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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMEF

E. _____ S. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 05-051116- -00010-0000

Fecha: 2011-07-01 17:08:39 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 412 REPOSRESRECU Folios: 24

Re: PI.-JANSSEN PHARMACEUTICA N.V.-Solicitud de Patente de Invención en Fase Nacional PCT para "COMPOSICIONES FARMACÉUTICAS QUE COMPRENDEN UN COMPUESTO FARMACOLÓGICO BÁSICO Ó ÁCIDO, UN SURFACTANTE Y UN ÁCIDO O UNA BASE HIDROSOLUBLE FISIOLÓGICAMENTE TOLERABLE, RESPECTIVAMENTE".-Clase A61K 9/48.-Expediente número 05-051.116.-

Yo, **ALICIA LLORED A RICAURTE**, mayor, vecina de esta ciudad, identificada con la cédula de ciudadanía número 39.690.713 de Usaquén, Representante Legal en Bogotá para todos los asuntos relacionados con la Propiedad Industrial de la sociedad **JANSSEN PHARMACEUTICA N.V.**, organizada y existente de conformidad con las leyes de Bélgica, domiciliada en Beerse, Bélgica, atentamente manifiesto a usted que interpongo **Recurso de Reposición** contra la Resolución número **30041** dictada por el señor Superintendente de Industria y Comercio el 31 de Mayo de 2011 y notificada por Edicto fijado el 10 de Junio de 2011, por medio de la cual se niega el privilegio de patente solicitado para la invención contenida en el expediente de la referencia.

I. FUNDAMENTOS DE HECHO

- (1) El 25 de Mayo de 2005 se presentó ante la División de Nuevas Creaciones de la Superintendencia de Industria y Comercio (en adelante **SIC**), una solicitud de Patente de Invención en Fase Nacional del Tratado de Cooperación en Materia de Patentes (PCT) para "COMPOSICIONES FARMACÉUTICAS QUE COMPRENDEN UN COMPUESTO FARMACOLÓGICO ACÍDICO RESPECTIVAMENTE BÁSICO, UN SURFACTANTE Y UNA BASE RESPECTIVAMENTE ÁCIDO HIDROSOLUBLE FISIOLÓGICAMENTE TOLERABLE", a nombre de **JANSSEN PHARMACEUTICA N.V.**, sociedad organizada y existente de conformidad con las leyes de Bélgica, y domiciliada en Beerse, Bélgica.
- (2) La solicitud mencionada fue admitida a trámite por ese Despacho en vista del cumplimiento de los requisitos establecidos en la ley, siéndole otorgado el número de radicación 05-051.116.
- (3) Posteriormente, en virtud del Artículo 38 de la Decisión 486, la **SIC** expidió el auto No. 13163, notificado por fijación en lista de 1 de Julio de 2005, y mediante el cual fue notificada al solicitante la objeción con respecto al título de la solicitud.

(4) El solicitante dio respuesta oportuna a dicho Oficio, modificando el título originalmente presentado por el nuevo título: "COMPOSICIONES FARMACÉUTICAS QUE COMPRENDEN UN COMPUESTO FARMACOLÓGICO BÁSICO O ÁCIDO, UN SURFACTANTE Y UN ÁCIDO O UNA BASE HIDROSOLUBLE FISIOLÓGICAMENTE TOLERABLE, RESPECTIVAMENTE".

(5) Posteriormente, el extracto de la solicitud de la referencia apareció publicado en la Gaceta de la Propiedad Industrial No. 575 de fecha 30 de Abril de 2007, surgiendo para el solicitante la carga de solicitar la realización del examen de patentabilidad de la invención cuya protección se pretende, así como la de pagar la correspondiente tasa administrativa. El solicitante, estando dentro del término previsto, mediante memorial de fecha 22 de Octubre de 2007 solicitó dicho examen, adjuntando el recibo de pago que acreditaba la cancelación de la tasa administrativa mencionada.

(6) En virtud del Artículo 45 de la Decisión Andina 486 de 2000, la SIC expidió el Concepto Técnico No. 3083, notificado por fijación en lista de 14 de Noviembre de 2008, mediante el cual le fueron notificadas al solicitante las objeciones formuladas por ese Despacho respecto del cumplimiento de los requisitos de Reivindicaciones relativas a un uso o a un uso distinto¹ y Novedad² con base en las enseñanzas del documento US 4,596,705 (D1).

(7) El solicitante dio respuesta oportuna al requerimiento realizado bajo el Artículo 45 de la Decisión Andina 486 el día 11 de Febrero de 2009, refiriéndose a cada una de las objeciones expuestas por ese Despacho en su Concepto Técnico No. 3083.

(8) No obstante lo anterior, y nuevamente en virtud del Artículo 45 de la Decisión Andina 486 de 2000, la SIC expidió el Concepto Técnico No. 1311, notificado por fijación en lista de Junio 4 de 2010 mediante el cual le fueron informadas al solicitante las objeciones formuladas nuevamente por ese Despacho respecto del cumplimiento de los requisitos de Claridad de la solicitud y Nivel Inventivo con base en las enseñanzas de los documentos WO 0122938 (D2), WO 9926607 (D3) y el libro titulado "Encyclopedia of Pharmaceutical Technology" (D4).

(9) El solicitante dio respuesta oportuna al requerimiento previamente mencionado el día 30 de Agosto de 2010, refiriéndose a cada una de las objeciones expuestas por ese Despacho en su Concepto Técnico No. 1311.

(10) Sin embargo, en respuesta a lo anterior la SIC expidió la Resolución número 30041, dictada por el señor Superintendente de Industria y Comercio, el 31 de Mayo de 2011 y notificada por Edicto fijado el 10 de Junio de 2011, mediante la cual se negó el privilegio de patente para el objeto reclamado, argumentando falta de claridad y

¹ Ibidem, Artículo 21.

² Ibidem, Artículo 16.

sustento en la descripción de las reivindicaciones, y Nivel Inventivo de la invención a la luz de las enseñanzas de los documentos WO 0122938 (D2), WO 9926607 (D3) y el libro titulado "Encyclopedia of Pharmaceutical Technology" (D4).

Son los hechos aquí enunciados los que dan lugar al presente recurso de reposición, sustentado en los siguientes:

II. FUNDAMENTOS DE DERECHO

Como fundamento de mi recurso me permito manifestar ante ese Despacho que el contenido de la Resolución número 30041 evidencia que durante el examen técnico realizado a la solicitud en cuestión de ninguna manera se consideró el alcance, objeto e importancia de la invención denegada en su verdadera dimensión, esto es, no se consideró el valioso aporte técnico que representan para el arte en cuestión las composiciones farmacéuticas que son reveladas en la solicitud denegada.

En efecto, a pesar de los argumentos proporcionados por el solicitante en respuesta al Concepto Técnico número 1311 de 4 de junio de 2010, en la Resolución impugnada ese Despacho mantiene las objeciones frente a la claridad y soporte en la descripción de las reivindicaciones, así como frente al nivel inventivo de la solicitud, argumentando básicamente que:

"...el capítulo reivindicatorio no revela de manera concisa el objeto de la solicitud y no se encuentra enteramente sustentado en la descripción, razón por la cual no cumple con los requisitos exigidos por el Artículo 30 de la Decisión Andina";

Así como también que:

"...la reivindicación 1, tal como se reivindica, es obvia, porque dicha persona, enfrentada al problema de "cómo obtener composiciones con mayor solubilidad y como consecuencia una mejor biodisponibilidad", incluiría vitamina E TPGS, ya revelada en D3, dentro de las composiciones que se enseñan en D2, logrando de esta forma la composición objeto de esta solicitud".

Por consiguiente, a continuación me permito demostrar ante ese Despacho que la presente invención **sí cumple con los requisitos de claridad y nivel inventivo** exigidos por los Artículos 30 y 18 de la Decisión 486 de 2000, respectivamente, toda vez que la información técnica divulgada por el solicitante en la descripción de la invención soporta plenamente las composiciones farmacéuticas establecidas en el capítulo reivindicatorio, las cuales a su vez se caracterizan porque **permiten de manera sorprendente mejorar la biodisponibilidad de los compuestos activos pobremente solubles en agua**, lo cual de ninguna manera habría podido ser

predicho por la persona medianamente versada en la materia a partir de las enseñanzas del estado de la técnica anterior.

1) Consideraciones frente a la claridad y el sustento descriptivo de la invención

Inicialmente me permito manifestar ante ese Despacho que el capítulo reivindicatorio de la solicitud de patente en cuestión encuentra pleno sustento en la descripción de la invención y también revela de manera clara y concisa las novedosas composiciones farmacológicas desarrolladas por el solicitante, toda vez que:

- Para el experto medio en la materia, -quien es la persona interesada en la información técnica contenida en la solicitud de patente en cuestión y quien puede ponerla en práctica-, los elementos de las composiciones farmacológicas reveladas en la presente invención son absolutamente evidentes, a saber: un compuesto farmacológico básico, vitamina E TPGS y un ácido hidrosoluble fisiológicamente tolerable, en donde la relación de ácido:compuesto farmacológico es de 1:1 a 100:1 en peso, ó un compuesto farmacológico ácido, vitamina E TPGS y una base hidrosoluble fisiológicamente tolerable, en donde la relación de base:compuesto farmacológico es de 1:1 a 100:1 en peso;
- Adicionalmente, con base en lo mencionado por el solicitante en la descripción de la invención, para la persona medianamente versada en la materia es claro que las composiciones farmacológicas allí reveladas permiten obtener una solución supersaturada de un agente activo que presente baja solubilidad en agua, siendo dicha conclusión el resultado de los diferentes ensayos realizados por el solicitante con diferentes agentes activos, tal y como se menciona en la descripción de la invención (donde se divulgan los resultados de las pruebas realizadas con dos agentes activos diferentes: 4-[[4-[[4-(2-cianoetenil)-2,6-dimetilfenil]amino]-2-pirimidinil]amino]-benzonitrilo e Itraconazol) y en los artículos científicos escritos por el mismo solicitante en relación con la invención en cuestión (R. Vandecruys, et al.; *Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug solutions and application for this system to solid formulation design*, Int'l J. Pharmaceutics 342 (2007) 168-175 and M.E. Brewster et al., *Comparative interaction of 2-hydroxypropyl- β -cyclodextrin and sulfobutylether- β -cyclodextrin with itraconazole: Phase-solubility behaviour and stabilization of supersaturated drug solution*, Eur. J. Pharma. Sci. 34 (2008) 94-103).
- De otro lado, considero de gran importancia señalar que en la reivindicación 1 de la solicitud en cuestión se menciona el límite inferior y el límite superior de la relación que debe existir entre el ácido ó la base y el compuesto

farmacológico dentro de las composiciones reveladas por el solicitante. En este sentido me permito aclarar que si bien en dicha reivindicación 1 aparece el término "al menos" como parte de la frase "...es de por lo menos 1:1 a 100:1", dicha expresión hace referencia únicamente al hecho de que es necesario que la relación ácido/base:compuesto farmacológico sea, como mínimo, 1:1, y como máximo, 100:1, más de ninguna manera puede ser entendido como un mecanismo para proteger cualquier otra relación por fuera del rango allí definido.

- Así mismo, con respecto a las expresiones "un compuesto farmacológico ácido" y "un compuesto farmacológico básico" que también son mencionadas en la reivindicación 1 de la solicitud en cuestión, me permito señalar que con base en el contexto de la invención y ante todo, teniendo en cuenta el problema técnico objetivo para el caso en cuestión, es claro que dichas expresiones de ninguna manera corresponden a una generalización inadecuada del alcance de la invención, toda vez que el desarrollo técnico logrado por el solicitante implica haber obtenido un mecanismo para mejorar la solubilidad en agua de los diferentes compuestos farmacológicos básicos y ácidos que muestran baja solubilidad en agua, y que por lo tanto presentan una biodisponibilidad baja.

Visto lo anterior, me permito manifestar que el capítulo reivindicatorio de la solicitud de patente en cuestión cumple a cabalidad con las disposiciones de patentabilidad establecidas en el Artículo 30 de la Decisión 486 de 2000, **toda vez que es claro, conciso y presenta pleno sustento en la descripción de la solicitud.**

2) Principales características de la invención denegada

De otro lado y antes de continuar con el análisis de nivel inventivo, inicialmente me permito realizar un resumen de las principales características de la materia revelada en la solicitud de patente denegada, con el fin de reiterar ante ese Despacho el verdadero objeto y la importancia de la invención en estudio:

La presente solicitud se refiere a **novedosas composiciones farmacéuticas que proveen un mejorado perfil de disolución para el compuesto activo, proporcionando una mayor solubilidad y por ende una mayor biodisponibilidad a dicho compuesto activo.**

En la actualidad, muchos compuestos farmacológicos -si bien poseen propiedades terapéuticas deseadas- se utilizan de forma ineficaz como consecuencia de su pobre solubilidad en agua. Al respecto, en el estado de la técnica anterior se conocen numerosos mecanismos para intentar mejorar la solubilidad y por ende la

biodisponibilidad de tales agentes activos, entre los que se cuentan la derivación molecular del fármaco, el aumento del área superficial del agente activo, el acomplejamiento del compuesto farmacológico y su mezcla con agentes surfactantes. Sin embargo, dichos mecanismos implican diversos inconvenientes técnicos, entre los que se cuentan la disminución en la eficiencia terapéutica, el aumento de la toxicidad, las dificultades de administración al paciente de la nueva forma de dosificación y la restricción de uso debido a las características fisico-químicas de los agentes activos.

Sin embargo, el solicitante de la invención en cuestión ha encontrado de manera sorprendente que la combinación de un compuesto farmacológico básico, vitamina E TPGS y un ácido hidrosoluble fisiológicamente tolerable, en donde la relación de ácido:compuesto farmacológico es de 1:1 a 100:1 en peso, ó la combinación de un compuesto farmacológico ácido, vitamina E TPGS y una base hidrosoluble fisiológicamente tolerable, en donde la relación de base:compuesto farmacológico es de 1:1 a 100:1 en peso, permite aumentar el perfil de disolución del compuesto farmacológico y por ende permite mejorar su biodisponibilidad.

Tal como se analizará en detalle más adelante dentro del presente escrito, el solicitante de la invención en cuestión ha logrado demostrar que las composiciones reveladas en la reivindicación 1 de la solicitud rechazada por ese Despacho permite obtener soluciones supersaturadas de compuestos terapéuticamente activos que difieren en características fisico-químicas como el peso molecular, el pKa, el volumen molecular, el log P y el área de superficie polar, razón por la cual dichas novedosas composiciones representan un importante avance en el estado de la técnica en cuestión.

3) Consideraciones técnicas frente a las enseñanzas del arte previo

Ahora bien, teniendo en cuenta las características técnicas mencionadas anteriormente, me permito manifestar ante ese Despacho que las composiciones farmacológicas reveladas en la solicitud de patente denegada presentan un inequívoco carácter inventivo con respecto a las enseñanzas de las anterioridades mencionadas en la Resolución número 30041, esto es, frente a los documentos **WO 0122938 (D2)**, **WO 9926607 (D3)** y el libro **"Encyclopedia of Pharmaceutical Technology"**. En este sentido, a continuación me permito realizar un análisis comparativo detallado entre la información revelada en la presente solicitud y las enseñanzas brindadas por dichas anterioridades.

3.1. Consideraciones técnicas frente al documento WO 0122938 (D2)

Básicamente, el documento D2 enseña composiciones farmacéuticas de compuestos antivirales que pueden ser administrados a mamíferos, -particularmente a seres humanos-, que sufren de alguna infección de carácter viral. Según los autores

de tal anterioridad, dichas composiciones comprenden partículas que pueden ser obtenidas al extruir por fusión una mezcla que comprende uno o más compuestos antivirales y uno o más polímeros adecuados solubles en agua y subsecuentemente moler dicha mezcla extruída.

