SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RAD: 13-302836- -7 **FECHA**: 2014-12-04 17:36:20
DEP: 2020 DIRECCION DE NUEVAS CREACIONES **EVE:** 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II **FOLIOS**: 7
ACT: 519 PRESENTACION

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
LUZ CLEMENCIA DE PÁEZ

Asunto: Radicación: 13-302836- -7
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 15164
Folios: 7

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/JP2012/065180				
Fecha de Presentación Internacional	07/06/2012				

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:	Rad. No. 13-302836-00000-0000	30/Diciembre/2013
Reivindicaciones:	Rad. No. 13-302836-00000-0000	30/Diciembre/2013
Dibujos:	Rad. No. 13-302836-00000-0000	30/Diciembre/2013
Oposiciones:	Rad. No. 13-302836-00003-0000	11/Abril/2014
Respuesta del solicitante:	Rad. No. 13-302836-00006-0000	24/Julio/2014
Prioridad:	US 61/494,088	07/Junio/2011

2. Análisis de la Prioridad

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

3. Objeto de la invención

Título de la solicitud: “Formulación liofilizada que contiene aripiprazol”.

El problema planteado en esta solicitud consiste en la necesidad de suministrar una

formulación de aripiprazol liofilizada con dispersabilidad mejorada.

Para resolver este problema técnico la solicitud en estudio proporciona una formulación de aripiprazol liofilizado obtenida por secado por aspersión y congelamiento.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A 61K9/16, 9/19.

4. **De la Solicitud de Patente**

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Si bien la reivindicación 1 no es clara porque define a la composición farmacéutica en términos del procedimiento para su elaboración, el cual no es una característica técnica esencial de la misma. Se solicita hacer la corrección correspondiente.

La reivindicación 1 no es clara cuando emplea la expresión “con el fin de congelar…” porque indica el uso de un paso en el proceso de obtención de la composición farmacéutica, el cual no es una característica técnica que defina a la invención. Se sugiere hacer la corrección correspondiente.

Las reivindicaciones 3 y 18 no son claras cuando emplean términos como “esencialmente”, “sustancialmente” porque no son limitativos, por lo cual hacen opcional a la característica y no limita el alcance de la reivindicación. Se sugiere incluir las características opcionales o preferentes en una reivindicación dependiente.

Las reivindicaciones 4-6, 14 y 15 no son claras cuando emplean los términos “aproximadamente”, “alrededor de”, entre otros, porque son imprecisos, por lo cual no permiten distinguir la invención del estado de la técnica. Se sugiere sustituirlo por un término preciso o un rango concreto.

La reivindicación 13 no es clara cuando señala que el aripiprazol está “en forma de monohidrato”, porque se refiere a una forma cristalina sin que esté debidamente caracterizada por el patrón de difracción de rayos X en mono cristal, el patrón de difracción de rayos X en polvo (DRXP) con por lo menos los 20 picos correspondientes a las intensidades de los haces difractados o a los espacios interplanares d_{hkl} según los índices de Miller (hkl) medidos de acuerdo con el ángulo de difracción de Bragg 2θ en el intervalo más representativo comprendido entre 5° hasta 90°, las espectroscopías Raman e IR, la espectroscopía RMN-C^{13}, o, los métodos de análisis térmico: TGA, DTA y DSC. Se sugiere caracterizar el pseudopolimorfo o eliminar dicha expresión del pliego reivindicatorio.

La reivindicación 19 no es clara cuando emplea las expresiones “denota buena dispersabilidad”, “forma una suspensión de aripiprazol homogénea cuando se reconstituye con agua”, porque se refiere a resultados a alcanzar, no definen a la composición porque la invención no queda definida por el resultado a alcanzar, puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Se sugiere reemplazarlas por las características esenciales, estructurales o funcionales, que contribuyen a la obtención de ese efecto.

5. **Análisis de la Oposición**

Argumentaciones de Lafrancol S.A.S.

Afirma la sociedad oponente que *“…las composiciones y formulaciones farmacéuticas que comprenden Aripiprazol liofilizado en polvo (…) son prácticas y eventos totalmente conocidos en la industria farmacéutica…”* Sustenta lo anterior con base en el texto “The

Merck Index", citando de este las patentes EP 367141 y US 5006528, que se refiere al principio activo aripiprazol. Además, señala que *"…la solicitud en comento pretende reivindicar combinaciones, los cuales no se consideran invenciones. En este sentido, la mezcla (Combinaciones) de productos (Moléculas) conocidos y su variación, bien sea en uso, forma, dimensión o de materia, NO se consideran invenciones por cuanto no son susceptibles de aplicación industrial, nivel inventivo y novedad, luego NO pueden acceder a beneficios patentarios (...) un efecto sinérgico nuevo no se considera inventivo como tal, dado que puede ser obvio para una persona versada en la materia…"* Finaliza diciendo que la solicitud carece de novedad y nivel inventivo frente a los documentos VAZHAPPILLY REMA ET AL: "Eicosapentatoic acid and docosahexanoic acid production potential of microalgae and their heterotrophic growth"; WEN Z-Y ET AL: "Heterotrophic production of eicosapentatoic acid by microalgae"; COHEN ZVI: "Production potential of eicosapentaenoic acid by Monodus subterraneus"; FR 294126; CHRON KOON TAN ET AL: "Screening of diatoms for heterotrophic eicosapentaenoic acid production"; JP 2007 236277 y WO 2007/047805, sin dar explicación alguna al respecto y sin aportar ninguno de estos últimos documentos.

Respuestas del Solicitante

La solicitante señala que *"…en el texto de la oposición no se realiza un análisis claro que permita determinar con facilidad por qué los documentos afectarían la novedad y/o el nivel inventivo de la presente solicitud…"* Sin embargo, la solicitante agrega que *"… ninguno de los documentos citados por el Examinador <sic> se refieren al aripiprazol y en su lugar, se refieren a ácidos graos insaturados. En particular, se refieren al ácido eicosapentaenoico (EPA) y al ácido docosahexanóico (DHA). Adicionalmente, los documentos citados no enseñan o sugieren una formulación de aripiprazol liofilizado y por lo tanto, la invención objeto de protección en la presente solicitud de patente es completamente diferente de la materia divulgada en las anterioridades citadas."*

Respuesta del Examinador Técnico

En cuanto al documento "The Merck Index", y la mención de las patentes EP 367141 y US 5006528, referenciadas en dicho documento, no resulta útil para afectar la novedad o el nivel inventivo de la invención, toda vez que no pertenecen al mismo campo técnico de la invención, es decir, no se refieren o siquiera mencionan composiciones farmacéuticas que comprendan aripiprazol, de tal manera que la simple mención del principio activo no es suficiente para ser tenido en cuanta como estado de la técnica para la presente invención. Aunado a lo anterior, no es clara la mención que la opositora hace a "combinaciones", cuando la invención no se refiere a ese tema.

