

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 14-146898- -4	FECHA: 2015-05-28 14:23:34
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 10
ACT: 519 TRASLADOINFORME	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
CARLOS R. OLARTE GARCIA

Asunto: Radicación: 14-146898- -4
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 5767
Folios: 10

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/US2013/021521				
Fecha de Presentación Internacional	15/01/2013				

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:	Radicado No. 14-146898-00000-0000	8/julio/2014
Reivindicaciones:	Radicado No. 14-146898-00000-0000	8/julio/2014
	Radicado No. 14-146898-00001-0000	8/septiembre/2014
	Radicado No. 14-146898-00002-0000	26/noviembre/2014
Prioridad:	US 61/587.280	17/enero/2012

2. Análisis de la Prioridad

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

3. Modificaciones aceptadas

Se acepta el nuevo capítulo reivindicatorio (Radicado No. 14-146898-00002-0000) porque no se amplía el objeto de la solicitud.

4. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el presente caso, se tiene en cuenta la totalidad del nuevo capítulo reivindicatorio (Radicado No. 14-146898-00002-0000) para el examen de patentabilidad.

Título de la solicitud: "NUEVAS FORMAS Y SALES DE UN DIHIDROPIRROLO [1,2-C]IMIDAZOLILO, INHIBIDOR DE ALDOSTERONA SINTASA O AROMATASA".

El problema planteado en esta solicitud consiste en la necesidad de suministrar sales del compuesto 6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il- 3- fluoro- benzonitrilo.

Para resolver este problema técnico la solicitud en estudio proporciona una sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro - benzonitrilo.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K31/407; A61P5/38; C07D487/04.

5. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1 y 2 reclaman una sal cristalina de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo, sin embargo no se realiza una caracterización apropiada de dicho sólido cristalino. Un polimorfo se puede caracterizar apropiadamente en una reivindicación, por sus parámetros físicoquímicos, 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 dhkl 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-C13
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.

La difracción de rayos X es la técnica más útil para el estudio de cristales 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. En consecuencia se recomienda al solicitante hacer una correcta caracterización del cristal desde la reivindicación independiente.

La expresión “aproximadamente” utilizada en la reivindicación 1 es ambigua y no limita el alcance de la solicitud. Se sugiere a la solicitante utilizar valores precisos.

Las reivindicación 7 y 8 caracteriza la forma cristalina indicando que puede tener al menos 4 a 9 de los picos de refracción de rayos X. Esta caracterización es inapropiada ya que como se indicó anteriormente, la solicitante debe caracterizar cada polimorfo por sus propiedades fisicoquímicas, siendo la más aceptada el espectro de difracción de rayos X, y para cada cristal se debe presentar los picos de mayor intensidad, los cuales se consideran los más representativos. Es pertinente señalar que menos de diez picos del espectro de difracción de rayos X son insuficientes para caracterizar de manera inequívoca un sólido cristalino de un compuesto dado. Esto es evidenciado por Bhattacharya¹ que provee un gráfico comparativo (fig. 11) mediante el cual superpone los difractogramas XRPD de dos polimorfos de ibandronato sódico. A través de esto Bhattacharya *et al.*, demuestran que diferentes polimorfos de un compuesto dado pueden compartir incluso tres picos principales de XRPD. De acuerdo con Bhattacharya, alrededor de diez de los picos más fuertes del XRPD junto con sus tolerancias son necesarios para identificar una forma cristalina única.

La reivindicación 10 caracteriza la forma cristalina haciendo referencia a la figura A. Esta caracterización no es aceptada debido a que un cristal debe caracterizarse mediante sus parámetros fisicoquímicos como se indicó anteriormente.

6. Unidad de invención (Art 25 D 486)

La Solicitud no cumple con el Requisito de Unidad de Invención porque se refiere a 2 Grupos Inventivos.

Los Grupos Inventivos están definidos por las siguientes características **esenciales**:

1° Una sal cristalina en la forma A de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo, procedimiento para su preparación y composiciones que la comprenden (Reiv. 1 a 10 y 12 a 13).

2° Una sal de nitrato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo, composiciones que la comprenden y método de obtención (Reiv. 11 y 12).

No se guarda un concepto común inventivo porque las sales reivindicadas no comparten una estructura esencial y sus propiedades son diferentes por lo que no se puede atribuir una actividad común.

7. 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: 17 de enero de 2012, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	WO2011088188	“Use of an adrenal hormone-modifying agent”	21/julio/2011
D2	H.G. Brittain. Segunda Edición, Informa Healthcare,NY,	“Polymorphism in Pharmaceutical Solids”	2009

1. H.G. Brittain. “Polymorphism in Pharmaceutical Solids”. Segunda Edición, Informa Healthcare,NY, 2009. Págs. 334 y 335.

	2009		
D3	Journal of Pharmaceutical Sciences 66, número 1, pp. 2 a 19.	Pharmaceutical salts	01/1977

D1² divulga la sal fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo la cual es útil para el tratamiento de la enfermedad de Cushing (Pág. 9, línea 30; pág. 10, línea 20).

D2 enseña que muchos compuestos son capaces de exhibir el fenómeno de polimorfismo, y pueden cristalizar en formas cristalinas con diferentes propiedades fisicoquímicas (Págs. 1 a 4, Tabla 2), la anterioridad da a conocer las diversas técnicas que permiten la obtención de diversos polimorfos (Págs. 15 a 19) y las metodologías que permiten la caracterización de los mismos (Págs. 318 a 430).

El documento D3³ enseña los efectos químicos, biológicos, físicos y económicos que pueden obtenerse al convertir un compuesto farmacéutico en una sal (Págs. 1 a 17).

8. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La reivindicación 1 consiste en una sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo.

Características esenciales	D1
Una sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo.	Una sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo (Pág. 9, línea 30 a Pág. 10, línea 20)

Las características esenciales de la composición de la sal cristalina de la reivindicación 1 habían sido divulgadas previamente en el documento D1, el cual enseña una sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo.

En consecuencia, las reivindicaciones 1 y 2 no son nuevas según lo divulgado en el documento D1.

Nivel inventivo (Art18 D486)

Las reivindicaciones 3 a 9 caracterizan la sal fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo por sus parámetros fisicoquímicos tales como TG/DTA, XRPD.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D2
La forma cristalina A de la sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo caracterizada por:	Una sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo (Pág.	Divulga la importancia de los polimorfos en la industria farmacéutica y las diferencias en las

2<https://www.google.com.co/patents/WO2011088188A1?cl=en&dq=WO2011088188&hl=es&sa=X&ei=sUJiVbS-PMmhgwTiy4HQAw&ved=0CB0Q6AEwAA>

3[] <http://onlinelibrary.wiley.com/doi/10.1002/jps.2600660104/abstract>

	9, línea 30 a Pág. 10, línea 20)	propiedades fisicoquímicas las cuales poseen aplicaciones en el procesamiento, formulación, y biodisponibilidad del fármaco (Pág. 2)
TG/DTA entre 209 y 212°C XRPD con picos característicos den 12.9, 16.3 y 20.4 grados.	No lo divulga	Da a conocer las diversas técnicas que permiten la caracterización de un polimorfo. (Págs. 318)

El análisis de nivel inventivo del producto reclamado se realizó mediante la aproximación problema-solución en polimorfos (Patenting Polymorphs at the European Patent Office⁴) posición que adoptada por la Superintendencia de Industria y Comercio para efectos del análisis de la patentabilidad de las formas polimórficas.

1. Determinación del estado de la técnica más cercano.

El documento del estado de la técnica más cercano es D1 que enseña el compuesto sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo (Pág. 9, líneas 30 a pág. 10, línea 20; pág. 10, línea 31 a pág. 11, línea 9; Reiv. 3 y 4), donde este agente es útil para tratar la enfermedad de Cushing.

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 la anterioridad no establece que la sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo pueda encontrarse en forma cristalina no se especifican los parámetros del espectro de difracción de rayos X ni de TG/DTA.

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

El Efecto Técnico que logra la nueva configuración cristalina de la sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo consiste en una menor higroscopicidad con respecto a la sal clorhidrato, sin embargo la sal nitrato es aún menos higroscópica.

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 identificar los polimorfos de la sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo conforme a las enseñanzas de D1?

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

De acuerdo con la información suministrada en la solicitud, la solubilidad de la forma cristalina de la sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c]

4 http://www.pcb.ub.edu/centrepatents/pdf/cursos/dillunsCP/papathoma_polymorphs.pdf

imidazol- 5- il)-3- fluoro – benzonitrilo tiene una menor higroscopicidad con respecto a la sal clorhidrato del mismo agente. Sin embargo, estos resultados no evidencian una ventaja o superioridad de la forma cristalina reivindicada, toda vez que no existen estudios comparativos frente al compuesto previamente divulgado en el estado de la técnica que da a conocer de la sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo. Del mismo modo, los resultados presentados corresponden a la determinación de una propiedad física como lo es la higroscopicidad y no corresponden a una ventaja real a la sal que ya era conocida.

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

La solución al problema técnico planteado por la invención reclamada se considera obvia porque la anterioridad D2 divulga que muchos sólidos con actividad farmacológica pueden exhibir polimorfismo, la existencia de diferentes estructuras cristalinas de los diversos polimorfos de una sustancia a menudo produce que estos sólidos exhiban una variedad de propiedades fisicoquímicas diferentes, muchas de las cuales se encuentran listadas en la Tabla 2 (Pág. 3). Debido a las diferencias en las dimensiones, forma, simetría, capacidad (número de moléculas), y volúmenes de vacíos de sus celdas unitarias, los diferentes polimorfos de una sustancia dada tienen diferentes propiedades físicas que surgen de las diferencias en el empaquetamiento molecular.

Estas diferencias en las propiedades físicas entre las formas cristalinas de un sistema polimórfico son extremadamente interesantes para los científicos farmacéuticos debido a que sus manifestaciones pueden algunas veces llevar a diferencias observables que poseen implicaciones para el procesamiento, formulación, y en la disponibilidad. Luego de que las fases espaciales de una sustancia han sido determinadas, y se ha establecido el alcance de las posibles formas polimórficas y solvatomórficas, se convierte en algo crítico el determinar los límites de estabilidad de las diferentes formas y la manera como estas pueden ser interconvertidas. Por lo menos, se debe determinar cuál forma cristalina es la más estable, y determinar la mejor forma a ser elegida para continuar con los desarrollos (Pág. 2). En consecuencia estos estudios son ampliamente conocidos y hacen parte del desarrollo farmacotécnicos.

Igualmente, el documento D2 enseña que las formas salinas de los fármacos son a menudo utilizadas para incrementar sus solubilidades en agua. La anterioridad publica los datos de mayor frecuencia de sales con contraiones básicos más comunes encabezando el listado las sales sódicas (Pág. 79 y 80). Es pertinente señalar que es sabido que las características químicas, biológicas, físicas y económicas de los agentes medicinales pueden ser manipuladas y optimizadas mediante la conversión a forma de sal. Las sales imparten propiedades únicas a los fármacos, los agentes formadores de sales se eligen a menudo empíricamente. De las muchas sales sintetizadas, la forma salina preferida se selecciona por los químicos farmacéuticos principalmente sobre una base práctica tal como: costo de las materias primas, la facilidad de cristalización, y el porcentaje de rendimiento. Del mismo modo el listado de sales farmacéuticas aceptadas por la FDA es ampliamente conocido, al igual que la predominancia de la formación de sales sódicas⁵.

En consecuencia, la formación de de sales con la finalidad de modificar las propiedades fisicoquímicas y termodinámicas de los compuestos farmacéuticos ya se había discutido en D2 (págs. 79 y 80), el cual divulga el catión sodio como farmacéuticamente aceptable y uno de los preferidos en la industria farmacéutica.

5 Journal of pharmaceutical sciences. Vol. 66 (1), January 1977. "Pharmaceutical Salts".

Igualmente, es conocido que una forma salina la mayor parte de las veces imparte mayor solubilidad en agua, es menos tóxica o disminuye la velocidad de disolución lo que podría beneficiar a los químicos y formuladores.

Adicionalmente, el documento D2 enseña diferentes métodos útiles en la determinación de la presencia de diferentes estructuras cristalinas de un polimorfo entre los que se encuentran la termogravimetría para estudiar la cinética de transición de fase de los solvatomorfos, el XRD que permite obtener información fundamental sobre la estructura cristalina de la sustancia, XRD en monocristal que se considera la descripción más fundamenta de un solvatomorfo y ayuda a explicar sus propiedades físicas, la espectroscopia de absorción de infrarrojo, la espectroscopia NIR, la espectroscopia de terahertz y la espectroscopia Raman (Pág. 318).

Por lo anterior, una persona del oficio normalmente versada en la materia, conociendo lo divulgado en el documento D1, divulga la sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo, se vería motivada a utilizar métodos como los revelados en D2 para identificar otras posibles formas cristalinas de la molécula en cuestión, que además pueden ser caracterizados por métodos cristalográficos, de espectroscopia vibracional (Pág. 318). De tal manera que no es posible concluir que la identificación de un nuevo derivado cristalino envuelva actividad inventiva, como quiera que la molécula misma ya había sido sintetizada con anterioridad y que la capacidad de formar sales y polimorfos es una propiedad intrínseca de dicho compuesto; es decir, la identificación del cristal de la sal fosfato de esta 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, comoquiera que no se encontró alguna prueba o ensayo que permitiera determinar las ventajas reales de los sólidos la sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo, que permita probar un efecto realmente inesperado asociado a una mejora en sus propiedades o actividad, más aun teniendo en cuenta que es conocido que los las formas salinas la mayor parte de las veces son más solubles en medio acuoso que su correspondiente forma anhidra según lo indica D2 (Pág. 79).

En este orden de ideas, es evidente que se pretende protección para un estado cristalino de la sal sódica del compuesto de fórmula (1), que aunque es nuevo por no encontrarse divulgada el espectro de difracción de rayos X y el TG/DTA reivindicado, se deriva de la naturaleza del estado sólido de esta molécula, propiedad alrededor de la cual se tiene amplio conocimiento, de acuerdo con la materia técnica que los documentos D1 y D2 ya enseñaban al momento de la presentación de la solicitud y que se discutió anteriormente.

En consecuencia, una persona del oficio normalmente versada en la materia, encontraría la sugerencia o motivación suficiente aplicando los métodos y conocimientos que se tiene sobre polimorfismo y formación de sales revelados en D2, a la sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo dada a conocer en D1, con el fin de determinar la existencia una forma cristalina adicional para este compuesto tal como la reivindicada.

En conclusión la sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo cristalina de la reivindicación 3 junto con sus dependientes y las composiciones que las contienen de la reivindicación 13 carecen de nivel inventivo.

La reivindicación 12 reclama un procedimiento para preparar una sal fosfato cristalina del compuesto 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3-

fluoro – benzonitrilo:

Características esenciales	D1	D3
Reiv. 12: Un procedimiento para preparar la sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo. comprendiendo dicho procedimiento los siguientes pasos: (a) proporcionar una solución de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo, ya sea en un disolvente polar prótico o aprótico; (b) adición de ácido fosfórico; (c) aislar la forma cristalina formada de una sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo.	Divulga el compuesto sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo (Pág. 9, línea 30 a Pág. 10, línea 20) Las sales farmacéuticamente aceptables se elaboran mediante la adición de ácidos orgánicos como el ácido fosfórico.	Procesos para la obtención de sales y sus formas cristalinas de un compuesto sólido. (Pág. 002). La formación de sales es una reacción ácido-base que implica una transferencia de protones o una reacción de neutralización y es por lo tanto controlada por factores que influyen en tales reacciones. (Pág. 003, col. izquierda).

Los documentos D1 y D2 se consideran el estado de la técnica más cercano a la invención definida en las reivindicaciones 12, porque D1 enseña el compuesto de sal de fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo (Pág. 9, líneas 30 a pág. 10, línea 20; pág. 10, línea 31 a pág. 11, línea 9; Reiv. 3 y 4), la anterioridad indica que es posible formar sales de adición ácido-base, donde dichas sales retienen la efectividad biológica para el tratamiento de la enfermedad de Cushing, entre las sales preferidas se encuentra la sal fosfato y divulga el proceso para obtener dicha sal que consiste esencialmente en la adición del ácido inorgánico preferido que en este caso es ácido fosfórico al fármaco que se encuentra en un solvente orgánico (Pág. 5).

La diferencia entre la invención, definida en D1 consiste en que la anterioridad no divulga la obtención de una forma cristalina de la sal fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo.

El efecto que logran los procesos reivindicados consiste en la obtención de formas cristalinas alternativas de la sal fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo desarrollar procedimientos para obtener sólidos cristalinos de la sal fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo divulgada en D1.

Por su parte, el documento D3 enseña que la formación de sales consiste en una reacción ácido-base con transferencia de un protón, o puede ser una reacción de neutralización y en teoría todo compuesto que exhiba características ácidas o básicas puede formar una sal (Pág. 003). La persona versada en la materia sabe que la forma salina modifica las propiedades físicas y farmacocinéticas de un activo, lo que es de gran utilidad para los formuladores (Pág.003, col. derecha). Por otra parte, la solicitud carece de información que permita establecer que las sales obtenidas mediante el proceso reclamado sean en alguna medida inesperadamente ventajosas con relación sal fosfato de 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo previamente divulgado en D1.

En consecuencia, la persona del oficio normalmente versada en la materia, frente a la necesidad de obtener sales cristalinas alternativas del compuesto 4 - (R) - (6,7- dihidro- 5H- pirrolo [1,2- c] imidazol- 5- il)-3- fluoro – benzonitrilo, utilizaría la

técnica de formación de sales por reacción ácido base, como lo sugieren la anterioridad D3, en el compuesto previamente divulgado en D1 para proporcionar así un proceso como el reclamado. En consecuencia la reivindicación 12 carece de nivel inventivo.

9. Conclusión

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

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO2011088188	1-2 3-10 y 12 a 13	Novedad Nivel inventivo
D2	H.G. Brittain. Segunda Edición, Informa Healthcare,NY, 2009	3-10 y 13	Nivel inventivo
D3	Pharmaceutical salts	12	Nivel inventivo

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 del día siguiente 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 de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

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.



JESUS FERNEL GARCÍA GARCÍA
Director de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-06-02

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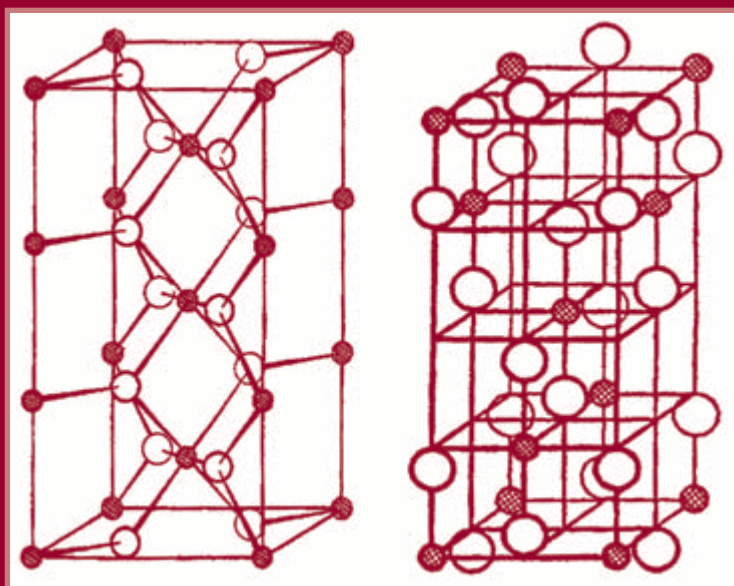
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DRUGS AND THE PHARMACEUTICAL SCIENCES

VOLUME 192

SECOND EDITION

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edited by

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Polymorphism in Pharmaceutical Solids

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Preface

It is now just about 10 years since the publication of the first edition of *Polymorphism in Pharmaceutical Solids*, which certainly received a positive reaction from workers in drug development. Since then, Joel Bernstein and Rolf Hilfiker have published their books on polymorphic phenomena, and the field has continued to expand both in the number of works published and also in the depth of their coverage. Some things have not changed, however, and the effects of crystal structure on the solid-state properties of a given system remains of paramount importance. As I stated in the preface to the first edition, the heat capacity, conductivity, volume, density, viscosity, surface tension, diffusivity, crystal hardness, crystal shape and color, refractive index, electrolytic conductivity, melting or sublimation properties, latent heat of fusion, heat of solution, solubility, dissolution rate, enthalpy of transitions, phase diagrams, stability, hygroscopicity, and rates of reactions are all strongly influenced by the nature of the crystal structure.

The content of the present edition of *Polymorphism in Pharmaceutical Solids* has expanded to reflect the larger scope of topics having interest to development scientists. The book is now divided into six main sections, the first dealing with thermodynamic and theoretical issues. Within this initial section, one will find updated chapters from the first edition, "Theory and Principles of Polymorphic Systems" and "Application of the Phase Rule to the Characterization of Polymorphic and Solvatomorphic Systems." Reflecting the growing trend in predictive science, a new chapter entitled "Computational Methodologies: Toward Crystal Structure and Polymorph Prediction" is now featured in this section.

The second section of the new edition features preparative methods for polymorphs and solvatomorphs, and the single chapter of the first edition has been split into two chapters entitled "Classical Methods of Preparation of Polymorphs and Alternative Solid Forms" and "Approaches to High-Throughput Physical Form Screening and Discovery." In the next section, one will find chapters relating to the structural properties of polymorphs and solvatomorphs, updating the chapters from the first edition, "Structural Aspects of Polymorphism" and "Structural Aspects of Solvatomorphic Systems." With greater interest developing about the advantageous properties of co-crystal systems, it was appropriate to expand the structural section to include a new chapter entitled "Pharmaceutical Co-crystals: A New Opportunity in Pharmaceutical Science for a Long-Known but Little-Studied Class of Compounds."

In the first edition, topics related to the characterization methods for polymorphs and solvatomorphs were covered in two chapters, but the growth in the field that has taken place in the past 10 years required far greater coverage of these

areas. Hence, the four chapters of the next section are entitled, "Thermoanalytical and Crystallographic Methods," "Vibrational Spectroscopy," "Solid-State Nuclear Magnetic Resonance Spectroscopy," and "Effects of Polymorphism and Solid-State Solvation on Solubility and Dissolution Rate." The chapter on solubility and dissolution is especially poignant, as it retains timeless and consequential contributions written by the late Professor David Grant for the analogous chapter in the first edition.

In the first edition, the phase interconversion of polymorphs and solvatomorphs was covered only from a processing viewpoint, but in the present edition, this important topic is now covered in two chapters, "Solid-State Phase Transformations" and "Effects of Pharmaceutical Processing on the Solid Form of Drug and Excipient Materials."

As in the first edition, the last section contains chapters that have been grouped together as special topics. The chapter "Structural Aspects of Molecular Dissymmetry" concerns structural variations that can arise from the existence of molecular dissymmetry, manifested primarily in marked differences in solid-state properties between solids composed of racemates relative to solids composed of separated enantiomers. Finally, as the amorphous state represents one polymorphic form potentially available to all compounds, this extremely important field is covered in great depth in a chapter entitled "Amorphous Solids."

Even though the scope of the second edition of *Polymorphism in Pharmaceutical Solids* is substantially increased relative to that of the first edition, there is simply no way that all developments in the field could have been covered in depth in a single volume. Beginning with a survey of papers published during 2004, I am writing annual reviews of polymorphism and solvatomorphism that attempt to summarize the state of the field during a given calendar year. Interested readers can easily find these in the literature.

In the present edition of *Polymorphism in Pharmaceutical Solids*, I have once again tried to bring together a single volume that contains a comprehensive view of the principles, practical concerns, and consequences of the existence of polymorphism and solvatomorphism. As with the previous edition, I hope that the new chapters will continue to suggest approaches that will stimulate work and encourage additional growth in this area of solid-state pharmaceuticals.

Harry G. Brittain

Contents

Preface . . . vii

Contributors . . . xi

PART I THERMODYNAMIC AND THEORETICAL ISSUES

1. Theory and Principles of Polymorphic Systems 1

Harry G. Brittain

2. Application of the Phase Rule to the Characterization of Polymorphic and Solvatomorphic Systems 24

Harry G. Brittain

3. Computational Methodologies: Toward Crystal Structure and Polymorph Prediction 52

Sarah (Sally) L. Price

PART II PREPARATIVE METHODS FOR POLYMORPHS AND SOLVATOMORPHS

4. Classical Methods of Preparation of Polymorphs and Alternative Solid Forms 76

Peter W. Cains

5. Approaches to High-Throughput Physical Form Screening and Discovery 139

Alastair J. Florence

PART III STRUCTURAL PROPERTIES OF POLYMORPHS AND SOLVATOMORPHS

6. Structural Aspects of Polymorphism 185

Harry G. Brittain, Stephen R. Byrn, and Eunhee Lee

7. Structural Aspects of Solvatomorphic Systems 233

Harry G. Brittain, Kenneth R. Morris, and Stephan X. M. Boerrigter

**8. Pharmaceutical Co-crystals: A New Opportunity
in Pharmaceutical Science for a Long-Known but Little-Studied
Class of Compounds 282**

Kapildev K. Arora and Michael J. Zaworotko

**PART IV CHARACTERIZATION METHODS FOR POLYMORPHS
AND SOLVATOMORPHS**

9. Thermoanalytical and Crystallographic Methods 318

Sisir Bhattacharya, Harry G. Brittain, and Raj Suryanarayanan

10. Vibrational Spectroscopy 347

Harry G. Brittain

11. Solid-State Nuclear Magnetic Resonance Spectroscopy 381

Patrick A. Tishmack

**12. Effects of Polymorphism and Solid-State Solvation
on Solubility and Dissolution Rate 436**

Harry G. Brittain, David J. R. Grant, and Paul B. Myrdal

PART V INTERCONVERSION OF POLYMORPHS AND SOLVATOMORPHS

13. Solid-State Phase Transformations 481

Harry G. Brittain

**14. Effects of Pharmaceutical Processing on the Solid Form
of Drug and Excipient Materials 510**

Peter L. D. Wildfong

**PART VI SPECIAL TOPICS RELATED TO POLYMORPHISM
AND SOLVATOMORPHISM**

15. Structural Aspects of Molecular Dissymmetry 560

Harry G. Brittain

16. Amorphous Solids 587

Lynne S. Taylor and Sheri L. Shamblin

Index . . . 631

Contributors

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Theory and Principles of Polymorphic Systems

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INTRODUCTION

With the discovery by Bragg that one could use the angular dependence of scattering of X rays from a crystalline solid to determine the structure of that solid (1), structural science has played a large role in the fields of chemistry and physics. Very early in the 19th century, it had become known that many compounds were capable of exhibiting the phenomenon of *dimorphism*, and could be crystallized into solids having different melting points and crystal habits. For example, the α - and β -forms of potassium ethyl sulfate were found to exhibit different solubilities and eutectic temperatures in their phase diagram (2). The existence of a thermally induced phase transition between the anhydrous and monohydrate forms of 5-nitrosalicylic acid was deduced from the temperature dependence of its solubility (3).

As the techniques of structure elucidation grew in their sophistication, the crystallographic basis of dimorphism became firmly established. The X-ray crystallographic technique enabled workers to determine the dimensions and angles associated with the fundamental building blocks of crystals, namely, the unit cell. At the same time it also became recognized that crystalline solids were not limited to one or two crystal forms, and that many solids were capable of being isolated in multitudes of crystalline forms.

During the very first series of studies using single-crystal X-ray crystallography to determine the structures of organic molecules, Robertson reported the structure of resorcinol (1,3-dihydroxybenzene) (4). This crystalline material corresponded to that ordinarily obtained at room temperature, and was later termed the α -form. Shortly thereafter, it was found that the α -form underwent a transformation into a denser crystalline modification (denoted as the β -form) when heated to about 74°C, and that the structure of this newer form was completely different (5). A summary of the unit cell parameters reported for both forms is provided in Table 1. The α -form features a relative open architecture that is maintained by a spiraling array of hydrogen bonding that ascends through the various planes of the crystal. The effect of the thermally induced phase transformation is to collapse the open arrangement of the α -form by a more compact and parallel arrangement of the molecules in the β -form. This structural change causes an increase in crystal density on passing from the α -form (1.278 g/cm³) to the β -form (1.327 g/cm³).

The term *polymorphism* has come to denote those crystal systems for which a substance can exist in structures characterized by different unit cells, but where each of the forms consists of exactly the same elemental composition. For a long time, the term *pseudopolymorphism* was used to denote other crystal variations where the crystal structure of the substance is defined by still other unit cells where these unit cells differ in their elemental composition through the inclusion of one or more

TABLE 1 Summary of the Unit Cell Parameters Associated with the two Polymorphs of Resorcinol (4,5)

Polymorphic form	α -form	β -form
Crystal class	Orthorhombic	Orthorhombic
Space group	Pna	Pna
Number of molecules per unit cell	$Z = 4$	$Z = 4$
Unit cell axis lengths	$a = 10.53 \text{ \AA}$	$a = 7.91 \text{ \AA}$
	$b = 9.53 \text{ \AA}$	$b = 12.57 \text{ \AA}$
	$c = 5.66 \text{ \AA}$	$c = 5.50 \text{ \AA}$
	$\alpha = 90^\circ$	$\alpha = 90^\circ$
Unit cell angles	$\beta = 90^\circ$	$\beta = 90^\circ$
	$\gamma = 90^\circ$	$\gamma = 90^\circ$

molecules of solvent, and more recently this term has become replaced by the term *solvatomorphism*. The crystallographic origins and consequences of polymorphism and solvatomorphism have been the focus of several monographs and reviews (6–12), recent annual reviews (13–15), and will be discussed in great detail in one of the later chapters in this book.

The existence of different crystal structures of the various polymorphs of a substance often causes these solids to exhibit a variety of different physical properties, many of which are listed in Table 2. Because of differences in the dimensions, shape, symmetry, capacity (number of molecules), and void volumes of their unit cells, the different polymorphs of a given substance have different physical properties arising from differences in molecular packing. Such properties include molecular volume, molar volume (i.e., molecular volume multiplied by Avogadro's number), density, refractive index along a given crystal axis, thermal conductivity, electrical conductivity, and hygroscopicity. Differences in melting points of the various polymorphs arise from differences of the cooperative interactions of the molecules in the solid state compared with the liquid state. Also observed are differences in spectroscopic properties, kinetic properties, and some surface properties. Differences in packing properties and in the energetics of the intermolecular interactions (i.e., thermodynamic properties) among polymorphs give rise to differences in mechanical properties.

These differences in physical properties among the crystal forms of a polymorphic system have become extremely interesting to pharmaceutical scientists because their manifestation can sometimes lead to observable differences that have implications for processing, formulation, and drug availability (16–21). For such situations, the regulatory concerns can often become critically important, and can determine the path of development for a given drug substance (22). Consequently, an entire field of characterization techniques for the evaluation of pharmaceutical solids has arisen, and its degree of sophistication continues to grow (23–29).

Once the phase space of a substance has been determined, and the scope of possible polymorphic or solvatomorphic forms is established, it becomes critical to determine the boundaries of stability for the different forms and how they might be interconverted. At the very least, one must determine which crystal form is the most stable state, because unless mitigating circumstances dictate otherwise, that form would be the one to be chosen for continued development.

TABLE 2 Physical Properties that Differ Among Crystal Forms of a Polymorphic System

Packing properties	Molar volume and density Refractive index Conductivity: electrical and thermal Hygroscopicity
Thermodynamic properties	Melting and sublimation temperatures Internal or structural energy Enthalpy Heat capacity Entropy Free Energy and Chemical Potential Thermodynamic Activity Vapor Pressure Solubility
Spectroscopic properties	Electronic state transitions Vibrational state transitions Nuclear spin state transitions
Kinetic properties	Dissolution rate Rates of solid-state reactions Stability
Surface properties	Surface free energy Interfacial tensions Crystal habit
Mechanical properties	Hardness Tensile strength Compatibility, tableting Handling, flow, and blending

THERMODYNAMICS OF POLYMORPHIC SYSTEMS

Before a discussion of the thermodynamics associated with systems capable of being crystallized in more than one form can be undertaken, a number of fundamental principles regarding the interactions that can take place in solid systems must be set out. In such discussions, one often uses thermodynamics to treat an *ideal* system, which may be taken as approximating some type of limiting condition. Real systems are often difficult to treat, but ideal systems are useful in that their boundaries can be used to deduce simple laws that are often sufficiently accurate to be practically useful. The following discussion has been distilled from several standard texts on thermodynamics and chemical equilibrium (30–34).

Systems are said to possess *energy*, and interacting systems exhibit simultaneous changes in observable properties that are accompanied by changes in energy. The energy of a system therefore implies the power to interact but also is a description of the results of interaction in terms of changed properties. To the thermodynamic scientist, these properties are usually descriptions in which the system exchanges energy with some standard system, although the properties can also be defined with respect to another member of the system. The changes of interest most pertinent to the present discussion involve changes in *potential* energy, or energy stored in a system as a result of how it came into that state. For example, the transformation of a substance from one physical phase to another involves the transfer of energy in the form of heat. Only changes or differences in energy are empirically measurable, because the

absolute energy of a system depends critically on the standard from which that energy might be measured.

Properties are identified as being *extensive* (dependent on the quantity of mass present) or *intensive* (independent of the amount of mass present), and the latter properties express a quality of the system rather than a quantity of something. For example, one may measure the amount of heat required to vaporize one gram of water, but dividing that amount of heat by that amount of water yields an intensive property that defines the substance called water. For every type of energy, there is a property whose difference between two systems determines whether energy will be exchanged and over which direction that energy will flow. Temperature, for example, is a measure of the intensity of heat in a system, and the value of this property with respect to the temperature of another system determines how much heat will flow and which system will be the donor of that heat.

It is concluded that the relative intensities of the various forms of energy in different systems determine whether interactions or exchanges of energy can take place between them. For a series of systems isolated from the universe, energy must flow until total equality in all forms of energy is attained. Consequently, to define a system one must be able to state the intensities of all significant forms of energy contained within that system. When this situation has been reached, the intensities of these energies existing within the system are grouped together in a class of properties denoted as *conditions*.

The conditions of a system can be controlled by manipulating the surroundings of the system. For example, unless a system is contained in a closed vessel, the pressure of ordinary chemical and physical transformations is fixed as the same as atmospheric pressure by virtue of the interaction of the system with open surroundings. As will be seen in the next chapter dealing with the Phase Rule, this stipulation results in reduced degrees of freedom and a limitation on the number of equilibria available to a system. In partly isolated systems, one may vary conditions by the deliberate introduction of one type of energy in order to observe the consequences of that addition in a linear manner.

In numerous experiments, it has been demonstrated that although energy can be converted from one form to another, it cannot be created or destroyed. This finding is the basis for the law of conservation of energy, which in turn, is the basis for the *first law of thermodynamics*: "The total energy of a system and its surroundings must remain constant, although it may be changed from one form to another." The energy of a system is seen to depend upon its pressure, volume, temperature, mass, and composition, with these five quantities being related by the *equation of state* for the system. Therefore, it is possible to assign a definite amount of energy to any given state of a system, which is determined only by the state itself and not by its previous history. If E_A represents the energy of the state A, and E_B is the energy of the state B, then the change in energy that accompanies the transformation of the system from A to B is independent of the path taken, and is given by:

$$\Delta E = E_B - E_A \quad (1)$$

The *internal energy* of the system, E , is a function of pressure, volume, and temperature, and includes all forms of energy other than those resulting from the position of the system in space. The actual magnitude of the internal energy is usually not known, but because thermodynamics is concerned primarily with changes in energy, the actual value of the internal energy is not significant.

When a system changes from one state to another, it may perform some type of external work, the magnitude of which is represented by w . If the work is done by the system, then w is positive, but if work is not done in the system, then w is negative. In addition, the system may absorb or evolve an amount of heat equal to q during the change, and q will always be positive if the system absorbs heat. According to the first law of thermodynamics, in order for the total energy of the system and surroundings to remain unchanged during the transition, it follows that the change in energy (ΔE) must be exactly equivalent to the heat q absorbed from the surroundings less the energy w lost to the surroundings in the form of external work:

$$\Delta E = q - w \quad (2)$$

For non-electrical thermodynamic processes that take place at constant pressure, the work term in equation (2) can be replaced by an expansion term, where P is the constant external pressure and ΔV is the increase of volume. If the amount of heat absorbed at constant pressure is represented as q_p , then with a slight rearrangement, one obtains:

$$q_p = \Delta E + P\Delta V \quad (3)$$

Because P and V are thermodynamic properties of the system, and because E depends only on the state of the system and not on its previous history, it follows that the quantity $(E + PV)$ is also dependent only the state of the system. This latter quantity is called the *enthalpy* (H) of the system:

$$H = E + PV \quad (4)$$

At constant pressure:

$$\Delta H = \Delta E + P\Delta V \quad (5)$$

Comparison of equations (3) and (5) indicates that the increase ΔH in the enthalpy of the system at constant pressure equals the heat absorbed under these conditions.

Thermochemistry deals with the changes in heat of a system that accompany chemical or physical transformations where reactants transition into products. Because different substances have different amounts of internal energy in the form of chemical energy, the total energy of the products of a reaction will differ from the total energy of the reactants. As a result, the reaction will be accompanied either by the liberation or consumption of heat. An *exothermic* reaction is one where heat is produced as a product of the reaction, while an *endothermic* reaction is one where heat is consumed as a reactant in the reaction.

If a system transformation is run under constant atmospheric pressure, then the amount of heat absorbed is identified as the *enthalpy of reaction*, and this quantity represents the difference in the enthalpies of the reaction products and the reactants. For example, the combustion of solid elemental graphite with gaseous elemental oxygen at 25°C (i.e., 298 K) to yield gaseous carbon dioxide is endothermic:



and the enthalpy of combustion equals -94.05 kcal/mol . It is generally postulated that elements are in their standard states (i.e., the stable forms at ambient conditions), and

therefore their respective enthalpies are set to zero. Because equation (6) depicts the formation of CO_2 from its constituent elements, the enthalpy of that reaction is termed the enthalpy of formation for CO_2 .

When the reaction under consideration involves a phase change, then the change in enthalpy is indicative of that reaction. For example, the *enthalpy of vaporization* of a substance is defined as the amount of heat required at constant pressure to vaporize one mole of that substance. One may determine the difference in enthalpy between two polymorphic forms of a compound by applying Hess's Law of constant heat summation, if the enthalpies of combustion for the two forms are known. The enthalpy of combustion for the reaction of diamond with oxygen equals -94.50 kcal/mol , and therefore the enthalpy of transition accompanying the conversion of diamond into graphite equals -0.45 kcal/mol .

Although the majority of chemical reactions that are exothermic in character will spontaneously go to completion under ordinary conditions, a number of reactions are known to require the absorption of heat and are still spontaneous. For example, the dissolution of most salts is endothermic, and yet their dissolution proceeds spontaneously as long as the equilibrium solubility is not exceeded. This simple observation demonstrates that enthalpy considerations are not sufficient to determine the spontaneity of a reaction, and that the definition of another parameter is required.

This additional state function is known as the *entropy* of the system, and has been given the symbol, S . One often encounters the explanation that entropy is a measure of disorder in a system, and that a spontaneous reaction is accompanied by an increase in entropy. Although apart from statistical mechanics it is difficult to define entropy, it is easier to define changes in entropy. Even though it is clear that spontaneous reactions are irreversible in nature, one can still break down the overall irreversible process into a series of infinitely small processes, each one of which is reversible in nature. The increase in entropy, dS , that accompanies an infinitesimal change equals the heat absorbed when the change is carried out in a reversible manner divided by the absolute temperature, T :

$$dS = \delta(q_{\text{REV}})/T \quad (7)$$

Because $\delta(q_{\text{REV}})$ has a definite value for a reversible, isothermal change, one can integrate equation (7) between the temperature limits of the initial and final states to obtain the entropy change for the process, ΔS .

It has proven expeditious to define other functions where the entropy is part of the determinant of spontaneity, one of these being:

$$A = E - TS \quad (8)$$

where the *work function*, A , equals the maximum amount of work obtainable when a system undergoes a change under reversible conditions. More useful to pharmaceuticals and issues of polymorphism is the *free energy*:

$$G = H - TS \quad (9)$$

It is not difficult to show that combination of equations (4), (8), and (9) yields the relation:

$$G = A + PV \quad (10)$$

When a system undergoes a transformation, that change takes place at constant temperature and then the free energy of the transition is given by:

$$\Delta G = \Delta H - T\Delta S \quad (11)$$

If the transformation is also conducted at constant pressure, then equation (5) can be substituted into equation (11) to yield:

$$\Delta G = \Delta E + P\Delta V - T\Delta S \quad (12)$$

Figure 1 shows the energy relationships for a hypothetical system where the enthalpy and the entropy of the system increase with increasing absolute temperature. According to the Third Law of Thermodynamics, the entropy of a perfect, pure crystalline solid is zero at absolute zero, enabling one to set the zero-point entropy of the system. The $(T \cdot S)$ product is seen to increase more rapidly with increasing temperature than does the enthalpy, and therefore the free energy will decrease with increasing temperature. This decrease also corresponds to the fact that the slope $(\delta G/\delta T)$, of the plot of G against T is negative according to the equation:

$$(\delta G/\delta T)_P = -S \quad (13)$$

Each polymorphic form of a substance will yield an energy diagram similar to that of Figure 1, and because each polymorph has its own distinctive crystal lattice, it is to be anticipated that the values of enthalpy, entropy, and free energy at a given temperature would be different among the various polymorphs. In discussions of the relative stability of polymorphs and the driving force for polymorphic transformations at constant temperature and pressure, the difference in free energy between the forms is the decisive factor, with the form exhibiting the lowest free energy being the most stable.

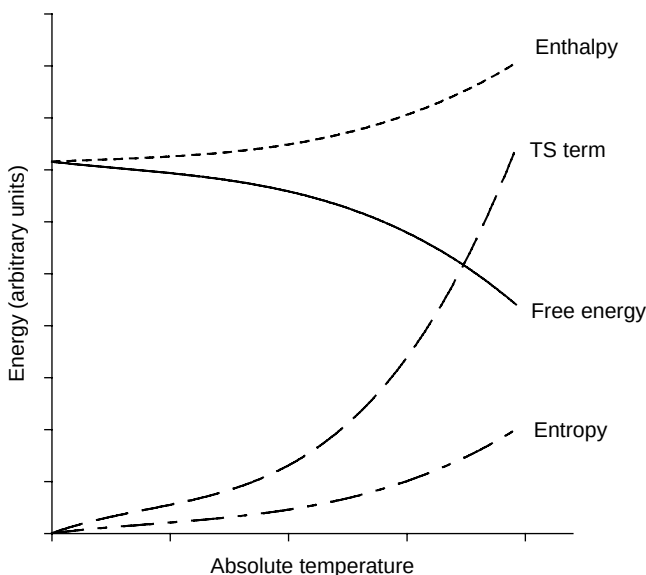


FIGURE 1 Temperature dependence of various thermodynamic functions.

Figure 2 shows the temperature dependence of the enthalpy and free energy for two different polymorphs, identified as Form-1 and Form-2. Because the temperature dependence of the free energies of the forms differs, at some temperature the respective curves cross and the two forms become isoenergetic. If the intersection point is determined under ambient conditions, the temperature is referred to as the *ordinary transition point* (T_{TR}). The fact that the free energies of the two polymorphs are equal implies that Form-1 and Form-2 are in equilibrium at that temperature.

Figure 2 shows Form-2 having an enthalpy that is higher than that of Form-1, so that the difference in enthalpies has the order $H_2 > H_1$ (i.e., ΔH is positive and the transition is endothermic in nature). Because at the transition temperature the difference in free energies of the forms equals zero, it follows that the difference in entropies will have the order $S_2 > S_1$. Equating the free energies of the two forms leads to the useful relation:

$$\Delta H_{\text{TR}} = T_{\text{TR}} \Delta S_{\text{TR}} \quad (14)$$

where $\Delta H_{\text{TR}} = H_2 - H_1$ and $\Delta S_{\text{TR}} = S_2 - S_1$ at the transition point. Through the use of differential scanning calorimetry, one may measure the enthalpy of the transition, and therefore calculate the entropy of the transition as long as the transition point is accurately determined. For this measurement to be accurate, the rate of temperature increase must be slow enough to allow Form-1 to completely transform into Form-2 over a span of a few degrees so as to achieve reversible conditions as closely as possible.

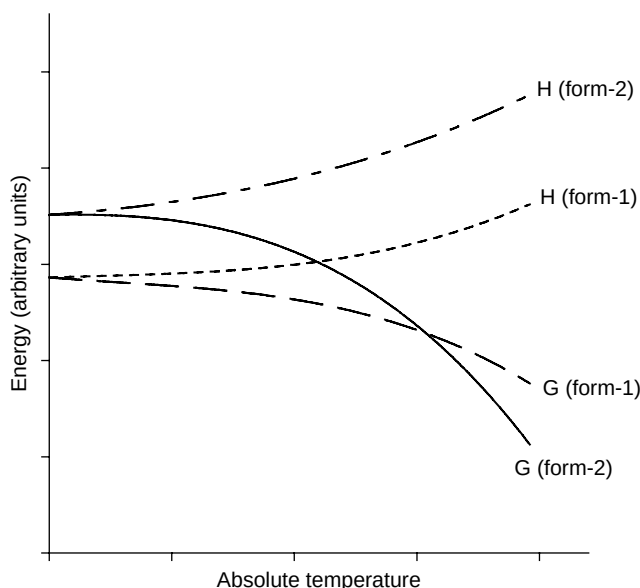


FIGURE 2 Temperature dependence of the enthalpy (H) and free energy (G) for two polymorphic crystal forms.

Figure 2 also shows that below the transition temperature, Form-1 has the lower free energy (i.e., $G_2 > G_1$), and therefore is more stable within that temperature range. On the other hand, above the transition temperature, Form-2 now has the lower free energy and is therefore more stable (i.e., $G_2 < G_1$). One concludes that under defined conditions of temperature and pressure, only one polymorph can be stable, and that all other polymorphs must be unstable. It is important to note that thermodynamics speaks to the relative energies and stabilities of polymorphs, but as will be discussed shortly, has nothing to say regarding the rates of these phase transformations. Diamond is thermodynamically unstable with respect to graphite, but the kinetics associated with that phase change are so infinitesimally slow that one refers to diamond as a metastable phase.

Equation (10) applies to the ideal systems discussed thus far, and differentiating both sides of the equation yields:

$$dG = dA + PdV + VdP \quad (15)$$

But dA is the maximum work of the expansion and must therefore be numerically equal to $-PdV$, so equation (15) reduces to:

$$dG = VdP \quad (16)$$

In order to integrate equation (16), one requires an equation of state defining V in terms of P . For one mole of an ideal gas, the law is simply:

$$V = nRT/P \quad (17)$$

where n is the number of moles of gas and R is the gas constant. Substitution of equation (17) into (16) and integrating yields:

$$\Delta G = G_2 - G_1 = RT \ln(P_2/P_1) \quad (18)$$

Equation (18) applies to any change of state or isothermal transfer of a substance from a region in which it has a vapor pressure P_1 to another region where its vapor pressure is P_2 .

Practically all substances do not behave as ideal gases, so the concept of *fugacity* has been developed for real materials. One way to understand fugacity is to see it as the tendency manifested by a substance to leave the phase where it exists and pass into every other phase to which it has access. Because for an ideal gas, the partial pressure equals the fugacity, it is clear that equation (18) is a limiting instance of a more general equation. One may therefore substitute the fugacities (f_i) of the substance in each phase for the partial pressures to obtain:

$$\Delta G = RT \ln(f_2/f_1) \quad (19)$$

As is typically the case for thermodynamics, it is useful to define the fugacity of a substance with respect to the fugacity of some standard state, which can be taken as f^0 . The ratio of the fugacity of a substance to that of the substance in the standard state has been termed the *activity* (a):

$$a = f/f^0 \quad (20)$$

so that:

$$G - G^0 = RT \ln(a) \quad (21)$$

As long as the reference state used to define G^0 and f^0 is the same, the quantities may be used interchangeably, so it follows that:

$$\Delta G = G_2 - G_1 = RT \ln(a_2/a_1) \quad (22)$$

The tendency of any one substance to be transferred from one phase or state to another at the same temperature depends on the properties of that substance, on the states involved, and on the temperature in question. However, neither the fugacity nor the activity are dependent upon the path or mechanism of transfer. At any specified temperature, these quantities can be considered to be governed by a property of the substance in the separate states. For many purposes, they are satisfactorily measured by the free energy of transfer or difference in molal free energy between the states. The molal free energy in any individual phase therefore comprises a measure of the escaping tendency of the substance in that phase relative to a standard state.

For dilute solutions, the activity is approximately proportional to the solubility, s , in any given solvent. One can then write an expression approximating the free energy difference between two polymorphic forms in terms of their respective equilibrium solubilities, or:

$$\Delta G \sim RT \ln(s_2/s_1) \quad (23)$$

If the dissolution of the polymorphic forms is conducted under transport-controlled sink conditions and under conditions of constant hydrodynamic flow, then the dissolution rate per unit surface area, J , is proportional to the solubility according to the Noyes–Whitney equation. One then can write another approximation for the free energy difference of two polymorphs as:

$$\Delta G \sim RT \ln(J_2/J_1) \quad (24)$$

Because the most stable polymorph under defined conditions of temperature and pressure has the lowest free energy content, it must therefore have the lowest values of fugacity, vapor pressure, thermodynamic activity, and solubility, and dissolution rate per unit surface area in any solvent.

ENANTIOTROPY AND MONOTROPY

In the preceding section, the general thermodynamics associated with systems was discussed, and methods were developed for determining the degree of spontaneity of a potential change were outlined. Implicit to the discussion was the understanding that the thermodynamic relations applied to systems undergoing reversible changes. In real crystals, however, a multitude of complicating factors introduce a degree of irreproducibility into the thermodynamic relations, thus limiting the scope of exact calculations in the understanding of real systems (35). Consequently, a number of more empirical concepts and rules have been developed to deal with actual polymorphic systems.

As described above, it is possible for polymorphic crystal forms to exhibit an ordinary transition point where one form can reversibly transform into another. Obviously, the temperature of this transition point must be less than the melting point of either polymorph or else the system would pass into the liquid state and no phase transition could be detected. For such systems, one polymorph will be

characterized by a definite range of conditions under which it will be the most stable phase, and the other form will be characterized by a different range of conditions under which it is the most stable phase. Polymorphic systems of this type are said to exhibit *enantiotropy*, and the two polymorphs are said to be enantiotropes of each other.

The free energy relationships between two enantiotropic polymorphs is illustrated in Figure 3, where now the enthalpy and free energy curves of the liquid (molten) state have been added. In the figure, Form-1 is shown as having a lower free energy content over the lower temperature range, while Form-2 is shown to have a lower free energy over a higher temperature range. For such an enantiotropic system, a reversible transition between forms can be observed at the transition temperature where the free energy curves cross and the forms are isoenergetic. The existence of enantiotropism in the system is indicated by the fact that the free energy curve for the liquid phase intersects the free energy curves for both polymorphs at a temperature that is higher than the temperature of the transition point.

Other systems exist where only one polymorph is stable at all temperatures below the melting point. As a result, all other polymorphs have no region of stability anywhere on a pressure–temperature diagram, and must be unstable with respect to the stable form. Polymorphic systems of this type are said to exhibit *monotropy*, and the two polymorphs are said to be monotropes of each other. The polymorph having the lowest free energy curve and solubility at any given temperature will necessarily be the most thermodynamically stable form.

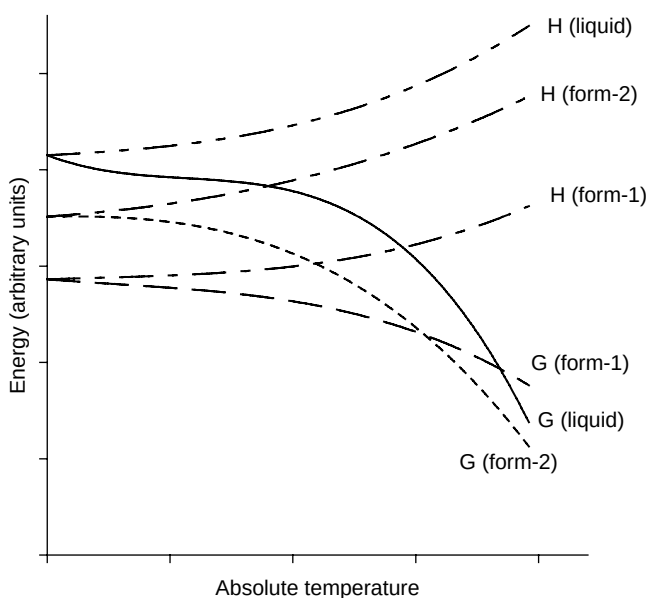


FIGURE 3 Temperature dependence of the enthalpy (H) and free energy (G) for two enantiotropic polymorphic crystal forms and their liquid (molten) state.

The free energy relationships between two monotropic polymorphs is illustrated in Figure 4, including the enthalpy and free energy curves of the liquid (molten) state. In this figure, Form-1 is shown as always having a lower free energy over the entire accessible temperature range, and Form-2 has a higher free energy over the same temperature range. The free energy curve of the liquid state crosses the free energy curves of both polymorphs at temperatures less than that of the transition point, and hence, there can be no temperature at which the two polymorphs would exhibit a reversible phase transition. For a monotropic system the free energy curves do not cross, so no reversible transition can be observed below the melting point.

The isolation of polymorphs that form an enantiotropic system requires careful control over the isolation conditions. For enantiotropic materials, one can always identify a set of conditions where one polymorph or the other is the most thermodynamically stable form, and if crystallization is performed under those conditions one can usually obtain the desired form. Owing to its superior stability under all accessible temperature and pressure conditions, the isolation of the most stable polymorph in a monotropic system can usually be achieved without great difficulty. Isolation of the less stable form, however, requires a kinetic trapping of the system under conditions where the polymorph is characterized as being metastable at best.

A number of rules have been developed that serve to aid in the elucidation of the relative order of stability of polymorphs, and to facilitate determination of the existence of enantiotropism or monotropism in a polymorphic system (36–41). Although a summary of these many thermodynamic rules is provided in Table 3, it should be noted that the most useful and generally applicable rules are the Heat of Fusion rule and the Heat of Transition rule.

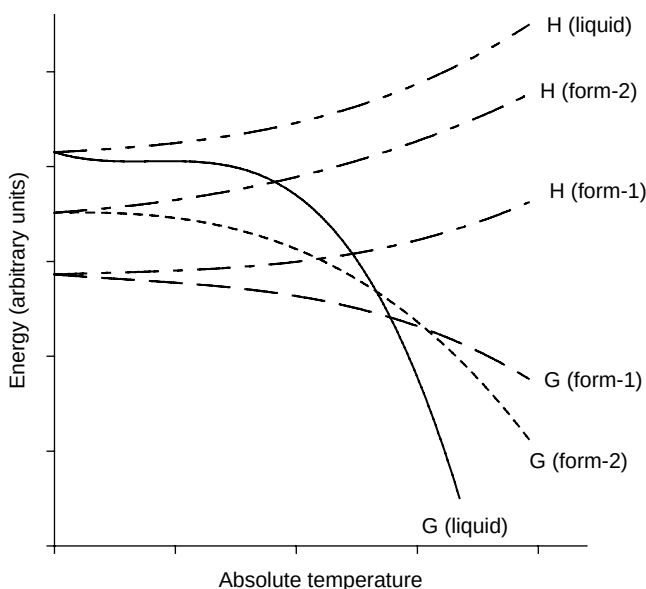


FIGURE 4 Temperature dependence of the enthalpy (H) and free energy (G) for two monotropic polymorphic crystal forms and their liquid (molten) state.

TABLE 3 Empirically Based Rules for Assigning the Nature of Phase Relationships in Polymorphic Systems^a

Rule	Enantiotropic system	Monotropic system
Fundamental definition	Form-1 is the most stable polymorphic form at temperatures below the transition point, while Form-2 is the most stable polymorphic form at temperatures above the transition point	Form-1 is the stable polymorph at all temperatures below that of the melting point
Heat of fusion	The enthalpy of fusion of Form-1 is less than the enthalpy of fusion of Form-2	The enthalpy of fusion of Form-1 is more than the enthalpy of fusion of Form-2
Heat of transition	The phase transition of Form-2 to Form-1 is endothermic	The phase transition of Form-2 to Form-1 is exothermic
Entropy of fusion	The melting points of both Form-1 and Form-2 is less than the temperature of the transition point	The melting point of the most stable polymorph is higher than the temperature of the transition point
Phase transformation reversibility	The phase transformation at the transition point is reversible	The phase transformation of Form-2 into Form-1 is irreversible
Solubility	Form-1 is the most soluble polymorphic form at temperatures below the transition point, while Form-2 is the most soluble polymorphic form at temperatures above the transition point	Form-1 is the most soluble polymorph at all temperatures below that of the melting point
Density	The density of Form-1 is less than the density of Form-2	The density of Form-1 is more than the density of Form-2

^aIn the table, the convention where Form-1 has a higher melting point relative to that of Form-2 has been used.

The *Heat of Transition Rule* states that, if the transition between polymorphic forms is endothermic in nature, then the two forms are related by enantiotropy. Conversely, if the phase transformation is exothermic, then the two polymorphic forms are related by monotropy. Burger and Ramberger based this rule on the fact that because ΔH and ΔS are ordinarily positive for a spontaneous reaction, the enthalpy curves will not intersect and the free energy curves can intersect only once (36). In favorable circumstances, the sign and magnitude of the enthalpy change can be determined using differential scanning calorimetry (DSC).

When the enthalpy of transition cannot be measured by DSC, the *Heat of Fusion Rule* should be applied next. This rule states that if the higher melting polymorph has the lower enthalpy of fusion, then the two forms are enantiotropes. Conversely, if the higher melting polymorph has the higher enthalpy of fusion, then the two forms are monotropes. Burger and Ramberger have pointed out that the difference between the enthalpies of fusion of a polymorphic pair does not

exactly equal the enthalpy of transition, and have provided an improvement to the difference in enthalpies of fusion based on the difference in heat capacities of the two forms (36).

The *Entropy of Fusion Rule* states that if the polymorph having the higher melting point has a lower entropy of fusion, then the two forms are related by enantiotropy (38). One may calculate the entropy of fusion (ΔS_F) from the enthalpy of fusion (ΔH_F) measured for a reversible phase transformation taking place as the transition point (T_{TR}) by applying equation (7) to the melting process:

$$\Delta S_F = \Delta H_F / T_{TR} \quad (25)$$

Equation (25) cannot properly be applied to the calculation of ΔS_F for a monotropic system, because monotropy is fundamentally irreversible in nature. However, if the form having the higher melting point had a higher entropy of fusion, then the two polymorphic forms would be related by monotropy. Yu has developed a method for inferring thermodynamic stability relationships from melting data, calculating the free energy difference and the temperature slope of ΔG between two polymorphs (40).

The *Solubility Rule* proceeds directly from equation (23), which relates the free energy difference between two polymorphic forms to the solubility ratio of these. Because the solubility of a solid phase is directly determined by its free energy, it follows that if one polymorph is the most soluble form at temperatures below the transition point, and the other form is the most soluble form at temperatures above the transition point, then the two polymorphs must be enantiotropes. Conversely, if one polymorph is the most soluble form at all temperatures below that of the melting point of either form, then the two polymorphs must be monotropes.

The *Density Rule* is probably the least reliable of the Burger and Ramberger rules (36), and states that the polymorph having the highest true density will be the more stable crystal form. The basis for this rule is the assumption that the most stable polymorphic form would have the most efficient crystal packing, and hence, the greatest amount of lattice energy. A number of exceptions have been observed to the density rule, among them the instance of resorcinol that was discussed earlier (4,5).

As an example of how the thermodynamic rules are used, consider the enantiotropically related system constituted by the two non-solvated polymorphs of auranofin (i.e., 5-triethylphosphine-gold-2,3,4,6-tetra-*o*-acetyl-1-thio—*D*-glucopyranoside) (42). Form-A was found to melt at 112°C, with the enthalpy of fusion being determined as 9.04 kcal/mol. Form-B was found to melt at 116°C, and its enthalpy of fusion was found to be 5.84 kcal/mol. According to the heat of fusion rule, because the higher melting form has the lower heat of fusion, the two polymorphs must be enantiotropically related and the difference in fusion enthalpies was calculated to be 3.20 kcal/mol. Using solution calorimetry, the enthalpy of solution for Form-A in 95% ethanol was found to be 12.42 kcal/mol, whereas the enthalpy of solution for Form-B in the same solvent system was found to be 9.52 kcal/mol. In dimethylformamide, the enthalpy of solution of Form-A was found to be 5.57 kcal/mol, whereas the enthalpy of solution for Form-B was found to be 2.72 kcal/mol. Thus, the enthalpy difference between the two forms was found to be 2.90 kcal/mol in 95% ethanol and 2.85 kcal/mol in dimethylformamide. The equilibrium solubility of Form-A in 25% aqueous polyethylene glycol 200 was found to be 0.65 mg/mL, whereas the equilibrium solubility of Form-B in the same

solvent system was found to be 1.30 mg/mL. The enantiotropic nature of the auranofin system is demonstrated that at room temperature Form-A is the most stable, whereas at elevated temperatures Form-B is the most stable.

NUCLEATION AND CRYSTAL GROWTH

Among the various methods one may use to prepare different polymorphs are crystallization from liquid solutions of various pure and mixed solvents, crystallization from the molten liquid state, suspension of less-soluble substances in pure and mixed solvents, thermal treatment of crystallized substances, exposure of solids to various relative humidities, sublimation, and crystallization from supercritical fluids. Typically, the first experiments performed in a preformulation study entail the attempted crystallization of polymorphic solids from solutions using various solvents and various temperature regimes (43,44). In these experiments, initially supersaturated solutions are prepared, and then the supersaturation is discharged by either slow or rapid cooling of the solution, evaporation of the solvent, addition of an anti-solvent to induce precipitation, chemical reaction between two or more soluble species, or variation of pH to produce a less soluble acid or base.

The crystallization process begins with the aggregation of molecules into clusters, and the continued addition of molecules to the clusters eventually results in the formation of tiny crystallites (45–48). The *critical nucleus* is obtained when the clusters of molecules have the smallest size capable of independent existence in the supersaturated phase, with these particles existing in a reversible state where they have an equal probability of growing into larger crystals or dissolving back in the solution phase. These critical nuclei are too small to be observed directly, and their structure is not known. Mullin has stated that the structure of a critical nucleus could be anything from a diffuse agglomeration of molecules to a miniature crystal that is perfect in form (46).

The typical theory of nucleation is based on the theory developed for the condensation of vapor into a liquid that has been extended to crystallization from the molten state. The formation of a liquid droplet or a solid particle in a homogeneous fluid requires the performance of work to obtain the end product. The total amount of work required to form a crystal nucleus, W_{TOT} , equals the amount of work required to form the surface (W_s) plus the amount of work needed to form the bulk of the particle (W_v):

$$W_{TOT} = W_s + W_v \quad (26)$$

Using the geometrical equations known for spherical particles, it can be shown that total work of equation (26) equals:

$$W_{TOT} = \frac{4}{3}\pi\sigma r^2 \quad (27)$$

where r is the radius of the particle and σ is the surface energy of the particle per unit area.

The increase in vapor pressure resulting from the decrease in size of a droplet can be estimated from the Gibbs–Thompson equation:

$$\ln(P_R/P^*) = \frac{2M\sigma}{RT\rho r} \quad (28)$$

where P_R is the vapor pressure over a droplet of radius r , P^* is the equilibrium vapor pressure of the liquid, M is the molecular weight, ρ is the liquid density, T is the absolute temperature, and R is the universal gas constant.

For solid particles, the pressure terms of equation (28) can be replaced by concentration equivalents. However, the ratio of the concentration of a particle having a radius equal to r (C_R) to the equilibrium solubility (C^*) is a measure of the degree of supersaturation (D) in the system:

$$D = C_R / C^* \quad (29)$$

In that case, equation (28) can be written as:

$$\ln(D) = \frac{2M\sigma}{RT\rho r} \quad (30)$$

or more usefully as:

$$r = \frac{2M\sigma}{RT\rho \ln(D)} \quad (31)$$

Substitution of equation (31) into equation (27) yields the important relationship:

$$W_{\text{TOT}} = \frac{16\pi M^2 \sigma^3}{3[RT\rho \ln(D)]^2} \quad (32)$$

According to equation (32), a saturated solution cannot spontaneously nucleate, because $\ln(D) = 0$, and the work required for nucleation would be infinite. The equation also indicates that any supersaturated solution can undergo spontaneously nucleation as long as a sufficient amount of energy is supplied to the system. Nucleation may be primary (not requiring pre-existing crystals of the crystallizing substance) or secondary (nucleation is induced by pre-existing crystals of the substance). Primary nucleation may be homogeneous (the nuclei of the crystallizing substance arise spontaneously in the medium), or heterogeneous (the nuclei comprise foreign solid matter, such as particulate contaminants, dust particles, or the walls of the container).

The change in free energy associated with the process of nucleation (ΔG_{TOT}) from a homogeneous solution is given by:

$$\Delta G_{\text{TOT}} = \Delta G_s + \Delta G_v \quad (33)$$

where ΔG_s is the excess free energy between the surface of the particle and the bulk of the particle, whereas ΔG_v is the excess free energy between a very large particle having $r = \infty$ and the solution in solution. ΔG_s is a positive quantity known as the surface excess free energy, ΔG_v is a negative quantity known as the volume excess free energy, and both quantities are functions of the radius of the particle.

Because the ΔG_s and ΔG_v terms contribute opposing contributions to the total free energy change as the radius of the nucleus increases, the free energy passes through a maximum (ΔG_{CRIT}) at a particle radius equal to the radius (r_{CRIT}) of the critical nucleus. This behavior has been illustrated in Figure 5, and the free energy of the critical nucleus can be calculated as:

$$\Delta G_{\text{CRIT}} = 4\pi\sigma r_{\text{CRIT}}^{2/3} \quad (34)$$

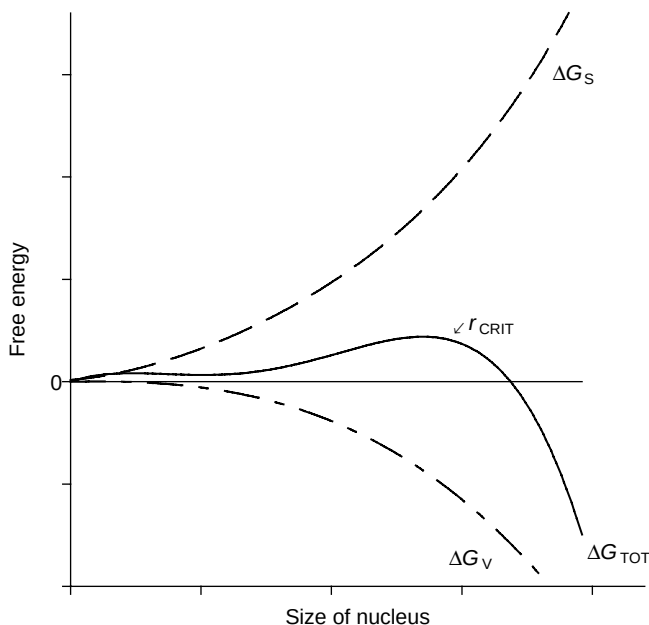


FIGURE 5 Dependence of the surface excess free energy (ΔG_s) and the volume excess free energy (ΔG_v), illustrating the existence of a critical nucleus having a diameter equal to r_{CRIT}

Spontaneous nucleation is therefore seen to be governed by the algebraic opposition of a volume term that favors the accretion of additional molecules from the supersaturated medium and a surface term that favors the dissolution of the molecular aggregates that would otherwise form nuclei (45–48). The molecules of the crystallizing substance tend to aggregate in the supersaturated medium under the influence of the volume term that tends to reduce the Gibbs free energy of the system.

For a substance capable of existing in two or more polymorphic forms, each polymorph would have its own characteristic ΔG_{TOT} as determined by its particular ΔG_v and ΔG_s properties, as well as its own characteristic value of r_{CRIT} and ΔG_{CRIT} . Within the limits imposed by their characteristic curves, the aggregates or embryos of the various polymorphs would compete for molecules in their relative attempts to grow into crystallites so that their free energies could decrease. Depending on the characteristics of the free energy curves and the properties of the solution, it is to be anticipated that the aggregate for which the critical activation energy is the lowest will form the first nucleus, and continued deposition of molecules on that nucleus would eventually yield the crystallization of that particular polymorph.

In order to form crystals from the nuclei, molecules of the crystallizing substance attach onto the nuclei until the crystallization medium is no longer supersaturated and the equilibrium solubility of the substance is reached. The small but definite increase of solubility with decreasing particle size for microscopic solid particles predicted by equation (30) does, however, account for the increase in the average particle size when crystals of various sizes are allowed to age in constant

with a saturated solution. This phenomenon, known as *Ostwald ripening*, occurs because a smaller particle, having a higher solubility, will dissolve in the unsaturated solution that is saturated with respect to a larger particle of lower solubility. Conversely, a larger particle having a lower solubility will grow in the supersaturated solution that is actually saturated with respect to a smaller particle of higher solubility. Larger particles will therefore grow at the expense of smaller particles and the concentration of the "saturated" solution will decrease asymptotically.

Because the most easily nucleated polymorph is the one whose critical nuclei are the easiest to form (i.e., they have the most favorable free energy characteristics), one frequently finds that a phase transformation accompanies an Ostwald ripening process. As the science of crystallization developed during the 19th century and workers learned that compounds could be obtained in more than one solid state form, a number of cases were documented where a metastable form of a compound crystallized first and subsequently transformed into a more stable form. These findings led Ostwald to propose his *Law of Stages*, which stated that a supersaturated state does not spontaneously transform directly into that phase that is the most stable of the possible states, but instead, transforms into the phase that is next more stable than itself (49). In thermodynamic terms, the crystal form most likely to be initially crystallized would be the one whose free energy was closest to the free energy of the dissolved state.

Stranski and Totomanov provided an explanation for this phenomenon developed in terms of the kinetics of transformation (50). In this model, the determining factors are the relative rates of nucleation and crystal growth for the stable and metastable forms. The differences between the various parameters may be such that at the working temperature, the rate of nucleation is greater for the metastable product. This situation would cause the metastable phase to preferentially nucleate. In another scenario, the rates of nucleation may be more or less the same for the two forms, but if the metastable phase has a higher rate of growth, then this form would eventually predominate in the isolated product.

One may also encounter the situation where nucleation of the stable form may have taken place to a small extent along with the nucleation and growth of the metastable form. Because the stable form would necessarily have a lower solubility, a process of solution-mediated phase transformation is set up where over time the metastable phase transforms into the stable phase. For the situation where no nuclei of the stable phase were formed, then for a phase transformation to occur nuclei of the stable form would have to be created. The most likely situation for formation of these nuclei would be that they would not be generated within the bulk solution, but would instead be formed on the surfaces of the metastable crystals.

One typically identifies those situations where two crystal forms are obtained in an isolated product as *concomitant crystallization*, the products as *concomitant polymorphs*, and the thermodynamics and kinetics of the phenomenon have been discussed in detail (51). For example, two orthorhombic polymorphs of 1-deoxy- α -D-tagatose have been crystallized from a mixed methanol/ethyl acetate solvent system (52). Form-II was obtained as hexagonal plates after allowing the mother liquor to stand for 16 hours, while Form-I crystallized as needles from the same solution after 72 hours. The two polymorphic forms were collected in approximately equal amounts from the crystallizing solution, and the single-crystal structures of these forms indicated that the polymorphism was derived from differences in hydrogen-bonding patterns.

Probably the best way to avoid the generation of concomitant polymorphs is through the introduction of seed crystals into a slightly supersaturated solution. As long as the seed crystals do not undergo a solution-mediated phase transformation of their own, the supersaturation in the crystallization medium is then discharged through growth onto the seeds. The implementation of this process requires knowledge of the temperature dependence of the equilibrium solubility and the spontaneous nucleation curve, and seeding is conducted in the concentration region between these boundaries (i.e., the *metastable zone*). The techniques for seeding a desirable polymorphic form during crystallization have been discussed in detail (46–48,53).

Another possibility where one may obtain stable or metastable crystal forms is where nucleation and subsequent crystal growth takes place on foreign surfaces, a process known as *epitaxial crystallization*. When surfaces, foreign nuclei, or appropriate seed crystals are present in a solution, these may favor the formation of a different form when the surfaces of the epitaxial agents present interfaces for which the structure closely matches the structure that would exist in a crystal of the new form (54,55). For example, Form-III of anthranilic acid was obtained by crystallization on glass coated with trimethoxysilane, Form-II was obtained when the crystallization took place on glass coated with chloro-triisobutylsilane, and a mixture of Form-II and Form-III was obtained if the crystallization was conducted on uncoated glass (56). It was concluded that the availability of hydrogen-bonding functionality at the nucleation surface played an important role in the polymorphic selectivity.

The various phenomena discussed in the preceding paragraphs amply demonstrate that one must exercise a considerable degree of control over the nucleation process and succeeding crystal growth processes if one seeks to obtain phase-pure materials. The crystal nucleation process has been discussed in detail (57), as has been the significance of controlling crystallization mechanisms and kinetics (58). These phenomena have also been critically examined with a view toward polymorph selection, and the crystal engineering that would be desirable in obtaining bulk drug substances having appropriate structures (59).

DISAPPEARING AND REAPPEARING POLYMORPHS

Over the years, stories have accumulated that summarize the failed attempts to reproduce previously reported crystallization products. When observed, the phenomenon is simultaneously frustrating and infuriating because modern physical science is often judged on the basis of its reproducibility. Dunitz and Bernstein addressed systems where a particular crystal form could not be obtained despite heroic efforts, concluding that control over nucleation and crystal growth processes was required (60). Crystallographers and preformulation scientists recognize the role of seeding in initiating nucleation, and many consider the disappearance of a metastable form to be a local and temporary phenomenon. These authors concluded that, "once a particular polymorph has been obtained, it is always possible to obtain it again; it is only a matter of finding the right experimental conditions."

In a subsequent work, Bernstein and Henck returned to the subject of transient polymorphs, examining this time certain systems where polymorphs had become elusive after a new polymorphic form was isolated (61). Through studies of the benzocaine:picric acid, *p*'-methylchalcone, benzophenone, and N-(N'-methylanilino) phthalimide systems, hot-stage microscopy was demonstrated to be of great use in the design of further experimentation that would yield the elusive polymorph.

The monoclinic polymorph of paracetamol (i.e., acetaminophen) is a commercially important form of the drug substance, despite its unsuitability in direct compression formulations. An orthorhombic crystal form of the drug substance had been characterized (62), but this polymorph could not be reproduced by several groups even though they followed the reported method of isolation. Eventually the experimental difficulties were overcome and a scalable process was reported that yielded the orthorhombic form in sufficient quantities for its characterization and formulation (63). The key to the successful process came through the use of appropriate seeding techniques to suppress the nucleation of the unwanted monoclinic polymorph, and rapid isolation of the product at low temperatures to suppress any phase transformation.

One example where a metastable polymorph was replaced by a more stable crystal form is that of meso-xylitol. In the early 1940s, two polymorphs of xylitol were described, with one being a metastable, hygroscopic, monoclinic form, melting at 61–61.5°C (64) and the other a stable orthorhombic form melting at 93–94.5°C (65). After a sample of the orthorhombic form was introduced into a laboratory in which the monoclinic polymorph had been prepared, the metastable spontaneously transformed into the stable form on exposure to the ambient environment. As part of a structural study of the orthorhombic polymorph, it was noted that “Attempts to obtain the lower melting monoclinic form from alcoholic solutions either at room temperature or close to 0°C have hitherto been unsuccessful. We invariably grow the orthorhombic crystals. It is interesting to note that although xylitol was first prepared as a syrup in 1891 there was no report of crystallization until 50 years later, when it was the metastable hygroscopic form that was prepared first. Having now obtained the stable form, it is difficult to recover the metastable crystals” (66).

The existence of two new polymorphic forms of 3-aminobenzenesulfonic acid (orthorhombic needles and monoclinic plates) have been reported, one of which had not been previously known (67). Form-I was suggested to be a disappearing polymorph, and the serendipitous discovery of Form-III resulted from the attempt to use tailor-made additives in order to re-obtain Form I. Although the attempt to prepare Form-I did not succeed, the study demonstrated the necessity to explore the polymorphic phase space as fully as possible even in simple systems.

A metastable form of benzamide was identified by Liebig and Woehler in 1832, but the structure of this unstable modification was determined much later (68). During reproductions of the historical experiments, rapid phase transformation was observed of the metastable form to the stable form, with the phase transformation being complete within 800 seconds. Ultimately, a high-resolution powder diffraction pattern of the metastable form was obtained by performing the crystallization in a sealed capillary, and subtracting the diffraction peaks of the stable form. Detailed evaluation of the structures of the stable and metastable polymorphs indicated that the phase transformation involved little structural rearrangement, and this fact was deduced as contributing to the difficulty of preparing phase-pure metastable crystals.

Three concomitant polymorphs of 1,3-bis(*m*-nitrophenyl)urea were reported in 1899 as yellow prisms (the α -form), white needles (the β -form), and yellow tablets (the γ -form), and a more detailed investigation of the system has been conducted (69). During work designed to prepare the γ -form, a new δ -form (that had the same color and habit as the β -form) and a monohydrate form were discovered, and the analysis suggested that the monohydrate was actually the reported γ -form.

It was also observed that despite the existence of considerable conformational differences in the molecules constituting the various crystal forms, the small degree of difference in the solid-state ^{13}C -NMR spectra of these forms indicated the existence of comparable environments for the NMR-active nuclei.

In their review, Dunitz and Bernstein pointed out that their examples of disappearing polymorphs involved molecules capable of adopting different conformations (60). These molecules would possess significant degrees of conformational freedom and molecular configurations that would facilitate the existence of equilibrium amounts of the different conformations in the solution, and solid-state effects would dictate which of these could be best able to build up into a crystal. It was noted that the rate of formation of nuclei of a stable polymorph could be significantly reduced by a low concentration of the required conformer, whereas another conformer might be incorporated in the nuclei of a metastable polymorph, which then underwent rapid growth. The phase interconversions accessible to systems of these types must be considered in the context of their enantiotropic or monotropic character, and therefore correctly designed preformulation studies of pharmaceutical compounds should resolve these kinetic and thermodynamic issues.

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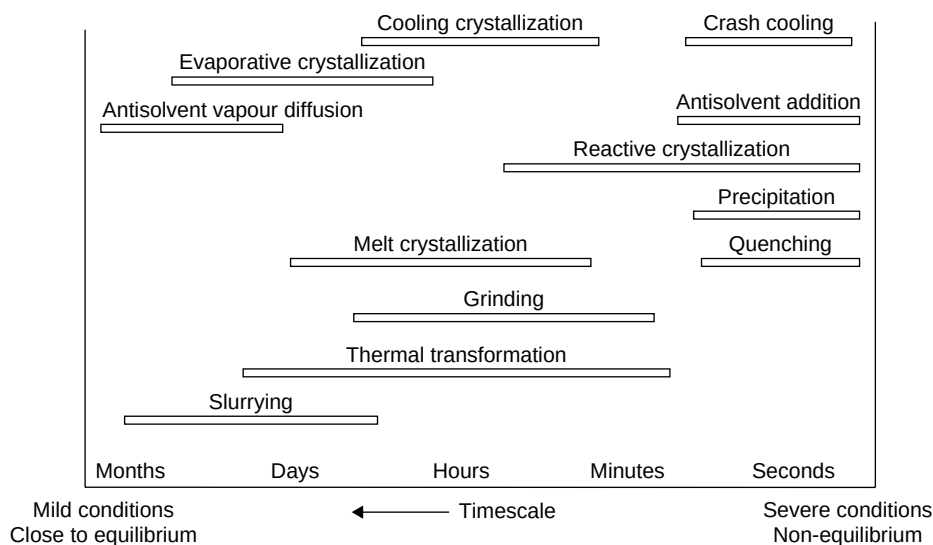


FIGURE 6 A selection of methods for producing crystalline forms. Longer running procedures favor stable polymorphs, whereas more rapid methods are more likely to yield less stable forms (35,36).

the antisolvent slowly, that is used in the slow growth of single crystals, whereas bulk antisolvent addition will cause a solute to crash out of solution, and is often used where other milder crystallization methods fail.

CRYSTALLIZATION FROM SOLUTION

As mentioned above, crystallization from solution is by far the commonest crystallization method used as a process for product recovery in the pharmaceutical industry. In this context, it simultaneously fulfills two essential functions:

1. Purification of the high-value pharmaceutical product, and separation from reaction by-products.
2. Consolidation of the product into a specified crystalline form with consistent and well-characterized physical properties.

Ideally, crystallization will be carried out starting with the solution from the final reaction step of product preparation or manufacture. Often, however, isolation of crude product is carried out using a method that maximizes yield but does not crystallize well, which does not purify effectively or give rise to a good crystalline form. In these cases, recrystallization is often employed to carry out the purification and consolidation functions.

Crystallization to produce specific polymorphs follows the same set of principles as those used in designing processes to manufacture crystalline products, and a basic understanding of these principles can greatly aid the design of a crystallization screen for polymorphs or of a process by which a particular polymorph,

stable or metastable, can be prepared consistently. Some of the general principles, applying to all solution crystallizations, will be outlined in the section “Cooling Crystallization,” for which they are easiest to formulate and understand. In the following sections, we will show how they can be extended to alternative methods of crystallization from solution.

Although following the same principles, crystallizations carried out for investigative purposes such as polymorph screening are subject to fewer constraints and limitations than processes in manufacture, although these limitations may subsequently come to bear if the process has to be adapted for production of the identified polymorph. For example, investigative crystallizations do not need high yields, because small quantities of a polymorph product can usually be isolated and identified. Also, it is possible to employ a diversity of methods in investigative work that are not scaleable and adaptable to larger scale, or for which such adaptations may be expensive. Conversely, there are also methods that are operated much more conveniently at larger scale, such as processes involving supercritical fluids.

Cooling Crystallization

Cooling crystallization from solution is usually the method of first choice, both for investigative crystallization in the laboratory and for manufacture at large scale. Its advantages are that it is easy to carry out and reproduce, it is generally well understood, and it may be reasonably scaled up in most cases. In practice, there are often limitations to cooling crystallization at large scale, in particular, the preference for natural cooling limits the rate at which cooling can be applied. In the laboratory, however, cooling rates can be set flexibly and accurately, depending on the purpose for which the crystallization is carried out and the product properties that are required.

There are a number of excellent texts that cover the principles of cooling crystallization comprehensively (37–39), and they are only outlined briefly here to serve the matter in hand, viz. the use of cooling crystallization to prepare polymorphs and other variable solid forms. Nevertheless, a sound understanding of the principles of crystallization is essential if solid-form searching is to be designed in an efficient and comprehensive manner. For a given crystallizing compound and solvent, the first requirement is knowledge of the solubility curve. This is best illustrated by reference to Figure 7, which shows the aqueous solubilities of some common inorganic salts as a function of temperature, from readily available data (40). CuSO_4 shows a steady increase in solubility with temperature, and may be crystallized by cooling the solution to give blue crystals of the stable pentahydrate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. Examination of the curve reveals that cooling a solution from 90°C to 10°C could result in recovery of 64% of the dissolved solid, if the crystallization proceeds to equilibrium. On the other hand, NaCl shows only a very slight increase in solubility with temperature, and cooling from 90°C to 10°C could produce only a maximum of 5% of the dissolved solute as solid. Cooling crystallization from water is unsuitable for NaCl, and industrial processes for its preparation rely on other methods, principally evaporative crystallization. The solubility curve for Na_2SO_4 shows a discontinuity at 32°C, which corresponds to the transition of the most stable solid phase at equilibrium with the solution from the decahydrate $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ to the anhydrous salt. Although the most stable phase at the crystallization temperature will be favored thermodynamically under these conditions, it will not necessarily be obtained for the reasons discussed below.

