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Reviews

Enantioenrichment by Crystallization

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Abstract:

The separation of enantiomers is of great interest to the pharmaceutical industry since more than 50% of pharmaceutically active ingredients are chiral, and 9 of the top 10 drugs have chiral active ingredients. One particular enantiomer is usually preferred over the racemic mixture. In general, two methods are utilized for the production of a chiral active pharmaceutical ingredient (API) at a large scale: asymmetric synthesis and separation of enantiomers by crystallization. Neither process guarantees a product with an enantiomeric excess (ee) meeting regulatory requirements but instead generates a chiral mixture enriched with the desired enantiomer. In these cases, further chiral purification is required. This ee upgrade process remains largely an art that has not been systematically discussed. Development of a crystallization method for an ee enhancement should include three steps: (1) determine the thermodynamically stable phase of the racemate (conglomerate, racemic compound, or pseudoracemate) at the temperature of interest, (2) obtain the key solubility data, and (3) design the crystallization process. This review paper is intended to summarize recent publications and our own work concerning these areas and provide insight into how the process can be streamlined.

1. Introduction

The importance of chirality in the pharmaceutical industry has been widely recognized. It is well established that one enantiomer generally exhibits biological activities different from those of the other enantiomer because the target receptors or enzymes are chiral.[1–6] In some cases, the inactive enantiomer can even elicit undesirable side effects, which can be avoided by the development of the pure enantiomer rather than the racemate. In addition, because the metabolic pathways are stereoselective, enantiomers often have distinct pharmacokinetic properties.[7–9] Bepta[10] reported a case where the higher solubility of an enantiomer compared to that of the racemic compound was utilized to improve a pharmaceutical formulation. Currently, more than half of all marketed drugs are chiral,[11] and 9 out of the top 10 drugs have chiral active ingredients.[12] The percentage of chiral drug is likely to increase with time since almost 70% of drug candidates worldwide are chiral compounds.[13]

In general, two methods are utilized for the production of a chiral active pharmaceutical ingredient (API): asymmetric synthesis and separation of enantiomers by chromatography or crystallization. The field of asymmetric synthesis enjoyed tremendous progress over the last few decades with the advent of asymmetric reactions and enantioselective catalysts.[14–16] However, such asymmetric processes do not always guarantee a product with an ee meeting regulatory requirements but instead generate chiral mixtures enriched with the desired enantiomer. Often times, further chiral purification will be required. Separation of enantiomers by chromatography has also advanced over the past few decades,[17–22] notably with the development of supercritical fluid chromatography (SFC)[12,18–22] and hybrid processes consisting of simulated moving bed (SMB) chromatography and crystallization.[23,24] However, the scale is often limited, and operational cost is typically high.

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Racemic resolution by crystallization began in 1848 when Pasteur observed crystals of sodium ammonium tartrate that were mirror images with respect to one another. Until now, separation of enantiomers by crystallization can be classified into two main categories: (1) use of a foreign chiral element to form diastereomers followed by fractional crystallization[25–29] or formation of a diastereoselective host–guest inclusion complex,[30] and (2) direct crystallization of one enantiomer from a racemic mixture, which includes the well-known "preferential crystallization" of pure enantiomers from conglomerate mixtures,[25,31–33] an unusual enantiomeric resolution referred to as "preferential enrichment",[33–35] and application of crystallization inhibitors to chiral separation in racemic compound-forming systems.[36–38] Again, these processes do not always guarantee a product with an ee meeting regulatory requirement, and in those cases further chiral purification will be required.

As discussed above, chiral purification is often required to produce an API that meets regulatory requirement regardless what chiral separation approach is utilized. To this day, this process remains largely an art that has not been systematically discussed. Crystallization is widely used at small and large scales to reject impurities, including an enantiomeric impurity. Development of a crystallization method for an ee enhancement should include three steps: (1) determine the thermodynamically stable phase of the racemate (conglomerate, racemic compound, or pseudoracemate) at the temperature of interest, (2) obtain the key solubility data, and (3) design the crystallization process. This review is intended to summarize recent publications concerning these areas and provide additional insight into how the process can be streamlined.

One landmark book titled *Enantiomers, Racemates, and Resolutions* by Jean Jacques, André Collet, and Samuel H. Wilen[25] was first published in 1981. In this book, the authors thoroughly discussed the properties of racemates and of their constituent enantiomers, as well as the principles that underlie chiral separations. The concepts discussed in this book are foundations for most of the work published in this area, including this review.

