# 晶云药物第二届晶型专题技术培训



# 如何展开药物多晶型研究？

**主讲人：**陈敏华博士，首席执行官
**Crystal Pharmatech**
苏州晶云药物科技有限公司
Email：sales@crystalpharmatech.com
电话：0512-69561921



Crystal Pharmatech
您的药物晶型研究和固态研发专家

# 提纲

- 什么是晶体和多晶型？
- 多晶型研究对药物开发的重要性
- 如何筛选和选择药物新晶型
- 多晶型的物理分析手段
- 实战讨论

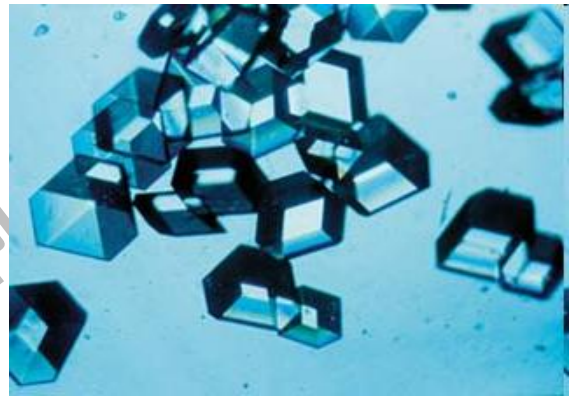
1. 如何确定两个无水晶型的热力学稳定性关系？
2. 如何进行晶型检测和混晶含量分析？
3. 如何制备球状晶体？

# 什么是晶体和多晶型？

# 什么是晶体？

- 晶体
  - a solid material whose constituent atoms, molecules, or ions are arranged in an orderly repeating pattern extending in all three spatial dimensions

- 药物晶体
  - Most drugs are developed as crystalline
    - Stability
    - Processability
    - IP protection



Insulin crystals grown in outer Space: From NASA

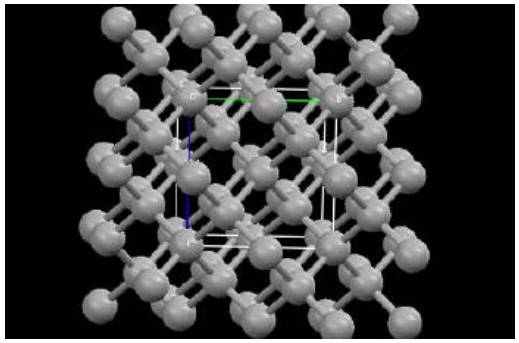
# 什么是多晶型？

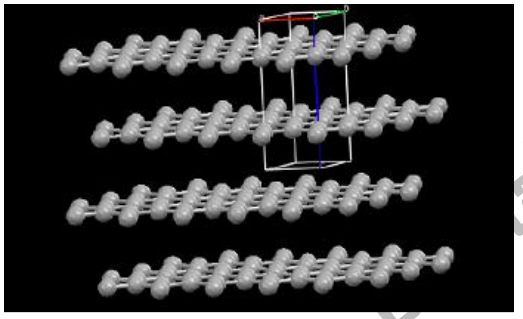
- Polymorphism（多晶型现象）
  - The ability of a solid material to exist in more than one form or crystal structure.

- Polymorphs （多晶型）
  - Solid phase
  - Same chemical composition
  - Different molecular arrangements

# 日常生活中的多晶型现象

- 金刚石和石墨

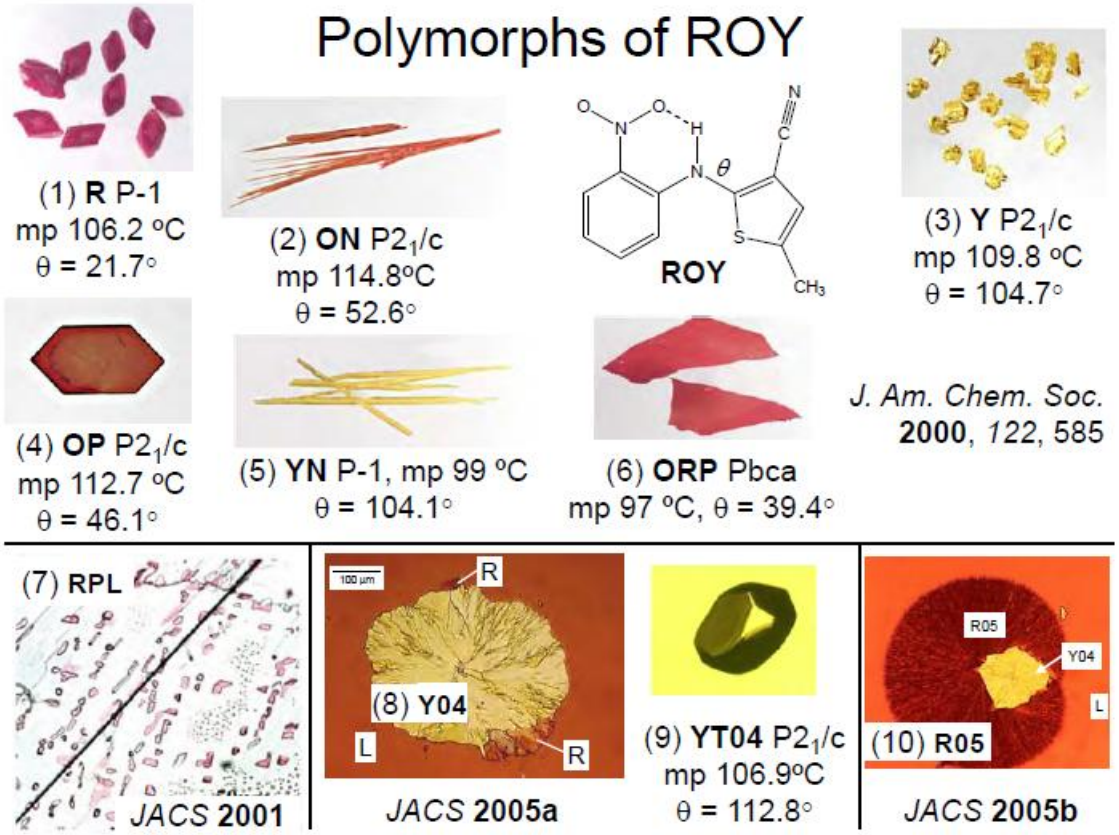




# 日常生活中的多晶型现象

- 巧克力（可可脂）
  - 可可脂是多种不同类型的甘油三酸酯组成的混合体，因此，从液态→固态时，随不同的温度条件，会出现多种晶型。可可脂晶型有γ、α、β’、β四种，其中γ、α、β’晶型均不稳定，它们的形成不利于巧克力的质量提高，β晶型稳定，β晶型在巧克力制品中数量越高，成品质量越稳定。调温的目的就是使物料产生最高比例的β晶型。

# ‘著名’的多晶型化合物ROY




*Courtesy of Professor Lian Yu, University of Wisconsin, Madison*

# 阿司匹林的多晶型现象

C&EN
CHEMICAL & ENGINEERING NEWS

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LATEST NEWS

NOVEMBER 17, 2005

## Aspirin Polymorph Found

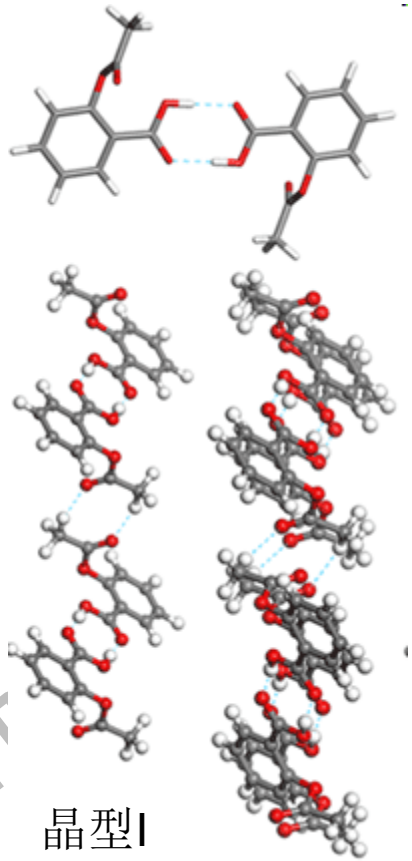
### Second crystal form was first predicted to exist in the 1960s

Celia Henry Arnaud

In the late 1960s, there were indications that aspirin might have a second crystalline form, but it continued to escape detection. Now, Michael J. Zaworotko, chemistry professor at the University of South Florida, Tampa, and coworkers at South Florida and TransForm Pharmaceuticals, Lexington, Mass., have found this elusive polymorph (*J. Am. Chem. Soc.* 2005, *127*, 16802).

This second form was obtained during cocrystallization experiments with aspirin and other compounds. Form II is kinetically stable at 100 K, but it converts back to form I at ambient conditions. Both forms contain a hydrogen-bonded carboxylic acid dimer (shown). But they differ in the way the dimers adjoin one another through their acetyl groups: The familiar form I assembles into dimers of dimers, whereas the new polymorph forms chains of dimers.

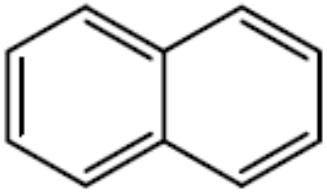
Chemical & Engineering News
ISSN 0009-2347

晶型I

晶型II

# “不存在”多晶型现象的分子



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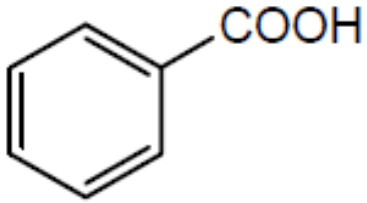
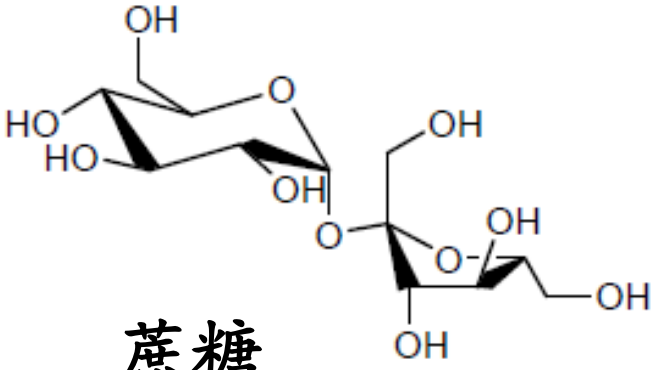



苯甲酸




蔗糖

# 晶型研究在药物开发中的重要性

# 药物多晶型研究的重要性

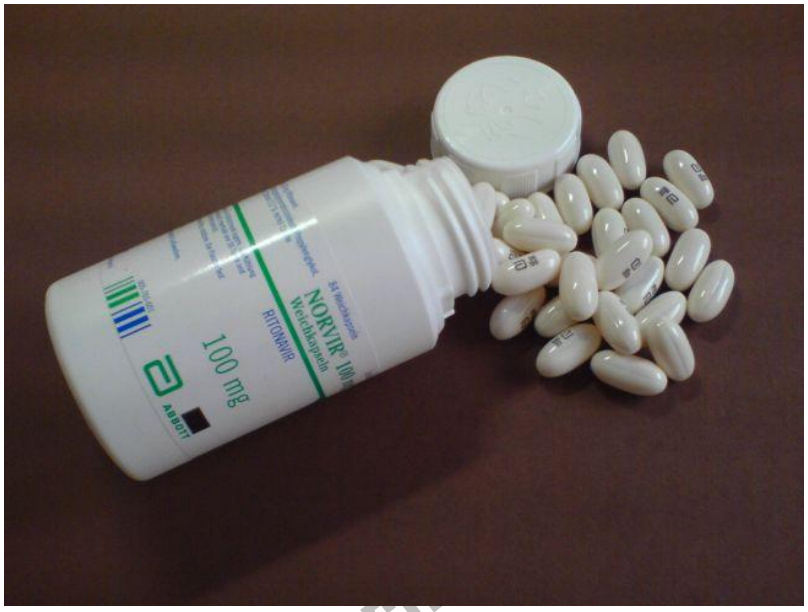
## Abbott Reports Production Problems For Norvir

Article | 28 July 1998

Print This ShareThis

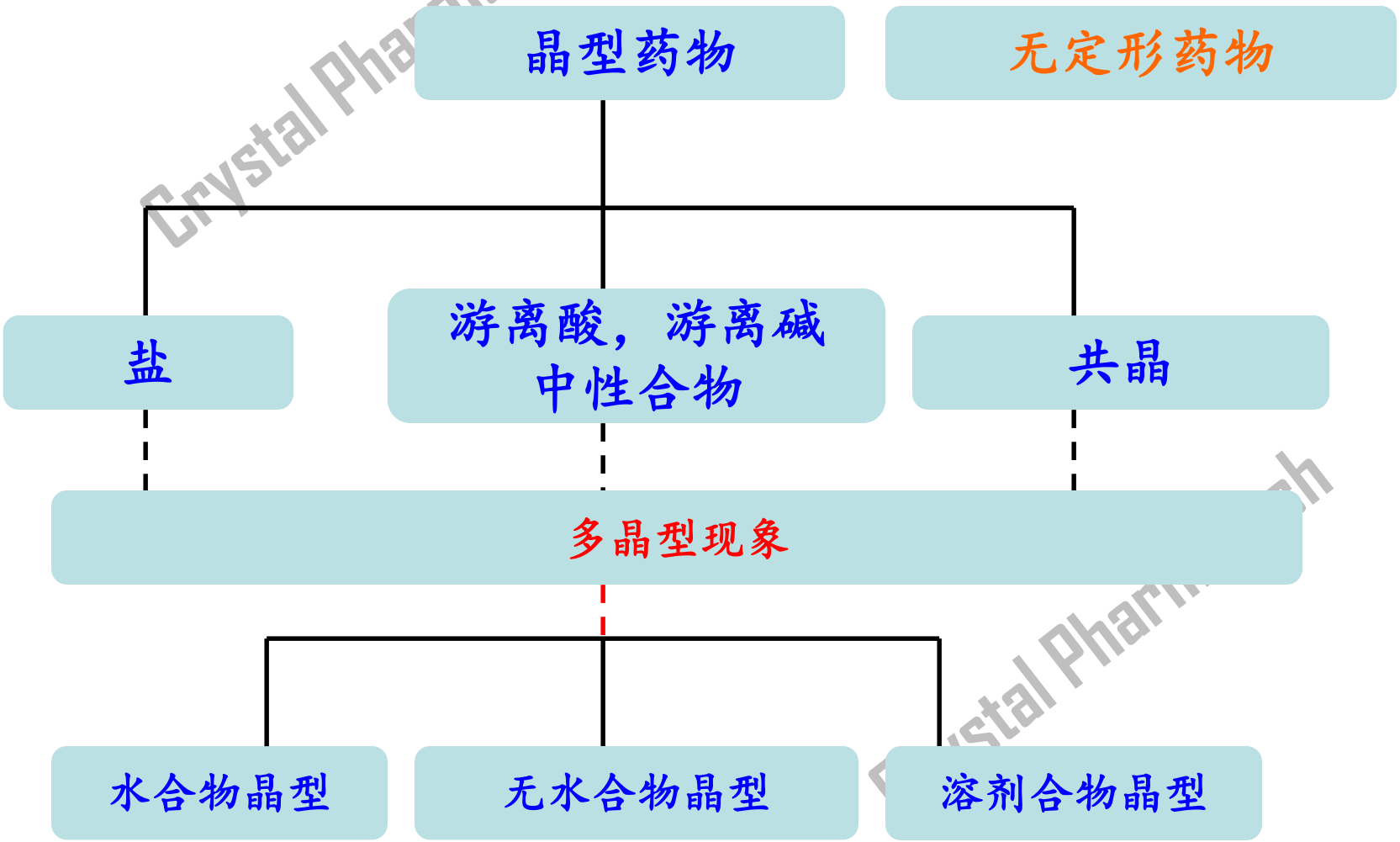
**Abbott Laboratories has reported that it is having difficultiesmaintaining production of its HIV protease inhibitor Norvir (ritonavir). The problems are related to its capsule formulation of the antiretroviral.**

"We have encountered an undesired formation of a Norvir crystalline structure that affects how the capsule form of Norvir dissolves," commented Arthur Higgins, senior vice president for pharmaceutical operations at Abbott. Although the problem has been identified, to date the company has been unable to come up with a solution.