En lo que tiene que ver con los demás documentos citados por la opositora, es importante resaltar que en ningún momento proporciona argumentos que den cuenta de la carencia de alguno de los requisitos de patentabilidad de la invención frente a dichos documentos, en otras palabras, la oposición no está fundamentada.

Sin embargo, al evaluar el contenido de los documentos VAZHAPPILLY REMA ET AL: "Eicosapentatoic acid and docosahexanoic acid production potential of microalgae and their heterotrophic growth"; WEN Z-Y ET AL: "Heterotrophic production of eicosapentatoic acid by microalgae"; COHEN ZVI: "Production potential of eicosapentaenoic acid by Monodus subterraneus"; FR 294126; CHRON KOON TAN ET AL: "Screening of diatoms for heterotrophic eicosapentaenoic acid production"; JP 2007 236277 y WO 2007/047805, ninguno pertenece al mismo campo técnico de la invención toda vez que se refieren al ácido eicosapentaenoico (EPA) y al ácido docosahexanóico (DHA), como bien lo identificó la solicitante, y no a composiciones que comprendan aripiprazol. Así las cosas, ninguno de los documentos citados por la opositora será tenido en cuenta en el examen de patentabilidad.

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, 07 de junio de 2011, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	WO2005/041937	Controlled release sterile injectable aripiprazole formulation and method	15/Mayo/2005
D2	Artículo	Improvement of dissolution rates of poorly water soluble apis using novel spray freezing into liquid technology	Septiembre/2002

D1[1] enseña una composición de aripiprazol obtenida mediante un proceso que comprende formar una suspensión estéril de aripiprazol, el agente suspensor, el agente de carga y el buffer, reducir el tamaño de partícula a un rango de 1-30micras y congelar la suspensión para obtener partículas liofilizadas (pág. 3, línea 27 a pág. 4, línea 9 y pág. 10, líneas y 1-3).

D2[2] da a conocer que la velocidad de disolución de fármacos poco solubles en agua puede ser aumentada mediante el empleo de la técnica de secado por aspersión y congelamiento, esto es, mediante la aspersión de una suspensión que comprende el activo en un vehículo a baja temperatura para su congelación, seguida del secado a presión reducida (resumen, págs. 1278-1280), lo que da lugar moléculas del principio activo dispersas homogéneamente en la matriz de excipiente (pág. 1279).

7. **Examen de Patentabilidad** (Art 14 D486)

Novedad (Art 16 D486)

La reivindicación 1 consiste en una formulación de aripiprazol liofilizado que comprende aripiprazol, un vehículo para el aripiprazol y agua para inyectables.

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

Características esenciales	D1
Formulación de aripiprazol liofilizado que comprende:	Formulación de aripiprazol liofilizado que comprende (pág. 3, línea 27 a pág. 4, línea 9 y pág. 10, líneas y 1-3):
aripiprazol	aripiprazol
un vehículo para el aripiprazol	un vehículo para el aripiprazol que comprende el agente suspensor, un agente de carga, un buffer y opcionalmente un regulador de pH
agua para inyectables	agua para inyectables

Las características esenciales de la composición de la reivindicación 1 habían sido divulgadas previamente en el documento D1 el cual enseña una composición liofilizada de aripiprazol que comprende aripiprazol, un vehículo para el aripiprazol que comprende el agente suspensor, un agente de carga, un buffer y opcionalmente un regulador de pH y agua (pág. 3, línea 27 a pág. 4, línea 9 y pág. 10, líneas y 1-3).

En consecuencia, la reivindicación 1 no es nueva, según lo divulgado en el documento D1.

Asimismo, el documento D1 enseña que el tamaño de partícula es de 1 a 30micrones, preferiblemente de 1 a 20 micrones, y más preferiblemente de 1 a 10 o 15 micrones (pág. 5, líneas 25-30). Que la cantidad de aripiprazol en la formulación liofilizada es superior a 50% p/p (ejemplos 1 y 2). Que la formulación liofilizada comprende

1 http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=2005041937A2&KC=A2&FT=D&ND=3&date=20050512&DB=worldwide.espacenet.com&locale=en_EP

2 Jiahui Hu, True L. Rogers, Judith Brown, Tim Young, Keith P. Johnston, Robert O. Williams; Improvement of dissolution rates of poorly water soluble apis using novel spray freezing into liquid technology; Pharmaceutical Research, Vol. 19, No. 9, Septiembre 2002; págs. 1278-1284.

aripiprazol y agua para inyección, así como uno o más agentes de suspensión, uno o más agentes de carga, uno o más reguladores de pH (pág. 2, líneas 23-29, pág. 3, líneas 15-22). Que el agente suspensor puede ser la sal sódica de carboximetilcelulosa, que el agente de carga puede ser manitol, que el buffer puede ser fosfato de sodio y el agente regulador de pH puede ser hidróxido de sodio (Tabla, pág. 16). También que el aripiprazol puede estar en forma monohidrato (pág. 10, líneas 27-31). Finalmente, es pertinente señalar que tanto el tamaño de partícula como la densidad volumétrica corresponden a propiedades intrínsecas de la composición.

Por lo tanto, las reivindicaciones dependientes 2 - 13, 19 y 20 no mencionan alguna característica que haga nueva a la composición de la reivindicación 1, por lo cual se considera que no son nuevas.

Nivel Inventivo (Art18 D486)

La reivindicación 14 consiste en un proceso para producir una formulación de aripiprazol liofilizado, que comprende los siguientes pasos: (e'-1) congelar por aspersión una suspensión de aripiprazol que tiene un tamaño medio de partícula variable en el rengo entre 1 y 10 micrones, para obtener partículas congeladas por aspersión; y (e'-2) secar las partículas congeladas por aspersión para obtener partículas secadas por aspersión y congelamiento.