Teniendo en cuenta lo anterior, a continuación me permito presentar ante ese Despacho las siguientes consideraciones técnicas frente a las enseñanzas de D2:

- En primer lugar considero de gran importancia señalar que dentro de las enseñanzas del citado documento D2 **no se menciona ni se sugiere que un compuesto como la Vitamina E TPGS mejore la solubilidad y aumente la biodisponibilidad de las sustancias farmacológicamente activas (ácidas o básicas)**, razón por la cual la información contenida en el citado documento D2 no afecta el nivel inventivo de la solicitud de patente en cuestión debido a que de acuerdo con lo mencionado en la descripción de la presente invención, **la inclusión específica de vitamina E TPGS es fundamental para que las formulaciones farmacológicas reveladas permitan aumentar de manera efectiva la solubilidad de los compuestos ácidos y básicos pobremente solubles en agua.**

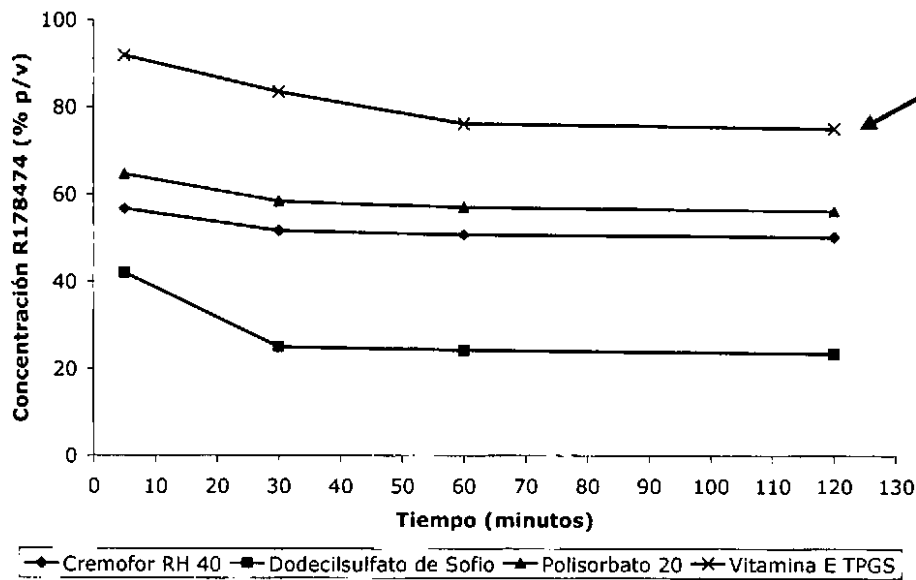
De conformidad con lo anterior, me permito dirigir la atención del señor Examinador a los siguientes resultados de ensayos de determinación del porcentaje de agente activo en solución en función del tiempo de dos diferentes compuestos farmacológicamente activos (R278474 e Itraconazol), a través de los cuales se demuestra el sorprendente efecto técnico de supersaturación que se presenta al utilizar Vitamina E TPGS como agente surfactante.

En primer lugar, se evaluó el porcentaje de compuesto activo en solución en función del tiempo empleando compuesto **R278474** (4-[[4-[[4-(2-cianoetenil)-2,6-dimetilfenil]amino]-2-pirimidinil]amino]-benzonitrilo), utilizando como sustancia ácida fisiológicamente tolerable ácido clorhídrico 0,01N, una temperatura de 37°C y una concentración de surfactante de 2,5% (los surfactantes evaluados fueron Cremophor RH40 [muestra 1], Dodecilsulfato de sodio [muestra 2], Polisorbato 20 [muestra 3] y vitamina E TPGS [muestra 4]).

Los resultados obtenidos de concentración (%p/v) del compuesto (R278474) farmacológicamente activo en función del tiempo fueron:

Tiempo (minutos)	Muestra 1	Muestra 2	Muestra 3	Muestra 4
5	56,67	42,00	64,59	91,75
30	51,60	25,00	58,40	83,50
60	50,70	24,20	57,00	76,25
120	50,30	23,50	56,20	75,25
pH final	2,11	5,97	2,10	2,36
Solución de DMF adicionada (mL)	0,37	0,46	0,37	0,53

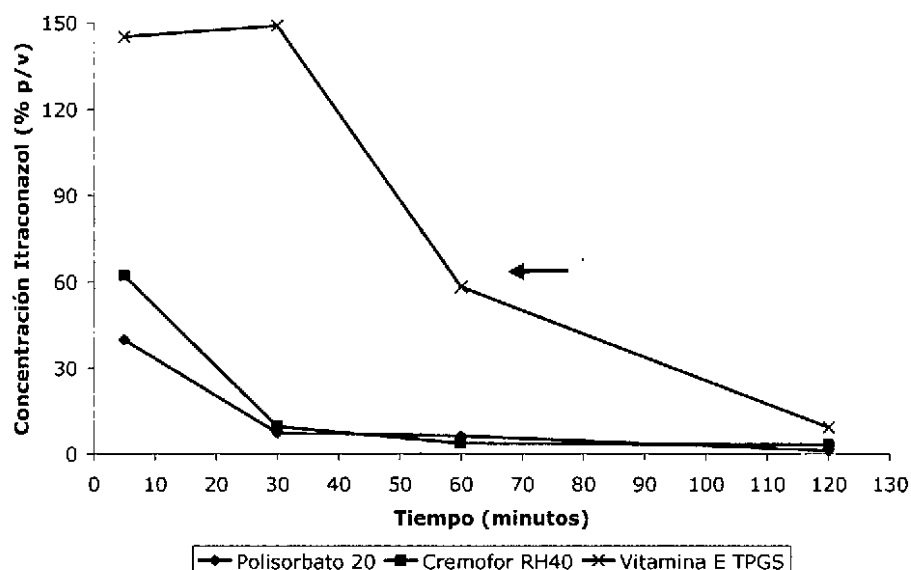
Perfil de solubilidad del compuesto R278474



De igual manera, se evaluó también el porcentaje de compuesto activo en solución en función del tiempo empleando el compuesto **Itraconazol**, utilizando como sustancia ácida fisiológicamente tolerable ácido clorhídrico 0,01N, una temperatura de 37°C y una concentración de surfactante de 2,5% (los surfactantes evaluados fueron Polisorbato 20 [muestra 1], Cremophor RH40 [muestra 2] y vitamina E TPGS [muestra 3]). Los resultados obtenidos de concentración (%p/v) del compuesto farmacológicamente activo en función del tiempo fueron:

Tiempo (minutos)	Muestra 1	Muestra 2	Muestra 3
5	39,90	62,20	145,10
30	7,30	9,60	149,00
60	6,24	3,69	58,20
120	1,08	3,04	9,15
pH final	1,98	2,00	1,99
Solución de DMF adicionada (mL)	0,10	0,17	0,32

Perfil de solubilidad de Itraconazol



De los resultados anteriores se concluye que la **vitamina E TPGS**, en efecto, permite alcanzar niveles muy altos de supersaturación comparados con otros surfactantes de uso común dentro del estado de la técnica y por lo tanto es el preferido para las formulaciones de la presente solicitud. En este punto, es preciso señalar que de la información revelada por el documento D2 no resulta obvia ni evidente la elección de vitamina E TPGS, máxime cuando dentro de las enseñanzas de dicho documento **no se menciona la inclusión de ningún surfactante dentro de las formulaciones**.

- De otro lado me permito resaltar que la anterioridad D2 no enseña ni sugiere a la persona medianamente versada en la materia a incluir explícitamente un ácido dentro de las formulaciones allí reveladas, toda vez que en dicha anterioridad únicamente se menciona la utilización de ácidos como parte del proceso de preparación de sales de adición ácidas de los compuestos de fórmula (I-A), (I-B) y (I-C), pero no como aditivos de carácter ya sea ácido o básico que formen un microambiente que mejore la solubilidad en agua del compuesto farmacológicamente activo.

Por lo tanto, en vista de los argumentos técnicos planteados previamente, me permito manifestar ante ese Despacho que la información técnica revelada en la solicitud de patente en cuestión presenta un inequívoco carácter inventivo frente a las enseñanzas del documento D2.

3.2. Consideraciones técnicas frente al documento WO 9926607 (D3)

Por su parte, el documento D3 básicamente divulga una matriz de TPGS/fármaco que tiene la capacidad de disolverse muy lentamente, proporcionando una liberación

controlada de dicho fármaco. A este respecto, los autores del citado documento D3 (página 16, líneas 8-19) señalan que:

"Adicionalmente, el nuevo sistema de transporte TPGS/medicamento de la presente invención proporciona una matriz que se disuelve lentamente que se absorbe en el fluido gastrointestinal en la matriz TPGS, donde un cristal líquido similar a un gel se forma. Este frente de gel forma un cristal líquido límite de una cierta viscosidad en la región donde la disolución del medicamento es la más alta. Este límite establecido entre el cristal líquido/fluido gastrointestinal tiene lugar cuando la velocidad de formación del cristal líquido iguala la velocidad de disolución del cristal en la interfase acuosa, por consiguiente se obtiene una liberación controlada del medicamento en el tracto gastrointestinal. De esta forma, la velocidad de disolución es controlada por la velocidad de disolución del cristal líquido de TPGS y la geometría de la forma de dosificación. Esto permite la liberación controlada de los medicamentos disueltos, que por lo general son lipofílicos o de medicamentos dispersos que son generalmente hidrofílicos".

Con base en lo anterior, considero de gran importancia señalar que las composiciones farmacológicas reveladas en la presente solicitud tampoco pueden derivarse de forma obvia a partir de las enseñanzas de la anterioridad citada, toda vez que en D3 de ninguna manera se hace mención a una sustancia hidrosoluble fisiológicamente tolerable (bien sea de naturaleza ácida o básica) que genere un microambiente que aumente la solubilidad de los compuestos farmacológicamente activos, y por ende su biodisponibilidad.

En este sentido, resulta de gran importancia tener en cuenta que en el documento D3 no existe motivación técnica alguna para que la persona medianamente versada en la materia sugiera una formulación farmacéutica que combine Vitamina E TPGS, un componente farmacológicamente activo y una sustancia hidrosoluble fisiológicamente tolerable de carácter ácido o básico que genere en la solución un microambiente que aumente la solubilidad y biodisponibilidad del medicamento, máxime cuando el problema técnico que resuelven los autores de D3 está relacionado con la liberación controlada de medicamentos que usualmente son de administración inmediata como la ciclosporina y no está relacionado con aumentar la solubilidad y biodisponibilidad de los compuestos farmacológicamente activos.

En consecuencia, es claro que la materia objeto de la presente invención no es obvia ni evidente a partir de las enseñanzas del documento D3, presentando por lo tanto un inequívoco carácter inventivo frente a dicha anterioridad.

3.3. Análisis del nivel inventivo de la presente solicitud frente a la combinación de los documentos D2 y D3

Ahora bien, a continuación me permito analizar el nivel inventivo de la solicitud en cuestión frente a la combinación de las enseñanzas de los documentos D2 y D3 señalados por ese Despacho en la Resolución No. 30041, no sin antes mencionar una serie de consideraciones frente al requisito de nivel inventivo establecidas en el artículo 18 de la Decisión Andina 486 de 2000.

a) Requisitos exigidos por el artículo 18 de la Decisión 486 de 2000:
Consideraciones frente al estudio del nivel inventivo

Inicialmente considero de gran importancia resaltar que el artículo 18 de la Decisión 486 de la Comunidad Andina establece lo siguiente en relación con el requisito de nivel inventivo que debe cumplir una solicitud de patente:

"Artículo 18.- Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica".

A éste respecto, resulta de gran importancia señalar que la apreciación del nivel inventivo -por su gran contenido de subjetividad- puede resultar muchas veces incierta o inexacta, lo cual puede llevar a confusiones y excesos. Por lo tanto, para evitar caer en lo anterior, al realizar la apreciación de nivel inventivo es necesario que el experto en la materia base su examen en una apreciación objetiva, teniendo en cuenta la realidad y circunstancias determinadas al momento en que surgió la invención y sin incluir en dicha apreciación factores meramente subjetivos.

En este sentido, considero relevante destacar que para realizar un verdadero juicio sobre el nivel inventivo de una invención es necesario ubicarse en el momento en que se realizó dicha invención, razón por la cual el experto encargado del caso debe retrotraerse al estado de la técnica existente en dicho momento y analizar desde esa óptica si para ese instante verdaderamente hubiera sido realmente posible llegar de manera obvia o evidente al objeto que se reivindica en una invención. Lo anterior para no caer en el error de comprender, analizar y juzgar una invención después de que ésta ha sido realizada, algo que en el ámbito mundial de las patentes se denomina un análisis de tipo "hindsight". Esta posición errónea es contemplada, por ejemplo, en el Manual Andino de Patentes, donde se establece que: *"se debe tener en cuenta que la búsqueda de anterioridades se efectúa a posteriori, tomando como punto de partida la misma invención. Por lo tanto el examinador debe realizar el esfuerzo intelectual de colocarse en la situación que ha tenido que afrontar el técnico con conocimientos medios en la materia en un momento en que la invención no era conocida, es decir, antes de la invención".*

De otro lado, atentamente me permito citar al profesor Paul Mathely³, quien señala que la altura inventiva de un invento es un concepto relativo que depende en gran parte del grado de habilidad de la persona ejecutante de la invención. Por lo tanto, para una cierta parte de la doctrina mundial, la altura inventiva puede ser confirmada mediante ciertos índices exteriores, entre los cuales puede destacarse aquel que corresponde al progreso o aporte técnico que pueda suministrar la invención al estado de la técnica, de tal suerte que se considera que una invención tiene altura inventiva si dicha invención representa un avance para el estado de la técnica.

b) Nivel inventivo de la solicitud frente a la combinación de los documentos D2 y D3

Visto lo anterior, a continuación me permito dirigir la atención de ese Despacho a una serie de argumentos técnicos mediante los cuales se demuestra que los ya referenciados documentos D2 y D3, ni aún en combinación, sugieren o motivan al técnico medianamente versado en la materia a obtener la materia objeto de la presente solicitud.

En primer lugar, señala ese Despacho que la persona medianamente versada en la materia tomaría las enseñanzas de las composiciones farmacéuticas reveladas en el documento D2 y de acuerdo con las enseñanzas del documento D3 incluiría en ellas la vitamina E TPGS, haciendo obvias las composiciones farmacológicas reveladas en la solicitud de patente en cuestión.

Sobre este particular, atentamente me permito señalar que:

i) El documento D2 no menciona ni sugiere que un compuesto como la vitamina E TPGS mejore la solubilidad y aumente la biodisponibilidad de sustancias farmacológicamente activas ácidas o básicas, así como tampoco enseña o sugiere la inclusión de un ácido dentro de las formulaciones allí reveladas, ya que únicamente menciona la utilización de ácidos como parte del proceso de preparación de sales de adición ácidas de los compuestos de fórmula (I-A), (I-B) y (I-C), pero no como aditivos de carácter ya sea ácido o básico que formen un microambiente que mejore la solubilidad en agua del compuesto farmacológicamente activo.

ii) Por su parte, a pesar de que el citado documento D3 menciona la vitamina E TPGS como uno de los componentes de las formulaciones allí reveladas, dicho componente es empleado para garantizar la liberación controlada del agente activo, pero de ninguna manera se menciona o se sugiere en dicha anterioridad que la vitamina E TPGS en combinación con: i) un compuesto farmacológico básico o ácido y ii) un ácido o una base hidrosoluble fisiológicamente tolerable aumente de forma

³ MATHELY Paul. Le droit français des brevets d'invention; Paris: Journal des notaires et des avocats, 1978; p. 125.

drástica la solubilidad y la biodisponibilidad del componente farmacológicamente activo.

En conexión con lo anterior, es claro que el técnico con conocimiento medio en la materia no encontraría ninguna sugerencia en las enseñanzas del documento D3 para incluir la vitamina E TPGS dentro de las enseñanzas reveladas en el citado documento D2, máxime cuando el efecto técnico alcanzado por el uso de la vitamina E TPGS en el documento D3 es el contrario al obtenido por los autores de la presente solicitud. Por lo tanto, la combinación de las enseñanzas reveladas de los documentos D2 y D3 no conduce de forma obvia ni evidente a la materia de la presente invención.

Adicionalmente, me permito dirigir la atención de ese Despacho a las enseñanzas del Manual Andino de Patentes, relevante publicación preparada por la Secretaría General de la Comunidad Andina; la cual con relación al estudio del nivel inventivo de las solicitudes de patente señala que:

*"... La pregunta a contestar es si teniendo en cuenta el estado de la técnica en su conjunto **existe alguna indicación** que lleve a la persona versada en la materia a modificar o adaptar el estado de la técnica más cercano para resolver el problema técnico, de tal forma que llegue a un resultado que estuviera incluido en el tenor de la(s) reivindicación(es). ..."⁴. (Resaltado fuera de texto)*

Por lo tanto, debido a que en los documentos D2 y D3 no existe alguna indicación técnica que motive al técnico medianamente versado en la materia a obtener la materia objeto de la invención en cuestión, es claro que la misma cumple a cabalidad con lo establecido en el artículo 18 de la Decisión Andina 486 de 2000.

