

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS PATENTES

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Abogado
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 Evento 378
 Actuación 519
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 Folios 77

Patente de Invención	☒	Patente de Modelo de Utilidad	☐
Vía PCT (Fase Nacional)	☒	Capítulo I	☒
		Capítulo II	☐
No. Solicitud Internacional	PCT/EP2007/060879		
Fecha de Presentación Internacional	12/Octubre/2007		

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 73 a 105	13/Abril/2009
Reivindicaciones:	Folios 106 a 107	13/Abril/2009
Dibujos:	Folios 109 a 110	13/Abril/2009
Prioridad:	EP 06122265.9	13/Octubre/2006

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud:
"Forma cristalina de 2-clor0-5-[3,6-dihidr0-3-metil-2,6-dioxo-4-(trifluorometil)-1-(2H)-pirimidinil]-4-fluoro-N-[[metil(1-metiletil)amino]sulfonil]benzamida".

El problema planteado en esta solicitud es suministrar nuevas formas de feniruracilo para composiciones solidas donde se presenta el caso de formulaciones polifásicas.

Para resolver este problema técnico la solicitud en estudio proporciona una forma de feniluracilo II cristalina, esencialmente exenta de disolventes.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A 01 N 43/48

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4. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

La reivindicación 14, que se refiere a un método para controlar el crecimiento de vegetación no deseada, **caracterizado** porque se deja actuar la forma II conforme a cualquiera de las reivindicaciones 1 a 5, sobre plantas, su entorno y/o sobre semillas. Es evidente que la llamada etapa de “se deja actuar la forma II”, corresponde a la forma de la forma cristalina II reivindicada en la solicitud, aunque en el preámbulo se reclame como método. Se le aclara al solicitante que un método cuya única etapa corresponde a una aplicación o a una interacción por contacto, dicha aplicación/interacción es un uso. Por lo tanto dicho método de la Reivindicación 14 no es patentable de acuerdo con el artículo 14.

5. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1-3, y 15-17, no son claras, puesto que no presentan de manera adecuada la caracterización de los cristales (polimorfos) reclamados, e sugiere al solicitante realice las modificaciones pertinentes, considerando la Guía del Examinador, Página 163:

Un polimorfo se puede caracterizar apropiadamente en una reivindicación, por sus parámetros fisicoquímicos, tales como:

1. *El patrón de difracción de Rayos X en mono cristal, que es específico de cada polimorfo, por lo cual, si la reivindicación caracteriza el polimorfo mediante este patrón, no se exigirá otro dato.*
2. *El patrón de difracción de Rayos X en polvo (DRXP), el cual debe presentar por lo menos 20 picos correspondientes a las intensidades de los haces difractados o a los espacios interplanares d_{hkl} según los índices de Miller (hkl) medidos de acuerdo con el ángulo de difracción de Bragg 2θ en el intervalo más representativo comprendido entre 5° hasta 90°.*
3. *Las espectroscopías Raman e IR.*
4. *La espectroscopía RMN- C^{13}*
5. *Los métodos de análisis térmico: TGA, DTA y DSC*

La Difracción de Rayos X es la técnica más útil para el estudio del polimorfismo en lo que atañe a la estructura cristalina, dado que los patrones de difracción de los diversos polimorfos presentan siempre notables diferencias, por lo que deben incluir los picos de intensidad más representativa para el caso del cristal reivindicado.

Adicional a lo anteriormente mencionado, se indica el pico de fusión de la modificación cristalina II, la cual no es una característica esencial de un polimorfo, se le sugiere describir de manera adecuada el polimorfo que desea reivindicar, siguiendo las pautas anteriormente mencionadas.

La reivindicación 1 y 5, mencionan las mismas características, se sugiere eliminar la reivindicación 5.

El termino “disolvente orgánico que esta esencialmente esta exento de agua” en la reivindicación 6, no es conciso y no permite diferenciar la invención del estado de la técnica, se sugiere restringir a un grupo mas específico de disolventes probados en el proceso para la preparación de la forma cristalina II.

La reivindicación 7 no muestra sustento en el capítulo descriptivo, puesto que no se muestran ejemplos de cristalización de todos los solventes usados en estos procesos, se sugiere que restrinja a lo realmente probado.

Se hace un especial llamado al solicitante puesto que las reivindicaciones relacionada en cuanto al proceso de cristalización de la forma cristalina II es muy general y no es conciso puesto, que para un técnico versado en la materia las características del proceso de cristalización descrito en las reivindicaciones 8 a 10, son las pautas mas generales y amplias en cuanto a cualquier proceso de cristalización, además, que al ser tan generales, no se establece de manera veras las condiciones especificas de cristalización para la forma cristalina II.

En la reivindicación 8, la expresión “y/o”, es impreciso, y no permite diferenciar la invención del estado de la técnica, Se sugiere sustituirlo por un término o rango concreto.

Descripción (Art 28 D 486)

Haciendo acápite a la Página 162 de la guía del examinador:

*La descripción debe divulgar el polimorfo de manera suficientemente completa para que la persona del oficio normalmente versada en la materia pueda reproducirlo.
Para cumplir con este criterio, debe haber una descripción detallada de al menos un modo de lograr el polimorfo, es decir, se debe divulgar con suficiente detalle todos los pasos esenciales y las condiciones experimentales, para que se pueda reproducir el polimorfo inventado por medio del proceso que permite su obtención.
Adicionalmente, en la descripción se deben divulgar los elementos esenciales del cristal por medio de técnicas que permitan caracterizarlo (DRX monocristal o en polvo y métodos de análisis térmico TGA, DTA y DSC) y que aporten información estructural del compuesto de forma complementaria (espectroscopía Raman e IR o espectroscopía RMN-C¹³), así como del problema técnico que pretende resolver la invención y de los demás elementos característicos asociados a la red cristalina, tal es el caso de las dimensiones de la unidad de celda y del hábito cristalino que le permitan a la persona versada en la materia comprender el aporte de la invención al estado de la técnica.*

De esta manera, la descripción no es clara y concisa, además porque no considera que todos los procesos de preparación divulgados, preparan los polimorfos reivindicados usando cristales semilla, de los cuales se debe describir el proceso para preparar los cristales semilla y las características de este ultimo (espectros).

De esta manera, para una persona del medio medianamente versada en la materia, no es posible reproducir la invención con la información suministrada por el solicitante.

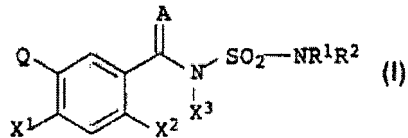
6. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la Fecha de Presentación de la Solicitud de Prioridad: Octubre 12 de 2007 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Item	Documento	Título	Fecha de Publicación*
D1	WO0183459	Uracil substituted phenyl sulfamoyl carboxamides	8/Noviembre/2001
D2	Advanced Drug Delivery Reviews 56 (2004) 275– 300	High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids	6/Octubre/2003
D3	Advanced Drug Delivery Reviews 48 (2001) 3–26	Crystalline solids	21/Diciembre/2000
D4	FARMACIA PRÁCTICA REMINGTON. VOL 2. 19ª Edición EDITORIAL MEDICA PANAMERICANA	Preformulación Polimorfismo Formación de sales.	1995

	Capítulo 83 Preformulación (Págs 2226-2231).	
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D1http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20011108&CC=WO&NR=0183459A2&KC=A2
divulga (abstract) divulga nuevos compuestos fenil uracil sulfamoil carboxiamida sustituidos de fórmula



donde los sustituyentes significan como se observa en el documento, y los cuales se emplean como herbicidas y para la desecación/defoliación de plantas.

D2 (Fls. 136-159)
divulga las características que se presentan en los diferentes tipos de sólidos cristalinos.

D3 (Fls. 160-185)
divulga las características que se presentan en los procesos de critalización para polimorfos, sales, co-cristales, y solvatos de sólidos farmacéuticos..

D4 (ver folios anexos 186-188)
divulga las pautas y características que deben tenerse en cuenta para procesos de recrystalización.

7. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La reivindicación 1 enseña un compuesto, **caracterizado** porque es una forma cristalina II, esencialmente exenta de disolvente, de 2-cloro-5-[3,6-dihidro-3-metil-2,6-dioxo-4-(trifluorometil)-1-(2H)pirimidinil]-4-fluoro-N-[[metil(1-metiletil)amino]sulfonil]benzamida. Adicional a esto la reivindicación 2 enseña que La forma cristalina II conforme a la reivindicación 1, es **caracterizada** porque en un difractograma de polvos por medio de rayos X, a 25°C y radiación Cu-Kα, muestra al menos dos de los siguientes reflejos, que se dan como valores 2θ:

6.3 ± 0.3°, 9.4 ± 0.3°, 10.9 ± 0.3°, 11.9 ± 0.3°, 12.6 ± 0.3°, 15.0 ± 0.3°, 15.8 ± 0.3°, 17.1 ± 0.3°, 20.0 ± 0.3°, 20.4 ± 0.3°, 24.7 ± 0.3°, 25.2 ± 0.3°, 26.2 ± 0.3°.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1
Modificación cristalina II, esencialmente exenta de disolvente, de 2-cloro-5-[3,6-dihidro-3-metil-2,6-dioxo-4-(trifluorometil)-1-(2H)pirimidinil]-4-fluoro-N-[[metil(1-metiletil)amino]sulfonil]benzamida <chem>CN(C)S(=O)(=O)NC(=O)c1cc(F)c(N2C(=O)C=C(C3C(=O)N(C)C3=O)C2=O)c(Cl)c1</chem> I.	<chem>CCN(C)S(=O)(=O)NC(=O)c1cc(F)c(N2C(=O)C=C(C3C(=O)N(C)C3=O)C2=O)c(Cl)c1</chem> [Ejemplo 101 Pág. 57]
un difractograma de polvos por medio de rayos X, a 25°C y radiación Cu-Kα, muestra al menos dos de los siguientes reflejos, que se dan como valores 2θ: 6.3 ± 0.3°, 9.4 ± 0.3°, 10.9 ± 0.3°, 11.9 ± 0.3°, 12.6 ± 0.3°, 15.0 ± 0.3°, 15.8 ± 0.3°, 17.1 ± 0.3°, 20.0 ± 0.3°, 20.4 ± 0.3°, 24.7 ± 0.3°, 25.2 ± 0.3°, 26.2 ± 0.3°.	No lo divulga

El análisis de nivel inventivo del producto reclamado se realizó mediante la aproximación problema-solución en polimorfos (Patenting Polymorphs at the

European Patent Office¹) posición que adoptada por la Superintendencia de Industria y Comercio para efectos del análisis de la patentabilidad de las formas polimórficas.

Al respecto la Dra Papathoma², ha señalado que para analizar la patentabilidad de un polimorfo se debe:

1. Determinar el arte previo más cercano.
2. Determinar la(s) diferencia(s) técnica(s) de la invención con respecto al estado de la técnica más cercano.
3. Determinar del efecto técnico obtenido por las diferencias técnicas de la invención con respecto al estado de la técnica más cercano.
4. Establecer el problema técnico objetivo a solucionar con base al estado de la técnica más cercano.
5. Comprobar que el problema técnico objetivo de la solicitud fue solucionado.
6. Establecer el problema técnico objetivo a solucionar con base al estado de la técnica más cercano.

Según la aproximación mencionada, el arte previo es normalmente el documento que divulga el mismo compuesto, ya sea en un cristal diferente o una forma no cristalina o no definida. Asimismo, se ha definido que para determinar el problema técnico se debe usar el criterio objetivo, basándose en lo que realmente está indicado en la solicitud y teniendo en cuenta el arte previo más cercano.

Ahora bien, según la doctora Papathoma, en el caso de polimorfo, se ha determinado que los problemas técnicos más comunes son: (i) Obtener una forma física alternativa de un compuesto conocido y que logre el mismo efecto técnico u (ii) obtener una forma alternativa de un compuesto conocido y que proporcione una propiedad mejorada o diferente (ej, biodisponibilidad, estabilidad, solubilidad, etc.).

De igual manera, señala que es conocido que: (i) Los polimorfos difieren en sus propiedades (ej. Punto de fusión, densidad, reactivada y estabilidad química, rango de solubilidad y disolución), (ii) la investigación sistemática de un compuesto para determinar si el mismo es propenso al polimorfismo es una rutina en los estudios de pre-formulación y (iii) cada compuesto tiene diferentes formas polimórficas y el número de formas conocidas depende del tiempo y dinero invertido en la investigación del compuesto.

En este orden de ideas, el nivel inventivo será reconocido si: (i) Se obtiene un efecto inesperado del polimorfo reivindicado sobre el arte previo más cercano o (ii) la práctica rutinaria era insuficiente para encontrar un polimorfo de una forma cristalina conocida.

Hechas las anteriores precisiones, procede esta Oficina a realizar el estudio de nivel inventivo bajo los criterios ya mencionados:

1. Determinación del estado de la técnica más cercano.

El documento del estado de la técnica más cercano es D1 porque enseña el compuesto feniluracilo [Ejemplo 101 Pág. 57]; es decir D1 ya divulga la existencia de feniluracilo en forma cristalina.

1 http://www.pcb.ub.edu/centredopatents/pdf/cursos/dillunsCP/papathoma_polymorphs.pdf.

2 Examinadora de patentes de la EPO del Área Química Pura y Aplicada

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2. Determinación de las diferencias técnicas de la invención con respecto al estado del estado de la técnica más cercano.

La solicitud se diferencia de D1 en que se divulga la modificación cristalina II de feniluracilo y porque presenta difractograma de polvos por medio de rayos X, a 25°C y radiación Cu-K α , muestra al menos dos de los siguientes reflejos, que se dan como valores 2 θ : $6.3 \pm 0.3^\circ$, $9.4 \pm 0.3^\circ$, $10.9 \pm 0.3^\circ$, $11.9 \pm 0.3^\circ$, $12.6 \pm 0.3^\circ$, $15.0 \pm 0.3^\circ$, $15.8 \pm 0.3^\circ$, $17.1 \pm 0.3^\circ$, $20.0 \pm 0.3^\circ$, $20.4 \pm 0.3^\circ$, $24.7 \pm 0.3^\circ$, $25.2 \pm 0.3^\circ$, $26.2 \pm 0.3^\circ$, mientras que el documento de D1 no reporta esa información, además, si bien es cierto que no se menciona los picos en D1, la información suministrada en la presente invención para caracterizar la modificación cristalina II es insuficiente para establecer una diferencia apreciable con algún polimorfo previamente conocido en el estado de la técnica.

3. Determinación del efecto técnico obtenido por las diferencias técnicas de la invención con respecto al estado de la técnica más cercano.

Teniendo en cuenta lo divulgado en el capítulo descriptivo, no existe evidencia que las diferencias estructurales entre el compuesto de D1 y el compuesto de la reivindicación 1 (modificación cristalina II) introduzcan un efecto técnico inesperado, que represente para la persona de nivel medio versada en la materia una mejora estabilidad en las formulaciones polifasicas.

4. Establecer el problema técnico objetivo a solucionar con base al estado de la técnica más cercano.

El Problema Técnico Objetivo de esta solicitud consiste en cómo proporcionar una modificación cristalina de feniluracilo alternativa que permita preparar formulaciones polifasicas con características diferentes/mejoradas a las ya conocidas en el estado de la técnica.

5. Comprobar que el problema técnico objetivo de la solicitud fue solucionado.

De acuerdo con la información suministrada en la descripción de la solicitud en donde se proporciona soportar el objeto de la solicitud se encuentra que:

- Preparaciones cristalinas de la forma cristalina II a partir de la I (Fls. 88 a 90).
- Como menciona el solicitante, "hay una mejora en la actividad herbicida en comparación a la forma cristalina I". (Fls. 102-105).

Según lo expuesto anteriormente, la solicitud menciona formas de cristalización y evalúa la actividad herbicida. Sin embargo a partir de esta información no es posible corroborar o evidenciar la solución al problema técnico objetivo planteado en la solicitud porque:

- La comparación técnica se realiza entre un cristal y su forma amorfa(según indica el solicitante), lo anterior no muestra igualdad entre las condiciones de ensayo o preparación del compuesto.
- Por otro lado, es conocido que de acuerdo a las características de forma y conformación espacial del cristal obtenido y posteriormente su tamaño de partícula, las características de la formulación de una suspensión pueden variar. Por tal razón es evidente para una persona normalmente versada en la materia que formulaciones donde se use un cristal (con una conformación espacial mas organizada) presentan mejores características de estabilidad para formulaciones polifasicas con respecto a una forma amorfa y menos

organizada.

Por lo tanto, se puede concluir que no existe un efecto técnico probado, porque no se demuestran las ventajas relacionadas con una mayor estabilidad en formulaciones polifásicas de la nueva forma cristalina II de feniluracilo reclamado en la actual solicitud y que había sido previamente sugerido en D1.

6. Evaluar si habría sido obvio solucionar el problema técnico objetivo por la invención reivindicada.

De tal manera que la solución al problema técnico planteado por la invención reclamada se considera obvia porque las anterioridades D2-D4, puestos que estos documentos divulgan:

D2: enseña que compuestos activos pueden existir en una variedad de distintas formas sólidas, incluyendo polimorfos, solvatos, hidratos, sales, co-cristales y sólidos amorfos. Cada forma muestra unas propiedades físico-químicas que pueden influir profundamente en la biodisponibilidad, fabricación, purificación, la estabilidad y otras características de rendimiento de la droga. El descubrimiento y caracterización de la diversas formas sólidas de compuestos, ofrecen varias opciones entre las cuales, seleccionar una forma que muestre el adecuado equilibrio de las propiedades críticas para el desarrollo específico de un producto (abstract Fl. 136).

D3: enseña que los compuestos orgánicos más bioactivas pueden existir en una o más formas cristalinas. Cuando se aplica a los sólidos, el adjetivo, cristalino, implica un cristal ideal en el que las unidades estructurales, denominadas células unitarias, se repiten regularmente y de forma indefinida en tres dimensiones en el espacio. Polimorfos cristalinos tienen la misma composición química pero diferentes estructuras cristalinas internas y, por lo tanto, poseen diferentes propiedades físico-químicas. Las estructuras cristalinas diferentes en polimorfos surgen cuando la sustancia cristaliza en diferentes disposiciones y/o conformaciones diferentes. La ocurrencia de polimorfismo es muy común entre las moléculas orgánicas. Enseña además que es muy importante elegir la forma más adecuada del compuesto cristalino en las etapas iniciales de desarrollo. (abstract Fl. 160)

D4: ya divulga que las diferentes formas cristalinas para un mismo compuesto difieren en sus propiedades fisicoquímicas tales como la solubilidad, estabilidad, velocidad de disolución, higroscopicidad, biodisponibilidad entre otras, y es además una práctica habitual en estudios de preformulación dentro de los cuales se incluyen rigurosos trabajos para determinar su presencia en los medicamentos con el fin de evitar su inestabilidad o falta de efectividad. Dentro de estos estudios, se investiga habitualmente (i) la cantidad de polimorfos que existen, (ii) el grado relativo de estabilidad de los diversos polimorfos, (iii) las gamas de estabilidad térmica de cada polimorfo, (iv) solubilidades, (v) método de preparación de cada forma, entre otros (Folio 186 Pág. 2227), y una vez establecido que el compuesto (en este caso aplicable a la modificación cristalina II de feniluracilo) presenta polimorfismo, existen procedimientos con los cuales el preformulador puede preparar las diversas formas en cantidades más grandes para su evaluación (Folio 186 Pág. 227 columna de derecha).

De tal manera, no es posible concluir que la identificación de la nueva forma cristalina envuelva actividad inventiva, como quiera que la molécula misma ya había sido sintetizada con anterioridad; es decir, la identificación de la nueva forma cristalina reclamada y caracterizada mediante la técnica de DRX, no representa un avance técnico real y tampoco un salto cualitativo a la regla técnica porque el efecto asociado no fue demostrado en la descripción de la solicitud, debido a que no se encontró alguna prueba o ensayo que permitiera determinar las ventajas reales de la

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forma cristalina de la modificación cristalina II de feniluracilo, con respecto a la forma cristalina ya conocida con anterioridad que permita probar, un efecto realmente inesperado asociado a una mayor estabilidad.

Puesto que las reivindicaciones dependientes 2-5 y 11-13 no mencionan características inventivas adicionales, tampoco pueden ser consideradas inventivas.

La reivindicación 6 indica un proceso para preparar de la forma cristalina II conforme a cualquiera de las reivindicaciones 1 a 4, caracterizado porque comprende:

- i) proporcionar una solución de 2-cloro-5-[3,6-dihidro-3-metil-2,6-dioxo-4-(trifluorometil)-1-(2H)-pirimidinil]-4-fluoro-N-[[metil-(1-metiletil)amino]sulfonil]benzamida en un disolvente orgánico que está esencialmente exento de agua,
- ii) lograr una cristalización de 2-cloro-5-[3,6-dihidro-3-metil-2,6-dioxo-4-(trifluorometil)-1-(2H)-pirimidinil]-4-fluoro-N-[[metil-(1-metiletil)amino]sulfonil]benzamida en un período de al menos 1 h.

El documento D1, considerado estado de la técnica cercano a la invención, divulga ya el feniluracilo, como se define el proceso de cristalización en la reivindicación 6, no hay características específicas adicionales que diferencien este proceso del proceso básico en un cristalización, la única diferencia que puede considerarse es el periodo de tiempo el cual según la reivindicación es de al menos 1 hora, sin embargo es bien conocido para un persona versada en la materia, la optimización de métodos de cristalización³, por medio de variaciones en las proporciones de los solventes, tiempos de cristalización y en el uso o empleo, si es necesario, de cristales semillas.

En consecuencia, la persona del oficio medianamente versada en la materia, realizaría ensayos para mejorar el método de cristalización, variando proporción de solvente, tiempos de cristalización o usando cristales semilla en el procedimiento de D1 para llegar así al objeto de la reivindicación 6 de la solicitud. Por lo cual se considera obvia.

Puesto que las reivindicaciones dependientes 7-10 no mencionan características inventivas adicionales, tampoco pueden ser consideradas inventivas.

8. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO0183459	1-14	Nivel Inventivo
D2	Advanced Drug Delivery Reviews 56 (2004) 275– 300	1-5 y 11-13	Nivel Inventivo
D3	Advanced Drug Delivery Reviews 48 (2001) 3–26	2-5 y 11-13	Nivel Inventivo
D4	FARMACIA PRÁCTICA REMINGTON. VOL 2. 19ª Edición EDITORIAL MEDICA	2-5 y 11-13	Nivel Inventivo

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9. Respuesta a los argumentos del solicitante

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante y presentarla por escrito

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.


JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

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Crystalline solids

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Abstract

Many drugs exist in the crystalline solid state due to reasons of stability and ease of handling during the various stages of drug development. Crystalline solids can exist in the form of polymorphs, solvates or hydrates. Phase transitions such as polymorph interconversion, desolvation of solvate, formation of hydrate and conversion of crystalline to amorphous form may occur during various pharmaceutical processes, which may alter the dissolution rate and transport characteristics of the drug. Hence it is desirable to choose the most suitable and stable form of the drug in the initial stages of drug development. The current focus of research in the solid-state area is to understand the origins of polymorphism at the molecular level, and to predict and prepare the most stable polymorph of a drug. The recent advances in computational tools allow the prediction of possible polymorphs of the drug from its molecular structure. Sensitive analytical methods are being developed to understand the nature of polymorphism and to characterize the various crystalline forms of a drug in its dosage form. The aim of this review is to emphasize the recent advances made in the area of prediction and characterization of polymorphs and solvates, to address the current challenges faced by pharmaceutical scientists and to anticipate future developments.

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Keywords: Crystallinity; Polymorphs; Hydrates; Solvates; Formulation; Drug substance; Phase transformation; Characterization

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1. Introduction

Most organic and inorganic compounds of pharmaceutical relevance can exist in one or more crystalline forms. When applied to solids, the adjective, *crystalline*, implies an ideal crystal in which the structural units, termed *unit cells*, are repeated regularly and indefinitely in three dimensions in space. The unit cell has a definite orientation and shape defined by the translational vectors, *a*, *b*, and *c*, and hence has a definite volume, *V*, that contains the atoms and molecules necessary for generating the crystal. Each crystal can be classified as a member of one of seven possible crystal systems or crystal classes that are defined by the relationships between the individual dimensions, *a*, *b*, and *c*, of the unit cell and between the individual angles, α , β , and γ of the unit cell [1,2]. The structure of a given crystal may be assigned to one of the seven crystal systems, to one of the 14 Bravais lattices, and to one of the 230 space groups [1]. All the 230 possible space groups, their symmetries, and the symmetries of their diffraction patterns are compiled in the International Tables for Crystallography [3].

The common crystalline forms found for a given drug substance are polymorphs and solvates. Crystalline polymorphs have the same chemical composition but different internal crystal structures and, therefore, possess different physico-chemical properties. The different crystal structures in polymorphs arise when the drug substance crystallizes in different crystal packing arrangements and/or different conformations. The occurrence of polymorphism is quite common among organic molecules, and a large number of polymorphic drug compounds have been noted and catalogued [4–7].

Solvates, also known as pseudopolymorphs, are

crystalline solid adducts containing solvent molecules within the crystal structure, in either stoichiometric or nonstoichiometric proportions, giving rise to unique differences in the physical and pharmaceutical properties of the drug. If the incorporated solvent is water, a solvate is termed a hydrate. Adducts frequently crystallize more easily because two molecules often can pack together with less difficulty than single molecules. While no definite explanations can be given, possible reasons include adduct symmetry, adduct-induced conformation changes, and the ability to form hydrogen bonds through the solvent molecules [2,8,9]. Desolvated solvates are produced when a solvate is desolvated and the crystal retains the structure of the solvate [10]. Desolvated solvates are less ordered than their crystalline counterparts and are difficult to characterize, because analytical studies indicate that they are unsolvated materials (or anhydrous crystal forms) when, in fact, they have the structure of the solvated crystal form from which they were derived [11].

Because different crystalline polymorphs and solvates differ in crystal packing, and/or molecular conformation as well as in lattice energy and entropy, there are usually significant differences in their physical properties, such as density, hardness, tabletability, refractive index, melting point, enthalpy of fusion, vapor pressure, solubility, dissolution rate, other thermodynamic and kinetic properties and even color [12]. Differences in physical properties of various solid forms have an important effect on the processing of drug substances into drug products [13], while differences in solubility may have implications on the absorption of the active drug from its dosage form [14], by affecting the dissolution rate and possibly the mass transport of the molecules. These concerns have led to an increased regulatory

interest in understanding the solid-state properties and behavior of drug substances. For approval of a new drug, the drug substance guideline of the US Food and Drug Administration (FDA) states that “appropriate” analytical procedures need to be used to detect polymorphs, hydrates and amorphous forms of the drug substance and also stresses the importance of controlling the crystal form of the drug substance during the various stages of product development [11]. It is very important to control the crystal form of the drug during the various stages of drug development, because any phase change due to polymorph interconversions, desolvation of solvates, formation of hydrates and change in the degree of crystallinity can alter the bioavailability of the drug. When going through a phase transition, a solid drug may undergo a change in its thermodynamic properties, with consequent changes in its dissolution and transport characteristics [15].