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

Características esenciales	D1	D2
Proceso para producir una formulación de aripiprazol liofilizado, que comprende los siguientes pasos:	Proceso para producir una formulación de aripiprazol liofilizado, que comprende (págs. 3y 4):	Proceso para producir una formulación de fármacos poco solubles liofilizada, que comprende los siguientes pasos (resumen):
(e'-1) congelar por aspersión una suspensión de aripiprazol que tiene un tamaño medio de partícula variable en el rengo entre 1 y 10 micrones, para obtener partículas congeladas por aspersión; y	- formar una suspensión estéril de aripiprazol, reducir el tamaño de partícula a un rango de 1-30micras y congelarla	- formar una suspensión del activo y congelarla por aspersión
(e'-2) secar las partículas congeladas por aspersión para obtener partículas secadas por aspersión y congelamiento;	- secar la torta de suspensión congelada	- secar las partículas congeladas

El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga una un proceso para elaborar una composición de aripiprazol que comprende formar una suspensión estéril de aripiprazol, el agente suspensor, el agente de carga y el buffer, reducir el tamaño de partícula a un rango de 1-30micras y congelar la suspensión para obtener partículas liofilizadas (pág. 3, línea 27 a pág. 4, línea 9 y pág. 10, líneas y 1-3).

La diferencia entre la invención, definida en la reivindicación 1 y el proceso del documento D1 consiste en la congelación de la suspensión se hace por aspersión, mientras que en la anterioridad se congela la suspensión sin aspersión de la misma.

El efecto que logra el incluir esta característica es que da lugar a composiciones de aripiprazol con dispersabilidad mejorada.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular como la necesidad de suministrar un proceso para obtener composiciones farmacéuticas de aripiprazol liofilizado con dispersabilidad mejorada.

Sin embargo, el documento D2 enseña que la velocidad de disolución de fármacos poco solubles en agua puede ser aumentada mediante el empleo de la técnica de secado por aspersión y congelamiento, esto es, mediante la aspersión de una suspensión que comprende el activo en un vehículo a baja temperatura para su

congelación, seguida del secado a presión reducida (resumen, págs. 1278-1280), lo que da lugar a una matriz que comprende el fármaco homogéneamente disperso (pág. 1279).

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría el paso de congelar por aspersión la suspensión del principio activo, divulgada en el documento D2, en la obtención de composiciones de aripiprazol enseñada en el documento D1, para llegar así al objeto de la reivindicación 14 de la solicitud, por lo cual se considera obvia.

En conclusión, la reivindicación 14 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Asimismo, el documento D1 enseña que la disminución del tamaño de partícula se puede hacer por molienda húmeda (pág. 10, líneas 5-9).

Por lo tanto, las reivindicaciones dependientes 15-18 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

8. Conclusión

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

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO2005/041937	1-13, 19 y 20	Novedad
D1	WO2005/041937	14-18	Nivel inventivo
D2	Artículo	14-18	Nivel inventivo

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

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

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

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

JESUS FERNEL GARCÍA GARCÍA
Director de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-12-09

Elaboró: Rubiela Pacanchique Vargas
Revisó: Gloria Jacqueline Alfonso Rozo

Improvement of Dissolution Rates of Poorly Water Soluble APIs Using Novel Spray Freezing into Liquid Technology

Jiahui Hu,[1] True L. Rogers,[1] Judith Brown,[1] Tim Young,[2,3] Keith P. Johnston,[3] and Robert O. Williams III[1,4]

Received May 16, 2002; accepted May 30, 2002

Purpose. To develop and demonstrate a novel particle engineering technology, spray freezing into liquid (SFL), to enhance the dissolution rates of poorly water-soluble active pharmaceutical ingredients (APIs).

Methods. Model APIs, danazol or carbamazepine with or without excipients, were dissolved in a tetrahydrofuran/water cosolvent system and atomized through a nozzle beneath the surface of liquid nitrogen to produce small frozen droplets, which were subsequently lyophilized. The physicochemical properties of the SFL powders and controls were characterized by X-ray diffraction, scanning electron microscopy (SEM), particle size distribution, surface area analysis, contact angle measurement, and dissolution.

Results. The X-ray diffraction pattern indicated that SFL powders containing either danazol or carbamazepine were amorphous. SEM micrographs indicated that SFL particles were highly porous. The mean particle diameter of SFL carbamazepine/SLS powder was about 7 μm. The surface area of SFL danazol/poloxamer 407 powder was 11.04 m^2/g. The dissolution of SFL danazol/poloxamer 407 powder at 10 min was about 99%. The SFL powders were free flowing and had good physical and chemical stability after being stored at 25°C/60%RH for 2 months.

Conclusions. The novel SFL technology was demonstrated to produce nanostructured amorphous highly porous particles of poorly water soluble APIs with significantly enhanced wetting and dissolution rates.

KEY WORDS: danazol; carbamazepine; spray freezing into liquid; dissolution; stability.

INTRODUCTION

Many potentially bioactive molecules have been rejected during the early stages of development because they are poorly water soluble and difficult to wet. Active pharmaceutical ingredients (APIs) with poor aqueous solubility often demonstrate low bioavailability when administrated orally due to the dissolution rate-limiting absorption in the gastrointestinal (GI) tract. Of particular interest is the poorly water soluble, highly permeable APIs discussed in the Biopharmaceutics Classification System (BCS Class II) (1), such as danazol and carbamazepine. The irregular and delayed absorption of danazol and carbamazepine has been attributed to slow dissolution rates (2,3). The physicochemical properties of the APIs play a significant role in controlling their dissolution from a dosage form. According to the Noyes-Whitney equation, the aqueous solubility of an API is a factor determining dissolution rate. Other factors include the particle size distribution, degree of crystallinity, as well as the presence of surfactants and other additives. Other physical properties such as wettability, density, and viscosity contribute to particle flocculation, flotation, and agglomeration, which further influence dissolution rates.

