Deracemization Controlled by Reaction-Induced Nucleation: Viedma Ripening as a Safety Catch for Total Spontaneous Resolution

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ABSTRACT

Viedma ripening was recently applied to a reaction enabling the conversion of achiral reactants in solution into enantiopure product crystals. Here we show that the configuration of the final product and the rate of deracemization is highly dependent on the initial crystal nucleation process or, if applied, seed crystals. Depending on the nucleation process, the transformation proceeds through total spontaneous resolution or Viedma ripening. Swift solid state deracemization can also be achieved using heating-cooling cycles as an alternative to Viedma ripening, provided that crystal nucleation results in a sufficiently high initial enantiomeric excess to trigger the deracemization process.

INTRODUCTION

Chiral molecules which racemize in solution and form racemic conglomerate crystals offer the possibility for complete deracemization, leading to enantiopure solids in high yield.¹ Such a transformation can be achieved in four different ways, being 1) total spontaneous resolution, 2) Viedma ripening, 3) Ostwald ripening and 4) temperature cycling.

Starting from a homogeneous solid-free solution, total spontaneous resolution leads to the crystallization of one enantiomer, provided that nucleation of the opposite enantiomer is inhibited.² This approach involves the nucleation of a single enantiopure crystal through careful slow crystallization from solution, as was shown by Havinga's pioneering work.^{3, 4} Havinga also suggested that crystallization under the presence of agitation could lead to the fracture of the initial crystal leading to smaller crystals (i.e. secondary nucleation), which grow larger, yet retain the chiral identity of the initial crystal.^{3, 4} The latter approach was studied in detail for the intrinsically achiral compound NaClO_3 and later for an intrinsically chiral amino acid derivative.^{5, 6}

A fundamentally different approach to reach single chirality is Viedma ripening, which involves the near-equilibrium transformation of an initial racemic mixture of crystals into an enantiopure solid state through grinding of a suspension under isothermal conditions.^{7, 8} Experimental conditions including attrition intensity, racemization rate, crystal size distribution and Ostwald ripening were found to affect the Viedma ripening process.⁹⁻¹² Ostwald ripening alone also leads to single chirality, but on considerably longer time scales.¹¹

During Viedma ripening, crystal growth and secondary nucleation are favored over primary nucleation. However, if a strong increase in supersaturation is applied to a Viedma ripening experiment, primary nucleation can still happen. This was observed during Viedma ripening of a

Naproxen derivative, as nucleation of the unwanted enantiomer also occurred because of an *in-situ* feed of both enantiomers to the suspension.¹³ Consequently, the enantiomeric excess (*ee*) of the enantiopure product in the solid state, which was applied at the start of the experiment, decreased. However, as a result of grinding, Viedma ripening led to an increase in *ee* to eventually give more of the enantiopure product in the solid state.

In other reports, Viedma ripening conditions were subjected to homogeneous solutions whilst cooling slowly to induce primary nucleation of which the chirality was further amplified through secondary nucleation and possibly also Viedma ripening.^{6, 14, 15} Because in these cases the *ee* was determined in the end, it is unclear to what extent secondary nucleation and Viedma ripening were involved.

Finally, in addition to Viedma ripening, an initial racemic mixture of enantiopure crystals can also undergo deracemization by applying repeated temperature cycles instead of grinding.¹⁶⁻¹⁸ In this way, the crystals partly dissolve during heating, while the remaining crystals grow during cooling.

Previously we reported the synthesis of enantiopure product **1** from the achiral reactants *p*-anisidine (**2**) and (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-2-one (**3**). (Figure 1).¹⁹ The achiral precursors reversibly reacted with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as an achiral catalyst in solution to give both enantiomers of the product, which in turn rapidly crystallized to form a racemic crystal-solution system. Due to the applied grinding, the initially racemic conglomerate crystals were subsequently deracemized through Viedma ripening in which the final configuration of the product was found to be randomly either pure (*R*)-**1** or pure (*S*)-**1**.

Figure 1. After combining the achiral reactants **2** and **3** in ethanol with DBU, precipitation of the product can lead to enantiopure **1** from start (total spontaneous resolution) or through deracemization (Viedma ripening or temperature cycling) in case both enantiomers precipitate.

