结晶技术在手性药物拆分和纯化中的应用

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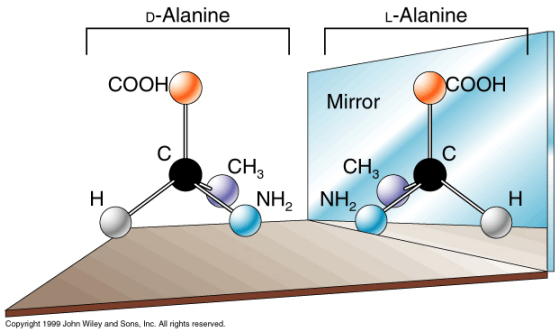

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提纲

- 手性药物拆分和纯化的重要性
- 结晶技术在手性药物拆分和纯化中的应用
- 实例分析: 对于不同种类的对映异构体和非对映异构体进行手性拆分不同策略的成功应用

手性药物的重要性

- 市场上50%的药物是手性分子
- 70%的正在研发的药物是手性分子
- 全球销量最好的10个药物中，9个是手性分子
- 对于手性药物来说，生产和制备光学纯的对映异构体非常重要，因为不同的对映异构体可能有非常不同的生物活性

手性化合物常用术语

Enantiomers: stereoisomers which are mirror images of each other (R vs. S)

Enantiomeric excess (ee) = (R-S)/(R+S) (0.92 or 92%)

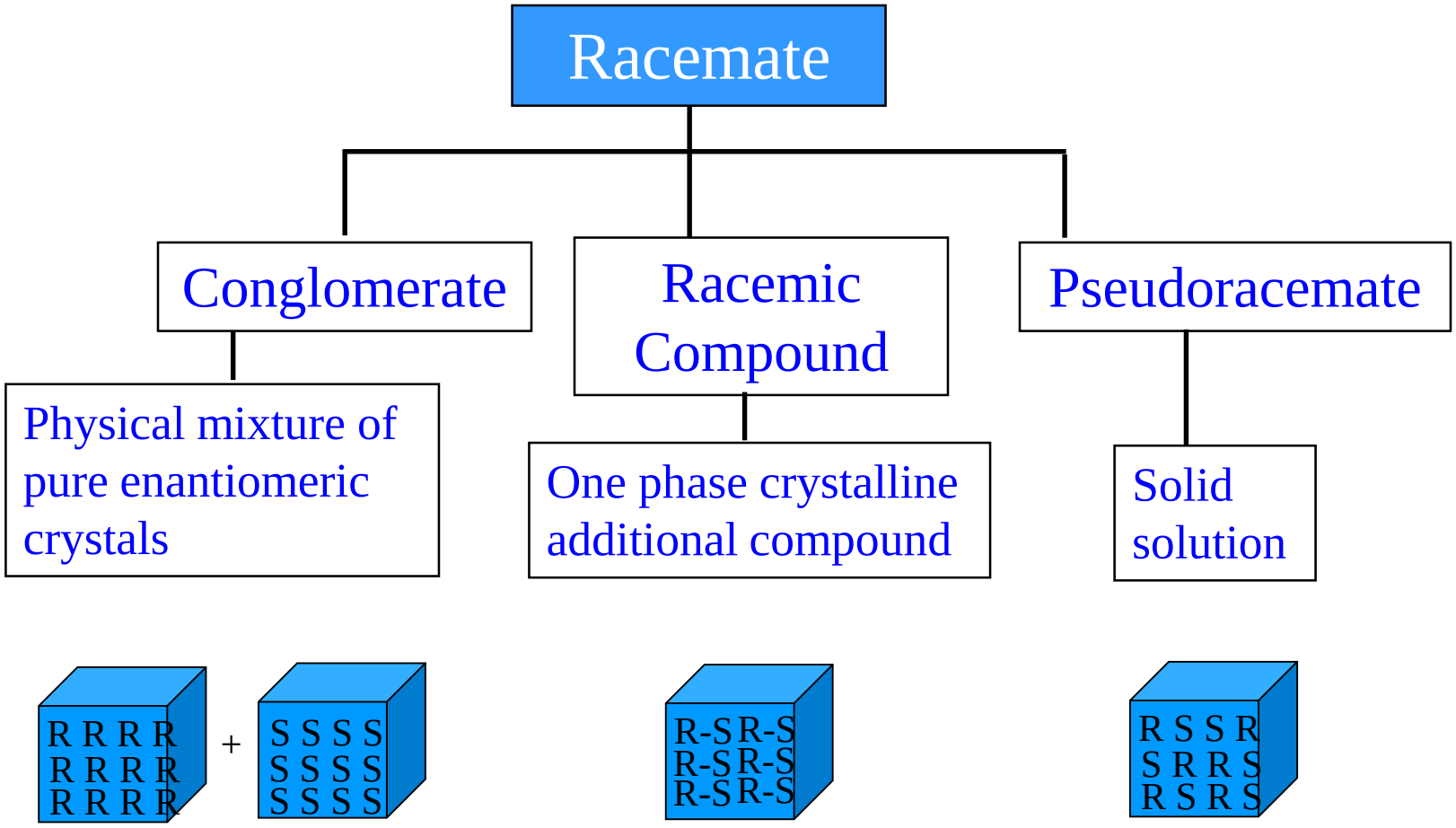
Racemate: an equimolar mixture of a pair of enantiomers

Diastereomers: stereoisomers which are not mirror images of each other

(R,S) vs. (R,R)

Diastereomeric excess (de)

对映异构体分类


Racemate
Conglomerate
Racemic Compound
Pseudoracemate
Physical mixture of pure enantiomeric crystals
One phase crystalline additional compound
Solid solution
R R R R
R R R R
R R R R
+
S S S S
S S S S
S S S S
R-S R-S
R-S R-S
R-S R-S
R S S R
S R R S
R S R S

美国药监局对于新的手性药物开发的指南

- Although it is now technologically feasible to prepare purified enantiomers, development of racemates may continue to be appropriate. However, currently available information suggests that the following should be considered in product development:
  - Appropriate manufacturing and control procedures should be used to assure stereoisomeric composition of a product, with respect to identity, strength, quality and purity. Manufacturers should notify compendia of these specifications and tests.
  - Pharmacokinetic evaluations that do not use a chiral assay will be misleading if the disposition of the enantiomers is different. Therefore, techniques to quantify individual stereoisomers in pharmacokinetic samples should be available early. If the pharmacokinetics of the enantiomers are demonstrated to be the some or to exist as a fixed-ratio in the target population, an achiral assay or an assay that monitors one of the enantiomers may be used, subsequently.

http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm122883.htm

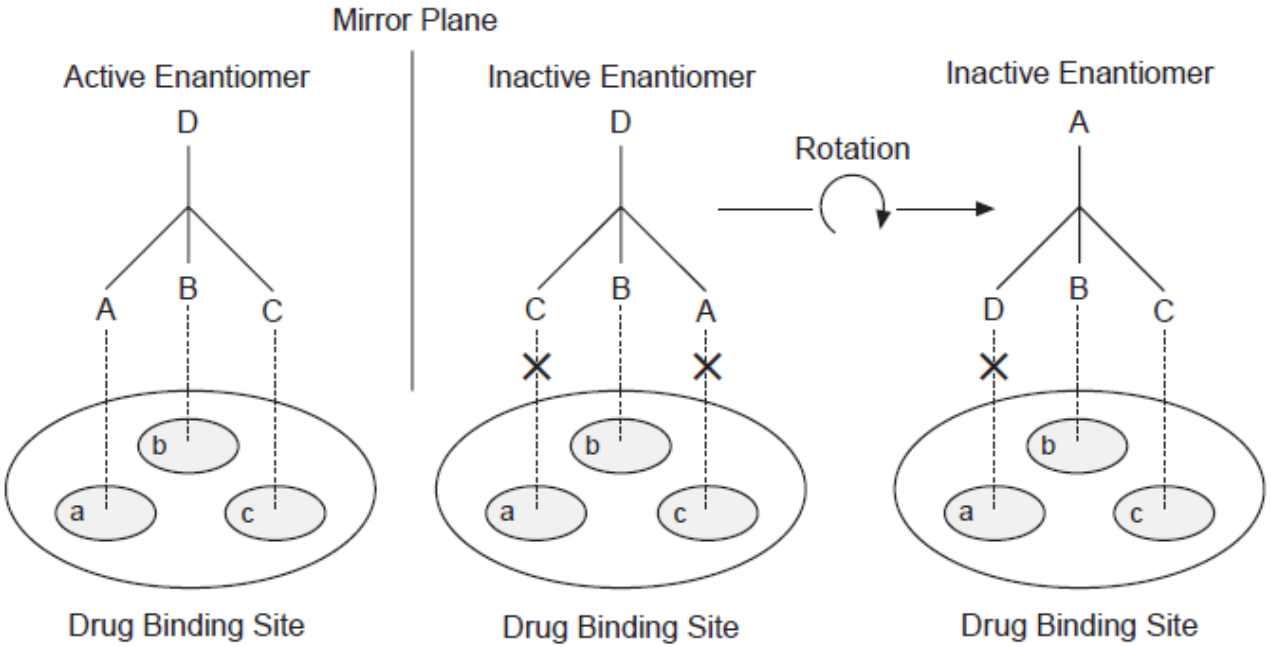
对映异构体具有相同活性的手性药物

- Both enantiomers of dobutamine are positive inotropes;
- Both ibuprofen enantiomers are anti-inflammatory agents;
- Both enantiomers of warfarin and phenprocoumon are anticoagulants;
- The enantiomers of bupivicaine both produce local anesthesia;

对映异构体具有不同生物活性的手性药物

- Granulocytopenia is related to the d-isomer of levodopa; vomiting is caused by the d-isomer of levamisole; and myasthenia gravis symptoms were no longer observed when the d-isomer was removed from d,lcarnitine.