Increasing the dissolution rate of poorly water soluble APIs is a significant challenge to pharmaceutical scientists. Technologies that have been commonly used to improve the dissolution rate of poorly water soluble APIs include mechanical milling, spray drying, precipitation, and freeze-drying. Spray drying is a widely used technology (4). However, because the spray drying process uses elevated temperatures, it is not always appropriate for use with thermolabile compounds. The process precipitation with a compressed fluid antisolvent (PCA), also referred to as SEDS or SAS, has been used to produce particles containing poorly water-soluble API and water-soluble excipients (5). In some cases this process can be limited by the lack of solvent systems compatible with compressed carbon dioxide that dissolve both hydrophilic and hydrophobic substances simultaneously. Although lyophilization or freeze-drying (6) is a promising technique for producing pharmaceutical powders, the freezing rate in some cases is too slow so that the solvent crystallizes as it is frozen.

The objective of this study was to develop and demonstrate the use of the novel spray freezing into liquid (SFL) particle engineering technology to enhance the dissolution rate of two poorly water soluble APIs, danazol and carbamazepine, and to determine the physicochemical stability of SFL powders.

SFL is a novel cryogenic atomization technology in which either an aqueous or an aqueous-organic cosolvent solution containing an API and pharmaceutical excipient(s) is atomized directly into a compressed liquid, such as compressed fluid CO_2, helium, propane, ethane, or the cryogenic liquids nitrogen, argon, or hydrofluoroethers. SFL technology was created by the adaptation of several atomization processes. SFL is derived in part from the PCA process, which utilizes liquid-liquid impingement between an organic or organic/aqueous feed solution through a nozzle that is submerged into compressed CO_2 fluid. As the two liquids collide, the high Reynolds and Webber numbers lead to intense atomization into micronized droplets. In PCA the solvent(s) must be miscible with compressed fluid CO_2 to produce dry particles from the microdroplets. The low solubility of water in CO_2 limits markedly the use of solvents containing water, which are often needed to dissolve hydrophilic excipients. Other limitations include the need for elevated pressures and the recovery of product from a high-pressure vessel. With the novel SFL technology, the solvents are frozen during the spray and are not required to be miscible with the cryogenic liquid in contrast to PCA. Liquid nitrogen is utilized as the cryogenic fluid in this study instead of CO_2 because of the ultra-rapid freez-

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ing rates resulting from its low boiling point of –196°C. The feed solution is atomized below the liquid surface in a dewar flask at atmospheric pressure. The suspended frozen powder can then be separated from the cryogenic continuous phase by sieve filtration or other means.

In spray freezing into vapor over liquid (SFV/L) (7–11), the feed solution is atomized through a nozzle positioned at a distance above the boiling refrigerant. The droplets may begin to solidify while passing through the vapor gap and then freeze completely as contact is made with the boiling refrigerant liquid. Spray-freezing into halocarbon vapor over liquid has been performed by Briggs and Maxwell and Adams *et al.* (7,8). The resulting particles ranged in diameter from 0.84 mm to 1.68 mm (7). Halocarbon refrigerants present problems. Chlorofluorocarbons are deleterious to the ozone layer, and the replacement hydrofluoroalkane refrigerants are expensive (12). In addition, both chlorofluorocarbons and hydrofluoroalkane are suitable solvents for lipophilic compounds, so API extraction into the halocarbon cryogen can result in low product yield (13).

Gombotz *et al.* and Gusman and Johnson developed spray freezing into nitrogen vapor over liquid technologies for the purpose of capturing frozen API particles following atomization (14,15). As the atomized droplets pass through the vapor gap above the liquid nitrogen, they may collide and coalesce. The solutes may precipitate and grow in the unfrozen liquid droplets as they cool and/or coalesce. The particle morphology is not quenched until the droplets are fully solidified on contact with the liquid nitrogen phase below the vapor. Each of these factors may broaden the particle size distribution.

In the novel SFL technology, the solution is sprayed below the surface of the cryogenic liquid phase to avoid particle growth in the vapor gap described above for the conventional SFV/L process. The liquid-liquid impingement that occurs as the feed solution impacts the cryogenic media results in intense atomization into fine microdroplets that freeze instantaneously. A schematic representation of the SFL apparatus is shown in Fig. 1. A pressurized syringe pump is used to propel the feed solution from the solution vessel through an insulated nozzle that is submerged beneath the surface of the cryogenic liquid. Nitrogen is the cryogen of choice because it is inexpensive, environmentally friendly, inert, and may be used at atmospheric pressure, unlike CO_2. Because of the ultra-rapid freezing rates achieved by atomizing the feed solution directly into liquid nitrogen, a cryogenic suspension containing the dispersed frozen microparticles is produced. The SFL micronized powder can then be separated from the liquid nitrogen by using a fine sieve to collect the powder. The frozen powder is then dried, in this study by lyophilization, to produce the dry SFL micronized powder.

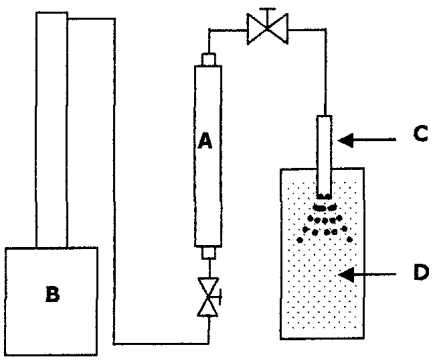

Fig. 1. Schematic diagram of the SFL apparatus illustrating the solution cell (A), high pressure pump (B), atomizing nozzle (C), and the cryogenic liquid cell (D).

The SFL process offers a variety of advantages relative to traditional technologies mentioned above. Because of intense atomization, the formation of high-surface area droplets and the low intrinsic temperature of liquid nitrogen, ultra-rapid freezing rates are achieved. As a result of the ultra-rapid freezing rates, the time for phase separation of solutes within the feed solution is minimized. Therefore, the API molecules may be dispersed homogeneously throughout the solidified excipient matrix of the frozen microparticle. After lyophilization, the dried microparticle retains the shape of the microdroplet, but is highly porous due to the channels created as the solvent(s) are removed. The API is molecularly dispersed within a homogeneous excipient matrix that composes the porous microparticle.

Aqueous media should immediately wet and dissolve the SFL microparticles due to the high surface area of the porous microparticles. The dissolution media will fill the pore channels and dissolve the API and hydrophilic excipients, which are utilized to enhance the aqueous dissolution of a lipophilic API.

