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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
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DEP: 2020 DIRECCION DE NUEVAS CREACIONES	**EVE:** 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	**FOLIOS**: 9
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
CARLOS REINALDO OLARTE GARCIA

Asunto: Radicación: 13-90547- -4
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 9964
Folios: 9

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/US2011/054841				
Fecha de Presentación Internacional	05/10/2011				

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. 13-090547-00000-0000	8/abril/2013
Reivindicaciones:	Radicado No. 13-090547-00000-0000	8/abril/2013
	Radicado No. 13-090547-00002-0000	20/junio/2013
Prioridad:	US 61/390,888	7/octubre/2010

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

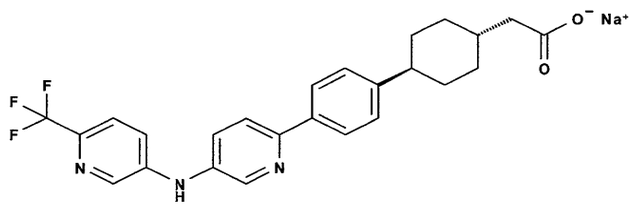
Las reivindicaciones modificadas (Radicado No. 13-090547-00002-0000) son aceptadas porque no amplían el objeto de la solicitud.

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. 13-090547-00002-0000) para el examen de patentabilidad.

Título de la solicitud: "NUEVAS FORMAS CRISTALINAS DE LA SAL SÓDICA DEL ÁCIDO (4-{4-[5-(6-TRIFLUORO-METIL-PIRIDIN-3-IL-AMINO)PIRIDINA-2-IL]-FENIL}-CICLOHEXIL)-ACÉTICO".

El problema planteado en esta solicitud consiste en proveer formas cristalinas del compuesto (1):



(II)

Para resolver este problema técnico la solicitud en estudio proporciona una sal cristalina de sodio del compuesto de fórmula (II) con un modelo de difracción de rayos X en polvo que comprende picos a 5,9 Y 17,0 grados 2θ (± 0.1 grado) (CuKα λ = 1.5418 Å).

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07D401/12; A61K 31/415; A61P 3/04.

5. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 carece de una caracterización adecuada los sólidos B, C, D, F, I, L, N y O, que permita establecer de manera clara el alcance de la solicitud e identificar de forma inequívoca cada uno de estos sólidos. Un polimorfo se puede caracterizar apropiadamente en una reivindicación, por sus parámetros fisicoquímicos, tales como:

1. El patrón de difracción de Rayos X en mono cristal, que es específico de cada polimorfo, por lo cual, si la reivindicación caracteriza el polimorfo mediante este patrón, no se exigirá otro dato.
2. El patrón de difracción de Rayos X en polvo (DRXP), el cual debe presentar por lo menos 20 picos correspondientes a las intensidades de los haces difractados o a los espacios interplanares 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.

Es pertinente señalar que dos picos del espectro de difracción de rayos X son insuficientes para caracterizar de manera inequivoca un sólido cristalino de un compuesto dado. Esto es evidenciado por Bhattacharya[1] provee un gráfico (fig. 11) en 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 tres picos principales de XRPD. De acuerdo con Bhattacharya, alrededor de diez de los picos más fuertes del XRPD son necesarios para identificar una forma cristalina única. Es consecuencia, dos picos de XRPD son insuficientes para caracterizar inequívocamente un polimorfo.

Las reivindicaciones 3 y 4 caracterizan la forma cristalina por un parámetro como lo es permanecer seco a 25°C y una humedad relativa en el rango de 0% a 80%. Esto no corresponde a características técnicas esenciales del producto ya que la forma adecuada de caracterizar un cristal es como se describió anteriormente. Del mismo modo, las expresión "permanecer seco" es ambigua ya que no existe un parámetro que permita establecer a qué se refiere el solicitante con la misma.

Las reivindicaciones 6, 8 y 9 hacen referencia a figuras lo cual no es aceptado.

La expresión "el cual comprende" utilizada en la reivindicación 12 no limita el alcance de la solicitud ya que abarca no solo el DMSO, una mezcla de tetrahidrofurano y metanol, sino cualquier otro sistema de disolventes ya que dicha expresión es de tipo abierto.

6. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: 7 de octubre de 2010, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	WO 2007/126957	"New Compounds"	8/noviembre/2007
D2	H.G. Brittain. Segunda Edición, Informa Healthcare,NY, 2009	"Polymorphism in Pharmaceutical Solids"	2009

El documento D1[2] divulga el compuesto de fórmula (II) (Pág. 106, líneas 11, 12. Ejemplos 5-1), sin embargo la anterioridad no especifica que este corresponda a la una forma cristalina y no divulga el XRPD de dicho sólido.

El documento 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).

7. Examen de Patentabilidad (Art 14 D486)

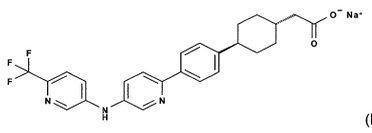
Nivel inventivo (Art18 D486)

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

2http://worldwide.espacenet.com/publicationDetails/biblio?CC=WO&NR=2007126957&KC=&locale=en_EP&FT=E

La reivindicación 1 consiste en una sal cristalina de sodio del compuesto de fórmula (II):

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

Características esenciales	D1	D2
Una forma cristalina de la sal de sodio del ácido (4-{4-[5-(6-trifluorometil-piridin-3-ilamino)-piridin-2il]-fenil}-ciclohexil)-acético de fórmula (II):  (II)	Divulga el compuesto sal de sodio del ácido (4-{4-[5-(6-trifluorometil-piridin-3-ilamino)-piridin-2il]-fenil}-ciclohexil)-acético (Ejemplo 5-1, Pág. 106, líneas 11 y 12).	Divulga la importancia de los polimorfos en la industria farmacéutica y las diferencias en las propiedades fisicoquímicas las cuales poseen aplicaciones en el procesamiento, formulación, y biodisponibilidad del fármaco (Pág. 2)
La modificación C presenta un patrón de difracción de rayos X de polvo con picos en 5.9 y 17.0 grados 2θ La modificación N presenta un patrón de difracción de rayos X de polvo con picos en 6.2, 8.3, 16.6, 19.6, 21.2, 25.0, 29.4 grados 2θ. La modificación O presenta un patrón de difracción de rayos X de polvo con picos en 4.3, 8.7, 15.2, 17.8, 18.5, 19.1, 19.6 grados 2θ (± 0,1 grados 2θ) (CuKα λ=1.5418 Å), o un patrón y un espectro 13C_RMN en estado sólido que comprende picos a un desplazamiento químico de 176,8 ppm y 168,4 ppm (± 0,2 ppm).	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 Office[3]) 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 la sal sódica del ácido (4-{4-[5-(6-trifluorometil-piridin-3-ilamino)-piridin-2il]-fenil}-ciclohexil)-acético (Ejemplo 5-1, Pág. 106, líneas 11 y 12), donde dichas sales retienen la efectividad biológica en el tratamiento de desórdenes asociados con DGAT1.