Hypothetical Interaction between the 2 Enantiomers of A Chiral Drug and Its Binding Site



[a]The active enantiomer has a 3-dimensional structure that allows drug domain A to interact with binding site domain a, B to interact with b, and C to interact with c. In contrast, the inactive enantiomer cannot be aligned to bind the same 3 sites simultaneously. The difference in 3-dimensional structure allows the active enantiomer to bind and have a biological effect, whereas the inactive enantiomer cannot.

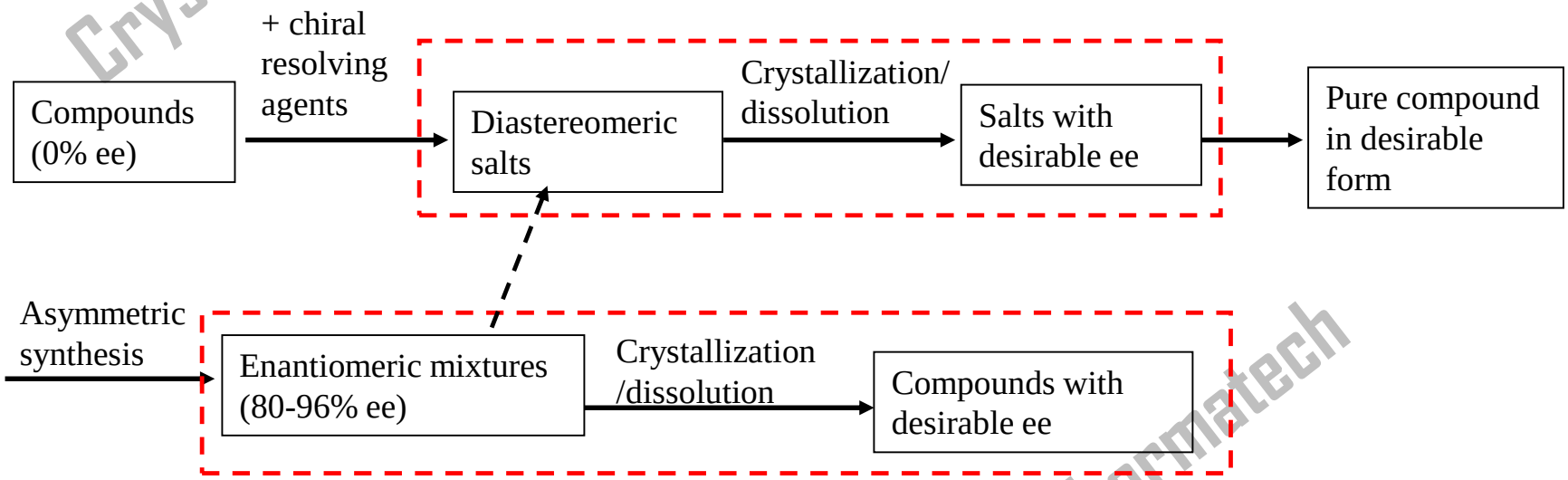
http://www.psychiatrist.com/pcc/pccpdf/v05n02/v05n0202.pdf

Some Examples of Chiral Psychiatric Drugs

Table 1. Selected Racemic Drugs Currently Used in Psychiatric Practice
Bupropion[a]
Citalopram[b]
Fluoxetine
Methylphenidate[b]
Thioridazine (and some other phenothiazines)
Tranylcypromine
Trimipramine
Venlafaxine
Zopiclone[a]

[a]Single-enantiomer formulation under development.
[b]Single-enantiomer form also available.

得到纯手性药物的主要途径



如何进行手性药物结晶提纯工艺的开发？

现状

- 开发工艺耗时太长，很多随机的实验
- 缺少对手性分子晶型和所属体系的系统了解
- 对手性结晶提纯的工艺的可重复性和可放大性没有把握，缺少对工艺风险的预测（缺乏对结晶工艺动力学和热力学的理解）

利用三元相图指导结晶技术在手性药物拆分和纯化中的应用

- 可以全面理解结晶提纯的原理和过程
- 了解通过结晶工艺提高手性纯度的可能性
- 确定最佳的结晶工艺条件，预测能够提纯到的最高手性纯度以及对应的产率

两篇利用三元相图指导手性药物结晶提纯工艺开发的文献

Organic Process Research & Development **2008**, *12*, 271–281

Purification of Partially Resolved Enantiomeric Mixtures with the Guidance of Ternary Phase Diagram

Alex M. Chen,*,† Yaling Wang,‡ and Robert M. Wenslow†

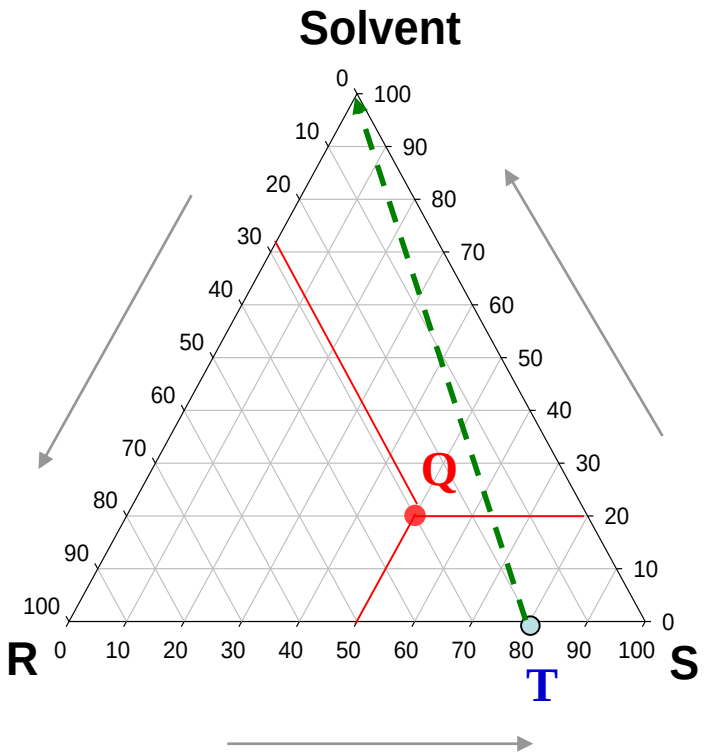
Organic Process Research & Development **2008**, *12*, 282–290