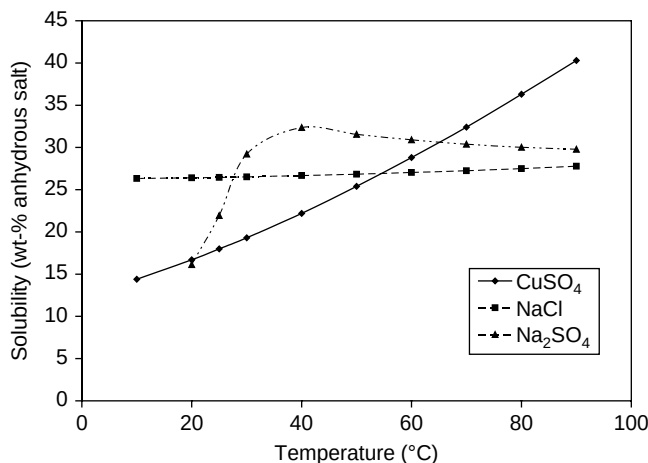


FIGURE 7 Solubility curves for the dissolution of simple inorganic salts in water (40). CuSO_4 may be crystallized from water by cooling, whereas NaCl may not. Na_2SO_4 exhibits a change in the most stable solid phase at 32°C , which is manifested as a discontinuity in the solubility curve.

Extensive solubility data is not usually available when approaching the crystallization of a new compound in form screening, but an indication as to whether cooling crystallization is a feasible option can be obtained from two data points corresponding to the solubilities at low and high temperature for the solvents under consideration. A compound that shows a marked solubility increase at the higher temperature may, in principle, be crystallized by cooling. For form screening purposes, the crystallization yield does not have to be high, and cooling crystallization may be employed provided that a reasonable supersaturation level, say 15% to 20%, can be achieved by cooling the solution. For production, yields will normally need to be higher than this, and in such cases cooling will have to be supplemented by other means of removing solid from solution, such as evaporation or an antisolvent.

The solubility curve is fixed by the thermodynamic relationship between the solid and the solution, and represents the condition that the chemical potentials of the dissolved solute and the solid at equilibrium with the solution are equal. The Gibbs Phase Rule stipulates that, for any given temperature, there will be a unique and determined concentration corresponding to equilibrium. The other component of the route map for a cooling crystallization is the metastable zone width (MZW), illustrated in Figure 8. An initially undersaturated solution at point A is cooled at a constant rate of temperature decrease, following the horizontal arrow. On crossing the solubility line at B the solution becomes supersaturated, but no solid separates at this point. At C, crystal nucleation occurs. Substantial crystallization occurs beyond this point by both the growth of the nuclei formed at C and by further nucleation and growth, and the conditions return toward equilibrium on the solubility line at D. Taking a range of temperature–concentration starting points A, represented as points below and to the right of the solubility curve in Figure 8, the supersolubility curve represents the locus of the points at which nucleation first

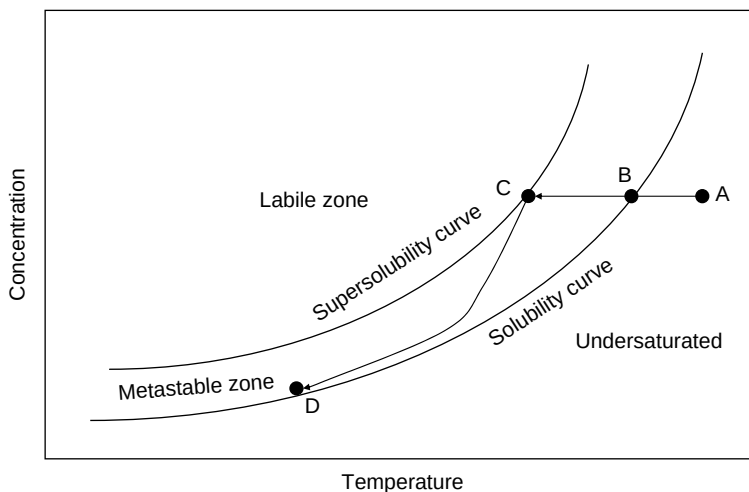


FIGURE 8 The metastable zone in cooling crystallization. Cooling a solution from A induces supersaturation at B, and nucleation at C. The region BC in which the solution is supersaturated but no nucleation occurs is the metastable zone. Beyond the nucleation point, crystal growth and further nucleation occurs until equilibrium is attained at D.

occurs for a given set of crystallization conditions, including cooling rate. The width of the metastable zone (MZW), represented by BC, is a measure of the difficulty in crystallizing a given substance due to the kinetics of inducing nucleation.

Unlike the solubility curve, the supersolubility curve and MZW are dependent on the conditions employed for crystallization, in particular, the cooling rate. Because crystal nucleation has a kinetic component associated with the ordering of molecular clusters into structured crystal nuclei, higher rates of cooling will give rise to wider metastable zones. MZW can vary from 2 to 5 K for simple inorganic salts to tens of degrees for complex organic molecules. The MZW represents the level of supersaturation driving force and departure from equilibrium at which nucleation and crystallization take place, and this can substantially affect polymorph selectivity. Operation close to equilibrium with narrow metastable zones will favor the formation of relatively stable solid forms, because stable crystalline solids are generally less labile than unstable and amorphous forms, and the lower driving forces and more extended timescales associated with working close to equilibrium generally favor their formation.

A further factor that can affect polymorph selectivity in cooling crystallization is enantiotropy, and this is illustrated in Figure 9 for the simple case of a dimorphic system in which the transition temperature T_i lies within the operating temperature range of the crystallization (41). Form II is stable below T_i , form I above T_i . Cooling from point A, the metastable zone of form I is traversed and crystallization is nucleated while the solution remains undersaturated with respect to form II. Point B represents the limit of this condition; the nucleation of form I occurs at the saturation point of form II. At C, the solution first becomes supersaturated with respect to form I, but in this case saturation with respect to form II occurs before the form I

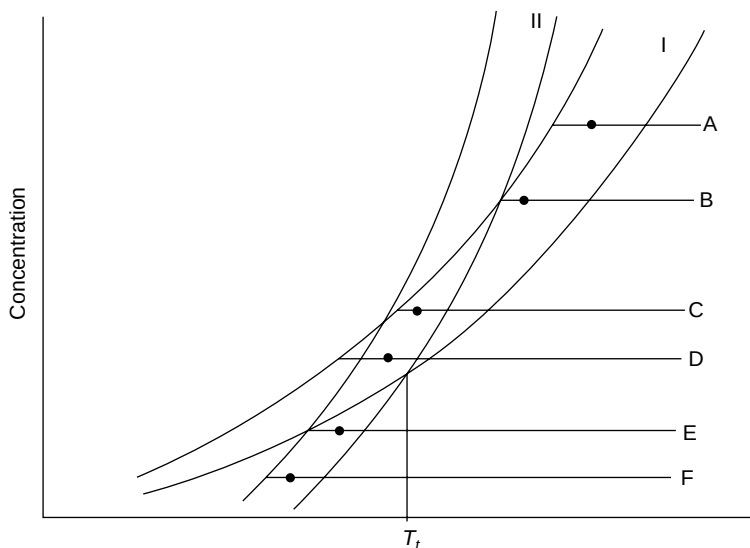


FIGURE 9 Solubility and metastable zones for the cooling crystallization of a dimorphic compound with a transition temperature within the crystallization region. Selecting the start and end temperatures A–F will determine the crystal forms obtained. *Source:* Adapted from Ref. (41). *Abbreviation:* T_t , transition temperature.

metastable zone limit is reached. Thus, crystallization of form I will occur from a solution that is also supersaturated with respect to form II, and there is the possibility of cross-seeding and producing a mixture of the two forms. At D, a mixture of forms is even more likely. Points E and F represent the converse of B and A, respectively, and indicate the conditions under which form II may be prepared. Figure 9 illustrates the importance of varying the crystallization conditions, in this case the temperature, in any search for polymorphs. Given that such searches are usually conducted to discover what forms can be prepared, the existence of characteristics such as enantiotropy will not be known at the time such searches are undertaken.

Figure 10 illustrates a situation where further unstable polymorphs exist along with the enantiotropic dimorphic relationship of Figure 9, and how these forms may be accessed by using extreme crystallization conditions, in this case cooling rate (41). Under slow cooling, forms III and IV will never occur because the solute will crystallize as forms I and/or II depending on the temperature conditions. Increasing the cooling rate will “stretch” the metastable zone to encompass a larger area of the concentration–temperature continuum. Unstable forms will have high solubilities, and this extension of the metastable zone, as shown in Figure 10, will eventually lead to the conditions for their crystallization falling within the metastable zones of the more stable forms. Under these conditions, the unstable forms are more likely to form because they are generally more labile than the more stable forms, as is reflected in their relatively narrow metastable zones within this area of the concentration–temperature plane.

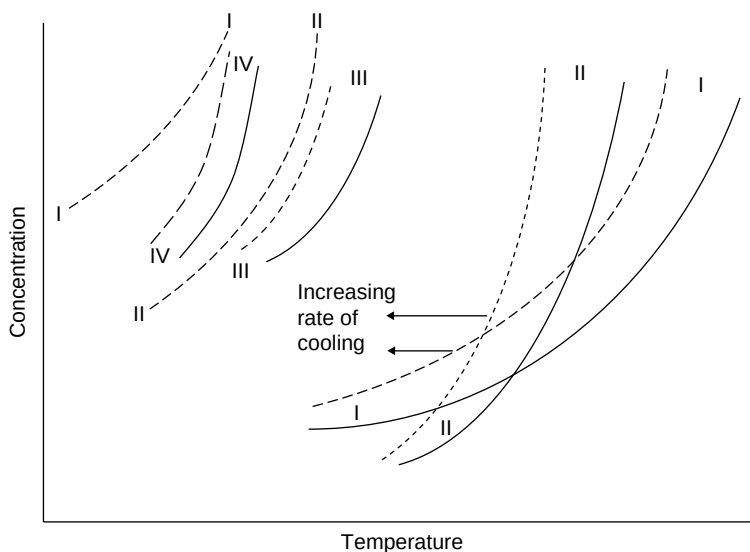


FIGURE 10 Crystallizing unstable polymorphs by crash-cooling. Increasing the rate of cooling broadens the metastable zones of the stable forms I and II, as shown by the dashed curves. This may eventually span the crystallization regions of unstable forms III and IV. Under these conditions, the unstable forms are more likely to occur because of their lability. *Source:* Adapted from Ref. (41).

Figures 9 and 10 demonstrate the importance of diversity in any polymorph screening program. Within the confines of a single crystallization method, variations in the conditions employed—temperature and cooling rate—can lead to different outcomes. Crystallizing under conditions close to equilibrium generally results in the more stable polymorphs; such conditions are usually “mild” in that they employ moderate driving forces sustained over longer periods. More “extreme” conditions induce crystallization further from equilibrium under large driving forces and generally favor unstable polymorphs and amorphous forms. Such extreme conditions are inherently unstable and often cannot be sustained over extended periods. The less labile and more stable polymorphs are thus denied the time scales of structural organization that they require, which works in favor of the less stable forms.

Effects of Solvent

Moderate solubilities with a steep increase with increasing temperature are the optimum conditions for cooling crystallization, with solubilities typically in the range 5 to 200 mg/mL at room temperature. If the solubility exceeds 200 mg/mL, the viscosity of the solution is likely to be high, and a glassy amorphous product may be obtained in preference to crystals. A useful preliminary test can be performed on 25 to 50 mg of sample, adding a few (5–10) drops of solvent: if the solid completely dissolves the solubility is probably too high. Also, highly viscous solvents are not usually conducive to efficient crystallization. Solvents with very low vapor pressures, such as DMSO or glycerol, are not good for process applications because of the difficulty of removing residues from the isolated products.

Pharmaceutical Co-crystals: A New Opportunity in Pharmaceutical Science for a Long-Known but Little-Studied Class of Compounds

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INTRODUCTION

Active pharmaceutical ingredients (APIs) are typically orally delivered as solid dosage forms that contain a single crystalline form of the API. This is an artifact of crystalline solids tending to be more stable and reproducible in the context of purification and processing when compared to other types of solid forms such as amorphous solids and solid solutions. Interestingly, the importance of crystal form selection during development of APIs has probably never been higher, and in the past five years pharmaceutical co-crystals have become part of a landscape that was previously occupied only by polymorphs, salts, and solvates/hydrates (Fig. 1) (1–4). Nevertheless, whereas there is a clear need for better understanding and control of crystalline forms in the context of drug development, the concepts of supramolecular synthesis (5–8) and crystal engineering (9–18) remain largely underexploited, and there are many basic questions about pharmaceutical co-crystals that remain unanswered, including their tendency to form polymorphs (19). Furthermore, a number of factors that are critically important in making an API viable as an oral dosage form remain difficult to predict or even control:

- *Dissolution rate and intrinsic solubility* of different crystal forms of an API are variable, and can strongly influence bioavailability. This is a particularly salient factor in light of the increasing prevalence of “BCS Class II” drugs (i.e., low-solubility, high-permeability APIs) (20).
- *Stability toward temperature and humidity* of an API is critically dependent upon crystal packing.
- *The unpredictability (i.e., lack of obviousness) of crystal structures* and physical properties means that there are opportunities and challenges in terms of obtaining and maintaining patent protection for an API.

In such a context, scientific developments that afford greater diversity in terms of the number of crystalline forms available for a given API and, therefore, an opportunity to customize the physical properties of an API with a process or clinical need, are obviously of profound relevance to the pharmaceutical industry. This chapter highlights how crystal engineering can afford such an opportunity to diversify the number of crystal forms known for an API, and to fine tune their physical properties of clinical relevance by reviewing the emerging field of pharmaceutical co-crystals. Particular emphasis is placed upon their history, their design, and

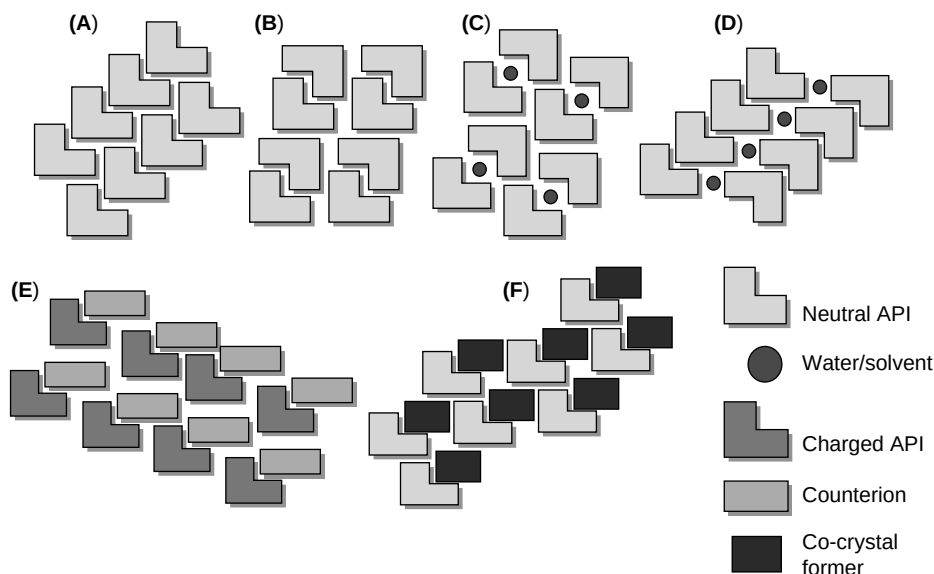


FIGURE 1 A schematic illustration of the classes of crystal form that might be exhibited by active pharmaceutical ingredients (APIs) (A) single component API; (B) polymorph of single component API; (C) hydrate or solvate of API; (D) polymorph of solvate or hydrate of API; (E) salt of API; (F) co-crystal of API.

their recent impact on preformulation strategies for development of dosage forms of APIs.

WHAT IS A CO-CRYSTAL?

That there is not yet a recognized definition of the term “co-crystal” has engendered a topical debate on the subject (21–27). In the Zaworotko research group we have been using the following operating definition: *A co-crystal is a stoichiometric multiple component crystal in which all components are solid under ambient conditions when in their pure form. These components consist of a target molecule or ion and a molecular co-crystal former(s) and when in a co-crystal they coexist at the molecular level within a single crystal* (28). This perspective has also been taken by Aakeröy (29). That all components are solids under ambient conditions has important practical considerations because synthesis of co-crystals can be achieved via solid-state methods, and chemists can execute a certain degree of control over the composition of a co-crystal because they can invoke molecular recognition, especially hydrogen bonding, during the selection of co-crystal formers. These features distinguish co-crystals from another broad group of multiple component compounds, solvates, and it should be noted that most molecular compounds are amenable to formation of co-crystals as they exist as solids under ambient conditions (30). An increasingly important subset of co-crystals is pharmaceutical co-crystals, that is, *co-crystals in which the target molecule or ion is an active pharmaceutical ingredient, API, and it bonds to the co-crystal former(s) through*

hydrogen bonds (31). The recent and rapid emergence of pharmaceutical co-crystals can be attributed to a number of factors including the following:

- *Design.* Our scientific understanding of the noncovalent forces that sustain molecular organic crystals, in particular co-crystals, has recently advanced to the extent that control over the stoichiometry and composition of co-crystals can be asserted, that is, by exploiting the strategy of crystal engineering (9–18,32–35) in the context of understanding and predicting hydrogen-bonding interactions. Crystal engineering is facilitated by the Cambridge Structural Database (CSD) (36), and the nature of APIs, that is, molecules or ions with exterior functional groups that engage in hydrogen bonding, makes all APIs inherently predisposed to formation of pharmaceutical co-crystals (31,37–39). This is not ordinarily the case for polymorphs and solvates for which high-throughput screening, which to a certain extent relies upon serendipity, tends to be relied upon rather than design, or for salts, which require an ionizable functional group.
- *Discovery.* That mechanochemistry can be utilized to synthesize co-crystals has been known since the first co-crystals were discovered in the 1840s by dry grinding (40), but it has only recently been realized and accepted that “solvent-drop” or “liquid-assisted” grinding are preferred methodologies (41–44). Indeed, it is fair to assert that co-crystals are readily accessible through solvent-free or solvent-reduced methods, although other techniques such as slurrying (45) and solution (46) are complementary.
- *Diversity.* It has become apparent that pharmaceutical co-crystals exhibit different physical properties compared to the pure crystal form(s) of APIs, that a given API might form co-crystals with dozens of co-crystal formers, and that some of these co-crystals might exhibit enhanced solubility (47–50) or stability to hydration (51). Therefore, pharmaceutical co-crystals represent an opportunity to significantly diversify the number of crystal forms of an API, and in turn, fine tune or even customize its physicochemical properties without the need for chemical (covalent) modification.
- *Development.* Whereas pharmaceutical co-crystals can be designed using crystal engineering strategies, this does not mean that details of their crystal structures or physical properties can be predicted before they have been measured. Therefore, there is no reason to assume that it will not be possible for pharmaceutical co-crystals of existing APIs to be patented as new crystal forms and, if they exhibit clinical advantages, then they are open to be developed as new drugs (52). This has potentially important implications for drug development because it abbreviates some aspects of drug development time lines and mitigates costs and risks related to discovery and toxicology of new APIs.
- *Delivery.* As mentioned earlier, being able to fine tune solubility can be a critical factor that influences the clinical performance of an API if its bioavailability is controlled by rate of dissolution. This is generally considered to be important for BCS Class II APIs (53), the most common classification (20,54–55) for the current generation of APIs.

History of Co-crystals

If one uses the criterion that all components of a co-crystal must be solid under ambient condition, then co-crystals represent a long known class of compounds.

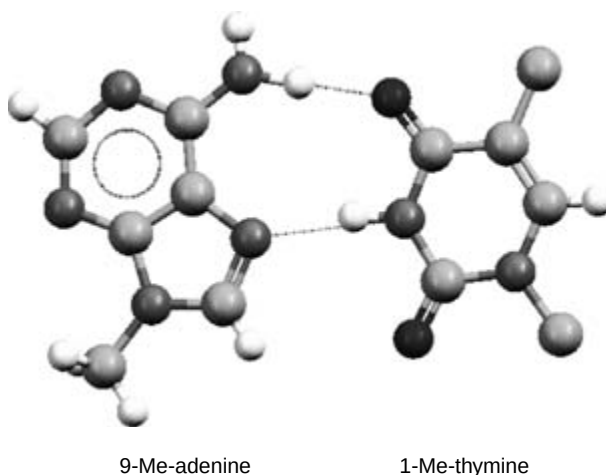


FIGURE 2 The Hoogsteen base-pairing that occurs in the 1:1 co-crystal of 9-methyladenine and 1-methylthymine.

The prototypal co-crystal is quinhedrone, which was reported in 1844 (40), and was further studied during the late 1800s (56). Co-crystals were subsequently described as *organic molecular compounds* (57). However, structural information on co-crystals was largely absent until the 1960s, when the term *complexes* was coined, and most recently, the term *heteromolecular crystals* was coined to describe a co-crystal formed between two hydrocarbons: cubane and fullerenes (58).

That complexes form readily via molecular recognition between complementary nucleic bases (59–60) is not just of relevance to biochemistry. Such interactions are key to understanding existing co-crystals and designing new co-crystals. Figure 2 illustrates the crystal structure of the co-crystal that forms when 9-methyladenine is crystallized in the presence of 1-methylthymine. This compound can be regarded as a prototypal co-crystal that illustrates how molecular recognition (i.e., hydrogen bonding) can indeed govern the existence and the composition of a co-crystal. It is therefore somewhat surprising, especially given the broad relevance and even application of interactions between nucleobases (61), that even today there is relatively little structural information on base pairing. Indeed, the August 2008 release of the CSD (36) contains structural information on 456,628 organic, metal–organic, and organometallic crystal structures and, if salts and solvates are excluded, there are less than 100 crystal structures involving nucleobases. Furthermore, there is essentially no information concerning the stability of base pairing in a competitive environment (i.e., when other hydrogen bonding groups are present). The situation with nucleobases represents a microcosm of co-crystals in general because there is not a great deal of structural information in the CSD on co-crystals. There are only two entries prior to 1960, and even today there are only ca. 2083 (0.46% of the CSD) hydrogen bonded co-crystals versus 50,019 hydrates (10.95% of the CSD). Therefore, it would be fair to summarize co-crystals as being a long known but little studied class of compounds.

History of Pharmaceutical Co-crystals

Pharmaceutical companies are always in search of a crystal form that is stable during handling, processing, and storage. However, such stability is not always at hand because APIs are prone to the formation of polymorphs that could be metastable or less soluble. Furthermore, it is at best difficult to predict beforehand how many polymorphs of an API might exist and under what experimental conditions these polymorphs will appear. The other commonly developed forms of active pharmaceutical ingredients include salts, solvates, hydrates and multi-component crystalline phases, pharmaceutical co-crystals, and of course, polymorphs can also occur with all these entities. The availability of multiple co-crystal formers for a given API is what makes pharmaceutical co-crystals special. The co-crystal former is a compound that is pharmaceutically acceptable or approved. The scope of available co-crystal formers is not yet set but even if it is limited to "generally regarded as safe" (GRAS) compounds or compounds that have already been approved by the FDA for use in formulation such as a "salt formers," there could be 100 or more possible co-crystal formers for an API. However, pharmaceutical co-crystals are not only of interest because they can afford crystal form diversity and improve physical properties of clinical relevance. They also represent an opportunity to patent and market clinically improved crystal forms of existing APIs, possibly as a new drug. It should therefore be unsurprising that much of the currently available literature on the subject is found in patents. Indeed, perhaps the earliest example of a pharmaceutical co-crystal was reported in a 1934 French patent that disclosed co-crystals of barbiturates with 4-oxy-5-nitropyridine, 2-ethoxy-5-acetaminopyridine, *N*-methyl- α -pyridone and α -aminopyridine (62). In 1995, Eli-Lilly patented complexes of cephalosporins and carbacephalosporins, a class of β -lactam antibiotics, with parabens and related compounds (63). Sildenafil citrate, the active pharmaceutical ingredient in Viagra[®], a \$1.5 bn/year drug that is used for the treatment of male erectile dysfunction and pulmonary arterial hypertension, is a BCS class II drug with low solubility. The co-crystal of sildenafil with acetyl salicylic acid (aspirin) was patented in 2007 (64), and it exhibits higher solubility under acidic conditions compared to the commercially available crystal form sildenafil citrate (Fig. 3). A more detailed discussion of this pharmaceutical co-crystal is presented in the section "Case Studies of Pharmaceutical Co-crystals."

In terms of the scientific literature there were few reports of pharmaceutical co-crystals until the past decade. However, Caira, demonstrated that "old" drugs such as sulfonamides could form co-crystals (65), and suggested their potential in pharmaceutical development. Pharmaceutical co-crystals have received much more attention in recent years, as evidenced by several review articles (28,31,38,39) and a feature article in the June 18th 2007 issue of *Chemical & Engineering News* (66). This cover story focused upon various aspects of active pharmaceutical ingredients including polymorphs, solvates, hydrates, pharmaceutical co-crystals, and patentability.

Design of Co-crystals

It is evident that many of the early co-crystals reported in the literature appear to have been the result of serendipity rather than design. However, the emergence of crystal engineering has changed this situation, and in recent years several groups have successfully exploited crystal engineering principles for design and synthesis of co-crystals, with particular emphasis upon exploitation of hydrogen bond

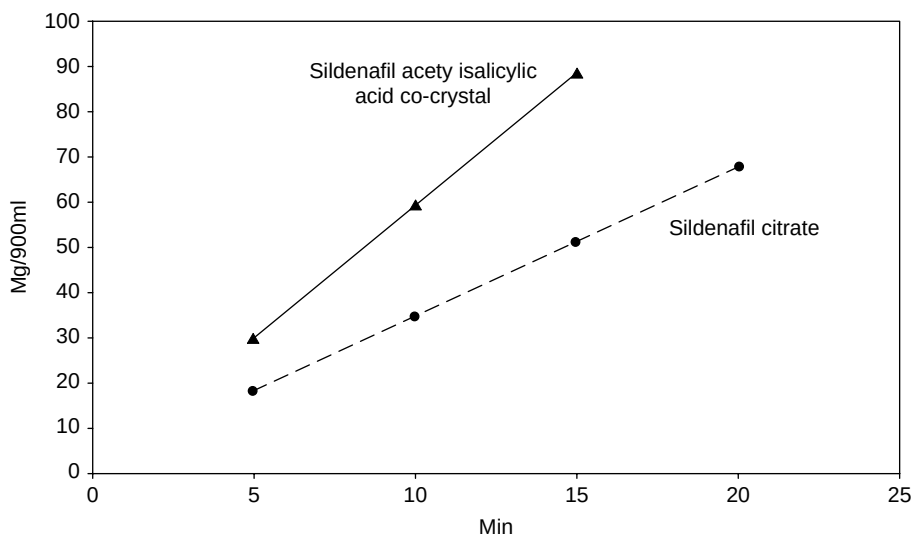


FIGURE 3 Intrinsic dissolution rate of the 1:1 sildenafil citrate:aspirin co-crystal versus sildenafil citrate.

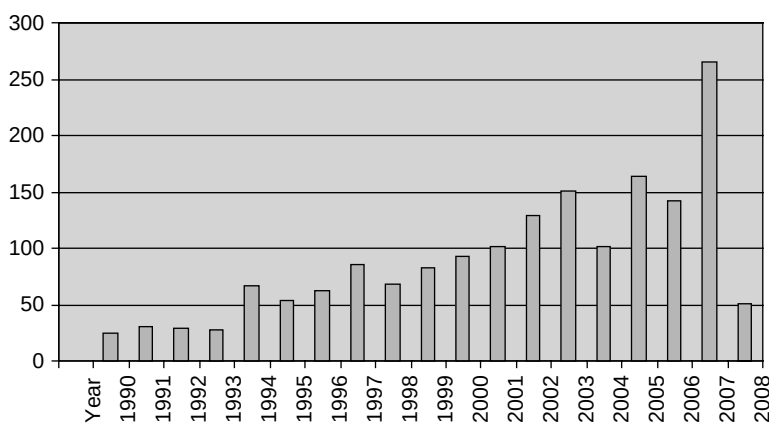


FIGURE 4 Hydrogen-bonded co-crystals formed between two or more compounds that are solid under ambient conditions deposited in the CSD from 1990 to 2008 [CSD Conquest 1.10 (August 2008 update)].

motifs (29,33,34,67–69). Figure 4 reveals that there has been a steady increase in the number of co-crystals reported from 1990–2008, a time period that coincided with the emergence of the paradigm of crystal engineering. Pepinsky introduced the concept of crystal engineering as far back as 1955 (32), and it was first implemented by Schmidt in the context of organic solid-state photochemical reactions (9). It is fair to assert that Etter popularized the term “co-crystal” in the 1980s (33), and at around

the same time Desiraju defined crystal engineering as “the understanding of intermolecular interactions in the context of crystal packing and in the utilization of such understanding in the design of new solids with desired physical and chemical properties” (11). Crystal engineering has now matured into a widely accepted paradigm for the preparation or supramolecular synthesis of new compounds. A salient feature of crystal engineered structures is that they are designed from first principles using the concepts of supramolecular chemistry and self-assembly (16,70–73).

A crystal engineering experiment typically involves CSD surveys followed by experimental work to prepare and characterize new compounds that are sustained by molecular recognition events or *supramolecular synthons* (5). A detailed understanding of the supramolecular chemistry of the functional groups present in a given molecule is a prerequisite for designing a co-crystal because it facilitates selection of appropriate co-crystal formers. However, when multiple functional groups are present in a molecule, the CSD rarely contains enough information to address the hierarchy of the possible supramolecular synthons. Furthermore, the role of solvent in nucleation of crystals and co-crystals remains poorly understood, and solvent can play a critical role in obtaining a particular co-crystal. However, the hierarchy of the supramolecular synthons that can occur for common functional groups such as carboxylic acids, amides, and alcohols with emphasis upon *supramolecular heterosynthons*, that is, non-covalent bonds between different but complementary functional groups (29,47,67b,e,f,74) is becoming better defined. Furthermore, it is becoming evident that such interactions are key to implementing a design strategy for co-crystals in which a target molecule forms co-crystals with a series of co-crystal formers that are carefully selected for their ability to form supramolecular heterosynthons with the target molecule.

How Are Co-crystals Prepared?

Synthesis of a co-crystal from solution might be thought of as counter-intuitive because crystallization is such an efficient and effective method of purification. However, if different molecules with complementary functional groups result in hydrogen bonds that are energetically more favorable than those between like molecules of either component, then co-crystals are likely to be thermodynamically (although not necessarily kinetically) favored. Supramolecular heterosynthons that seem to favor formation of co-crystals are exemplified by carboxylic acid–pyridine (29a,67b,e,75), carboxylic acid–amide (67g,76), and alcohol–pyridine (67f,77) (Fig. 5).

Single crystals involving these supramolecular heterosynthons are commonly discovered via slow evaporation from a solution containing stoichiometric amounts

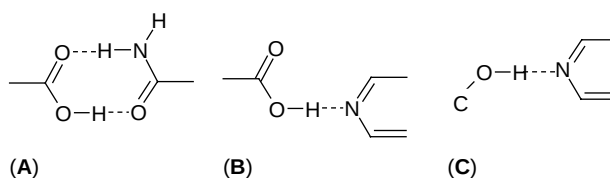


FIGURE 5 (A) Acid–amide, (B) acid–pyridine, and (C) alcohol–pyridine supramolecular heterosynthons.

of the components (co-crystal formers); however, sublimation, growth from the melt, slurring, and grinding of two or more solid co-crystal formers in a ballmill are also suitable methodologies. Indeed, dry grinding was used as far back as the 19th century (40,56), and the recently developed technique of solvent-drop grinding, addition of a small amount of suitable solvent to the ground mixture to accelerate co-crystallization, appears to be a particularly fruitful methodology (41–44). The crystal form that is obtained is typically (45,74a), but not always, (4a) independent of the synthetic methodology. However, that co-crystals can often be prepared in a facile one-step manner using “green chemistry” approaches (78) does not mean that their synthesis and isolation is routine:

- As mentioned above, a detailed understanding of the supramolecular chemistry of the functional groups present in a given molecule is a prerequisite for designing a co-crystal because it facilitates selection of appropriate co-crystal formers. However, when multiple functional groups are present in a molecule the CSD rarely contains enough information to address the hierarchy of the multiple possible supramolecular synthons.
- Mismatched solubility between the components of a co-crystal can preclude their generation from solution if the co-crystal formers are present in a stoichiometric ratio.
- The role of solvent in nucleation of crystals and co-crystals remains poorly understood, and choice of solvent can be crucial in obtaining a particular co-crystal.

Why are Co-crystals Broadly Relevant?

Co-crystals are attractive to solid-state chemists because they offer opportunities to modify the composition of matter and the chemical and/or physical properties of a molecular species without the need to make or break covalent bonds. Thus far, there are several notable applications for co-crystals, all of which have direct or indirect relevance to pharmaceuticals:

- *Non-covalent derivatization* (79,80) was coined in the context of modifying the stability of Polaroid film (80,81). In this particular context, reduced solubility of a co-crystal reduced solubility and diffusion of an active ingredient. The net effect was greater stability and shelf life. Such a situation could be relevant to drug formulation, especially transdermal formulations, but has not to our knowledge yet been applied as such.
- *Solid-state synthesis*. Crystal engineering was first implemented in the context of photodimerization in the solid-state (9), and there are multiple examples of co-crystal formers having been used as templates to control the orientation of photoreactive molecules (Fig. 6) (67d,77c). Such a situation affords a level of control that cannot be achieved in the absence of the co-crystal former or in solution, and can lead to 100% yield and no waste. Co-crystals in which both co-crystal formers are reactants have also been exploited for solid-state synthesis. Etter et al. reported the first example of such a reaction in 1989 (82), a nucleophilic substitution reaction, and more recently, Cheney et al. have reported co-crystal controlled solid-state synthesis of imides, C^3S^3 , via condensation of amines and acid anhydrides (83).
- *Chiral resolution*. Whereas spontaneous resolution of racemic mixtures into racemic conglomerates, “Pasteur crystallization,” represents one of the best

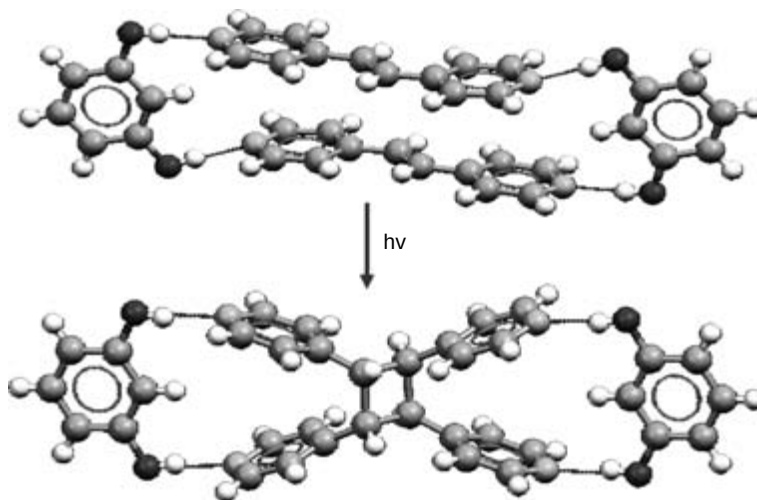


FIGURE 6 The co-crystal controlled (2+2) photodimerization of a resorcinol co-crystal of a substituted olefin.

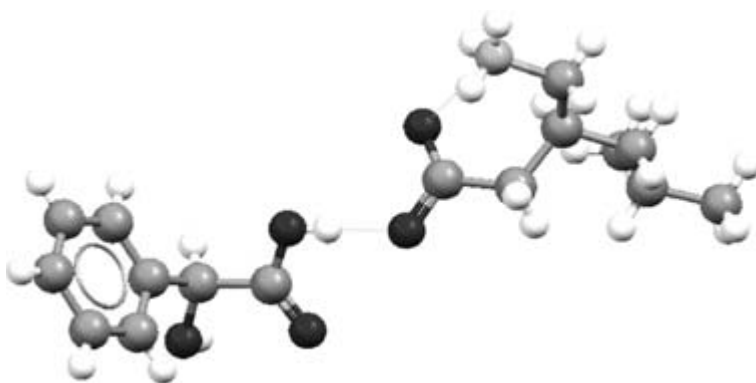


FIGURE 7 1:1 co-crystal of (S)-3-(ammoniummethyl)-5-methylhexanoate and (S)-mandelic acid.

known crystallization experiments (84), it is not the typical outcome to such an experiment. Rather, racemates are generally considered to be the outcome in around 90% of crystallizations of racemic mixtures (85). Chiral co-crystal formers offer an enhanced possibility of separating enantiomers by fractional crystallization because the resulting co-crystals are diastereomeric in nature. For example, (S)-mandelic acid has been used to resolve racemic pregabalin on an industrial scale (86), and it is able to resolve racemic 2-aminobutyric acid (87a) and phenylalanine (87b) through fractional crystallization. On the other hand, racemic mandelic acid can be resolved by (S)-alanine and R-cysteine (88). A diastereomeric co-crystal of (S)-mandelic acid is illustrated in Figure 7.

- Many of the crystal engineering principles and protocols that have been exploited for the generation of pharmaceutical co-crystals might also be invoked for the design and isolation of other types of co-crystal. In this context, molecular targets that would be particularly attractive and amenable for study because of technological opportunities and their hydrogen-bonding capabilities include the following: nucleobases (drug design and detection of oligonucleotides), molecules with high polarizability (new classes of NLO material), explosives/propellants (reformulation, enhanced thermal stability, enhanced properties), agrichemicals (reformulation, enhanced properties), renewable substrates (solid-state, polymer, and green chemistry), and volatile organics (air pollution).

CASE STUDIES OF PHARMACEUTICAL CO-CRYSTALS

An early example of studies that are related to pharmaceutical co-crystals was a series of studies conducted in the 1950s by Higuchi and his coworkers, who studied complex formation between macromolecules and certain pharmaceuticals (89). For example, complexes of polyvinylpyrrolidone (PVP) with sulfathiazole, procaine hydrochloride, sodium salicylate, benzylpenicillin, chloramphenicol, mandelic acid, caffeine, theophylline, and cortisone were isolated (89–90). However, these compounds would not be classified as pharmaceutical co-crystals according to the criteria applied herein. Whitesides et al. addressed the use of substituted barbituric acid, including barbital and melamine derivatives, to generate supramolecular “linear tape,” “crinkled tape,” and “rosette” motifs sustained by robust supramolecular synthons with three-point hydrogen bonding (34). Despite their success in co-crystal formation, the focus of these studies was not so much the physical properties of the resulting co-crystals but rather the supramolecular functionality of barbitals and their complementarity with melamine. Nevertheless, these studies illustrated the potential diversity of crystal forms that can exist for a particular API, as more than 60 co-crystals were structurally characterized in this series of studies. Herein we shall focus upon case studies that exemplify the formation of pharmaceutical co-crystals with altered physical properties of clinical relevance.

Pharmaceutical Co-crystals of Carbamazepine (Tegretol®)

Carbamazepine (CBZ) has been in use for over three decades for the treatment of epilepsy and trigeminal neuralgia even though it has multiple challenges associated with its oral drug delivery, including a small therapeutic window, autoinduction of metabolism, and dissolution limited bioavailability. A CSD analysis on CBZ reveals that it has 45 entries that include four fully characterized polymorphs (91), a dihydrate (92), 14 solvates (acetone, furfural, DMSO, trifluoroethanol, DMF, NMP, nitromethane, acetic acid, formic acid, butyric acid, formamide, trifluoroacetic acid, tetrahydrofuran, *N,N'*-dimethyl acetamide) (93,96a), two ammonium salts (94), a solid solution with dihydrocarbamazepine (95), and 16 co-crystals including co-crystal hydrate/solvates and co-crystal polymorphs (67g,96). Most recently, Hilfiker et al. (97), at Solvias AG, identified three new forms and a dioxane solvate using high-throughput screening and Childs et al. (45) demonstrated the preparation of 27 unique solid phases of carbamazepine utilizing 18 carboxylic acids as co-crystal formers and four different screening methods. CBZ probably has more reported co-crystals than any other API, and some of these co-crystals have also been studied in terms of their dissolution and bioavailability. CBZ is therefore an

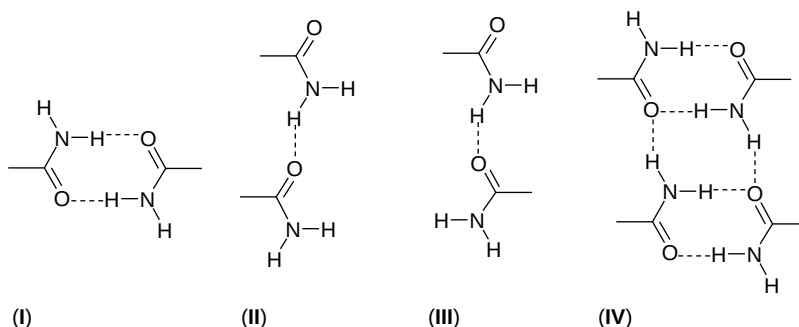


FIGURE 8 The four supramolecular homosynths typically exhibited by primary amides.

appropriate starting point for case studies. CBZ exhibits a simple molecular structure in terms of its hydrogen-bonding capabilities because it possesses a single hydrogen-bonding moiety, a primary amide. The supramolecular homosynths typically exhibited by primary amides are presented in Figure 8.

The primary amide dimer, I, like the carboxylic acid dimer, is a well-documented supramolecular homosynthon. A survey of the CSD reveals that there are 1,464 crystal structures that consist of at least one primary amide functional group, and I is exhibited in 589 of these structures even in the presence of competing hydrogen bond donors/acceptors. However, this represents a raw set of data, and a more refined search of the occurrence of this moiety in the absence of competing hydrogen bond donors and/or acceptors revealed 61 crystal structures that contain an amide moiety in the absence of other hydrogen bond donor or acceptor groups. Fifty-two (85 %) of these structures exhibit I and 9 (15 %) exhibit catemer motif II, suggesting that I is favored over II when no competing groups are present. Once the dimer is formed between the amide moiety, the anti-hydrogen atom either forms a hydrogen bond to an adjacent amide so as to form molecular tapes or sheets or it hydrogen bonds to a different functional group via a supramolecular heterosynthon. However, this is not the case with CBZ, in which the anti-hydrogen atom is not involved in hydrogen bonding, presumably due to steric constraints imposed by the azepine ring of CBZ (Fig. 9). That the CBZ dimer does not engage its peripheral hydrogen-bonding capabilities represents one avenue for crystal engineering and makes CBZ an excellent candidate for formation of pharmaceutical co-crystals. A second strategy involves breakage of the dimer motif and formation of supramolecular heterosynths. Both strategies have been successful, and have afforded a number of CBZ co-crystals that exhibit improved physicochemical properties. Several of these co-crystals are described herein.

Carbamazepine:Nicotinamide 1:1 Co-crystal (1a) (96a)

In the pharmaceutical co-crystal of CBZ and nicotinamide, **1a**, CBZ molecules form supramolecular homosynthon I around a crystallographic inversion center. These CBZ dimers H-bond to the *syn* positions of nicotinamide amide groups, and the aromatic nitrogen atoms of the nicotinamide molecules are not involved in hydrogen bonding (Fig. 10).

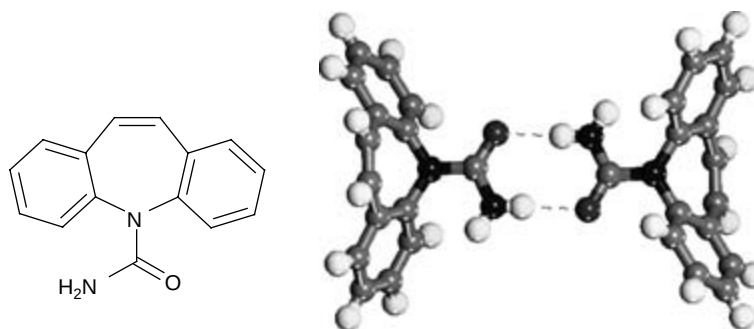


FIGURE 9 Molecular structure of carbamazepine (CBZ; *left side*) and the amide dimer (I; *right side*) that occurs in pure CBZ polymorphs.

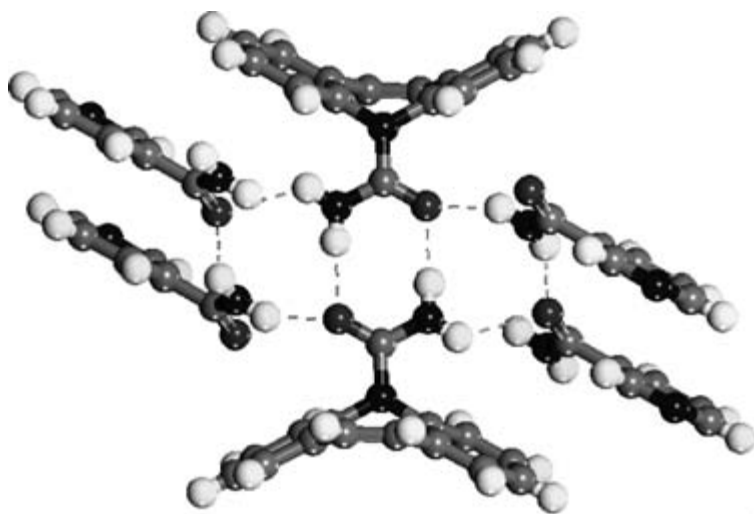


FIGURE 10 Illustration of the interaction between CBZ dimers and nicotinamide. Nicotinamide links the CBZ dimers by forming hydrogen-bonded tapes.

Rodriguez-Hornedo and coworkers have studied CBZ to develop an approach called reaction crystallization for the rapid formation of co-crystals in microscopic and macroscopic scales under ambient conditions (40). Reaction crystallization exploits solubility differences between the API, co-crystal former and co-crystal, and involves analysis of the ternary phase diagram. **1a** was selected as a model system to study the reaction pathways and kinetics in both aqueous and organic media. The phase solubility diagram for **1a** as a function of the concentration of co-crystal former in EtOH was reported, and the co-crystallization process of **1a** using anhydrous CBZ form III and nicotinamide in EtOH was monitored by Raman spectroscopy (Fig. 11). This formation of **1a** was the result of dissolution of

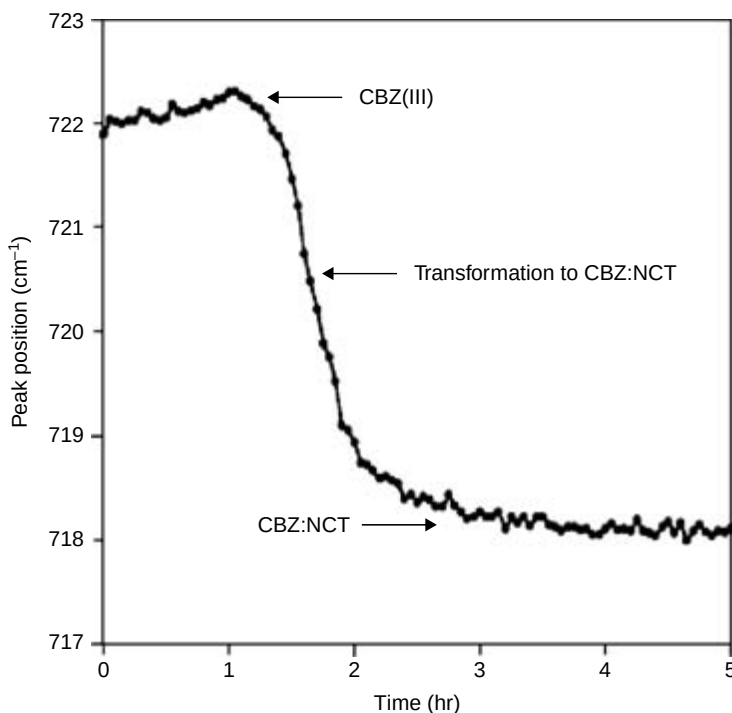


FIGURE 11 Raman peak position with respect to time showing the slurry conversion or transformation of solid phase CBZ(III) to co-crystal **1a** at 25°C after adding 0.16 M solution of nicotinamide in ethanol to CBZ(III).

anhydrous CBZ form III, leading to supersaturation of co-crystal **1a**. In situ preparation of **1a** using covered depression slides on a polarizing optical light and Raman microscope by addition of a few drops of solvent such as ethanol, ethyl acetate, or 2-propanol to the solid API and co-crystal former was also described. This study suggests broadly applicable screening methods for the preparation of co-crystals.

The factors that can control amorphous phase-mediated co-crystallization using **1a** as a model system were also addressed in order to evaluate whether amorphous phases lead to co-crystal formation and if there is a relation between temperature and crystallization pathways (98). The results suggested that amorphous phases can indeed generate co-crystals, and that temperature affects the crystallization pathways in significant ways. A new phase of **1a** (Form II) was identified during the thermal analysis.

Rodriguez-Hornedo et al. also developed a mathematical model to describe the solubility of co-crystals by taking into consideration the equilibria between co-crystal, co-crystal components, and solution complexes with **1a** as a model [Fig. 12(A)] (99). Nicotinamide solutions with varying concentrations were prepared using solvents such as ethanol, 2-propanol, or ethyl acetate [Fig. 12(B)]. The co-crystal solubility was measured by suspending co-crystals in these solutions, and it was observed that the solubility of co-crystal decreases with the increase in nicotinamide concentration.

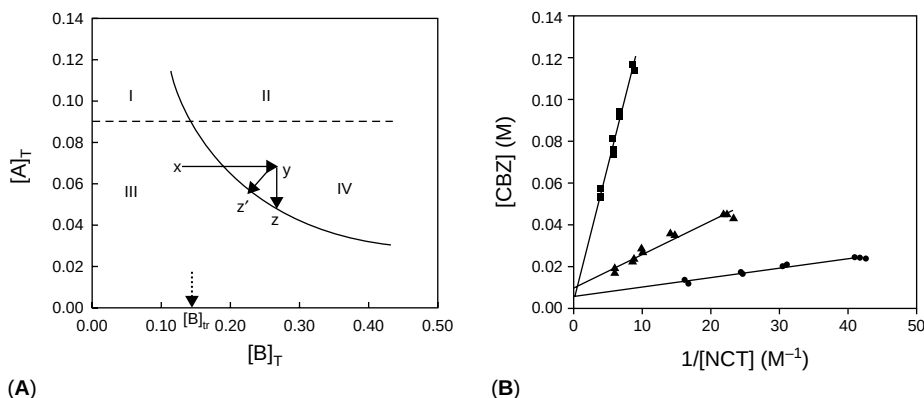


FIGURE 12 (A) Effect of co-crystal former B concentration on the solubility of co-crystal AB (*solid line*) and the solubility of single-component crystal (for instance API) A (*dashed line*). The transition of the co-crystal former concentration $[B]_{lr}$ occurs when the solubility of A equals the solubility of AB. (B) Total CBZ concentration in equilibrium with co-crystal, **1a**, at 25°C as a function of the inverse total nicotinamide concentration in ethanol (*top*), 2-propanol (*middle*), and ethyl acetate (*bottom*), showing the linear dependence as per the prediction.

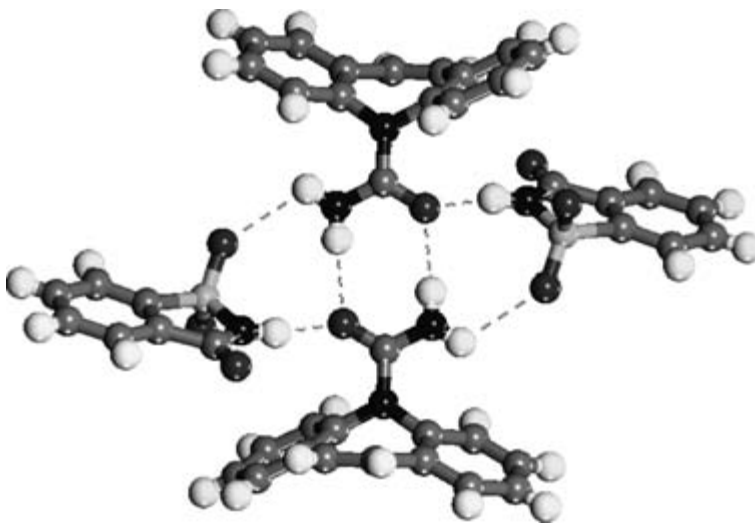


FIGURE 13 Illustration of the interactions between CBZ dimers and saccharin (**1b**).

Carbamazepine:Saccharin 1:1 Co-crystal (**1b**) (96a)

1b exhibits a structure similar to that of **1a** in that CBZ molecules form supramolecular homosynthon I related by inversion symmetry. The saccharin molecules serve as both hydrogen-bond donors (N–H moiety) and acceptors (S=O group) by forming hydrogen bonds with both the carbonyl and N–H moieties of CBZ. The carbonyl and the second S=O of saccharin molecules are not involved in hydrogen bonding (Fig. 13).

Almarsson and coworkers (50) studied **1b** with regard to multi-gram scale-up, propensity for crystal polymorphism, physical stability, in vitro dissolution, and oral bioavailability in comparison with the marketed form III of CBZ (Tegretol®). **1b** was scaled up to 30g with a conventional cooling crystallization process from alcohol solution without seeding. The physical stability of the co-crystal **1b** was found to be superior compared to that of solvates of CBZ and similar to that of the marketed form of CBZ. It was also found that co-crystal **1b** is resistant to hydrate formation over a period of 15 days under direct exposure to ICH storage conditions. The oral pharmacokinetics (PK) of co-crystal **1b** were studied in dogs and compared with the marketed form of Tegretol®. The study was conducted at a dose equivalent to 200 mg of anhydrous CBZ, and was administered orally in a two-way cross-over design to four fasted beagle dogs. The study suggested that co-crystal could serve as a practical alternative to anhydrous CBZ in oral formulation because the bio-availability appears to be improved. Other benefits of the co-crystal **1b** include the following: (i) it has better solubility compared to the CBZ dihydrate or pure CBZ forms; (ii) it is stable in water for >24 hours; (iii) it has similar chemical stability compared to other forms of CBZ; (iv) although pure CBZ has four polymorphs co-crystal is not overtly polymorphic (1200 screen CRYSTALMAX experiments that include high-throughput screening, neat grinding, solvent-drop grinding with various solvents, and slurry conversion in different solvents). In general, if CBZ represents a microcosm of issues related to crystal form selection of APIs then pharmaceutical co-crystals could indeed represent a viable approach for improving the diversity and performance of API crystal forms.

Polymorphs of 1a and 1b (96d)

Utilization of functionalized cross-linked polymers as heteronuclei has been used as effective strategy for polymorph discovery of active pharmaceutical ingredients, and CBZ co-crystal polymorphs have been discovered using such an approach. Matzger and coworkers demonstrated that **1a** and **1b** are polymorphic when grown from solution in the presence of polymer heteronuclei. The single-crystal X-ray structure of form II of **1b** revealed the formation of supramolecular heterosynthons between CBZ and saccharin molecules as shown in Figure 14, in contrast to the amide–amide dimers observed in Form I.

Polymorphs of the Carbamazepine:Isonicotinamide 1:1 Co-crystal (1c) (100)

Horst and coworkers investigated two polymorphs of **1c** through a solvent-mediated transformation process in which a dry mixture of the pure components was suspended in ethanol. Co-crystals with two different morphologies and stabilities were obtained. Metastable, needle-like crystals (Form II) were obtained first followed by stable plate-like single crystals (Form I). Single-crystal X-ray structure determination revealed that Form I is constituted of chains of alternating dimers of CBZ and dimers of isonicotinamide, whereas the powder X-ray diffraction pattern of Form II suggests that it is isostructural to the known 1:1 stoichiometric co-crystal of CBZ and nicotinamide (i.e., centrosymmetric CBZ dimers hydrogen bonded to chains of nicotinamide molecules). Figure 15 compares the crystal structures of the two polymorphs of **1c**.

The ternary phase diagram reveals that excess isonicotinamide favors formation of co-crystals, whereas 1:1 stoichiometry affords either CBZ crystals or mixtures of CBZ and co-crystals. Generally APIs are slightly or moderately soluble

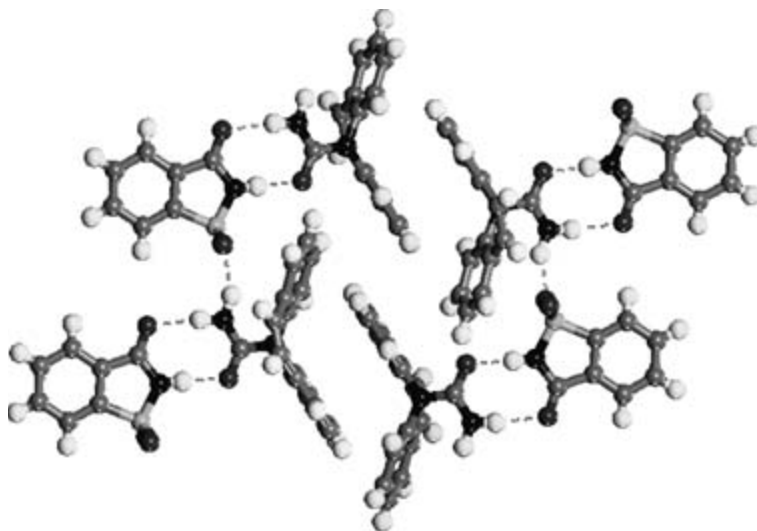


FIGURE 14 Molecular packing in CBZ:saccharin form II co-crystal.

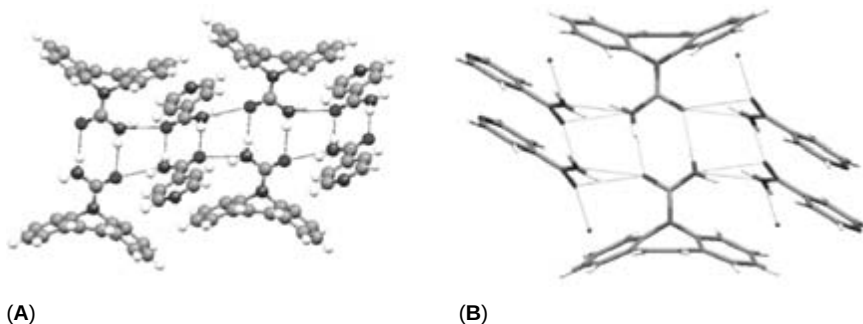


FIGURE 15 Single-crystal X-ray structure of CBZ:isonicotinamide co-crystals: (A) Form I; (B) proposed structure of Form II, showing the principal hydrogen bonding synthons.

in solvents acceptable in pharmaceutical industries. Taking this into consideration a typical schematic phase diagram for an API (A), a co-crystal former (B), and solvent is as shown in Figure 16. This phase diagram highlights how solvent-mediated transformations such as reaction crystallization can offer a simple, flexible, and adaptable method for identifying the presence of co-crystal polymorphs.

Pharmaceutical Co-crystals of Fluoxetine Hydrochloride (Prozac®) (48)

Fluoxetine hydrochloride (fluoxetine HCl) is the API found in the common anti-depressant drug Prozac®. It is a solid under ambient conditions, and only one crystalline phase is known. Childs et al. (48) demonstrated the preparation of co-crystals of

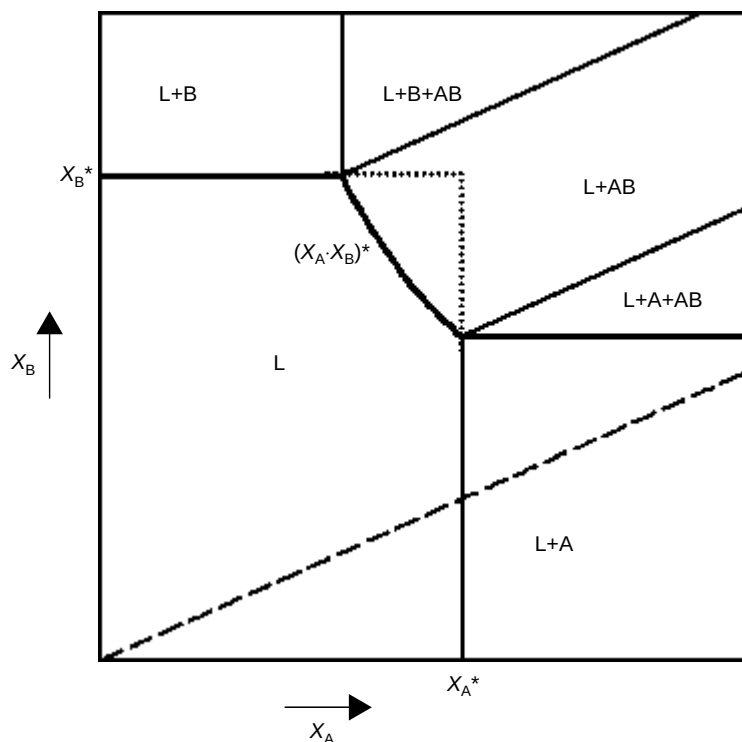


FIGURE 16 A schematic phase diagram for slightly soluble components and co-crystal. In the region L+AB co-crystallization occurs and ultimately equilibrium is reached between the AB co-crystal and the solution. In the regions L+A+AB and L+B+AB both the co-crystal and a pure component crystal phase are formed and the system moves to the three-phase equilibrium point where the two solubility lines meet. The dashed line indicates a 1:1 stoichiometric solution.

fluoxetine HCl using pharmaceutically acceptable carboxylic acids that form hydrogen bonds with the chloride ions. That chloride is perhaps the most preferred anion for generating salts of APIs makes co-crystals of fluoxetine HCl prototypal for many APIs. Fluoxetine HCl was co-crystallized with benzoic acid (1:1), succinic acid (2:1), and fumaric acid (2:1) via traditional evaporation techniques. For all three co-crystals, the carboxylic acid was found to hydrogen bond to the chloride ion, which in turn, interacted with the protonated amine. Powder dissolution experiments were carried out in water for the three novel co-crystals, resulting in a spread of dissolution profiles (Fig. 17). The fluoxetine HCl:benzoic acid co-crystal was found to have a decrease in aqueous solubility of ca. 50%, and the fluoxetine HCl:fumaric acid co-crystal had only a slight increase in aqueous solubility. However, the fluoxetine HCl:succinic acid co-crystal exhibited an approximately two-fold increase in aqueous solubility after only five minutes. The dissolution profile is consistent with the complex between succinic acid and fluoxetine HCl falling apart in solution to generate its pure components after about one hour. An intriguing aspect of this study is that by simply hydrogen bonding a hydrochloride salt of an API with similar

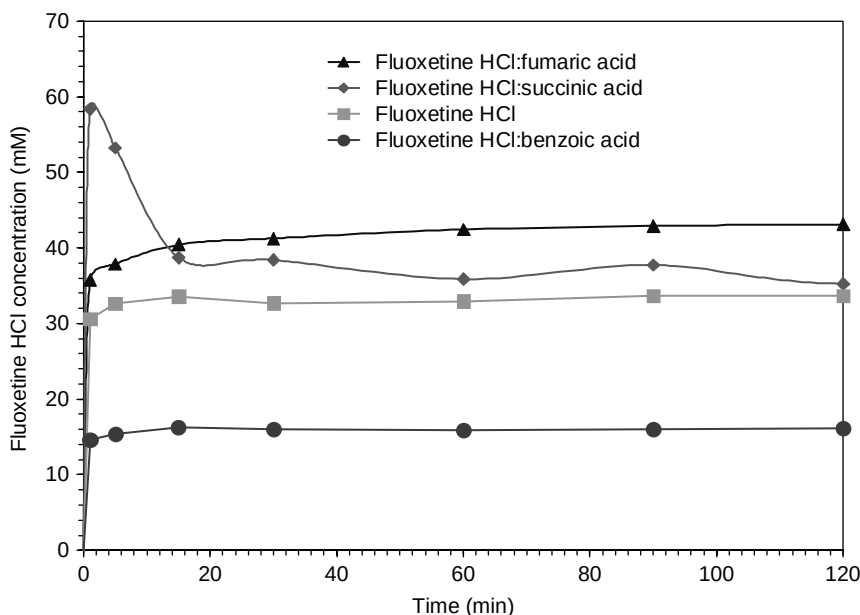


FIGURE 17 Powder dissolution profile for various forms of fluoxetine HCl illustrate how intrinsic dissolution can be modified through co-crystallization.

co-crystal formers one can generate distinctively different dissolution profiles in a manner that could not be achieved through, for example, particle size control.

Pharmaceutical Co-crystals of Itraconazole (Sporanox®) (47)

Itraconazole (Fig. 18) is an extremely water-insoluble (aqueous solubility is estimated at ~ 1 ng/mL at neutral pH and ~ 4 μ g/mL at pH 1) (101) triazole antifungal agent. It is administered both orally and intravenously. The oral administration is formulated with the amorphous form of itraconazole coated on the surfaces of sucrose beads of 0.4–0.5-mm diameter and marketed as Sporanox® capsules. In addition, co-administration of acidified HP- β -cyclodextrin beverages with Sporanox® capsules is required to achieve the maximal absorption of the API, even though such a co-administration can cause diarrhea.

Intriguingly, a survey of the patent literature revealed that there is no data available on crystalline salts of itraconazole, even though salt formation using itraconazole and an acidic salt former would seem to be a logical approach to improve the absorption properties of the API. Crystalline phases of itraconazole can be crystal engineered by introduction of additional molecules to match hydrogen-bond donors and acceptors. The 2:1 co-crystal of itraconazole and succinic acid is shown in Figure 19, and it reveals hydrogen bonding via carboxylic acid-triazole supramolecular heterosynthons. The aqueous dissolution of itraconazole co-crystals was studied; two of the co-crystals exhibit a dissolution profile more akin to the Sporanox® form than to the crystalline form of pure API. In a further pharmacokinetic study of itraconazole co-crystals, it was revealed that the co-crystal formulation of the API

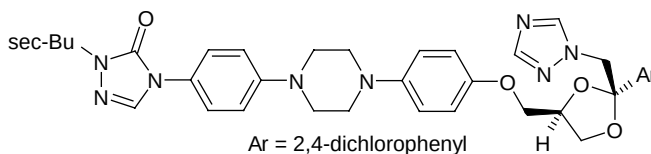


FIGURE 18 Molecular structure of itraconazole.

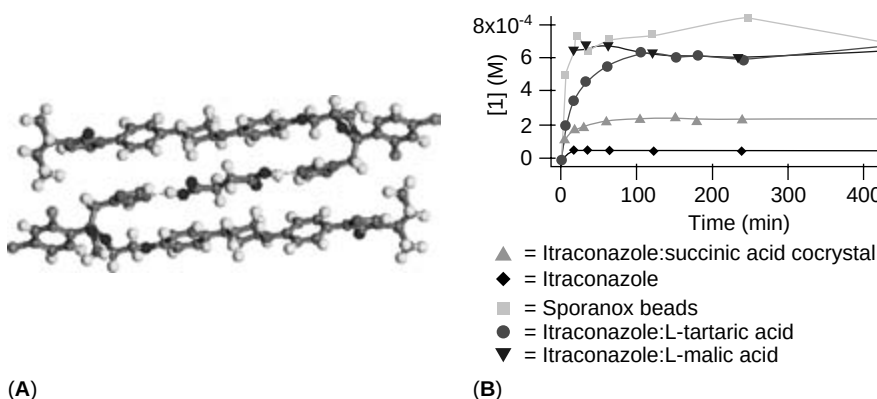


FIGURE 19 (A) The 2:1 co-crystal of itraconazole and succinic acid forms a trimeric unit. (B) Dissolution profiles in 0.1N HCl at 25°C.

gives similar oral bioavailability to the Sporanox[®] form in the animal trial using a dog model.

Pharmaceutical Co-crystals of Sildenafil (Viagra[®]) (64)

Sildenafil, 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4-3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulphonyl]-4-methylpiperazine [Fig. 20(A)] is a drug utilized in the treatment of a number of disorders including pulmonary arterial hypertension, stable, unstable, and variant angina, congestive heart failure, atherosclerosis, conditions of reduced blood vessel patency, peripheral vascular disease, bronchitis, chronic and allergic asthma, as well as male erectile dysfunction and female sexual disorders. Sildenafil is available as a citrate salt form, and has been commercially developed and marketed by Pfizer under the trademark Viagra[®]. Although it is available as a salt form it is moderately soluble in water and thus exhibits erratic or incomplete absorption, low bioavailability, and slow onset of action. It has been observed that sildenafil in a pharmaceutical co-crystal form could provide an improved solubility of the API under acidic conditions. In addition, such an improvement of solubility of sildenafil could be particularly advantageous for its orally administrable formulation. Sildenafil has been successfully co-crystallized with acetylsalicylic acid (1:1 molar ratio) by slurry or under reflux conditions. The crystal structure of the co-crystal of sildenafil and acetylsalicylic acid has been determined

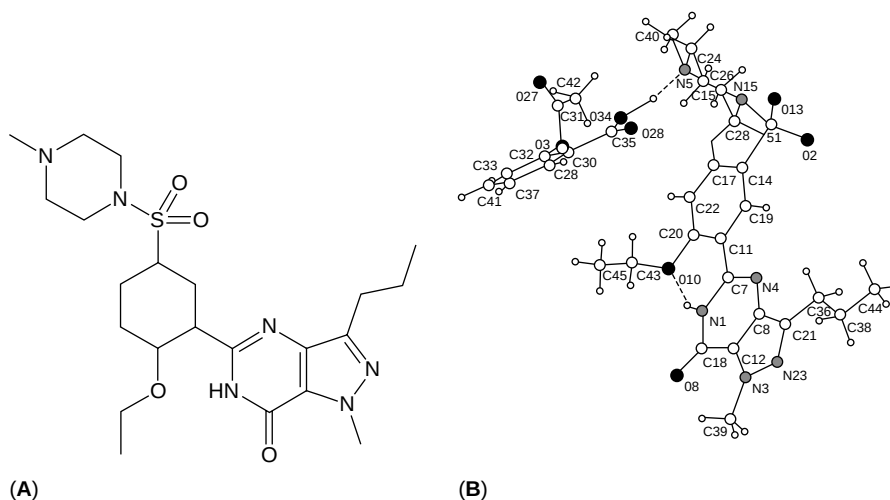


FIGURE 20 (A) Molecular structure of sildenafil. (B) Crystal structure of 1:1 sildenafil:acetylsalicylic acid co-crystal.

by single-crystal X-ray diffraction [Fig. 20(B)], and in addition, the composition of matter was confirmed by powder X-ray diffraction and infrared spectrometry. The melting point of the co-crystal is approximately 143°C, and it remains thermodynamically stable up to ca. 165°C. An intrinsic dissolution study in simulated gastric body fluid (pH = 1.2) shows that the co-crystal exhibits an intrinsic dissolution rate (IDR) of ca. 11.75 mg·min⁻¹ cm⁻² versus 6.64 mg·min⁻¹ cm⁻² for sildenafil citrate under the same conditions.

Pharmaceutical Co-crystals of 2-[4-(4-Chloro-2-Fluorophenoxy)Phenyl]Pyrimidine-4-Carboxamide (SCB), a Sodium Channel Blocker (49)

2-[4-(4-Chloro-2-fluorophenoxy)phenyl]pyrimidine-4-carboxamide (SCB) is a BCS class II drug that belongs to a class of sodium channel blockers and was developed as a potential drug candidate for the treatment or prevention of surgical, chronic, and neuropathic pain. SCB was observed to exhibit low in vitro dissolution, and upon oral administration to dogs it showed low in vivo plasma concentration. Efforts to increase the bioavailability of SCB by forming salts, solvates, hydrates, polymorphs, or amorphous material failed to produce a viable solid form for drug formulations. Hence, co-crystallization was used to improve the pharmaceutical properties of SCB. Co-crystal screening was carried out employing two methods: melt crystallization or highly saturated solution. A total of 26 carboxylic acids were screened out of which a co-crystal containing SCB and glutaric acid in 1:1 molecular ratio was identified and characterized by single-crystal X-ray analysis (Fig. 21), **5.5** can be scaled up in gram quantities, is non-hygroscopic, and chemically and physically stable to thermal stress.

The intrinsic dissolution rate profile suggests that the co-crystal increases the in vitro rate of dissolution by 18 times when compared to the pure API. Single-dose

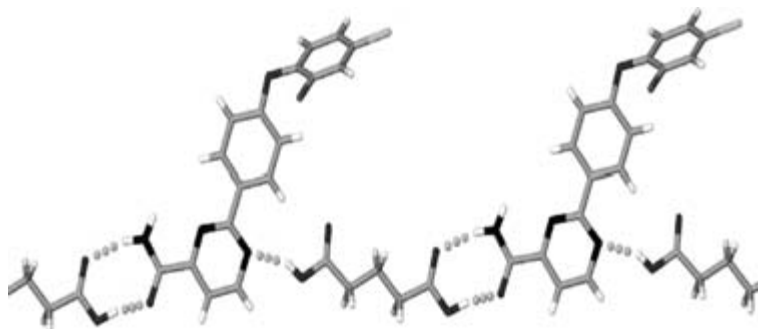


FIGURE 21 Hydrogen bonding in co-crystal **5** showing the interactions between the carboxylic acid groups of glutaric acid molecules and the amide and pyridine groups of the drug molecules, SCB.

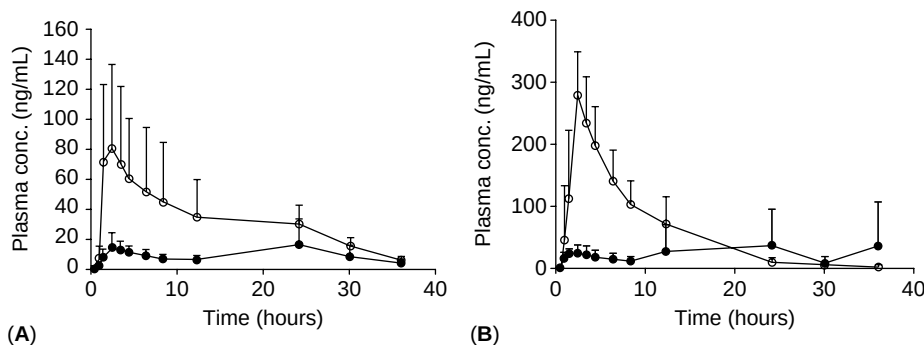


FIGURE 22 Dog plasma concentration with time for (A) 5 mg/kg dosing and (B) 50 mg/kg of API, SCB (solid circles) and co-crystal (open circles). Error bars show +1 standard deviation.

bioavailability studies on dogs confirmed that the co-crystal improved the plasma area under the curve (AUC) values by three times at both low (5 mg/kg) and high dose (50 mg/kg) as shown in Figure 22.

Pharmaceutical Co-crystals of “AMG 517” (102)

Transient receptor potential vanilloid 1 or TRPV1 is a nonselective ligand-gated cation channel that can be activated by a wide variety of external and internal stimuli such as a temperature greater than 43°C, an acidic medium or a neurotransmitter such as anandamide, or the capsaicin-active components in chilli pepper. TRPV1 receptors are found in central and peripheral nervous systems, and they are responsible for transmission and modulation of pain along with the integration of diverse painful stimuli. AMG 517 is a transient receptor potential vanilloid 1 antagonist that was developed by Amgen for the treatment of acute and chronic pain. The free base of AMG 517 is associated with problems such as insolubility in water and buffers at physiological pH. AMG 517 has three isolated forms: a monohydrate (stable for at

least three years at ambient temperature and humidity), a metastable nonsolvated form, and a stable free base. In addition numerous salts and solvates have been prepared, isolated, and characterized. However, the salts disproportionate in aqueous medium and convert into the hydrated form of AMG 517. Bak and coworkers (102) investigated 10 co-crystals of AMG 517 with the following carboxylic acids: sorbic acid, benzoic acid, *trans*-cinnamic acid, 2,5-dihydroxybenzoic acid, glutaric acid, glycolic acid, *trans*-2-hexanoic acid, 2-hydroxycaproic acid, L(+)-lactic acid, and L(+)-tartaric acid. The hydrogen bonding in two co-crystals is as shown in Figure 23. The physicochemical properties such as particle size, solubility, stability, hygroscopicity, thermal behavior, and structural characteristics of these co-crystals were studied in detail.

Solubility experiments on the AMG 517:sorbic acid co-crystal showed that the co-crystal has better initial solubility compared to AMG 517; however, it converts back to a form of the free base hydrate after prolonged slurrying in FaSIF (fasted simulated intestinal fluid, pH 6.8). Pharmacokinetic studies of the co-crystal in rats using 10% (w/v) Pluronic F1081 in OraPlus1 suspensions revealed that a 30 mg/kg dose in suspension had comparable exposure to a 500 mg/kg dose of the free base. Further solubility measurements on AMG 517 co-crystals with glutaric acid, glycolic acid, *trans*-2-hexanoic acid, lactic acid, and benzoic acid showed that the co-crystals reach a maximum solubility within 1–2 hours and, similar to the sorbic acid co-crystal, the dissolution profile indicates conversion of the co-crystals to AMG 517 hydrate.

Correlations between the melting point of the co-crystal formers and the co-crystals and between the melting point and solubility of the co-crystals were also addressed. The melting point of all co-crystals was between the co-crystal formers and AMG 517, and linear regression indicated that the melting points of co-crystal formers and co-crystals are directly proportional with 78% correlation. However, the melting point and solubility of the co-crystals are related with only 55% correlation.

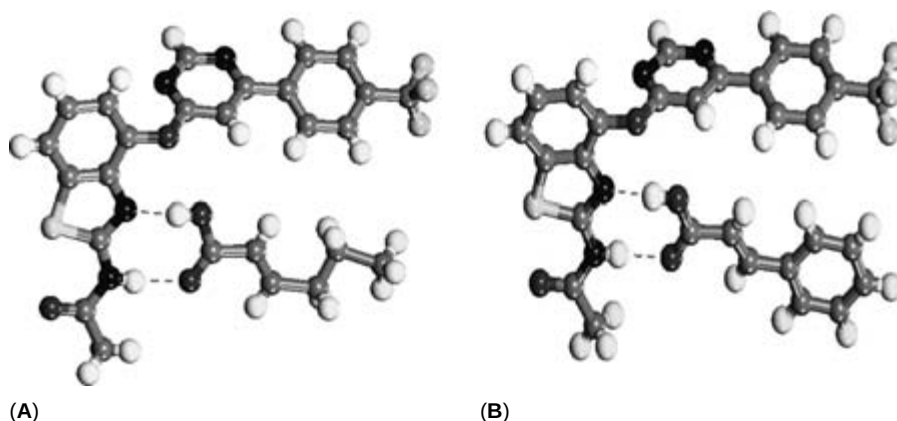


FIGURE 23 Molecular recognition between AMG 517 and (A) *trans*-cinnamic acid (B) *trans*-2-hexanoic acid.

Co-crystals of Melamine and Cyanuric acid (103)

In early 2007 there was a major pet food recall by the FDA based on the receipt of numerous complaints regarding the deaths of animals after ingestion of a variety of pet food products. It was reported that the majority of these deaths were associated with acute renal failure in these animals. Melamine, 2,4,6-triamino-1,3,5 triazine, was the primary suspect for contamination in the tainted pet food products because melamine had been intentionally added to raise the apparent protein content in the pet food. However, melamine is considered relatively nontoxic [LD_{50} values of 3100mg/kg (rat male) and 3900mg/kg (rat female)] and oral administration of 125mg/kg body weight of melamine to dogs leads to diuretic effects but is not fatal. Indeed, the quantity of melamine observed in the pet food was not at the lethal dosage. In the course of the pet food recall investigation, cyanuric acid, another relatively nontoxic compound, was also identified in the pet food as a co-contaminant. Although melamine and cyanuric acid are relatively safe individually, no data could be found in the literature that determines the potential toxicity of melamine and cyanuric acid in combination. From the crystal engineering viewpoint, melamine and cyanuric acid (1:1 molar ratio) form an extensive two-dimensional networks in the solid state based on robust three-point molecular recognition (Fig. 24) and

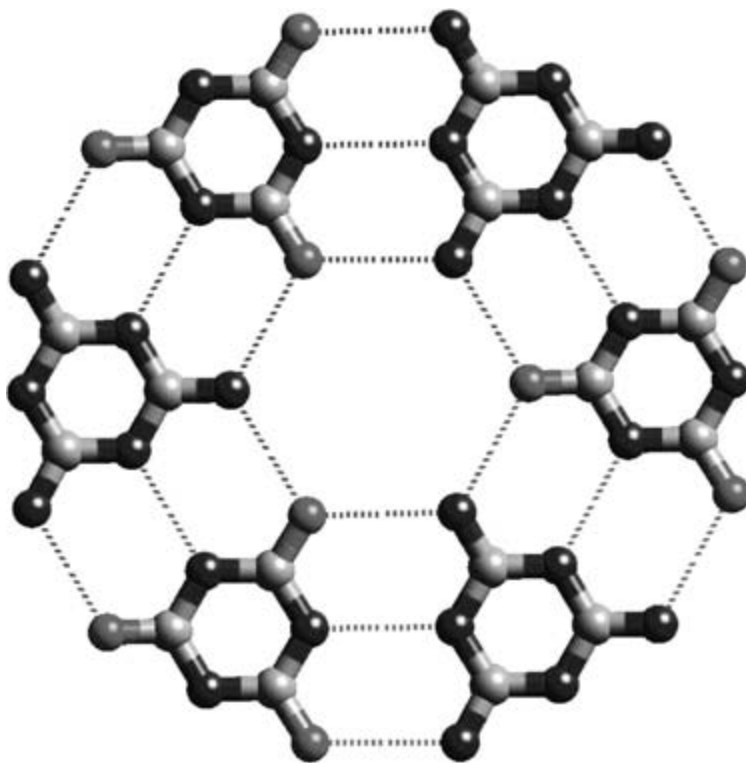


FIGURE 24 The rosette network formed between melamine and cyanuric acid.

the resulting melamine:cyanuric acid co-crystal is, perhaps unsurprisingly, highly insoluble in water.

Puschner et al. investigated the toxicity of melamine and cyanuric acid individually and also in combination when administered to cats, and demonstrated clinical pathologic changes, gross or histologic lesions, associated with the contaminants in the recalled pet food. The oral administration of 181 mg/kg body weight of melamine alone at 0.5% and 1% of their diet to three cats for 11 days did not show any evidence of renal failure. Similar results were obtained on administration of cyanuric acid alone in the diet at 0.2%, 0.5%, and 1% dosage respectively. However, it was observed that a single oral exposure of cats to the combination of melamine and cyanuric acid at a dosage as low as 32 mg/kg body weight leads to acute renal failure. A study conducted at the Bergh Memorial Animal Hospital in New York revealed that co-crystals blocked the tubes leading from the kidneys to the bladder in one cat. Thus, it seems clear that the formation of a low solubility co-crystal of melamine and cyanuric acid is responsible for these incidents. This case study of melamine:cyanuric acid co-crystals might be the first example of co-crystals altering clinically relevant physical properties in a negative manner.

POLYMORPHISM IN CO-CRYSTALS AND PHARMACEUTICAL CO-CRYSTALS

Polymorphism (19,104–105), the existence of different crystal forms of the same compound, is long recognized from a scientific perspective. Mitscherlich introduced the concept of isomorphism, an important development in structural chemistry, in 1819 (106), and in 1932 Wöhler and Liebig (107) described dimorphism of benzamide, perhaps the first example of a polymorphic organic substance. That polymorphism can impact physical properties is perhaps best exemplified in elements, where it is known as “allotropy,” as exemplified by carbon, which exhibits three crystal forms with dramatically different physical properties: graphite, diamond, and fullerenes. The phenomenon of polymorphism is now well appreciated both academically and industrially because it impacts preparation and formulation of specialty chemicals, pharmaceuticals, dyes, explosives, and foods (108). McCrone’s definition (109) of polymorphism, that is, “a solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of the molecules of that compound in the solid state,” is apt in the context of this chapter’s focus upon structural chemistry.