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 el compuesto la sal sódica obtenida se encuentre en forma de cristal y no se especifican los parámetros del espectro de difracción de rayos X.

3 http://www.pcb.ub.edu/centredepatents/pdf/cursos/dillunsCP/papathoma_polymorphs.pdf

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 sódica del ácido (4-{4-[5-(6-trifluorometil-piridin-3-ilamino)-piridin-2il]-fenil}-ciclohexil)-acético consiste en que algunos de estos sólidos poseen una menor higroscopicidad.

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 desarrollar polimorfos de la sal sódica del compuesto de fórmula (II) 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, las modificaciones C, O y N poseen una menor higroscopicidad cuando el %HR es inferior al 80% con relación a la forma A. Sin embargo, es pertinente aclarar que los resultados presentados por la solicitante no evidencian una ventaja o superioridad de las formas cristalinas de la sal sódica reivindicadas, toda vez que no existen estudios comparativos frente al compuesto previamente divulgado en el estado de la técnica o en relación con la forma salida que igualmente se encuentra previamente sugerida. Del mismo modo, los resultados presentados corresponden a la determinación de una propiedad física como lo es la higroscopicidad que de la cual se sabe que varía entre diversos polimorfos, esto no corresponden a una ventaja real frente al fármaco base el cual ya era conocido y cuya actividad no varía en relación con sus polimorfos.

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) y entre las cuales se encuentra la higroscopicidad. 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 polimorficas 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 trabajo rutinario del formulador.

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 sódicas[4].

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 enseña el catión sodio como farmacéuticamente aceptable y uno de los preferidos en la industria farmacéutica. 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 fundamental de un polimorfo 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, el cual divulga la sal sódica del compuesto ácido (4-{4-[5-(6-trifluorometil-piridin-3-ilamino)-piridin-2il]-fenil}-ciclohexil)-acético, 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 los cristales de la sal de sodio del ácido (4-{4-[5-(6-trifluorometil-piridin-3-ilamino)-piridin-2il]-fenil}-ciclohexil)-acético 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 de la sal sódica del compuesto de formula (II), 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 polimorfos poseen propiedades fisicoquímicas diferentes entre las que se encuentra la higroscopicidad.

4 Journal of pharmaceutical sciences. Vol. 66 (1), January 1977. “Pharmaceutical Salts”.

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 divulgado el espectro de difracción de rayos X y el ^{19}F-RMN de los polimorfos reivindicados, estas nuevas configuraciones sólidas se derivan 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 a la sal del sodio del ácido (4-{4-[5-(6-trifluorometil-piridin-3-ilamino)-piridin-2il]-fenil}-ciclohexil)-acético, con el fin de determinar la existencia formas cristalinas alternativas para este compuesto tal como la reivindicada.

En conclusión los polimorfos de la reivindicación 1 y las composiciones que los contienen carecen de nivel inventivo.

La reivindicación 12 reclama un proceso para hacer la modificación C:

Características esenciales	D1	D2
Reiv: 12: Un proceso para hacer la modificación C que consiste en los siguientes pasos: (a) disolver el compuesto de fórmula (II) en un sistema de disolventes, el cual comprende dimetilsulfóxido (DMSO) o una mezcla de tetrahidrofurano y metanol; (b) adicionar (i) un disolvente el cual es seleccionado del grupo que comprende acetonitrilo, tolueno, y metil-t-butil éter, o (ii) una mezcla de agua y un disolvente seleccionado del grupo que comprende acetonitrilo, tolueno y metil-t-butil éter, donde el contenido de agua es 0.25 a 3% en volumen; o (iii) un anti-disolvente como el heptano: (e) filtrar la mezcla obtenida al final del paso (b): y (d) opcionalmente, secar los cristales.	Divulga el compuesto base de fórmula (1) (compuesto No. 3085). enseña que el mismo puede encontrarse en forma de sales de adición, como la sal sódica (Col. 9, líneas 20 a 30).	La cristalización a partir de una solución es el método de cristalización más utilizado como proceso para recuperación de producto en la industria farmacéutica. Divulga la técnica de cristalización por enfriamiento (Pág. 89) y algunos de los solventes de cristalización comúnmente utilizados (Pág. 95) y establece que este proceso se fundamenta en el conocimiento de las curvas de solubilidad donde el crecimiento cristalino y la futura nucleación ocurre cuando el equilibrio es alcanzado en D (Págs. 90 y 91)

Los documentos D1 y D2 se consideran el estado de la técnica más cercano a la invención definida en la reivindicación 12, porque D1 enseña la sal sódica del ácido (4-{4-[5-(6-trifluorometil-piridin-3-ilamino)-piridin-2il]-fenil}-ciclohexil)-acético, y declara que estos agentes pueden poseer polimorfos (Ejemplo 5-1, Pág. 106, líneas 11 y 12, Pág. 7).

La diferencia entre la invencion, definida en la reivindicación 12 consiste en que la anterioridad no divulga los procesos para obtener la forma cristalina C de la sal sódica del ácido (4-{4-[5-(6-trifluorometil-piridin-3-ilamino)-piridin-2il]-fenil}-ciclohexil)-acético.

El efecto que logran los procesos reivindicados consiste en la obtención de formas sólidas alternativas del activo de fórmula (II).