Declaración rendida por el Dr. Marcus E. Brewster

De otro lado, considero de gran importancia resaltar ante ese Despacho que de conformidad con lo mencionado en la declaración rendida en el año 2009 por el Doctor Marcus E. Brewster (uno de los inventores de la solicitud de patente en cuestión) ante la Oficina de Patentes y Marcas de los Estados Unidos de América (**ANEXO I**), el efecto de supersaturación logrado mediante las composiciones farmacológicas reveladas en la presente invención fue probado con alrededor de 25 moléculas terapéuticamente activas básicas ó ácidas que presentan diferencias fisicoquímicas en cuanto a su peso molecular, volumen molecular, pKa y área superficial polar, encontrándose en todos los casos que dichas formulaciones conteniendo dicho compuesto farmacológico básico ó ácido en combinación con vitamina E TPGS y un ácido o una base hidrosoluble fisiológicamente tolerable permiten lograr un promedio alto de supersaturación del compuesto activo en comparación con formulaciones que

⁴ Secretaría General de la Comunidad Andina "Manual para el examen de solicitudes de patentes de invención en las oficinas de propiedad industrial de los países de la Comunidad Andina". Lima, Perú 2004. Página 77.

comprenden otro tipo de agentes surfactantes (ver **ANEXO II** → R. Vandecruys, et al.; *Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug solutions and application for this system to solid formulation design*, Int'l J. Pharmaceutics 342 (2007) 168-175).

Así mismo, a través de dicha declaración el Dr. Marcus E. Brewster señala que en el mismo artículo científico previamente mencionado se divulgan los resultados experimentales de las pruebas realizadas para demostrar que las formulaciones sólidas que son preparadas de acuerdo con lo especificado en el capítulo reivindicatorio de la solicitud de patente en cuestión permiten lograr hasta un 100% de biodisponibilidad del agente activo (ver página 174, columna 1 del ANEXO II).

Por último, el Dr. Brewster menciona en su Declaración que, tal y como se encuentra demostrado en el artículo científico "M.E. Brewster et al., *Comparative interaction of 2-hydroxypropyl- β -cyclodextrin and sulfobutylether- β -cyclodextrin with itraconazole: Phase-solubility behaviour and stabilization of supersaturated drug solution*, Eur. J. Pharma. Sci. 34 (2008) 94-103" (**ANEXO III**), una composición sólida o semi-sólida que comprende un compuesto farmacéutico básico ó ácido, TPGS y un ácido o base soluble en agua y fisiológicamente tolerable, permite lograr perfiles de supersaturación y por ende de biodisponibilidad para dicho agente activo ácido ó básico que resultan ser completamente inesperados a la luz de las enseñanzas del arte previo.

Visto lo anterior, atentamente me permito manifestar ante ese Despacho que las composiciones farmacológicas reveladas en la solicitud de patente en cuestión presentan un inequívoco carácter inventivo frente a la combinación de las enseñanzas de los documentos D2 y D3, cumpliendo por lo tanto a cabalidad con las disposiciones de patentabilidad establecidas en el artículo 18 de la Decisión 486 de 2000.

3.4. Consideraciones técnicas frente al documento "Encyclopedia of Pharmaceutical Technology" (D4)

De otra parte, manifiesta ese Despacho que los procesos reclamados en las reivindicaciones 24 a 51 no tienen nivel inventivo debido a que los procesos de disolución, evaporación, granulado y extrusión, entre otros, son ampliamente conocidos por la persona con conocimientos medios en formulaciones farmacéuticas.

En respuesta a lo anterior inicialmente me permito aclarar ante ese Despacho que de conformidad con lo establecido en el Manual Andino de Patentes:

"Si una reivindicación independiente es nueva e inventiva, también lo son sus reivindicaciones dependientes. De la misma manera, si un producto es nuevo e

inventivo también lo serán las reivindicaciones de proceso que necesariamente llevan a ese producto y a las reivindicaciones de producto”⁵.

Teniendo en cuenta lo anterior, es claro que habiendo demostrado el indudable nivel inventivo de las composiciones farmacéuticas de la solicitud en cuestión, el método para producir tales composiciones también resulta ser inventivo a la luz de las enseñanzas del arte previo.

3. **RESPUESTA A LOS ARGUMENTOS PRESENTADOS EN LA RESOLUCIÓN IMPUGNADA**

De conformidad con los anteriores argumentos, me permito señalar ante ese Despacho que las afirmaciones presentadas en la Resolución impugnada carecen de toda relevancia para desvirtuar la novedad de la presente solicitud, tal como me permito señalar a continuación:

- En la Resolución número 30041 de la SIC se afirma que: *“...con el sólo ejemplo descrito no es suficiente para que una persona normalmente versada en la materia pueda reproducir sin problema alguno la invención, ni tampoco se logren obtener con éxito todas las realizaciones que están comprendidas dentro del alcance del capítulo reivindicatorio”.*

En respuesta a lo anterior me permito mencionar una vez más ante ese Despacho que con base en lo mencionado por el solicitante en la descripción de la invención, para la persona medianamente versada en la materia es claro que las composiciones farmacológicas allí reveladas permiten obtener una solución supersaturada de un agente activo que presente baja solubilidad en agua, siendo dicha conclusión el resultado de los diferentes ensayos realizados por el solicitante con diferentes agentes activos, tal y como se menciona en la descripción de la invención (donde se divulgan los resultados de las pruebas realizadas con dos agentes activos diferentes: 4-[[4-[[4-(2-cianoetenil)-2,6-dimetilfenil]amino]-2-pirimidinil]amino]-benzonitrilo e Itraconazol) y en los artículos científicos escritos por el mismo solicitante en relación con la invención en cuestión (R. Vandecruys, et al.; *Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug solutions and application for this system to solid formulation design*, Int'l J. Pharmaceutics 342 (2007) 168-175 and M.E. Brewster et al., *Comparative interaction of 2-hydroxypropyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin with itraconazole: Phase-solubility behaviour and stabilization of supersaturated drug solution*, Eur. J. Pharma. Sci. 34 (2008) 94-103).

- De otro lado, en la Resolución impugnada se afirma que: *“...la anterioridad D3 (WO 9926607) describe una manera de solucionar el problema técnico*

⁵ Secretaría General De La Comunidad Andina. “Manual para el examen de solicitudes de patentes de invención en las oficinas de propiedad industrial de los países de la Comunidad Andina”; Lima, 2004; página 80.

objetivo de la solicitud, es decir "cómo obtener composiciones con mayor solubilidad y como consecuencia una mejor biodisponibilidad, alternativas a las ya divulgadas, en D2", mediante una composición antiviral que contiene vitamina E TPGS, como tenso activo (surfactante), para mejorar la solubilidad del compuesto activo y su biodisponibilidad...".

Con respecto a lo anterior me permito aclarar que, contrario a lo afirmado por ese Despacho en la Resolución No. **30041**, el documento D3 de ninguna manera divulga un método para obtener composiciones farmacéuticas con mayor solubilidad y mejor biodisponibilidad. Contrario a esto, **D3 está dirigido a proporcionar una matriz de TPGS/fármaco que tiene la capacidad de disolverse muy lentamente, proporcionando una liberación controlada de dicho fármaco, es decir, dicha anterioridad se encuentra dirigida a resolver un problema técnico completamente diferente a aquel abordado por el solicitante de la invención en cuestión.**

Una vez aclarado lo anterior, considero de gran importancia señalar una vez más ante ese Despacho que la persona medianamente versada en la materia no encuentra en el documento D3 alguna indicación técnica que le sugiera que una composición que comprenda vitamina E TPGS en combinación con: i) un compuesto farmacológico básico o ácido y ii) un ácido o una base hidrosoluble fisiológicamente tolerable, es capaz de aumentar de forma drástica la solubilidad y por ende la biodisponibilidad del componente farmacológicamente activo.

Por lo tanto, atentamente solicito a ese Despacho la reconsideración de las objeciones realizadas a éste respecto.

- Finalmente, en la Resolución número **30041** de la SIC se afirma que:
- "...utilizar diferentes proporciones del ingrediente activo (un compuesto activo básico: (4-[[4-amino-5-bromo-6-(4-ciano-2,6-dimetilfenoxi)-2-pirimidinil]amino]-benzonitrilo) y del excipiente ácido cítrico, es una labor de optimización de composiciones que corresponde a la labor diaria de una persona del oficio normalmente versada en la materia técnica".*

En respuesta a lo anterior considero de gran importancia mencionar que en el arte previo no existía consideración técnica alguna que le sugiriera a la persona medianamente experta algún mecanismo para lograr obtener una solución supersaturada de un compuesto terapéuticamente activo ácido ó básico, y de esta forma mejorar su biodisponibilidad. Por lo tanto, considero pertinente mencionar que las afirmaciones del señor Examinador demuestran que el análisis realizado no se enfocó de ninguna manera en el momento en que el solicitante de la invención en cuestión planteó el objetivo de obtener un mecanismo efectivo para mejorar la biodisponibilidad de compuesto activos básicos ó ácidos pobremente solubles en agua, es decir, el señor Examinador realmente no tuvo en cuenta la imperiosa necesidad que existe de que el examen de patentabilidad que lleve a cabo lo realice haciendo el

esfuerzo intelectual de colocarse en la situación afrontada por el técnico con conocimientos medios en la materia antes de que el solicitante de la presente invención diera a conocer sus sorprendentes y ventajosos hallazgos técnicos.

III. CONCLUSIONES

Con base en los argumentos técnicos mostrados previamente, me permito manifestar ante ese Despacho que la materia revelada en el capítulo reivindicatorio de la solicitud de patente en cuestión es clara, concisa, se encuentra plenamente sustentado en la descripción y presenta un inequívoco carácter inventivo frente a las enseñanzas de los documentos citados en la Resolución número **30041** aquí impugnada, cumpliendo de esta manera con las disposiciones establecidas en los artículos 30 y 18 de la Decisión 486 de 2000, y por lo tanto demostrando ser merecedora del privilegio de patente requerido.

En efecto, a lo largo del presente escrito se ha demostrado que la capacidad de las composiciones farmacológicas reveladas en la presente invención para aumentar la solubilidad y por ende la biodisponibilidad de agentes activos ácidos ó básicos pobremente solubles en agua, de ninguna manera habría podido ser insinuada por la persona medianamente versada en la materia a partir de la combinación de las enseñanzas de los documentos WO 0122938 (D2) y WO 9926607 (D3), toda vez que dichas anterioridades de ninguna manera mencionan dicho problema técnico, así como tampoco sugieren los componentes y las proporciones de éstos que son necesarios para lograr tal aumento de biodisponibilidad.

En consecuencia y en aras de restablecer los derechos vulnerados al solicitante, tal y como fue explicado a lo largo del presente escrito, atentamente solicito a ese Despacho considerar los argumentos expuestos anteriormente y revocar en su totalidad la Resolución No. **30041**.

IV. ANEXOS

Con el fin de soportar los argumentos mencionados a lo largo de este escrito me permito anexar los siguientes documentos para su referencia:

- (i) La declaración del inventor Marcus E. Brewster con su respectiva traducción.
- (ii) El artículo científico de Roger Vandecruys et. al.; y
- (iii) El artículo científico de Marcus E. Brewster et. al.

Finalmente me permito recordarle a ese Despacho que el documento que acredita mi calidad de representante legal en Bogotá para asuntos relativos a la Propiedad Industrial de la sociedad peticionaria, fue presentado ante dicha entidad con mi solicitud de 25 de Mayo de 2005.

JOSE LLORED CAMACHO & CO.

Calle 72 No. 5-83 Piso 5, Bogotá, D.C. Tel: 6069700 Fax 6069701

V. DOMICILIO

Mi dirección es Calle 72 No. 5-83, Piso 6º., Bogotá D.C., Colombia.

Del Señor Superintendente,



ALICIA LLORED RICAURTE

T.P. 53.215

NOTARIA 41 DEL CIRCULO DE BOGOTÁ D.C.
DEPARTAMENTO DE CUNDINAMARCA
REPUBLICA DE COLOMBIA

El Notario 41 del círculo de Bogotá, da fe de que compareció personalmente: Alicia Lloreda Ricaurte
identificada (e) con: C.C. 39.690.713 y tarjeta
profesional de abogado No. 53215 del Consejo
Superior de la Judicatura, quien autentica la firma puesta por ella
(el) mismo en este escrito.
(Art. 84 C. P. C. Decreto. 2782/89 mod. 89)

Bogotá D.C. 22 JUN. 2011

ALICIA LLORED RICAURTE
C.C. No. 39.690.713, de Usaquén

Notary Seal
Mónica R. Camacho
C.C. 39.690.713



DOCKET NO.: JANS-0086(PRD2017USPCT)
Application No.: 10/536,542
Office Action Dated: March 17, 2009

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Vandecruys, et al.

Confirmation No.: 7079

Application No.: 10/536,542

Group Art Unit: 1618

Filing Date: May 26, 2005

Examiner: Jake Minh Vu

For: Compositions Comprising a Basic Drug Compound, a Surfactant, and a
Physiologically Tolerable Water Soluble Acid

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION PURSUANT TO 37 C.F.R. 1.132

I, Marcus E. Brewster, hereby declare as follows:

1. I am a Distinguished Research Fellow at Johnson & Johnson Pharmaceutical Research and Development (J&J PRD) based in Beerse, Belgium. I am currently the Head of ChemPharm Research and Early Development for Europe as well as the Chief Scientific Officer for the Chemical and Pharmaceutical division and have worked with Johnson & Johnson for 12 years. I am a Fellow of the American Association of Pharmaceutical Scientists, a former member and co-chairman of the Board of Scientific Advisors of the Controlled Release Society (2007-2009) as well as the current co-chairman of the Symposium/Workshop Committee, an Associate Editor of the Journal of Pharmaceutical Sciences (drug delivery and biopharmaceutics), a member of the Editorial Board of Die Pharmazie, a former theme editor for Advanced Drug Delivery Reviews, a member of various scientific societies and organizing boards (including the European Symposium for Controlled Drug Delivery and the International Symposium on Cyclodextrins) and the recipient of various recognitions including the J&J Excellence in Science Award (in 1998 and 2006), an Innovative Analytical Research Prize presented by FACCS (2003) and a PARC Prize for

Innovation in Pharmaceutical Analysis (2007). I have published over 245 peer-reviewed journal articles, book chapters and proceeding, have co-edited a monograph on solvents systems and their use for AAPS Press/Springer, presented over 350 meeting abstracts and was named as inventor or co-inventor on approximately 75 patents. I have also delivered more than 40 plenary lectures and 50 other invited presentations. I received my B.S. from Mercer University in 1978 and his Ph.D. from the University of Florida in 1982 in the field of Pharmaceutical Sciences. I was a visiting scientist at the Weizmann Institute of Science from 1996 to 1997.

2. I am an inventor of the above-referenced patent application. It is my understanding that the claims of the present application are directed to solid or semi-solid pharmaceutical compositions comprising a basic drug compound, vitamin E TPGS ("TPGS"), and a physiologically tolerable water-soluble acid. I also understand that methods of making these compositions are also claimed.
3. Under my direction and control, experiments were performed comparing the supersaturation effect of, for example, formulations of the present invention, on twenty-five developmental candidates having varying physicochemical properties, including molecular weight, TPSA, log P, pKa, and molecular volume. The results of these experiments were published in R. Vandecruys, et al., *Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug solutions and application of this system to solid formulation design*, Int'l J. Pharmaceutics 342 (2007) 168-175 ("Vandecruys").
4. TPGS tended to give a higher average supersaturation as compared to Cremophor RH40 and Polysorbate 20. Vandecruys at Table 2; page 172, col. 2. As this effect was seen over a range of compounds having varying physicochemical properties, I have concluded that this effect is general. That TPGS consistently gave higher average supersaturation results as compared to Cremophor RH40 and Polysorbate 20 is surprising and unexpected in view of what was known in the art.