Crystallization of polymorphs is governed by a combination of thermodynamic and kinetic factors (110). The occurrence of polymorphs often follows Ostwald’s rule of stages (111), which states that the meta-stable form is often obtained first and the stable form crystallizes subsequently. Depending upon the arrangement of molecules and/or conformation adopted by the functional groups present in the molecule, polymorphs may be classified into several categories: that is, conformational polymorphs (112), concomitant polymorphs (107,113) structural polymorphs (114), and configurational polymorphs (115). Nevertheless, a CSD analysis tabulated in Table 1 reveals that polymorphism is not prevalent in either single-component crystals (1,882/134,086) or in co-crystals (40/2,083).

This chapter focuses upon pharmaceutical co-crystals, and we present herein a structural analysis of selected co-crystals that exhibit polymorphism in addition to the polymorphic co-crystals of CBZ discussed earlier in the section “Pharmaceutical Co-crystals of Carbamazepine (Tegretol®).”

TABLE 1 Cambridge Structural Database Statistics of Polymorphic Single and Multicomponent Crystal Structures

	Number of entries	Percentage of organic structures
Organic crystal structures	186,053 ^a	100 ^d
Single-component molecular organic structures	134,086	72.1 ^d
Single-component polymorphic structures	1,882 ^b	1.4 ^e
Hydrates	13,843	7.4 ^d
Co-crystals	2,083 ^c	1.1 ^d
Polymorphic co-crystals	40	1.9 ^f

CSD August 2008 update, 456,628 entries.

^aStructures containing Na, Li, K, As were excluded.

^bOnly one refcode was counted for each polymorphic compound.

^cCo-crystals sustained by strong hydrogen bonds.

^dPercentages with respect to organic crystal structures only.

^{e,f}Percentages with respect to 134,086 and 2083 sub-totals, respectively.

1:1 Co-crystals of Caffeine and Glutaric Acid (EXUQUJ–EXUQUJ01)

The crystal structures of the polymorphic forms of 1:1 co-crystals of caffeine and glutaric acid were described by Jones et al. (42) in 2004. Both crystal forms, rods and blocks, were obtained concomitantly from a solution containing a 1.3:1 ratio of caffeine and glutaric acid, respectively. The “rods” are monoclinic (Form I), whereas the “blocks” are triclinic (Form II). Both Forms I and II exhibit identical secondary architecture because they form sheets resulting from hydrogen bonds (O–H...N) between one of the –COOH moieties of glutaric acid and the free aromatic nitrogen atom (N_{arom}) of caffeine. The two polymorphs exhibit differences in the torsion angle of the methylene carbon atoms of glutaric acid (Fig. 25). The authors noted how solvent-drop grinding could be used to afford a co-crystal polymorph exclusively: dry grinding and solvent-drop grinding using nonpolar solvents such as *n*-hexane, cyclohexane, or heptane yielded only Form I; solvent-drop grinding using polar solvents such as chloroform, dichloromethane, acetonitrile, and water produced only Form II.

2:1 Co-crystals of 4-Cyanopyridine and 4,4'-Biphenol (TEHNAW–TEHNAW01)

Zaworotko et al. (116) reported that crystallization of a 2:1 ratio of 4-cyanopyridine and 4,4'-biphenol in methanol:ethyl acetate afforded concomitant polymorphs of the 2:1 co-crystal of 4-cyanopyridine and 4,4'-biphenol. The single-crystal X-ray structures revealed that Form I (irregular hexagonal plates) and Form II (parallel-piped plates) exhibit conformational differences in the 4,4'-biphenol molecules (Fig. 26), but O–H...N supramolecular heterosynthon sustain both forms, suggesting that O–H...N(pyridine) supramolecular heterosynthons are favored over competing O–H...N(cyano) supramolecular heterosynthons. Form I crystallized in *C2/c* with half a 4,4'-biphenol molecule and one 4-cyanopyridine molecule in the asymmetric unit, whereas Form II crystallized in *P2₁/n* with four 4-cyanopyridine and two 4,4'-biphenol molecules in the asymmetric unit. Form I can be obtained by dissolving Form II in MeOH, EtOAc, or acetone where Form II can be obtained by solvent drop grinding of the starting materials or Form I.

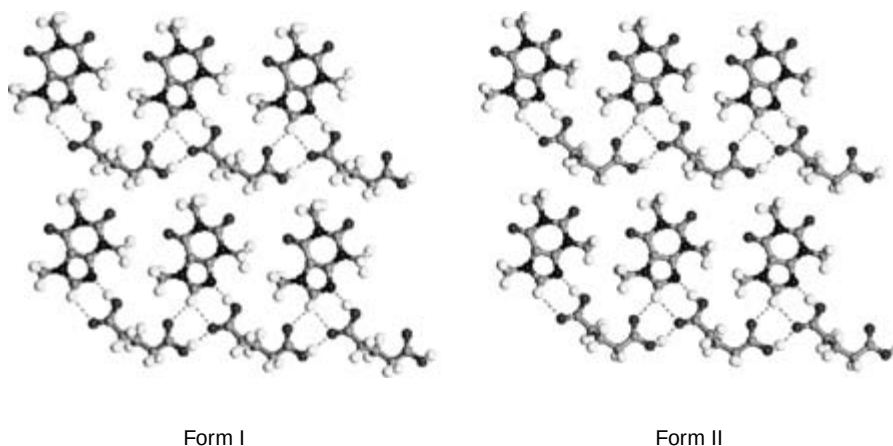


FIGURE 25 Crystal structures of monoclinic (Form I) and triclinic (Form II) forms of caffeine:glutaric acid co-crystals. Although the crystal packing is similar there is a difference in the torsion angle of the methylene carbon atoms of glutaric acid.

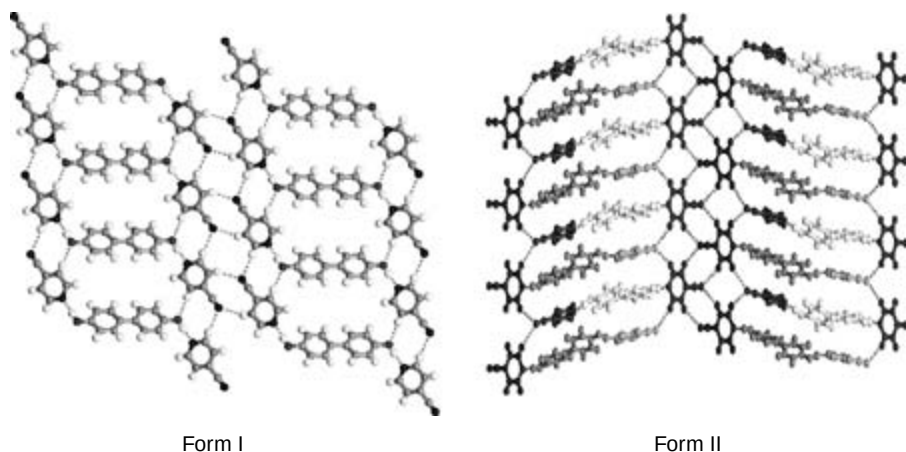


FIGURE 26 Crystal packing of Form I and Form II of the 2:1 co-crystal of 4-cyanopyridine: 4,4'-biphenol.

1:1 Co-crystals of Hydroquinone/*p*-Benzoquinone (QUIDON–QUIDON02) (117–119)

Quinhydrone is a 1:1 co-crystal of hydroquinone and *p*-benzoquinone. It is the prototypical co-crystal that was first reported in 1844, and it is also the earliest entry of a co-crystal in the CSD for which atomic coordinates are available. Quinhydrone exists in two polymorphic crystal forms: monoclinic and triclinic. The monoclinic form ($P2_1/c$ space group, QUIDON02) was reported by Matsuda et al. in 1958.

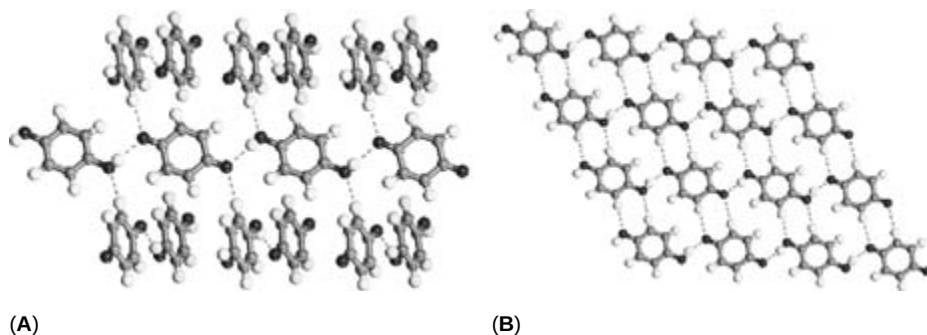


FIGURE 27 Crystal structures of the (A) monoclinic and (B) triclinic forms of quinhydrone.

The crystal structure reveals that both hydroquinone and *p*-benzoquinone molecules reside on an inversion center generating linear tapes through O—H...O and C—H...O hydrogen bonds. Such tapes are further connected to generate a 3D network as illustrated in Figure 27(A). In the triclinic polymorph (*P*-1 space group, QUIDON), H-bonded tapes are also formed; however, the tapes are packed parallel to generate molecular sheets [Fig. 27(B)]. The crystal packing of Forms I and II is therefore different although the supramolecular synthons remain the same.

Polymorphism in the 1:1 Co-crystal of *N,N*-Bis(4-bromophenyl)melamine/5,5-Diethylbarbituric acid (JICTUK01–JICTUK10)

In 1994, Whitesides and coworkers (34) reported polymorphism in 1:1 co-crystals of *N,N*-bis(4-bromophenyl) melamine and 5,5-diethylbarbituric acid. Form I crystallized in a monoclinic crystal system (*P*2₁/*n*, JICTUK01), whereas Form II crystallized in a triclinic system (*P*-1, JICTUK10). Both polymorphs form linear tapes through three-point molecular recognition involving N—H...O and N—H...N hydrogen bonds. Once again, the same supramolecular synthons are observed in both crystal forms; however, the rotation of the phenyl ring along the C—N bond in Form II generates a different arrangement of linear tapes. (Fig. 28).

1:1 Co-crystals of Oxalic Acid and Iso-nicotinamide (ULAWAF01–ULAWAF02)

The crystal structures of the polymorphs of the 1:2 co-crystal of oxalic acid and iso-nicotinamide were reported by Wilson and coworkers in 2007 (120). The co-crystal exist in two forms: Form I crystallizes in the monoclinic space group *C*2/*c* as earlier reported by Nangia and coworkers (75b), whereas Form II crystallizes in the triclinic space group *P*-1. These polymorphs can be obtained concomitantly from 1:1 ethanol:water. The fundamental difference between the two polymorphs is a *cis*/*trans* isomerism observed in the hydroxyl groups of oxalic acid. Form I exhibits the *cis*-configuration, whereas Form II contains the *trans*-configuration. The oxalic acid and iso-nicotinamide molecules form trimeric units sustained through O—H...N hydrogen bonds. These trimeric units are, in turn, connected through centrosymmetric amide dimers between iso-nicotinamide molecules, and thereby forming tapes that H-bond to form sheets. The overall crystal packing in both Form I and Form II can be described as stacked sheets (Fig. 29).

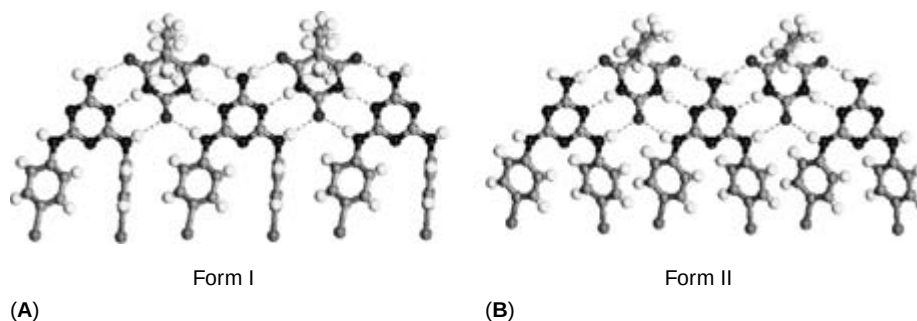


FIGURE 28 Linear tapes as observed by *N,N*-bis(4-bromophenyl)melamine and 5,5-diethylbarbituric acid co-crystals: (A) JICTUK01; (B) JICTUK10. The supramolecular synthons are identical in both forms and only salient difference between the structures lies with the orientation of the phenyl rings.

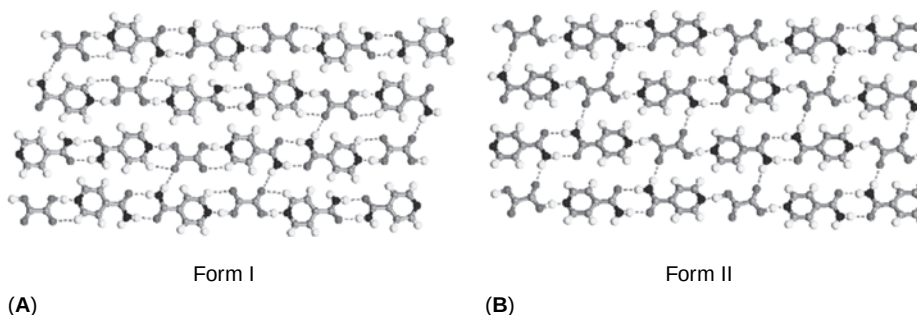


FIGURE 29 Crystal structures of Form (A) I and (B) Form II of the 1:1 oxalic acid:iso-nicotinamide co-crystal.

In summary, the series of polymorphic co-crystals presented herein is representative of the limited information within the CSD that addresses polymorphism in co-crystals and indicates that polymorphism in co-crystals is not typically the result of competing supramolecular synthons. However, the paucity of data precludes sweeping conclusions about polymorphism in co-crystals. That co-crystals are attracting wider interest means that there will likely be an increase in the number of systematic studies devoted to co-crystals and a corresponding increase in the number of polymorphic co-crystals. Intriguingly, this increase in the number of studies on co-crystals has afforded several recent reports of failed co-crystallizations that have instead resulted in new polymorphs of long known compounds such as benzidine and aspirin. Bernstein and coworkers (121) recently reported four new forms of benzidine obtained accidentally in the attempts to co-crystallize benzidine with different co-crystal formers. Zaworotko and coworkers (96b) first obtained Form II of aspirin during co-crystallization experiments involving aspirin and the antiseizure compound levetiracetam from hot acetonitrile. Such generation of new polymorphs could be a result of the creation of a new medium for crystallization via

the presence of co-crystal formers, and if so, it has implications for high-throughput screening as a methodology for discovering new polymorphs.

CONCLUSIONS

The pharmaceutical industry has only recently paid more than passing attention to pharmaceutical co-crystals, but there are several conclusions that can already be drawn:

- New pharmaceutical co-crystals should be patentable as novel crystal forms because their crystal structures are not obvious until prepared.
- New pharmaceutical co-crystals can be generated via rational design and multiple synthetic approaches for nearly all APIs by using crystal engineering strategies.
- Pharmaceutical co-crystals significantly diversify the number of crystal forms available for an API and always modify the physical properties of the API, thereby offering an opportunity to solve clinical problems through control of physicochemical properties of the API.

However, this does not mean that pharmaceutical co-crystals will become commonplace in marketed drug products because there remain practical handicaps that must be overcome such as the costs associated with the significant amounts of additional experimental research that are required and the risks associated with a patent and regulatory landscape that is presently in flux. As stated by Remenar (66), "If making a co-crystal of a specific molecule helps to enable an application or solve a problem, then it's the right thing to do, but you don't design a molecule in the real world simply because you are looking for a co-crystal strategy." This is probably a realistic assessment of where matters stand today in terms of pharmaceutical co-crystals and new drug development. However, there are certain niches where pharmaceutical co-crystals might be expected to find a role in drug development including high-throughput solid-state synthesis, new formulations of existing APIs, and processing of "difficult" APIs that are hard to crystallize and/or purify.

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Sisir Bhattacharya**Department of Pharmaceutics, University of Minnesota, Minneapolis, Minnesota, U.S.A.***Harry G. Brittain***Center for Pharmaceutical Physics, Milford, New Jersey, U.S.A.***Raj Suryanarayanan***Department of Pharmaceutics, University of Minnesota, Minneapolis, Minnesota, U.S.A.***INTRODUCTION**

The different physical forms of a compound can exhibit pronounced differences in physicochemical properties. The term “physical form” encompasses polymorphs, solvates (also referred to as solvatomorphs), the non-crystalline (amorphous) form, and partially crystalline forms. Although numerous analytical techniques are available to characterize the physical forms of pharmaceutical compounds, this chapter will focus on thermal and crystallographic characterization techniques. While discussing the analytical techniques and their applications, we will use the term “polymorphism” in its broadest sense, with the understanding that with appropriate modifications, the techniques can often be extended for the characterization of the other physical forms of pharmaceuticals. Although crystallography (through crystal structure) provides the most definitive evidence of polymorphism, thermoanalytical, spectroscopic, and microscopic techniques are often used in a complementary fashion for the characterization of polymorphs.

Comprehensive and in-depth discussions of different thermoanalytical techniques can be found in the literature (1,2), and this chapter will specifically discuss the applications of thermoanalytical and crystallographic techniques in the characterization of pharmaceuticals. The specific thermoanalytical techniques to be discussed are differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and hot-stage microscopy (HSM). Several recent reviews and book chapters have covered this topic, and the examples presented are designed to complement the information in the literature (1–4). In the second part of the chapter, applications of X-ray powder diffractometry (XRD) (5,6) are provided. Finally, applications of variable-temperature XRD (VT-XRD) are discussed, specifically to monitor phase transitions as a function of temperature.

THERMOANALYTICAL TECHNIQUES

Thermal analysis methods can be conveniently defined as those techniques in which some property of the analyte is measured as a function of an externally applied temperature. In most applications, one effects a linear increase in the temperature of the sample while the property of interest is recorded on a continuous basis. Thermoanalytical methods are used to monitor endothermic processes (i.e., melting, boiling,

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sublimation, vaporization, desolvation, solid–solid phase transitions, and chemical degradation) as well as exothermic processes (i.e., crystallization and oxidative decomposition). Thermal methods are routinely used for the characterization of polymorphs, and in light of the experimental simplicity, often constitute the first line of techniques used to understand the polymorphic space of a system.

Differential Scanning Calorimetry (DSC)

In DSC, which is the most widely used technique for thermal characterization of pharmaceuticals, the analyte is subjected to a controlled temperature program and the temperature and the heat flow associated with a thermally induced transition is measured.

DSC has been developed along two lines: power-compensation DSC and heat-flux DSC. In power-compensation DSC, separate heating elements are in contact with the sample and reference cells, with the rate of heating by these elements being controlled and measured. Thus the sample and reference are maintained at the same temperature and the heat flow required to accomplish this is measured. The other methodology is that of heat-flux DSC, where the sample and reference cells are heated by the same element, and one monitors the direction and magnitude of the heat being transferred between the two.

A detailed theory of DSC is outside the scope of this chapter and has been discussed elsewhere (1,2). Briefly, the basic principle behind a change in the baseline of a typical DSC plot (power vs. temperature), signifying a thermal event, is a change in the heat capacity of the system under investigation. The basic equation (4) describing the relationship between the heat flux (Q) associated with a thermal event and the heat capacity (C_p) of the system under investigation is given by:

$$(dQ/dt) = C_p(dT/dt) \quad (1)$$

where dQ/dt represents power at the constant heating or cooling rate, dT/dt . DSC plots are obtained as the differential rate of heating [in units of watts (joules/second) or calories/second] against temperature, and thus represent direct measures of the heat capacity of the sample. The area under a DSC peak is directly proportional to the heat absorbed or evolved by the thermal event, and integration of these peak areas yields the heat of reaction (in units of calories/seconds · gram or joules/seconds · gram).

The effectiveness of DSC to detect various polymorphic forms is illustrated in Figure 1 (7). Profiles 1, 2, and 3 (*from top*) show the reversible transitions between the enantiotropically related forms I and III. The fourth DSC curve shows the melting of form II followed by crystallization and melting of form I. The bottom DSC profile is a heating curve of a mixture of forms II and III, which shows the respective melting endotherms but no transition to form I. This was tentatively attributed to the absence of form I seeds in the mixture of forms II and III, which were concomitantly crystallized as opposed to the pure forms (profiles 1–4), which were crystallized separately using different methods.

Standard Reference Materials (SRMs) are available from the National Institute of Standards and Technology for calibration of temperature and enthalpy of fusion for differential scanning calorimeters. Indium (SRM 2232; melting temperature = 156.6°C; enthalpy of fusion = 28.5 J/g), tin (SRM 2220; melting point = 232.0°C; enthalpy of fusion = 60.2 J/g), and gallium (SRM 2234; melting temperature = 29.8°C;

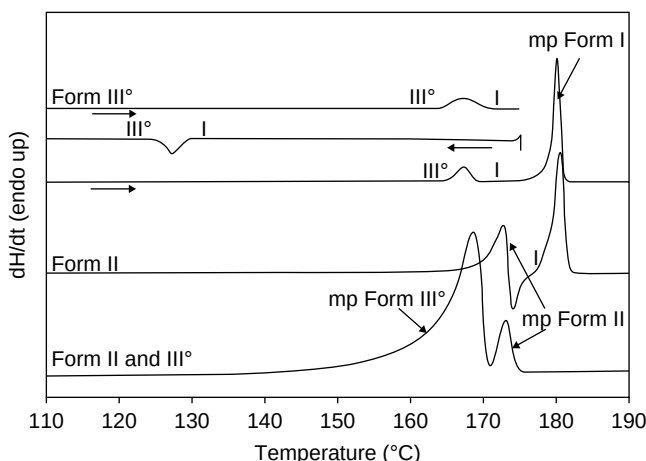


FIGURE 1 DSC profiles of cinacalcet hydrochloride polymorphs. The first (heating), second (cooling), and third (heating) profiles (*from top*) show the solid–solid transition (i.e., III° $\rightarrow$ I); the fourth profile shows melting of form II followed by crystallization and melting of form I; the fifth profile is a heating curve of a mixture of form II and form III° showing respective melting endotherms but no transition to form I signifying absence of form I seeds. *Note:* The direction of endotherm is *up*. *Source:* From Ref. (7).

enthalpy of fusion = 80.1 J/g) are suitable as calibration standards over the range of temperatures of interest in pharmaceutical analyses. Because DSC is also used at sub-ambient temperatures for the characterization of frozen solutions, mercury (SRM 2225; melting temperature = -38.9°C ; enthalpy of fusion = 11.5 J/g) is a suitable calibration standard. Heat capacity calibration in a DSC is usually carried out with synthetic sapphire (SRM 720), copper (SRM RM5), polystyrene (SRM 705a), or molybdenum (SRM 781D2).

Depending on the protocol used for temperature programming, a DSC experiment could be classified as being conventional, modulated, or accelerated (also known as Hyper-DSC®). In the conventional mode, the heating rate is constant and typically ranges from $0.1^{\circ}\text{C}/\text{min}$ to $20^{\circ}\text{C}/\text{min}$. The majority of DSC experiments are carried out in the conventional mode and may require optimization of the sample pan configuration (discussed later) and the experimental parameters to obtain the desired information (3). In Table 1, the effects of operational parameters are outlined.

In modulated DSC (MDSC), a changing (modulated) heating rate is superimposed on a constant average heating rate. The modulation is described by a frequency, determined by specific amplitude ($\pm^{\circ}\text{C}$) and period (seconds), such as $\pm 0.5^{\circ}\text{C}$ every 60 seconds, with the choice of the amplitude and period being dictated by the nature of the transition (8–10). The advantage of MDSC over conventional DSC is that it can separate the reversible heat flow from the non-reversible heat flow (Fig. 2). Reversible heat flow corresponds to events that respond to the changing heating rate (e.g., heat capacity change, melting). On the other hand, non-reversible heat flow events (e.g., crystallization or decomposition) are not affected by changing heating rates.

TABLE 1 Influence of Operational Parameters on the Outcome of DSC Experimental Results

	Effects
Sample size	
Large	Advantages (i) Non-homogeneous samples (ii) Detects “weak” transitions Disadvantages (i) Broad peaks (ii) Temperature of the event may be inaccurate (iii) Poor resolution (iv) Requires slow heating rate
Small	Advantages (i) Sharp peaks (ii) Increased resolution (iii) Permits faster heating rate Disadvantages (i) Decreased sensitivity
Heating rate	
Fast	Advantages (i) Increased sensitivity Disadvantages (i) Decreased resolution (ii) Decreased temperature accuracy
Slow	Advantages (i) Increased resolution Disadvantages (i) Decreased sensitivity

Source: Adapted from Ref. (2).

MDSC has found limited utility in the characterization of polymorphs (11,12). However, the inherent capability of this technique to separate overlapping melting and crystallization events has been demonstrated for the polymorphs of tristearin (13,14). MDSC is especially suited for the characterization of amorphous materials (15,16), and has enabled the quantification of amorphous content in a partially crystalline material (17). Because crystallinity differences could affect material performance, it is important that analysts are aware of low levels of lattice disorder in highly crystalline materials that were brought about by processing and storage conditions. Saklatvala et al. investigated two batches of an investigational crystalline drug, which showed pronounced differences in their stability and water sorption profiles, and MDSC enabled detection and quantification of the amorphous content in the batch characterized by the lower chemical stability and the increased propensity to take up water (18).

Hyper-DSC[®] uses accelerated heating rates (up to 500°C/min) in order to selectively investigate phase transitions. The high heating rates result in selective inhibition of transitions that would normally take place over the longer timescales

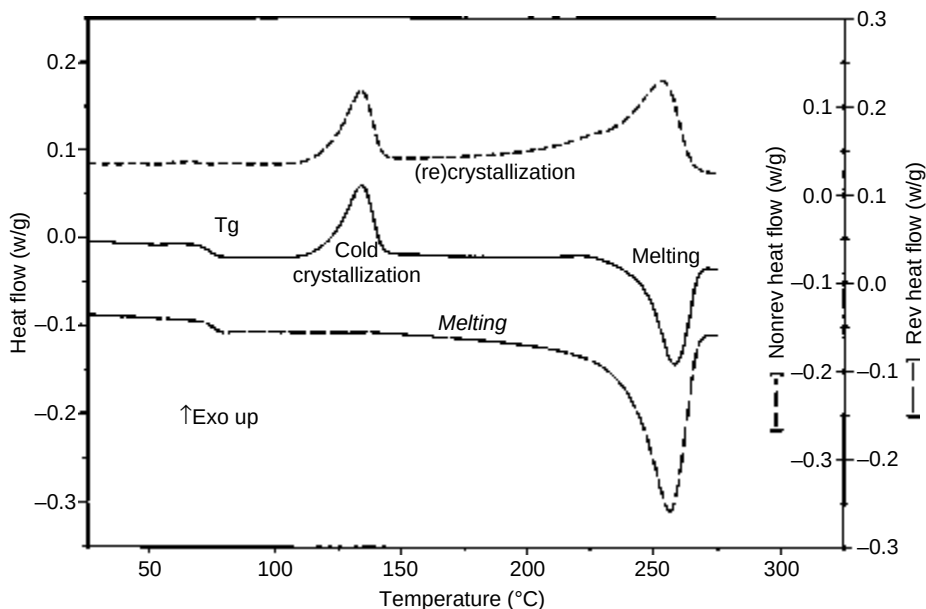


FIGURE 2 Modulated DSC profiles of quenched polyethylene terephthalate, showing the non-reversible (*uppermost trace*), total (*middle trace*), and reversible (*lowest trace*) heat flow curves. The exothermic recrystallization event is hardly discernible in the total heat flow but is prominent in the non-reversible heat flow. *Source:* From Ref. (66).

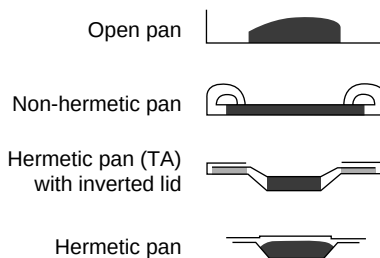


FIGURE 3 Diagrammatic representation of different DSC pan configurations. *Source:* From Ref. (9).

of conventional heating rates. A case in point are the polymorphs of carbamazepine, where a heating rate of 250°C/min was used to prevent simultaneous crystallization of form I during the melting of form III, thereby allowing determination of the enthalpy of fusion of form III (19).

In a typical DSC application, the sample is encapsulated in a pan and the lid is crimped, where the lid can be crimped either in a non-hermetic or in a hermetic manner (Fig. 3). In the hermetically crimped pan, a pinhole can be made in the lid to relieve pressure during the acquisition of the DSC curve. To minimize the head space, the lid can be inverted and then hermetically crimped.

The pan configuration can influence the outcome of a DSC experiment. This is especially true for solvates, because the desolvation kinetics, as well as the physical form of the product phase, can be profoundly influenced by the sample environment. In an open pan, there is no physical barrier to the escape of liberated solvent, and the purge gas (typically nitrogen) will facilitate removal of the liberated solvent. The reasonably unrestricted nature of a non-hermetically crimped pan will also permit ready escape of the liberated solvent. At the other extreme, the liberated solvent cannot escape in a hermetically crimped pan. In a sealed pan with a pinhole, the rate of vapor loss will become appreciable only when the vapor pressure exceeds atmospheric pressure. Different pan configurations, which result in varied desolvation conditions, have been used to generate different polymorphs of anhydrous carbamazepine and trehalose from their respective hydrates (20,21).

Pressure DSC (PDSC) enables DSC experiments to be carried out at elevated pressures as high as 1000 psi. PDSC is useful for the separation of overlapping dehydration and vaporization events that are observed following the dehydration of hydrates. Although the temperature of dehydration is likely to be unaffected by pressure, the boiling temperature of water is pressure dependent and can be ascertained from the Clausius–Clapeyron equation. In addition, the potential interaction of the liberated water with the anhydrous product phase can also be ascertained. Han and Suryanarayanan (22) used PDSC to successfully resolve overlapping dehydration and vaporization transitions observed when carbamazepine dihydrate was heated (Fig. 4, *upper left panel*). The conventional DSC of carbamazepine exhibited two overlapping endotherms over the temperature range of 85°C to 100°C, attributed to dehydration followed by vaporization, which were followed by the melting of non-solvated carbamazepine around 189°C. When the pressure was increased, only a single endotherm attributable to dehydration was observed at approximately 90°C. Interestingly, several thermal events were observed between the dehydration and the melting temperatures.

A limitation of DSC is that although it reveals the existence of thermally induced transitions, the nature of these transitions can be difficult to determine. In an effort to simulate the PDSC experiments, Han and Suryanarayanan (22) heated carbamazepine dihydrate in a “sealed” environment. Following dehydration, the released water caused an increase in pressure by being retained in the holder, although the pressure was uncontrolled. As shown in the *upper right panel* of Figure 4, the carbamazepine polymorphic transitions were further characterized by VT-XRD. The formation of β -carbamazepine at $\sim 90^\circ\text{C}$ was evident in the diffraction patterns on the basis of peaks observed at scattering angles of 13.1, 15.3, and $18.7^\circ 2\theta$. Based on the disappearance of the characteristic peaks of carbamazepine dihydrate (at 8.9 and $12.3^\circ 2\theta$), dehydration appeared to be complete by 100°C . Around 160°C , several characteristic peaks of γ -carbamazepine were observed, indicating a $\beta \rightarrow \gamma$ solid–solid phase transition. Based on the decrease in the intensities of the β -carbamazepine peaks, and the increase in the intensities of the γ -carbamazepine peaks, the $\beta \rightarrow \gamma$ phase transition was accelerated as the temperature was increased to 180°C . These VT-XRD results aided in the interpretation of the DSC results. When DSC was conducted under elevated pressure, usually four endotherms were observed. The first endotherm at $\sim 90^\circ\text{C}$, as explained earlier, is attributed to dehydration. The two overlapping endotherms at $\sim 120^\circ\text{C}$ and 125°C are attributed to the $\beta \rightarrow \gamma$ carbamazepine phase transition and to the vaporization of water. The melting of γ -carbamazepine occurred at $\sim 189^\circ\text{C}$. This scheme is

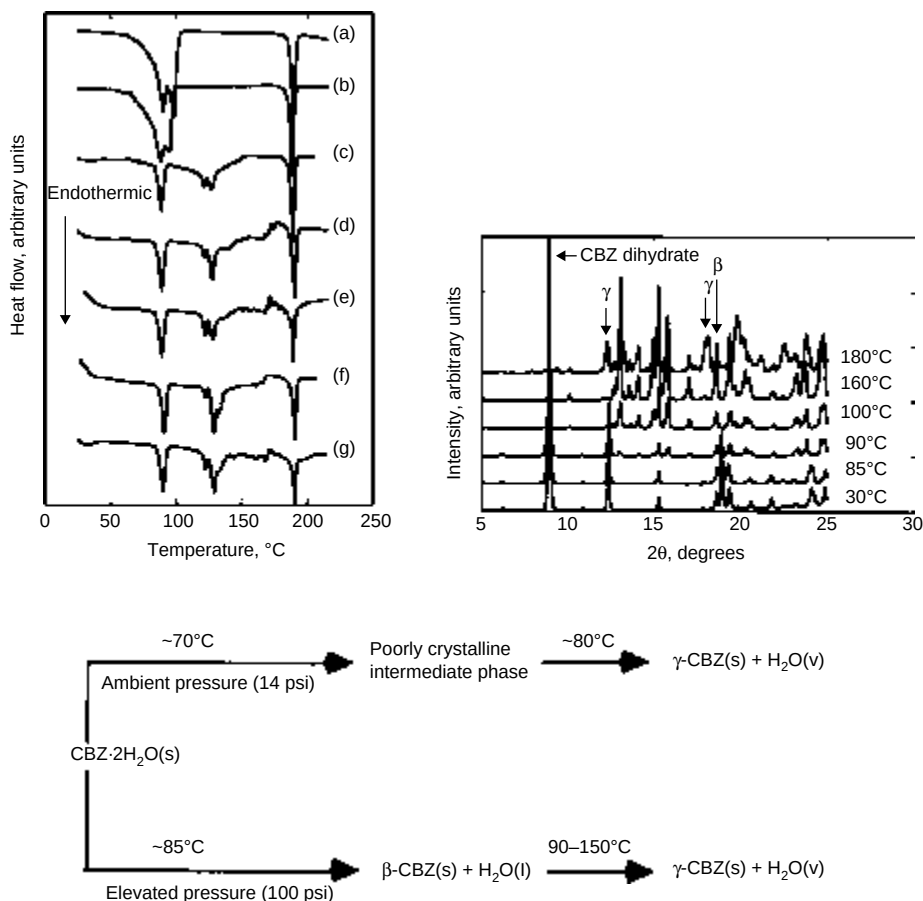


FIGURE 4 (Upper left panel) DSC of carbamazepine dihydrate at different pressures: (a) ambient pressure in conventional DSC cell, (b) ambient pressure in PDSC cell, (c) 100 psi, (d) 200 psi, (e) 300 psi, (f) 400 psi, (g) 600 psi. (Upper right panel) VT-XRD of carbamazepine dihydrate. Peaks characteristic of the dihydrate as well as anhydrous β and γ forms are shown. (Lower panel) Schematic showing carbamazepine dihydrate transitions under ambient and elevated pressures. Source: From Ref. (22).

illustrated in Figure 4 (lower panel) and shows the transitions of carbamazepine dihydrate at ambient and elevated pressures.

An emerging technique that could be considered as a variant of DSC is microthermal analysis, which combines the principles of DSC and scanning probe microscopy. Microthermal analysis was used to identify local regions of different polymorph content in a 50:50 mixture of two cimetidine polymorphs (23). This technique also has the potential to map a tablet surface for polymorph content uniformity.

Thermogravimetry

Thermogravimetry (TG, and also known as thermogravimetric analysis or TGA) is a technique where one uses a very sensitive balance to continuously determine the

weight of the analyte as a function of temperature or time. This simple technique provides valuable information that can aid in the identification and characterization of the thermal events associated with thermally induced reactions of solvates. For example, several hydrates of nedocromil sodium were identified and profiled using TG as one of the characterization tools (24). As the sample was heated, the weight loss occurred in a stepwise fashion, which facilitated calculation of the hydrate stoichiometries based on the percent weight loss at each step.

As discussed elsewhere in this book, there are different ways in which water can associate with solids. In authentic hydrates, water is incorporated in the crystal lattice, whereas in amorphous and partially crystalline materials, water can associate with disordered regions in the lattice. Water can also be adsorbed and also exist as "bulk water" in incompletely dried solids. TG analysis can also be used to distinguish between some of these states of water in solids. Compared with dehydration, desorption (adsorbed as well as bulk) typically occurs at lower temperatures, although these two processes can overlap. As shown in Figure 5, for carbamazepine dihydrate, when the TG experiment is carried out under isothermal conditions, there can be sequential loss of sorbed and lattice water (25).

TG analysis performed under isothermal conditions can be used to study the kinetics of phase transitions in solvatomorphs (26,27). Alkhamis et al. (26) carried out model-independent isoconversional analysis of the isothermal desolvation of the monohydrate and ethyl acetate solvate of fluconazole. One potential shortcoming of this technique is that a separate and direct technique might be required to confirm the cause of weight loss. In order to address this inadequacy, the TG instrument can be interfaced with additional instrumentation capable of identifying the vapor released upon heating. For example, TG has been used in combination with infrared absorption spectroscopy to understand the thermal behavior of a hydrate (28). In this study, two different thermal events resulting in weight loss were shown to be due to separate dehydration and decomposition processes.

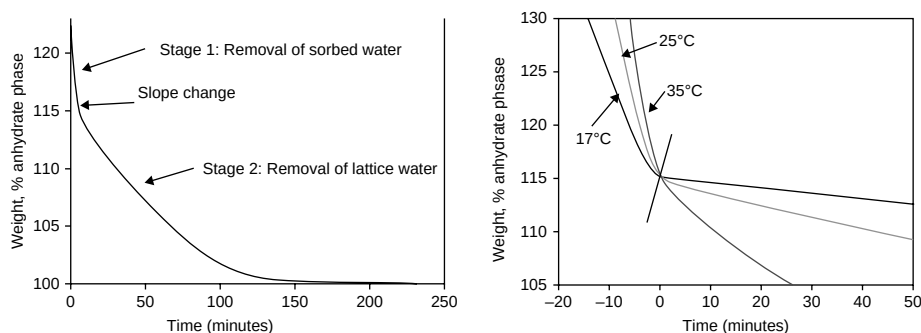


FIGURE 5 (Left panel) Dehydration of a wet sample of carbamazepine dihydrate at 0% RH (25°C). The plotted sample weight is based on the dry sample weight after complete dehydration. The first and second stages of dehydration are separated by the point of slope change. (Right panel) Effect of temperature on desorption and dehydration kinetics of carbamazepine dihydrate shown at three representative temperatures. For the purpose of comparison, the x-axis origin is the time point of transition from desorption to dehydration (point of slope change in left panel). Source: From Ref. (25).

Temperature calibration of TG instrumentation can be accomplished using melting point standards or the Curie point transition of a paramagnetic material (ASTM E1582) (29). Although not considered a “reference standard,” calcium oxalate monohydrate is frequently used to validate the performance of a TG system (30).

Hot-Stage Microscopy

Visualization of solids using a microscope often provides unique insights not afforded by other techniques. The visual observation of the analyte, placed on a temperature controlled stage (“hot stage”) of a microscope, forms the basis of HSM. For the characterization of polymorphs and solvates, HSM, with DSC and TG analyses, forms an optimal routine three-pronged approach. Vitez et al. (31) have briefly outlined the historical evolution of HSM from being merely a melting point determination tool to being an integral part of polymorph characterization. The practice and application of HSM has been reviewed and discussed in recent publications (3,32).

One of the primary advantages of HSM is the small amount of sample needed, which, given the limited availability of an active pharmaceutical ingredient (API) in the discovery phase, often represents a very important consideration. When characterizing solvates, the analyte is typically dispersed in silicone oil in order to aid in the visualization of the thermally induced release of any included solvent. As shown in Figure 6, a desolvation process is usually manifested by loss of transparency (perceived as a darkening of the crystals) (33), and in the formation of bubbles (specifically for hydrates) in the oil (34,35). Caution should be exercised in data interpretation because this observation could be potentially complicated by the release of entrapped air from the solids or by the simple presence of air bubbles in the dispersion medium. In addition, crystals may also darken as a result of decomposition.

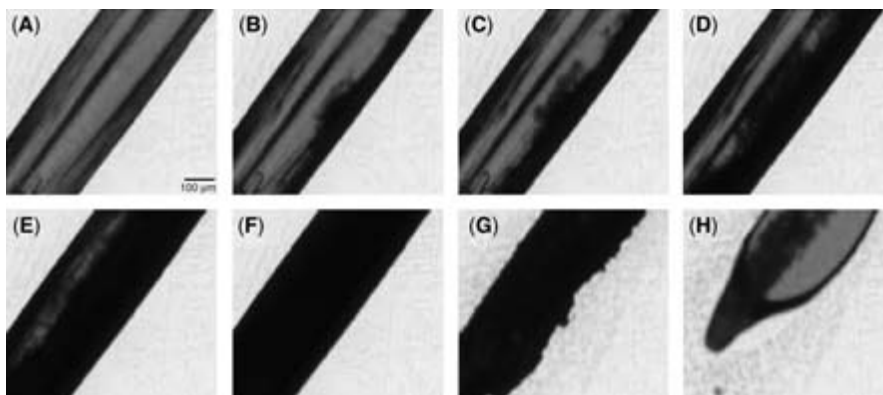


FIGURE 6 Hot-stage photomicrographs of toluene solvate of picryltoluidene, taken at various temperature values: (A) 25°C; (B) 35°C, onset of desolvation from crystal edges; (C) 45°C; (D) 55°C; (E) 65°C; (F) 80°C, completely desolvated anhydrous form; (G) 162.5°C, melting onset of the anhydrous form; (H) 163°C, remaining crystal of the anhydrous form in the melt. Source: From Ref. (33).

In some instances, desolvation may be accompanied by a change in crystal habit. For example, Tonder et al. used HSM to study the desolvation of different niclosamide solvates (36). Although the solvate crystals were characterized by different morphologies, desolvation resulted in the appearance of crystals having the same habit as that of the anhydrate form. In addition, the desolvated crystals were all observed to melt over the same temperature range as did the anhydrate, which was taken as confirming their transformation to the anhydrate phase.

Crystallization from the amorphous state can be demonstrated by the appearance of birefringence. In order to explain DSC observations, Bottom monitored the crystallization of a metastable spherulitic polymorph of sulfapyridine from the amorphous state through a solid-state phase transition to the stable needle-shaped form (12). Grooff et al. (37) used HSM as one of the tools to characterize crystallization of a metastable polymorph of nifedipine from the amorphous state. In this work, two other polymorphs were identified, and an enantiotropic relationship between two of the polymorphs was confirmed by direct observation of a reversible solid-state transition between the two crystal forms.

Microscope stage attachments are commercially available having a wide temperature range (less than -150°C to higher than 350°C) and excellent temperature stability ($\pm 0.1^{\circ}\text{C}$). In addition, most modern hot-stage systems are equipped with a high-resolution camera capable of rapid image collection, and which is controlled by a computer interface. All these accessories have drastically reduced the subjectivity associated with microscopy and have increased the efficiency of data collection. Consequently, HSM has evolved into one of the primary techniques that is used for the routine identification and characterization of pharmaceutical solids.

CRYSTALLOGRAPHIC CHARACTERIZATION TECHNIQUES

The technique of XRD is exceedingly important in the study of polymorphs and solvatomorphs because it represents the primary method whereby one can obtain fundamental information about the structure of a crystalline substance. The methodology is highly suited for the differentiation of crystal forms, as it would be only by pure coincidence that two compounds might form crystals having identical XRD patterns due to identical three-dimensional spacing of planes in all directions. One example of accidental isostructure is the trihydrate phases of ampicillin and amoxicillin (38), but such instances are uncommon. Typical applications of XRD methodology include the determination of crystal structures, evaluation of polymorphism and solvate structures, evaluation of degrees of crystallinity, and the study of phase transitions.

Every crystal consists of exceedingly small fundamental structural units, which are repeated indefinitely in all directions. In 1830, Hessel conducted a purely mathematical investigation of the possible types of symmetry for a solid figure bounded by planar faces, and deduced that only 32 symmetry groups were possible for such objects. The same conclusion was reached by Bravais in 1949, and Gadolin in 1867. These 32 crystallographic point groups are grouped into six crystal systems, denoted as triclinic, monoclinic, orthorhombic, tetragonal, trigonal, hexagonal, and cubic. Each crystal system is characterized by unique relationships existing among the crystal axes and the angles between these, and this information is summarized in Table 2.

TABLE 2 Characteristics of the Six Crystal Systems

System	Description
Cubic	Three axes of identical length (identified as a_1 , a_2 , and a_3) intersect at right angles.
Hexagonal	Four axes (three of which are identical in length, and denoted as a_1 , a_2 , and a_3) lie in a horizontal plane, and are inclined to one another at 120°. The fourth axis, c , is different in length from the others, and is perpendicular to the plane formed by the other three.
Tetragonal	Three axes (two of which are denoted as a_1 and a_2 , and are identical in length) intersect at right angles. The third axis, c , is different in length with respect to a_1 and a_2 .
Orthorhombic	Three axes of different lengths (denoted as a , b , and c) intersect at right angles. The choice of the vertical c axis is arbitrary.
Monoclinic	Three axes (denoted as a , b , and c) of unequal length intersect such that a and c lie at an oblique angle, and the b axis is perpendicular to the plane formed by the other two.
Triclinic	Three axes (denoted as a , b , and c) of unequal length intersect at three oblique angles.

Bragg (39) explained the diffraction of X rays by crystals using a model where the atoms of a crystal are regularly arranged in space, and that they can be regarded as lying in parallel sheets separated by a definite and defined distance. Then he showed that scattering centers arranged in a plane act like a mirror to X rays incident on them, so that constructive interference would occur in the direction of specular reflection. Within a given family of planes, defined by a Miller index of (hkl) and with an inter-planar distance d , each plane produces a specular reflectance of the incident beam. If the incident X rays are monochromatic (having wavelength equal to λ), then for an arbitrary glancing angle of θ , the reflections from successive planes are out of phase with one another. This yields destructive interference in the scattered beams. However, through the systematic variation of analysis angles, a set of values for θ can be found so that the path difference between X rays reflected by successive planes will be an integral number (n) of wavelengths, and then constructive interference would occur. One ultimately obtains the expression known as Bragg’s law that explains the phenomenon:

$$2d\sin \theta = n\lambda \tag{2}$$

Unlike the diffraction of light by a ruled grating, the diffraction of X rays by a crystalline solid leads to constructive interference (i.e., reflection) only at critical Bragg angles. When reflection does occur, it is stated that the plane in question is reflecting in the n th order, or that one observes n th order diffraction for that particular crystal plane. Therefore, one will observe an X-ray scattering response for every plane defined by a unique Miller index of (hkl).

Single-Crystal X-Ray Diffractometry

Single-crystal XRD involves an assignment of the arrangement of atoms based on an electron density map generated by diffraction of a pure, regular, and adequately large crystal (40). Structural information derived from an XRD study of a single

crystal is the most fundamental description of a polymorph or solvatomorph, and helps to explain its physical properties.

Generally, polymorphic structures can be classified into one of two main categories. The first is associated with molecules that can only exist as a rigid grouping of atoms that can be stacked in different motifs to occupy the points of different lattices (i.e., packing polymorphism). One of the best-known instances of packing polymorphism is the allotropic system of carbon, namely graphite and diamond. As shown in Figure 7, in diamonds each carbon atom is tetrahedrally surrounded by four equidistant neighbors, and the tetrahedra are arranged to give a cubic unit cell. Graphite is composed of planar hexagonal nets of carbon atoms, which can be arranged to yield either a hexagonal unit cell (the α -form) or a rhombohedral unit cell (the β -form).

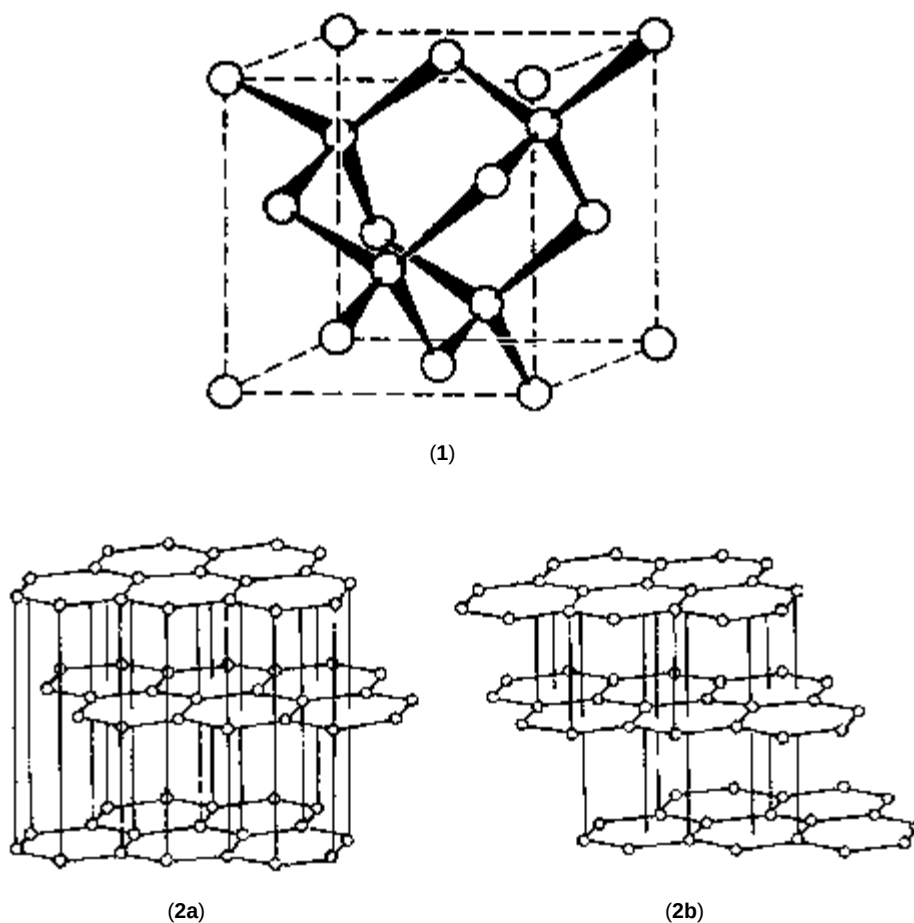


FIGURE 7 Crystal structure of (1) diamond, showing the tetrahedral coordination of each carbon atom. Also shown are the crystal structures of the two polymorphs of graphite, specifically (2a) the hexagonal α -form, and (2b) the rhombohedral β -form.

The other category is associated with molecules that are capable of existing in conformationally different arrangements, each of which can crystallize in its own characteristic structure. This latter behavior has been termed conformational polymorphism (41), and the probucol system represents an extreme example of a compound where the polymorphism arises from the packing of different conformers (42). Although both polymorphs were found to be monoclinic, the unit cells belonged to different space groups and the molecular conformations of the title compound were quite different (Fig. 8). In form II, the C–S–C–S–C chain is extended,

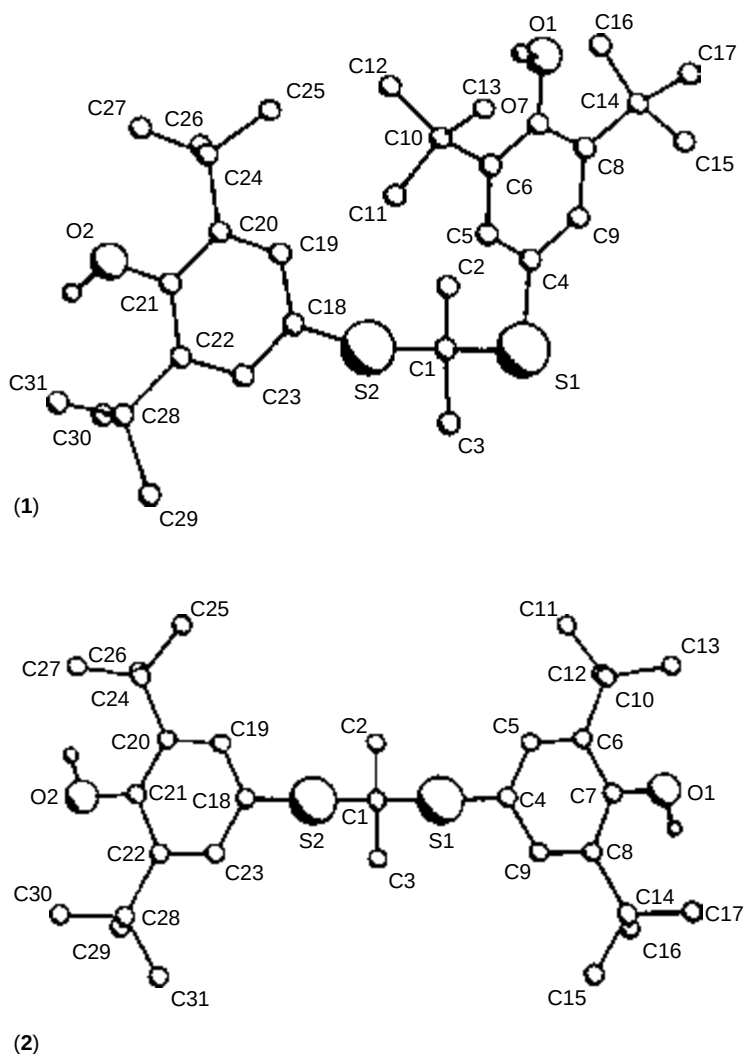


FIGURE 8 Conformation of the probucol molecule existing in (1) form I and in (2) form II. The figure was adapted from data contained in Ref. (42).

and the molecular symmetry approximates C_{2v} . This symmetry is lost in the structure of form I, where the torsion angles about the two C–S bonds deviate significantly from 180° . The extended conformer was shown to be less stable relative to the bent conformer, as simple grinding was sufficient to convert form II into form I.

Not all instances of conformational polymorphism are as dramatic as the probucol system, and often different conformers of a single sidechain are able to pack into different crystalline arrangements. For instance, the two polymorphs of *p*-(1*R*,3*S*)-3-thioanisoyl-1,2,2-trimethylcyclopentane carboxylic acid were found to be associated with different conformations of the carboxylate group (43). Torsion about a single C–N bond was shown to be the origin of the polymorphism detected for lomeridine dihydrochloride (44). Finally, relatively small differences in molecular conformation were detected for the two polymorphic and four solvated crystalline forms of spironlactone (45).

The analysis of single-crystal XRD data is divided into three parts (46–49). The first of these is the geometrical analysis, where one measures the exact spatial distribution of X-ray reflections and uses these to compute the size and shape of a unit cell. The second part entails a study of the intensities of the various reflections and uses this information to determine the atomic distribution within the unit cell. Finally, one looks at the X-ray diffraction pattern to deduce qualitative information about the quality of the crystal or the degree of order within the solid. This latter analysis may permit the adoption of certain assumptions that may aid in the solving of the crystalline structure.

A determination of the internal structure of a crystal requires the specification of the unit cell dimensions (axis lengths, and angles between these), and measurement of the intensities of the diffraction pattern of the crystal. For a given lattice, regardless of the content of the unit cell, the directions of reflection are the same. The experimental determination of these directions is used to deduce the reciprocal lattice of the crystal, which unambiguously yields the crystal lattice. In addition, the relative intensities diffracted by different planes depend on the contents of the unit cell. Their measurement leads to the determination of the crystal structure factor, and these data permit the determination of the atomic structure of crystals.

Crystallographic characterization of the physical form of an API may be used to understand its performance during pharmaceutical processing. One well-known case is that of polymorphic forms I and II of acetaminophen (also known as paracetamol). The crystal structures of both forms have been determined and the associated implications on their physical properties have been discussed (50). Monoclinic acetaminophen form I cannot be formulated into tablets by direct compression, but the presence of slip planes (illustrated in Fig. 9) in the orthorhombic form II allows its direct compression due to a plastic deformation process. This property is an immense advantage considering that direct compression is a cost-effective and time-efficient method of tablet production.

Crystallographic information is critical to understand dehydration of solvates that have channels in their crystal structures. As mentioned earlier, such solvatomorphs undergo desolvation under certain conditions, leading to the formation of isomorphic desolvates that tend to retain the three-dimensional structures of the parent compound (51,52).

Variable temperature and atmospheric-induced dehydration was characterized in form I of a small molecule developmental drug that was presented as a sodium salt and a trihydrate (53). It was shown that at 25°C , the compound lost 0.5% w/w

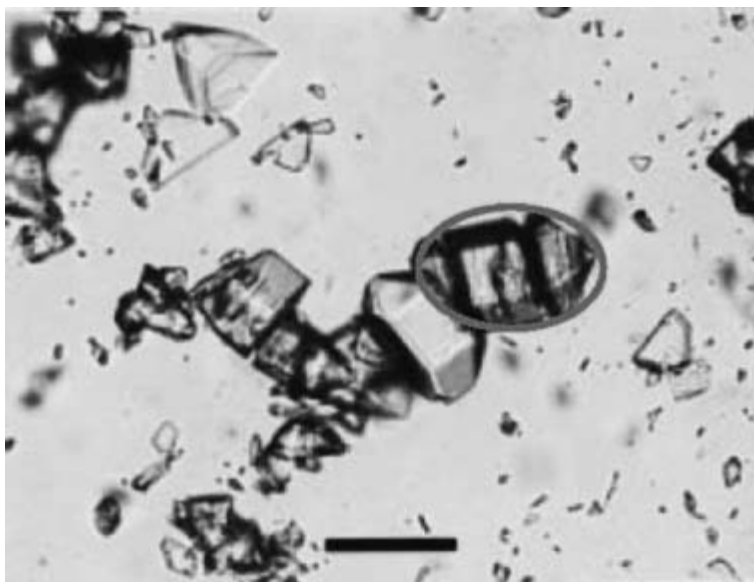


FIGURE 9 Pressure-induced formation of cleavages (*indicated by the ellipse*) in an acetaminophen form II crystal perpendicular to its length, showing the evidence of the existence of slip planes. *Source:* From Ref. (41).

water when the relative humidity was decreased from 90% to 15%, and that this water loss did not cause any change in the XRD pattern. A single-crystal XRD study revealed the existence of channels in the form I crystal structure (illustrated in Fig. 10), where one of the water molecules was weakly bound by hydrogen bonds and where the other two water molecules were bound to the sodium cation. Formation of the isomorphic dehydrate was attributed to the escape of some weakly bound water molecules through the channel, which did not compromise the integrity of the structure. Further decrease in relative humidity into the range of 5% to 15% resulted in a reversible phase transformation into form II, and at relative humidities below 5%, XRD showed that the compound transformed into the semi-crystalline form III. A combination of techniques was used to show that form II was formed as a result of the escape of both weakly and strongly bound water molecules, which caused a partial collapse of the channels. Further dehydration of the solid resulted in disordered anhydrate form III.

The interplay between intramolecular conformational flexibility and intermolecular hydrogen bonding may influence the properties of a polymorph (54), such as in the classic example of ritonavir (55). In 1998, the commercial form of this drug substance (tradename of Norvir®, and marketed as a semi-solid formulation contained in capsules) had to be reformulated because the newer lots failed to meet established dissolution specifications. This was attributed to the sudden appearance of a polymorph (form II), which was much less soluble than the parent polymorph (form I) in the hydro-alcoholic medium of the formulation. Single-crystal XRD studies showed that both the hydrogen bonding capability and the packing density of

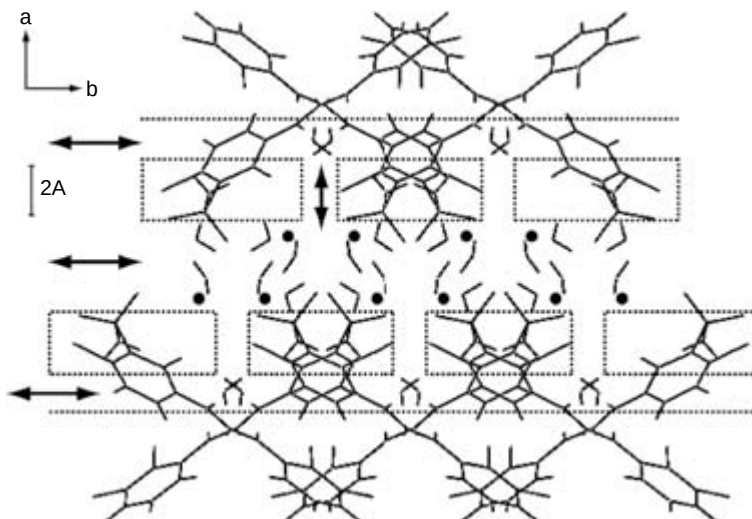


FIGURE 10 Top view along *c*-axis showing the crystal packing of form I of a developmental drug, the sodium salt of *N*-(3-(aminosulfonyl)-4-chloro-2-hydroxyphenyl)-*N'*-(2,3-dichlorophenyl) urea. Arrows indicate potential motion of water along the channels (*dashed lines*). Sodium atoms are represented by dark circles. *Source:* From Ref. (44).

form II was less than that of form I, which could be taken to suggest a greater stability of form I. However, the molecular conformation of ritonavir in form II was more favorable for crystal packing, and made this form the more stable polymorph.

It has been shown elsewhere that a thermodynamically stable polymorph may be characterized by the presence of unstable molecular conformations, such as the thermodynamically stable polymorph of oxybuprocaine hydrochloride, which was shown to have both stable and unstable molecular conformations in its crystalline forms (56).

X-Ray Powder Diffractometry

Although the solving of a crystal structure from an analysis of single-crystal diffraction provides the most fundamental structural understanding of polymorphic solids, the need for suitable single crystals and the degree of complexity associated with data analysis precludes this technique from being used on a routine basis for batch characterization. During routine synthesis, most drug substances are obtained as micro-crystalline powders, for which it is usually sufficient to establish only the physical form (usually polymorphic identity) of the solid, and to verify that the isolated compound is indeed of the desired structure. For these reasons, and in light of the experimental simplicity, the technique of XRD is the predominant tool for the study of polycrystalline materials (57–60), and is eminently suited for the routine characterization of polymorphs and solvates.

A properly prepared sample of a powdered solid will present a substantially random selection of all possible crystal faces and the diffraction patterns will therefore provide information regarding all possible spacings (atomic or molecular) in

the crystal lattice. To measure a powder pattern, a randomly oriented powdered sample is prepared in an effort to expose all the planes in the lattice. The scattering angle is determined by slowly rotating the sample and measuring the angle of diffracted X rays (typically using a scintillation detector) with respect to the angle of the incident beam. Alternatively, the angle between sample and source can be kept fixed, while moving the detector to determine the angles of the scattered radiation. Knowing the wavelength of the incident beam, the spacing between the planes (identified as the d -spacings) is calculated using Bragg's Law.

The XRD pattern will therefore consist of a series of peaks detected at characteristic scattering angles. These angles, and their relative intensities, can be correlated with the computed d -spacings to provide a full crystallographic characterization of the powdered sample. After indexing all the scattered lines, it is possible to derive unit cell dimensions from the powder pattern of the substance under analysis. For routine work, however, this latter analysis is not normally performed, and one typically compares the powder pattern of the analyte to that of reference materials to establish the polymorphic identity. Because every compound produces its own characteristic powder pattern owing to the unique crystal structure, powder XRD is clearly the most powerful and fundamental tool for polymorphic identity of an analyte.

The United States Pharmacopeia contains a general chapter on XRD (61), which sets the criterion that identity is established if the scattering angles in the powder patterns of the sample and reference standard agree to within the calibrated precision of the diffractometer. It is noted that it is generally sufficient that the scattering angles of the ten strongest reflections obtained for an analyte agree to within either ± 0.10 or $\pm 0.20^\circ 2\theta$, whichever is more appropriate for the diffractometer used. Older versions of the general test contained an additional criterion for relative intensities of the scattering peaks, but it has been noted that relative intensities may vary considerably from that of the reference standard, making it impossible to enforce a criterion based on the relative intensities of corresponding scattering peaks.

For identification purposes, it is usually convenient to identify the angles of the 10 most intense scattering peaks in a powder pattern, and to then list the accepted tolerance ranges of these based on the diffractometer used for the determinations. Useful tabulations of the XRD patterns of a number of compounds have been published by Koundourellis and coworkers, including 12 diuretics (62), 12 vasodilators (63), and 12 other commonly used drug substances (64).

The literature abounds with countless examples that illustrate how powder diffraction has been used to distinguish between polymorphs. It is safe to state that one could not publish the results of a phase characterization study without the inclusion of XRD data. Nowhere is this more crucial than in intellectual property controversies, where the different XRD patterns of polymorphs are used for patentability purposes. For example, two polymorphic forms of ibandronate sodium have been the subject of two United States patent applications (65), and although the XRD patterns of these are somewhat similar (Fig. 11), a sufficient number of differences in scattering peak angles exist so as to permit their identification. In the patent applications, some of the claims define the two polymorphs on the basis of five characteristic XRD scattering peaks, which enables one to tabulate the data and determine whether or not a given sample of ibandronate sodium is within the scope of these XRD claims.

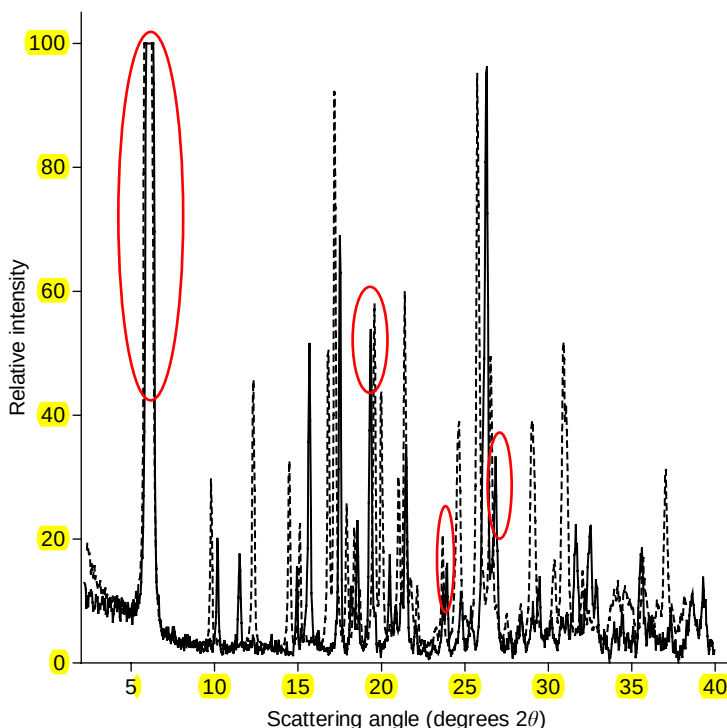


FIGURE 11 X-ray powder diffraction patterns of ibandronate sodium, Form A (*solid trace*) and Form B (*dashed trace*). The patterns have been adapted from data in United States patent applications 2006/0172975 and 2006/0172976 (65).

Once the characteristic XRD patterns of one or more analytes have been established, it is usually possible to develop methods for phase quantification. The methodology is based on the premise that each component will contribute to the overall scattering by an amount that is proportional to its weight fraction in a mixture, and that the powder pattern of each analyte contains one or more peaks whose scattering angle is unique to that analyte. Quantitative analysis typically requires the use of reference standards that contribute known scattering peaks at appropriate scattering intensities. This can be achieved through the use of internal standards, where one would mix reference materials such as elemental silicon or lithium fluoride into the sample matrix.

Although simple intensity correction techniques can be used to develop suitable quantitative XRD methods, the introduction of more sophisticated data acquisition and handling techniques can greatly improve the quality of the developed method. For instance, improvement of the powder pattern quality through use of the Rietveld method has been used to evaluate mixtures of two anhydrous polymorphs of carbamazepine and the dihydrate solvatomorph (66). The method of whole pattern analysis developed by Rietveld (67) has found widespread use in crystal structure refinement and in the quantitative analysis of complex mixtures.

Using this approach, the detection of analyte species was possible even when their concentration was less than 1% in the sample matrix. It was reported that good quantitation of analytes could be obtained in complex mixtures even without the requirement of calibration curves.

Well-characterized and stable (both physically and chemically) Standard Reference Materials are available from the National Institute of Standards and Technology (NIST) (68). These materials are used both to calibrate powder XRD equipment and to measure the instrument sensitivity. Silicon powder (SRM 640c) is typically used for the calibration of *d*-spacing or line position, and also can be used as an internal reference for quantitative applications. NIST SRM 674b is a set of four metal oxides (CeO_2 , Cr_2O_3 , TiO_2 , and ZnO) that are also used as internal standards in quantitative work.

The use of parallel beam optics as a means for determining the polymorphic composition in powder compacts has been evaluated (69). In this study, compressed mixtures of known polymorphic composition were analyzed in transmission mode, and the data processed using profile fitting software. The advantage of using transmission, rather than reflectance, was that the results were not sensitive to the geometrical details of the compact surfaces, and that spurious effects associated with preferential orientation were minimized.

The effects of preferred orientation in XRD analysis can be highly significant, and are most often encountered when working with systems characterized by plate-like or tabular crystal morphologies. A viable XRD sample is one that is free from the deleterious effects of preferential orientation, and any sample packing effect that serves to introduce a non-random pattern of crystal faces can strongly affect the observed intensities and any quantitative results. The problem has been investigated for the three needle-like polymorphs of mannitol, and it was found that through the use of small particles, the preferential orientation effects were held to a minimum, enabling quantification of the polymorphs around the 1% level (70).

The use of powder XRD has been investigated to determine whether one may use pattern indexing as another tool for polymorph screening studies (71). In this work, data were collected on six compounds using two diffractometers that employed transmission geometry, primary monochromatic radiation, and a position-sensitive detector. The data were found to exhibit good angular resolution, and therefore lattice parameters were easily obtained using the indexing program DICVOL-91. The extent of preferred orientation in each pattern was estimated using the DASH implementation of the March–Dollase function. It was concluded that the combination of experimental techniques used would be highly effective compared to just one technique that would require a large number of samples per day with the aim of obtaining the type of high-quality data necessary for pattern recognition and indexing.

Practical complications associated with preferential orientation effects have been discussed in detail (72). A number of sample-packing methods were considered (vacuum free-fall, front-faced packing vs. rear-faced packing, etc.), but the best reduction in preferential orientation was achieved by using materials having small particles that were produced by milling. Through the use of sieving and milling, excellent linearity in diffraction peak area as a function of analyte concentration was attained. The authors deduced a protocol for development of a quantitative XRD method consisting of (a) calculation of the mass attenuation coefficient of the drug substance, (b) selection of appropriate diffraction peaks for quantification,

(c) evaluation of the loading technique for adequate sample size, (d) determination of whether preferred orientation effects can be eliminated through control of the sample particle size, (e) determination of appropriate milling conditions to obtain reproducibility in peak areas, and (f) generation of calibration curves from physical mixtures.

Profile-fitting analysis has been combined with powder XRD to develop a quantitative method to determine the relative amounts of two prazosin hydrochloride polymorphs in their powder mixtures (73). Powder patterns of the pure α - and δ -polymorphs were initially obtained, and then patterns of calibration samples containing the α -form at concentrations ranging from 0.5% to 10% w/w in bulk δ -form. As is evident from Figure 12, the α -form exhibits a strong scattering peak at $27.5^\circ 2\theta$ where the δ -form exhibits little scattering, thus making this peak suitable for use in developing a quantitative method for phase composition. Using mathematical profile fitting, the background intensity level of the δ -form and the integrated intensity of peaks due to the α -form were determined, enabling a detection limit of 0.5%.

Powder XRD and diffuse reflectance infrared absorption spectroscopy analyses were used to develop two independent quantitative phase composition methods

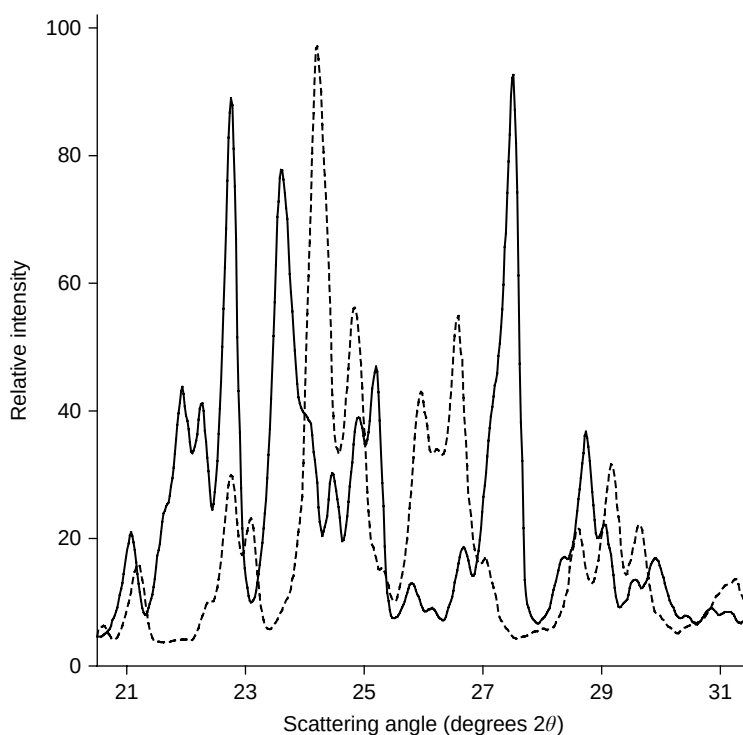


FIGURE 12 Expanded X-ray powder diffraction patterns of prazosin hydrochloride, α -form (solid trace) and δ -form (dashed trace), illustrating the peak at $27.5^\circ 2\theta$ that was used for quantitation purposes. The patterns have been adapted from data in Ref. (73).

for the determination of quantities of cefepime dihydrochloride dihydrate in samples of cefepime dihydrochloride monohydrate (74). For the XRD method, over a working concentration range 2.5% to 15% w/w, the limit of detection and quantification of the dihydrate in monohydrate were respectively 0.75% and 2.5% w/w. For the infrared method, a working range of 1.0% to 8.0% w/w was established with a minimum quantifiable level of 1.0% w/w and a limit of detection of 0.3% w/w dihydrate in monohydrate.

When reference samples of the pure amorphous and pure crystalline phases of a substance are available, calibration samples of known degrees of crystallinity can be prepared by mixing these reference samples. Establishment of a calibration curve (XRD response vs. degree of crystallinity) permits quantification of crystallinity in unknown samples. In one such study, a quantitative method was developed by beginning with commercially available crystalline digoxin and ball-milling of this substance to obtain various samples having various degrees of amorphous phase (75). Calibration mixtures were prepared as a variety of blends prepared from the 100% crystalline and 0% crystalline materials, and acceptable linearity and precision was obtained in the calibration curve of XRD intensity versus actual crystallinity.

In the absence of standard materials having known degree of crystallinity, one may determine the degree of crystallinity from the simple relation:

$$\text{DOC} = (A_c/A_T)(100) \quad (3)$$

where A_c is the integrated intensity attributable to the crystalline regions, and A_T is the total diffracted intensity. The evaluation of A_c and A_T is illustrated in Figure 13, where the reported XRD pattern of lenalidomide Form H (76) is shown along with its associated amorphous background. One would obtain A_c as the sum of the areas of the peaks above the dashed line, whereas A_T would be measured as the total area under the curve.

Determination of Crystal Structures from Powder Diffraction Data

As discussed above, a powder pattern will consist of a series of peaks having varying intensities, detected at various scattering angles. Through the indexing process, the d -spacings computed from the values of the scattering angles are assigned to diffraction from the crystal planes defined by their Miller (hkl) indices to provide a full crystallographic characterization of the powdered sample. After the indexing procedure is complete, it is possible to derive unit cell dimensions and other crystallographic information from a high-resolution powder pattern of a substance, eventually developing an approximate structural model that can be refined against experimental powder XRD data (structure refinement) (77–81). This procedure is best conducted on diffraction data collected using a high-flux synchrotron source (82).

Of the three crystal forms of telmisartan, the structure of solvated Form C was determined using single-crystal XRD and the structures of non-solvated forms A and B were determined from high-resolution powder XRD data using the method of simulated annealing for structure solution followed by Rietveld refinement (83). With 13 degrees of freedom (three translational, three orientational, and seven torsion angles), the structure solution was accomplished in approximately two hours of computer time, demonstrating that the crystal packing and the molecular conformation

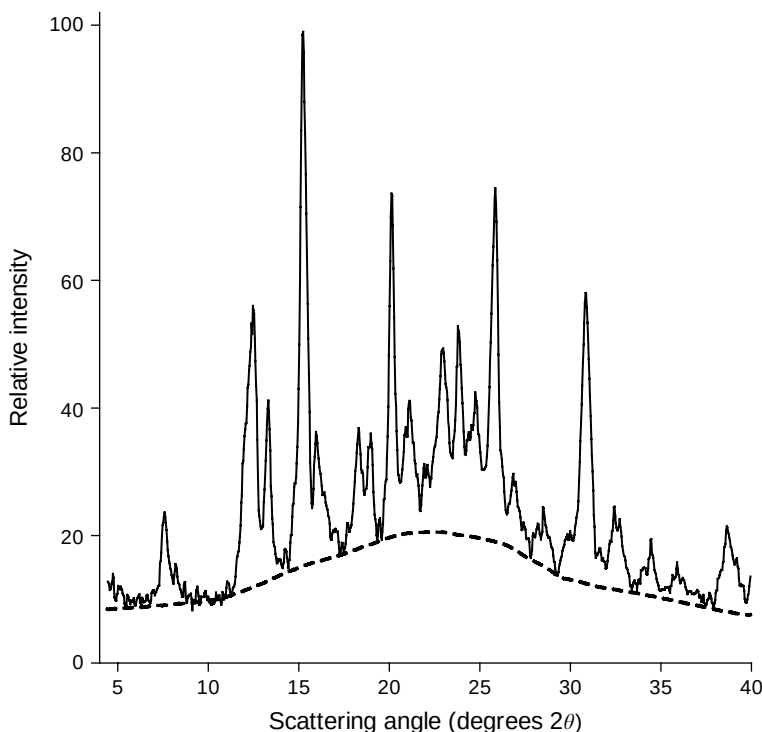


FIGURE 13 XRD pattern of lenalidomide form H (*solid trace*), and an estimation of the amorphous background (*dashed line*). The powder pattern of form H was adapted from data in Ref. (74).

of medium-sized pharmaceutical compounds could be solved quickly and routinely from high-resolution powder XRD data.

Other examples where structural properties have been derived from powder XRD data include enalapril maleate form II (84) and bupivacaine base (85).

Variable-Temperature X-Ray Diffractometry

XRD, performed on a hot stage, enables one to obtain powder patterns at elevated temperatures, and permits the deduction of structural assignments for thermally induced phase transformation reactions. By conducting VT-XRD studies, one can bring the system to conditions that had been indicated to be of interest during the conduct of thermal analysis studies. XRD systems equipped with hot-stage sample holders have been described, with this instrumentation being capable of obtaining powder patterns at temperatures of pharmaceutical interest (86,87). VT-XRD studies conducted under isothermal conditions can also be used for the calculation of activation energies of solid-state reactions.

VT-XRD is ideally suited to explain potentially complex solid-state transformations involving pharmaceutical hydrates. For example, Terakita et al. examined the dehydration behavior of calcium benzoate trihydrate, a system where the solid state transformations involved, in addition to crystalline and amorphous phases,

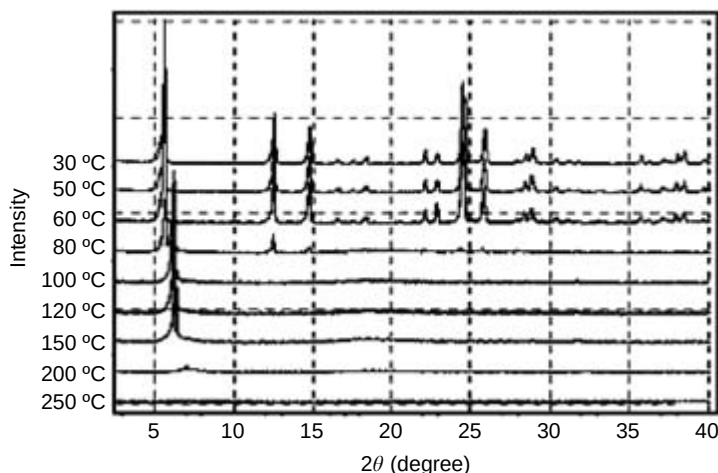


FIGURE 14 VT-XRD patterns obtained during the drying of calcium benzoate trihydrate, showing the gradual dehydration to a mesophase (100°C), which eventually culminated in the formation of an amorphous phase (250°C). *Source:* From Ref. (88).

a mesophase (88). As the temperature was increased, the trihydrate crystal form gradually formed a mesophase, as indicated by a sharp low-angle peak. As illustrated in Figure 14, further heating resulted in complete amorphization and the disappearance of all XRD peaks. A ground sample of the trihydrate was found to contain traces of calcium benzoate monohydrate, which was confirmed by VT-XRD to have formed by a recrystallization process. In this work, VT-XRD was shown to function both as an aid in the identification of crystalline phases, as well as in decoding the mechanism of their formation. Understanding such transformations and the knowledge of the conditions under which phase transitions occur, could aid in optimizing process parameters, such as the drying temperature of granules.

By virtue of their higher free energy compared to their more stable counterparts, metastable polymorphs of drug substances can significantly affect drug release from a dosage form. For example, theophylline monohydrate was observed by VT-XRD to dehydrate to the anhydrous form via an intermediate metastable anhydrate (89). The dissolution performance of tablets containing stable and metastable anhydrous theophylline was compared, where surprisingly the dissolution rate of the metastable form was found to be slower than that of the stable form. This phenomenon was shown to be due to the relatively rapid conversion of the metastable anhydrate form to the monohydrate form in the dissolution medium.

Isomorphic desolvates, formed by desolvation of solvates, represent the type of lattice structures that can be inherently unstable owing to their molecular packing. Consequently, such materials tend to be quite reactive and prone to instability, especially during their secondary processing into drug products (90). Miroshynk et al. used VT-XRD to investigate processing-induced phase transformations in erythromycin dihydrate (91). Dehydration resulted in shifts in the XRD peak positions, where some peaks shifted to higher angles owing to lattice expansion, whereas other peaks shifted to lower angular values as a result of lattice contraction.

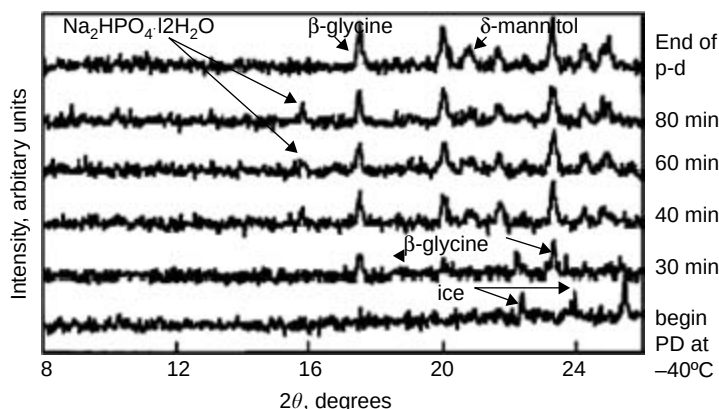


FIGURE 15 In situ VT-XRD patterns obtained during the primary drying (PD) of a frozen aqueous solution consisting of buffer salts, mannitol, and glycine. The solution was first cooled to -70°C at $2^{\circ}\text{C}/\text{min}$, which was then followed by heating to the primary drying temperature (-40°C) at $5^{\circ}\text{C}/\text{min}$. Primary drying was carried out for 4 hours. Source: From Ref. (92).

At higher temperatures, the XRD patterns indicated that the solids rearranged to form anhydrous erythromycin.