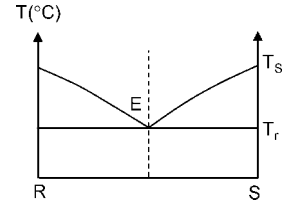

Figure 1. **Binary phase diagram of a conglomerate system.**

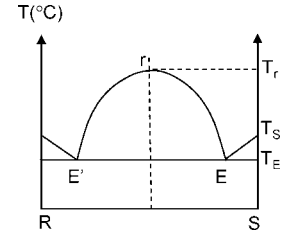

Figure 2. **Binary phase diagrams of a racemic system.**

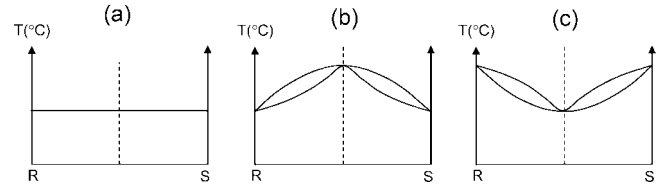

Figure 3. **Binary phase diagrams of a pseudoracemate system: (a) type I, (b) type II, and (c) type III.**

2. Determination of the Most Thermodynamically Stable Phase of Racemate

2.1. Type of Crystalline Racemates. Three types of racemates (an equimolar mixture of two enantiomers whose physical state is unspecified or unknown) are defined by Roozeboom.[25] A conglomerate is a mechanical mixture of crystals of the two pure enantiomers. Figure 1 displays the binary melting point diagram of a conglomerate system. It has been estimated that only 5–10% of the organic racemates exist as conglomerates. The most common type of racemate, a racemic compound, corresponds to a crystalline racemate in which the two enantiomers are present in equal quantities in a well-defined arrangement within the same crystal lattice. Figure 2 illustrates the binary melting point diagram of a racemic compound system. Pseudoracemate refers to the formation of a solid solution between the two enantiomers coexisting in an unordered manner in the crystal. Figure 3a, b, and c represents binary melting point diagrams of the three classic cases of solid solutions, types I, II, and III, where the two constituents are enantiomers.[39,40] In the case of type I, mixtures of the two enantiomers in all proportions melt at the same temperature as the pure enantiomers. In the case of type II, the phase diagram exhibits a maximum melting point for the racemate, and in the case of type III, a minimum melting point. Unlike a racemic compound, which is a unique compound requiring an equal quantity of two enantiomers, a pseudoracemate is only a special case of a continuous series of heterochiral solid solutions. Formation of a solid solution of enantiomers over the entire range of composition is rare. Much more common are conglomerate and racemic compounds with partial solid solution

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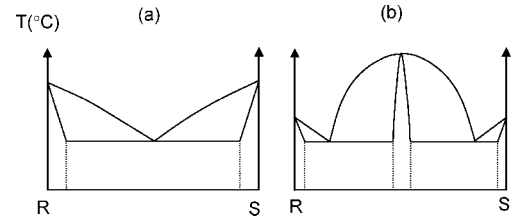

Figure 4. **Binary phase diagrams showing the formation of solid solutions of enantiomers in a limited range of concentration: (a) conglomerate and (b) racemic compound.**

formation,[41] particularly in the vicinity of either pure enantiomer or of the racemic compound.[25] Figure 4 illustrates two examples of binary melting point diagrams for these systems. Neau[42] reported that the free base of bevantolol and propranolol form a racemic compound at high ee and a pseudoracemate in the vicinity of the racemic mixture.

The occurrence of racemic compounds is believed to be less frequent for salts than for neutral compounds. Jacques[43] conducted a survey of more than 500 organic chiral compounds and found that the occurrence of a conglomerate is 2–3 times more frequent for salts than for covalent racemic species. Li[44] included 25 chiral pharmaceutical compounds in his studies, and among them, 19 racemic species are racemic compounds, corresponding to a 76% frequency, lower than 90% among organic chiral compounds. This was partially explained by the sample collection, of which seven were salts. This lower frequency of racemic compounds may reflect their occurrence among chiral pharmaceuticals because many drugs are formulated as salt forms.

It is also possible that one compound exists as a racemic compound as the stable form at one temperature but a conglomerate at another. Li[44] provided thermodynamic basis for chiral systems that display a transformation from a racemic compound to a racemic conglomerate at certain temperatures.

Just as any other crystalline material, polymorphism is everything but rare for chiral compounds. Srčič,[45] Lamm,[46] and Rollinger[47] published their work on the polymorphism and racemate identification of felodipine, a dihydropyridine calcium channel blocker. Burger[48] reported polymorphism of nitrendipine (NTD), another calcium channel antagonist of the 1,4-dihydropyridine type. When a chiral system is referred to as belonging to a racemic compound, conglomerate, or pseudoracemate system (in other words, the racemic compound, conglomerate, or pseudoracemate is the most stable phase under the concerning conditions), it is assumed one only deals with one specific crystal form of each racemate type (racemic compound, conglomerate, and pseudoracemate). If polymorphism is observed for any of the three racemate types, the relative thermodynamic stability of the racemate types may depend on which polymorph of a particular racemate type one is dealing with. Obviously, combining polymorphism and racemate type can make the cases overwhelming. We suggest dealing with these two problems separately. In other words, the most thermodynamically stable form under the relevant conditions is determined first, and then the phase diagram of the racemate system is constructed using only the stable form.

