approach a start-up phase ($t=0$) was considered with a total flow rate of 14.4 mL/min (corresponding to 2.16 g/h total solid crystal mass) and $ee(F)_0 = 0.1$. In subsequent runs ($t=1-10$) $ee(F)_t$ was set to 0 and the mass flow rate was adapted to account for the recycle stream. For the process conditions chosen and a single passage through the temperature cycler the k_N value has been predetermined during the parameter study ($k_N = 0.097$). Based on the exponential Eq. 2, and assuming k_N to be constant during multiple recycle runs, an explicit solution can be found by specifying a value for the recycle ratio Y (see Eq. 3). Corresponding values of the enantiomeric excess in the product stream after t residence times $ee(P)_t$ were calculated for ten residence times in the temperature cycling unit and a constant feed rate.

$$\begin{aligned} ee(P)_1 &= ee(F)_0 \cdot e^{k_N \cdot N} \\ ee(P)_t &= Y \cdot ee(P)_{t-1} \cdot e^{k_N \cdot N} \end{aligned} \quad (3)$$

Results

Results of four experiments are shown in Fig. 7 as curves of the enantiomeric excess over time (represented as overall time divided by the residence time per single run through the temperature cycling unit). For comparison, calculation results (dotted black lines) from suitable recycle ratios have been chosen to align with

the experimental results. During experiments 1–3 the ultrasound probe in the collecting vessel was activated to induce breakage of the crystals to be recycled. This method enabled us to operate the reactor with recycling ratios close to the calculated value using the predetermined value of k_N for a single run. However, as soon as the ultrasound has been switched off, this resulted in a completely different E profile: While the enantiomeric excess in the collecting vessel is similar to experiments with ultrasound for ~ 2 residence times, in the following a deviation towards lower values becomes apparent. After five residence times, the enantiomeric excess was already below the initial value and then was washed out quickly. After eight residence times, no enantiomeric excess could be detected in the collecting vessel. We assume that the reason for this behaviour is the shift towards larger particle sizes induced by temperature cycling. Therefore, the (mass-related) enantiomeric excess of the desired enantiomer after mixing feed (small crystals) and recycle stream (larger crystals) does not correspond to the excess in available surface area, which is crucial for the speed of deracemization. Furthermore, crystals of the counter enantiomer as well have reached a size that is above the critical size for size-dependent solubility. These will no longer dissolve in the process and therefore slow down the overall deracemization rate. In our setup, ultrasound proved to be able to compensate for this imbalance by inducing crystal breakage. Microscope images of the feed crystals and product crystals before and after ultrasound treatment are shown in Fig. 8.

Fig. 6 Schematic representation of the continuous setup with recycle stream

Fig. 7 Development of the enantiomeric excess at the outlet of the temperature cycling unit. All recycle experiments were started with a scalemic suspension with $ee(0) = 0.1$, which was replaced by a racemic suspension after reaching steady state. Simultaneously, the recycle stream was activated ($t = 0$). For comparison, calculation results of the mass balances are shown using a k_N value of 0.097 and $ee(0) = 0.32$. Dashed lines are included to guide the eye

While other experimental studies in the field have used different model systems showing different rates of racemization, crystal growth and dissolution, etc., calculating the productivity can give an idea about the process efficiency [30]. Generally, the productivity is defined as the solid crystal mass m delivered per total volume of the crystallizer V_{total} and the average residence time t_{res} . In our setup, the experimentally determined minimum recycle ratio required to achieve enantiopurity was $Y = 0.37$. The resulting maximum product mass flow at steady state is 1.36 g/h, which corresponds to a volume-related productivity of $9.1 \text{ kg/m}^3 \cdot \text{h}$.

We assume, that by tuning process conditions the productivity of our setup could be further increased. The temperature of the cold water bath likely plays a crucial role, since here racemization kinetics in solution, as well as crystal growth kinetics are affected. According to the Arrhenius equation kinetics of all phenomena involved in the process of deracemization are faster at higher temperatures [22]. Therefore, operating the process at higher overall temperatures could further enhance its productivity.

In our setup, two opposing effects are likely to overlap: while the greatest temperature difference resulted in the greatest deracemization speed, the temperature of the cold water bath is also lowest here. As long as the substance of interest is thermally stable, the temperatures of the water baths can be increased while keeping

the temperature swing constant (or adapt it according to the solubility curve).

Conclusion

In the present study the feasibility of complete chiral resolution via the use of a tubular temperature cyclers with recycle stream was demonstrated for a racemic mixture of crystals. While comprehensively exploring the design space of such a process is out of reach due to the large amount of different parameters [21], here our aim was to capture those which show the biggest influence on the deracemization rate. Without doing extensive optimization, in the present study we were able to show, that the benefit of very fast heating and cooling rates achieved in such tubular reactors is effective also when it comes to deracemization.

To our knowledge we are the first to realize a continuous flow approach, which is capable of resolving a racemic mixture of crystals to an enantiopure state. Moreover, we show that the process benefits from very fast heating and cooling rates together with short residence times for each segment. A competitive productivity could be achieved, since the process is condensed to the most relevant mechanisms which are fast dissolution at high undersaturations and fast growth at high

Fig. 8 Microscope images of NMPA crystals filtered from the feed (left), crystals filtered after a single passage through the temperature cyclers (middle), and crystals filtered after single passage followed by ultrasound treatment (right). The white bar indicates a length of 50 μm

supersaturations, while keeping nucleation to a minimum or avoiding it completely.

Low shear forces are present in such tubular reactors at laminar flow conditions, and hence particle breakage is hardly found in corresponding setups [15, 31, 32]. Hence, the results of our parameter study offer an indication, that crystal breakage in fact is not a prerequisite for the underlying mechanism of temperature cycling-induced deracemization.

For achieving enantiopurity and hence during recycle experiments, though, it became apparent that multiple passages through the temperature cycling unit lead to a significant decrease in deracemization rate. Our present results, together with conclusion from one of our recent publications [19] confirm that this is due to crystal ripening-induced shift of the PSD to larger sizes. We could show that this imbalance in particle size between those recycled and those coming from the fresh feed strongly affects the deracemization rate, due to their differences in specific surface area. The advanced deracemization of the recycled particles is not reflected by the relative available surface area. Therefore, the autocatalytic effect on deracemization rate is reduced and k_N decreases gradually in the course of multiple runs through the temperature cycling unit. Only via ultrasound-induced comminution the PSD of the recycled material could be reduced, enabling deracemization rates similar to those found for pre-milled particles.

Although symmetry breaking is limited to conglomerates and depends on racemization in the dissolved state, by establishing a robust continuous process this could help paving the way towards enantioselective pharmaceuticals.

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Declarations

Conflict of interest Not applicable.

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