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Resolution of (±)-Imeglimin-2,4-dichlorophenylacetate Methanol Solvate by Preferential Crystallization

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Abstract:

The resolution of this derivative was optimized at 2-L scale in methanol by using two preferential crystallization modes (auto-seeded polythermic programmed preferential crystallization, hereafter AS3PC, and seeded isothermal preferential crystallization, hereafter SIPC) and by tuning the starting temperature. The results evidenced that the AS3PC mode is more efficient than the SIPC mode, and the higher the starting temperature, the higher the productivity. Despite a careful tuning of the operating conditions and a stable conglomerate offering a full chiral discrimination in the solid state, the enantiomeric excess of the crude solid obtained by preferential crystallization remained lower than 90%. However, this can be improved up to 99% by a single recrystallization of the methanol solvate of the imeglimin-2,4-dichlorophenylacetate since it crystallizes as a stable conglomerate without any detectable partial solid solution.

Introduction

Imeglimin (Figure 1) belongs to the class of dihydro-1,3,5-triazine derivatives. The (*R*) enantiomer of this molecule exhibits a good ability to reduce the level of glucose in blood and is used to cure the diseases caused by insulin resistance.[1]

Since no efficient enantioselective synthesis exists, both enantiomers must be separated.

Among several resolution methods, preferential crystallization (hereafter PC) is a valuable alternative which necessitates a compound crystallizing as a (preferably stable) conglomerate. The main advantages of PC over the conventional resolution methods are the following:[2]

- the theoretical yield is quantitative since the mother liquor can be recycled
- there is no need for chiral resolving agent
- the two enantiomers are easily purified without loss of enantiomeric excess since they crystallize as a conglomerate.

Since none of the nonsolvated and solvated imeglimin phases crystallize as a conglomerate, a prescreening of salts using achiral acids was carried out by using second harmonic generation (SHG).[3] During this screening, the methanol solvate of the imeglimin-2,4-dichlorophenylacetate was spotted as crystallizing as a conglomerate. It highlights the importance of the nature of the solvent(s) during the conglomerate screening.[4] Indeed, the formation of a solvated phase gives a substantial increase in the probability to crystallize a conglomerate since one-third of the organic compounds are said to crystallize as a solvate.[5] Moreover, once a solvate is detected, organic compounds give usually several of them, increasing furthermore the chance to spot the spontaneous resolution.[6] Therefore, the screening of conglomerates should be made dual in such a way that, for every achiral counterion, different solvents and mixtures of solvents should be tested. This raises the problem of efflorescent solvates which should be tested via SHG detection in suspension in equilibrium with their mother liquors. The last evolution of the conglomerate prescreening setup by using SHG takes that possibility into account.[3]

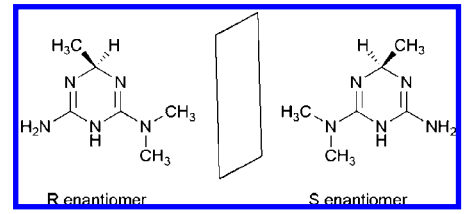

Figure 1. **Chemical formula of imeglimin.**

The aims of this study are the following; (i) to assess the possibility to resolve the racemic mixture of the API via PC of its 2,4-dichlorophenylacetate methanol solvate, (ii) to make the first steps of the optimization of the resolution of this salt by PC with different modes (auto-seeded polythermic programmed preferential crystallization, hereafter AS3PC and seeded isothermal preferential crystallization, hereafter SIPC) and by changing other operating conditions.

Structural and Thermodynamic Features of the (+)- and (−)-Imeglimin-2,4-dichlorophenylacetate Methanol Solvates. Prior to the resolution attempts via PC, the methanol solvate of the imeglimin-2,4-dichlorophenylacetic acid salt was investigated.

