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Enabling Direct Preferential Crystallization in a Stable Racemic Compound System

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Supporting Information Placeholder

ABSTRACT: The preparative resolution by preferential crystallization (PC) of proxiphylline has been achieved despite the existence of a stable racemic compound. This is enabled through the careful selection of a solvent in which both the racemic compound and the metastable conglomerate possess a low nucleation rate. Induction time (IT) measurements in isobutyl alcohol show that a highly supersaturated solution ($\beta = 2.3$) remains clear for almost 1 hour at 20 mL scale, revealing a slow nucleation rate. Seeding the supersaturated solution with the pure enantiomer triggered its crystallization and isothermal and polythermic modes of PC were successfully implemented. Alongside the reported case of diprophylline, this study opens opportunities to broaden the application of PC towards slowly crystallizing racemic compound.

Keywords: Preferential Crystallization, Nucleation Inhibition, Proxiphylline, Metastable Conglomerate.

1. INTRODUCTION

In the field of pharmaceutical industry, around 56% of the marketed drugs are chiral compounds,¹ of which *ca.* 80% are formulated as racemic mixtures, *i.e.* equimolar mixtures of both enantiomers.² Although two enantiomers have the same physicochemical properties, their biological activities (including toxicity) can be different.³ Nowadays, the biological activity of each antipode has to be established and, if different, any new chiral active pharmaceutical ingredient has to be developed from the most active enantiomer.⁴ The two main strategies to obtain pure enantiomer are: (i) the direct manufacturing of a single enantiomer by means of asymmetric synthesis⁵ and (ii) the separation of the enantiomers from their racemic mixture, sometimes combined with the racemization of the undesired enantiomer (*i.e.* the distomer).⁶

To access to pure enantiomers, different methods exist. The desired enantiomer can be directly synthesized by asymmetric synthesis or by starting from a stock of readily available enantiopure sources (chiral pool synthesis⁷). Such synthesis is often complex, expensive and require many different reagents and processing stages. Enantiopure compound can also be achieved by various

separation methods, including chiral chromatography⁸ and enzymatic resolution.⁹ However, crystallization techniques remain the preferred route at the industrial scale for their high selectivity and low cost. Besides Pasteurian Resolution and Deracemization,⁶ Preferential Crystallization¹⁰ (PC hereafter) is an attractive process with high selectivity and efficiency. Indeed, successive crystallization of the two enantiomers –supposed here non-racemizable during the crystallization– allows them to be separated quantitatively. Such PC processes actually consist of out-of-equilibrium stereoselective crystallizations with a high degree of chiral discrimination, triggered by seeding a racemic or slightly enriched supersaturated solution with single enantiomer seed crystals.¹¹ The implementation of PC is simplified by the knowledge of the heterogeneous equilibria between both enantiomers in the solid state since it is commonly thought that PC is only applicable when the racemic mixture crystallizes as a conglomerate, *i.e.* a physical mixture of enantiopure crystals. For chiral compounds giving rise to a stable racemic compound (*i.e.*, a 1:1 defined compound between both enantiomers) or solid solution (*i.e.*, a single solid phase which does not discriminate between enantiomers), PC is assumed to be non-applicable. This strongly limits the scope of PC as only roughly 5% of racemic mixtures crystallize as conglomerates whereas the vast majority of around 95% exist as racemic compounds. Therefore, the strategy for the implementation of PC in case of racemic compound forming systems is usually to convert the racemic compound into a diastereomer solid or a conglomerate by using a crystallization partner in order to form a salt,¹² a solvate¹³ or a cocrystal.¹⁴ The scope of PC would obviously be widened if this resolution method could be implemented for chiral compounds forming stable racemic compounds.

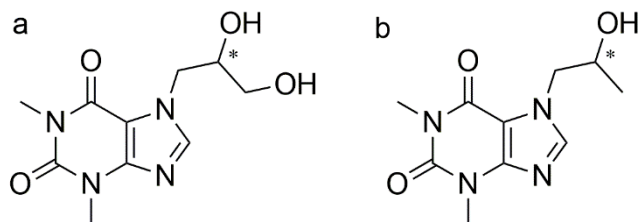
In 2013, we reported that the chiral compound Diprophylline (Scheme 1a, DPL hereafter)¹⁵ exhibits a rich solid state landscape with no less than four crystal forms, including a stable racemic compound, two metastable solid solutions and a metastable conglomerate. In the course of this study, we found that a solution of racemic DPL in polar solvents such as water, DMSO and DMF can remain highly supersaturated for several hours before any spontaneous nucleation. This large metastable zone width (MSZW hereafter), in which the nucleation of the stable form (and any other form) is hindered, served the successful implemen-

tation of PC, leading to a pure enantiomer product by using this unconventional process.

Beside the careful analysis of chiral discrimination mechanism¹⁶ it can be presumed that the key parameter for the successful implementation of PC despite the absence of a stable conglomerate is the use of a solvent in which spontaneous nucleation is strongly delayed, giving rise to a large MSZW, even at high supersaturation. This feature allows the use of metastable equilibria associated to the conglomerate whereas the stable equilibria of the racemic compound can be overpassed. Since the solubility of the conglomerate in such situation is necessarily larger than that of the racemic compound, the concentration should be high enough to reach a value at which there exists a sufficient supersaturation with respect to the conglomerate.