Reviews

Enantioenrichment by Crystallization

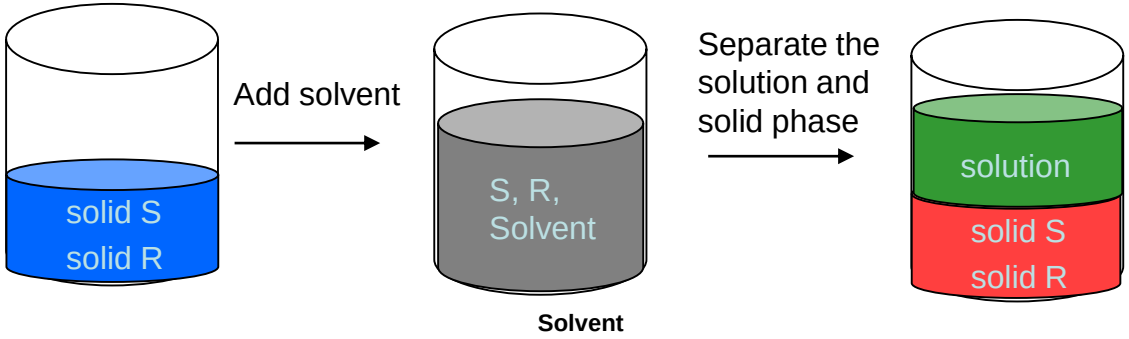
Yaling Wang*,† and Alex M. Chen‡

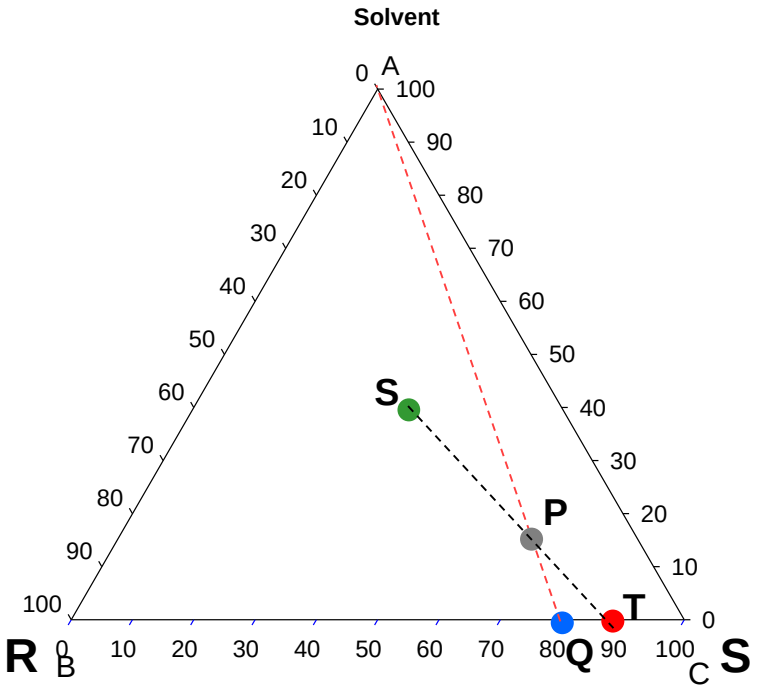
三元相图的基础知识



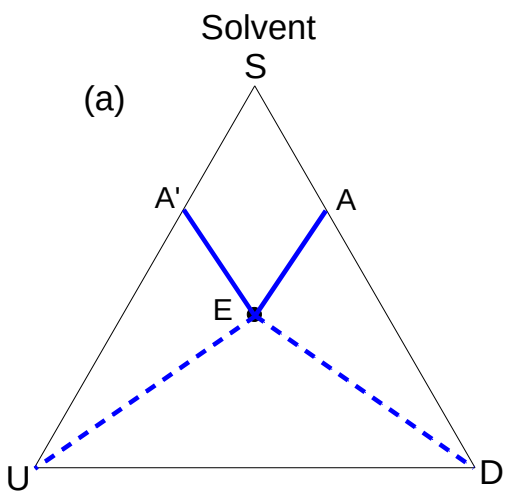
- **Q** represents 50% S, 20% solvent and 30% R.
- **T** represents 80% S, 0% solvent and 20% R.
- Any point on the dashed line will represent a system with same ratio of S to R.

用三元相图示意手性分子结晶/溶解提纯的工艺流程



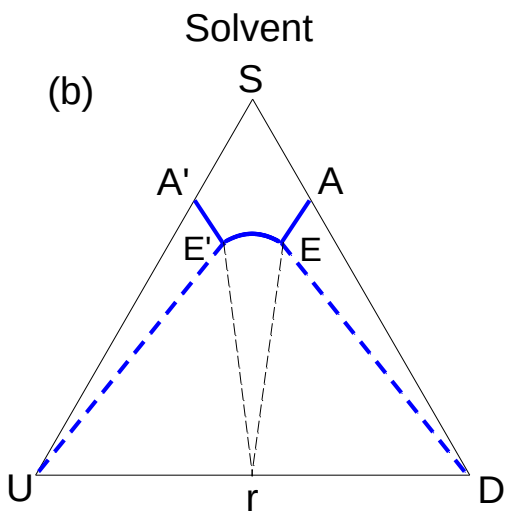


对映异构体的三元相图



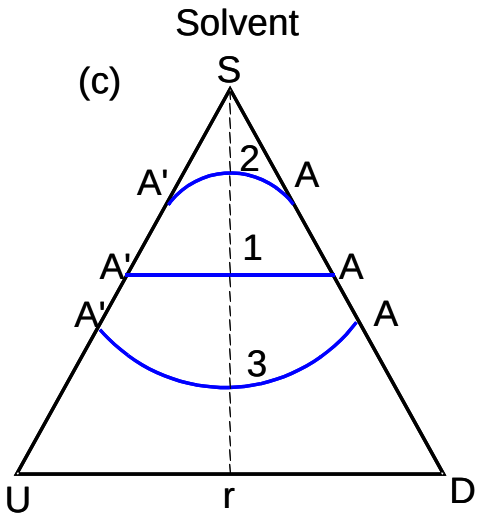
Conglomerate

（外消旋混合物）



Racemic compound

（外消旋化合物）



Pseudoracemate

（外消旋固体溶液）

Conglomerate系统的三元相图

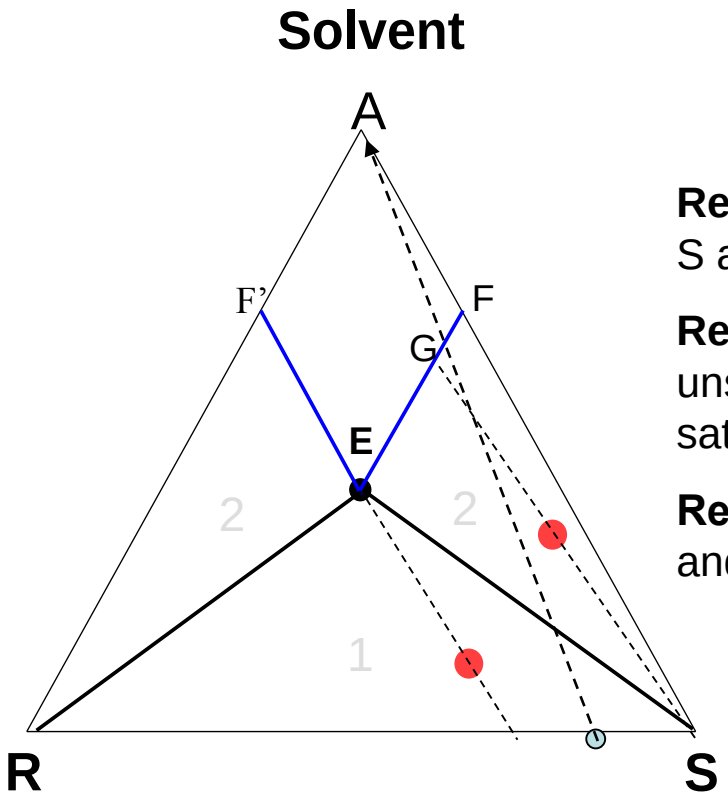
Gibbs Phase Rule:

$$\text{var}\,iance = C - \Pi + 2$$

C=3
Fix Pressure

$$\text{var}\,iance = C - \Pi + 1$$

Variance in Grey



Region ERS: Both R and S are saturated.

Region EFS: R is unsaturated and S is saturated.

Region AF'E'EF: Both R and S are unsaturated.

Blue solid line represents the saturated solution curve

Racemic Compound系统的三元相图

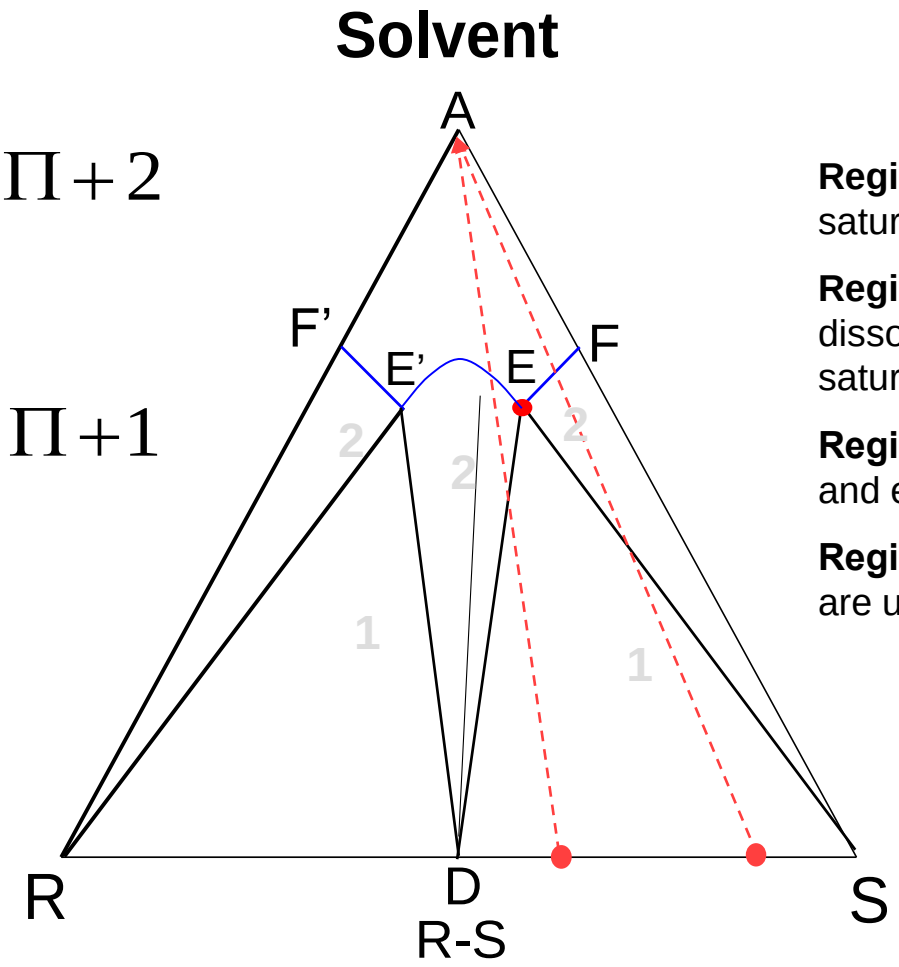
Gibbs Phase Rule:

