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Technical Notes

Efficient Fast Screening Methodology for Optical Resolution Agents: Solvent Effects Are Used To Affect the Efficiency of the Resolution Process

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Abstract:

An efficient and fast screening methodology for optical resolution agents through the classical crystallization of the corresponding diastereomeric salts is described. In this contribution, we demonstrate that the determination of the eutectic composition by chiral analysis of the corresponding mother liquor (ML) obtained under appropriate experimental conditions provides us with a very fast screening methodology. We also demonstrate that solvent can have a profound effect not only on the efficiency of the resolution process by modifying the eutectic composition but also on the ease of crystallization.

Introduction

Crystallization is one of the most universally useful approaches to separate stereoisomers. Separation of racemic mixtures by crystallization of the corresponding diastereomeric salts (*p* and *n*)[1] providing actively pure enantiomers continues to be an important synthetic strategy in the pharmaceutical industry. The diastereomeric salts are prepared by reaction of an ionizable racemate with a chiral resolving agent (acid or base), forming in the vast majority eutectic mixtures. Sometimes, direct preferential crystallization can be effected, provided the racemate is a conglomerate.[2] Well-known examples of this latter case are the manufacture of α-methyl-dopa[3] and chloramphenicol.[4]

To speed up the discovery of an efficient resolution agent, a fast screening methodology[5] is proposed in this report. The eutectic composition is the key data to generate and will be used as a guide to select the more efficient resolution agent. The eutectic composition governs the efficiency[6] of the resolution process by crystallization under thermodynamic equilibrium. This value will be assessed from the chiral analysis of the mother liquors obtained after a successful crystallization of the corresponding diastereomeric salts, provided they form eutectic mixtures (solid solutions or 1:1 double salts may happen in some cases).[2] The screening will be performed with a collection of resolution agents in various solvents as well as their combination with water to maximize the likelihood of crystallization and resolution. The experimental requisite is to form the salts in as concentrated a solution as possible to ascertain that the chiral analysis of the mother liquors will provide the eutectic composition as shown by the ternary solubility phase diagram.[7] If too much solvent is added, then more of the desired stereoisomer will be solubilized than is warranted through the eutectic formation. In these conditions, chiral analysis of the mother liquors will give wrong results about the eutectic composition.

Most of the time, selection of an efficient resolution from a reported screening experiment[8] relies on the thermal analysis (DSC) of the obtained crystals to assess the eutectic composition. However, these crystals may not be representative of the molecular species involved in the resolution process due to the occurrence of polymorphs, solvates, or hydrates. Moreover, thermal analyses are not always obvious to interpret, since they are often complicated by thermal decomposition or by occurrence of polymorphism.[9]

Varying the solvent for performing a screening resolution is not as common as it is when screening a synthetic reaction. During optical resolution screening, advantages can be taken from variation of solvents as it may affect the eutectic composition as well as the crystallization behavior through the formation of hydrates or solvates. Although initially not recognized[10] as a powerful method to affect the efficiency of a resolution, more and more literature data show the

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(1) Letter *p* is used to designate the diastereomers resulting from reaction of the two constituents having like sign of rotation, and the letter *n*, to designate the diastereomers formed from constituents of unlike sign (ref 2).

(2) Jacques, J.; Collet, A.; Willen, S. *Enantiomers, Racemates and Resolution*; Wiley: New York, 1981.
(3) Reinhold, D. F.; Firestone, R. A.; Gaines, W. A.; Chemerda, J. M.; Sletzinger, M. J. *J. Org. Chem.* **1968**, *33*, 1209.
(4) Amiard, G. *Experientia* **1959**, *15*, 1.

(5) **Methodology**: a matrix of screw-capped vials is assembled. Each row contains one of the 12 or more selected resolving agents with four replicates per column. The racemic mixture is added to each vial (typical quantities are 50 mg of racemic mixture, 1 mol equiv of resolution agent in 10 mL of solvent). To every vial in a column is added the solvent. Typically, alcohols, acetone, AcOEt, and THF as well as their combinations with H_2O (10% v:v) are used. The vials are magnetically stirred or shaken at room temperature for at least 24 h to reach the thermodynamic equilibrium. The vials containing solid are filtered and both the crystal and the mother liquors analyzed for de assessment (HPLC, CZ, GC, polarimetry, etc...).