As the reaction commences in a homogeneous solution, Viedma ripening is preceded by the nucleation of the product as the result of a steady increase in supersaturation. As long as only one enantiomer nucleates, total spontaneous resolution leads to single chirality. On the other hand, when both enantiomers precipitate in any proportion, Viedma ripening or temperature cycling still will lead to deracemization of the solid state.

Here we investigate the effect of nucleation on solid state deracemization using the reversible reaction system as outlined in Figure 1. We show that three crystal-solution approaches can be applied to reach single chirality, depending on the conditions. The nucleation process not only determines the final configuration of the product, but also controls the deracemization rate.

EXPERIMENTAL SECTION

Chemicals, solvents and glass beads ($\varnothing = 1.5 - 2.5$ mm) were purchased from Sigma-Aldrich and used as received. PTFE-coated octahedral magnetic stirring bars (length 25 mm, $\varnothing$ 10 mm), PTFE-coated oval magnetic stirring bars (length 20 mm, $\varnothing$ 10 mm) and PTFE-coated double crossheaded magnetic stirring bars (height 8 mm, $\varnothing$ 10 mm) were acquired from VWR. Compound (E)-4-(3,4-dimethoxyphenyl)but-3-en-2-one (**3**) (98% pure) was purchased from Alfa Aesar and used as received. Round-bottom flasks were bought from Duran Group. $^1\text{H-NMR}$ experiments were recorded on a 300 MHz spectrometer.

Viedma ripening experiments involving different concentrations (Figure 2). Experiments involving different concentrations (0.9 – 1.5 M) were carried out by filling a scintillation flask with the achiral reactants *p*-anisidine (**2**) (138.5 mg, 1.13 mmol – 231.0 mg, 1.88 mmol), (E)-4-(3,4-dimethoxyphenyl)but-3-en-2-one (**3**) (232.0 mg, 1.13 mmol – 386.6 mg, 1.88 mmol) and DBU (84.0 μL , 0.57 mmol – 140.0 μL , 0.94 mmol) which were dissolved in EtOH (2.5 mL). The homogeneous solution was stirred at 800 r.p.m. using an oval magnetic stirring bar in the presence of glass beads ($\varnothing = 1.5 - 2.5$ mm, 7 g) until solids were formed. The solid state *ee* and the concentration of the product (**1**) were monitored over time using chiral HPLC and $^1\text{H-NMR}$ analysis respectively.

Viedma ripening experiments in the presence of seed crystals (Figure 3). A solution of 1.5 M was prepared by dissolving *p*-anisidine (**2**) (231.0 mg, 1.88 mmol), (E)-4-(3,4-dimethoxyphenyl)but-3-en-2-one (**3**) (387.0 mg, 1.88 mmol) and DBU (140.0 μL , 0.94 mmol) in ethanol (2.5 mL) in a scintillation flask. To this homogenous solution were added enantiopure seed crystals (0.6 mg, 0.10 mmol – 68.6 mg, 10.00 mmol). The suspension was ground in the presence of glass beads ($\varnothing = 1.5 - 2.5$ mm, 7 g) using an octahedral stirring bar at 700 r.p.m. and samples were taken regularly.

Temperature cycling experiments (Program 4, Figure 4). To a 5 mL thermostatted flask, equipped with a double crossheaded magnetic stirring bar, was added *p*-anisidine (**2**) (154.0 mg, 1.25 mmol), (E)-4-(3,4-dimethoxyphenyl)but-3-en-2-one (**3**) (258.0 mg, 1.25 mmol) and ethanol (2.5 mL). During the experiments, the solutions were continuously stirred. After complete dissolution, DBU (93 μ L, 0.62 mmol) was added to the homogenous solution and the solution was kept at 30 °C over a period of 5 minutes. The homogenous solution was slowly cooled to 10 °C over a period of 12 hours to allow sufficient precipitation of the product. The suspension was subsequently subjected to repeated temperature cycles of 60 minutes each: the suspension was first heated to 30 °C over a period of 10 minutes. Once at 30 °C, this temperature was maintained for 5 minutes after which the suspension was cooled to 10 °C over a period of 30 minutes. Finally, the suspension was kept at 10 °C for 15 minutes and samples were taken at this point to determine the ee of the product.