In a ternary system (two solid phases and a liquid phase, as in a crystallization system), assignment of a racemate type can be more complex if a solvate (including hydrate) of an enantiomer or racemic compound forms. Since a stable solvate has a lower solubility in the constituent solvent than the anhydrous form, the relative stability of the conglomerate and the racemic compound (at least one of them is solvate) can be different from that of the binary system. In addition, the relative stability of a solvate and anhydrous form or another solvate with lower degree of solvation may change at a certain temperature and the most stable racemate type can change at this temperature as well. Again, we suggest determining the most stable form for each racemate type before determining the most stable racemate type.

2.2. Identification of a Racemate. Characterization of a racemate is prerequisite for the design of a resolution and purification process. X-ray powder diffraction (XRPD) and solid-state nuclear magnetic resonance spectroscopy (SSNMR) are the two most powerful and widely used techniques for structural characterization of crystalline material. Identical XRPD patterns and SSNMR spectra for pure enantiomer and racemate generally suggest that the racemate is a conglomerate. However, in a pseudoracemate, the molecules of both enantiomers coexist in a common unit cell that is similar to or a slightly distorted version of that of the constituent enantiomers.[49] The extent of this distortion is often too small to be detected by routine instrumentation. Therefore, similar XRPD patterns and SSNMR spectra cannot exclude the formation of a pseudoracemate, although it occurs much less frequently than conglomerate formation. A different XRPD pattern or a different SSNMR spectrum of the racemate with respect to the constituent enantiomers indicates the racemate has a different crystal structure (Figures 5 and 6). This difference can stem from the formation of a racemic compound or a different crystal form of the constituent enantiomers.

Construction of the binary phase diagram from measurements of the melting temperatures of the racemate and of the corresponding enantiomers has traditionally been used for identifying the type of the racemate.[42,50,51] There are several points worth mentioning regarding this approach: (1) a good mixing between the two components is imperative for eutectic formation; (2) if a polymorphic transformation occurs for any of the species involved during the course of the experiments, the results can be misleading; (3) the establishment of the phase diagram for type II and type III pseudoracemates poses an experimental problem due to the difficulty in measuring

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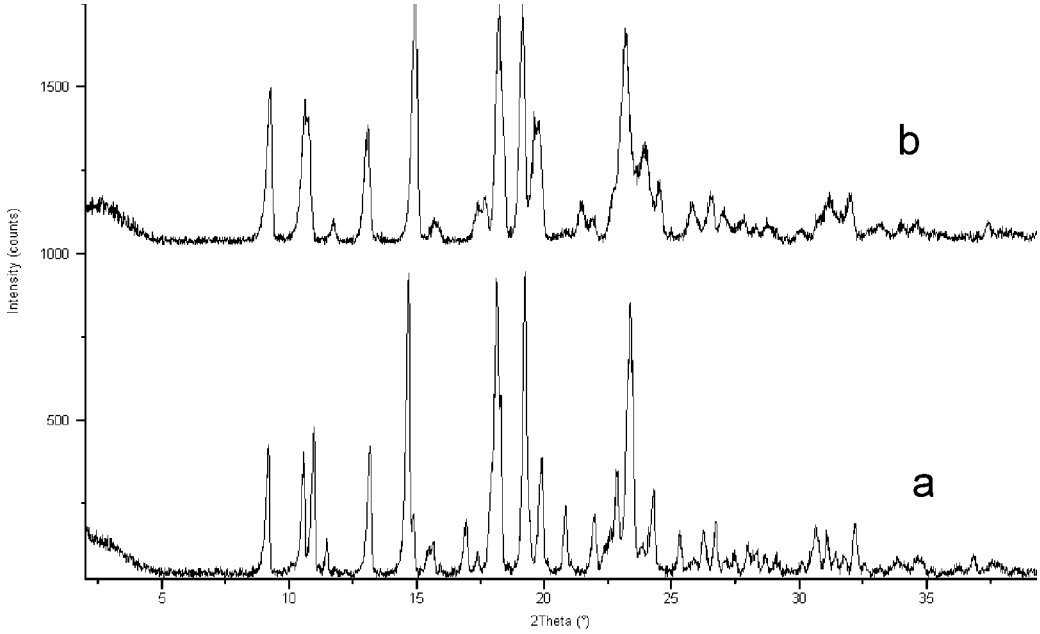

Figure 5. **XRPD patterns of (a) the *S* enantiomer and (b) the racemate.**[52]