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 sódica del del ácido (4-{4-[5-(6-trifluorometil-piridin-3-ilamino)-piridin-2il]-fenil}-ciclohexil)-acético divulgado en D1.

Sin embargo, una persona versada en la materia sabe que el uso de diferentes solventes en un proceso de recristalización permite la obtención de diferentes formas cristalinas[5], además el documento D2 enseña que la cristalización a partir de una solución es el método más común de cristalización utilizado como un proceso para la recuperación de producto en la industria farmacéutica, este cumple de manera simultánea dos funciones esenciales: 1) la purificación de un producto farmacéutico de gran valor, y la separación de sus productos de reacción. 2) la consolidación del producto en una forma cristalina específica con propiedades fisicoquímicas consistentes y bien caracterizadas (Pág. 88).

D2 enseña que el primer paso para realizar recristalización por enfriamiento consiste en identificar la curva de solubilidad del activo. De acuerdo con la figura 8 de identificación de zona metaestable en cristalización por enfriamiento la cristalización sustancial ocurre luego del punto C tanto por el crecimiento del núcleo formado en este punto como por la posterior nucleación y crecimiento, y las condiciones regresan al equilibrio en la línea de solubilidad en el punto D al disminuir la temperatura (Págs. 90 y 91). Del mismo modo, D2 indica que varios efectos en la cristalización y obtención del polimorfo son mediados por la selección del solvente, el más simple es el efecto de la temperatura ya que esta se encuentra limitada por el punto de ebullición y por el perfil de solubilidad del soluto (Pág. 94). Esto sustenta que la técnica de recristalización para obtención de polimorfos ha sido ampliamente estudiada y se fundamenta en las propiedades de solubilidad del fármaco.

En consecuencia, la persona del oficio normalmente versada en la materia, enfrentada a la necesidad de obtener nuevas estructuras sólidas del compuesto de fórmula (II) utilizaría las técnicas de recristalización para obtener polimorfos, según lo revelado en D2, por lo que resulta obvio diseñar una secuencia de solubilización –recristalización en diferentes solventes de acuerdo con la curva de solubilidad de activo en un rango de temperatura acorde con el punto de ebullición del solvente seleccionado, con el ánimo de obtener el polimorfo de interés, más aún si se conoce el fundamento teórico para la obtención de cristales por diferencias en solubilidad y modificando parámetros adicionales como temperatura, velocidad de enfriamiento, agitación, presión, evaporación etc., con la expectativa razonable de encontrar un ordenamiento cristalino como el reivindicado. Por lo cual la reivindicación 12 corresponde la aplicación de una tecnología conocida y se considera obvia.

8. Conclusión

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

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO 2007/126957	1 – 12	Nivel inventivo
D2	H.G. Brittain. Segunda Edición, Informa Healthcare,NY, 2009	1 – 12	Nivel inventivo

5 FARMACIA PRÁCTICA REMINGTON VOL,2, 19a Edición EDITORIAL MEDICA PANAMERICANA Pagina 2229,

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.

JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-08-19

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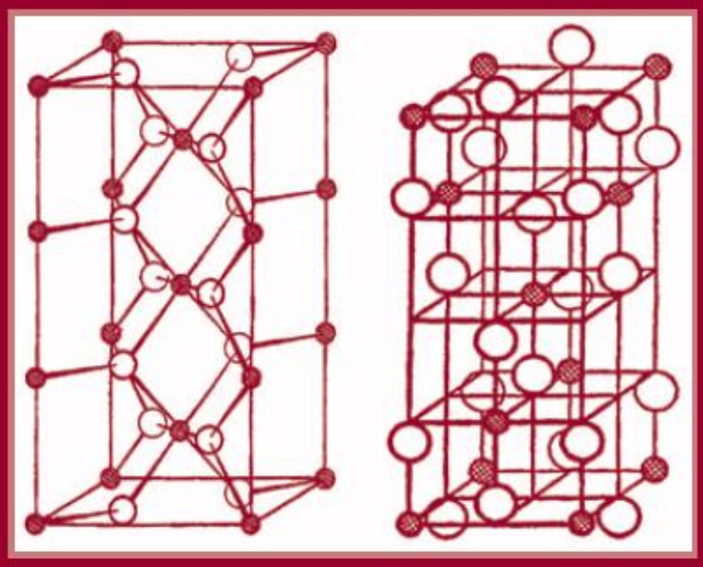
DRUGS AND THE PHARMACEUTICAL SCIENCES VOLUME 192

SECOND EDITION

Polymorphism in Pharmaceutical Solids



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1 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/cm^3) to the β-form (1.327 g/cm^3).

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	P*na*	P*na*
Number of molecules per unit cell	Z = 4	Z = 4
Unit cell axis lengths	*a* =10.53 Å	*a* = 7.91 Å
	b = 9.53 Å	*b* = 12.57 Å
	c = 5.66 Å	*c* = 5.50 Å
Unit cell angles	α = 90°	α = 90°
	β = 90°	β = 90°
	γ = 90°	γ = 90°

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
	Compactibility, tabletting
	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

Solvates also include crystal structures where the solvent is not incorporated stoichiometrically into the crystal structure, but is accommodated by affinity at the surface, or in voids or channels in the structure (Fig. 1B). Where solvent is located at the surfaces, it may usually be driven off at temperatures slightly above the solvent boiling point, and the thermal stability will be the primary factor determining whether or not the structure is disrupted. Solvation by incorporation into voids or channels is more akin to solid solution formation, whereby the solvent acts as a solute in the host matrix of the crystalline substrate. In these cases, desolvation may or may not result in structural disruption, depending on both the thermal stability and the extent of the mechanical effects of removing solvent molecules from the depths of the lattice structure. In these cases, desolvation usually occurs at temperatures considerably higher than the solvent boiling point (often up to 30 K higher), because of capillary effects in channels and geometric blocking of solvent release. It should be noted, however, that the arrangement of solvent molecules in channels also occurs quite commonly in stoichiometric solvatomorphs (15), by virtue of the crystallographic geometry, and the identification of solvent molecules within channels does not, in itself, characterize the solvate type.