First of all, single crystals of both pure enantiomer and racemic mixture were obtained and analyzed by X-ray diffraction (Table 1 and Figure 2). The crystallographic data from the two structural determinations are reported in Table 1. Furthermore, the crystal stemming from the racemic mixture exhibited the enantiomorphic space group $P4_3$, while the one extracted from the pure enantiomer was its counterpart $P4_1$. In addition,

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Table 1. **Crystallographic data**

compound reference	crystal obtained from racemic mixture	crystal obtained from pure *R* enantiomer
CCDC no.	785357	785358
crystal system	tetragonal	
a/Å	8.881(5)	8.887(5)
c/Å	25.112(5)	25.152(5)
unit cell volume/Å^3	1980.6(2)	1986.5(2)
calculated density	1.316	1.312
temperature/K	300	
space group	$P4_3$	$P4_1$
no. of formula units per unit cell, Z,Z'	4, 1	
absorption coefficient, μ/mm^{-1}	0.351	0.350
no. of reflections measured	15717	15947
no. of independent reflections	4053	4046
no. of reflections with $I > 2\sigma(I)$	2627	3334
R_{int}	0.0505	0.0212
final R_1 values ($I > 2\sigma(I)$)	0.0595	0.0468
final $wR(F^2)$ values ($I > 2\sigma(I)$)	0.1395	0.1216
final R_1 values (all data)	0.0912	0.0552
final $wR(F^2)$ values (all data)	0.1553	0.1275
coodness of fit on F^2	0.981	0.984
electronic residual density (e·Å^{-3})	0.274 and −0.246	0.328 and −0.284
absolute structure parameter	0.07(1)	0.03(7)

the presence of chloride atoms allows the absolute configuration of the chiral atom C2 to be assigned.[7] Therefore, these data evidence that this phase crystallizes as a conglomerate, which is the first condition to be fulfilled for the use of PC.

The asymmetric unit is constituted by one molecule of imeglimin, one molecule of 2,4-dichlorophenylacetic acid, and one molecule of methanol. Inside the asymmetric unit, strong interactions are established: hydrogen bonds between the oxygen atom O1S of the methanol and the protonated amine N2 of the imeglimin molecule on one side and between the two oxygen atoms, O1A and O2A, from the carboxylate anion and the two protonated amines, N2 and N1, respectively, on the other side (Figure 2). The geometry of these interactions is summarized in Table 2.

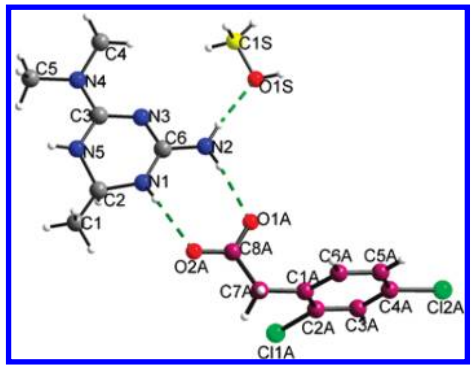

Figure 2. **Asymmetric unit of the (*R*)-imeglimin-2,4-dichlorophenylacetetate, methanol solvate. The molecular entities have their carbon atoms represented in different colours: the methanol in yellow, the carboxylate in purple, and the imeglimin molecule in grey.**

Along [001] direction, the nitrogen atom N5 carrying the positive charge and the carboxylate moiety with the oxygen atom O1S establish another ionic bond so that the building units formed by the asymmetric units are connected and wrapped around the 4_1 or 4_3 screw axes leading to periodic bond chains (Figure 3). Two adjacent periodic bond chains along [100] or [010] directions are connected by hydrogen bonds established between the oxygen atom O2A from the anion and the hydroxyl moiety of the methanol. This interaction takes place along the 2_1 screw axis (Figure 4). Every methanol molecule interacts within the neighbouring PBC either along the *a* direction or along the *b* direction.

Figure 5 shows the TGA-DSC profile of the pure enantiomer. This phase exhibits a complex behavior on heating. At circa 40 °C, the methanol molecules start to be released. The weight and the exchange of heat versus temperature indicate a change in the mechanism of desolvation before half way. The second step overlaps an exothermic phenomenon concomitant with the degradation of the compound. Therefore, the variation of mass is just indicative because the desolvation cannot be completely separated from the degradation. These data indicate a poor thermal stability of the solvate and show that its long-term storage necessitates a solid−vapor equilibrium under a substantial partial pressure of methanol. This latter point could be an issue in the case of SIPC process because the (+) and (−) seeds must be stored before use. Therefore, making suspensions of the seeds in methanol is a good option to avoid an inoculation of a phase different from the desired pure solvate.

Since the conglomerate is a methanol solvate, it was natural to envisage the primary tests of PC in methanol.