Various pharmaceutical processes during drug development significantly influence the final crystalline form of the drug in the dosage form. The various effects of pharmaceutical processing on drug polymorphs, solvates and phase transitions have been described in detail by Brittain and Fiese [16] and will be discussed in later chapters. Briefly, processes such as lyophilization and spray drying may lead to the formation of the amorphous form of drug, which tends to be less stable and more hygroscopic than the crystalline product. Also, processing stresses, such as drying, grinding, milling, wet granulation, oven drying and compaction, are reported to accelerate the phase transitions in pharmaceutical solids. The degree of polymorphic conversion will depend on the relative stability of the phases in question, and on the type and degree of mechanical processing applied. Keeping these factors in mind, it is desirable and usual to choose the most stable polymorphic form of the drug in the beginning and to control the crystal form and the distributions in size and shape of the drug crystals during the entire process of development. The presence of a metastable form during processing or in the final dosage form often leads to instability of drug release as a result of phase transformation [17].

Crystallization plays a critical role in controlling the crystalline form and the distribution in size and shape of the drug. The significance of crystallization

mechanisms and kinetics in directing crystallization pathways of pharmaceutical solids and the factors affecting the formation of crystals have been reviewed in detail by various researchers [12,18,19]. A crystalline phase is created as a consequence of molecular aggregation processes in solution that lead to the formation of nuclei, which achieve a certain size during the nucleation phase to enable growth into macroscopic crystals to take place during the growth phase. The factors affecting the rate and mechanisms by which crystals are formed are: solubility, supersaturation, rate at which supersaturation and desupersaturation occur, diffusivity, temperature, and the reactivity of surfaces towards nucleation. The various forces responsible for holding the organic crystalline solids together, such as nonbonded interactions and hydrogen bonding, have been discussed in detail by Byrn et al. [2] and Etter [20].

Various analytical methods are being currently used to characterize the crystalline form of the drug during the various steps of processing and development. These methods have been reviewed recently in detail by many authors [7,10,21–25]. The single most valuable piece of information about the crystalline solid, including the existence of polymorphs and solvates, is the molecular and crystalline structure, which is determined by single-crystal X-ray diffractometry [2]. Powder X-ray diffractometry provides a “fingerprint” of the solid phase and may sometimes be used to determine crystal structure. Once the existence of polymorphism (or solvate formation) is definitely established by single-crystal and powder X-ray diffractometry, spectral methods, such as Fourier transform infrared absorption (FTIR) spectroscopy, Fourier transform Raman scattering (FT Raman) spectroscopy, solid-state nuclear magnetic resonance (SSNMR) spectroscopy, ultraviolet and visible (UV–Vis) and/or fluorescence spectroscopy [23] may be employed for further characterization. Of special significance are thermal methods, such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and optical microscopy using a hot stage [24]. These methods are almost always employed for further characterization. Modulated (temperature) differential scanning calorimetry (MDSC) in combination with DSC and optical microscopy are able to identify the glass

transition of amorphous forms with much greater clarity and allow unique insights into the glass transitional and polymorphic behavior of drug substances [26].

Because solid-state NMR spectroscopy can be used to study crystalline solids, as well as pharmaceutical dosage forms, this powerful method is finding increasing application in deducing the nature of polymorphic variations [27], such as variations in hydrogen bonding network and molecular conformations among polymorphs [28,29] and for the determination of molecular conformations and mobility of drugs in mixtures and dosage forms [2]. Solid-state ^{13}C -NMR in conjunction with the techniques, known as high power proton decoupling, cross polarization (CP), and magic-angle spinning (MAS) offers information not obtained readily by other techniques. Recently, two-dimensional ^{13}C -solid-state NMR spectroscopy has been used to study the three conformational polymorphs of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile [30]. Use of two-dimensional NMR and total suppression of spinning side bands (TOSS) pulse sequences allowed the separation of isotropic and anisotropic chemical shifts for the three forms. This is a very powerful method for analyzing differences in the chemical environment and is finding increased application in the study of conformational polymorphism.

With advances in analytical methods, the current focus of research in the solid-state area is to understand polymorphism and pseudopolymorphism at the molecular level. Knowledge of the crystal packing arrangements and the various intermolecular forces involved in the different packing arrangements will help in the prediction and preparation of the most stable polymorphs of a given compound well in advance, to avoid surprises during product development. A current emphasis is on the development of software to predict crystal structures of polymorphs from molecular structures. A thorough understanding of the physicochemical properties of polymorphs and solvates (hydrates) is of primary importance to the selection of a suitable crystalline form and development of a successful pharmaceutical product. Bray et al. [31] have shown that, by thorough characterization of four different crystalline forms of L-738,167, a fibrinogen receptor antagonist by various analytical

techniques, it was possible to determine the suitability of one or two forms for the development of pharmaceutical oral dosage forms.

The present review aims to emphasize the recent advances made in the area of prediction and characterization of polymorphs and solvates, attempts to address the current challenges and problems faced by pharmaceutical scientists and intends to anticipate future development. This review does not attempt to provide solutions to the problems but attempts to review comprehensively the advances made in recent years to help address these problems.

2. Recent advances in the identification, prediction and characterization of polymorphs

2.1. Types of polymorphism

Based on differences in the thermodynamic properties, polymorphs are classified as either enantiotropes or monotropes, depending upon whether one form can transform reversibly to another or not. In an enantiotropic system, a reversible transition between polymorphs is possible at a definite transition temperature below the melting point. In a monotropic system, no reversible transition is observed between the polymorphs below the melting point. Four useful rules have been developed by Burger and Ramburger [32,33] to determine qualitatively the enantiotropic or monotropic nature of the relationship between polymorphs. These rules are the heat of transition rule, heat of fusion rule, infrared rule and density rule.

If, by use of the above rules, it is established that the polymorphs of a particular drug are enantiotropic or monotropic, then the next goal is to define the thermodynamically stable (or metastable) domain of each crystalline phase of a substance as a function of temperature. The plot of the Gibbs free energy difference, ΔG , against the absolute temperature, T , gives the most complete and quantitative information on the stability relationship of polymorphs [22], with the most stable polymorph having the lowest Gibbs free energy. The ΔG between the polymorphs may be obtained using several techniques operating at

different temperatures, such as solubility [34] and intrinsic dissolution rate. Yu [35] has derived thermodynamic equations to calculate ΔG between two polymorphs and its temperature slope from the melting data. This method is essentially an extension of the heat of fusion rule, which is based on statistical mechanics. Extrapolating ΔG to zero gives an estimate of the transition temperature, from which the existence of monotropy or enantiotropy is inferred. The integration of different types of data provides the ΔG vs. T curve over a wide temperature range and allows the consistency between techniques to be checked [22]. Another approach to establish the order of stability among various polymorphs has been studied using pressure versus temperature plots, e.g., for sulfanilamide and piracetam [36]. This approach is based upon Ostwald's principle of least vapor pressure, according to which the stable polymorph exhibits the lowest vapor pressure. The accuracy of this approach to establish the stability hierarchy among the polymorphs has been shown to be very much dependent on the accuracy of the experimental data.

In recent years, the main focus of research has been the characterization of polymorphs arising from structural differences in the crystal lattice. It has been established for some time that organic molecules are capable of forming different crystal lattices through two different mechanisms. One of the mechanisms is termed *packing polymorphism*, and represents instances where conformationally relatively rigid molecules can be assembled into different three-dimensional structures through the invocation of different intermolecular mechanisms. The other mechanism is termed *conformational polymorphism* and arises when a nonconformationally rigid molecule can be folded into different arrangements, which subsequently can be packed into alternative crystal structures. The distinction between *packing polymorphism* and *conformational polymorphism* is somewhat artificial because different packing arrangements impose different conformations on the molecules, however slight, and different conformations will inevitably pack differently. The structural aspects associated with polymorphs have been reviewed recently [2], as have the analogous features of solvate and hydrate systems [9]. In the next

section, the results of some more recent investigations are discussed.

2.2. Packing polymorphism

An investigation into the structures and charge densities of two polymorphs of *p*-nitrophenol has been performed with the aim of deducing the different modes of inter-molecular hydrogen bonding that lead to the formation of the two structures shown in Fig. 1a and b [37]. A detailed analysis of the charge density of the two forms indicates charge migration from the benzene ring region to the nitro and hydroxyl groups that accompanies the transformation of one form into the other. In addition, polarization of the oxygen lone-pair electrons was found to be substantially larger in the crystal forms than in the free molecule, resulting in considerably larger dipole moments in the solid state.

During the study of a new crystal form (form I) of

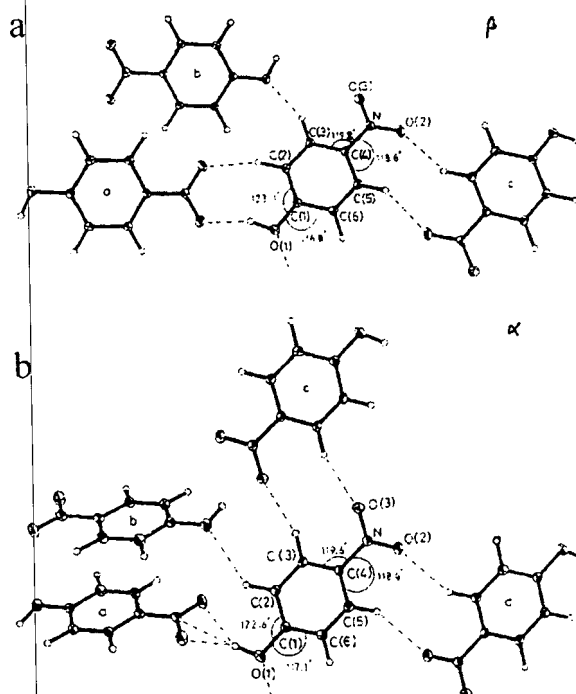


Fig. 1. Molecular packing diagrams of the (a) β polymorph of *p*-nitrophenol, (b) α polymorph of *p*-nitrophenol, showing 50% probability displacement ellipsoids ([37], reproduced with the permission of the American Chemical Society).

chlordiazepoxide, it was found that the heat of transition between the two forms (forms I and II) is rather modest, and kinetic factors permit the existence of the metastable phase [38]. Both structures contain four crystallographically independent molecules linked in dimers through hydrogen bonding, but the dimers are packed differently to yield the two crystal forms. Because the dimers in the fundamental units are spaced differently in the two forms, it was proposed that the solid-state enantiotropic transformation entailed rearrangement of the dimer units.

A different approach has been taken during an evaluation of the different structures formed by sulfathiazole [39]. Using a graph set approach to classify the known structural differences and similarities among the various forms, it became possible to identify packing motifs common to three of the four crystal structures. Fig. 2 shows the unit cells of the polymorphs I, II, III and IV, where molecules are paired as hydrogen-bonded dimers. At the end of the process, the authors were able to deduce possible links between the observed patterns of hydrogen bonding, processes of nucleation, and the crystal growth observed from a number of solvent systems. Interestingly, the analysis did not indicate a relationship between the appearance of a particular polymorph from solution and the growth of its fastest

growing surface. Rather, it appeared as if the different solvents affected the process of polymorph formation through their effects on nucleation of the various forms.

2.3. Conformational polymorphism

The conformational polymorphism of the two forms of piroxicam pivalate has been studied in detail [40]. This compound is distinctive in that the high-melting form (polymorph 1) contains an unanticipated array of associated molecules bound as centrosymmetric dimers through hydrogen bonding, with the amido nitrogen atom acting as the donor and the pyridine nitrogen as the acceptor (Scheme 1, structure I). The low-melting form (polymorph 2) contains molecules of two distinct conformational states coexisting in the same crystal (Fig. 3), but linked through different hydrogen bonding arrangements. This latter finding represents another unusual aspect of the crystallography of the substance.

The inclusion of different solvent molecules in a crystal lattice can lead to the existence of different packing patterns, and has also been found to influence the molecular conformation of paroxetine hydrochloride in two solvate forms [41]. One form

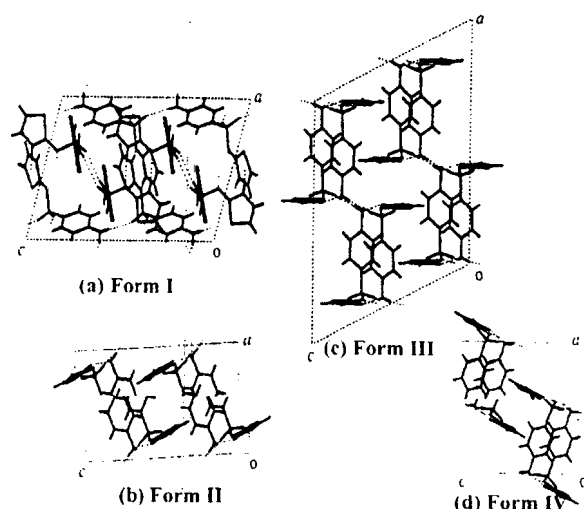
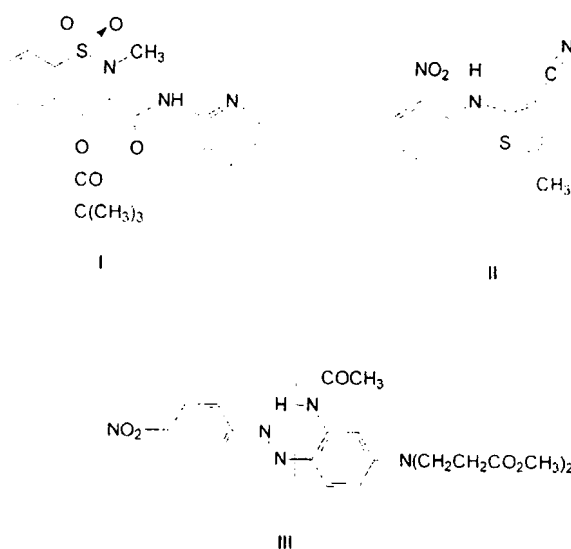


Fig. 2. Unit cells of four polymorphs (I, II, III and IV) of sulfathiazole showing hydrogen bonds, with the dimer structure clearly discernible [39], reproduced with the permission of the Royal Society of Chemistry).



Scheme 1. Molecular structure of piroxicam pivalate (I) [40], 5-methyl-2-[2-(nitrophenyl)amino]-3-thiophenecarbonitrile (II) [30], 2'-acetamido-4'-[N,N-bis(2-methylcarbonyl)ethyl]amino]-4-nitroazobenzene (III) [48].

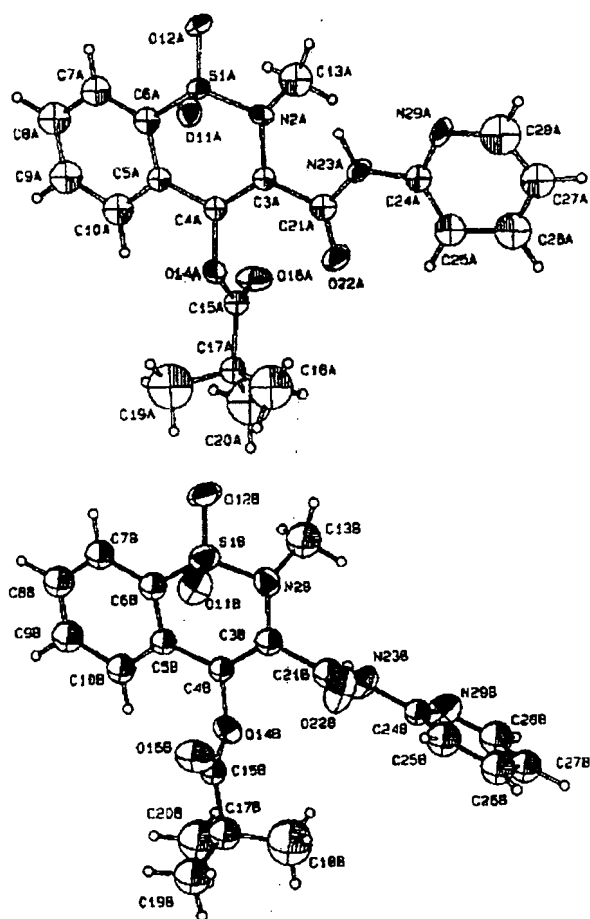


Fig. 3. Conformations of the two independent molecules of piroxicam pivalate (I) in polymorph 2. Thermal ellipsoids are drawn at the 40% probability level, and H atoms are shown as spheres of arbitrary size ([40], reproduced with the permission of the American Pharmaceutical Association).

was obtained as a hemihydrate, and the other as the solvate of isopropanol (2-propanol). In the unit cell of the hemihydrate, one finds two protonated paroxetine and two chloride ions together with one water molecule. Interestingly, the two paroxetine molecules are conformationally nonequivalent, and exhibit a number of different bond angles and torsion angles. In the other form, the unit cell contains one protonated paroxetine molecule, one chloride ion, and one isopropanol molecule disordered along a molecular channel. Furthermore, the conformation of the paroxetine molecule in the isopropanol solvate is different from either molecular conformation observed in the

hemihydrate phase. Crystals of the isopropanol solvate decomposes in the open air at room temperature, because the isopropanol molecules are released easily through the channel. The hemihydrate is relatively stable.

In an impressive fundamental study, the polymorphism of 5-methyl-2-[2-(nitrophenyl)amino]-3-thiophenecarbonitrile (Scheme 1, structure II) has been catalogued [42,43] and discussed in detail [2]. This compound was crystallized as six solvent-free polymorphs, each of which differed in the mode of packing and in molecular conformation. The different conformers yielded sufficient perturbations on the respective molecular orbital so that a variety of crystal colors (red, orange, and yellow) were observed. To obtain a more detailed evaluation of the relative stability, the authors considered a partitioning of polymorphic energy differences into lattice and conformational contributions, and were able to deduce general trends that appeared valid in the absence of hydrogen bonding. The act of crystallization was found to feature an interplay of opposing forces, with perpendicular molecular conformations being favored in fluid solutions, while a preference for planar/high dipole conformers existed in most crystal forms, as shown in Fig. 4 [42]. The unusual polymorphism displayed by this system may result from one or more of the following factors: the preference for perpendicular conformations in solutions, the preference for planar/high dipole conformers in crystals, the formation of inter- and intramolecular hydrogen bonds, and the thermodynamic tendency towards low energy and high entropy.

2.4. Phase transformations in the solid state

Studies of phase transformations in the solid state are important, because the sudden appearance or disappearance of a crystalline form can threaten process development, and can lead to serious pharmaceutical consequences if the transformation occurs in the dosage forms. Hence, an understanding of the kinetics and mechanism of phase transformations is of practical importance. The rearrangement of molecules into a new structure during phase transformation may or may not involve a solvent or vapor phase. To explain the mechanism of solid–solid

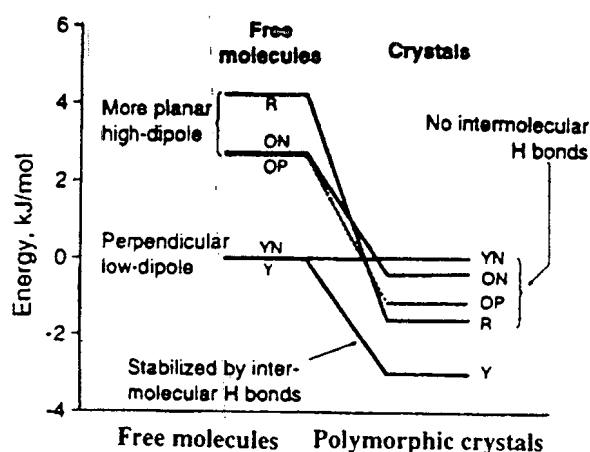


Fig. 4. Comparison of conformational energies and crystal energies of the various polymorphs of 5-methyl-2-[2-(nitrophenyl)amino]-3-thiophenecarbonitrile (II). Form R (red prisms, m.p. 106.2°C), form Y (yellow prisms, m.p. 109.8°C), form OP (orange plates, m.p. 112.7°C), form ON (orange needles, m.p. 114.8°C), form YN (yellow needles, m.p. not measurable) [42], reproduced with the permission of the American Chemical Society).

physical transition, four steps have been proposed: (a) molecular loosening in the initial phase; (b) formation of an intermediate solid solution; (c) nucleation of the new solid phase and (d) growth of the new phase [2]. In an interesting study, Skwierczynski [44] has proposed a two-environment model to describe the decomposition reaction kinetics of a crystalline solid, aspartame. The decomposition reaction of aspartame is a simple unimolecular thermally-induced aminolysis and the reaction proceeds under anhydrous conditions, i.e., water is not a reactant [45]. This model links the chemistry of the solid-state reaction with the molecular mobility of the reactant as the reaction proceeds. The advantage of this model is that it can be used to determine the shelf life of a product from kinetic data gathered at elevated temperatures. Apart from solid–solid physical transformations, solution-mediated physical transformations among polymorphs are also known to occur in processes, such as wet granulation and during dissolution testing.

While the majority of studies have probed the equilibrium properties of polymorphic and solid-state solvated systems, relatively few have been concerned with the dynamics of phase transformation. Byrn et

al. [2] have reviewed briefly the aspect of polymorphic interconversions and the factors affecting the transformations. In one study, the contribution of hydrogen bonding to the $\alpha \rightarrow \beta$ phase change of resorcinol has been detailed [46]. The α form is more stable than the β form at room temperature, but is less dense than the β form. The transition of $\alpha \rightarrow \beta$ at an estimated transition temperature of 337 ± 1 K is accompanied by an increase in crystal density, with the structure shifting from an open array of molecules (linked through hydrogen bonding) to a denser structure resembling molecular crystals. Through the use of a simple potential model, it was concluded that, during the phase transformation, the energy of the hydrogen bonds decreases along with the extent of such bonding. The energy liberated by this process is almost offset by the enhanced Van der Waals energies associated with the increase in crystal density, and consequently the transition enthalpy is rather small. Accompanying the shifts in hydrogen bonding is a number of effective proton transfers, altering the covalent and ionic portions of the crystal. It was also learned that the increase in entropy produced from the redistribution of protons was of the same order of magnitude as the entropy of the phase transition.

A number of spectroscopic techniques have been used to study the processes associated with a polymorphic transition of 2-(2,4-dinitrobenzyl)-3-methylpyridine [47]. The two interconverting structures coexisted over a temperature range of at least 8–9°C. The phase change was associated with a molecular tautomerization that translated through the collective changes of a large number of molecules, yielding domains having definite short-range order. The slowly evolving spectroscopy that took place above the transition temperature was interpreted as the annealing of domains into a long-range ordered system. The process of phase transformation appeared to consist of an initial fast redistribution of the mole ratio of the coexisting phases, followed by a much slower process involving a macroscopic relaxation of the system. Although local thermodynamic equilibrium was thought to exist in individual domains, the magnitude noted for the temperature range of the phase transition was proposed to arise from nonequilibrium conditions existing among the various types of domain.

A combination of solid-state ^{15}N -NMR spectroscopy and X-ray crystallography was used to study polymorphic transitions in an azobenzene dyestuff, 2'-acetamido-4'-[*N,N*-bis(2-methoxycarbonyl)ethylethyl]amino]-4-nitroazobenzene (Scheme 1, structure III) [48]. This work established that the structure of one polymorph was disordered, and that the process of phase transformation entailed a crankshaft-type motion of the azo linkage. The ORTEP plots of the two molecular conformations for the X-ray structure determination of structure III at 293 K are shown in Fig. 5. Selective polarization inversion and band shape-fitting experiments were used to deduce the thermodynamic parameters of the exchange process.

Raman spectroscopy was used to study the effect of pressure on the phase transitions in hexamethylbenzene and hexa(methyl- d_3)benzene [49]. The form II $\rightarrow$ form III transition of the partially deuterated substance was found to take place at a lower pressure relative to that of the analogous hexamethylbenzene compound, which was attributed to differences in the energies of the intramolecular methyl torsional vibration in the two crystal forms. In another study performed by the same group, the effects of both temperature and pressure on the phase transitions of tetrafluoro-1,4-benzoquinone were considered [50]. In this system, the changes in en-

vironmental conditions were found to influence a number of intermolecular and intramolecular vibrational modes, yielding conformational changes that in turn produced the observed phase transitions.

2.5. Prediction of polymorphs

The main challenge in managing the phenomenon of multiple solid forms of a drug is the inability to predict the number of forms that can be expected in a given case. This prediction would involve quantification of the myriad intermolecular forces within any proposed crystal structure as well as the ability to postulate the likely packing modes for a given molecule in all its configurations [10]. Accurate theoretical prediction of polymorphs from studies of molecular dynamics and crystal structure generation would be of outstanding importance in drug research [36].

More research is now being directed towards developing computational tools to understand the nature of polymorphism and to predict polymorphic forms at an early stage in the drug development process. The recent developments in computational chemistry allow the prediction of possible polymorphic forms based only on the molecular structure of the drug. The Polymorph Predictor, from Molecular Simulations, is currently the only commercial software package that can predict the possible polymorphs of an organic compound from its molecular structure [51]. The package developed by Karfunkel and co-workers [52–54] uses a Monte Carlo simulated annealing approach to generate thousands of possible crystal packing alternatives for a given molecule. Each of the unique crystal structures is then subjected to a lattice energy minimization to obtain the relative stability ranking of the various packing possibilities and the resulting lowest-energy structures are the potential polymorphs. This method has been successfully employed to generate known polymorphs of primidone (Fig. 6A and B) and progesterone, starting from the molecular structures alone [55]. It has also been used to predict polymorphs for a range of small molecules and to predict unknown polymorphic structures of 4-amidinoin-danone guanyldiazide, a selective inhibitor of *S*-adenosylmethionine decarboxylase [56], and of aspirin [57].

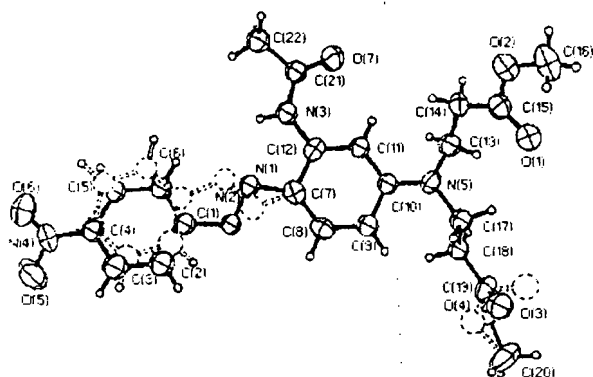


Fig. 5. ORTEP plots of the two molecular conformations (1 and 2) for the X-ray structure determination of 2'-acetamido-4'-[*N,N*-bis(2-methoxycarbonyl)ethylethyl]amino]-4-nitroazobenzene (Scheme 1 Structure) at 293 K. Thermal ellipsoids are shown at 30% for clarity, with conformer 2 being represented by the solid lines and Conformer 1 by the dotted lines ([48], reproduced with the permission of the American Chemical Society).