5. TPGS also provided better stability of the formed supersaturated solution than either Polysorbate 20 or Cremophor RH40. Vandecruys at Table 3; page 173, col. 1. As this effect was seen over a range of compounds having varying physicochemical properties, I have concluded that this effect is general. That TPGS consistently provided better stability results as compared to Cremophor RH40 and Polysorbate 20 is surprising and unexpected in view of what was known in the art.
6. Solid formulations were also prepared to assess the oral bioavailability of Compound 1 using the dog as a model. Vandecruys at page 174, col. 1. Compound 1 is a poorly soluble weak base with a water solubility of 0.002 mg%. *Id.* at 173 at col. 1. Blends of drug compound with either PEG 400, Cremophor RH40, or TPGS were prepared. *Id.* at 174 col. 1. The oral bioavailability of the drug from each formulation was determined by comparing blood levels with those obtained using an IV dose of the compound. *Id.* at page 174, col 1.
7. The results of the dog bioavailability study are shown in Table 5 of Vandecruys. As set forth therein, solubilizing the compound in PEG 400 increased the oral bioavailability of the drug compound to about 30%. Solubilizing the compound in Cremophor RH40 resulted in an oral bioavailability of about 60%. Surprisingly and unexpectedly in view of what was known in the art, using TPGS, an oral bioavailability of about 100% was achieved.
8. Also under my direction and control, experiments were performed comparing the effect on supersaturation of, for example, TPGS, Tween 20 and Cremophor RH40, for itraconazole. The results of these experiments were published in M.E. Brewster et al. *Comparative interaction of 2-hydroxypropyl- β -cyclodextrin and sulfobutylether- β -cyclodextrin with itraconazole: Phase-solubility behaviour and stabilization of supersaturated drug solutions* Eur. J. Pharma. Sci. 34 (2008) 94-103 ("Brewster"). As shown in FIG. 10, formulations of itraconazole with TPGS exhibited very good levels and stability of supersaturation. See also Table 1 supporting robustness of the technique used.

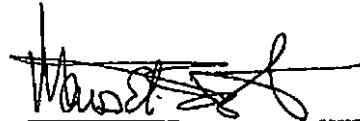
DOCKET NO.: JANS-0086(PRD2017USPCT)
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PATENT

9. Based on the foregoing, I concluded that solid or semi-solid compositions comprising a basic drug compound, TPGS, and a physiologically tolerable water-soluble acid exhibit extent and stability of supersaturation, and oral bioavailability profiles that are unexpected.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 15 September 09



Marcus E. Brewster

EXPEDIENTE NO.: JANS-0086(PRD2017USPCT)
Solicitud No.: 10/536,542
Resolución de fecha: Marzo 17 de 2009

PATENTE

**EN LA OFICINA DE MARCAS Y PATENTES DE
LOS ESTADOS UNIDOS DE AMERICA**

En re a la solicitud de:

Vandecruys, et al.

Confirmación No.: 7079

Solicitud No.: 10/536,542

Unidad Técnica de Grupo: 1618

Fecha de presentación: Mayo 26 de 2005 Examinador: Jake Minh Vu

**Para: Composiciones que comprenden un Compuesto Fármaco Básico,
un Surfactante, y un Ácido Soluble en Agua Fisiológicamente Tolerable.**

Comisionado de patentes

P.O. Box 1450

Alexandria, VA 22313-1450

Señor:

DECLARACIÓN EN CUANTO A 37 C.F.R. 1.132

Yo, Marcus E. Brewster, por medio de la presente declaro lo siguiente:

1. Soy Miembro Distinguido de Investigación en Johnson & Johnson Pharmaceutical Research and Development (J&J PRD) de base en Beerse, Bélgica. Actualmente soy el Jefe de ChemPharm Research and Early Development para Europa así como el Director Científico para la división Química y Farmacéutica y he trabajado con Johnson & Johnson por 12 años. Soy miembro de la Asociación Americana de Científicos Farmacéuticos, ex miembro y co-presidente de la Junta de Consejeros Científicos de la Sociedad de Liberación controlada (2007-2009) así

EXPEDIENTE NO.: JANS-0086(PRD2017USPCT)

PATENTE

Solicitud No.: 10/536,542

Resolución de fecha: Marzo 17 de 2009

como actual co-presidente del Comité del Simposio/taller, un Editor Asociado del Journal of Pharmaceutical Sciences (liberación de fármaco y biofarmacéuticos), un miembro de la Junta Editorial de Die Pharmazie, un ex editor de tema para Advanced Drug Delivery Reviews, un miembro de varias sociedades científicas y juntas organizadoras (incluyendo el Simposio Europeo para Liberación Controlada de Fármaco y el Simposio Internacional sobre Ciclodextrinas) y destinatario de varios reconocimientos incluyendo el Premio de Excelencia en Ciencia J&J (en 1998 y 2006), un Premio de Investigación Analítica Innovadora presentado por FACCS (2003) y un premio PARC por Innovación en Análisis Farmacéutico (2007). He publicado más de 245 artículos para revistas arbitradas, capítulos de libros y procedimientos, he co-editado una monografía sobre sistemas solventes y su uso para AAPS Press/Springer, he presentado más de 350 resúmenes de reuniones y fui nominado como inventor o co-inventor en aproximadamente 75 patentes. También he dictado más de 40 conferencias plenarias y otras 50 presentaciones como invitado. Recibí mi Licenciatura en Ciencias en Mercer University en 1978 y Doctorado de la University of Florida en 1982 en el campo de Ciencias Farmacéuticas. Fue científico visitante en el Weizman Institute of Science de 1996 a 1997.

2. Soy inventor de la solicitud de patente referenciada anteriormente. Entiendo que las reivindicaciones de la presente solicitud están dirigidas a composiciones farmacéuticas sólidas o semisólidas que comprenden un compuesto fármaco básico, vitamina E TPGS ("TPGS"), y un ácido soluble en agua fisiológicamente tolerable. También entiendo que también se reivindican métodos para preparar estas composiciones.
3. Bajo mi dirección y control se realizaron experimentos que comparan el efecto de la supersaturación de, por ejemplo, formulaciones de la

presente invención, sobre veinticinco candidatos en desarrollo que tienen propiedades fisicoquímicas diferentes, incluyendo peso molecular, TPSA, log P, pKa y volumen molecular. Los resultados de estos experimentos se publicaron en R. Vandecruys, et al., *Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug Solutions and application of this system to solid formulation design*. Int'l J. Pharmaceutics 342 (2007) 168-175 ("Vandecruys").

4. TPGS tendió a dar una supersaturación promedio mayor comparado con Cremophor RH40 y Polysorbato 20. Vandcruys en la Tabla 2; página 172, col. 2. Como este efecto se observó en un rango de compuestos que tienen propiedades fisicoquímicas diferentes, he concluido que este efecto es general. El hecho que TPGS produjo consistentemente resultados de supersaturación promedio mayores comparados con Cremophor RH40 y Polysorbato 20, es sorprendente e inesperado en vista de lo que se conoce en la técnica.
5. TPGS también produjo mejor estabilidad de la solución supersaturada formada que cualquiera de Polysorbato 20 o Cremophor RH40. Vandecruys en la Tabla 3; página 173, col. 1. Como este efecto se observó en un rango de compuestos que tienen propiedades fisicoquímicas diferentes, he concluido que este efecto es general. El hecho que TPGS produjo consistentemente resultados de estabilidad mejores comparados con Cremophor RH40 y Polysorbato 20, es sorprendente e inesperado en vista de lo que se conoce en la técnica.
6. También se prepararon formulaciones sólidas para evaluar la biodisponibilidad oral del Compuesto I usando el perro como un modelo. Vandecruys en la página 174, col. 1. El Compuesto I es una base débil

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poco soluble en agua de 0.002%mg. *Id.* en 173 en col. 1. Se prepararon mezclas de compuesto fármaco con cualquiera de PEG 400, Cremophor RH40 o TPGS. *Id.* en 174 en col. 1. La biodisponibilidad oral del fármaco en cada formulación se determinó comparando los niveles en sangre con los obtenidos usando una dosis IV del compuesto. *Id.* en 174, col. 1.

7. Los resultados del estudio de biodisponibilidad en perro se muestran en la Tabla 5 de Vandecruys. Como allí se indica, solubilizar el compuesto en PEG 400 aumentó la biodisponibilidad oral del compuesto fármaco hasta aproximadamente 30%. Solubilizar el compuesto en Cremophor RH40 produjo una biodisponibilidad oral de aproximadamente 60%. Sorprendentemente e inesperadamente, en vista de lo que se conoce en la técnica, al usar TPGS, se logró una biodisponibilidad oral de aproximadamente 100%.
8. También bajo mi dirección y control se realizaron experimentos que comparan el efecto de la saturación de, por ejemplo, TGPS, Tween 20 y Cremphor RH40, por itraconazole. Los resultados de estos experimentos se publicaron en M.E. Brewster et al. *Comparative interaction of 2-hydroxypropyl- β -cyclodextrin and sulfobutylether- β -cyclodextrin with itraconazole: Phase-solubility and stabilization of supersaturated drug solutions* Eur. J. Pharma. Sci. 34 (2008) 94-103 ("Brewster"). Como se muestra en la FIG. 10, las formulaciones de itraconazole con TPGS mostraron niveles muy buenos y estabilidad de supersaturación. Ver también Tabla 1 que respalda la solidez de la técnica usada.
9. Con base en lo anterior, concluí que las composiciones sólidas o semisólidas que comprenden un compuesto fármaco básico, TPGS y un ácido soluble en agua fisiológicamente tolerable, muestran extensión y

estabilidad de supersaturación y perfiles de biodisponibilidad oral que son inesperados.

10. Declaro además que todas las afirmaciones presentes de mi propio conocimiento son verdaderas y se cree que todas las afirmaciones hechas sobre información y creencia son verdaderas; y además que estas declaraciones se hicieron con el conocimiento que declaraciones falsas intencionadas y similares así hechas son punibles por multa o prisión, o ambas, bajo § 1001 del Título 18 del Código de los Estados Unidos, y que tales declaraciones falsas intencionadas pueden poner en peligro la validez de la solicitud o cualquier asunto patentario de la misma.

Fecha: Septiembre 15, 09

(Firma Ilegible)

Marcus E. Brewster



Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug solutions and application of this system to solid formulation design[☆]

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Received 6 December 2006; accepted 8 May 2007

Available online 13 May 2007

Abstract

Assessing the effect of excipients on the ability to attain and maintain supersaturation of drug-based solution may provide useful information for the design of solid formulations. Judicious selection of materials that affect either the extent or stability of supersaturating drug delivery systems may be enabling for poorly soluble drug candidates or other difficult-to-formulate compounds. The technique suggested herein is aimed at providing a screening protocol to allow preliminary assessment of these factors based on small to moderate amounts of drug substance. A series of excipients were selected that may, by various mechanisms, affect supersaturation including pharmaceutical polymers such as HPMC and PVP, surfactants such as Polysorbate 20, Cremophor RH40 and TPGS and hydrophilic cyclodextrins such as HPBCD. Using a co-solvent based method and 25 drug candidates, the data suggested, on the whole, that the surfactants and the selected cyclodextrin seemed to best augment the extent of supersaturation but had variable benefits as stabilizers, while the pharmaceutical polymers had useful effect on supersaturation stability but were less helpful in increasing the extent of supersaturation. Using these data, a group of simple solid dosage forms were prepared and tested in the dog for one of the drug candidates. Excipients that gave the best extent and stability for the formed supersaturated solution in the screening assay also gave the highest oral bioavailability in the dog.

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Keywords: Supersaturation; Excipient; Cellulose polymers; PVP; Surfactants; Cyclodextrins; Co-solvents

1. Introduction

The ability to generate formulations with relevant orally bioavailability depends on a number of factors including solubility, permeability and metabolic stability. Absorbability is related to the first two factors whose importance has been recognized in

the guise of the biopharmaceutical classification system (BCS) (Dressman et al., 1998; Amidon et al., 1995). This approach bins drugs and drug candidates into four categories based on their solubility and permeability properties. The manner in which new drug candidates are selected often relies on high throughput screening techniques. These immobilized receptor techniques tend to sub-select for compounds with undrug-like attributes including high lipophilicity, poor aqueous solubility and high molecular weights (Lipinski et al., 1997; Lipinski, 2001). Retrospective analysis completed in the late 1980s suggested that many drug failures (~40%) were related in some way to the poor biopharmaceutical properties (Prentis et al., 1988). Reanalysis of drug candidate failures in 2004 indicated that some progress had been made with regard to screening out drug candidates with poor drugability elements but poor drug solubility and, by virtue of the Noyes–Whitney relationship, poor dissolution rates continue to be an important consideration in drug candi-

[☆] A preliminary account of this work has been presented in poster form: Peeters J, Vandecruys R, Brewster M., 2003. The use of supersaturation studies in early pharmaceutical development. 2003 American Association of Pharmaceutical Scientists Annual Meeting and Exposition, Salt Lake City, UT, USA, 26–30 October 2003; Vandecruys R, Peeters J, Brewster M., 2006. Supersaturation studies to improve drug formulations. 2006 American Association of Pharmaceutical Scientists Annual Meeting and Exposition, San Antonio, TX, 29 October–2 November 2006.

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date attrition (Kola and Landis, 2004). Numerous methodologies have been suggested and practically applied to improve the ability to market drug candidates whose development is limited by drug solubility, dissolution rate and by virtue of Pick's First Law, absorbability. These include the use of particle size manipulation via micronization and nanonization, the use of wetting agents, the use of complexing agents such as cyclodextrins and the preparation of high energy drug states related to polymorphic or amorphous transformations (Liu, 2000; Kim and Park, 2004; Merisko-Liversidge et al., 2003; Forster et al., 2002; Humberstone and Charman, 1997; Davis and Brewster, 2004).

One suggested path forward is the use of a spring and parachute approach wherein a technique such as solid dispersions or self-emulsifying systems allow rapid dissolution of the poorly water-soluble drug at supersaturated concentration. A formulation component which hinders nucleation or crystal growth then acts as a parachute to stabilize the metastable supersaturated systems (Guzmán et al., 2004; Gao and Morozowich, 2006; Gao et al., 2004). An example of this stratagem is the capsule-based dosage form for the antifungal compound, Sporanox (itraconazole) (Peeters et al., 2002). Itraconazole is associated with very poor formulation properties including a low aqueous solubility (estimated at ~ 1 ng/mL at neutral pH), a $\log P > 5$ and a melting point of 167 °C. The successful preparation of an oral formulation was based on the development of a solid solution of the drug in a polymeric matrix based on HPMC. This system was prepared by a solvent method in which the drug and polymer were dissolved in a common solvent and coated onto an inert sugar sphere. In this formulation, dissolution of the water-soluble HPMC phase was associated with release of the itraconazole at concentrations above its saturation solubility. The co-dissolving HPMC acted as an inhibitor of drug nucleation and crystal growth such that supersaturated concentrations were maintained long enough for significant absorption and oral bioavailability. The maximum fraction absorbed for this formulation was $\sim 85\%$ and oral bioavailability as high as $\sim 55\%$ (Brewster et al., 2004). Other examples are available for solid dispersions prepared by melt extrusion and spray drying (Kohori et al., 1999; Crew et al., 2005; Appel et al., 2006). In addition, nanoparticulate systems can give supersaturated systems based on the greater solubility of small versus large particles (Müller et al., 1999; Lindfors et al., 2006; Wu and Nancollas, 1998).

The optimisation of system features that will allow for sustained supersaturation and by extension improved oral bioavailability is the stimulus for the current study. A number of research efforts have pointed to methods for solubilizing, that is increasing the saturation solubility, of poorly water-soluble drugs and drug candidates. Some of these have lead to marketed products (Strickley, 2004). Supersaturation approaches to enhance topical and transdermal therapies have been widely reported (Raghavan et al., 2001, 2003; Davis and Hadgraft, 1991; Moser et al., 2001a,b; Hadgraft, 1999). Less information is available on techniques to stabilize formed high energy solutions intended for oral application especially in a manner which could be useful in early formulation design and screening. Armed with such data the selection of excipient may be optimized. Herein we suggest an approach for testing excipients

with regard to their ability to attain and maintain supersaturation. This approach was implemented and results on a number of early drug candidates are discussed. Finally, a case study is described where early solid formulations are generated based on excipient behaviour in the supersaturation tests.