$$\text{var}\,iance = C - \Pi + 2$$

C=3
Fix Pressure

$$\text{var}\,iance = C - \Pi + 1$$

Variance in Grey



Blue solid line represents the saturated solution curve

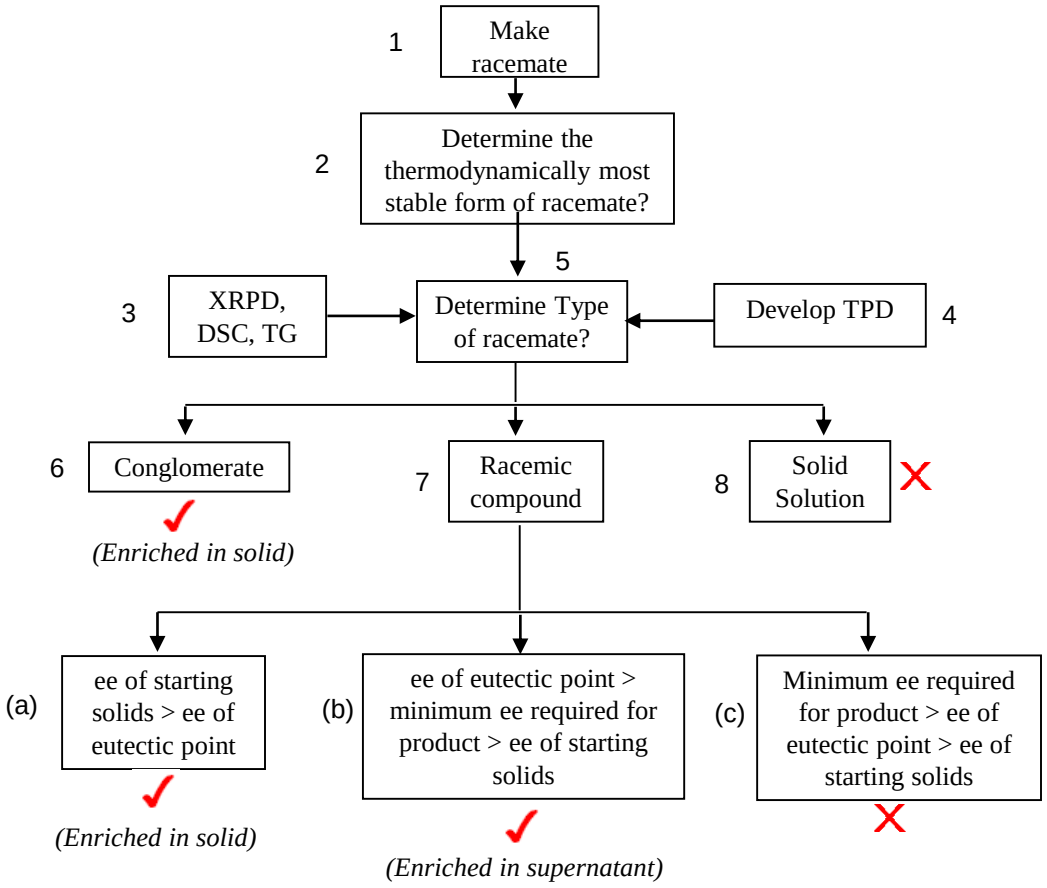
Region EDS: Both R-S and S are saturated.

Region EFS: R-S completely dissolves in solution and S is saturated.

Region E'DE: R-S is saturated and either R or S is unsaturated.

Region AF'E'EF: Both R-S and S are unsaturated.

对映异构体的结晶提纯工艺开发流程



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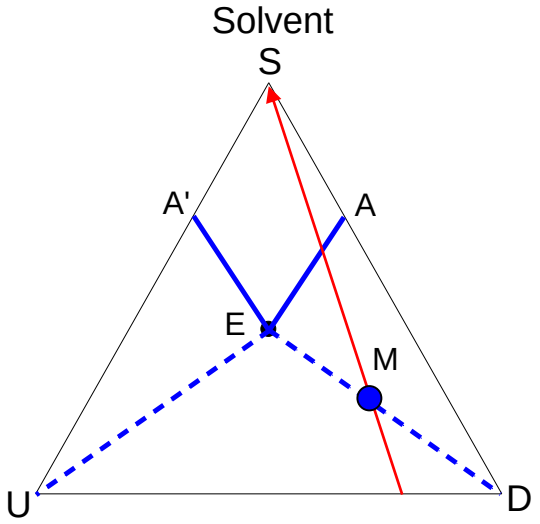
三元相图的深入理解：Conglomerate

Conglomerate System

$$V_{min} = \frac{1}{2 \cdot [U]_{eu}} (1 - ee_0) \quad (1)$$

$$Yield_{max} = \frac{2ee_0}{1 + ee_0} \quad (2)$$



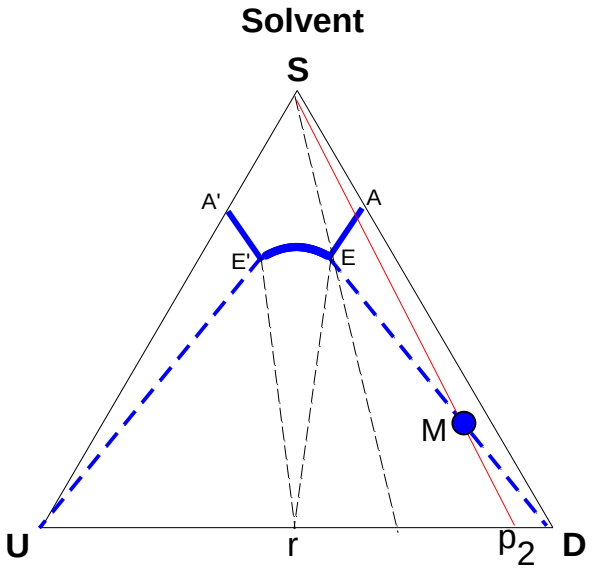
$$Yield_{(at\ V \geq Vmin)} = 1 - \left(2V \cdot [D]_{pure} + \frac{[D]_{eu} - [D]_{pure}}{[U]_{eu}} \cdot (1 - ee_0) \right) \cdot \left(\frac{1}{1 + ee_0} \right) \quad (3)$$

Racemic compound system

(when the starting solid has ee higher than that of eutectic)

$$V_{min} = \frac{1}{2 \cdot [U]_{eu}} (1 - ee_0) \qquad (4)$$

$$Yield_{max} = 1 - \frac{(1 - ee_0)(1 + ee_{eu})}{(1 + ee_0)(1 - ee_{eu})} \qquad (5)$$



$$\text{Yield}_{(\text{at } V \geq V_{min})} = 1 - \left(2V \cdot [D]_{pure} + \frac{[D]_{eu} - [D]_{pure}}{[U]_{eu}} \cdot (1 - ee_0) \right) \cdot \left(\frac{1}{1 + ee_0} \right) \qquad (6)$$

V_{min} represents the volume of solvent (expressed in ml of solvent) to be added to one milligram of solid to reach point M.
$[D]_{pure}$ represents the solubility of desired enantiomer in mg of solid per ml of solvent.

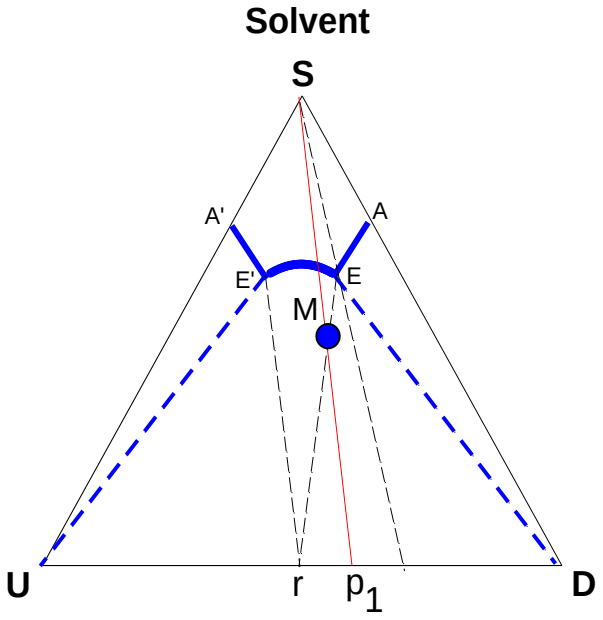
Racemic compound system

(when the starting solid has ee lower than that of eutectic)

$$V_{max} = \left(\frac{ee_0}{[D]_{eu} - [U]_{eu}} \right) \quad (7)$$

$$\text{Yield}_{(\text{at } V=V_{max})} = \frac{ee_0 (1 + ee_{eu})}{ee_{eu} (1 + ee_0)} \quad (8)$$

$$\text{Yield}_{(\text{at } V \le V_{max})} = \frac{[D]_{eu}}{0.5 + 0.5 ee_0} \cdot V \quad (9)$$



V_{max} represents the volume of solvent (expressed in ml of solvent) to be added to 1 mg of solid to reach point M.
ee_0 represents the ee of starting solids whose ee is to be upgraded.
ee_{eu} represents the ee of eutectic point.
$[D]_{eu}$ and $[U]_{eu}$ represent the concentration of desired enantiomer and undesired enantiomer (mg of solids per ml of solvent) at the eutectic point respectively.
V represents the volume of solvent (in ml) added to one mg of solid.