"True" solvates are not polymorphic with the corresponding non-solvated forms, because the chemical constitutions are different. The term "solvatomorph" can be used to designate the fact that they represent alternative structures that are not polymorphic (14). As a general rule, solvates are not favored for pharmaceutical development, except for hydrates and occasionally ethanolates, because of the toxicity and undesirability of ingesting most solvents. Hydrates are often, although by no means universally, less soluble in aqueous media than the corresponding anhydrous forms (4).

Salt forms of drug substances are often employed to enhance their aqueous solubilities. Table 1 shows published data on the number of drug salts available in the 1990s (16). It can be seen that the number of salts with acidic counter-ions (anions) represents about 75% of the total from both sources. There are several reasons for this. First, most APIs contain basic nitrogen functions in their free base forms. Second, the list of anions that are suitable in terms of both appropriate pK_a values and pharmacological safety is much more extensive than the corresponding list of bases. Many nitrogenous bases exert biological and pharmacodynamic effects, and most inorganic cations are either toxic or exert essential biological functions, and in this latter case the dosing of additional quantities may restrict the scope of administration of the drug. Even so, the proportion of cations that are inorganic is considerably higher than the corresponding ratio for anions in both data sets recorded in Table 1. Table 2 gives the frequency distribution of the most common

TABLE 1 Reported Number of Drugs Available as Salts, and the Distribution of Acidic (Anionic) and Basic (Cationic) Counter-ions (16)

Source	No. of drug salts		No. of counter-ions (CIs)	
	with acid CI	with basic CI	Acidic	Basic
Index Nominum, 1995	1346	474	108	37
Rote Liste, 1999 (Germany)	612	208	55	21

TABLE 2 Distributions of Salts with the Most Frequently Occurring Acidic (Anionic) and Basic (Cationic) Counter-ions

Acidic (anionic) counter-ions			Basic (cationic) counter-ions		
Anion	Percentage of total		Cation	Percentage of total	
	Ref. (16)	Ref. (17)		Ref. (16)	Ref. (17)
Hydrochloride	36.2	42.1	Sodium	14.8	17.5
Sulfate	5.2	10.1	Potassium	2.9	4.9
Tartrate	3.3	2.6	Calcium	3.6	1.7
Hydrobromide	4.4	1.4	Magnesium	1.6	
Maleate	3.3	2.3	Meglumine	1.0	
Mesylate	3.3	1.2	Ammonium	0.9	
Phosphate	2.0	3.1	Aluminium	0.8	
Acetate	1.8	3.2	Zinc	0.5	
Citrate	3.0	0.8	Piperazine	0.5	
Pamoate	1.5	0.3	Tromethamine (Tris)	0.5	
Nitrate	1.3	0.8	Lithium	0.5	
Lactate	1.1	1.0	Choline	0.4	
Fumarate	1.1	0.6	Diethylamine	0.4	
Hydroiodide	1.3		4-Phenylcyclohexylamine	0.3	
Methylsulfate	1.1		Benzathine	0.3	
Succinate		1.1			
Propionate		1.0			
Carbonate		0.5			
Gluconate		0.5			
Aspartate		0.3			
Saccharate		0.3			
Besylate		0.3			
Valerate		0.3			

salt-forming acidic and basic counter-ions, as percentages of the total (acids + bases), calculated using data from two sources (16,17). It is clear from these data which counter-ions are used predominantly, although the detailed figures vary according to the data that has been sampled for the two compilations.

Figure 2 shows examples of the solubilization by salt formation of two candidate drug compounds, both of which were virtually insoluble as free bases, with data points effectively lying on the baselines of the charts (18). On solubility grounds alone, Figure 2A shows a clear preference for the edisylate (ethanedisulfonate) salt, although the solubility of 1.7 mg/mL is not high in absolute terms. Much higher solubilities are exhibited by the salts in Figure 2B, up to 10 mg/mL for the three most soluble forms. A recent investigation of salts with the saccharate anion has identified solubility enhancements of 600, 40, and 3, respectively, for halperidol, mirtazepine, and quinine (19). However, it cannot be assumed that all salts will confer enhanced solubility, and salts can often have lower solubilities than the corresponding free bases. Salt screens are frequently carried out to identify the most suitable counter-ions, as will be described below.

revert to crystalline forms over a period of time. The rate at which such changes occur is of critical importance, and methods developed for stabilizing and formulating amorphous drugs are usually designed to minimize and slow down such interconversions, both in storage and in use. A result such as that in Figure 5C, with interconversion over 20 minutes at 45°C, indicates that such reductions in solubility could occur in vivo (37°C) over the time scale of absorption of the drug, which could end up deposited in the digestive tract as a poorly soluble and slowly absorbed crystalline form.

OVERVIEW OF METHODS FOR PRODUCING SOLID FORMS

The ways in which the various solid forms above may be prepared will be outlined in the following sections in a method-based structure, whereby those methods employed most extensively will be described first and in most detail. The last 10 years or so has seen an explosion in the range and types of method used to prepare solid forms that has been fuelled by the burgeoning interest in polymorphism and solid form diversity and the consequent requirement to prepare and manufacture targeted solid forms consistently and reliably. The traditional crystallization methods have been developed by understanding and controlling their functions, and have been augmented by a number of innovative new techniques to improve both their form selectivity and the range of polymorphs and other solid forms that can be prepared (35).

For preparing crystalline forms, crystallization from solution is by far the commonest method used in the pharmaceutical industry today. Crystallization from melts is rarely employed, mainly because of the limitation of thermal stability of API compounds and the small scales of production. Pharmaceutical production is usually at relatively small scale, with processes carried out batchwise in multipurpose plants under conditions that are specific for a given product and manufacturing process. Furthermore, synthesized organic compounds are usually prepared in a solution, and crystallization is most commonly employed as the method by which solid material is recovered from the solution of the final synthesis step. Although the logistics and procedural details are different, the same underlying principles govern crystallization as a production method and its use in investigative work, including polymorph searching and single-crystal preparation for X-ray structure determination. Moreover, the production of a specific polymorph identified in such investigations may require the method by which it was obtained to be translated to a preparative or production environment, and this knowledge transfer will be greatly assisted by a sound understanding of the principles that underlie the method by which it was obtained.