**公司**：美国雅培
**药名**：利托那韦（Norvir）
**剂型**：胶囊
**教训**：1998年，晶型发生变化，药品退出市场，直接经济损失上亿美元

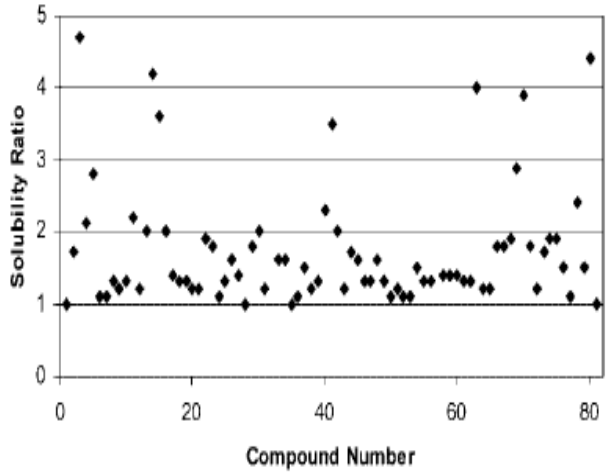
# 固态药物的分类



# 晶型研究的重要性

- 同一个药物的不同晶型具有不同的物理和化学性质，这些性质会影响药物的：
  - 物理和化学稳定性(Stability)
  - 溶解度，溶出率和生物利用度(Bioavailability)
  - 工艺可开发性(Processability)

Solubility ratios for polymorphs (n=81).




*Pudipeddi and Serajuddin., Journal Of Pharmaceutical Sciences, Vol. 94, No. 5, May 2005*

**我们需要选择一个最适合开发的药物固相进行开发**

# FDA指南

- “Polymorphic forms of a drug substance can have different chemical and physical properties, including melting point, chemical reactivity, apparent solubility, dissolution rate, optical and mechanical properties, vapor pressure, and density. … Thus, polymorphism can affect the quality, safety, and efficacy of the drug product.”

*Guidance for Industry by Office of Generic Drugs (OGD), Center for Drug Evaluation and Research (CDER) of the FDA.*

# FDA指南

- “We recommend that ANDA applicants investigate whether the drug substance in question can exist in polymorphic forms. Polymorphic forms in the context of this guidance refer to crystalline and amorphous forms as well as solvate and hydrate forms….”

*Guidance for Industry by Office of Generic Drugs (OGD), Center for Drug Evaluation and Research (CDER) of the FDA.*

# 原料药开发中的晶型问题

- 怎样开发一个结晶工艺得到单一晶型？
- 如何开发和放大仿制药亚稳态晶型的结晶工艺？
- 如何控制药物晶型的颗粒度大小和形态？
- 如何避免结晶过程中的杂质和溶剂残留？
- 如何应对晶体的静电现象和流动性差的问题？
- 晶型纯度的定量和定性分析有哪些手段？
- 如何展开药物晶型的质量研究？
- 晶型的控制对药物稳定性，比色度，溶出度的影响？
- 如何确定晶型的热力学和动力学稳定性？
- 如何提高仿制药的仿制精度？
- 在没有单一晶型对照品的情况下，混晶的判断？



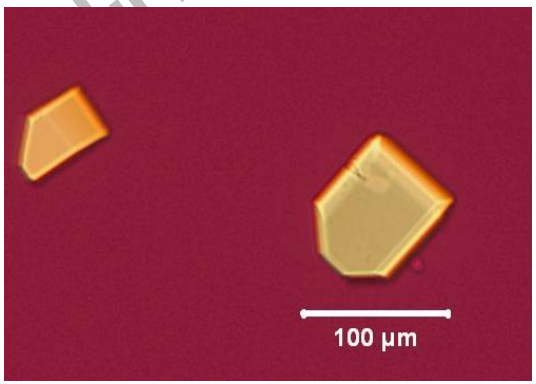
# 制剂开发中的晶型问题

- 制剂中药物晶型的确认以及单一晶型及混晶的确认和分析？
- 如何监控制剂中晶型的相互转变？
- 晶型的粒径分布对溶出率，生物利用度，及制剂工艺的影响？
- 如何预测和评估晶型在制剂中的化学和物理稳定性？
- 晶型在湿法制粒过程是否稳定？
- 如何预测和评估无定形药物在制剂中的重结晶风险？
- 如何确定盐类晶型在制剂中是否发生歧化反应？
- 如何判定晶型对药物溶出度，生物利用度的影响？
- 晶型在制剂中的结块现象如何研究和解决？
- 如何判定晶型的生物等效性？

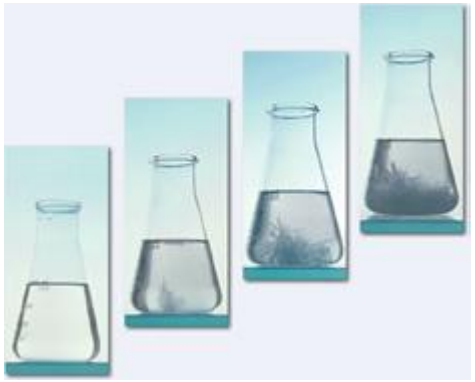
# 如何筛选和选择药物新晶型

# 如何找到一个药物的新晶型？

- "The number of polymorphs discovered for a chemical is proportional to the effort/time spent", by Walter McCrone




单晶培养

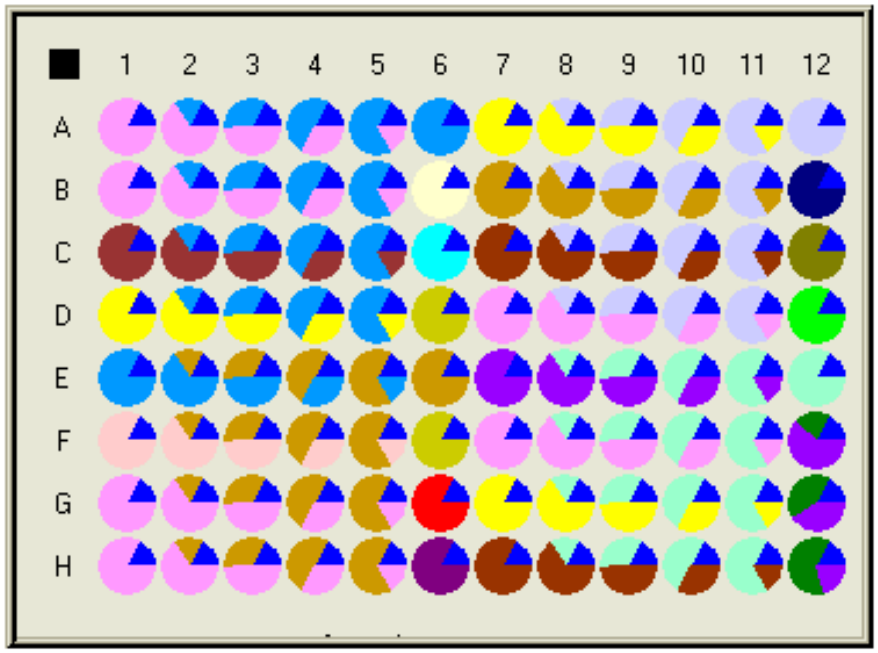


手动筛选

机器筛选

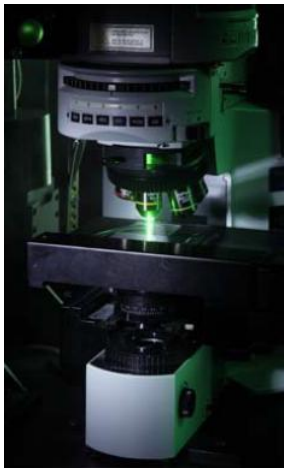
- Discovered in other experiments (unintentionally)
  - During storage, formulation development, process scale-up, manufacturing etc.
- Molecular modeling

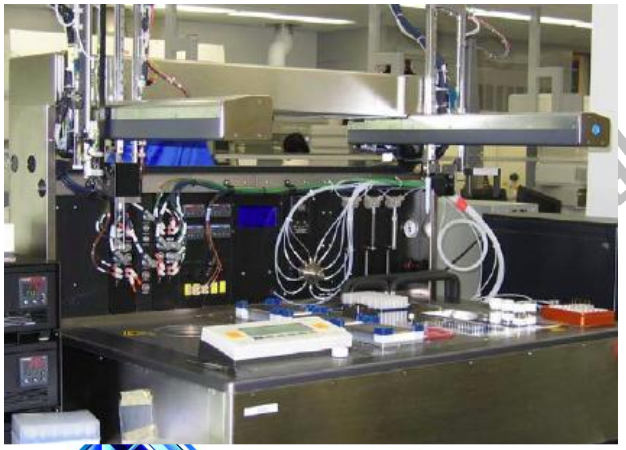
# 药物晶型筛选中的溶剂设计

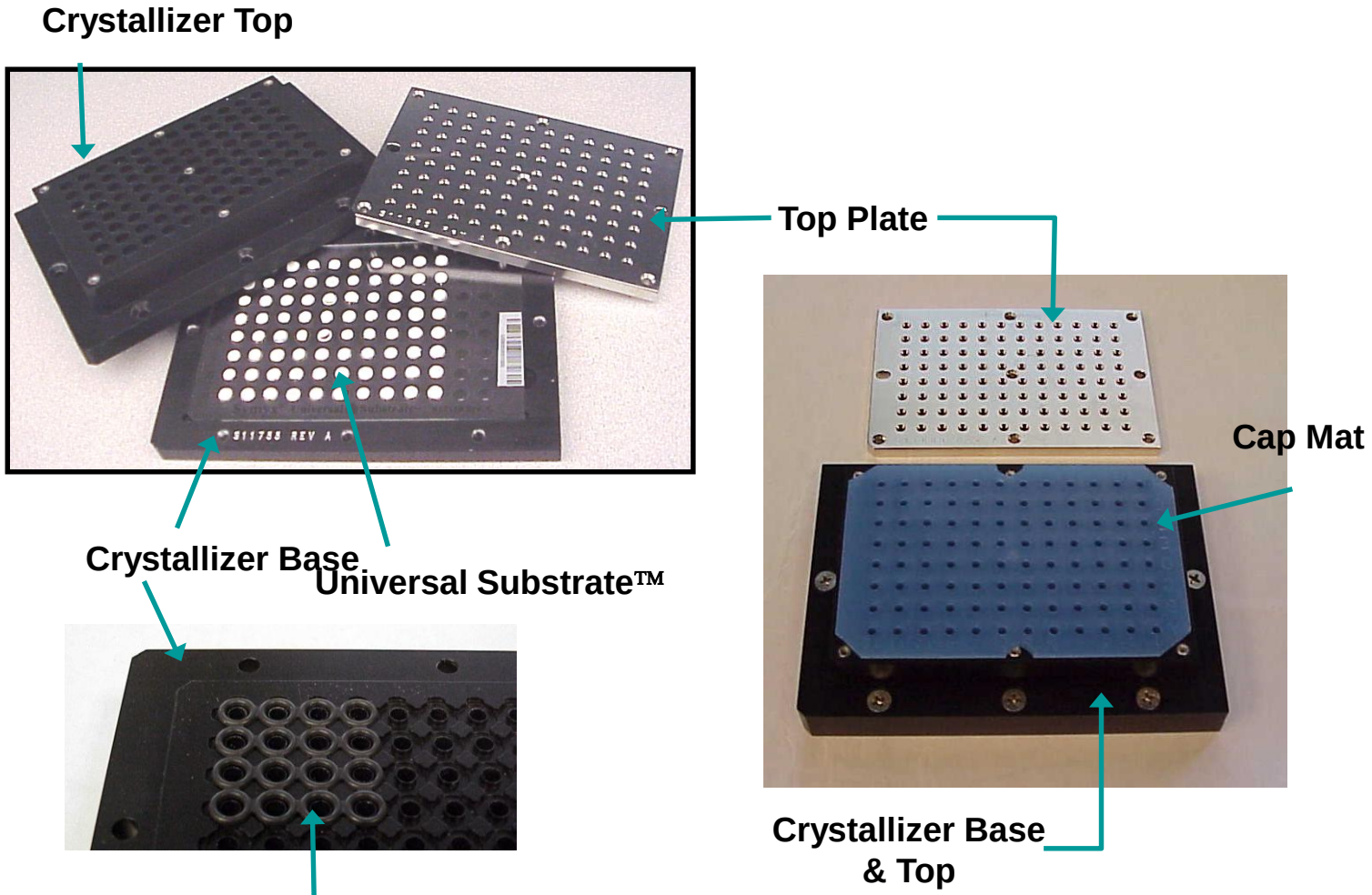

在晶云药物，晶型筛选的溶剂会根据药物分子的特性进行优化设计

# 高通量晶型筛选常用仪器

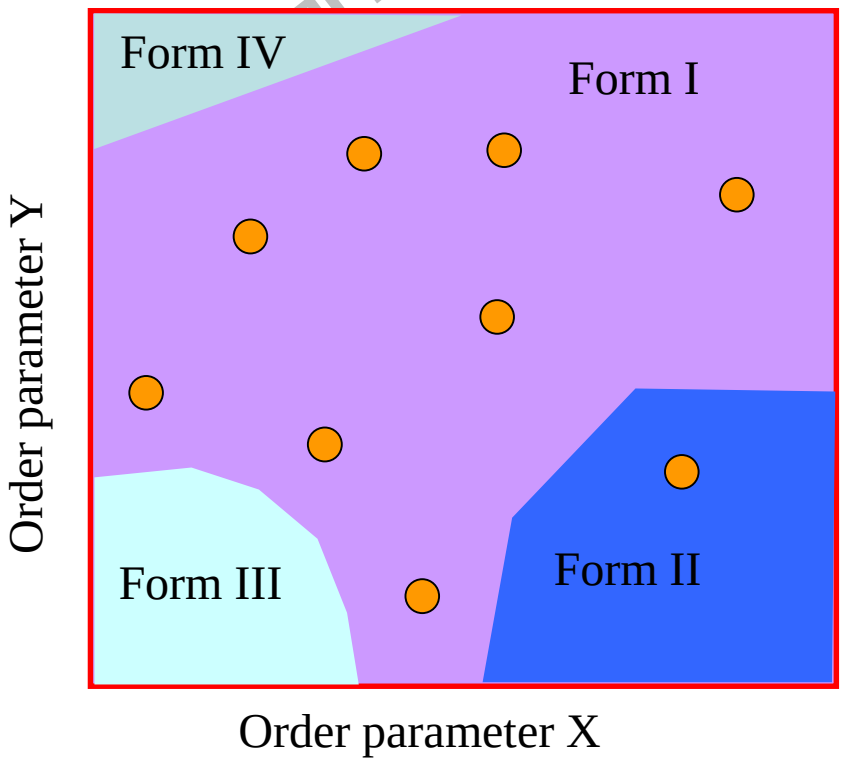


# 高通量晶型筛选的常用设备

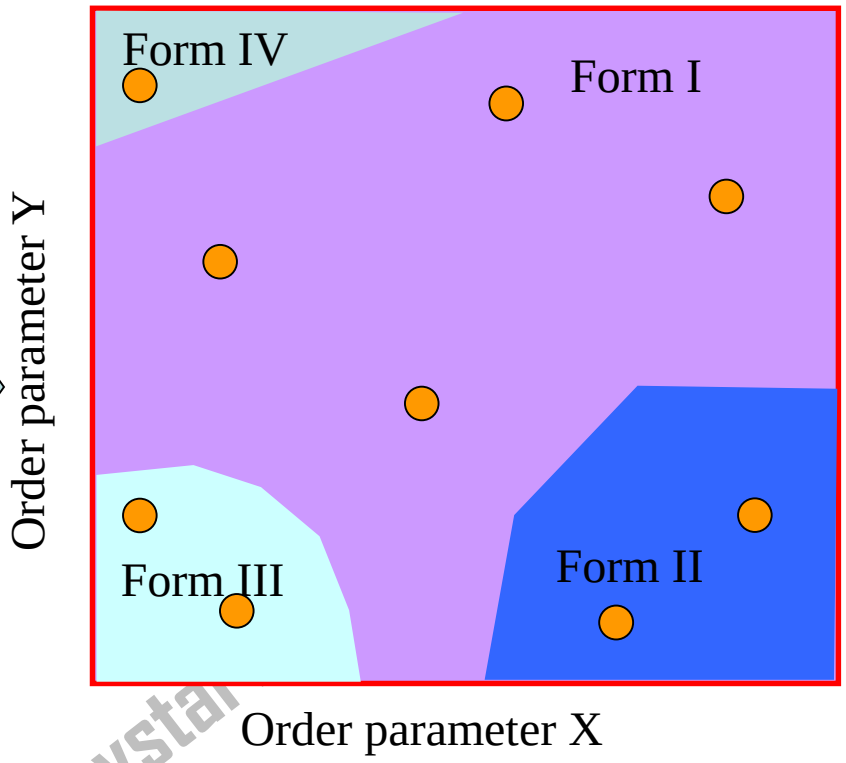


# 优化晶型筛选的策略

传统筛选




晶云药物筛选策略：
提高找到所有晶型的可能性

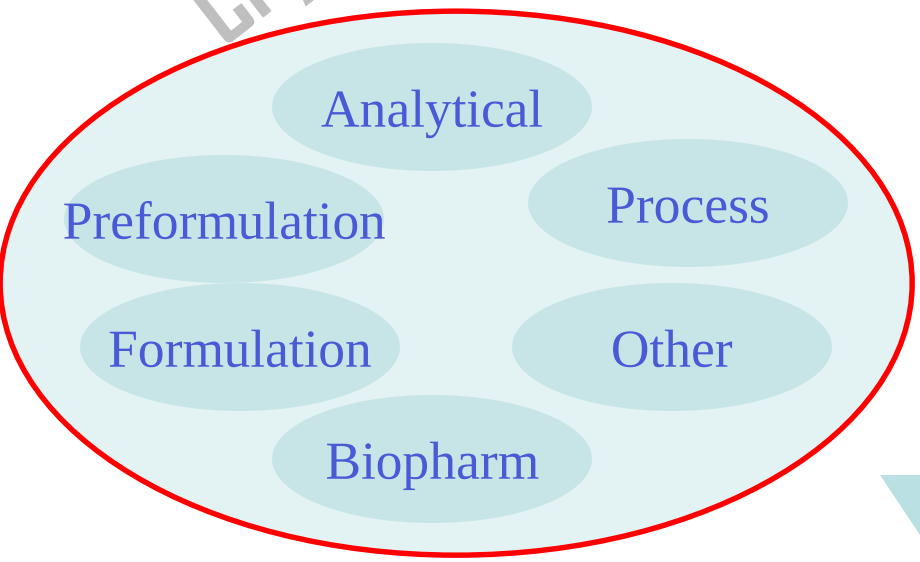



关键点：如何在药物用量，筛选时间和实验数量不变的情况下，合理选择实验空间，提高找到所有晶型的可能性。

# 如何选择一个最佳的晶型进行开发？



Polymorphs Discovered
发现所有可能的晶型




- Stability (chemical & physical)
- Bioavailability (Solubility & Dissolution)
- Processability (API, Formulation)