案例分析

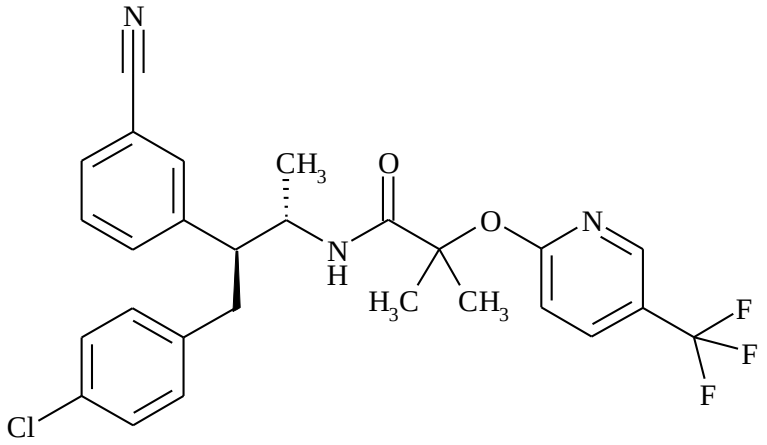
案例分析1: A racemic compound system

(with high ee for eutectic point)

Compound I

Desired enantiomer: (S, S)

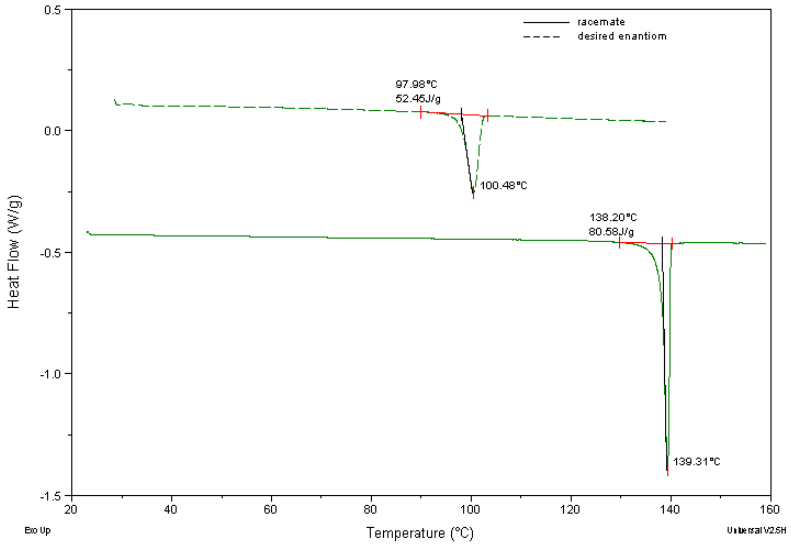
Undesired enantiomer: (R, R)



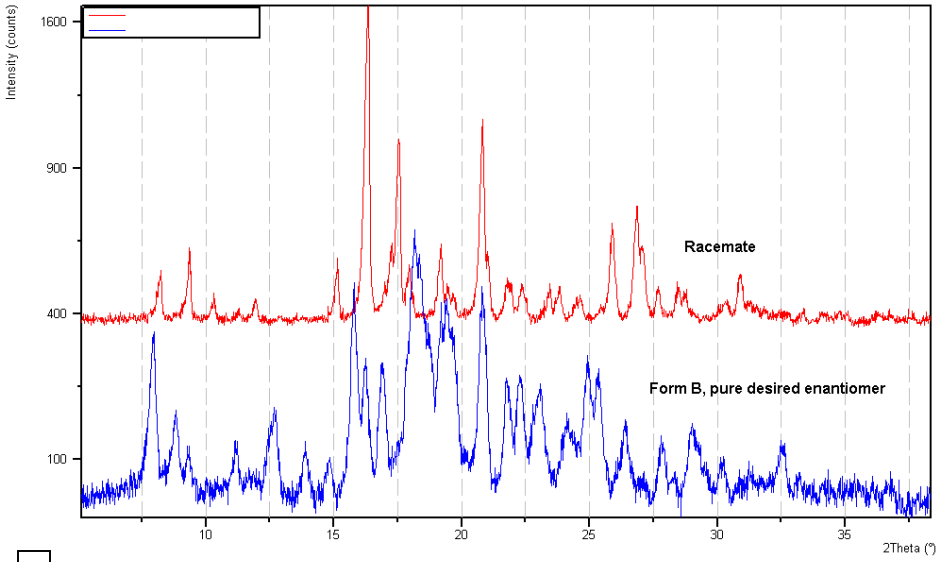
By asymmetric synthesis, API solids with 92-96% ee can be produced, how to upgrade the ee to above 98%?

纯对映异构体 vs. 消旋体的物理性质

Different DSC



Different XRPD



Most likely, the racemate is racemic compound.

To be confirmed by solubility ternary phase diagram...

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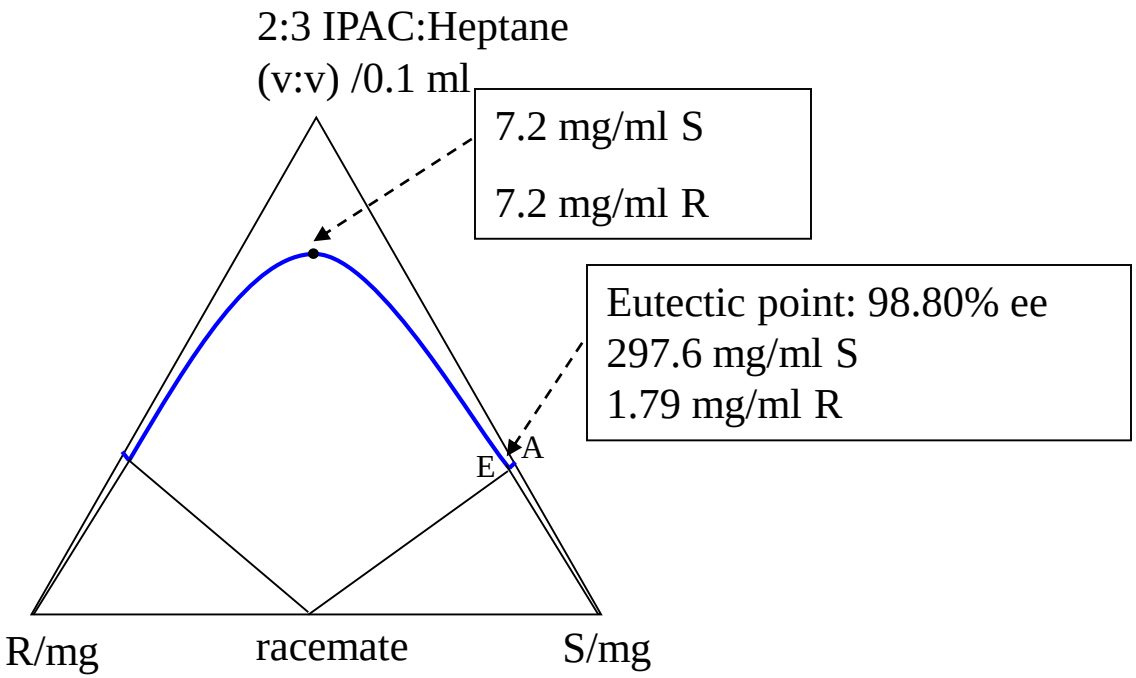
ee upgrade of Compound I Form B