MATERIALS AND METHODS

Chemicals

Danazol USP and carbamazepine USP were obtained as micronized powders. A polyoxyethylene-*b*-polyoxypropylene-*b*-polyoxyethylene triblock copolymer (Pluronic F127; Poloxamer 407), polyvinylpyrrolidone (PVP) K-15, sodium lauryl sulfate (SLS) NF, sodium taurocholate, lecithin NF, potassium chloride NF, and sodium hydroxide NF were purchased from Spectrum Quality Products Inc. (Gardena, CA, USA). Tetrahydrofuran (THF), methanol, acetonitrile, and acetic acid were obtained from EM Industries Inc. (Gibbstown, NJ, USA). Purified water was obtained from an ultrapure water system (Milli-QUV plus, Millipore S. A., Molsheim Cedex, France).

SFL Processing

SFL feed solutions were prepared according to the following procedure. Danazol or carbamazepine was dissolved in THF. Poloxamer 407, PVP K-15, and/or SLS were dissolved in water. The aqueous and organic solutions were then mixed to obtain danazol/excipient blend and carbamazepine/excipient blend SFL feed solutions.

A schematic diagram of the SFL apparatus is shown in Fig. 1. The SFL feed solution was placed into the solution cell (A). A constant pressure of 4000 PSI from the ISCO syringe pump (B) (Model 100 DX, ISCO Inc., Lincoln, NE, USA) provided a flow rate of 11 mL/min for the SFL feed solution. The solution cell was connected to an insulated nozzle (C), which was positioned to atomize the SFL feed solution beneath the surface of the cryogenic liquid (D). In these experiments liquid N_2 was used as cryogenic liquid. The atomizing nozzle used was composed of polyetheretherketone tubing

with an inner diameter of 63.5 μm. The SFL feed solutions were then sprayed through the nozzle and atomized directly into the liquid N_2 phase. Frozen particles formed instantaneously. The frozen particles were collected and lyophilized in a Labconco freeze dryer (Freeze dryer 5, Labconco Corporation, Kansas City, MO, USA) for 48 h.

To investigate the influence of the ultra rapid freezing rate in conjunction with atomization in the SFL technology, a lyophilized mixture formed directly by conventional lyophilization process was used as a control (LM control). The lyophilized mixture was made of an API and excipients frozen at −78°C and lyophilized for 48 h. The compositions of the lyophilized mixtures were identical to the SFL powders. The influence of the excipients on the physicochemical properties of the SFL powders was further studied by comparison with a physical mixture of API and excipients (PM control). The physical mixtures were of the same composition as the SFL API powders. The micronized APIs were also used as a control. The SFL powders and control samples were stored in glass vials over desiccant in a vacuum desiccator at room temperature before the characterization measurement.

Powder X-Ray Diffraction

Powder X-ray diffraction (XRD) was conducted using $CuK\alpha_1$ radiation with a wavelength of 1.54054 Å at 40 kV and 20 mA from a Philips 1720 X-ray diffractometer (Philips Analytical, Natick, MA, USA). The sample powders were placed in a glass sample holder. Samples were scanned from 5 to 45° (2θ) at a rate 0.05°/s. For comparative purposes the three highest values for relative line intensity and their corresponding line position 2θ were compared for the API samples (16).

Scanning Electron Microscopy (SEM)

A HITACHI S-4500 field emission SEM (Hitachi Instruments Inc., Irvine, CA, USA) was used to examine the surface morphology of each sample powder. The sample was fixed to a SEM stage with double-sided adhesive tape and gold sputter coated.

Particle Size Distribution

The particle size distribution of the sample powders was determined using the method based on a time-of-flight measurement (Aero-Disperser®, Amherst Process Instrument, Amherst, MA, USA). The mean particle diameter and span index were determined for the SFL powers and controls.

Surface Area Measurement

A Nova 3000 surface area analyzer (Quantachrome Corporation, Boynton Beach, FL, USA) was used to determine N_2 sorption at 77.40° K. The surface area per unit powder mass was calculated from the fit of adsorption data to the Brunauer, Emmett, and Teller (BET) equation (17).

Contact Angle Measurement

Compacts of sample powders were prepared at a 500 kg compression force using a Carver Laboratory Press (Model M, ISI Inc., Round Rock, TX, USA) with flat-faced 6 mm diameter punches. A droplet of purified water or fed state simulated intestinal fluid (FeSSIF) media (3 μl) was placed onto the surface of compact and observed using a low power microscope. The contact angle was determined by measuring the tangent to the curve of the droplet on the surface of the compact using a goniometer (Model No.100-00-115, Ramè-Hart Inc., Mountain Lakes, NJ, USA).

Dissolution Studies

The amount of API dissolved, as a function of time, was determined using USP Apparatus 2 method (Vankel 7000, Vankel Technology Group, Cary, NC, USA). All dissolution studies were conducted at sink conditions. The FeSSIF media was prepared according to Dressman *et al.* (18). SFL danazol/poloxamer 407 powders or control samples containing approximately 3 mg danazol were added to 900 mL of FeSSIF media (37°C). The paddle speed was 50 rpm. A 5-mL aliquot was taken at each time point and filtered through a 0.45-μm filter then diluted with acetonitrile, filtered through a 0.45-μm filter again, and analyzed by HPLC (19,20). Purified water (900 mL) was used for the carbamazepine as the dissolution media and the same operating parameters discussed for danazol. Dissolution profile for SFL carbamazepine/excipients powders were studied as well as their controls.

Stability Study

Stability studies were conducted at 25°C/60%RH. Sample powders were stored in the capped glass vials (20 mL) and characterized as a function of exposed time.

Statistical Analysis

The data were compared using a Student's *t* test of the two samples assuming equal variances to evaluate the differences. The significance level ($\alpha = 0.05$) was based on the 95%probability value ($p < 0.05$).