Assuming that a solvent fulfilling the above criteria can be identified, the applicability of PC might be envisaged for any chiral compound crystallizing as a stable racemic compound, which would considerably expand the scope of chiral resolution by PC. The associated strategy should therefore involve a screening procedure aiming at identifying a solvent in which a large MSZW can be reached at the working temperature, via the measurement of induction times.¹⁷

Scheme 1. Chemical structure of (a): (RS)-DPL and (b): (RS)-PXL. The chiral center is denoted with a star.



The present paper reports on the application of direct PC of the racemic compound proxyphylline. The selected compound is proxyphylline (PXL hereafter, Scheme 1b) a xanthine-type bronchodilator drug from the series of theophylline derivatives that acts as a cardiac stimulant, vasodilator and bronchodilator¹⁸ closely related to DPL. This work gives a proof-of-concept for PC of a stable racemic compound and describes a basic process design procedure including a solvent selection procedure for PXL as well as an approach to identify optimal PC process conditions in the selected solvent.

2. MATERIALS AND METHODS

2.1. Materials. Racemic 7-(2-Hydroxypropyl) theophylline (purity >98.0%) was purchased from TCI EUROPE (Zwijndrecht-Belgium) and used as received. HPLC grade solvents and other reagents were purchased and used as received.

2.2. Synthesis of the Pure Enantiomer of Proxyphylline. Enantiopure proxyphylline was synthesized by analogy with a published procedure¹⁹ from theophylline and enantiopure propylene oxide. A mixture of anhydrous theophylline (5 g, 27.75 mmol), (*R*)-propylene oxide (purity 99 %, ee 97%) (5 g, 86.08 mmol, 6 mL) and a catalytic amount of triethylamine (1 g, 7.17 mmol, 1 mL) in methanol (40 mL) was stirred for 3 h at reflux until complete dissolution. After cooling the mixture, the solvent was evaporated under reduced pressure. Then, 20 mL of methanol were added, and the flask was stored in the fridge (-18 °C) for 4 h until crystallization. The obtained solid was filtered off and washed with cold ethanol (15 mL) yielding the desired product (*S*)-7-(2-Hydroxypropyl) theophylline, (*S*)-PXL, as a white crystalline solid (4 g, 16.78 mmol, yield = 61%) - mp 149.5 °C [Ref.²⁰ 142-150 °C and Ref.²¹ 150.5-151.5 °C]; $[\alpha]_D^{20} = -53^\circ$ ($c = 1.00$

mg/mL, CHCl₃) {Ref.¹⁹ : $[\alpha]_D^{20} = -54^\circ$ ($c = 1.00$ mg/mL, CHCl₃), 98% ee}. ee_{HPLC} = (-) 99.9 %.

¹H NMR (MHz) $\delta = 1.28$ (d, 3H), 3.42 (s, 3H), 3.60 (s, 3H), 4.07-4.3 (m, 2H), 4.45-4.53 (m, 1H), 7.62 (s, 1H).

2.3. Chiral HPLC. Purity of the obtained enantiomer was determined with chiral high-performance liquid chromatography (C-HPLC) using a CHIRALPAK IC column (DAICEL group, Chiral Technologies Europe), 250 × 4.6 mm. The mobile phase was a heptane:ethanol mixture (7.5:2.5, v:v), and the flow rate was 1 mL/min. The used wavelength for UV detection was 273 nm at 20 °C. Under these conditions, retention times of 15 and 18 min were obtained for (*R*)-PXL and (*S*)-PXL respectively. An offline C-HPLC analysis was used to monitor the enantiomeric excess (ee) of the liquid phase as function of time during the PC.

2.4. X-Ray diffractometry (XRPD). X-ray Powder Diffraction (XRPD) analyses were performed at room temperature using a D8 Discover diffractometer (Bruker analytic X-ray Systems, Germany) with Bragg-Brentano geometry. The instrument is equipped with a copper anticathode (40 kV, 40 mA, K α radiation ($\lambda = 1.5418$ Å)), and a lynx eye linear detector. The diffraction patterns were recorded with a scan rate of 0.04° (2 θ) in the angular range of 3-30° 2 θ , with a counting time of 4s per step.

2.5. Solubility Measurements. The solubilities of racemic PXL in 22 different solvents were measured at 20 °C by the standard gravimetric method.²² A suspension of racemic PXL was prepared in a solvent and stirred at 700 rpm. Temperature was controlled using a thermostated double-jacket glass vessels. After equilibration for more than 2 hours, the suspension was filtered, the solid phase in equilibrium with the solution was verified by XRPD and the saturated liquid phase was weighed before and after evaporation. The solubility s^* was calculated in weight percent (w%) by equation 1 with m the mass of the dissolved solid and m_s the mass of the saturated solution.

$$s^* = \frac{m}{m_s} \times 100 \quad \text{Equation 1}$$

2.6. Induction Time Measurements. The induction time (t_i) *i.e.* the time required to detect spontaneous crystallization in a supersaturated solution, was determined at different supersaturation ratios and in different solvents. For each solvent/supersaturation couple, a suitable mass of racemic PXL was dissolved in a suitable mass of solvent by heating. The solution was then introduced in 4 different 1.5 mL glass vials equipped with magnetic stirrers. The vials were then placed in a Crystal16 (Technobis, The Netherlands)¹⁷ and stirred (800 rpm) 10 °C above their saturation temperature for at least 90 min. The clear solutions were then cooled down to 20 °C with a cooling rate of 5 °C/min. The moment at which the set temperature (20 °C) was reached is taken as time zero (t_0). The vials were kept at 20 °C until spontaneous crystallization was detected via the decrease of light transmission through the sample. The difference between the moment when the transmissivity started to decrease and t_0 was taken as t_i . Once the different samples crystallized, the vials were reheated above their clear point and the procedure was repeated to ensure statistical reproduction.