2. Materials and methods

Research compounds were obtained from Johnson & Johnson Pharmaceutical Research and Development, Beerse, Belgium. All compounds demonstrated a purity of $>95\%$. The $\log P$ and pK_a of the drug candidates were determined experimentally while other parameters (topological polar surface area (TPSA) and molecular volume (MolVol)) were calculated using the ADME Predictor software package (Simulations Plus, Lawrenceville, CA). The following excipients were used in the studies: hydroxypropyl cellulose (HPC) 150–700 mPa.s (Aqualon Belgium N.V., Doel-Beveren, Belgium), hydroxypropylmethyl cellulose (HPMC) 2910 5 mPa.s (Aqualon, Hercules, Zwijndrecht, The Netherlands), Polyox NF 100K (Dow Chemical Company, Midland, MI, USA), polyvinylpyrrolidone-co-vinyl acetate (PVP VA)64 (BASF AG, Ludwigshafen, Germany), polyvinylpyrrolidone (PVP) K30 (BASF AG, Ludwigshafen, Germany), Cremophor RH40 (BASF, Hamoir, Belgium), Polysorbate 20 (Codibet NV, Zaventem, Belgium), α -tocopherol polyethylene glycol succinate (TPGS) (Eastman Chemical Company, Anglesey, UK), PEG4000 (Sigma-Aldrich, Bornem, Belgium), 2-Hydroxypropyl- β -cyclodextrin (HP β CD) was obtained from Roquette (Lestrem, France) and was characterized by a degree of substitution of 4.2 based on an FT-IR method (Michaud and Icart, 2001). Additionally, for the solid dosage forms the following excipients were used: lactose (DMV International, Veghel, The Netherlands), citric acid (Merck, Darmstadt, Germany), sodium lauryl sulfate (SLS) (Cognis Benelux, Hoofddorp, The Netherlands), silicone dioxide (Degussa, Frankfurt, Germany), PEG400 (Functional Chemicals, Mittenz, Switzerland), PEG20000 (Sigma-Aldrich, Bornem, Belgium), sugar spheres (Hans Werner, Tornesch, Germany), microcrystalline cellulose (MCC) (FMC, Philadelphia, USA), croscarmellose sodium (FMC, Philadelphia, USA), magnesium stearate (Univar Benelux, Brussels, Belgium). Other materials and solvents were obtained from Sigma-Aldrich (Bornem, Belgium) or Janssen Pharmaceutica (Beerse, Belgium).

2.1. Supersaturation assay

A co-solvent/solvent quench-based approach was used to generate the drug in a supersaturated state. In most cases, the drug of interest was dissolved to a concentration of 100 mg/mL in *N,N*-dimethylformamide (DMF). Dimethylacetamide (DMA) was also used in a few experiments and in isolated incidences, a 50 mg/mL solution was applied based on the limited solubility of the drug candidate. In the majority of cases the final organic content in the dissolution media ranged between 1% and 5% (v/v) although there were a few compounds where the final media contained $>5\%$ (v/v) of the organic component. A dissolution vessel

AL

was prepared using a 20 mL glass vial with stopper. Into the vial was placed 10 mL of the media of interest (0.01N HCl, USP pH 4.5 buffer, USP pH 6.8 buffer or water) with or without 2.5% (w/v) of the excipient of interest. The vial was equilibrated at 37°C in a water bath (Variomag Tele module 20P) and stirred at 600 rpm using a magnetic stir bar (2 cm × 0.55 cm). The organic solution of the compound was added drop-wise using a small volume pipet into the stirring solution until a precipitate was just noticeable visually. At 5, 30, 60 and 120 min post-drug-addition, a small volume of the dissolution medium was withdrawn, filtered through a 0.5 µm Millex-LCR (Millipore Corp.) filter and the concentration determined using Beer's Law with an Agilent 8543 UV spectrophotometer. The pH of the systems was measured throughout the sampling exercise (Sentron-type pH meter (Titan)). In assessing the excipients, a supersaturation index was defined based on the ratio of the initial drug concentration in the excipient-based dissolution vessel as a function of that in the dissolution media that did not contain the excipient. In addition the physical stability of the solution was assessed over time with a $\Delta\%$ defined as the extent to which the drug precipitated from C(5 min) to C(120 min). In addition, initial supersaturation associated with addition of the drug-containing organic solvent to media without excipients was inferred based on the initial concentration values as well as the change in concentration of these systems over time.

2.2. Formulation based on the supersaturation studies

Six simple formulations were used in the animal studies including (1) a simple drug blend in a gelatin capsule, (2) a PEG400 solution, (3) a drug dispersion in HPMC E5 coated onto sugar spheres and placed in a gelatin capsule, (4) an HPBCD coprecipitate pressed into a tablet, (5) a mixture of the drug and Cremophor RH40 with additional excipients filled into a gelatin capsule, (6) a mixture of TPGS and other excipients filled into a gelatin capsule. In all cases red cap-red body capsules from Capsugel (Belgium) were used. The amount of drug in the formulations was adjusted to the weight of the dogs. In the descriptions, generic preparation methods for either 50 mg (1 unit per dog) or 25 mg (2 units per dog) nominal weight formulations are given. For the drug blend in capsule, 25 mg drug, 0.59 mg sodium lauryl sulfate, 0.59 mg silicon dioxide and 300 mg granulated lactose were mixed and filled into a size 1 capsule. The PEG400 solution contained 40 mg of Compound 1 in 1.0 mL of PEG400. The bead-based concept contained 25 mg Compound 1, sugar spheres (25–30 mesh, 236 mg), 75 mg of HPMC E5 and 13.5 mg of PEG20000 used as a seal coat. Drug and HPMC were dissolved in a mixture of 2040 mg of dichloromethane and 360 mg of ethanol. The solution was sprayed on the sugar beads using an Uni-Glatt coater with 6 in. Wurster insert (Uni-Glatt, Glatt, Germany) at a spraying rate of 15–20 g/min and an inlet temperature of 50–60°C. In a second step, PEG20000 was dissolved in a mixture of 72.9 mg dichloromethane and 48.6 mg ethanol and sprayed on top of the first layer. The total batch size was approximately 1600 g. The coated sugar beads were filled in the hard gelatin capsules (size 0) by hand. The HPBCD-based formulation contained

25 mg of Compound 1, 75 mg of HPBCD, 14 mg of citric acid monohydrate, 243 mg of granulated lactose, 60 mg of microcrystalline cellulose, 30 mg of croscarmellose sodium and 2.3 mg of magnesium stearate. The drug, cyclodextrin and citric acid were mixed in acetone and ethanol after which the solvent was removed. The co-precipitate was blended with the other listed excipients and hand pressed into a tablet. For the concept based on Cremophor RH40, the drug (50 mg), the surfactant (250 mg) and citric acid monohydrate (500 mg) were dissolved in ethanol after which the solvent was removed and the residue manually loaded into a size 00 capsule. The last concept was similarly prepared by blending the drug (50 mg), TPGS (250 mg) and 25 mg of HPMC E5 in ethanol, removing the solvent and then loading the residue into a size 0 capsule. The latter two concepts are based on a glass thermoplastic approach (Brewster et al., 2003). The dissolution of the capsules was assessed using a USP II apparatus using 600 mL of 0.01N HCl medium equilibrated at 37°C. The paddle stirred at 75 rpm and data are given as the average of two units.

2.3. Bioavailability in the dog

All animal studies were completed according to relevant Belgium Law (18 October 1991) as approved by the European Convention on the protection of vertebrates that are used for experimental and other scientific purposes, Annex A and B as drawn up in Strasbourg (18 March 1986) and the Royal Decree of 14 November 1993 covering the protection of laboratory animals. All experiments were planned and completed under the auspices of the Institutional Animal Care and Use Committee (IACUC). Male Beagle dogs were obtained from Harlan, Germany and maintained in the resident Jansen Pharmaceutica dog colony. Their body weight ranged from 11 to 13.4 kg at the start of the study. Dogs were healthy as judged from routine blood examinations (haematological and biochemical parameters) and certified by the attending veterinarian. All animals were observed prior to, at the time of dosing and up to 24 h post-dosing. Dogs had free access to water and food through the experimental period. Fed animals were dosed using a cross-over design with a washout period of 14 days. Solid formulations were dosed orally. Oral solutions were administered by gavage using a stomach tube. The IV arm of the study was completed using a dose of 1.25 mg/kg and a vehicle containing 75:25 PEG:water. Blood samples were obtained from the jugular vein (3 mL collected onto EDTA) just prior to dosing and at the following time points thereafter: 0.5, 1, 2, 4, 6, 8, 24, 32, 48, 72 and 96 h. Blood samples were centrifuged at room temperature at 1900 × g for 10 min to separate the plasma. The plasma was transferred to a second test tube and frozen within 2 h of sampling at –20 °C. Samples were analysed using a validated LC–MS/MS method. Plasma samples (0.1 mL) were subjected to solid phase extraction (Bond Elut solid phase columns, 130 mg, SPE (Varian Corp)). Columns were conditioned with 3 mL methanol, 3 mL water and 1 mL acetic acid (1 M). Plasma was applied to column and washed with subsequent aliquots of water, acetic acid and methanol. The compound was eluted from the column with 3 mL methanol/ammonium

hydroxide (25% aqueous) at a ratio of 98:2. The extract was evaporated to dryness and reconstituted with 150 µL of a 50:50 mixture of ammonium formate (0.01 M) and methanol. Twenty microliters of the sample was then applied to a 10 cm × 4.6 mm i.d. reversed phase HPLC column (3 µm Hypersil C18 BDS) and eluted with a mobile phase containing 40% 0.01 M ammonium formate and 60% methanol flowing at 0.8 mL/min (before splitting). LC-MS/MS analysis was completed using an API-3000 system (Applied Biosystems) which was dedicated to the HPLC (Agilent). The lower limit of quantification was 1 ng/mL. Individual plasma concentration-time profiles were subjected to a non-compartmental pharmacokinetic analysis using WinNonlin software (Pharsight Corp., Mountain View, CA, USA). The area under the plasma curve (AUC) from time 0 to 24 or 96 h was calculated using the linear up/down trapezoid rule. Oral bioavailability was reported as the ratio of (AUC_{oral}/AUC_{iv}) × 100.

3. Results and discussions

Excipients which stabilize formed supersaturated solutions may be important additives to solid formulations. A method was developed to screen for such excipients based on a co-solvent approach. Twenty-five developmental candidates were screened using this assay. Physicochemical properties including molecular weight, TPSA, log *P*, p*K*_a and molecular volume for the screened materials are presented in Table 1. The compounds cover a reasonable chemical space with MW ranges between 327 and 721, TPSAs between 46 and 198 Å², log *P*s between 1.76 and >5 and molecular volumes between 273 and 602 Å³. The effect of various excipients on the extent of supersaturation

is given in Table 2 while the stability of the formed supersaturated solution in the presence of the specified excipient is given in Table 3. The excipients were selected based on a variety of potential mechanisms which may impact the ability of the material to nucleate or to arrest crystal growth. The exact mechanisms associated with nucleation and crystal growth are not well-described. Crystal growth is believed to take place in three steps (Macic and Grant, 1986; Rodríguez-Hernández and Murphy, 1999; Raghavan et al., 2001):

- diffusion of the molecule from the bulk media to the solid crystal interface;
- the adsorbed molecule, through a surface reaction, becomes part of the crystal lattice and the heat of crystallization is released;
- the heat of crystallization is conducted to the bulk media.

Materials that may inhibit nucleation or crystal growth have been reported. These materials have several potential actions including:

- altering bulk properties such as surface tension or saturation solubility;
- changing the adsorption layer at the crystal-medium interface;
- selectively adsorbing to the crystal interface thereby blocking crystal growth;
- being adsorbed into growth layers and thereby disrupting growth layers across the surface;
- adsorbing into surface imperfections causing rough surfaces to become flat;

Table 1
Compound properties, dissolution media and initial and final concentrations in the dissolution media

Compound	MW	TPSA (Å ²)	log <i>P</i>	p <i>K</i> _a	MolVol (Å ³)	Media	C(5 min) (mg%)	C(120 min) mg%	Δ%
1	366.4	97	4.8	5.6	352	0.01N HCl	18	16	−11
2	373.4	80	1.58	1.86	306	0.01N HCl	18	14	0
3	436.5	107	4.97	4.9	392	0.01N HCl	41	8	−80
4	453.5	150	3.9	3.53	385	0.01N HCl	0.05	0.03	−40
5	558.7	108	3.9	Multiple	559	pH 6.8	5.5	0.21	−96
6			1.76	2.68		0.01N HCl	34	10	−71
7	547.4	109	4.49	6	373	pH 6.8	0.3	<0.001	−
8	441.4	131	3.05	10.42	340	0.01N HCl	7.3	1.5	−75
9	353.4	82	2.13	2.2/3.9	308	0.01N HCl	92	9	−90
10	413.5	47	2.72	8.18	322	0.01N HCl	0.23	<0.001	−
11	327.5	49	4.27	—	336	Water	38	38	0
12	573.7	198	3.29	2.2	490	pH 6.8	71	4	−94
13	504.5	102	5.03	5.72	410	0.01N HCl	<0.05	<0.05	−
14	686.8	49	4.43	7.2/3.1	602	pH 6.8	9	4	−56
15	573.7	108	3.29	2.2	490	0.01N HCl	17	18	6
16	720.9	108	4.94	Multiple	686	0.01N HCl	3	7	130
17	676.3	130	4.88	7.5/3.6	585	pH 4.5	13	3	−77
18	427.5	97	2.4	8.26	303	0.01N HCl	397	—	—
19	344.4	94	2.9	—	306	0.01N HCl	<0.01	<0.01	—
20	371.4	54	2.9	2.1/8.5	343	0.01N HCl	0.4	0.4	0
21	499.4	50	5.11	2.8	324	0.01N HCl	<0.001	<0.001	—
22	555.5	46	3.14	9.1	466	0.01N HCl	351	364	4
23	572.6	59	>5	6.3	487	pH 6.8	<0.001	<0.001	—
24	394.4	154	2.15	3/9	273	0.01N HCl	5	5	0
25	639.8	84	>5	8.5	609	0.01N HCl	<0.001	<0.001	—

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Table 2
The extent of supersaturation observed for various compounds in the presence of 2.5% (w/v) of various excipients

Compound	HPC	HPMC E5	PolyOx	PVPVA	PVPK30	RH40	Poly 20	TPGS	HPBCD	PEG4000
1		12.3						5.1		
2									16	
3										
4	8	12		6	6	26	23	48		
5				5		7.5		16	4.4	
6	7			7	5.1				7.2	
7		5		57	20	57	17	33	20	
8						73	46	84	15	
9				10		73	10	10	6.7	
10						15	22	70	91	
11						56	53	6	11	
12										
13		6.5								
14						48	13	45	11	
15						24.5	19	42	10	
16				6	6.3	9.2	51	23.5	18	
17							30	31	14	
18										
19		9				12	34			
20				6.4	2.5	55	45	85	40	
21										
22										
23										
24										
25										

Supersaturation ratio: (white) 0–4.9; (light gray) 5–9.9; (medium gray) 10–99; (dark gray) 100–1000; (black) >1000.

- altering the surface energy of the crystal face which may change the level of solvation.

Rheological polymers such as HPMC and PVP are thought to interact through a number of mechanisms including adsorbing to the crystal (via hydrogen bonding) and collecting at the growing crystal-bulk media interface and thereby providing diffusion resistance (Raghavan et al., 2001). Some reports also suggest that these polymers can form complexes with the drug of interest, increase their saturation solubility and therefore reduce the extent of supersaturation (Rodríguez-Hernández and Murphy, 1999; Strickley, 2004). Surfactants can solubilize materials via micelle formation but can also alter the surface tension at the crystal-medium interface (Constantinides et al., 2006; Rangel-Yagui et al., 2005). Cyclodextrins can solubilize material through the formation of dynamic inclusion complexes (Lofsson et al., 2004; Lofsson and Brewster, 1996; Thompson, 1997; Rajewski and Stella, 1996). Additional data suggest that cyclodextrins can also inhibit nucleation and crystal growth through non-complex based mechanisms which may be similar to those associated with the pharmaceutical polymers described above (Brewster et al., 2006; Torres-Labandeira et al., 1990; Uekama et al., 1992). PEG4000 or Polyox may affect supersaturated solutions through various mechanisms (Li et al., 2006; Urbanetz and Lippold, 2005).