Table 2. **Characteristics of the hydrogen bonds**

	d(H···A) (Å)		*d*(D···A) (Å)		DHA angles (deg)	
	$P4_3$	$P4_1$	$P4_3$	$P4_1$	$P4_3$	$P4_1$
N(1)−H(1)···O(2a)	2.08	2.08	2.867(4)	2.878(3)	152.2	153.6
N(2)−H(2b)···O(1a)	1.97	1.99	2.796(5)	2.803(3)	160.1	158.5
N(2)−H(2a)···O(1s)	2.06	2.07	2.916(5)	2.920(4)	173.7	172.2
N(5)−H(5)···O(1a)	1.97	1.99	2.819(4)	2.831(3)	167.3	163.9
O(1s)−H(1s)···O(2a)	1.88	1.87	2.679(4)	2.683(3)	164.1	170.4

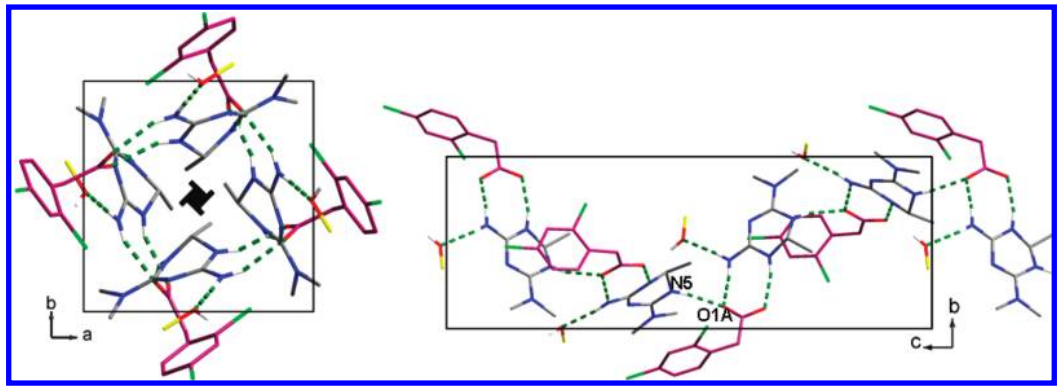

Figure 3. **Strong hydrogen bonds are observed between the three entities leading to periodic bond chains coiled round the 4_1 or 4_3 screw axes. (a) Projection along *c* axis; (b) Projection along the *a* axis.**

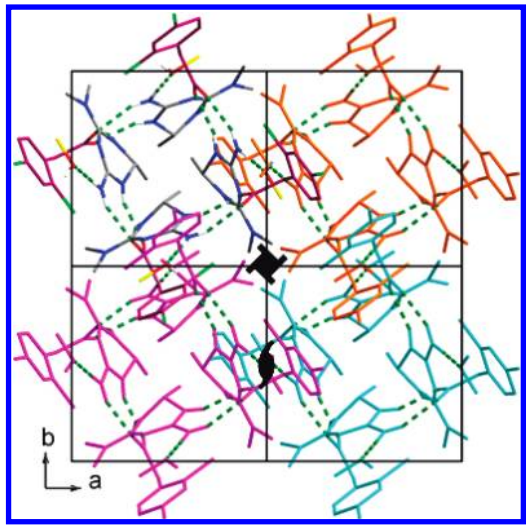

Figure 4. **The periodic bond chains are interconnected by hydrogen bonds established between the methanol and the carboxylate anion.**

Table 3 collects the solubility of the racemic mixture in methanol as a function of temperature. An isothermal section ($T = 30$ °C) of the ternary phase diagram [(+)-imeglimin-2,4-dichlorophenylacetate/(−)-imeglimin-2,4-dichlorophenylacetate/methanol] is presented in Figure 6. The boundaries of the stable domains were determined by means of discontinuous isoperibolic thermal analysis (DITA) measurements.[8] The upper points belong to the solubility curves, whereas the points that are lined up belong to the tie-lines that limit the two-phase domains and the three-phase domain. The extrapolation of these two tie-lines down to the point representative of the methanol solvates reveals that there is almost no detectable partial solid solution at room temperature.