The solvents that gave the longest t_i values were also tested at a larger scale of 10-20 mL. For this purpose, a suitable amount of solvent was added to a known amount of racemic PXL and the mixture was stirred for at least 1h at high temperature to ensure complete dissolution. Then the solution was cooled down to the crystallization temperature T_c (10 or 20 °C) and the t_i value was taken as the time lapse between the moment at which the solution reached T_c and the moment at which spontaneous crystallization occurred, detected visually. Each measurement was repeated at

least three times using the same solution. Systematically, the racemic compound of PXL crystallized.

2.7. Determination of ternary isotherms between (R)-PXL, (S)-PXL and isobutyl alcohol. In order to determine isothermal ternary phase diagrams, the solubility values were determined in isobutyl alcohol at 25 and 10 °C using the regular gravimetric method for (i) the stable racemic compound of PXL (RC-PXL), (ii) the pure enantiomer (R)-PXL (PE-PXL), (iii) the doubly saturated solution containing (R)-PXL and RC-PXL, and (iv) the equimolar mixture of (R)- and (S)-PXL (*i.e.*, the metastable conglomerate (MC-PXL)). Concerning (iii), the enantiomeric composition of the dry extract was measured by chiral HPLC. For (iv), the solubility of the metastable conglomerate was determined by stirring an equimolar mixture of (R) and (S)-PXL (prepared manually) in a small volume of isobutyl alcohol for a few minutes under a controlled constant temperature. The solid phase in equilibrium with the saturated solution was verified systematically by XRPD.

2.8. Preferential Crystallization. Preferential Crystallization (PC) experiments using (RS)-PXL were performed in isobutyl alcohol using a 50 mL glass tube equipped with a magnetic stirring bar. The temperature was controlled by a cryothermostat (+/- 1°C). Seeding was performed once the solution reached the desired T_c in order to start the preferential crystallization. Seeds used in these experiments were obtained by recrystallization from methanol. The seeds were dried and manually ground with a mortar and a pestle (Figure S2 presents a microscopy picture of the seed material). Seeding was performed by direct introduction of the solid particles in the solution. The absence of agglomerates was controlled systematically.

The enantiomeric excess in the liquor and in the solid phase during the process was monitored by offline chiral chromatography. Vacuum filtration was performed at the end of each PC and the solid phase was washed with cold heptane (at around -10°C). The purity and solid-state form of the collected crystals were determined by respectively chiral chromatography and XRPD. Several cycles of PC were performed in two modes:^{10,23} Seeded Isothermal Preferential Crystallization (SIPC) and Seeded Polythermic Programmed Preferential Crystallization (S3PC).

3. RESULTS AND DISCUSSION

3.1. Solvent Selection. The starting assumption of this work is that the key parameter for the successful implementation of PC for a stable racemic compound-forming system is the selection of a solvent in which the spontaneous nucleation of the stable crystal form as well as the metastable conglomerate are kinetically inhibited. If a highly supersaturated solution can be maintained for a sufficiently long period of time in this solvent, seeding with a single enantiomer would mainly promote its growth whereas the stable racemic compound or the counter enantiomer would not nucleate. Obviously, in the case of a racemic forming system, PC can only be envisaged if the solution is also supersaturated with reference to the metastable conglomerate.

In a preliminary step, a large collection of solvents was tested for suitable solubility. Those solvents for which the PXL solubility at 20 °C lies between 1.5 and 50 w% were further investigated by determining induction time t_i at 1 mL scale (see Figure S3 and Table S1 in supporting information). For samples showing large t_i values at 1 mL scale, tests were made at larger scale (10 or 20 mL), at a supersaturation ratio $1.5 \leq \beta \leq 2.5$ in respect to the stable racemic compound (Table 1). Spontaneous nucleation of RC-PXL occurred much more rapidly at larger scales and this type of dependency of the nucleation rate with crystallizer size has been discussed elsewhere.²⁴

3.2. Design of Preferential Crystallization processes. Despite t_i values larger than 180 and 100 minutes found in water and 1-propanol respectively, preliminary PC attempts were unsuccessful due to the primary heterogeneous crystallization and nucleation of the stable racemic compound immediately after seeding with crystals of pure enantiomer. This spontaneous crystallization was detected by means of X-ray powder diffraction (XRPD) of the solid phase sampled during the experiment. These solvents were therefore discarded. This shows that a large metastable zone width is necessary but not sufficient to ensure the success of PC under these particular circumstances. Based on the promising t_i results, it was chosen to further investigate the resolution process in isobutyl alcohol (IBA).