**Desired Polymorph**
选择出最佳的晶型进行开发

# 晶型的专利问题

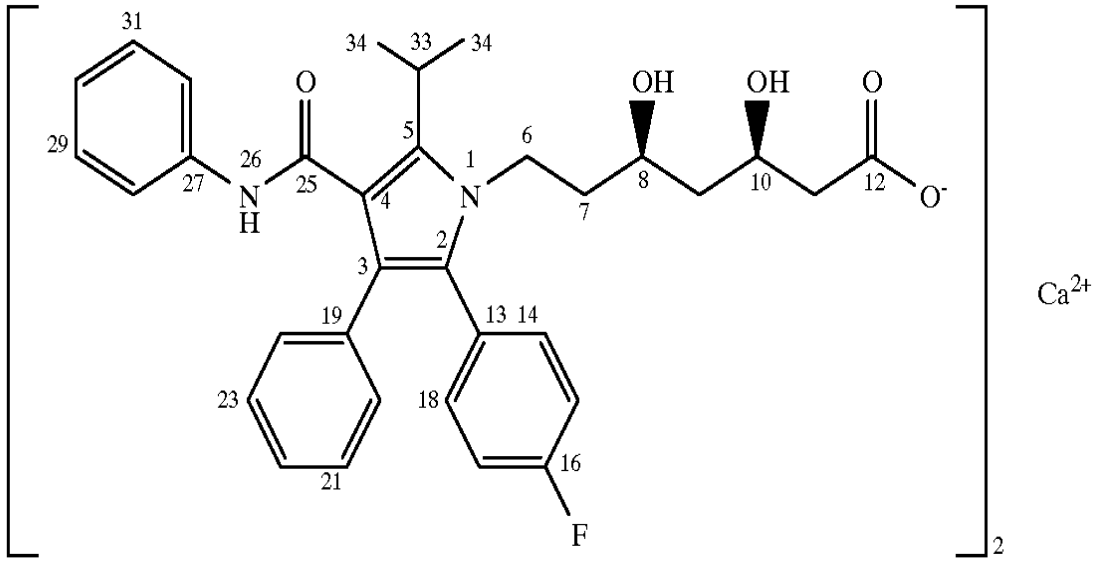
# 申请晶型专利保护的重要性

- 创新药公司: a way to extend the life of its intellectual property right
- 仿制药公司: a way to enter the game early:
  - prepare to enter generic market without undue delay;
  - enjoy independently protectable IP right

# 申请专利基本要求

- Patentable subject matter (could vary depending on country);
- Novelty (at least some aspect is new);
- **Non-obviousness** (U.S.), inventive step (EP), inventiveness (China); and
- Utility (U.S.), industrial application (EP), practical applicability (China).

# 申请专利策略：
# 阿托伐他汀案例分析




**Atorvastatin Hemi-Calcium Salt**
**Sales > $ 12B/yr**

# 阿托伐他汀专利案例分析

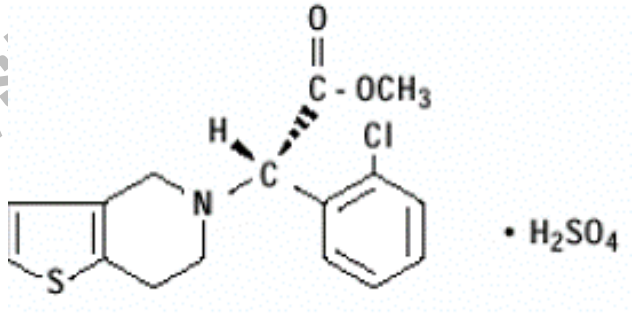
**Atorvastatin (Pfizer):**

- US Pat. No. 4,681,893: original patent on composition of matter (expired in **March 2010**)
- US Pat. No. 5,273,995: hemi-calcium salt of the (R)-enantiomer ("**Lipitor**") (expired in **June 2011**)
- US Pat. No. 5,969,156: crystalline hydrate Forms I, II, IV (to expire in **2014**)
- US Pat. No. 6,121,461: Form III (to expire in **2014**)
- US Pat. No. 6,605,729: claims Crystalline Forms V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, XVII, XVIII, and XIX (to expire in **2022**)

# 不同层次的专利保护

- New Chemical Entity
- Enantiomer
- Salt Form
- Crystal Form (Polymorph)
- Formulation
- Process

# Sanofi-Synthelabo v. Apotex, Inc. 470 F.3d 1368 (Fed. Cir. 2006)




硫酸氢氯吡格雷

U.S. Pat. No. 4,847,265

(bisulfate salt)

**Prior Art:**

U.S. Pat. No. 4,529,596

(free base)

Clopidogrel: Plavix® (bisulfate salt)

2010 Global Sale: $ 9.4 B/yr

# Sanofi-Synthelabo v. Apotex, Inc. (cont'd)



**District Court Findings:**

- The '596 patent does not describe clopidogrel bisulfate.
- The '596 patent does not enable a person of ordinary skill in the art to make clopidogrel bisulfate without undue experimentation.

# Sanofi-Synthelabo v. Apotex, Inc. (cont'd)

- **Enantiomer Not Obvious** over disclosure of the racemate in the prior art due to the unexpected properties of clopidogrel bisulfate:
  - high pharmacological activity
  - low toxicity
- **Salt Not Obvious** because extensive experimentation was required to arrive at that particular compound (evidence: a named inventor tested 20 different salts before discovering that bisulfate had the most desirable properties)

# 晶型的固态分析方法

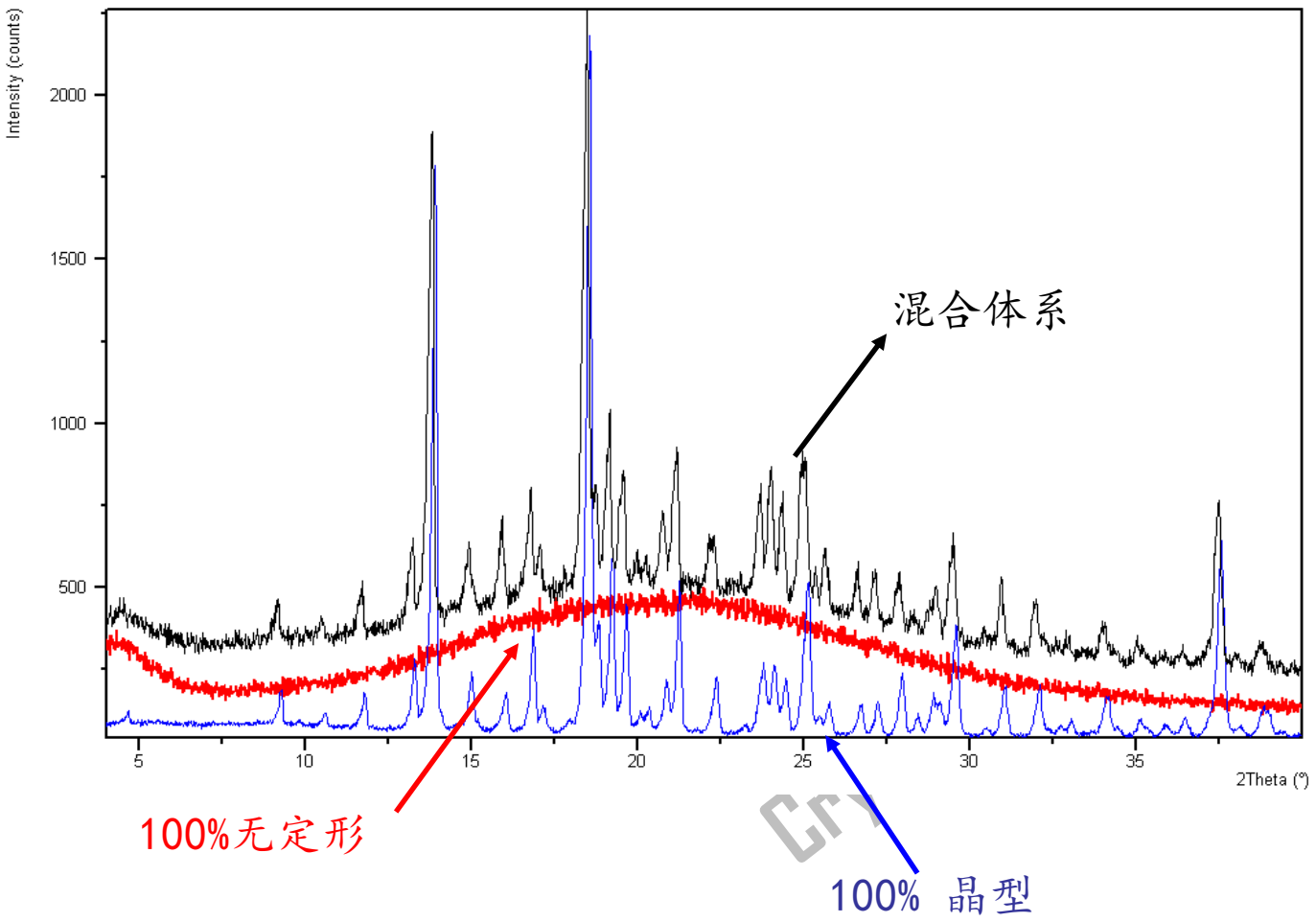
# 固态表征方法

*All variants of spectroscopy, microscopy, scattering etc. are used*

*Structure, order and dynamics of the solid state probed*

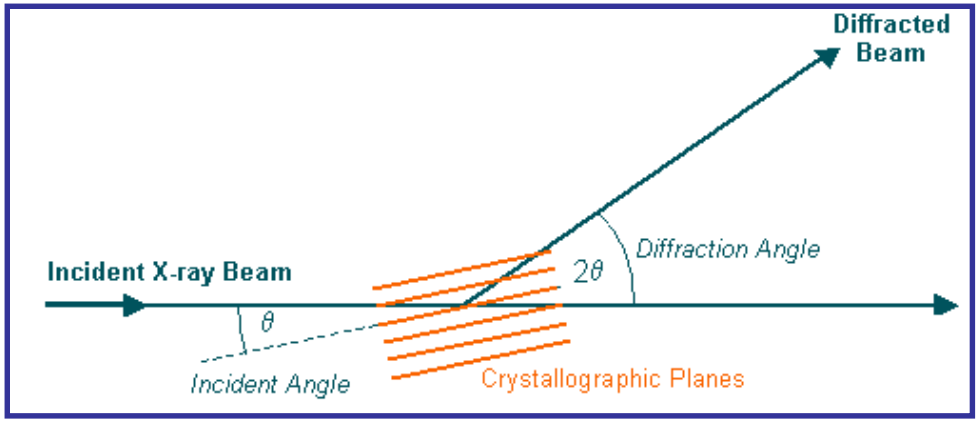
| | |
|---|---|
| Spectroscopy | **ssNMR**, IR, **Raman**, Terahertz… |
| Microscopy | SEM, Optical, AFM… |
| Scattering | **X-ray**, **Laser**, … |
| Thermal analysis | **DSC**, **TGA**, Microcalorimetry, DMA, DEA… |
| Physio-Mechanical analysis | Bulk density, Solubility, Surface area, PSD… |
| Computer Modeling | Quantum, DFT, MD/MC, … |