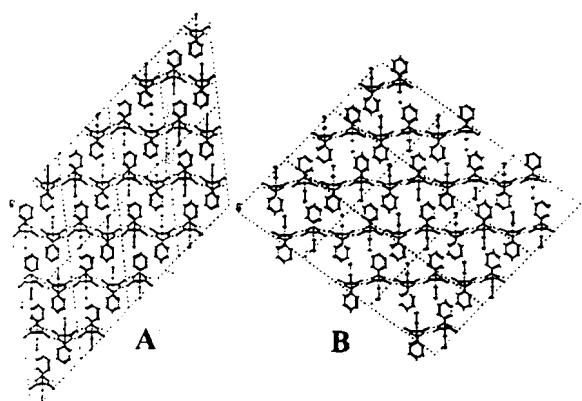


Fig. 6. Comparison of the crystal structure of primidone A (A) versus the most likely packing arrangement, frame 7 (B) ([55], reproduced with the permission of Elsevier Science).

The theoretical predictions of lattice energies, entropies, morphologies and polymorphs should stimulate experimental activities and vice versa. The current crystal-modeling efforts have the potential of producing more quantitative tools for bridging structures and properties, which could help in creating solid forms with desired properties [22]. There are many limitations in using computational methods for predicting polymorphs theoretically. The first limitation is that the *ab initio* screening is useful only for nonionic rigid molecules. For more complex systems, the method is very useful for generating plausible crystal structures, but it is not accurate enough to determine which of these possible structures can actually be crystallized [58]. In addition, the limitations in computer power can restrict the use of this method for predicting polymorphs of complex molecules. An issue of concern is that the existing methods only predict the lattice energies, which relate to internal energies or enthalpies of the crystals. However, the relative thermodynamic stability of polymorphs is determined by the Gibbs free energy, which is a linear function of both enthalpy and entropy. Predictions of the relative stability of polymorphs will be more accurate when the entropies, as well as lattice energies, are considered. Application of molecular dynamics may enable the entropies to be calculated. Hence, no general method is currently available for the prediction or interpretation of the properties of complicated polymorphic or pseudopolymorphic systems.

2.6. Directing the crystallization of specific polymorphs

Complementing the different computational methods for predicting the stable polymorphs of a given compound, various experimental methods are also being employed extensively to control the type of polymorph formed during the crystallization process. Many studies have reported the role of additives in controlling the outcome of the crystallization process. Some of the preselected additives are capable of inhibiting the nucleation and/or growth of the unwanted polymorphs. For the first time, the role of reaction by-products in controlling polymorph appearance of a drug has been reported [59]. This drug is sulfathiazole that is known to exist as polymorphs, forms I, II, III and IV, that differ in the hydrogen-bond network. Form I was found to be different from the other three forms as a result of a different hydrogen bonding at the aniline moiety of the molecule. From studies of the hydrogen-bonding pattern, it was predicted that the ethamido derivative of sulfathiazole could selectively control the formation of form I over other forms by entering the growing face of form I without disrupting the structure (Fig. 7a). Because a similar effect was not possible with the other forms, incorporation of the ethamido derivative in the other forms should inhibit their growth (Fig. 7b). Experimentally, it was shown that the ethamido by-product stabilized form I over the other polymorphs. This study clearly shows that the combination of crystal morphology and the hydrogen-bond network analysis of the different polymorphs offer a new and powerful approach to understanding and controlling polymorph appearance and stability in the presence of additives.

A similar approach was also applied to stabilize a metastable α conformational polymorph of L-glutamic acid using additives [60]. Methods such as DREIDING and TRIPOS force fields were used to select appropriate additives which could mimic the α and β conformations. Four additives were chosen for this study of which two were present exclusively in the β conformation and theoretically should selectively inhibit the crystallization of the β phase and thus stabilize the metastable α phase. Experimentally, it was proven that the additives, by virtue of their conformation, were able to selectively inhibit the

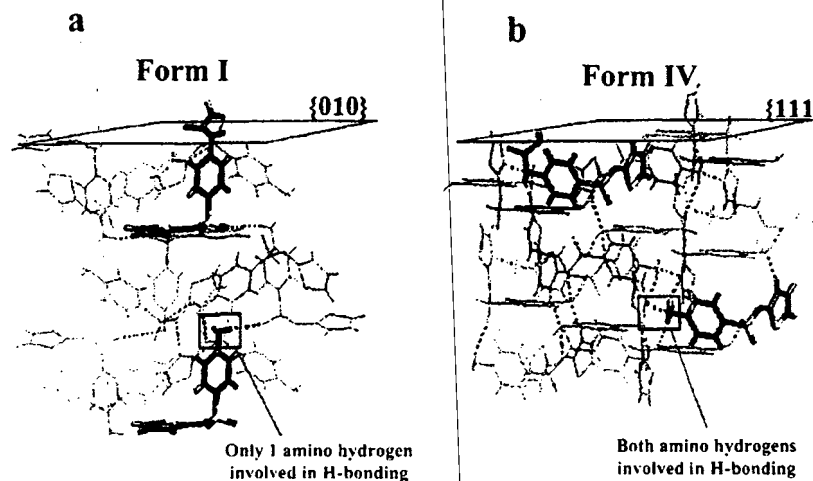


Fig. 7. Possible binding interaction of ethamidosulfamide in the fastest growing faces of (a) form I and (b) form IV of sulfathiazole ([59], reproduced with the permission of Elsevier Science).

appearance of the stable β polymorph of L-glutamic acid by interfering with either the nucleation rates or the growth rates and thus stabilize the metastable form. These studies demonstrate clearly that the molecular packing and intermolecular hydrogen bonds are the main features, which make possible the conformational discrimination. The use of conformational mimicry to stabilize the metastable structures of conformational polymorphs now offers a powerful tool for the prediction and development of robust processes for the control of polymorphic systems.

2.7. Characterization of polymorphs using a combination of analytical techniques

The common techniques often fail to differentiate definitively between two structurally similar polymorphs. Hence more advanced techniques or a combination of techniques need to be used to avoid errors of interpretation and in the identification of polymorphs [24]. Combinations of techniques are being employed currently for the characterization of crystalline pharmaceutical solids. For example, conventional single-crystal X-ray diffractometry and polarized microscopy were of no use in distinguishing the two forms I and II of roxifiban, a very promising cardiovascular drug, because of the relatively small crystallite sizes of the polymorphs. Hence, transmission electron microscopy (TEM) and

synchrotron X-ray diffraction techniques were employed to characterize the unit cells of the two forms. By coupling the highly resolved synchrotron powder X-ray diffraction data shown in Fig. 8, with in-

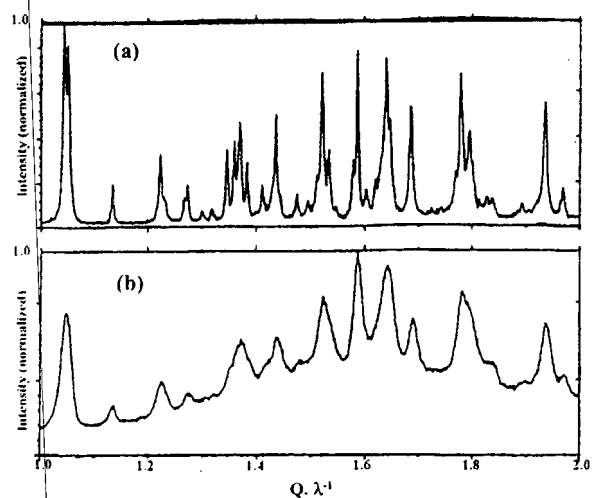


Fig. 8. A partial comparison of (a) a synchrotron pattern of polymorph II of roxifiban, collected using a wavelength of 1.00006 Å with (b) a conventional X-ray diffraction pattern using $\text{CuK}\alpha$ radiation in a region where there are many overlapping peaks. The patterns are plotted as a function of $Q = 2\pi/d = 4\pi \sin \theta/\lambda$ to remove the effects of different wavelengths ([61], reproduced with the permission of the American Pharmaceutical Association).

formation obtained from TEM diffraction patterns, the unit cell parameters of the two forms of roxifiban were determined [61]. Similarly, the three modifications, I, II and III, of the nonsteroidal antiinflammatory drug, tiaprofenic acid, could not be distinguished by the two traditional spectroscopic methods, FTIR and FT-Raman spectroscopy. The modifications can only be distinguished by a combination of thermoanalytical and powder X-ray diffractometric methods [62].

Another example, to which a combination of techniques has been successfully applied to identify the various conformational polymorphs of a drug, is the characterization of the solid forms of neotame [29]. Neotame, *N*-(3,3-dimethylbutyl)-L-aspartyl-L-phenylalanine methyl ester, a new high-potency sweetener exists in the following phase-pure crystalline forms: monohydrate, the most stable crystalline form of neotame under ambient conditions, a methanol + water solvate [63], a methanol solvate [64], an amorphous anhydrate [29] and a crystalline anhydrate (form A; [65]). The authors conducted a systematic study of the conversion of the monohydrate under vacuum to a mixture of anhydrate forms followed by the reconversion of the anhydrate to the monohydrate upon exposure to moisture under ambient conditions. No significant changes were observed in the powder X-ray diffraction patterns during part of the reconversion process, suggesting that no change in lattice structure had occurred. However, the solid-state ^{13}C -CP-MAS NMR spectra, indicated the presence of several forms of neotame during the reconversion (Fig. 9). This discrepancy in the results between the two techniques was attributed to the conformational change of neotame molecules during reconversion, without significant change in unit cell parameters. This example indicates that both solid-state ^{13}C -CP-MAS NMR spectroscopy and powder X-ray diffractometry are needed to analyze mixtures of solid forms of conformationally flexible molecules, such as neotame.

A combination of solid-state ^{13}C -NMR spectroscopy and single crystal X-ray diffractometry also has been used to examine the solid-state tautomerism of acetohexamide [66,67]. Polymorphism of the anti-diabetic drug acetohexamide has been investigated by numerous techniques. On the basis of FTIR data, form A of acetohexamide has been proposed to exist

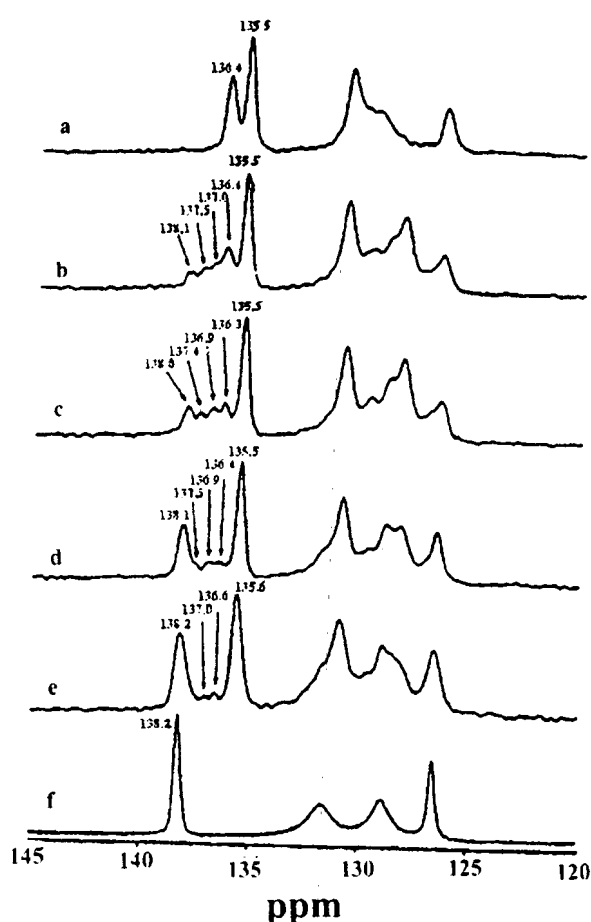


Fig. 9. Resonance signal of the phenyl carbon (C-15) attached to the side chain in the ^{13}C -CP-MAS NMR spectra of neotame anhydrate: (a) sample generated by placing the original monohydrate under vacuum (~ 1 Torr) for 3 days; (b–e) sample after being sealed in a jar for 2, 4, 6 and 8 days, respectively; (f) sample after being exposed to a relative humidity environment of 84% for 12 days ([29], reproduced with the permission of the American Chemical Society).

in the enol-tautomeric state, whereas form B has been proposed to be in the keto-tautomeric state. Using NMR and crystal structure data it was firmly established that both these acetohexamide polymorphic forms are present in the keto-form. Hence the combination of solid-state NMR spectroscopy and X-ray crystallography provided strong evidence that both forms of acetohexamide exist in the keto-tautomeric state and are truly polymorphic.

3. Recent advances in the identification and characterization of hydrates and solvates

3.1. Introduction to solvates and hydrates

It has been estimated that approximately one-third of the pharmaceutically active substances are capable of forming crystalline hydrates [68]. The water molecule, because of its small size, can easily fill structural voids and because of its multidirectional hydrogen bonding capability, is also ideal for linking a majority of drug molecules into stable crystal structures [2]. The mere presence of water in a system is not a sufficient reason to expect hydrate formation, because some compounds, though they are soluble in water, do not form hydrates. It is the activity of water in the medium that determines whether a given hydrate structure will form. Solvates may be formed when a pure organic solvent or a mixture of solvents is used as the solvent for crystallizing the compound. Guillory [69] has discussed the various methods of preparation of hydrates and solvates in detail. Because solvates behave similarly to hydrates, common analytical techniques can be used for characterization of solvates and hydrates.

3.2. Structural aspects

Crystalline hydrates, based on their structure may be classified into three categories. The first category (class 1) are the isolated site hydrates, where the water molecules are isolated from direct contact with other water molecules by intervening drug molecules, e.g., cephadrine dihydrate. The second category (class 2) are channel hydrates where the water molecules included in the lattice lie next to other water molecules of adjoining unit cells along an axis of the lattice, forming channels through the crystal, e.g., ampicillin trihydrate. The channel hydrates can be subclassified into two subcategories. One category comprises the expanded-channel or nonstoichiometric hydrates, which may take up additional moisture in the channels when exposed to high humidity and for which the crystal lattice may expand or contract as the hydration or dehydration proceeds effecting changes in the dimensions of unit cells, e.g. cromolyn sodium. The other subcategory comprises

the planar hydrates, which are channel hydrates in which water is localized in a two-dimensional order, or plane, e.g., sodium ibuprofen. The third category (class 3) of crystalline hydrates are the ion-associated hydrates, in which the metal ions are coordinated with water, e.g., catteridol calcium [8.9].

In this section, some examples of nonstoichiometric hydrates and their characterization will be discussed in detail because these forms pose a special challenge in dosage form development due to unpredictability of water content in the crystals. Following the work of Cox et al. [70] the unusual water uptake and formation of nonstoichiometric hydrates of cromolyn sodium was reinvestigated using single crystal X-ray diffractometry, PXRD, as well as by molecular modeling [71]. Cromolyn sodium, an antiasthmatic drug, exists as two liquid crystalline phases and a crystalline hydrate phase that sorbs and liberates water continuously and reversibly to give a continuous range of nonstoichiometric hydrates [70]. The changes in the PXRD patterns of the crystalline hydrate phase of cromolyn sodium in response to the surrounding relative humidity (RH) were explained in the light of the molecular and crystal structure of cromolyn sodium. Single crystal X-ray diffractometry indicated the space group for cromolyn sodium as *P*1, a chiral space group, even though the molecule itself is achiral. The crystal structure of cromolyn sodium with five or six water molecules per cromolyn sodium molecule, solved at room temperature by Hamodrakas et al. [72], revealed the positions of only one sodium ion and two water molecules and showed that the second sodium ion and the other water molecules are disordered. Recently, the single crystal structure of cromolyn sodium at 76% RH, with 6.44 molecules of water was solved at 173 K by Chen et al. [71]. This work showed that the second undetermined sodium ion is disordered over three sites and that four of the eight water positions are partially occupied. Comparison of the crystal structures determined by Hamodrakas et al. [72] and Chen et al. [71] indicated that the cromolyn anion is flexible. In particular, the bond and torsional angles of the 2-hydroxypropane linking the two cyclic moieties, changed to accommodate lattice expansion or contraction resulting from water sorption and desorption by the crystals. As water is taken up, the relative occupancies of the sites of the

second sodium ion and that of water molecules change. As a result, the triclinic structure with $\alpha > 90^\circ$ approaches the monoclinic form with $\alpha \approx 90^\circ$. To summarize, the presence of large water channels, the flexibility of the 2-hydroxypropane link, the disorder of the second sodium ion (Fig. 10) and the disorder of the surrounding water molecules in the crystal lattice explain the reversible and nonstoichiometric water sorption and desorption by cromolyn sodium. This study emphasizes the importance of the detailed single crystal structure in explaining many unusual physico-chemical properties of drug hydrates.

The muscarinic agonist, LY297802 tartarate [i.e., (+)-3-[3-(butylthio)-1,2,5-thiadiazol-4-yl]-1-azabicyclo[2.2.2]octane monohydrogentartrate}, was also found to exhibit an unusual tendency to form nonstoichiometric hydrates of variable, but specific composition, ranging from 0 to 0.5 mol of water [73]. Solid-state ^{13}C -NMR spectroscopy, in conjunction with moisture sorption analysis and X-ray crystallography was used to provide unique insights into nonstoichiometric moisture sorption behavior. The PXRD patterns of the drug exposed to different RH values (0 to 75%), indicated neither a peak shift nor the presence of any new peaks, suggesting that

the anhydrous and the hydrated forms of the drug are isomorphic. Fig. 11 shows the significant changes in the SSNMR peaks on exposure to different relative humidities and temperature, indicating that water incorporated into the crystal lattice changes the local chemical environment and causes the observed NMR changes. The incorporation of water into the crystal lattice of the drug was also confirmed by X-ray crystallography. The considerable hygroscopicity of the drug was rationalized in terms of the similar crystal structures of the hydrated and nonhydrated forms, and hence no significant structural modifications are needed for the reabsorption of water into the solids. The rates of dehydration and rehydration are largely determined by the size of the water channels and the strength of the hydrogen-bonding interactions that bind the water molecules in the channels.

Another interesting study with different solvated forms of L-lysine monohydrochloride (LH) was conducted by Bandyopadhyay et al. [74]. LH was found to form: a pure methanol solvate at water activity, $a_w < 0.34$, with methanol activity, $a_m > 0.7$; a dihydrate at $a_w > \sim 0.65$ with $a_m < 0.45$; and mixed solvates at intermediate values of a_w and a_m . It was found that the dihydrate and the mono-

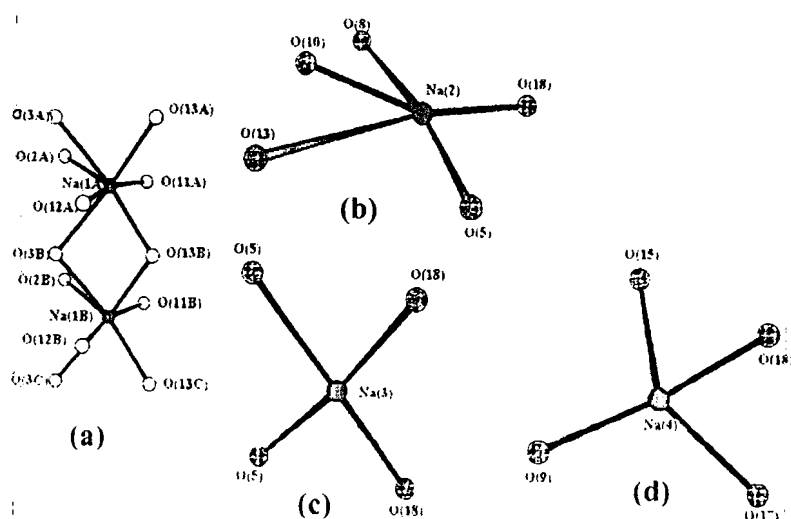


Fig. 10. In hydrated cromolyn sodium, coordination environment of: (a) the first (ordered) sodium ion, Na(1), shown in two neighboring unit cells (A and B); and the second (disordered) sodium ion at the three partially occupied sites, (b) Na(2), (c) Na(3), and (d) Na(4). The striped circles represent the sodium sites. The open circles represent the oxygen atoms coordinated to Na(1). The dotted (gray) circles represent the oxygen atoms coordinated to Na(2), Na(3), or Na(4) ([71], reproduced with the permission of the American Pharmaceutical Association).

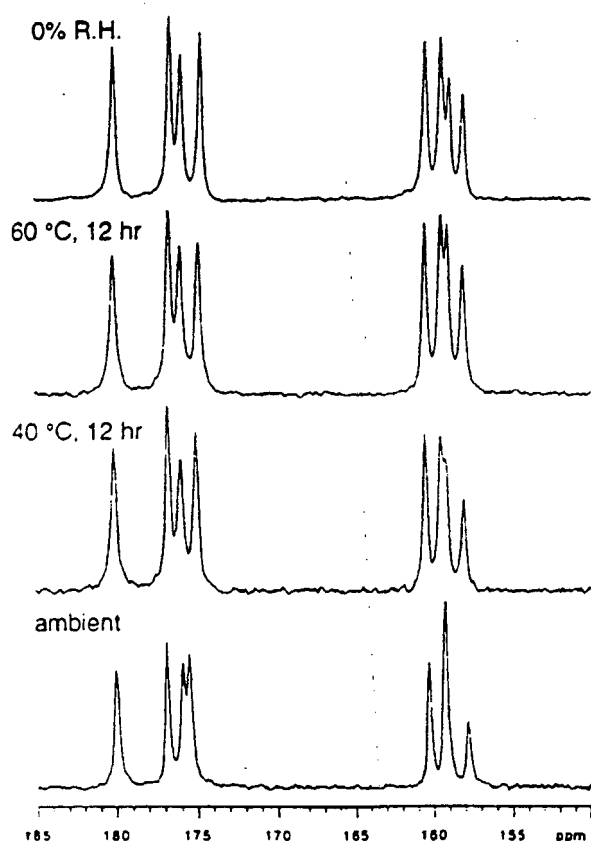


Fig. 11. Solid-state ^{13}C -NMR spectra of LY297802 tartrate ((+)-3-[3-(butylthio)-1,2,5-thiadiazol-4-yl]-1-azabicyclo[2.2.2]octane monohydrogentartrate) after storage at 0% humidity and before and after drying at elevated temperatures ([73], reproduced with the permission of the American Pharmaceutical Association).

methanol solvated forms of LH possessed similar crystal structures, similar PXRD patterns, but differed in the crystal habit. The crystal structures of LH hydrate and methanol solvate indicate that one molecule of methanol in the methanol solvate occupies approximately the same volume as the two water molecules in the dihydrate. During dehydration in the presence of methanol in the crystallization medium, the loss of one or more water molecules from the crystal lattice was compensated by the gradual uptake of methanol into the crystal structure to satisfy the hydrogen bonding pattern within the lattice with minor rearrangements, giving rise to mixed solvates. The similarities of the crystal lattices of the dihydrate and monomethanol solvate explain the similarity of the PXRD patterns.

3.3. Phase transformation of hydrates and solvates

Phase changes due to hydration/dehydration and solvation/desolvation of pharmaceutical compounds during processing or in the final product may result in an unstable system that would effect the bioavailability of drug from solid dosage forms. Various types of phase changes are possible in solid-state hydrated or solvated systems in response to changes in environmental conditions, such as relative humidity, temperature and pressure. For example, some hydrated compounds may convert to an amorphous phase upon dehydration and some may convert from a lower to a higher state of hydration yielding forms with lower solubility. Alternatively, a kinetically favored but thermodynamically unstable form may be converted during pharmaceutical processing to a more stable and less soluble form [8]. The phase transitions in hydrates and solvates can occur at various stages of dosage form development. Morris [9] has discussed the behavior of hydrates during processing, handling and storage of formulations in detail.

The phase transformations associated with exposure to water, such as during solubility measurements, wet granulation processes, dissolution studies and accelerated stability tests are likely to occur via solution mediation. Solution mediated phase transformations depend upon the solution phase to provide the mobility necessary to rearrange in the most stable form and hence are much faster than solid-state transformations. The rate of a solution-mediated transformation is proportional to the solubility of the species involved. Temperature, pressure and relative humidity may increase the rate of phase transformation of hydrates by inducing mobility in the system.

Solution-mediated phase transformations have been reported for many hydrate systems, such as theophylline crystals [17], eprosartan mesylate [75] and nedocromil sodium [76]. Ghosh and Grant [77] have addressed a common problem associated with the characterization of solvates which centers around the determination of solubilities of solvates and of nonsolvates that undergo phase transformation in the presence of an interacting solvent, such as solvation of nonsolvates in the solvent of crystallization or the desolvation of solvates in water. A thermodynamic cycle analogous to Hess's law but based on free

energies has been developed to predict the theoretical solubilities of 1,2-dialkyl-3-hydroxy-4-pyridones, which form 1:1 formic acid solvates in the presence of formic acid, and of the 1:1 formic acid solvates which produce the corresponding unsolvated compounds in the presence of water. A good correlation was obtained between the solubility values measured by the standard extrapolation method and that calculated by means of the thermodynamic cycle.

Apart from identifying and characterizing the phases during various stages of drug development, it is very important to gain an understanding of the dehydration/hydration mechanisms and kinetics. Many models have been developed to account for the dehydration kinetics of the crystalline hydrates [78]. Nucleation is the most significant phenomenon in determining the transformation kinetics, that is, the rate of formation of a new phase [8]. The dehydration kinetics to some extent will also depend upon the class of the hydrate system to which the drug belongs, particle size and morphology. The practical applications of understanding the dehydration kinetics, as indicated by Morris [9], are mainly the determination of the conditions for allowable exposure of bulk drug substances during development and processing, proper packaging, allowable temperature ranges for shipping, storage, and labeling of the final product, and the initial selection of a form for development.

3.4. Prediction of the formation of hydrates and solvates

Predicting the formation of solvates or hydrates of a compound and the number of molecules of water or solvent incorporated into the crystal lattice of a compound is complex and difficult. Each solid compound responds uniquely to the possible formation of solvates or hydrates and hence generalizations cannot be made for a series of related compounds. Certain molecular shapes and features favor the formation of crystals without solvent; these compounds tend to be stabilized by efficient packing of molecules in the crystal lattice, whereas other crystal forms are more stable in the presence of water and/or solvents. There may be too many possibilities so that no computer programs are currently available

for predicting the crystal structures of hydrates and solvates.

3.5. Characterization of hydrates and solvates

The common methods for the characterization of hydrates and solvates are polarized light microscopy and hot stage microscopy, DSC, TGA, Karl Fischer titrimetry, single-crystal X-ray diffractometry, powder X-ray diffractometry, and infrared spectroscopy. These methods have been reviewed in detail [21] and will also be discussed in detail in later chapters.

Pressure DSC is gaining increasing popularity in the study of solvates and hydrates where dehydration reactions occur above or near the boiling point of water. Using conventional DSC, it is very difficult to measure the heats of dehydration and heat of vaporization separately, but if one conducts DSC experiments at elevated pressures, the two processes may be completely separated. The advantage of using pressure DSC is that the pressure can be precisely controlled and the solids can be subjected to a controlled temperature program while under substantially elevated temperatures. The influence of elevated pressures on the solid-state behavior of carbamazepine dihydrate was studied by Han and Suryanarayanan [79]. In Fig. 12 it is shown that pressure DSC can separate the dehydration and vaporization endotherms of carbamazepine dihydrate during its conversion to the anhydrate form. Also the technique permitted the water liberated on dehydration to remain in intimate contact with the anhydrous phase formed which could significantly influence its solid-state properties.