Although the typical use of VT-XRD is at elevated temperatures, the technique can also be used for work under sub-ambient conditions. For example, Pyne et al. characterized freeze-dried buffered aqueous systems of mannitol and glycine, and found that δ -mannitol and β -glycine crystallized during primary drying (92). By monitoring the intensities of the characteristic peaks of δ -mannitol and β -glycine both during primary and secondary drying, it was possible to determine the rate of crystallization of these solutes. Interestingly, one of the buffer salts, disodium hydrogen phosphate, crystallized as its dodecahydrate phase at the start of primary drying, but became dehydrated to an amorphous anhydrate at the end of the drying cycle (Fig. 15). It is important to note that conventional XRD of the final lyophile would not have revealed the crystallization of the buffer salt. The selective crystallization of disodium hydrogen phosphate only became evident by monitoring the phase transitions that took place *during* the freeze-drying process.

While attempting to simulate pharmaceutical processing steps, it is important to consider the effect of sample geometry on the phase transition kinetics. The relative quantities of stable and metastable anhydrous theophylline were found to be significantly different when theophylline monohydrate granules were dried in a microscale fluid bed dryer and in the sample stage of a powder X-ray diffractometer, with these differences being attributed primarily to differences in sample geometry (93). Although the VT-XRD sample bed was stationary, resulting in a slow drying rate, the sample in the fluid bed drier was dried rapidly due to fluidization.

XRD studies conducted at elevated temperatures can also be run under controlled water vapor pressures, using humidified nitrogen to control the vapor pressure. Han et al. proposed this technique to rapidly evaluate the physical stability of pharmaceutical hydrates, and studied the isothermal dehydration kinetics

of amoxicillin trihydrate at 68°C and at different water vapor pressures (94). The product they obtained was a poorly crystalline anhydrate. From these kinetic studies, the transition water vapor pressure of amoxicillin trihydrate was determined to be approximately 10.5 Torr at 68°C.

In addition to being extremely useful in the study of phase transformations, VT-XRD can also be used to obtain information on substance decomposition during a variable-temperature study. The commercially available form of aspartame is hemihydrate form II, which is known to transform into hemihydrate form I when milled, but a 2.5-hydrate species is also known (95,96). Through the use of VT-XRD, it was found that when either hydrate form was heated to 150°C, they dehydrated into the same anhydrous phase. Further heating of the anhydrate to 200°C resulted in a thermally induced cyclization, forming 3-(carboxymethyl)-6-benzyl-2,5-dioxopiperazine. The 2.5-hydrate was shown to dehydrate to hemihydrate form II when heated to 70°C, and this dehydration product was shown to undergo the same decomposition sequence as directly crystallized hemihydrate form II.

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INTRODUCTION

In addition to the crystallographic techniques of X-ray diffraction and optical crystallography, methodologies based on molecular spectroscopy have become extremely important for the characterization of polymorphs and solvatomorphs (1). These methods become particularly useful when information must be obtained from a small amount of sample in a short period of time, and because most of the methods are non-destructive in nature, the analyzed material can be recycled for additional study once the measurements are complete. A number of techniques have been developed for the characterization of compounds in the solid state, and those based on probing the periodic motion of atoms and groups of atoms in molecules in solids will form the scope of this chapter.

The vibrational patterns existing in molecules are characterized by repetitious departures and returns about the center of gravity, and these correlated motions are termed the vibrational modes of the molecule. Energies associated with the lowest energy vibrational modes of a chemical compound will lie within the range of 400 to 4000 cm^{-1} , a spectral region of the electromagnetic spectrum denoted as the mid-infrared. Transitions among states associated with these vibrational modes can be observed directly through their absorbance in the mid-infrared region of the spectrum, or alternatively through an inelastic scattering of incident energy via the Raman effect. Transitions involving the excitation of multiple vibrational states are known as overtone bands or combination bands, and these are observed in the near-infrared (NIR) region of the spectrum (4000–13,350 cm^{-1}).

Infrared absorption spectroscopy is an enormously useful technique for the physical characterization of solids having pharmaceutical interest. When the crystallography of a given molecule sufficiently perturb the frequencies of vibrational modes relative to the energies of the modes for a free molecule, one can use changes in the spectra as a means to study the solid-state chemistry of the system. For example, being especially sensitive to changes in molecular conformation between different solid-state forms, infrared absorption spectra are frequently used to evaluate the polymorphic space of a drug substance system. In addition, the technology can be very useful in studies of solvent molecules contained within a solvatomorph.

The vibrational energy levels of a molecule in its solid form can also be evaluated using Raman spectroscopy. Because most compounds of pharmaceutical interest are characterized by low or no molecular symmetry, the same bands observed in the infrared absorption spectrum will also be observed in the Raman spectrum. However, the fundamentally different nature of the selection rules associated with the Raman effect leads to the existence of significant differences in intensity among peaks as measured by the two methods. In general, symmetric

vibrations and non-polar groups yield the most intense Raman scattering bands, whereas antisymmetric vibrations and polar groups yield the most intense infrared absorption bands.

The spectral features observed in the NIR region of the electromagnetic spectrum are associated with overtones and combinations of the same fundamental bands detected in infrared absorption or Raman spectroscopies. Some of the spectral features that have found the greatest utility are those functional groups that contain unique hydrogen atoms. For example, studies of water in solids can be easily performed through systematic characterization of the characteristic --OH band, usually observed around 5170 cm^{-1} . The determination of hydrate species in an anhydrous matrix can also be easily performed using near-IR analysis.

In this chapter, a brief overview of the necessary theoretical bases for all three types of vibrational spectroscopy will be presented, and it should be noted that more extensive details regarding molecular spectroscopy (2–6) and vibrational spectroscopy (7–17) are available. The utility of vibrational spectroscopy in the physical characterization of pharmaceutical compounds has been reviewed many times (18–32).

Motion of Atoms in Molecules

Because all forms of vibrational spectroscopy have their origin in the patterns of motion executed by the atoms and groups in the molecule, it is clear that the energies associated with these motions provide the basis to an understanding of the observed spectra. Consequently, it is appropriate to briefly consider the nature of these motions, and the manner in which energy can become distributed among the various modes. For this part of the discussion, the perturbing effects of other molecules in a condensed state will not be considered, but instead, only the state of a molecule will be considered, which is essentially free to move through space. The perturbing effects of the solid state on the vibrational properties of the component molecule in a crystal will be the focus of illustrative examples.

The number of degrees of freedom that any body may possess is related to the number of independent coordinates that are needed to specify its position in space. For instance, a sphere that is able to move randomly in space would require the specification of all three of the Cartesian coordinates (X , Y , and Z) to define its position. One therefore states that this free object has three degrees of freedom. However, if the object were constrained to move only in the XY -plane, then the value of the Z -coordinate would be fixed and only the X and Y coordinates would have to be specified during the motion. In that case, the object would have only two degrees of freedom. If it is further required that the object move along a line in this plane, then one would only have to specify the coordinate along which the object moved (i.e., one degree of freedom).

If one now considers a molecule consisting of N atoms, it is clear that one must specify the three X , Y , and Z coordinates for each atom, and therefore, the total number of degrees of freedom possible for this molecule would be equal to $3N$. At the same time, the atoms in the molecule are bound, and this bonding will affect how the $3N$ degrees of freedom would be distributed among the different types of motion available to the molecule. In a molecule, each atom is not free to move independently of the others, but instead, the molecule must translate and rotate as a whole. For non-linear molecules, three degrees of freedom are associated with translational motion, and three more degrees of freedom are associated with

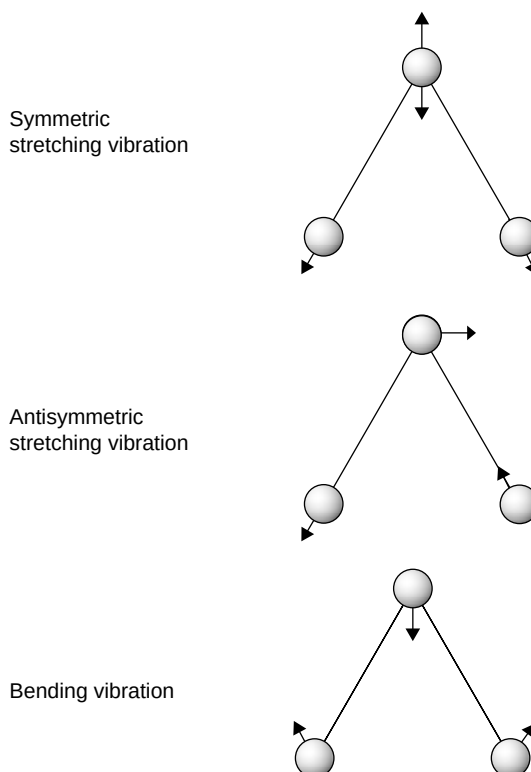


FIGURE 1 Motions of the atoms in each of the genuine vibrational modes of a nonlinear triatomic molecule.

rotational motion, so for polyatomic molecules consisting of N atoms, there will be $(3N - 6)$ degrees of freedom due to the various types of internal motion known as the vibrational modes.

Consider the case of a non-linear triatomic molecule, for which the number of authentic vibrations will therefore equal $[3(3) - 6]$. The three normal vibrational modes of motion are illustrated in Figure 1. To preserve the pure vibrational nature of the symmetric stretching motion, the central atom must move an equal amount in the direction opposite to the motion of the end atoms, so that the net result of the simultaneous motions of the end and central atoms is a symmetric stretching of the bonds. The atomic motions for the asymmetric stretching vibration gives rise to a compression of one bond and a stretching of the other bond, whereas the atomic motions for the bending vibration yield an opening and closing of the bond angle.

The Harmonic Oscillator Model and Spectroscopy Associated with Fundamental Vibrational Modes

A full understanding of vibrational spectroscopy requires a solution of the wave functions associated with vibrational motion, which are usually developed as quantum

mechanical expressions independent of the expressions for electronic motion. For a simple diatomic molecule, the Hamiltonian operator for nuclear motion is given by the sum of the kinetic and the potential energy operators, and so the Schrödinger equation for nuclear motion can be written as:

$$\{T_N + V(r)\}\psi = E\psi \quad (1)$$

where T_N is the kinetic energy operator, $V(r)$ is the potential energy function, and it is understood that ψ is the wave function for nuclear motion. For the diatomic molecule A-B, equation (1) becomes:

$$\frac{1}{m_A} \nabla_A^2 \psi + \frac{1}{m_B} \nabla_B^2 \psi + \frac{2}{\hbar^2} \{E - V(r)\} \psi = 0 \quad (2)$$

where m_A and m_B are the masses of the two atoms comprising the molecule.

After elimination of terms associated with translation of the nuclei, the motion of the atoms is determined by the effect of the potential field directed along the internuclear axis. Mathematically, this situation is equivalent to that of a single particle moving in the field defined by $V(r)$, where the mass of this equivalent particle is given by the reduced mass (μ) of the two atoms:

$$\mu = \frac{m_A m_B}{m_A + m_B} \quad (3)$$

The wave equation now becomes:

$$\nabla^2 \psi + \frac{2\mu}{\hbar^2} \{E - V(r)\} \psi = 0 \quad (4)$$

After the wave function is transformed into spherical polar coordinates, it can be factored into radial and angular functions that each consist of a single variable. The usual procedure is to define the radial distribution function, $S(r)$, and after much work the radial portion of the wave equation becomes:

$$\frac{dS}{dr^2} + \frac{2\mu}{\hbar^2} \{E - V(r)\} S = 0 \quad (5)$$

Equation (5) cannot be considered further until one chooses a form for the potential function, but highly useful solutions are obtained if $V(r)$ is assumed to have the form associated with simple harmonic motion:

$$V(r) = \frac{1}{2} k r^2 \quad (6)$$

In equation (6), k is the force constant associated with the magnitude of the restoring force on the particle.

The mathematical solution to the harmonic oscillator wave function is beyond the scope of this chapter, but has been amply described in numerous texts on quantum mechanics (33–37). At the end of the process, one obtains expressions for the energies (E_v) of the vibrational wave functions that are functions of the reduced mass, the bond force constant, and a quantum number v for each wave function:

$$E_v = \eta \{k/\mu\}^{1/2} (v + \frac{1}{2}) \quad (7)$$

Because the classical vibrational frequency (ν_{OSC}) of a harmonic oscillator is:

$$\nu_{\text{OSC}} = (1/2\pi) \{ k / \mu \}^{1/2} \quad (8)$$

it follows that the energies (in units of ergs) of the vibrational states are also equal to:

$$E_v = h \nu_{\text{OSC}} (v + 1/2) \quad (9)$$

It is more typical to express the energy of a given vibrational state in units of reciprocal centimeters (cm^{-1}), which is obtained by dividing equation (9) by the speed of light:

$$G_v = \frac{h \nu_{\text{OSC}}}{c} (v + 1/2) \quad (10)$$

Defining the oscillator energy as ω (in units of cm^{-1}), one obtains the expression:

$$G_v = \omega (v + 1/2) \quad (11)$$

For most molecules, ω will have values in the range of 200 to 4000 cm^{-1} .

In order for a molecule to absorb infrared energy to promote the system from the ground $E(v = 0)$ ground state to an excited $E(v \neq 0)$ excited states, the molecule must possess a permanent dipole moment. The intensity of this electric dipole transition is determined by the magnitude of the transition probability, $\mathbf{P}_{ij}$, which is calculated from the lower vibrational state ($\mathbf{S}_i$) to the upper vibrational state ($\mathbf{S}_j$):

$$\mathbf{P}_{ij} = \int \mathbf{S}_i \boldsymbol{\mu} \mathbf{S}_j d\tau \quad (12)$$

For a molecule having a permanent dipole moment, it can be shown that equation (12) will equal zero unless the following harmonic oscillator selection rule is met:

$$\Delta v = \pm 1 \quad (13)$$

The energetics of vibrational energy levels is such that the ground vibrational state of a given molecule will be characterized by $v = 0$. The implication of the harmonic oscillator selection rule is that the only allowed vibrational transition would be $\mathbf{S}_0 \rightarrow \mathbf{S}_1$, which is known as the *fundamental* vibrational transition. In the harmonic oscillator model, transitions from $\mathbf{S}_0$ to higher excited states $\mathbf{S}_v$ are forbidden.

In the harmonic oscillator model, therefore, the energy of the $E(v = 0)$ state is given by:

$$E_0 = \frac{\hbar}{2} \{ k / \mu \}^{1/2} \quad (14)$$

and the energy of the $E(v = 1)$ state is given by:

$$E_1 = \frac{3\hbar}{2} \{ k / \mu \}^{1/2} \quad (15)$$

Therefore, energy of the $(v = 0) \rightarrow (v = 1)$ transition is given by:

$$E_1 - E_0 = \hbar \{k/\mu\}^{1/2} \quad (16)$$

or:

$$G_1 - G_0 = \omega \quad (17)$$

According to equation (17), the energy of a vibrational transition may be seen to be partially defined by the magnitude of the force constant (which is interpreted as a measure of the bond strength). In particular, it can be shown that one can calculate a value for the force constant of a harmonic oscillator using the relation:

$$k = \mu(4\pi^2\omega^2c^2) \quad (18)$$

where the reduced mass μ is give in units of grams/molecule, ω is in units of cm^{-1} , and the speed of light c equals 2.9979246×10^{10} cm/sec.

The energy of a vibrational transition is also dependent on the reduced mass of the atoms involved in the vibrational motion. Using the average bond stretching force constants of Wilson, Decius, and Cross [p. 175 of Ref. (17)], and the relation:

$$\omega = \frac{\{k/\mu\}^{1/2}}{2\pi c} \quad (19)$$

it is possible to calculate the transition energies for the fundamental vibrational transitions of some chemically relevant bond types. For instance, the energy of the C-H stretching mode is calculated to be 3444 cm^{-1} , and the energy of the C-C stretching mode is calculated to be 1189 cm^{-1} . In addition, the energy of the N-H stretching mode is calculated to be 3425 cm^{-1} , and the energy of the N-N stretching mode is calculated to be 1044 cm^{-1} . Finally, the energy of the O-H stretching mode is calculated to be 3737 cm^{-1} , and the energy of the C-C stretching mode is calculated to be 950 cm^{-1} .

From this analysis, the general trend emerges that the stretching frequencies of molecules containing a hydrogen atom will be much higher than the stretching frequencies of molecules that do not contain a hydrogen atom. The spectroscopic consequence of this trend (which will be developed further in forthcoming sections) is that vibrational transitions associated with atom-hydrogen stretching modes will be observed at much higher energies than will be the transitions of other functional groups.

Even for polyatomic molecules, the harmonic oscillator model can be adequately used to describe their fundamental vibrational transitions, because the vibrational spectra of complicated molecules can often be assumed to be summations of a number of harmonic oscillators. The concept of group frequencies is based on the fact that many vibrational wave functions are be essentially localized on the atoms of a functional group and its nearest neighbors. Because the bond force constants are fairly constant from molecule to molecule, and because the reduced masses often are comparable as well, it follows that the vibrational frequency of a given functional group is determined primarily by the identity of the atoms

involved. This concept has led to the concept of *group frequencies*, which have been summarized in several monographs (38–40).

Returning to the example of the non-linear triatomic molecule exhibiting three modes of vibration, the symmetric stretching mode will be identified as ν_1 , the “scissors” bending mode as ν_2 , and the anti-symmetric stretching mode as ν_3 . Because the individual vibrational modes are independent of each other, the vibrational wave function describing the states of this non-linear triatomic molecule will be:

$$\Psi(\nu_1, \nu_2, \nu_3) = \Psi(\nu_1)\Psi(\nu_2)\Psi(\nu_3) \quad (20)$$

The ground state of this non-linear triatomic molecule will be characterized by $\Psi(\nu_1)$, $\Psi(\nu_2)$, and $\Psi(\nu_3)$ each being in their respective $v = 0$ states, and its wave function will be abbreviated as Ψ_{000} . The lowest energy excited states will be characterized by two of the $\Psi(\nu_1)$, $\Psi(\nu_2)$, or $\Psi(\nu_3)$ functions each being in the $v = 0$ state and the third function being in the $v = 1$ state. These three possible excited states would be identified by the functions Ψ_{100} , Ψ_{010} , and Ψ_{001} .

Because the harmonic oscillator selection rule is $\Delta v = \pm 1$, it follows that the only possible fundamental vibrational spectroscopic transitions for the non-linear triatomic molecule water are:

$$\Psi_{000} \rightarrow \Psi_{100} \text{ (the } \nu_1 \text{ mode)} \quad (21)$$

$$\Psi_{000} \rightarrow \Psi_{010} \text{ (the } \nu_2 \text{ mode)} \quad (22)$$

$$\Psi_{000} \rightarrow \Psi_{001} \text{ (the } \nu_3 \text{ mode)} \quad (23)$$

For water in the gas phase, it has been found that the energy of the ν_1 vibrational mode equals 3652 cm^{-1} , the energy of the ν_2 mode equals 3756 cm^{-1} , and the energy of the ν_3 mode equals 1545 cm^{-1} . However, as shown in Figure 2, in bulk water these frequencies are substantially shifted to 1634 , 3262 , and 3341 cm^{-1} , respectively, owing to the extensive degree of hydrogen bonding.

The Anharmonic Oscillator Model and Spectroscopy Associated with Overtones and Combinations of Fundamental Vibrational Modes

Because the harmonic oscillator selection rule states that only transitions for which $\Delta v = \pm 1$ are allowed, and because, the majority of molecules will be in the $v = 0$ state at room temperature, only fundamental transitions can be observed in either the infrared absorption or Raman spectra. A more realistic description of the energy level sequence can be achieved by using potential energy functions that allow for molecular distortion in excited vibrational states. The inclusion of anharmonic effects serves to weaken the $\Delta v = \pm 1$ selection rule, and permits transitions to higher quantum states. The development of appropriate technology has permitted the development of a wide variety of applications in the pharmaceutical and other industries.

The most widely used anharmonic potential energy function is the Morse potential:

$$V(r) = D_e [1 - e^{-a(r - r_e)}]^2 \quad (24)$$

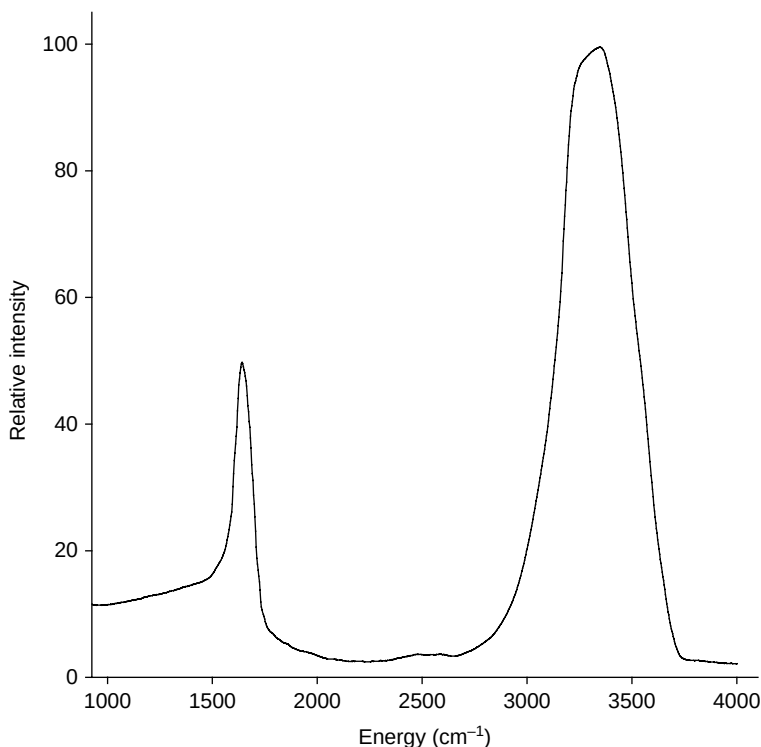


FIGURE 2 Infrared absorption spectrum of bulk water, obtained using the attenuated total reflectance sampling mode.

where D_e is the energy required to dissociate the bond. The rigorous approach would then be to substitute this form for the potential energy function back into the Hamiltonian equation, and to then solve the resulting Schrödinger equation in the usual manner. However, this approach is not trivial, and hence, more empirical approaches are usually taken.

A more practical approach toward vibrational spectroscopy is to expand the harmonic oscillator equation in an infinite power series based on $(v + \frac{1}{2})$ to include anharmonic effects:

$$G'_v = \omega_e(v + \frac{1}{2}) - \omega_e X_e(v + \frac{1}{2})^2 + \omega_e Y_e(v + \frac{1}{2})^3 + \dots \quad (25)$$

where ω_e is the harmonic oscillator constant, and where X_e and Y_e are anharmonic constants. The dissociation energy is given by the sum:

$$D_e = \sum \Delta G'(v + \frac{1}{2}) \quad (26)$$

where the summation is performed over the range of $v = 0$ to the highest vibrational level for which the bond in the molecule is still intact. Usually the $\omega_e X_e$ term is

considerably larger than the $\omega_e Y_e$ term, and thus most experimentally determined vibrational spectra can be fitted to the simplified equation:

$$G'_v = \omega_e(v + \frac{1}{2}) - \omega_e X_e(v + \frac{1}{2})^2 \quad (27)$$

For most molecules, the $\omega_e X_e$ term will be positive in character, so the separation between successively higher excited states will decrease as v increases. In this simplified model, the separation between the G'_v state and the G'_{v+1} state will be given by:

$$\Delta G'_v = \omega_e - \omega_e X_e(2v + 2) \quad (28)$$

In this simplified model of the anharmonic oscillator, the dissociation energy is given by:

$$D_e = \omega_e / 4X_e \quad (29)$$

Equation (28) indicates that progressions of experimentally observed vibrational transitions can be considered as being composed of a harmonic and an anharmonic part. But at ambient conditions the majority of molecules will be in the $v = 0$ state because the thermal energy available at 25°C is not sufficient to raise more than a few percent of molecules into the $v = 1$ state. This gives rise to the reality that for a transition from $v = 0$ to $v = 1$, the contribution from the anharmonic terms is relatively minor; therefore, fundamental transitions can be adequately treated using the harmonic oscillator model.

With the inclusion of anharmonic effects in vibrational energy states, the strict harmonic oscillator selection rule of $\Delta v = \pm 1$ becomes relaxed and transitions from the ground vibrational state to higher vibrationally excited states to gain some degree of allowedness. Because the overtone transitions can only take place through relaxation of the primary selection rule transitions, where $\Delta v = 2, 3, 4$, etc., will be less intense than the corresponding fundamental transition. Nevertheless, overtone transitions are easily detected, and are observed in the NIR region of the electromagnetic spectrum (41–45).

Returning to the non-linear triatomic molecule, once the effects of anharmonicity are factored into the transition moment calculations, almost any type of vibrational excitation becomes possible. One could observe sequences of simple *overtone* transitions, such as:

$$\Psi_{000} \rightarrow \Psi_{200}, \Psi_{300}, \Psi_{400}, \text{ etc.} \quad (30)$$

$$\Psi_{000} \rightarrow \Psi_{020}, \Psi_{030}, \Psi_{040}, \text{ etc.} \quad (31)$$

$$\Psi_{000} \rightarrow \Psi_{002}, \Psi_{003}, \Psi_{004}, \text{ etc.} \quad (32)$$

or any one of a number of *combination* transitions, some of which can be written as:

$$\Psi_{000} \rightarrow \Psi_{110}, \Psi_{101}, \Psi_{011}, \text{ etc.} \quad (33)$$

$$\Psi_{000} \rightarrow \Psi_{120}, \Psi_{201}, \Psi_{021}, \text{ etc.} \quad (34)$$

TABLE 1 Overtone and Combination Vibrational Transitions of Water in the Vapor Phase

Upper State			Energy (cm ⁻¹)	Wavelength (nm)	Intensity
ν_1	ν_2	ν_3			
0	1	1	5,332.0	1875.5	Medium
0	2	1	6,874.0	1454.8	Weak
1	0	1	7,251.6	1379.0	Medium
1	1	1	8,807.1	1135.4	Strong
2	0	1	10,613.1	942.2	Strong
0	0	3	11,032.4	906.4	Medium
2	1	1	12,151.2	823.0	Medium
3	0	1	13,830.9	723.0	Weak
1	0	3	14,318.8	698.4	Weak

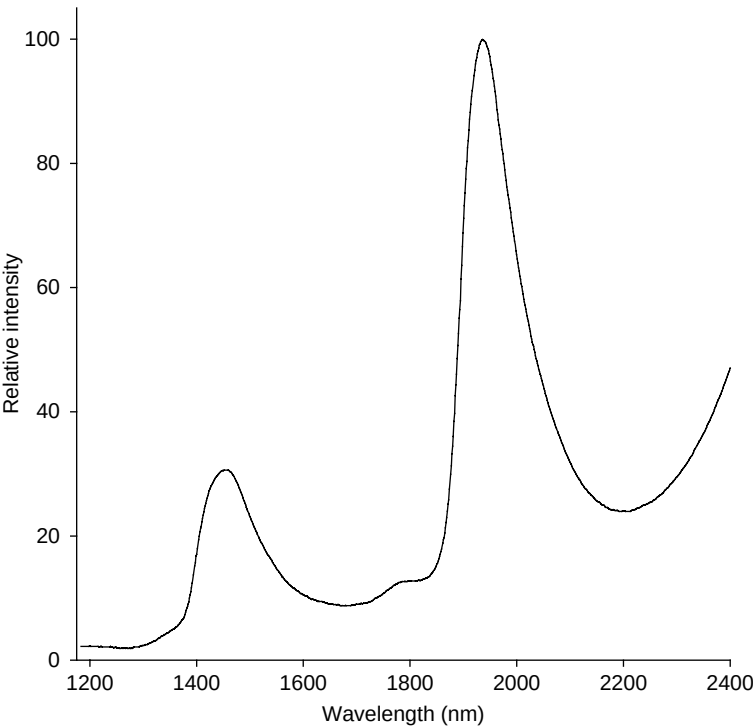


FIGURE 3 NIR absorption spectrum of bulk water, obtained using the reflectance sampling mode.

A summary of the overtone and combination bands of water in the vapor phase is given in Table 1, and a portion of its liquid phase absorption spectrum is shown in Figure 3. For bulk water, the moderate band observed at 1455 nm can be assigned to the $\psi_{000} \rightarrow \psi_{101}$ transition, the weak band at 1806 nm can be assigned to the $\psi_{000} \rightarrow \psi_{021}$ transition, and the strong band at 1936 nm can be assigned to the $\psi_{000} \rightarrow \psi_{011}$ transition.

INFRARED ABSORPTION SPECTROSCOPY FOR THE CHARACTERIZATION OF POLYMORPHS AND SOLVATOMORPHS

The acquisition of high-quality infrared spectra on solid materials has been made possible using Fourier transform technology, because the use of this methodology minimizes transmission and beam attenuation problems. Essentially all FTIR spectrometers use a Michelson interferometer, where infrared radiation entering the interferometer is split into two beams: one of which follows a path of fixed distance before being reflected back into the beam splitter, and the other traveling a variable distance before being recombined with the first beam. The recombination of these two beams yields an interference pattern, where the time-dependent constructive and destructive interferences have the effect of forming a cosine signal. Each component wavelength of the source yields a unique cosine wave, having a maximum at the zero pathlength difference (ZPD) and which decays with increasing distance from the ZPD. The radiation in the central image of the interference pattern impinges on the detector, and intensity variations in the recombined beam become measurable manifest as phase differences. If the component cosine waves can be resolved, then the contribution from individual wavelengths can be observed. The frequency domain spectrum is obtained from the interferogram by performing the Fourier transformation mathematical operation. More detailed descriptions of the components of a FTIR spectrophotometer are available (46–48).

Numerous modes of sampling may be used with FTIR spectrophotometers, although only a few of these are appropriate for the study of polymorphs and solvatomorphs. The classic alkali halide pellet method is probably the least useful for this type of work, as the sample may undergo so a solid-state transformation due to the pressure used to form the pellet, or one may encounter halide exchange between the KBr or KCl matrix and the sample (49). The use of mineral oil mulls avoids the problems noted for the pellet technique, but the mineral oil itself exhibits a number of intense absorption bands (2952, 2923, 2853, 1458, and 1376 cm^{-1}) that may overlap important absorption bands associated with the sample and obscure important spectral regions. Use of diffuse reflectance sampling avoids all of the problems just mentioned (50,51), but usually requires dilution of the analyte with either KBr or KCl at a level of 1% to 5% w/w. The diffuse reflectance technique lends itself to studies of polymorphic composition because it is non-invasive, its use causes no changes in polymorph character due to its inherent limited sample handling, and the technique can be used for quantitative purposes.

Probably the most useful sampling method for the study of polymorphs and solvatomorphs uses attenuated total reflectance accessories (52,53). In the ATR technique, infrared radiation is passed through a crystal at an angle less than the critical angle, which causes the light to undergo total internal reflection. At each such reflection, the radiation penetrates a small distance beyond the crystal surface, and if an analyte is in physical contact with the crystal, then the internally reflected

energy will be attenuated at those frequencies corresponding to changes in molecular vibrational states. The advantage of the ATR approach is that it requires effectively no sample preparation, because one simply clamps the analyte onto the surface of the crystal with moderate pressure to ensure a sufficient degree of optical contact. Because the internal reflectance process does not permit the infrared beam to pass very deeply into the sample, it is typical to determine the composition of an analyte up to a sampling depth in the range of 5 to 10 μm .

Not surprisingly, the sensitivity of infrared absorption spectroscopy to subtle changes in crystal structure have led to its application in a wide variety of investigations of polymorphic solids, often in conjunction with Raman spectroscopic studies. For example, FTIR spectra of different polymorphs of mepivacaine hydrochloride were obtained in Nujol mulls and via ATR sampling, with the ATR method providing better distinction between the polymorphs (54). This has been illustrated in Figure 4, where the infrared absorption spectra of mepivacaine hydrochloride (Form-II) is seen to be superior when obtained using the attenuated total reflectance

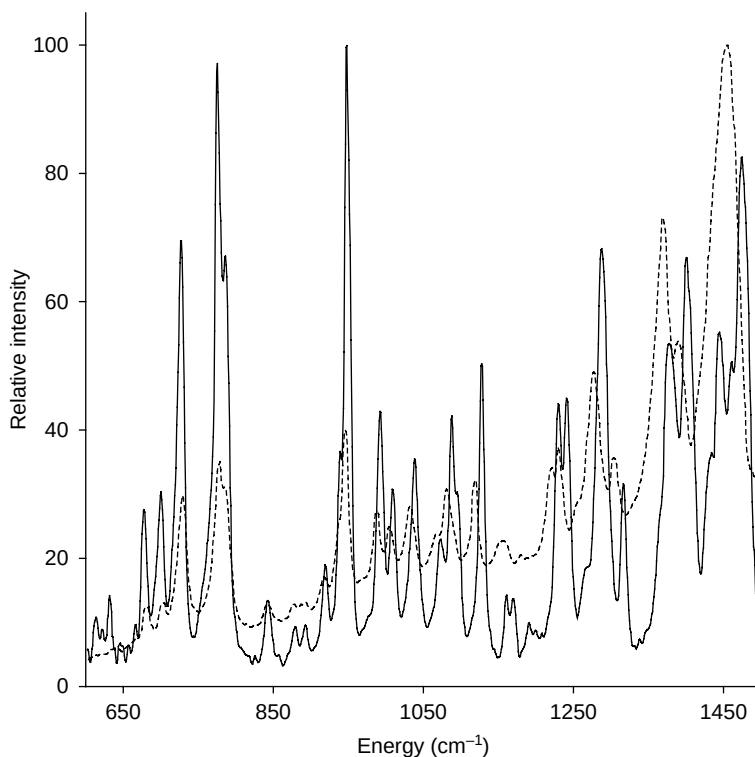


FIGURE 4 Infrared absorption spectra of mepivacaine hydrochloride, Form-II, obtained using the attenuated total reflectance (*solid trace*) and Nujol mull (*dashed trace*) sampling modes. Each spectrum has been normalized so that the relative intensity of the most intense band equals 100%, and the normalized spectra were generated from data given in Ref. (54).

sampling mode as opposed to a Nujol mull. When coupled with artificial neural network methods, a diffuse reflectance FTIR spectroscopic method was developed that permitted the simultaneous quantification of three polymorphs of carbamazepine in ternary mixtures (55).

FTIR-ATR spectroscopy was used to characterize the salt-induced crystallization of a metastable polymorph of flufenamic acid, and to characterize the product obtained after a subsequent interface-mediated polymorphic transition (56). The FTIR-ATR method was also used to characterize the fluconazole products obtained using the supercritical antisolvent process, with the technique being able to identify the polymorphic forms produced as a result of variation of operating conditions such as temperature, pressure, and solvent type (57). Three concomitant polymorphs of 1,3-bis(*m*-nitrophenyl)urea had been reported in 1899 as yellow prisms (the α -form), white needles (the β -form), and yellow tablets (the γ -form), and FTIR-microscopy was used during a more detailed investigation of the system (58). In this work, complete assignments for the absorption bands associated with hydroxyl, amide, nitro, and benzene-ring functional groups were developed in order to obtain a deeper understanding of the conformational differences in the molecules constituting the various crystal forms.

Five differently colored solid-state forms of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile, where the color of the polymorphic crystal forms is related to changes in the energies of molecular orbitals of the systems have been studied using infrared absorption spectroscopy (59). The spectra for three of the solid-state forms were characterized by frequency shifts originating from the structural differences, with the nitrile stretching frequency varying by approximately 10 cm^{-1} each for the yellow, orange, and red solid-state forms of the compound. In a study of the amorphous salt formed by the co-precipitation of cimetidine and diflunisal, solid-state infrared absorption spectroscopy was used to prove the existence of the salt species (60). The prominent carbonyl absorption band observed at 1650 cm^{-1} in crystalline diflunisal could not be observed in the spectrum of the amorphous salt, but a new peak was noted at 1580 cm^{-1} that was assigned to an asymmetric stretching mode of a carboxylate group.

Diffuse reflectance sampling was used to study the anhydrides and hydrates of ampicillin and nitrofurantoin, with the presence of water in the hydrate crystal forms causing new bands to appear in the infrared absorption spectrum (61). On the other hand, FTIR spectroscopy was not useful in distinguishing between the hydrate forms of diclofenac sodium as the frequencies in the spectra of the polymorphic tetrahydrates were nearly identical, and only differed in band relative intensities (62). The utility of combined FTIR and solid-state ^{13}C nuclear magnetic resonance studies enabled the deduction that although the water molecules of topotecan hydrochloride trihydrate were intrinsic parts of the crystal lattice, the pentahydrate possessed additional structural channels where water could hydrogen bond to specific portions of the topotecan molecules (63).

Fluconazole has been isolated in a number of solvated and non-solvated forms, and infrared spectroscopy has proven to be an important tool in the characterization of these. In one study involving two non-solvated polymorphs (Forms I and II) and several solvatomorphs (the $1/4$ -acetone solvate, a $1/7$ -benzene solvate, and a monohydrate), the infrared spectra of the different forms showed differentiation in bands associated with the triazole and 2,4-difluorobenzyl groups, and in the propane backbone (64). In another study, the diagnostic infrared spectral

characteristics of non-solvated Form-III and two solvatomorphs (the $\frac{1}{4}$ -ethyl acetate solvate, and a monohydrate) were used to demonstrate the novelty of the new forms relative to those in the literature (65). In yet another work, the kinetics associated with the dehydration and desolvation of fluconazole solvatomorphs were studied by three physical methods, and then used to understand the mechanisms of the processes involved (66).

It has been found that the carbonyl frequency of niclosamide anhydrate and two of its monohydrates was particularly sensitive to the crystal form of the drug substance, and hence, infrared spectroscopy could be used to demonstrate the conformational state of the compound in the solvatomorphs (67). Similarly, infrared spectra obtained in the fingerprint and high-frequency regions for four non-solvated polymorphs of tenoxicam, and of its acetonitrile, dioxane, dimethyl formamide, ethyl acetate, acetone, and isopropanol solvates, facilitated a differentiation between the various crystal forms (68). A combination of thermogravimetric analysis and infrared absorption spectroscopy was used to develop a quantitative method for the determination of pantoprazole sodium monohydrate in bulk quantities of the sesquihydrate solvatomorph (69).

Because the pattern of vibrational modes is usually sufficiently perturbed by crystallographic differences, infrared absorption spectroscopy can be profitably used to study the phase transformation process between polymorphic forms of a substance. For example, infrared absorption spectroscopy has been used to study the solution-mediated phase transformation of metastable β -glycine to the stable α -phase, making use of disappearance of the weak band at 1660 cm^{-1} and shifts in energy for the anti-symmetric stretch of the carboxyl group ($1580\text{--}1590\text{ cm}^{-1}$), the deformation of the NH_3^+ group ($1515\text{--}1500\text{ cm}^{-1}$), and the $\text{O}=\text{C}=\text{O}$ group bend (around 700 cm^{-1}) (70). In another work, characteristic absorption bands around 1145 to 1165 cm^{-1} were found to be useful in understanding the effect of experimental variables on the solution-mediated Form-II to Form-I phase transformation of buspirone hydrochloride (71).

Infrared spectroscopy has been used to follow the isothermal transformation of mefenamic acid Form-I into Form-II at a sufficient number of temperatures so as to calculate the activation energy for the process (72). The value of 71.6 kcal/mol , obtained using the infrared spectroscopic method, was significantly smaller than the previously reported value of 86.4 kcal/mol that had been determined using of differential scanning calorimetry. Conventional FTIR spectroscopy in conjunction with FTIR micro-spectroscopy has been used to study the polymorphic interconversion of famotidine metastable Form-B into stable Form-A during grinding in a ceramic mortar (73). The particle size reduction that resulted from the grinding process was also found to lead a decrease in the transition temperature for the Form-B to Form-A phase transformation.

Changes in infrared absorption spectra have also been used to study the phase transformation process between solvatomorphs. For example, granulation of anhydrous lactose with water results in conversion of the anhydrate phase to the monohydrate phase, as well as a change from the β -anomer to the α -anomer. Figure 5 shows the FTIR-ATR spectra obtained in the high-frequency region for different stages in the solution-mediated conversion of lactose anhydrate to lactose monohydrate phase of lactose monohydrate, where it is clear that the absorbance band at 3522 cm^{-1} could be used to follow the kinetics of formation of the monohydrate (74).

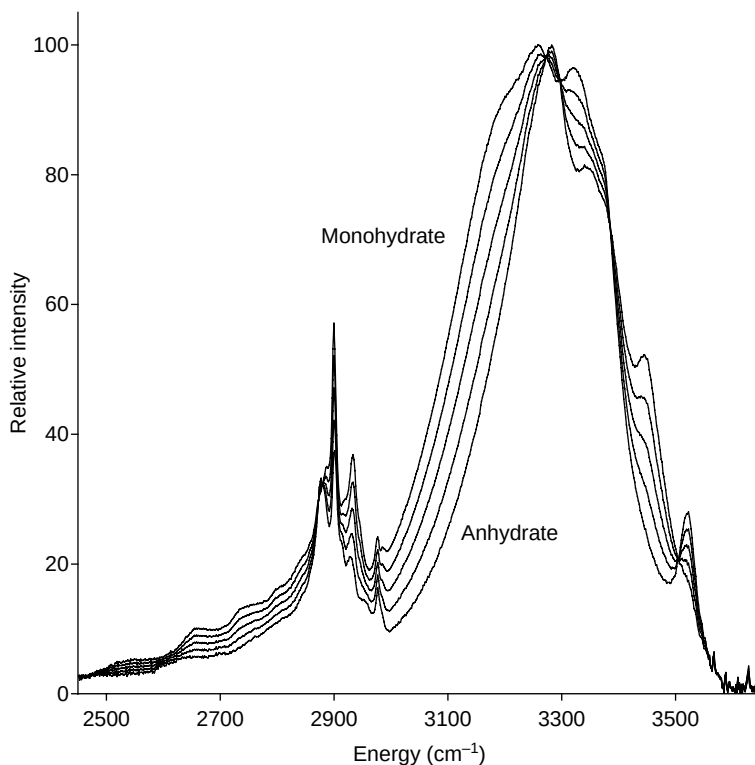


FIGURE 5 FTIR-ATR spectra obtained in the high-frequency region of lactose anhydrate (*lowest trace*), and during various stages of its solution-mediated phase transformation into the monohydrate phase (*uppermost trace*). H.G. Brittain, unpublished results (70).

Trehalose dihydrate undergoes a dehydration transition around 100°C, and the infrared spectra obtained during this process were found to change significantly over the 1500 to 1800 cm⁻¹ region (75). For example, the band intensities at 1640 and 1687 cm⁻¹ decreased sharply around 65°C, but remained relatively constant at higher temperatures. During the rehydration process where trehalose anhydrate became trehalose dihydrate, the liquid-like water became solid-like water over the same temperature range. It was postulated that this phase transition could be related to the protective effect of trehalose in preserving protein stability.

NIR SPECTROSCOPY FOR THE CHARACTERIZATION OF POLYMORPHS AND SOLVATOMORPHS

Because the energy separation of the ground vibrational state and the lowest vibrational excited states is large relative to kT at room temperature, molecules at ambient temperature will reside almost entirely in their ground vibrational states. According to the harmonic oscillator selection rule of $\Delta v = \pm 1$, only the fundamental vibrational transitions that are studied by ordinary infrared absorption spectroscopy

or Raman spectroscopy will be allowed. As long as the harmonic oscillator selection rules can be considered as being valid, vibrational transitions corresponding to $\Delta v = \pm 2$, $\Delta v = \pm 3$, $\Delta v = \pm 4$, etc., or combinations of these, are not allowed.

However, the harmonic oscillator model should be considered as being the first term in a solution of the vibrational Hamiltonian equation where the potential term can also include anharmonic effects. As discussed above, the strict $\Delta v = \pm 1$ selection rule can be relaxed by the inclusion of anharmonicity, and the relaxation of this fundamental selection rule enables the observation of transitions from the ground vibrational state to higher vibrationally excited states. However, because the fundamental selection rule is merely relaxed and not broken, these overtone transitions will generally be less intense than their fundamental transition.

The NIR region of the spectrum is generally considered to span 750 nm ($13,350\text{ cm}^{-1}$) to 2500 nm (4000 cm^{-1}). Through the design of appropriate instrumentation, the overtone and combination transitions can be detected and quantitated, with these being observed in the NIR region of the spectrum (76–83). In general, the spectral features of greatest utility entail overtone transitions associated with functional groups that contain unique hydrogen atoms. For example, NIR spectroscopy has been successfully used to develop methods for moisture determination, whole tablet assay, and powder blending (84–87). Owing to the usually broad nature of the absorption bands, the raw spectra themselves tend not to be most useful in the study of polymorphs and solvatomorphs, but when the spectra are differentiated and the resulting derivative spectra combined with multi-component analysis, real analytical utility emerges.

NIR spectroscopy has been shown to be capable of differentiating between polymorphs of sulfathiazole and sulfamethoxazole, as well as hydrates of ampicillin and lactose (88). For example, the raw absorption spectra of sulfathiazole Form-I and Form-III are shown in Figure 6 along with their corresponding second derivative spectra. It is evident that use of the derivative spectra eliminates the sloping baseline of the raw absorption spectra, readily identifying bands at 1376 and 1528 nm that were useful in differentiating between the polymorphic forms. These spectra differences were exploited to develop a quantitative analytical method for determination of the amount of Form-III in Form-I that was characterized by recovery errors less than 3%.

Although NIR spectroscopy can definitely be used as a means of polymorph identification, its real power lies in the ability of workers to readily develop quantitative assays for phase composition. The quantification of sulfathiazole Form-I and Form-III in binary mixtures was addressed in another work, here using univariate, multiple linear regression, and partial least-squares regression methods to generate the validation set (89). Quantitative methods for the two polymorphs of bicifadine hydrochloride have been developed using differential scanning calorimetry coupled with thermogravimetric analysis, X-ray powder diffraction, infrared absorption spectroscopy, and NIR spectroscopy, with the NIR methods being found to yield the best accuracy and ease of operation (90). The utility of Raman and NIR spectroscopies combined with multivariate analysis for the quantitation of ternary mixtures of α -, γ -, and amorphous indomethacin was investigated, with comparable root-mean-square errors of prediction being obtained for the two methods (91).

Distinctions between the solvated and non-solvated forms of a drug substance are often easy to detect using NIR spectroscopy, making the technique a valuable part of a multi-disciplinary study such as that carried out on the baclofen anhydrate

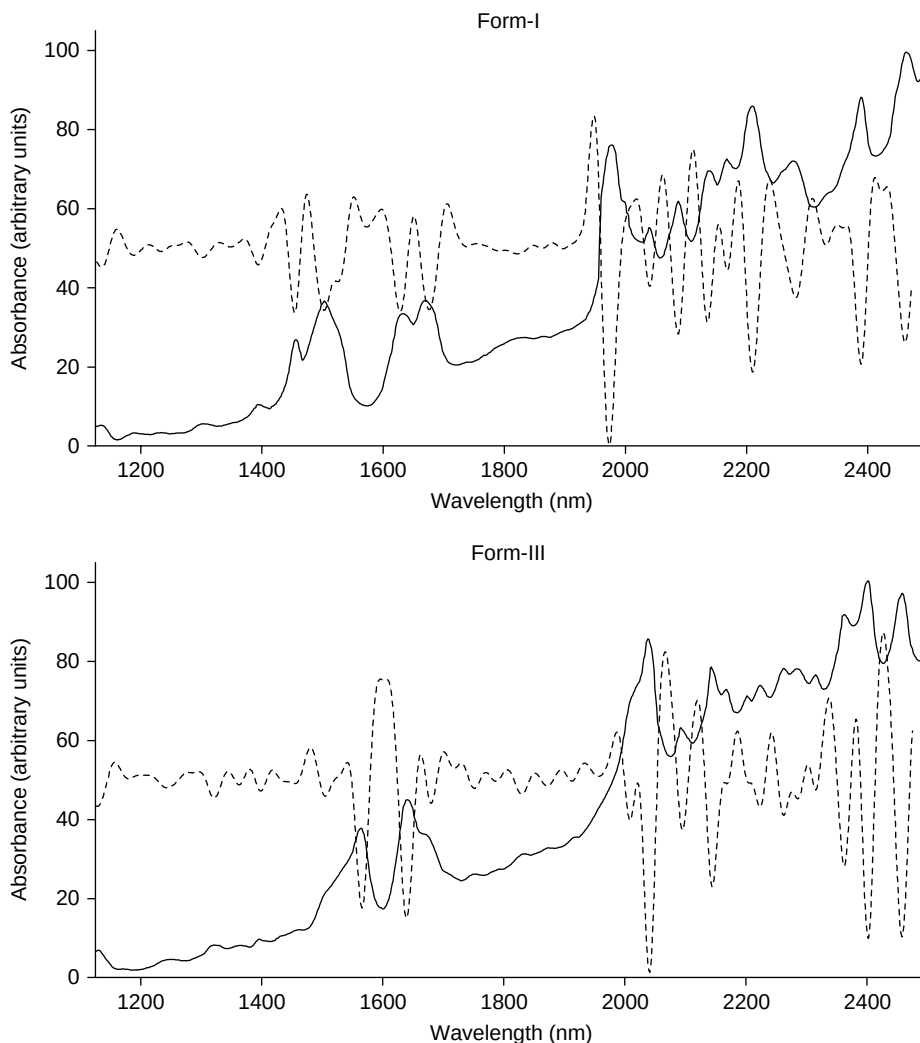


FIGURE 6 NIR spectra obtained for sulfathiazole Form-I (*upper spectra*) and Form-III), showing both the raw absorption spectrum (*solid traces*) and the corresponding second derivative spectrum (*dashed traces*) for each. The spectra are shown in arbitrary units, and have been adapted from Ref. (88).

and monohydrate (92). The factors associated with the quantitative analysis of anhydrate/hydrate powder mixtures have been discussed (93), and these issues illustrated in work establishing the phase boundaries in the anhydrate/hydrate system of caffeine (94). The interconversion between the anhydrate, monohydrate, and dihydrate forms of azithromycin have been studied, with the spectral region associated with the first overtone of water (1800–2200 nm) being most useful (95). In another work, the monohydrate/dihydrate composition of magnesium

stearate samples was established using NIR spectroscopy and multivariate calibration (96).

NIR spectroscopy was used to monitor the degree of conversion between the stable Form-A of a new chemical entity and the metastable Form-B produced at the elevated temperature and humidity conditions of its wet granulation (97). Because a reference method was not available for quantitation of Form-B in bulk quantities of Form-A, a calibration set was developed from the NIR spectra of the drug substance, the premix blend, and wet granulated samples, because narrow spectral regions unique to Form-B were found that were insensitive to differences in physical properties between the premix blend and wet granulation. The final univariate method was used for either for off-line, or for on-line, monitoring of phase conversion during the wet granulation process.

In-line NIR spectroscopy was used to demonstrate that the process-induced transformation of erythromycin A dihydrate to its dehydrated form did not take place easily during pellet manufacture via extrusion/spheronization and drying, although partial phase transformation was noted for pellets dried at 60°C (98). NIR spectroscopy was one of several techniques used to obtain quantitative determinations of phase compositions of a developmental compound in its bulk drug substance and in compressed tablets, with a partial least-squares regression algorithm being used to obtain good multivariate calibration (99).

TERAHERTZ SPECTROSCOPY FOR THE CHARACTERIZATION OF POLYMORPHS AND SOLVATOMORPHS

The ability to acquire absorption spectra in the far-infrared region of the spectrum (10–450 cm^{-1}) became much easier to obtain when dispersive technology was replaced by Fourier transform methodology. Initial applications of far-infrared spectroscopy included studies of pure rotational spectra, skeletal bending modes, molecular motion in four-, five-, six, and seven-membered rings, torsional vibrations, vibrational modes involving heavy atoms, and weak intermolecular interactions (100). Other investigational work involved studies of organic crystalline solids, where the translational and librational modes of molecular crystals below 250 cm^{-1} could be evaluated. In one such study, the four lattice modes of crystalline films of elemental nitrogen and carbon monoxide at 10 K were studied over the range of 20 to 250 cm^{-1} , and it was determined that although the translational modes were moderately strong, the librational modes were weak in intensity (101).

Fairly recently, it has been recognized that the developing field of terahertz (THz) spectroscopy can also be used to study low-frequency modes that hitherto had been the domain of far-infrared spectroscopy. Because 1 THz is equivalent to 33.33 cm^{-1} , it turns out that the spectral region of interest (i.e., 3–400 cm^{-1}) corresponds to frequencies of 0.09 to 12 THz. With the development of appropriate instrumentation, the technique has been applied to the study of a wide variety of materials (102,103), and has been used to study the crystallinity of materials having pharmaceutical interest (104–106). With the introduction of the attenuated total reflectance sampling mode (107), it is anticipated that application of THz spectroscopy to the characterization of polymorphs and solvatomorphs will become even more widespread.

The incident THz radiation is obtained as femtosecond pulses that are generated by the excitation of charge carriers in a semiconductor material through the

use of ultra-short pulses of light that is higher in energy than the bandgap of the semiconductor. These pulses can be detected with an ultra-fast antenna receiver, providing simultaneous amplitude and phase information over a wide frequency range in a single measurement. It is to be noted that this type of measurement is based on frequency (in the form of the instantaneous value of the electric field itself) and not on an intensity measurement of the type that would be used during the conduct of conventional far-infrared spectroscopy. The frequency-dependent absorption coefficient and index of refraction of the analyte are obtained from the attenuation, delay, and distortion of the femtosecond THz pulse transmitted through the sample. Additional details of the different modes of measurement technology are available in the book by Dexheimer (103).

The utility of pulsed THz spectroscopy as a tool for the differentiation of the two polymorphs of ranitidine hydrochloride has been demonstrated (108). As shown in Figure 7, the THz spectra of the two crystal forms are quite different, and the authors concluded that the region around 1.10 THz (36.7 cm^{-1}) was the most useful for identification of the polymorphic form contained in tablet formulations. It was also noted that the plastic bags commonly used for drug substance storage

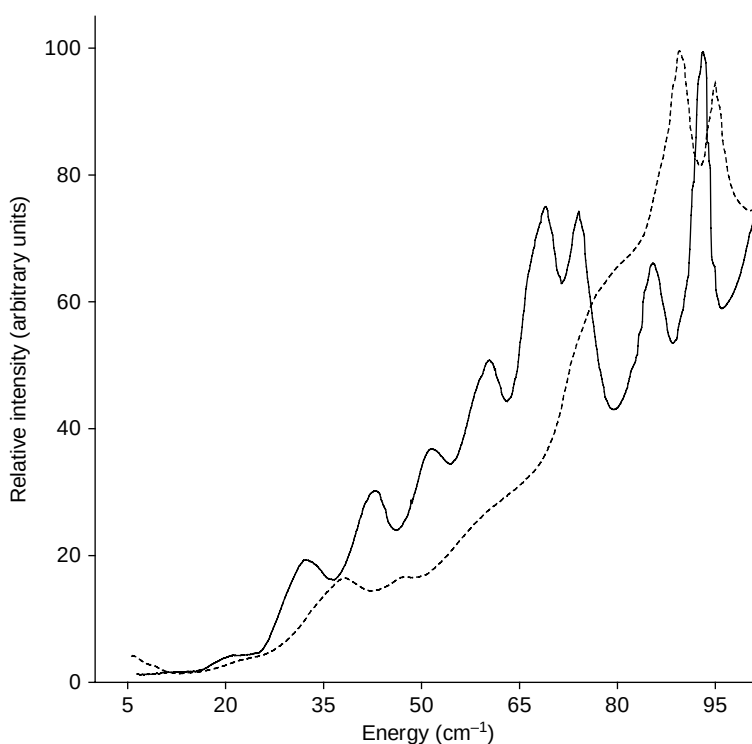


FIGURE 7 THz pulsed spectra of ranitidine hydrochloride, Form-I (*solid trace*), and Form-II (*dashed trace*). Each spectrum has been normalized so that the relative intensity of the most intense band equals 100%, and the normalized spectra were generated from data given in Ref. (104).

were transparent to THz radiation, thus making it possible to use the technology for substance identification without having to remove a sample for analysis.

The utility of pulsed THz spectroscopy for quantitative analysis of phase composition was demonstrated for various forms of carbamazepine, enalapril maleate, indomethacin, and fenoprofen calcium (109). Distinct THz spectra were measured for the various crystal forms owing to the unique patterns of lattice vibrations associated with each compound type and crystal form, and these differences were exploited to develop quantitative methods of analysis. Harmonic lattice dynamics calculations were performed on the four polymorphs of carbamazepine in order to derive assignments for the bands observed in the THz spectra, and to understand the observed trends in frequencies of similar hydrogen bond vibrations in the polymorphs (110). In fact, the sensitivity of THz spectroscopy toward differences between crystal structures has led to its inclusion as one of the identification tools in a polymorph screening study (111).

With the inclusion of a hot-stage in a THz spectrometer, acquisition of temperature-dependent spectra in real time could be used to study a variety of thermally induced phenomena. The methodology was first applied to a study of the phase transition of carbamazepine Form-III to Form-II, as well as the solid-state transformation under isothermal conditions below the melting point (112). When heated between 20°C and 160°C, the spectral features of Form-III broadened, decreased in intensity, and underwent a shift to lower energies. Further heating to 180°C led to melting of Form-III and recrystallization to Form-I, with the THz spectra reflecting the formation of the phase form. Sequences in the disappearance of Form-III spectral features, and in the appearance of Form-I features, indicated that the conversion mechanism entailed more than one step.

The far-infrared spectroscopic characteristics of five polymorphs of sulfathiazole have been studied by THz pulsed spectroscopy and thermal analysis, and then variable-temperature spectroscopic studies were conducted to monitor thermally induced phase transitions among the different forms (113). Time-domain THz spectroscopy was used to obtain the defining characteristics of two anhydrous polymorphs of theophylline and its monohydrate, and then variable-temperature studies were used to monitor phase transformations (114). Details of the phase transformation processes associated with the hydration and dehydration of theophylline have also been studied using THz pulsed spectroscopy (115).

THz time-domain spectroscopy has been used to study the anhydrous and hydrate forms of caffeine, theophylline, glucose, and ampicillin, with the aim of demonstrating the utility of this method as a process analytical tool in production and quality control (116). Crystallization and relaxation phenomena in amorphous carbamazepine have been studied using THz pulsed spectroscopy, and the technique was shown to be capable of determining glass transition temperatures (117).

Raman Spectroscopy for the Characterization of Polymorphs and Solvatomorphs

With the introduction of Fourier-transform methodology into measurement instrumentation, the use of Raman spectroscopy as a tool to solve problems having pharmaceutical interest has become widespread, and the technique is now as essential to the study of polymorphs and solvatomorphs as infrared absorption spectroscopy. Comparable to the use of attenuated total reflectance in FTIR spectroscopy, the sampling modes used in the acquisition of Raman spectra are non-destructive

in nature, and spectra can even be obtained in a non-invasive manner using fiber optics. Unlike diffuse reflectance sampling in FTIR spectroscopy, analytes are not required to be diluted with a spectroscopically inert filler before study. A number of detailed reviews are available regarding the use of Raman spectroscopy in areas of pharmaceutical interest (20–29,31,32).

When substances are irradiated with intense beams of monochromatic radiation, the majority of the photons are scattered at the same frequency as that of the incident beam (i.e., elastic or Rayleigh scattering). However, it has been found that a small amount of the incident light will also be scattered at non-resonant frequencies, some at frequencies more than and some at frequencies less than the frequency of the Rayleigh line. The differences in frequency between the incident radiation and the shifted frequencies correspond to the frequency of molecular vibrations of the molecules in the sample. The scattering peaks observed at lower frequencies relative to the Rayleigh line are known as the *Stokes* bands, and the scattering peaks observed at higher frequencies are known as the *anti-Stokes* bands. Owing to their stronger intensity, most Raman spectroscopic studies use only the Stokes peaks.

For those molecular systems having non-degenerate ground electronic states that are irradiated with intense beams of electromagnetic radiation whose energy does not permit a transition among electronic states, the theory states that the intensities of vibrational Raman transitions are determined by the matrix elements of the electronic polarizability. Therefore, only molecular transitions characterized by a change in polarizability will exhibit an allowed Raman transition. Because the magnitude of the frequency shifts of Raman bands relative to the frequency of the incident beam is determined by the energy differences of the associated vibrational states, the intensity of a given Raman band is related to the transition probability the transition, and as long as the harmonic oscillator model can be used to describe the system, the usual selection rule of $\Delta v = \pm 1$ will hold.

The intensity of scattered light will depend on the magnitude of the induced dipole moment, $\mathbf{P}$, which can be defined in analogy with equation (12) for a transition between states i and j that are defined by the wavefunctions ψ_i and ψ_j , and which is given by the matrix formed from the integrals:

$$\mathbf{P}_{ij} = \int \psi_i \mathbf{P} \psi_j d\tau \quad (35)$$

The polarizability operator, $\mathbf{P}$, being a matrix quantity, requires that the elements of transition moment along each of the Cartesian axes will be:

$$\mathbf{P}_X^{ij} = E_X \int \psi_i a_{XX} \psi_j d\tau + E_Y \int \psi_i a_{XY} \psi_j d\tau + E_Z \int \psi_i a_{XZ} \psi_j d\tau \quad (36)$$

$$\mathbf{P}_Y^{ij} = E_X \int \psi_i a_{YX} \psi_j d\tau + E_Y \int \psi_i a_{YY} \psi_j d\tau + E_Z \int \psi_i a_{YZ} \psi_j d\tau \quad (37)$$

$$\mathbf{P}_Z^{ij} = E_X \int \psi_i a_{ZX} \psi_j d\tau + E_Y \int \psi_i a_{ZY} \psi_j d\tau + E_Z \int \psi_i a_{ZZ} \psi_j d\tau \quad (38)$$

E_X , E_Y , and E_Z are the amplitudes of the incident electromagnetic radiation along each Cartesian direction.

The three diagonal matrix elements of the transition moment ($\int \psi_i a_{XX} \psi_i d\tau$, $\int \psi_i a_{YY} \psi_i d\tau$, and $\int \psi_i a_{ZZ} \psi_i d\tau$) correspond to the Rayleigh scattering, whereas the six off-diagonal elements ($\int \psi_i a_{XY} \psi_j d\tau$, $\int \psi_i a_{XZ} \psi_j d\tau$, $\int \psi_i a_{YX} \psi_j d\tau$, $\int \psi_i a_{YZ} \psi_j d\tau$, $\int \psi_i a_{ZX} \psi_j d\tau$, and $\int \psi_i a_{ZY} \psi_j d\tau$) correspond to the Raman scattering. The $\psi_i \rightarrow \psi_j$ transition will be

Raman allowed if at least one of the six off-diagonal elements does not equal zero. Such determinations can be qualitatively made using symmetry-based group theory, but for low symmetry organic molecules all fundamental (i.e., $\Delta\nu = \pm 1$) Raman transitions will be allowed.

Although Raman spectra can be obtained using dispersive technology, the best spectra are acquired through the use of Fourier transform technology because FT-Raman systems can provide superior wavelength accuracy and minimization of any background fluorescence. In a typical system, a gas or diode laser is used to irradiate the sample, the sample is positioned in the laser beam, and the scattered radiation collected either in the 180° backscattering or the 90° right-angle configuration. The scattered photons are passed into an interferometer equipped with laser line filtering, and ultimately quantitated by a suitable detector. More detailed discussions of the spectrometer hardware can be found in the literature (13–16,118).

With appropriate collection optics, Raman spectroscopy can be performed on very small samples, with solid samples of approximately 25 to 50 mg often being merely filled into metal or glass sample holders. Samples that are darkly colored can present practical difficulties because such materials tend to absorb the exciting energy and degrade. In such cases, one must dissipate the heat, which can be accomplished by reducing the laser power or by spinning the sample to avoid the irradiation of a single point.

As in the case of infrared absorption spectroscopy, the bands in a Raman spectrum can be assigned through the use of group frequencies correlation tables. Significant insight can be obtained from the compilations of functional group vibrational frequencies associated with infrared absorption spectroscopy (38–40), but of greater utility is the compilation of group frequencies specific to Raman spectroscopy (119). Because the transition moment of a Raman transition depends on the magnitude of polarizability, it follows that the vibrational modes exhibiting the strongest intensities in a Raman spectrum will be those that are associated with functional groups that are characterized by high degrees of polarizability. Examples of such functionalities include the C–S, S–S, C–C, N=N, and C=C groups.

Although excitation of a given vibrational mode gives rise to infrared absorption bands and Raman scattering bands having identical frequencies, the fact that the infrared absorption process is an electric dipole transition and the Raman process is based on changes in polarizability leads to the observation of differences in relative spectral intensities for a given material. This is illustrated in Figure 8, where the infrared absorption and Raman spectra of racemic ibuprofen in the fingerprint region are compared (120). Most notable is the complete absence of a peak associated with the asymmetric C=O stretching mode in the Raman spectrum that is very prominent at 1707 cm^{-1} in the infrared absorption spectrum. Also noteworthy are the differing phenyl ring vibrational modes, one of which is observed at 779 cm^{-1} in the infrared absorption spectrum, and another which is observed at 827 cm^{-1} in the Raman spectrum.

As was the case for the other vibrational spectroscopic methods discussed above, Raman spectroscopy can be profitably used to study polymorphic and solvatomorphic solids when the differing crystal structures results in a perturbation of the pattern of molecular vibrations. This is often the case, but the question arises as to how different the energies of Raman peaks associated with the same group vibration in two different crystal forms should be in order to be useful. This question was addressed through the use of descriptive statistics and analysis of variance

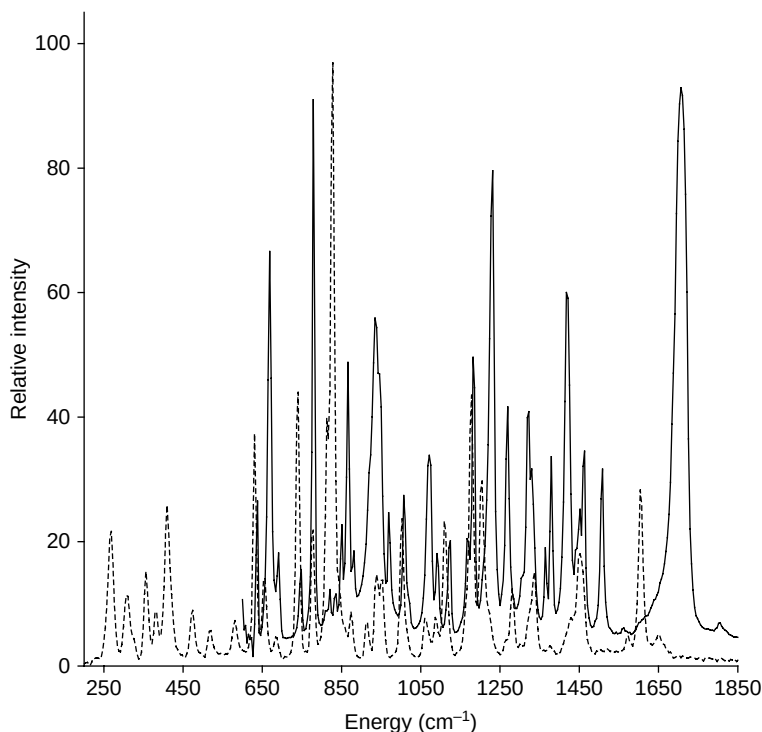


FIGURE 8 Fingerprint region infrared absorption spectrum of racemic ibuprofen (*solid trace*), and the fingerprint region Raman spectrum of the same compound (*dashed trace*). Each spectrum has been normalized so that the relative intensity of the most intense band equals 100%. H.G. Brittain, unpublished results (120).

methods for the development of a guideline that signify when Raman spectral differences could reliably signify the existence of different crystal structures for the same substance (121). From this analysis, it was proposed that the shift in the energy of a Raman peak would need to exceed 1.6cm^{-1} to indicate the existence of polymorphism.

Nevertheless, the shifts commonly observed among polymorphic substances are frequently sufficiently large to permit the use of Raman spectroscopy as a triage method in screening protocols. In one such study, Raman spectroscopy was used to measure the amount of dissolved carbamazepine in a given solvent system, and subsequently to determine whether Form-I or Form-III crystallized upon cooling of the solutions (122). The advantage of the Raman method was that the analyses could be performed in sample volumes of 35 or $100\mu\text{L}$, and use of these small volumes allowed faster approaches to equilibrium through the use of smaller quantities of drug substance. The Raman method has been shown to be applicable to high-throughput studies conducted in multi-well plates (123) and in studies of epitaxial-induced crystallization (124).

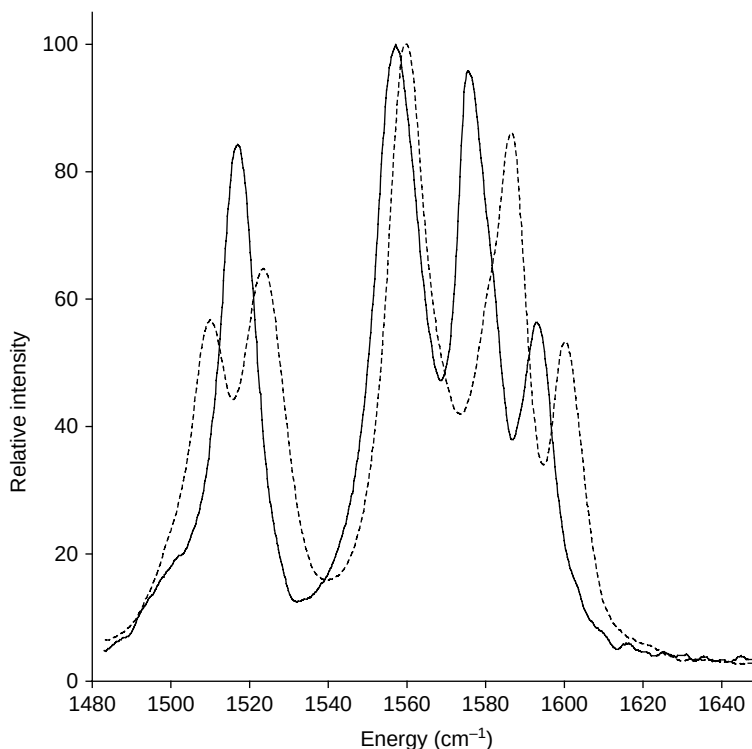


FIGURE 9 Raman spectra obtained in the high-frequency region for olanzapine Form-1 (*solid trace*) and Form-2 (*dashed trace*). Each spectrum has been normalized so that the relative intensity of the most intense band equals 100%, and the normalized spectra were generated from data given in Ref. (125).

A combination of infrared and Raman spectroscopic investigations was used to characterize two polymorphs of olanzapine, with spectral assignments being deduced for all observed bands in the two solid-state forms in order to obtain insight into the crystalline structures (125). As illustrated in Figure 9, the spectrum of Form-1 in the high-frequency region is characteristic of a hydrogen-bonded molecule, and the changes in the spectrum of Form-2 were determined to be consistent with atoms involved directly or indirectly to hydrogen bonds associated with the N-H group and not with any type of conformational difference between the molecules in the two crystal structures.

Schmidt has used Raman spectroscopy to study the polymorphic structures formed by benzocaine, butambene, and isobutambene as part of a program to characterize local anesthetic drugs having the general formula of lipo-CO-hyd, where the lipophilic end is mostly phenyl, CO is a negatively charged linkage (usually ester or amide), and the hydrophilic group (hyd) is usually a secondary or tertiary amine (126). Three polymorphs of sibnadet hydrochloride were found to exhibit varying degrees of dynamic disorder in a terminal phenyl group, with the

polymorphism being largely governed by skeletal symmetry changes rather than alterations in hydrogen-bonding patterns (127).

Raman spectroscopy has been found to be extremely useful for the quantitative analysis of phase composition. For example, two polymorphs of buspirone hydrochloride were characterized by a full range of techniques, with unique peaks in the Raman spectrum and chemometric analysis being used to develop quantitative methods for phase composition in mixtures of the two forms (128). A related approach was used to develop a quantitative method for the polymorphs of paracetamol in mixtures (129). An analytical method has been developed to determine the phase composition in mixtures of the two polymorphs of *N*-[5-chloro-4-[(4-chlorophenyl)cyanomethyl]-2-methylphenyl]-2-hydroxy-3,5-diiodobenzamide and its amorphous form, with these being quantitatively distinguished using Raman spectroscopy and a constrained linear regression model (130). The effect of particle size on quantitative phase determinations using Raman spectroscopy was studied for the case of flufenamic acid, noting that the intensity of Raman scattering tended to decrease with increasing particle size (131).

The use of X-ray powder diffraction in the study of amorphous solids is hampered by the lack of a useful response, but the vibrational transitions of such materials are still visible in both infrared absorption and Raman spectroscopies. Both techniques were used to evaluate the hydrogen-bonding patterns in a series of crystalline and amorphous dihydropyridine calcium channel blocker compounds, where it was learned that although significant variations existed in the various crystalline forms, similar patterns were detected for the amorphous states (132). The crystalline γ and amorphous modifications of indomethacin have been studied using vibrational spectroscopic and density functional theory computational methods, where it was deduced that the drug substance in the amorphate existed primarily in dimeric structures that were similar to those existing in the crystalline γ -form (133).

Raman spectroscopy has proven to be very useful as well in the study of the polymorphic content of drug substances in their formulations. For example, a detailed series of calibration samples was used to develop a method based on Raman spectroscopy for determination of the phase composition of the methyl ester of 5-*p*-fluoro-benzoyl-2-benzimidazolecarbamic acid in two formulations (134). The difficulty in this analysis lay in the fact that the drug substance was capable of existing in three polymorphic forms, and that a full statistical package based on the intensities of 78 selected bands was required to differentiate the forms.

The characterization of solvatomorphs by Raman spectroscopy is based on crystallographic effects on the energies of molecular vibrations. In addition to the known monohydrate and sesquihydrate crystal forms of pantoprazole sodium, two additional hydrate forms were studied using a full complement of spectroscopic characterization techniques, with both infrared absorption and Raman spectroscopies being capable of distinguishing between the various solvatomorphs (135). Four hydration states (an anhydrate, a monohydrate, a hemi-pentahydrate, and a variable hydrate containing four to six waters) have been reported for risedronate sodium, with the Raman spectra of these forms being dominated by peaks associated with the substituted pyridine ring of the compound (136). Comparison of structures obtained by X-ray diffraction indicated the existence of strong intermolecular out-of-plane hydrogen bonding between the pyridine ring and an adjacent phosphate

group was capable of impacting the ring vibrational modes in the monohydrate and variable hydration forms.

Owing its ready adaptability as an in situ method of characterization, Raman spectroscopy has been shown to be useful in the study of solution-mediated phase transformation reactions. For example, a number of methods (including Raman spectroscopy) were used to follow the phase change of the metastable α -form of (*L*)-glutamic acid into its stable β -form, and as an aid develop the first-order kinetics for nucleation and growth kinetics of both polymorphs in a population balance model (137). The anhydrate/monohydrate equilibria of citric acid in aqueous solutions have been investigated with the aim of demonstrating the process analytical technology capabilities of in situ Raman spectroscopy (138). Flufenamic acid was used as the model system in a study of the use of in situ Raman spectroscopy as a method for the determination of transformation kinetics and transition temperatures, with good agreement being reported between results obtained by the spectroscopic method and those obtained using the more conventional van't Hoff approach (139).

The solution-mediated aqueous transformation of anhydrous carbamazepine to its dihydrate phase has proven to be a good model system for the use of Raman spectroscopy as a means to study the kinetics, because certain bands in the Raman spectra are diagnostic of the phase identity (Fig. 10). The thermodynamics associated with the system were studied in ethanol–water mixtures by measuring the solubility of both forms over the temperature range of 0°C to 60°C using a Raman immersion probe to establish the phase composition (140). In a related work, the Raman immersion probe was used to determine that crystallization of the stable form represented the rate-determining step (141). Raman spectroscopy was used to follow the kinetics associated with the conversion of the Form I, II, and II anhydrides to the dihydrate, where it was found that the morphology of the starting material seemed to determine the rates of reaction (142). The Raman technique, in combination with partial least squares regression analysis, was found to be the most robust method for the detection and quantification of mixtures of the carbamazepine solvatomorphs, with the possible biasing influences of particle size, morphology, mixing, and surface effects being evaluated (143).

Owing to its ease of acquisition, and non-invasive nature of sample handling, Raman spectroscopy has proven to be an ideal technique for the in situ study of thermally induced phase transformations. For example, variable-temperature Raman spectroscopy was used to study the low-temperature phase transitions in a biphenyl–fullerene single crystal, where three biphenyl vibrational modes and the C–H stretching mode were indicative of the phase transition, and changes in the planarity of the biphenyl molecule appeared to accompany the phase change (144). The behavior of most of the C_{60} -fullerene vibrational modes could be correlated with either of the transitions, indicating changes in its site symmetry. In contrast, Raman spectroscopy was used to demonstrate that single crystals of different polymorphs of pentacene did not undergo any phase transformation reactions over the temperature range of 79 K to 300 K (145).

Details of the phase transformation processes associated with the hydration and dehydration of theophylline have also been studied using Raman spectroscopy, because the bands in the high-frequency region were found to be especially sensitive toward the phase identity (146). The thermally induced dehydration of

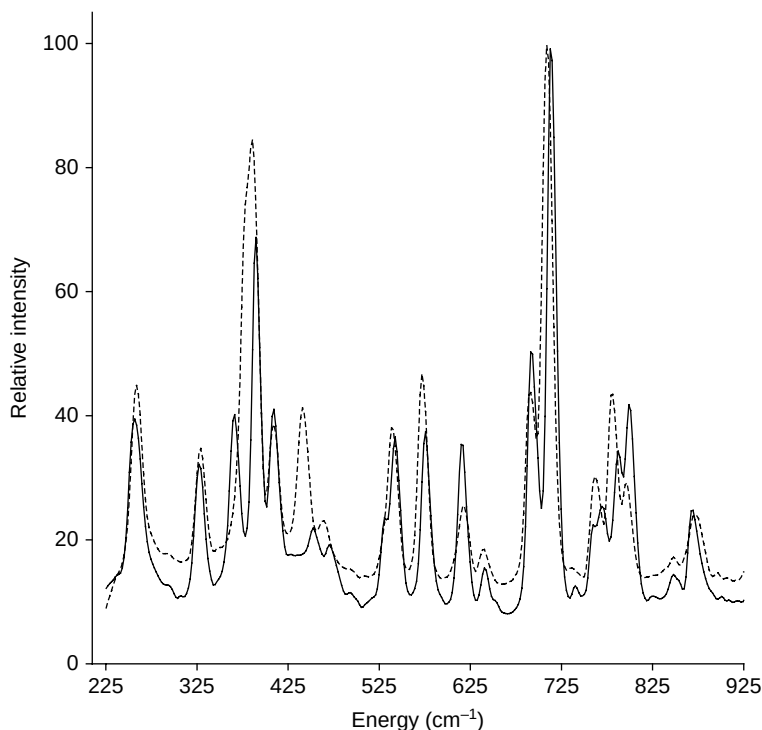


FIGURE 10 Raman spectra of carbamazepine anhydrate (*solid trace*) and carbamazepine dihydrate (*dashed trace*) over the lowest frequency region. Each spectrum has been normalized so that the relative intensity of the most intense band equals 100%. H.G. Brittain, unpublished results (120).

erythromycin A dihydrate has been studied using variable-temperature Raman spectroscopy, because the spectroscopic method was able to distinguish between the isomorphic dihydrate and its dehydrated product and being sufficiently responsive so as to be useful as an in-process technique for control over a drying process (147).

The use of principal component analysis and Raman spectroscopy in studies of the dehydration phenomena associated with various hydrated compounds, where it was found that the approach was effective in the fast screening of accumulated information, and its use facilitated the identification of critical temperatures or critical time points in the dehydration reactions (148). A Raman spectrometer interfaced with a moisture sorption gravimetric analyzer has been used to study modes of water–solid interactions in sulfaguanidine, cromolyn sodium, ranitidine hydrochloride, amorphous sucrose, and silica gel (149). Once again, principal components analysis was used to determine the genuine trends in the Raman data and facilitate the generation of information related the various types of interactions.

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Solid-State Nuclear Magnetic Resonance Spectroscopy

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INTRODUCTION

Solid-state NMR (SSNMR) spectroscopy has been used to study polymorphism beginning with the analysis of hydroquinone by Ripmeester almost 30 years ago (1). The characterization of pharmaceutical polymorphs by SSNMR spectroscopy has become more common in the last 10 to 15 years as the technology has improved and NMR spectrometers have become easier to use, particularly for non-specialists. SSNMR spectroscopy provides unique information due to its selectivity and specificity compared to other analytical techniques, which makes it very valuable for characterization of pure compounds and well as mixtures.

SSNMR spectroscopy has also found an important role in establishing intellectual property in the pharmaceutical industry because it is critical to know the specific solid forms in which most drug substances exist. SSNMR spectroscopy tends to be the primary technique to establish the solid form of a drug in a formulated product due to its high specificity and relatively limited interference from excipients compared to X-ray powder diffraction (XRPD), and vibrational spectroscopy (IR or Raman). Therefore, it is prudent to have all solid forms characterized by SSNMR spectroscopy in addition to the typical XRPD data as part of a patent package. In general, ^{13}C cross-polarization magic angle spinning (CP/MAS) is sufficient for characterizing the solid form, but SSNMR spectroscopy of other nuclei might also be important for a particular drug.

A number of recent reviews have appeared about the use of SSNMR spectroscopy to analyze polymorphs and other solid forms (2–7). Three of the more extensive recent reviews covering many aspects of SSNMR spectroscopy of pharmaceutical compounds are by Harris (2), Medek (3), and Tishmack et al. (4). The reader is referred to the book chapter by Medek for a thorough review of many aspects of pharmaceutical SSNMR spectroscopy in addition to a theoretical discussion of NMR spectroscopy (3). The review by Harris is particularly interesting for solid-state structural determination, analysis of hydrates, and amorphous solids (2). The review by Tishmack et al. is a general overview of SSNMR spectroscopic analyses of pharmaceuticals through approximately 2002 (4).

In this chapter, the theory and applications of SSNMR spectroscopy will be discussed primarily in the context of pharmaceutical compounds. SSNMR spectroscopy is considered a necessary analytical characterization technique for every pharmaceutical solid that is marketed. The International Committee of Harmonization (ICH) specifically refers to SSNMR spectroscopy along with IR and Raman spectroscopic techniques as necessary for characterizing polymorphs in drug substances and drug products (8). It has been well established that characterization of pharmaceuticals generally requires multiple analytical techniques (e.g., diffraction, spectroscopy,

thermal analysis, microscopy, dissolution, etc.) to adequately understand their behavior with respect to production, stability, and bioavailability. Therefore, many of the literature references cited in this chapter use SSNMR spectroscopy as only one of several analytical techniques to study the compound(s) of interest to the authors. One of the difficulties in using SSNMR spectroscopy is that acquisition and interpretation of spectra usually requires a reasonably good understanding of the underlying physics. This may be the main reason that a relatively high percentage of the pharmaceutical literature examples use SSNMR spectroscopy as a secondary technique with minimal or even incorrect interpretation of the results. Hopefully this situation will improve in the future as SSNMR spectroscopy becomes even more powerful in analyzing solid materials.

The section on NMR theory in this chapter is not highly mathematical or extensive given that there are many excellent texts, reviews, and monographs on NMR theory that include details specific to SSNMR spectroscopy (9–23). Recent reviews by Baldus (9) and Reichert (10) cover general applications of SSNMR spectroscopy to many solid systems. Laws et al. wrote a particularly thorough review of SSNMR spectroscopy that is highly recommended (11). The SSNMR spectroscopy book by Duer is a useful reference for beginners and more advanced spectroscopists (12), and two older books are also excellent references for most aspects of SSNMR spectroscopy (13,14). The books by Friebolin (15), Chandrakumar (16), Harris (17), and Becker (18) are texts on general NMR spectroscopy with chapters devoted to the solid state. These books also provide reasonable reviews of the basic theory of NMR spectroscopy. Traficante's chapter is a basic introduction to NMR spectroscopy that is generally easy to understand (19). The older books by Carrington and McLachlan (20), Slichter (21), and Abragam (22) are fundamental resources on NMR spectroscopy with a much more mathematically detailed treatment of essentially every aspect of the quantum mechanical basis of the technique. Grant and Harris edit a large encyclopedia covering all aspects of NMR spectroscopy that is periodically updated with new information (23).