Determination of solid state ee of 1. Samples for the determination of the solid state ee were obtained through centrifugation. Typically, three drops of suspension were taken from the experiment by means of a Pasteur pipette and were brought into an Eppendorf vial. Centrifugation was carried out at 14,000 r.p.m. for 1 minute after which the mother liquid was carefully removed from the solids. About 0.1 mg of the solids was dissolved in 2-propanol (1.5 mL) in an HPLC vial. A drop of DMSO was added to the HPLC vial to ensure complete dissolution of the crystals. The samples were subjected to chiral HPLC analysis using the following conditions: HPLC column Chiralpak AD-H (250 $\times$ 4.6 mm ID), injection volume 10 μ L, eluent *n*-heptane/2-propanol (80/20 v/v%), flow 1 mL/min-1, room temperature, λ = 254 nm. Retention times: (*R*)-**1** 15.8 min, (*S*)-**1** 19.1 min, *p*-anisidine (**2**) 7.3 min, ketone (**3**) 7.3 min.

Determination of the fraction of 1. A single drop of solution was taken from the experiment and the solvent was allowed to evaporate at room temperature. The residue was dissolved in CDCl₃ (0.5 mL) and analyzed by ¹H-NMR. The chemical shifts of compounds **1** and **3** are reported in literature.¹⁹ From the spectra, the mass fraction of **1** was determined with respect to the total amount of reaction components (**1-3**).

Solubility measurements. A suspension was prepared by adding either compound **1**, **2** or **3** (~700 mg) to a solution of ethanol (3 mL). Glass beads (5 g) were added and the suspensions were magnetically stirred with an octahedral stirring bar at 600 rpm at r.t. for about 16 hours. The suspensions were filtered over an Acrodisc HPLC syringe filter and the resulting filtrates were collected. The mass of compounds **1-3** were measured after complete removal of the solvent to give the following solubilities: enantiopure product **1** (1 wt%), reactant **2** (44 wt%) and reactant **3** (7 wt%).

RESULTS AND DISCUSSION

The fraction of product **1** (with **1+2+3** in total), in both solution and the solid state, was followed in time during crystal nucleation and deracemization. Three typical cases involving different initial reactant concentrations are shown in Figure 2. As the fraction of the product was determined by solution-phase NMR, some racemization during analysis possibly took place. Therefore, the fraction of the product should be regarded as an estimate.

The solid state *ee* was also monitored over time. At a high concentration (1.5 M), precipitation proceeds almost immediately after the start of the experiment. Initially only one of the enantiomers precipitates but as the reaction progresses, the other enantiomer crystallizes as well

leading to a decrease in ee after which Viedma ripening completely restores the ee to 100% in 5 days.

Figure 2. The solid-phase ee of **1** (symbols) and the fraction of **1** (bars) as a function of time for three experiments starting with different reactant concentrations. Note that the fraction of **1** was not determined at days 6 and 8. The lines are a guide to the eye.

Previously, we showed that experiments involving a lower initial concentration of achiral reactants lead to the formation of a smaller number of crystals.¹⁹ This in turn leads to a shorter deracemization time as less crystals have to undergo deracemization. However, crystal nucleation affects the deracemization rate in a more complex way. In some experiments nucleation occurred less gradually, resulting in the rapid precipitation of both enantiomers (1.0 M, Figure 2). Although in this case less crystals have to undergo deracemization, still complete deracemization was realized only after 8 days due to the low initial ee . The low initial ee requires

Viedma ripening to go through the complete sigmoidal-type increase in *ee* whereas with a high initial *ee*, deracemization starts in the fast exponential part of the process.

Finally, single chirality can be realized from the beginning of the experiment through total spontaneous resolution (0.9 M, Figure 2). Note that the fraction of the final product in the solid state in this case is lower as compared to the experiments conducted using higher initial reactant concentrations.

A typical observation in our experiments is the small decrease in mass% of product after day 3. This could be explained by the increasing solubility of the product crystals as the result of sampling: Each time a sample is taken, the total volume is being reduced whereas the attrition intensity remains the same. Overall this leads to a higher attrition intensity and thus smaller crystals which tend to dissolve faster. In solution the product partially splits up into its starting components, which overall leads to a somewhat smaller amount of product.

The final configuration of the product was always the same as the configuration of the initially formed crystals.