As a general principle, slow crystallization and transformation processes operated under mild conditions with moderate driving forces are more likely to produce stable crystalline polymorphs, whereas rapid processes employing dynamic and extreme conditions with large driving forces will produce metastable and unstable crystalline forms and amorphous solids. This principle is illustrated in Figure 6 for a selection of preparation methods, compiled by the author based on a recent similar idea (35,36). The relationship between the speed at which a process operates and the outcome is well represented by the antisolvent mediated processes, using bulk addition and diffusion. These operate on the same principle, but with very different rates of antisolvent addition. Diffusion is a method that introduces

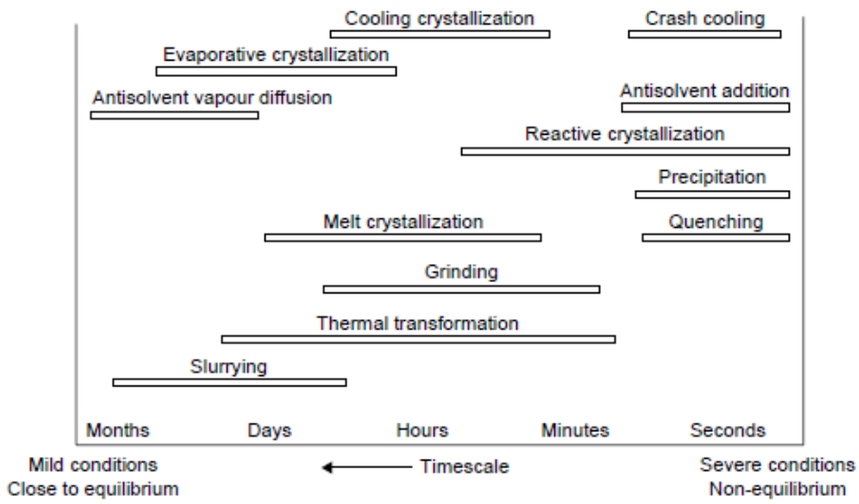

FIGURE 6 A selection of methods for producing crystalline forms. Longer running procedures favor stable polymorphs, whereas more rapid methods are more likely to yield less stable forms (35,36).

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 $CuSO_4 \cdot 5H_2O$. 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 $Na_2SO_4 \cdot 10H_2O$ 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.

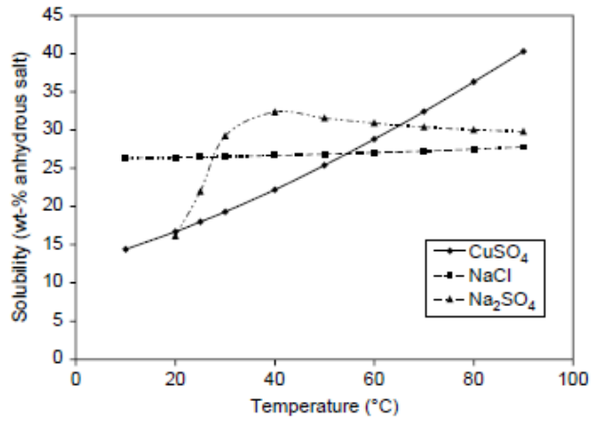

FIGURE 7 Solubility curves for the dissolution of simple inorganic salts in water (40). $CuSO_4$ may be crystallized from water by cooling, whereas NaCl may not. Na_2SO_4 exhibits a change in the most stable solid phase at 32°C, which is manifested as a discontinuity in the solubility curve.

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

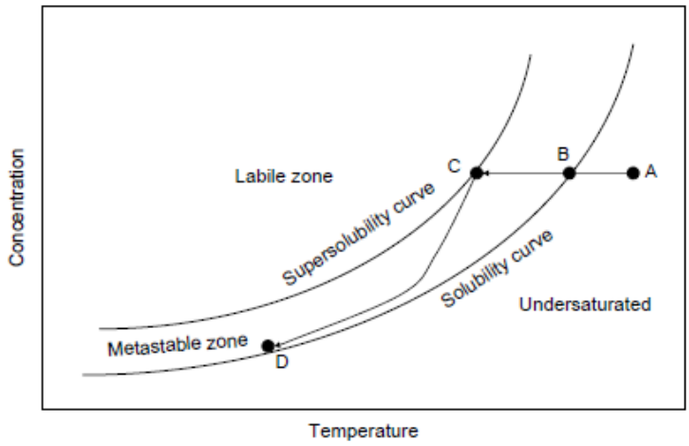

FIGURE 8 The metastable zone in cooling crystallization. Cooling a solution from A induces supersaturation at B, and nucleation at C. The region BC in which the solution is supersaturated but no nucleation occurs is the metastable zone. Beyond the nucleation point, crystal growth and further nucleation 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_t lies within the operating temperature range of the crystallization (41). Form II is stable below T_t, form I above T_t. 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

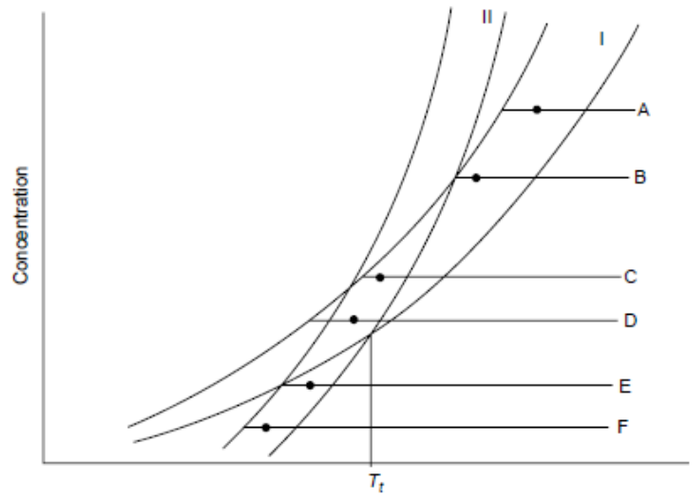

FIGURE 9 Solubility and metastable zones for the cooling crystallization of a dimorphic compound with a transition temperature within the crystallization region. Selecting the start and end temperatures A–F will determine the crystal forms obtained. *Source*: Adapted from Ref. (41). *Abbreviation*: T_t,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