GENERAL THEORY OF NMR SPECTROSCOPY

NMR spectroscopy derives from the nuclear spin angular momentum, which is based on the number of protons and neutrons of an atom. Nuclear spin (I) is quantized in multiples of the Planck constant ($\hbar$), $I = 0, 1/2, 1, 3/2, 2$, etc. Nuclei with even numbers of protons and neutrons have zero spin and thus no NMR signal such as ^{12}C and ^{16}O . Half integer nuclear spins result when the nucleus of an element has either an even or odd number of protons and an odd number for its mass. Integer nuclear spins result if a nucleus has an odd number of protons and an even mass. Most elements have a isotope that can be studied by NMR spectroscopy, but it is not always the isotope with the highest natural abundance. Unfortunately, nuclei with spins other than one-half have a quadrupole moment and thus an electric field gradient that will interact with the nuclear dipole moment and lead to broad peaks in most cases. Spin one-half nuclei such as ^1H , ^{13}C , ^{15}N , ^{19}F , and ^{31}P are studied most often because they have better resolution and sensitivity compared to quadrupolar nuclei. However, ^{13}C and ^{15}N have relatively low natural abundances (1.1% and 0.37%, respectively) and correspondingly lower sensitivity than the other commonly studied spin one-half nuclei. Some of the positive and negative aspects of NMR spectroscopy are summarized in Table 1.

TABLE 1 Summary of Some Advantages and Disadvantages of Solid-State NMR Spectroscopy

NMR property	Advantages	Disadvantages
High field strength	Better sensitivity. Possibly better resolution. Decreased effect of quadrupolar nuclei.	Longer T_1 relaxation times. Instrument cost and upkeep. Increased CSA effects. Increased anisotropic bulk magnetic susceptibility.
Very weak transition energies	Highest resolution spectroscopy. High specificity.	Lowest sensitivity spectroscopy. Larger samples may be required.
Multiple nuclei	High selectivity for compounds containing chosen nucleus.	Compounds without the chosen nucleus are not observed.
Signal is directly proportional to the number of nuclei producing it	Intrinsically quantitative technique.	Quantification efficiency depends on nuclear relaxation properties.
T_1 and $T_1\rho$ relaxation times	Dynamic and spatial properties can be measured.	Long T_1 increases total data collection time. A different T_1 for each nuclei or molecule requires careful optimization for each case particularly for mixtures.
T_2 relaxation time	Dynamic properties can be measured.	Short T_2 causes broader peak. Potential loss of signal during longer pulse sequences.
Chemical shift	Structural analyses are possible.	Difficult to predict accurately. Extensive overlap in complex molecules.
Coupling	Structural analyses are possible.	Complicates spectrum with peak broadening or splitting.
Quadrupolar nuclei	Comprise most of periodic table.	Not easy to observe due to broad peaks and rapid relaxation times. Special techniques may be required to obtain spectra.
Multi-dimensional NMR	Increased resolution. Correlations between multiple different nuclei possible. Bonding and spatial information for structural analyses.	Time consuming to obtain data. Significant skill usually required.

NMR active nuclei also have a magnetic quantum number (m_I), which has $2I + 1$ possible energy states (i.e., $m_I = I, I - 1, \dots, -I$) that determine the orientations of the magnetic moments in a static magnetic field ($\mathbf{B}_0$). For a spin one-half nucleus, there are two possible energy states, $m_I = -1/2, +1/2$ that are either approximately anti-parallel or parallel to the magnetic field. The nuclear magnetic moment (μ_0) is directly proportional to the nuclear spin: $\mu_0 = \gamma \hbar [I(I + 1)]^{1/2}$, where the spin angular momentum is $P = \hbar [I(I + 1)]^{1/2}$ and $\hbar = h/2\pi$ (Fig. 1). The magnetogyric ratio (γ) is a constant relating the magnetic moment to the spin angular momentum of a particular nucleus, and it determines the direction of precession of a magnetic moment in a magnetic field. The magnetic moment and spin angular momentum are usually aligned parallel to each other, but there are exceptions such as for ^{29}Si and ^{15}N . An anti-parallel orientation will affect certain NMR experiments such as those that involve the nuclear Overhauser effect (NOE) or CP. The interaction energy between the magnetic moment and the magnetic field is given by the equation: $E = -\mu_z \mathbf{B}_0$, and the energy difference between adjacent energy levels is $\Delta E = -\gamma \hbar \mathbf{B}_0$ or $\Delta E = h\nu$, where ν is the radiation frequency. Transitions occur between energy levels by applying the appropriate radiofrequency electromagnetic radiation given by the equation, $h\nu = |\gamma \hbar \mathbf{B}_0 \Delta m_I|$. The selection rule for transitions is $\Delta m_I = \pm 1$. Substituting in the appropriate values gives the resonance frequency or Larmor frequency of the nucleus, $\nu_L = |\gamma \mathbf{B}_0 / 2\pi|$. The vertical bars in the equation mean absolute value to show that the Larmor frequency of a particular nucleus is always positive.

Electrons in molecules shield and deshield nuclei and lead to different resonant frequencies (chemical shifts) for each nucleus in a unique environment. The chemical shielding effect for a nucleus is, $\nu_i = \gamma \hbar \mathbf{B}_0 (1 - \sigma_i)$, where σ_i is the shielding constant for the nucleus, and $\mathbf{B}_0$ is the applied magnetic field corrected for the effect of bulk magnetization, which depends on the shape of the sample and its bulk

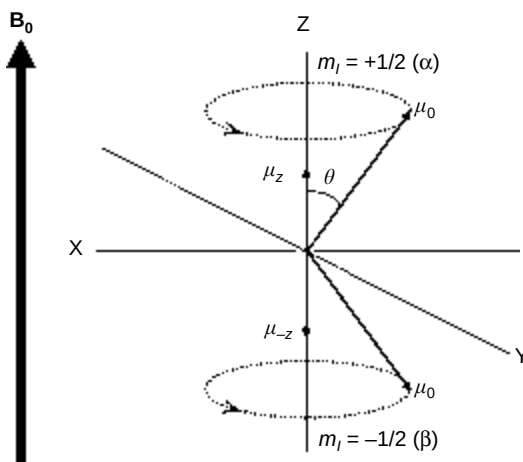


FIGURE 1 Spin one-half nuclear magnetic moments in a magnetic field ($\mathbf{B}_0$). The angle θ is 54.74° for spin one-half nuclei. μ_z is the z-component of the nuclear magnetic moments in $\mathbf{B}_0$. The α -state is lower energy and aligned parallel to $\mathbf{B}_0$, and the higher energy β -state is aligned anti-parallel to $\mathbf{B}_0$.

susceptibility. The electrons in molecular bonds also allow nuclear spins to interact or couple with each other, which results in fine splittings of the peaks produced by coupled spins. This is referred to as scalar coupling or J-coupling, and it is considered indirect because it is mediated by electrons. Dipolar coupling is referred to as direct coupling because it depends on the orientation and separation of nuclear dipoles that interact directly through space and not through bonding electrons. Dipolar coupling is a much stronger interaction than scalar coupling, and may cause significant peak broadening in solids. Dipolar coupling is not directly observed in NMR spectra of liquids or gases because it is averaged to zero by rapid motions. However, magnetic relaxation and the NOE rely on dipolar coupling, so it is a very important property for NMR structure determination in liquids. Other coupling mechanisms are also possible, but they are usually not important for spin one-half nuclei in common NMR samples.

The Boltzmann distribution of spins primarily determines the sensitivity for NMR spectroscopy. The NMR absorption and emission processes (i.e., transitions) between energy levels occur with equal probability, but the slight excess of spins in the lower energy state leads to absorption occurring more often. Each transition between energy states is a result of a spin flip from the α to β or β to α state. The equilibrium spin population ratio is: $n_{\beta}/n_{\alpha} = e^{-\Delta E/kT}$, where $n_{\alpha,\beta}$ are the spin populations in the α -state (lower energy) or β -state (higher energy) for the spin one-half case, k is the Boltzmann constant ($1.3806503 \times 10^{-23}$ J/K), ΔE is defined previously, γ for ^1H is 26.7515×10^7 rad $\cdot\text{T}^{-1}\cdot\text{s}^{-1}$ and for ^{13}C is 6.7283×10^7 rad $\cdot\text{T}^{-1}\cdot\text{s}^{-1}$, the Planck constant ($h = h/2\pi$) is $1.054571596 \times 10^{-34}$ J $\cdot\text{s}$, and $\mathbf{B}_0$ is the magnetic field measured in tesla (kg $\cdot\text{s}^{-2}\cdot\text{A}^{-1}$) (24). In a magnetic field of 9.4 tesla at ambient temperature ($T \sim 298$ K), for every one million ^1H nuclei in the β -state, there will be one million and sixty-four in the α -state. The difference between the spin populations is graphed in Figure 2 for various magnetic field strengths represented by the corresponding ^1H frequency. The very small energy difference is the reason that NMR is inherently insensitive relative to other spectroscopic techniques. Low natural abundance also contributes to the decreased sensitivity of most nuclei relative to ^1H . A long T_1 relaxation time or short T_2 relaxation time for some nuclei may also adversely affect sensitivity. Relaxation properties will be discussed in more detail later. Dipolar coupling to the electric field gradient of quadrupolar nuclei or to unpaired electrons in paramagnetic systems can cause significant peak broadening and shifting that decreases the spectral resolution and also reduces the sensitivity. The weak transitions of NMR spectroscopy are also the reason that it has much higher resolution than any other spectroscopic techniques.

The magnetization vector in the positive z -axis (M_z) resulting from a summation of all the excess nuclear magnetic moments in the low energy state can be manipulated by modern NMR pulse sequences. Figure 3 shows what happens when a radiofrequency pulse (B_1) is applied perpendicular to M_z . M_z is equivalent to the equilibrium magnetization, M_0 , when the time between pulses is sufficiently long to allow full relaxation of the particular nuclei being observed. The z component of the magnetization will rotate into the x - y plane if B_1 is applied for an appropriate length of time (i.e., a 90° pulse). The magnetization will then rotate in the x - y plane but not necessarily as a single vector, because each nucleus in a molecule may have a slightly different frequency (chemical shift) and may interact with other nuclei (coupling). This will break up the single magnetization vector into multiple vectors rotating at various rates orthogonally to the main field. The magnitude of each vector will also

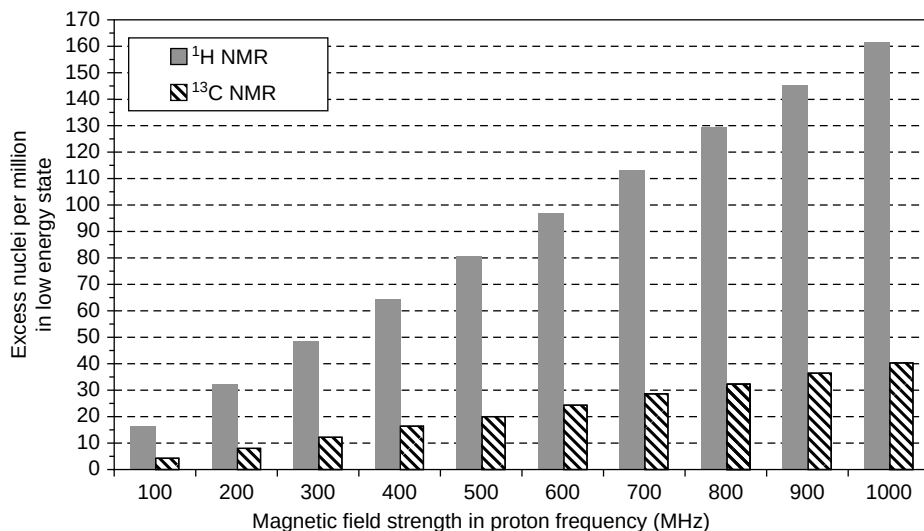


FIGURE 2 Bar graphs comparing the effect of the magnetic field strength on the number of excess nuclei in the lower energy state for two spin one-half nuclei. The effect of natural abundance is not taken into account for ^{13}C .

decrease over time due to interactions with electrons and nuclei in the sample that lead to relaxation. This is observed in the detector of the NMR spectrometer as a free induction decay (FID) where the signal intensity usually decays exponentially as the vectors dephase in the x - y plane due to random spin flips (T_2 relaxation) and as the equilibrium magnetization is re-established in the z -axis (T_1 relaxation) primarily through dipolar coupling. There is also another type of relaxation ($T_1\rho$) that occurs when the magnetization vector is continuously irradiated or spin-locked with a B_1 field that is typically in the kilohertz frequency region. The spin-lock is to achieve the Hartmann-Hahn matching condition, which is used in CP for solids or total correlation spectroscopy (TOCSY) and rotating frame Overhauser effect spectroscopy (ROESY) for liquids. In a solid, the time it takes for ^{13}C nuclei (or other low abundance nuclei) to be cross-polarized by ^1H nuclei (T_{CH}) is important because the ^1H $T_1\rho$ relaxation time determines how long the polarization will persist. Rapid ^1H $T_1\rho$ relaxation times may eliminate the sensitivity advantage that CP introduces. The ^{13}C T_1 and $T_1\rho$ relaxation times are usually not a significant factor for CP because they are almost always much longer than the ^1H relaxation times. The effects from relaxation due to the ^1H nuclei will occur long before those from ^{13}C nuclei can affect the magnetization. Accurate measurement of SSNMR relaxation times can be very important, and several of the most common techniques are compared by Frye (25). Figure 4 compares some of the effects of NMR parameters on the observed signal at various field strengths. Although it is generally beneficial to have a higher magnetic field strength, relaxation times may increase enough to adversely affect some samples and NMR experimental techniques. Higher field strength reduces the peak

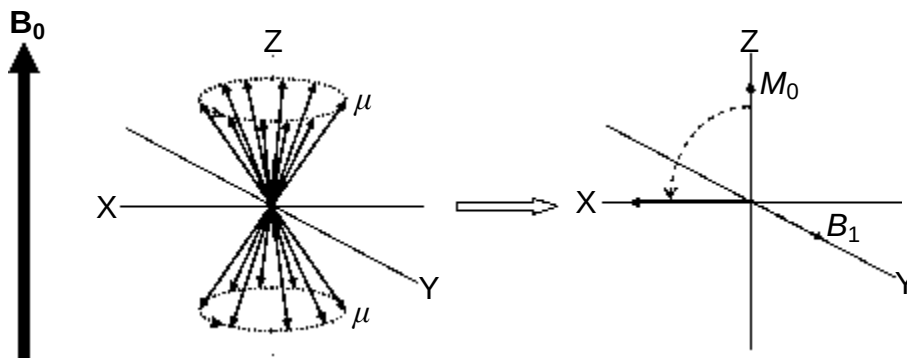


FIGURE 3 The magnetic moments for the excess nuclear spins in the lower energy state of a spin one-half nucleus in a magnetic field (B_0) produce net magnetization (M_0) along the positive z-axis. A radiofrequency pulse (B_1) applied along the positive y-axis rotates the z-axis magnetization vector into the positive x-axis where it will begin to precess in the x,y-plane according to its Larmor frequency. If the nucleus exists in multiple unique environments as in a molecule, the magnetization vector will divide into vectors for each Larmor frequency corresponding to each chemical shift. Scalar coupling will also cause further separation of each vector in the x,y-plane, but usually this will be a much smaller effect because the frequency differences between peaks of multiplets are typically small relative to chemical shift differences.

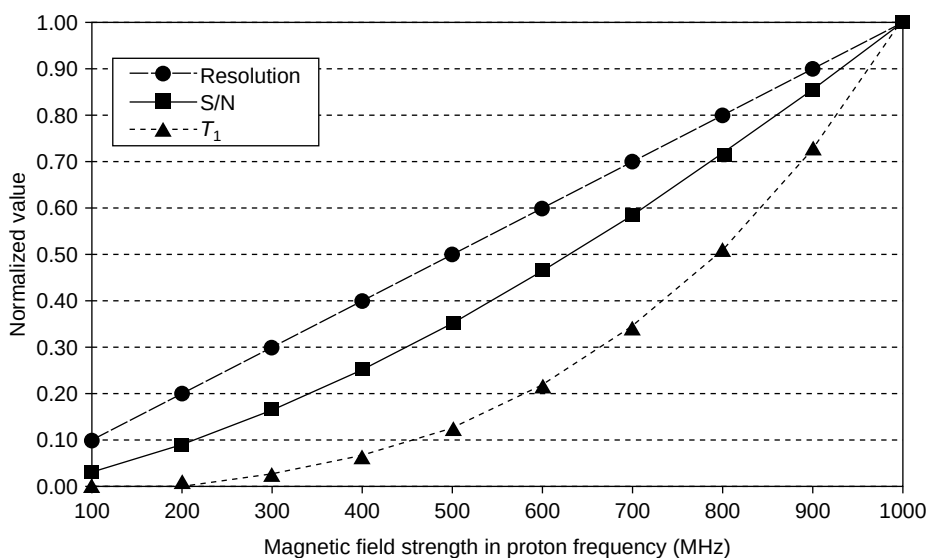


FIGURE 4 A graph of the effect of field strength on resolution, signal-to-noise ratio, and T_1 relaxation time. Although the resolution is expected to increase linearly with field strength, anisotropic bulk magnetic susceptibility, paramagnetic species, and quadrupolar coupling may significantly reduce this. In this comparison, the increase in the T_1 relaxation time has the largest deviation from linearity as the field strength increases.

broadening effect from quadrupolar nuclei, but it increases the longitudinal relaxation time (T_1) as well as the effects of chemical shielding anisotropy (CSA) and anisotropic bulk magnetic susceptibility (ABMS). These effects are particularly important for SSNMR spectroscopy.

SOLID-STATE NMR SPECTROSCOPY

NMR of solids presents significant problems compared to liquids. The rapid motions in liquids remove two effects that are largely responsible for the broad peaks typically observed in NMR spectra of solids: CSA and dipolar coupling. The T_2 relaxation time is also significantly shorter in solids, which increases the intrinsic peak widths. One of the main goals of SSNMR spectroscopy is to obtain spectra similar to what is observed for liquid-state NMR spectroscopy of the material in a solution. Figure 5 shows the structure of a simple organic molecule (DTPPAA) that has been used by the author to test pulse sequences for both liquid-state and SSNMR spectroscopy. The NMR spectra of DTPPAA are compared in Figure 6. Note that the chemical shifts are very close in the solid-state and liquid-state NMR spectra for DTPPAA with only one set of unresolved peaks (C14 and C17) in the SSNMR spectrum. This makes DTPPAA reasonably useful as a standard to test SSNMR pulse sequences and compare the results to the corresponding liquid-state NMR analyses. However, crystal packing forces and/or conformational changes will usually result in significant differences between liquid-state and SSNMR spectra.

For powdered solids, the nuclear magnetic moments are in fixed random orientations that result in broad peaks because both the CSA and dipolar coupling tensors have a $3\cos^2\theta - 1$ term, where θ is the angle of the tensor relative to the direction of $\mathbf{B}_0$. These phenomena will produce broad overlapped peaks in a SSNMR spectrum of a molecule with relatively few carbons. This would make SSNMR spectroscopy of limited use unless there was a means of reducing or eliminating the effects of CSA and dipolar coupling.

The isotropic component of chemical shielding averages to the isotropic frequency, ν_{iso} , in liquids due to rapid molecular motion, and the anisotropic component (CSA) vanishes. The CSA in solids depends on the orientation of nuclear magnetic moments with respect to the direction of $\mathbf{B}_0$. Nuclei of molecules in powdered solids have a distribution of parallel to perpendicular orientations relative to $\mathbf{B}_0$, and the peak shapes will depend on the crystallographic site symmetry at the nucleus (Fig. 7). Many different nuclei in a molecular solid will result in overlapping CSA patterns and a featureless spectrum with little useful information.

Dipolar coupling between spin one-half nuclei depends on the orientation of the dipolar vector between the nuclei relative to $\mathbf{B}_0$ as well as the distance between the nuclei. The distance dependence of dipolar coupling is very powerful for structural analysis of solids and partially ordered materials. The strength of the local field at each nucleus depends on the parallel to perpendicular orientations of dipolar vectors in the $\mathbf{B}_0$ field and gives rise to a distribution of resonance frequencies that produces peak shapes similar to axial CSA (Fig. 8). The two mirror image peaks are due to the two spin one-half transitions separated by the dipolar coupling constant (D_{CH}). The isotropic chemical shifts are identical for the two spin states and will be observed when the dipolar vector is at an angle of 54.74° relative to $\mathbf{B}_0$. There is no isotropic component of the dipolar coupling tensor, so it averages to zero in liquids due to rapid molecular motions, and has no direct effect on the spectrum. As for CSA,

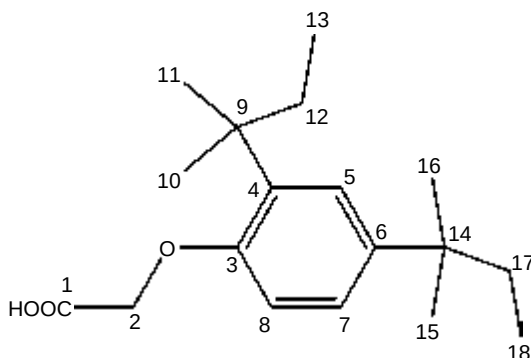


FIGURE 5 Chemical structure of (2,4-di-*tert*-pentylphenoxy)-acetic acid (DTPPAA) with numbering used for the assignments shown in Figure 6.

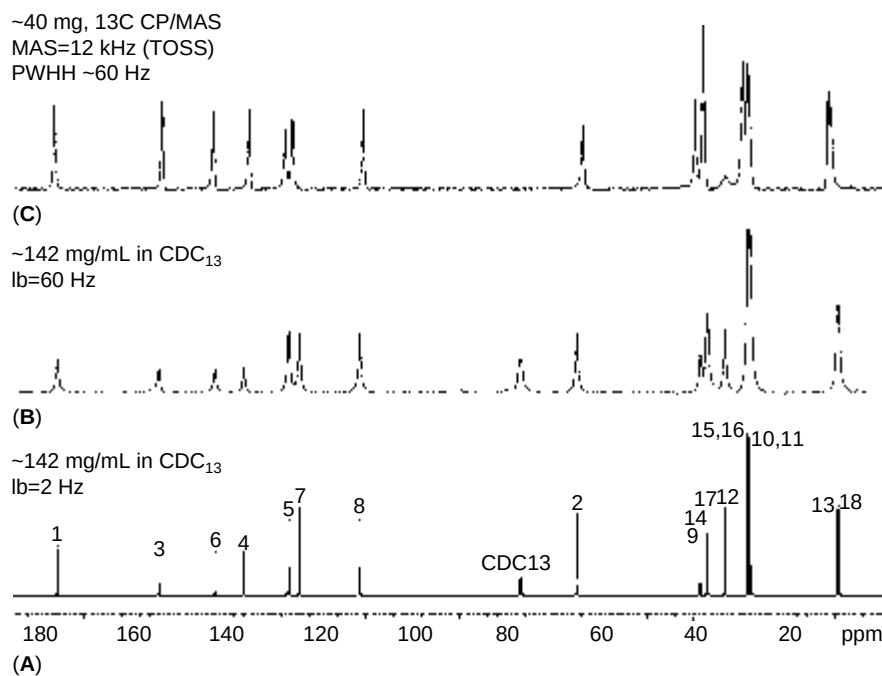


FIGURE 6 ^{13}C NMR spectra of DTPPAA at 9.4 T: (A) in CDCl_3 solution, (B) spectrum (A) with 60 Hz exponential multiplication, and (C) SSNMR spectroscopy of crystalline DTPPAA. The peak positions are similar for this molecule in the solid and liquid states. The peak labels in spectrum (A) are the resonance assignments for the structure in Figure 5. *Abbreviations:* CP/MAS, cross polarization magic angle spinning; TOSS, total side band suppression.

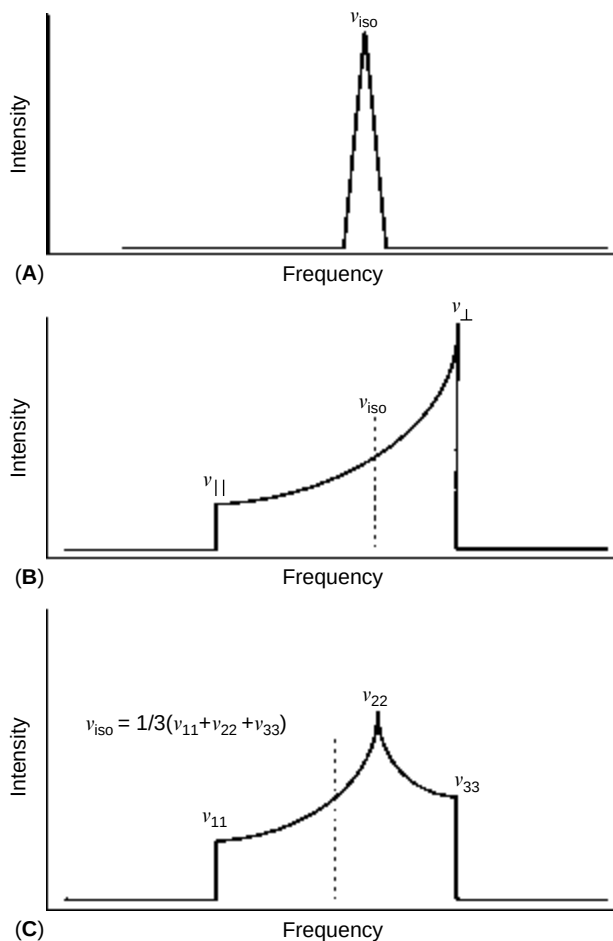


FIGURE 7 Representations of CSA powder patterns for (A) cubic, (B) axial, and (C) lower crystallographic site symmetry for a spin one-half nucleus relative to the magnetic field. Cubic symmetry results in only the isotropic frequency. For axial symmetry, the magnetic moment of the nucleus can vary between parallel and perpendicular orientations. The probability is much greater for a nuclear magnetic moment being in a perpendicular orientation, which results in the higher peak intensity for this case. The isotropic frequency for the axial case is a weighted average of the two extremes. The lower symmetry case results in a more complex peak shape, where ν_{22} will vary in position somewhere between the highest frequency (ν_{11}) and the lowest frequency (ν_{33}) with an isotropic frequency (ν_{iso}) as shown in the *bottom* diagram.

multiple dipolar coupled spins in a solid would result in many broad overlapped resonances and little useful information in the spectrum. Figure 9 shows the orientation dependence of dipolar coupling for two spin one-half nuclei in a solid. The results are similar for CSA except that only one peak will be present in each spectrum (i.e., the solid line spectrum of panel Fig. 9a and the corresponding peaks in Fig. 9b and c that align with frequencies for the parallel and perpendicular orientations.

The orientation dependence of CSA and dipolar coupling provides a means to reduce or eliminate their effects in SSNMR spectra. Andrew et al. discovered that rotating solid samples at 54.74° (the magic angle) relative to the magnetic field significantly reduced the peak widths in SSNMR spectra (26,27). Figure 10 shows the basic procedure for reducing CSA by rapid spinning at the magic angle, and it works essentially the same for dipolar coupling. In a powdered solid, all possible orientations of angles θ and β are present, but the angle of the rotor (θ_R) in the probe can be set by the operator.

Magic angle spinning (MAS) will cause modulations in the spectrum that extend out from the isotropic chemical shift position of each nucleus at integral multiples of the spinning speed. These spinning side bands move farther out and decrease in intensity as the spinning speed increases. The intensity of the isotropic peak increases with the spinning rate while the spinning side bands decrease until they are eliminated when the rate of rotation exceeds the CSA. An example of MAS for glycine (Fig. 11) is shown in Figure 12. Note the different CSA powder patterns for the carbonyl and methylene resonances in spectrum (a) of Figure 12. For ^{13}C SSNMR, the carbonyl resonance has a relatively large CSA (~ 150 ppm), whereas aliphatic carbons have significantly smaller CSA (~ 60 ppm). The CSA increases with field strength (e.g., for ^{13}C 100 ppm is ~ 10 kHz at 9.4 T and 15 kHz at 14.1 T), which means that faster spinning is required at higher field strengths to reduce the spinning side bands to the same extent. Smaller diameter rotors are required to achieve faster spinning speeds, but the reduced sample quantity results in lower sensitivity that may not be compensated by the higher field strength. If necessary, spinning side bands can be suppressed by using the total side band suppression (TOSS) technique first introduced by Dixon (28–33). In the typical four-pulse TOSS sequence, the 90° pulse width for the X-nucleus must be reasonably accurate for good suppression of the side bands. TOSS is particularly useful for removing overlapping side bands when the spinning speed is not fast enough to push them out past the peaks of

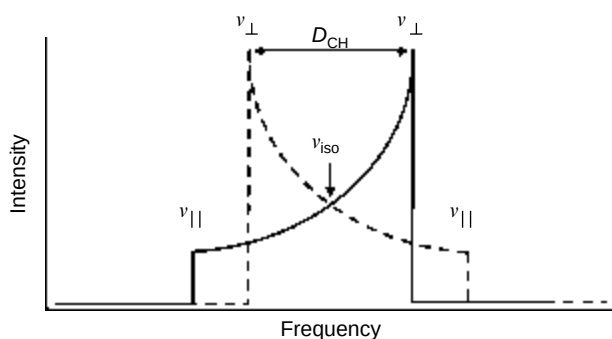


FIGURE 8 A diagram of a heteronuclear dipolar coupling powder pattern for the simple case of two coupled spins (e.g., ^1H and ^{13}C). The solid and dashed lines represent the two spin states for either of the spin one-half nuclei. Note the similarity of one peak of this pattern to the axial symmetry for CSA. The dipolar vectors also vary between the parallel and perpendicular orientations with respect to the magnetic field. The dipolar coupling constant is D_{CH} .

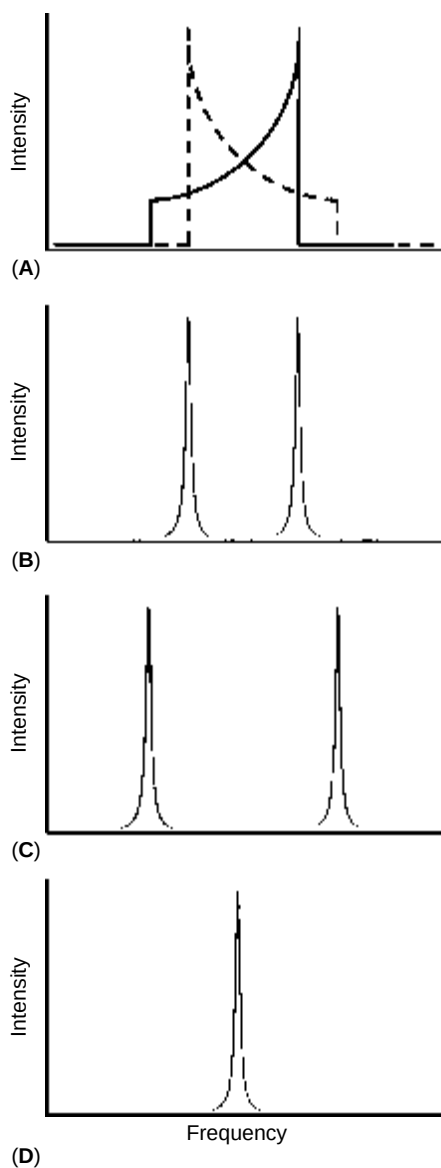


FIGURE 9 Representations of dipolar coupling for a two spin case: (A) powder pattern for randomly oriented dipolar vectors, (B) perpendicular orientation, (C) parallel orientation, (D) 54.74° orientation (the magic angle). Spectra (B)–(D) would be obtained for a single crystal positioned as specified.

interest such as with larger diameter rotors or at high magnetic field strengths. The ^{13}C CP/MAS spectrum (c) of Figure 6 was obtained with TOSS to eliminate potential overlap of the small isotropic peak at approximately 34 ppm with spinning side bands, which also helped confirm that it was a real peak.

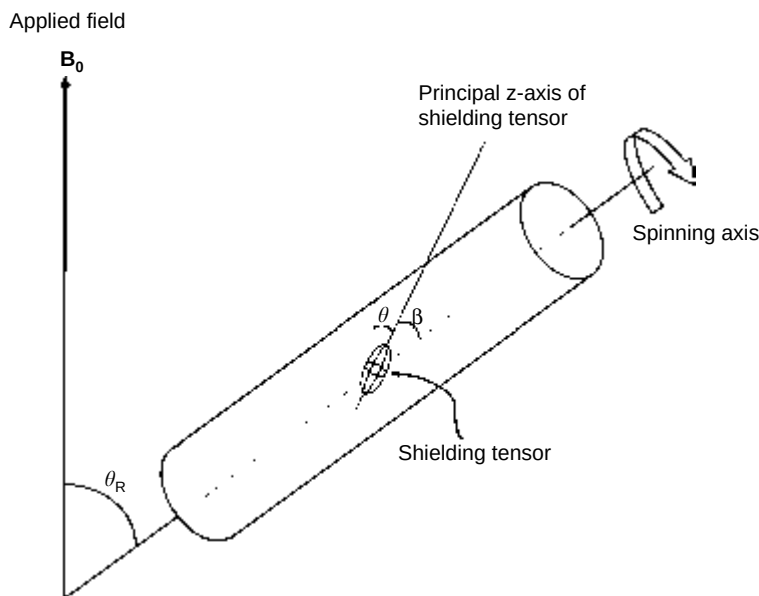


FIGURE 10 Spinning a sample rapidly at the magic angle ($\theta_R = 54.74^\circ$) will average the CSA (and the dipolar coupling) to zero because of $3\cos^2\theta - 1$ dependence of their orientation with respect to the magnetic field. Adapted from Ref. (12).

As with CSA, MAS will eliminate dipolar coupling only if it is significantly faster than the strength of the dipolar interaction (i.e., tens of kHz). Otherwise, it scales the dipolar coupling in proportion to the spinning speed and gives broader peaks. MAS will usually remove the homonuclear dipolar coupling between ^{13}C nuclei because it is much weaker than $^1\text{H}/^{13}\text{C}$, $^1\text{H}/^1\text{H}$, $^{19}\text{F}/^{13}\text{C}$, or $^{19}\text{F}/^1\text{H}$ dipolar couplings (11). High-power ^1H decoupling is used to dramatically narrow the peaks in ^{13}C and ^{15}N detected SSNMR spectra. Simultaneous high-power ^1H and ^{19}F decoupling can also be used if both nuclei are present in the molecule of interest. Both scalar (through-bond) and dipolar (through-space) coupling are removed by decoupling. The decoupling power necessary to remove the dipolar interaction in solids is substantially greater than that used for broadband proton decoupling of the scalar coupling in liquid samples. Continuous wave decoupling was originally used, but dramatically improved results are possible with newer modulated techniques that will be discussed later.

Nuclei with low sensitivity (e.g., ^{13}C and ^{15}N) can be difficult to obtain spectra with good signal to noise in a reasonable time. Pines et al. first used the CP technique to increase the sensitivity of low natural abundance spins (34). This technique relies on dipolar coupling to transfer polarization from ^1H to ^{13}C nuclei, and its effectiveness is thus extremely dependent on the separation between the ^1H and ^{13}C nuclei in a solid. The enhancement of the ^{13}C signal is roughly proportional to the magnetogyric ratios of the nuclei. The high natural abundance and strong magnetic moment of protons results in an approximate four-fold enhancement of the ^{13}C signal or 10-fold enhancement of the ^{15}N signal. Because the ^1H T_1 relaxation time is

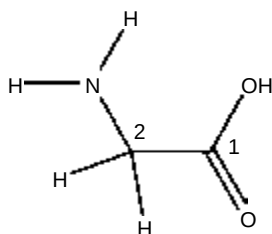


FIGURE 11 Chemical structure of the amino acid glycine.

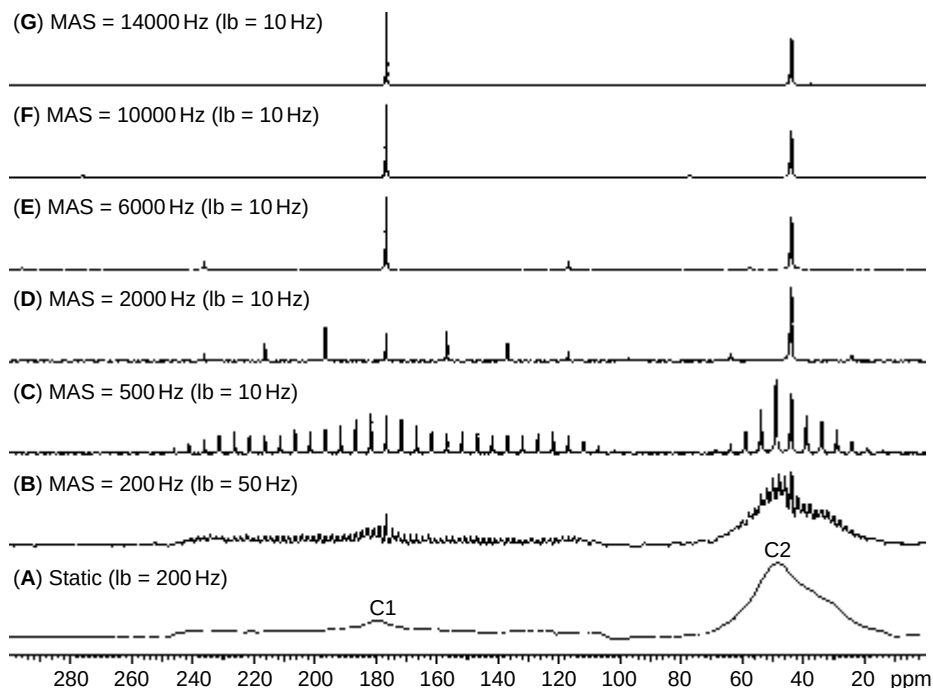


FIGURE 12 The effect of MAS on the ^{13}C SSNMR spectrum of glycine at 9.4T. The peaks of the static pattern in (A) are broken up into spinning side bands that are separated by integral multiples of the spinning speed in spectra (B)–(G). The isotropic frequency is not readily apparent until the spinning speed is fast enough to reduce the spinning side bands significantly (2 kHz for methylene, 6 kHz for carbonyl). Note that the spinning side bands for the methylene resonance are essentially eliminated at 6 kHz because this is approximately equivalent to the CSA ($60 \text{ ppm} \times 100 \text{ Hz/ppm} = 6000 \text{ Hz}$). The spinning speed would need to be approximately 15 kHz to eliminate the spinning side bands for the carbonyl resonance, which has a CSA of $\sim 150 \text{ ppm}$. *Abbreviation:* MAS, magic angle spinning.

usually much shorter than those of ^{13}C or ^{15}N , CP permits the acquisition of more scans in less time because the proton spin polarization determines how efficiently the X-nuclei are cross-polarized.

Stejskal and Shaefer first used a combination of magic angle spinning, high-power ^1H decoupling, and CP to obtain high-resolution ^{13}C SSNMR spectra (35,36). This CP/MAS technique is the standard procedure for obtaining high-resolution NMR spectra of solids. The effects of these three techniques on the ^{13}C SSNMR spectrum of glycine (Fig. 11) are shown in Figures 12 and 13. The CSA powder patterns are readily observed for the carbonyl and methylene carbon resonances of the static spectrum (a) of Figure 12. The enhancement due to CP is also apparent in spectra (b) and (d) of Figure 13. The high-power decoupling has the most dramatic effect on reducing the peak widths in the spectra (c) and (d) of Figure 13. An efficient modulated ^1H decoupling method [SPINAL-64 (57)] was used whenever decoupling was required because it is significantly better than the continuous wave decoupling procedure that was first used for ^{13}C CP/MAS spectra and is still used in many of the literature examples discussed in section "Solid-Form Characterization" on p. 398.

The basics of acquiring 1D SSNMR spectra has been demonstrated in an excellent article by Bryce et al., which is highly recommended to both novices and experienced NMR spectroscopists (37). Several things are important to clarify regarding this useful review. First, the authors state that glycine polymorphism may cause problems with using it as a standard for chemical shift referencing, CP, and sensitivity. However, the glycine polymorphs have such different relaxation properties, and it would not be difficult to determine if there was a polymorphic transformation in a glycine standard sample. The metastable α -glycine has significantly shorter relaxation times, which means that phase transformation is not likely to go unnoticed when obtaining SSNMR spectra (38). In this author's hands, the α -glycine polymorph appears to be stable for years under ambient storage conditions and in regular use as a ^{13}C CP/MAS SSNMR reference standard for signal-to-noise measurement, Hartmann-Hahn matching for CP, as a chemical shift reference, and to optimize ^1H decoupling (including at nonambient temperatures, -40°C to $+80^\circ\text{C}$). The same sample of solid α -glycine has shown no detectable phase transformation or degradation affecting its ^{13}C CP/MAS spectrum for at least four years of regular use. Second, spectra C and D of Figure 7 in the review by Bryce et al. appear to be the same, which does not show the effect of two pulse phase modulated (TPPM) decoupling for spectrum D as the authors intended (37). Therefore, one should expect different results using the stated conditions. The peak for the methylene carbon of phenylacetic acid would actually be much like in spectrum B but with greater intensity due to the variable amplitude CP (VACP) in addition to the TPPM decoupling. The peak for the carbonyl resonance may not be narrowed as dramatically by TPPM, but it would likely be similar to what is shown in spectrum B of Figure 7 in the review by Bryce et al. (37).

Taylor has also written a more specific summary of the basic experimental set up procedures for ^{13}C CP/MAS SSNMR spectroscopy (39), and an interesting ^{13}C SSNMR spectroscopy study of the effect of low temperatures (-60°C) on the polymorphs of glycine (40). The changes in relaxation times (longer ^1H T_1 and shorter ^1H $T_{1\rho}$) for the glycine polymorphs at low temperature combine to make it significantly more difficult to acquire high sensitivity spectra in a reasonable amount of time. The carbonyl resonance is affected more dramatically because the magnitude of the changes in relaxation times is greater. This makes glycine somewhat problematic

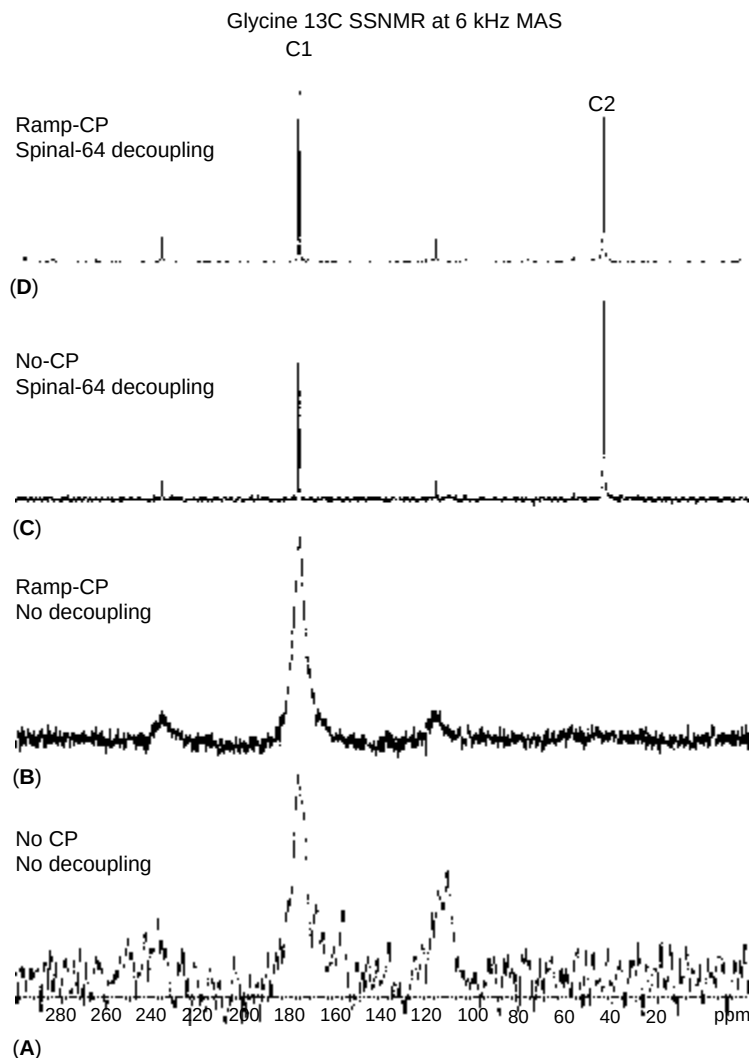


FIGURE 13 The effects of high-power ^1H decoupling and CP are shown for ^{13}C SSNMR spectra of glycine at 9.4T and 6 kHz MAS. The MAS speed is too slow to reduce the dipolar coupling enough to observe the methylene resonance (C2) in spectra (A) and (B). CP dramatically increases the signal-to-noise ratio [compare spectra (A/B) or C/D]]. High-power ^1H decoupling is required to get the narrowest peaks in spectra (C) and (D). The apparent peak at approximately 115 ppm in spectrum (A) is due to the transmitter artifact partially overlapping a broad spinning side band combined with the very low signal-to-noise ratio of the spectrum. *Abbreviations:* CP, cross-polarization; MAS, magic angle spinning; RAMP-CP, ramped amplitude cross polarization; SSNMR, solid-state nuclear magnetic resonance.

as a reference standard to optimize the spectrometer at low-temperature conditions. However, this is not a significant problem in practice until the temperature is approximately -50°C , especially if amplitude- or frequency-modulated CP (41–48) and modulated decoupling are used (49–59).

Setting the magic angle accurately is obviously very important for obtaining the highest resolution SSNMR spectra. Frye and Maciel demonstrated the use of potassium bromide as a useful means of setting the magic angle for ^{13}C SSNMR spectroscopy (60). More recently, Antonijevic and Bodenhausen have used deuterated α -oxalic acid to set the magic angle to within 0.01° by minimizing the residual ^2H quadrupolar coupling (61). Barich et al. have shown that 3-methylglutaric acid (MGA) can be used to set the magic angle, optimize the Hartmann-Hahn match for CP, determine the signal-to-noise ratio, and as chemical shift reference for ^{13}C SSNMR spectroscopy (62). The two carboxylic acid carbonyl resonances for MGA are used to set the magic angle because carbonyl groups have a large CSA, which makes them very sensitive to the magic angle setting. However, adamantane is still the best molecule to use for optimizing the field homogeneity due to its very narrow ^{13}C SSNMR peak width (<0.05 ppm).

As noted earlier, SSNMR spectroscopy requires additional procedures compared to liquid-state NMR spectroscopy to obtain high-resolution spectra. The three procedures currently used are high-power ^1H decoupling, magic angle spinning, and CP. These have advanced in several ways since their inception. Magic angle spinning for spin one-half nuclei has been extended to double rotation (DOR) and dynamic angle spinning (DAS) for quadrupolar nuclei (63). Spinning speeds have also increased from several kHz when MAS was initially developed, to tens of kHz now. This is particularly helpful for ^1H and ^{19}F SSNMR, because these nuclei have strong dipolar coupling (40–50 kHz) compared to ^{13}C (10–20 kHz) and high natural abundance. Very fast spinning will reduce the dipolar coupling to give narrower peaks. High-power proton decoupling has progressed from continuous wave to various frequency and phase modulated decoupling methods (49–59). One of the best ^1H decoupling methods currently available for common use is the small phase incremental alternation (SPINAL-64) decoupling procedure (57), which is based on supercycles of the TPPM decoupling of Bennett et al. (59). TPPM decoupling itself was a dramatic improvement over existing decoupling techniques. CP has also advanced from the original continuous polarization to VACP and ramped amplitude (RAMP-CP) polarization techniques. RAMP-CP is more efficient than VACP and dramatically better than standard CP, particularly for rapidly spinning samples (>10 kHz). The result from using these techniques is higher sensitivity and resolution SSNMR spectra for all of the common nuclei. A minor drawback to using these modern techniques is that they require more complicated pulse sequences and additional effort to optimize them for obtaining spectra.

Temperature control for the sample is important for studying a variety of solid-state properties such as reactivity, relaxation properties, and molecular dynamics. Accurate temperatures in a SSNMR rotor have been significantly more difficult to obtain and determine than for liquids NMR (see Ref. (4) and references therein). Methanol or ethylene glycol is typically used for temperature calibration in liquid-state NMR spectroscopy, but they can work well for SSNMR spectroscopy in an appropriately sealed rotor. Antonijevic and Bodenhausen note that liquid methanol was used to calibrate the temperature in the rotor for their study (61). Methanol is used primarily for ambient and lower temperatures, whereas ethylene glycol is

used for ambient and higher temperatures. The overlap around room temperature is useful for connecting calibration curves for the entire temperature range of a probe. A significant advantage of using methanol and ethylene glycol is that only one or two samples are required to accurately determine an entire temperature calibration curve simply by measuring the frequency difference between the two peaks. The disadvantage is that a modified pulse sequence may be required to measure the temperature under similar CP/MAS conditions that will be used for solid samples. This is because the temperature calibration for either methanol or ethylene glycol must be determined with a ^1H pulse sequence, and liquids will not give NMR signals with CP as do solids.

SOLID-FORM CHARACTERIZATION

Molecular polymorphism can be divided into several types. Conformational polymorphism occurs when a molecule exists in two or more different conformations in a crystalline solid. Packing polymorphism occurs when a single conformation of a molecule is arranged in one of several unique orientations that have different long-range order. Polycrystalline powders are the typical samples for SSNMR spectroscopy and usually have a crystallite size in approximately the micrometer range (i.e., microcrystalline). Amorphous solids are essentially random arrangements of molecules, but they have some short-range order relative to each other because molecular motions are restricted in the solid state. Only the rapid tumbling of molecules in liquids or gases results in truly amorphous material. The molecules of amorphous solids can also have multiple conformations, which might suggest that these conformational differences would be observable by SSNMR spectroscopy or other techniques. However, the different conformations and the random spatial order of an amorphous solid tend to be indistinguishable by SSNMR spectroscopy because they have similar peak broadening effects on the spectrum. Nanocrystalline solids may also be distinguished from amorphous solids by SSNMR spectroscopy. Nanocrystallites will typically appear almost identical to microcrystallites or macrocrystallites (single crystals) by SSNMR spectroscopy using the typical CP/MAS technique. If nanocrystalline solids have significant disorder due to defects, they may be difficult to distinguish from amorphous solids.

The majority of the reports on polymorphism that are reviewed in this chapter were published within approximately the last eight years. Many published reports in the pharmaceutical literature use SSNMR spectroscopy as a secondary technique to analyze solid forms, and the interpretation tends to be rather minimal even when the results are quite interesting or potentially critical to a study. The complexity of NMR spectroscopy can be daunting at times, but the body of literature available on data acquisition and interpretation is extensive and reasonably easy to understand even for a novice (37,39). The value of SSNMR spectroscopy is significant for pharmaceutical solid-form analysis, and should be used to its greatest advantage as a complementary technique to XRPD, vibrational spectroscopy, microscopy, and thermal analyses. Hopefully, the examples provided in this chapter will demonstrate both the strengths and weaknesses of SSNMR spectroscopy in characterizing pharmaceutical solid forms.

Conformational polymorphism, where distinct conformations of a molecule result in different crystal forms, has been studied for decades (64,65). SSNMR spectroscopy is useful here because it is very sensitive to molecular conformations and

short-range order in solids. Griesser et al. studied conformational polymorphism in oxybuprocaine hydrochloride with spectroscopy, X-ray diffraction, and thermal analyses, and found it to have either a less stable bent conformation (U-type) or a more stable straight conformation (I-type) in the crystal forms (66). This local anesthetic drug has three polymorphs: modification I with one U-type conformer per asymmetric unit, modification III with two U-type conformers per asymmetric unit, and modification II° with one U-type and one I-type conformer in the asymmetric unit. Modification II° is the most stable polymorph below approximately 90°C, modification I is more stable above this temperature, whereas modification III is metastable over the temperature range studied. Both ^{13}C and ^{15}N SSNMR spectroscopy were useful in determining the number of molecules per asymmetric unit, and molecular mobility was studied using variable temperature analyses and the dipolar dephasing technique. The dipolar dephasing NMR experiment can be used to analyze molecular motion because dipolar coupling is reduced for more mobility for more mobile regions of a molecule.

Vogt et al. studied the two enantiotropic polymorphs of {4-(4-chloro-3-fluorophenyl)-2-[4-(methoxy)phenyl]-1,3-thiazol-5-yl} acetic acid by ^1H , ^{13}C , ^{15}N , and ^{19}F SSNMR spectroscopy (67). A thorough SSNMR spectroscopy study was used to help understand the structures of each polymorph determined by X-ray diffraction. The solid-state transition temperature of the polymorphs was $\sim 30^\circ\text{C}$. Form I was thermodynamically more stable below 35°C , but form II was more stable to milling and had lower hygroscopicity. The single-crystal structure of form I was determined, but form II did not produce suitable single crystals. The structure of form II was determined using XRPD, and various SSNMR spectroscopy analyses were used to provide additional restraints for the structure determination. Two 2D NMR pulse sequences that rely on dipolar coupling, ^1H DQ-BABA (double quantum back-to-back) and ^1H - ^{13}C HETCOR (heteronuclear correlation), were used to determine conformational features of both polymorphs. The 2D PASS (phase-adjusted spinning sidebands) pulse sequence was used to determine chemical shielding tensors (CSTs) for ^{13}C resonances of form II to help refine the calculated structure. The orientation dependence of CSA is useful in providing unique structural details to support diffraction analyses. The authors suggest that, with additional work on other polymorphs, it may be possible to use SSNMR spectroscopy rather than the Burger-Ramberger IR rule for predicting the relative stability of polymorphs (68,69). The degree of peak overlap in the spectral region sensitive to hydrogen bonds is not nearly the problem for SSNMR spectroscopy as it is for IR spectroscopy. This study is an excellent example of using multinuclear, multidimensional SSNMR spectroscopy as a powerful technique to thoroughly characterize polymorphs. However, many of the analyses and the interpretation require a high level of expertise in NMR spectroscopy.

Maccaroni et al. studied the solid-state form interconversions for two linezolid (Fig. 14) polymorphs by ^{13}C SSNMR, XRPD, and thermal methods (70). Most of the SSNMR peaks were assigned for the two polymorphs by comparison to the ^{13}C NMR assignments of linezolid in a CDCl_3 solution. However, this procedure will not necessarily lead to correct assignments for the solid state unless the conformations are very similar or the electronic environment around each nucleus is coincidentally the same as shown for DTPPAA in Figure 6. It appears that the spectra of both polymorphs were acquired with the same parameters (e.g., contact time, pulse delay, number of scans), but form IV obviously has a longer ^1H T_1 relaxation time based on

the much lower signal-to-noise ratio in the spectrum (Fig. 15). The peak widths also are somewhat broader for form IV, which may result from unresolved peaks of two very similar molecules per asymmetric unit, as the authors state. The authors also note that the lowest frequency methyl carbon resonance of form IV is split into two peaks (nearly overlapping), which may be consistent with two molecules per asymmetric unit. However, this could also be explained by conformational exchange due to restricted rotation about the amide bond, which is quite well known for liquid-state NMR spectroscopy. The small symmetrical splitting of C2 (~170 ppm) in form II is likely due to conformational exchange rather than coupling to ^{14}N , because the quadrupolar broadening effect is not very large at this field strength (~9.4 T), and only some peak broadening is likely to be observed instead. The ^{13}C - ^{14}N coupling usually produces asymmetrical splitting at lower field strengths where it is more readily observed (71–75). The ^{19}F atom directly bonded to the aromatic carbon (C9) produced significant broadening for this peak in the spectrum of form II due to the relatively strong scalar and dipolar coupling. The C8 resonance also shows broadening as do the C7 and C10 peaks to a lesser extent (Fig. 15). This is because ^{19}F decoupling is not commonly used simultaneously with ^1H decoupling for ^{13}C CP/MAS SSNMR spectroscopy unless the spectrometer and probe are specifically designed to do so. The MAS speed would need to be much faster than 7 kHz to reduce ^{19}F - ^{13}C dipolar coupling significantly. The effect of ^{19}F coupling shows that for form II, C9 should be assigned to the broader peak at 154.9 ppm, and C6 should be assigned to the peak at 157.2 ppm. The C6 and C13–C16 resonances were apparently not observed for form IV supposedly because of broadening due to their proximity to oxygen and nitrogen in the molecule. However, the assignments for the C6 and C9 resonances are also swapped for form IV, which means that the C9 resonance is broadened (by ^{19}F coupling) into the baseline noise in this spectrum that already has a very low signal-to-noise ratio relative to the spectrum of form II (Fig. 15). This alternative interpretation of the spectrum of form IV is supported by the missing C13–C16 resonances (also broad in the spectrum of form II), which are likely broadened due to inefficient ^1H decoupling. Although the experimental details for the SSNMR spectroscopy data acquisition are very minimal in this report, it is quite likely that the older continuous wave ^1H decoupling scheme rather than one of the modern modulated decoupling methods was used to acquire the SSNMR spectra. The contact time (1 millisecond) is also relatively short, and may partly account for the low sensitivity of some quaternary carbons in the spectrum of form IV. The difficulties in correctly interpreting SSNMR spectra to compare solid forms can be reduced by using modern fast MAS and modulated decoupling or simultaneous ^1H and ^{19}F decoupling along with efficient CP. One or more of the various spectral editing methods would have been particularly useful in confirming the assignments in this case (76).

Two polymorphs of buspirone hydrochloride were compared using ^1H and ^{13}C SSNMR spectroscopy (observed and calculated values), XRPD, Fourier transform spectroscopy, infrared spectroscopy (FT-IR), and differential scanning calorimetry (DSC) (77). The single-crystal X-ray structure was determined for form I and found to be very similar to previous work. The authors used XRPD and DSC for quantitative analysis of mixtures of polymorphs and compared the results to previous quantitative FT-IR data. The XRPD patterns were difficult to use for quantification in this case. FT-IR spectroscopy gave superior quantitative data based on the summaries in Table 1 of their report (77), although the authors did not discuss

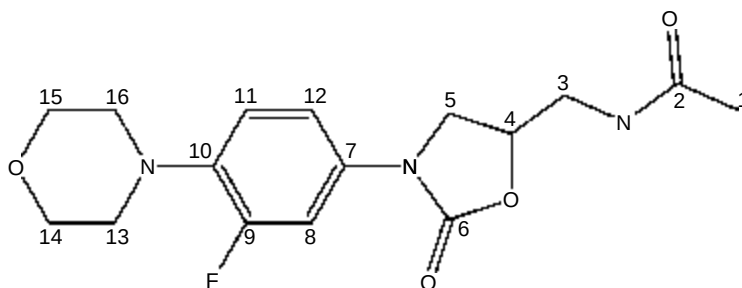


FIGURE 14 Chemical structure of linezolid. Adapted from Ref. (70).

this further. The ^{13}C SSNMR spectra clearly differentiated between the two forms, but form I appeared to have a longer ^1H relaxation time based on its relatively low signal-to-noise ratio. This may be why the authors were not able to obtain a ^1H SSNMR spectrum of form I, and also suggests that quantification by SSNMR spectroscopy may not be very practical in this case. The calculated ^1H and ^{13}C NMR chemical shifts were in generally good agreement with the observed values. The authors used the comparisons to determine which portions of buspirone hydrochloride had the greatest effect on the SSNMR spectroscopy differences observed for the two polymorphs. These comparisons with calculated chemical shifts are useful if the resonance assignments have not been previously obtained from a liquid-state NMR spectrum of the same molecule. Although calculated NMR chemical shifts have progressed greatly in recent years, it is still difficult to unambiguously determine them for cases when the molecular conformations are very close.

A recent study of donepezil hydrochloride used XRPD, SSNMR, vibrational spectroscopy, and thermal analyses to characterize five polymorphs, two hydrates, and an amorphous form (78). This work demonstrates the utility of multiple analytical techniques for characterizing pharmaceuticals. Unfortunately, each technique was not used to analyze every sample, which prevents a complete comparison of them. In general, the spectroscopic techniques could differentiate the solid forms. The authors apparently found three forms of a trihydrate ("hydratemorphism") based on DSC, FT-IR, and water vapor sorption analyses. However, none of these hydrate forms were analyzed by any of the three most powerful solid-state techniques (XRPD, SSNMR spectroscopy, and Raman spectroscopy). These additional analyses would have greatly enhanced their case for "hydratemorphism." The authors also observed that hydrate I had slightly higher water content by Karl Fischer titration than a monohydrate, and one of three proposed reasons was that a small amount of amorphous material may cause this. The Karl Fischer results are explained by the observation of weak broad peaks in the SSNMR spectrum due to disordered material, and the XRPD pattern showed some diffuse scattering typical of disordered material. An interesting observation is that the carbonyl resonances in the ^{13}C SSNMR spectra are shifted to higher frequency for the hydrates, which is expected for hydrogen bonding with water.

Masuda et al. examined the α and γ forms of indomethacin by XRPD and ^{13}C CP/MASSNMR spectroscopy (79). They were able to determine that α -indomethacin

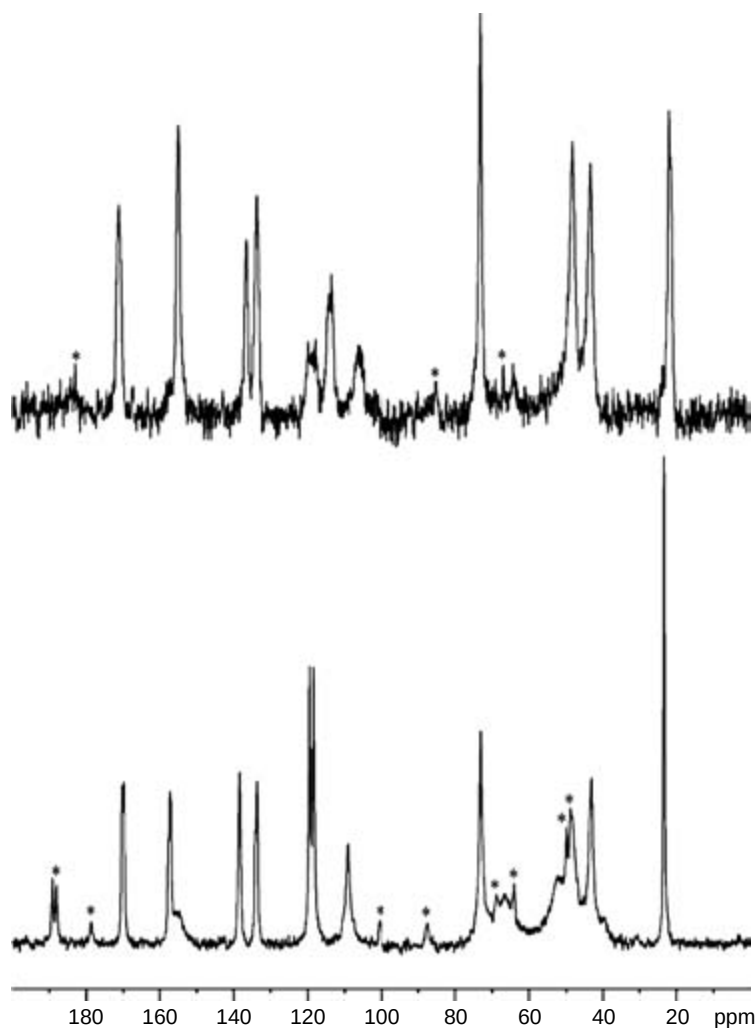


FIGURE 15 ^{13}C CP/MAS spectra of linezolid form II (*bottom*) and form IV (*top*) obtained at 9.4 T and 7 kHz MAS. Asterisks indicate spinning sidebands. Adapted from Ref. (70).

has three molecules per asymmetric unit while γ -indomethacin has only one based on the peak splitting (Fig. 16). However, the methoxy group (C12) and the aromatic carbon to which it was bonded (C5) of α -indomethacin also showed a slight splitting of the highest frequency peak at low temperature (203 K), which seemed inconsistent with three molecules per asymmetric unit. The variable temperature SSNMR analyses (at 203, 298, and 343 K) showed that this splitting was due to conformational exchange for one molecule in the asymmetric unit (Fig. 17). The flexible methoxy group (C12) of one molecule in the asymmetric unit is also in two slightly different conformations resulting in two nearly overlapping signals, and this apparently affects the aromatic carbon (C5) to which the methoxy group is bonded. The other

methyl group (C13) also shows some conformational flexibility, but it results in a broader peak rather than splitting. This is a good example of the sensitivity of SSNMR spectroscopy to molecular conformations of solid forms. Conformational flexibility is typically seen in an X-ray crystal structure as an enlarged thermal ellipsoid. The low temperature (<300 K) SSNMR spectrum shows that the methoxy carbon atom for one of the molecules in the asymmetric unit become "conformationally trapped" in one of two different environments. Above 300 K the methoxy group is apparently mobile enough to cause coalescence of the peaks. It was not clear from the report if temperature cycling or varying the cooling rate resulted in the same final spectrum at ambient temperature. This could demonstrate whether the two conformations became equally populated, or if they could be trapped unequally in the different conformations.

Apperley et al. also studied the solid forms of indomethacin as well as nifedipine by ^1H and ^{13}C SSNMR spectroscopy and ^1H T_1 and $T_1\rho$ relaxation analyses (80). The ^{13}C SSNMR spectroscopy peak widths of crystalline nifedipine were significantly narrower than for crystalline indomethacin, which suggests that molecular mobility is higher for nifedipine. The authors showed that amorphous nifedipine recrystallizes upon grinding while amorphous indomethacin does not. The onset points for the glass transition temperatures (T_g) of indomethacin and nifedipine are $\sim 42^\circ\text{C}$ and $\sim 45^\circ\text{C}$, respectively. Nifedipine recrystallized at 55°C into a mixture of two different polymorphs (forms I and II), which converted almost completely to form I at 70°C .

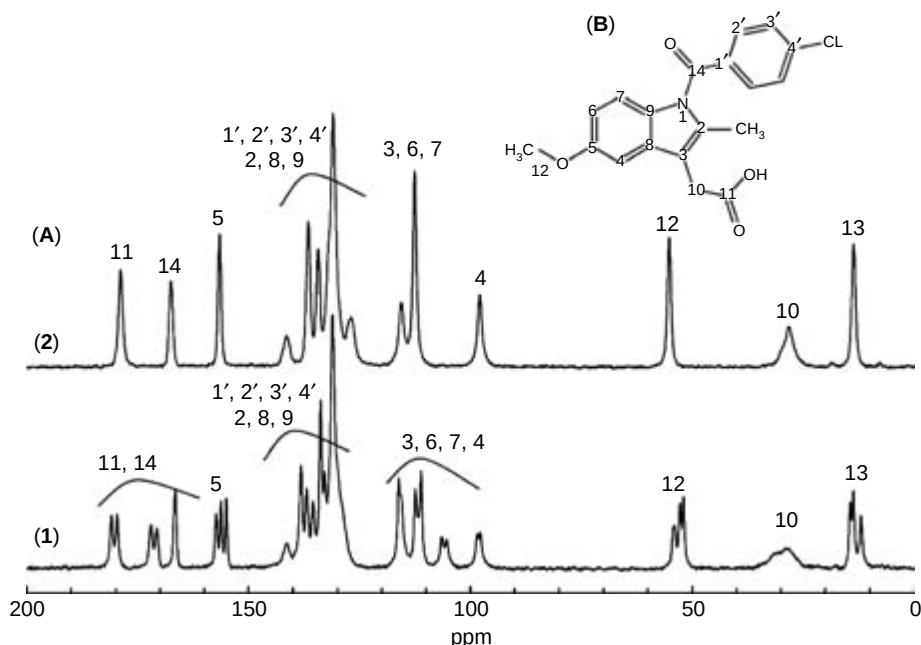


FIGURE 16 ^{13}C CP/MAS spectra of indomethacin at 9.4T and 15 kHz MAS: (A1) α -form, (A2) γ -form, and (B) chemical structure of indomethacin. Adapted from Ref. (79).

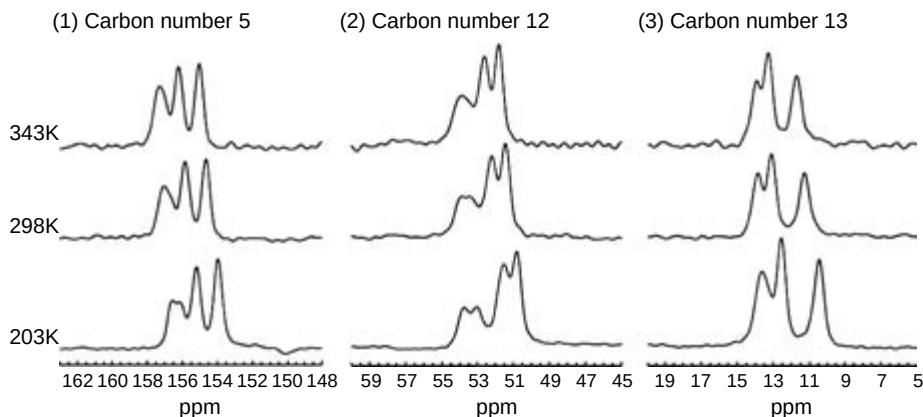


FIGURE 17 Expanded regions of the variable temperature ^{13}C CP/MAS spectra of α -indomethacin. The three groups of carbon resonances show conformational disorder for the highest frequency peak(s) in each region. The spectra also show that there are three molecules per asymmetric unit. Adapted from Ref. (79).

Nifedipine form II is a metastable polymorph. The ^{13}C SSNMR spectrum of amorphous indomethacin showed sharpening of peaks as the temperature was increased up to 80°C , but it did not recrystallize because the temperature was not increased high enough for this to occur in the ^{13}C SSNMR spectroscopy part of the study. The ^1H SSNMR spectra and relaxation measurements indicate that amorphous nifedipine has greater mobility below its glass transition temperature than indomethacin, and nifedipine tends to recrystallize first to a metastable form. The quadrupolar broadening effect of ^{35}Cl and ^{37}Cl was also examined in indomethacin at five different field strengths ranging from 4.7 to 18.8 T (50–200 MHz for ^{13}C). The quadrupole moment of both chlorine nuclides are much larger than for ^{14}N , which results in significantly more peak broadening as well as more complex splitting patterns. This can lead to much greater difficulty in SSNMR spectral interpretation for molecules having chlorine atoms unless high magnetic field strengths are used.

Schmidt et al. performed a series of studies on the polymorphism of local anesthetic drugs using spectroscopic, diffraction, microscopic, and thermal analyses (81–85). SSNMR spectroscopy was not a major part of any of these studies, but it provided unique complementary information for these compounds. In Part V (81), the authors showed ^1H SSNMR spectra in a study of hydrogen-bonding differences between modification II $^\circ$ and hydrated modification I, and ^{13}C SSNMR spectroscopy could easily differentiate modification II $^\circ$ from modification I and its hydrate. Modification I and its hydrate both appeared to have two molecules per asymmetric unit, which was confirmed by the single-crystal X-ray structures. Part IV (82) used ^1H , ^{13}C , and ^{15}N SSNMR spectroscopy to analyze polymorphism and molecular mobility in falcaine hydrochloride and isomorphous dyclonine hydrochloride. The SSNMR spectra showed that the polymorphs of both compounds have only one

molecule per asymmetric unit. Modification I of both falicaine hydrochloride and dyclonine hydrochloride had a significant amount of the corresponding modification II°. High-temperature ^{13}C SSNMR spectroscopy of the mixture of falicaine hydrochloride modifications I and II° showed that the aromatic ring is rotating at a rate that is intermediate on the NMR time scale at ambient temperature. This causes broadening of the protonated ring carbons for modification I such that they are not easily observed in the ^{13}C SSNMR spectrum. The authors state that a similar process is occurring for modification I in the dyclonine hydrochloride mixture, and this can be readily determined by the broad peaks near the baseline at the corresponding aromatic chemical shift positions in the ^{13}C CP/MAS spectrum. In Part VII (83), the author mentions that the ^{13}C SSNMR spectra of the three polymorphic systems did not have significant differences, although no spectra were shown for any of them. Only XRPD, thermal analyses, and microscopic data were shown for the polymorphs, but no IR, Raman, or SSNMR spectroscopic data was presented. A potentially interesting study would be to see if this were a series of polymorphs that can only be distinguished by specific analytical techniques. In Part VIII (84), the authors used ^1H and ^{13}C SSNMR spectroscopy to study several solid forms of hydroxyprocaine hydrochloride. The ^1H SSNMR spectroscopy was used to analyze the hydrogen bonding of the hydrate, and ^{13}C SSNMR spectroscopy was used to differentiate the two polymorphs and determine that the hydrate had two molecules per asymmetric unit. Part XI (85) also used ^1H SSNMR spectroscopy to study hydrogen-bonding differences for several hydrates. The relatively high chemical shifts of carboxylic acid protons made them useful for characterizing hydrogen bonds in several of these materials even though ^1H SSNMR peaks are typically very broad without very fast MAS or special pulse sequences like combined rotation and multiple pulse spectroscopy (CRAMPS).

Rubin-Preminger et al. studied polymorphs of ethambutol dihydrochloride diastereomers by single crystal X-ray diffraction, XRPD, SSNMR, and thermal analyses (86,87). The ^{13}C SSNMR spectroscopy was consistent with the crystal structure data showing that there was half a molecule per asymmetric unit for each form. Ethambutol dihydrochloride is a symmetrical molecule, and this property is maintained in the solid state resulting in the half molecule per asymmetric unit. The *S,S*-diastereomer has four polymorphs with two pairs that are enantiotropically related. The *R,S*-diastereomer has two enantiotropically related polymorphs. Variable temperature ^{13}C SSNMR spectroscopy of the *S,S*-diastereomer showed a solid-state phase transformation of a mixture of forms III and IV to form III at $\sim 40^\circ\text{C}$ and then back to form IV upon cooling again (87). A similar solid-state phase transformation occurs for form II to form I above $\sim 70^\circ\text{C}$. The ^{13}C SSNMR spectra in these reports were very similar due to the similarity of the crystal structures for the polymorphs of each diastereomer. However, SSNMR spectroscopy was still sensitive and specific enough to easily distinguish the subtle differences among the solid forms.

Moynihan and O'Hare used IR, Raman, and SSNMR spectroscopic analyses to study two polymorphs of paracetamol (acetaminophen) (88). The authors note that IR and SSNMR spectra were useful for distinguishing the polymorphs, but the Raman spectra were not. The commercial form was monoclinic, and an orthorhombic form was produced as a mixture by melt quenching of the monoclinic material. A ^{13}C SSNMR spectrum of the orthorhombic form was obtained by subtracting the spectrum of the monoclinic form. The ^{13}C SSNMR spectra in this report use TOSS to suppress the spinning sidebands. The small out-of-phase peaks in each spectrum

are likely due to inefficient side band suppression from incorrectly setting the ^{13}C pulse width as noted in section "Solid-State NMR Spectroscopy" on p. 385.

Shaibat et al. used ^{13}C SSNMR spectroscopy to study polymorphs of the potential anti-leukemia compound $\text{Cu(II)(8-quinolinol)}_2$ (89). The paramagnetic copper atom increased the peak widths and shifted some of them to higher frequency for these molecules. The authors used relatively fast MAS of 20 kHz or more to decrease the peak broadening in the spectra. The interconversion of the polymorphs upon heating was also monitored by ^{13}C SSNMR. This study was a useful demonstration of SSNMR spectroscopy for characterizing molecular complexes having strong paramagnetic centers that cause peak broadening. Typically paramagnetic compounds have been very difficult to analyze by NMR spectroscopy because of the very broad peaks.

Enright et al. studied two anhydrous polymorphs of caffeine (Fig. 18) with single-crystal X-ray diffraction and SSNMR spectroscopy at low- and high-field strengths (90). The high-field ^{13}C CP/MAS SSNMR spectra had much better resolution compared to the low-field data because the interaction with the quadrupolar ^{14}N nucleus was dramatically reduced (Fig. 19). The anhydrous polymorphs appeared to be more disordered compared to the monohydrate, and the low-temperature anhydrous form had multiple molecules per asymmetric unit (Fig. 20). These results were confirmed by the single-crystal structures of each form.

Sheth et al. used ^{13}C SSNMR spectroscopy to study conversion of piroxicam polymorphs after cryogenically grinding them into "amorphous" solids (91). The "amorphous" material recrystallized to the original crystalline form during storage at 25°C and 0% relative humidity. This is not surprising given that forms I and II after cryogrinding for various times show different XRPD patterns for the most disordered forms, which are actually nanocrystalline piroxicam of the corresponding polymorph (92). The disordered nanocrystallites act as seeds for conversion back into larger crystallites of the same polymorph. SSNMR spectroscopy was not very useful in this case because the high degree of disorder in the cryoground samples resulted in broad peaks that masked the small amount of nanocrystalline material.

Harris et al. used ^1H and ^{13}C SSNMR spectroscopy to study the structures of four carbamazepine polymorphs, a dihydrate, and acetone and dioxane solvates (93).

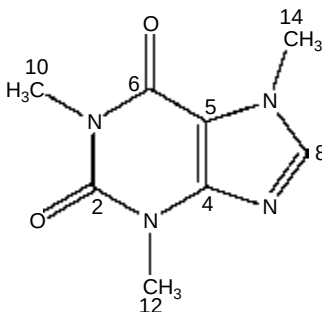


FIGURE 18 Chemical structure of caffeine with the numbering system as used for the assignments of Figure 19.

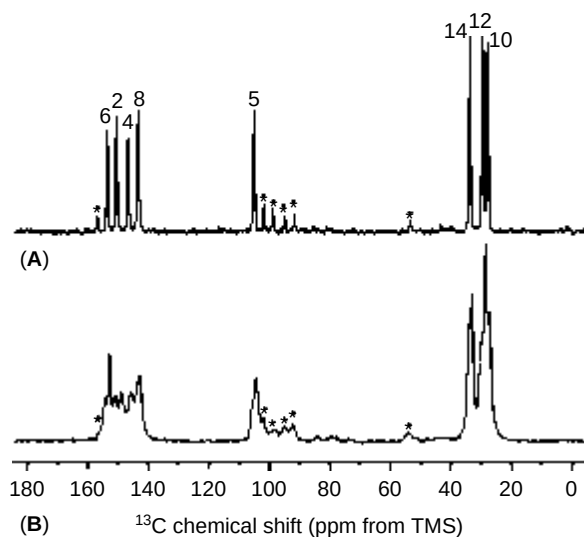


FIGURE 19 ^{13}C CP/MAS spectra of caffeine monohydrate: (A) 226.3 MHz (21.1 T) and 11.7 kHz MAS; (B) 50.3 MHz (4.7 T) and 2.6 kHz MAS. Asterisks indicate spinning sidebands. The assignments in spectrum (A) of the structure in Figure 18 are based on a spectrum obtained by liquid-state NMR spectroscopy. Adapted from Ref. (90).

The number of molecules per asymmetric unit was accurately determined for each case when compared to the crystal structures with only the triclinic form (with four molecules) having more than one. Computational analyses of the shielding constants of the P-monoclinic form were compared to the observed values and found to be reasonably close. The torsion angles for the P-monoclinic and trigonal forms of carbamazepine were also calculated and compared. The single-crystal X-ray structure of the dihydrate was also re-examined and determined to be monoclinic rather than orthorhombic as previous work had found, possibly due to micro-twinning.

Portieri et al. used ^{13}C and ^{15}N SSNMR, ^1H and ^{15}N T_1 and $T_1\rho$ relaxation data, and CSA to study polymorphs of sulfanilamide (94). The α , β , and γ forms were easily distinguished by ^{13}C SSNMR. The ^1H T_1 values for the α and γ forms were more than 10 times longer than for the β form, but the ^1H $T_1\rho$ values for the α and β forms were about twice as long as that of the γ form. This indicated that the solid-state molecular mobility was different for each polymorph. The authors note that the relaxation differences are large enough to result in potentially misleading information for mixtures of polymorphs. The peak intensities for the form with the shorter ^1H T_1 relaxation time may be relatively enhanced compared to the other if the SSNMR spectroscopy analyses are not set up to properly account for the differences. This is the reason that care should be used if one anticipates that mixtures of solid forms might be present in a sample to be analyzed by SSNMR spectroscopy. The ^{13}C SSNMR spectroscopy of each form obtained at this relatively low field strength (~ 50 MHz for ^{13}C) clearly showed the quadrupolar splitting of the carbon bonded to the quadrupolar ^{14}N nucleus. Selective ^{15}N -labeling at one of the two nitrogens was used to enable

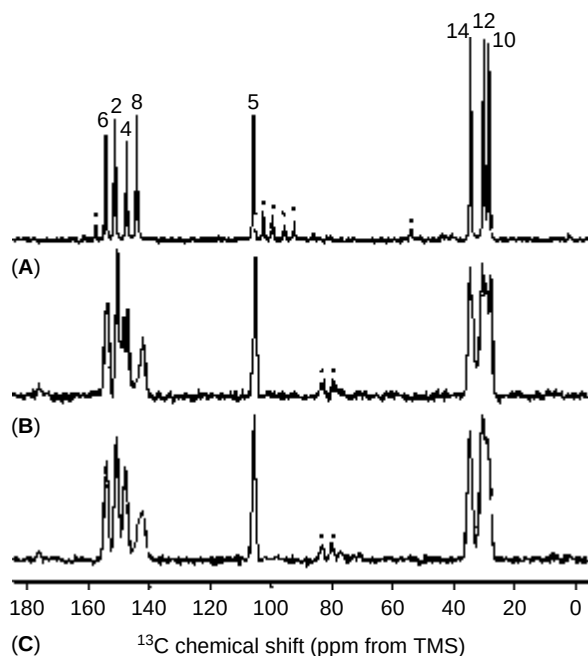


FIGURE 20 ^{13}C CP/MAS spectra of caffeine solid forms at 226.3 MHz (21.1 T): (A) caffeine monohydrate, (B) low-temperature anhydrous form, and (C) high-temperature anhydrous form. Asterisks indicate spinning sidebands. The spectra for the two anhydrous forms were acquired with 16 kHz MAS. Adapted from Ref. (90).

easier analysis of the nitrogen NMR parameters. The ^{15}N T_1 times for the secondary amine nitrogen in all three polymorphs were 100 seconds or longer as measured by the method of Torchia (95). The sulfonamide nitrogen T_1 relaxation times for all three polymorphs were less than three seconds. The $T_{1\rho}$ relaxation times for the secondary amine were 0.64 milliseconds for both the α and β forms, and 1.5 milliseconds for the γ form. The $T_{1\rho}$ relaxation times for the sulfonamide group were ~ 4 to 10 milliseconds. These results indicate that the mobility is substantially different in specific parts of this small molecule. The measured chemical shielding parameters were different for each nitrogen but almost the same among the three polymorphs. The calculated shielding parameters for ^{13}C and ^{15}N were reasonably useful for analyzing the differences in the polymorphs but also show that improvements in computational methods are still needed to accurately determine these values for detailed understanding of solid molecules.