# 晶型和无定形的XRPD图谱对比



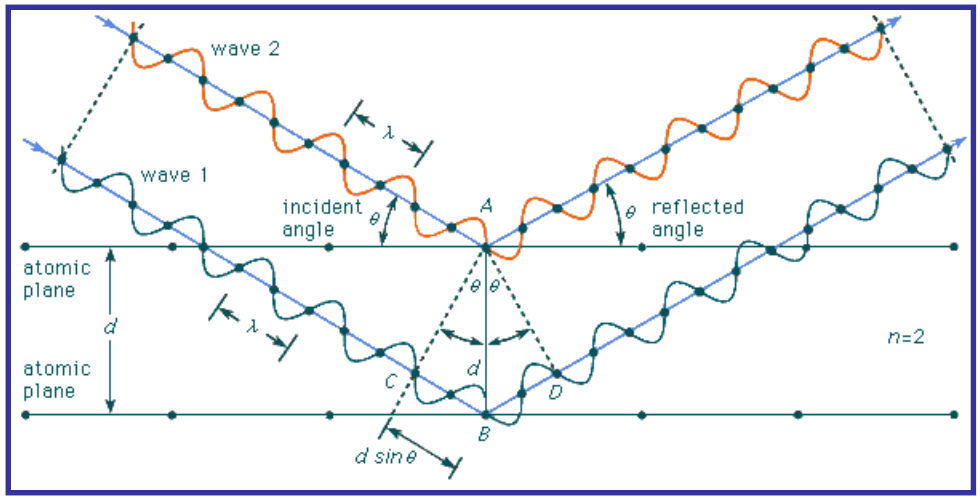
# X射线衍射：实验原理

**Basic set-up**




**Bragg's law:**

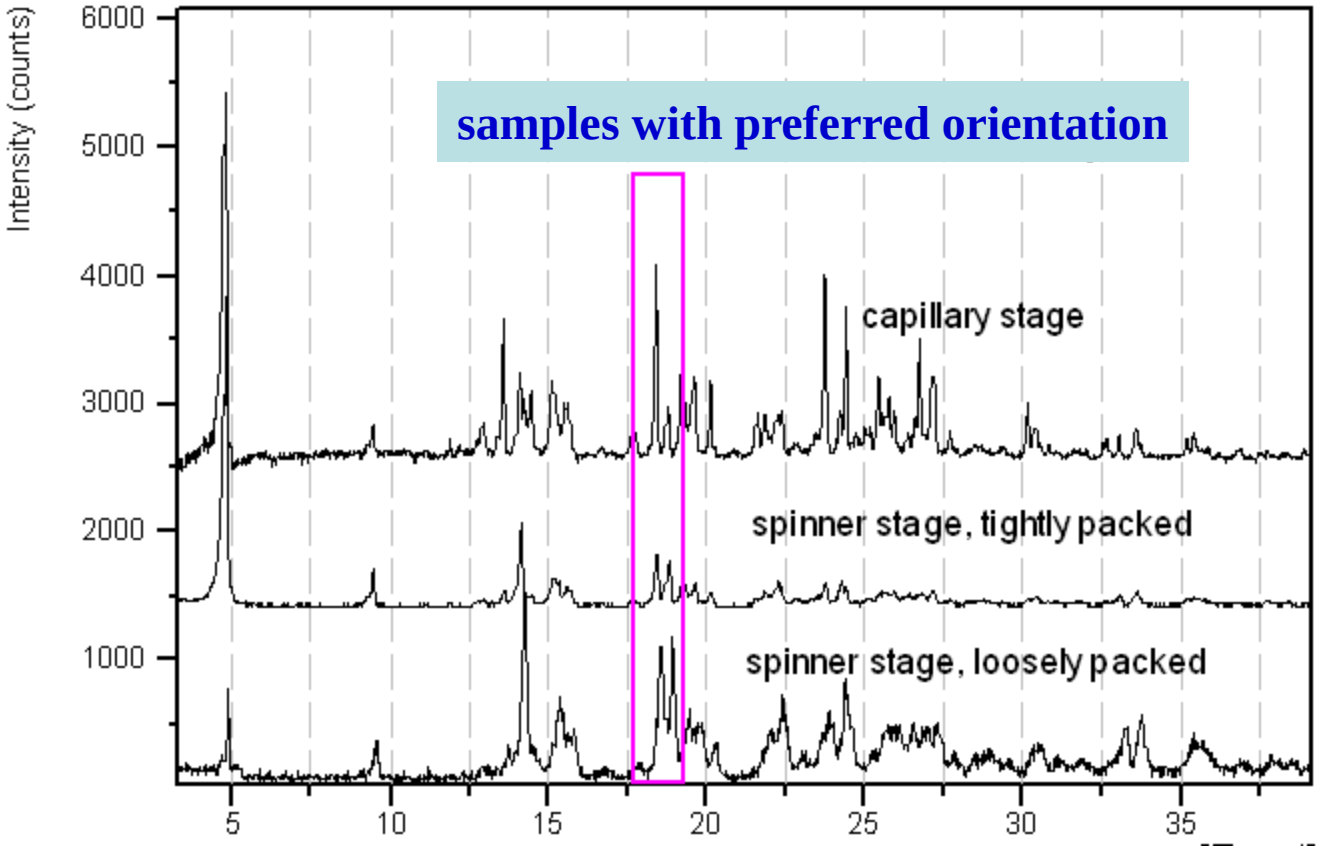
$$n\lambda = 2d\sin\theta$$



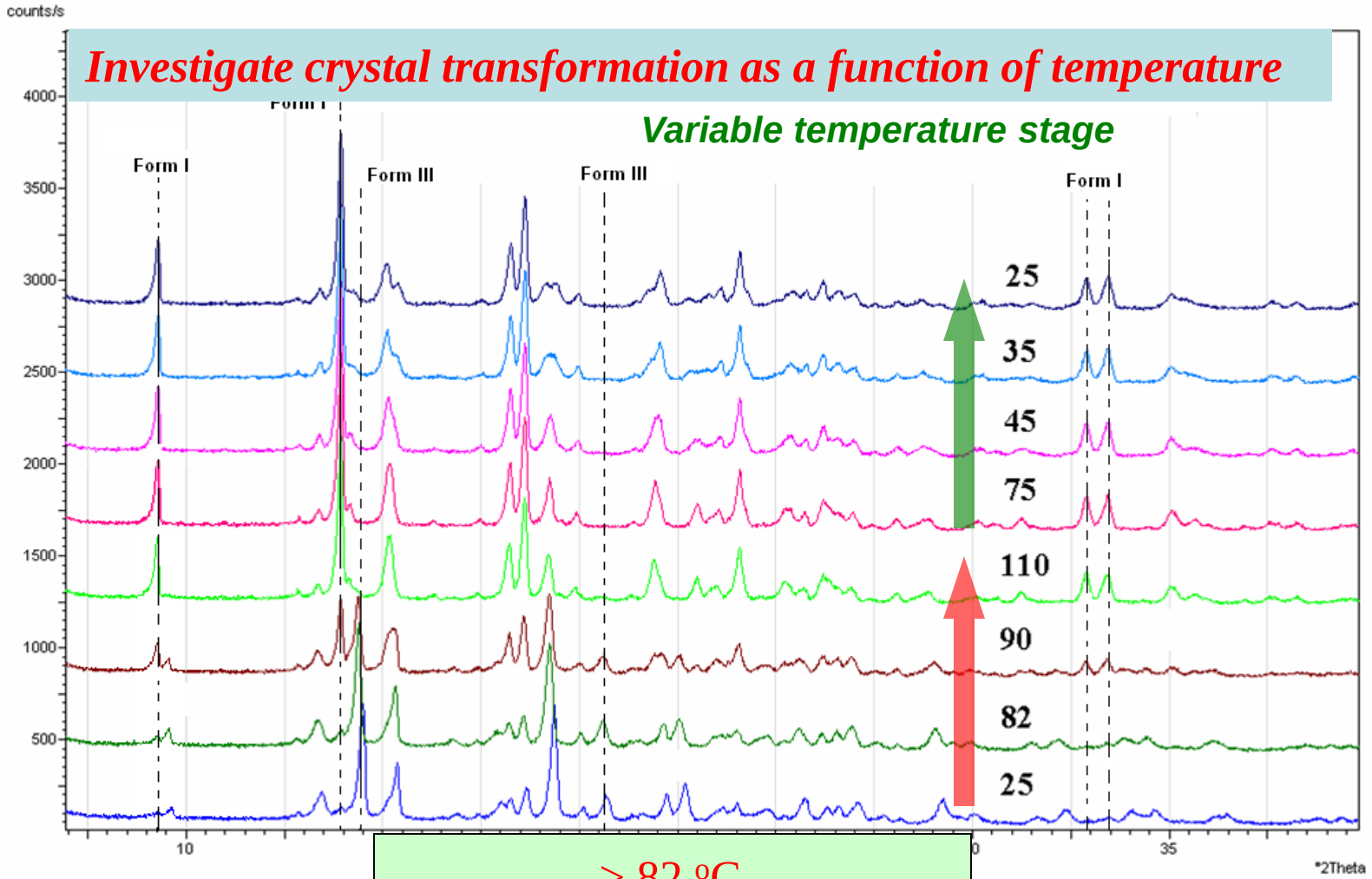

d: Interplanar spacing
l: wavelength of X-ray
q: Incident angle

# 晶型的择优取向现象

毛细管粉末衍射（Capillary XRPD）技术能够有效去除晶型的择优取向问题



# 不同温度下晶型转变的研究

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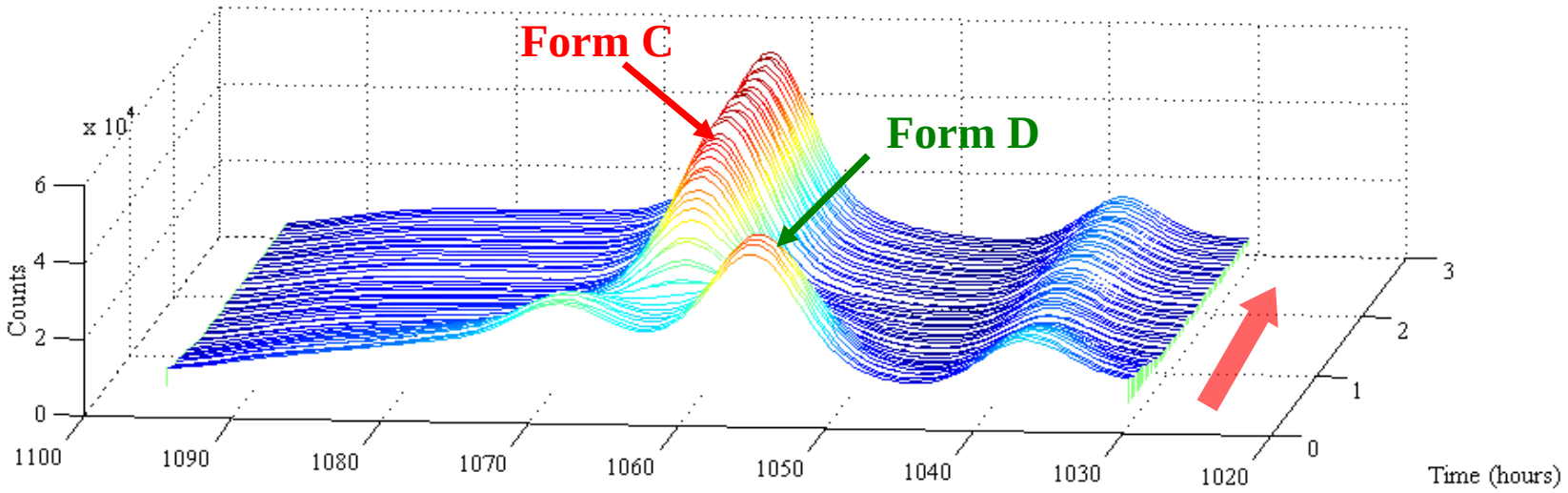
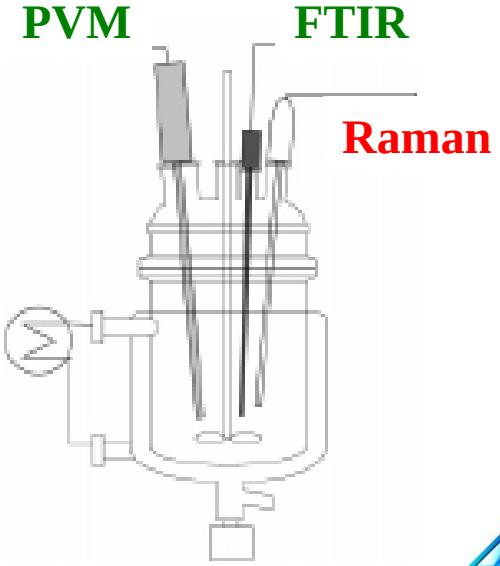
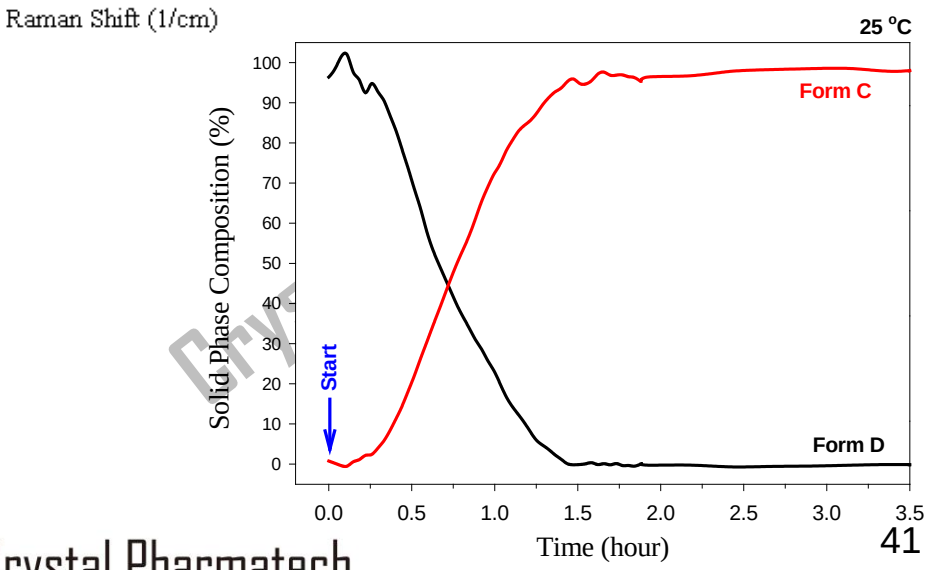
您的药物晶型研究和固态研发专家

# 使用Raman在线检测晶型的转化



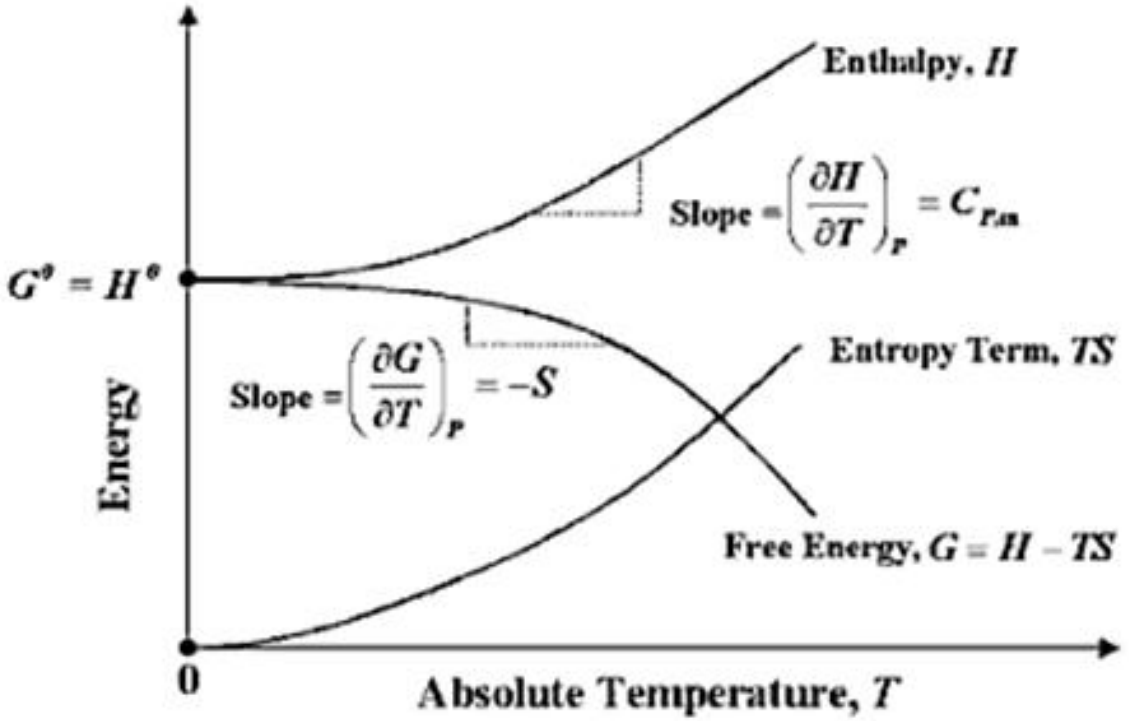



# 实战讨论一：如何确定两个无水晶型的热力学稳定性关系？

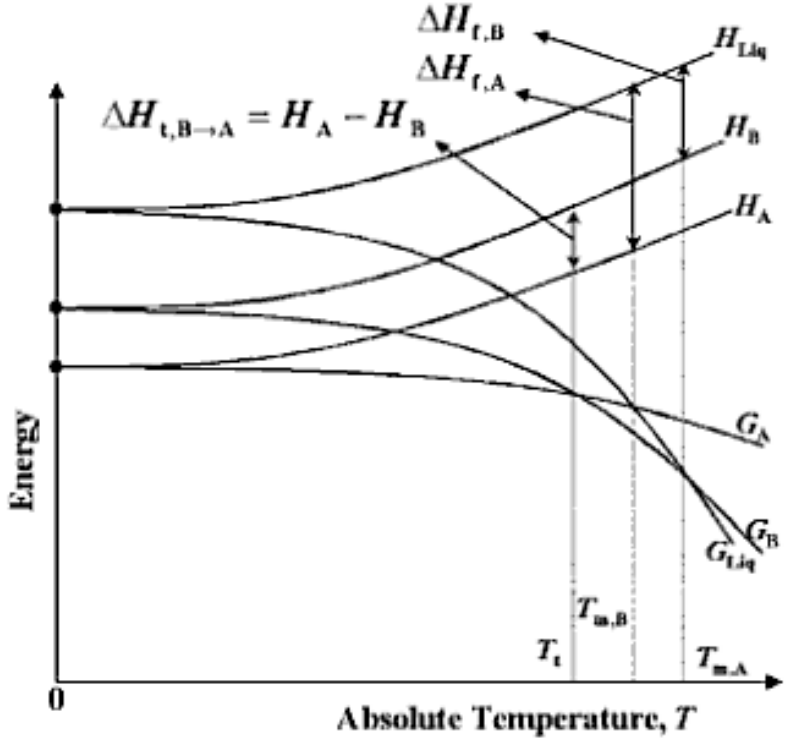
# 三种基本方法

- DSC Analysis
- Van’t Hoff plot (with solubility measurement)
- Slurry experiments