Table 1. Induction time for (RC)-PXL in different solvents.

| Solvent | T_c (°C) | S^* (w%) | $\beta^{[c]}$ | t_i (min) |
|------------------|---------------|---------------|---------------|--------------------|
| Methanol | 20 | 11.7 | 1.5 | 20 ^[a] |
| 2-butanol | 20 | 4.6 | 2.3 | 20 ^[b] |
| Ethanol | 20 | 5.5 | 1.5 | 20 ^[b] |
| 1,4-dioxane | 20 | 6.6 | 1.5 | 15 ^[b] |
| Tetrahydrofuran | 20 | 5.6 | 1.5 | 5 ^[a] |
| DMSO | 20 | 21.4 | 2 | 15 ^[b] |
| Ethyl acetate | 20 | 1.8 | 1.7 | 2 ^[a] |
| Acetonitrile | 20 | 6 | 2 | 2 ^[a] |
| Water | 20 | 47.7 | 2.5 | 180 ^[a] |
| 1-propanol | 20 | 3.2 | 2 | 100 ^[a] |
| Isobutyl alcohol | 20 | 2.9 | 2.3 | 60 ^[a] |
| | 10 | 2.1 | 2.3 | 60 ^[a] |

^[a]20 mL scale, ^[b] 10 mL scale, ^[c] maximum supersaturation ratio tested.

3.2.1. Solubility Measurements and Ternary Isotherms (R)-PXL/(S)-PXL/IBA. Knowledge of the ternary phase diagram is needed in order to rationalize any resolution procedure by PC. PXL can exist as a pure enantiomer crystal form (PE-PXL) and, at the racemic composition, either as a stable racemic compound (RC-PXL) or as a metastable conglomerate (MC-PXL). The solubility curves of the three crystal forms in the solvent IBA are shown in Figure 1 (the corresponding solubility values are given in Table S2).

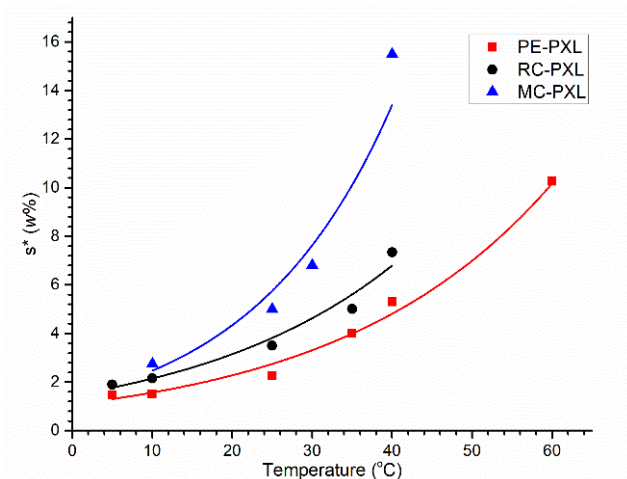


Figure 1. Solubility curves of the three crystal forms of PXL in IBA as function of temperature. PE-PXL: pure enantiomer of PXL, RC-PXL: racemic compound, MC-PXL: metastable conglomerate. Colored lines are a guide to the eye.