Lee et al. used liquid-state ^{13}C NMR of several amphetamines to help assign the ^{13}C SSNMR spectra (96). The various amphetamines were easily distinguished by SSNMR spectroscopy. The authors also dry mixed 3,4-methylenedioxy-*N*-methyl-amphetamine-HCl [(*R,S*)-MDMA·HCl] with lactose monohydrate to mimic “Ecstasy” tablets. This simple mixing process involved rotating a vial of the two components without compression or shear forces, yet there was a very noticeable difference in the

^{13}C SSNMR spectrum compared to that of the pure drug. The authors noted shifts of about +5.5 ppm for C8 (methylene) and -6.2 ppm for C10 (methyl). Significant shifts of about 2.6 ppm for C1 and 1.3 ppm for C5 (both aromatic carbons) as well as a distinct increase in peak widths for the amphetamine in the mixture can also be seen in the spectra. A spectrum of pure lactose monohydrate was not shown for comparison, so the effect of mixing on it could not be determined. The authors also noted that the peaks shifted toward the positions of those observed in D_2O and attributed this to potential hydrogen bond formation in the solid mixture. The peak broadening may also be due to ABMS (138–140). However, the other amphetamines did not show similar spectral changes upon mixing with lactose monohydrate, so there appears to be a specific intermolecular interaction, such as hydrogen bonding, resulting in a conformational change that occurs with (*R,S*)-MDMA·HCl and causes both peak broadening and shifting.

Reutzelt-Edens et al. used spectroscopic, diffraction, thermal, and water vapor sorption analyses to study three anhydrides, a dihydrate, and an acetic acid solvate of the developmental compound, LY33470 HCl (97). All of the forms had one molecule per asymmetric unit. An unusual observation was that form I had a much lower dissolution rate than the dihydrate. The liquid-state ^{13}C NMR spectrum was used to assign the peaks for the solid forms, but the peak shifting observed in the solid state was such that these assignments are tentative without supporting solid-state editing techniques or correlation spectroscopy. The peak of the fluorinated aromatic carbon was very near the amide carbonyl peak, and the large ^{19}F – ^{13}C coupling constant (~150–375 Hz or ~1.5–3.7 ppm at 9.4 T field strength in this study) caused overlap with the amide resonance. The authors assigned three or four peaks in this region, although no more than two distinct peaks and a small shoulder were observable for any of the spectra they reported. The largest separation between the peaks above 160 ppm (excluding acetic acid) is about 3.3 ppm, which is within the range for ^{19}F – ^{13}C coupling. The field strength used for the analyses was high enough that the proposed residual ^{13}C dipolar coupling to the quadrupolar ^{14}N nucleus of the amide group could not be responsible for this larger splitting, but may produce the shoulders observed for forms I, II, and the dihydrate. Restricted rotation about the amide bond is also a potential source of additional peaks that may be asymmetric depending on the conformational exchange rate. Therefore, the amide carbonyl and fluorinated aromatic carbon resonances appear to be overlapped with ambiguous chemical shift assignments without further information. A good example of the effects of ^{19}F – ^{13}C coupling is shown for flurbiprofen by Antonioli and Hodgkinson (98).

Wang et al. studied four polymorphs of a fluorinated NK1 receptor agonist referred to as Compound A (Fig. 21) using variable temperature XRPD and ^{13}C SSNMR spectroscopy as well as differential scanning calorimetry, thermogravimetry, and microscopy (99). They found two sets of enantiotropically related polymorphs. Forms III and IV (Fig. 22) are the high-temperature polymorphs of forms I and II (Fig. 23), respectively. The highest frequency carbon resonance was due to the fluorinated aromatic carbon at ~160 to 165 ppm, which formed an asymmetrical doublet similar to that observed in the spectrum of LY334370 HCl (97) but without any possible overlap from amide carbonyl peaks or spinning side bands that could lead to misinterpretation (Figs. 22 and 23). The two trifluoromethyl carbon resonances (~120–130 ppm) were very broad in forms I and II (Fig. 23), but less so in forms III and IV (Fig. 22). The high-temperature forms of compound A appear to be conformational polymorphs of the low-temperature forms given the similarity of most

peak positions and the potential for flexibility for the methyl carbons and morpholine ring. The authors suggested that form I may have multiple molecules per asymmetric unit based on doublets at ~48 and ~100 ppm, but this could also be a conformational difference in the morpholine ring system (~48 ppm for N-CH₂ and ~100 ppm for O-CH-O) given that no other peak splittings were observed in the spectrum of form I to support this hypothesis. However, the spectrum of form II shows four peaks between 20 ppm and 30 ppm corresponding to the single methyl group, indicating that it is in four different environments in the unit cell (Fig. 23). This could result from conformational exchange for the mobile methyl group, but no variable temperature SSNMR spectra of form II were obtained to help clarify this.

Isotopic labeling is a very useful means of enhancing the sensitivity of NMR spectroscopy, particularly for biological molecules. It is used much less often for small molecules due to the difficulty in synthesizing molecules with isotopes at specific locations. Booy et al. specifically ¹³C-labeled two ethynyl and one methyl carbon of a steroid (Org OD 14) as a means to enhance the sensitivity for detecting two polymorphs in formulations having 0.5% or 2.5% API loads (100). From the ¹³C SSNMR spectra, form I had two molecules per asymmetric unit, and form II had one. Under the conditions used by the authors for ¹³C SSNMR, the natural abundance steroid peaks were easily observed at 2.5% loading along with the much larger peaks from the isotopic labels. The polymorphic form was also readily determined from the natural abundance peaks at this loading level. For the 0.5% loading, the peaks of the natural abundance steroid were just visible above the noise in the baseline, and it was very difficult to determine the polymorphic form except by using the peaks of the labeled material. A spectrum of the pure labeled sample showed both sharp ethynyl carbon peaks overlapped on broader peaks, and this was also observed for

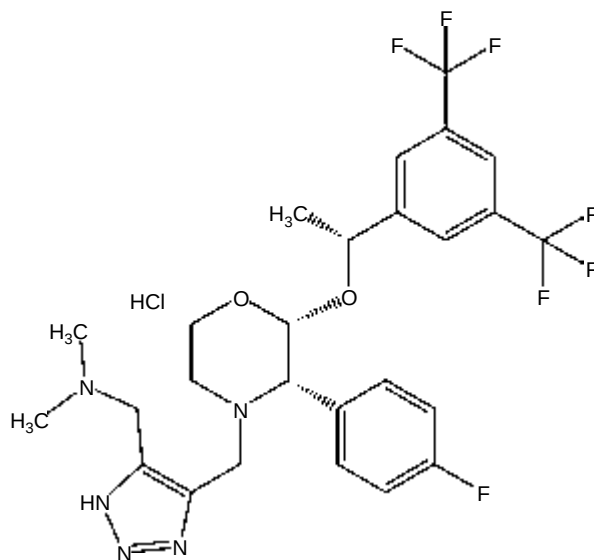


FIGURE 21 Chemical structure of Compound A. Adapted from Ref. (99).

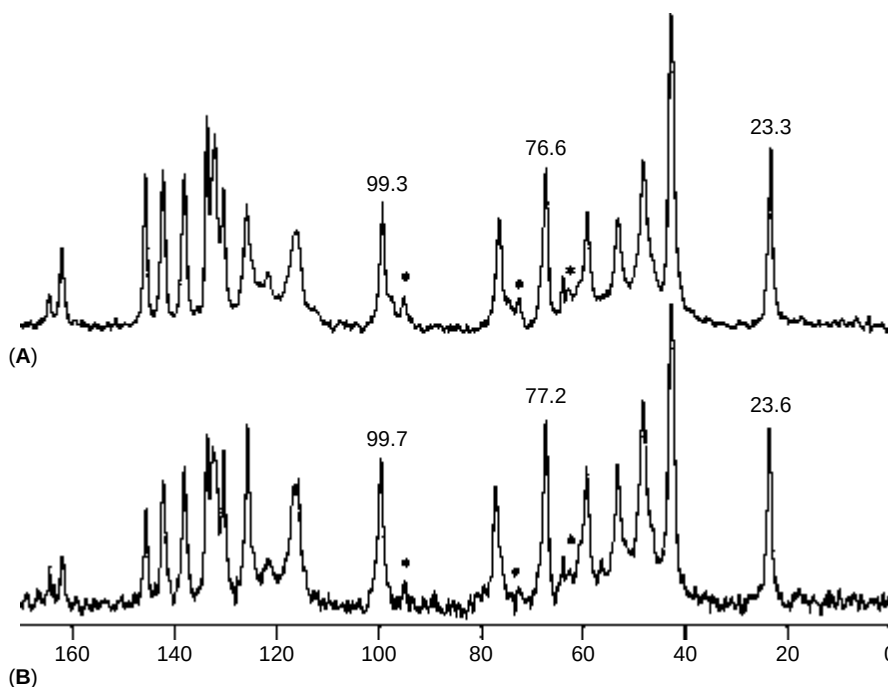


FIGURE 22 ^{13}C CP/MAS spectra of Compound A at 9.4T and 7 kHz MAS: (A) form III and (B) form IV. Asterisks indicate spinning sidebands. Adapted from Ref. (99).

the spinning side bands. Although the authors attribute this to ^{13}C – ^{13}C dipolar coupling, it is more likely due to some disordered ^{13}C -labeled material, because spinning at 8 kHz would dramatically reduce or eliminate the relatively small homonuclear ^{13}C coupling as noted in section “Solid-State NMR Spectroscopy” on p. 385. Synthetic isotopic labeling methods can be very useful for characterizing solid forms in low load formulations as long as the time and expense required for labeling the API is justified. It may eventually be a required technique if high potency pharmaceuticals become more prevalent as the current trend indicates.

Olejniczak et al. used 1D and 2D ^{13}C and ^{15}N SSNMR, CSA tensor analysis, and theoretical calculations to analyze polymorphs of *N*-benzoylphenylalanine (101). The carbonyl carbons were ^{13}C -labeled, and the amide nitrogen was ^{15}N -labeled. This permitted rapid 2D ^1H – ^{13}C , ^{13}C – ^{15}N , and ^1H – ^{15}N HETCOR analyses of the molecule. CSA tensor analyses (^{13}C and ^{15}N) and $T_1\rho_{\text{H}}$ measurements were used to estimate the relative strengths of the hydrogen bonds. The authors noted that ^{15}N CSA tensor values were less reliable than the ^{13}C values for evaluating hydrogen bonding. The ^{15}N chemical shift tends to be very sensitive to the effects of chemical bonding and coupling, which makes them somewhat unpredictable even in relatively simple molecules. Polymorphs I and II each had two molecules per asymmetric unit. Polymorph III was only obtained as a mixture with one or more other polymorphs that were not isolated and studied. The isotopic labeling as done in this study can be further exploited for structural

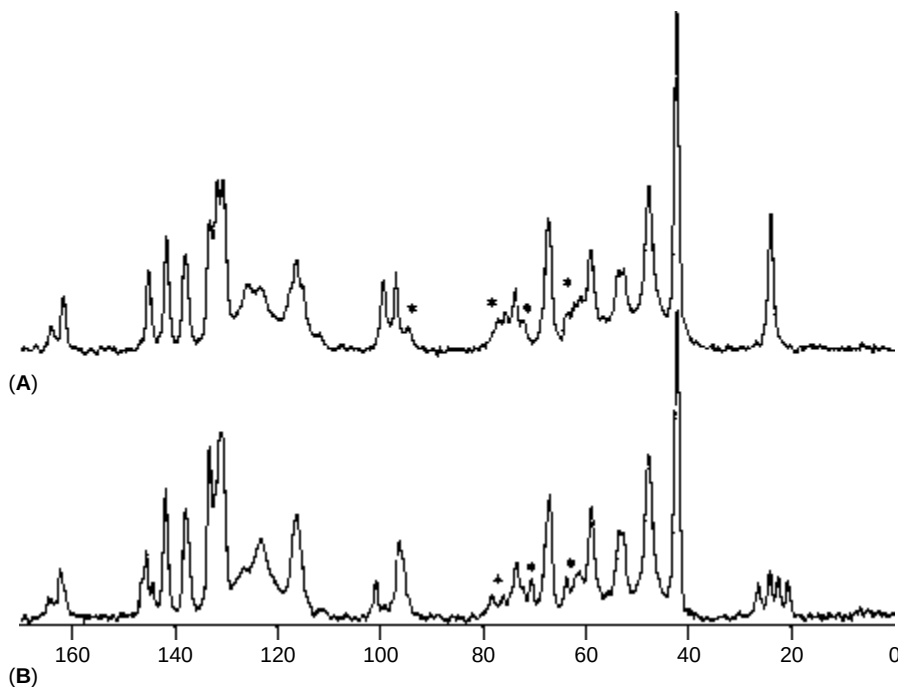


FIGURE 23 ^{13}C CP/MAS spectra of Compound A at 9.4T and 7 kHz MAS: (A) form I and (B) form II. Asterisks indicate spinning sidebands. Adapted from Ref. (99).

analysis by using rotational echo double resonance (REDOR) to determine accurate internuclear distances within molecules (102,103).

Zhou et al. used both ^1H MAS and ^2H MAS SSNMR spectroscopy to study what they called “isotopomeric polymorphism” in a complex of pentachlorophenol with 4-methylpyridine (104). This complex could also be considered a co-crystal. They found that the fully protonated material only produces the triclinic polymorph with a strong hydrogen bond, whereas the 55% or 90% deuterated material forms the disordered monoclinic polymorph with a weaker hydrogen bond. The reason for deuterium causing weaker hydrogen bonding is not clear. It would be interesting to correlate the deuterium and proton covalent bond strengths with intermolecular hydrogen (or deuterium) bond strengths to determine what controls the resulting solid form. Neutron diffraction analyses and IR spectroscopy would be useful analytical techniques to study this further.

In some cases, SSNMR spectroscopy is more useful than XRPD in distinguishing solid forms. Othman et al. used ^1H and ^{13}C SSNMR spectroscopy (CP and direct polarization) and XRPD to study four polymorphs and four isomorphous solvate hydrates of finasteride (105). The solvate hydrates were readily distinguished by SSNMR spectroscopy but not by XRPD. The solvents in the crystal structures do not change the packing pattern significantly, but they influence the local electronic environments that affect the NMR chemical shifts. The solvent molecules (other than

water) also have unique chemical shifts that are observed in the SSNMR spectra. This study further emphasizes the need to use multiple analytical techniques when characterizing solid forms, because there is no reliable way to predict which analytical technique will be appropriate for properly characterizing solid materials. XRPD may be the gold standard for studying solid materials, but spectroscopic techniques provide unique data that complements or occasionally surpasses the value of XRPD.

Apperley et al. used ^{13}C and ^{15}N SSNMR spectroscopy to study hydrogen bonding in sildenafil citrate (active ingredient of ViagraTM) upon exposure to water vapor (106). One of the citrate carbonyl peaks shifted to higher frequency upon exposure to water vapor over a seven-day period. The other two citrate carbonyl peaks did not change. The authors interpreted this as one citrate carboxyl group forming a hydrogen bond with water. The chemical shifts of the propyl and ethoxy groups of sildenafil also changed upon exposure to water vapor, which the authors suggest is due to water molecules occupying sites close to these groups and presumably changing the electronic environment surrounding these nuclei. However, the chemical shift changes may also result from modification of molecular mobility, and thus the conformations in the crystal, due to incorporation of water. The ^{15}N SSNMR spectroscopy chemical shifts of sildenafil varied by 0.2 to 1.5 ppm upon exposure to water vapor over seven days, which are relatively small changes that suggest weak interactions with the water molecules. The N2 and N3 nitrogens are bonded in sildenafil, which resulted in the typical 1:2 doublet peaks due to the interaction of the ^{15}N dipole with the electric field gradient of the ^{14}N nuclear quadrupole. The authors determined residual dipolar coupling constants of 33 Hz for $^{14}\text{N3}\text{--}^{15}\text{N2}$ and 37 Hz for $^{14}\text{N2}\text{--}^{15}\text{N3}$. The ^{15}N dipolar dephasing experiment showed that N26 was the only protonated nitrogen of sildenafil. The N5 peak was slightly suppressed by the dipolar dephasing experiment, which was interpreted to be a hydrogen bond at this position. The ^{13}C and ^{15}N SSNMR spectroscopy data are consistent with one citrate carbonyl donating a proton to N26 of sildenafil to form a salt, while a second citrate carbonyl is free to interact with water introduced into the sample. The third citrate carboxylate may have a $\text{p}K_{\text{a}}$ that significantly reduces the exchange rate of its proton, which results in essentially no observed chemical shift changes for the corresponding carbonyl resonance upon exposure to water vapor.

Rafilovich et al. used a number of analytical techniques including ^{13}C SSNMR spectroscopy to study polymorphs of 1,3-bis(*m*-nitrophenyl)urea (107). This is the compound where concomitant polymorphs were first discovered. The authors found a new polymorph, designated δ , and a monohydrate that they believe was previously reported as the γ form. They were able to obtain only the β form as a pure phase. The α and δ forms have one molecule per asymmetric unit, and the monohydrate and β forms have half a molecule per asymmetric unit based on the X-ray diffraction data. This was generally supported by the ^{13}C SSNMR spectra. However, the spectrum of the α form did not show the expected 13 peaks, although the seven peaks observed were substantially broader than those in the spectra of the other forms. This may be indicative of some disorder in the crystal structure that may be sufficient to broaden the peaks and prevent observation of all of them. The authors were not able to obtain a ^{13}C SSNMR spectrum of the δ form, which was expected to show new peaks as a mixture with the β form. Only peaks from the β form were observed, so it was not certain whether any of the δ form was present or if it was indistinguishable from the β form. Small splittings for one peak due to ^{13}C dipolar coupling to the quadrupolar ^{14}N nucleus was observed for the monohydrate and β

forms that were analyzed at ~ 7.1 T but not for the α form that was analyzed at ~ 11.7 T, which is consistent with the field strength effect on dipolar coupling to quadrupolar nuclei. Some small chemical shift differences were observed in the ^{13}C SSNMR spectra that are consistent with the differences in hydrogen bonding among the polymorphs. An interesting result noted by the authors was the hydrogen bonding interactions observed for this molecule in acetone solution where a small amount of added water caused specific shifting of several peaks in the spectrum. This demonstrates that water specifically interacts with 1,3-bis(*m*-nitrophenyl)urea in the liquid-state, which is analogous to hydrates of solid forms.

Dong et al. prepared polymorphs of neotame anhydrate and studied them by XRPD, spectroscopy, microscopy, water vapor sorption, and thermal analyses (108). Forms A, D, E, and G were characterized more fully than the three forms that were more difficult to make in adequate quantities (B, C, and E). The polymorphs were readily distinguished by XRPD, FT-IR, and ^{13}C SSNMR spectroscopy. The ^{13}C SSNMR spectra of forms D and F had broader peaks, indicating some disordered material was present, which was also consistent with the XRPD data. Form D also appeared to have two polymorphs per asymmetric unit based on its ^{13}C SSNMR spectrum. The ^{13}C SSNMR spectra were obtained at a magnetic field strength of 7.1 T, so splitting due to dipolar coupling of ^{13}C with the ^{14}N nucleus was observed for the amide carbonyl resonance.

A recent brief communication by Griffin et al. demonstrated the use of ^1H double quantum combined rotation and multiple pulse (DQ CRAMPS (109)) NMR spectroscopy to distinguish the anhydrous and monohydrate pharmaceutical compounds in a tablet with $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ as the primary excipient (110). The identity of the compound and the loading level were not reported by the authors. However, the ^1H DQ CRAMPS analysis showed that the anhydrous form was present in the tablet, which would have been difficult to determine using 1D ^1H SSNMR due to overlap of some key resonances. The authors did not show a corresponding spectrum of the monohydrate in the same formulation, which would help validate this potentially useful technique.

Zhou and Rienstra used ^1H detected fast-MAS HETCOR to study acetaminophen and ibuprofen in tablet formulations (111). Fast MAS of 40 kHz was used with a 1.6 mm rotor that could hold about 6 mg of material. The fast MAS reduced the ^1H - ^1H dipolar coupling without using homonuclear decoupling. A major advantage of this technique was that it did not require the tedious calibration procedures typical of CRAMPS experiments as used by Griffin et al. (110). The authors also used the technique to study hydrogen bonding and molecular motions. The ^1H detected HETCOR was much faster than the usual ^{13}C detected version particularly for acetaminophen (Fig. 24), which had a proton T_1 relaxation time about 30 times longer than that of ibuprofen (Fig. 25). The spectra in Figure 24 also show that it is important to acquire enough scans to obtain all of the correlations particularly for compounds with long relaxation times.

Harris et al. studied two anhydrides, a monohydrate, a higher hydrate, and an acetic acid solvate of terbutaline sulfate by ^1H , ^{13}C , and ^{15}N SSNMR spectroscopy as well as XRPD, single-crystal XRD (hydrates only), and thermal analyses (112). The ^1H and ^{13}C chemical shifts were also calculated and compared to the observed values. Modification A had broader ^{13}C SSNMR spectroscopy peaks that were attributed to disorder or crystal defects for this form, and it has only one molecule per asymmetric unit. Modification A also appeared to be contaminated with modification B. Based

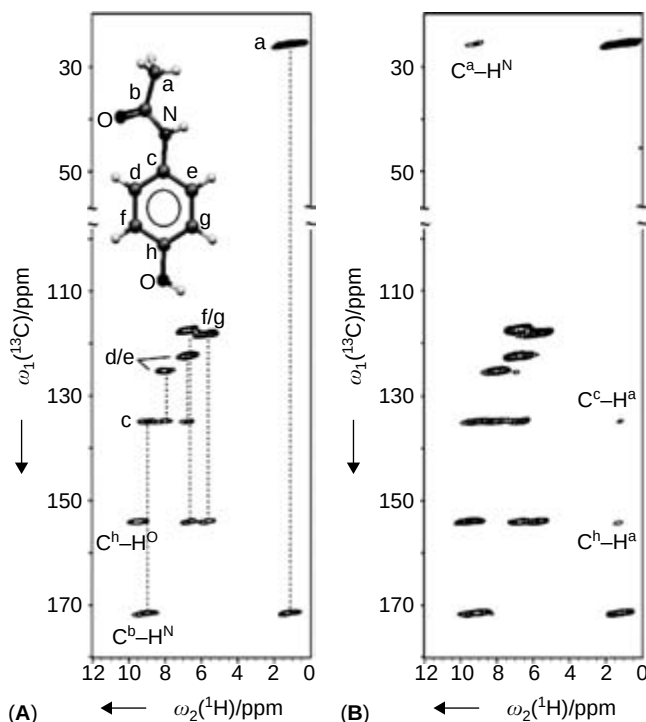


FIGURE 24 (A) ^1H detected ^{13}C - ^1H HETCOR spectra at 17.6T and 40kHz MAS for 6mg of a formulation containing 5.5 mg of acetaminophen (~93% load) with assignments for the labeled acetaminophen crystal structure shown in the inset of spectrum (A). Spectrum (A) was acquired in six hours with two scans per row, and spectrum (B) was acquired in 18 hours with six scans per row. The recycle delay for both spectra was 35 seconds. The ^1H T_1 relaxation time for acetaminophen was 63 seconds. Note the higher sensitivity in spectrum (B) that provided additional correlations. Adapted from Ref. (111).

on the peak splitting observed in their ^{13}C SSNMR spectra, modification B, the monohydrate, and the acetic acid solvate each have two molecules per asymmetric unit. The ^{13}C chemical shifts for each molecule in the asymmetric unit of modification B were computed, but the authors were not able to assign them to the independent molecules in this case. The ^1H SSNMR data and computational calculations indicated that strong hydrogen bonds may be present in all the forms, but further work is required to obtain better evidence for this.

Martin-Islán et al. used XRPD, FT-IR, ^{13}C SSNMR spectroscopy, and thermal analyses to study sodium pravastatin (113). The authors found a new polymorph (designated M) for this compound by very slow evaporation from a solution of 2-propanol. They also observed that sodium pravastatin tends to crystallize out as mixtures of multiple polymorphs (concomitant polymorphs). The polymorphs also tend to be disordered, which is typical of some statins due to the flexibility of the alkyl groups connected to the ring structures. Therefore, it is difficult to determine

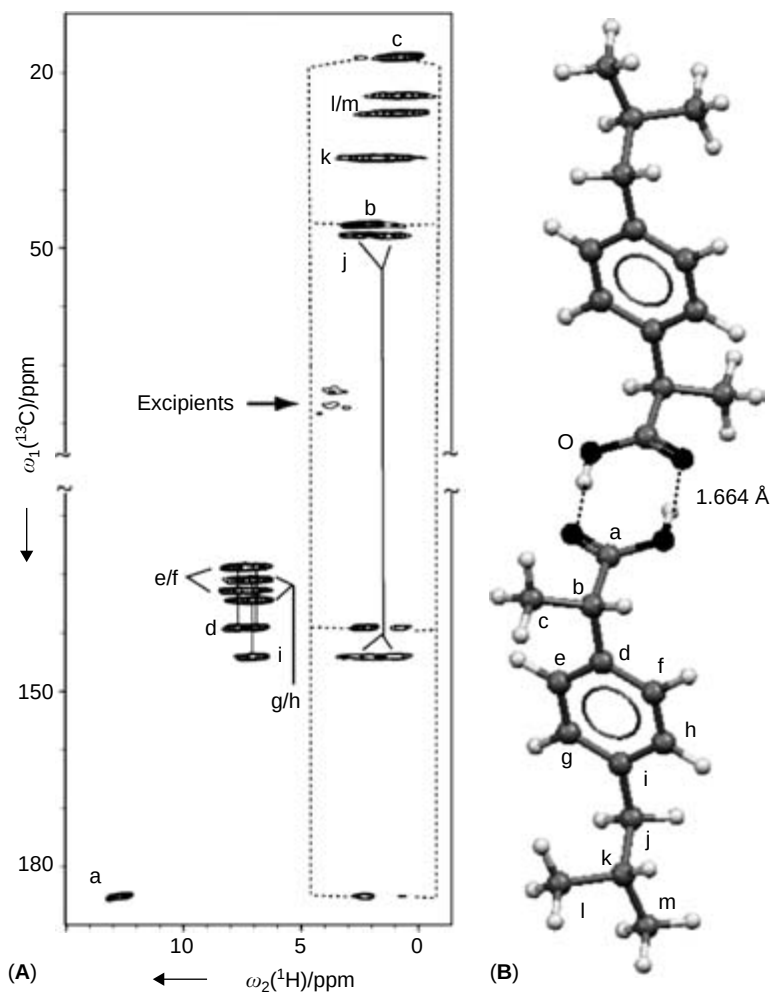


FIGURE 25 (A) ^1H detected ^{13}C – ^1H HETCOR spectrum at 17.6T and 40kHz MAS for 6mg of a formulation containing 3.8mg of ibuprofen (~63% load) with assignments for the labeled ibuprofen crystal structure shown in (B). The spectrum was obtained in 33 minutes with two scans per row. The ^1H T_1 relaxation time for ibuprofen was 1.7 seconds. Adapted from Ref. (111).

the phase purity by most analytical methods unless truly pure phases can be obtained and compared. Without a crystal structure available for sodium pravastatin, no calculated XRPD patterns were possible. However, the authors used the crystal structure of *tert*-octylammonium pravastatin to build a model structure for sodium pravastatin, although they did not try to use this to calculate an XRPD pattern to compare with any of the forms they analyzed. The authors suggested that previously reported polymorphs of sodium pravastatin may actually be mixtures due to its propensity to crystallize out in multiple forms quite readily.

Maurin et al. studied two enantiotropically related polymorphs of roxifiban by XRPD, spectroscopy, and thermal analyses (114). The FT-IR spectra were essentially identical for both forms, and the FT-Raman spectra were almost indistinguishable. The standard DSC data for each form were the same, but the modulated DSC data showed some differences. The isothermal microcalorimetry data also showed differences between the two forms. The XRPD and ^{13}C SSNMR spectroscopy data easily differentiated both forms, although some peak regions were almost identical for the SSNMR spectra indicating that only part of the molecule experienced a change in electronic environment between the two polymorphs. Form I is the stable form above 132°C, and the *n*-butyl group of roxifiban showed splitting for three of the four aliphatic carbon resonances compared to form II. The splitting of the aliphatic resonances in the spectrum of form I is not necessarily indicative of two molecules per asymmetric unit because roxifiban has several amide groups that may have restricted rotation resulting in conformational exchange. Variable-temperature SSNMR spectroscopy would be useful in clarifying this issue, and single-crystal structures of both forms would show whether there was greater disorder for the aliphatic region of form I.

SSNMR Spectroscopy of Quadrupolar Nuclei in Pharmaceuticals

Several studies have shown that nuclear quadrupolar resonance (NQR) spectroscopy can be useful in analyzing polymorphs (115,116). Blinc et al. studied polymorphs of five sulfa drugs by using polarization enhancement of the ^{14}N NQR signal from protons (115). All of the drugs and their polymorphs were readily distinguished by this technique. Quantification was possible because the signal was directly proportional to the amount of sample that contains nitrogen, but it requires 10 mg or more of the compound in a sample. The authors also note that tablets can be analyzed without removing them from the bottle.

Pérez et al. used ^{35}Cl NQR spectroscopy without enhancement to analyze chlorpropamide and sodium diclofenac (116). Chlorpropamide had two polymorphs with one molecule per asymmetric unit in each, and variable temperature ^{35}Cl NQR spectroscopy revealed a third low-temperature form with a phase transition at -85°C. Quantification was also demonstrated in this study in which the limit of detection was near 10 mg and the limit of quantification limit was approximately 20 mg. NQR is not very useful with disordered or amorphous materials because the typically broad peaks even for crystalline materials become too broad to observe reliably.

NQR spectroscopy would not be a generally applicable technique for studying pharmaceuticals, but it can be useful in certain cases.

SSNMR Spectroscopy and Structure Determination

Structural determination in the solid state has been primarily done using diffraction techniques because of their atomic resolution. SSNMR spectroscopy is potentially very powerful for determining the conformation of a molecule in the solid state, but it is practically useless for determining long-range order in solids due to its strong dependence on the local electronic environment surrounding each nucleus. The conformations of molecules in the asymmetric unit and interactions with nearest neighbors provide useful information about different solid forms. There are numerous NMR pulse sequences used for structural determinations in liquid solutions, and some of them have been modified for use in the solid state. The orientationally

dependent properties of dipolar coupling and CSA have also been exploited to determine structure of solids at relatively high resolution. The various SSNMR spectroscopy techniques can also be applied to disordered solids to analyze their structures, although the broader peaks can make the data interpretation much more difficult. The rapid developments occurring in SSNMR spectroscopy will hopefully overcome some of the current limitations, particularly the broad peaks of ^1H SSNMR spectroscopy. Theoretical computations of chemical shifts in solid samples is also quite common as shown in several previous examples in section "Solid-Form Characterization" on p. 398, but improvements are required to obtain more accurate chemical shifts particularly for very similar conformations that may exist in the unit cell.

Harper et al. compared the ^{13}C detected HETCOR and 2D INADEQUATE experiments for analyzing the different molecules in the asymmetric units α -santonin, catechin, and calcium acetate (117). The resolution in the proton dimension of the HETCOR analysis tends to be low, which can make it difficult to assign the resonances appropriately. The ^{13}C – ^{13}C INADEQUATE experiment has very low sensitivity (e.g., several days of data acquisition) but can show the carbon-to-carbon connections of a small molecule. Although the INADEQUATE experiment takes significantly longer than the HETCOR analysis, it usually has better resolution because there is no ^1H dimension.

Harris et al. used the refocused INADEQUATE (Figs. 26 and 27) and the HETCOR (Figs. 28 and 29) SSNMR spectroscopy experiments to determine most of the ^1H and ^{13}C resonance assignments for the two different molecules in the asymmetric unit for oxybuprocaine hydrochloride modification II° (118). This required a significant amount of data acquisition time as the INADEQUATE experiment took five days, whereas the HETCOR took 17 hours. The authors also observed unusually low chemical shifts for one of the ethyl amine groups ($\text{CH}_3\text{CH}_2\text{NH}^+$) that is likely due to the γ -*gauche* shielding effect.

Harper et al. used CST data to study a polymorph of paclitaxel with two molecules per asymmetric unit (119). Although they obtained useful data from the CST results for use with the XRPD structural refinement, they could not assign the specific peaks to one or the other molecule in the asymmetric unit. Paclitaxel is quite complex compared to most of the molecules so far discussed, which is why it is much more difficult to interpret its SSNMR spectra.

Harris et al. used the ^{13}C – ^{13}C INADEQUATE experiment to assign the resonances of the two molecules in the asymmetric unit of the α form of testosterone (120). The SSNMR spectroscopy information was also used to test the accuracy of computed chemical shifts. Although the chemical shift calculations were reasonably close to the observed values, they were not accurate enough to differentiate between the molecules in the asymmetric unit.

Mifsud et al. used ^1H – ^1H DQ-CRAMPS, ^1H – ^{13}C INEPT-HSQC, and computation of ^1H and ^{13}C chemical shifts to study crystalline penicillin G (121). The resonance assignments were determined using through-space ^1H – ^1H correlations from the DQ-CRAMPS technique and the through-bond ^1H – ^{13}C correlations from the INEPT-HSQC technique. The computational results worked reasonably well for both ^1H and ^{13}C chemical shifts, although the aromatic resonances are probably the least reliable considering that five to six peaks are observed in a rather narrow chemical shift range. The variety of SSNMR techniques currently available allows one to analyze solids in many ways, which will lead to improved computational predictions of NMR properties and much better structural determinations of solids.

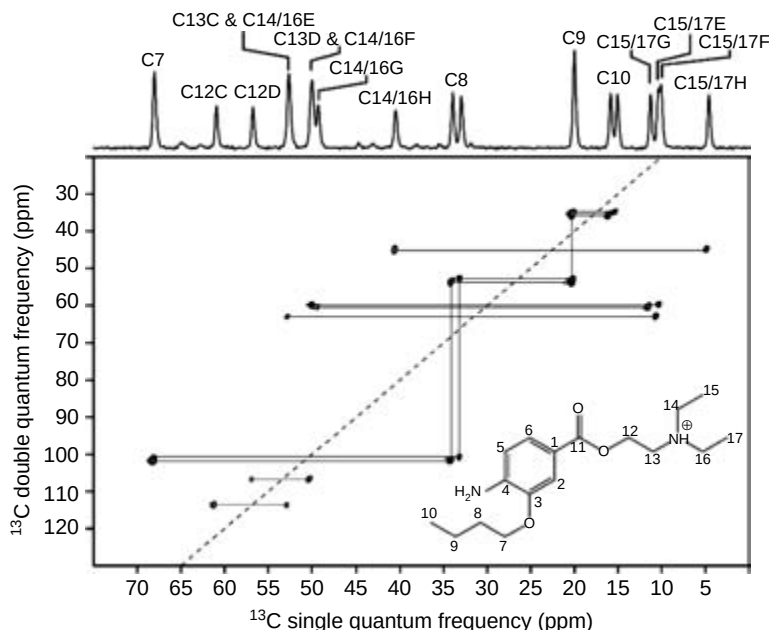


FIGURE 26 Solid-state ^{13}C INADEQUATE spectrum (low-frequency region) of oxybuprocaine hydrochloride (inset) modification II° at 11.7T and 10 kHz MAS. The connections are shown for the two independent molecules in the asymmetric unit, and the ^{13}C resonance assignments are labeled in the 1D spectrum. Adapted from Ref. (118).

SSNMR Spectroscopy of Biopharmaceuticals

Biopharmaceuticals have been a rapid growth area in the pharmaceutical industry. Biopharmaceuticals are usually large molecules produced by living organisms, and most of those currently developed as pharmaceuticals tend to be proteins, particularly monoclonal antibodies. However, biopharmaceuticals can also be oligonucleotides, polysaccharides, and lipids with beneficial pharmacological properties. SSNMR spectroscopy is a very useful technique for studying crystalline and amorphous forms of proteins. Most pharmaceutical proteins are delivered in the lyophilized form primarily because protein crystals usually need a significant amount of liquid water to remain crystalline. The value of crystalline protein pharmaceutical formulations has been discussed in the literature (122). Production of crystalline formulation rather than the typical amorphous formulation may be beneficial for manipulating the stability or bioavailability of a biopharmaceutical.

Several studies have shown that SSNMR spectroscopy readily differentiates protein polymorphs (123,124). Five polymorphs of the protein GB1 (β 1 immunoglobulin binding domain of protein G, 56 residues, ~6202 Da) were analyzed by ^{13}C SSNMR spectroscopy and XRPD (123). SSNMR spectroscopy showed the characteristic sharp peaks for the crystalline forms (Figs. 30 and 31). Although there was significant overlap due to the number of carbons in the molecule, the resolution was sufficient to easily identify the polymorphs.

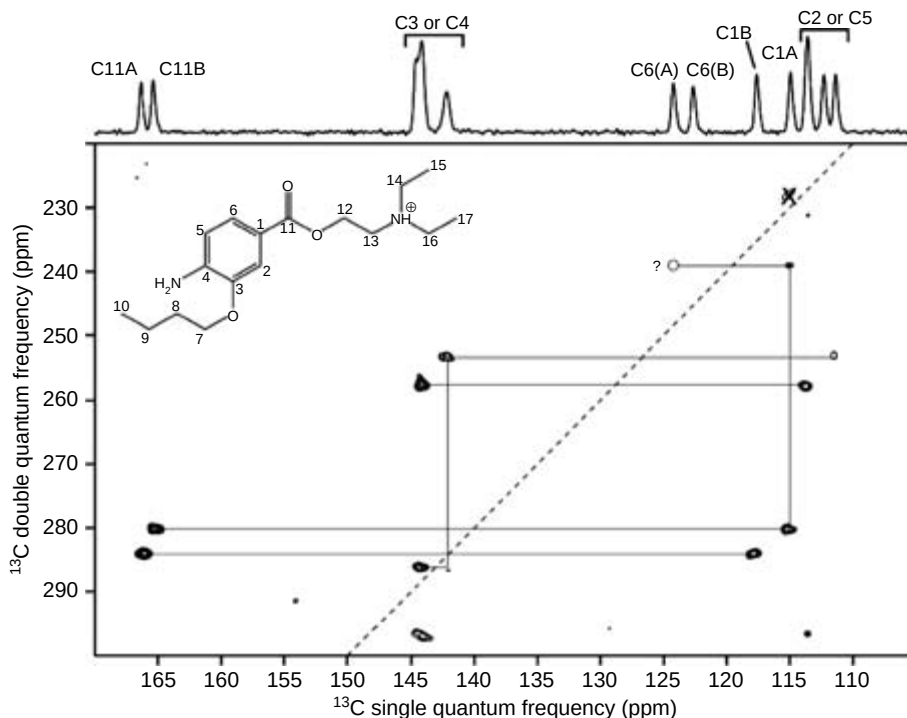


FIGURE 27 Solid-state ^{13}C INADEQUATE spectrum (high-frequency region) of oxybuprocaine hydrochloride (inset) modification II° at 11.7 T and 10 kHz MAS. The connections are shown for the two independent molecules in the asymmetric unit, and the ^{13}C resonance assignments are labeled in the 1D spectrum. Adapted from Ref. (118).

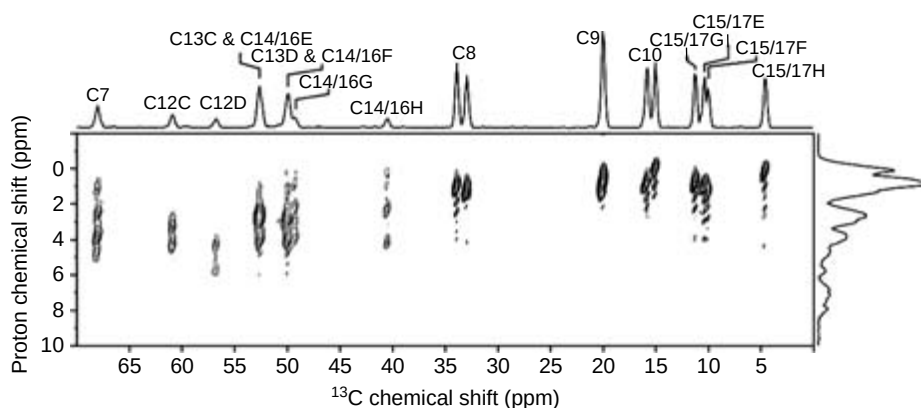


FIGURE 28 Solid-state ^{13}C - ^1H HETCOR spectrum (low frequency region) of oxybuprocaine hydrochloride modification II° at 11.7 T and 10 kHz MAS. The ^{13}C resonance assignments are labeled in the 1D spectrum. Adapted from Ref. (118).

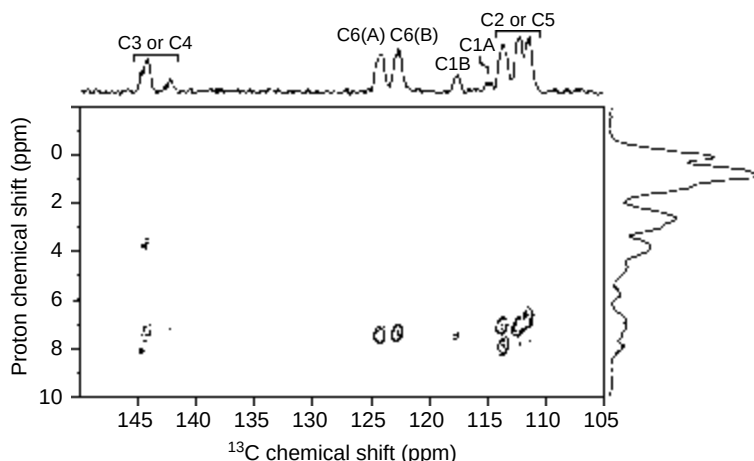


FIGURE 29 Solid-state ^{13}C - ^1H HETCOR spectrum (high-frequency region) of oxybuprocaine hydrochloride modification II° at 11.7T and 10kHz MAS. The ^{13}C resonance assignments are labeled in the 1D spectrum. Adapted from Ref. (118).

Martin and Zilm analyzed five proteins with MW between ~8 and 56 kDa by ^{13}C SSNMR spectroscopy and XRPD (Figs. 32 and 33) (124). They showed that microcrystalline and nanocrystalline proteins produced essentially identical SSNMR spectra for cases where the nanocrystals were 100 nm or larger (Fig. 33). The authors also demonstrated some potentially useful procedures for making batch quantities of submicrometer size crystals and preparing small samples of wet crystals for SSNMR spectroscopy.

The need for molecular-level characterization of biopharmaceutical solids is likely to increase in the coming years as more of these molecules are developed to treat a wide variety of diseases. SSNMR spectroscopy of biopharmaceuticals may be a useful extension of liquid-state NMR spectroscopy of these large molecules. NMR spectroscopy of proteins, oligonucleotides, and polysaccharides in aqueous or organic solutions provides a detailed fingerprint of the molecule, which is a very powerful tool for comparing biopharmaceuticals. SSNMR spectroscopy is particularly useful for characterizing biopharmaceuticals because amorphous forms can be also analyzed and usually have unique spectra. Comparability of biologicals in the solid state has the potential to be a major application of SSNMR spectroscopy in pharmaceutical development.

Quantification with SSNMR Spectroscopy

The difficulties in performing quantitative SSNMR spectroscopy have been discussed by Harris (125). Stephenson et al. have reviewed quantitative X-ray diffraction, vibrational spectroscopy, and SSNMR spectroscopy (126). The main problem for quantitative SSNMR spectroscopy is ensuring that the relative peak areas are accurate for the components in the sample. For CP methods (e.g., ^{13}C CP/MAS) where the sensitivity enhancement is provided by ^1H to ^{13}C magnetization transfer, there are three main relaxation processes that need to be considered (127): (1) The CP time

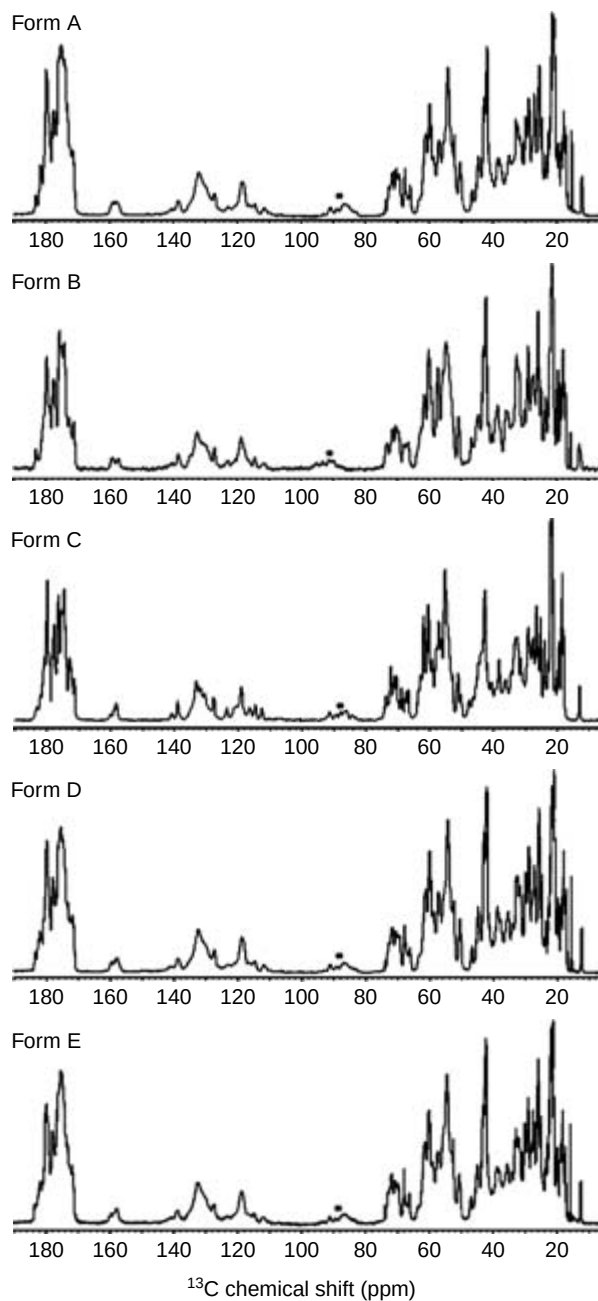


FIGURE 30 ^{13}C CP/MAS spectra of five forms of microcrystalline GB1. The spectra of forms A, D, and E were acquired at 11.7 T and 11.11 kHz MAS. The spectrum of form B was acquired at 14.1 T and 13.3 kHz MAS. The spectrum of form C was acquired at 17.6 T and 16.67 kHz MAS. Asterisks indicate spinning sidebands. Adapted from Ref. (123).

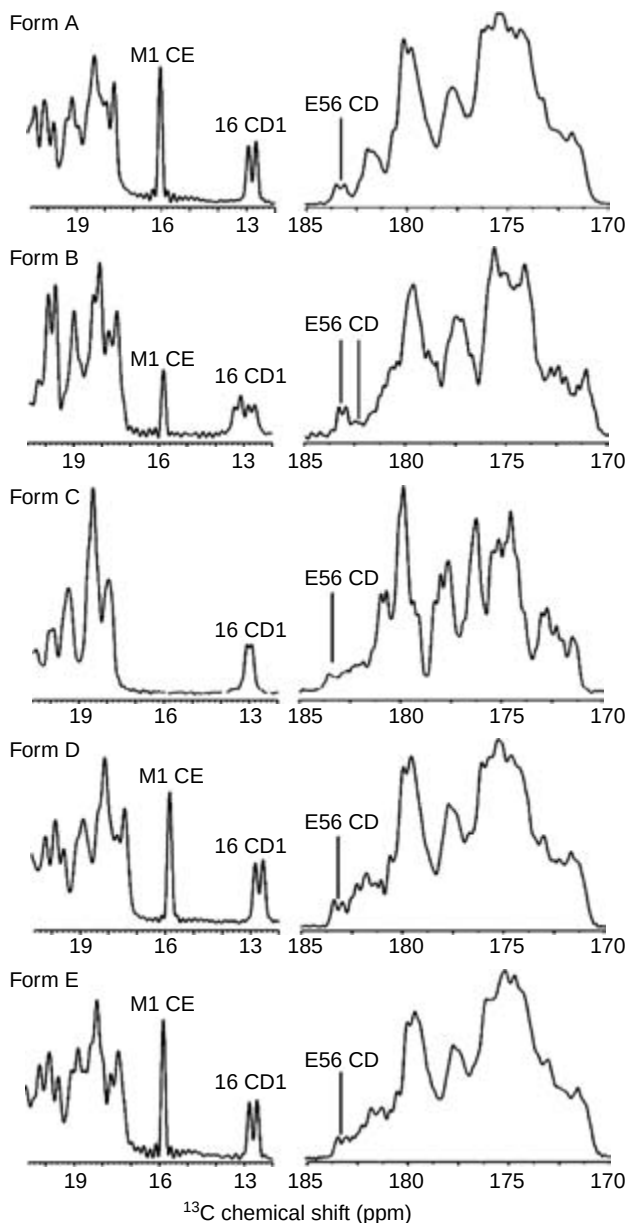


FIGURE 31 Expanded regions of the ^{13}C CP/MAS spectra of five forms of microcrystalline GB1. The carbonyl region is on the *right*, and the methyl region is on the *left*. Note that spectra of forms A, D, and E have similarities (labeled in each spectrum), but they are distinctly different and represent unique solid forms of GB1. Adapted from Ref. (123).

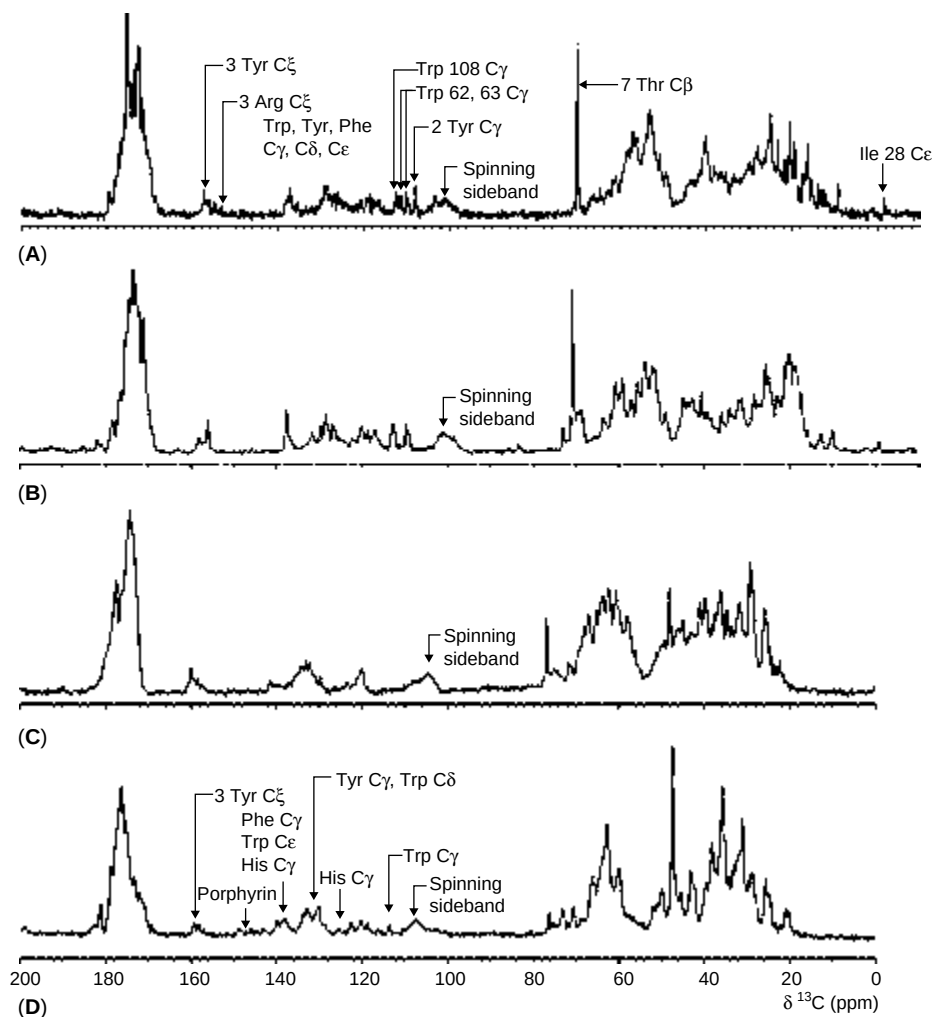


FIGURE 32 ^{13}C CP/MAS spectra of crystalline (A) lysozyme, (B) streptavidin, (C) ribonuclease A, and (D) cytochrome c. Some possible assignments are labeled. Adapted from Ref. (124).

(T_{CH}), (2) the rotating frame spin-lattice relaxation time for protons ($T_{1\rho_{\text{H}}}$), and (3) the spin-lattice relaxation time for protons ($T_{1\text{H}}$). The corresponding ^{13}C relaxation times ($T_{1\rho_{\text{C}}}$ and $T_{1\text{C}}$) usually do not have a significant effect on the observed signal for CP spectra. The T_{CH} determines how rapidly each carbon resonance gets enhanced by ^1H polarization transfer. Relatively immobile carbons with directly bonded protons will be polarized most rapidly. The $T_{1\rho_{\text{H}}}$ determines how rapidly the rotating frame ^1H magnetization decreases, and it competes with T_{CH} during the CP process. This means that if $T_{1\rho_{\text{H}}}$ is rapid, then T_{CH} will not be able to enhance some or all of the ^{13}C resonances sufficiently for CP to be useful. The $T_{1\text{H}}$ determines how rapidly the equilibrium magnetization of each proton is re-established, and

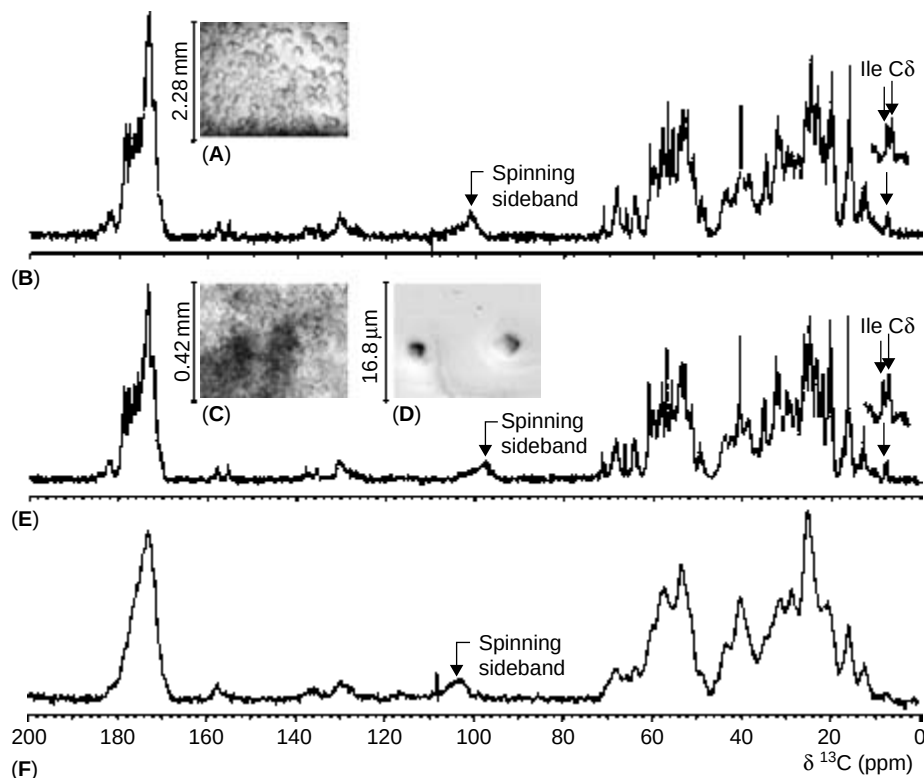


FIGURE 33 ^{13}C CP/MAS spectra of ubiquitin at 18.8 T and 14.5 kHz MAS: (B) microcrystalline protein, (E) nanocrystalline protein, and (F) lyophilized protein. Micrographs of the microcrystalline (A) and nanocrystalline (C and D) proteins are inset: (A) 5 $\times$ magnified light microscope image of microcrystalline protein, (B) 225 $\times$ magnified light microscope image of nanocrystalline protein, and (C) 4000 $\times$ magnified electron microscope image of two larger crystallites in the nanocrystalline protein sample. Adapted from Ref. (124).

therefore, how long one must wait between pulses. Figure 34 shows how the length of the delay between pulses affects the recovery of magnetization. A delay of approximately three times the relevant T_1 value is required for greater than 90% of the magnetization to recover. Because protons are the most prevalent atom in pharmaceutical solids and have strong dipolar coupling, spin polarization will be rapidly distributed over the entire molecule (spin diffusion). Spin diffusion usually results in a single T_{1H} relaxation time for a compound. Even if an internal standard is used, some corrections would be necessary to obtain quantitative data from CP/MAS spectra due to the potential differences in relaxation times.

Direct polarization ^{13}C MAS experiments can be used for quantitative SSNMR analyses, but this requires waiting at least five times the T_{1C} between scans for greater than 99% of the magnetization to recover. Carbon-13 relaxation times can be several to many minutes long, making it impractical to obtain good signal averaging in a reasonable amount of time for many solid materials.

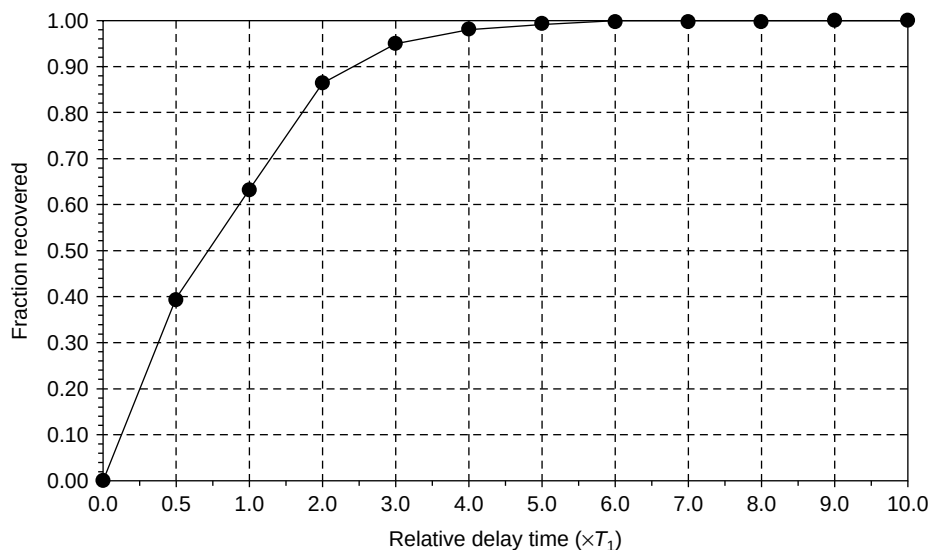


FIGURE 34 Recovery of magnetization (M_z) as a function of the amount of time allowed for relaxation.

Formoterol fumarate dihydrate was quantified at 0.45% in lactose by ^{13}C SSNMR, and the dihydrate to anhydrate ratio could be quantified to 2% in lactose (128). It is typical for quantification of polymorph mixtures to be more difficult than quantification of a single polymorph mixed with excipients because most active pharmaceutical compounds are well resolved from excipients but much less so from a polymorph.

Ziarelli et al. have discussed quantitative ^{13}C and ^{23}Na MAS SSNMR spectroscopy using an unusual external electronic signal (ERETICTM) as a quantification standard (129). They also show that using the center portion of the rotor is necessary to obtain the most accurate results, which has been previously discussed by Campbell et al. (130).

Offerdahl et al. quantified different forms of neotame by ^{13}C CP/MAS SSNMR spectroscopy (131). Their technique requires the determination of T_{CH} and $T_1\rho_{\text{H}}$ of the pure forms, obtaining spectra of mixtures using at least five different contact times that are $>5 \times T_{\text{CH}}$, and calculating the $T_1\rho_{\text{H}}$ for each component from a plot of the peak area versus contact time. The resulting graph gives a straight line with the y-intercept equal to the quantity of the particular component in the sample. The authors found a quantification limit of ~1% to 2%, and that both polymorphs contained a significant amount of amorphous material.

Miscellaneous Applications of SSNMR Spectroscopy

SSNMR spectroscopy can be used to analyze essentially any solid with NMR active nuclei. Small organic molecules are the typical compounds used for active pharmaceuticals, although larger proteins are being developed at a rapid rate.

Less commonly used as pharmaceuticals are carbohydrates, lipids, and nucleic acids. Tautomerization, ABMS, solid-state dynamics, hydrogen bonding, and pK_a s can also be analyzed by SSNMR. All of these applications have potential uses in characterizing pharmaceutical solids, and some examples of these are discussed in this section.

Paradowska et al. studied seven crystalline methyl glycopyranosides by ^{13}C SSNMR spectroscopy and XRPD (132). Sugars tend to be structurally very similar and potentially difficult to differentiate by spectroscopic techniques. However, the ^{13}C SSNMR spectra were very different for each of the sugars in this study. One of the sugars had two molecules per asymmetric unit, whereas another was apparently contaminated with an unknown sugar that may have two molecules per asymmetric unit. The authors used XRPD and calculated patterns from the single-crystal structure to confirm the results from SSNMR spectroscopy.

Yoshinari et al. studied the effect of water on the β and δ polymorphs of mannitol by DSC, scanning electron microscopy (SEM), XRPD, FT-IR, ^{13}C SSNMR spectroscopy, and water vapor sorption analyses (133). SSNMR spectroscopy was used to confirm the δ polymorph because the crystal structure had not been obtained for it. Upon exposure of the δ polymorph to 97% relative humidity overnight, it completely converted to the β polymorph. The ^1H and ^{13}C T_1 relaxation times were obtained for the β and δ polymorphs. The ^1H T_1 relaxation time was quite long as is typical for sugars (~ 4 and ~ 8 minutes for β and δ , respectively), and the ^{13}C T_1 relaxation times were as much as 15 times longer than the ^1H T_1 relaxation times. Surprisingly, the authors used a very short relaxation delay (0.1 second) to obtain the ^{13}C SSNMR spectroscopy. This would require many more scans and more time to obtain useful data given that the relaxation delay was $\sim 0.05\%$ of shortest ^1H T_1 , and 0.02% of the longest ^1H $T_1(0)$. For most pure compounds with long ^1H T_1 relaxation times, a ^{13}C CP/MAS with an acceptable signal-to-noise ratio can be obtained using a relaxation delay of 0.5 to 1.0 times the ^1H T_1 relaxation time and relatively few scans (≤ 100). A very useful alternative for obtaining spectra of nuclei with long relaxation times is the flip-back pulse sequence (134).

Colsenet et al. used ^{13}C CST analysis and ^1H SSNMR spectroscopy to study hydrogen bonding in malonic acid by lyophilization of solutions at various pH values (135). The $\delta_{11'}$, $\delta_{22'}$, and δ_{iso} chemical shift tensor values had significant changes with pH, whereas the δ_{33} value did not change very much. The authors also calculated the pK_a s of the carboxylate groups with ^{13}C SSNMR spectroscopy determinations of the acid to base ratios in the solid samples.

Mirmehrabi et al. used X-ray diffraction, ^{13}C SSNMR, FT-IR, scanning electron microscopy, and thermal analysis to study tautomerization in two forms of ranitidine hydrochloride (136). Tautomers undergo a configurational change in bonding to give a different isomer of the molecule, and these isomers exist in an equilibrium state among the various configurations depending on the conditions. This is in contrast to the more common conformational changes without bond breaking that lead to polymorphism. A change in protonation state is the most common tautomeric process because it requires less energy to break and reform bonds with electronegative atoms such as oxygen and nitrogen. The authors performed the ^{13}C SSNMR spectroscopy at 9.4 T with a 3.2 mm rotor and relatively fast MAS at 20 kHz. Both forms I and II appeared to have one molecule per asymmetric unit. The spectrum of form II showed significant peak broadening especially in the aliphatic region, which is consistent with a disordered crystalline solid. The single-crystal X-ray structure

confirmed the disorder in form II, but no crystal structure was obtained for form I because only small crystals (1–10 μm) could be grown. The higher degree of order in form I was obvious from the ^{13}C SSNMR spectra, although this was not confirmed by another technique in their study. The highest frequency carbon resonance (C15) was noticeably broader than the other resonances (particularly for form I) because C15 was bonded to two nitrogens. There was no asymmetrical splitting for this carbon resonance because the field strength was high enough to reduce the coupling with the ^{14}N quadrupole.

Novoa de Armas et al. studied two polymorphs of ranitidine base with XRPD, hot-stage microscopy, DSC, thermogravimetry, FT-IR spectroscopy, and ^{13}C SSNMR spectroscopy (137). The XRPD data was used to determine the crystal structure of forms I and II. The SSNMR spectra easily showed the differences between the solid forms, and that there was no indication of a mixture of tautomers as previously observed for the hydrochloride salt (136). The authors state that only X-ray crystallography can determine which tautomer is present, but SSNMR spectroscopy could also be used to do this, although it would probably be much more difficult. SSNMR analysis of the tautomers might require ^{15}N labeling for ^{15}N CP/MAS or REDOR analysis (102,103).

Barich et al. studied peak widths of ibuprofen in various formulations by ^{13}C SSNMR spectroscopy, XRPD, and SEM (138). The authors note that they could not make an amorphous form of ibuprofen, and it has no known polymorphs. The effect of ABMS was tested by mixing ibuprofen with materials that had different ABMS such as talc, hydroxypropyl methyl cellulose, and croscarmellose sodium (139,140). The concentration of the material and its particle size both affected the observed SSNMR peak widths of ibuprofen. The authors note that peak broadening observed after tableting may be due to reduction in the particle size or elimination of air from the voids. This is because air has much lower magnetic susceptibility than the surrounding solid. The authors also note that SSNMR spectroscopy can be used to study crystalline domain size, although they did not have any specific examples of this important issue in their report. The domain size of nanocrystalline materials is important for differentiating them from amorphous or disordered solids, such as solid dispersion formulations. The distance-dependent properties of NMR spectroscopy are particularly useful for studying domain sizes in solids, but this topic is outside the scope of this chapter.

Adam-Berret et al. used ^{13}C SSNMR spectroscopy to analyze the metastable α and stable β polymorphs of five triacylglycerols important in the food industry (141). The ^1H T_1 and T_2 relaxation times were determined at 9.4 T, whereas variable-temperature low-resolution time-domain NMR spectroscopy at 0.47 T was also used to measure the dipolar second moments of the polymorphs. They were able to distinguish the different triacylglycerol polymorphs by their different relaxation times (due to mobility differences) as well as by the chemical shift differences.

SSNMR spectroscopy can be a powerful analytical tool for partially ordered systems. Crowley et al. studied mesophases resulting from solid–solid transformations of lipids using variable-temperature ^{13}C SSNMR spectroscopy and T_1 relaxation times (142). They found that α -oleic acid and both forms of propranolol oleate were conformationally disordered crystalline phases.

Hughes and Harris used ^{13}C SSNMR spectroscopy to monitor crystallization of the α and γ polymorphs of ^{13}C -labeled glycine from H_2O and D_2O solutions (143). Both H_2O and D_2O show nucleation of α -glycine, but this converts to

γ -glycine for the D₂O solution (>80% within ~6 hours). The greater CP efficiency of α -glycine (~3-fold) was not a significant factor in observing the transformation, although the quantification would be unreliable without specifically accounting for the differences.

Xu and Harris monitored the dehydration of sodium acetate trihydrate during ¹³C SSNMR spectroscopic analyses at several spinning rates, and confirmed the resulting solid form by XRPD (144). Their dehydration process produced form I from the trihydrate when all other studies had shown that the β form results from dehydration. A mixture of forms I and β are observed at lower spinning speed (3 kHz), and the ratio of form I increased at higher spinning speed (7 kHz). The final product after about five days was a mixture of forms I and β . Form I had previously been obtained by vacuum drying of the β form. However, the authors demonstrated with ¹³C SSNMR spectroscopy that both form I and β are simultaneously produced during the drying process rather than an initial transformation of the trihydrate to the β form followed by dehydration to form I. Pure form β , obtained by dehydration, was also rotated at 3 and 7 kHz and did not convert to form I, which demonstrates that a transformation of form β to form I was not occurring.

SUMMARY

SSNMR spectroscopy is a versatile analytical technique for characterizing a wide variety of solid materials. Differentiating polymorphs is only one potential application, but it is critical to pharmaceutical development. SSNMR spectroscopy particularly stands out for mixture analysis, studying dynamic processes, and conformational analysis. It should be used routinely as one of the analytical techniques for characterizing pharmaceuticals as part of regulatory submissions. SSNMR spectroscopy is particularly suitable for patent applications of formulated pharmaceuticals because of its high specificity compared to XRPD or vibrational spectroscopy.

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Effects of Polymorphism and Solid-State Solvation on Solubility and Dissolution Rate

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INTRODUCTION

It has now been amply demonstrated that the different lattice energies (and entropies) associated with different polymorphs or solvates give rise to measurable differences in the physical properties (density, color, hardness, refractive index, conductivity, melting point, enthalpy of fusion, vapor pressure, etc.). Even the explosive power of cyclotetramethylene-tetranitramine depends on which of its four polymorphs is being used (1). We have seen in previous chapters that the different lattice energies of polymorphs or solvates give rise to different solubilities and dissolution rates. If the solubilities of the various solid forms are sufficiently different, they can be very important during the processing of drug substances into drug products (2), and may have implications for the adsorption of the active drug from its dosage form (3). These concerns have led to an increased regulatory interest in the solid-state physics of drug substances, and in their characterization (4–10).

That the crystal structure can have a direct effect on the solubility of a solid can be understood using a simple model. For a solid to dissolve, the forces of attraction between solute and solvent molecules must overcome the attractive forces holding the solid intact and the solvent aggregates together. In other words, the solvation free energy released upon dissolution must exceed the lattice free energy of the solid plus the free energy of cavity formation in the solvent for the process to proceed spontaneously. The balance of the attractive and disruptive forces will determine the equilibrium solubility of the solid in question (which is an exponential function of the free energy change of the system). The enthalpy change and the increase in disorder of the system (i.e., the entropy change) determine the Ostwald free energy change. Because different lattice energies (and enthalpies) characterize different crystal structures, the solubility of different crystal polymorphs (or solvate species) must differ as well. Finally, the act of dissolution may be endothermic or exothermic in nature, so that measurements of solution calorimetry can be used to provide important information on the substance under study. The most common solvent media used in the characterization of polymorphs or solvates are liquids or liquid mixtures that give rise to liquid solutions of the solute (11) and that constitute the focus of this chapter.

The effect of polymorphism becomes especially critical on solubility because the rate of compound dissolution must also be dictated by the balance of attractive and

disruptive forces existing at the crystal–solvent interface. A solid having a higher lattice free energy (i.e., a less stable polymorph) will tend to dissolve faster, because the release of a higher amount of stored lattice free energy will increase the solubility and hence the driving force for dissolution. At the same time, each species would liberate (or consume) the same amount of solvation energy, because all dissolved species (of the same chemical identity) must be thermodynamically equivalent. The varying dissolution rates possible for different structures of the same drug entity can, in turn, lead to varying degrees of bioavailability for different polymorphs or solvates. To achieve bioequivalence for a given drug compound usually requires equivalent crystal structures in the drug substance, although exceptions are known to exist.

The dissolution rate and solubility in a solvent medium are one of the most important characteristics of a drug substance, because these quantities determine the bioavailability of the drug for its intended therapeutic use. Solubility is defined as the equilibrium concentration of the dissolved solid (the solute) in the solvent medium, and is ordinarily a function of temperature and pressure.

EQUILIBRIUM SOLUBILITY

The capacity of any system to form solutions has limits imposed by the phase rule of Gibbs:

$$F + P = C + 2 \quad (1)$$

where F is the number of degrees of freedom in a system consisting of C components with P phases. For a system of two components and two phases (e.g., solid and liquid) under the pressure of their own vapor and at constant temperature, F equals zero. If one of the phases consists solely of one component (a pure substance), the equilibrium solubility at constant temperature and pressure is a fixed quantity that is given as the amount of solute contained in the saturated solution in a unit amount of the solvent or solution.

For any case in which F is zero, a definite reproducible solubility equilibrium can be reached. Complete representation of the solubility relations is accomplished in the phase diagram, which gives the number, composition, and relative amounts of each phase present at any temperature in a sample containing the components in any specified proportion. Solubilities may therefore be expressed in any appropriate units of concentration, such as the quantity of the solute dissolved (defined mass, number of moles) divided by the quantity either of the solvent (defined mass, volume, or number of moles) or of the solution (defined mass, volume, or number of moles). Jacques et al. have provided a compilation of the expressions for concentration and solubility (12).

Determination of Equilibrium Solubility

Methods for the determination of solubility have been thoroughly reviewed (11,13–15), especially with respect to the characterization of pharmaceutical solids (16). Solubility is normally highly dependent on temperature, so the temperature must be recorded for each solubility measurement in addition to the precise nature of the solvent and the solid phase at equilibrium. Plots of solubility against temperature are commonly used for characterizing pharmaceutical solids, and have been extensively discussed (11,17,18). Frequently (especially over a relatively

narrow temperature range), a linear relationship may be given either by a van't Hoff plot:

$$\ln X_2^{\text{sat}} = (-a/RT) + c' \quad (2)$$

or by a Hildebrand plot:

$$\ln X_2^{\text{sat}} = (b/R)\ln T + c'' \quad (3)$$

In equations (2) and (3), X_2^{sat} is the mole fraction solubility of the solid solute at an absolute temperature T , a is the apparent molar enthalpy of solution, b is the apparent molar entropy of solution, and c' and c'' are constants. The combined equation, attributed to Valentiner, has been used by Grant et al. (17) in the form:

$$\ln X_2^{\text{sat}} = (-a/RT) + (b/R)\ln T + c''' \quad (4)$$

This three-parameter equation enables solubility to be simulated and correlated quite accurately over a wide temperature range (e.g., 60°C).

As implied in the previous paragraph, the validity of the afore-mentioned equations requires that each crystal phase is stable with respect to the absence of any phase conversion during the determination of equilibrium solubility. Quite different methods of analysis must be considered if the substance under study is known to undergo a solution-mediated phase transformation.

Two general methods, the analytical method and the synthetic method (16), are available for determining solubility. In the analytical method, the temperature of equilibration is fixed, whereas the concentration of the solute in a saturated solution is determined at equilibrium by a suitable analytical procedure. The analytical method can be either the traditional, common batch agitation method, or the more recent flow column method. In the synthetic method, the composition of the solute-solvent system is fixed by appropriate addition and mixing of the solute and solvent, then the temperature at which the solid solute just dissolves or just crystallizes is carefully bracketed.

Another technique that has recently gained attention is that of the potentiometric titration method (19–21). The results of this technique have been favorably compared to that of the analytical shake flask method (20). In order to utilize this method a compound must be ionizable with some appreciable solubility. If compound solubility is too low, then cosolvents may be utilized; however, this complicates and can confound analysis. Fioritto et al. investigated the pH-metric method for determining the solubility and conversion of eight polymorphic compounds (22). Llinas et al. have used a potentiometric method referred to as the "chasing equilibrium" method (23) to determine the inter-conversion and solubility properties of hydrate polymorphs of sparfloxacin (24).

It is usually not difficult to determine the solubility of solids that are moderately soluble (greater than 1 mg/mL), but the direct determination of solubilities much less than 1 mg/mL is not straightforward. Problems such as slow equilibrium resulting from a low rate of dissolution, the influence of impurities, the apparent heterogeneity in the energy content of the crystalline solid (25), and analytical variability, can lead to large discrepancies in reported values. For example, reported values of the aqueous solubility of cholesterol range from 0.25 to 2600 mg/mL (26).

Metastable Solubility

Because only one member of a family of polymorphs or solvates can be the most thermodynamically stable form under a given set of environmental conditions defined by the phase rule, one frequently finds that one form spontaneously converts to another form during the time required to establish equilibrium solubility. The existence of an unexpected metastable solubility can lead to important (and possible undesirable) consequences. For digoxin, unexpectedly high solubility values and abnormally high dissolution rates have resulted in the overdosing of patients before the phenomenon of its solid-state phase conversion was properly understood and controlled (27). This phenomenon was caused by higher energy crystals resulting from a greater density of crystal defects rather than by polymorphism.

Any metastable phase will have a higher free energy than would a thermodynamically more stable phase, and will undergo a phase transformation to the more stable phase once the activation energy barrier is overcome. Often the barrier to phase transformation is merely the improbability of a suitable nucleation step. Hence, only fortuitously unfavorable kinetics permitted a determination of the equilibrium solubility of the various higher energy phases discussed in the preceding section.

Conversions of a metastable phase into a more stable phase may include the transformation of one polymorphic phase into another, the solvation of an anhydrous phase, the desolvation of a solvate phase, the transformation of an amorphous phase into a crystalline anhydrate or solvate phase, the degradation of a crystalline anhydrate or solvate phase to an amorphous phase, or in the case of digoxin, the conversion of imperfect (less crystalline, more amorphous) crystals with a high density of defects into more perfect (more crystalline) crystals with a lower density of defects. Although it is straightforward to determine the equilibrium solubility of a phase that stabilizes with respect to conversion, the measurement of solubilities of metastable phases that are susceptible to conversion is not a trivial matter.

Because determinations of the solubility of solid materials are often made by suspending an excess of the compound in question in the chosen solvent or other dissolution medium, the application of this equilibrium method to a metastable phase will result in a determination of the solubility of the stable phase. One of the attempts to measure the solubility of a metastable polymorph was made by Milosovich, who developed a method based on the measurement of the intrinsic dissolution rates (IDR) and used it to deduce the relative solubilities of sulfathiazole Forms I and II (28). This method assumes that the IDR is proportional to the solubility, the proportionality constant being the transport rate constant that is constant under constant hydrodynamic conditions in a transport-controlled dissolution process. Nogami et al. have also used intrinsic dissolution rate models for characterizing compounds that have metastable states with relatively fast conversion times (29,30).

Ghosh and Grant have developed an extrapolation technique to determine the solubility of a crystalline solid that undergoes a phase change upon contact with a solvent medium (31). They proposed a thermodynamic cycle analogous to Hess's law, but based on free energies, and used this cycle to predict the theoretical solubility of solvates in water. In the model systems to which the technique was applied, good agreement was obtained between the solubility values measured

by equilibration (and derived from an extrapolation method in a mixed solvent system), and those derived from the extrapolation method and calculated by means of the thermodynamic cycle.

A light-scattering method has recently been described for the determination of the solubility of drugs, and its application to solubility evaluations of metastable phases has also been demonstrated (32). Using this technique to deduce solubility data for theophylline anhydrate (metastable with respect to the monohydrate phase in bulk water at room temperature), agreement with the most reliable literature data was excellent. The light-scattering method appears to be most useful in the determination of solubility data for metastable crystal phases in dissolution media in which they spontaneously and rapidly convert into a more stable crystal phase.

Because the solubility determination of metastable polymorphs is often challenging due to conversion kinetics, it is useful to pursue theoretical calculations based on pure component properties (e.g., transition temperatures, entropy, and enthalpy of melting). Indeed, the rigorous derivation of polymorph ideal solubility from first principals can be considered and is detailed elsewhere (18,33,34). A practical consideration when using the ideal solubility equation is to assume that polymorphs have similar heat capacity terms, and that the change in the heat capacity from the solid to the liquid state is negligible. Using these heat capacity assumptions, Mao et al. (34) have shown that for a monotropic polymorph system, the relationship between the solubility of polymorph 1 (X_1) and polymorph 2 (X_2) is,

$$\log \frac{X_1^{(i)}}{X_2^{(i)}} \approx \frac{\Delta H_{m2} - \Delta H_{m1}}{RT} + \frac{\Delta S_{m2} - \Delta S_{m1}}{R} \quad (5)$$

where ΔH_m and ΔS_m are the enthalpy and entropy of melting, respectively.

For the enantiotropic systems, the difference in solubility between two forms is

$$\ln \frac{X_1^{(i)}}{X_2^{(i)}} \approx \frac{\Delta S_t(T_t - T)}{RT} \quad (6)$$

where T_t is the transition temperature and ΔS_t is the entropy of transition.

Mao et al. found that the calculated solubility ratios based on good thermal data were in good agreement for representative enantiotropic and monotropic experimental data. From their work they provided a guide for calculating polymorph solubility ratios based on differential scanning calorimetry (DSC) thermal observations. A modified guide is given in Table 1.

Apparent Solubilities of Polymorphic Systems

The thermodynamic relationships examined in involving polymorphism and solubility has been applied to the methylprednisolone system (35). The solubilities of the two polymorphs of this steroid were determined at various temperatures in water, decyl alcohol, and dodecyl alcohol. Because the chemical potential and thermodynamic activity of the drug in the solid state and in each saturated solution is constant, the solubility ratios for the two forms (which may be found in Table 2) were found to be independent of the solvent. The enthalpy, entropy, and temperature

TABLE 1 Decision Scheme for Choosing a Solubility Ratio Equation Based on Different DSC Thermal Observations

Observations				Methodology		
Transition observed?	Melting point	Heat of event ^a	Solubility	Relationship ^b	Transition temperature ^a	Equation ^c
No	$T_2 > T_1$	$\Delta H_{m2} > \Delta H_{m1}$	$S_1 > S_2$	Monotropic	n.a.	Mono (M)
Yes	No T_1	$\Delta H'_t$ seen	$S_1 > S_2$	Enantiotropic	$T_t \leq T'_t$	Enant (E)
	T_2 seen	No ΔH_{m1} ΔH_{m2} seen			$T_t < T'_t$	
Yes	No T_1	$\Delta H'_t$ seen	$S_2 > S_1$	Enantiotropic	$T_t \leq T'_t$	Enant (E)
	T_2 seen	No ΔH_{m1} ΔH_{m2} seen			$T < T'_t$	
No	$T_2 > T_1$	$\Delta H_{m1} > \Delta H_{m2}$	$S_2 > S_1$	Enantiotropic	$T_t > T$	Mono (M)
No	$T_2 > T_1$	$\Delta H_{m1} > \Delta H_{m2}$	$S_1 > S_2$	Enantiotropic	$T_t < T$	Mono (M)

Adapted from Ref. (34).
^a T_t is the equilibrium transition temperature and T'_t is the solid–solid transition temperature observed by DSC ($\Delta H'_t$ is the heat at T'_t).
^bRelationship represents the actual thermodynamic relationship between the polymorphs.
^cEquation refers to the equation that can be used in practice to estimate the solubility ratio based on the data observed.

TABLE 2 Equilibrium Solubility and Solubility Ratios of the Polymorphs of Methylprednisolone in Different Solvent Systems (35)

Temperature (°C)	Solubility (mg/mL)		
	Form I	Form II	Solubility ratio (II/I)
In Water			
30	0.09	0.15	1.67
39	0.12	0.20	1.67
49	0.16	0.26	1.63
60	0.21	0.33	1.57
72	0.30	0.43	1.43
84	0.43	0.55	1.28
In Decyl Alcohol			
30	2.9	4.8	1.66
39	3.5	5.7	1.63
49	4.3	6.9	1.60
60	5.5	8.6	1.56
72	8.3	11.9	1.43
84	–	–	–

of transition calculated from the data were 1600 cal/mol, 4.1 cal/K·mol, and 118°C, respectively.

Solubility determinations were used to characterize the polymorphism of 3-(((3-(2-(7-chloro-2-quinolinyl)-(E)-ethenyl)-phenyl)-((3-dimethylamino-3-oxopropyl)-thio)-methyl)-thio)-propanoic acid (36). The solubility of Form II was

TABLE 3 Equilibrium Solubility of Phenylbutazone Polymorphs, at Ambient Temperature in Different Solvent Systems (37)

Solvent system	Solubility (mg/mL)				
	I	II	IV	V	III
pH 7.5 Phosphate buffer	4.80	5.10	5.15	5.35	5.9
Above buffer with 0.05% Tween 80	4.50	4.85	4.95	5.10	5.52
Above buffer with 2.25% PEG 300	3.52	5.77	5.85	6.15	6.72

Note: The polymorphs are listed in order of increasing free energy at ambient temperature.

found to be higher than that of Form I in both isopropyl alcohol (IPA, solubility ratio approximately 1.7 from 5°C to 55°C) and in methyl ethyl ketone (MEK, solubility ratio approximately 1.9 from 5°C to 55°C), indicating that Form I is the thermodynamically stable form in the range of 5°C to 55°C. An analysis of the entropy contributions to the free energy of solution from the solubility results implied that the saturated IPA solutions were more disordered than were the corresponding MEK solutions, in turn, indicating the existence of stronger solute-solvent interactions in the MEK solution. This finding corroborated results determined for the enthalpy with respect to the relative idealities of the saturated solutions.