The final configuration of the product can be controlled by adding seed crystals of the product to the initially achiral homogeneous solution (Figure 3). As the solubility of the enantiopure product **1** (1 wt%) in ethanol is much smaller than both reactant **2** (44 wt%) and reactant **3** (7 wt%), the seed crystals hardly dissolve. In all cases, enantiopure **1** is obtained with the same configuration as that of the seeds. The seed crystals failed to completely inhibit the formation of the other enantiomer but Viedma ripening restores the *ee* to give the enantiopure product in all experiments. Nevertheless, a higher amount of enantiopure seed crystals more effectively suppresses nucleation of the other enantiomer. This results in a higher initial *ee* but

leads to a slower deracemization process as there is more solid. Therefore, the overall time to reach single chirality is roughly the same for different amounts of seed crystals.

Figure 3. Viedma ripening experiments starting with 1.5 M of achiral reactants with different amounts of initially added enantiopure seed crystals of the product. The lines are a guide to the eye.

These results show that two consecutive processes are involved in the transformation of an achiral homogeneous solution into enantiopure crystals: First, crystals that are formed undergo secondary nucleation, resulting in an initial non-zero *ee*. Secondly, the initial *ee* reaches 100% through Viedma ripening, a safety catch that corrects for the accidental crystallization of the opposite enantiomer. Both processes rely on vigorous grinding conditions. The question arises whether vigorous grinding is required for complete deracemization and whether mere temperature cycling could give the same result. To investigate this, we also performed deracemization experiments involving temperature cycling (Figure 4) as an alternative to attrition. In this we used a reactant concentration of 1.0 M, corresponding to the situation of Figure 2.

Figure 4. Temperature cycling experiments starting with 1.0 M of achiral reactants. a) Different temperature programs were used of which program 4 leads to complete solid state deracemization. b) The *ee* of the solids plotted against time for three typical experiments using program 4. The lines are a guide to the eye.

First, we tested the effect of several temperature programs, involving rapid heating and slow cooling, on our reaction system (Figure 4a). Experiments involving temperature programs 1 and 2 failed to maintain a solid state, while the temperature differences in temperature program 3 did not lead to deracemization due to insufficient dissolution of the crystals. Temperature program 4 did lead to complete deracemization of the solid state provided that the setup was cooled to 10 °C prior to the start of the experiment to allow the formation of a sufficient amount of solid product. Intriguingly, despite the absence of attrition and the rapid formation of tiny crystals during the start-up precipitation, significant initial *ee* values of up to 26% were found (Run 1, Figure 4b). Subjecting temperature program 4 to the slurries resulted in complete deracemization within 5 days in most cases. However, some experiments resulted in a solid state with an *ee* of 0% (Run 2, Figure 4b).

CONCLUSIONS

In conclusion, this study shows that the mechanism and time needed for the complete transformation of a solid-free racemic solution into an enantiopure solid product largely depends on the nucleation stage. Typically, both enantiomers of the product precipitate after which Viedma ripening is required as a safety catch for complete deracemization. Total spontaneous resolution can also lead to single chirality provided that the increase in supersaturation is sufficiently slow. The final configuration of the product can be controlled using seed crystals of the product. Temperature cycling also leads to complete deracemization provided that initial precipitation proceeds to give sufficiently enantioenriched solids. More generally, the presented deracemization mechanisms should also hold for other systems that are able to undergo the transformation from a solid-free racemic or achiral solution to crystals of single chirality.

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Notes

The authors declare no competing financial interest.

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TOC Graphic

In deracemization experiments, the configuration of the final product and the deracemization rate is highly dependent on the initial crystal nucleation process or, if applied, seed crystals. Viedma ripening works as a safety catch for total spontaneous resolution since crystals of the unwanted enantiomer will be transformed into the desired form.

Reviewer 1:

Recommendation: Publish after minor revision.

In this paper the authors show the importance of the nucleation stage on the mechanism and time needed for the transformation of a solution into an enantiopure state and how Viedma ripening could be required as a safety catch for complete deracemization. This is an interesting work with useful implications in engineering tools in pharmaceutical industry and for that the manuscript deserves to be published in the JCG&D.

Comments:

Page 2, line 48-54

"Viedma ripening, which involves the near-equilibrium transformation of an initial racemic mixture of crystals.....Although Viedma ripening starts with crystals in a supersaturated solution, in some experiments primary nucleation still happened. "
There is an apparent contradiction between "near-equilibrium" and "supersaturated solution" where there is even primary nucleation

We agree with the reviewer and clarified the contradiction in the introduction accordingly:

"During Viedma ripening, crystal growth and secondary nucleation are favored over primary nucleation. However, if a strong increase in supersaturation is applied to a Viedma ripening experiment, primary nucleation can still happen. This was observed during Viedma ripening of a Naproxen derivative, as nucleation of the unwanted enantiomer also occurred because of an in-situ feed of both enantiomers to the suspension."

