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Innovative Chiral Resolution Using Enantiospecific Co-Crystallization in Solution

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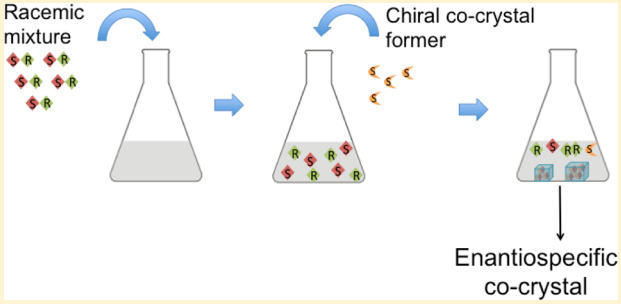
Supporting Information

ABSTRACT: A large number of active pharmaceutical ingredients (API) are chiral. Most of them are synthesized as racemic mixtures, and a chiral resolution step is introduced somewhere along the production process. In this study, we have used the specific hydrogen bonding interactions present in co-crystals to develop a new resolution technique. As these interactions are strongly direction dependent, we highlighted that an enantiopure API only forms a co-crystal with one of two enantiomers of a chiral co-crystal former (or co-former). Unlike salts, a diastereomeric pair cannot be obtained. This enantiospecific behavior of co-crystal candidates suggests that a racemic mixture of this candidate can be resolved through a co-crystallization in solution, which hitherto has not been observed yet. As a study system, we chose (*RS*)-2-(2-oxopyrrolidin-1-yl)butanamide, as the *S*-enantiomer is an API and no viable salts of this compound have been identified. The only known resolution technique for this compound is, therefore, based on chiral chromatography. Because of enantiospecific interactions with an *S*-mandelic acid coformer, we were able to selectively co-crystallize the *S*-enantiomer in acetonitrile. This enantiospecific co-crystallization in solution has been thermodynamically verified, by construction of ternary phase diagrams at different temperatures. Initial results not only validate our innovative resolution technique through co-crystallization but also furthermore already showed high efficiency, as 70% of the *S*-enantiomer could be separated from the racemic mixture in a single co-crystallization step.



Identification of enantiospecific co-crystal formation between two chiral compounds led to the development of an innovative chiral resolution technique, using an enantiospecific co-crystallization in solution. Unlike classical chiral resolution, the chiral resolving agent only co-crystallizes with one of the two enantiomers, leading to a novel technique that allows obtaining high yields in a single crystallization step and that furthermore can be extended to the series of compounds that do not or not easily form salts.

Chiral resolution is common practice in the biomedical and pharmaceutical industry given the large number of chiral drug candidates.[1,2] In most cases, only one enantiomer shows biological activity,[3] while the other has either no physiological effects or in the worst case scenarios shows undesirable or even toxic effects.[4] Separation of both enantiomers is, therefore, a crucial step in the production process, either to ensure a higher activity for a reduced dosage or to avoid unwanted side effects.

Currently, two common industrially applied techniques for the separation of two enantiomers are diastereomeric salt formation[3−8] and chiral chromatography.[9−11] The first is a relatively less costly process but can only be successfully applied when the compound of interest can easily be converted into a salt. In this case, addition of an enantiopure resolving agent leads to the formation of two distinct diastereomeric salts, which show dissimilar physical properties, allowing a chiral resolution through crystallization. Albeit being relatively expensive, the second technique, enantiomeric resolution through chiral chromatography, is used for an important number of compounds and seems to be the only viable technique for those compounds that do not or not easily form salts.[12]

Here we introduce an innovative type of chiral resolution through enantiospecific co-crystallization in solution. This technique has the advantage of being economically and environmentally more interesting compared to chromatography and can furthermore be applied to compounds that do not or not easily form salts. To show the potential of this technique, we decided to use a model pharmaceutical compound which does not easily form salts and which up to now required chiral chromatography for effective resolution. The optimization of the final process is not discussed here and will be described elsewhere.

In this study, 2-(2-oxopyrrolidin-1-yl)butanamide (**1**) was chosen as model pharmaceutical compound. This compound shows two polymorphic forms with form I being the most stable one.[13−17] The S enantiomer of this compound, **S-1**, shows nootropic activity and is marketed under the name levetiracetam, as an anticonvulsant used to treat epilepsy.[18,19] The R-enantiomer, **R-1**, does not show any biological effect.[20]

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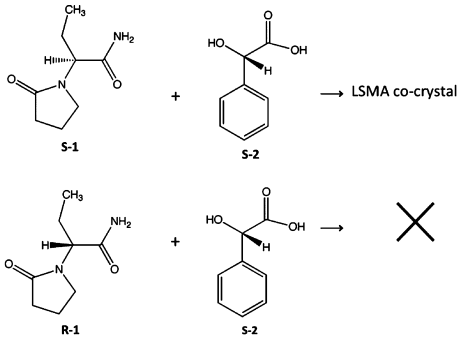
Although efforts have been made toward an enantiopure synthesis,[21] the enantiopure compound is still commonly obtained from a racemic mixture, resolved using chiral chromatography. Classical chiral resolution through crystallization cannot be used as an alternative, as the compound does not easily form salts.