The combined physical analytical techniques of thermogravimetry and infrared spectroscopy (TG/IR) can permit identification of the solvent incorporated into the crystal lattice. This combined technique has been used to study formulated products, such as capsules and tablets [80].

4. Current challenges and future directions

4.1. Origins of the challenges

A series of flow charts and decision trees have been presented and discussed [11,22] that can be

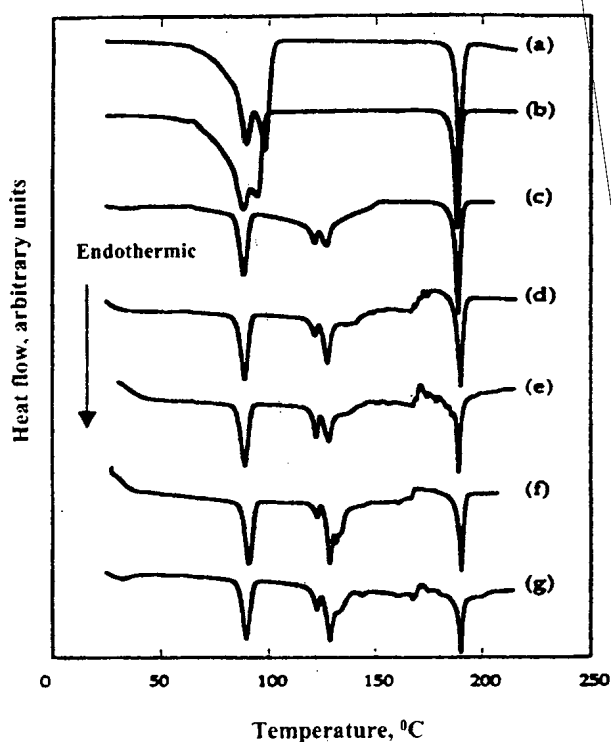


Fig. 12. Differential scanning calorimetry (DSC) curves of carbamazepine dihydrate at different pressures: (a) at atmospheric pressure in a conventional DSC cell, (b) at atmospheric pressure in a pressure DSC (PDSC) cell, (c) 100 p.s.i., (d) 200 p.s.i., (e) 300 p.s.i., (f) 400 p.s.i., and (g) 600 p.s.i. (1 p.s.i. = 6.9 kPa) ([79], reproduced with the permission of Elsevier Science).

used by investigators to characterize the polymorphs and solvates of compounds under development or for registration with regulatory authorities. Due to the complex and nonconventional behavior of various organic drug molecules, there are many opportunities for research and development in the area of characterization of polymorphs and solvates. Some of the problems which are commonly encountered during characterization of crystalline solids and which need to be addressed are: disorder in the crystal lattice due to pharmaceutical processing leading to conversion of a crystalline phase to an amorphous material or phase conversion from one form to the other; quantitating the amount of single polymorph in a mixture of polymorphs; identifying the solid form of the active ingredient in the formulated product, particularly when the drug is a minor component in the presence of numerous other materials (excipi-

ents); and the issues of disappearing polymorphs and the appearance of new polymorphs. In the following sections we will address some of these issues and some of the studies that have addressed these problems.

4.2. Phase transformations during processing

The effects of pharmaceutical processing on the crystalline state of drug polymorphs and solvates have been discussed recently by Brittain and Fiese [16]. Exposure to changes in temperature, pressure, relative humidity and comminution are encountered during processes such as drying, granulation, milling and compression. The stresses applied to crystals during pharmaceutical processing can cause defects in their crystal lattices, and contribute to lattice disorder, thus affecting the physical properties of the resulting powder [81]. This problem has been discussed in detail by Byrn et al. [10]. Arising from different degrees of crystalline disorder, the difficulty in reproducing materials with the same properties is a major concern in the pharmaceutical industry.

Milling, the last processing step in the production of bulk drug substance to reduce particle size, is often accompanied by a decrease in crystallinity due to the creation of lattice defects, beginning at the surface. The defects created by mechanical activation of the solid on the surface can migrate, transform, and change their number and nature. If the defects in the mechanically activated crystal heal to produce a crystal lattice different from the initial lattice, then a polymorphic transformation has taken place. Milling-induced polymorphic changes have been observed for many small drug molecules, such as fostedil, chloramphenicol palmitate, indomethacin and phenylbutazone [16]. Polymorphic transformation of the dipeptide sweetener, aspartame hemihydrate, can occur during milling [82]. Polymorph II of aspartame hemihydrate was found to transform to form I during ball-milling or on heating for 30 min at 160°C in the presence of steam as shown in the X-ray diffraction pattern (Fig. 13). The susceptibility of form II of aspartame hemihydrate to transform to form I has been attributed to the less symmetric crystal structure of form II compared to that of form I as studied by spectroscopic methods.

Some authors, such as Hüttenrauch [81] have

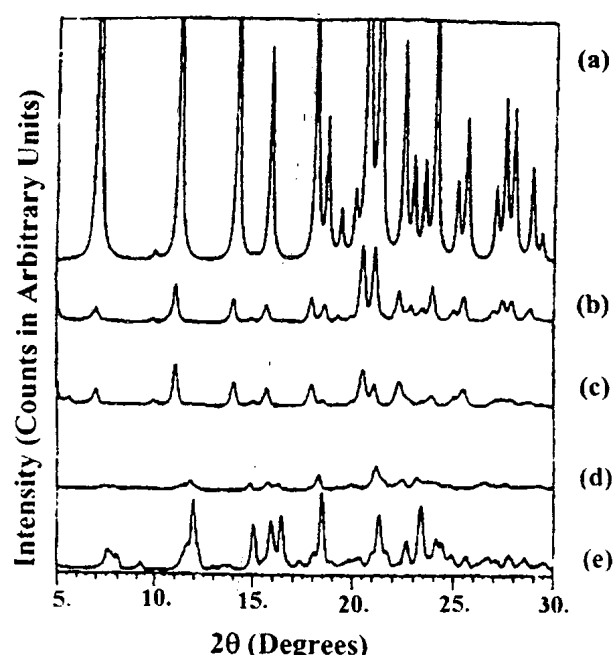


Fig. 13. Powder X-ray diffraction patterns of aspartame hemihydrate: (a) theoretical powder pattern calculated from the crystal structure of aspartame hemihydrate (form I) determined by Hatada et al., (1985) [99], (b) experimental powder pattern of the ball-milled aspartame hemihydrate (form I), (c) experimental powder pattern of aspartame hemihydrate that had been heated for 30 min at 160°C in the presence of steam (form I), (d) experimental powder pattern of aspartame hemihydrate after compression at 250 MPa for 1 min (form II), and (e) aspartame hemihydrate as received (form II) ([82], reproduced with the permission of the American Pharmaceutical Association).

suggested that the trauma to crystals during grinding may lead to a decrease in crystallinity, which should improve the compression capacity and dissolution rate of the drug molecules. This hypothesis was tested by studying the morphology, crystalline state, compression capacity and dissolution properties of native and ground crystals of aspirin and lactose monohydrate [83]. No significant increase in compression capacity was observed when native and ground crystals were compared. Only a slight increase in the dissolution rate was observed for ground aspirin crystals which was attributed to surface defects due to grinding resulting in improved crystal wetting.

The effect of roller compaction on lattice defects and phase change has been examined for aspirin [84]. The water vapor sorption isotherm obtained for aspirin on roller compaction indicated much more

water uptake than had been reported previously for other crystalline samples of aspirin under similar conditions. Various possibilities were suggested for the unusual water uptake of aspirin on roller compaction, such as formation of a polymorphic form of aspirin with a much greater affinity for water, formation of a crystalline hydrate in the aspirin sample, or significant reduction in particle size of the aspirin particles thereby providing an increased specific surface area for water vapor adsorption. It is also possible that roller compaction disrupted the crystalline order of some part of the aspirin crystals forming amorphous regions, which then take up relatively large quantities of water into their bulk structure. This example clearly indicates that processes, such as roller compaction, can introduce considerable disorder in the surface of crystals leading to a marked increase in the tendency to sorb water vapor.

Thermal activation, like mechanical activation during processing, also results in a high-energy state of crystals that may reorganize into a different lattice arrangement resulting in a phase change. The thermal stability of drug substances is important, because formulations are often dried at elevated temperatures after wet granulations so that the tablets may contain small regions of high temperature (hot spots) during compression. Various examples have shown that a change of temperature may influence the stability of drug molecules [16]. The effect of low temperatures, such as during freeze-drying, on the crystalline form of the drug has also been studied. The formation of a new mannitol hydrate during freeze-drying has been reported [85]. The formation of a crystalline hydrate by an excipient during freeze-drying may have several practical consequences, such that the difficulty of removing bound water from the crystal lattice can significantly limit the drying rate, while the residual water that is not removed by freeze-drying may be a potential threat to product stability if it is released during storage. The mannitol hydrate formed during freeze-drying survived the typical drying cycle and converted to the anhydrous polymorph of mannitol upon heating.

Spray drying has also been shown to lead to loss of crystallinity in materials, by a combination of processes involving rapid solidification of dissolved material and solid-state transitions due to milling effects in the atomiser. Spray drying leads to conver-

sion of a crystalline phase to an amorphous state and, because the amorphous state is metastable with respect to the crystalline form, phase transformations are likely to occur within the shelf life of the pharmaceutical product, resulting in loss of quality and potency in the product [86].

In view of the significant effects that the state of disorder in crystalline solids caused by pharmaceutical processing can have on the properties of pharmaceutical solids, it is important to be able to assess the extent of disorder in a solid quantitatively down to very low levels. Various methods have been used to measure the percent disorder, such as using predetermined mixtures, measurements of X-ray powder diffraction, density and heats of crystallization which revealed limits of detectability down to about 10%. Using water vapor sorption measurements under very carefully controlled conditions, it was possible to detect disorder as low as 1% in milled samples of sucrose [87]. A comparison of the four methods mentioned above for estimating the percent disorder of milled samples of sucrose gave very consistent results, once the underlying factors that make these techniques sensitive to the concentration of amorphous content were recognized and taken into account.

4.3. Degree of crystallinity

The previous section has emphasized that many pharmaceutical processes lead to a decrease in crystallinity of drug phases. Various studies have concluded that the formation of amorphous material during processing is highly undesirable. The amorphous material, being in a thermodynamically metastable state, is susceptible to reconversion to the crystalline state, affecting many physico-chemical characteristics of the drug. A later chapter provides detailed coverage of amorphous materials. An estimation of the degree of crystallinity of a sample before and after processing poses one of the larger challenges facing the pharmaceutical field. Powder X-ray diffractometry is still the commonly used method for determining the degree of crystallinity, though this method suffers from some limitations due to peak broadening, amorphous halo, and preferred orientation which make interpretation and quantitation difficult. DSC may not be a sensitive method for measuring crystallinity due to crystalliza-

tion of the amorphous content at elevated temperatures and the effects of differences in heat capacity. Solution calorimetry has been proposed as an accurate method for analysis of percent crystallinity [11,88,89]. A decrease in the endothermic enthalpy of solution indicates a decrease in the crystallinity of the sample. However, differences in surface area produced by grinding or by other processing techniques can also result in changes in the heat of wetting of a sample. Judicious choice of solvent can be employed to reduce such surface effects, which themselves contribute to the observed crystallinity of the sample.

Near infrared (NIR) spectroscopy is another technique being used to measure the degree of crystallinity, and has also proved useful in studies of the polymorphism and water content of sugars. The NIR spectrum of a sample contains both physical and chemical information. Being noninvasive, nondestructive and operable at room temperature, the method is a valuable tool with which to assess changes in the amorphous and crystalline state of lactose [90]. NIR has been used to follow the changes in the amorphous state, the onset of crystallization, and the changes between α - and β -lactose, which accompany the onset of crystallization. In another study, the nucleation and crystallization kinetics of amorphous lactose was investigated by gravimetry in an automated vacuum moisture balance. The combination of isothermal and nonisothermal activation energies allowed the investigation of both crystal growth and nucleation mechanisms and led to the separation of activation energies for nucleation and growth [91].

4.4. Characterization of mixtures of polymorphs

Another common problem encountered during drug development is quantitative control of the proportion of polymorphic forms present in a mixture. According to the US FDA regulations, the method of analysis for the proportion of forms must be validated, and also the proportion of forms must remain within stated limits through the retest date of the drug substance and potentially throughout the shelf life of the product. This is a very onerous requirement, especially if the forms have a tendency to interconvert. Byrn et al. [11] suggested that the best way to deal with this problem is probably by

developing methods to prepare only one crystal form and maintaining this form throughout processing. Powder X-ray diffractometry is often a useful method to determine the percentages of polymorphs in a mixture. However, the detection limit is variable from case to case, and is sometimes as high as 15%. It is therefore important to develop sensitive analytical methods with a lower limit of detection.

Attenuated total reflectance (ATR) FTIR spectroscopy has been shown to be valuable for the quantitative analysis of the polymorphic content of bulk pharmaceutical materials. The feasibility of using ATR-FTIR for the qualitative and quantitative analysis of mixtures of pharmaceutical polymorphs has been studied using three known polymorphs of ganciclovir as a model compound [92]. Definitive identification and quantitation of all three polymorphs could be achieved using ATR-FTIR spectroscopy in conjunction with partial least-squares modeling. This technique has many advantages, such as speed, nondestructiveness, relative ease of use, and most important, no sample pretreatment before measurement.

Raman spectroscopy is another technique that is being widely used to quantitatively estimate the percentage of one polymorph in a mixture of polymorphs. FT-Raman spectrometry offers many advantages, the most prominent being, minimal sample preparation, sensitivity to polymorphism, and noninterference from water. Two polymorphs of fluconazole were characterized using FT-Raman spectroscopy and principal components regression using cross validation provided quantitative analysis of the percentage of one polymorphic form in the mixture of other forms [93]. A novel sample holder was developed whereby the sample is held in an NMR tube which is rotated around its axis and at the same time moved up and down. This method of sample presentation leads to a large increase in the volume studied and is important for inhomogeneous samples for which sub-sampling is a problem. Possible degradation of the sample through heating by the laser can also be avoided [94].

X-Ray powder diffractometry is still the common method for the quantitative estimation of polymorphs in a mixture of polymorphs. This method requires that at least one high-intensity peak unique to each form is available for intensity measurements and that

the plot of the peak intensity ratio as a function of the weight ratio of the components should result in a straight line. Modern computer controlled X-ray powder diffractometers now permit quantitative analysis of multicomponent mixtures using the complete powder diffraction profile rather than a limited amount of low-angle integrated intensity data. Artificial neural networks (ANNs) in quantitative X-ray powder diffractometry were used successfully to identify and quantify the two known modifications of ranitidine hydrochloride even when the weight fraction of one polymorph in the mixture was as low as 0.01 [95]. ANNs have been used mostly in problems of pattern recognition and modeling, and is therefore useful in deciphering the pattern in diffraction data from polymorphic mixtures. The ANNs model predicted concentration precisely, accurately, and with minimal bias through a wide range of ratios of the two known ranitidine hydrochloride polymorphic forms in a mixture (Fig. 14). This method minimizes the problems associated with preferred orientation and overlapping X-ray lines. The same group of researchers has shown the potential of ANNs in combination with DRIFT spectroscopy to analyze the polymorphic purity of crystalline ranitidine hydrochloride as a bulk drug and as an active ingredient of

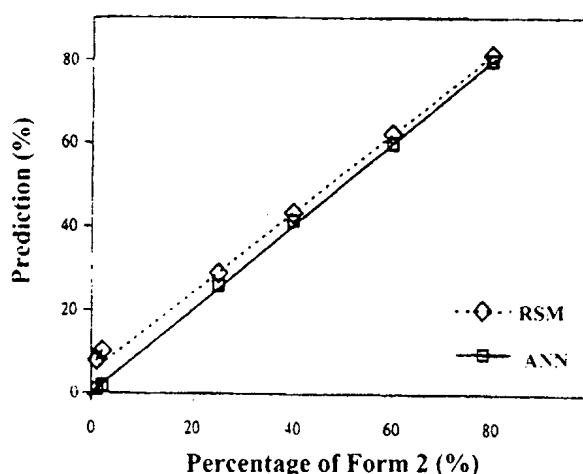


Fig. 14. Predicted concentrations of the two polymorphic forms (form 1 and 2) of ranitidine hydrochloride by the response surface methodology (RSM), a statistical modeling method, and by the artificial neural networks (ANNs) method, plotted against measured percentage of form 2 ([95], reproduced with the permission of Elsevier Science).

a tablet formulation. Simultaneous identification and quantification of all the ingredients in the tablet formulation was possible. This study has shown that the complex problem of quantifying a drug in mixtures containing two or more components with overlapping spectra can be solved by the DRIFT-ANNs technique [96].

Another technique, which is gaining popularity in the quantitative analysis of mixtures of polymorphs, is solid-state ^{13}C -CP-MAS NMR spectroscopy. This method was preferred for quantitative analysis of polymorphic mixtures of the herbicide, pendimethalin, which exists as two polymorphs with different colors and crystal habits [27]. ^{13}C -NMR provided the most sensitive and definitive evidence of the transition from the yellow to the orange form. This method enabled as little as 2% of orange pendimethalin to be determined in a sample consisting mostly of the yellow polymorph. It is the least invasive of the instrumental methods and can be used to detect the ratio of the two polymorphs in solid formulations.

A related challenge faced by the pharmaceutical industry is the determination of the polymorphic nature of the drug in the presence of excipients in a dosage form especially when the active drug is present as a low percentage of the overall formulation [97]. This problem can be addressed by developing sensitive techniques with lower limits of detection or by using a combination of techniques.

5. Conclusions

In order to save time and cost it is very important to choose the most suitable form of the crystalline drug in the initial stages of drug development. In recent years a good deal of research has been directed towards achieving this goal. Systematic isolation and early characterization of the largest number of possible forms of a drug reduces the chances of surprises at the late production stage due to identification of a new crystalline form or phase change. With the development of more sophisticated computational tools, the main focus of many investigators is to be able to predict all the possible forms of a drug from its molecular structure. Understanding the origins of the multiple solid forms of a drug

molecule, either due to differences in packing arrangement or conformation of the molecules, becomes the first step in prediction. Single crystal X-ray diffractometry and solid-state NMR spectroscopy are two techniques that are gaining increased application in determining the various crystal structures and the origins of polymorphism and pseudopolymorphism of a particular drug. When crystal structures can be calculated with certainty, it will be possible to predict the various polymorphs of a compound and this information could be used to guide experimental studies. This goal may be difficult to achieve owing to the complex molecular structures of new organic molecules and the presence of several molecules in each asymmetric unit, but the future development of improved force fields and increased computational speeds, may make it achievable.

Improved experimental methods leading to more accurate and detailed phase diagrams are also finding increased use in determining the stability of various polymorphs. It is important to make every effort to prepare and to identify the most stable polymorph in order to guide the selection of the optimal form for development. The emergence of sensitive methods and the use of combination techniques, facilitate the identification and the more accurate characterization of the various polymorphs of a drug molecule. One of the main analytical challenges faced by the pharmaceutical analyst is the development of better quantitative methods for identifying a single polymorph in a mixture of polymorphs and for determining the percentages of amorphous or crystalline content of the drug. More and more sensitive methods are being developed to address this problem.

An increased understanding of the phenomenon of polymorphism should enable pharmaceutical scientists to gain control over the crystallization process in order to selectively obtain the desired polymorph or suppress the growth of an undesired one. Phase changes during processing and scale-up are a problem, which may be avoided by carefully designed initial small-scale studies. The availability of detailed structural data, combined with strategic design of substrates and additives, has led to significant advances in the control over the polymorphs obtained in a particular crystallization [98]. With all the information available from these initial studies, it

should be possible to design and to select processing conditions which would give a desired polymorph and maintain the desired form throughout the various stages of drug processing and manufacture.

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High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids

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Abstract

The concepts of high-throughput (HT) screening and combinatorial synthesis have been integrated into the pharmaceutical discovery process, but are not yet commonplace in the pharmaceutical development arena. Emerging strategies to speed pharmaceutical development and capture solid form diversity of pharmaceutical substances have resulted in the emergence of HT crystallization technologies. The primary type of diversity often refers to polymorphs, which are different crystal forms of the same chemical composition. However, diverse salt forms, co-crystals, hydrates and solvates are also amenable to study in HT crystallization systems. The impact of form diversity encompasses issues of stability and bioavailability, as well as development considerations such as process definition, formulation design, patent protection and regulatory control. This review highlights the opportunities and challenges of HT crystallization technologies as they apply to pharmaceutical research and development.

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Keywords: High-throughput; Crystallization; Polymorph; Solvate; Salt; Co-crystal

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1. Introduction

Active pharmaceutical ingredients (APIs) are frequently delivered to the patient in the solid-state as part of an approved dosage form (e.g., tablets, capsules, etc.). Solids provide a convenient, compact and generally stable format to store an API or a drug product. Understanding and controlling the solid-state chemistry of APIs, both as pure drug substances and in formulated products, is therefore an important aspect of the drug development process. APIs can exist in a variety of distinct solid forms, including polymorphs, solvates, hydrates, salts, co-crystals and amorphous solids. Each form displays unique physicochemical properties that can profoundly influence the bioavailability, manufacturability, purification, stability and other performance characteristics of the drug [1]. Hence, it is critical to understand the relationship between the particular solid form of a compound and its functional properties. Discovery and characterization of the diversity of solid forms of a drug substance provide options from which to select a form that exhibits the appropriate balance of critical properties for development into the drug product. Importantly, the desired properties may vary with each mode of delivery (i.e., oral, pulmonary, parenteral, transdermal, etc.), such that the solid form may differ for each optimized dosage form. Given these options, the choice and design of pharmaceutical solid forms can be critically important to successful drug development.

Solid form discovery and design depends on the nature of the molecule of interest and type of physical property challenges faced in its development. The preferred solid form is generally the thermodynamically most stable crystalline form of the compound [1,2]. However, the stable crystal form of the parent compound may exhibit inadequate solubility or dissolution rate resulting in poor oral absorption, particularly for water-insoluble compounds. In this case, alternative solid forms may be investigated. For ionizable compounds, preparation of salt forms using pharmaceutically acceptable acids and bases is a common strategy to improve bioavailability [1,3,4].

Like the parent compound, pharmaceutical salts may exist in several polymorphic, solvated and/or hydrated forms.

Most APIs and their salts are purified and isolated by crystallization from an appropriate solvent during the final step in the synthetic process. A large number of factors can influence crystal nucleation and growth during this process, including the composition of the crystallization medium and the process(es) used to generate supersaturation and promote crystallization [1,5–13]. The most notable variables of composition and processing are summarized in Table 1. Solid form screening is used to understand the effects that these variables have on the polymorphic outcome of a crystallization experiment, so that a robust process can be identified to produce the desired crystal form. Traditionally, the study of solid form diversity of active compounds has relied on the use of a variety of common process methods for generation of new forms, coupled with modern characterization methods for analysis of the solids produced [2,14]. Most often, however, a combination of solvent recrystallization (cooling or evaporative, as well as slurry conversion) and thermal analysis (e.g., hot stage microscopy, differential scanning calorimetry) are employed for initial form screening. Such methods are inherently slow and only allow exploration of a small fraction of the composition and process space that can contribute to form diversity. Before suggesting a form for development, scientists may have carried out only a few dozen crystallization experiments and possibly prepared a handful of different salts of a compound. The main reasons for the limited number of experiments are the constraints on availability of compound and scientists' analytical capacity in a given time frame, and they are therefore often forced to make form selection decisions on incomplete data. Accordingly, it is not surprising that unexpected and undesired outcomes can, and do, occur later on in development.

Despite more than a century of research [15], the fundamental mechanisms and molecular properties that drive crystal form diversity, specifically the nucleation of polymorphic forms, are not well under-

Table 1
Crystallization composition and processing variables [1,2,8]

Composition type		Process variables ^a				
Polymorph/ solvates	Salts/ co-crystals	Thermal	Anti-solvent	Evaporation	Slurry conversion	Other variables
▪ Solvent/ solvent combinations ▪ Degree of supersaturation ▪ Additive type	▪ Counter-ion type ▪ Acid/base ratio ▪ Solvent/ solvent combinations	▪ Heating rate ▪ Cooling rate ▪ Maximum temperature	▪ Anti-solvent type ▪ Rate of anti- solvent addition ▪ Temperature of anti-solvent addition	▪ Rate of evaporation ▪ Evaporation time ▪ Carrier gas	▪ Solvent type ▪ Incubation temperature ▪ Incubation time	▪ Mixing rate ▪ Impeller design ▪ Crystallization vessel design (including capillaries, etc.)
▪ Additive concentration	▪ Degree of super-saturation ▪ Additive type and concentration ▪ pH ▪ Ionic strength	▪ Incubation temperature(s) ▪ Incubation time	▪ Time of anti- solvent addition	▪ Surface-volume ratio	▪ Thermal cycling and gradients	

^a Applicable to all types of screens.

stood [13,16]. As a result, predictive methods of assessing polymorphic behavior of pharmaceutical compounds by ab initio calculations remain a formidable challenge. Even in cases where the existence of a crystalline form is predicted, the stability relative to other crystalline packing arrangements has been difficult to estimate with accuracy [17]. Moreover, the prediction of packing structures for multicomponent (e.g., solvates, hydrates, co-crystals) or ionic systems is not yet possible [17]. Due to these limitations, solid form discovery remains an experimental exercise, where manual screening methods are employed to explore form diversity of a compound.

Control over solid form throughout the drug development process is of paramount importance. Reliable preparation and preservation of the desired form of the drug substance must be demonstrated, and has become increasingly scrutinized by regulatory agencies as more sensitive and quantitative solid-state analytical methods have become available [18]. Many strategies to influence and control the crystallization process to produce the solid form of interest have been reported. Some examples include stereochemical control using tailor-made auxiliaries [19–21], targeted solvent recrystallization [22–24], and templating using a variety of surfaces (e.g., organic single crystal substrates [25], surfaces of metastable crystal faces [25,26], inorganic crystal

surfaces [27] and polymeric materials [28]). Recent studies have also begun to uncover the role of reaction byproducts and other impurities in determining polymorphic outcome and crystal properties [29–32], and in fact, it has been shown that in some cases such species can stabilize metastable crystal forms [33,34]. In addition, new processing methods continue to be developed to improve discovery and characterization of new forms, including precipitation by supercritical fluid [35,36], laser induced nucleation [37–39] and capillary crystallization [40–42]. However, there remains a lack of fundamental understanding of the nucleation process and the specific factors that contribute to crystallization of diverse forms of a compound [13,21,23]. In order to fully control the crystallization process, the link between the physical or chemical processes that influence nucleation and crystal growth needs to be better established. It is in this area that new experimental methodologies have the potential to enable development of this knowledge base.