RESULTS AND DISCUSSION

Crystallinity

The crystallinity greatly impacts the solubility and dissolution rate of poorly water-soluble APIs (21). The chemical potential of a metastable high-energy amorphous state can be markedly larger than that of an equilibrium crystal. This higher chemical potential can lead to a substantially greater local solubility of the API near the interface and thus a faster mass transfer rate into the dissolution media. Therefore, the crystallinity of an API may be reduced to enhance the dissolution rate. Powder XRD patterns of SFL powders and control samples are presented in Figs. 2 and 3. Micronized bulk danazol had a similar X-ray diffraction pattern to that reported by Liversidge and Cundy (22). The high peak intensities of bulk danazol indicated a high degree of crystallinity. The physical mixture and lyophilized mixture showed the characteristic diffraction peaks (16) of danazol at 15.8, 17.2, and 19.0 (2θ). The X-ray pattern of the SFL danazol/poloxamer 407 powder exhibited a significant reduction in peak intensity for danazol. The diffraction peaks of danazol were not evident whereas those of poloxamer 407 at 19.3 and 23.4 (2θ) were present. This absence of peaks indicated that danazol contained in SFL danazol/poloxamer 407 powder was in an amorphous state. Similarly, lack of crystallinity was also

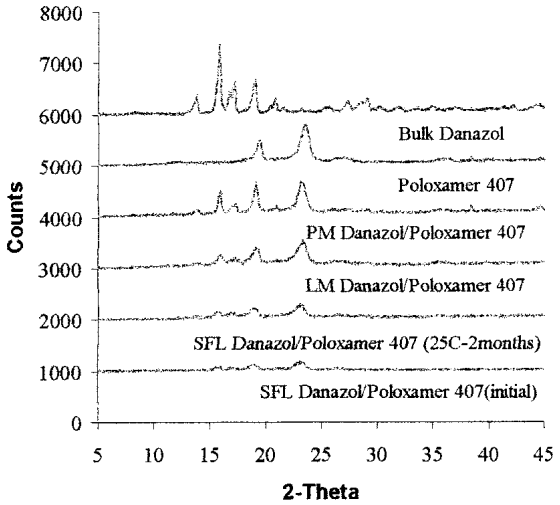

Fig. 2. Powder X-ray diffraction patterns of micronized danazol, PM danazol/poloxamer 407, LM danazol/poloxamer 407, and SFL danazol/poloxamer 407.

found for the SFL carbamazepine/SLS powder (Fig. 3). The absence of characteristic peaks of carbamazepine (23) at 15.3, 25.0, and 27.6 (2θ) indicated an amorphous state. The controls (either physical mixture or lyophilized mixture) exhibited crystalline diffraction peaks of carbamazepine. For both danazol and carbamazepine the powders produced by SFL technique exhibited very little crystallinity, in contrast with the significant crystallinity for powders made by the conventional lyophilization process. Apparently, the freezing rates in the SFL technique were fast enough to trap the API in an amorphous state without allowing time for crystallization. Ultra-rapid freezing may be expected from the high surface area of the atomized droplets and the rapid heat transfer from the droplets to the liquid nitrogen.

Particle Size Distribution and Morphology

The particle size of an API is another important parameter controlling the dissolution rate. Decreasing the particle size increases the surface area, which enhances the dissolution rate (24). The particle size distribution for the SFL powders and controls (Table I) were determined in an Aero-Disperser® based on time-of-flight measurements. The mean particle diameter of the SFL danazol/poloxamer 407 powder was 7.10 μm. In contrast, the mean particle diameters of the danazol and lyophilized mixture were 18.27 and 18.86 μm, respectively. Similar results were found for the SFL carbamazepine/SLS powder. The mean particle diameter of the SFL carbamazepine/SLS powder was 7.11 μm, which was smaller than that of the controls. The span index is used to describe the polydispersity in a given particle size distribution and is defined as (D90-D10)/D50, where D10, D50, and D90 are the respective particle sizes at 10, 50, and 90% cumulative percentage undersize (25). The span index of carbamazepine was 2.94, indicating high polydispersity. For the SFL powder the polydispersity was decreased markedly down to 1.31. The conventional lyophilization process reduced the mean particle diameter only slightly compared to the micronized APIs. However, for both danazol and carbamazepine, the SFL technique produced nanostructured particles with narrow particle size distributions. Various factors contributed to the limited particle growth during SFL processing. The atomized droplets were immediately frozen upon the rapid freezing rate, and the rapid freezing rates limited the time for particle growth. Also, the surfactant in the unfrozen domains in the sprayed droplets inhibits particle coalescence and crystal growth.

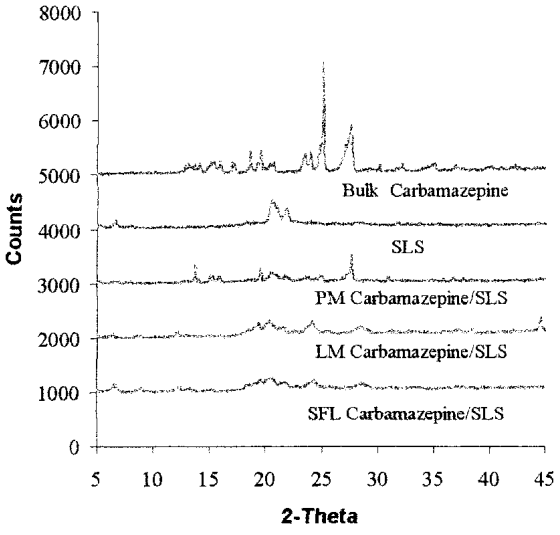

Fig. 3. Powder X-ray diffraction patterns of micronized carbamazepine, PM carbamazepine/SLS, LM carbamazepine/SLS, and SFL carbamazepine/SLS.

To further investigate the effect of the SFL technique on the morphology of APIs, sample powders were examined by SEM. The SEM micrograph of the micronized bulk danazol (Fig. 4a) illustrates large crystalline particles. The SEM micrograph of the lyophilized control particles (Fig. 4b) indicates a smooth surface when compared with the bulk danazol. In contrast, the SFL danazol/poloxamer 407 particles (Fig. 4c) had a highly porous morphology. The surface area of the SFL danazol/poloxamer 407 powder was 11.04 m^2/g, which was a significant increase over the lyophilized mixture (1.27 m^2/g). The high surface area confirmed the highly porous structure of SFL powders observed by SEM. Such high surface area indicated that the SFL powder particles were highly porous, similar to the SFV/L produced protein particles reported by Maa *et al.* (4) and Costantino *et al.* (26). Similar results for SFL carbamazepine/excipients powders were observed in the SEM analysis and surface area measurement. For example, the SEM micrograph of the SFL carbamazepine/SLS sample demonstrates a fine powder consisting of porous microparticles. Powder made by the conventional lyophilization process was larger and less porous relative to the SFL powder. The surface area of SFL carbamazepine/SLS powder was 12.81 m^2/g, which was greater than that of the lyophilized mixture (2.33 m^2/g).