In a recent paper, however, we found levetiracetam to show a high efficiency toward co-crystal formation.[22] Pharmaceutical co-crystals are alternative crystalline solid forms comprising at least two components — an API and a co-former (or co-crystal former) — that are solid under ambient conditions. As no proton transfer occurs between both components, co-crystals are clearly different from salts and most often rely on a highly directional intermolecular hydrogen bonding pattern for their formation.[23,24] Over the past decade, the main crystal engineering efforts focused on the development of alternative pharmaceutical solid forms allowing to improve physicochemical properties of a drug, without altering its initial biological functionality.[25−29]

The enantioselectivity behavior of the co-crystallization was already reported for the formation of co-crystal[30] and salt co-crystal products.[31] These contributions led to the suggestion that co-crystallization could be used to obtain alternate chiral resolution of racemic compounds but which until now has not been observed yet. Up to now, only a chiral enrichment in solution due to the formation of a co-crystal solid solution was observed.[32] Here we introduce a concrete example of chiral resolution from a racemic mixture via an enantiospecific co-crystallization in solution.

In our work, we showed that **S-1** effectively co-crystallizes with either *S*-mandelic acid, **S-2**, or *S*-tartaric acid, leading in both cases to a 1:1 co-crystal.[22] Astonishingly, **S-1** does not form a co-crystal with *R*-mandelic acid, **R-2**, nor with *R*-tartaric acid, implying that unlike salts, a diasteriomeric pair of co-crystals cannot be obtained (Scheme 1). This difference in

Scheme 1. (Top) S-1 Co-Crystallizes with S-2, Leading to a 1:1 Co-Crystal (LSMA Co-Crystal). (Bottom) R-1 Does Not Form a Co-Crystal with S-2



behavior is probably due to the presence of highly directional hydrogen bonding patterns observed in co-crystals in contrast to the less directional nature of ionic bonds in salts. This fascinating behavior, already referred to by Thorey et al. in 2010,[30] suggests again that a co-crystallization in solution can be enantiospecific and could be used as a novel resolution tool. To confirm the nonexistence of a diasteriomeric co-crystal pair, melting phase diagrams, shown in Figure 1, were constructed for the **S-1**−**S-2** and **S-1**−**R-2** pairs. As shown in Figure 1a, the **S-1**−**S-2** system shows two asymmetric eutectic temperatures, a characteristic of a binary co-crystal melting diagram,[33] while the single eutectic point shown in the **S-1**−**R-2** system confirms the nonexistence of a co-crystal under standard conditions (Figure 1b). Similar diagrams are obtained for the **S-1** and *R*/*S*-tartaric acid systems and are available in the Supporting Information. These diagrams are obtained from differential scanning calorimetry (DSC) measurements on a series of mixtures of **S-1** and coformer under different proportions.

If **S-1** selectively co-crystallizes with **S-2**, obviously the R enantiomer, **R-1**, will selectively co-crystallize with **R-2**. On the basis of this assumption, addition of **S-2** to a racemic solution of **RS-1** could, under specific conditions, lead to an effective co-crystallization of the LSMA co-crystal, hence allowing for the recovery of the biologically active enantiomer through enantiospecific co-crystallization. In this work, acetonitrile was chosen as crystallization solvent, as this latter is a good solvent for both components. Three components **S-1**, **R-1**, and **S-2** are initially dissolved in the solvent. In total, the system therefore involves five variables: the three components, the solvent, and the temperature. As our present goal is to develop a novel chiral resolution through enantiospecific co-crystallization and not to optimize such a process, we have decided, for simplicity reasons, to fix the temperature and the molar percentage of solvent. The system reduces to a three-variable system, which can be represented in a ternary phase diagram such as the hypothetical diagram presented in Figure 2. In an isothermal plane, each top of the triangle corresponds to a suspension of the pure compound in the appointed molar percentage of solvent. The middle of the lower base represents a suspension of the racemic compound in the solvent. The ternary phase diagram graphically shows which solid phase is thermodynamically stable in suspension for a given total composition. Depending on the total composition, a solid−liquid suspension will lead either to a pure solid phase or a mixture of solid phases as presented in Figure 2. A change in temperature induces a shift of the thermal stability zones, as indicated by the red curves in Figure 2.