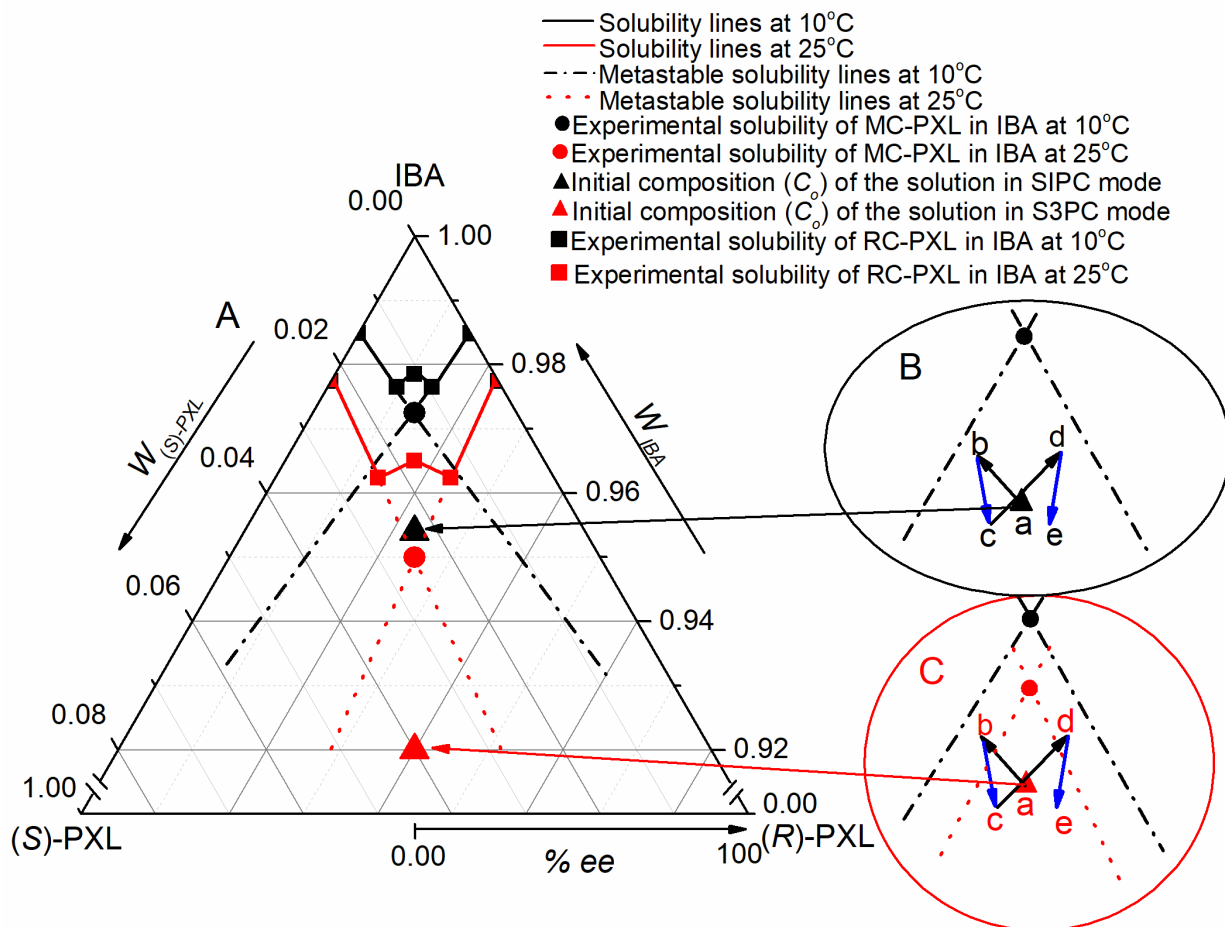


Figure 2. A: Ternary solubility phase diagram including the metastable solubility lines (dashed lines) for (*R*) and (*S*)-PXL in IBA at 10 and 25 °C used as T_c for SIPC and S3PC respectively (W is for weight fraction). B: Performance of PC process for enantioseparation in SIPC mode, C: Performance of PC process in S3PC mode. Signification of (a,b,c,d and e) is described in the text.

As expected, the s^* of the metastable conglomerate MC-PXL in IBA is higher than that of the racemic compound RC-PXL and the solubility difference substantially increases with temperature. For instance, at 10°C s^* of MC-PXL is 1.3 times higher than that of RC-PXL and roughly twice that of PE-PXL. At 40 °C the s^* of MC-PXL becomes twice higher than that of RC-PXL and three times that of PE-PXL.

These solubility data were used for the construction of the ternary isothermal sections at 10 and 25 °C (Figure 2) (temperature

used for PC processes) and to plot the metastable solubility lines representing the virtual crystallization limits. The slope of the solubility curves of the enantiomers can be described using the molar solubility ratio α_{mol} , defined as the solubility of the racemic conglomerate divided by the solubility of the pure enantiomer. As discussed by Jacques et al.,²⁵ the PC process is less efficient when α_{mol} is higher than 2. In the present case, α_{mol} ratio drops from 2.8 at 40°C to 1.8 at 10°C which indicates that PC of RC-PXL in IBA is more favorable at lower temperature.

Based on the ternary isothermal sections, two processes of PC have been used to resolve PXL in IBA: Seeded Isothermal Preferential Crystallization (SIPC) and Seeded Programmed Polythermic Preferential Crystallization (S3PC).¹⁰ In Figure 2 the initial concentration of RC-PXL in IBA (C_o) used in this work is represented with black and red triangles respectively for SIPC and S3PC modes. Under these conditions, the solution is supersaturated at the crystallization temperature (10 or 20 °C) regarding both RC-PXL and MC-PXL.

3.2.2. Preferential Crystallization of PXL by SIPC. As evidenced in Table 1, a supersaturated solution of racemic PXL in IBA with a supersaturation ratio of $\beta = 2.3$ at 20 mL scale and at 10 °C takes at least 1h to crystallize spontaneously as RC-PXL which prompted us to perform a resolution procedure using the SIPC mode. Four SIPC experiments were monitored as function of time with various seed mass using the conditions in Table 2. The principle of SIPC, is presented in Figure 2.B which depicts the envisaged evolution of the composition of the mother liquor during the process (it is exemplified by the SIPC experiment using 150 mg of seeds). The starting solution saturated with RC-PXL at $T_s = 35$ °C ($s^* = 5$ w%) is indicated as a black triangle in Figure 2. After rapid cooling to $T_c = 10$ °C ($s^* = 2.1$ w%, $\beta = 2.3$ with reference to RC-PXL and 1.8 with reference to MC-PXL), the supersaturated solution is seeded with 150 mg of fine particles of the pure *R* enantiomer. This induces the stereoselective crystallization of this enantiomer while the enantiomeric excess of the mother liquor evolves from $ee_a = 0$ % (point a in Figure 2.B) to $ee_b = (-) 5.10$ % (point b in Figure 2.B) within 20 minutes. At (b), the system is filtered resulting in, 212.2 mg of crystals with an enantiomeric purity of 91.3%. The liquor is then compensated with 47.73 mg of RC-PXL and with solvent, thus moving from point b to point c ($ee_c = (-) 3.80$ %). After homogenization of the system at 35 °C, the liquor, enriched in (*S*) enantiomer is cooled to 10 °C and seeded with 150 mg of (*S*)-DPL to complete the cycle and move from (-) 3.15 % ee_c to (-) 0.15 % ee_d (point d in Figure 2.B) within 30 minutes, 201.0 mg of solid was collected with an ee of (+) 89.7 %. To move from point d to point e (Figure 2.B), the liquor is again compensated with 30.29 mg of RC-PXL and solvent, homogenized at 35 °C than cooled down to 10 °C to start a new cycle. The process can thus be continuously cyclized.