There is reason to believe that the already complicated landscape of pharmaceutical solid forms will become even more complex in the future. It is now increasingly appreciated that hydrogen bonded co-crystal structures between active agents and molecules other than water or solvent can be prepared. For example, co-crystals of aspirin, *rac*-ibuprofen and

rac-flurbiprofen have been prepared by disrupting the carboxylic acid dimers using 4,4'-bipyridine [43]. These structures are formally molecular compounds (or co-crystals) but do not involve formation of covalent bonds or charge transfer from or to the active substance. Recent demonstrations of these principles with drug compounds have been published [43–45].

Exploration of a given compound's polymorphs, hydrates, solvates, salts, co-crystals and combinations of all of these appears intractable by conventional experimental methods, and as the number of potential methods for exploring and controlling crystal form diversity continue to expand, existing strategies will become increasingly inadequate. In an effort to understand form diversity in a more comprehensive manner, high-throughput (HT) crystallization systems have recently been developed. This methodology uses a combinatorial approach to solid form generation, where large arrays of conditions and compositions are processed in parallel. Experiments are performed at small scale to reduce the material demand and to afford the largest number of conditions possible. The large number of crystallization trials performed in these experiments reflects the reality that nucleation rate has an extremely non-linear dependence on the experimental conditions, and as such, the probability of a chance occurrence of a particular form is increased by a HT approach. Supersaturation (solubility) and induction time of the various possible solid forms are independently controlled by these conditions, resulting in highly non-linear time dependence of crystallization. In addition, the combinatorial approach permits exploration of a chemical continuum, where use of many solvent mixtures may allow one to assess what underlying physical or chemical processes are required to produce a particular solid form. Once a variety of conditions that can be used to produce a given crystal form on the microscale are identified in the HT screen, scale-up studies are typically conducted to optimize the process for laboratory scale production.

In this review, the development and application of novel HT crystallization technologies for exploration of solid form diversity are discussed. The operational features of a fully integrated, automated HT crystallization system are presented, highlighting the design requirements for hardware and software components, as well as general specifications for consumables.

Case studies are used to illustrate the benefits and capabilities of the approach, including salt selection in early lead optimization (ELO) and pre-clinical development, polymorph and solvate screening in highly polymorphic systems, comprehensive discovery of crystal forms to reduce the risk of late displays of polymorphism, comparison of experimental and predictive methods of solid form discovery, and engineering of co-crystals. The need for post-screening characterization of crystal forms to enable ranking and selection of the most suitable form for development is briefly reviewed. Finally, the implications of HT crystallization technologies on the future of solid form screening processes, intellectual property protection and regulatory compliance are discussed.

2. Development of high-throughput crystallization technologies

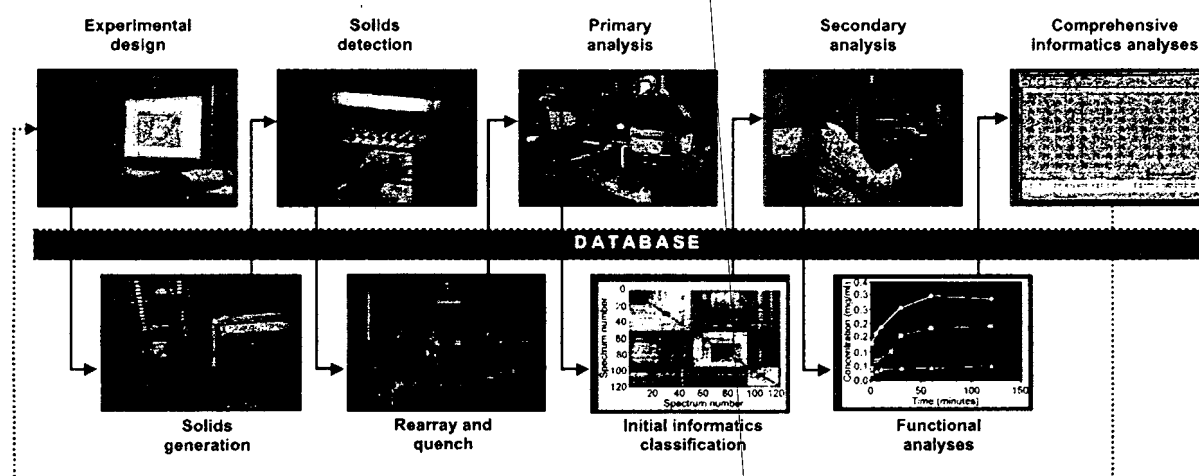
HT crystallization systems have been developed to more rapidly and comprehensively explore the multi-parameter space that contributes to solid form diversity [40,46–51]. In its simplest description, HT crystallization can be broken down into three key experimental steps: *design* of experiment (DOE), *execution* of experimental protocols and *analysis* of data. Systems designed to carry out these experiments generally consist of both hardware and software components that drive and track experimentation, and permit data storage, retrieval and analysis. Such systems should be designed to be flexible and scalable to ensure that a variety of experimental procedures can be carried out either serially or concurrently. Thus, the system can be employed at various stages of drug development, where differences exist in the quality and quantity of compound available. While it is highly desirable to have the ability to mine and model experimental data, and to use the subsequent knowledge to guide further experiments, not all HT crystallization systems are equipped with these features. In Section 3, the hardware and software considerations for design and development of a fully integrated, informatics-driven HT crystallization system are described.

While the concepts of HT screening are widely applied in the pharmaceutical industry, most notably in the drug discovery arena [52], the application of

HT approaches to drug development, in particular solid form screening, are just beginning to be realized. These latter approaches, however, are more akin to HT experimentation than HT screening. Hence, several important distinctions, which reflect on the design of HT experimental systems, need to be made. First, the goal of HT screening is to get a small number of successful outcomes, which are then passed on to the next stage of development. Little effort is typically made to learn why certain outcomes were positive and why others were negative. In contrast, HT experimentation, such as HT crystallization, is carried out with the goal of having each point in the experiment produce multiple types of data that can be interpreted, and the interpretation used to guide the experimental process to a successful conclusion. Second, unlike traditional HT screening assays where experiments are generally conducted under constant experimental conditions, HT crystallization experiments for solid form discovery are best conducted using a variety of process methods, each having varying experimental conditions (e.g., temperature variations as a function of time) over the course of the experiment. These additional process variables permit maximal diversity in the experimental space, increasing the likelihood that comprehensive coverage will be achieved. Finally, there is a distinction to be made in terms of relative “hit rates”. In both HT screening and HT crystallization, a “hit” can be

thought of as a set of conditions that gives rise to a desired result. In HT screening, the desired result is typically an activity, or potency, that exceeds a pre-defined threshold. In HT crystallization, a hit is defined as the formation of a solid. The typical observed hit rate of HT screening is on the order of 0.1% of the total number of samples analyzed. In contrast, HT crystallization experiments can yield hit rates ranging from tens of percents to nearly 100%, depending on the type of experiment and the process mode(s) used. For example, while only a handful of compounds from a selection of thousands may exhibit the required potency, 10–50% of crystallization trials may yield solids. In fact, the range of wells that yield solids is very wide, depending on process mode and experimental time scale, as will be discussed in subsequent sections. The impact of these differences is manifested in the design and operational requirements of HT experimentation systems.

A fully integrated HT crystallization system consists of a number of components, including experimental design and execution software, robotic dispensing and handling hardware, automated high-speed micro-analytical tools, end-to-end sample tracking and integrated cheminformatics analysis software for data visualization, modeling and mining. A schematic overview detailing the workflow of such a system is depicted in Scheme 1 [53]. These features are supported by a comprehensive informatics foun-



Scheme 1. A schematic illustration of the workflow of a fully integrated HT crystallization system [53].

dation that is used to handle the large quantities of data generated. Specifically, informatics tools are used to design statistically relevant and diverse experiments, drive the automation hardware to perform the specified operations, and provide an analytical function to analyze, compare and sort the results of experiments. An important feature of these systems is the ability to mine and model experimental data and use the knowledge generated to guide further experiments. These functions are supported by use of a relational database that provides a mechanism of communication between system components.

When designing a HT crystallization experiment, or set of experiments, a large variety of parameters of composition and process are involved. Experimental designs must be aimed at covering a large multifactorial parameter space, with the goal of determining which experimental factors affect the desired outcome. In practice, it is desirable to place constraints on the experimental space, making common statistical design methods such as full or partial factorial designs inappropriate or impractical. For example, hardware limitations, including minimum and maximum dispense volumes or masses and accessible temperature ranges, as well as constraints related to chemical compatibility (i.e., reactivity of components, miscibility, etc.) or toxicity limits of components (if appropriate), need to be considered. Thus, alternative DOE methods that can accommodate such constraints are required. D-optimal design [54,55] is an example of a DOE algorithm that can take a set of constraints, such as the ones described above, in combination with a target analytical model and determine the optimal set of experimental points to test. Another commonly used DOE algorithm is diversity generation, with which the experimentalist selects a set of pertinent chemical properties and uses the algorithm to evenly spread experimental points over the chosen property space. In addition, some systems utilize a solubility calculator tool to estimate the solubility of the API in the given solvent/additive mixture. The calculated information is then used to select the appropriate concentration of API in each mixture so that it is supersaturated with respect to the reference phase at the harvest temperature. Here, the driving force for crystallization can also be varied by tailoring the composition of each sample based on the API solubility in that mixture. With such DOE tools, experiments may be designed to effective-

ly and simultaneously explore the diverse composition and process space described in Table 1.

Ideally, DOE algorithms should also incorporate prior knowledge or experimental results, which have been stored in a database as a set of rules or models, to limit an experimental space to have certain predicted characteristics. For example, over the course of time, a regression model may be developed between a set of known or calculated chemical properties and a parameter of experimental interest. The model could be used during the design of a new experiment in order to test only those chemicals that are predicted to give a desirable result. Since a large number of factors need to be considered during experimental design, the DOE interface available to the scientist must not only be flexible and easy to use, but must also offer tools that aid design efficiency and effectiveness and permit input of scientific knowledge generated over time.

At the end of the experimental design process, the resulting set of experimental conditions is translated into a series of commands for the HT systems, and stored in a relational database for later retrieval by the software that controls the automation. When an experiment is activated, the overall operation of the automation systems is managed by the HT informatics system, which is responsible for physical operation of the HT platforms as well as data tracking and storage.

Execution of experimental commands is carried out by automated laboratory equipment that comprises the HT crystallization system. Specialized automated systems perform several of the functions in a sequence of events that make up the experiment. Each station is controlled through an interface to the informatics system that ensures the samples are processed at the correct stations, in the correct order, with the selected experimental parameters being followed. Parameters of operation are recorded, including the time at which an action is taken. After execution of the experimental steps, the software interface retrieves any pertinent information generated by the automated platform, such as assay results or operational parameters, stores these data in the relational database, and updates the status of the experiment to reflect the completion of operations.

In general, the hardware required for a HT crystallization system is comprised of four major functional elements: sample preparation, solids generation, solids detection and sample analysis. Sample preparation

involves adding the compound of interest (API) to the diverse set of conditions used to conduct crystallization studies. Typically, the API is dispensed as a solution in a suitable solvent, followed by solvent removal to yield the solid API. Solvent removal can be achieved by passive evaporation or by controlled active evaporation (e.g., use of a vortex dryer). Alternatively, the API can be delivered in the solid state with suitable powder handling systems. Depending on the amount of saturation desired, the crystallization vessel used, and the API's solubility in solvents or solvent mixtures of interest, API masses ranging from a few hundreds of micrograms to several milligrams will be present in each vessel. Once the API has been delivered to the crystallization vessels (tubes, vials or microwell plates), combinations of solvents and/or additives are added to each vessel. By taking advantage of the power of combinatorial approaches, large numbers of unique combinations can be dispensed from manageable sets of starting materials.

Compatibility of equipment components (syringes, dispense tips, tubing, etc.) and consumables (plates, tubes, etc.) with solvents and other compounds is a key hurdle faced in the development of combinatorial crystallization for small molecules. Unlike protein crystallization systems [56,57], which are commonly based on the sitting-drop method in aqueous media, small molecule crystallization employs a range of crystallization additives and processes. The additives include organic solvents with varying properties (e.g., alcohols, acetone, hexane, ethyl acetate, etc.), water, acids, bases and co-crystal formers, as well as other compounds (e.g., small molecule templating agents, surfactants, pharmaceutical excipients, etc.). This wide range of materials needs to be handled by appropriate liquid handling techniques to enable the combinatorial assembly previously mentioned. Ideally, liquid transfers are achieved using multichannel pipettors with individually controllable channels. Depending on the crystallization vessel design, the volumes of reagents dispensed will be as low as a few microliters to as high as several hundred microliters.

Potential for cross-contamination and tendency toward unwanted solvent evaporation from crystallization wells are challenges that need to be addressed in a HT crystallization system. A large number of the solvents used to crystallize small molecules have high

vapor pressure under ordinary laboratory conditions. Sealing of the crystallization vessels is key to being able to control composition during crystallization from these solvents. Due to solvent fugacity, vessels need to be protected from ingress of the components of neighboring wells. These problems have been solved by different means, such as sealing of individual tubes with a Teflon-backed crimp seal [40] or O-rings/gasket seals and clamped covers [47,51].

HT crystallization must enable several process modes that are compatible with the compound (e.g., chemical stability, thermal stability, etc.). In some cases, multiple modes of operation may be combined. The most common modes of solids generation will be discussed below, including thermal cooling crystallization, anti-solvent and evaporative crystallization. Less common process modes include melt crystallization, flash or quench cooling and template-directed crystallization. It is important to note that generation of maximal diversity in solid form requires multiple modes of operation [6,18,58].

In thermally induced cooling crystallization, samples created in the sample preparation process described above are subjected to temperature ramps. Prior to beginning the temperature ramp, samples are exposed to an elevated temperature for a short period of time in order to dissolve the API in the crystallization medium. Although dissolution can be achieved most simply by diffusion and convection from the heating process, addition of external energy can speed up the process (e.g., sonication). Samples may be optically inspected (see Fig. 1) and vessels that contain undissolved solids can be flagged in the database for further analysis. For instance, undissolved samples may be treated as slurry conversion experiments and monitored over time for crystal form changes. The thermal cycle is then initiated, using controlled cooling to induce supersaturation. In this mode of crystallization, samples continually experience an under cooling and, based on the level of supersaturation in the vessel, may recrystallize at a given temperature after a period of time. Thermal crystallization tends to generate a cumulative number of samples that are produced over time in a fashion approximating a square root function, as illustrated in Fig. 2. This means that initially there is a small bolus of "hits", after which the rate of crystallization tails off over a period of time, typically in days to weeks. This results in a manageable hit rate

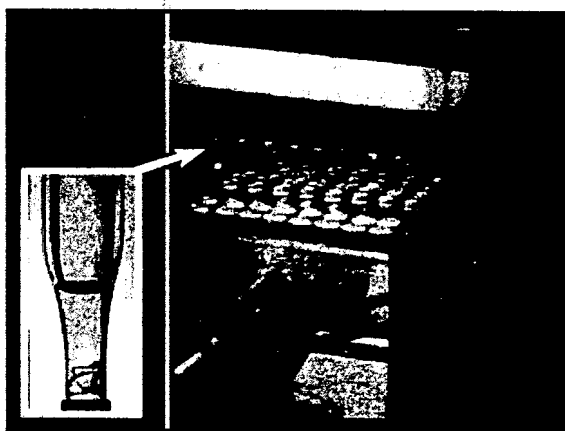


Fig. 1. Photo of optical inspection station. (Inset shows close up of crystallization vessel that contains crystals.) (Courtesy of Trans-Form Pharmaceuticals, 2002.)

for analysis, on the order of approximately 10% in aggregate. This mode of solids generation has the lowest throughput rate, typically, because experiments span days to weeks, with system residence times of months being possible.

In contrast, anti-solvent addition, also known as “crash-out” (or “drown out”) crystallization, relies on the fact that an API is soluble to varying degrees in the crystallization medium, but is largely insoluble in a particular solvent or solvents (e.g., the anti-solvent). As a result, this mode of crystallization can operate at high-throughput rates, with samples being turned around hourly. When crystallization vessels containing API in reagent mixtures are exposed to aliquots of anti-solvent, nearly all vessels will contain API that has precipitated out of solution. This creates a challenge to the analytical process, as the near 100% hit rate leads to a large bolus of samples. There are, however, advantages to this mode of solids generation, such as the ability to produce microfine crystallites and amorphous solids, should they be desired.

Lastly, evaporative crystallization can be carried out on the combinatorial array of samples. This mode of operation relies on gradually increasing the concentration of API in the vessel to achieve supersaturation and to increase the degree of supersaturation (by preferential evaporation) in order to induce crystallization. Concentration of samples can be achieved either passively or actively by controlled flow of inert gas while maintaining temperature. With evaporative

methods, differential rates of solvent loss from mixtures result in unknown composition of the crystallization medium at the time of crystal nucleation. In addition, the degree of supersaturation changes over the course of the experiment, often resulting in the appearance of multiple crystal forms. The evaporative mode of solids generation typically produces throughput and hit rates intermediate between the thermal and anti-solvent processes.

As suggested above, in appropriately configured HT crystallization systems, several process modes may be used in series or in parallel [40]. Frequently, the preparation of replicate plates (in some systems “daughter” plates [47,51]) is necessary for parallel processing by different process modes. Systems may be additionally equipped with the ability to serially process sample arrays using different process modes [59]. This feature is particularly attractive for cases where only small quantities of sample are available, increasing the drive to generate useful information from every sample. Here, samples may be processed by optimal modes first (e.g., thermal crystallization), then a secondary process step can be applied to maximize the hit rate. Another example where this feature is useful is in the case of salt selection, especially in early drug discovery. Upon the addition of salt forming acids or bases, the solubility of the compound is modulated by in-situ salt formation, often resulting in reduced or non-existent driving forces for crystallization (e.g., subsaturation) of the salt species, particularly in polar

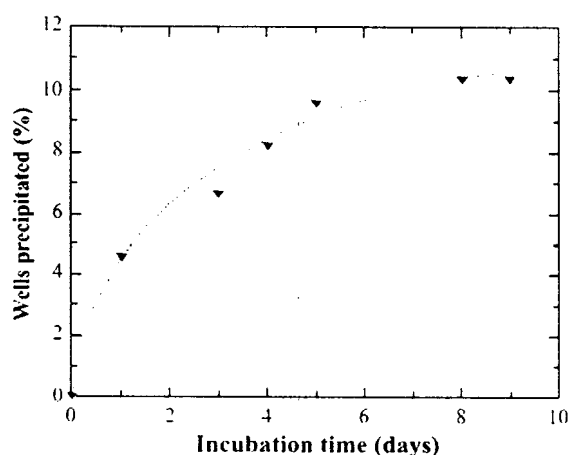


Fig. 2. Typical rate of appearance of solids during a thermally driven HT crystallization experiment [65].

solvents. It should be noted that rapid onset of supersaturation can be experienced in any of the process modes discussed and can result in oiling out or precipitation of amorphous solids, rather than generation of crystalline solids. Thus, it is important to monitor and control the crystallization conditions throughout the experiment.

In general, the percentage of wells that yield solids varies, depending on process mode and experimental time scale. For example, evaporative modes usually result in a solid in virtually every vessel, while slow undercooling results in far fewer (on the order of low percents). The differences in hit rates between these process methods arise in part from the differences in the supersaturation attained. For evaporative crystallization, supersaturation is achieved in all cases as the concentration of the active compound is continuously increased as solvent is evaporated. In contrast, the composition of wells processed by thermal crystallization is fixed. In some cases, because there is limited data on the precise state of supersaturation for each of the large variety of experimental compositions and potential crystal forms, some wells may remain subsaturated during the process. For these wells, additional process steps, such as partial evaporation or anti-solvent addition, may be employed to generate supersaturation to yield a solid. In contrast, as mentioned previously, a fraction of the wells may not go fully into solution at elevated temperatures. In this case, the temperature of the system may be raised to achieve full dissolution, additional solvent may be added to solubilize residual solids or the samples may simply be monitored for slurry conversion over time. To overcome these challenges, we have developed a solubility calculator tool using group contribution theory to estimate the solubility of the reference solid phase at specified temperatures in each solvent composition. These data are then used at the DOE step to define the viable concentrations of the active compound for crystallization (i.e., minimum concentration required to achieve saturation and maximum solubility limit or concentration) in each solvent mixture. Additionally, the timescale of the experiment has a significant impact on the observed hit rate. Hit rates will approach 100% for viable crystallization conditions in the limit of infinite time, but in practice most experiments are conducted over days to weeks, so observed hit rates reflect this temporal influence. In fact, similar

behavior is observed in manual experimentation. Note that only some HT crystallization systems are configured to permit selective sampling of “hits”, providing the ability to further incubate un-crystallized samples to monitor for slow growing crystal forms.

Solids detection can be achieved by examining each sample using machine vision systems. Samples may be monitored over time to detect precipitation in vessels that were previously devoid of solids. This simple, yet robust process can rapidly and non-destructively determine state changes in the crystallization vessels and signal when a particular vessel or set of vessels is ready for solid-state analysis. Depending on the sample array configuration, the signaling of “hits” results in harvesting of samples by one of two approaches. In the “cherry-picking” approach, only those samples that have been flagged as containing solids are selected for further processing [40]. In contrast, using a sacrificial approach the entire plate must be moved forward after a predetermined fraction of the samples in that array have produced precipitates [47,51]. The latter, of course, can be carried out without an online detection system. Here, samples can be processed in batches, without regard to whether there are actually solids present in a vessel. This simple process approach is effective, but has significant limitations, the primary of which being that samples are destroyed after a fixed amount of time regardless of their state. Hence, it is advantageous to employ an online detection and harvest system so that samples can be differentially and asynchronously processed, with only those vessels containing solids undergoing analysis [40,60].

Sample analysis is the final action in execution of the HT crystallization process. Depending on the mode of operation and the choice of analytical measurements employed, this process may involve several steps. Most HT crystallization systems use Raman spectroscopy and/or powder X-ray diffraction (PXRD) for primary analysis of harvested solid-state samples. Both techniques have advantages and disadvantages in terms of their ability to discriminate between forms of a solid (i.e., polymorphs, salt forms, solvates, hydrates) [1,14,61]. The rate of generation of samples for analysis likely dictates which technique is used for the primary approach. Generally speaking, Raman spectroscopy can be employed in a more rapid fashion than PXRD, since acquisition times for Raman are considerably less dependent on sample size, as is depicted in

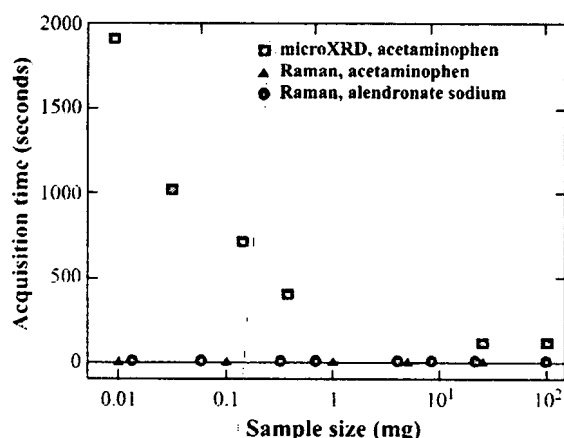


Fig. 3. Comparison of acquisition times of Raman and X-ray powder diffraction data as a function of mass of API [65]. (Data collected on D/Max Rapid, Contact Rigaku/MS, 9009 New Trails Drive, The Woodlands, TX, USA 77381-5209).

Fig. 3. In addition, plate-based PXRD methods are susceptible to problems with preferred orientation effects, which may prevent accurate classification of samples. As a result, Raman spectroscopy methods are often used as a primary means of characterization in HT crystallization systems. Although one disadvantage of the Raman technique is interference due to fluorescent samples, the wavelength of the excitation laser can be changed to the near-IR to reduce fluorescence of problematic samples. Recent advances in PXRD instrumentation, brought on by the increasing demands of HT crystallization, make it possible to achieve similar analysis timescales with PXRD and Raman, on the order of less than one minute per sample depending on the capabilities of particular instruments used. Clearly, the best option is to employ both methods for initial sample evaluation, which can be realized with the appropriate informatics structure, as described in Section 3.

Once the primary solid-state characterization data are collected and stored, samples are generally classified into groups (or bins) that display similar characteristics (e.g., Raman spectra or powder X-ray diffraction patterns) using informatics tools. A variety of methods can be used to accomplish the binning. For instance, Raman spectra may be compared (based on relevant features or over the entire spectral range) and clustered using calculated similarity measures, such as Tanimoto coefficients. In one method [40,60,61], each Raman

spectrum, which represents the contents of an individual well at a given time, is filtered to remove background and to accentuate Raman peaks and shoulders. Peaks are then located and assigned a wavenumber using standard derivative methods and the amplitude of each peak is calculated. These data are used to calculate a similarity (or distance) measure related to the Tanimoto coefficient, from which the Raman spectra are binned into groups of similar samples using a classification algorithm such as hierarchical clustering. This method often uses peak positions, rather than amplitudes to discriminate between different patterns in order to reduce the significance of potential preferred orientation effects, which can result in modulation of relative peak intensity for certain crystallographic planes. The window over which two peaks are considered to be at the same position (e.g., 1 cm^{-1} wavenumber), as well as a minimum height for a filtered peak to be considered for clustering, can be selected by the user, allowing regions of interest (e.g., spectral ranges) to be explored in greater detail. With appropriate settings, a Raman spectrum that has only one peak or feature in a slightly different location than observed in other patterns can be differentiated and binned as unique, indicating a different or new crystal form. During clustering, each spectrum is assigned an arbitrary number, i.e., a sorted spectrum number, for ease of tracking, and the resultant clusters are graphed as shown in Fig. 4, where the red-colored regions repre-

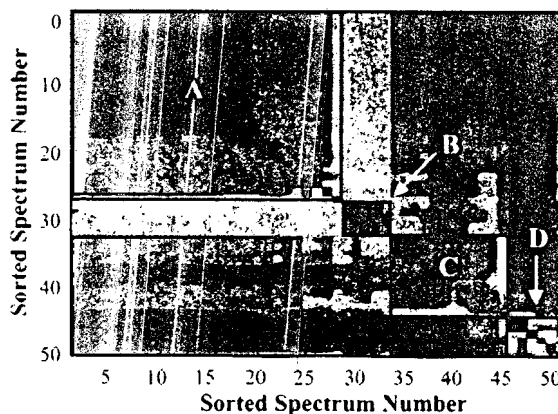


Fig. 4. Raman cluster diagram showing n -by- n matrix of sorted spectrum numbers for all samples resulting from the HT polymorph screen of Ritonavir. Clusters are indicated by warm-colored (red) regions, which have been outlined to guide the eye, and indicate different solid forms [65].

sent bins of similar samples. Alternatively, the results from several analytical methods such as Raman and PXRD can be used to simultaneously classify samples.