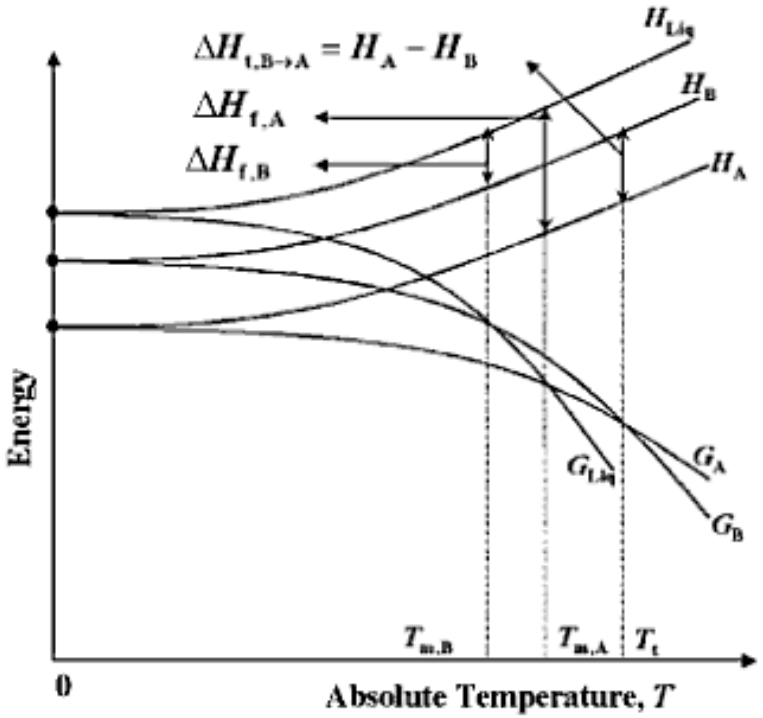
# Energy-Temperature Diagram of a Crystalline Solid Under Constant Pressure



# Energy-Temperature Plots for an Enantiotropic and a Monotropic System

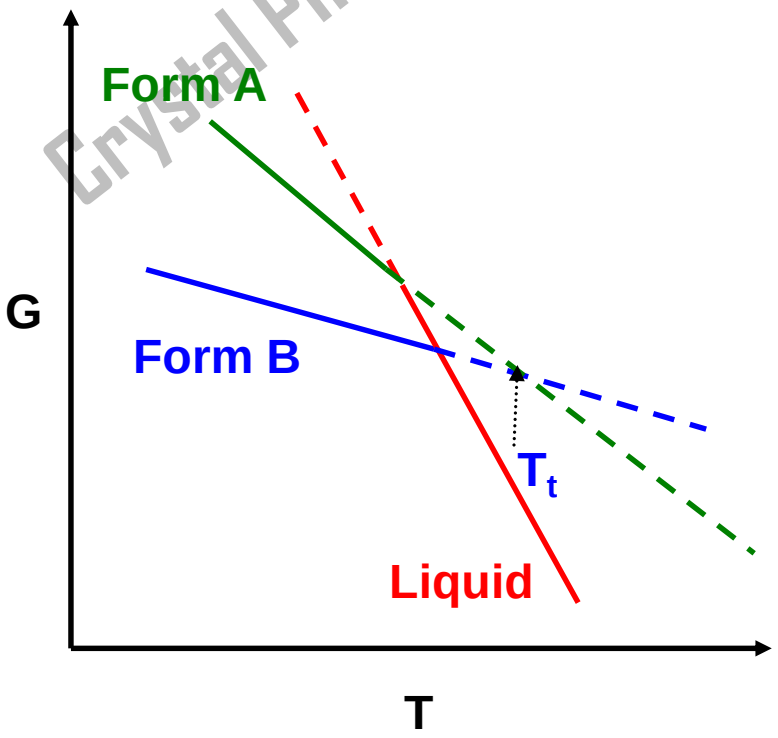



Enantiotropic



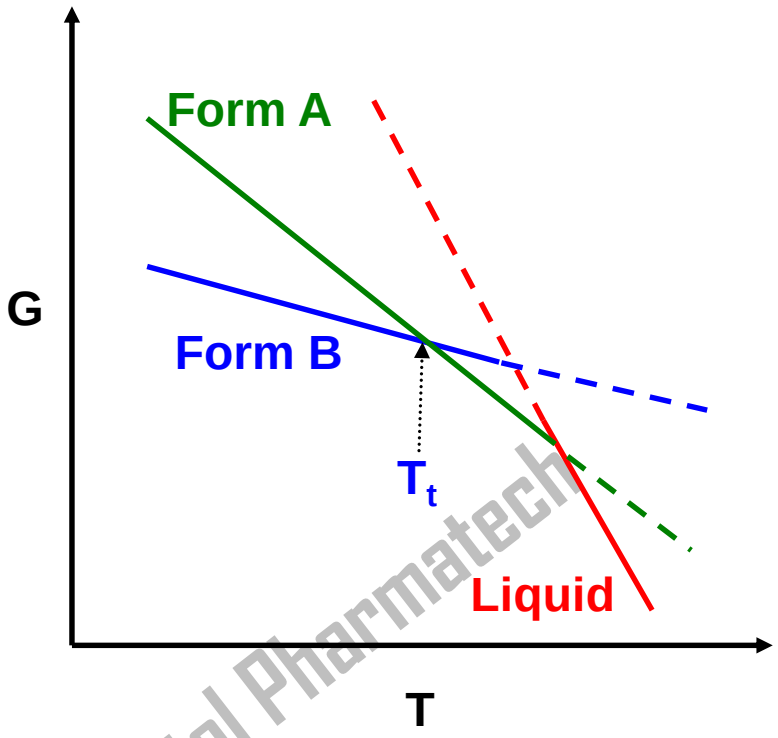

monotropic

# 晶型间的相互转变




**Monotropic**

**单变关系**




**Enantiotropic**

**互变关系**

# 晶型间的相互转变

- 晶型A比晶型B熔点更高，融化吸收热量更大→晶型A和B是单变关系，在任何温度下A都比B稳定

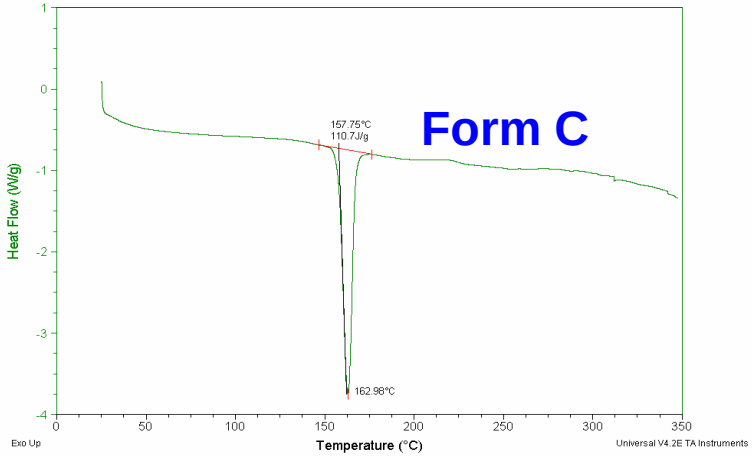
- 晶型A比晶型B熔点更高，融化吸收热量更小→晶型A和B是互变关系，晶型A在高温下比晶型B稳定，但在某个转化温度晶型B更稳定

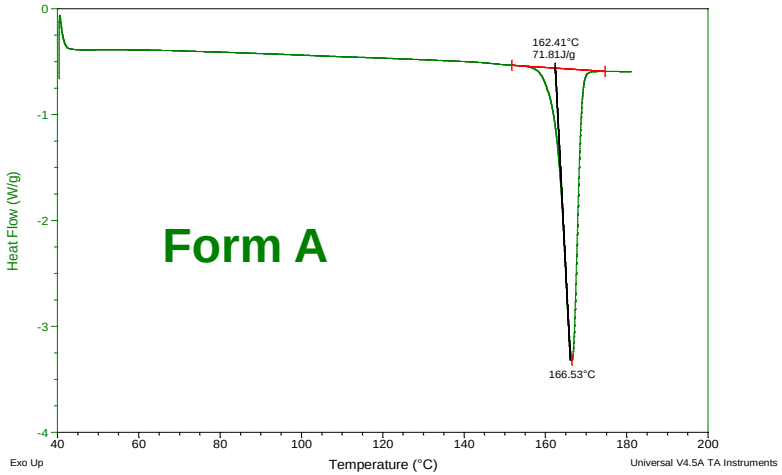
# 晶型间的相互转变

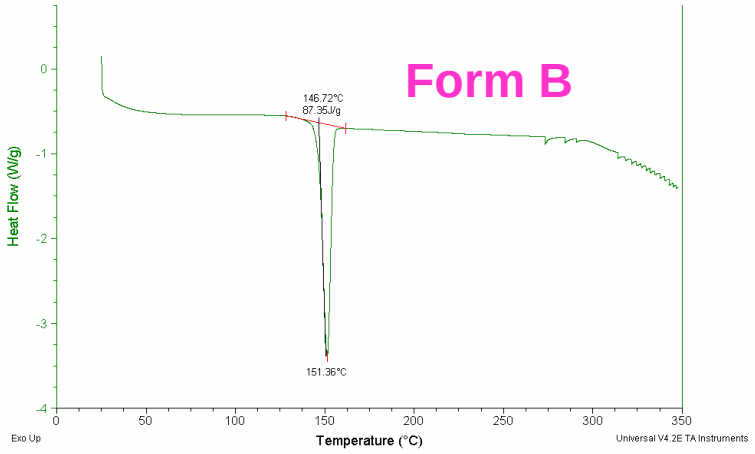
- 如果晶型A转化成B是一个吸热过程，则A和B是互变关系

- 如果晶型A转化成B是一个放热过程，则A和B是单变关系，B在所有温度下都比A稳定

# 用DSC数据推测晶型的热力学稳定性



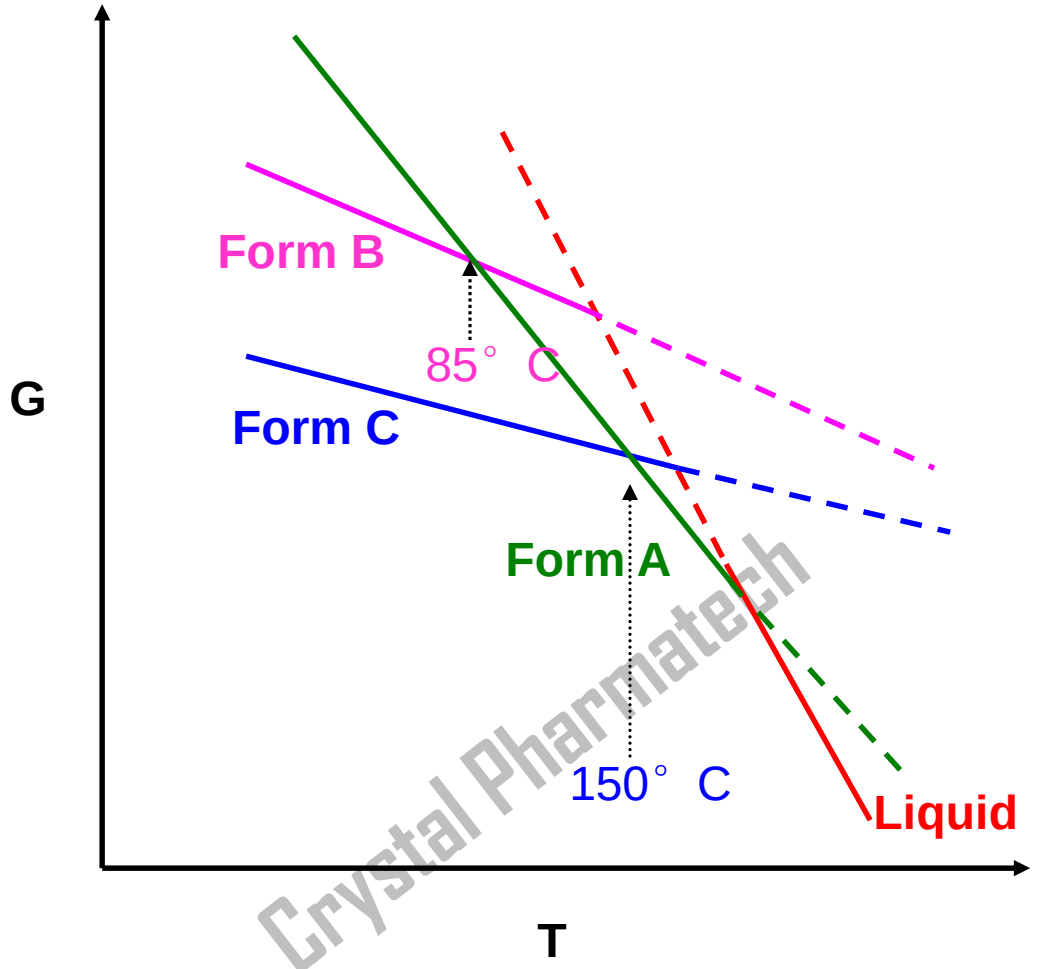

| 晶型 | 熔点(°C) | 熔融热 (J/g) |
|---|---|---|
| A | 162.4 | 71.80 |
| B | 146.7 | 87.40 |
| C | 157.8 | 110.70 |

# 用DSC数据推测晶型的热力学稳定性

$$T_t \approx T_{m,A}\frac{\left(\Delta H_{m,A}/\Delta H_{m,B}-1\right)-yK}{\left(\Delta H_{m,A}/\Delta H_{m,B}-(1+y)\right)-yK}$$

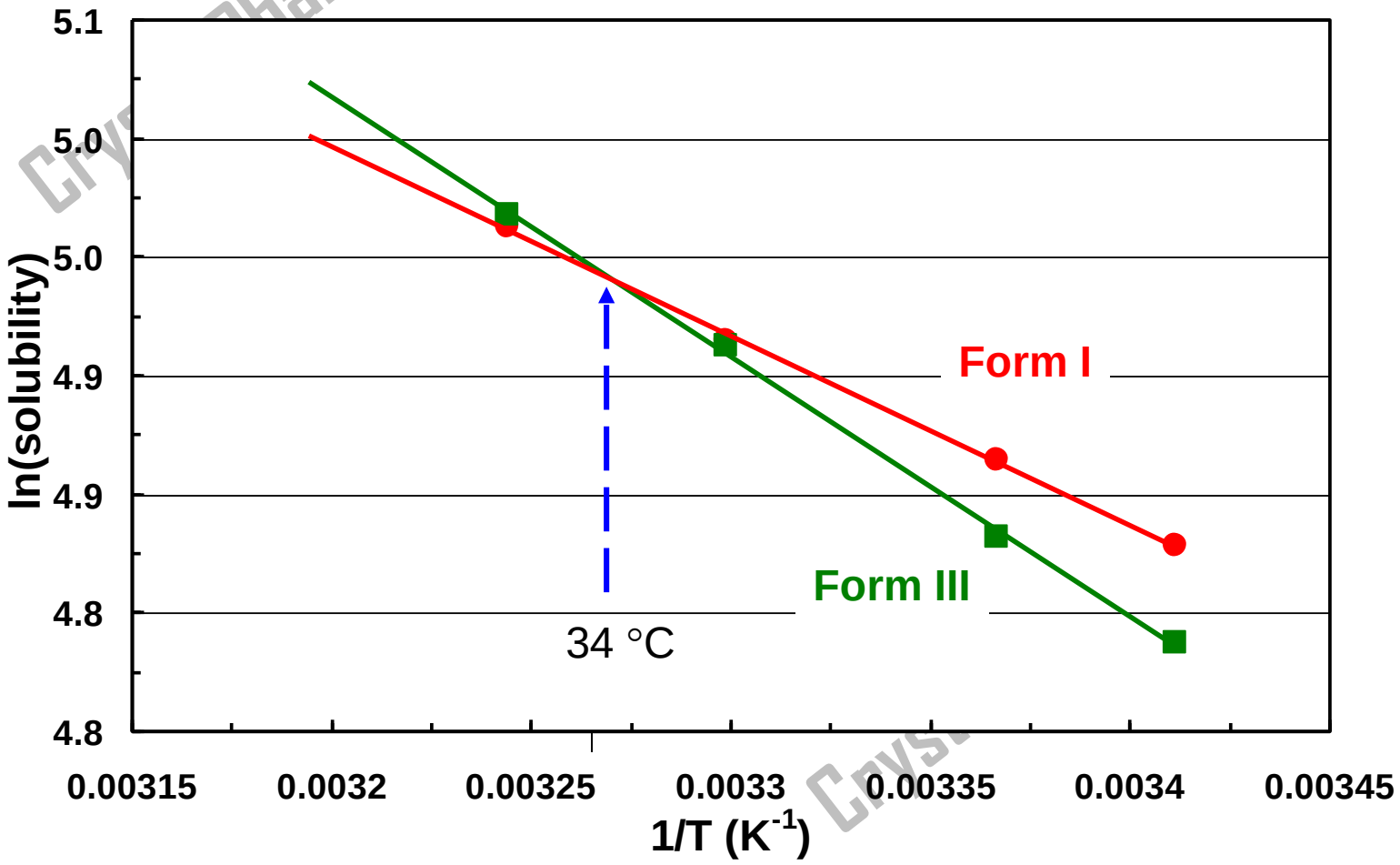
$$y \equiv \frac{T_{m,A}-T_{m,B}}{T_{m,B}} = \frac{T_{m,A}}{T_{m,B}}-1$$

$$K \equiv \left(C_{p,L}-C_{p,B}\right)T_{m,B}/\Delta H_{m,B}$$




The relative thermodynamic stability between the three forms was confirmed by solubility measurement.