The cellulosic, PEO/PEG and PVP-based polymers had variable effects on the formed supersaturated solution. HPC gave

generally poor results with two compounds (Compounds 4 and 6) giving solubility increases in the 5–10-fold range and one compound (Compound 25) providing a significant increase over the baseline. In this case, the absolute value of the drug concentration in the supersaturated solution was small (11 mg%). HPMC was somewhat more conducive for the formation of supersaturated solutions with 7 hits, 4 in the range of 5–10-fold (Compounds 7, 10, 13 and 19), 2 in the range of 10–20-fold (Compounds 1 and 4) and in one instance the excipient provided for a 280-fold increase in concentration (Compound 21). Again in this case, the absolute values of the formed solution was low (0.5 mg%) and the enhancement was related to the very poor solubility of the compound in the dissolution media without excipients. Polyox and, not unexpectedly, PEG4000 were the worst excipients in forming supersaturated solutions in that there was not a single example of a concentration increases above three-fold or so. The surfactants, as a class, better supported the formation of supersaturated solutions. There were 17-hits in the case of both Cremophor RH40 and Polysorbate 20 and 20-hits for TPGS. TPGS also tended to give a higher average supersaturation compared to the other two materials. Compounds that gave the highest absolute values in the supersaturation test for TPGS included Compound 9, 885 mg%, Compound 17, 710 mg% and Compound 8, 614 mg%. HPBCD also provided for useful values with 18 of the 25 compounds demonstrating supersaturation ratios >5. The highest ratios were obtained for Compounds 13, 21, 23 and 25 while the highest absolute values were available

Table 3
The stability of formed supersaturated solution in the presence of 2.5% (w/v) of various excipients

Compound	HPC	HPMC E5	PolyOx	PVPVA	PVPK30	RH40	Poly20	TPGS	HPBCD	PEG4000
1	247			36						
2						42	45	70		
3	205									
4		67		73	67					
5		75				56	80	71		
6							65			
7	35			35						
8	30	60			31		50	60		
9				34						
10		70	21	44			40	44	33	
11										
12						27	27	63		
13	60		54	27	30			58		
14				63						74
15							20			
16						74		70		
17	68				72		43			
18										
19								30		
20		38	46		57	41		20		43
21								51		
22					28					
23								77		
24								21	21	37
25				53		56				

Δ (%): (ΔΔΔΔ) 0–25%; (ΔΔΔ) 26–75%; (ΔΔΔΔΔ) >75%.

for Compound 9, 625 mg%; Compound 25, 421 mg%; Compound 11, 414 mg% and Compound 6, 244 mg%. These data are in contrast to other reports which suggest that cyclodextrins were poor excipients in supporting supersaturation and, in some cases, accelerated crystallization (Dias et al., 2003; Ma et al., 1996; Iervolino et al., 2000). Simple correlation between supersaturation ratios and individual physicochemical parameters given in Table 1 was poor suggesting complex interactions between the excipients and the drug candidates.

An indication of the stability of the formed supersaturated systems is provided in Table 3. Taken in isolation, the data suggest that HPC provides stable solution in 12 out of 25 instances while there were 14 hits for HPMC. Polyoxy and PEG4000 provided stable solutions in 8 and 15 cases, respectively. The surfactants could be stratified based on stability with TPGS > Polysorbate 20 > Cremophor RH40. These data cannot, however, be used in and of themselves and should be combined with the information in Table 2. That is information on both the extent and stability of the supersaturated solutions is needed to select appropriate excipients for subsequent development. To this point, an example may be illustrative.

Compound 1 is a poorly soluble weak base with a water solubility of 0.002 mg% and solubilities in 0.01N HCl of 1.9 mg% and 0.1N HCl of 1.3 mg%. When a DMA solution is added to 0.01N HCl, a supersaturated solution is generated at 78 mg% (i.e., nine-fold higher than the equilibrium solubility in 0.01N HCl) which is maintained through 120 min with an 11% decrease

in concentration. Table 4 gives the time versus concentration data obtained in the supersaturation assessments. In evaluating the first line of Tables 2 and 3, the excipient that gave the highest extent of supersaturation was HPMC which gave a 12-fold increase over the solubility generated in the medium without excipient and greater than 100-fold increase over the equilibrium solubility in 0.01N HCl and TPGS where a five-fold improvement over the medium and an almost 50-fold increase over the

Table 4
Concentration (in mg%) of Compound 1 over time in a dissolution media (0.01N HCl) containing 2.5% (w/v) of various excipients

Time (min)	0.01N HCl	HPC	HPMC E5	PVPVA	PVPK30
5	18	38	232	31	16
30	16	24	27	22	14
60	16	20	21	20	13
120	16	20	20	18	13
Final pH	1.99	2.30	2.38	2.29	2.30
Final % organic solvent	3.4	2.9	2.4	3.1	2.9

Time (min)	RH40	Poly20	TPGS	HPBCD	PEG4000
5	57	65	92	28	25
30	52	58	84	25	22
60	51	57	76	24	21
120	50	56	75	24	20
Final pH	2.11	2.10	2.36	2.0	2.29
Final % organic solvent	3.7	3.7	5.3	2.7	2.6

Table 5
Biopharmaceutical properties and oral bioavailability of various early solids concepts for Compound 1

Formulation concept	Dissolution ^a (%)	C _{max} (ng/mL)	t _{max} (h)	AUC (ng h/mL)	Oral BA (%)
Drug-in-capsule	47	21	4	308	1.3
PEG400 solution	—	341	4	8,359	31
HPMC E5 coated bead	94	449	2	11,778	38
HPBCD-based tablet	88	761	6	18,129	58
RH40-based capsule	78	816	3	16,981	58
TPGS-based capsule	98	1145	5	31,008	106

^a Dissolution at 30 min (see Section 2).

equilibrium solubility was observed. In assessing stability, the worst excipient was HPMC with compound levels falling 90% over the 120 min experiment time course. The concentration of drug obtained using TPGS as the excipients were sustained with an 18% loss between the 5 and 120 min time point.

Solid formulations were prepared to optimize the oral bioavailability of Compound 1 using the dog as a model. The formulations that were prepared included a simple drug blend in capsule, a PEG400 solution, a capsule containing beads onto which was deposited a solid dispersion of the compound in HPMC (Verreck *et al.*, 2004), a tablet based on a co-precipitate of the drug with HPBCD, the compound dissolved in Cremophor RH40 and filled into a gelatin capsule and the drug formulated with TPGS and filled into a gelatin capsule. The amount of drug was adjusted such that dogs received 5 mg/kg of the compound. The oral bioavailability of the drug from each concept was determined by comparing blood levels with those obtained using an IV dose of the compound. The data are presented in Table 5. The bioavailability of the compound out of a simple blend was poor consistent with its high crystallinity (m.p. = 256 °C), low solubility, high log *P* and weakly basic nature. Solubilizing the compound in PEG400 increases the bioavailability to 30% but the poor effect of PEG400 on supersaturation suggests further improvements might be possible. To that end, a concept based on HPMC E5 was assessed. The oral bioavailability of this concept was slightly better than that associated with the PEG400 solution. That is while HPMC may give a good effect on supersaturation extent, the compound so formulated may rapidly precipitate negating any advantage. The cyclodextrin tablet increased the bioavailability in the dog to almost 60% meaning the modest solubility increase (1.6-fold versus medium without excipient and 15-fold versus equilibrium solubility in 0.01N HCl) together with the stabilizing effect of the excipient combined to give a useful solid formulation. Similar results were obtained with Cremophor RH40 which was comparable to the cyclodextrin with respect to both the extent and stability offered to the formed supersaturated solutions. The solid concept with the best performance was based on TPGS where a bioavailability of 100% was achieved. In this case, it is possible that the combination of the increased extent of supersaturation as well as the resulting stability was optimal with regard to the compound assessed. In this limited sample size, it may be concluded that while generating supersaturated solutions at increased concentrations is important, the stability of the formed systems may be the main driver in this excipient-based formulation approach.

4. Conclusions

The data collected herein suggest that a knowledge of how excipients interact with developmental drug candidates may be useful in designing oral solid dosage forms. Two factors seem to be important including the extent to which the excipient can increase supersaturation and the stability of the formed systems. Such data is useful in preparing systems based on solid dispersions, nanoparticles or the amorphous form of the drug. In all of these cases, the high energy form of the drug may dissolve at supersaturated concentration which may, from both a thermodynamic as well as a concentration point of view, be enabling for difficult-to-formulate materials. In the example cited, information on supersaturating excipients was useful in designing simple solids. The approach described is a useful place to start to generate possible excipients with added value in a solid formulation. It is just a start however. Follow-up information should include assessing synergies between different excipients or excipient types. In addition, it may be possible to select a polymeric carrier which also has good supersaturating properties as in the case of HPMC and itraconazole (Verreck *et al.*, 2003; Six *et al.*, 2003) or HPBCD and itraconazole (Ramhali *et al.*, 2003).

Acknowledgement

The authors are indebted to our colleagues in Global Preclinical Development for pharmacokinetic and bioanalytical support.

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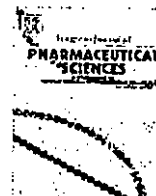
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Comparative interaction of 2-hydroxypropyl- β -cyclodextrin and sulfobutylether- β -cyclodextrin with itraconazole: Phase-solubility behavior and stabilization of supersaturated drug solutions

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ARTICLE INFO

Article history:

Received 19 October 2007

Received in revised form

10 December 2007

Accepted 1 February 2008

Published on line 26 February 2008

Keywords:

Cyclodextrin

Solubility

Itraconazole

Supersaturation

Co-solvent/solvent quench approach

ABSTRACT

Cyclodextrins can increase the apparent solubility and dissolution rate of poorly water-soluble drug candidates improving their biopharmaceutical performance. The current data assess the ability of hydrophilic cyclodextrins to solubilize compounds via stabilization of supersaturated drug solutions presumably by inhibition of nucleation and arresting crystal growth. To these points, the effects of 2-hydroxypropyl- β -cyclodextrin (HPBCD) and sulfobutylether- β -cyclodextrin (SBE β CD) on equilibrium solubility was assessed via phase-solubility analysis as were the interactions of these excipients on drug solubility under conditions favoring supersaturation. Phase-solubility analysis indicated that different profiles were generated as a function of the cyclodextrin examined and the pH of the complexing medium. When kinetic solubility measurements were completed, the cyclodextrins were found to stabilize concentrations of itraconazole significantly in excess of their equilibrium solubility when supersaturated solutions were formed using the co-solvent/solvent quench approach. These solutions were stable over 240 min falling in concentration at the 24 h time point of the experiment unlike those formed using surfactants and other polymers which demonstrated a rapid decrease in concentration over time. These data suggest that hydrophilic cyclodextrins might be useful formulation adjuncts in supersaturating drug delivery systems.

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1. Introduction

Cyclodextrins are functional excipients that have gained widespread use and attention because of their ability to sol-

ubilize, and in some instances stabilize, poorly water-soluble drug candidates enabling both oral and parenteral formulation (Fig. 1) (Davis and Brewster, 2004; Loftsson et al., 2004a; Loftsson and Brewster, 1996; Thompson, 1997; Rajewski and

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doi:10.1016/j.ejps.2008.02.007

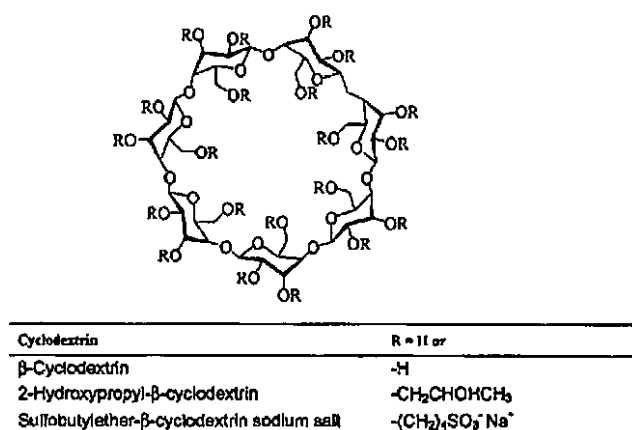


Fig. 1 – Chemical structure of β -cyclodextrin, 2-hydroxypropyl- β -cyclodextrin (HP β CD) and sulfobutylether- β -cyclodextrin (SBE β CD).

Stella, 1996). In addition, these materials can convert oils and viscous liquids into free flowing powders and can suppress vapor pressure and therefore can reduce unaesthetic smells and tastes (Brewster et al., 1989). In the case of solubilization and for many of the other property improvements observed, the mechanism suggested is the formation of dynamic inclusion complexes in solution between the cyclodextrin and the compound of interest (Szejtli and Osa, 1996; Szejtli, 1988). This interaction occurs because of the specialized architecture of the cyclodextrin molecule wherein the material takes the form of a truncated cone or torus with the primary hydroxyl functions oriented to the narrower end of the torus and the secondary hydroxyl function oriented towards the wider end (Lofstsson and Brewster, 1996; Brewster et al., 1989). This makes the exterior of the molecule relatively hydrophilic while the cavity interior is relatively hydrophobic so that the starch derivative has some degree of water solubility. At the same time, the interior lipophilic cavity allows appropriately sized lipophiles to interact resulting in solubilization.

While complexation describes many of the pharmaceutically and biopharmaceutically relevant attributes of cyclodextrins, some observations suggest that other factors are also involved. Lofstsson et al. (2002, 2004b) found, for example, that in addition to complexation, other non-complex related phenomena can contribute to drug solubilization including the formation of aggregates in solution as well as surface active properties of the drug-cyclodextrin system. In the model developed, solubilization of the drug is a function of both complexation and non-complex-based association and was based on discrepancies between ideal and observed phase-solubility behavior.

The current work looks to explain several observations that seem to be in contradiction to pure complex-driven effect of cyclodextrins using itraconazole as a probe molecule. Itraconazole is a broad-spectrum antifungal agent which is marketed in an oral and i.v. solution containing HP β CD (Sporanox®) in the US, Europe and the Far East (Davis and Brewster, 2004). Certain hydrophilic cyclodextrins appear to have differential effects, both qualitatively and quantitatively,

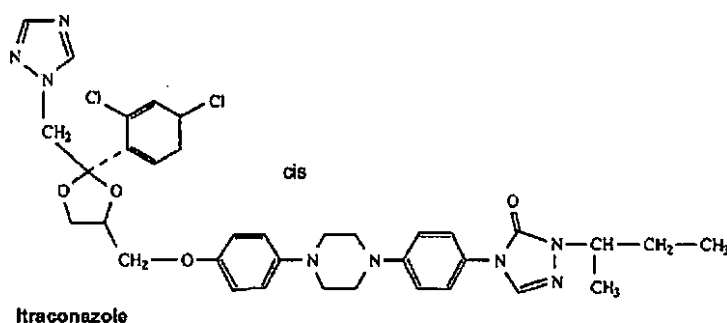


Fig. 2 – Chemical structure of itraconazole.

on the solubilization profile on itraconazole (Fig. 2). Brewster et al. (2007) reported that solubilization of crystalline itraconazole in 20% w/v HP β CD in an acidic medium was associated with a day 1 solubility value of 350 μ g/mL which increased slowly over time to give a day 28 value of 500 μ g/mL. Conversely, amorphous itraconazole generated 11.6 mg/mL at a day 1 measurement which slowly decreased to 2 mg/mL at day 28. One explanation for these observations is the possible effect of HP β CD as a stabilizer of the formed supersaturated system via nucleation and crystal growth inhibition. The ability of cyclodextrin to act in this capacity has been suggested by several authors (Loftsson et al., 2004c; Pedersen, 1997; Uekama et al., 1992; Ma et al., 1996; Xiang and Anderson, 2002; Torres-Labandeira et al., 1991; Dias et al., 2003; Iervolino et al., 2000, 2001).

2. Materials and methods

2.1. Materials

Crystalline and amorphous itraconazole was obtained from Johnson & Johnson Pharmaceutical Research and Development, Beerse, Belgium. The following excipients were used in the studies: hydroxypropyl methylcellulose (HPMC) 2910 5mPas (Aqualon, Hercules, Zwijndrecht, the Netherlands), Cremophor RH40 (BASF, Hamoir, Belgium), Polysorbate 20 (Codibei NV, Zaventem, Belgium), α -tocopheryl succinate esterified to polyethylene glycol 1000 (TPGS) (Eastman Chemical Company, Anglesey, UK). HP β CD was obtained from Roquette (Lestrem, France) and was characterized by a degree of substitution of 4.2 based on an FT-IR method (Michaud and Icart, 2001). SBE β CD was obtained from CyDex Pharmaceuticals, Inc. (Lenexa, KS, USA). Phosphate-citrate buffers were used for pHs 4 and 7 media. The pH 7 buffer contained a mixture of a 0.1 M citric acid solution and 0.2 M disodium hydrogen phosphate solution (17.65:82.35 v/v) while the pH 4 buffer contained a 51.45:38.55 v/v mixture of the citrate and phosphate components.