Phenylbutazone has been found capable of existing in five different polymorphic structures, characterized by different X-ray powder diffraction patterns and melting points (37). The equilibrium solubilities of all five polymorphs in three different solvent systems are summarized in Table 3. Form I exhibits the highest melting point (suggesting the least energetic structure at the elevated temperature), whereas its solubility is the lowest in each of the three solvent systems studied (actually demonstrating the lowest free energy). These findings indicate that Form I is the thermodynamically most stable polymorph both at room temperature and at the melting point (105°C). However, identification of the sequence of stability for the other forms at any particular temperature was not straightforward. Following one common convention, the polymorphs were numbered in the order of decreasing melting points, but the solubility data does not follow this order. This finding implies that the order of stability at room temperature is not the same as that at 100°C, and emphasizes that only measurements of solubility can predict the stability order at room temperature. If different polymorphs are not discovered in the same study, they are ordinarily numbered according to the order of discovery to avoid re-numbering those discovered earlier. Ostwald's rule of stages, discussed in Chapter 1, explains why metastable forms are often discovered first.

Gepirone hydrochloride was found to exist in at least three polymorphic forms, whose melting points were 180°C (Form I), 212°C (Form II), and 200°C (Form III) (38). Forms I and II, and Forms I and III, were deduced to be enantiotropic pairs in the sense that their G versus T curves crossed. Form III was found to be monotropic with respect to Form II, because the G versus T curves did not cross below their melting points, and because there was no temperature at which Form III was the most stable polymorph. The solubility data illustrated in Figure 1 were used to estimate a transition temperature of 74°C for the enantiotropic Forms I and II, whereas the reported enthalpy difference was 4.5 kcal/mot at 74°C and 2.54 kcal/mot at 25°C.

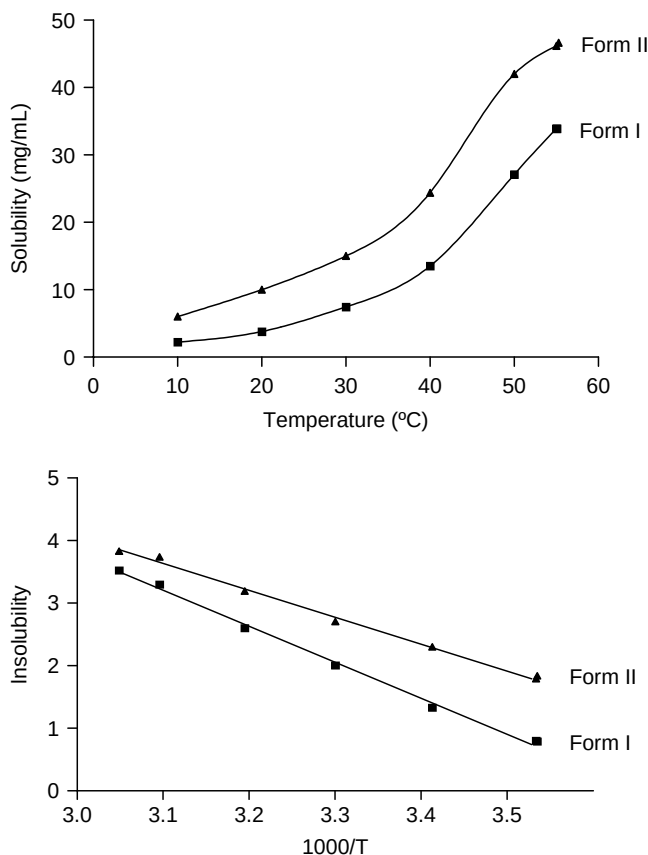


FIGURE 1 Temperature dependence of the equilibrium solubilities of two polymorphic forms of gepirone hydrochloride (38).

The most stable polymorph below 74°C was Form I, whereas Form II was the most stable above 74°C.

The effect of solvent composition on the solubility of polymorphs was investigated with cimetidine (39). Both forms exhibited almost identical melting points, but Form B was found to be less soluble than Form A, identifying it as the most stable polymorph at room temperature. The two forms were more soluble in mixed water–isopropanol solvents than in either of the pure solvents, reflecting the balance between the solvation of the molecules by water and isopropanol in determining the activity coefficient of the solute, and hence, the solubility. At constant temperature, the difference in the Gibbs free energy and the solubility ratio were constant, independent of the solvent system.

The equilibrium solubilities of two polymorphs of an experimental anti-viral compound were used to verify the results of solubility ratio predictions made on the basis of melting point and heat of fusion data (40). Even though the solubilities of Forms I and III were almost equal in three different solvent systems, the theoretically

calculated solubility ratio agreed excellently with the experimentally derived ratios in all of the solvent systems studied. The highest melting form (Form I) was found to be more soluble at room temperature, indicating that an enantiotropic relationship existed between Forms I and III.

It is well established that the temperature range of thermodynamic stability (and certain other quantities) can be determined from measurements of the equilibrium solubilities of the individual polymorphs (41). In one such study, the two polymorphic forms of 2-[[4-[[2-(1*H*-tetrazol-5-ylmethyl)-phenyl]-methoxy]-phenoxy]methyl]-quinoline were found to exhibit an enantiotropic relationship, because their *G* versus *T* curves intersected with Form I melting at a lower temperature than did Form II (42). Form I was determined to be the more thermodynamically stable form at room temperature, although the solubility of the two forms was fairly similar. The temperature dependence of the solubility ratio of the two polymorphs afforded the enthalpy of transition (Form II to Form I) as +0.9 kcal/mol, whereas the free energy change of this transition was -0.15 kcal/mol.

Aqueous suspensions of tolbutamide were reported to thicken to a non-pourable state after several weeks of occasional shaking, whereas samples of the same suspensions that were not shaken showed excellent stability after years of storage at ambient and elevated temperature (43). Examination by microscopy revealed that the thickening was due to partial conversion of the original plate-like tolbutamide crystals to very fine needle-shaped crystals. The new crystals were identified as a different polymorphic form, and did not correspond either to a solvate species or to crystals of a different habit. The crystalline conversion was observed to take place in a variety of solvents, the rate of conversion being faster in solvents where the drug exhibited appreciable solubility. Because the conversion rate in 1-octanol was relatively slow, use of this solvent permitted an accurate solubility ratio of 1.22 to be obtained (Form I being more soluble than Form III).

The polymorphism and phase interconversion of sulfamethoxydiazine (sulfameter) have been studied in detail (44). This compound can be obtained in three distinct crystalline polymorphs, with the metastable Form II being suggested for use in solid dosage forms on the basis of its greater solubility and bioavailability (45). However, the formulation of Form II in aqueous suspensions was judged inappropriate because of the fairly rapid rate of transformation to Form III. This behavior is illustrated in Figure 2, which shows that seeding of a Form II suspension with Form III crystals greatly accelerates the phase conversion. It was subsequently learned that phase conversion could be retarded by prior addition of various formulation additives, possibly permitting the development of a suspension containing the metastable Form II (46). Although there are many examples of the conversion of a metastable polymorph to a stable polymorph during the dissolution process, some of them seminal (47,48), the use of tailor-made additives to inhibit the crystallization of a more stable polymorph is relatively recent (49-52).

Table 4 provides a practical perspective of the differences in solubility that may be observed for compounds that exhibit different polymorphic states. The experimental data in Table 4 is a non-exhaustive list of solubility ratios (solubility of metastable/solubility of stable form) for 30 compounds abstracted from the scientific literature. When many of the reports offer solubilities at multiple equilibration temperatures, representative values nearest to 25-37°C are presented. Acemetacin, acetohexamide, cyclopenthiiazide, and oxyclozamide on this list are the only compounds that show solubility ratios of greater than 3.0. Most metastable phases have

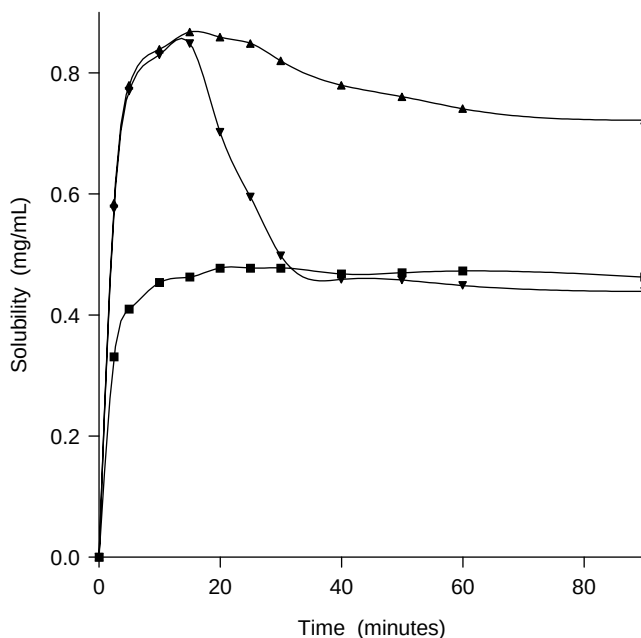


FIGURE 2 Effect on the solubility of sulfamethoxydiazine Form II by seeding with crystals of Form III. Shown are the dissolution profiles of Form II ($\blacktriangle$), Form III ($\blacksquare$), and Form II seeded with Form III after 20 minutes elapsed time ($\blacktriangledown$) (46).

apparent solubilities that are 25% to 100% greater than the polymorph with the lowest solubility. This results in a free energy of transition (ΔG_{tr}) of 132–410 cal/mol. As can be seen in the case of buspirone hydrochloride, the magnitudes of the solubilities vary considerably by solvent; however, because of the independence of the solubility ratio from the solvent identity, the ratio is virtually constant.

Pudipeddi and Serajuddin (81) have compiled 81 solubility ratios for 55 different drugs having polymorphic crystal forms, and their results are seen to parallel those of Table 4. Figure 3 combines the data from Table 4 with those of Pudipeddi and Serajuddin to offer a more comprehensive evaluation of observed solubility ratios. The 128 compounds utilized in Figure 3 are presented in order of increasing solubility ratios. Eighty-one percent of the polymorphs have solubility ratios between 1 and 2, whereas 9% of them have ratios greater than 3.5. Although these relative increases in solubility may be characterized as modest, for water-insoluble drugs exhibiting dissolution rate-limited absorption, this difference can be important for therapeutic activity.

Apparent Solubilities of Systems Having Solvate Phases

When the hydrates or solvates of a given compound are stable with respect to phase conversion in a solvent, the equilibrium solubility of these species can be used to characterize these systems. For instance, the equilibrium solubility of the trihydrate phase of ampicillin at 50°C is approximately 1.3 times that of the more stable

TABLE 4 Representative Examples of Solubility Differences Between Polymorphs

Drug	Solvent/Temp.	Solubilities	Ratio	Ref.
Acetohexamide	Distilled water	I 27 µg/mL	II/I = 1.2	53
	37°C	II 32 µg/mL		
	0.1 N HCl	V 31.4 µg/mL	V/I = 3.7	54
	30°C	IV 16.3 µg/mL	IV/I = 1.9	
		III 10.4 µg/mL	III/I = 1.2	
Acemetacin		I 8.4 µg/mL		
	Butanol	I 9.18 mM	II/I = 1.7	55
	20°C	II 15.33 mM	IV/I = 2.1	
		IV 19.00 mM	V/I = 2.8	
		V 25.65 mM	III/I = 4.7	
		III 43.44 mM		
Acetazolamide	pH 7 Phosphate buffer	A 2.04 mg/mL	B/A = 1.1	56
	25°C	B 2.28 mg/mL		
Auranofin	25% aq. PEG 200	A 0.55 mg/mL	B/A = 2.5	57
	37°C	B 1.35 mg/mL		
Benoxaprofen	pH 7 Phosphate buffer	I 230 µg/mL	I/II = 1.5	58
	25°C	II 150 µg/mL		
Buspirone HCl	Distilled water	I 101.1 g/100 g	II/I = 1.9	59
	20°C	II 190.0 g/100 g		
	Isopropanol	I 0.43 g/100 g	II/I = 1.9	
	20°C	II 0.80 g/100 g		
	60:40	I 84.93 g/100 g	II/I = 1.6	
	Water:isopropanol	II 133.02 g/100 g		
	20°C			
Carbamazepine	2-Propanol	I 11.16 mg/mL	I/II = 1.2	60
	26°C	II 9.27 mg/mL		
{4-(4-Chloro-3-fluorophenyl)-2-[4-(methyloxy)phenyl]-1,3-thiazol-5-yl} acetic acid	Acetonitrile	I 10.6 mg/mL	II/I = 1.2	61
	8.9°C	II 13.3 mg/mL		
	Acetonitrile	I 20.0 mg/mL	I/II = 1.2	
	43.1°C	II 16.0 mg/mL		
Cyclopenthiiazide	Distilled water	I 34.7 µg/mL	I/III = 2.0	62
	37°C	II 61.8 µg/mL	II/III = 3.6	
		III 17.2 µg/mL		
Difenoxin HCl	1% aq. tartaric acid	I 4.5 mg/100 mL	I/II = 1.5	63
	37°C	II 3.1 mg/100 mL		
Famotidine	Methanol	B 11 mg/mL	B/A = 1.8	64
	37°C	A 6 mg/mL		
Frusemide	pH 5 Acetate buffer	II 57.1 mg/100 mL	II/I = 1.6	65
	37°C	I 35.2 mg/100 mL		
Gepirone HCl	<i>n</i> -Pentyl alcohol	II 10.01 mg/mL	II/I = 2.6	38
	20°C	I 3.79 mg/mL		
Glibenclamide	Distilled water	II 1.06 mg/100 mL	II/I = 1.6	66
	37°C	I 0.66 mg/100 mL		
Glycine	Distilled water	α 226.8 g·kg ⁻¹	α/γ = 1.2	67
	25°C	γ 202.1 g·kg ⁻¹		
Indomethacin	Distilled water	α 0.87 mg/100 mL	α/γ = 1.3	68
	35°C	γ 0.69 mg/100 mL		

(Continued)

TABLE 4 Representative Examples of Solubility Differences Between Polymorphs (*Continued*)

Drug	Solvent/Temp.	Solubilities	Ratio	Ref.
Mefloquine HCl	Distilled water 37°C	E 5.1 mg/mL D 4.3 mg/mL	E/D = 1.2	69
Meprobamate	Distilled water 25°C	II 6.2 mg/mL I 3.3 mg/mL	II/I = 1.9	70
MK571	Isopropyl alcohol 25°C	II 0.390 mg/mL I 0.228 mg/mL	II/I = 1.7	36
	Methyl ethyl ketone 25°C	II 2.40 mg/mL I 1.24 mg/mL	II/I = 1.9	
Nateglinide	pH 6.8 Phosphate buffered saline 25°C	H 1663.6 ug/mL B 1192.5 ug/mL S 1553.6 ug/mL	H/B = 1.4 S/B = 1.3	71
Nimodipine	Distilled water 25°C	I 0.036 mg/100 mL II 0.018 mg/100 mL	I/II = 2.0	72
5-Nor-Me	Ethanol 27.8°C	R 2.83 mg/mL O 3.06 mg/mL	O/R = 1.1	73
Oxyclozamide	0.1% aq. tween 80 25°C	III 109 ppm I 73 ppm I 28 ppm	III/I = 3.9 II/I = 2.6	74
Phenylbutazone	pH 7 Phosphate buffer 36°C	I 288.7 mg/100 mL II 279.9 mg/100 mL III 233.6 mg/100 mL IV 213.0 mg/100 mL	I/IV = 1.4 II/IV = 1.3 III/IV = 1.1	75
Phenobarbital	Distilled water 25°C	II-Ba 1.39 mg/mL II 1.28 mg/mL III-Cy 1.17 mg/mL	II-Ba/III = 1.2 II/III = 1.1	76
Piretanide	pH 1.2 37°C	B 13.3 mg/100 mL A 8.3 mg/100 mL	B/A = 1.6	77
Retinoic acid	30:70 Water:methanol 37°C	I 0.097 mg/100 mL II 0.129 mg/100 mL	II/I = 1.3	78
Seratrovast	pH 8 Phosphate buffer 25°C	I 0.543 mg/mL II 0.817 mg/mL	II/I = 1.5	56
Sulfanilamide	Ethanol 39–40°C	Ortho 21.4 g/1000 g Mono 14.0 g/1000 g	O/M = 1.5	79
Tolbutamide	Distilled water 37°C	I 14.61 mg/100 mL III 13.03 mg/100 mL	I/III = 1.1	80
	Octanol 30°C	I 23.54 mg/mL III 19.33 mg/mL	I/III = 1.2	

anhydrate phase at room temperature (82). However, below the transition temperature of 42°C, the anhydrate phase is more soluble and is therefore less stable. These relationships are illustrated in Figure 4.

Amiloride hydrochloride can be obtained in two polymorphic dihydrate forms, A and B (83). However, each solvate dehydrates around 115°C to 120°C, and the resulting anhydrous solids melt at the same temperature. However, form B was found to be slightly less soluble than form A between 5°C and 45°C, indicating that it is the thermodynamically stable form at room temperature. The temperature

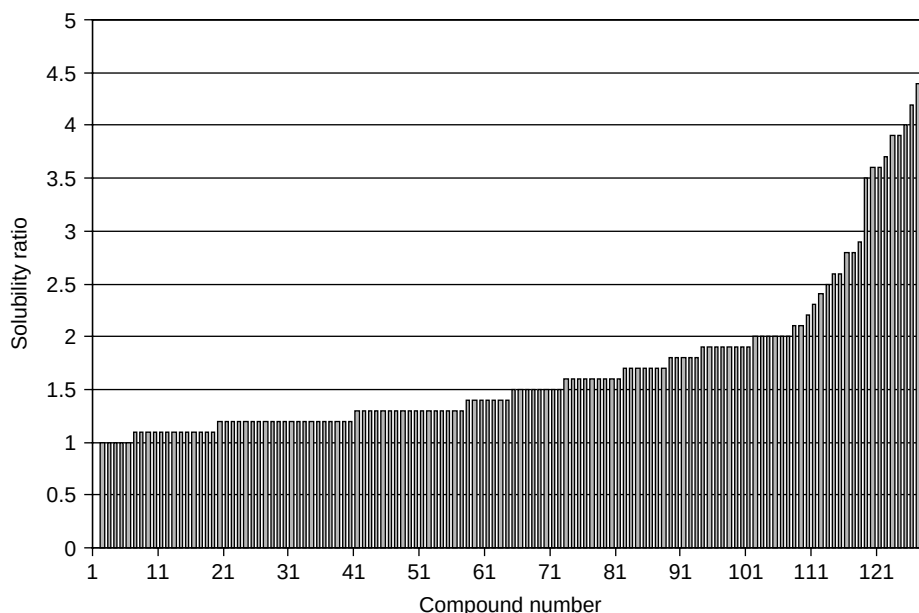


FIGURE 3 One hundred twenty-eight literature solubility ratios (metastable form/stable form) for polymorphic systems.

dependencies of the solubility data were processed by the van't Hoff equation to yield the apparent enthalpies of solution of the two polymorphic dihydrates.

The solubility of polymorphic solids derived from the anhydrate and monohydrate phases of tranilast crystals were evaluated, as were materials processed from them to enhance in vitro availability and micromeritic properties (84). Agglomerates of monohydrate phases I, II, or III were produced using different crystallization solvents and procedures. Monohydrate Form I transformed directly to the stable α -form upon dehydration, whereas Forms II and III dehydrated to the amorphous and β phases, respectively. The apparent equilibrium solubilities of monohydrate Form II and the amorphous form were much higher than those of the α and β forms due to their high surface energies. The solubilities of tranilast hydrate phases exceeded those of the anhydrate phases, which runs counter to the commonly observed trend and suggests that the anhydrate/hydrate transition temperatures are below the temperature of measurement. An analogous situation applies to the anhydrate and trihydrate phases of ampicillin (82) discussed above. The trihydrate phase is more soluble than the anhydrate phase at 50°C, because the transition temperature (42°C) is lower.

Carbamazepine is known to exist in both an anhydrate and a dihydrate form, with the anhydrate spontaneously transforming to the dihydrate upon contact with bulk liquid water (85). The anhydrous phase is reported to be practically insoluble in water, but this observation is difficult to confirm owing to its rapid transition to the dihydrate phase. The rates associated with the phase transformation process have been studied, and appear to follow first-order kinetics (86). Interestingly, the only difference in pharmacokinetics between the two forms was a slightly higher

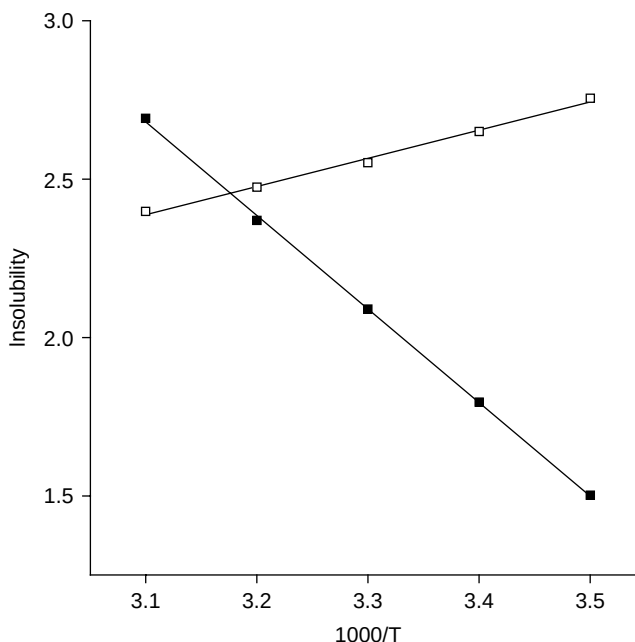


FIGURE 4 The van't Hoff plot for the anhydrate (□), and trihydrate (■) phases of ampicillin in water (82).

absorption rate for the dihydrate (87). The slower absorption of anhydrous carbamazepine was attributed to the rapid transformation to the dihydrate, accompanied by a fast growth in particle size. Comparison of the bioavailabilities of different polymorphs of a given drug suggest that significant differences are found only when the polymorphs differ significantly in Gibbs free energy deduced from the ratio of solubilities or intrinsic dissolution rates, as in the case of chloramphenicol palmitate.

A monohydrate phase of metronidazole benzoate exhibited solubility properties different from those of the commercially available anhydrous form (88). The monohydrate was found to be the thermodynamically stable form in water below 38°C. The enthalpy and entropy changes of transition for the conversion of the anhydrate to the monohydrate were determined to be -1200 cal/mol and $-3.7 \text{ cal/K}\cdot\text{mol}$, respectively. This transition was accompanied by a drastic increase in particle size, and caused physical instability of oral suspension formulations. These findings were taken to imply that any difference in bioavailability between the two forms could be attributed to changes in particle size distribution, and not to an inherent difference in the *in vivo* activity at body temperature.

Recognizing that the hydration state of a hydrate depends on the water activity, in the crystallization medium, Zhu and Grant investigated the influence of solution media on the physical stability of the anhydrate, trihydrate, and amorphous forms of ampicillin (89). The crystalline anhydrate was found to be kinetically stable in the sense that no change was detected by powder X-ray diffraction for at least five days in methanol/water solutions over the whole range of water activity ($a_v = 0$ for pure

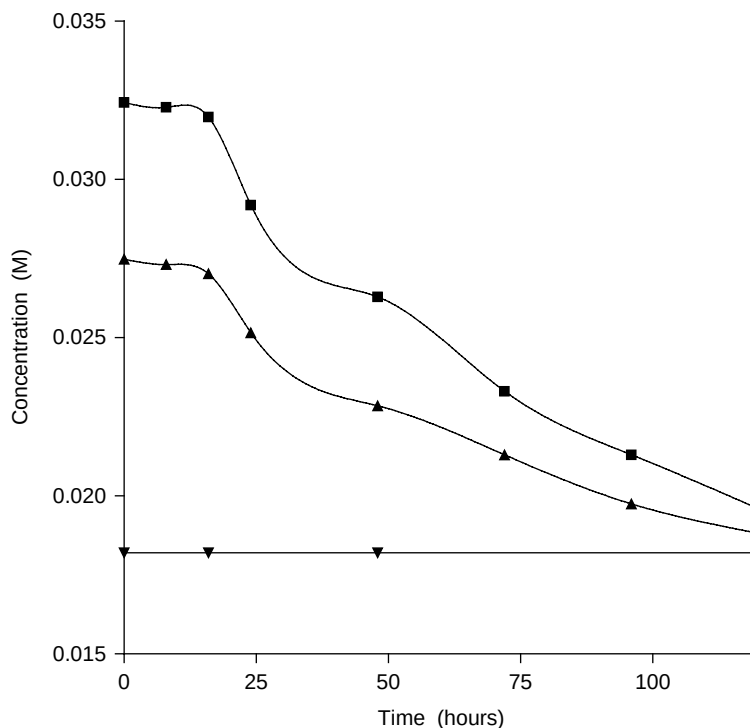


FIGURE 5 Conversion of ampicillin anhydrate to the trihydrate phase at various water activities after seeding with the trihydrate. Shown are the concentration–time data at $a_w = 1.0$ (■), $a_w = 0.862$ (▲), and $a_w = 0.338$ (▼). The curves were adapted from data originally presented in Ref. (89).

methanol to $a_w = 1$ for pure liquid water). However, addition of trihydrate seeds to ampicillin anhydrate suspended in methanol/water solutions at $a_w \geq 0.381$ resulted in the conversion of the anhydrate to the thermodynamically stable trihydrate. The trihydrate converted to the amorphous form at $a_w \leq 0.338$ in the absence of anhydrate seeds, but converted to the anhydrate phase at $a_w \leq 0.338$ when the suspension was seeded with the anhydrate. These trends are illustrated in Figure 5. The metastable amorphous form took up water progressively with increasing a_w from 0.000 to 0.338 in the methanol/water mixtures. The most significant finding of this work was that water activity was the major thermodynamic factor determining the nature of the solid phase of ampicillin, which crystallized from methanol/water mixtures.

Perhaps the most studied example of phase conversion in the presence of water concerns the anhydrate to monohydrate transition of theophylline. It had been noted in a very early work that the anhydrous phase would convert to the monohydrate phase within seconds of exposure of the former to bulk water (90). The conversion to the monohydrate phase was also demonstrated to take place during wet granulation (91), and could even occur in processed tablets stored under elevated humidity conditions (92). The difficulty in determining the equilibrium solubility of theophylline anhydrate is evident in the literature, which reports a wide range of values (93–95). Better success has been obtained in mixed solvent

TABLE 5 Representative Examples of Solubility Differences Between Solvated Systems

Drug	Solvent/Temp.	Crystal forms and solubilities	Ratio	Ref.
Ampicillin	Water	Anhydrate 10.1 mg/mL	A/T = 1.3	99
	Not given	Trihydrate 7.6 mg/mL		
Calcium gluceptate	Distilled water	Anhydrate 1.3 molal	A/I = 18.6	100
	22°C	I (3.5 hydrate) 0.07 molal		
DMHP	Distilled water	DMHP anhydrate 0.109 M	F/A = 8.2	31
	25°C	DMHP formate 0.894 M		
Formoterol fumarate	Water	A (anhydrate A) 4.7 mM	A/D = 3.1	101
	RT	B (anhydrate B) 3.5 mM	B/D = 2.3	
		C (anhydrate C) 7.5 mM	C/D = 5.0	
		D (dihydrate) 1.5 mM		
Furosemide	Water*	Dioxane solvate (X) 25.9 µg/mL	X/A = 1.3	102
	37°C	DMF solvate (D) 24.7 µg/mL	D/A = 1.2	
		Form I anhydrate 19.8 µg/mL		
GK-128	pH 4.0 acetate buffer	Anhydrate I 16.4 mg/mL	A/M = 2.2	103
	25°C	Hemihydrate 9.3 mg/mL	H/M = 1.2	
		Monohydrate 7.6 mg/mL		
Glibenclamide	Distilled water	Pentanol solvate	P/II = 31.8	66
	37°C	33.7 mg/100 mL	T/II = 2.4	
		Toluene solvate 2.5 mg/100 mL		
		Form II nonsolvate		
		1.06 mg/100 m		
Lamivudine	Distilled water	I (0.2 hydrate) 84.9 mg/mL	II/I = 1.2	104
	25°C	II (anhydrate) 98.1 mg/mL	I/II = 1.6	
	Ethanol	I (0.2 hydrate) 18.5 mg/mL		
	25°C	II (anhydrate) 11.4 mg/mL		
Mebendazole	Water	DMA solvate (X) 5.82 µg/mL	X/A = 1.8	105
	Not given	DMF solvate (D) 4.86 µg/mL	D/A = 1.5	
		Anhydrate 3.16 µg/mL		
Paroxetine HCl	Distilled water	I (hemihydrate) 4.9 mg/mL	II/I = 1.7	106
	20°C	II (anhydrate) 8.2 mg/mL		
Piroxicam	0.1 N HCl	A (anhydrate) 11.90 mg/L	B/A = 1.0	107
	25°C	B (monohydrate) 12.30 mg/L	B/A = 1.4	
	0.1 N HCl	A (anhydrate) 10.58 mg/L		
	30°C	B (monohydrate) 14.64 mg/L		
Sulfamethoxazole	0.1 N HCl	A (anhydrate) 119.3 mg/100 mL	A/B = 1.2	108
	25°C	B (monohydrate)		
		97.5 mg/100 mL		
Sparfloxacin	0.15 M KCl	A (anhydrate) 5600 µg/mL	A/B = 33.5	24
	25°C	B (trihydrate) 167 µg/mL		
Theophylline	pH 6 phosphate buffer,	Anhydrate 12 mg/mL	A/M = 2.0	109
	25°C	Monohydrate 6 mg/mL		

systems, as in the work of Zhu and Grant (97), analogous to the experiments with ampicillin (89). However, the data obtained in water-rich solutions was distorted by the formation of the monohydrate phase (96,97,98).

Table 5 provides literature solubility data for some representative solvated systems. The experimental data in Table 5 is a non-exhaustive list of solubility

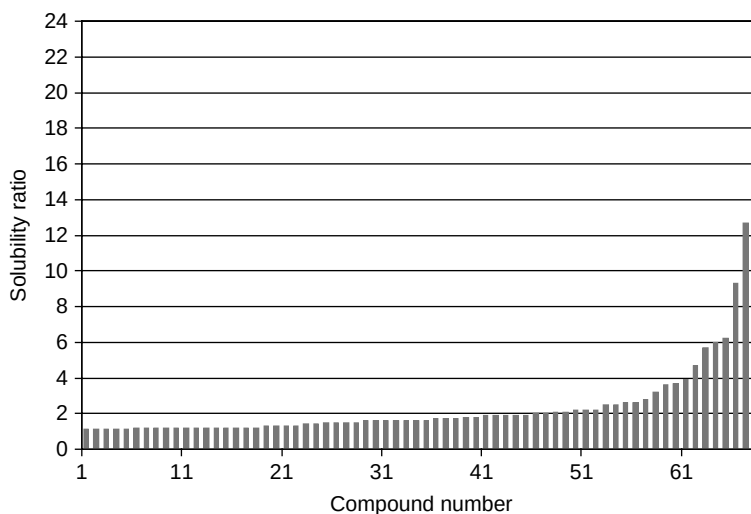


FIGURE 6 Sixty-eight literature solubility ratios (metastable form/stable form) for metastable solvated systems.

ratios (solubility of metastable/solubility of stable form) for 14 different compounds. In general, the solubility ratios for the solvated systems are greater than those of the polymorphic systems. In the case of glibenclamide and sparflaxacin, metastable solids have a 30-fold increase in solubility. Figure 6 combines the data from Table 5 and additional solvated system identified by Pudipeddi and Serajuddin (81). The 68 data points, representing 34 different compounds in Figure 6 are presented in order of increasing solubility ratios. Sixty-nine percent of the polymorphs have solubility ratios between 1 and 2, whereas 15% have ratios greater than 3.5.

SOLUTION CALORIMETRY

The practice of thermochemistry involves measurement of the heat absorbed or evolved when a chemical or physical reaction occurs. Such work entails the determination of the amount of heat, q , in the First Law of Thermodynamics:

$$dE = \delta q - \delta w \quad (7)$$

$$dH = \delta q + VdP \quad (8)$$

The usual practice for solution calorimetry is to conduct the studies at constant pressure, so that the enthalpy change equals either the heat evolved (for an exothermic change) or the heat absorbed (for an endothermic change). The principal chemical requirement for calorimetry is that the measured heat change must be assignable to a definite process, such as the dissolution of a solute in a solvent medium.

Enthalpies of Solution

When a solute is dissolved in a solvent to form a solution, there is almost always absorption or evolution of heat. According to the principle of Le Chatelier, substances that absorb heat as they dissolve must show an increase in solubility with an increase in temperature. Those that evolve heat upon dissolution must become less soluble at higher temperatures.

The heat change per mole of solute dissolved varies with the concentration, c , of the solution that is formed. It is useful to plot the total enthalpy change, ΔH , at constant temperature against the final molar concentration. This type of curve increases rapidly at low solute concentrations, but levels off at the point when the solution is saturated at the temperature of the experiment. The magnitude of the enthalpy change at a given concentration of solute divided by the corresponding number of moles of that solute dissolved represents the increase in enthalpy per mole of solute when it dissolves to form a solution of a particular concentration. This quantity is called the *molar integral heat of solution* at the given concentration. The integral heat of solution is approximately constant in dilute solution, but decreases as the final dissolved solute concentration increases.

For hydrated salts and salts that do not form stable hydrates, the integral heat of solution is ordinarily positive, meaning that heat is absorbed when these substances dissolve. When the anhydrous form of a salt capable of existing in a hydrated form dissolves, there is usually liberation of heat energy. This difference in behavior between hydrated and anhydrous forms of a given salt is attributed to the usual negative change in enthalpy (evolution of heat) associated with the hydration reaction.

Because the heat of solution of a solute varies with its final concentration, there must be a change of enthalpy when a solution is diluted by the addition of solvent. The *molar integral heat of dilution* is the change in enthalpy resulting when a solution containing one mole of a solute is diluted from one concentration to another. According to Hess's law, this change in enthalpy is equal to the difference between the integral heats of solution at the two concentrations.

The increase of enthalpy that takes place when one mole of solute is dissolved in a sufficiently large volume of solution (which has a particular composition), such that there is no appreciable change in the concentration, is the *molar differential heat of solution*. When stating a value for this quantity, the specified concentration and temperature must also be quoted. Because the differential heat of solution is almost constant in very dilute solutions, the molar differential and integral heats of solution are equal at infinite dilution. At higher concentrations, the differential heat of solution generally decreases as the concentration increases.

The *molar differential heat of dilution* may be defined as the heat change when one mole of solvent is added to a large volume of the solution at the specified concentration. The difference between the integral heats of solution at two different concentrations corresponds to the heat of dilution between these two concentrations. The heat of dilution at a specified concentration is normally obtained by plotting the molar heat of solution at various concentrations against the number of moles of solvent associated with a definite quantity of solute, and finding the slope of the curve at the point corresponding to that particular concentration. Because of the approximate constancy of the molar integral heat of solution at small concentrations, such a curve flattens out at high dilutions, and the differential heat of dilution then approaches zero.

The molar differential heats of solution and dilution are examples of partial molar properties, which are important thermodynamic quantities that must be used whenever systems of variable composition, such as solutions, are involved.

Principles Underlying Partial Molar Quantities

A solution that is deduced to be ideal of the chemical potential (μ_i) of every component is a linear function of the logarithm of its mole fraction (X_i), according to the relation:

$$\mu_i = \mu_i^* + RT \ln X_i \quad (9)$$

where μ_i^* is the (hypothetical or actual) value of μ_i when X_i equals unity, and is a function of temperature and pressure. A solution is termed ideal only if equation (9) applies to every component in a given range of composition (usually corresponding to dilute solutions), but it is not necessary that the relation apply to the whole range of composition. Any solution that is approximately ideal over the entire composition range is termed perfect solutions, although there are relatively few such solutions known. However, because a given solution may approach ideality over a limited composition range, it is worthwhile to develop the equations further.

When substance i is present both as a pure solid and as a component of an ideal solution, the condition of equilibrium may be stated as:

$$\mu_i^s = \mu_i^* + RT \ln X_i \quad (10)$$

where μ_i^s is the chemical potential of the pure solid, and X_i is the mole fraction in the solution. Rearranging, one finds:

$$\ln X_i = (\mu_i^s / RT) - (\mu_i^* / RT) \quad (11)$$

According to the phase rule, this two-component, two-phase, system is characterized by two degrees of freedom. One concludes that both the temperature and pressure of the solution can be varied independently. Because the pressure on the system is normally held fixed as that of the atmosphere during solubility studies, integration of equation (11) yields:

$$(\delta \ln X_i / \delta T)_p = (H_i - H_i^s) / RT^2 \quad (12)$$

H_i is the partial molar enthalpy of the component in the ideal solution, and H_i^s is its enthalpy per mole as the pure solid. The equation may therefore be rewritten as:

$$(\delta \ln X_i / \delta T)_p = \Delta H_i / RT^2 \quad (13)$$

where ΔH_i is the heat absorbed (at constant temperature and pressure) when one mole of the component dissolves in the ideal solution. As stated above this quantity is the differential heat of solution, and is given by:

$$\Delta H_i = H_i - H_i^s \quad (14)$$

Provided that the solution remains ideal up to $X_i = 1$, and because H_i is independent of composition in the region of ideality, H_i is the same as the enthalpy per mole of the pure liquid component. ΔH_i is equal to its molar heat of fusion, which was formerly termed the molar latent heat of fusion. It is noted, however, that these quantities refer to the temperature at which the solution having mole fraction X_i is in equilibrium with the pure solid.

If one now assumes that ΔH_i is independent of temperature over a narrow temperature range, then equation (13) can be integrated at constant pressure to yield:

$$\ln(X_1/X_2) = (\Delta H_i/R)\{(1/T_2) - (1/T_1)\} \quad (15)$$

where X_1 and X_2 refer to the solubilities (expressed as mole fractions) of the solute at temperatures T_1 and T_2 , respectively. If equation (15) remains approximately valid up to a mole fraction of unity, this situation corresponds to that where pure liquid solute is in equilibrium with its own solid at the melting point. In that case, equation (15) yields:

$$\ln X = (\Delta H_i/R)\{(1/T_m) - (1/T)\} \quad (16)$$

where X is the solubility at temperature T , and T_m is the melting point of the solute. ΔH_i is the heat of solution, but by the nature of the assumptions that have been made is also equal to the latent heat of fusion (ΔH^f) of the pure solute.

Because the number of energy levels available to take up thermal energy is greater in the liquid state than in the solid state, the heat capacity of a liquid frequently exceeds that of the same substance in the solid state. As a result, ΔH^f must be assumed to be a function of temperature. If one assumes the change in heat capacity to be constant over the temperature range of interest, then one can use the relation:

$$\Delta H_i = \Delta H_R + \Delta C_p(T_i - T_R) \quad (17)$$

where ΔH_R is the heat of solution at some reference temperature, T_R . This situation has been treated by Grant and coworkers (17), who have provided the highly useful equation (8) for the treatment of solubility data over a wide range of temperature values. Equation (8) was originally derived by Valentiner by substituting the expression for ΔH_i [equation (17)] into the differential form of the van't Hoff [equation (13)], and integrating. In equation (8), a is equal to ΔH_R when T_R equals 0 K, while b is equal to ΔC_p .

The determination of solubility data over a defined temperature range can therefore be used to calculate the differential heat of solution of a given material. For instance, the data illustrated in the bottom half of Figure 1 indicate that equation (16) can be used to deduce a value for the molar differential heat of solution of gepirone. In addition, the fact that Forms I and II yield lines of different slopes indicates the existence of unique values of the molar differential heats of solution for the two polymorphs. One can subtract the differential heats of solution obtained for the two polymorphs to deduce the heat of transition (ΔH_T) between the two forms:

$$\Delta H_T = \Delta H_S^B - \Delta H_S^A \quad (18)$$

where ΔH_s^A and ΔH_s^B denote the differential heats of solution for polymorphs A and B, respectively.

The validity of the assumption regarding constancy in the heats of solution for a given substance with respect to temperature can be made by determining the enthalpy of fusion (ΔH_F) for the two forms, and then taking the difference between these:

$$\Delta H'_T = \Delta H_F^B - \Delta H_F^A \quad (19)$$

where $\Delta H'_T$ represents the heat of transition between forms A and B at the melting point. The extent of agreement between ΔH_T and $\Delta H'_T$ can be used to estimate the validity of the assumptions made.

For example, the heats of fusion and solution have been reported for the polymorphs of auranofin (57), and these are summarized in Table 6. The similarity of the heats of transition deduced in 95% ethanol (2.90 kcal/mol) and dimethylformamide (2.85 kcal/mol) provides a fair estimation of the thermodynamics associated with this polymorphic system.

Because of the temperature dependence of the various phenomena under discussion, and because of the important role played by entropy, discussions based purely on enthalpy changes are necessarily incomplete. One can rearrange equation (9) to read:

$$\mu_i^* - \mu_i^s = -RT \ln X_i \quad (20)$$

where the left-hand side of the equation represents the difference in chemical potential between the chemical potential of i in its pure solid and the chemical potential of this species in the solution at a defined temperature and pressure. This difference in chemical potential is by definition the molar Gibbs free energy change associated with the dissolution of compound i , so one can write:

$$\Delta G_s = -RT \ln X_i \quad (21)$$

TABLE 6 Heats of Solution and Fusion Measured for the Polymorphs of Auranofin (57)

	Heat of solution, 95% ethanol (kcal/mol)	Heat of solution, dimethylformamide (kcal/mol)
Form A	12.42	5.57
Form B	9.52	2.72
Differential heat of solution	2.90	2.85
	Heat of fusion (kcal/mol)	
Form A		9.04
Form B		5.85
Differential heat of formation		3.20

where ΔG_s is the molar Gibbs free energy of solution. By analogy with equation (12), the molar Gibbs free energy associated with the transformation of polymorph A to B is given by:

$$\Delta G_T = \Delta G_s^B - \Delta G_s^A \quad (22)$$

$$= RT \ln(X_A/X_B) \quad (23)$$

where X_A and X_B are the equilibrium solubilities of polymorphs A and B, respectively, expressed in units of mole fraction.

Finally, the entropy of solubility (ΔS_s) is obtained from the relation:

$$\Delta S_s = \{\Delta H_s - \Delta G_s\}/T \quad (24)$$

For basic thermodynamic understanding of the solubility behavior of a given substance, ΔG_s , ΔH_s , and ΔS_s must be determined. Similarly, a basic thermodynamic understanding of a polymorphic transition requires an evaluation of the quantities ΔG_T , ΔH_T , and ΔS_T associated with the phase transition.

To illustrate the importance of free energy changes, consider the solvate system formed by paroxetine hydrochloride, which can exist as a non-hygroscopic hemihydrate or as a hygroscopic anhydrate (106). The heat of transition between these two forms was evaluated both by DSC ($\Delta H'_T = 0.0 \text{ kJ/mol}$) and by solution calorimetry ($\Delta H_T = 0.1 \text{ kJ/mol}$), which would indicate that both forms are isoenthalpic. However, the free energy of transition (-1.25 kJ/mol) favors conversion of the anhydrate to the hemihydrate, and such phase conversion can be initiated by crystal compression or by seeding techniques. Because the two forms are essentially isoenthalpic, the entropy increase that accompanies the phase transformation is responsible for the decrease in free energy and may therefore be viewed as the driving force for the transition.

Methodology for Solution Calorimetry

Any calorimeter with a suitable mixing device and designed for use with liquids can be applied to determine heats of solution, dilution, or mixing. To obtain good precision in the determination of heats of solution requires careful attention to detail in the construction of the calorimeter. The dissolution of a solid may sometimes be a relatively slow process and requires efficient and uniform stirring. Substantial experimental precautions are ordinarily made to ensure that heat input from the stirrer mechanism is minimized.

Most solution calorimeters operate in the batch mode, and descriptions of such systems are readily found in the literature (110,111). The common practice is to use the batch solution calorimetric approach, in which mixing of the solute and the solvent is affected in a single step. Mixing can be accomplished either by breaking a bulb containing the pure solute, allowing the reactants to mix by displacing the seal separating the two reactants in the calorimeter reaction vessel, or by rotating the reaction vessel and allowing the reactants to mix (111). Although the batch calorimetric approach simplifies the data analysis, there are design problems associated with mixing of the reactants. Guillory and coworkers have described the use of a stainless steel ampoule whose design greatly facilitates batch solution calorimetric

analyses (112). This device was validated by measurement of the enthalpy of solution of potassium chloride in water, and the reproducibility of the method was demonstrated by determination of the enthalpy of solution of the two common polymorphic forms of chloramphenicol palmitate in 95% ethanol.

Applications of Solution Calorimetry

Solution calorimetric investigations may be classified into studies that focus entirely on enthalpic processes and studies that seek to understand the contribution of the enthalpy change to the free energy change of the system. Although the former can prove to be quite informative, only the latter permit the deduction of unequivocal thermodynamic conclusions about relative stability.

Although heats of solution data are frequently used to establish differences in enthalpy within a polymorphic system, they cannot be used to deduce accurately the relative phase stability. According to equation (12), the difference between the differential heats of solution of two polymorphs is a measure of the heat of transition (ΔH_T) between the two forms. Because enthalpy is a state function (Hess's Law), this difference must necessarily be independent of the solvent system used. However, conducting calorimetric measurements of the heats of solution of the polymorphs in more than one solvent provides an empirical verification of the assumptions made. For instance, ΔH_T values of two losartan polymorphs were found to be 1.72 kcal/mol in water and 1.76 kcal/mol in dimethylformamide (113). In a similar study with moricizine hydrochloride polymorphs, ΔH_T values of 1.0 and 0.9 kcal/mol were obtained from their dissolution in water and dimethylformamide, respectively (114). These two systems, which show good agreement, may be contrasted with that of enalapril maleate, where ΔH_T was determined to be 0.51 kcal/mol in methanol and 0.69 kcal/mol in acetone (115). Disagreements of this order (about 30%) suggest that some process, in addition to dissolution, is taking place in one or both solvents.

In systems characterized by the existence of more than one polymorph, the heats of solution have been used to deduce the order of stability. As explained above, the order of stability cannot be deduced from enthalpy changes but only from free energy changes. If the enthalpy change reflects the stability, then the polymorphic change is not driven by an increase in entropy, but by a decrease in enthalpy. The heat of solution measured for cyclopenthiazide Form III (3.58 kcal/mol) was significantly greater than the analogous values obtained for Form I (1.41 kcal/mol) or Form II (1.47 kcal/mol), identifying Form III as the polymorph with the greater enthalpy, but not necessarily the most stable polymorph at ambient temperature (62).

In the case of the anhydrate and hydrate phases of norfloxacin (116), the dihydrate phase was found to exhibit a relatively large endothermic heat of solution relative to either the anhydrate or the sesquihydrate. Both urapidil (117) and dehydroepiandrosterone (118) were found to exhibit complex polymorphic/solvate systems, but the relative enthalpy of these could be deduced through the use of solution calorimetry. As an example, the data reported for urapidil (117), which have been collected into Table 7, show that the form with the lowest heat of solution implies the highest enthalpy content, which would therefore be the least stable form. These deductions have merit because the rank order of enthalpy changes corresponded to that of the free energy changes.

It is invariably found that the amorphous form of a compound is less stable than its crystalline modification, in the sense that the amorphous form tends to

TABLE 7 Heats of Solution for the Various Polymorphs and Solvates of Urapidil (117)

Crystalline form	Heat of solution (kJ/mol)
Form I	21.96
Form II	24.26
Form III	22.98 (estimated)
Monohydrate	44.28
Trihydrate	53.50
Pentahydrate	69.16
Methanol solvate	48.39

crystallize spontaneously, indicating that the amorphous form has the greater Gibbs free energy. As discussed in Chapter 1, the amorphous form is more disordered, and must therefore have a greater entropy than does the crystalline form. Hence, the enthalpy of the amorphous form is also greater. The heat of solution of amorphous pirtanide in water was found to be 12.7 kJ/mol, whereas the heat of solution associated with Form C was determined to be 32.8 kJ/mol (119). The authors calculated the heat of transformation associated with the amorphous-to-crystalline transition to be -20.1 kJ/mol. Any facile transformation of the two phases was obstructed by the significant activation energy (145.5 kJ/mol).

As emphasized above, a basic thermodynamic understanding of a polymorphic system requires a determination of the free energy difference between the various forms. The two polymorphs of 3-amino-1-(*m*-trifluoromethylphenyl)-6-methyl-1*H*-pyridazin-4-one have been characterized by a variety of methods, among which solubility studies were used to evaluate the thermodynamics of the transition from Form I to Form II (120). At a temperature of 30°C, the enthalpy change for the phase transformation was determined to be -5.64 kJ/mol. From the solubility ratio of the two polymorphs, the free energy change was then calculated as -3.67 kJ/mol, which implies that the entropy change accompanying the transformation was -6.48 cal/K·mol. In this system, one encounters a phase change that is favored by the enthalpy term, but not favored by the entropy term. However, because the overall free energy change (ΔG_T) is negative, the process takes place spontaneously, provided that the molecules can overcome the activation energy barrier at a significant rate.

A similar situation has been described for the two polymorphic forms of 2-[[4-[[2-(1*H*-tetrazol-5-ylmethyl)phenyl]-methoxy]phenoxy]methyl]quinoline (42). The appreciable enthalpic driving force for the transformation of Form II to Form I (-0.91 kcal/mol) was found to be partially offset by the entropy of transformation (-2.6 cal/K·mol), resulting in a modest free energy difference between the two forms (-0.14 kcal/mol).

In other instances, an unfavorable enthalpy term was found to be compensated by a favorable entropy term, thus rendering negative the free energy change associated with a particular phase transformation. Latnividine can be obtained in two forms, one of which is a 0.2-hydrate obtained from water or from methanol that contains water, and the other which is non-solvated and is obtained from many non-aqueous solvents (104). Form II was determined to be thermodynamically favored in the solid state. Solubility studies of both forms as a function of solvent

TABLE 8 Thermodynamic Parameters for Lamivudine in Various Solvents (104)

	Form I	Form II
Solvent = Water		
ΔG_{sol} (cal/mol)	2990	2950
ΔH_{sol} (cal/mol)	5720	5430
ΔS_{sol} (cal/deg·mol)	9.2	8.3
Solvent = Ethanol		
ΔG_{sol} (cal/mol)	3180	3460
ΔH_{sol} (cal/mol)	5270	4740
ΔS_{sol} (cal/deg·mol)	7.0	4.3
Solvent = <i>n</i> -Propanol		
ΔG_{sol} (cal/mol)	3120	3610
ΔH_{sol} (cal/mol)	5350	5000
ΔS_{sol} (cal/deg·mol)	7.5	4.7

and temperature were used to determine whether entropy of enthalpy was the driving force for solubility. Solution calorimetric data indicated that Form I would be favored in all solvents studied on the basis of enthalpy alone (Table 8). In higher alcohols and other organic solvents, Form I exhibited a larger entropy of solution than did Form II, compensating for the unfavorable enthalpic factors and yielding an overall negative free energy for the phase change.

Shefter and Higuchi considered the thermodynamics associated with the anhydrate/hydrate equilibrium of theophylline and glutethimide (90). For both compounds, the free energy change for the transformation from the anhydrate to the hydrate was negative (hence, indicating a spontaneous process), the favorable enthalpy changes being mitigated by the unfavorable entropy changes. In this work, the free energy was calculated from the solubilities of the anhydrate and hydrate forms, whereas the enthalpy of solution was calculated from the temperature dependence of the solubility ratio using the van't Hoff equation. The entropy of solution was evaluated using equation (24).

A similar conclusion was reached regarding the relative stability of the monohydrate and anhydrate phases of metronidazole benzoate (88). The enthalpy term (−1.20 kcal/mol) favored conversion to the monohydrate, but the strong entropy term (−3.7 cal/K·mol) essentially offset this enthalpy change. At 25°C, the overall ΔG_{T} of the transition was still negative, favoring the monohydrates, but only barely so (−0.049 kcal/mol). This difference was judged to be too small to result in any detectable bioavailability differences.

KINETICS OF SOLUBILITY: DISSOLUTION RATES

Evaluation of the dissolution rates of drug substances from their dosage forms is extremely important in the development, formulation, and quality control of pharmaceutical agents (16,121–123). Such evaluation is especially important in the characterization of polymorphic systems owing to the possibility of bioavailability differences that may arise from differences in dissolution rate that may themselves arise from differences in solubility (4). The wide variety of methods for determining

the dissolution rates of solids may be categorized either as batch methods or as continuous-flow methods, for which detailed experimental protocols have been provided (124).

Factors Affecting Dissolution Rates

The dissolution rate of a solid may be defined as dm/dt , where m is the mass of solid dissolved at time t . To obtain dm/dt , the following equation, which defines concentration, must be differentiated:

$$m = Vc_b \quad (25)$$

In a batch dissolution method the analyzed concentration (c_b) of a well-stirred solution is representative of the entire volume (V) of the dissolution medium, so that:

$$dm/dt = V(dc_b/dt) \quad (26)$$

In a dissolution study, c_b will increase from its initial zero value until a limiting concentration is attained. Depending on the initial amount of solute presented for dissolution, the limiting concentration will be at the saturation level, or less than this.

Batch dissolution methods are simple to set up and to operate, are widely used, and may be carefully and reproducibly standardized. Nevertheless, they suffer from several disadvantages (16). The hydrodynamics are usually poorly characterized, a small change in dissolution rate will often create an undetectable and immeasurable perturbation in the dissolution time curve, and the solute concentration may not be uniform throughout the solution volume.

In a continuous flow method, the volume flow rate over the surface of the solid is given by dV/dt , so that differentiation of equation (25) leads to:

$$dm/dt = c_b(dV/dt) \quad (27)$$

where c_b is the concentration of drug dissolved in the solvent that has just passed over the surface of the solid drug.

Continuous-flow methods have the advantages that sink conditions may be easily achieved, and that a change in dissolution rate is reflected in a change in c_b (16). At the same time, they require a significant flow rate that may require relatively large volumes of dissolution medium. Should the solid be characterized by a low solubility and a slow dissolution rate, c_b will be small and a very sensitive analytical method would be required.

The diffusion layer theory is the most useful and best known model for transport-controlled dissolution, and satisfactorily accounts for the dissolution rates of most pharmaceutical solids. In this model, the dissolution rate is controlled by the rate of diffusion of solute molecules across a thin diffusion layer. With increasing distance from the surface of the solid, the solute concentration decreases in a non-linear manner across the diffusion layer. The dissolution process at steady state is described by the Noyes-Whitney equation:

$$dm/dt = k_D A(c_s - c) \quad (28)$$

where dm/dt is the dissolution rate, A is the surface area of the dissolving solid, c_s is the saturation solubility of the solid, and c is the concentration of solute in the bulk solution. The dissolution rate constant, k_D , is given by D/h , where D is the diffusion constant. The hydrodynamics of the dissolution process have been fully discussed by Levich (125).

It has been shown (16) that the dissolution rates of solids are determined or influenced by a number of factors, which may be summarized as follows:

1. Solubility of the solid, and the temperature.
2. Concentration in the bulk solution, if not under sink conditions.
3. Volume of the dissolution medium in a batch-type apparatus, or the volume flow rate in a continuous flow apparatus.
4. Wetted surface area, which consequently is normalized in measurements of intrinsic dissolution rate.
5. Conditions in the dissolution medium that, together with the nature of the dissolving solid, determine the dissolution mechanism.

The conditions in the dissolution medium that may influence the dissolution rate can be summarized as:

1. The rate of agitation, stirring, or flow of solvent, if the dissolution is transport-controlled, but not when the dissolution is reaction-controlled.
2. The diffusivity of the dissolved solute, if the dissolution is transport-controlled. The dissolution rate of a reaction-controlled system will be independent of the diffusivity.
3. The viscosity and density influence the dissolution rate if the dissolution is transport-controlled, but not if the dissolution is reaction-controlled.
4. The pH and buffer concentration (if the dissolving solid is acidic or basic), and the pKa values of the dissolving solid and of the buffer.
5. Complexation between the dissolving solute and an interactive ligand, or solubilization of the dissolving solute by a surface-active agent in solution. Each of these phenomena tends to increase the dissolution rate.

Applications of Dissolution Rate Studies to Polymorphs and Hydrates

Historically, batch-type dissolution rate studies of loose powders and compressed disks have played a major role in the characterization of essentially every polymorphic or solid-state solvated system (35,82,90). Stagner and Guillory used these two methods of dissolution to study the two polymorphs and the amorphous phase of iopanoic acid (126). As evident in the loose powder dissolution data illustrated in the *upper half* of Figure 7, the two polymorphs were found to be stable with respect to phase conversion, but the amorphous form rapidly converted to Form I under the dissolution conditions. In the powder dissolution studies, the initial solubilities of the different forms followed the same rank order as did their respective intrinsic dissolution rates, but the subsequent phase conversion of the amorphous form to the stable Form I appeared to change the order. The amorphous form demonstrated a 10-fold greater intrinsic dissolution rate relative to Form I, whereas the intrinsic dissolution rate of Form II was 1.5 times greater than that of Form I.

The nature of the dissolution medium can profoundly affect the shape of a dissolution profile. The relative rates of dissolution and the solubilities of the two polymorphs of 3-(3-hydroxy-3-methylbutylamino)-5-methyl-*as* triazino-[5,6-*b*]-indole

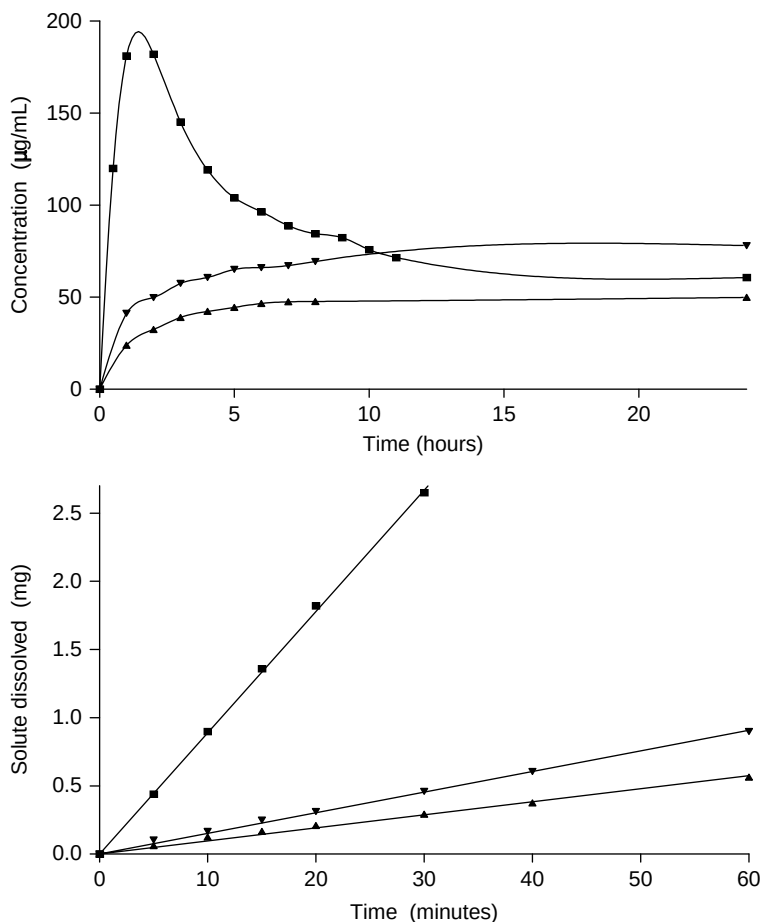


FIGURE 7 Loose powder dissolution (*upper set of traces*) and intrinsic dissolution (*lower set of traces*) profiles of iopanoic acid. Shown are the profiles of Form I ($\blacktriangle$), Form II ($\blacktriangledown$), and the amorphous form ($\blacksquare$). The plots were adapted from data originally presented in Ref. (126).

were determined in artificial gastric fluid, water, and 50% ethanol solution (127). In USP artificial gastric fluid, both polymorphic forms exhibited essentially identical dissolution rates. This behavior has been contrasted in Figure 8 with that observed in 50% aqueous ethanol, where Form II has a significantly more rapid dissolution rate than Form I. If the dissolution rate of a solid phase is determined by its solubility, as predicted by the Noyes–Whitney equation, the ratio of dissolution rates would equal the ratio of solubilities. Because this type of behavior was not observed for this triazinoindole drug, the different effects of the dissolution medium on the transport rate constant may be suspected.

The solubilities of the two polymorphs of difenoxin hydrochloride have been studied, as well as the solubility of tablets formed from mixtures of these

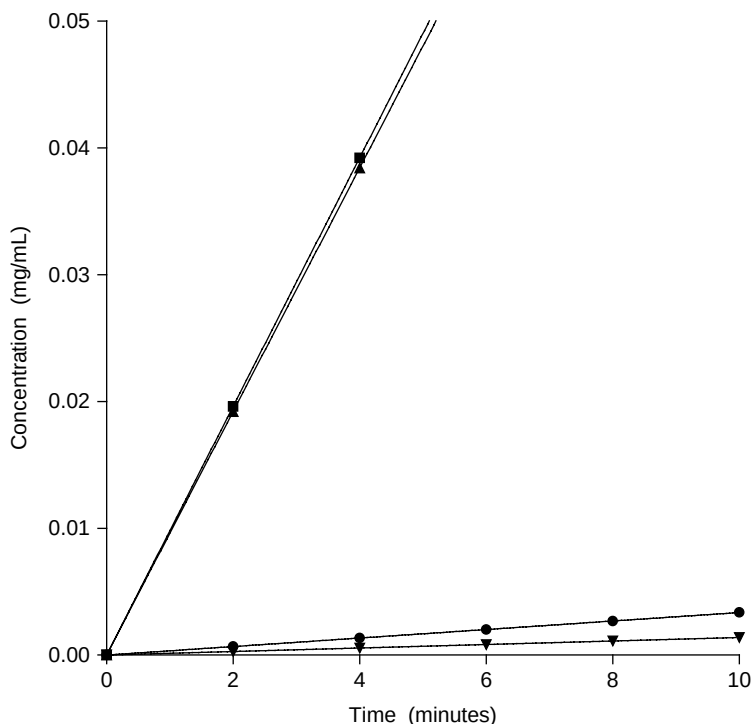


FIGURE 8 Initial stages of the dissolution of the two polymorphs of 3-(3-hydroxy-3-methylbutylamino)-5-methyl-as triazino-[5,6-*b*]-indole in different media. Shown are the profiles obtained for Form I (■) and Form II (▲) in simulated gastric fluid, as well as the profiles of Form I (●) and Form II (▼) in 50% aqueous ethanol. The traces were adapted from data originally presented in Ref. (127).

polymorphs (63). Form I was found to be more soluble than was Form II, and the solubilities of materials containing known proportions of Forms I and II reflected the differences in the solubilities of the pure forms. Likewise, the dissolution rate of difenoxin hydrochloride from tablets was determined by the ratio of Form I to Form II. In these studies, no solid-state transformation of the more soluble form to the less soluble form was observed. In addition, micronization proved to be a successful method for improving the dissolution of tablets prepared from the less soluble polymorph.

Stoltz and coworkers have conducted extensive studies on the dissolution properties of the hydrates and solvates of oxyphenbutazone (128,129). They compared the dissolution properties of the benzene and cyclohexane solvates with those of the monohydrate, hemihydrate, and anhydrate forms, and then compared their findings with results reported in the literature. The powder dissolution rates of the solvates proved to be comparable to those of the hemihydrate and the anhydrate, but superior to that of the monohydrate. This trend is illustrated in Figure 9, which confirms the usual observation that increasing degrees of hydration results in slower dissolution

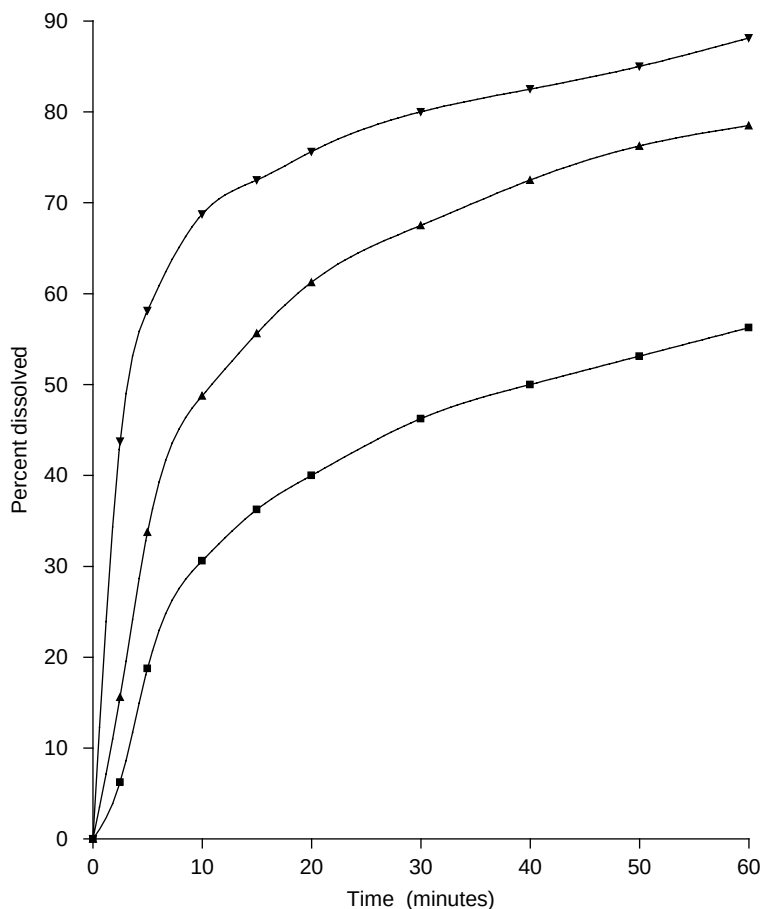


FIGURE 9 Powder dissolution profiles obtained for oxyphenbutazone anhydrate (▼), hemihydrate (▲), and monohydrate (■). The curves were adapted from data originally presented in Ref. (128).

rates. This observation differed from that previously reported by Matsuda and Kawaguchi, who reported powder dissolution rates in simulated intestinal fluid that were in the sequence hemihydrate > monohydrate > anhydrate (130). The reversed order in the dissolution rates of the former work (128) was attributed to the presence of a surfactant in the dissolution medium, which apparently overcame the hydrophobicity of the crystal surfaces of the anhydrate form. In terms of the Noyes–Whitney equation these results may be explained by the influence of the surface active agent in increasing either the wetted surface area, or the transport rate constant, or both quantities.

It has been noted from the earliest dissolution work (90) that, for many substances, the dissolution rate of an anhydrous phase usually exceeds that of any corresponding hydrate phase. These observations were explained by thermodynamics, where it was reasoned that the hydrates possessed less activity and

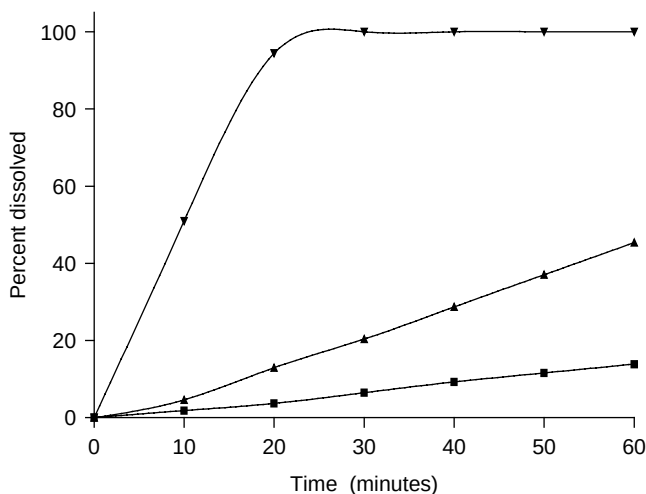


FIGURE 10 Dissolution profiles of erythromycin anhydrate (■), monohydrate (▲), and dihydrate (▼). The plots were adapted from data originally presented in Ref. (133).

would be in a more stable state relative to their anhydrous forms (131). This general rule was found to hold for the previously discussed anhydrate/hydrate phases of theophylline (93,95,97), ampicillin (89), metronidazole benzoate (88), carbamazepine (85,87), glutethimide (132), and oxyphenbutazone (129), as well as for many other systems not mentioned here. In addition, among the hydrates of urapidil, the solubility decreases with increasing crystal hydration (117).

Since the mid-1970s, a number of exceptions to the general rule have been found. For example, Figure 10 shows that the hydrate phases of erythromycin exhibit a reverse order of solubility where the dihydrate phase exhibits the fastest dissolution rate and highest equilibrium solubility (133). More recent examples include the magnesium, zinc, and calcium salts of nedocromil, for which the intrinsic dissolution rate increases with increasing water stoichiometry of their hydrates (134). The explanation for this behavior is that the transition temperatures between the hydrates are below the temperature of the dissolution measurements and decrease with increasing water stoichiometry of the hydrates. Consequently, the solubilities, and hence the intrinsic dissolution rates, increase with increasing stoichiometry of water in the hydrates. Acyclovir was recently found to be capable of forming a 3:2 drug/water hydrate phase, which exhibited an almost instantaneous dissolution relative to the more slowly dissolving anhydrous form (135). This latter finding implies a substantial difference in Gibbs free energy between the two forms.

Intrinsic Dissolution Rates

It should be recognized that the final concentration measured using the loose powder dissolution method is the equilibrium solubility, and that the initial stages of this dissolution are strongly affected by the particle size and surface area of the dissolving solids. For this reaction, many workers have chosen to study the dissolution

of compacted materials, where the particle size and surface area are regulated by the process of forming the compact.

In the disk method for conducting intrinsic dissolution studies, the powder is compressed in a die to produce a compact. One face of the disk is exposed to the dissolution medium, and rotated at a constant speed without wobble. The dissolution rate is determined as for a batch method, whereas the wetted surface area is simply the area of the disk exposed to the dissolution medium.

It is good practice to compare the powder X-ray diffraction patterns of the compacted solid and of the residual solid after the dissolution experiment with that of the original powder sample. In this manner, one may test for possible phase changes during compaction or dissolution.

The dissolution rate of a solid from a rotating disc is governed by the controlled hydrodynamics of the system, and has been treated theoretically by Levich (125). In this system, the intrinsic dissolution rate (J) may be calculated using either of the following relations:

$$J = 0.620D^{2/3}\nu^{-1/6}(c_s - c_b)\omega^{1/2} \quad (29)$$

or

$$J = 1.555D^{2/3}\nu^{-1/6}(c_s - c_b)W^{1/2} \quad (30)$$

where D is the diffusivity of the dissolved solute, ω is the angular velocity of the disc in radians per second, ν is the kinematic viscosity of the fluid, c_b is the concentration of solute at time t during the dissolution study, and c_s is the equilibrium solubility of the solute. The dependence of J on $\omega^{1/2}$ has been verified experimentally (136).

Equations (29) or (30) enable the diffusivity of a solute to be measured. These relations assume the dissolution of only one diffusing species, but because most small organic molecules exhibit a similar diffusivity (of the order 10^{-5} cm²/sec in water at 25°C), it follows that J depends on the $2/3$ power of D . Consequently, the errors arising from several diffusing species only become significant if one or more species exhibit abnormal diffusivities. In fact, diffusivity is only weakly dependent on the molecular weight, so it is useful to estimate the diffusivity of a solute from that of a suitable standard of known diffusivity under the same conditions. In most cases, the diffusivity predictions agree quite well with those obtained experimentally (137).

Intrinsic Dissolution Rate Studies of Polymorphic and Hydrate Systems

Under constant hydrodynamic conditions, the intrinsic dissolution rate is usually proportional to the solubility of the dissolving solid. Consequently, in a polymorphic system, the most stable form will ordinarily exhibit the slowest intrinsic dissolution rate. For example, a variety of high-energy modifications of frusemide were produced, but the commercially available form was found to exhibit the longest dissolution times (138). Similar conclusions were reached regarding the four polymorphs of tegafur (139) and (*R*)-*N*-[3-[5-(4-fluorophenoxy)-2-furanyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (140). However, it is possible that one of the less stable polymorphs of a compound can exhibit the slowest dissolution rate, as was noted in the case of diflunisal (141).

TABLE 9 Intrinsic Dissolution Rates for the Various Polymorphs of Aprazolam at Different Spindle Speeds (142)

Crystalline form	IDR, 50 RPM ($\mu\text{g}/\text{min}/\text{cm}^2$)	IDR, 75 RPM ($\mu\text{g}/\text{min}/\text{cm}^2$)
Form I	15.8	21.8
Form II	18.4	21.9
Form V	20.7	27.3

Intrinsic dissolution rate studies proved useful during the characterization of the two anhydrous polymorphs and one hydrate modification of alprazolam (142). The equilibrium solubility of the hydrate phase was invariably less than that of either anhydrate phase, although the actual values obtained were found to be strongly affected by pH. Interestingly, the intrinsic dissolution rate of the hydrate phase was higher than that of either anhydrate phase, with the anhydrous phases exhibiting equivalent dissolution rates. The IDR data of Table 9 reveal an interesting phenomenon, where discrimination between some polymorphs was noted at slower spindle speeds, but not at higher rates. Thus, if one is to use IDR rates as a means to determine the relative rates of solubilization of different rates, the effect of stirring speed must be investigated before the conclusions can be judged genuine.

Intrinsic dissolution rate investigations can become complicated when one or more of the studied polymorphs interconverts to another during the time of measurement. Sulfathiazole has been found to crystallize in three distinct polymorphic forms, two of which are unstable in contact with water (143) but which convert only slowly to the stable form (i.e., are kinetically stable) in the solid state. As may be seen in Figure 11, the initial intrinsic dissolution rates of these are all different, but as Forms I and II convert into Form III, the dissolved concentrations converge. Only the dissolution rate of Form III remains constant, which suggests that it is the thermodynamically stable form at room temperature. Aqueous suspensions of Forms I or II each converted into Form III over time, supporting the conclusions of the dissolution studies.

Suitable manipulation of the dissolution medium can sometimes inhibit the conversion of one polymorph to another during the dissolution process, thus permitting the measurement of otherwise unobtainable information. In studies on the polymorphs of sulfathiazole and methylprednisolone, Higuchi, who used various alcohols and additives in the dissolution medium to inhibit phase transformations, first employed this approach (144). Aguiar and Zelinier were able to thermodynamically characterize the polymorphs formed by chloramphenicol palmitate and mefenamic acid through the use of dissolution modifiers (145). Furthermore, the use of an aqueous ethanol medium containing 55.4% v/v ethanol yielded adequate solubility and integrity of the dissolving disc during studies conducted on digoxin (146).

One area of concern associated with intrinsic dissolution measurements is associated with the preparation of the solid disc by compaction of the drug particles. If a phase transformation is induced by compression, one might unintentionally measure the dissolution rate of a polymorph different from the intended one. This situation was encountered with phenylbutazone, where Form III was transformed to the most stable modification (Form IV) during the initial compression step (75).

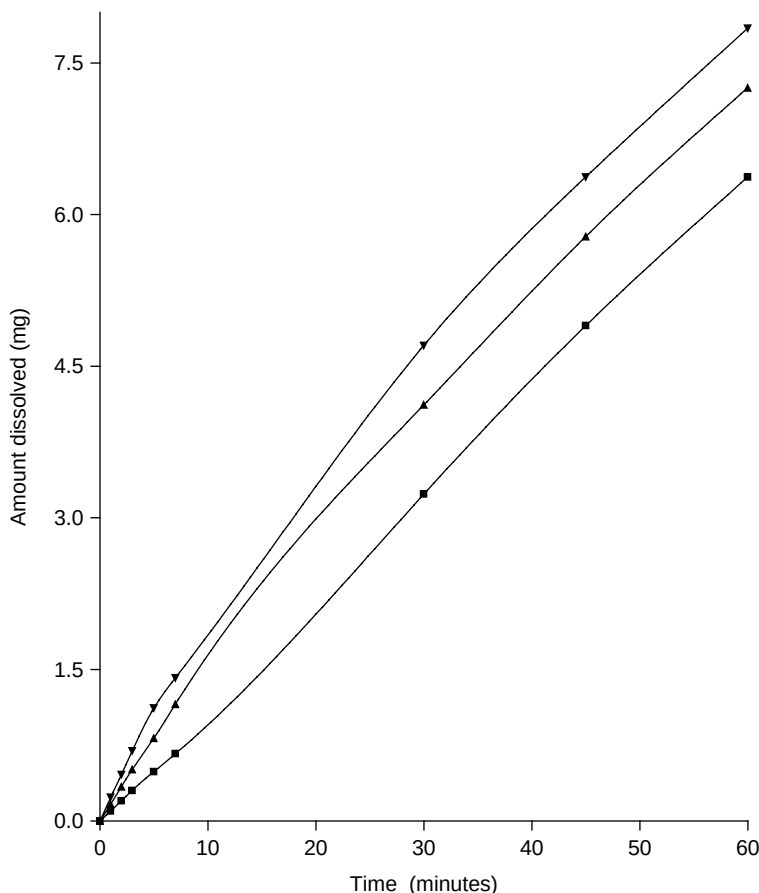


FIGURE 11 Dissolution profiles obtained for sulfathiazole Form I ($\blacktriangle$ -), Form II ($\blacktriangledown$ -), and Form III ($\blacksquare$ -) in water at 37°C. The figure has been adapted from data originally presented in Ref. (143).

One interesting note concerns the aqueous dissolution rates of solvate forms, where the solvent bound in the crystal lattice is not water. As noted earlier, the dissolution rate of an anhydrous phase normally exceeds that of any corresponding hydrate phase, but this relation is not usually applicable to other solvate species. It has been reported that the methanol solvate of urapidil exhibits a heat of solution approximately twice that of any of the anhydrate phases, and that it also exhibits the most rapid dissolution rate (147). Similarly, the pentanol and toluene solvates of glibenclainide exhibit significantly higher aqueous dissolution rates and aqueous equilibrium solubility values when compared to either of the two anhydrous polymorphs (66). The acetone and chloroform solvates of sulindac yielded intrinsic dissolution rates that were double those of the two anhydrate phases (148). These trends would imply that a non-aqueous solvate phase could be considered as being a high-energy form of the solid with respect to dissolution in water.

The most usual explanation of these phenomena is that the negative Gibbs free energy of mixing of the organic solvent, released during the dissolution of the solvate, contributes to the Gibbs free energy of solution, increasing the thermodynamic driving force for the dissolution process (90). This explanation, due to Shefter and Higuchi, was originally derived from observations on the higher dissolution rate of the pentanol solvate of succinylsulfathiazole than of the anhydrate (90). Prior addition of increasing concentrations of pentanol to the aqueous dissolution medium reduced the initial dissolution rate of the pentanol solvate. This reduction was attributed to a less favorable (less negative) Gibbs free energy of mixing of the released pentanol in the solution that already contained pentanol. In this way, the Gibbs free energy of solution was rendered less favorable (less negative), reducing the thermodynamic driving force for dissolution of the solvate. Thermodynamic characterization of the various steps in the dissolution of solvates and evaluation of their respective Gibbs free energies (and enthalpies) has been carried out by Ghosh and Grant (31).

CONSEQUENCES OF POLYMORPHISM AND SOLVATE FORMATION ON THE BIOAVAILABILITY OF DRUG SUBSTANCES

In those specific instances where the absorption rate of the active ingredient in a solid dosage form depends upon the rate of drug dissolution, the use of different polymorphs would be expected to affect the bioavailability. One can imagine the situation in which the use of a metastable polymorph would yield higher levels of a therapeutically active substance after administration owing to its higher solubility. This situation may be either advantageous or disadvantageous depending on whether the higher bioavailability is desirable or not. On the other hand, unrecognized polymorphism may result in unacceptable dose-to-dose variations in drug bioavailability, and certainly represents a drug formulation not under control.

The trihydrate/anhydrate system presented by ampicillin has received extensive attention, with conflicting conclusions from several investigations. In one early study, Poole and co-workers reported that the aqueous solubility of the anhydrate phase was 20% higher than that of the trihydrate form at 37°C (149). They also found that the time for 50% of the drug to dissolve *in vitro* was 7.5 and 45 minutes for the anhydrate and trihydrate forms, respectively (150). Using dogs and human subjects, these workers then determined *in vivo* blood levels, following separate administration of the two forms of the drug in oral suspensions or in capsules. The anhydrous form produced a higher maximum concentration of ampicillin ($C_{\max}$) and an earlier time to reach maximum concentration ($T_{\max}$) in the blood serum relative to the trihydrate form. This behavior was more pronounced in the suspension formulations. In addition, the area under the curve (AUC) was found to be greater with the anhydrous form, implying that the anhydrous form was more efficiently absorbed.

Since the early works just discussed, an interesting discussion on the comparative absorption of ampicillin has arisen. Some workers have concluded that suspensions and capsules containing ampicillin anhydrate exhibit superior bioavailabilities than analogous formulations made from the trihydrate (151,152). For instance, in a particularly well-controlled study, Ali and Farouk (152) obtained the clear-cut distinction between the anhydrate and the trihydrate, which is illustrated in Figure 12. However, others have found that capsules containing either form of

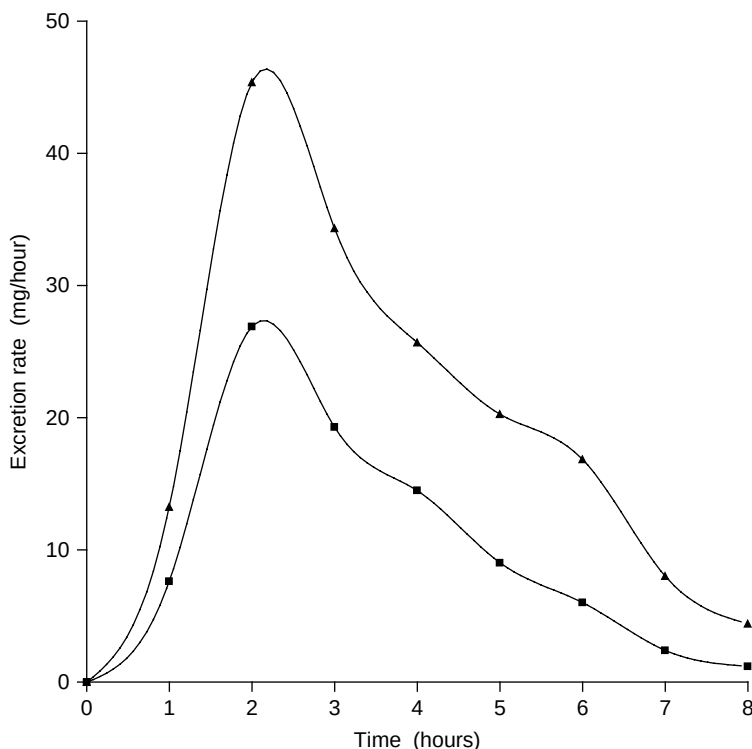


FIGURE 12 Ampicillin urinary excretion rates at various times after separate administration of the two forms. Shown are the profiles obtained for the anhydrous ($\blacktriangle$) and trihydrate phases ($\blacksquare$). The figure has been adapted from data provided in Ref. (152).

ampicillin yielded an essentially identical bioavailability (153–155). These conflicting observations indicate that the problem is strongly affected by the nature of the formulation used, and that the effects of compounding can overshadow the effects attributed to the crystalline state.

Chloramphenicol palmitate has been shown to exist in four crystal modifications, and the effect of two of these on the degree of drug absorption has been compared (156). After oral ingestion of Forms A and B, the highest mean blood levels were obtained with suspensions containing only Form B. In mixed dosage forms, the blood levels of the drug were found to bear an inverse relationship with the fraction of Form A. This finding explained the previous report, which noted that a particular suspension formulation of chloramphenicol palmitate exhibited an unsatisfactory therapeutic effect (157). A study of various commercial products indicated that the polymorphic state of the drug in this formulation was uncontrolled, consisting of mixtures of the active polymorph B and the inactive polymorph A.