Regardless of the choice of primary analytical method, and in keeping with traditional methodologies for solid form screening, it is necessary to further characterize the solids generated in HT crystallization systems to accurately determine their solid form and properties. Most HT systems integrate multiple analytical methods as part of the screening process. These so-called secondary analytical methods often include thermal property measurement (e.g., melting point) and optical microscopy (for crystallinity, habit, etc.). Depending on how the samples are processed and the degree of computerized support, these techniques may be applied to all samples, or a subset of selected samples. For systems that analyze all samples by secondary techniques, several HT plate-based methods for optical microscopy and melting point determination have been developed [47,51]. It is important to note that, in this case, all samples are destroyed during characterization of the melting point. When replicates are retained, the functional properties such as dissolution rate and hygroscopicity can be analyzed using either manual or HT methods. (For more information on functional analysis, see Section 4 on post-screening analyses and form selection.)

With the aid of informatics tools, the data sets obtained can be used to generate information about the experimental space. Software interfaces that allow access to the data permit classification and regression analysis to be performed. The results are displayed in high-dimensional visualization tools that can be used to guide further experiments toward optimizing processes to make each form. For instance, sample composition and processing information can be linked to the resulting crystal form and morphology. Correlation of trends between experimental factors and the products can lead to hypotheses that can be used to direct the design of follow-up experiments. An example of this was reported by Peterson et al. [40], where the knowledge gained from iterative experiments was used to drive new experimental designs, which ultimately yielded the desired outcome, i.e., the isolation and characterization of the highly unstable form III of acetaminophen (paracetamol).

While these new methodologies provide unprecedented capabilities for solids form discovery, it is clear

that there remains a need for some level of manual processing, particularly in the case of detailed form characterization such as single crystal structure determination, scale-up of the desired form and understanding the effects of downstream processing on potential form conversion. HT methods provide the landscape of possible forms and their properties and should be used in conjunction with traditional methods to enable rapid, efficient selection of the optimal form for development.

3. Applications of high-throughput crystallization screening in pharmaceutical research and development: case studies

HT technologies offer unprecedented capabilities for form discovery and characterization. Potential applications range across the entire pharmaceutical value chain, including screening of active molecules in discovery during ELO, form selection for preclinical candidates, final form optimization for early clinical candidates, process chemistry development of crystallization processes for bulk drug and intermediates, as well as identification of new or enabling solid forms for product life cycle management. While numerous impact points have been identified, only limited information on the use and performance of HT form screening systems is available in the literature, indicating that the benefits of these new methodologies have just begun to be realized. In the following sections, case studies on the application of HT crystallization systems are reviewed. Special attention is given to the implications of new form discoveries.

3.1. High-throughput salt selection

Preparation of salt forms of an active compound is commonly used to modulate physicochemical properties. In most cases, the goal is to increase solubility (or dissolution rate) to improve bioavailability or to enhance the manufacturability of poorly soluble ionizable compounds [1,3,4]. Salts may also be employed to increase chemical stability [3] or to reduce the solubility of a given compound for certain applications (e.g., sustained release dosage forms) [62]. Thus, it is important to consider the route of administration and

dosage form requirements when selecting a salt form for development. Since the choice of counter-ion affects the properties of salt forms [3,4], salt selection studies involve the preparation of a number of different salts using a variety of pharmaceutically acceptable acids or bases with differing properties (e.g., acidity/basicity, molecular size, shape, flexibility, etc.). The relevant physicochemical properties of each salt are characterized, including degree of crystallinity, hygroscopicity, aqueous solubility, crystal habit, and physical and chemical stability. Based on these properties of the salt forms, their suitability for development can be evaluated. Several strategies for streamlining and optimizing salt selection procedures have been reported, including in-situ techniques for ranking the solubility of salts [63], tiered approaches in which the least time-consuming studies are carried out first and used to remove from consideration salts that are not viable [64]. One issue not readily considered by existing strategies is the polymorphism and solvate forming behavior of the different salt forms of a compound, which could be used as an additional criterion when more than one salt may be viable, but the degree of polymorphism and solvate formation of each may become a criterion for form selection.

HT crystallization technologies have been used to more rapidly and comprehensively identify the range of salt forms that may be prepared for a given compound or series of compounds, and characterize their crystal form diversity (polymorphs, solvates, hydrates). However, only a few studies have been published or presented. Several HT salt selection studies on well-characterized pharmaceutical compounds have been carried out to demonstrate the power of these technologies in solid form discovery. For example, in a small HT study (i.e., 96 wells) on the antibacterial sulfathiazole, salt formation was explored using varying stoichiometric ratios of pharmaceutically acceptable organic and mineral bases in an array of solvent conditions [65]. The screen resulted in the rapid identification and characterization of 10 salt forms and showed that the salts exhibited a range of melting points depending on the counter-ion type and stoichiometric ratio. Similar HT salt selection experiments on caffeine and naproxen resulted in the identification of numerous salts of each compound [47,50,51].

In the discovery phase, HT crystallization has been used to identify soluble salt forms of compounds

during ELO to facilitate early animal dosing, thereby providing the ability to uncover underlying chemical and/or biological responses elicited by candidate molecules, including toxicity or efflux [46,59]. Such information permits rapid identification of problematic compounds or scaffolds, allowing resources to be directed to projects with greater opportunity for success. HT crystallization can facilitate selection of leads that are more likely to survive preclinical development. HT crystallization has been used successfully to identify multiple new salt forms and the polymorphs and solvates of each compound belonging to two discovery programs using less than 200 mg of compound per screen [59]. Approximately 150–200 experiments were performed on each compound using a library of pharmaceutically acceptable acids or bases with an array of solvent compositions and process conditions. Each screen resulted in discovery of multiple new salt forms, and in some cases polymorphs and solvates. Interestingly, similar salt types were identified for each compound in a given series, as illustrated in Fig. 5, where the frequency of occurrence is plotted as a function of counter-ion for each discovery series. Clear trends in the degree of solid form diversity of salt forms, including polymorphism and solvation behavior, were also evident within each compound series. These data indicate the potential for identifying salts suitable for most compounds tested in a particular scaffold or series, based on analysis of only a portion of the series, i.e., a platform-based approach to salt selection, provided the chemistry surrounding the ionizable functionality is not significantly altered during further structure–activity relationship (SAR) development. Furthermore, solubility measurements of each salt form in physiologically relevant fluids allowed ranking of salt forms in a given series, and comparison of salts between series was also possible. The average turnaround time per screen was approximately 2 weeks, such that feedback on the physicochemical properties of each compound was provided to the medicinal chemists on a similar time scale as potency, selectivity and metabolism screens.

Salt selection is normally part of the standard preformulation studies carried out during preclinical development, where rapid identification of the possible salts of a compound and their properties can facilitate product development. To further facilitate

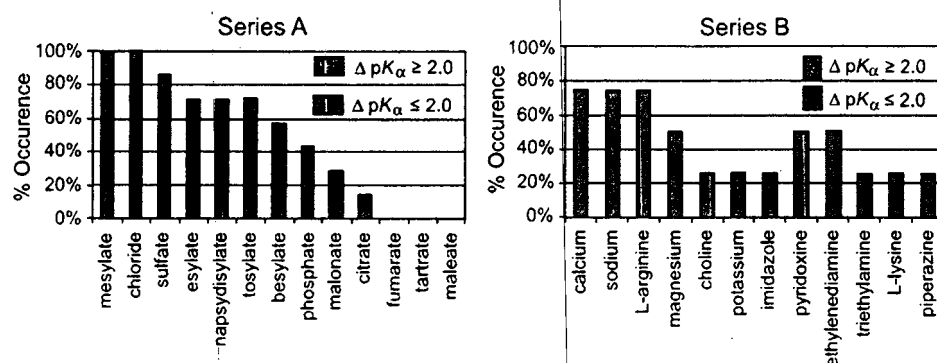


Fig. 5. Frequency of occurrence (%) plotted as a function of the counter-ion of the salt for compounds from discovery series A and B [59].

such studies, a microplate technique capable of investigating an array of conditions has been developed to determine which counter-ion and solvent conditions can be used to prepare crystalline salts of the compound [66]. Each plate is prepared by first depositing approximately 0.5 mg of compound into each well using an appropriate amount of stock solution. The counter-ion type is systematically varied along the rows of the plate and different crystallization solvents are deposited down the columns of the plate. Crystallization is monitored by optical microscopy over the course of the evaporative crystallization, which can be accelerated by flowing a stream of dry nitrogen over the plate. Once salt forms are identified, they are scaled up for more detailed characterization.

The microplate approach was demonstrated by Bastin et al. [66] through several examples, however little detail of the specific screening protocol and results was provided. All three of the reported examples are on compounds that are weak bases with pK_a between 4.1 and 5.3. Only a small number of stable, crystalline salts could be prepared for the two very weak bases (i.e., $pK_a < 4.25$), as opposed to the larger variety found for the stronger base. In each case, the salt forms were scaled-up for more detailed analysis and comparison to the respective free base compound to determine the optimal form for development. This approach provides a useful mechanism for preliminary, small-scale salt formation studies. Both the crystallization media and process modes accessible by the technique are somewhat limited, resulting in a narrow exploration of experimental conditions for salt

formation. For example, only solvents compatible with plate materials can be used, thereby reducing the probability that a crystalline phase can be identified. In addition, current protocols only provide for evaporative crystallization, likely due to difficulties with sealing of the plates. In this case, the composition of the crystallization medium is not well controlled. The utility of HT crystallization in ELO, although demonstrated by initial reports of feasibility, is less well documented than the use of HT on later stage compounds.

3.2. Solid form discovery in highly polymorphic systems

The statement by the late Walter McCrone in 1965 that "the number of forms of a given molecule is proportional to the time, money and experiments spent on that compound" [67] has gained credence in recent years, as illustrated by the significant increase in reported crystal form diversity of pharmaceutical solids. Depending on when alternative solid forms of a compound are identified, the appearance of a novel form may or may not be a welcomed discovery. Occurrence of a new form in research or early development is potentially enabling. At later stages, the appearance of new forms, particularly stable ones that are not bioequivalent or deemed unprocessable, can have catastrophic consequences for product performance as well as regulatory compliance (e.g., control of crystal form). Additionally, recent rulings on the use of alternative, commercially viable solid forms not protected by patents from

innovator companies have opened the market to generic competition [68–79]. In order to mitigate these risks, and to save time and reduce costs, many pharmaceutical companies have begun to re-evaluate their strategies for solid form screening and are looking to HT crystallization technologies to address the needs for more rapid and comprehensive exploration. In this section, the application of HT crystallization to highly polymorphic systems is reviewed, including specific cases of compounds exhibiting latent polymorphism.

Polymorphic systems are quite common among many types of organic crystals [7]. For the purposes of this review, compounds exhibiting more than three polymorphic forms will be classified as being “highly polymorphic”. While only a handful of well-known organic compounds are considered for practical purposes to be non-polymorphic, e.g., aspirin [80,81], sucrose and naphthalene [7], it should be stressed that one will never be able to exclude the possibility of polymorphs appearing, even a century after the initial discovery of the compound. So far, no polymorphs of aspirin have been found, despite the proposal by Payne et al. [80] that polymorphic forms may exist. In contrast, acetaminophen form III was observed by Burger in 1982 using thermal microscopy [82], but it took another 20 years for a crystal structure to be proposed [40]. Many reports exist on the polymorphic nature of specific drug compounds with one or two alternative packing modes for the same chemical composition. However, literature examples of compounds with more than three packing modes are considerably rarer, as will be summarized shortly. It should be noted that the increased number of reports on highly polymorphic compounds in recent years is likely the result of enhanced screening practices and more sensitive characterization techniques.

Highly polymorphic compounds present several challenges in drug development. First, the generation of different forms is often not a simultaneous event, but rather a gradual evolution of form diversity leading to the branding of a compound as being highly polymorphic. Consequently, once more than one form is identified, concern is raised that additional forms may eventually be discovered. For instance, the 13 polymorphs of phenobarbitone evolved over ca. 13 years [7], and a fourth polymorph of carbamazepine was reported in 2002, a full two decades after the

publication of the structures of the initial three forms [83]. Second, selection of the preferred form of a highly polymorphic compound for development demands a complex set of thermodynamic and kinetic investigations, due to the geometric increase in the number of stability relationships that need to be established. More complexity arises when some polymorphic pairs are enantiotropic, exhibiting a switch in the identity of the stable form as a function of temperature. Third, concerns over bio-performance and the impact of a large number of polymorphs on processing lead to regulatory issues that need to be addressed. Decision trees [58] have been established to aid scientists in assessing the impact of polymorphic change and have been incorporated into the ICH guidelines [84]. Lastly, the analytical challenge of monitoring polymorph content in the dosage form increases as the number of possible forms grows, particularly with low dose compounds where the concentration of drug in the formulation is small.

The literature on highly polymorphic pharmaceuticals is relatively sparse, but several examples of compounds known to have four or more polymorphic forms are available in the literature and are summarized in Table 2. In addition to these drug examples, the pharmaceutical ingredients mannitol and aspartame have been shown to exhibit 4 and 5 polymorphs, respectively [7]. The phenomenon in inactive exci-

Table 2
Examples of highly polymorphic drug compounds in the literature

Compound	Number of reported polymorphs	Other forms	Reference(s)
Phenobarbitone	13		[7,p.255]
Cimetidine	7	Hydrates	[7,p.73]
‘ROY’	7	7th form found after the initial publication	[111,112]
Sulfathiazole	5	Numerous solvates	[113]
Carbamazepine	4	Dihydrate and numerous solvates	[28,45,83,85]
MK-996	9	Hydrate	[87]
MK-A	4	2 hydrates and numerous solvates	[86]

pipients may well be under-appreciated due to lack of study.

In general, pharmaceutical polymorphism is likely to be underreported in the literature, since much of the polymorphism research is carried out in companies. As a result of growing interest in the subject and advances in techniques to study polymorphism, it is expected that reports of extreme form diversity will grow. Conferences on the subject, such as the ACS ProSpectives symposium, reflect the appreciation for the complexities introduced by the appearance of polymorphism in important materials such as pharmaceuticals. Work has recently commenced to understand the opportunities and challenges of using HT technologies in pursuit of rapid identification and characterization of the large number of forms presented by highly polymorphic compounds. Three published case studies and two examples that are in press at the time of this review will be highlighted.

Form IV of carbamazepine was reportedly discovered as the result of crystallization trials in the presence of hydroxypropyl cellulose HPC [83]. Subsequent to this publication, Lang et al. [28] published the use of polymers to influence polymorphic form using a 96-well plate system for the screening of polymorphs of carbamazepine and acetaminophen. In all, 84 different polymers were employed to direct nucleation. Form IV of carbamazepine was found to crystallize from methanol in the presence of hydroxypropyl cellulose, poly(4-methylpentene), poly(*R*-methylstyrene) or poly(*p*-phenylene ether-sulfone). Using the same approach, the monoclinic and orthorhombic forms I and II, respectively, of acetaminophen were also isolated. While observation of metastable form III was not reported in this study, the strategy of employing polymeric additives is of interest, as it can direct the course of crystallization and because polymeric impurities may be in contact with a drug substance and/or formulation at various points in development.

Another approach, reported by Anquetil et al. [85], identified selective conditions for the crystallization of carbamazepine polymorphs forms I and III, as well as the dihydrate, from methanol and/or methanol/water solutions by thermal processing in a microliter cell format (i.e., 35–100 μ l). Optical laser trapping was used in situ to target the microcrystals for real-time form analysis using Raman spectroscopy. The crystal-

lization process was monitored optically and with Raman spectroscopy as a function of temperature and time. The study revealed the conversion of form I to form III, as evidenced by a change in characteristic crystal habit from needles to prisms. Raman spectroscopy on the solution phase measured the saturation solubility of each crystal form produced. Although only several experiments were carried out in this study, the authors advance the microfluidic cell format as a potentially viable system for HT polymorph screening.

A third report details the use of in situ Raman spectroscopy to optimize process conditions. The compound MK-A has four anhydrous polymorphs and several other forms, including two hydrates and numerous solvates [86]. The study gives an example of the complex thermodynamic relationships (monotropic and enantiotropic pairs) that can exist in highly polymorphic systems and demonstrates the power of in-situ methods for monitoring the crystallization process.

The angiotensin-II antagonist MK-996 is an example of a highly polymorphic compound (Table 2) [87]. The structure of MK-996, depicted in Fig. 6, contains seven rotatable bonds, the conformations of which could lead to many configurations for crystal packing. HT crystallization experiments with MK-996 in 96-well arrays comprising over 1500 discrete recrystallization trials from a set of 21 solvents or solvent mixtures yielded 186 solids, which were harvested over a period of 7 days [87]. PXRD analysis of these solids suggested the presence of at least 18 distinct

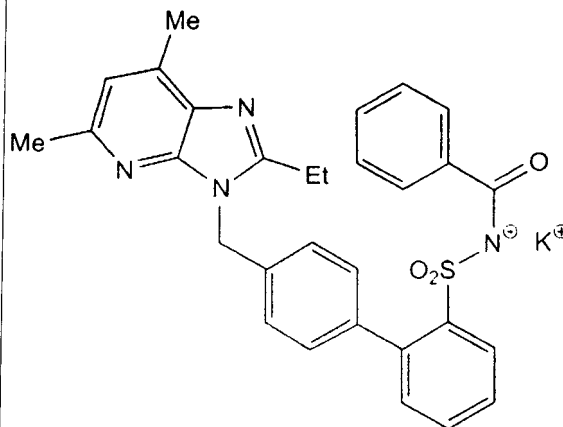


Fig. 6. The molecular structure of the angiotensin-II antagonist MK-996 [87].

forms, some resulting from solvent-mediated recrystallization. A hydrate (originally named form I), obtained by slurry conversion in the presence of aqueous solvent mixtures in the HT experiments, was the form previously selected for pharmaceutical development. Importantly, a form (form D) reported by the innovator [87] to be a “disappearing polymorph” [88] once form I appeared, was also found in the HT screen. Clearly, sufficient experimentation with rationally selected diverse conditions affords the possibility to regenerate elusive forms.

Sertraline HCl, the active ingredient in the antidepressant Zoloft®, is found in various crystal forms. The molecular structure for Sertraline HCl is illustrated in Fig. 7. Information on various solid phases can be found in patent disclosures filed by several companies [89–92]. Survey of these documents, which published between 1992 and 2001, reveals data for 27 purported crystal forms of Sertraline HCl, including 17 polymorphs, 4 solvates, 6 hydrates and the amorphous solid. Further analysis and comparison of characterization data for the various forms presented in the patents revealed that mixtures have been mistaken for real polymorphs on at least two occasions, and at least two polymorphs were disclosed more than once (by different workers each time). In addition, the hydrate forms reported were not readily identified as polymorphic and many of the forms are likely transient, e.g., only identified by variable-temperature and humidity-controlled XRD. With the help of HT crystallization, the extent of true polymorphism of the HCl salt was estimated at eight forms so far [92]. Two new solvates were also found in the HT studies. Care should be taken in isolation of such forms, particularly at small to intermediate scale, as desolvation of solvates due to aggressive drying

during processing may cause one to overlook solvated forms [93]. Comparing the results of the HT study to the congruence of historical data, one can conclude that HT screening gives rise to relevant forms of the drug in a time frame of weeks rather than years. One metastable form, polymorph IV, remained elusive in the hands of the authors [92]. The lack of observation of form IV may be due to a subtle purity difference between early batches at Pfizer and the materials available for testing in the HT screen. Clearly, impurity effects should be explored further [32].

To date, HT studies on highly polymorphic materials highlight the importance of varying processing conditions (including solvent conditions, degree of supersaturation, method of crystallization, desolvation of solvates, inclusion of additives, thermal microscopy, etc.) to find as many forms as possible. It has been shown that multiple process modes, including HT processing, coupled with detailed follow-up characterization studies of form stability, facilitate insight into crystal form diversity [40]. Such a multimode strategy becomes valuable in the quest for the most comprehensive dataset possible for a given pharmaceutical material.

Undoubtedly, the definition of highly polymorphic materials and their frequency will evolve in the age of HT crystallization [40,60] and with the aid of ever improved solid-state analytical capabilities [18,94,95]. The value of employing multiple processing techniques to elucidate as many crystal forms as possible will be demonstrated, as it is expected that no single technique will generate all forms of a given compound. Without doubt, HT crystallization strategies will be used, as a complement to other techniques, to identify issues of polymorphism early, thus allowing drug development scientists to react appropriately to information on form diversity of their compounds.

3.3. Avoiding latent polymorphism

Very few cases of latent polymorphism have been reported in the literature. It is likely that many more instances of the phenomenon have occurred, but unless product development was slowed, product performance was impacted, or generic competition was threatened, a spotlight is not usually cast on the issue. As an example of a public polymorph issue, form 2 of ranitidine hydrochloride was discovered 2–

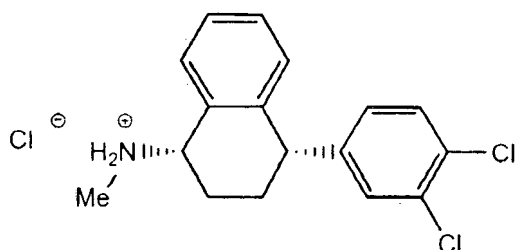


Fig. 7. The molecular structure of the selective serotonin reuptake inhibitor (SSRI) sertraline HCl.

3 years into development but it was (and is) the form still marketed by GlaxoSmithKline [75,76,96]. Paroxetine hydrochloride hemihydrate, the active ingredient in Paxil®, was discovered during development after only an anhydrate had been known for a number of years [97]. The hemihydrate is the form marketed by the innovator, but recent litigations have occurred between the innovator company and generic competition around the anhydrate form.

One of the most recognized cases of latent polymorphism occurred with Abbott Laboratories' Norvir®. Two years after entry into the market, a previously unknown, but thermodynamically more stable, polymorph of the active ingredient (Ritonavir) appeared. This new form (form II) was approximately 50% less soluble in the hydroalcoholic formulation vehicle, resulting in poor dissolution behavior and eventual withdrawal of the original Norvir® capsule from the market [98]. At some considerable cost, a new formulation of Norvir® using form II was eventually developed and launched [99]. In a recent HT crystallization study on Ritonavir, a total of five forms were found: both known polymorphs and three previously unknown forms [99]. The HT polymorph screen, which consisted of 2000 experiments was carried out with less than 2 g of the API and used multiple, and sometimes combined, process methods. The three new forms were described as a metastable polymorph, a crystalline solvate and a non-stoichiometric hydrate. Interestingly, the solvate was easily converted to form I via the hydrate phase using a simple washing procedure, and provided an unusual route to prepare the form I "disappearing polymorph" [88]. Since the crystals of form I prepared using this method retained the small needle morphology of the solvate, the authors suggest that the process may offer a potential strategy for particle size and morphology control. The results of this study emphasize the need for more comprehensive studies of form diversity in the early stages of drug development to avoid risks of form conversion downstream, and highlight the advantage of combining parallel HT crystallization experimentation with detailed physico-chemical analyses to identify the diversity of solid forms in which a given molecule can exist. Clearly, late stage discovery of new forms or form conversion can have serious competitive and regulatory implications (e.g., process control), especially in cases where the new forms are not bioequivalent.

3.4. *Prediction of crystallization and polymorphism: applications to pharmaceutical form studies*

Crystal structure prediction is a challenging area of research. Due to the overwhelming influence of packing forces in determining crystal structure, it remains extremely difficult to predict the structural impact of subtle conformational effects and weak interactions between adjacent molecules in a crystalline arrangement. Although significant progress has been made in the last decade, crystal structures are by and large not reliably predictable from first principles [88]. While this important area of theoretical research is too large a topic to be considered in detail here, a brief overview of the successes and challenges will be presented, and the potential for using HT crystallization as a validation to aid model development will be highlighted. For a more detailed discussion on polymorph and crystal structure prediction, refer to the article by Price [100] in this issue.

Polymorph prediction of pharmaceuticals is thwarted by the complexity of active pharmaceutical molecules. The number of degrees of freedom in torsion angles and the molecule count in the unit cell (which can be deduced by such techniques as solid-state NMR [94]) are frequently too great to allow computations on a reasonable time scale. Additionally, predictions are typically carried out one space group at a time. This limitation is mitigated by the fact that over 90% of the organic compounds in the Cambridge Structural Database (CSD) [101] crystallize in only a few space groups [100]. We know of only one example where predictions have been extended to multicomponent systems [102]. The prevalence of multicomponent systems, some of which have charge transfer (salts) and many of which exist as hydrates, solvates or mixed hydrate/solvates, essentially limits the usefulness of the prediction methods to neutral compounds. Various other technical issues remain as the science of crystal structure prediction matures [100]. Some of these issues were highlighted in two blind tests that were conducted in recent years to determine the accuracy and robustness of crystal structure prediction [103]. In the latest round, 17 methods were used to predict structure, yielding only three correct predictions [104]. For one of the compounds used in the study, experimental characterization of a second, more stable, polymorph provided the key to the correct prediction by three participating

research groups. The structure could have easily been overlooked, leading to the misinterpretation of the results as an apparent failure of the computational methods. Thus, compounds that are amenable to structure prediction are not always studied experimentally to the extent necessary to ensure that the relevant forms have in fact been discovered and characterized ahead of computational studies.

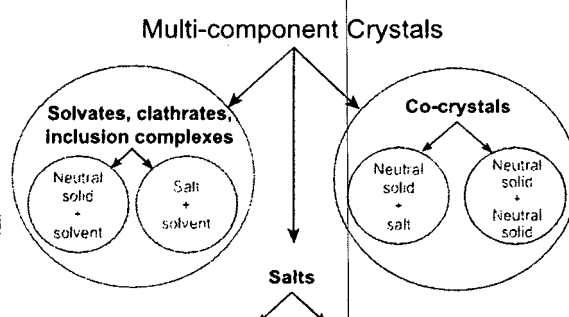
Despite the challenges, a few methods have been developed that allow structure prediction of small, relatively rigid organic compounds with only a few functional groups in several important space groups [17,105,106]. Polymorph Predictor™ has been implemented within the commercial software Cerius2 (C2 Polymorph by Accelrys). In general, current prediction methods generate large ensembles of different packing arrangements along with calculations of relative energetics. In reality, many of the calculated structures are not observed, giving the appearance of over-prediction of polymorphism. This was apparently the case with acetaminophen (paracetamol) [107]. In their study of the drug, Beyer et al. [107] calculated 14 structures, 2 of which were the known monoclinic (stable) and orthorhombic forms. The remaining 12 structures were considered as candidates for the metastable form, III, which had been observed by thermal microscopy methods [82] but for which diffraction data were unavailable. Using calculations of mechanical properties and morphology, Beyer et al. separated the 12 energetically feasible structures into two groups, based on the likelihood of each structure to exist as a stable form. Shortly after the publication of the prediction study, the experimental powder pattern of form III became available [40]. Rietveld refinement and comparison of the experimental diffraction results with the theoretical powder patterns published by Beyer et al. yielded a monoclinic structure solution for form III. This structure is in fact part of the prediction set, but was considered an unlikely contender based on its extreme plate-like morphology. The potential for complementarity of HT crystallization and polymorph prediction is evident from these studies. In one sense, polymorph prediction can serve as a yardstick for “risk assessment” when it comes to form diversity, but inevitably one will require experimental data to assess the scope of polymorphism that can be elicited and the precise relative stabilities of different crystalline arrangements.