2.2. Phase-solubility analysis

Complexes were prepared by sonicating an excess of itraconazole in various concentrations of aqueous HP β CD or SBE β CD ranging from 0 to 20% w/v prepared in several systems (water, 0.1N HCl, 0.01N HCl and pHs 4 and 7 citrate-phosphate buffer) (Peeters et al., 2002). After 10 min of sonication, the suspensions were equilibrated for 3 days at which time, a small volume of the supernatant was withdrawn and filtered through a 0.45 μ m polyvinylidene difluoride (PVDF) membrane (Nihon Millipore). Samples were then diluted with 0.01N HCl and analyzed by UV (at 254 nm) using a Hewlett Packard 8451B diode array spectrophotometer. Experiments were completed in triplicate. The solubility and chemical stability of itraconazole was also assessed by HPLC in certain experiments. The systems configuration included a Varian LC 9010 solvent pump, a Varian 9096 autosampler fitted with a 10 μ L sample loop and a Varian 9065 Polychrom diode array detector (for itraconazole, λ = 268 nm) dedicated to a Compac Descpro PC. Samples were eluted on an RP 18

Hypersil ODS column (10 cm $\times$ 4.0 mm i.d., 3 μ m particle size) using a flow rate of 1.6 mL/min and a mobile phase composition of ammonium acetate (0.5%):methanol:acetonitrile (35:14:51). Dibutyl phthalate served as the internal standard. Under these conditions, the retention time of itraconazole was 4.0 min and for the internal standard, 4.62 min. To avoid adsorption of the drug on the filters and transferring devices, the membranes and syringes were treated using a saturated solution of itraconazole in 0.01N HCl. The syringes and filters were flushed with 3 mL of the itraconazole solution four times prior to analytical sample handling. Itraconazole is chemically stable under the conditions of the analysis. The stoichiometry of various phase-solubility isotherms is estimated by curve-fitting to various polynomials to generate trial values as presented in the appropriate figures.

2.3. Solubilization by surfactants

An excess of itraconazole was added to water adjusted to pH 5 containing various percentages of TPGS (2.5, 5, 10, 15, 20% w/v) and the sample were equilibrated for 10 days. Samples were filtered through a 0.45 μ m PVDF membrane and analysed by HPLC as described in the previous section.

2.4. Supersaturation testing

Several types of experiments were completed all of which were based on the co-solvent method (Raghavan et al., 2001, 2003; Davis and Hadgraft, 1991). In this approach, a drug in a solvent providing good solubility is added to a second solvent in which the drug has little or no solubility. This creates a situation where highly supersaturated solutions can be almost instantaneously formed and is similar, in form, to the techniques used to assess kinetic solubility. In a preliminary assessment, a solution of itraconazole was prepared in dimethylformamide (DMF) (50 mg/mL). The solution was then added dropwise into the dissolution media until a precipitate formed. Dissolution was completed according to the USP (apparatus II) using 300 mL of 0.1N HCl equilibrated at 37°C with or without 500 mg HPMC. The solution is stirred at 150 rpm. At 5, 30, 60 and 120 min after addition of the solution, a small volume of the suspension is removed from the vial, filtered and the concentration determined by UV as described above. For the second type of experiment, a screening assay was developed. In this instance the 50 mg/mL solution of itraconazole in DMF was added in small aliquots over a prescribed time period into 10 mL of dissolution solvent, in this case 0.01N HCl with (2.5% w/v) and without the excipient to be tested. Just at the point when a precipitate was observable, the addition of the drug solution was stopped and at 5, 30, 60 and 120 min thereafter the dissolution bath was sampled, the sample filtered and assayed spectrophotometrically to provide the concentration of itraconazole remaining in solution as described in the section above. The third group of experiments, which extended the characterization of TPGS, HP β CD and SBE β CD-based systems, were similarly completed however they used an n = 3 and provided additional time points at 180, 240 and 1440 min to better characterize the longevity of the supersaturated system.

3. Results

3.1. Phase-solubility analysis

Phase-solubility analysis of itraconazole in the presence of the two cyclodextrins was completed in turn (Loftsson et al., 2002, 2005; Peeters et al., 2002; Higuchi and Connors, 1965; Brewster and Loftsson, 1999, 2007). The total solubility (S_{Total}) of a drug as a function of cyclodextrin concentration is given by:

$$S_{\text{Total}} = S_0 + m[D_m\text{CD}]$$

where S_0 is the drug concentration in the absence of cyclodextrin and m refers to the stoichiometry of the drug-cyclodextrin complex. If one cyclodextrin molecule interacts with one drug molecule, complex characteristic can be assessed in part using phase-solubility analysis such that the slope of the phase-solubility relationship can be given by:

$$\text{Slope} = \frac{S_0 K_{1:1}}{S_0 K_{1:1} + 1}$$

where $K_{1:1}$ is the stability constant for the complex. Higher order complexes require other techniques for the calculation of their stability constants. Historically, higher order complexation (that is the interaction of one drug with a number of cyclodextrin units) has been suggested by a positive deviation from linearity for the phase-solubility relationship and deconvolution of the curve can give numerical solutions for the stability constants (Higuchi and Connors, 1965; Brewster and Loftsson, 1999). For a second-order relationship, fitting the data to a quadratic model is appropriate:

$$S_{\text{Total}} = S_0 + K_{1:1}S_0[\text{CD}] + K_{1:1}K_{1:2}S_0[\text{CD}]^2$$

however this model assumes that the total CD concentration is estimated by the free (i.e., uncomplexed) CD concentration. If this assumption does not hold, the paradigm suggested by Higuchi and Kristiansen should be used (Peeters et al., 2002; Higuchi and Kristiansen, 1970). For 1:3 interactions, a third-order curve-fitting is appropriate. To account for the concentration of bound cyclodextrin in these cubic relationships, Peeters et al. (2002) suggested a non-linear optimization technique based on an iterative Nelder-Mead approach.

The relationships between the two solubilizers and drug in water (pH ~6.5), pHs 7 and 4 buffer, 0.01N HCl (pH ~2) and 0.1N HCl (pH ~1.5) are given in Figs. 3–7. In water, the isotherm gives an A_p -type profile which can suggest the formation of higher order complexes at higher cyclodextrin concentrations. In addition, the fact that it is difficult to identify an initial linear segment of the isotherm may also suggest non-inclusion complex related phenomena including cyclodextrin aggregation or surfactant-based effects (Loftsson et al., 2004b). In any case, there is a significant effect of HP β CD on solubility of itraconazole such that the drug concentration increases from ~1 ng/mL in the absence of cyclodextrin to 4 μ g/mL at 2.5% w/v HP β CD and 330 μ g/mL at 20% w/v HP β CD (Fig. 3). For SBE β CD an A_p -type profile is also observed. Various groups have reported that this strongly suggests the intervention of non-inclusion complex effects since coulombic repulsion discourages higher order complexation based on charge interaction for this negatively charged cyclodextrin (Thompson,

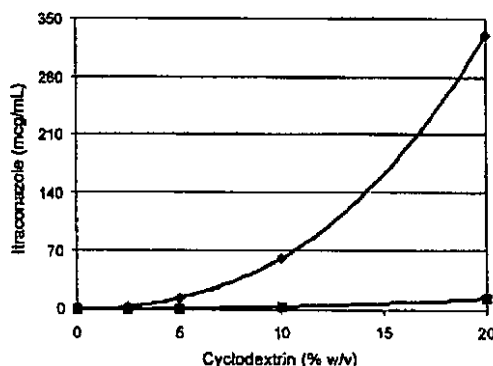


Fig. 3 – Effect of HP β CD (diamonds, blue line) and SBE β CD (squares, pink line) on the solubility of itraconazole in water ($n=3$). The calculated lines represent fitting of the data to a third-order model which was optimal in this case based on goodness-of-fit and correlation coefficients. For HP β CD, $r^2 > 0.999$ and for SBE β CD, $r^2 > 0.999$. Note that error bars are within the symbol.

1997; Loftsson et al., 2002; Okimoto et al., 1996; Zia et al., 1997). The apparent equilibrium solubility of itraconazole in 2.5% SBE β CD was measured at 2 μ g/mL while that in 10% w/v cyclodextrin the value was 14 μ g/mL. In a buffered media at pH 7, the data was qualitatively similar (Fig. 4). At pH 4, HP β CD continued to provide for better relative solubility as a function of cyclodextrin concentration although the effect was lower (21-fold increase for HP β CD compared to SBE β CD at pH 7 and 8-fold at pH 4 at 10% w/v cyclodextrin) (Fig. 5). Curve-fitting suggested that complexation for itraconazole with both HP β CD and SBE β CD at pHs 7 and 4 was best using a cubic equation consistent with published work on itracona-

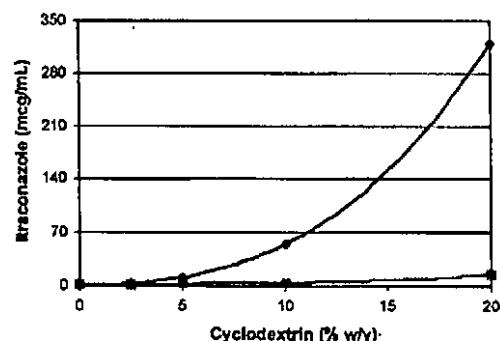


Fig. 4 – Effect of HP β CD (diamonds, blue line) and SBE β CD (squares, pink line) on the solubility of itraconazole in pH 7 phosphate-citrate buffer ($n=3$). The calculated lines represent fitting of the data to a third-order model which was optimal in this case based on goodness-of-fit and correlation coefficients. For HP β CD, $r^2 > 0.999$ and for SBE β CD, $r^2 = 0.998$. Note that error bars are within the symbol.

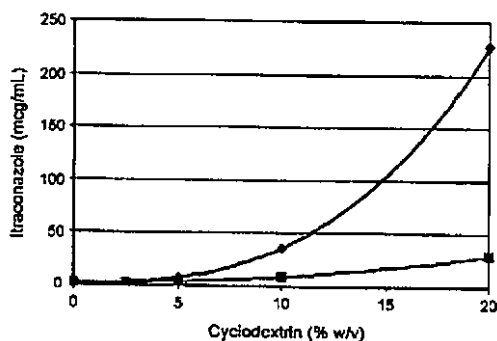


Fig. 5 – Effect of HPβCD (diamonds, blue line) and SBEβCD (squares, pink line) on the solubility of itraconazole in pH 4 phosphate-citrate buffer ($n=3$). The calculated lines represent fitting of the data to a third-order model which was optimal in this case based on goodness-of-fit and correlation coefficients. For HPβCD, $r^2 > 0.999$ and for SBEβCD, $r^2 > 0.999$. Note that error bars are within the symbol.

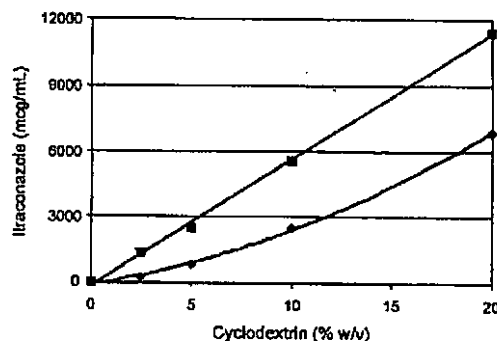


Fig. 7 – Effect of HPβCD (diamonds, blue line) and SBEβCD (squares, pink line) on the solubility of itraconazole in 0.1N HCl ($n=3$). The calculated lines represent fitting of the data to a second-order model in the case of HPβCD ($r^2 > 0.999$) and a first-order model in the case of SBEβCD ($r^2 > 0.999$) which was optimal in this case based on goodness-of-fit and correlation coefficients. Note that error bars are within the symbol.

zole and HPβCD (Peeters et al., 2002). When 0.01 or 0.1N HCl was used as the complexation medium, the relative order of the cyclodextrin with regard to solubilization switched such that the SBEβCD become the better solubilizer. This, along with the effects seen at pH 4, is consistent with the work of Zia et al. (2001) wherein ionization of bases tended to reduce interactions with HPβCD while association constants with SBEβCD were less effected. Furthermore, increasing ionic strength tended to reduce complexation efficiency between SBEβCD and charged basic drugs due to charge shielding which minimizes the coulombic attraction. In any case, at 10% w/v cyclodextrin, SBEβCD solubilized approximately 1600 and 5500 μg/mL itraconazole at 0.01 and 0.1N HCl, respec-

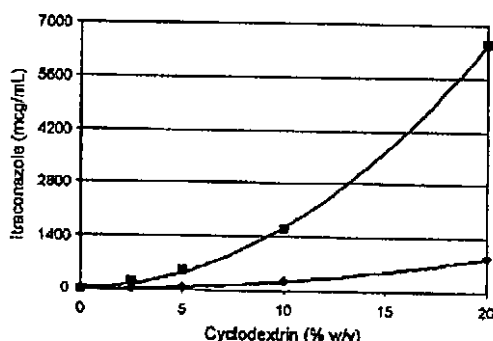


Fig. 6 – Effect of HPβCD (diamonds, blue line) and SBEβCD (squares, pink line) on the solubility of itraconazole in 0.01N HCl ($n=3$). The calculated lines represent fitting of the data to a second-order model which was optimal in this case based on goodness-of-fit and correlation coefficients. For HPβCD, $r^2 > 0.999$ and for SBEβCD, $r^2 > 0.999$. Note that error bars are within the symbol.

tively, while the corresponding values for HPβCD were 253 and ~2500 μg/mL itraconazole, respectively. At pH 2 (0.01N HCl) the isotherms for both HPβCD and SBEβCD were best fit to a quadratic function (Fig. 6). This behavior had been previously reported for HPβCD by Peeters et al. (2002) and Miyake et al. (1999). At pH 1, the isotherm for HPβCD could best be fit to a quadratic function while that for SBEβCD was linear (Fig. 7). The effect of decreasing pH on the stoichiometry of complexes for HPβCD with weak bases has been suggested to be related to changes in structural affinity as a function of protonation (Peeters et al., 2002; Johnson et al., 1994). The SBEβCD data are interesting. As indicated above, the geometry and structure of this highly anionic cyclodextrin argues against the formation of higher order complexes especially given the conformational nature of the itraconazole molecule (Loftsson et al., 2002; Peeters et al., 2002; Zia et al., 2001). Having said that there seems to be a change in isotherm curvature for the SBEβCD–itraconazole interaction in going from pHs 7 and 4, to pH 2 (0.01N HCl) to systems studied in 0.1N HCl. Whether this is related to non-complex features of cyclodextrin solubilization or other effects still needs to be investigated. It is possible that the low pH or higher ionic strength would not only change the drug but also the manner in which the cyclodextrin interacts with the drug. The pK_a of the SBEβCD is at least 2 units lower than the pH of the media at 0.1N HCl but charge shield or other effects might be involved as suggested above.

3.2. Solubilization by surfactants

Surfactants can enhance the solubility of poorly water-soluble lipophiles through micelle formation and other mechanisms (Rangel-Yagui et al., 2005; Torchilin, 2001). Solubilization of a drug by a surfactant is given by the following equation:

$$S_{\text{total}} = S_0 + k(C_{\text{surfactant}} - \text{CMC})$$

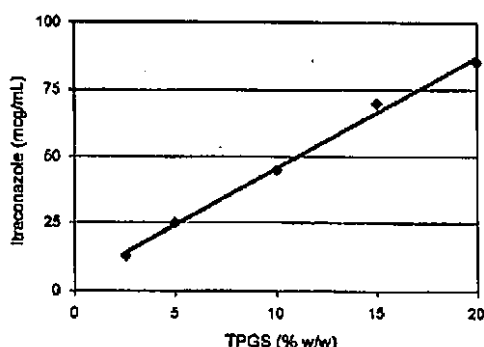


Fig. 8 – Effect of TPGS on the solubility of itraconazole.

where S_{Total} is the total concentration of drug solubilized, S_0 is the solubility of drug in the absence of the solubilizer, $C_{\text{Surfactant}}$ is the concentration of surfactant, CMC is the critical micellar concentration and κ is defined as:

$$\kappa = \frac{S_{\text{micelle}}}{C_{\text{micelle}}}$$

where S_{micelle} is the drug concentration within the micelle and C_{micelle} is the molar concentration of surfactant (Loftsson et al., 2002; Yalkowsky, 1999). If the CMC is significantly lower than the concentration of surfactant used, the solubility relationship simplifies to:

$$S_{\text{Total}} = S_0 + \kappa C_{\text{Surfactant}}$$

The interaction of itraconazole with TPGS is illustrated in Fig. 8. The CMC of TPGS is 0.02 w/w% meaning that the concentrations used in this study were all well above the CMC (Constantinides et al., 2006). The data indicate a linear relationship between the solubilizer and drug ($r^2 = 0.995$) with equilibrium solubilities of 13 $\mu\text{g/mL}$ at 2.5 w/w% and 85 $\mu\text{g/mL}$ at 20 w/w% TPGS. The κ value is 8.95×10^{-4} .