Experimental ternary phase diagrams have been obtained through screening of different total compositions in acetonitrile at a given temperature. A set of supersaturated solutions were created by dissolving all solids at higher temperatures. All solutions were then placed at the defined temperature and seeded with a mixture of all possible solid states, that is, **RS-1** form I, **S-1**, **S-2**, and the LSMA co-crystal. After two weeks, the system was assumed to have reached equilibrium. For each initial composition, the liquid phase was then analyzed using both achiral and chiral high-performance liquid chromatography (HPLC) to determine not only the solution concentration but also the enantiomeric excess (ee). The isolated solid phase was investigated using X-ray powder diffraction (XRPD), which allows distinguishing between the different possible solid forms.

The final aspect of ternary phase diagrams will depend on the nature and amount of solvent, as well as the temperature.[34] Under the conditions considered here, certain zones of the hypothetical ternary diagram presented in Figure 2 do not appear experimentally at 9 °C and −10 °C. The zones appearing will depend on the amount of solvent, as well as the temperature. The zone in which **S-1** is the only stable form in suspension (zone III)[35] is not observed. Consequently, the zones in which the co-crystal and the **(RS)-1** phase are stable become adjacent, thereby enclosing a zone in which their mixture is stable in suspension, zone (II + IV), as shown in Figure 3.

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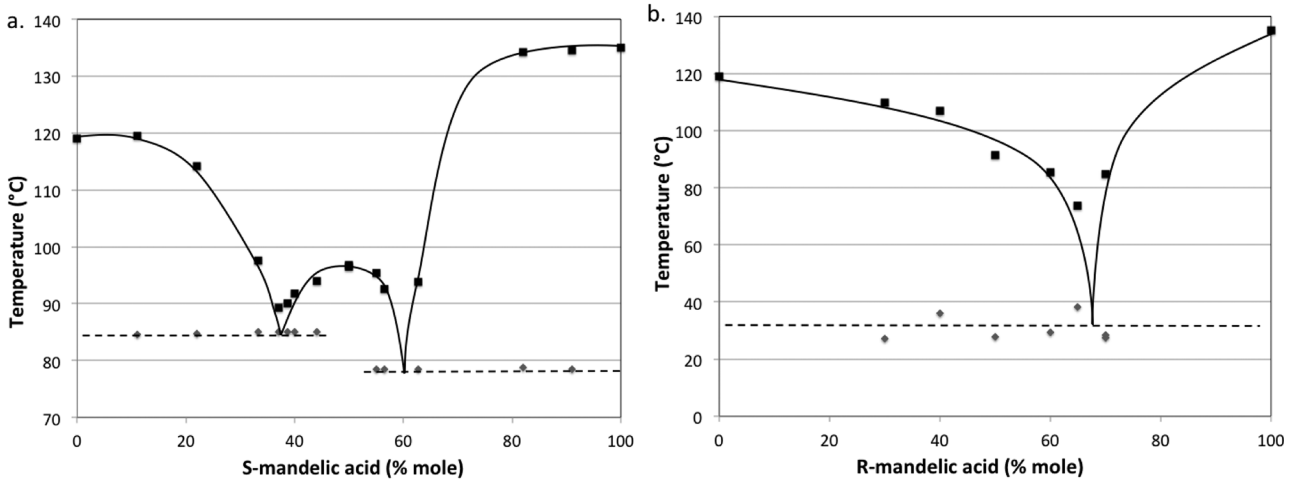

Figure 1. (a) Binary melting phase diagrams of **S-1** and **S-2** exhibit two asymmetric eutectic points indicating co-crystal formation. (b) Binary melting phase diagrams of **S-1** and **R-2** exhibit a single eutectic melting point indicating the absence of co-crystal formation. Liquidius (black square) and eutectic melting temperatures (gray diamond) are shown. Black lines and dashed gray lines are fitted through experimental data.

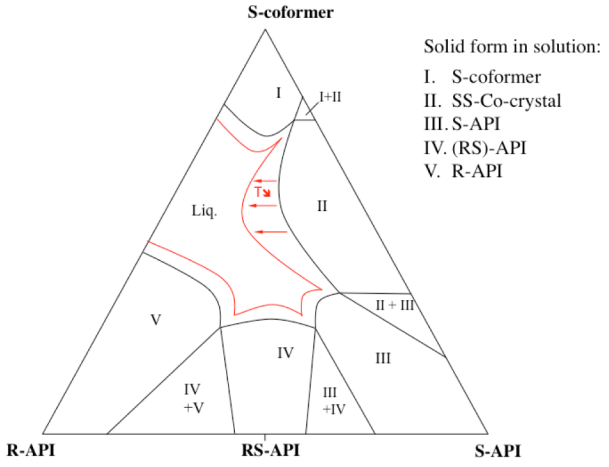

Figure 2. Hypothetical ternary phase diagram of an enantiospecific co-crystal system. The red curve shows the effect of a decrease in temperature.