Opportunities do exist for current use of predictions in solid form discovery. For instance, certain hydrogen-bonding motifs or molecular layer types may be observed in predicted structures. Such information can be used to aid the design of crystallization experiments. It might be desirable to employ a particular type of interaction with salt selection or co-crystal formation by the strategic selection of crystallization conditions, solvents, additives and processing methods [22,23]. In addition, since transient or metastable crystalline species may be difficult to characterize accurately, one may use predicted structures to estimate various physical data. For example, powder diffraction patterns may be used to assist the accurate description of these metastable forms [40]. Continued development of theoretical methods coupled with validation of the predictions by extensive crystallization screening will lead to better models and computational methods. At present, experimental methods must still be relied upon to assess the potential form diversity of a given compound. It will be important to concurrently push the limits on theoretical prediction and HT crystallization, in order to advance our understanding of the nature and extent of polymorphism in pharmaceutical compounds.

3.5. Engineering of co-crystals

Co-crystals of drugs and drug candidates represent a new type of material for pharmaceutical development. They are part of a broader family of multicomponent crystals that also includes salts, solvates, clathrates, inclusion crystals and hydrates as shown in Scheme 2. The primary difference between solvates and co-crystals is the physical state of the isolated pure components: if one component is a liquid at room temperature, the crystals are designated as solvates; if both components are solids at room temperature, the crystals are designated as co-crystals. While at first glance these differences may seem trivial, they have profound impact on preparation, stability and ultimately on the ability to develop products.

In general, it is usually easier to initially prepare solvates than co-crystals, and indeed, solvates are often found as by-products of polymorph and salts screens. Co-crystals have been prepared by melt-crystallization, grinding and recrystallization from solvents [1]. Sol-



Scheme 2. Types of multicomponent crystals.

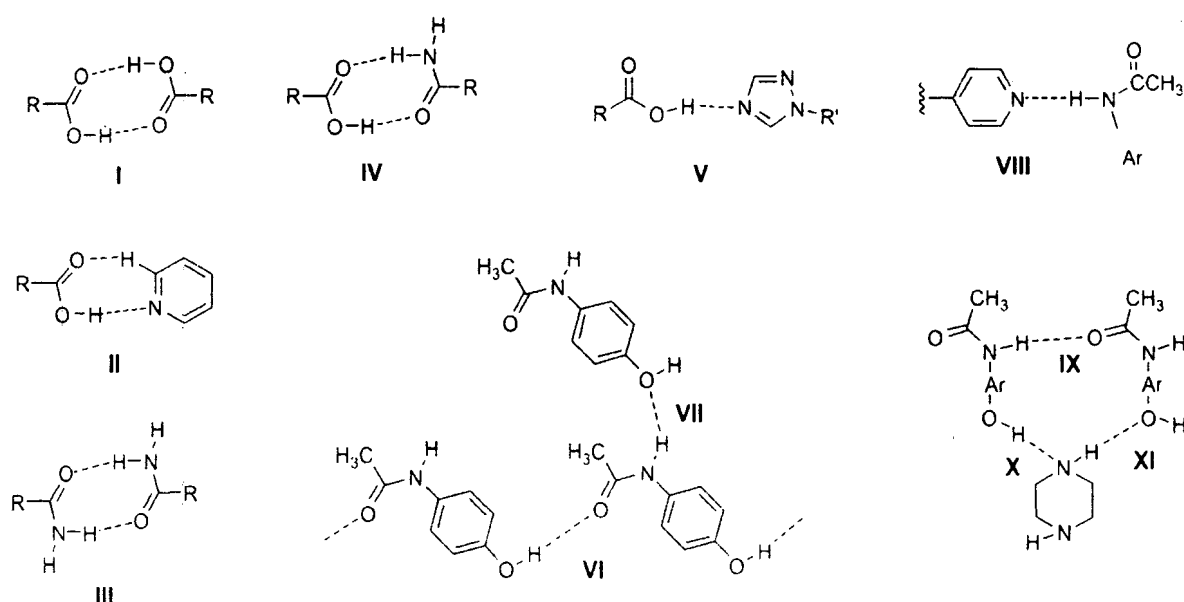
vent systems for co-crystals must dissolve all components, but must not interfere with the interactions necessary for co-crystal formation. The need to try many solvent combinations and the availability of multiple co-crystal formers creates a diversity that is ideally suited for exploration by HT systems.

Co-crystals have the potential to be much more useful in pharmaceutical products than solvates or hydrates. The number of pharmaceutically acceptable solvents is very small, and because solvents tend to be more mobile and have higher vapor pressure, it is not unusual to observe dehydration/desolvation in solid dosage forms. Solvent loss frequently leads to amorphous compounds, which are less chemically stable and can crystallize into less soluble forms. In contrast, most co-crystal formers are unlikely to evaporate from solid dosage forms, making phase separation and other physical changes less likely.

Examples of co-crystals have existed in conductive organic crystals, non-linear optical crystals, dyes, photographic materials pigments and agrochemicals for some time [7]. Two recent papers by Fleischman et al. [43, 45] emphasize the importance of understanding “supramolecular synthons” in synthesizing co-crystals containing pharmaceutical agents. For example, the ability to insert 4,4'-bipyridine between the carboxylic acid dimers of aspirin, *rac*-ibuprofen and *rac*-flurbiprofen was recently reported [43]. The three examples clearly demonstrate the generality of the use of a pyridine-carboxylic acid heterosynthon II

(Scheme 3) to replace a dicarboxylic acid dimer homosynthon I. A second study focused on finding multiple solvates and co-crystals of carbamazepine [45]. Carbamazepine polymorphs crystallize as amide dimers, each of which ties up the polar amide functional groups through homosynthon III. Crystal structures shows that each dimer contains a peripheral H-bond donor and acceptor pair that remain unused due to geometric constraints imposed by the drug molecule. Simple H-bond acceptor solvents like acetone and DMSO insert themselves to fill voids between the adjacent pairs of dimers [45]. Multiple co-crystals formers having hydrogen bond acceptors likewise insert themselves into the void. The homosynthon can also be broken to form heterosynthon IV, an amide-carboxylic acid dimer [45]. This was achieved to form solvates with acetic, formic and butyric acids, and co-crystals with trimesic and nitro-isophthalic acid.

A recent study of adducts of acetaminophen (paracetamol) with ethers and amines provides additional examples of supramolecular synthons for co-crystal formation [108]. While amide-amide homosynthon could have formed, both known forms of the pure material consist of linear head-to-tail chains held together through motif VI; the chains are cross-linked through synthon VII. The linear chain structure is preserved in co-crystals with 4,4' bipyridine, but the cross-linking interaction VII is replaced by VIII, in which the 4,4' bipyridine is hydrogen bonded to the amide hydrogen. The chains remain cross-



Scheme 3. Supramolecular synthons observed in co-crystals.

linked but only through pi-stacking interactions between 4,4' bipyridine pairs on neighboring chains. In co-crystals with piperazine, the acetaminophen forms head-to-head chains through IX. Each chain is joined to the next through a layer of piperazine molecules that interact through heterosynthons X and XI. The paper also includes many solvates that will not be reviewed here, but their synthons should be applicable to co-crystal formation.

The above studies focused on demonstrating the use of supramolecular synthons to create novel crystalline phases. The variety of structures observed provides hope that some forms will have superior performance in pharmaceutical dosage forms. However, the studies stop short of providing data on the physical properties, such as solubility, necessary to evaluate their utility. Furthermore, only the saccharin and nicotinamide co-crystals of carbamazepine represent pharmaceutically acceptable co-crystals. Crystals containing two drugs may appear to be a good technique for making combination products of two drugs, but unless the two drugs are dosed only in stoichiometric ratios consistent with the co-crystal composition, such crystals would still need to be co-formulated with at least one of the bulk drugs in order to satisfy the clinical requirements.

We recently reported on the discovery and dissolution properties of pharmaceutically acceptable co-crystals consisting of hydrogen-bonded trimers of two molecules of *cis*-itraconazole and one molecule of a 1,4-dicarboxylic acid resulting from a HT crystallization screen [44]. The crystal structure of the succinic acid co-crystal (Fig. 8) revealed an unanticipated interaction between the triazole of itraconazole and the carboxylic acid (heterosynthon V in Scheme 3). The extended succinic acid molecule fills a pocket, bridging the triazole groups. The interaction between the 1,4-diacid and the strongest base on itraconazole (piperazine) is absent in the co-crystal structure. Other 1,4-diacids including fumaric acid, L-malic acid and L-, D- and DL-tartaric acids also yielded co-crystals with itraconazole, but co-crystals could not be made from maleic acid with Z-regiochemistry, or from 1,3- or 1,5-dicarboxylic acids. Hence, geometric fit appears to be more important than acid-base chemistry in directing crystallization of the compounds of itraconazole with 1,4-dicarboxylic acids.

Identification of multiple crystal forms of the same drug with acceptable solubility, dissolution rate and stability enables selection of the optimal form for dosage form development. To demonstrate this feature, the dissolution of itraconazole co-crystals in

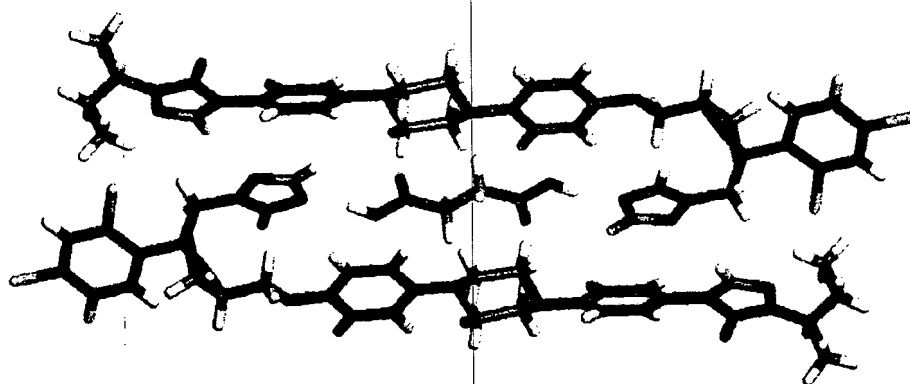


Fig. 8. Trimer unit of the itraconazole succinic acid co-crystal from single crystal X-ray structure (from [44], with permission).

aqueous medium was studied to assess their potential impact on bioavailability of the drug from a solid dosage form. Fig. 9 compares the dissolution profiles of the co-crystals into 0.1 N HCl to those of crystalline itraconazole-free base (95 % of all crystalline particles < 10 μm) and commercial Sporano[®] beads (amorphous itraconazole). The malic acid co-crystal rivals the dissolution of the commercial product. In general, the co-crystals behave more similarly to Sporano[®] than the crystalline-free base. The co-crystal forms achieve and sustain 4- to 20-fold higher concentrations than that achieved from the crystalline-free base. The practical implication is significant, since the ability to form a supersaturated solution, even transiently, can have dramatic impact on absorption and bioavailability.

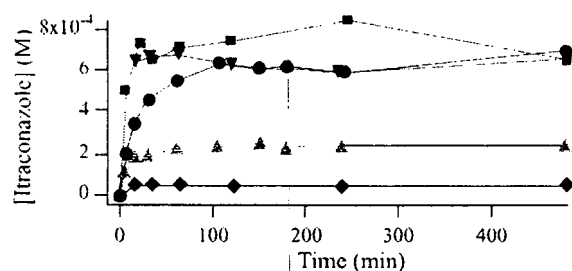


Fig. 9. Dissolution profiles into 0.1 N HCl at 25 °C plotted as itraconazole concentration ([itraconazole]) as a function of time for Sporano[®] beads (■), crystalline itraconazole-free base (◆) and co-crystals of itraconazole with L-malic acid (▼), L-tartaric acid (●) and succinic acid (▲) (from [44], with permission).

Co-crystals represent a class of pharmaceutical materials of interest, both in terms of projected diversity and applicability. The study of co-crystals, along with polymorphs, solvates, salts and hydrates, is perfectly suited to HT crystallization experimentation and should be considered part of the form selection processes.

4. Post-screening analyses and form selection

Several functional characteristics must be considered in the selection of a suitable crystal form for a pharmaceutical dosage form. HT crystallization has the potential to create a larger pool of crystal forms for which functional parameters, such as dissolution rate, chemical stability, flow and compressibility, must be determined and compared. Strategies to accomplish ranking of the numerous forms must be devised. An example is the adaptation of HT for solubility measurement. The plot in Fig. 9 illustrates results of a plate-based kinetic dissolution assay in which various forms of a compound were placed in simulated gastric fluid and monitored for dissolution as a function of time. The schematic in Fig. 10 shows how such an analysis can be accomplished in a 96-well filter plate. The concentration at a given time point is determined after filtration of the suspension by quantification using either UV or HPLC with UV detection.

While the entire plate is filtered at one time, different time points can be achieved by timing the addition of dissolution medium such that the aliquot

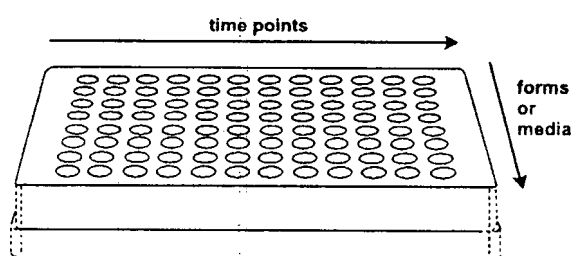


Fig. 10. Schematic of a 96-well dissolution filter plate.

for the longest time point desired is dispensed first and the shortest one comes last. Instead of varying the form along one axis of the plate, one can choose to study the dissolution of a single form into several different media (see Fig. 10). Equilibrium solubility can be determined in a variety of solvents and at different temperatures using a similar principle to the dissolution plate. A demonstration has been provided using automated React-IR analysis [109]. Other functional parameters, such as solid-state stability and thermal properties, can be adapted to HT. Such systems for ranking the stability of forms generated from HT crystallization await publication and review at a future date.

5. Summary and outlook

HT crystallization methodologies are capable of screening hundreds or thousands of crystallization conditions in parallel using small amounts of compound for the identification and characterization of diverse forms of active pharmaceutical ingredients. As demonstrated by numerous case studies from several stages of pharmaceutical development, such technologies have begun to show promise in enabling more comprehensive exploration of solid form diversity. The technologies are likely to provide a landscape of potential operating conditions from which scientists and engineers can design robust and scalable processes for transfer to manufacturing.

The ability to conduct extensive crystallizations with small amounts of material using a variety of solvents, additives and conditions necessarily generates large sets of data. However, the information by itself is of limited value, unless it can be properly analyzed. In order to extract maximum knowledge

from the studies, it is essential to have the ability to design experiments, track samples in the process, collect the data in a relational database, and mine the information using statistical techniques and models in property space that assist the scientist to maximize the value of the data. Such models attempt to fit an output variable to physical properties or descriptors using techniques similar to those used in traditional quantitative structure activity relationships (QSAR). These models can be *carefully* extended to mixtures containing compounds that were not included in the original experiments if validation suggests that the models are sufficiently stable. Significant models that are found in the analysis of the data can be stored in the database for later retrieval and use to direct iterative experiments. The power of this approach becomes increasingly more visible when several properties are being co-optimized, as can be very important in the pharmaceutical development process where such properties as oral bioavailability, stability and processability need to be reconciled. The availability of a map of conditions that lead to the formation of different forms (salts, hydrates, solvates, polymorphs, co-crystals) of the drug can be valuable to the process chemists or engineers as they develop scalable processes to produce materials suitable for development and registration.

For many years, the value of composition of matter (CoM) patents on new chemical entities, including where appropriate, pharmaceutically acceptable salts, has been well appreciated. However, it is only within the last decade or so that the application of CoM patents has been significantly extended to cover all forms of the compound, including hydrates, solvates, co-crystals and polymorphs. Unlike salts, which for the most part can be prophetically claimed based on an understanding of the chemical structure of the compound and its ionization constants, the existence and identity of hydrates, solvates, co-crystals and polymorphs have defied prediction. Therefore, in order to obtain patent protection on these forms, some of which may have significantly different properties and relevance as development candidates, it is essential to prepare them, identify conditions for making them and evaluate their properties as valuable new pharmaceutical materials.

In general, discrete crystal forms are considered non-obvious and patentable. Given the diversity and greater complexity of chemical structures of today's

drug candidates [110], coupled with the advanced technology to identify novel forms, it is common to find multiple forms of drugs [61], some similar, some dramatically different in terms of their in vivo performance. These forms are all candidates for separate intellectual property protection. Therefore, it is incumbent on the innovator of a new drug candidate to identify and patent these forms in order to optimally protect their investment in the compound. Recent case studies suggest that identifying and patenting all forms of new chemical entities should be a primary strategy of all innovators of novel drugs. In this regard, the use of HT crystallization technologies for rapid, comprehensive discovery and characterization of solids form diversity offers significant advantages for the development of a strong intellectual property position.

With the advent of HT crystallization methods, appreciation for the landscape of physical form for drug development has begun to change. Use of these systems has the potential to facilitate drug development by saving valuable time in selecting the optimal physical or chemical form of a given compound. HT systems that generate rich datasets offer the ability to develop a more fundamental understanding of the crystallization process, based on knowledge generated from large numbers of experiments on diverse compounds. Having such information at an early stage minimizes the risk of process modifications resulting in form changes and provides the opportunity to gain more comprehensive intellectual property coverage. In addition, comprehensive form data help address important regulatory questions related to the number of solid forms of an API and the relationships between them.

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Forma I
Suspensión inicialForma I
Suspensión después de las 6 horas

Fig. 83-4. Microfotografías que ilustran el cambio de tamaño de los cristales para una suspensión de la Forma I de una droga experimental.

de una nueva entidad química. Si se presume que el pH gástrico oscila entre 1 y 3 y el del intestino delgado entre 5 y 8, en la mayoría de los casos las drogas ácidas (pK_a 3) se absorberán con mayor rapidez en el estómago, mientras que las drogas más básicas (pK_a 8) lo harán en el tracto intestinal. Sin embargo, existen excepciones. Algunos compuestos poseen bajos coeficientes de partición o se encuentran altamente ionizados dentro de la totalidad de espectro de pH fisiológico, pero de todos modos se asocian con un buen grado de biodisponibilidad.

Polimorfismo

Un polimorfo es una fase cristalina sólida de un compuesto dado resultante de la posibilidad de como mínimo dos ordenamientos distintos de las moléculas del compuesto en el estado sólido. La molécula propiamente dicha puede ser de distinta forma en ambos polimorfos, pero esta condición no es necesaria y, en realidad, ciertos cambios de forma conllevan a la formación de moléculas diferentes y por lo tanto no representan polimorfismos. Los isómeros geométricos o los tautómeros, aun cuando sean interconvertibles en forma reversible, no pueden ser considerados polimorfos, aunque pueden comportarse en forma muy similar y crear confusiones.

Un criterio seguro para la clasificación de un sistema como polimórfico es el siguiente: dos polimorfos mostrarán diferencias en la estructura de los cristales pero serán idénticos en los estados líquidos o de evaporación. Al igual que los polimorfos, los isómeros dinámicos funden a diferentes temperaturas pero arrojan productos fundidos de distinta composición. Con el curso del tiempo, cada uno de estos productos fundidos llega a una mezcla en equilibrio de los dos isómeros con composiciones dependientes de la temperatura. Algunos casos publicados de polimorfismo indudablemente representan isomerismo dinámico, dado que ambos sistemas se comportan en forma muy similar.

El polimorfismo es la capacidad de cualquier elemento o compuesto para cristalizar en la forma de más de una especie cristalina, por ejemplo, el carbón en la forma de diamante cúbico o grafito hexagonal. Los distintos polimorfismos de un compuesto dado en general son diferentes en estructura y propiedades como los cristales de dos compuestos distintos. La solubilidad, el punto de fusión, la densidad, la dureza, la forma de los cristales, las propiedades ópticas y eléctricas, la presión de vapor, la estabilidad, etc., varían según la forma polimórfica. En general debería ser posible obtener distintas formas cristalinas de una droga que muestre polimorfismo y de esa manera modificar las propiedades de ese compuesto. Para ello se requiere un conocimiento del comportamiento de los polimorfos. Existen numerosas revisiones relacionadas con el polimorfismo; además, en la bibliografía aparecen numerosas indicaciones de la importancia del polimorfismo en los productos farma-

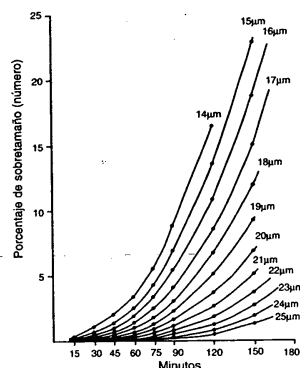


Fig. 83-5. Cambio en el recuento acumulativo en función del tiempo para una suspensión acuosa de la Forma I de una droga experimental.

céuticos. Se llevaron a cabo estudios extensivos de polimorfismo con esteroides, barbitúricos, antihistamínicos y sulfonamidas. La preformulación generalmente incluye estudios rigurosos para determinar la presencia de polimorfos en las nuevas drogas preparadas para las investigaciones preliminares en animales de experimentación. Algunos de los parámetros investigados en forma rutinaria consisten en la cantidad de polimorfos presentes, el grado relativo de estabilidad de los diversos polimorfos, la presencia de un estado vídrioso, la estabilización de las formas metaestables, los rangos de estabilidad térmica para cada polimorfo, las solubilidades, el método de preparación de cada forma, los efectos de la micronización o la preparación de tabletas y la interacción con los componentes de la formulación.

La tarea inicial del preformulador consiste en determinar si la sustancia química evaluada existe en más de una forma cristalina. Por lo general se utilizan los siguientes procedimientos para inducir la cristalización en forma metaestable:¹

1. Fundir completamente una pequeña cantidad del compuesto en un portaobjeto y observar la solidificación entre polares cruzados. Si después de la congelación espontánea se produce una transformación espontánea o inducida por la siembra o el raspado, es probable que del compuesto existan como mínimo dos formas polimórficas. Es esencial prevenir la nucleación de la forma estable induciendo un sobreenfriamiento. Este puede inducirse mediante el uso de

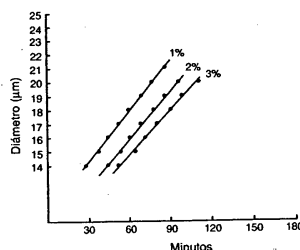


Fig. 83-6. Velocidad de crecimiento de la Forma I de una droga experimental en suspensión acuosa.

una muestra pequeña, manteniendo el fundido durante aproximadamente 30 segundos a aproximadamente 10°C por arriba del punto de fusión; separar en forma cuidadosa el compuesto evitando el shock físico antes de observarlo y enfriarlo con rapidez.

2. Calentar una muestra del compuesto sobre una placa caliente y observar si durante el calentamiento se produce una transformación sólido-sólido.

3. Sublimar una pequeña cantidad del compuesto e intentar inducir la transformación entre el sublimado y la muestra original mezclando ambos compuestos en una gota de solución saturada de uno de ellos. Si ambos son polimorfos, el más estable de los dos será insoluble y crecerá a expensas de la forma metaestable más soluble. Este proceso continuará hasta que la forma metaestable sea transformada completamente en la forma estable. Si las muestras son polimorfos, una de ellas puede disolverse pero la otra no aumentará. Si ambas formas son idénticas no se observará ninguna modificación.

4. Mantener el exceso de compuesto en una pequeña cantidad de solvente cerca de su punto de fusión. Aislar el sólido suspendido. Es importante adoptar precauciones a fin de mantener la temperatura durante este paso. Evaluar el material aislado con una muestra original utilizando el procedimiento descrito antes en el paso 3.

5. Recristalizar el compuesto de la solución mediante enfriamiento rápido y observar una fracción del material precipitado suspendido en una gota del licor madre. La gota ulteriormente puede ser sembrada con el compuesto original para evaluar la transformación de fase de la solución. Si el precipitado es un polimorfo diferente debe observarse una transformación de fase en la solución.

Una vez establecida la presencia de polimorfismo se cuenta con procedimientos que permiten al preformulador preparar las diversas formas en mayores cantidades para una evaluación ulterior y adaptación para la incorporación en formas farmacéuticas.

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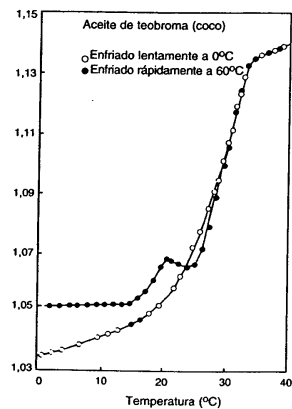


Fig. 83-7. Curvas dilatométricas; aceite de teobroma (coco) con enfriamiento lento y rápido.

Una vez demostrado que un compuesto existe en más de una forma cristalina se dispone de numerosas técnicas para identificar las distintas fases polimórficas presentes. Cada una de estas técnicas pueden ser adecuadas para identificar la fase, pero existe una combinación de métodos que permite aislar e identificar cada modificación cristalina. Para confirmar la presencia de más de una forma cristalina de un compuesto es recomendable identificar las modificaciones presentes mediante más de un método. El uso de un solo método para confirmar la presencia de polimorfos puede generar confusiones.

Microscopía. La cristalografía óptica se utiliza en la identificación de polimorfos. Los cristales existen en formas isotrópicas y anisotrópicas. Cuando se examinan los cristales isotrópicos, la velocidad de la luz es la misma en todas las direcciones, mientras que los cristales anisotrópicos se asocian con dos o tres velocidades luminosas o índices de refracción diferentes. Este método requiere el servicio de un cristalógrafo con experiencia. Los sistemas de registro con video posibilitaron grabar los eventos visualizados durante el calentamiento y el enfriamiento, lo que proporciona un registro permanente que puede ser examinado en forma repetida.

Métodos en platina caliente. El microscopio de polarización, equipado con una platina caliente o fría, es sumamente útil para la investigación de polimorfos. Un observa-

dor experimentado puede determinar rápidamente si existen polimorfos: el grado de estabilidad de las formas metaestables; las temperaturas de transición y los puntos de fusión; los índices de transición en diversas condiciones térmicas y físicas, y establecer si el polimorfismo es una vía hacia una forma de dosificación óptima.

Difracción de polvo con rayos X. Los materiales cristalinos en forma de polvo dan patrones de difracción con rayos X característicos que consisten en picos en ciertas posiciones y con intensidades variables. Cada patrón cristalino es característico para un polimorfo determinado. Este método posee la ventaja con respecto a otras técnicas de identificación en que la muestra se examina en la forma en la que es presentada. Se recomiendan precauciones para reducir y mantener el control del tamaño de las partículas. Se requiere una muestra muy pequeña y el método es no destructivo. Este método fue utilizado por varios investigadores para identificar polimorfos en sustancias farmacéuticas.