Table 3. **Experimental values of the methanol solvate of imeglimin-2,4-dichlorophenylacetate versus temperature in pure methanol**

temperature (°C)	solubility (mass %)
0	6.7
5	7.7
20	11.5
26	14.4
29	16.6
40	24.8

Anyway, the existence of narrow domains of partial solid solutions would not have prohibited the application of PC.[9] However, it often limits the productivity of the resolution, the enantiomeric excess of the solid as well as the ease of enhancing the enantiomeric excess of the crude crops.

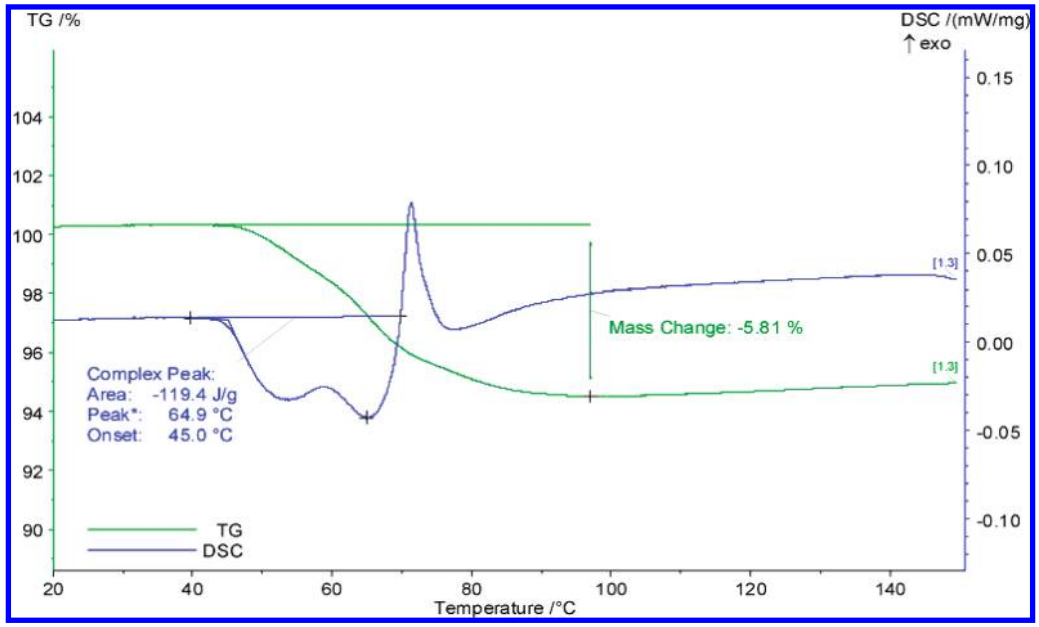

Figure 5. **TGA-DSC of the pure enantiomer.**

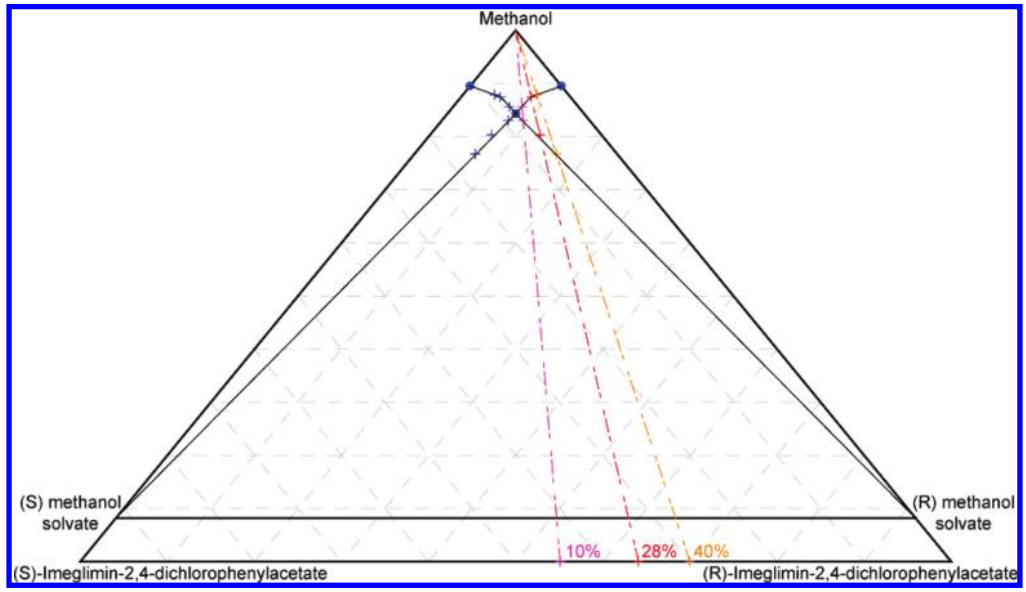

Figure 6. **Isothermal section of ternary phase diagram [(+)-salt/(−)-salt/methanol] at 30 °C (the blue circles correspond to solubility data carried out by gravimetry; the colored crosses correspond to the DITA experiments).**