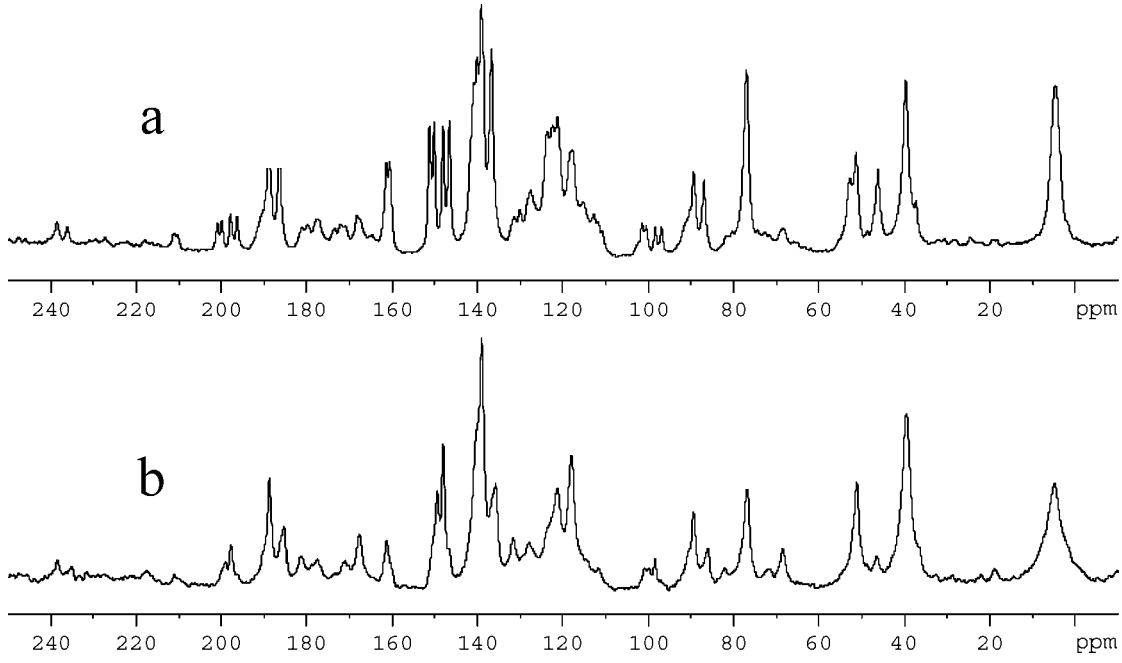

Figure 6. **SSNMR of (a) the *S* enantiomer and (b) the racemate.**[52]

accurately the melting point range of a given mixture since true liquid–solid equilibria are practically impossible to establish.[47] Nevertheless, the phase diagrams constructed for a conglomerate system and a pseudoracemate system should be different enough to distinguish the two systems.

Construction of a ternary solubility phase diagram of a racemate system is another way to identify the type of the racemate. Figures 7, 8, 9 represent the ternary phase diagrams of a conglomerate, racemic compound, and three types of pseudoracemate systems, respectively, assuming no solvate formation. Jacques[25] described the procedure to construct ternary solubility phase diagrams in great detail. Since the tremendous advancement of chiral high pressure liquid chromatography (HPLC) in recent years, the determination of chiral composition of supernatants has been made faster and more accurate.

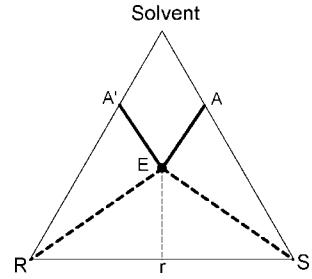

Figure 7. **Ternary solubility phase diagram of conglomerate system.**

Construction of experimental ternary phase diagrams for the type II and III solid solutions presents the same practical difficulties as those described earlier for the binary phase diagrams. The composition of the solid phase and that of the

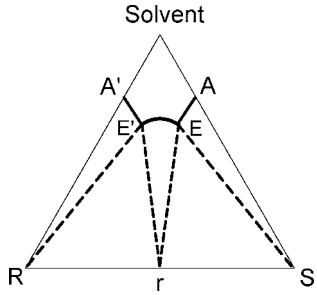

Figure 8. **Ternary solubility phase diagram of a racemic compound forming system.**

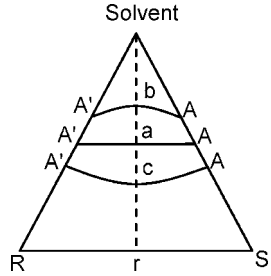

Figure 9. **Ternary solubility phase diagram of pseudoracemate system: (a) type I, (b) type II, and (c) type III.**

liquid phase are interdependent; thus, establishment of a true solubility equilibrium would require that there is a continuous change in the composition of the solid phase as a whole concomitantly with that of the solution during the process of crystallization, which is kinetically unachievable. The consequence is that the crystals of the solid solution will not be homogeneous and the phase diagram will only have qualitative significance. Nevertheless, the phase diagram generated can be used for system identification.

In the case of one or two starting materials forming solvate(s) with the solvent, the ternary phase diagrams of the conglomerate and racemic compound will change accordingly (Figures 10 and 11, respectively). Since the solubility of the solvate changes as a function of temperature at a rate very different from that of the anhydrate, the relative stabilities of the racemic compound and conglomerate are more likely to reverse as temperature changes.