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benefit of varying the solvent during the search for a resolution agent.[11]

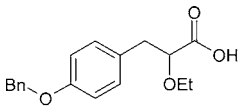
However, in some cases, mixtures of diastereomers might form solid solutions. In this case, analysis of the mother liquors could not be used to assess the efficiency of the resolution agent. The separation of the diastereomers will be more difficult, not predictable, and only feasible if the difference in solubility between salts *p* and *n* is large.

Solid solutions may also occur during optical upgrade of enriched diastereomeric salts. In this case, a salt change or crystallization of the optically enriched free base or acid might overcome the problem.

Knowledge of the eutectic point properties (composition and solubility) will allow the experimenter to establish optimum conditions for a resolution process. The maximum theoretical yield of diastereomerically pure salt obtainable by crystallization of the racemic mixture is given by Max % Yield = $100(0.5 - x_{eu})/(1 - x_{eu})$, where x is the molar fraction of the less soluble diastereomer, x_{eu} is the eutectic composition.[12] The solubility of the eutectic will be used to define the minimum amount of solvent needed to perform the resolution.

This optical resolution screening methodology was exemplified on compounds **1** and **2**.[13]

The scale-up was demonstrated with **2**.[14]



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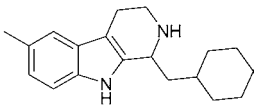

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Results and Discussion

The chiral analysis of the resulting mother liquors from the crystallization of **1** and **2** are summarized in Tables 1 and 2. To demonstrate the solvent effect on the resolution efficiency of **2**, as well as the effect on the crystallization behavior, four solvents; *i*-PrOH, *i*-PrOH/H_2O, acetone, and acetone/H_2O, along with a collection of chiral acids, have been used.

The results in Tables 1 and 2 show that several potential resolution agents (eutectic composition ≠ 50:50) have been discovered for **1** and **2** by applying the screening resolution methodology.

Optically impure diastereomeric salts (Table 1) of **1** ascertain that the chiral analysis of the mother liquor (ML) provide the eutectic composition.

Moreover, for **2** the solvent effect on the eutectic composition has been demonstrated with several resolution agents (entries 3, 6, 7, 9, and 11, Table 2).

Table 1. **Eutectic composition (%*S*:%*R*)[15] from *i*-PrOH ML analysis (chiral HPLC[16]) and enantiomeric composition (%*S*:%*R*) of obtained salts of 1**

entry	chiral bases	ML	cryst.[17]
1	*a*	18:82	56:44
2	*b*	*r*	–
3	*c*	–	–
4	*d*	13:87	67:33
5	*e*	–	–
6	*f*	–	–
7	*g*	–	–
8	*h*	62:38	43:57
9	*i*	–	–
10	*j*	5:95	71:27
11	*k*	–	–
12	*l*	–	–
13	*m*	77:23	39:61
14	*n*	–	–
15	*o*	70:30	44:56
16	*p*	–	–
17	*q*	33:67	47:53

[a] Cinchonine. [b] (1*S*,2*S*)-(+)-2-Amino-1-phenyl-1,3-propanediol. [c] (*S*)-levamisole. [d] (D)-(−)-*threo*-2-Amino-1,4-nitrophenyl-1,3-propanediol. [e] Quinine. [f] Quinidine. [g] (1*R*,2*S*)-(−)-Ephedrine. [h] (*R*)-2-Amino-2-phenylethanol. [i] (1*R*,2*S*)-Norephedrine. [j] (L)-(−)-2-Amino-3-phenylpropanol. [k] Dehydroabiethylamine. [l] (L)-Proline. [m] (*R*)-1-(4-Methylphenyl)ethylamine. [n] (D)-(+)-α-Methylbenzylamine. [o] (*S*)-(−)1-(2-Naphthyl)ethylamine. [p] (L)-Cinchonidine. [q] (D)-Alalinol. [r] No crystallization occurred.

Table 2. **Eutectic composition (%*S*:%*R*)[18] from ML analysis (chiral HPLC[19]) of 2[20]**

entry	chiral acids	*i*-PrOH	*i*-PrOH/H_2O	acetone	acetone/H_2O
1	*a*	27:73	24:76	27:73	25:75
2	*b*	*m*	78:22	–	–
3	*c*	51:49	46:54	31:69	50:50
4	*d*	–	60:40	–	–
5	*e*	–	31:69	–	30:70
6	*f*	53:47	35:65	47:53	35:65
7	*g*	57:43	49:51	6:94	50:50
8	*h*	53:47	55:45	43:57	50:50
9	*i*	50:50	50:50	35:65	50:50
10	*j*	50:50	–	51:49	–
11	*k*	28:72	*n*	19:81	47:53
12	*l*	24:76	68:32	28:72	28:72