Table 4 lists solvents commonly used in the crystallization of pharmaceuticals, in decreasing order of their boiling points. Comprehensive polymorph screening will involve a larger and wider range of solvents that are not necessarily safe and suitable for use in processing but rather give the maximum range and diversity of interactions and effects on the solute that is being crystallized. Data from a search of the CCDC database (in March 2008) of their frequency of occurrence is also given, but this information must be treated with some caution. Although the searches were carried out using the common synonyms of the solvents (e.g., 2-butanone/methyl ethyl ketone/MEK, chloroform/trichloromethane), the "hits" include structures in which the "solvent" moiety forms part of the crystal structure, and in these cases may or may not be considered part of the solvent. For pyridine and cyclohexane, these structures or their derivatives occur very frequently in the crystal structures prepared, and their categorization as a "solvent" does not seem appropriate. THF (tetrahydrofuran) also occurs frequently in this way, and most of the solvents in Table 4 participate in at least a few of the structures reported. The distinction between this type of structure and a solvate, where the solvent molecule forms part of the crystal structure but is deemed to constitute a distinct and separate molecular species, can be a fine one. Also, Table 4 has been compiled using all entries in the database, many of which are organometallic, and how representative this selection is of pharmaceutical products is difficult to judge. Searching with exclusions does not seem to be justified, as organometallics arise quite often as therapeutic compounds, for example, as cytotoxic compounds used in cancer chemotherapy.

The need to utilize a diversity of solvents in polymorph and other solid-form screening was recognized early on (42), and the selection of suitable lists of solvents has now become a matter of considerable scientific sophistication. In the 1990s, the selection of suitable solvents was approached predictively using group contribution methods such as UNIFAC (43), and this has now evolved into more sophisticated packages (44) such as the NRTL–Segment Activity Coefficient (SAC) model that is now implemented in commercially available software (45). Mixtures of solvents are commonly employed to modify and optimize the solvent properties, including solute solubility, polarity, and hydrogen-bonding donor and acceptor properties.

Several effects on crystallization and polymorph selection can be mediated by the solvent selection, the simplest of which is an effect of temperature. The temperature range in which a solvent can be used is limited by its boiling point (Table 4), and by the solubility profile of the solute. In enantiotropic systems, the polymorph that is crystallized may depend on the crystallization temperature by the principles discussed in the section "Cooling Crystallization" and Figure 9. McCrone, writing in 1957 (9), describes the use of high-boiling solvents such as benzyl alcohol and nitrobenzene for the recrystallization of metastable forms on a hot stage. For the API buspirone hydrochloride, a higher or a lower melting form resulted according to whether the crystallization was carried out above or below 95°C (46). Recrystallization from xylene (boiling point 137–140°C) converted the lower boiling form to the higher boiling form. This can be explained by enantiotropy, whereby the lower melting form becomes the more stable below the transition temperature, but crystallization is strongly influenced by the kinetics of nucleation and crystal growth and does not always give rise to the most stable form.

The freeze-crystallization of mannitol was found to be influenced by both the initial mannitol concentration and by the rate of freezing (47). In the range of 2.5–15%, the δ-polymorph was favored by higher concentrations, whereas the

9 Thermoanalytical and Crystallographic Methods

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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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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 ±0.20° 2θ, 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.

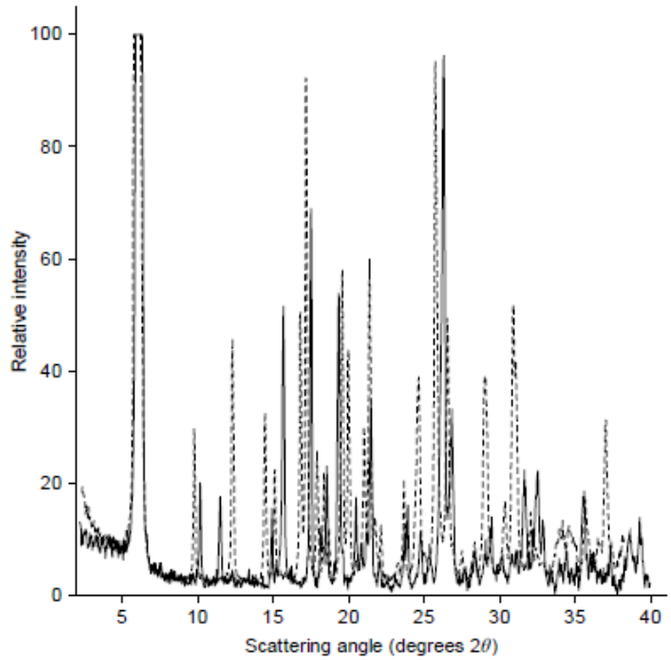

FIGURE 11 X-ray powder diffraction patterns of ibandronate sodium, Form A (*solid trace*) and Form B (*dashed trace*). The patterns have been adapted from data in United States patent applications 2006/0172975 and 2006/0172976 (65).

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.

For example, it has been observed that milled melt-quenched samples crystallize much more readily than do unmilled samples (82). It has been suggested that mechanical stress such as milling introduces "high energy sites" or nuclei, which can lead to crystallization.

Another factor that needs to be considered is the importance of the surface area of the powder to the phenomenon of surface crystallization. It has been demonstrated that the growth rate of crystals at the surface is much faster than the bulk growth rate (81). The implication of this observation is that solids with a higher surface area may be more prone to crystallization, and thus this might be one reason that milled samples crystallize faster.

As described above, it is very difficult to predict the crystallization tendency of amorphous solids, and therefore, experimental measurements of physical stability need to be made. Crystallization rates are normally measured using techniques such as XRPD, DSC, or spectroscopy. Given that crystallization may be slow under ambient conditions, it is obviously desirable to use accelerated stability testing conditions to evaluate the crystallization potential of an amorphous formulations. There are several factors that may complicate extrapolations of crystallization rates at high temperatures/relative humidities to ambient conditions. The crystal phase resulting from devitrification may not be the same for the different experimental conditions. For example, indomethacin crystallizes predominantly as the gamma polymorph at lower temperatures and relative humidities, and as the alpha form at higher temperatures and relative humidities (83,84).