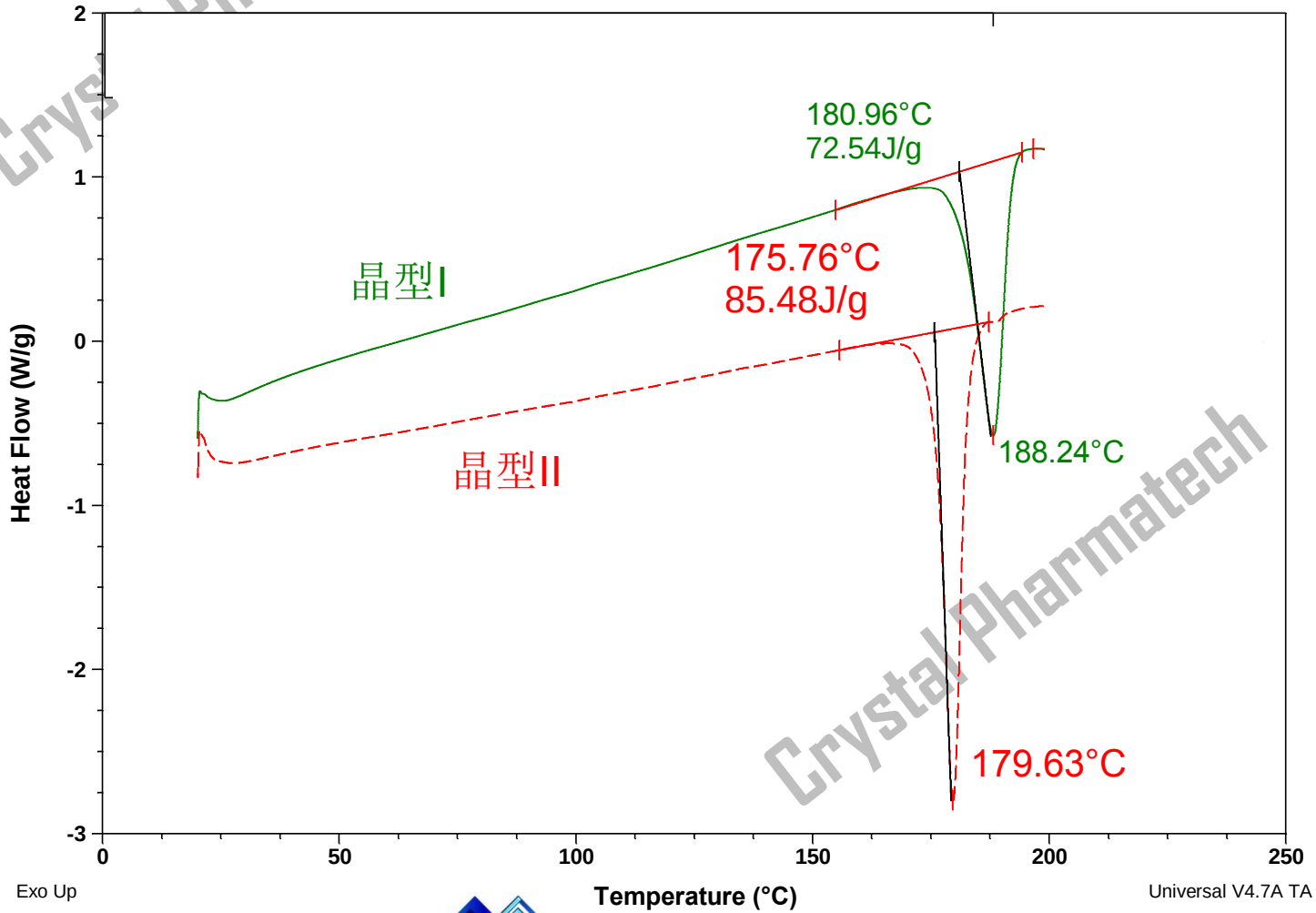
# Van't Hoff Plot



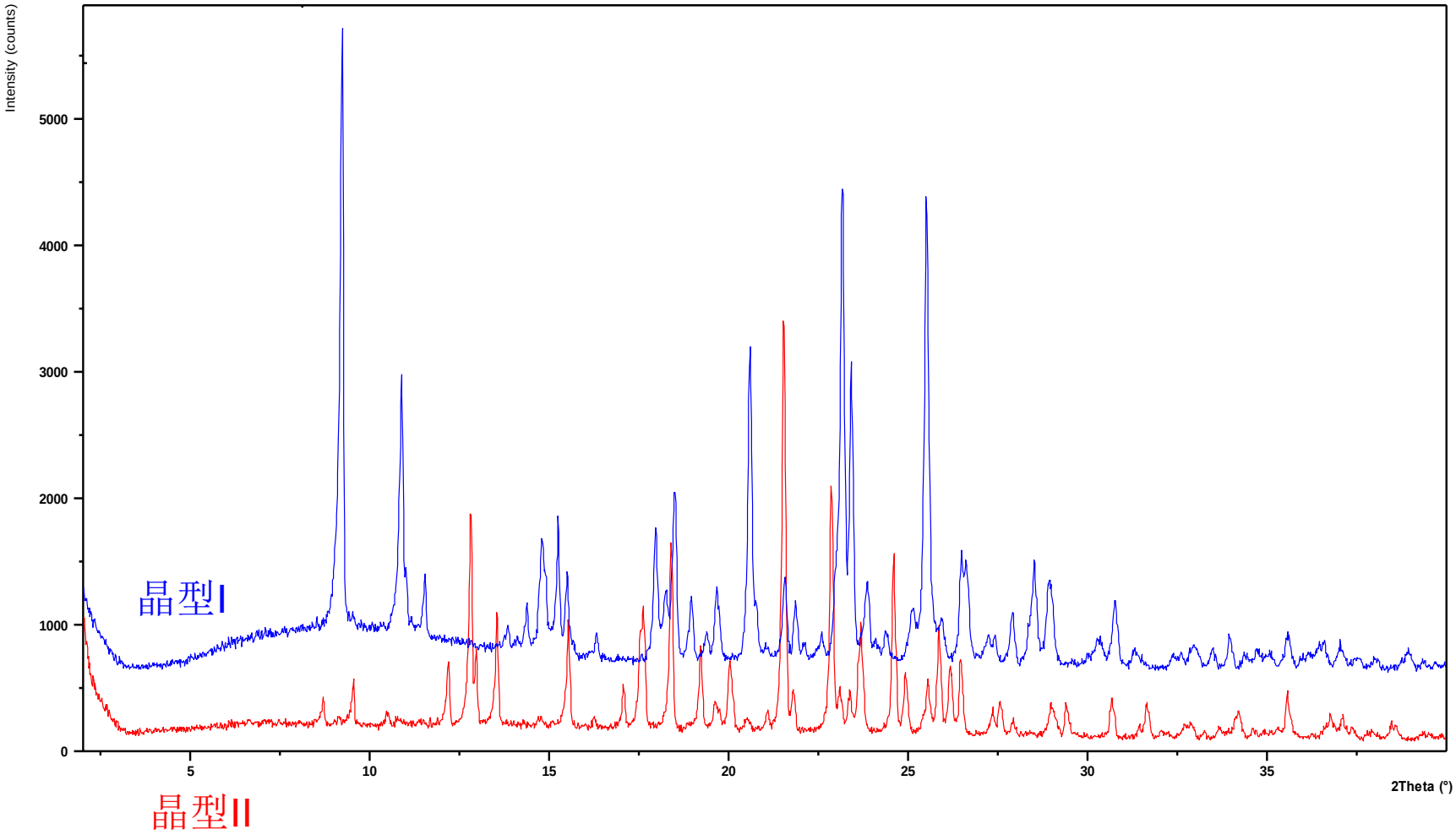
# 多晶型热力学稳定性关系小测试

- 硫酸氢氯吡格雷晶型I vs. 晶型II

# 晶型I vs. 晶型II



# 硫酸氢氯吡格雷XRPD对比

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# 实战讨论二：晶型检测和混晶含量分析

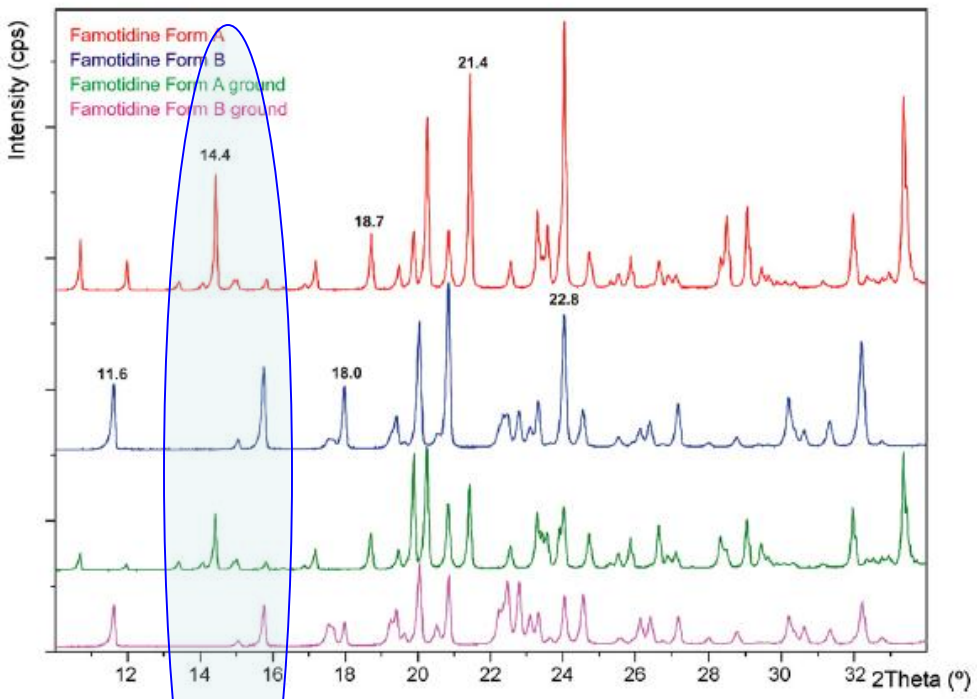
# 晶型检测和混晶含量分析

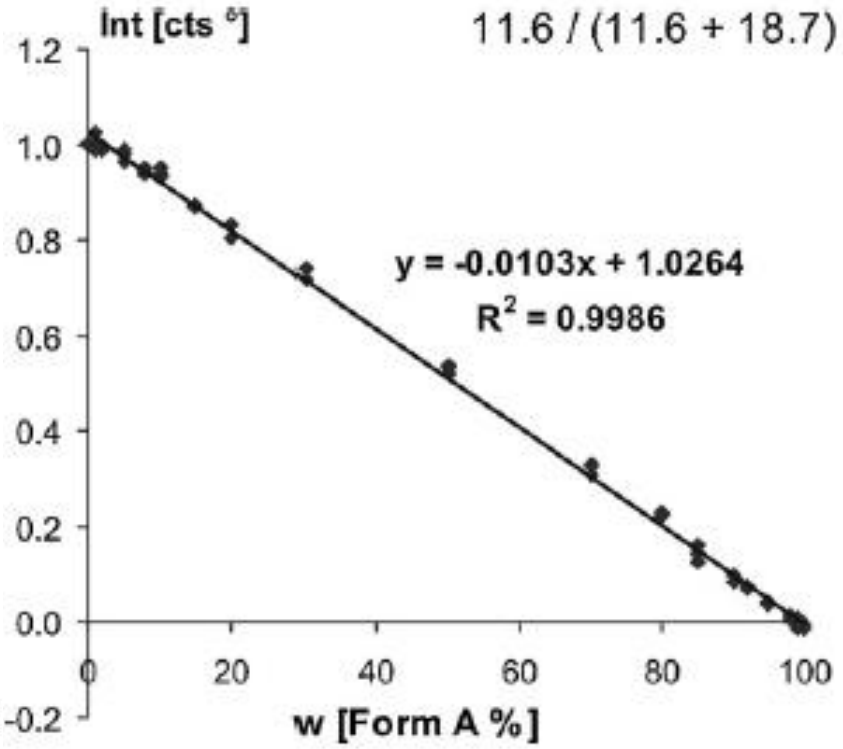
- Why is it important?
  - Quality assurance for your API or formulation
    - Deliver the correct pure polymorph
    - Ensure process robustness
  - Study the kinetics of polymorph transition
    - Process development
    - Storage condition specification
  - IP consideration

- Techniques
  - XRPD, DSC, Raman/IR, ssNMR, DVS, etc

# 用XRPD进行晶型的检测和定量分析

famotidine polymorphs:

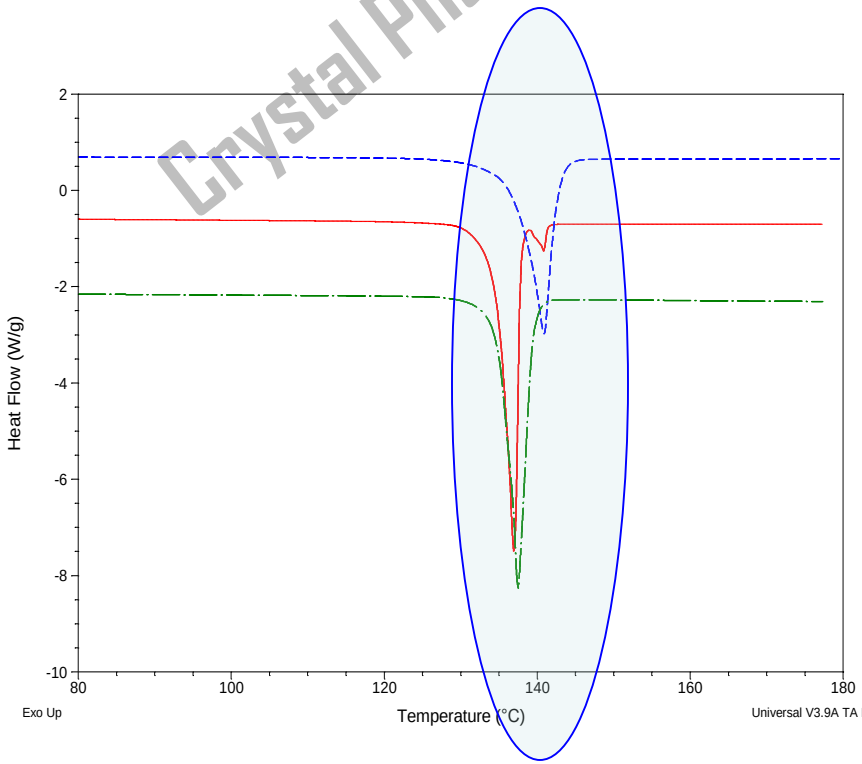


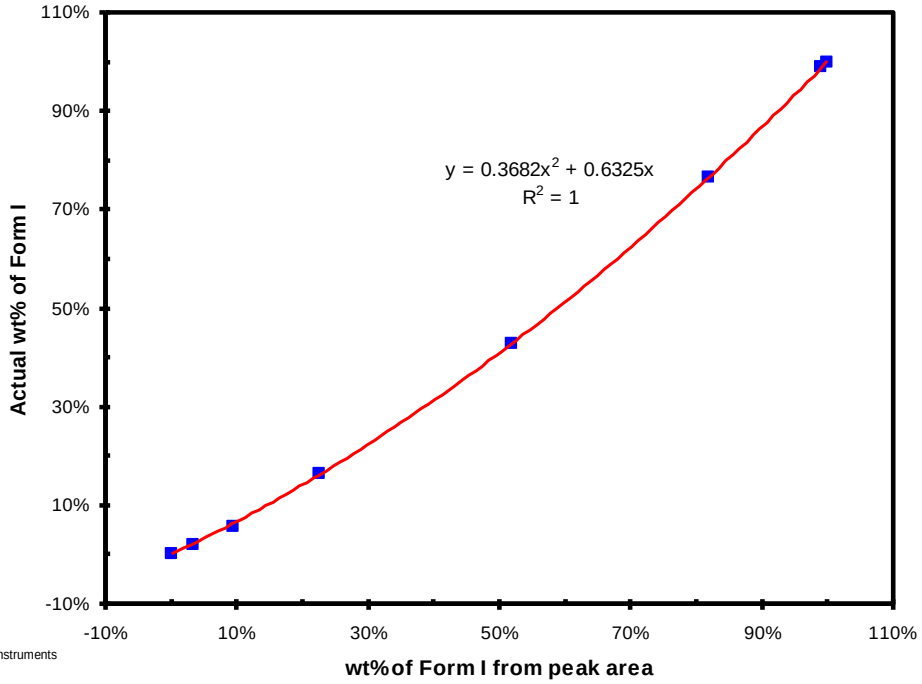




XRPD is able to quantify ~3wt% Form B in a mixture of Form A and B.