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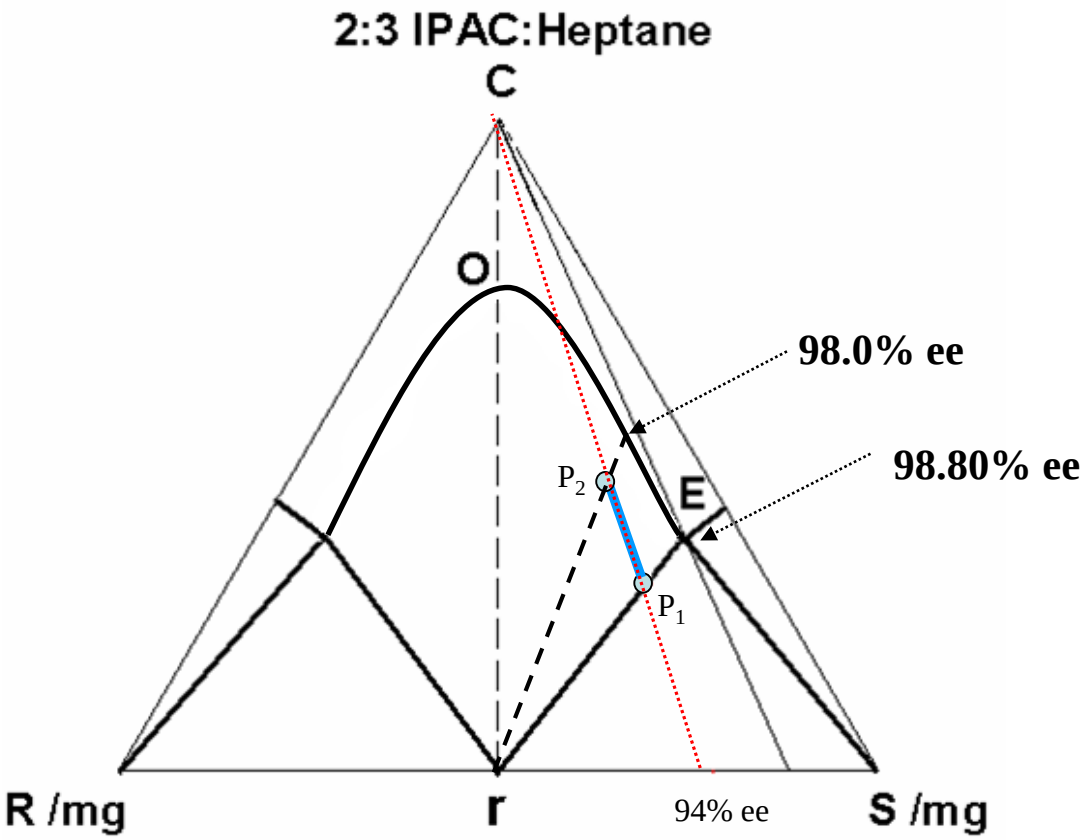
Ternary phase diagram of Form B in 2:3 IPAC:Heptane (v:v) at 25 C



Optimal process to upgrade ee in 2:3 IPAC:Heptane (v:v) at 25 C

Feasibility and strategy:

The ee of eutectic point (98.80%) is higher than that of starting solids (~ 94%-96%). Therefore, the ee can be upgraded to a maximum value of 98.80% in the supernatant by dissolution.

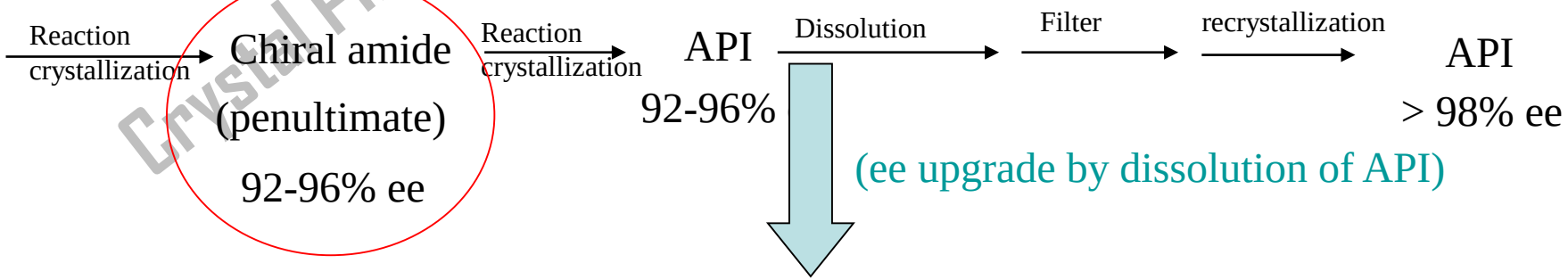
Determine the optimal solvent/solid ratio and the corresponding yield:

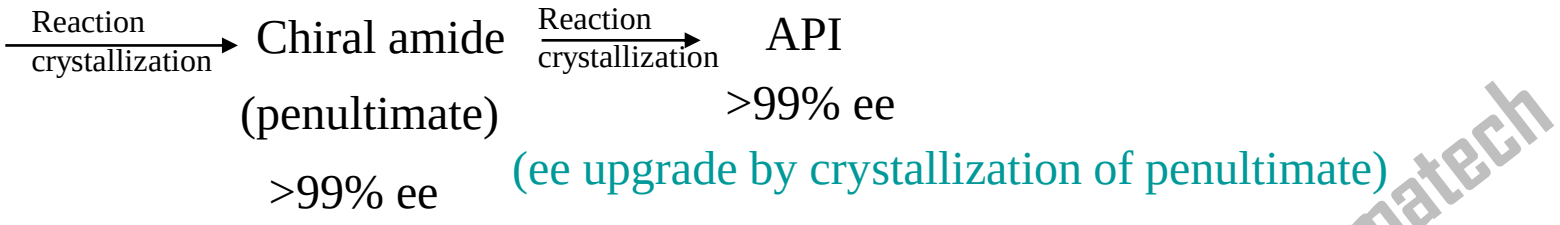
$$V_{max} = \left(\frac{ee_0}{(D)_e - (U)_e} \right) = 0.00338\ ee_0$$

$$\text{Yield}_{V \le V_{max}} = \frac{297.6}{0.5 + 0.5 ee_0} \cdot V$$

Based on the above equations, given a starting mixture with ee of 94%, V_{max} is 0.00318 and yield is 97.6% at $V=V_{max}$. This means by adding 0.00318 ml of 2:3 (v:v) IPAC: Heptane solvent to 1 mg of starting enantiomeric mixture with 94% ee at 25.0 C, at equilibrium, the ee will be upgraded to 98.80% with a yield 97.6%.

中间体新晶型的发现改变了手性分子的结晶提纯工艺





ee upgrade in chiral amide crystallization step was achieved when a new anhydrous crystalline form of chiral amide enantiomer was discovered. Prior to the discovery of the anhydrous crystalline form, chiral amide crystallized as a mixture of MTBE solvate and amorphous, with no ee upgrade.

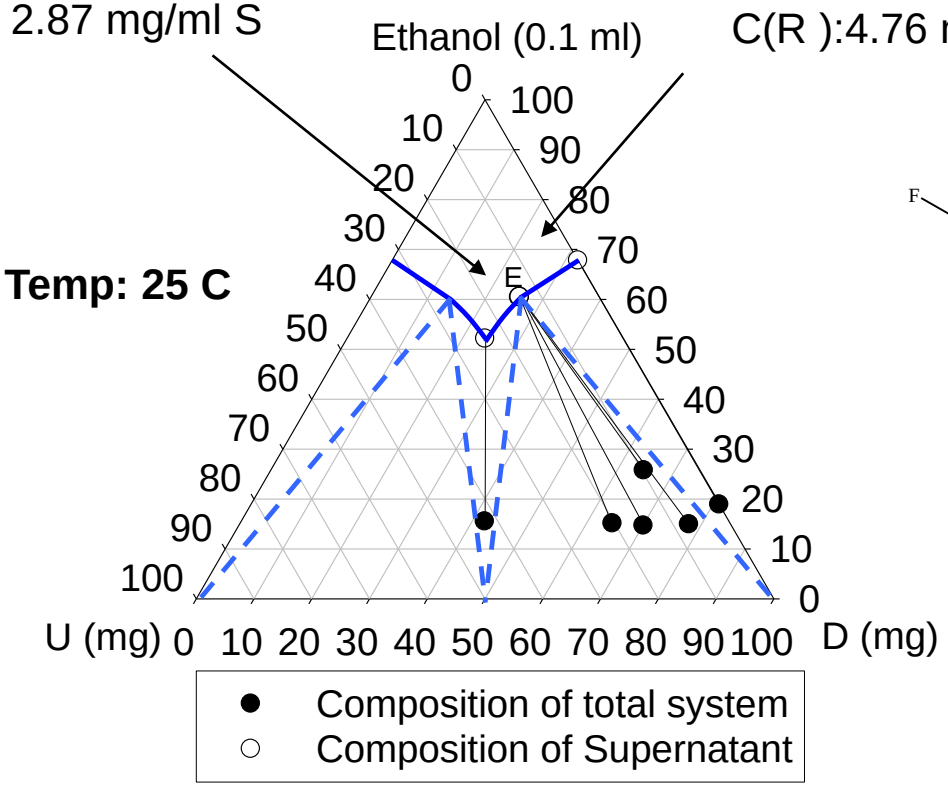
New ee upgrade process was implemented in the manufacturing process.