Page 3 line 3-8

"Consequently the initially enantiopure solid state could not be maintained and a decrease in enantiomeric excess (ee) was observed. However, as a result of grinding, Viedma ripening led to complete deracemization to eventually give an enantiopure product. "
Perhaps a bit confusing wording... initially enantiopure solid state and finally enantiopure product.

To avoid confusion, we used the same words in an improved context:

"Consequently, the enantiomeric excess (ee) of the enantiopure product in the solid state, which was applied at the start of the experiment, decreased. However, as a result of grinding, Viedma ripening led to an increase in ee to eventually give more of the enantiopure product in the solid state."

Page 3, line 22-25

"Finally, in addition to Viedma ripening, an initial mixture of racemic crystals can also undergo deracemization by applying repeated temperature cycles instead of grinding"
Perhaps it is better to say that the mixture is racemic and crystals enantiopure.

We would like to thank the reviewer for this correct observation and we have changed it as follows:

“racemic mixture of enantiopure crystals”

Additional Questions:

Originality: Good

Technical Quality: Excellent

Clarity of Presentation: Excellent

Importance to Field: Good

Reviewer 2:

Recommendation: Publish after minor revision.

Comments:

This MS reports what seem to me to be carefully conducted experiments that are quite relevant to designing methods for preparing solids of a single chirality from racemic or achiral materials where the desired product crystallizes as a racemate. I don't see anything of striking novelty with respect to fundamental mechanisms. As the authors mention, secondary nucleation from seeds of a single chirality (generated spontaneously in small number or added artificially) is well known, as is the much more recent phenomenon of Viedma ripening by grinding or temperature cycling. In that sense there is nothing fundamentally new here, but it is still quite relevant to see how these phenomena play out in a specific, well-studied case. As in many crystallization processes, reproducibility is challenging. For example, Figure 4b shows that in Run 1 it took 24 temperature cycles to go from 30 to 70% e.e., while in Run 3 it seemed to take only half as many cycles.

I found Figure 2 confusing. Especially the "bars: fraction of 1 (mass %)" axis. If I understand the horizontal gray "solids/solution" line properly, the bar length above the line corresponds to the mass of solid 1, and the length below to the mass of 1 in solution (each expressed as a % of the total mass of reagents). How can the ultimate % in solution be the same starting from 0.9 M (or 1.0 M) and 1.5 M?

We assume that the solution fraction remains the same for all the experiments. However, we have no experimental evidence for this. Therefore we removed the horizontal bar from Figure 2 in the revised manuscript. This also leads to a less confusing figure.

If the ultimate concentration in solution in both cases is the same (saturated), shouldn't the % in the latter case be only 2/3 as large? It is also interesting that the % of solid seems in all cases to overshoot initially. Is this thought to be experimentally significant?

We think that the decline in mass% after the initial “overshoot” can be attributed to the increasing solubility of the product crystals as the result of sampling. We’ve added the following sentences to the revised manuscript to elaborate on this:

“A typical observation in our experiments is the small decrease in mass% of product after day 3. This could be explained by the increasing solubility of the product crystals as

the result of sampling: Each time a sample is taken, the total volume is being reduced whereas the attrition intensity remains the same. Overall this leads to a higher attrition intensity and thus smaller crystals which tend to dissolve faster. In solution the product partially splits up into its starting components, which overall leads to a somewhat smaller amount of product.”

This is hard to judge, because no indication is given of the uncertainty in the graphed values. I think it would be good to give somewhere an estimate of these uncertainties.

It is very difficult to determine an estimate for the uncertainty of the graphed values because solution-phase racemization still takes place to a certain degree during H-NMR analysis. To account for the uncertainty, we added the following sentence to the revised manuscript:

“As the fraction of the product was determined by solution-phase NMR, some racemization during analysis possibly took place. Therefore, the fraction of the product should be regarded as an estimate.”

Additional Questions:

Originality: Fair

Technical Quality: Good

Clarity of Presentation: Good

Importance to Field: Good