Table 4. **Initial conditions for the PC experiments**

$m_{(\pm)}$ (g)	m_{MeOH} (g)	$C_{(\pm)}$ (%)	m_{enant} (g)/$ee_{initial}$ (%)	$T_{ini\ AS3PC}$ (°C)	cooling rate (°C·min^{-1})	$T_{ini\ SIPC}$ (°C)	T_{final} (°C)
284	1424	16.6	28.1/9	29.9	0.26	36.0	16

Table 5. **Results of the SIPC and AS3PC modes**

batch	mode	duration (min)	$m_{crude\ crops}$ (g)	OP (%)	$m_{pure\ crops}$ (g)	ee_f (%)
1	AS3PC	70	86.1	83.6	71.8	10.4
2	AS3PC	70	100.8	71.2	71.7	8.2
3	AS3PC	70	92.3	83.6	77.1	12.7
4	AS3PC	66	94.7	73.5	77.6	7.7
5	AS3PC	65	97.7	86.7	84.7	13
6	AS3PC	69	93	82.5	76.7	10.8
average	**AS3PC**	**68**	**94**	**80**	**77**	**11**
standard deviation	AS3PC	2	5	6	5	2
7	SIPC	85	105.3	67	70.5	7.8
8	SIPC	91	121.7	56	68.1	6.5
9	SIPC	88	118.5	63	74.6	8
average	**SIPC**	**88**	**115**	**62**	**71**	**7**
standard deviation	SIPC	3	9	6	3	1

Resolution by Preferential Crystallization

PC consists of alternate crystallizations of both enantiomers; it has been comprehensively described in several recent communications.[2,10] Let us just recall that this resolution method can be carried out via different variants:

The Seeded isothermal Preferential Crystallization (SIPC mode), in which a nearly racemic undersaturated solution is cooled down to a temperature where both enantiomers are supersaturated within their metastable zones. This doubly supersaturated solution is then seeded with enantiopure crystals that are initially in excess to trigger a stereoselective crystallization.

The Auto Seeded Polythermic Programmed Preferential Crystallization (AS3PC mode) in which, at a precise high temperature, a small initial enantiomeric excess (usually around 10% e.e.) is partially dissolved and thus partially present as crystals in the suspension (providing the so-called auto-seeding). An adapted cooling ramp is then applied which leads to a stereoselective crystallization as long as the temperature remains in the metastable zone of the antipode.

In order to compare these two modes, the application of PC to the methanol solvate of Imeglimin – 2,4-dichlorophenylacetate was carried out at 2 liter scale. The starting conditions are presented in Table 4.

Six successive crystallizations were performed using the AS3PC mode, followed by three crystallizations using the SIPC mode (Table 4).

These sets of experiments (Table 5) show that the AS3PC method leads to better optical purities and yields than the classical SIPC method. The benefit of the autoseeded process can also be highlighted by monitoring, in-line, the progress of the crystallizations, by means of focused beam reflectance measurements (FBRM). This technique consists in measuring the number of chord length per time unit (between 0.8 and 1000 μm). These chords result from the duration of the reflection

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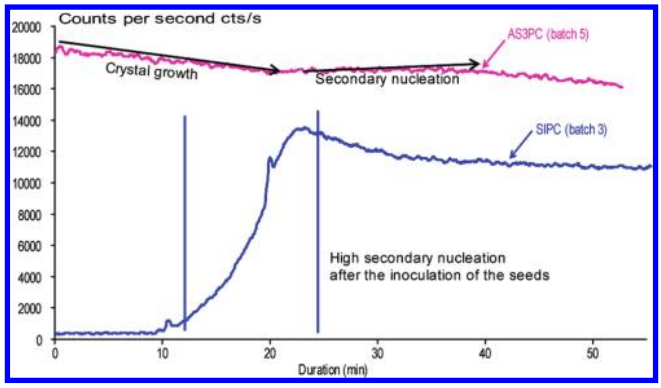