Besides binary and ternary phase diagrams, other approaches can also be used to determine the type of a racemate along with XRPD and SSNMR. For example, in order to determine if a different XRPD pattern displayed by a racemate stems from the formation of a racemic compound or from a different polymorph of the enantiomer, a roughly 1:1 mixture of the racemate and one of the constituent enantiomer can be swished

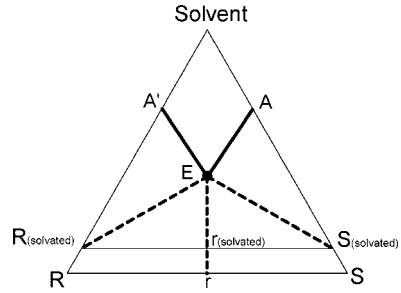

Figure 10. **Ternary solubility phase diagram of conglomerate system where the enantiomers form solvates.**

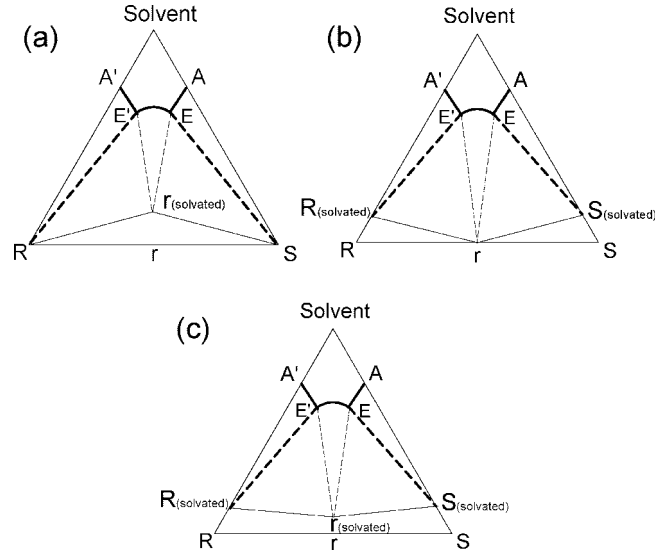

Figure 11. **Ternary solubility phase diagram of a racemic compound forming system: (a) the racemic compound forms a solvate, (b) the enantiomers form solvates, and (c) both the enantiomer and the racemic compound form solvates.**

in a suitable solvent to find out if this will lead to a conversion of one to the other by XRPD. If the XRPD pattern remains unchanged after a few days of mixing, it suggests the racemate is a racemic compound[52] and it is more stable than the corresponding conglomerate, and if the XRPD of the slurry shows the same pattern as the racemate, it indicates the racemate is the conglomerate of a new polymorph of the original enantiomer and the new form is more stable. If the XRPD pattern of the slurry represents the XRPD pattern of the pure enantiomer, it means the racemate is either a racemic compound that is less stable than the conglomerate or a conglomerate of a new polymorph of the enantiomer and this new polymorph is less stable than the original form. In some cases, no appreciable polymorph conversion is observed even after two forms are mixed for a few days because of low solubility of the two forms in the solvent, close solubility values between the two forms, surface poisoning by impurities, or just slow growth of the more stable form in the chosen solvent. Therefore, in order to increase the confidence level of concluding the existence of a stable racemic compound, more than one solvent system in which the enantiomer has appreciable solubility (a few milligrams per gram of solvent) should be used and a long enough equilibration time (a few days at least) should be given. If the second enantiomer is available, a conversion observed when mixing the racemate with a 1:1 mixture of two enantiomers is sufficient evidence to conclude the existence of a stable racemic compound.

2.3. Determination of the Relative Thermodynamic Stability of Racemates. A robust crystallization process often functions under a thermodynamic equilibrium. For chiral purification, it is important to identify the most thermodynamically stable racemate.

Li[44] introduced the method of using the melting temperatures of enantiomer and racemic compound to determine the relative

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stability of the different racemate species. On the basis of the equation of Gibbs free energy for formation of the racemic compound, it was concluded that when the racemic compound melts at a higher temperature than its enantiomers, the formation of the racemic compound is always thermodynamically favorable at the melting temperature of the enantiomer and is likely to remain thermodynamically favorable over a wide temperature range below the melting temperature. When the racemic compound melts at a temperature close to or no more than 30 °C lower than its enantiomers, the formation of the racemic compound is likely thermodynamically favorable at the melting temperature of the racemic compound, and the relative stability of the racemic compound and its enantiomers may reverse at a lower temperature. Furthermore, when the racemic compound melts at a temperature about or more than 30 °C lower than its enantiomers, the formation of the racemic compound is less thermodynamically favorable at and below the melting temperature of racemic compound. Among 19 chiral compounds examined in Li's study, 18 melted at temperatures higher than (T^{r}_{A} − 30 °C), and the other melted at 31 °C lower than its enantiomers. Another interesting observation reported in Li's work is that 18 out of the 19 racemic compounds had a heat of fusion higher than those of their corresponding enantiomers. The other one showed a heat of fusion slightly lower than that of its enantiomer, possibly due to experimental error. It should be noted that the difference between the heat of fusion of a racemic compound and its enantiomer is not the same as the heat of formation of a racemic compound, which is actually negative for most of the racemic compounds studied by Li[44] and by Jacques.[25]

Solubility measurement is a valuable tool to determine directly the relative stability of a racemic compound and corresponding conglomerate at the temperature of operation. If the solubility of a racemic compound (defined as the total amount of racemic compound dissolved in a unit volume of solvent) is lower than the solubility of the corresponding conglomerate (defined as the total amount of conglomerate (ee of 0%) dissolved in a unit volume of solvent), the racemic compound is the more stable phase under the experimental conditions. Otherwise, the conglomerate is the more stable phase.