Table 2. Starting Experimental Conditions for SIPC. m_{IBA} : mass of isobutyl alcohol, m_{RC} : initial mass of RC-PXL dissolved in solvent, C_o : initial total concentration of the solution, T_s : initial saturation temperature, T_c : constant crystallization temperature, $s_{RC10^\circ C}^*$: solubility of RC-PXL at 10 °C in IBA, $s_{MC10^\circ C}^*$: solubility of MC-PXL at 10 °C in IBA, Y_{theo} : theoretical yield defined as $\frac{C_o - C_{MC10^\circ C}}{2 C_o} (\times 100)$.

| Conditions | SIPC |
|----------------------|------|
| m_{IBA} (g) | 17 |
| m_{RC} (mg) | 894 |
| C_o (w%) | 5.0 |
| T_s (°C) | 35 |
| T_c (°C) | 10 |
| $s_{RC10^\circ C}^*$ | 2.1 |
| $s_{MC10^\circ C}^*$ | 2.7 |
| Y_{theo} (%) | 20 |

In order to determine the suitable balance between seed mass and duration before filtration, the impact of the seed amount on

the entrainment effect was assessed by using various amounts of seeds ranging from 50 to 150 mg. The enantiomeric excess values in the solution as a function of time are shown in Figure 3a. After seeding with (*S*) enantiomer seed crystals, the solution is progressively enriched in the counter (*R*) enantiomer. This is a clear indication that an out-of-equilibrium stereoselective crystallization of (*S*)-PXL is occurring in spite of the existence of the stable racemic compound.

One can also see that the higher the seed mass, the higher and the faster the liquor gets enriched in the opposite enantiomer until the seed mass reaches 100 mg. This can be either because (*S*) enantiomer crystallize faster from solution due to larger seed crystal surface area, or because less (*RS*) is formed when more seeds are added, so that less (*R*) is removed from solution.

In the profile given in Figure 3a (red curve), one can see that the entrainment starts after seeding with 80 mg of (*S*)-seeds, yet after 60-70 minutes the ee of the liquid phase decreases. This probably results from the heterogeneous nucleation of RC-PXL, and possibly of the counter enantiomer. Therefore, one could expect that the filtration window of the process could be as high as 60 to 80 minutes since the enantiomeric excess of the liquor is then maximized.²⁶ However, the optical purity in the solid phase in Figure 3b indicates a gradual decrease of the crystal enantiomeric purity with process time. XRPD analyses of the crystals filtered at 15 and 30 minutes reveals that this is due to the spontaneous crystallization of the racemic compound RC-PXL after approximately 20 minutes (Figure S4) This shows that the introduction of PE-PXL facilitates the formation of RC-PXL crystals, probably through a heterogeneous nucleation mechanism. Consequently, the filtration window was set at 20 or 30 minutes rather than 60-80 min to compromise between the purity and the mass of the collected solid.

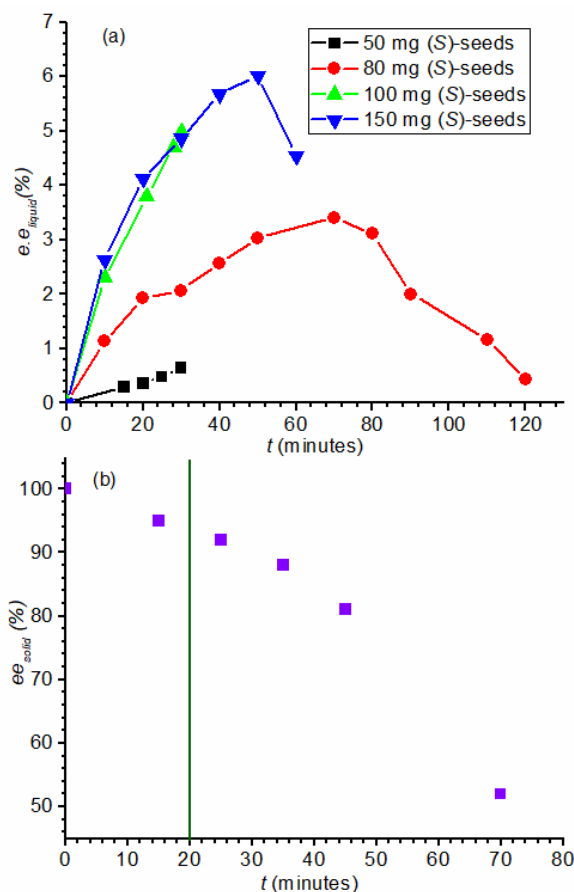


Figure 3. Evolution of enantiomeric excess of preferential crystallization in SIPC mode starting from racemic conditions monitoring (a): the liquid phase enriched with (*S*)-PXL after seeding with 50 mg, 80 mg, 100 mg and 150 mg of (*S*)-PXL and (b) the solid phase with 80 mg of (*S*)-seeds. The vertical green line crossing the point $t = 20$ minutes taken as filtration window shows the time required to produce (*S*)-PXL with good purity with steadily increasing the excess of (*R*)-PXL in the liquid phase.