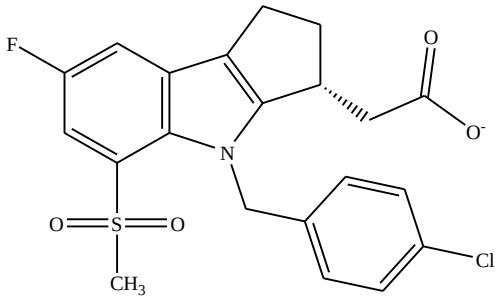
案例分析2：A racemic compound system

(with low ee for eutectic point)

ee upgrade of Compound II DIPA Salt

Eutectic point: ee: 30.3%
C(R): 5.36 mg/ml R
C(S): 2.87 mg/ml S





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Optimal process to upgrade ee of DIPA Salt in ethanol at 25 C

Feasibility and strategy:

ee of eutectic point is 30.3%, lower than that of starting solids (92% to 96% ee). Therefore, ee can be upgraded to 100% in the solid phase by adding V_{min} or more amount of solvents to the solids.

Determine the optimal solvent/solid ratio and the corresponding yield:

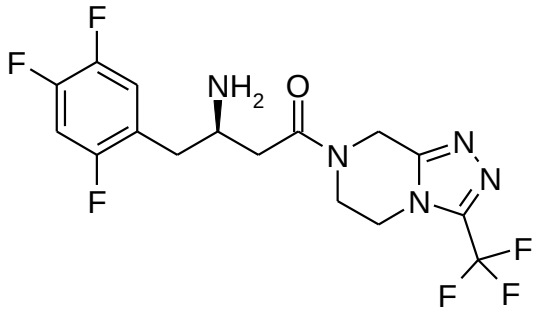
$$V_{min} = \frac{1}{2 \cdot [U]_{eu}}(1 - ee_0) = 0.174\ (1\text{-}ee_0)$$

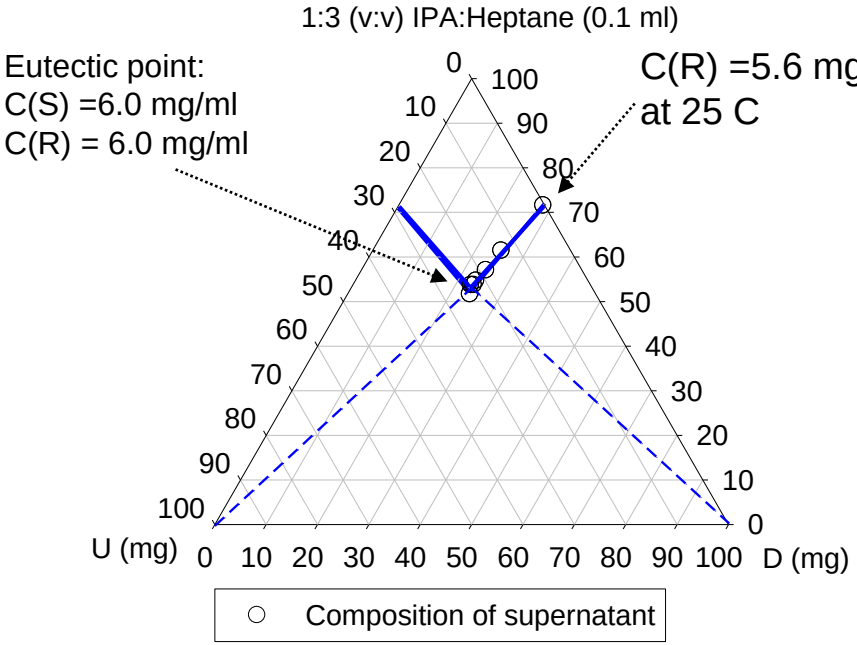
$$Yield_{max} = 1 - \frac{(1-ee_0)(1+ee_{eu})}{(1+ee_0)(1-ee_{eu})}$$

Based on above, given a starting enantiomeric mixture with 94% ee, V_{min} can be determined to be 0.010 and the yield is 94.4 % at $V=V_{min}$. This means that by adding 0.010 ml of EtOH to 1 mg of starting enantiomeric mixture with 94% ee at 25.0 C, at equilibrium, the ee of D will be upgraded to 100% in the solid phase with a yield of 94.4 %.

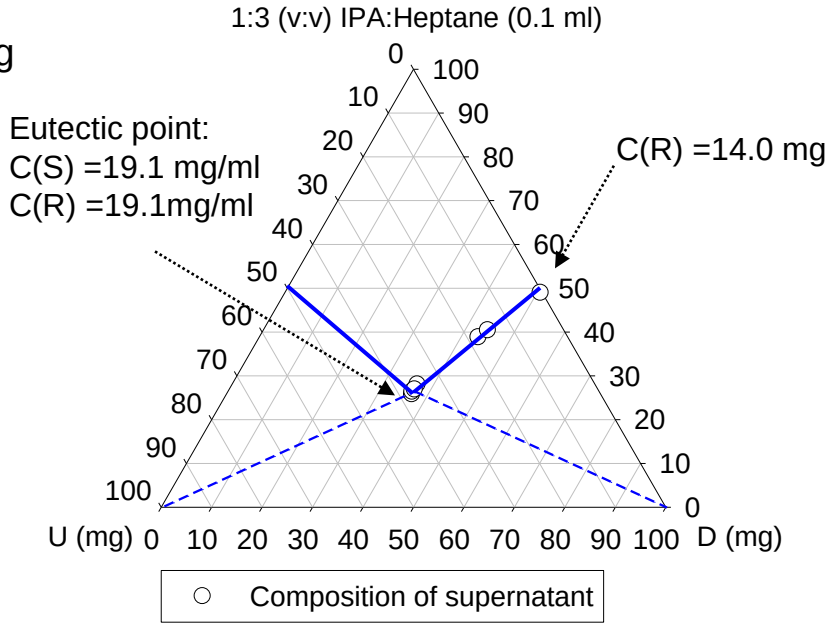
案例分析三: A conglomerate System

ee upgrade of *compound III* freebase





25.0 C



45.0 C

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Optimal process to upgrade ee of Compound III freebase in 1:3 (v:v) IPA:Heptane at 25 C or 45 C

Feasibility and strategy:

ee can be upgraded to 100% in the solid phase by adding V_{min} or more solvents to the starting solid.

Determine the optimal solvent/solid ratio and the corresponding yield:

$$V_{min} = \frac{1}{2 \cdot [U]_{eu}} (1 - ee_0) = 0.113\ (1\text{-}ee_0)$$

$$\text{Yield}_{max} = \frac{2ee_0}{1 + ee_0}$$

Based on the TPD at 45 C, the solubility of pure conglomerate is 14.0 mg of R and 14.0 mg of S per ml of solvent and that of pure enantiomer R is 10.4 mg per ml of solvent. Therefore, similar V_{min} and yield at $V \geq V_{min}$ can be calculated based on equations (7) and (8).

非对映异构体的三元相图

Gibbs Phase Rule:

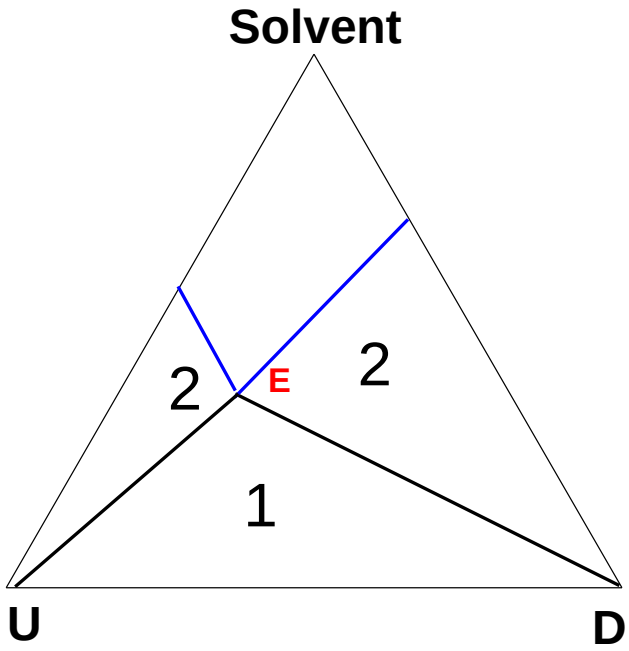
$$\text{var}\,iance = C - \Pi + 2$$

C=3

Fix Pressure

$$\text{var}\,iance = C - \Pi + 1$$

Variance in Bold



The ternary phase diagram is not symmetric due to different solubility of two diastereomers.

Define the optimal ratio and predict the yield based on the ternary phase diagram

$$V_{min} = \frac{1}{2 \cdot [U]_{eu}} (1 - de_0)$$

$$\text{Yield}_{(at\ V \geq Vmin)} = 1 - \left(2V \cdot [D]_{pure} + \frac{[D]_{eu} - [D]_{pure}}{[U]_{eu}} \cdot (1 - de_0) \right) \cdot \left(\frac{1}{1 + de_0} \right)$$

$$\text{Yield}_{(at\ V=Vmin)} = 1 - \frac{(1 - de_0)(1 + de_{eu})}{(1 + de_0)(1 - de_{eu})}$$

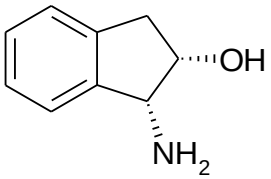
Yield is defined as the ratio of desired diastereomer in the solid phase to the desired diastereomer in the total system.
de represents the diastereomeric excess.