3. Measurement of Solubility

Crystallization is widely used for chiral purification. Development of such a crystallization method involves solvent screening, temperature selection, and definition of system composition. It is more complicated than dealing with achiral compound since the solubilities of enantiomers and the racemate depend not only on the solvent and temperature but also on the system composition. The ternary solubility phase diagram is extremely valuable during this process. However, constructing phase diagrams in different solvents at various temperatures is time-consuming and also requires a large quantity of compound. Perhaps for this reason, the application of ternary solubility phase diagrams in upgrading ee from partially resolved mixtures has been neglected in the pharmaceutical industry. Luckily, recent developments have shown that it is actually not necessary to experimentally construct ternary phase diagrams in multiple solvents and temperatures.

3.1. Eutectic Composition as a Function of Solvent Composition and Temperature. For a conglomerate system, the eutectic ee is always zero despite solvent changes or temperature variations.

For a racemic compound system, the process of rejecting the undesired enantiomer becomes the rejection of the racemic compound. In one of the authors' recent works,[52] it was demonstrated that although the ternary solubility phase diagram helps to understand the system and the rational design of the crystallization process, the eutectic ee is the key to assess the feasibility of a crystallization method and to predict the ee and yield of the product. Furthermore, the eutectic ee is independent of solvent composition in dilute solutions (where Henry's Law is obeyed, which is applicable to most crystallization solutions) if no solvate is formed. This implies that once the eutectic ee is determined in one solvent system, there is no need to screen other systems hoping for a significantly different eutectic ee value. If a significantly different ee is desired, the effort should be focused on looking for a solvent that may form a solvate with one or two of the solids.[53] In the same work, it was also proven that the eutectic ee changes as a function of temperature as illustrated by the following equations:

$$(\ln K_{eu})_{T_2} = (\ln K_{eu})_{T_1} + \int_{T_1}^{T_2} \left(\frac{2}{RT^2}\right)\left\{\left\{\left(\Delta H_f^{(T_m)_s}\right)_s - \left(\Delta H_f^{(T_m)_r}\right)_r\right\} + \int_{T}^{(T_m)_s} [(C^s)_s - (C^s)_r]dT + \int_{(T_m)_s}^{(T_m)_r} [(C^l) - (C^s)_r]dT + \left\{\frac{1}{2}(\mu_{ss} - \mu_{RS})\right\}\right) \quad (1)$$

In dilute solutions:

$$K_{\mathrm{eu}} = \frac{a_S}{a_R} \approx \frac{[S]}{[R]} \quad (2)$$

$$\mathrm{ee}_{\mathrm{eu}} = \frac{[S]-[R]}{[S]+[R]} * 100 = \frac{\frac{[S]}{[R]}-1}{\frac{[S]}{[R]}+1} * 100 = \frac{K_{\mathrm{eu}}-1}{K_{\mathrm{eu}}+1} * 100 \quad (3)$$

where K_{eu} was defined as the eutectic constant; $(\Delta H_{\mathrm{f}}^{(T_{\mathrm{m}})_S})_S$ and $(\Delta H_{\mathrm{f}}^{(T_{\mathrm{m}})_{\mathrm{r}}})_{\mathrm{r}}$ are the enthalpy of fusion at the melting temperature $(T_{\mathrm{m}})_S$ and $(T_{\mathrm{m}})_{\mathrm{r}}$ of enantiomer (S) and racemic compound (r), respectively; $(C^s)_S$, $(C^s)_{\mathrm{r}}$, and (C^l) are heat capacities of solid enantiomer (S), solid racemic compound (r), and liquid (r), respectively; and μ_{SS} and μ_{RS} are pair potential energy between (S) and (S) and (R) and (S), respectively. From above equations, the rate at which the eutectic constant and the eutectic ee change with temperature can vary greatly from one system to another depending on the value of the second term on the right side of eq 1. The system studied in this work showed significantly

(53) Klussmann, M.; White, A. J. P.; Armstrong, A.; Blackmond, D. G. *Angew. Chem., Int. Ed.* **2006**, *45*, 7985–7989.

different eutectic ee at different temperatures. In another case, Lorenz[54] reported the eutectic ee of the mandelic acid system was insensitive to changes of temperature.

3.2. Measurement of Solubility. Solubility of a solid compound is traditionally obtained by measuring the concentration of a saturated solution which is in equilibrium with the solid phase of this compound. The same approach is applicable for obtaining the solubility of a pure enantiomer. However, in an industrial setting, it is quite common that only chiral mixtures with high ee, but not pure enantiomer, are available for solubility measurements. In this case, the solubility of the pure enantiomer can still be obtained by measuring the concentration of the enriched enantiomer in a saturated solution if enough solvent is used to keep the other enantiomer (in the case of conglomerate system) or the racemic compound in solution. The higher the ee of the supernatant, the more accurate is the approach. An ee above 90% is generally recommended.