Table I. Effect of Composition and Process on the Particle Size Distribution

Sample	D10	D50	D90	Span index
Bulk danazol	7.14	18.27	29.56	1.23
Slowly frozen control	7.27	18.86	31.13	1.27
SFL danazol/poloxamer 407	1.54	7.10	10.20	1.22
Bulk carbamazepine	15.56	39.70	132.33	2.94
Slowly frozen control	4.58	26.71	46.74	1.58
SFL carbamazepine/SLS	1.33	7.11	10.61	1.31

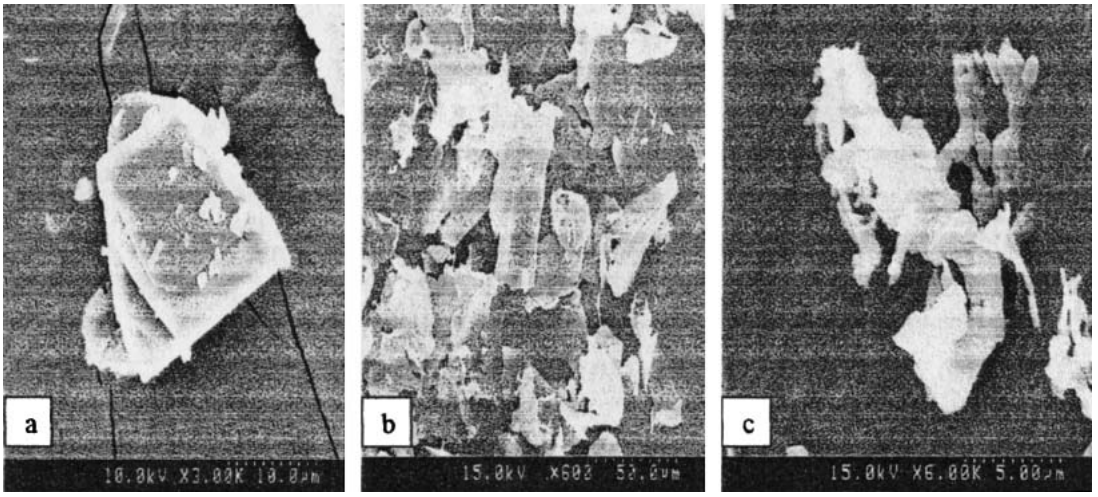

Fig. 4. SEM micrographs of micronized danazol control (Fig. 4a), LM danazol/poloxamer 407 (Fig. 4b) and SFL danazol/poloxamer 407 (Fig. 4c).

Wettability

The importance of wettability on dissolution rate—that is, the area of contact between a powder and dissolution media—has been well studied (27). To characterize wettability, the contact angle was used to determine the interfacial tension present between the compact of API powders and liquid intercontact angle values for the SFL powders, and controls are reported in Table II. The limits in the contact angle are 0° for complete wetting and 180° for no wetting (28). The mean value of contact angle for the SFL danazol/poloxamer 407 powder was only 25° against the FeSSIF media, which was significantly lower than that of the SFL danazol (55°), lyophilized mixture (34°), and physical mixture (58°) ($p < 0.05$). The mean value for SFL carbamazepine/SLS powder was 24° against purified water, which was significantly lower than for the physical mixture (52°) and SFL carbamazepine ($p < 0.05$). The significant reduction of contact angle θ for the SFL powders compared with the controls indicated the presence of a hydrophilic solid surface. Specifically, the contact angle is described by the equation: $\cos \theta = f_1 \cos \theta_1 + f_2 \cos \theta_2$ (29), where f_1 and f_2 are the bulk volume fractions of the API and excipient. The contact angle of SFL powders was well below that of the physical mixture, indicating that the solid surface of the SFL powder was enriched in the hydrophilic excipient.

Table II. Effect of Composition and Process on the Contact Angle of Danazol and Carbamazepine Powders

Samples	Contact angle degrees (FeSSIF media)
PM danazol/poloxamer 407	58
LM danazol/poloxamer 407	34
SFL danazol	55
SFL danazol/poloxamer 407	25
	Contact angle degrees (purified water)
PM carbamazepine/SLS	52
LM carbamazepine/SLS	29
SFL carbamazepine	45
SFL carbamazepine/SLS	24

This enrichment may be formed during the SFL process. As the API and excipient precipitated from the concentrated unfrozen aqueous phase, and the precipitate was surrounded by hydrophilic solvent. The hydrophilic solvent attracts the hydrophilic excipient molecules preferentially to the surface of the particles. The lower contact angle of the SFL powders compared with the lyophilized mixture may be due to the surface roughness. The rougher surface for the SFL powders relative to the lyophilized mixture as depicted in the SEMs and the higher surface areas may be expected to decrease the contact angle as observed. So, both preferential enrichment of the powder surface with the hydrophilic excipient and the surface roughness lower the contact angle and favor wetting of the SFL micronized powder relative to the controls.

Dissolution

The profiles presented in Figs. 5 and 6 illustrate the dissolution of the SFL powders and controls for danazol and carbamazepine, respectively. The FeSSIF media used for the danazol dissolution studies reportedly simulates *in vivo* gas-

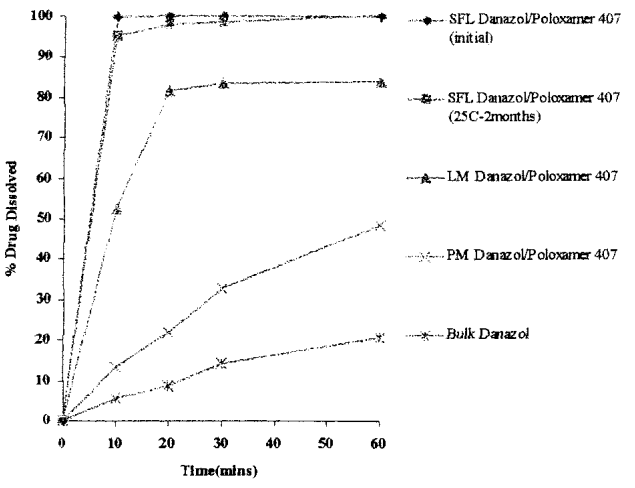

Fig. 5. Dissolution profiles of micronized danazol, PM danazol/poloxamer 407, LM danazol/poloxamer 407, and SFL danazol/poloxamer 407.