3.3. Supersaturation assays

To evaluate supersaturation, an assessment was completed for itraconazole in the presence and absence of HPMC. The equilibrium solubility of itraconazole at room temperature in 0.1N HCl is 6 and <1 $\mu\text{g/mL}$ in 0.01N HCl. In a first series of experiments, the hypothesis that HPMC stabilize supersaturated solutions of itraconazole was assessed. For this purpose, 300 mL of 0.1N HCl was used as the dissolution medium with or without added HPMC. Using the co-solvent approach, a solution of 50 mg/mL itraconazole in DMF was added to the media to generate a supersaturated solution of itraconazole at approximately 400 $\mu\text{g/mL}$ (66-fold increase over the equilibrium solubility) (Raghavan et al., 2001, 2003; Davis and Hadgraft, 1991). These levels were not physically stable and the drug concentration were reduced by 25% at the 120 min time point (Fig. 9). The total volume of DMF added to the dissolution vessel was <1%. When HPMC is added, the supersaturation levels are maintained through 120 min suggesting that this excipient improves the physical stability of the formed super-

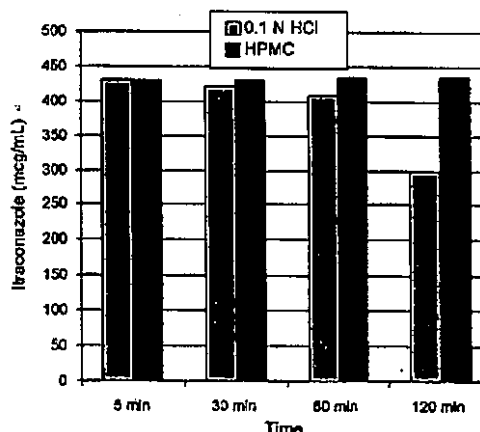


Fig. 9 – Supersaturation data for itraconazole in either 300 mL 0.1N HCl or 300 mL 0.1N HCl with 500 mg HPMC. Equilibrium solubility of itraconazole at 0.1N HCl is 7 $\mu\text{g/mL}$.

saturated solution. Having said that there was no effect on the level of supersaturation. HPMC and FVP have been shown to act as a solubilizer for various compounds including acetazolamide, prazepam and sulfamethoxazole via polymer-drug complexation (Loftsson et al., 1996). In this instance, solubilization of itraconazole by these polymers is negligible.

While this approach was useful in assessing the effect of excipients on supersaturation, it was cumbersome, required large volumes of dissolution medium and thus did not lend itself to screening. To address these issues, a downsized supersaturation test was developed to assess the effect of various excipient on both the extent of supersaturation and the stability of the formed supersaturated solutions (Vandecruys et al., 2006, 2007). The approach was again based on the co-solvent/solvent quench method but the assay was completed using 10 mL of a 0.01N HCl dissolution medium. This medium was selected based on both physiological relevance (it is similar to SGF without pepsin) and breadth of application since this was developed as a general screening tool. In the assay, three surfactants (Tween20, RH40, TPGS), two hydrophilic cyclodextrins (HP β CD and SB β CD) and PEG4000 were used. Data from a screening assay are presented in Fig. 10. In the media without additives, addition of itraconazole in DMF generated a supersaturated phase with concentration of 17 $\mu\text{g/mL}$. There was little if any effect of PEG4000 with supersaturation values and durations being similar to the blank. Addition of surfactants to the dissolution media allowed higher levels of itraconazole to be generated such that 400, 520 and 1450 $\mu\text{g/mL}$ were obtained in the presence of Tween20, Cremophor RH40 and TPGS, respectively. These values are supersaturated relative to the equilibrium values for itraconazole in media containing these surfactants. For TPGS, for example, 2.5% w/w of the excipient generates solubility for itraconazole of 13 $\mu\text{g/mL}$. The physical stability of kinetic systems was limited with the original supersaturation concentration in the Tween20 system reduced by 83% at 30 min and >95% by 120 min. Cremophor gave similar

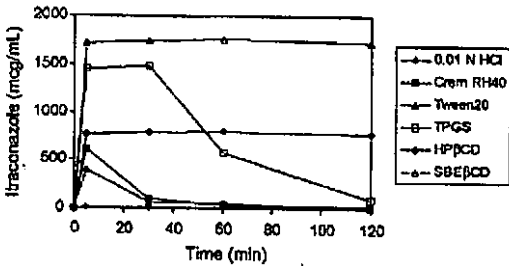


Fig. 10 – Supersaturation data for itraconazole in the presence of various excipients. Equilibrium solubility at the same excipient concentration (2.5% w/v) for TPGS was 13 µg/mL, HPβCD was 21 µg/mL and SBEβCD was 219 µg/mL.

results. TPGS was associated with a somewhat more robust profile with maintenance of supersaturation through 30 min, a 60% decline in concentration by 60 min but a greater than 90% decline by 120 min.

The cyclodextrins tested gave a different profile. The addition of 2.5% w/v HPβCD into the dissolution media resulted in a measured concentration of 770 µg/mL which represented an increase above both the sample without the excipient (16 µg/mL) as well as the solubility determined at equilibrium in phase-solubility analysis (21 µg/mL). Note that the organic solvent (DMF) at the concentrations present in the samples (3.3% w/v in the worst case) did not affect the solubility of itraconazole (data not shown). Furthermore, the concentration of drug in the system was maintained throughout the time course of the experiment. The effect of the SBEβCD was even greater with itraconazole concentrations increased to 1700 µg/mL and good stability over the 120 min experiment. Again these levels were in excess of those generated in the absence of an excipient or at equilibrium in 0.01N HCl containing 2.5 w/v % SBEβCD (206 µg/mL). Interestingly, even though SBEβCD is a better complexing agent for itraconazole in 0.01N HCl, the extent of supersaturation is greater for the HPβCD system (36 vs. 8-fold). This may suggest that mechanisms other than simple complex formation are responsible for the stabilizing effects which may include inhibition of nucleation

and crystal growth. Based on these screening experiments, more detailed assessments were completed on the supersaturation effects of TPGS, HPβCD and SBEβCD as presented in Table 1. These data, completed in triplicate, give an indication of the robustness of the technique in that a comparison of Fig. 10 and Table 1 suggest reasonable concordance. The patterns generated were similar with regard to supersaturation effects. In addition, the stability of the formed supersaturated systems was followed over a 24 h time course rather than 120 min. The itraconazole concentrations in media containing TPGS were stable through 60 min (rather than 30 min in the screening experiment) however the concentration in solution at 120 min were only 6% of that at 60 min. Again, the cyclodextrins better support supersaturation with minimal loss through 240 min followed by a decline such that the 24 h sampling point was 8% of the 240 min values in the case of HPβCD and 12.5% in the case of the SBEβCD. Interestingly, the standard deviations associated with supersaturated concentrations of itraconazole increase at the time point(s) just prior to solution “collapse” as seen at 60 min for TPGS, 180 and 240 min for HPβCD and at 240 min for SBEβCD. At 24 h, the values for itraconazole were 13-fold and 40-fold higher in the HPβCD and SBEβCD systems, respectively, when compared to the solvent blank.

4. Discussion

As reviewed by Macie and Grant (1986), the actual process of nucleation is not known with any degree of certainty but a nucleus must form to serve as a center for deposition of solute from the supersaturated system allowing for crystal growth. Crystal growth is believed to take place in three steps including (1) diffusion of the molecule from the bulk media to the solid crystal interface, (2) the adsorbed molecule, through a surface reaction, becomes part of the crystal lattice and the heat of crystallization released and (3), the heat of crystallization is conducted to the bulk medium.

The rate at which nucleation and crystallization takes place is determined by a number of thermodynamic, kinetic and molecular recognition phenomenon (Miller et al., 2007). A solid phase will crystallize out of solution if the chemical potential of the solid phase is less than that of the dissolved phase. In order for crystallization to proceed, supersaturation must

Table 1 – Effect of selected excipients at 2.5% w/v on the concentration ± S.D. (n = 3) and solution physical stability of itraconazole added in a DMF solution from 5 min to 24 h post-addition

Time (min)	Itraconazole (µg/mL)			
	0.01N HCl	TPGS	HPβCD	SBEβCD
5	15.6 ± 0.1	1365 ± 13.5	775.0 ± 6.1	1739.3 ± 13.2
30	15.5 ± 0.3	1373 ± 16.8	778.7 ± 3.2	1731.0 ± 4.4
60	15.1 ± 0.2	1215 ± 114	772.8 ± 2.8	1718.3 ± 23.6
120	15.1 ± 0.2	79.2 ± 3.6	774.3 ± 5.8	1713.0 ± 5.3
180	10.1 ± 1.3	66.2 ± 3.2	757.0 ± 17.8	1683.7 ± 17.0
240	5.8 ± 0.4	61.9 ± 3.2	736.7 ± 15.2	1379.7 ± 373.2
1440	4.3 ± 0.1	45.3 ± 2.9	58.3 ± 2.2	173.7 ± 3.5
Final pH	2.02	2.17	2.10	2.29
DMF% in final solution	0.03%	2.9%	1.5%	3.3%

occur as this is the driving force for nucleation and crystal growth. The rate of nucleation is generally expressed by the following equation:

$$J = N_0 \nu \exp \left(\frac{-16\pi v^2 \gamma_{12}^3}{3(k_B T)^3 (\ln(c/s))^2} \right)$$

where J is the number of nuclei formed per unit time per unit volume, N_0 is the number of molecules of the crystallizing phase per unit volume, ν is the frequency of transport through the nucleus–liquid interface, v is the molecular volume of the crystallizing solute, γ is the interfacial energy per unit area, k_B is the Boltzmann constant, T is temperature and c/s is the extent of supersaturation. As described by Miller et al. (2007), solubility, interfacial tension and viscosity are the solvent-related features most likely to affect nucleation.

Materials that may inhibit nucleation or crystal growth have been reported (Raghavan et al., 2001, 2003; Gao et al., 2004; Terayama et al., 2004). These materials have several potential actions including altering bulk properties such as surface tension or saturation solubility, changing the adsorption layer at the crystal–medium interface, selectively adsorbing to the crystal interface thereby blocking crystal growth, being adsorbed into growth layers and thereby disrupting growth layers across the surface, adsorbing into surface imperfections causing rough surfaces to become flat and altering the surface energy of the crystal face.

The effect of pharmaceutical polymers such as methyl cellulose (MC), HPMC and PVP has traditionally been ascribed to several of the effects described above (Raghavan et al., 2001). Simonelli et al. (1970) suggested that the inhibitory effect of PVP on the nucleation and growth of sulfathiazole was associated with the polymer forming a porous net around growing crystals such that crystal growth was relegated to the formed pores in the polymeric network. Others have suggested that the polymers adsorb to the crystal thereby inhibiting the addition of molecular units from the bulk solution (Raghavan et al., 2001). Additional mechanisms that have been forwarded include the suggestion that these polymers can solubilize the drug of interest through complexation thereby reducing the degree of supersaturation (Rodríguez-Hornedo and Murphy, 1999; Strickley, 2004). Raghavan et al. (2001, 2003) correlated the hydrogen bond donor and acceptor properties of hydrocortisone acetate, HMPC and PVP to explain the differential inhibitory effect of the polymers on drug nucleation and crystal growth. The working theory developed included the interaction of the polymer in the unstirred water layer separating the growing crystal and the bulk media. Specifically, the polymers can hydrogen bond to growth sites on the crystal thereby blocking growth. In addition, even though these polymer may be rejected at the crystal interface, they may, for a variety of reasons, accumulate in the unstirred water layer and increase resistance to diffusion. These effects were predicted to be higher for those faces of the growing crystal that have a higher hydrogen bonding potential meaning that the habit of the crystals may change along with their growth rates. Yokoi et al. (2005) suggested HPMC and sugar esters (sucrose fatty acid esters) exerted differential effects on the stabilization of aqueous suspensions of cefditoren pivoxil based on the their nature and extent of surface binding. Thus, the inter-

action of HPMC was such that crystal growth was selectively inhibited where the sugar esters were predicted to inhibit both nucleation and crystal growth.

In the case of hydrophilic cyclodextrins, Xiang and Anderson (2002) found that the generation of supersaturated solutions of a novel anti-cancer agent (Silatecan) was possible by converting a precursor to the lactone at an appropriate pH in a SBECSD solution. These systems were stable for up to 3 days after preparation and could be lyophilized and successfully reconstituted. Uekama et al. (1992) have also assessed the ability of CD to inhibit crystal growth in drug formulations. In a spray-dried dispersion of nifedipine and HPBCD, crystal growth was minimized such that neither dissolution changes nor attenuation of oral bioavailability in dogs were observed in aged material compared to freshly prepared dispersions. Furthermore, Torres-Labandeira et al. (1991) found that supersaturated solutions of pancratistatin could be prepared in HPBCD solutions. In their preparation procedure, the drug was treated with ammonia after which the aqueous ammoniacal solution was removed by freeze-drying. Reconstitution of the powder allowed for solutions as high as 9 mg/ml to be prepared. While precipitation was observed in these samples, the use of polyethylene containers as well as HP-CD provided for a significantly prolonged latency period prior to precipitation. Iervolino et al. (2000) reported that HPBCD inhibits nucleation of ibuprofen in supersaturated PG-water systems and distinguished this effect from that of HPMC which was effective in preventing crystal growth. Dias et al. (2003) suggested a stabilizing effect of HPBCD–HPMC systems on supersaturated diclofenac solutions. On the other hand, Ma et al. (1996) found that HPBCD accelerated crystal growth of norethindrone acetate in a supersaturated acrylic adhesive.

The suggested mechanism for inhibition of nucleation and crystal growth observed in the case of pharmaceutical polymers may also apply to the chemically modified hydrophilic cyclodextrins examined. Specifically, cyclodextrins solubilize itraconazole by inclusion complex formation with possible contribution from non-complex-based mechanisms. This increases the chemical potential of the drug in solution, increases the apparent saturation solubility and decreases the extent of supersaturation (Iervolino et al., 2000, 2001; Strickley, 2004). As noted, this effect alone does not seem capable of accounting for the effect on the formed supersaturated solution based on the magnitude of the measured changes on apparent solubility. On the other hand, Iervolino et al. (2000) suggested that the interaction of ibuprofen with HPBCD changes the metastable zone as a function of drug–cyclodextrin interaction and solubilization. The cyclodextrins may also interact with the growing crystal in a manner analogous to HMPC with hydrogen bonding to sites associated with crystal growth as well as accumulation in the unstirred water layer resulting in an increase in diffusional resistance secondary to viscosity or even the ability of the cyclodextrin to complex with drug monomers inhibiting efficient mass transfer at the interface. In some cases, the preparation method can promote the formation of supersaturated systems as in cases where ammonia or volatile acids are added to facilitate complexation and then removed via lyophilization (Loftsson et al., 2004c). Pedersen (1997) suggested that supersaturation was more likely when a molecular

complex was assessed compared to freeze-dried or ground materials containing mixture of the drug and cyclodextrin based on the rate of cyclodextrin dissolution. For complexes, the dissolution rate is limited meaning that drug release as a function of cyclodextrin availability provides for supersaturation in contrast to other systems where the rate of cyclodextrin dissolution is larger providing a greater opportunity for solution complexation and an increase in the saturation solubility of the drug.

Loftsson et al. (2007) has also suggested that the manner by which excipient alter the cohesive nature of water may effect induction time and nucleation. To this point, materials that enhance water structure (i.e., kosmotropes) may stabilize supersaturated solution while those reducing water structure (i.e., chaotropes) may have the opposite effect. Cyclodextrin have been suggested to be sugar-like in their effect acting as a kosmotrope at higher concentrations. Finally, certain types of cyclodextrin nanoparticles can be formed using techniques similar to the solvent quench approach used herein (Duchêne et al., 1999). Importantly, these techniques have been applied to amphiphilic cyclodextrin derivatives in which the hydroxyl functions are appended with alkyl ethers, esters or amides would seem less relevant to hydrophilic cyclodextrins such as HP β CD and SB β CD. Having said that, the aggregation of hydrophilic cyclodextrins has been suggested in aqueous systems (Brewster and Loftsson, 2007).

5. Conclusions

Cyclodextrins have long been valued for their pharmaceutical properties which enable the formulation of a number of biopharmaceutically challenging drugs and drug candidates. The basis for these effects were generally attributed to the ability of cyclodextrins to form dynamic inclusion complexes in solution. Recent data suggest that some of the solubilizing potential of the cyclodextrins may be related to non-inclusion complex related phenomena such as cyclodextrin aggregation or surfactant-like properties inherent in these systems. A third possible action for these useful excipients may be their ability to stabilize formed supersaturated solutions. Such effects may be very useful in the preparation of dosage forms that are designed to operate under carrier-controlled dissolution (i.e., solid dispersions) and give rise to supersaturation and improved oral bioavailability.

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