*Zoltan et al, Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 338–346*

# 用DSC对晶型检测和定量分析

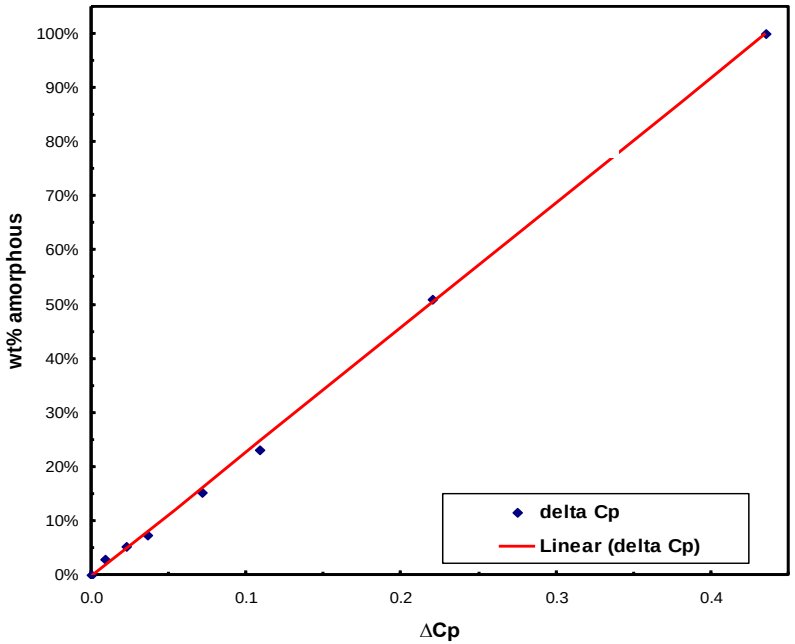


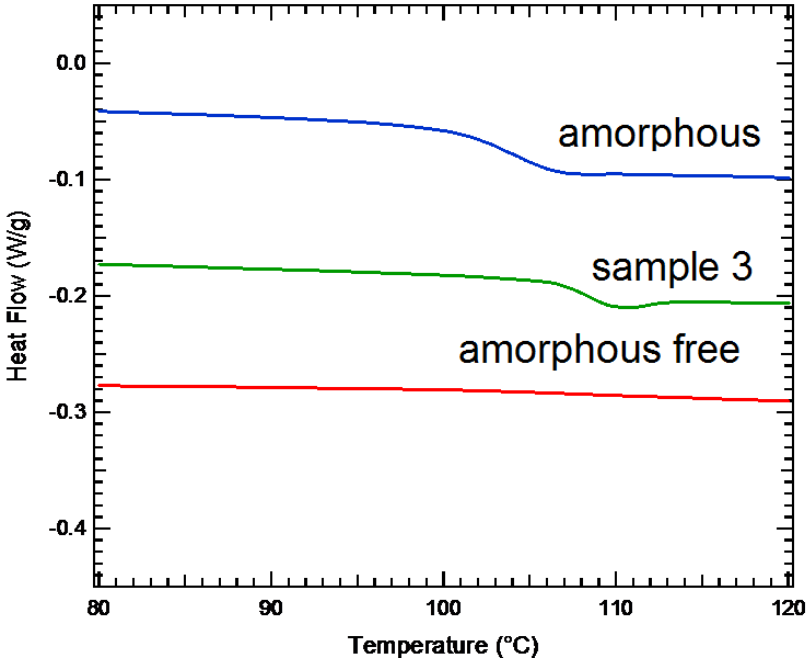




Limit of detection (LOD): < 1%

Limit of quantification (LOQ): < 1%

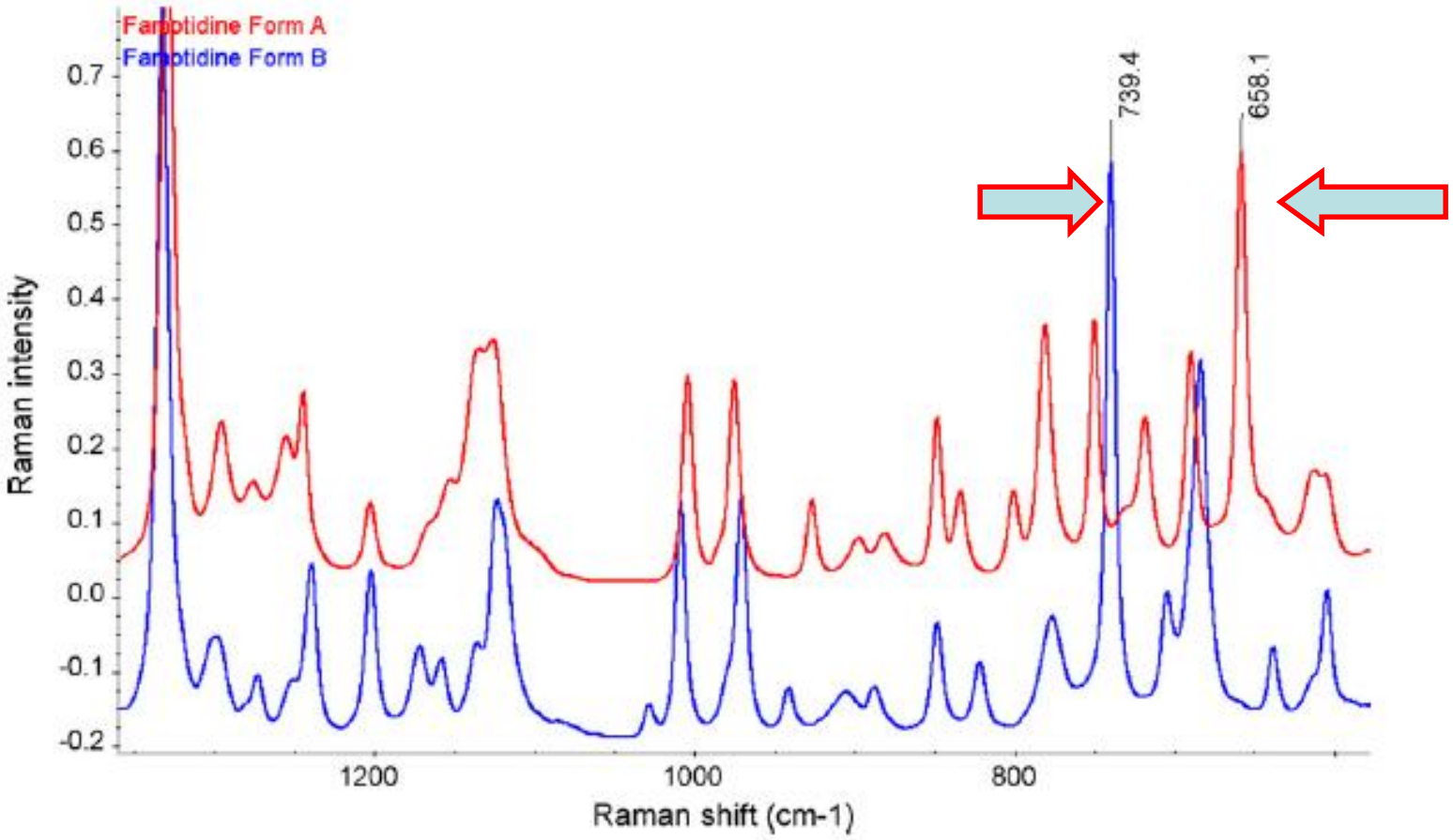
# 用DSC/mDSC对无定形成分检测和定量分析



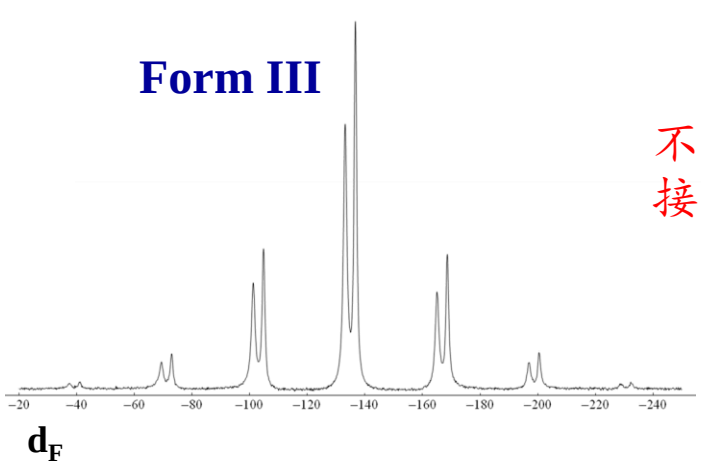



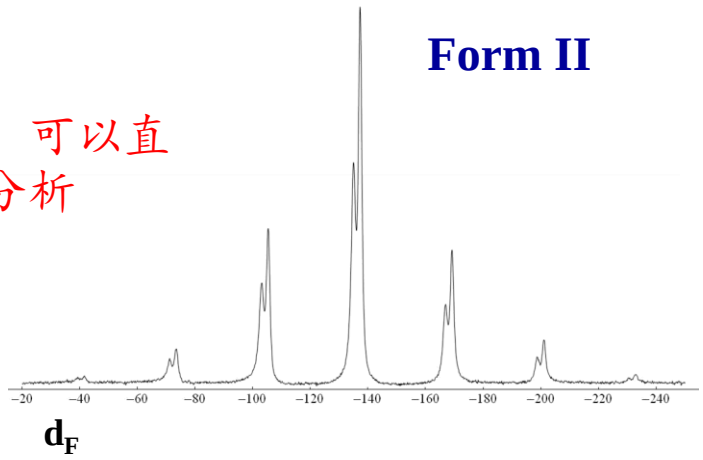

| Sample | Amorphous content (wt%) | std (wt%) | # of measurements |
|---|---|---|---|
| Sample 1 | **16.4** | 2.0 | 3 |
| Sample 2 | **23.2** | 2.9 | 3 |
| Sample 3 | **37.8** | 2.0 | 3 |
| Sample 3 stability study at 25° C/60%RH for 2 month | **39.6** | 2.7 | 3 |

# 使用拉曼光谱对不同晶型的检测和定量分析



# 固态核磁共振对混晶的检测和定量分析

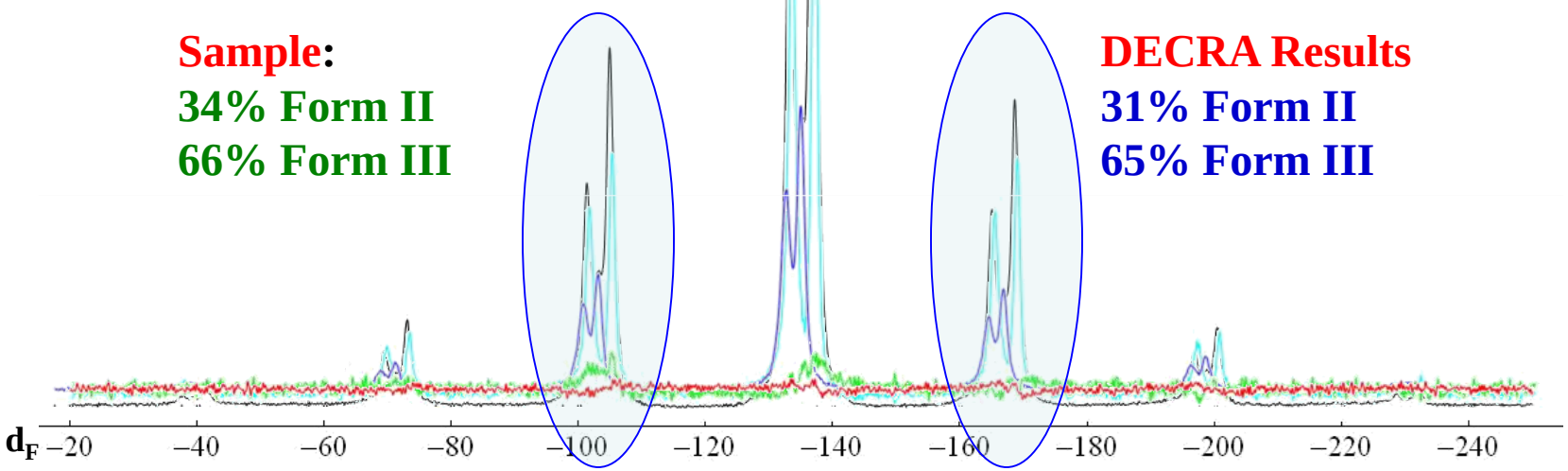



不需要参照样品，可以直接进行混晶定量分析

**Sample:**
**34% Form II**
**66% Form III**

**DECRA Results**
**31% Form II**
**65% Form III**

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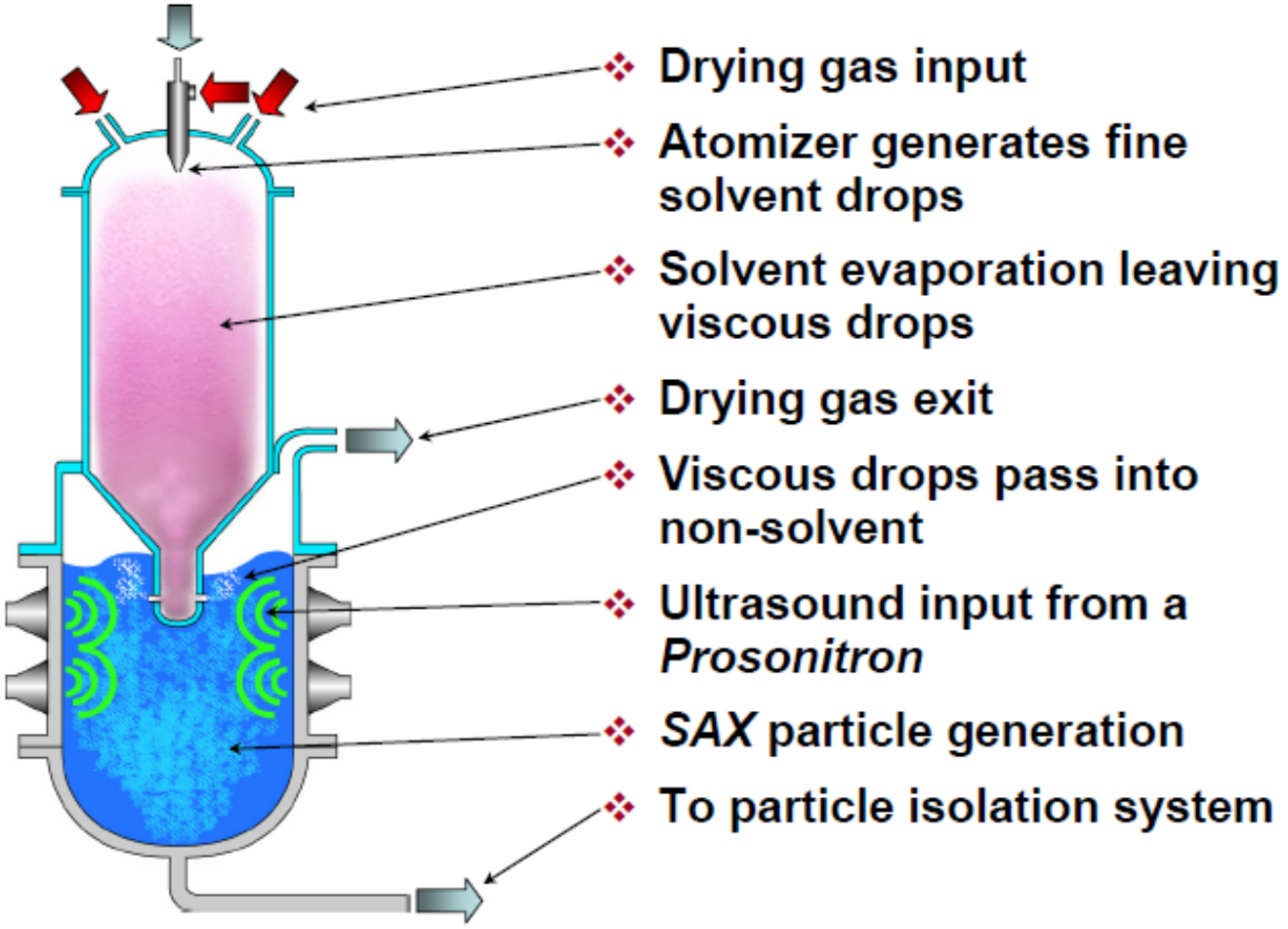
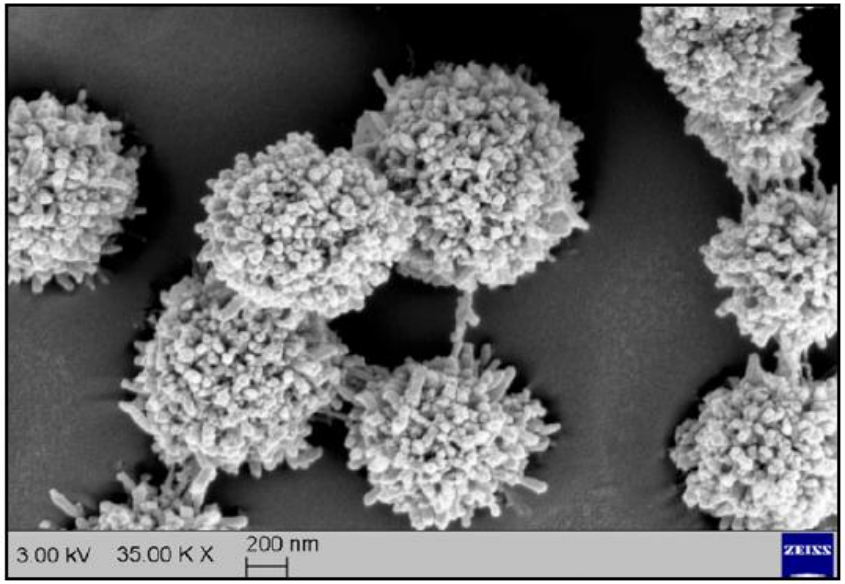
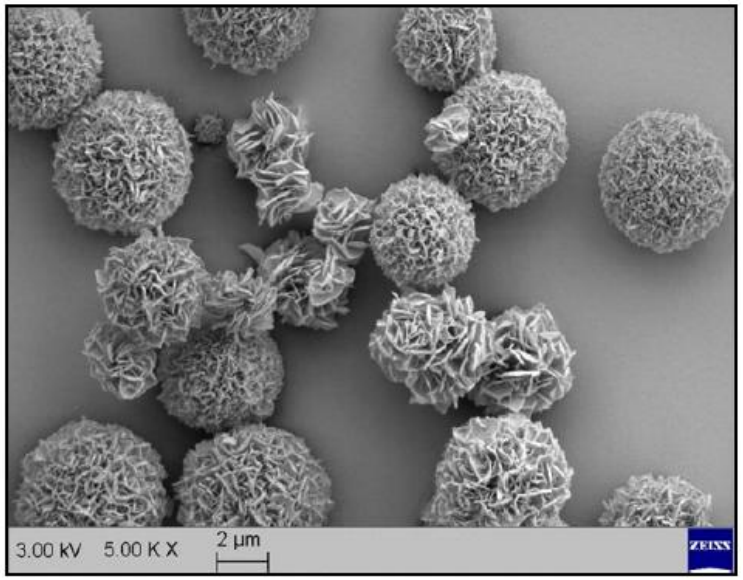
# 实战讨论三：如何制备球状晶体？