[a] (+)-10-Camphorsulfonic acid. [b] (*R*)-2-Methyl-2-(4-nitroimidazoyl)-2-(4-methoxyphenyl)acetic acid. [c] (*S*)-(−)-Malic acid. [d] L-(−)-Dibenzoyl-L-tartaric acid monohydrate. [e] (+)-*o*,*o*′-di-*p*-Toluyl-D-tartaric acid. [f] (+)-1,2,3-Dioxophosphorinane-5,5-dimethyl-2-hydroxy-4-phenyl-2-oxide. [g] L-(+)-Tartaric acid. [h] (*S*)-(+)-Mandelic acid. [i] (1*R*,3*S*)-(+)-Camphoric acid. [j] (1*R*,3*R*,4*R*,5*R*)-(+)-quinic acid. [k] (*S*)-(+)-2-Pyrolidone-5-carboxylic acid. [l] L-(+)-Lactic acid. [m] No crystallization occurred. [n] ML not available.

A strong solvent effect on the eutectic composition is observed when the crystallization of **2** with resolution agent *g* is performed in acetone (entry 7, Table 2). This extraordinary effect is very likely due to the formation of an acetone solvate[21] by the (L)-tartrate salts *p* or *n* or both. Addition of water to the acetone has a detrimental effect on the eutectic composition, very likely by breaking the acetone solvate[22] (entry 7, Table 2). Attempting optical resolution in this binary solvent (acetone/water) would have been totally inefficient as compared to that performed in pure acetone.

Entry 6 (Table 2) shows the favorable effect of water addition on the eutectic composition for the resolution agent *f*. The same eutectic value is found in both aqueous *i*-PrOH and acetone.

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With respect to the ease of crystallization, several examples show the positive or negative effect of water addition. For instances, a positive water addition effect on the crystallization is observed (entries 2, 4, and 5, Table 2). In each case, addition of water favors the crystallization of the salts. This is very likely due to the formation of hydrated species having a lower solubility.

On the other hand, we also observe a negative water addition effect on the crystallization (entry 10, Table 2), which might be linked with an increase of the solubility often observed when working with binary solvents.[23]

The remaining trials (entries 1, 8, 12, Table 2) show no difference either on the eutectic composition[24] or on the crystallization behavior, whatever the solvent.

According to the screening results (Table 2), the resolution of **2** with L-(+)-tartaric acid (entry 7) would have been the most efficient (Max % Yield = (100(0.5 − 0.06)/(1 − 0.06)) = 46.8%) in acetone. However, experimentally this was shown to be very impractical due to the low solubility of the eutectic (8.6×10^{-4} g/mL) at room temperature. Scale-up of the resolution of **2** was previously done in EtOH with (+)-10-camphorsulfonic acid (eutectic composition identical to that found in *i*-PrOH). On the basis of the eutectic value (Table 2, entry 1), we can calculate the Max % Yield = (100(0.5 − 0.27)/(1 − 0.27)) = 31.5%. In the initial experiment, resolution of **2** to give (*S*)-**2** (*S*)-CSA was obtained in 39.1% yield with a de of 82.8%.[14] Optical upgrade of this salt in EtOH would have been done with a theoretical yield of 88.2%, thus affording an overall yield of resolution of 34.5%. This matches closely with the 31.5% maximum yield derived by the calculation above.

In practice, the optical upgrade and final pharmaceutical salt formation of (*S*)-**2** was performed with L-(+)-tartaric acid in EtOH. This demonstrates that an inefficient chiral agent could be used to optically upgrade an enriched enantiomeric mixture.

Conclusions

This work has demonstrated a powerful screening methodology for optical resolution of racemic mixtures through the crystallization of their corresponding diastereomeric salts. The eutectic composition and its solubility are the only data necessary to establish optimal experimental conditions for the resolution experiment. In parallel to changing the resolution agent, this work has also pointed out the importance of varying the solvent during the screening experiment. The likelihood that the efficiency of the optical resolution of molecules, which are prone to form solvates or hydrates, will be affected by changing the solvent is high. This has been demonstrated with the resolution of **2**. Only three examples (Table 2) of the twelve showed no solvent effect.

Although it is unpredictable how the solvent change will affect the outcome of a resolution, it provides the experimenter an additional tool to manipulate in a simple and cheap manner the eutectic composition as well as the crystallization behavior for the same resolution agent.