Figure 7. **Chord size distribution versus time for the AS3PC and SIPC batches.**

flashes after illumination of the particles by the spinning focused laser beam. In view to monitor early steps after the nucleation, we mainly focused our interest on the smallest detectable chords (diameter ≈ 0.8−15.6 μm), which result from a growth of very fine particles and predominantly from an offspring of new particles created by means of secondary nucleation. Figure 7 depicts the evolution versus time of chord number in the range (0.8−15.6 μm) during the implementation of AS3PC for run 5 and run 3 for SIPC. A global analysis of the number of counts due to 0.8−15.6 μm chords in the two processes shows, as expected, only small variations in case of AS3PC which corresponds to a decrease of the count chords, relative to crystal growth of enantiomer particles initially in suspension. A secondary nucleation is then visible associated with a slight increase of chords counts.

By contrast, the driving force in the SIPC process is created by a rapid cooling of the homogeneous solution from 36 °C to the final temperature (16 °C) without any spontaneous nucleation. As shown in Figure 7, a high secondary nucleation is induced without any control after the inoculation of seeds. Surprisingly, the number of small chords remained lower during SIPC process than during AS3PC process; usually, the opposite situation is observed. This led to postulate that in the SIPC process particles are not isolated but are associated in aggregates which then lowers the number of small chords. Even if the mass of the crops was moderate, the morphology of these aggregates was not well adapted to a successful filtration.

Comparisons between yields and optical purities (OP) of the crude crops obtained via AS3PC and SIPC processes give evidence of the interest of the autoseeding and smooth conditions in secondary nucleation and crystal growth.[10] Nevertheless, AS3PC results were poorer than those ordinarily obtained for a stable conglomerate without partial solid solution. The maximum mass of pure dried salt recovered was 77 g in average in 68 min, associated to a productivity of 1.1 g of pure enantiomer per minute at 2-L scale. The classical process SIPC leads to only 0.81 g/min of pure enantiomer at 2-L scale.

In order to improve the productivity of the system and the performances of preferential crystallization, further attempts of entrainment were carried out in methanol at a higher range of temperature using the AS3PC mode (Tables 6 and 7). These results evidenced that the increase of the starting temperature significantly improved the mass of pure enantiomer collected at each filtration. The productivity was just enhanced to 2.6 g.min^{-1} at 2-L scale. However, the enantiomeric excess of the mother liquor at the time of the filtration (ee_f) remained identical at high and low temperatures, indicating that the stereoselective crystallization efficiency was not directly impacted by the starting temperature. Thus, the productivity enhancement was primarly due to the increase of the solubility at the starting temperature (i.e., the higher the mass of racemic mixture, the higher the productivity as soon as the entrainment effect remains constant) and also the shorter crystallization duration.

Table 7. **Summary of the AS3PC experiments at high temperature**

batch	duration (min)	$m_{crude\ crops}$ (g)	OP (%)	$m_{pure\ crops}$ (g)	ee_f (%)
1	49	137.3	80	110	11.8
2	39	194.1	64	122	9.7
3	38	166.0	70.5	117	11.2
4	50	140.2	78	109	10.0
5	50	158.7	80.5	128	12.3
average	**45**	**159**	**75**	**117**	**11**
standard deviation	6	23	7	8	1

Table 8. **Solubility of the racemic methanol solvate of the imeglimin-2,4-dichlorophenylacetate at 40°C in pure methanol and in the azeotropic mixture methanol/toluene 71/29 wt %)**

solvent	$s(\pm)$ (wt %) @ 40 °C
pure methanol	24.8
MeOH/toluene (71/29 wt %)	25.9

Most of the organic compounds are temperature sensitive. In that case, the temperature increase is often limited. The increase of the solubility at the starting temperature can also be obtained by using another medium which here needs to be a mixture of solvents including methanol. The composition of the medium must be within the domain of stability of the methanol solvate. A cosolvent screening was carried out by measuring the solubility of the racemic mixture at room temperature in various azeotropic mixtures: methanol/solvent. The solubility of the racemic mixture is slightly higher in the mixture methanol/toluene (71/29 wt %) than in pure methanol (Table 8). In addition, the methanol solvate is a stable phase in that particular binary mixture of solvents.