The experimental results for 4 consecutive SIPC cycles are given in Table 3 and shows that PC of PXL is successful in these conditions since high enantiomeric purity crops can be produced. These results indicate that the increase in seed mass increases the deposition rate of the pure enantiomer, which is the amount of crystalline material in units of gram/second that is deposited, due to the higher surface area offered by the larger amount of seeds and also to the slow nucleation kinetics of RC-PXL during the 20 first minutes. This significantly increases the enantiomeric excess

Table 3. Experimental results of the SIPC cycles in IBA at 10 °C. ee_o (%): initial enantiomer excess in the liquid phase, W_{seeds} : mass of seeds, t_{end} : duration time in minutes, $W_{c.s.}$ and $O.P_{c.s.}$: weight and optical purity of the collected solid with seeds included, $W_{P.E.}$: weight of produced pure enantiomer, Y_{exp} : experimental yield, ee_f (%): enantiomeric excess of the liquid phase at the end of the process.

| Run Num. | ee_o (%) | W_{seeds} (mg) | t_{end} (min) | $W_{c.s.}$ (mg) | $O.P_{c.s.}$ (%) | $W_{P.E.}$ (mg) | Y_{exp} (%) | ee_f (%) |
|----------|-------------------|------------------|-----------------|-----------------|-------------------|-----------------|---------------|-------------------|
| 1 | 0.00 | (<i>R</i>) 50 | 20 | 65.5 | (<i>R</i>) 89.3 | 8.49 | 4.74 | (<i>S</i>) 1.30 |
| 2 | (<i>S</i>) 1.00 | (<i>S</i>) 50 | 30 | 57.6 | (<i>S</i>) 90.7 | 2.60 | 1.45 | (<i>R</i>) 0.30 |
| 3 | 0.00 | (<i>R</i>) 80 | 20 | 108.0 | (<i>R</i>) 92.9 | 19.86 | 11.10 | (<i>S</i>) 2.25 |
| 4 | (<i>S</i>) 1.70 | (<i>S</i>) 80 | 30 | 110.5 | (<i>S</i>) 90.2 | 18.87 | 10.55 | (<i>R</i>) 0.11 |
| 5 | 0.00 | (<i>R</i>) 100 | 20 | 145.3 | (<i>R</i>) 91.7 | 32.84 | 18.36 | (<i>S</i>) 4.29 |
| 6 | (<i>S</i>) 3.64 | (<i>S</i>) 100 | 30 | 140.6 | (<i>S</i>) 91.6 | 28.38 | 15.87 | (<i>R</i>) 0.20 |
| 7 | 0.00 | (<i>R</i>) 150 | 20 | 212.2 | (<i>R</i>) 91.3 | 43.73 | 24.45 | (<i>S</i>) 5.10 |
| 8 | (<i>S</i>) 3.80 | (<i>S</i>) 150 | 30 | 201.0 | (<i>S</i>) 89.7 | 30.29 | 16.94 | (<i>R</i>) 0.15 |

3.2.3. Preferential Crystallization of PXL by S3PC. Compared to SIPC, the S3PC process is assumed to provide advanced control over the supersaturation profile and therefore to favor a gentler crystallization behavior, reducing the likelihood of spontaneous nucleation. Figure 2 C) depicts a cyclic operation of the PC process in S3PC mode to produce both enantiomers for the first two runs. A supersaturated solution was prepared at 25 °C ($C_o = 8$ w%), resulting in a supersaturation ratio of $\beta = 2.3$ at 25°C for the racemic compound RC-PXL which is identical to that applied for SIPC. Therefore, the supersaturation with reference to MC-PXL is 1.6 slightly lower than that employed for SIPC ($\beta_{SIPC}=1.8$) which creates a moderate variation of *ca.* 10 % compared to SIPC. Starting at a lower supersaturation for MC-PXL constitutes an advantage for S3PC and thus the driving force of the crystallization is controlled by the cooling profile.

The data of two successive S3PC cycles carried out by recycling the mother liquor are reported in Table 4. Based on our SIPC results we have chosen seed masses in the range of 80-100 mg. In the first cycle and starting from a racemic composition (point (a) in Figure 2.C) the solution is seeded with 100 mg PE-PXL ((*R*)-PXL) and linearly cooled down to 10 °C. A cooling rate of 0.75 °C/min was applied. The solution moves from $ee_a = 0\%$ (point a in Figure 2.C) to $ee_b = 3.3\%$ (point b in Figure 2.C) and is then filtered to collect 160.0 mg of crude crops with optical purity of 92.0%. After compensation with 47.0 mg of RC-PXL and with solvent, a new run can be performed (points c, d and e in Figure 2.C) with a cooling profile of 0.375 °C/min. A slower cooling profile was adapted in this case because we found that at least 40 minutes of cooling are required in order to get a solution enriched

of the liquid phase, thus accelerating the resolution process. Herein, it can be concluded that the seed mass affects the resolution rate and the yield of the produced solid in a SIPC mode. It can also be deduced from these data that the crystallization behavior of PXL during the SIPC process is driven by the crystal growth and by the secondary nucleation of PE-PXL. This assumption is supported by SEM observations made on seed crystals before and after entrainment from which it can be seen that PE-PXL has crystallized on the surface of the seeds (Figure S5). This prompted us to perform the resolution by implementing a controlled cooling profile with the aim of improving the efficiency of the process.

with the opposite enantiomer (*i.e.* the required starting condition for the next run). Such a slow cooling profile, however, systematically resulted in the concomitant spontaneous nucleation of RC-PXL. Compared to SIPC for which the nucleation of RC-PXL was sufficiently delayed at 10 °C, the higher nucleation rate of this phase in S3PC might be related to the higher concentration in solution (8 w% for S3PC vs 5 w% for SIPC as C_o).