The solubility of a racemic compound can be obtained in the traditional way as well if pure racemic compound with an ee of 0% is available. Again, quite often only material with low ee (a mixture of racemic compound and one enantiomer) is available. Then the solubility of racemic compound can be calculated from the following equation:[52]

$$S_r = 2\sqrt{[S] \times [R]} \tag{4}$$

where S_r is the solubility of racemic compound, and [*S*] and [*R*] are the concentrations of *S* and *R* in the supernatant, respectively. This approach is valid only when the racemic compound is the only solid phase in equilibrium with the saturated solution. In other words, the enriched enantiomer has to be kept in solution. In this case, the lower the ee of the supernatant, the more accurate is the approach. In one study,[52] the solubility results generated using material with 17% ee (the ee of the supernatants were 30%) agreed well with the data generated using pure racemic compound.

Solubility data at the eutectic point for a conglomerate system (equivalent to the solubility of the conglomerate) can be determined experimentally by measuring the total concentration in the saturated solution in equilibrium with solids of both enantiomers. The solubility of each enantiomer should be half of this value. Although "Meyerhoffer's double solubility rule"[55] is useful to estimate the total solubility at the eutectic point (which is double the solubility of the pure enantiomer), the estimation is usually not accurate enough to be used for process design. The validation of this rule is based on the assumption that the saturated solution of pure enantiomer and of conglomerate mixture are ideal, or at least that the activity coefficient of the enantiomer remains the same in the saturated solution of pure enantiomer and of the conglomerate mixture. However, this may not be true because a saturated solution of pure enantiomer and that of conglomerate can impose a very different chemical environment for the enantiomer.

Solubility data at the eutectic point for a racemic compound forming system, hence the eutectic ee, can be determined by measuring the concentrations of each enantiomer in a saturated solution which is in equilibrium with a solid mixture of one enantiomer and the racemic compound. Owing to the advancement of chiral HPLC in the past years, satisfactory results can be obtained fairly easily and with a reasonable amount of material if an appropriate solvent is chosen. M. Klussmann[53] proposed to predict eutectic ee of a racemic compound from the solubility of the pure enantiomer and the racemic compound from the following equation:

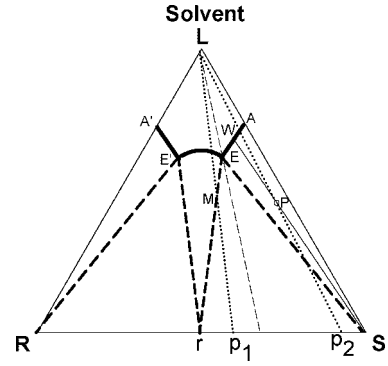

Figure 12. **Ternary solubility phase diagram of a racemic compound forming system in an achiral solvent L.**

$$ee_{eu} = \frac{1 - \frac{a^2}{4}}{1 + \frac{a^2}{4}} * 100 \tag{5}$$

where *a* is the ratio of the solubility of a racemic compound to that of the constituent pure enantiomer. Similar to Meyerhoffer's double solubility rule, this method assumes the saturated solution of pure enantiomer and of pure racemic compound are ideal. In our experience, this approach is useful for a quick estimation of eutectic ee, but in many cases, is not accurate enough to be used for crystallization design.

4. Design of Crystallization Process

Once the eutectic composition (and thus the eutectic ee as well, in the case of a racemic compound) at the temperature of interest is determined, and the solubility of the pure enantiomer is obtained, a rational design of the separation process can be carried out. A recent work by the authors[56] discussed in great detail the process design and demonstrated this approach in several industrial cases.

4.1. Racemic Compound Forming System. *4.1.1. Starting Material Has an ee Lower Than the Eutectic ee.* Figure 12 shows a ternary phase diagram for a racemic system at temperature T in an achiral solvent L when no solvates are formed. Points A and A′ represent the composition of the solutions saturated with pure enantiomer *S* and *R* respectively. Points E and E′ (eutectic points) correspond to the solution compositions when the three phases exist in equilibrium: racemic compound (r), one enantiomer (*S* or *R*), and the

(54) Lorenz, H.; Sapoundjiev, D.; Seidel-Morgenstern, A. *J. Chem. Eng. Data* **2002**, *47*, 1280–1284.
(55) Meyerhoffer, W. *Ber. Dtsch. Chem. Ges.* **1904**, *37*, 2604–2610.
(56) Chen, A. M.; Wang, Y.; Wenslow, R. M. *Org. Process Res. Dev.* **2007**, *12*, 271–281.