Espectroscopia infrarroja. Este procedimiento es útil para la identificación de los polimorfos. Deben utilizarse muestras sólidas, dado que los polimorfos de un compuesto presentan espectros idénticos en solución. La técnica puede utilizarse para la identificación cualitativa y cuantitativa.

Métodos térmicos. Se utilizaron las técnicas de calorimetría de rastreo diferencial y el análisis térmico diferencial para la identificación de polimorfos. En ambos métodos, la pérdida o la ganancia de calor resultante de transiciones físicas o químicas que se producen en una misma muestra se registran en función de la temperatura a medida que la sustancia es calentada con una velocidad uniforme. Las transiciones de las fases producen cambios entálpicos, endotérmicos y exotérmicos. Por ejemplo, la fusión, la sublimación, la transición sólido-sólido y la pérdida de agua generalmente provocan efectos endotérmicos, mientras que la cristalización produce efectos exotérmicos. El análisis térmico permite calcular los parámetros termodinámicos para los sistemas evaluados. Es posible obtener calores de fusión y determinar el índice de conversión de los polimorfos.

Dilatometría. La dilatometría mide el cambio de volumen causado por efectos térmicos o químicos. Ravin y Higucho¹⁰ utilizaron la dilatometría para seguir el comportamiento de fusión del aceite de teobroma (cacao) a través de la determinación de su volumen específico rápidamente calentado y enfriado en función del aumento de la temperatura. La presencia de la forma metaestable se expresó por una contracción en el rango de la temperatura de 20 a 24°C. Este fenómeno se ilustra en la figura 83-7. La dilatometría es un método sumamente impreciso, muy engorroso e insume mucho tiempo. La dilatometría no se utiliza en forma generalizada.

El polimorfismo a veces se estudia mediante resonancia magnética con protones, resonancia magnética nuclear y microscopía electrónica.

Los polimorfos pueden clasificarse en uno de dos tipos: 1) *enantiotrópico*: una forma polimórfica puede convertirse reversible en otra a través de una modificación de la temperatura o la presión, por ejemplo, el azufre y 2) *monotrópico*: una forma polimórfica es inestable en cualquier condición de tempera-

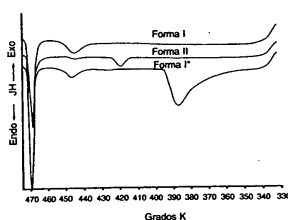


Fig. 83-8. Termogramas para las Formas I, I* e II de SK&F 30097.

tura y presión, por ejemplo, estearatos de glicerilo. En condiciones dadas de temperatura y presión solamente una forma polimórfica será termodinámicamente estable. Sin embargo, pueden existir otras formas metaestables en las mismas condiciones. Estas formas metaestables se convertirán en estructuras de cristales estables con el transcurso del tiempo. La primera indicación de la importancia de una transformación polimórfica en un sistema farmacéutico se observó con la novobiocina. Se notó que la forma amorfa de novobiocina se absorbía bien; sin embargo, cuando el compuesto era preparado en suspensión se observaba una reversión de la forma metaestable en una forma cristalina más estable que determinaba una absorción deficiente.

Una vez determinado que una droga existe en más de una forma cristalina es necesario establecer las condiciones en las cuales puede producirse cada una de esas formas. De este modo será posible mantener condiciones de cristalización adecuadas en los diferentes lotes para garantizar una materia prima uniforme y aceptable. El disolvente de recristalización, la velocidad de cristalización y otros factores pueden determinar que predomine una forma de cristal. Durante la investigación preliminar para establecer estas condiciones es necesario monitorear las formas preparadas. Por ejemplo, durante los trabajos preliminares con un derivado del indol se utilizaron métodos tales como calorimetría con rastreo diferencial, análisis con rayos X y análisis infrarrojo para establecer la presencia de polimorfos y determinar que la preparación de estos compuestos podía llevarse a cabo en forma satisfactoria. Las figuras 83-8, 83-9 y 83-10 ilustran los datos respectivos para arribar a esta conclusión. Cuando se muestra la presencia de polimorfos deben planificarse trabajos experimentales para determinar si las propiedades difieren o no en magnitud suficiente como para alterar el comportamiento farmacéutico o biológico.

Las pruebas de disolución pueden utilizarse en una fase inicial para revelar diferencias en las solubilidades de equilibrio aparentes siempre que se utilice

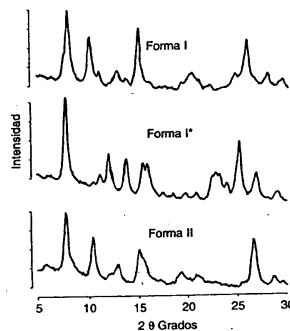


Fig. 83-9. Difractogramas de rayos X para las Formas I, I* e II de SK&F 30097.

un sistema de disolventes discriminativos. La figura 83-11 ilustra los datos de disolución para dos polimorfos de un derivado indólico que demostraron una disolución similar en el medio utilizado; sin embargo, cuando se utilizó un medio de disolución discriminativo se logró demostrar diferencias en sus características de disolución. Este fenómeno se ilustra en la figura 83-12. A partir de los datos presentados para el derivado indólico, se llegó a la conclusión de que no existirían diferencias apreciables en la disponibilidad de las dos formas si se administraran por

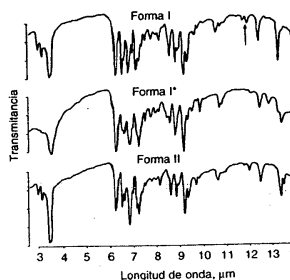


Fig. 83-10. Espectros infrarrojos de las Formas I, I* e II de SK&F 30097.

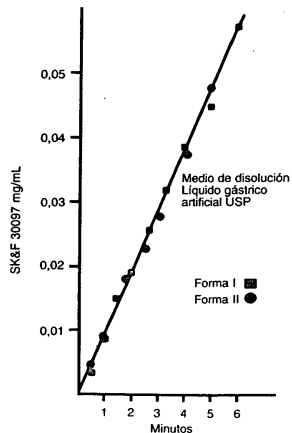


Fig. 83-11. Comportamiento de disolución de las Formas I y II de SK&F 30097 en el líquido gástrico artificial.

vía oral en una forma farmacéutica sólida. Pruebas ulteriores en animales de experimentación confirmaron esta conclusión. La ecuación de Nernst relaciona el aumento de la velocidad de concentración con la estabilidad de un sólido en disolución y se expresa de la siguiente manera:

$$\frac{dc}{dt} = \frac{AD}{Vh} (C_s - C_t) \quad (3)$$

en donde A es el área de la interfase de disolución del sólido, D es el coeficiente de difusión del soluto en el disolvente, V es el volumen del disolvente, h es el espesor de la capa de difusión y C_s y C_t representan la concentración del soluto en condiciones de saturación en el tiempo t, respectivamente. La ecuación se reduce a:

$$\frac{dc}{dt} = \frac{AD}{Vh} C_s \quad (4)$$

para las condiciones experimentales en donde C_s > C_t. Dado que D es una propiedad de la molécula de soluto y del disolvente, este parámetro es independiente de la forma en estado sólido. Las condiciones experimentales pueden ser seleccionadas de manera que A, V y h se mantengan constantes para medir las

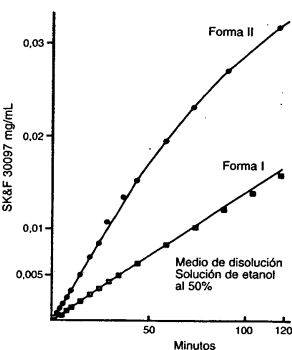


Fig. 83-12. Comportamiento de disolución de las Formas I y II de SK&F 30097 en una solución de etanol al 50%.

velocidades de disolución de las distintas formas polimórficas. Entonces, la velocidad de disolución es directamente proporcional a C_s; la solubilidad de saturación y las diferencias en la solubilidad puede relacionarse con sus energías libres.

Se determinó el comportamiento de solubilidad y disolución de varios polimorfos del palmitato de cloramfenicol. Las figuras 83-13 y 83-14 ilustran los datos obtenidos a diversas temperaturas. El comportamiento de disolución muestra que los máximos valores obtenidos representaron buenas aproximaciones de la solubilidad de las diversas formas. En consecuencia, la obtención de datos a diversas temperaturas permitiría calcular las cantidades termodinámicas implicadas en la transición de la forma metaestable a la forma estable. En la figura 83-15 se ilustra un gráfico en el que se relacionan los datos de solubilidad en función de la temperatura en un trazado típico de van't Hoff. La relación en línea recta permite calcular los calores de la solución para las diversas formas y además, por extrapolación, determinar un valor aproximado de la temperatura de transición para las distintas formas. Estos valores se encuentran en el cuadro 83-1.⁷

A temperatura y presión constantes, pueden calcularse las diferencias de energía libre entre los polimorfos mediante la siguiente fórmula:

$$\Delta G_f = RT \ln \frac{C_s \text{ Polimorfo A}}{C_s \text{ Polimorfo B}} \quad (5)$$

Esta ecuación relaciona la solubilidad, C_s, de las formas polimórficas a una temperatura dada, T, con

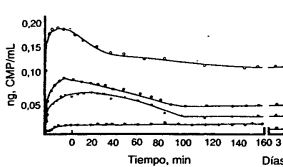


Fig. 83-13. Curvas de disolución del polimorfo C del palmitato de cloramfenicol en alcohol i-butílico al 35% y en agua a 30°C, 20°C, 15°C y 6°C. Símbolos: 30°C, ○; 20°C, □; 15°C, △; 6°C, ◇.

las diferencias de energía libre, ΔG_f. El cuadro 83-1 también incluye las diferencias de energía libre calculadas para los polimorfos. Los cambios de entalpía también pueden determinarse para las diversas transiciones mediante la sustracción del calor de solución derivado de la forma estable del de la forma metaestable. Además, en presencia de una temperatura dada T, la entropía para la transición de polimorfos puede evaluarse mediante la siguiente relación:

$$\Delta S_f = \frac{\Delta H_{B-A} - \Delta G_f}{T} \quad (6)$$

Los valores calculados para las transiciones también se encuentran en el cuadro 83-1. En presencia de la temperatura de transición, ΔG_f es igual a cero y es posible calcular la entropía ignorando el término energía libre en la ecuación 6.

Las relaciones termodinámicas comentadas parten de la premisa de que se cumple la ley de Henry. El conocimiento de estas relaciones termodinámicas permite al preformulador seleccionar en forma más racional la forma polimórfica más energética de la droga investigada para estudios farmacológicos ulteriores y además contar con una evaluación preliminar de su probable estabilidad.

Cuando un grupo de preformulación investiga de manera inadecuada formas polimórficas de una droga pueden surgir problemas durante la etapa de desarrollo. El crecimiento de cristales en suspensiones

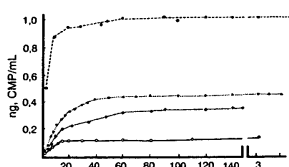


Fig. 83-14. Curvas de disolución de los polimorfos A y B del palmitato de cloramfenicol en alcohol i-butílico al 35% y en agua a 30°C y 38°C. Símbolos: polimorfo A, 30°C, ○; polimorfo B, 30°C, □; polimorfo A, 38°C, ◇; polimorfo B, 38°C, ◇.

asociadas con alteraciones en la uniformidad, el aspecto o la biodisponibilidad; las transformaciones producidas durante la molienda o la granulación que alteran las características físicas y biológicas; una respuesta farmacológica inadecuada, y la estabilidad química deficiente representan problemas típicos que pueden ponerse de manifiesto después.

Solubilidad

Cuando se manejan nuevas drogas es sumamente importante conocer algo acerca de sus características de solubilidad, especialmente en sistemas acuosos, dado que estos compuestos pueden tener alguna solubilidad acuosa insuficiente para inducir una respuesta terapéutica. Además, la información de la solubilidad es esencial para el desarrollo de un método de prueba discriminativo para la solución. Cuando una droga tiene una solubilidad acuosa menor de 1 mg/mL dentro del rango de pH fisiológico (1 a 7) puede existir un problema de biodisponibilidad y deben iniciarse estudios de preformulación para minimizar este inconveniente. La solubilidad de equilibrio de la droga debería determinarse en un disolvente o sistema de disolvente que no ejerza ningún efecto tóxico en el animal de experimentación. Para ello se coloca un exceso de droga en un frasco junto con el solvente. El frasco se agita a temperatura constante y la cantidad

Cuadro 83-1. Valores termodinámicos calculados para los polimorfos A, B y C del palmitato de cloramfenicol⁷

Polimorfo	Temperatura de transición (°C) a la forma A	Calor de la solución, kcal/mol	ΔG _f , cal/mol ^a	ΔS _{sol} , esu	ΔS _{trans} , esu ^a
A	—	21.8	—	—	—
B	88	15.4	-774	-18	-17
C	50	17.2	-465	-13	-14

^a Calculado para la conversión al polimorfo A.

888-0188

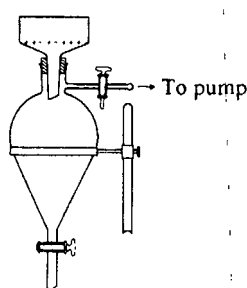


Fig. 2.79

used; here the funnel should be of a size appropriate to the amount of solid to be finally collected. When the receiver is about two-thirds full, atmospheric pressure is restored by suitably rotating the 'three-way' stopcock; the filtrate may then be removed by opening the tap at the lower end. The apparatus is again exhausted and the filtration continued.

For the suction filtration of small quantities of solid (less than 5 g) contained in a small volume of liquid, a small conical Buchner funnel, known as a Hirsch funnel, is employed (see Fig. 2.43(b)); the filtrate is collected either in a small filter flask or in a test tube with side-arm, the arrangement being illustrated in Fig. 2.43(f). A small sintered funnel, or a slit-sieve funnel may also be employed. The procedure for filtration is similar to that already given for the Buchner funnel.

Small volumes of solution (up to 2 ml in one operation) may conveniently be filtered through a dropping (Pasteur) pipette into the constriction of which has been rammed a small piece of paper tissue (about 3 cm square). The pipette is supported vertically and the solution is added from a second Pasteur pipette. Pressure to accelerate the filtration process may then be applied from a rubber bulb attached to the top of the pipette.

This method is particularly useful for the preparation of solutions of samples for spectroscopic examination where it is important to remove all insoluble impurities. The method may be adapted for the decolourisation of small samples by incorporating a short column of decolourising carbon above the paper plug (cf. Section 2.20).

2.20 RECRYSTALLISATION TECHNIQUES

Solid organic compounds when isolated from organic reactions are seldom pure; they are usually contaminated with small amounts of other compounds (impurities) which are produced along with the desired product. The purification of impure crystalline compounds is usually effected by crystallisation from a suitable solvent or mixture of solvents.

The purification of solids by crystallisation is based upon differences in their solubility in a given solvent or mixture of solvents. In its simplest form, the crystallisation process consists of: (i) dissolving the impure substance in some suitable solvent at or near the boiling point; (ii) filtering the hot solution from particles of insoluble material and dust; (iii) allowing the hot solution to cool

thus causing the dissolved substance to crystallise out; and (iv) separating the crystals from the supernatant solution (or mother-liquor). The resulting solid, after drying, is tested for purity (usually by a melting point determination, Section 2.33, but also by spectroscopic methods, Chapter 3, or by thin-layer chromatography, Section 2.31), and if found impure is again recrystallised from fresh solvent. The process is repeated until the pure compound is obtained; this often means until the melting point is unchanged, but confirmation by the other methods specified above is desirable.

The theory underlying the removal of impurities by crystallisation may be understood from the following considerations. It is assumed that the impurities are present in comparatively small proportion – usually less than 5 per cent of the whole. Let the pure substance be denoted by A and the impurities by B, and let the proportion of the latter be assumed to be 5 per cent. In most instances the solubilities of A (S_A) and of B (S_B) are different in a particular solvent; the influence of each compound upon the solubility of the other will be neglected. Two cases will arise for any particular solvent: (i) the impurity is more soluble than the compound which is being purified ($S_B > S_A$) and, (ii) the impurity is less soluble than the compound ($S_B < S_A$). It is evident that in case (i) several recrystallisations will give pure sample of A, and B will remain in the mother-liquors. Case (ii) can be more clearly illustrated by a specific example. Let us assume that the solubilities of A and B in a given solvent at the temperature of the laboratory (15°C) are 10 g and 3 g per 100 ml of solvent respectively. If 50 g of the crude material (containing 47.5 g of A and 2.5 g of B) are dissolved in 100 ml of the hot solvent and the solution allowed to cool to 15°C , the mother-liquor will contain 10 g of A and 2.5 g (i.e. the whole) of B; 37.5 g of pure crystals of A will be obtained.

The most desirable characteristics of a solvent for recrystallisation are as follows:

1. A high solvent power for the substance to be purified at elevated temperatures and a comparatively low solvent power at the laboratory temperature or below.
2. It should dissolve the impurities readily or to only a very small extent.
3. It should yield well-formed crystals of the purified compound.
4. It must be capable of easy removal from the crystals of the purified compound, i.e. possess a relatively low boiling point.

It is assumed, of course, that the solvent does not react chemically with the substance to be purified. If two or more solvents appear to be equally suitable for recrystallisation, the final selection will depend upon such factors as ease of manipulation, toxicity, flammability and cost.

Some common solvents available for the recrystallisation are collected in Table 2.8, broadly in the order of decreasing polarity. Their purification is included in Section 4.1.

The use of ether as a solvent for recrystallisation should be avoided wherever possible, partly owing to its great flammability and partly owing to its tendency to creep up walls of the containing vessel, thus depositing solid matter by complete evaporation instead of preferential crystallisation. Carbon disulphide, b.p. 46°C , should *never* be used if an alternative solvent can be found; it has a dangerously low flash point and forms very explosive mixtures with air.

Other recrystallisation solvents include tetrahydrofuran (THF), b.p. $65\text{--}66^\circ\text{C}$;

Table 2.8 Common solvents for recrystallisation

Solvent	b.p. (°C)	
Water (distilled)	100	To be used whenever suitable
Methanol*	64.5	Flammable; toxic
Ethanol	78	Flammable
Industrial spirit	77-82	Flammable
Rectified spirit	78	Flammable
Acetone	56	Flammable
Ethyl acetate	78	Flammable
Acetic acid (glacial)	118	Not very flammable, pungent vapours
Dichloromethane (methylene chloride)*	41	Non-flammable; toxic
Chloroform*	61	Non-flammable; vapour toxic
Diethyl ether	35	Flammable, avoid whenever possible
Benzene*†	80	Flammable, vapour highly toxic
Dioxane*	101	Flammable, vapour toxic
Carbon tetrachloride*	77	Non-flammable, vapour toxic
Light petroleum	40-60	Flammable‡
Cyclohexane	81	Flammable

* CAUTION: The vapours of these solvents are toxic and therefore recrystallisations involving their use must be conducted in an efficient fume cupboard; excessive inhalation of any vapour should be avoided. For notes on cumulative toxic effects refer to Section 2.3.

† Toluene is much less toxic than benzene and should be used in place of the latter whenever possible.

‡ Other fractions available have b.p. 60-80, 80-100 and 100-200 °C; when the boiling point exceeds 120 °C the fraction is usually called 'ligroin'. Pentane, b.p. 36 °C, and heptane, b.p. 98 °C, are also frequently used recrystallisation solvents.

butan-2-one (ethyl methyl ketone), b.p. 80 °C; 1,2-dichloroethane* (ethylene chloride), b.p. 84 °C; acetonitrile* (methyl cyanide), b.p. 80 °C; toluene*†, b.p. 110 °C; pyridine*, b.p. 115.5 °C; chlorobenzene*, b.p. 132 °C; cellosolve* (2-ethoxyethanol), b.p. 134.5 °C; dibutyl ether, b.p. 141 °C; 1,1,2,2-tetrachloroethane*, b.p. 147 °C; dimethylformamide* (DMF; formdimethylamide), b.p. 153 °C; dimethyl sulphoxide, b.p. 189 °C (d); nitrobenzene*, b.p. 209.5 °C; and ethyl benzoate, b.p. 212 °C.

The following rough generalisations may assist the student in the selection of a solvent for recrystallisation, but it must be clearly understood that numerous exceptions are known (for a further discussion see Section 9.2):

1. A substance is likely to be most soluble in a solvent to which it is most closely related in chemical and physical characteristics.
2. In ascending a homologous series, the solubilities of the members tend to become more and more like that of the hydrocarbon from which they may be regarded as being derived.
3. A polar substance is more soluble in polar solvents and less soluble in non-polar solvents. The solvents in Table 2.8 have been listed broadly in order of decreasing polar character.

* CAUTION: The vapours of these solvents are toxic and therefore recrystallisations involving their use must be conducted in an efficient fume cupboard; excessive inhalation of any vapour should be avoided. For notes on cumulative toxic effects refer to Section 2.3.

† Toluene is much less toxic than benzene and should be used in place of the latter whenever possible.

In practice the choice of a solvent for recrystallisation must be determined experimentally if no information is already available. About 0.1 g of the powdered substance* is placed in a small test tube (75 × 11 mm or 110 × 12 mm) and the solvent is added a drop at a time with continuous shaking of the test tube. After about 1 ml of the solvent has been added, the mixture is heated to boiling, due precautions being taken if the solvent is flammable. If the sample dissolves easily in 1 ml of cold solvent or upon gentle warming, the solvent is unsuitable. If all the solid does not dissolve, more solvent is added in 0.5 ml portions, and again heated to boiling after each addition. If 3 ml of solvent is added and the substance does not dissolve on heating, the substance is regarded as sparingly soluble in that solvent, and another solvent should be sought. If the compound dissolves (or almost completely dissolves†) in the hot solvent, the tube is cooled to determine whether crystallisation occurs. If crystallisation does not take place rapidly, this may be due to the absence of suitable nuclei for crystal growth. The tube should be scratched below the surface of the solution with a glass rod; the fine scratches on the walls (and the minute fragments of glass produced) may serve as excellent nuclei for crystal growth. If crystals do not separate, even after scratching for several minutes and cooling in an ice-salt mixture, the solvent is rejected. If crystals separate, the amount of these should be noted. The process may be repeated with other possible solvents, using a fresh test tube for each experiment, until the best solvent is found; the approximate proportions of the solute and solvent giving the most satisfactory results should be recorded.

If the substance is found to be far too soluble in one solvent and much too insoluble in another solvent to allow of recrystallisation, *mixed solvents* or '*solvent pairs*' may frequently be used with excellent results. The two solvents must, of course, be completely miscible.‡ Recrystallisation from mixed solvents is carried out near the boiling point of the mixture. The compound is dissolved in the solvent in which it is very soluble, and the hot solvent, in which the substance is only sparingly soluble, is added cautiously until a slight turbidity is produced. The turbidity is then just cleared by the addition of a small quantity of the first solvent and the mixture is allowed to cool to room temperature; crystals will separate. Pairs of liquids which may be used include: alcohols and water; alcohols and toluene; toluene and light petroleum; acetone and light petroleum; diethyl ether and pentane; glacial acetic acid and water; dimethylformamide with either water or toluene.

When the best solvent or solvent mixture and the appropriate proportions of solute and solvent have been determined by these preliminary tests or have been obtained from reference books containing solubility data,^{39, 40} the solid substance is placed in a round-bottomed flask of suitable size fitted with a reflux condenser (Fig. 2.54) and slightly less than the required quantity of solvent is added together with a few pieces of porous porcelain to prevent 'bumping' (see

* With practice the student should be able readily to perform trial recrystallisations with much smaller quantities of material (e.g. 5 mg) using a small ignition or centrifuge tube and correspondingly smaller quantities of solvents.

† If the crude substance contains an insoluble impurity, difficulty may be experienced at a later stage in estimating how much solute has crystallised from the cold solution. The hot solution should therefore be filtered into another tube through a very small fluted filter paper contained in a small short-stemmed funnel. The solution must always be clear before cooling is attempted.

‡ Solvent pairs selected from the extremes of the list Table 2.8 are not usually sufficiently miscible to be satisfactory, e.g. methanol and light petroleum.

Section 2.24). The mixture is heated to boiling on a water bath (if the solvent boils below 80 °C) or with an electric heating mantle, and more solvent is added down the condenser until a clear solution, apart from insoluble impurities*, is produced. If the solvent is not flammable, toxic or expensive, recrystallisation may be carried out in a conical flask, into the neck of which a funnel with a short stem is inserted, which is heated on an electric plate.

FILTRATION OF THE HOT SOLUTION

The boiling or hot solution must be rapidly filtered before undue cooling has occurred. (If a flammable solvent has been used, all flames in the vicinity must be extinguished.) This is usually done through a fluted filter paper (see below) supported in a relatively large funnel with a short wide stem; separation of crystals in and clogging of the stem is thus reduced to a minimum. The funnel should be warmed in an electric or steam oven before filtration is started, when it should be supported in a conical flask of sufficient size to hold all the solution; the conical flask is stood on an electric hotplate or steam bath and the filtrate is kept boiling gently so that the warm solvent vapours maintain the temperature of the solution undergoing filtration, and thus prevent premature deposition of crystals on the filter or in the neck of the funnel. If solid does separate out on the filter it must be scraped back into the first flask, redissolved and refiltered. The filtered solution is covered with a watch- or clock-glass, and then set aside to cool undisturbed. If large crystals are desired, any solid which may have separated from the filtered solution should be redissolved by warming (a reflux condenser must be used for a flammable solvent), the flask wrapped in a towel or cloth, and allowed to cool slowly. If small crystals are required, the hot saturated solution should be stirred vigorously and cooled rapidly in a bath of cold water or of ice. It should be noted that large crystals are not necessarily purer than small ones; generally very impure substances are best purified by slow recrystallisation to give large crystals, followed by several rapid recrystallisations to give small crystals.

If large quantities of hot solution are to be filtered, the funnel (and fluted filter paper) should be warmed externally during the filtration. The heating mantle illustrated in Fig. 2.47(c) is particularly suitable, using the lower heating element; no flames should be present while flammable solvents are being filtered through this funnel. When dealing with considerable volumes of aqueous or other solutions which do not deposit crystals rapidly on cooling, a Buchner funnel preheated in an oven may be used for filtration (see Section 2.19). The filter paper should be of close-grained texture and should be wetted with solvent before suction is applied; the solution may then be poured on to the filter.

PREPARATION OF A FLUTED FILTER PAPER

The filter paper is first folded in half and again in quarters, and opened up as shown in Fig. 2.80(a). The edge 2,1 is then folded on to 2,4 and edge 2,3 on to 2,4, producing, when the paper is opened, new folds at 2,5 and 2,6. The folding is continued, 2,1 to 2,6 and 2,3 to 2,5, thus producing folds at 2,7 and 2,8 respectively (Fig. 2.80(b)); further 2,3 to 2,6 giving 2,9, and 2,1 to 2,5 giving 2,10 (Fig. 2.80(c)). The final operation consists in making a fold in each of the eight segments – between 2,3 and 2,9, between 2,9 and 2,6, etc. – in a direction *opposite* to

* The undissolved material will be readily recognised if preliminary solubility tests have been correctly interpreted.