# Solution Atomization and Crystallization by Sonication



# 形态和大小的控制



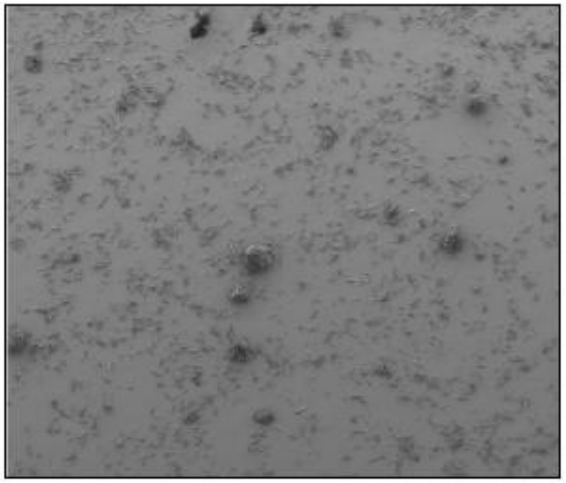




- 单一晶型
- 颗粒大小在0.5-3um之间
- 球状的聚集体颗粒
- 非常均匀的粒径分布
- 很好的流动性

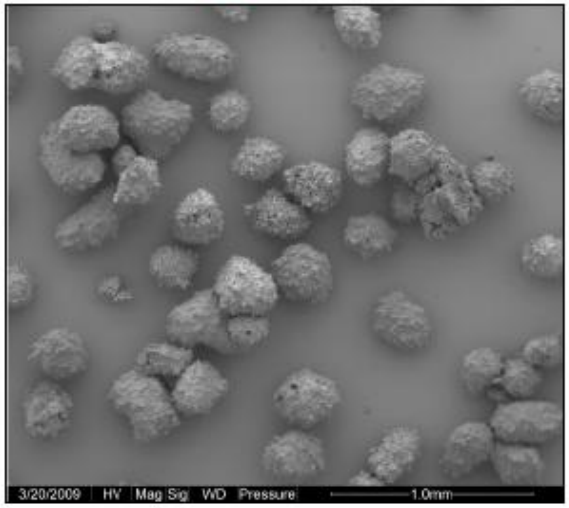
*Courtesy of Dr. Horspool, Pfizer*

# 通过原料药形态的控制来改善制剂工艺

- Formulation Difficulties with sticking and powder flow
- API properties limited formulations to 100 mg/tablet
- Spherical particles give two options
  - –A 200 mg capsule with little (if any) excipient
  - –A 200 mg capsule tablet formulation manufactured successfully



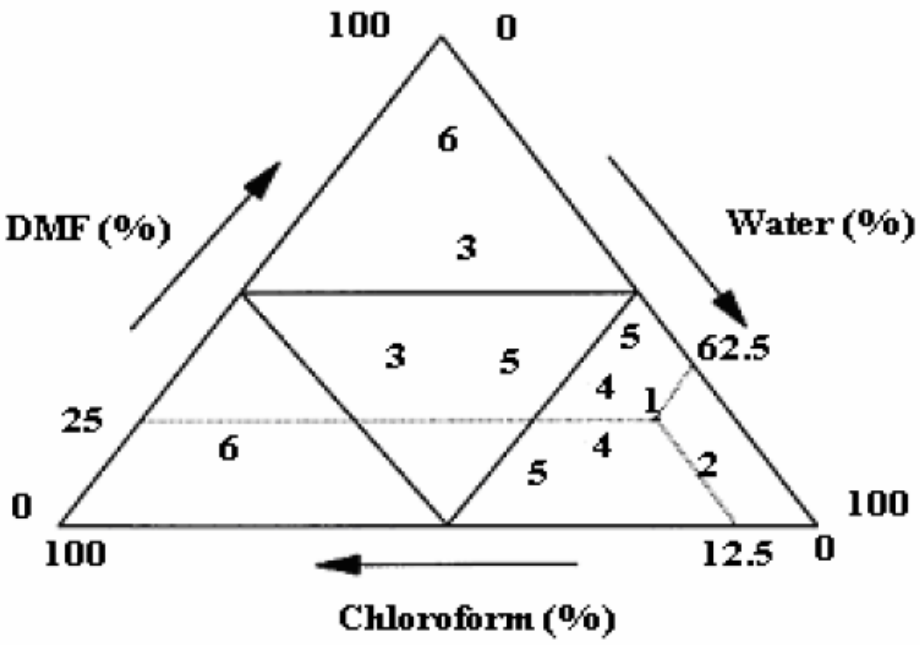
Non-Engineered




Engineered

*Courtesy of Dr. Horspool, Pfizer*

# Spherical Agglomeration of Indomethacin（吲哚美辛）




**Preparation of spherical agglomerates of indomethacin:**

Indomethacin (5 g) was dissolved in 25 ml of N,N-dimethylformamide heated at $45^0$ until a clear solution was obtained. The drug solution was quickly poured in to 62.5 ml of water maintained at $20^0$, under continuous stirring at 500 rpm with a propeller. When fine crystals of indomethacin begun to precipitate (5 min), 10 ml of chloroform was added. After 10 min 2.5 ml of chloroform was added again. After 30 min of stirring spherical agglomerates were formed and were separated from the solution by filtration. Spherical agglomerates were dried at $45^0$ for 12 h.

*Reference: International Journal of Pharma. Research & Development, 2010*

# Effect of Variables on Formulation of Spherical Agglomerates of Indomethacin

**Table 1: effect of variables on formulation of spherical agglomerates of indomethacin**

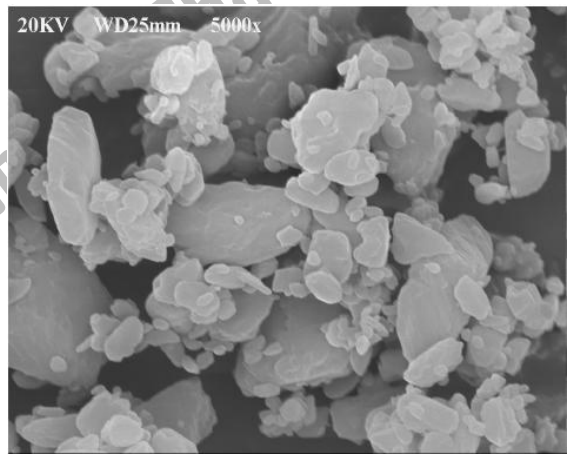
| Parameter | Variables | Observation |
|---|---|---|
| Conc. of bridging liquid (Chloroform) | 2%<br>8%<br>12.5% | No agglomeration<br>No agglomeration<br>Agglomeration |
| Agitation speed | 300±25<br>400±25<br>500±25<br>600±25<br>700±25 | Clumps<br>Spherical & large<br>Spherical<br>Spherical & small<br>Irregular shape & small |
| Agitation time | 20 min<br>30 min | Incomplete agglomerates<br>Spherical agglomerates |
| Temperature | $5\pm1^0$<br>$20^0$<br>$45\pm1^0$ | No agglomeration<br>Spherical agglomerates<br>Very large agglomerates |
| Mode of addition of bridging liquid | Whole at a time<br><br>Drop wise | Crystals of irregular geometry<br>Spherical agglomerates |

# Micromeritic Properties of Indomethacin Commercial Sample vs. Spherical Agglomerates

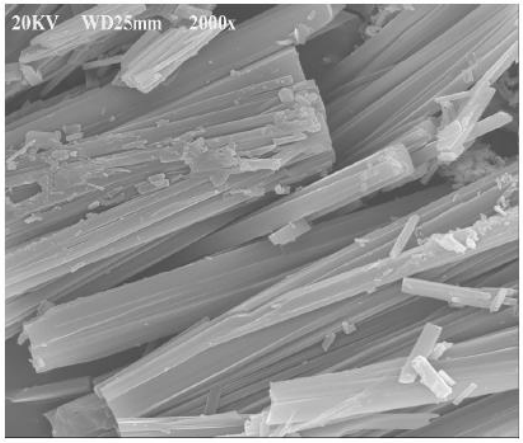


| Properties | Commercial sample | Recrystallized Sample | Spherical agglomerates |
|---|---|---|---|
| Particle size(µm) | 5-10 | 10-15 | 830 |
| Flow rate (g/s) | No flow | No flow | 7.75 |
| Angle of repose | 38.88 | 36.157 | 28.72 |
| Tapped density(g/ml) | 0.9259±0.009 | 0.5409±0.04 | 0.1937±0.04 |
| Bulk density(gm/ml) | 0.6664±0.0084 | 0.394±0.08 | 0.1857±0.006 |
| Carr's index | 27.57 | 27.15 | 7.41 |
| Mechanical strength ( % ) | - | - | 0.8974±0.65 |

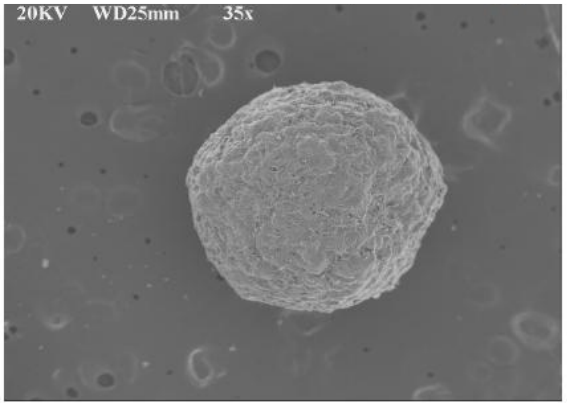
# Spherical Agglomeration of Indomethacin by Solvent Change Method



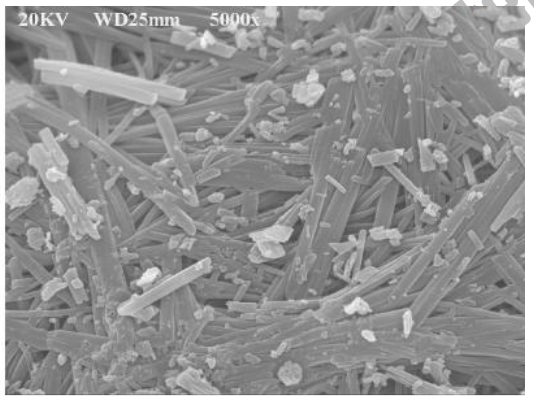

Commercial A




Recrystallized Sample B

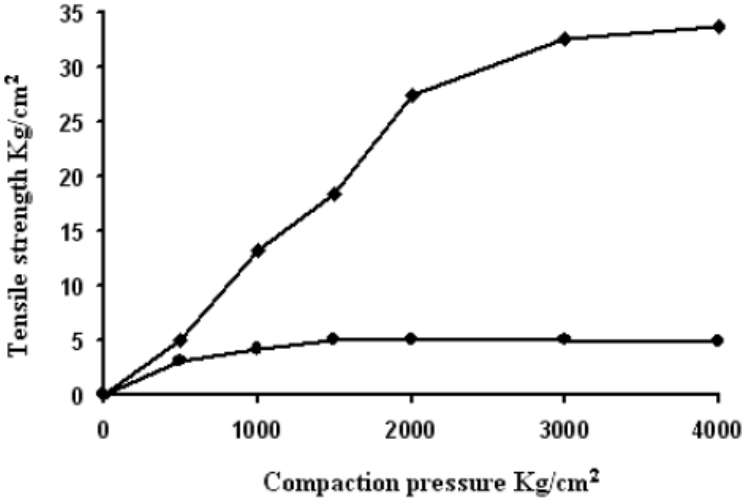


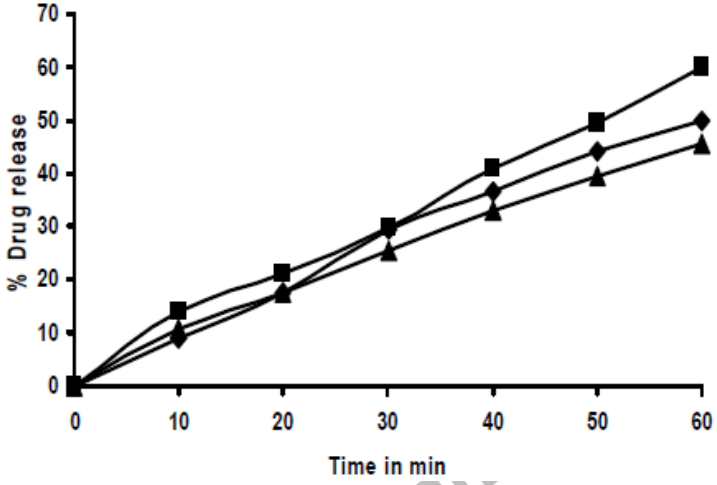

Spherical agglomerate




Surface of spherical agglomerate

# Improved Compressibility and Dissolution



# 药物晶型了解程度的小测试

- 什么是药物多晶型？
- 晶型 vs. 无定形
- 晶型重要性对于仿制药公司 vs. 创新药公司
- 晶型筛选 vs. 选择
- 晶型筛选策略 vs. 晶型工艺开发策略
- 无水化合物 vs. 水合物 vs. 溶剂化和物
- 如何在合适的药物发展阶段，投入合适的精力，展开足够的晶型研究？

# 总结

- 晶型研究和控制在药物研发中至关重要，将直接影响药物的**生产工艺，稳定性**以及**生物利用度**
- 开发新的药物晶型，及时申请专利，是突破创新药公司对于药物晶型的专利保护，提早将仿制药推向市场的关键
- 对多晶型相互稳定型的深刻理解是开发出优化结晶工艺的关键
- 利用多种固态分析手段，原料药或者制剂中不同的晶型的含量可以进行准确检测和量化

# 谢谢大家！