案例分析四: 非对映异构体的手性提纯

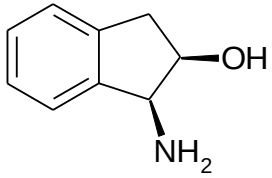
Compound IV API

API has three chiral centers.

CAI



(1R,2S)-(+)-cis-



(1S,2R)-(-)-cis-

MW=149.19

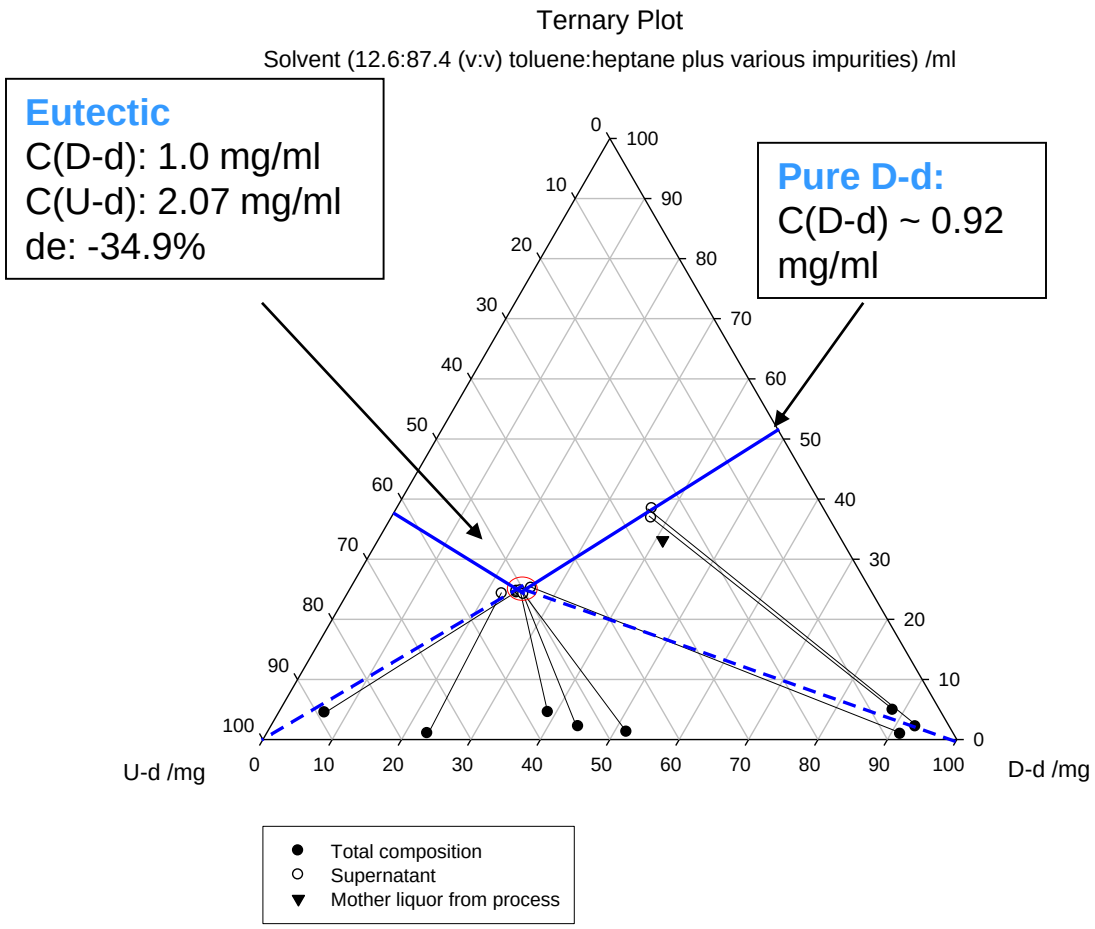
Four possible isomers of compound IV CAI salt

API(R,R,R)-CAI(R,S): **D-d**
API(S,S,S)-CAI(R,S): **U-d**
API(R,R,R)-CAI(S,R): **D-u**
API(S,S,S)-CAI(S,R): **U-u**

Assuming the crude free acid has ee ~ 92% and CAI salt has ee ~ 99%, then upon salt formation, there will be:

D-d: 95.52%
U-d: 3.98%
D-u: 0.48%
U-u: 0.02%

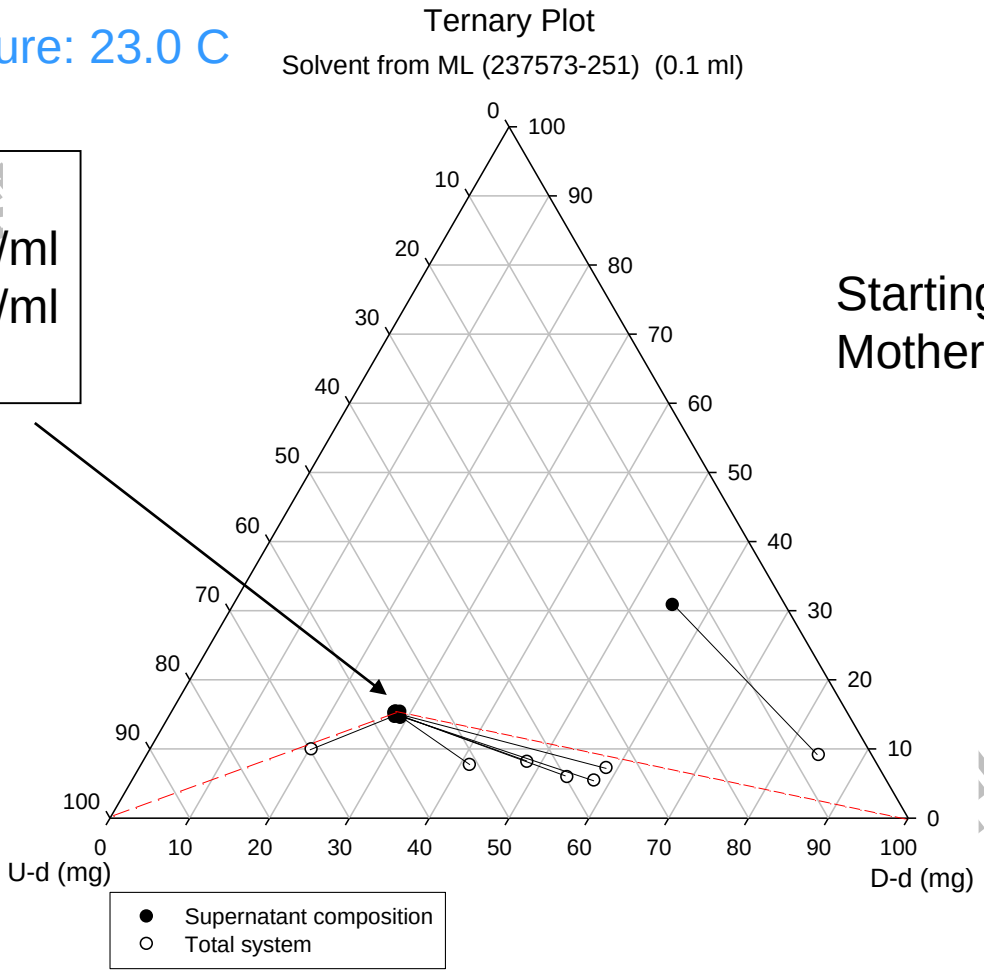
Temperature: 23.0 C



Starting Solvent:
Washed mother liquor (22.8% de, 2.02 g/l)

Temperature: 23.0 C

Eutectic:
D-d: 19.0 mg/ml
U-d: 37.7 mg/ml
de: -33.04%



Starting solvent:
Mother liquor (61.5% de, 17.7 g/l)

通过结晶提纯的可行性和策略

- Eutectic de of the diastereomeric system is relatively independent of the solvent composition/impurity concentration and it is very low ~ -34%.

- Low eutectic de suggests thermodynamically the undesired diastereomer can be rejected in the supernatant and pure desired diastereomer can be obtained in the solids upon crystallization.

- The optimal solvent/solid ratio and the yield can be pre-defined and calculated.

总结

- 三元相图是理解手性药物结晶提纯工艺的强有力的工具。
- 根据对映异构体或者非对映异构体系统的三元相图，一个手性药物分子通过结晶或者溶解的方法得到提纯的可行性可以立刻确定，同时三元相图可以协助我们找到最优化的结晶工艺条件。
- 手性药物的手性提纯可以在原料药最后一步的结晶工艺中实现，也可以通过中间体的结晶工艺实现。有时通过对手性药物中间体的结晶，可能可以将一个很难提纯的手性药物通过很简单的方法得以提纯。
- 手性药物的结晶提纯有时会受到结晶动力学的很大影响，不要忽略。