Sulfamethoxydiazine has been shown to exist in a number of polymorphic forms, which exhibit different equilibrium solubilities and dissolution rates (158). Form II, the polymorph with the greater thermodynamic activity, was found to

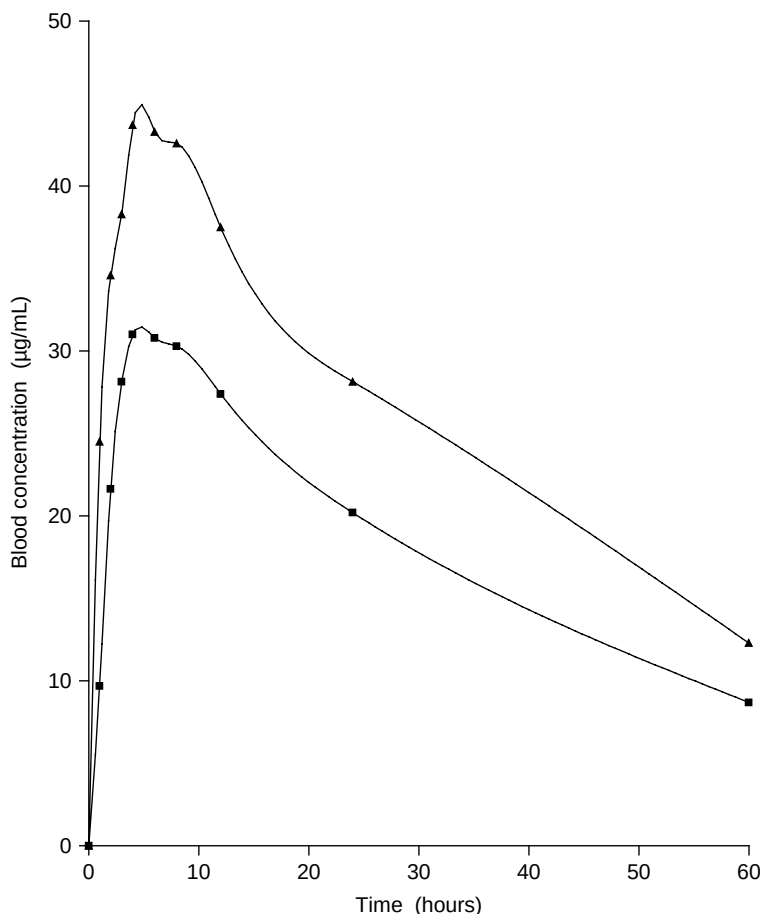


FIGURE 13 Mean concentrations of sulfamethoxydiazine in blood as influenced by the polymorphic state of the drug substance. Shown are the profiles of Form II ($\blacktriangle$) and Form III ($\blacksquare$). The figure has been adapted from data provided in Ref. (159).

yield higher blood concentration than those of Form III, which is stable in water (159). This relationship has been illustrated in Figure 13. Although the urinary excretion rates during the absorption phase confirmed the different drug absorption of the two forms as previously observed, the extent of absorption (as indicated by 72-hour excretion data) of the two forms was ultimately shown to be equivalent (160).

Fluprednisolone has been shown to exist in seven different solid phases, of which six were crystalline and one was amorphous (161). Of the crystalline phases, three were anhydrous, two were monohydrates, and one was a *tert*-butylamine solvate. The *in vitro* dissolution rates of the six crystalline phases of fluprednisolone were determined and compared with *in vivo* dissolution rates derived from pellet implants in rats (162). The agreement between the *in vitro* and *in vivo* dissolution

rates was found to be quite good, but the correlation with animal weight loss and adrenal gland atrophy was only fair. These results can be interpreted to indicate that, for fluprednisolone, differences in dissolution rates of the drug did not lead to measurable biological differences.

Erythromycin base is reported to exist in a number of structural forms, including an anhydrate, a dihydrate, and an amorphous form (163,164). The commercially available product appears to be a partially crystalline material, containing a significant amount of amorphous drug (165). From studies conducted in healthy volunteers, it was learned that the anhydrate and dihydrate phases were absorbed faster and more completely than was either the amorphous form or the commercially available form (166). These observations were reflected in two pharmacokinetic parameters ($C_{\max}$ and AUC).

Azlocillin sodium can be obtained either as a crystalline form or as an amorphous form, depending on the solvent and method used for its isolation (167). The antibacterial activity of this agent was tested against a large number of reference strains, and in most cases, the crystalline form exhibited less antibacterial activity than did the amorphous form. Interestingly, several of the tested microorganisms also proved to be resistant to the crystalline form.

Whether the different polymorphs or solvates of a given drug substance will lead to the existence of observable differences in the adsorption, metabolism, distribution, or elimination of the compound clearly cannot be predicted a priori at the present time. It is certainly likely that different crystal forms of highly soluble substances should be roughly bioequivalent, owing to the similarity of their dissolution rates. An effect associated with polymorphism that leads to a difference in bioavailability would be anticipated only for those drug substances whose absorption is determined by the dissolution rate. However, the literature indicates that, even in such cases, the situation is not completely clear. Consequently, when the existence of two or more polymorphs or solvates is demonstrated during the drug development process, wise investigators will determine those effects that could be associated with the drug crystal form and will modify their formulations accordingly.

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REVIEW ARTICLE

Pharmaceutical Salts

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Keyphrases □ Pharmaceutical salts—general pharmacy, physicochemical properties, bioavailability, pharmaceutical properties, toxicology, review □ Salts, pharmaceutical—general pharmacy, physicochemical properties, bioavailability, pharmaceutical properties, toxicology, review □ Physicochemical properties—dissolution, solubility, stability, and organoleptic properties of pharmaceutical salts, review □ Bioavailability—formulation effects, absorption alteration and pharmacokinetics of pharmaceutical salts, review □ Toxicology—pharmaceutical salts, review

CONTENTS

<i>Potentially Useful Salts</i>	2
<i>Physicochemical Studies</i>	4
Dissolution Rate	5
Solubility	7
Organoleptic Properties	8
Stability	9
Miscellaneous Properties	10
<i>Bioavailability</i>	10
Formulation Effects	11
Absorption Alteration	11
Pharmacokinetics	13
<i>General Pharmacy</i>	14
Pharmacological Effect	14
Dialysis	14
Miscellaneous	14
<i>Toxicological Considerations</i>	15
Toxicity of Salt Ion	15
Toxicity of Salt Form	15
<i>Conclusions</i>	15
<i>References</i>	16

The chemical, biological, physical, and economic characteristics of medicinal agents can be manipulated and, hence, often optimized by conversion to a salt form. Choosing the appropriate salt, however, can be a very difficult task, since each salt imparts unique properties to

Salt-forming agents are often chosen empirically. Of the many salts synthesized, the preferred form is selected by pharmaceutical chemists primarily on a practical basis: cost of raw materials, ease of crystallization, and percent yield. Other basic considerations include stability, hygroscopicity, and flowability of the resulting bulk drug. Unfortunately, there is no reliable way of predicting the influence of a particular salt species on the behavior of the parent compound. Furthermore, even after many salts of the same basic agent have been prepared, no efficient screening techniques exist to facilitate selection of the salt most likely to exhibit the desired pharmacokinetic, solubility, and formulation profiles.

Some decision-making models have, however, been developed to help predict salt performance. For example, Walkling and Appino (1) described two techniques, "decision analysis" and "potential problem analysis," and applied them to the selection of the most suitable derivative of an organic acid for development as a tablet. The derivatives considered were the free acid and the potassium, sodium, and calcium salts. Both techniques are based on the chemical, physical, and biological properties of these specific derivatives and offer a promising avenue for developing optimal salt forms.

Information on salts is widely dispersed throughout the pharmaceutical literature, much of which addresses the use of salt formation to prolong the release of the active component, thereby eliminating various undesirable drug properties (2-6). This review surveys literature of the last 25 years, emphasizing comparisons between the properties of different salt forms of the same compound. Included also is a discussion of potentially useful salt forms. Our purpose is twofold: to present an overview of the many different

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133

Table I—FDA-Approved Commercially Marketed Salts

Anion	Percent ^a	Anion	Percent ^a
Acetate	1.26	Iodide	2.02
Benzenesulfonate	0.25	Isethionate ^c	0.85
Benzoate	0.51	Lactate	0.76
Bicarbonate	0.13	Lactobionate	0.13
Bitartrate	0.63	Malate	0.13
Bromide	4.65	Maleate	3.03
Calcium edetate	0.25	Mandelate	0.36
Camphylate ^b	0.25	Mesylate	2.02
Carbonate	0.38	Methylbromide	0.76
Chloride	4.17	Methylnitrate	0.38
Citrate	3.03	Methylsulfate	0.88
Dihydrochloride	0.51	Mucate	0.13
Edetate	0.25	Napsylate	0.25
Edisylate ^c	0.38	Nitrate	0.64
Estolate ^d	0.13	Pamoate (Embonate)	1.01
Ethylate ^e	0.13	Pantothenate	0.25
Fumarate	0.25	Phosphate/diphosphate	3.16
Glucopate ^f	0.13	Polygalacturonate	0.13
Glucuronate	0.51	Salicylate	0.88
Glutamate	0.25	Stearate	0.25
Glycyllysarilate ^g	0.13	Subacetate	0.38
Hexylresorcinate	0.13	Succinate	0.38
Hydrabamine ^h	0.25	Sulfate	7.46
Hydrobromide	1.90	Tannate	0.88
Hydrochloride	42.98	Tartrate	3.54
Hydroxynaphthoate	0.25	Teclate ⁱ	0.13
		Triethiodide	0.13
Cation	Percent ^a	Cation	Percent ^a
Organic:		Metallic:	
Benzathine ^k	0.66	Aluminum	0.66
Chloroprocaine	0.33	Calcium	10.49
Choline	0.33	Lithium	1.64
Diethanolamine	0.98	Magnesium	1.31
Ethylenediamine	0.66	Potassium	10.32
Meglumine ^l	2.29	Sodium	61.97
Procaine	0.66	Zinc	2.95

^a Percent is based on total number of anionic or cationic salts in use through 1974. ^b Camphorsulfonate. ^c 1,2-Ethanedithiolate. ^d Lauryl sulfate. ^e Ethanesulfonate. ^f Glucoheptonate. ^g *p*-Glycollamidophenylarsionate. ^h *N,N'*-Di(dehydroabietyl)ethylenediamine. ⁱ 2-Hydroxyethanesulfonate. ^j 5-Chlorotheophyllinate. ^k *N,N'*-Dibenzylethylenediamine. ^l *N*-Methylglucamine.

to assemble data that will provide, for the student and practitioner alike, a rational basis for selecting a suitable salt form.

POTENTIALLY USEFUL SALTS

Salt formation is an acid-base reaction involving either a proton-transfer or neutralization reaction and is therefore controlled by factors influencing such reactions. Theoretically, every compound that exhibits acid or base characteristics can participate in salt formation. Particularly important is the relative strength of the acid or base—the acidity and basicity constants of the chemical species involved. These factors determine whether or not formation occurs and are a measure of the stability of the resulting salt.⁷

The number of salt forms available to a chemist is large; surveys of patent literature show numerous new salts being synthesized annually. Various salts of the same compound often behave quite differently because of the physical, chemical, and thermodynamic properties they impart to the parent compound. For example, a salt's hydrophobicity and high crystal lattice energy can affect dissolution rate and, hence, bioavailability. Ideally, it would be desirable if one could predict how a pharmaceutical agent's properties would be affected by salt formation.

Tables I and II list all salts that were commercially marketed through 1974. The list was compiled from all agents listed in "Martindale The Extra Pharmacopoeia,"

26th ed. (7). Table I categorizes all salt forms approved by the Food and Drug Administration (FDA), while Table II lists those not approved by the FDA but in use in other countries. (Only salts of organic compounds are considered because most drugs are organic substances.) The relative frequency with which each salt type has been used is calculated as a percentage, based on the total number of anionic or cationic salts in use through 1974. Because of simple availability and physiological reasons, the monoprotic hydrochlorides have been by far the most frequent choice of the available anionic salt-forming radicals, outnumbering the sulfates nearly six to one. For similar reasons, sodium has been the most predominant cation.

Knowledge that one salt form imparts greater water solubility, is less toxic, or slows dissolution rate would greatly benefit chemists and formulators. In some cases, such generalizations can be made. Miller and Heller (8) discussed some properties associated with specific classes of salt forms. They stated that, in general, salt combinations with monocarboxylic acids are insoluble in water and lend themselves to repository preparations, while those of dicarboxylic acids confer water solubility if one carboxylic group is left free. Pamoic acid, an aromatic dicarboxylic acid, is an exception since it is used as a means of obtaining prolonged action by forming slightly soluble salts with certain basic drugs. Saia *et al.* (9) reviewed the use of this salt form in preparing sustained-release preparations. More recently, latentiation of dihydrostreptomycin (10)

134

Table II—Non-FDA-Approved Commercially Marketed Salts

Anion	Percent ^a
Adipate	0.13
Alginate	0.13
Aminosalicylate	0.25
Anhydromethylenecitrate	0.13
Arccoline	0.13
Aspartate	0.25
Bisulfate	0.13
Butylbromide	0.13
Camphorate	0.13
Digluconate	0.13
Dihydrobromide	0.13
Disuccinate	0.13
Glycerophosphate	0.55
Hemisulfate	0.13
Hydrofluoride	0.13
Hydroiodide	0.25
Methylenebis(salicylate)	0.13
Napadisylate ^c	0.13
Oxalate	0.25
Pectinate	0.13
Persulfate	0.13
Phenylethylbarbiturate	0.13
Picrate	0.13
Propionate	0.13
Thiocyanate	0.13
Tosylate	0.13
Undecanoate	0.13
Cation	Percent ^a
Organic:	
Benethamine ^c	0.33
Clemizole ^d	0.33
Diethylamine	0.33
Piperazine	0.93
Tromethamine ^e	0.33
Metallic:	
Barium	0.33
Bismuth	0.93

^a Percent is based on total number of anionic and cationic salts in use through 1974. ^b 1,5-Naphthalenedisulfonate. ^c N-Benzylphenethylamine. ^d 1-*p*-Chlorobenzyl-2-pyrrolidin-1'-ylmethylbenzimidazole. ^e Tris(hydroxymethyl)aminomethane.

using pamoic acid resulted in the formation of a delayed-action preparation. Numerous studies using pamoate salts are dispersed throughout the literature (11–15).

Alginic acid also has been used to prepare long-acting pharmaceuticals. Streptomycin alginate was prepared (16) and shown to be effective in sustained-release preparations. A striking example of a long-acting alginate salt is that of pilocarpine. When dispersed in sterile water and dried to a solid gel, this compound was found useful in the preparation of long-acting ophthalmic dosage forms (17). While liquid preparations of the alginate and hydrochloride salts possess similar miotic activity, studies showed that solid pilocarpine alginate flakes constricted pupil size more effectively and increased the duration of miosis significantly when compared with the liquid preparations. Solid dose pilocarpine may be more uniformly available, because it diffuses more slowly through the gel matrix which holds the drug in reserve. In contrast, drops of the commonly employed solution dosage form release the dose immediately to the conjunctival fluid.

Málek *et al.* (18) devised a unique way of prolonging action through salt formation; they showed that the distribution of several antibiotics could be markedly altered by merely preparing macromolecular salts. Since macromolecules and colloidal particles have an affinity for the lymphatic system, streptomycin, neomycin, viomycin, and

streptothricin were combined with high molecular weight compounds such as polyacrylic acids, sulfonic or phosphorylated polysaccharides, and polyuronic derivatives. Parenteral administration of these compounds produced low blood levels of the antibiotic for long periods, while lymph levels were high. (In comparison, streptomycin sulfate gave high blood levels but low lymph levels.) This alteration in distribution caused the streptomycin to prolong its passage through the body, since lymphatic circulation is quite slow.

The appropriate choice of a salt form has been found to reduce toxicity. It can be rationalized that any compound associated with the normal metabolism of food and drink must be essentially nontoxic. The approach of choosing organic radicals that are readily excreted or metabolized opened up a new class of substances from which to select a salt form. For example, certain salts of the strong base choline have proven to be considerably less toxic than their parent compound. The preparation and properties of choline salts of a series of theophylline derivatives were reported (19), and it was shown that choline theophyllinate possessed a greater LD₅₀ than theophylline or its other salts (20). It was postulated that this agent would be less irritating to the GI tract than aminophylline, because "its basic constituent, choline, is an almost completely nontoxic substance of actual importance to the physiologic economy." This evidence led to the preparation of choline salicylate (21) as an attempt to reduce the GI disturbances associated with salicylate administration. Clinical studies indicated that choline salicylate elicited a lower incidence of GI distress, was tolerated in higher doses, and was of greater benefit to the patient than was acetylsalicylic acid (aspirin).

Amino acids and acid vitamins also have been used as salt-forming agents. Based on the evidence that coadministration of amino acids with aminoglycoside antibiotics reduced their toxicity, a series of amino acid salts of dihydrostreptomycin was prepared (22). In all but one case, the acute toxicities of these salts were lower than the toxicity of the sulfate. The ascorbate and pantothenate also were synthesized and shown to be less toxic than the sulfate. Of the salts prepared, the ascorbate had the highest LD₅₀.

The vitamins most commonly used for forming salts exhibiting reduced toxicity are ascorbic and pantothenic acids. Keller *et al.* (23) were the first to use pantothenic acid as a means of "detoxifying" the basic streptomycin antibiotics. Parenteral administration of the pantothenates of streptomycin and dihydrostreptomycin had a significantly reduced incidence of acute neurotoxicity in cats as compared with the sulfates. Subsequent studies (24–28) supported this finding and showed that the pantothenates of neomycin and viomycin also are less toxic. The ascorbate of oleandomycin was synthesized and its pharmacological properties were reported (29). Upon intramuscular injection in rats, it produced less irritation than the phosphate.

p-Acetamidobenzoic acid, an innocuous metabolite of folic acid present in normal blood and urine, has been used in preparing salts. In particular, it yields stable salts with amines that otherwise tend to form hygroscopic products with conventional acid components (30).

Often the salt form is chosen by determining a salt

135

component that will pharmacologically antagonize an unfavorable property or properties exhibited by the basic agent. Salts of *N*-cyclohexylsulfamic acid are an example of the practical application of this approach. *N*-Cyclohexylsulfamic acid salts, better known as cyclamates, have a characteristic sweet, pleasing taste. Although presently under investigation by the FDA for potentially carcinogenic properties, salts incorporating this compound can render unpleasant or bitter-tasting drugs acceptable. For example, the cyclamates of dextromethorphan and chlorpheniramine exhibit greatly improved bitterness thresholds compared to commonly occurring salts (31). Furthermore, their stability in aqueous solution was described as good when maintained at a pH not greater than 4.

N-Cyclohexylsulfamic acid salts of thiamine hydrochloride and lincomycin also have been synthesized. Thiamine *N*-cyclohexylsulfamate hydrochloride was reported to have a more pleasant taste than other thiamine salts while having an equal or greater stability (32). Lincomycin cyclamate, shown to possess an enhanced thermal stability over its hydrochloride, was prepared (33) to test the hypothesis that reduced lincomycin absorption in the presence of small quantities of cyclamates was due to a simple metathetic reaction. However, this assumption was found not to be true. An extensive study of the preparation and characterization of cyclamic acid salts of several widely used classes of drugs including antihistamines, antibiotics, antitussives, myospasmolytics, and local anesthetics was reported (34, 35).

Various salts of penicillin and basic amine compounds have been formulated in an effort to produce a long-acting, nonallergenic form of penicillin. Since antihistamines appear to mitigate the symptomatology of penicillin reactions in some patients, coadministration of the two has been advocated. The preparation of the benzhydralamine salt of penicillin was an attempt to produce a repository form of penicillin with antiallergic properties (36). Blood levels achieved with this salt were comparable to those of penicillin G potassium; however, its antiallergic properties were not evaluated. In fact, the investigators noted that antihistamines can actually cause sensitization at times and stated that "despite their occasionally favorable influence on the symptoms of penicillin sensitivity, they contribute directly to the potential of drug sensitivity when co-administered with penicillin."

Silver salts of sulfanilamide, penicillin, and other antibiotics have been prepared and represent cases where the species (ions) are complementary. When aqueous solutions of the salts were applied topically to burned tissue, they yielded the combined benefits of the oligodynamic action of silver and the advantages of the antibacterial agents (37).

The use of 8-substituted xanthines, particularly the 8-substituted theophyllines, as salt-forming agents was first reported in the preparation of a series of antihistamine salts (38-41). Synthesis of these xanthine salts was an attempt to find a drug to counteract the drowsiness caused by the antihistamines with the stimulant properties of the xanthines. When an electronegative group is introduced into the xanthine molecule at the 8-position, the electron-drawing capacity of the substituent results in the creation of an acidic hydrogen at position 7. Thus, these

moderately strong acidic compounds can undergo salt formation with various organic bases.

The 8-halotheophyllines were the first group of xanthines studied as potential salt-forming agents. Since the report on the preparation of the 8-chlorotheophylline salt of diphenhydramine (42), synthesis of the 8-halotheophyllinates of a number of organic bases has been attempted. The 8-chlorotheophylline salts of quinine, ephedrine, and strychnine were prepared and characterized (43). These salts were less water soluble than the corresponding free alkaloidal bases. In a similar report, the 8-chlorotheophyllinates of three synthetic narcotics, meperidine, levorphanol, and metopon, were prepared (44).

Pharmacological and clinical studies involving the 8-bromotheophylline pyrilamine salt revealed the unusual diuretic properties associated with the 8-halotheophylline portion of the compound (45, 46). This finding initiated an investigation into the preparation of a soluble 8-bromotheophylline salt of high diuretic activity. With readily available amines, over 30 salts were synthesized and screened for diuretic activity (47). When tested against theophylline salts of the same amines, the 8-bromotheophyllinates showed greater activity in every case.

With the successful formation of 8-halotheophyllinates of organic bases, Morozowich and Bope (48) proposed that, if the halogen moiety was replaced with a more electro-negative substituent such as a nitro group, a more acidic compound would be formed. Presumably, more stable salts would result and precipitation of the free xanthine derivative in the stomach would be less likely to occur. On this premise, they successfully prepared pharmacologically effective 8-nitrotheophyllinates of several pharmaceutically useful bases.

Duesel *et al.* (19), in their study of choline theophyllinate, prepared the 8-chloro-, 8-bromo-, and 8-nitrotheophylline salts of choline. Oral toxicity studies in mice showed that the LD₅₀ of the 8-nitrotheophyllinate was much greater than that of either 8-halotheophylline. In fact, it remained nonlethal at doses as high as 5 g.

Polygalacturonic acid, a derivative of pectin, has been used to prepare quinidine salts exhibiting reduced toxicity (49, 50). The compound possesses special demulcent properties and inhibits mucosal irritation. The rationale for use of this agent is to reduce the ionic shock to the GI mucosa resulting from the flood of irritating ions liberated by rapid dissociation of the conventional inorganic quinidine salts. Studies have shown that it is four times less toxic orally than the sulfate. This difference was attributed to the slower release of quinidine from the polygalacturonate.

Other compounds reported to be potentially useful as pharmaceutical salt forms are listed in Table III.

PHYSICOCHEMICAL STUDIES

Biological activity of a drug molecule is influenced by two factors: its chemical structure and effect at a specific site and its ability to reach—and then be removed from—the site of action. Thus, a knowledge of the physicochemical properties of a compound that influence its absorption, distribution, metabolism, and excretion is essential for a complete understanding of the onset and duration of ac-

136

Table III—Potentially Useful Salt Forms of Pharmaceutical Agents

Salt-Forming Agent	Compound Modified	Modification	Reference
Acetylaminoacetic acid	Doxycycline	Solubility	51
N-Acetyl-L-asparagine	Erythromycin	Solubility, activity, stability	52
N-Acetylcystine	Doxycycline	Combined effect useful in pneumonia	53
Adamantoic acid	Alkylbiguanides	Prolonged action	54
Adipic acid	Piperazine	Stability, toxicity, organoleptic properties	55
N-Alkylsulfamates	Ampicillin	Absorption (oral)	56
	Lincomycin	Solubility	57
	Cephalexin	Stability, absorption	58
Anthraquinone-1,5-disulfonic acid	Various alkaloids	Prolonged action	59, 60
Arabinogalactan sulfate (arabino)	Cephalosporins	Toxicity	61
Arginine	α -Sulfobenzylpenicillin	Stability, hygroscopicity, toxicity	62
	Erythromycin	Solubility	63
Aspartate	Tetracycline	Gastric absorption	64
Betaine	7-(Aminoalkyl)theophyllines	Activity, prolonged prophylactic effect	65
Bis(2-carboxychromon-5-yl-oxy)alkanes	Metformin	Toxicity	66
Carnitine	Propoxyphene	Organoleptic properties	67
4-Chloro- <i>m</i> -toluenesulfonic acid	Heptaminol	Prolonged action	68
Decanoate	Thiamine	Stability, hygroscopicity	69
Diacetyl sulfate	Ampicillin	Prolonged action	70, 71
Dibenzylethylenediamine	Cephalosporins	Reduced pain on injection	72
Diethylamine	Tetracycline	Activity	73
Diguanicyl phosphate	Vincamine	Organoleptic properties	74
Dioctyl sulfosuccinate	Kanamycin	Toxicity	75
Embonic (pamoic) acid	2-Phenyl-3-methylmorpholine	Toxicity	76
	Tetracycline	Solubility	77
Fructose 1,6-diphosphoric acid	Erythromycin	Solubility	77
	Tetracycline	Solubility	77
Glucose 1-phosphoric acid, glucose 6-phosphoric acid	Erythromycin	Solubility	52
L-Glutamine	Erythromycin	Solubility, activity, stability	78
Hydroxynaphthoate	Bephenium	Toxicity	79
2-(4-Imidazolyl)ethylamine	Prostaglandin	Prolonged action	80
Isobutanolamine	Theophylline	Stability	81
Lauryl sulfate	Vincamine	Organoleptic properties	82
Lysine	α -Sulfobenzylpenicillin	Toxicity, stability, hygroscopicity	83
	Cephalosporins	Solubility	84
Methanesulfonic acid	Pralidoxime (2-PAM)	Toxicity, stability, hygroscopicity	85
N-Methylglucamine	α -Sulfobenzylpenicillin	Reduced pain on injection	86
	Cephalosporins	Toxicity, faster onset of action	87
N-Methylpiperazine	Phenylbutazone	Reduced pain on injection	88
Morpholine	Cephalosporins	Organoleptic properties	89
2-Naphthalenesulfonic acid	Propoxyphene	Prolonged action	90
Octanoate	Heptaminol	Organoleptic properties	91
Probenecid	Pivampicillin	Prolonged action	92
Tannic acid	Various amines	Activity	93
Theobromine acetic acid	Propoxyphene	Organoleptic properties	94
3,4,5-Trimethoxybenzoate	Tetracycline	Prolonged action	95
	Heptaminol	Absorption (oral)	96
Tromethamine	Aspirin	Physical state	97
	Dinoprost (prostaglandin F ₂)		

tion, the relative toxicity, and the possible routes of administration (2).

In a review in 1960, Miller and Holland (92) stated that "different salts of the same drug rarely differ pharmacologically; the differences are usually based on the physical properties." In a subsequent review (93), Wagner expanded upon this statement, asserting that, although the nature of the biological responses elicited by a series of salts of the same parent compound may not differ appreciably, the intensities of response may differ markedly.

The salt form is known to influence a number of physicochemical properties of the parent compound including dissolution rate, solubility, stability, and hygroscopicity. These properties, in turn, affect the availability and formulation characteristics of the drug. Consequently, the pharmaceutical industry has systematically engaged in extensive preformulation studies of the physicochemical properties of each new drug entity to determine the most suitable form for drug formulation. Published information concerning such studies, however, is sparse. Preformulation studies have been outlined, and the influence of the salt form on the volatility and hygroscopicity of an agent

In one such study, methylpyridinium-2-aldoxime (pralidoxime) salts were investigated (95). This study set out to prepare a salt with water solubility adequate to allow intramuscular injection of a low volume (2-3 ml) therapeutic dose. The original compound, the methiodide, had the disadvantages of limited aqueous solubility and high potential toxicity, since its high iodide content could result in iodism. On the basis of physiological compatibility, better water solubility, favorable stability, and relatively high percentage of oxime, the chloride salt of pralidoxime was selected for therapeutic administration; it was claimed that "the anion used to form the salt can confer physical properties of importance and significance for the formulation and administration of the compound" (95).

Some physicochemical properties of a series of mineral acid salts of lidocaine also were determined (96). While the hydrochloride and hydrobromide were more hygroscopic, they were more soluble in a number of solvents than the nitrate, perchlorate, phosphate, or sulfate salts.

Dissolution Rate—The dissolution rate of a pharmaceutical agent is of major importance to the formulator. In many cases, particularly with poorly soluble drugs, this characteristic best reflects the bioavailability of the com-

137

pound. As a rule, a pharmaceutical salt exhibits a higher dissolution rate than the corresponding conjugate acid or base at an equal pH, even though they may have the same equilibrium solubility. The explanation for this result lies in the processes that control dissolution.

Dissolution can be described by a diffusion layer model¹ in terms of an equation developed by Nernst and Brunner (97):

$$\frac{dW}{dt} = \frac{DS}{h} (C_s - C) \quad (\text{Eq. 1})$$

where W is the mass of the solute dissolved at time t , dW/dt is the rate of mass transfer per unit time, D is the solute molecule diffusion coefficient, S is the surface area of the dissolving solid, h is the diffusion layer thickness, C is the concentration of the drug in the bulk solution at time t , and C_s is the saturation solubility of the solute in the diffusion layer.

The driving force for dissolution in Eq. 1 is the difference between the saturation solubility of the drug and the concentration of the drug in the bulk fluid. If the drug is not rapidly absorbed after it dissolves, then C , the concentration in the bulk solution, approaches C_s and the dissolution rate is retarded. When this occurs, absorption is "absorption rate" limited (or "membrane transport" limited). If the absorption rate is rapid (or if the absorption mass transfer coefficient is much larger than DS/h of Eq. 1), however, C becomes negligible compared to C_s and dissolution occurs under "sink" conditions. Absorption is then said to be dissolution rate limited, which is what occurs with most poorly soluble drugs. In either case, an increase in C_s , as in salt formation, increases dissolution.

Salts often speed dissolution by effectively acting as their own buffers to alter the pH of the diffusion layer, thus increasing the solubility of the parent compound, C_s , in that layer over its inherent solubility at the pH of the dissolution medium. Hence, dissolution is controlled by solubility in the diffusion layer which, in turn, is determined by the pH of that layer. The influence of K_{sp} on the solubility term, C_s , and dissolution rate, should an accumulation of ions be allowed to occur, will be treated later.

Nelson (98), in a study of theophylline salts, was the first to show the correlation between diffusion layer pH and dissolution rate. The major impact that this study had on the pharmaceutical sciences was its conclusion that, if other factors remained constant, the dissolution rate of a compound determined the rate of buildup of blood levels with time and the maximum levels obtained. Those salts of the acidic theophylline with high diffusion layer pH's had greater *in vitro* dissolution rates than those exhibiting a lower diffusion layer pH. And, indeed, the rank order of dissolution rates correlated well with clinically determined blood levels. Presumably, the higher pH in the diffusion layer retards hydrolysis of the salt, thereby maintaining the anionic charge of the theophyllinate ion. This report led to many additional studies which illustrate the influence of the salt form on dissolution and the beneficial effects of changing nonionized drugs into salts.

Juncher and Raaschou (99) demonstrated that the rank order of peak blood levels of penicillin V, obtained upon

administration of three different salts and the free acid, was the same as the rank order of their rates of dissolution *in vitro*. While the investigators ascribed these differences to the solubility properties of the salts, their experiments actually compared dissolution rates, not solubilities. The relative order of dissolution rates and mean maximal blood levels was: potassium salt > calcium salt > free acid > benzathine salt.

Nelson (100) determined dissolution rates for several weak acids and their sodium salts in media whose pH's represented GI fluids. In all cases, the sodium salt dissolved more rapidly than the free acid. This finding resolved the misconception that absorption of drugs is related only to solubility in the appropriate medium; rather, solubility affects absorption only to the extent that it affects dissolution rate. Absorption of drugs is a dynamic process, and the ultimate solubility of a drug in fluid at absorption sites is of limited consequence since absorption prevents the attainment of saturated solutions. Therefore, dissolution rate, more than solubility, influences absorption since it is a preceding process.

In two subsequent studies, Nelson and coworkers further illustrated the effects of changing nonionized drugs into salts. A report concerning tolbutamide (101), a weak acid, showed that the initial dissolution rate of tolbutamide sodium was approximately 5000 times more rapid than the free acid in acidic media and 300 times more rapid in neutral media. This difference, measured *in vitro*, reflected the differences observed between the free acid and the salt when administered to human subjects. Oral administration of tolbutamide sodium produced an immediate drop in blood sugar comparable to that produced by intravenous injection of the salt, while the slowly dissolving tolbutamide produced a smooth, sustained fall in blood sugar (102).

Correlation of urinary excretion rates and dissolution rates of tetracycline and some of its acid salts also was demonstrated by Nelson (103). The salts that exhibited greater *in vitro* dissolution rates showed greater urinary excretion rates, indicating more rapid absorption.

Benet (104), in a discussion of the biopharmaceutical basis for drug design, referred to the influence of the salt form on dissolution. He compared the dissolution rates of tetracycline and tolbutamide and their salts, as reported in the studies previously cited, and explained why the rates differ at the pH's exhibited by physiological fluids.

Although salt formation usually increases the dissolution rate of a drug, studies with aluminum acetylsalicylate (105, 106), warfarin sodium (107), and benzphetamine pamoate (108) showed that administration of the salt slowed dissolution of the drug and subsequent absorption compared to the nonionized form. This decrease appeared to result from precipitation of an insoluble particle or film on the surface of the tablet. Such a phenomenon decreases the effective surface area and prevents deaggregation of the particles. Theoretical considerations of the processes controlling dissolution of an acid salt of a base (108) and the sodium salt of a weak acid (109, 110) in reactive media have been discussed.

Tablet processing and various formulation factors can decrease the dissolution rate of a salt in human gastric juice over its nonionized form (111). Granulation and tableting slowed the dissolution rate of phenobarbital sodium to

¹The authors recognize the existence of other models; this one was chosen simply

138

decrease but had the opposite effect on phenobarbital. Therefore, as a tablet dosage form, the dissolution rate of the sodium salt was slower than that of the free acid. These results were attributed to differences in the disintegrating properties of the tablets; in some instances, rapid dissolution may in fact be a problem for very soluble drugs.

Others have illustrated a phenomenon that decreases the dissolution rate of a salt below that of its nonionized form. Lin *et al.* (112) studied the relationship between salts and biological activity by dissolving three salts and the free base of an experimental antihypertensive in water, 0.1 N HCl, and pH 7.2 phosphate buffer. The dissolution rate of the monohydrochloride salt was lower than that of the free base in 0.1 N HCl and higher than the free base in both water and phosphate buffer. These authors ascribed this variation to the common ion effect and substantiated it experimentally. Although the biological activity of the monohydrochloride was greater than that of the free base, the implications of altered absorption characteristics on the activity of any other hydrochloride salt in GI fluids must be considered. Similar results also were reported for hydrochloride salts of some tetracyclines (113).

Some consideration must be given to the influence of salt formation on oral toxicity, which often reflects the relationship between the *in vivo* dissolution rate and the appearance of drug in the circulation (114, 115). Morozowich *et al.* (114) showed that the relative toxicities of a series of salts of a drug reflect the rate of absorption, providing the salt-forming agents themselves are relatively nontoxic. They stated that "when absorption is rate-limited by dissolution of the salt in the gastrointestinal tract, as will be the case with slowly soluble salts, the toxicity of a slowly dissolving salt will most probably be lower than that of a more rapidly dissolving salt." The implications of salt formation on toxicology will be discussed under *Toxicological Considerations*.

Several reviews dealt with the influence of the dissolution rate on drug availability and, in particular, salt effects (116, 117). Other reports illustrating the influence of salts and salt form on dissolution rate are listed in Table IV.

Solubility—Knowledge of the solubility characteristics of a pharmaceutical agent is essential, because solubility is usually an important factor in the pharmacokinetic profile, the chemical stability, and, ultimately, the formulation of the drug. As discussed previously, it is certainly a primary factor in controlling dissolution rates. The solubility of a compound depends basically upon the physical and chemical properties of the solute; *e.g.*, a lower melting point for a compound within a series reflects a decreased lattice energy, which would suggest a higher solubility. Solubility depends as well upon such elements as temperature, pressure, solvent properties (such as resulting pH), and, to a lesser extent, the state of subdivision of the solute.

An important solvent property which is often overlooked involves the common ion effect; in particular, hydrochloride salts of drugs often exhibit less than desirable solubility in gastric juice because of the abundance of chloride ions. The equilibrium involved is shown in Scheme I.

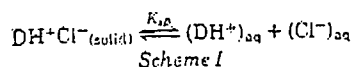


Table IV—Additional References on Salt Form and Dissolution Rate

Topic	Reference
Dissolution rate of mixtures of weak acids and tribasic sodium phosphate	118
Physiological availability and <i>in vitro</i> dissolution characteristics of some solid dosage formulations of aminosalicylic acid and its salts	119
Biopharmaceutics, rate of dissolution: chronological bibliography	120
Biopharmaceutics: rate of dissolution <i>in vitro</i> and <i>in vivo</i>	121
Dissolution tests and interpretation of anomalies observed in the dissolution process of sulfaquinoxaline based on salt formation	122
Influence of the dissolution rate of lithium tablets on side effects	123
Dissolution kinetics of drugs in human gastric juice	124
Comparison of dissolution and absorption rates of different commercial aspirin tablets	125
<i>In vitro</i> dissolution rates of aminorex dosage forms and their correlation with <i>in vitro</i> availability	126

considered as a means of increasing a compound's water solubility. As with dissolution rates, however, salt formation does not always confer greater solubility. Liberally dispersed throughout the pharmaceutical literature are studies that compare the solubilities of different salt forms of the same compound with those of its free acid or base (Table IV). Selection of the salt form exhibiting the desired solubility properties is critical, since these properties often dictate the formulation characteristics of the drug.

Phase solubility techniques were used to study the formation of complex salts of triamterene (127). The results indicated that the organic acid salts of basic drugs, such as amines, were more soluble in water than the corresponding inorganic (halide) salts. This consideration is important in the synthesis and selection of a salt form that will exhibit enhanced bioavailability and desirable formulation characteristics.

The hydrogen-ion concentration can significantly affect the solubility of a salt. Anderson (128) discussed the influence of pH on the solubility of pharmaceuticals. Mathematical relationships between pH and solubility of therapeutically useful weak acids and bases and their salts were given along with some considerations in the formulation of solutions of these particular agents.

An extensive study on the solubility interrelationships of the hydrochloride and free base of two pharmaceutically useful amines was reported (129). Mathematical equations describing the total solubility at an arbitrary pH in terms of the independent solubilities of the hydrochloride and free base species and the dissociation constant of the salt were derived and fitted to experimental data with good results. This report elucidated the point that, while the solubility of the amine hydrochloride generally sets the maximum obtainable concentration for a given amine, the solubility of the free base and the pKa determine the maximum pH at which formulation as a solution is possible (assuming that the desired concentration exceeds the free base solubility). Shifting the pH-solubility profile to higher pH values for formulation purposes may require increasing the solubility of the free base. This increase might be accomplished by using an appropriate cosolvent. Since the dissociation characteristics of carboxylic acids and other acidic organic species are similar to those of organic hydrochlorides, it is expected that the pH-solubility profiles

of these organic acids, although reversed, can be characterized theoretically using the same treatment.

Several reports showed that the structure of an organic salt-forming radical influences the solubility of the resulting salt. The water solubilities of 16 salts (carboxylates, sulfates, sulfamates, and phosphates) of the weak base erythromycin were dependent on the size of the alkyl group of the acid (130). In a study with *N*-alkylsulfamates of lincomycin (66), a similar phenomenon was observed: solubility of these salts decreased as the size of the alkyl group attached to the acidic function increased.

Senior (131), in a study on the formulation and properties of the antibacterial chlorhexidine, determined the water solubilities of 35 salts and the free base. He found that inorganic salts had remarkably low solubilities while those of the lower aliphatic acids proved to be somewhat more soluble. Hydroxylation of the acid increased solubility, since salt formation with polyhydroxy acids, particularly the sugar acids, conferred extensive water solubility to the molecule.

Several investigators reported the influence of the solubility of a drug on its formulation and subsequent availability from the dosage form. In a discussion of the preparation and formulation of epinephrine salts in an aerosol system using liquefied gas propellants, Sciarra *et al.* (132) pointed out that the solubility characteristics of the agent are important in two respects. First, the solubility of the therapeutically active ingredient in the various propellants is an important consideration if the product is to be used for either local action in the lungs or systemic therapy. Second, the solubility of the drug in extracellular fluids plays an important role in selection of the compound. The bitartrate, malate, maleate, and fumarate salts of epinephrine were prepared and subjected to solubility and stability studies. While all salts had similar partition coefficients, the solubility of the maleate in several propellants and its stability in formulated aerosols made it the drug form of choice.

Ephedrine hydrochloride was more rapidly released than the free base from theobroma oil suppositories containing different surfactants (133). This enhanced rate of release (dialysis) was ascribed primarily to the greater aqueous solubility of the hydrochloride, which solubilized faster from the oil-in-water emulsion, whereas the ephedrine alkaloid base tended to remain behind in the oil phase.

The solubility of the active ingredient in ointment bases can dramatically influence its diffusion properties. A study of salicylic acid and its sodium salt showed that the diffusion of both was very low from hydrophobic bases, whereas the solubility of the drug significantly affected the diffusion from hydrophilic bases. The more soluble sodium salicylate diffused much faster from these latter bases than did salicylic acid (134).

Additional references on the relationship of salt form and solubility are listed in Table V.

Organoleptic Properties—Modern medicine requires that a pharmaceutical formulation be efficacious, safe, stable, and acceptable to the patient. Of primary importance in the development of oral dosage forms is taste acceptability. This factor presents no major problems when the drug is to be administered as a tablet or a capsule and swallowed as a unit but is clearly a prominent factor in

Table V—Additional References on Salt Form and Solubility

Topic	Reference
Influence of solubility on the rate of GI absorption of aspirin	135
Effect of dosage form upon the GI absorption rate of salicylates	136
Physical-chemical properties of polyene macrolide esters and their water-soluble salts	137
Isolation and reaction products of orotic acid and amines and their solubility in water	138
Solubility and stability of erythromycin salts	139
Studies on pharmaceutical preparations of orotic acid: water-soluble properties of orotic acid salts	140
Solubility of antibiotics in 24 solvents	141, 142
Solubility of antibiotics in 26 solvents	143

patient acceptability when it is to be administered as a liquid, chewable tablet, or lozenge.

Since taste is a chemical sense, a substance must be dissolved if it is to elicit a taste sensation—either by taking it as a solution or by its dissolving in the saliva. Therefore, one method used to minimize undesirable organoleptic properties of pharmaceuticals involves the preparation of a poorly soluble salt form of the drug such that the saturation concentration is less than the taste threshold.

Erythromycin estolate (lauryl sulfate) has approximately one-twelfth the solubility of the free base, is tasteless, and is useful in the formulation of oral suspensions (144). A study on erythromycin salts showed that the bitterness level was dependent on two properties: (a) the water solubility of the salt, which is dependent on the size of the alkyl group attached to the acid function; and (b) the strength of the acid used to form the salt, i.e., the stability of the salt (130). The stearyl sulfamate salt possessed the most desirable organoleptic properties.

Many problems concerned with formulation and stability of topical and oral pharmaceuticals containing bacitracin have been overcome by incorporating bacitracin into the formulation as its zinc salt. One distinct advantage over the parent compound is its lack of taste, caused by its relative insolubility. Thus, it is the preferred drug form for preparations where taste is a factor (145). Taste panel evaluations of the comparative bitterness of bacitracin zinc and bacitracin indicate that the taste of the zinc salt is more easily masked and that the presence of a bitterness-masking adjuvant, such as sucrose, increases the bitterness threshold ratio differences between the two compounds even further (146).

Propoxyphene napsylate, nearly water insoluble, is only slightly bitter to the taste as compared to the highly water-soluble hydrochloride (147) and can be readily formulated into a flavored aqueous suspension. The taste of these suspensions can be improved significantly by the addition of a common ion (sodium or calcium napsylate) to depress solubility further.

A newer approach to the improvement of drug palatability has been to form insoluble salts with ion-exchange resins. Several investigators described and tested the practical application of this method (148–150). Spross *et al.* (149) outlined the conditions necessary for improving the palatability of a drug by adsorbing it onto an ion-exchange resin without appreciably modifying its pharmacological effects. They found that: (a) the degree of drug release from the ion-exchange adsorbate depends on the

equivalent quotient between the electrolytes in the surrounding fluid and the ionic drugs, (b) the amount of ions is far less in the saliva than in the gastric juice (the temporary electrolyte contents can be estimated at 0.05 mEq in the saliva and at 10 mEq in the gastric juice), and (c) the exchange rates should allow the equilibria to be attained within a fairly short period. Insoluble drug resins formed between dextran gel² cation exchangers and several basic drugs were in many cases much more pleasant tasting than their parent compounds. Furthermore, release of the drug from the ion-exchange adsorbate was quite rapid and complete under conditions prevailing in the GI tract.

Similar findings were reported using a polymethacrylic acid ion-exchange resin (150). In addition, coating the adsorbate particles with a 4:1 ethylcellulose-hydroxypropyl methylcellulose mixture further reduced bitterness. While *in vitro* release from the uncoated resinate was rapid and complete, release from coated adsorbates varied with the extent of coating.

Another approach to improving the taste properties of pharmaceutical agents is to prepare a pleasant-tasting soluble salt of a poor-tasting parent drug. This approach often can be very difficult, however, since solubilization of the parent compound usually imparts its unpleasant taste to the preparation. Nevertheless, some success has been reported using the artificial sweeteners cyclamate sodium and saccharin.

As described earlier, formation of *N*-cyclohexylsulfamate salts of several drug substances has produced better tasting derivatives with enhanced solubility properties (31, 32). The physicochemical and toxicological properties of benzalkonium saccharinate and a series of saccharinates of other quaternary ammonium compounds were reported (151). While conventional quaternary ammonium compounds have a very bitter taste, their saccharin analogs are sweet.

Potassium salts frequently possess an unpleasant taste and a metallic aftertaste. The palatability of some potassium salts in flavored vehicles was reported (152); while the salts had similar taste thresholds at effective therapeutic levels, all potassium salts exhibited inferior palatability.

Table III includes several samples of other salts that exhibit an improved taste relative to their free acid or base forms.

Stability—The chemical and physical stability of a pharmaceutical must be known, because it can influence the choice of dosage form, the manufacturing and packaging, and the therapeutic efficacy of the final preparation. Systematic determination of the thermal stability, solution stability (at various pH's), and light sensitivity of a drug and its derivatives, both alone and in the presence of common additives, provides essential input toward selecting the most suitable derivative and dosage form.

Depending on the route of degradation, different salt forms impart different stability characteristics to the parent drug by various mechanisms. Most commonly used are sparingly soluble salts which, when used in the formulation of suspensions, reduce the amount of drug in solution and, hence, its degradation. Differences in hygroscopicity of several salts influence the stability of the

drug in the dry state. In some cases, the salt-forming radical itself enhances the stability of the parent agent.

The stability of penicillin G and its salts has been widely studied due to the drug's therapeutic importance and its characteristic instability. Schwartz and Buckwalter (153) described some of the stability characteristics of this antibiotic, stating that, with present techniques, a solution of penicillin cannot be made stable for more than 2 weeks, even at refrigerator temperatures. They also discussed the use of suspensions of sparingly soluble amine salts in aqueous vehicles as a means of "allowing marketing of a 'ready-made' penicillin product." Procaine, benzathine, and hydrabamine salts are marketed, and their acceptable stability as aqueous suspensions is based mainly on their insolubility and the minimization of degradation in solution.

A theoretical treatment of the solubility of these salts was presented in which equations were derived for calculating the solubility as a function of pH and the pH of minimum solubility (154, 155). These equations are based on the mass action law and its relationship to the ionization constants of the amine and the penicillin and the solubility of the salt in water. Since the salt in solution is partially dissociated, further suppression of the solubility may be achieved by the common ion effect. Swintosky *et al.* (156) demonstrated this effect with penicillin G procaine by adding procaine hydrochloride to the preparation and further enhancing its stability. The 8-chlorotheophylline salt (or complex) of penicillin G was reported to be water soluble, yet stable in solution (157). Since 8-chlorotheophylline is acidic, it has been postulated that a buffer effect could account for the stabilization of this "salt."

While penicillin G procaine is more stable in aqueous vehicles, it is less thermally stable than the sodium or potassium salts, decomposing if heated much above 60°. The sodium and potassium salts are known to withstand heating up to 100° for 4 days with little loss in potency (158). This behavior might well be due to differences in melting points—*viz.*, 106° for penicillin G procaine and ~215° dec. for the potassium salt.

Since hydrolysis of penicillin is dependent on moisture content, preparations in which moisture is rigorously excluded are quite stable in the dry state. A study on the effect of moisture on penicillin salts found the calcium salt to be less hygroscopic than the sodium salt and, hence, more stable in moist atmospheres (159). Similarly, penicillin G potassium is also much less hygroscopic than penicillin G sodium and has become the preferred form for marketing in the dry state (160).

Several studies reported the relative stabilities of thiamine salts, particularly the hydrochloride and the mononitrate (161–163). The mononitrate is observed to be less hygroscopic and is accordingly much less water soluble than the hydrochloride. Investigations of various preparations including compressed tablets, multivitamin capsules, and dry-filled vitamin B complex capsules at various temperatures showed that the mononitrate was more stable than the hydrochloride (164, 165). The stabilities of numerous thiamine salts were studied in aqueous solution and in dry powder preparations with various excipients (166, 167). In aqueous solution, the resulting pH was the chief factor controlling hydrolysis and oxidative decomposition of thiamine salts; their stability as powder

² Sephadex.

141

preparations was related to their aqueous solubility, with the sparingly soluble salts being more stable (and presumably less hygroscopic).

An orally administered drug must be stable in acidic solution because it generally must pass intact through the acidic environment of the stomach if it is to exhibit therapeutic blood levels. The advantage of erythromycin esolate over the free base lies in its low solubility in gastric juice, which enables it to be administered with food without any decrease in attained blood levels. The salt is more stable in the stomach because it remains undissolved. Therefore, it retains its potency even when exposed to acidic environments for extended periods (144).

In a study on the preparation and characterization of lincomycin cyclamate (33), it was noted that the cyclamate salt had an enhanced thermal stability over the hydrochloride. In a subsequent report (168), differential thermal analysis and thermogravimetric analysis showed that while the hydrochloride easily undergoes thermal degradation, the cyclamate anion confers a considerably greater thermal stability on the lincomycin moiety.

Mullins and Macek (169) showed that the physical and chemical stability of the calcium salt of novobiocin makes it the form of choice for the formulation of a liquid preparation of the antibiotic. The amorphous calcium novobiocin salt proved to be tasteless, yet fully biologically active and perfectly stable in aqueous suspension. Neither the sodium salt nor the free acid is suitable; the sodium salt cannot be formulated in a liquid due to its chemical instability, while the crystalline free acid is not absorbed from the GI tract. Amorphous novobiocin is absorbed but is metastable in solution and slowly converts to the unabsorbed crystalline form.

Other reports of alterations in stability characteristics due to salt formation are listed in Table VI.

Miscellaneous Properties—The salt form has been reported to influence other physicochemical properties of a drug substance. Studies illustrating the effect of the salt-forming radical on surface tension, deaggregation behavior, and ion-pair extraction have appeared.

The influence of the anion on the absorption processes of two charged species, dextromethorphan and tetracycline, was studied in the rat stomach (186, 187). A linear relationship existed between the rate of absorption from buffer solutions of the anions under investigation and their surface tensions. Thus, the absorption process was related to the surface activity of the various salts and not to their lipid solubilities. This change of surface activity with the buffer (or salt) species is similar to the results reported in a study of the surface activity of various phenothiazine salts (188).

The antibacterial chlorhexidine possesses surface activity. A study of the colloidal properties of some chlorhexidine salts showed that the counterion can affect the critical micelle concentration of a surface-active agent, and this effect was usually associated with a change in micellar size (189).

The deaggregation behavior of a relatively insoluble acid and its sodium salt was studied, and deaggregation was postulated to be a possible rate-limiting step in the absorption of a drug from a dosage form (190). While no direct comparisons of the two forms were made, inspection of the data shows that the deaggregation rate of the salt

Table VI—Additional References on Salt Form and Stability

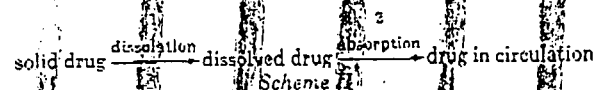
Topic	Reference
Stability of chlorhexidine solutions	170
Stability of chlorhexidine when autoclaved	171
Anhydrotetracycline and 4-epianhydrotetracycline in marketed tetracycline and aged tetracycline products	172
Solid-state stability of some crystalline vitamin A compounds	173
Physicochemical studies on the stability of penicillin salts	174
Light sensitivity of tetracyclines	175
Hygroscopic properties, thermostability, and solubility of oleandomycin salts	176
Stability of orotic acid and its amine salts in aqueous solution	177
Some factors influencing the stability of tablets (aspirin)	178
Stability of aqueous solutions of sodium aminosalicylate	179
Hygroscopic properties of various preparations of erythromycin	180
Physicochemical studies on the decomposition of aminosalicylic acid and its salts	181
Stabilities of aqueous solutions of 2-diethylaminoethyl-3-methyl-2-phenylvalerate hydrochloride and its methobromide	182
Investigation of some properties of penicillin G salts	183
Stability of ferrous iron tablets on storage	184
Stability of aspirin aluminum compounded with antacids	185

was considerably more rapid than that of the free acid in equivalent dosage forms. Therefore, if absorption is dependent on the dissolution rate, which in turn is dependent on the deaggregation rate, the salt should produce the highest and earliest blood levels. On the other hand, it is possible that hygroscopic (and deliquescent) salts can absorb atmospheric moisture, cause a sticky surface, and inhibit deaggregation.

Higuchi and coworkers presented an extensive study on the physicochemical basis of the ion-pair extraction of pharmaceutical amines. Distribution ratios of dextromethorphan (191) and chlorpheniramine (192) between an organic layer and water were highly dependent on the concentration and nature of the anion present. Less hydrophilic anions yielded more readily extractable ion-pairs. A study of the thermodynamic properties, enthalpy, free energy, and entropy, involved in the extraction equilibria of dextromethorphan ion-pairs indicated that the entropy change associated with transfer of the different anions between phases is the main controlling factor in the extraction process (193).

BIOAVAILABILITY

Most drugs prescribed in the United States are administered in solid and polyphasic dosage forms. Consequently, dissolution of the drug must precede the absorption process. The simplest model that adequately describes this process is shown in Scheme II.



Since the dissolution rate is generally slow for drugs with poor solubility, Step 1 is frequently rate limiting in the overall absorption process. As a result, the onset, intensity, duration of pharmacological activity, and, hence, bioavailability are affected by changes in dissolution rate. As discussed previously, administering a salt of the parent drug often proves to be an effective means of altering dissolution rate and absorption.

142

Table VII—Additional References on Bioavailability and Formulation Effects

Topic	Reference
Effects of various substances on the absorption of tetracycline in rats	197
Effects of dosage form upon the GI absorption rate of salicylates	136
Determination of <i>in vivo</i> and <i>in vitro</i> release of theophylline aminoisobutanol in a prolonged-action system	193
Ion-exchange resin salts for oral therapy: carbinoxamine	193
Latentiation of dihydrostreptomycin by pantoate formation	10
Solid-state ophthalmic dosage systems in effecting prolonged release of pilocarpine in the cul-de-sac	17
Absorption of erythromycin: various pharmaceutical forms	200
Comparative study of the absorption of drugs from old and new rectal preparations	201

Formulation Effects—Choice of the salt form of a drug may have a pronounced effect on the formulation of the parent compound. For example, Fenton and Warren (194) found there was no release of medicament from proflavine cream BPC, a water-in-oil emulsion containing 0.1% proflavine as the hemisulfate salt. They also investigated the release of various salts of proflavine with aliphatic carboxylic acids from water-in-oil cream emulsions. Salts formed with the water-soluble, oil-insoluble "lower" acids, such as formic and acetic acids, showed very poor release from a water-in-oil cream. The "higher" acid salts (e.g., *n*-valeric, caproic, cyclohexanecarboxylic, and caprylic) all showed increased diffusion from similar emulsions since these salts are soluble in both water and oil. Their release was even greater from oil-in-water emulsions, however, in agreement with their preferential oil solubility. The *n*-valerate salt provided the most effective water-in-oil cream. The primary factor responsible for diffusion of proflavine from a water-in-oil cream is the low hydrophilic-lipophilic balance conveyed to the salt by the acid. This finding illustrates the desirability of carefully selecting the salt anion of a cationic drug in lieu of the nature of the dosage form.

Studies of the effect of formulation on the bioavailability of warfarin sodium relative to warfarin yielded interesting results (107, 195). Absorption of warfarin upon administration of the sodium salt as a lactose-base tablet was no better than that from a similar formulation of the free acid. In fact, absorption was further depressed when the salt was formulated with starch instead of lactose. Later results indicated that the *in vitro* water dissolution rate of a warfarin sodium tablet was 350 times that of a slowly dissolving warfarin tablet formulation, yet the latter exhibited rapid and complete absorption *in vivo*. The virtual in-

calcium carbaspirin from several suppository bases was studied in dogs (196). The absorption of aspirin aluminum from either cocoa butter or a polyethylene glycol base was poor. While the maximum salicylate levels produced by aspirin and calcium carbaspirin from the cocoa butter base occurred at a later time than from the other bases studied, minimal plasma levels were exhibited by a polysorbate 61 base formulation. The highest peak and largest area under the blood level curve were seen with calcium carbaspirin in a vegetable fatty acid glyceride base. The poor absorption of aspirin aluminum from suppositories was not unexpected since it is poorly soluble. Furthermore, as pointed out for aspirin aluminum tablets (105, 106), an insoluble aluminum compound may form on the surface of the dissolving drug, further impeding its dissolution rate and bioavailability.

Additional references on bioavailability and formulation effects are given in Table VII.

Absorption Alteration—Several years ago, clinicians claimed that certain salts of theophylline were therapeutically preferable to other salts or to the free acid (202–204). For example, Schluger *et al.* (204) found higher blood theophylline levels after administering uncoated tablets of theophylline ethylenediamine than were observed with enteric-coated tablets of choline theophyllinate. These results were at variance with the *in vivo* work of Gagliani *et al.* (202), who found that the oral ingestion of choline theophyllinate produced significantly higher blood levels than the ethylenediamine salt. This apparent discrepancy could be explained by the formulation effects of tablet coating, etc., which was not discussed in the work of Gagliani *et al.* In another study, a slightly more rapid rise in blood concentration and a greater area under the curve were observed with theophylline isopropanolamine than with theophylline ethylenediamine (203). It was suggested that the difference was a result of the greater water solubility of the isopropanolamine salt.

The *in vitro* dissolution rates of the choline and isopropanolamine salts of theophylline have been observed to be three to five times greater than the ethylenediamine salt, depending on the dissolution medium (98). It has been suggested that these differences in dissolution rate are consistent with, and offer an explanation for, the clinical results.

In a comparative study of the absorption of ampicillin trihydrate and ampicillin potassium following oral administration (205), the potassium salt yielded 37% higher peak levels and a larger area under the curve. Only 36% of the administered ampicillin trihydrate was absorbed while 53% of the potassium salt was absorbed. Determining the percentage of each drug eliminated in the urine showed that 39% was eliminated following administration of the

143

multiple dosing (207). This finding is explained by the fact that this salt is acid stable and alkaline dissociable, permitting its passage through the acid of the stomach both in fasting and nonfasting subjects (209). Accordingly, the antibacterial activity remained essentially the same when this form of erythromycin was given, regardless of the state of fasting of the subject (209).

On first inspection, the higher serum levels attained with erythromycin estolate suggest that the salt form is more readily absorbed. Also critical to efficacy, however, is the volume of distribution of the drug, since extensive binding to plasma proteins can render a drug unavailable for activity at the biophase. Therefore, the significance of blood level data greatly depends on the measure of the free or unbound fraction of total antibiotics in the blood, which more directly indicates probable therapeutic benefit.

Wiegand and Chun (210) showed that, despite the higher blood levels attained with erythromycin estolate (after correction for half-life differences), the stearate salt produced seven times more free drug in serum than did the estolate salt. This finding explained the higher total tissue levels observed on administration of the stearate. They attributed the difference to a greater serum protein binding of the intact erythromycin estolate, proving that its higher serum levels did not necessarily reflect more efficient absorption.

Marked differences have been observed following oral administration of various salts of penicillin. Penicillin G potassium has been compared with penicillin G benzathine (benzethacil) (211, 212), penicillin G hydrabamine (213), penicillin V (214), penicillin G procaine (212), and penicillin G ammonium (215). As anticipated, penicillin G potassium produced higher and earlier blood levels than penicillin G benzathine (211, 212). Furthermore, tablets of penicillin G potassium buffered with sodium citrate yielded higher peak levels than unbuffered penicillin G potassium. While the absorption of buffered tablets was apparently not significantly affected by food intake, the unbuffered tablets yielded lower average levels and irregular absorption under similar conditions.

When penicillin G potassium was compared with penicillin G hydrabamine (213), a less soluble salt, blood levels similar to those produced by penicillin G benzathine were observed. When equivalent doses were administered, the penicillemia that occurred with penicillin G hydrabamine was only one-third or one-fourth as great and was of shorter duration than that produced by penicillin G potassium.

Budolfson *et al.* (212) found that peak concentrations of penicillin G potassium were three to four times those obtained with penicillin G procaine and five to six times those of penicillin G benzathine, indicating a more rapid rate of absorption. The therapeutic action was related to the maximum concentration attained, but it also depended on the persistence of penicillemia, which was greater with penicillin G potassium than with the other two compounds. The authors suggested that the lower blood levels attained with the relatively insoluble penicillin G benzathine were caused by its destruction in the GI tract prior to absorption. This suggestion seems unlikely, however, since the drug should not degrade very rapidly as an undissolved suspension.

V produces higher blood penicillin levels than corresponding doses of penicillin G. As a result, extensive investigations have been conducted with various salts of penicillin V (99, 214-220). For instance, tablets, capsules, and oral suspensions of penicillin V acid produced significantly higher blood concentrations than comparable penicillin G preparations (214). In the same study, the average serum levels produced by the benzathine salt of penicillin V were significantly higher than comparable doses of penicillin G benzathine for the first 4 hr but lower thereafter, again illustrating the value of using an insoluble salt to prolong blood levels of an acid-unstable compound. In another study (215), penicillin V acid was shown to produce higher and more prolonged plasma concentrations than either penicillin G potassium or ammonium, whose properties were comparable.

Plasma levels were correlated with dissolution rates of various forms of penicillin V (99). As solid dosage forms, the readily soluble potassium and calcium salts produced earlier and higher blood levels in fasting subjects than either the free acid or its benzathine salt. On the other hand, when the potassium and benzathine salts were administered orally as solutions, absorption was the same, implying that the poor solubility of the benzathine salt was responsible for the inferior blood levels obtained from its solid dosage forms. Other studies found that, while the potassium salt is 40% better absorbed than the free acid in fasting subjects, both forms produce therapeutic levels when administered with food (218).

Experiments with fistulated dogs indicated that penicillin V is absorbed primarily from the stomach. Therefore, it is not surprising that the potassium salt should show higher blood levels on oral administration since it is the most soluble salt in gastric pH (216). In accordance with this observation, it was also reported that the benzathine salt exhibited higher serum levels in patients with gastric achlorhydria (pernicious anemia) than in patients with normal gastric function (219). Differences in gastric emptying time may also explain this result.

Several studies compared the absorption of tetracycline and its salts (103, 221, 222). For example, serum concentrations in dogs and humans showed that a phosphate complex salt of tetracycline was absorbed more rapidly and gave higher blood levels during the first 6-8 hr than did tetracycline hydrochloride (221). The total amount of drug absorbed was about twice as great with the former compound.

Another study (222) suggested that tetracycline base produced higher blood levels than tetracycline hydrochloride. However, a subsequent investigation (223) showed that, in the absence of adjuvants or fillers, tetracycline hydrochloride and tetracycline base were absorbed equally well. Results obtained in this same study indicated that tetracycline hydrochloride encapsulated with citric acid produced higher serum concentrations than tetracycline hydrochloride mixed with hexametaphosphate or the phosphate complex salt of tetracycline.

In a study comparing urinary excretion rates and *in vitro* dissolution, the absorption of tetracycline and tetracycline phenolsulfonaphthaleinate was rate limited by their dissolution rates, whereas tetracycline hydrochloride absorption was rate limited by the absorption process itself (103).

Several lincomycin salts were studied for their comparative availability (224). In particular, the blood levels obtained with the relatively water-insoluble hexadecylsulfamate salt were compared to those of the hydrochloride following oral administration. Higher and extended whole blood and serum concentrations were obtained in mice, rats, and dogs with the hexadecylsulfamate. However, subcutaneously administered lincomycin did not produce significantly different fractions absorbed, regardless of which salt was administered. It is not known whether the greater area under the curve with oral administration of lincomycin hexadecylsulfamate is due to greater absorption from the GI tract, slower renal clearance, or greater enterohepatic circulations.

Salts of streptomycin, neomycin, viomycin, and streptothricin have been formed with: (a) polyacrylic acids, (b) sulfonic or phosphorylated polysaccharides, and (c) natural polycarboxyl acids from a series of polyuronic substances and polysaccharide derivatives containing carboxyl groups (18). The report indicated that these salts were absorbed from the injection locus primarily by the lymph system. Blood levels from the salts were generally lower but were maintained for a longer time than the equivalent amount of antibiotic alone, and higher concentrations of longer duration may actually be produced in the lymphatic drainage.

The influence of salt formation on the onset and duration of pharmacological activity also was illustrated with tolbutamide and several of its salts (101). Following oral administration, the sodium salt produced a rapid decrease in blood sugar level followed by a rapid recovery. By contrast, the free acid of tolbutamide caused a slow and prolonged drop in blood sugar level, a preferred effect since the chance of hypoglycemic shock would be lessened. This finding also illustrates the often overriding influence of the actual disease state on the choice of drug form.

Additional references on the implications of salt formation on absorption are listed in Table VIII.

Pharmacokinetics—Because of the various new properties that are usually imposed on a compound by salt formation, the pharmacokinetics of the drug necessarily change as a function of these properties.

For example, a pharmacokinetic evaluation comparing ampicillin sodium and potassium with ampicillin trihydrate was performed after oral administration to beagle dogs (243). The absorption rate constants of the sodium and potassium salts, which were similar, proved significantly greater than the rate constant of ampicillin trihydrate, resulting in earlier, higher peak concentrations with two to three times higher serum concentrations during the 1st hr. Yet, any differences between the fraction absorbed for the three products were not statistically significant. Apparently, although dissolution of the ampicillin trihydrate was the rate-limiting step in its absorption, the overall extent of bioavailability remained unaffected.

An interesting study of the biliary excretion of erythromycin base and erythromycin estolate was reported (244). The biliary excretion of erythromycin base was high, while that of erythromycin estolate was much lower; preferential secretion of erythromycin in the bile could partially account for the lower serum levels exhibited by the base. However, the proportion of the ingested dose secreted in the bile was small, and the total amounts in-

144
Table VIII—Additional References on Bioavailability and Absorption Alteration

Topic	Reference
Blood levels produced by three theophylline-containing elixirs	225
Naproxen oral absorption characteristics	226
Effect of food on absorption of a new form of erythromycin propionate	227
Effect of the anion on the absorption of tetracycline from the rat stomach	186
Blood levels following oral administration of different preparations of novobiocin	228
Absorption of iopanoic acid and its sodium salt	229
Oral absorption of secobarbital (quinabarbital) and its sodium salt	230
Absorption rate of barbiturates in humans	231
Morphine and atropine mucate	232
Excretion of buphenium salts in urine of human volunteers	233
Polymethylene bis(isothiuronium) salts: antituberculosis activity	234
Prolonged antitussive action of a resin-bound noscapine preparation	235
Pharmacology of sulfapyridine and sulfathiazole	236
Evaluation of plasma concentrations of propoxyphene utilizing a hybrid principal component analysis of variance technique: equimolar doses	237
Antrycide, a new trypanocidal drug	238
Pralidoxime methanesulfonate: plasma levels and pharmacokinetics after oral administration to humans	239
Intestinal absorption of pralidoxime and other aldoximes	240
Blood plasma levels and elimination of salts of pralidoxime (2-PAM) in humans after oral administration	241
Enhancement of GI absorption of a quaternary ammonium compound by trichloroacetate	242

involved were not sufficient to account entirely for the differences in serum concentrations attained. Undoubtedly, the protein binding studies of Wiegand and Chun (210) (discussed under *Absorption Alteration*) more satisfactorily explain the difference in serum concentrations.

Often, salt formation can be used to modify drug absorption and dose tolerance favorably. For example, aminosalicic acid exhibits a short half-life and, therefore, requires large and frequent doses which may cause gastric irritation. Consequently, different chemical forms such as salts have been prepared (119, 245-247) to reduce the incidence of gastric irritation, increase absorption, and prolong blood levels.

Aminosalicic acid is an interesting example in other ways; considerable confusion about this drug exists because many fail to recognize its nonlinear pharmacokinetics. Several definitive studies were reported regarding the absorption of the acid and its sodium, potassium, and calcium salts from solution, suspension, and tablet formulations (245, 246). Comparison of the relative bioavailabilities of aminosalicic acid suspended in water and its salts dissolved in water showed that, while differences in rate of absorption were found to exist, absorption of both the acid and its salts was essentially complete. Absorption of the free acid from tablets reached only 77% of the dose, whereas that of the tableted salts was rapid and complete.

Regardless of formulation, the area under the plasma concentration-time curve of unmetabolized drug from free acid administration was less than that for the salts. This result was attributed to concentration-dependent metabolism during absorption: when the rate of absorption is high, the metabolic processes become saturated and more unmetabolized drug remains in the blood; conversely,

145

Table IX—Additional References on Bioavailability and Pharmacokinetics

Topic	Reference
Pharmacodynamics of fosfomycin (phosphonomycin) after intravenous administration to humans	248
Pharmacodynamics of phosphonomycin after oral administration to humans	249
Comparative studies on distribution, excretion, and metabolism of ³ H-hydroxyzine and its ¹⁴ C-methiodide in rats	250
Pharmacokinetics of ampicillin trihydrate, ampicillin sodium, and dicloxacillin sodium following intramuscular injection	251
Physiological disposition of fenopropfen in humans: pharmacokinetic comparison of calcium and sodium salts administered orally	252

when the absorption rate is low, as for the free acid, a higher percentage of drug is metabolized.

Additional references regarding bioavailability and pharmacokinetics are presented in Table IX.

GENERAL PHARMACY

Pharmacological Effect—Chlorpromazine hydrochloride and quaternary chlorpromazine chloride were investigated with respect to their effects on the central nervous system (CNS) (253). The quaternized compound was less potent and more toxic in rodents than the parent tertiary compound.

Naloxone, an effective opiate antagonist, is generally used as the hydrochloride salt; however, the drug has a very short duration of action. The mucate salt was prepared to extend its duration of action, since mucic acid is only slightly soluble in water (254). In a test on the blocking of morphine activity in mice, however, the mucate salt did not differ in duration from the hydrochloride. These investigators assumed the same receptor site for naloxone as for morphine and, since Heron's (232) work suggested that the receptor had a greater affinity for morphine mucate than for the free base, it also should have a greater affinity for naloxone mucate. The results disproved this hypothesis. Furthermore, this theory implies that intact salt reaches the receptor, which is highly unlikely, regardless of whether the drug is administered as a solution or as a suspension.

A series of salts of 9-aminoacridine and its derivatives was prepared and screened for antifungal and antibacterial activity (255–257). By using salts of fatty acids, the antifungal action was found to parallel the length of the carbon chain of the anion, with maximal activity occurring with acridine caproate, undecylate, and undecylenate (where undecylenic acid also exhibits some intrinsic antifungal activity) (255). This result appears reasonable, because these salts would be more lipid soluble and could be expected to pass through the cell wall of the infecting organism more readily, probably as an ion-pair.

The efficacy of bases or salts as topical anesthetics for relieving cutaneous itch, burning, and pain in unbroken skin has also been examined (258). In these experiments, itching and pricking were induced by an alternating current of low amperage and voltage applied to the skin or by exposure of the skin to UV light. Interestingly, aqueous solutions of salts of the local anesthetics did not alleviate itching or burning in any of the subjects, although satu-

Table X—Additional References on General Pharmacy and Pharmacological Effect

Topic	Reference
Differential excretion of bromide and chloride ions and its role in bromide retention	259
Pharmacological study of calcium methionate	260
Synthesis and <i>in vitro</i> fungistatic evaluation of some <i>N</i> -substituted amides and amine salts of sorbic acid	261
Antiamelic studies on clomoxiquin [5-chloro-7-[(3-diethylaminopropyl)amino]methyl]-5-quinolinol] <i>in vitro</i> and in experimentally infected animals	262
Adjunctive value of oral prophylaxis with the oximes pralidoxime (2-PAM) lactate and pralidoxime methanesulfonate to therapeutic administration of atropine in dogs poisoned by inhaled sarin vapor	263
Pralidoxime (2-hydroxyiminomethyl- <i>N</i> -methylpyridinium) methanesulfonate and atropine in the treatment of severe organophosphate poisoning	264
Efficacy and limitations of oxime-atropine treatment of organophosphorus anticholinesterase poisoning	265
Antitussive activity of enoxolone (glycyrrhetic acid) and its derivatives	266
Pharmacological properties of glycyrrhetic acid hydrogen succinate (disodium salt)	267
Ganglionic blocking activity of diastereomeric dimethylaminobornyl acetates and their methiodides	268
A new potent nonnarcotic antitussive, 1-methyl-3-[bis(2-thienyl)methylene]piperidine: pharmacology and clinical efficacy	269

alcohol, and 10% glycerol were claimed to be effective. Such transport phenomena across the stratum corneum are often dependent on the polarity of the drug and vehicle and on the binding of the drug to keratin.

Additional references on pharmacological effects can be found in Table X.

Dialysis—Dialysis through a cellophane membrane of the hydrochloride or sodium salts has been studied with several drugs (270). In many cases, it appeared that the ionic form of the drug was bound to the membrane whereas the nonionized form was not. Ephedrine hydrochloride presented an interesting example, however, since it dialyzed considerably faster than its corresponding base. It was theorized that the chloride ion dialyzed rapidly, enhancing the rate of dialysis of the ephedrine ion. Accordingly, when chloride ion was present on both sides of the membrane, the observed rate of dialysis for the ephedrine ion was comparable to the ephedrine base.

The diffusion of sodium chloride through a lipoprotein interface was very slow, especially if calcium chloride was present on both sides of the interface (271). In the presence of low concentrations of choline chloride or carbamylcholine chloride, the diffusion of sodium chloride is more rapid. Apparently, choline salts are able to increase the permeability of the lipoprotein to salts, which may relate to the physiological action of choline salts.

Miscellaneous—Release rates were determined for aminophylline, ephedrine alkaloid, and ephedrine hydrochloride from theobroma oil suppositories containing nonionic surfactants (133). While surfactants with hydrophilic-lipophilic balance (HLB) values less than 11 only minimally affected release rate, rates increased with surfactants of HLB values greater than 11. Under optimal conditions, aminophylline was faster than ephedrine hydrochloride which, in turn, was superior to the ephedrine base.

Willis and Banker (272) reported on the formation of polymer-drug salts as an approach to the physicochemical design of dosage forms. Poly(methyl vinyl ether/maleic

anhydride) salts of methapyrilene were prepared and tested with the free base for *in vitro* dissolution and dialysis. Their dissolution and dialysis rates were not appreciably different from the free drug or its hydrochloride salt. Various poly(methyl vinyl ether/maleic anhydride) hemiester salts of methapyrilene exhibited substantially slower release than the polymer ether salts, hydrochloride salt, or free base forms. Polymer-drug salts thus appear to have promise.

A series of metallic salts of ederic (ethylenediamine-tetraacetic) acid were tested *in vitro* to determine their effect on blood coagulation (273). The results showed that only the dipotassium and disodium salts had any effect on coagulation. It was theorized that the lack of anticoagulant activity resulted from an almost complete suppression of ionization of the heavy metal salts.

Interesting research regarding the angina-preventive effect of some chromone-2-carboxylate salts showed a direct correlation between biological activity and pKa of the salt-forming amines (274).

Lin *et al.* (112) investigated the relationship between salt form and biological activity of a given antihypertensive. While the intrinsic dissolution rates of the dihydrochloride and disulfate salts were many fold greater than the monohydrochloride, the hypotensive potencies of the salts did not differ significantly from one another in an anesthetized dog study. Yet, when administered to renal hypertensive dogs, the dihydrochloride and disulfate salts produced greater hypotensive effects than did the monohydrochloride.

TOXICOLOGICAL CONSIDERATIONS

Toxicity of Salt Ion—Any discussion regarding the toxicity of salts of a drug must consider the pharmacological properties of the cation or anion used to form the salt as well as those of the free drug, since any of these may produce toxic effects. The toxicology of several ions that are commonly used to form salts and that are relevant to this review were discussed in depth (275).

Toxicity from ingestion of calcium salts of drugs is rare. If hypercalcemia occurs, however, calcium deposits in the kidney can bring on a reduction of renal function. The principal toxic effects of lithium also involve the kidneys. When small amounts of lithium are taken, no apparent damage occurs; yet large amounts of the metal can lead to irreversible damage. An apparent correlation was observed between lithium dosage and sodium intake (276). When lithium dosage was low or sodium intake was high, rats were able to excrete all lithium given and sustained a reversible polyuria. Conversely, if large amounts of lithium were administered to the rats or if their sodium intake was lowered, they incurred irreversible kidney damage. Ammonium ion, although it serves a major role in maintenance of the acid-base balance of the body, can be toxic in high concentrations and initiate CNS derangements.

Sulfate ions given orally tend to be minimally absorbed and may act as a laxative. The nitrate ion is irritating to the GI tract, causing nausea and gastric distress. Also, intestinal bacteria may convert the nitrate ion to nitrite which oxidizes hemoglobin to methemoglobin. The citrate ion, an intermediary in carbohydrate metabolism, can form a soluble complex with calcium which is poorly dissociable

and rarely causes any toxic reactions. While tartrate ions are usually absorbed minimally from the GI tract, high concentrations reaching the circulation can cause renal damage.

Acetate and lactate ions are normal metabolites and appear to be well tolerated in relatively large amounts. Iodide and bromide ions can produce conditions known as iodism and bromidism, respectively. Bromide intoxication occurs quite frequently, since bromides are used as ingredients in some nonprescription preparations (277-280). Bromide is slowly excreted by the kidney (its half-life is 12 days) and tends to accumulate when taken for prolonged periods or when used by patients with decreased renal function (277).

Toxicity of Salt Form—Provided the salt-forming agents are nontoxic, the relative toxicities of a series of salts of a compound are often observed to reflect directly their absorption rates. For example, the toxicities of dibromide, dichloride, diiodide, and dimethylsulfate salts of quina-pyramine³, a trypanocidal drug, were determined (239). The sparingly soluble halogen salts were much less toxic subcutaneously or intramuscularly than the freely soluble dimethylsulfate, yet all salts showed about equal toxicity upon intravenous administration. The difference in toxicities obviously resulted from rapid absorption of the methylsulfate compared to the slowly absorbed, poorly soluble halogen salts. Similar reasoning has been used to explain the acute oral toxicity of propoxyphene hydrochloride in rodents, which is twice that of equimolar doses of the napsylate salt (281).

Several salts of benzphetamine and etryptamine were prepared as potential sustained-release formulations (114). The water solubility, *in vitro* dissolution rates at pH 1.0 and 7.2, and the median lethal times (LT₅₀) were determined for each salt. Both the LT₅₀ and LD₅₀ (determined on only a few salts) increased as the *in vitro* dissolution rate at pH 7.2 decreased. While dissolution at pH 1.0 did not correlate well with toxicity, the LT₅₀'s were inversely related to the square root of the dissolution rates at pH 7.2.

Toxicity studies comparing iopanoic acid, a cholecystographic contrast medium, with its sodium salt (115) showed that the salt form has 10-fold greater toxicity. The LD₅₀'s of the free acid and the salt were 22 and 2.32 g/kg, respectively. It was postulated that the free acid precipitated from the sodium salt upon its reaction with gastric hydrochloric acid. The fine, amorphous particles of precipitated acid had a greatly increased surface area and, therefore, dissolved more rapidly than even fine crystals of the free acid. The faster and more complete drug absorption then resulted in increased toxicity.

Salts exhibiting greater water solubility than their parent compounds or less soluble salts are not always more toxic. For example, various inorganic and organic ions were used to prepare salts of methyl pyridinium-2-aldoxime that would have greater water solubility and would eliminate undesirable side effects due to the iodide ion (95). Even though the aqueous solubility of the majority of these salts was many times greater than the iodide, their toxicity on a molar basis was not significantly different, with the exception of the dihydrogen phosphate salt which was 15%

³ Aritycyde.

147

Table XI—Additional References on Toxicological Considerations

Topic	Reference
Toxicity and absorption of 2-sulfanilimidopyridine and its soluble sodium salt	235
Sorbic acid as a fungistatic agent for foods: harmlessness of sorbic acid as a dietary component	236
Toxicity and distribution of erythromycin	287
Further toxicological studies with erythromycin	253
Pharmacology and toxicology of erythromycin estolate	239
Erythromycin propionate (propionylerythromycin): a review of 20,525 case reports for side-effect data	290
New class of antibiotic salts of reduced toxicity	22
GI intolerance to oral iron preparations	291
Comparative toxicology of iron compounds	292
Influence of the dissolution rate of lithium tablets on side effect	123
Toxicity and tissue distribution studies on the hydrochloride, bismuth iodide complex, and a resinate of emetine	293
Bacitracin zinc in pharmaceutical preparations	145
New approach to quaternary ammonium compounds	151
Pharmacology of choline theophyllinate	284

more toxic. Further research with oximes revealed that other salts are also toxic (282).

GI bleeding is a common toxic effect of aspirin for a large percentage of the population. Consequently, a search was initiated for an aspirin derivative that would not induce GI blood loss (283). All compounds prepared, however, including the sodium and calcium salts, caused GI hemorrhage with a severity similar to aspirin.

Polyene antibiotics are potent antifungal agents but bear considerable toxicity. Even though the methyl ester hydrochlorides of these compounds are more soluble, they retain almost all of their antifungal activity and, more significantly, show a uniform decrease in toxicity compared to their parent compounds (284).

Additional references on toxicological considerations of salt formation are given in Table XI.

CONCLUSIONS

Salt formation is a means of altering the physical, chemical, and biological characteristics of a drug without modifying its chemical structure. Clearly, the salt form can have a dramatic influence on the overall properties of the parent compound. At present, selecting a salt form that exhibits the desired combination of properties is a difficult semiempirical choice. Pharmaceutical scientists now recognize these facts and are beginning to study the effects of different salt forms on the physicochemical properties, bioavailability, and toxicity of drug substances.

Although now only a few generalizations are available to predict the effect of particular salt forms on the characteristics of a drug, perhaps in time it will be possible to evolve increasingly more powerful generalizations regarding the effect of a salt on the properties of its parent compound. In addition, we predict that polymer-drug salts will have a revolutionary effect on future trends in drug therapy, particularly in the areas of reducing drug toxicity and in controlling the release profile of novel drug delivery systems.

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