This shows that the used conditions are not suited for S3PC. Additional experiments should be designed to implement this process under smoother conditions in order to assess the enantiomeric selectivity of S3PC and to reach an advanced control over the primary heterogeneous nucleation of RC-PXL.

Table 4. Two successive S3PC cycles of PXL in IBA. ee_o (%): initial enantiomer excess in the liquid phase, W_{seeds} : mass of seeds, t_{end} : duration time in minutes, $W_{c.s.}$ and $O.P_{c.s.}$: weight and optical purity of the collected solid with seeds included, $W_{P.E.}$: weight of produced pure enantiomer, Y_{exp} : experimental yield calculated compared to the theoretical yield of 33.125%, ee_f (%): enantiomeric excess of the liquid phase at the end of the process.

| Run Num. | ee_o (%) | W_{seed} (mg) | t_{end} (min) | $W_{c.s.}$ (mg) | $O.P_{c.s.}$ (%) | $W_{P.E.}$ (mg) | Y_{exp} (%) | ee_f (%) |
|----------|-------------|-----------------|-----------------|-----------------|------------------|-----------------|---------------|-------------|
| 1 | 0.00 | 100 (R) | 20 | 160.0 | (R) 92.0 | 47.20 | 9.96 | (S) 3.30 |
| 2 | (S) 2.30 | 100 (S) | 40 | 210.2 | (S) 81.1 | 70.47 | 14.97 | (R) 1.53 |
| 3 | (R) 1.40 | 80 (R) | 40 | 134.2 | (R) 81.2 | 28.57 | 6.02 | (S) 0.40 |
| 4 | (S) 0.20 | 100 (S) | 40 | 156.0 | (S) 82.6 | 28.85 | 6.08 | (R) 1.85 |

4. DISCUSSION

Preferential Crystallization (PC) is assumed to be non-applicable when a racemic mixture crystallizes as a stable racemic compound. In this work, it is demonstrated that this limitation can be overcome for proxiphylline. Even though scale-up was not performed and various experimental parameters might still be optimized, we obtained a proof of concept for implementation of PC in a system that crystallizes as a stable racemic compound. The key step of the development of PC in a metastable conglomerate system is the selection of a solvent in which the nucleation rate of the racemic compound is sufficiently low. A solvent selection method was therefore developed, mainly consisting of induction time measurements and preliminary tests of the entrainment effect. During the implementation of the PC process using both isothermal and polythermal modes, several critical factors were identified: (i) large MSZW that should exceed the ratio of solubility of conglomerate and racemic compound, and thus the absence of spontaneous nucleation of the racemic compound after seeding (ii) fast growth rate of enantiopure compound, (iii) optimal mass and quality of seeds. It is also noteworthy that a large MSZW is a required but insufficient criterion towards resolution since our results show that PC cannot be performed from two solvents exhibiting long IT (water and 1-propanol).

5. CONCLUSION

We show that it can be envisaged to widen the scope of preferential crystallization (PC) to racemic compound forming system. By inhibiting the nucleation of the stable racemic compound, it is possible to apply PC by seeding the supersaturated solution in the metastable zone with the pure enantiomer. The success of the entrainment effect depends on the nature of the solvent, the initial supersaturation, the initial enantiomeric excess in the solution and particularly on the seed amount. The large MSZW is necessary but does not appear to be the only factor enabling PC in this type of system since seeding with pure enantiomer can also trigger the primary heterogeneous nucleation of the racemic compound. Therefore, process optimization is mandatory for successful implementation of the method proposed here, and further studies are in progress to optimize process parameters such as stirring mode, supersaturation, temperature, cooling profile, mixture of solvents, and also seed quality. Although the method developed herein should be validated using more chiral compounds, the present work suggests new perspectives toward a possible enlargement of the application field of PC.

LIST OF SYMBOLS AND ABBREVIATIONS

T (°C) Temperature

| | |
|----------|--|
| α | Solubility ratio |
| β | Supersaturation |
| $w\%$ | Weight percent |
| t_i | Induction time |
| T_c | Crystallization temperature |
| T_s | Saturation temperature |
| C_o | Initial concentration |
| PC | Preferential crystallization |
| DMSO | Dimethyl sulfoxide |
| DMF | Dimethylformamide |
| IBA | Isobutyl alcohol |
| MSZW | Metastable zone width |
| ee | Enantiomeric excess |
| RC | Racemic compound |
| PE | Pure enantiomer |
| MC | Metastable conglomerate |
| DPL | Diprophylline |
| PXL | Proxiphylline |
| SIPC | Seeded isothermal preferential crystallization |
| S3PC | Seeded polythermic programmed preferential crystallization |

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

¹H NMR spectrum, Solubility and induction time (t_i) values in large scale, t_i values in 1 mL scale, solubility of proxiphylline in isobutyl alcohol, SEM pictures, X-ray diffraction patterns (PDF)

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Notes

The authors declare no competing financial interests.

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