saturated solution. When the starting material, represented by point P_1, has an ee_0 lower than the eutectic ee_{eu}, the desired enantiomer (*S*) can be enriched in the liquid phase, and the ee of the product will be the same as the ee of the eutectic point (ee_{eu}) if the system composition remains in region rES. The highest yield (defined here as the ratio of the amount of desired enantiomer *S* in the supernatant to the amount of *S* in the total system) for a product with ee_{eu} is obtained when the system composition is shifted from P_1 to M, the intersection point of lines LP_1 and rE.

$$\text{yield}_{max} = \frac{ee_0(1 + ee_{eu})}{ee_{eu}(1 + ee_0)} \tag{6}$$

The volume of solvent (expressed in milliliters) to be added to 1 mg of solid to reach point M can be calculated by the following equation:

$$V_{max} = \left(\frac{ee_0}{[S]_{eu} - [R]_{eu}}\right) \tag{7}$$

where $[S]_{eu}$ and $[R]_{eu}$ represent the concentration of *S* and *R* (mg of solids per mL of solvent) at the eutectic point, respectively. The subscript "max" is used to emphasize that this is the maximum volume to add without compromising the purity of the product.

When less solvent is added, $V < V_{max}$, the yield can be calculated by:

$$\text{yield} = \frac{[S]_{eu}}{0.5 + 0.5ee_0} \cdot V \tag{8}$$

4.1.2. Starting Material Has an ee Higher Than the Eutectic ee. In Figure 12, when the starting material, represented by point P_2, has an ee_0 higher than the eutectic ee_{eu}, pure enantiomer (*S*) can be obtained in the solid phase if enough solvent is added to move the system composition into region AES. The minimum volume of solvent required can be calculated by the following equation:

$$V_{min} = \frac{1}{2[R]_{eu}}(1 - ee_0) \tag{9}$$

The maximum yield is:

$$\text{yield}_{max} = 1 - \frac{(1 - ee_0)(1 + ee_{eu})}{(1 + ee_0)(1 - ee_{eu})} \tag{10}$$

When more solvent is added, $V > V_{min}$, the yield can be calculated by:

$$\text{yield} = 1 - \left(2V \cdot [S]_{pure} + \frac{[S]_{eu} - [S]_{pure}}{[R]_{eu}} \cdot (1 - ee_0)\right) \cdot \left(\frac{1}{1 + ee_0}\right) \tag{11}$$

where $[S]_{pure}$ represents the solubility of *S* in milligrams of solid per milliliter of solvent.

4.2. Conglomerate System. For a conglomerate system, the minor enantiomer can be dissolved in the supernatant and the pure product is retained in the solid phase. The minimum amount of solvent required to dissolve all of the minor enantiomer is given by the equation below:

$$V_{min} = \frac{1}{2[R]_{eu}}(1 - ee_0) \tag{12}$$

which corresponds to the maximum yield of pure enantiomer *S*:

$$\text{yield}_{max} = \frac{2ee_0}{1 + ee_0} \tag{13}$$

and at $V > V_{min}$, the yield can be calculated by the following equation:

$$\text{yield} = 1 - \left(2V \cdot [S]_{pure} + \frac{[S]_{eu} - [S]_{pure}}{[R]_{eu}} \cdot (1 - ee_0)\right) \cdot \left(\frac{1}{1 + ee_0}\right) \tag{14}$$

4.3. Solid Solution-Forming System. For a solid solution-forming system, a slight ee upgrade can be achieved in the case of type II and type III pseudoracemates by dissolution or crystallization, but no ee upgrade can be achieved in the case of type I. Because of the difficulties in achieving true equilibrium in the case of type II and type III pseudoracemates, upgrading ee for a pseudoracemate system is likely not reproducible and is not recommended for real production.

5. Discovery of the Thermodynamically Most Stable Racemate

It is clear from the discussion above that the definition of the system, racemic compound, conglomerate, or pseudoracemate drives the design of the chiral purification and determines the outcome of the process. Therefore, it is imperative to determine the most stable racemate. This is dependent on the discovery of the most stable phases of the enantiomer and racemic compound. It is not uncommon that a crystalline racemic compound is not the first racemate observed, even though it may be thermodynamically more stable than the corresponding conglomerate or pseudoracemate, similar to the phenomena that a thermodynamically more stable crystal form is not always observed before a less stable crystal form. In order to minimize the possibility of late stage discovery of a more stable racemic compound, it is important to make efforts to crystallize the racemic compound, if it has not been observed yet, with similar approaches used for polymorph screens. In addition, polymorph screens of enantiomer and racemic compound are also important since the relative stability of the conglomerate versus racemic compound can change if a more stable form of either enantiomer or racemic compound is discovered.

6. Conclusions

Chiral purification of partially resolved enantiomeric mixtures is an important part of most API manufacturing processes.

The crude material is usually purified through crystallization of the desired enantiomer or dissolution of the desired enantiomer followed by crystallization. The process design should follow three sequential steps: (1) determine the stable phase of the racemate (conglomerate or racemic compound) at the temperature of interest, (2) obtain the key solubility data, and (3) design the crystallization process. This review discussed the basics related to these three steps and summarized representative work in these areas.

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