From an industrial point of view, this is very important as it increases the potential of using inexpensive resolution agents commonly used in the pharmaceutical industry.

Furthermore, this work has also demonstrated the simplicity of generating eutectic compositions from ML as compared to those generated by thermal analysis on isolated 1:1 diastereomeric salt mixture, followed by a calculation using the Schröder−Van Laar equation.[25]

An additional advantage over the methodology reported by Dyer et al.,[8] is that it will also provide the solvent for performing the resolution process.

This methodology can be easily automated to allow a fast and reliable experimentation.

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(14) **Scale-up of resolution of 2.** The free base **2** is liberated from its hydrochloride salt by slurring at reflux **2**·HCl (3.199 kg, 91.62% w/w) with K_2CO_3 (2.540 kg) in EtOH (46 kg) during 8 h. After completion of reaction, the **2** ethanolic solution is cooled to room temperature and percolated on a SiO_2 (5.150 kg) bed. The SiO_2 is rinsed with EtOH (4.16 L) and the wash combined with the ethanolic solution of **2**. At that point, the dilution of the reaction is adjusted to (1 g of **2**/23 mL of EtOH) by concentration under reduced pressure. To that reaction mixture, (+)-10-camphorsulfonic acid (2.153 kg, 1 mol equiv vs **2**) are added at room temperature. The mixture was stirred under reflux for 2 h, cooled to room temperature, and stirred for 2 h. After filtration, the precipitate was washed with EtOH (4.6 L) and dried under reduced pressure at a temperature between 40 and 50 °C to give 1.849 kg of (*S*)-**2** (*S*)-CSA salt (yield = 39.1%, de = 82.8%). **Purification of (*S*)-2 and final salt formation.** The L-tartrate salt was selected as final pharmaceutical salt for **2**. Although the optical resolution of **2** with L-tartaric acid in EtOH was not efficient, the optical upgrade of partly resolved **2** was done with L-tartaric acid in EtOH. To a suspension of (*S*)-**2** (*S*)-CSA (2.165 g, de: 95%) in toluene (43 mL), were added H_2O (4 mL) and NaOH 30% (0.93 mL). The reaction mixture was stirred at 40 °C until dissolution. After cooling at room temperature, the organic layer was separated and evaporated to dryness. The residue (*S*)-**2** was dissolved in EtOH (12 mL) and added dropwise to a solution of L-tartaric acid (0.696 g) in water (106 mL). Then, the mixture was heated at reflux for 1 h and stirred at room temperature for 16 h. The crystals were filtered, rinsed twice with water (2 × 10 mL), and dried under reduced pressure for 20 h to yield (*S*)-**2**-(L)-tartaric acid monohydrate (1.623 g, 86%, de > 99%, $[\alpha]^{20}_{365}$ −78.8° (*c* = 2, MeOH)).

(15) The absolute configuration of the crystallized salt (*S*)-**1** (D)-alalinol salt was proven by X-ray analysis. It allows establishing the correspondence between the HPLC elution time and the *R* and *S* configurations of both enantiomers.

(16) Chiral HPLC analytical conditions: Chiralcel-OJ column, 250 mm × 4.5 mm, eluting with heptane/EtOH, 95/5, + 0.1% TFA, flow rate 1 mL/min, at 25 °C, detection at 220 nM.

(17) Optically impure diastereomeric salts ascertain that the chiral analysis of the mother liquors provide the eutectic composition.

(18) The (*S*)-**2** (*S*)-CSA salt crystallizes from EtOH as proven by X-ray analysis. It allows establishing the correspondence between the HPLC elution time and the *R* and *S* configurations of both enantiomers.

(19) Chiral HPLC analytical conditions: Chiralcel-OD column, 250 mm × 4.5 mm, eluting with heptane/ethanol, 90/10, + 0.1% DEA, flow rate 1 mL/min, at 20 °C with detection at 240 nM.

(20) Similar eutectic values to those found in *i*-PrOH have been measured for salts of **2** with (+)-10-camphorsulfonic and L-(+)-tartaric acid in EtOH.

(21) Salts (phosphate, HCl) of (*S*)-**2** have been shown to form acetone solvates.

(22) Evidence of a crystal form change was given by Raman spectroscopy analysis performed during water addition on (L)-tartrate salt of **2**, previously crystallized in acetone.

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(24) This is the normal behaviour expected when the solvent does not take part in the eutectic species.

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