In addition, the temperature dependence of the crystallization rate may not follow a simple Arrhenius-type relationship. This may result from crystallization of different polymorphs as mentioned above or from a non-Arrhenius dependence of molecular mobility on temperature as discussed earlier. Additional factors that have to be considered are changes in growth mechanism close to T_g as discussed previously and the effects of water content, which will be discussed in more detail below.

Based on the preceding discussion, it is clearly very difficult to predict precise crystallization rates under a given set of experimental conditions either from data obtained under accelerated stability conditions or from some experimentally measured property of the amorphous solid. However, given that different compounds have very different crystallization tendencies, it is still useful to assess crystallization tendency under a variety of conditions. Clearly, a compound that is stable under extreme conditions will be a viable candidate for formulation in an amorphous state. For compounds having a moderate tendency to crystallize, a measure of the effect of moisture and temperature on crystallization tendencies may help to determine if packaging could reduce the crystallization tendency and provide storage stability.

STABILIZATION AGAINST CRYSTALLIZATION: AMORPHOUS SOLID DISPERSION TECHNOLOGY

Single-component amorphous drugs often display physical instability over the intended shelf life of the product, and so polymeric crystallization inhibitors are frequently added to improve the physical stability. In order to be an effective inhibitor of bulk crystallization, the polymer must form an intimate mixture with the drug substance, resulting in a miscible, single-phase, amorphous solid dispersion. Molecular mixtures of amorphous solids are also generated in freeze-dried

biological products where sugars are added to improve the stability of proteins, and for polymeric film coatings where small molecules are added to the coating to act as plasticizers and to improve mechanical properties.

Several methods can be used to prepare amorphous solid dispersions, which involve destroying the crystal structure of the drug and mixing the resultant liquid with the polymer. One common method entails dissolution of crystalline drug and polymer in a solvent, followed by drug substance solvent removal. This process is typically carried out using techniques such as rotary evaporation, spray drying, freeze drying, air drying, or vacuum oven drying. Another common method entails heating the crystalline drug and polymer mixture to a temperature above the melting point of the drug and processing the mixed molten mass by means of melt extrusion. It has also been reported that solid dispersions can sometimes be prepared by co-milling the drug and the polymer (85). If crystallization is avoided during the preparation stage, and the drug and polymer remain intimately mixed, the system is said to have formed a miscible, single-phase, amorphous solid dispersion.

If miscibility is achieved, the behavior of the drug in the solid dispersion will be altered relative to that of the pure amorphous drug, and the properties of the solid dispersion will depend on the individual component properties as well as on the composition. There are several polymers that can be used to form solid dispersions, although it has been observed that some drug–polymer combinations are miscible whereas other systems are immiscible. From a theoretical perspective, molecular level mixing will only be achieved if the intermolecular interactions between the drug and the polymer are sufficiently favorable to overcome the cohesive interactions in the pure amorphous phases and crystallization of either component is avoided.

Derivatized cellulose polymers such as hydroxypropyl methylcellulose and the vinylpyrrolidone family of polymers [including poly(vinylpyrrolidone) and poly(vinyl-pyrrolidone-co-vinyl acetate)] have been found to be miscible with a variety of drug substances. In contrast, polymers or drugs containing strong cohesive interactions may not mix with chemically dissimilar components. For example, the ionic polymer, Eudragit E100 (which is a cationic methacrylate polymer), is only partially miscible with the itraconazole (86). Ibuprofen has been found to be largely immiscible with dextran (87), which is able to form extensive cohesive hydrogen bonding interactions.

ESTABLISHING MISCIBILITY

Determining the miscibility of the binary system can be challenging. For miscible systems, it would be expected that the glass transition temperature of the amorphous mixture is intermediate to that of the pure component T_g values. Thus, if a high T_g polymer is mixed with a low T_g drug substance, the T_g of the dispersion will increase with an increasing amount of polymer. An example is shown in Figure 17, where the T_g as a function of polymer concentration is shown for the felodipine–PVP solid dispersion system.

Assuming that two components are miscible, there are theoretical ways to predict the T_g of the mixture as a function of composition. The Gordon–Taylor equation is one of the most commonly used approaches for pharmaceutical systems (89), and assumes that the two components are miscible at all compositions and that the free volumes of the components are additive (90). The T_g of mixture, T_{g12}, is then defined as

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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

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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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Table I—FDA-Approved Commercially Marketed Salts

Anion	Percent[a]	Anion	Percent[a]
Acetate	1.26	Iodide	2.02
Benzenesulfonate	0.25	Isethionate[i]	0.88
Benzoate	0.51	Lactate	0.76
Bicarbonate	0.13	Lactobionate	0.13
Bitartrate	0.63	Malate	0.13
Bromide	4.68	Maleate	3.03
Calcium edetate	0.25	Mandelate	0.38
Camsylate[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
Esylate[e]	0.13	Pantothenate	0.25
Fumarate	0.25	Phosphate/diphosphate	3.16
Gluceptate[f]	0.13	Polygalacturonate	0.13
Gluconate	0.51	Salicylate	0.38
Glutamate	0.25	Stearate	0.25
Glycollylarsanilate[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	Teoclate[j]	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	10.82
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-Ethanedisulfonate. [d] Lauryl sulfate. [e] Ethanesulfonate. [f] Glucoheptonate. [g] *p*-Glycollamidophenylarsonate. [h] *N,N'*-Di(dehydroabietyl)ethylenediamine. [i] 2-Hydroxyethanesulfonate. [j] 8-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.

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. Saias *et al.* (9) reviewed the use of this salt form in preparing sustained-release preparations. More recently, latentiation of dihydrostreptomycin (10)

Table II—Non-FDA-Approved Commercially Marketed Salts

Anion	Percent[a]
Adipate	0.13
Alginate	0.13
Aminosalicylate	0.25
Anhydromethylenecitrate	0.13
Arecoline	0.13
Aspartate	0.25
Bisulfate	0.25
Butylbromide	0.13
Camphorate	0.13
Digluconate	0.13
Dihydrobromide	0.13
Disuccinate	0.13
Glycerophosphate	0.58
Hemisulfate	0.13
Hydrofluoride	0.13
Hydroiodide	0.25
Methylenebis(salicylate)	0.13
Napadisylate[b]	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	0.33
Tromethamine[e]	0.33
Metallic:	
Barium	0.33
Bismuth	0.33

[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 streptothrycin 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 LD_{50} 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 LD_{50}.