Starting from the mixture presented in Table 9, six crystallizations were carried out via the AS3PC mode (Table 10).

Despite a larger mass of racemic mixture at the starting point, the productivity in the azeotropic mixture is lower than that in pure methanol (1.6 g.min^{-1}). In particular, the final enantiomeric excesses are lower, indicating that the stereoselective crystallization in the azeotropic mixture is less effective than in pure methanol. It can be due to the modifications of the crystal growth mechanism and stereoselectvity because of toluene.[11]

Table 6. **Initial conditions of the PC experiments at high temperature**

$m_{(\pm)}$ (g)	m_{MeOH} (g)	$C_{(\pm)}$ (%)	m_{enant} (g)/$ee_{initial}$ (%)	$T_{ini\ AS3PC}$ (°C)	cooling rate (°C·min^{-1})	T_{final} (°C)
406	1470	21.6	32.1/7.3	39	0.30	21

Table 9. **Initial conditions of the PC experiments in the azeotropic mixture methanol/toluene**

$m_{(\pm)}$ (g)	$m_{MeOH/toluene}$ (g)	$C_{(\pm)}$ (%)	m_{enant} (g)/$ee_{initial}$ (%)	$T_{ini\ AS3PC}$ (°C)	cooling rate (°C·min^{-1})	T_{final} (°C)
489	1440	25.3	48.3/9.0	40.0	0.23	26

Table 10. **Summary of the AS3PC experiments in the azeotropic mixture methanol/toluene**

batch	duration (min)	$m_{crude\ crops}$ (g)	OP (%)	$m_{pure\ crops}$ (g)	ee_f (%)
1	64	123.7	71	87.8	7.4
2	60	110.2	−73	80.4	7.6
3	59	127.0	72	91.4	4.6
4	54	130.2	−74	96.3	12.0
5	61	141.7	76	107.6	4.7
6	53	142.3	−73	103.8	7.4
average	**58.5**	**129**	**73**	**95**	**7**
standard deviation	**4**	12	2	10	**3**

Whatever the procedure used and despite the absence of partial solid solution, the enantiomeric excess of the collected solid remained lower than 90%. This could be attributed to several reasons, such as the existence of a metastable racemic compound[12] or lamellar epitaxy,[13] but the more probable reason seems to be here the adsorption of the counter enantiomer on the crystal surfaces. Indeed, the nucleation of the opposite enantiomer was observed on the edges of enantiopure single crystals during crystal growth experiments.[11] However, the enantiomeric purification of the solids stemming from the PC experiments can be easily achieved since the methanol solvate crystallizes as a conglomerate. Starting from crude crops collected at the end of an AS3PC experiment (ee_{solid} = 82.5%), a single recrystallization in pure methanol led to enantiopure (higher than 99% ee) solid with a yield of 94%.

Conclusion

The methanol solvate of the imeglimin-2,4-dichlorophenyl-acetic acid salt crystallizes as a stable conglomerate without any detectable partial solid solution. Out of a methanolic solution this phase is on the verge of efflorescence. The resolution in methanol via the autoseeded and classical process of preferential crystallization was achieved at 2 L scale.

The comparison between the two processes show that different mechanisms are involved in terms of secondary nucleation and crystal growth, and gives evidence of the interest of the auto seeded method in terms of productivity, final enantiomeric excess (ee_f) and optical purity of the crude crops.

The productivity was significantly improved by increasing the starting temperature, even if the efficiency of the PC, characterized by the (ee_f) of the mother liquor at he end of the entrainment, remained identical.

The modification of the medium, i.e. using the azeotropic mixture methanol/toluene (71/29 wt %) led to a slightly higher solubility of the racemic mixture at the starting temperature. However, the productivity was not improved because of a limited entrainment effect.

Even in the best case, the enantiomeric excesses of the crude crops remained modest in comparison to those obtained with ordinary stable conglomerate deprived of partial solid solutions. A 'polishing' step in thus necessary; this study shows that a single recrystallization can improve the enantiomeric excess up to 99% with a good yield.

Nevertheless, further studies are necessary to understand the mechanism(s) that lowers the optical purity of the solids during the entrainment.

Acknowledgment

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Supporting Information Available

Additional experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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