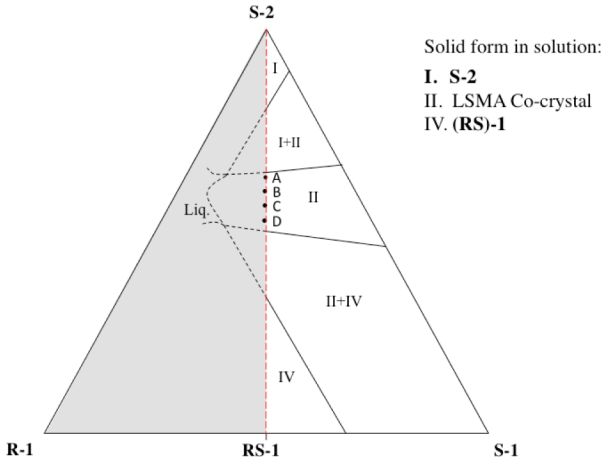

Figure 3. Schematic ternary phase diagram of **R-1**/**S-1**/**S-2** system in acetonitrile, based on experimental results at −10 °C. The experimental results used to create this phase diagram are given in the Supporting Information.

For the separation of **S-1** from a racemic mixture using enantiospecific co-crystallization, two conditions need to be satisfied. First, the chosen conditions of crystallization (nature and amount of solvent, and temperature) need to allow co-crystal formation. Furthermore, the zone (II) (or alternatively (I + II))[35] needs to cross the racemic composition line (red-dotted line in Figure 3), as is the case here.

To establish the efficiency of our novel resolution technique, four initial racemic mixtures were selected along the racemic composition line. These points (denoted A, B, C, and D in Figure 3) were considered at 9 °C, 3 °C, and −10 °C. The initial composition, as well as the final composition of all phases, is shown in Table 1. At 9 °C, only one condition (point C) led to an effective enantiospecific co-crystallization, with the LSMA co-crystal being the only stable form in suspension (zone II). The remaining solution becomes enriched in **R-1** (ee = 7.92%), and 14.67% of the initial amount of **S-1** can be recovered in the solid phase. No crystallization is observed for

Table 1. Results of Enantiospecific Co-Crystallization in Acetonitrile from an Initial Racemic Mixture of RS-1

	initial concentration				
	RS-1 mol %	**S-2** mol %	final solid state in suspension (XRPD analysis)	% ee in solution	% **S-1** recovered in solid phase (yield)
			At 9 °C		
A.	39.6	60.4		0	0
B.	41.5	58.5		0	0
C.	42.7	57.3	LSMA	7.92	14.67
D.	45.7	54.3		0	0
			At 3 °C		
A.	39.7	60.3	LSMA	24.66	39.56
B.	41.3	58.7	LSMA	22.90	37.26
C.	42.5	57.5	LSMA	24.22	38.99
D.	45.7	54.2	LSMA	27.80	43.50
			At −10 °C		
A.	39.9	60.1	LSMA	50.30	66.93
B.	41.5	58.5	LSMA	49.38	66.11
C.	42.3	57.7	LSMA	53.38	69.60
D.	45.7	54.3	LSMA	52.66	68.99

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the three remaining conditions (A, B, and D). For experiments at 3 °C and −10 °C, all conditions led to the enantiospecific co-crystallization of the LSMA co-crystal (zone II). As indicated schematically in Figure 2, lowering of the crystallization temperature is expected to lead to a more efficient resolution. This is confirmed by the results shown Table 1, with the ee in the remaining solution increasing up to 27.80% and 53.4% at respectively 3 °C and −10 °C. Choosing these latter conditions, 69.60% of the initial amount of **S-1** can be selectively recovered in the solid phase.

These results not only demonstrate the feasibility of our novel resolution technique but also show its potential strength, as up to 70% yield was obtained in a single resolution step for a compound, which cannot be separated using classical chiral crystallization resolution techniques. Optimization of the process conditions should allow increasing the yield even further.

In this study a novel resolution technique was developed using enantiospecific co-crystallization in solution. Unlike classical chiral resolution using a pair of diasteriomeric salts, the resolution technique developed here is enantiospecific, the difference most likely being due to the highly directional nature of the hydrogen bonding pattern in co-crystals. The added chiral resolving agent specifically co-crystallizes with only one of the two enantiomers of interest and is unable to form a co-crystal with the other. This novel resolution technique is particularly useful, as it leads to high effective yields in a single resolution step and can furthermore be used for the series of compounds that do not or not easily form salts, and which up to now, required chiral chromatography for effective resolution.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Materials and Methods; binary melting phase diagrams of levetiracetam and *R*/*S*-tartaric acid; ternary phase diagrams at 9 °C and at −10 °C. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(35) For a convenient reading, we will call zone X, the zone in which the solid state X is the stable phase in suspension.