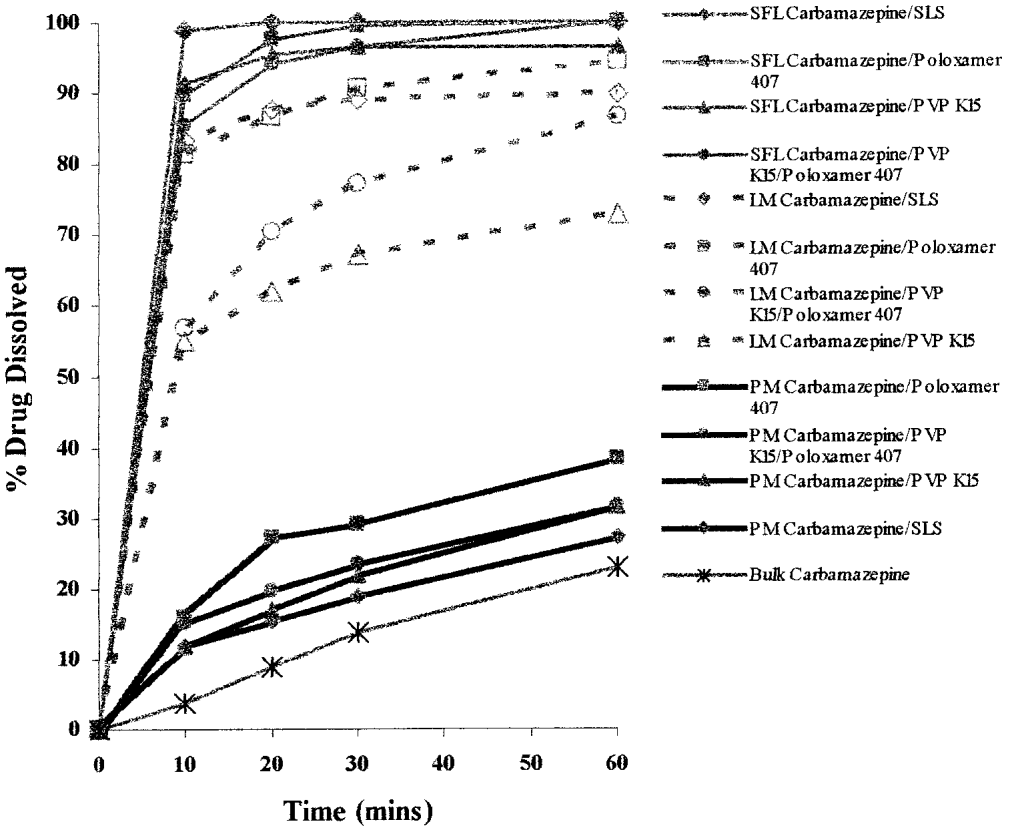

Fig. 6. Dissolution profiles of SFL carbamazepine/SLS, SFL carbamazepine/poloxamer 407, SFL carbamazepine/PVP K15, SFL carbamazepine/poloxamer 407/PVP K15, and their respective controls.

trointestinal fluid; low levels of surfactants are recommended to be included in the dissolution media to give a better correlation between *in vitro* and *in vivo* data (18). The rate of dissolution of micronized bulk danazol (Fig. 5) was slow; only 21% of the danazol dissolved in 60 min. The dissolution of the physical mixture was also quite slow, only about 48% in 60 min. The conventional lyophilization process increased the dissolution rate considerably, with 80% danazol dissolved in 60 min. However, the amount dissolved reached 99% in only 10 min for the SFL danazol/poloxamer 407 formulation. Major improvements in the dissolution rates were also found for SFL carbamazepine/excipient powders relative to the controls (Fig. 6). For example, the dissolved carbamazepine from SFL carbamazepine/SLS powder in 10 min was 98%, a profound enhancement when compared with 4% for the micronized bulk carbamazepine. The difference in the dissolution rate of SFL carbamazepine powders prepared by various excipients was negligible in 60 min and only slightly different in 20 min. Each of the excipients was successful in producing extremely rapid dissolution.

The increased dissolution rate of the SFL powders is due in part to the amorphous nature of the API, the reduction in particle size, the increase in porosity and resulting enhanced surface area, and intimate dispersion of the drug and hydrophilic excipient. The excipient may increase the local equilibrium concentration of the API in the boundary layer about the particles. The rapid freezing rate resulting from the intense atomization led to porous nanostructured particles with high amorphous API fraction and large surface area. This morphology is quite different from the low porosity, low surface area semicrystalline cakes produced by the conventional lyophilization process. At the same time, the enhanced wettability or better contact between the surface of the solid and the liquid increased the dissolution of the SFL powder. Also, the porous channels of the API/excipient matrix created in the SFL process allowed the dissolution media to easily penetrate into the particles and facilitate dissolution.

Stability

A stability study was conducted for the SFL danazol/poloxamer 407 at 25°C/60%RH for 2 months to examine any changes in crystallinity or other properties of the SFL powders. The X-ray diffraction pattern (Fig. 2) of SFL danazol/poloxamer 407 powder exhibited no change in peak intensity of danazol, but a slightly increased peak intensity of poloxamer 407. Despite the high humidity, the danazol crystallinity was unchanged over 2 months. The dissolution results (Fig. 5) demonstrated that there was only a 5% difference in the dissolution profiles between the initial and 2-month samples, which was calculated by the similarity factor f_2 for the dissolution profile comparison using the method reported by Shah (30). The slightly decreased initial release of danazol may be due to the change in crystallinity of poloxamer 407.

CONCLUSIONS

The novel SFL technology was demonstrated to produce free-flowing powder of nanostructured particles containing danazol or carbamazepine. The SFL powders exhibited sig-

nificantly enhanced dissolution rates compared to the powders formed by the conventional lyophilization process. In addition, an amorphous structure, high surface area and increased wettability of the flowable SFL powders are predominant characteristics, so the novel SFL technology is an effective particle engineering process for pharmaceutical development and manufacturing to improve dissolution rates of poorly water soluble APIs for oral delivery systems.

ACKNOWLEDGMENTS

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