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 streptomyces 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

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 electronegative 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 LD_{50} 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-

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
Anthraquinone-1,5-disulfonic acid	Cephalexin	Stability, absorption	58
Arabogalactan sulfate (arabino)	Various alkaloids	Prolonged action	59, 60
Arginine	Cephalosporins	Toxicity	61
	α-Sulfobenzylpenicillin	Stability, hygroscopicity, toxicity	62
Aspartate	Erythromycin	Solubility	63
Betaine	Tetracycline	Gastric absorption	64
Bis(2-carboxychromon-5-yloxy)alkanes	7-(Aminoalkyl)theophyllines	Activity, prolonged prophylactic effect	65
Carnitine	Metformin	Toxicity	66
4-Chloro-*m*-toluenesulfonic acid	Propoxyphene	Organoleptic properties	67
Decanoate	Heptaminol	Prolonged action	68
Diacetyl sulfate	Thiamine	Stability, hygroscopicity	69
Dibenzylethylenediamine	Ampicillin	Prolonged action	70, 71
Diethylamine	Cephalosporins	Reduced pain on injection	72
Digusiacyl phosphate	Tetracycline	Activity	73
Dioctyl sulfosuccinate	Vincamine	Organoleptic properties	74
Embonic (pamoic) acid	Kanamycin	Toxicity	75
	2-Phenyl-3-methylmorpholine	Toxicity	76
Fructose 1,6-diphosphoric acid	Tetracycline	Solubility	77
	Erythromycin	Solubility	
Glucose 1-phosphoric acid, glucose 6-phosphoric acid	Tetracycline	Solubility	77
	Erythromycin	Solubility	
L-Glutamine	Erythromycin	Solubility, activity, stability	52
Hydroxynaphthoate	Bephenium	Toxicity	78
2-(4-Imidazolyl)ethylamine	Prostaglandin	Prolonged action	79
Isobutanolamine	Theophylline	Stability	80
Lauryl sulfate	Vincamine	Organoleptic properties	81
Lysine	α-Sulfobenzylpenicillin	Toxicity, stability, hygroscopicity	62
	Cephalosporins		61
Methanesulfonic acid	Pralidoxime (2-PAM)	Solubility	82
N-Methylglucamine	α-Sulfobenzylpenicillin	Toxicity, stability, hygroscopicity	62
	Cephalosporins	Reduced pain on injection	72
N-Methylpiperazine	Phenylbutazone	Toxicity, faster onset of action	83
Morpholine	Cephalosporins	Reduced pain on injection	72
2-Naphthalenesulfonic acid	Propoxyphene	Organoleptic properties	84
Octanoate	Heptaminol	Prolonged action	68
Probenecid	Pivampicillin	Organoleptic properties	85
Tannic acid	Various amines	Prolonged action	86, 87
Theobromine acetic acid	Propoxyphene	Activity	88
3,4,5-Trimethoxybenzoate	Tetracycline	Organoleptic properties	89
	Heptaminol	Prolonged action	68
Tromethamine	Aspirin	Absorption (oral)	90
	Dinoprost (prostaglandin $F_{2\alpha}$)	Physical state	91

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-

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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 model[1] in terms of an equation developed by Nernst and Brunner (97):

$$\frac{dW}{dt} = \frac{DS}{h}(C_s - C) \qquad \text{(Eq. 1)}$$

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 caused the dissolution rate of phenobarbital sodium to

[1] The authors recognize the existence of other models; this one was chosen simply

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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.

$$DH^+Cl^-_{(solid)} \overset{K_{sp}}{\rightleftharpoons} (DH^+)_{aq} + (Cl^-)_{aq}$$

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 *in vitro* 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 *in vitro* and *in vivo*	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
In vitro dissolution rates of aminorex dosage forms and their correlation with *in vitro* 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

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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.

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

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 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 resinates formed between dextran gel[2] 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.

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 estolate 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 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.

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

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.

$$\text{solid drug} \xrightarrow{\text{dissolution}} \text{dissolved drug} \xrightarrow{\text{absorption}} \text{drug in circulation}$$

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.

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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	156
Determination of *in vivo* and *in vitro* release of theophylline aminoisobutanol in a prolonged-action system	198
Ion-exchange resin salts for oral therapy: carbinoxamine	199
Latentiation of dihydrostreptomycin by pamoate 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 insolubility of warfarin in acidic gastric fluids precluded its 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

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.

Budolfsen *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).

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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 streptothrycin 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 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.

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 (quinalbarbitone) and its sodium salt	230
Absorption rate of barbiturates in humans	231
Morphine and atropine mucate	232
Excretion of bephenium 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

Often, salt formation can be used to modify drug absorption and dose tolerance favorably. For example, aminosalicylic 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.

Aminosalicylic 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 aminosalicylic 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,

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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 fenoprofen 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 *in vitro* fungistatic evaluation of some *N*-substituted amides and amine salts of sorbic acid	261
Antiamebic studies on clamoxyquin [5-chloro-7-[[(3-diethylaminopropyl)amino]methyl]-8-quinolinol] *in vitro* 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-*N*-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 (glycyrrhetinic acid) and its derivatives	266
Pharmacological properties of glycyrrhetinic 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 edetic (ethylenediaminetetraacetic) 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 quinapyramine[3], a trypanocidal drug, were determined (238). 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 (LT_{50}) were determined for each salt. Both the LT_{50} and LD_{50} (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 LT_{50}'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 LD_{50}'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] Aritrycide.

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Table XI—Additional References on Toxicological Considerations

Topic	Reference
Toxicity and absorption of 2-sulfanilimidopyridine and its soluble sodium salt	285
Sorbic acid as a fungistatic agent for foods: harmlessness of sorbic acid as a dietary component	286
Toxicity and distribution of erythromycin	287
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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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